IDENTIFICATION OF QTLs AND GENES FOR DROUGHT TOLERANCE USING LINKAGE MAPPING AND ASSOCIATION MAPPING APPROACHES IN CHICKPEA (*Cicer arietinum*)

THESIS SUBMITTED TO
OSMANIA UNIVERSITY FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN GENETICS

Spurthi Nagesh Nayak

DEPARTMENT OF GENETICS
OSMANIA UNIVERSITY, HYDERABAD

2010

CERTIFICATE

This is to certify that Ms. Spurthi Nagesh Nayak has carried out the research work embodied in the present thesis entitled "**Identification of QTLs and genes for drought tolerance using linkage mapping and association mapping approaches in chickpea (*Cicer arietinum*)**" for the degree of Doctor of Philosophy under the joint-supervision of Dr. Rajeev K Varshney, Principal Scientist, Applied Genomics Laboratory, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru and Prof. P B Kavi Kishor, Department of Genetics, Osmania University, Hyderabad.

This is an original research work carried out at ICRISAT and is satisfactory for the award of Doctor of Philosophy. Any part of this work has not been submitted for the award of any degree or diploma of any other University or Institute.

Dr. Rajeev K Varshney _____

Supervisor

Prof. P B Kavi Kishor _____

Co-supervisor

DECLARATION

I hereby declare that the research work presented in this thesis entitled "**Identification of QTLs and genes for drought tolerance using linkage mapping and association mapping approaches in chickpea (*Cicer arietinum*)**", has been carried out under the supervision of Dr. Rajeev K Varshney at International Crops Research Institute for Semi-Arid tropics (ICRISAT), Patancheru and co-supervision of Prof. P B Kavi Kishor at Department of Genetics, Osmania University, Hyderabad.

This work is original and no part of the thesis has been submitted earlier for the award of any degree or diploma of any University.

Date: **(Spurthi Nagesh Nayak)**

Place: Hyderabad

ACKNOWLEDGMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or other contributed and extended their valuable assistance in the preparation and completion of this study.

First and foremost, I am sincerely obliged and indebted to my supervisor ***Dr. Rajeev K Varshney****, Principal Scientist-Applied Genomics, ICRISAT and Leader Theme1-Comparative and Applied Genomics (CAG), Generation Challenge Programme for his peer supervision, constructive comments, enthusiastic discussions, endless support, encouragement and able direction throughout my research project. It was indeed a rare privilege for me to work under his able guidance. I also thank him from bottom of my heart for the critical evaluation and emending suggestions in the preparation of scientific papers for journals and in achieving the final form of dissertation.*

I wish to record my profound gratitude and sincere thanks to the personification of generosity and kindness to my co-supervisor ***Prof. P B Kavi Kishor****, Department of Genetics, Osmania University for his expedient advice, ever-encouraging suggestions, timely help, invaluable support, kind concern and consideration regarding my academic requirements during this tenure of research work.*

I take this opportunity to thank ***Dr. Dave Hoisington*** *for giving me opportunity to work in one of the best labs of International standards. With respect, regards and immense pleasure, I wish to acknowledge and express sincere thanks from my heart thanks to several scientists including* ***Dr. Jayashree Balaji*** *for the valuable suggestions during the sequence analysis;* ***Dr. Junichi Kashiwagi*** *and* ***Dr. L Krishnamurthy*** *for providing the phenotypic data;* ***Dr. T Nepolean*** *for his help during mapping;* ***Dr. H D Upadhyaya*** *and* ***Dr. P M Gaur*** *for providing the genetic material used in the present study.*

It is my immense pleasure to render sincere regards to all the collaborators of the project especially ***Dr. Doug Cook*** *(University of California, Davis, USA),* ***Dr. Peter Winter*** *(GenXPro/ University of Frankfurt, Germany),* ***Dr. Dominique This*** *(CIRAD, Montpellier, France) for their valuable support and co-operation to complete this invaluable piece of work. I also thank* ***Dr. Pawan Kulwal*** *(MPKV, Rahuri) and* ***Dr. Joy K Roy*** *(NABI, Mohali) for their help rendered during analysis. I take this opportunity to thank* ***Dr. Sabhyata Bhatia*** *(NIPGR, New Delhi) for kindly providing the microsatellite markers used in the present study. I render special thanks* ***Dr. RR Mir****, Visiting Scientist, ICRISAT for his help in finalizing the thesis.*

With immense pleasure, I express my cordial thanks to Mrs. Seetha Kannan, Mrs. Manjula B, Mr. Prasad KDV and Mr. Murali Krishna Y for their administrative help. Words are less to express my gratitude to Mr. A Gafoor, Mr. K Eshwar and Mr. G Somaraju for teaching me the laboratory techniques at the beginning of the research work. I shall be failing in my duty if I do not express my cordial thanks to all my labmates and friends especially Srivani, Pavana, Nicy, Sivakumar, Mahendar, Ramu, Rajaram, Abhishek, Jalaja, Gautami, Susmitha-Krishna, Prathima, Ramesh, Anuja, Neha, Rachit, Ramani, Krithika and *Sunita for their support during my happiness and hard times. I also thank all the friends and staff at Department of Genetics and the Principal Office of Osmania University, Hyderabad for their kind help and co-operation.*

I also thank the Bioinformatics and Biometrics unit for the assistance given at the time of need. Assistance rendered by the members of Central Support Lab, library and Learning Systems Unit at ICRISAT is gratefully acknowledged.

*I feel blessed to have my husband **P Srinivasa Reddy** in my life, who was the constant inspiration for me to carry out the research and gave emotional support whenever I had difficult times. I feel indebted to my parents in-law **Mr. Venkatanarayana** and **Mrs. Varalakshmi** for their constant support and encouragement throughout the tenure of research work.*

*I avail this opportunity to thank my parents who are the Almighty's most treasured gift to me. I feel scanty of words to magnitude the boundless love and tireless sacrifice and affection showed on me by my parents, **Mr. Nagesh** and **Mrs. Nagaveni** and affection of my sister **Savita** and brother **Manjunath** that I could attain academic heights to accomplish my doctoral degree. And I express my deepest adoration to them for teaching me the etiquettes of life.*

The Council of Scientific and Industrial Research (CSIR), New Delhi, India is greatly acknowledged for providing me fellowship to carry out my research work. I also acknowledge the financial support from Generation Challenge Programme (GCP) to the ADOC and Tropical Legumes1 (TLI) projects where I worked in.

I convey my whole hearted thanks to many of my well wishers and other friends requesting their forgiveness for not mentioning them here by name.

Date:
Place: Hyderabad **(Spurthi N Nayak)**

CONTENTS

ABBREVIATIONS

˚C	:	degree Celsius
µl	:	microliter
ABA	:	Abscisic acid
ADOC	:	Allelic Diversity at Orthologous Candidate genes
AFLP	:	Amplified Fragment Length Polymorphism
ANOVA	:	Analysis of Variance
ASR	:	Abscisic acid Stress and Ripening
BAC	:	Bacterial Artificial Chromosome
BES	:	BAC-end Sequences
BIBAC	:	Binary Bacterial Artificial Chromosome
BLAST	:	Basic Local Alignment Search Tool
bp	:	base pair
CaM	:	Cicer arietinum Microsatellites
CAP2	:	Cicer Apetala gene 2
CAPS	:	Cleaved Amplified Polymorphic Sequences
cDNA	:	complementary DNA
CIM	:	Composite Interval Mapping
cM	:	centiMorgan
COS	:	Conserved Orthologous Set
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DArT	:	Diversity Array Technology
dCAPS	:	derived Cleaved Amplified Polymorphic Sequences
DDBJ	:	DNA Data Bank of Japan
DIVEST	:	Diversity Estimator
DNA	:	Deoxyribonucleic Acid
DREB	:	Drought Responsive Element Binding proteins
EMBL	:	European Molecular Biology Laboratory
EREBP	:	Ethylene Responsive Element Binding Protein
EST	:	Expressed Sequence Tag
GCP	:	Generation Challenge Programme
GSS	:	Genome Survey Sequences
ICCM	:	ICRISAT Chickpea Microsatellites

ICRISAT	:	International Crops research Institute for the Semi-Arid Tropics
Indel	:	Insertion deletion
INRA-CNG	:	Institut National de la Recherche Agronomique-Centre National de Génotypage
ISSR	:	Inter Simple Sequence Repeats
JCVI	:	J. Craig Venter Institute
kbp	:	kilo base pairs
LD	:	Linkage Disequilibrium
LDW	:	Leaf Dry Weight
LG	:	Linkage Group
LOD	:	Logarithm of odds (base 10)
LnPD	:	Natural logarithm of probability of data
MAS	:	Marker-Assisted Selection
Mb	:	Million bases
MISA	:	MIcroSAtellite
mM	:	milliMolar
MSA	:	Multiple Sequence Alignment
NCBI	:	National Center for Biotechnology Information
NIPGR	:	National Institute for Plant Genome Research
ng	:	nanograms
NLS	:	Nuclear Localization Sequence
PAGE	:	Polyacrylamide Gel Electrophoresis
PCR	:	Polymerase Chain Reaction
PIC	:	Polymorphism Information Content
QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RD	:	Rooting Depth
RDW	:	Root Dry Weight
RFLP	:	Restricted Fragment Length Polymorphism
RGA	:	Resistance Gene Analogues
RIL	:	Recombinant Inbred Line
RL	:	Root Length
RLD	:	Root Length Density
RSA	:	Root Surface Area
RT	:	Root Dry Weight/Total Dry Weight ratio

RV	:	Root Volume
SCAR	:	Sequence Characterized Amplified Region
SDW	:	Shoot Dry Weight
SIM	:	Simple Interval Mapping
SNP	:	Single Nucleotide Polymorphism
SMA	:	Single Marker Analysis
SPS	:	Sucrose Phosphate Synthase
SSCP	:	Single Strand Conformational Polymorphism
SSR	:	Simple Sequence Repeats
SSRIT	:	Simple Sequence Repeat Identification Tool
StDW	:	Stem Dry Weight
STMS	:	Sequence Tagged Microsatellite Sites
SuSy	:	Sucrose Synthase
TIGR	:	The Institute for Genome Research

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Low levels of polymorphism and lack of sufficient numbers of molecular markers such as microsatellite or simple sequence repeats (SSRs) are the main constraints in chickpea improvement. Hence to increase the number of SSR markers, 1,655 novel SSRs were developed from SSR-enriched genomic library (311) and mining the BAC-end sequences (1,344). These markers, along with already available markers were tested for polymorphism on parental genotypes of the inter-specific (ICC 4958 × PI 489777) and intra-specific mapping population (ICC 4958 × ICC 1882). As a result, a comprehensive inter-specific genetic map of 621 marker loci, spanning a genetic distance of 984.11cM was prepared. For identification of QTLs for drought tolerance traits, an intra-specific map (segregating for drought tolerance related traits) consisting of 230 SSR loci, spanning 466.95cM genetic distance was constructed after screening 2,409 SSR markers. The QTL analysis detected 47 significant QTLs for the ten root traits, of which seven were major QTLs (>20% phenotypic variation). The QTL analysis revealed the presence of a "QTL hot-spot" region explaining 49.9% phenotypic variation was detected. For undertaking association mapping for drought tolerance, two approaches namely candidate gene sequencing and genome-wide scanning approaches were used on the reference set comprising of 318 chickpea genotypes. In case of the candidate gene sequencing approach, five candidate genes associated with drought tolerance were selected namely, chickpea Apetala2 (*CAP2*-which is the homolog of *DREB2A*), abscisic acid stress and ripening hormone (*ASR*), sucrose synthase (*SuSy*), sucrose phosphate synthase (*SPS*) and *ERECTA* genes. Highest nucleotide diversity was observed in case of *ERECTA* followed by *ASR* gene and the lowest for *CAP2* gene. Association analysis based on candidate gene sequencing showed the association of two genes (*ASR* and *CAP2* promoter) with drought tolerance related traits. Apart from this, the genome-wide association studies using 1,157 DArT markers showed the significant association of 26 DArT markers with eight drought tolerance related traits.

In summary, developed genomic resources such as SSR markers and genetic maps will be useful for chickpea genetics and breeding applications. Moreover, markers and genes associated with QTLs for drought tolerance related traits will be useful for molecular breeding for drought tolerance in chickpea improvement.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a self-pollinated diploid (2n = 2x = 16) annual legume with a genome size of ~738 Mbp (Arumuganathan and Earle 1991). Chickpea (*Cicer arietinum* L.), the third most important cultivated grain legume in the world after soybean and beans (FAOSTAT 2009). It is the member of the *Leguminosae* family, which includes 18,000 species, grouped into 650 genera (http://www.ildis.org/Leguminosae). Chickpea is commonly called as gram, Bengal gram or garbanzo bean is mainly cultivated in the Indian subcontinent, West Asia, the Mediterranean, North Africa and the America (Croser et al. 2003). Over 95% of chickpea production area and consumption occur in developing countries, most of which are rainfed, with India contributing the largest share (71%), followed by Pakistan (10%), Iran (5%) and Turkey (4%) (FAOSTAT database-http://faostat.fao.org/site/567/default.aspx#ancor, 2009).

Among grain legumes, chickpea is rich in nutritional compositions and does not contain significant quantities of any specific major anti-nutritional factors. On an average, chickpea seed contains 23% of highly digestible protein, 64% crude fiber, 6% soluble sugar and 3% ash. The mineral component is high in phosphorous (343mg/100g), calcium (186mg/100g), magnesium (141mg/100g), iron (7mg/100g) and zinc (3mg/100g) (Williams and Singh 1987). There are two market types of cultivated chickpea namely, *desi* (with small and brown seeds) and *kabuli* (with bold and cream coloured seeds). In India, *desi* type of chickpea, accounts for nearly 90% of total area under cultivation and remaining (10%) cultivated area being occupied by *kabuli* type (Pundir et al. 1985).

Chickpea is a hardy, deep-rooted dry land crop and can grow to full maturity despite conditions that would prove fatal for most of the crop plants. It is grown on marginal land and rarely receives fertilizers or protection from diseases and insect pests (Singh and Reddy

1991). Nearly 90% of the crop is cultivated under rainfed conditions mostly on receding soil moisture, the region is called semi-arid tropics (SAT). Currently, production of chickpea is very low (6.5 mt, FAO 2009) and has stagnated for past several years. This can be attributed to a number of biotic and abiotic stresses that reduced the yield and yield stability, mainly *Ascochyta* blight, *Fusarium* wilt, *Helicoverpa* pod borer, *Botrytis* grey mold, drought and cold. The estimated yield losses due to abiotic stress (6.4 mt) are much more than loss due to biotic stress (4.8 mt) (Ryan 1997). Among various factors effecting yield in chickpea, drought is a major constraint causing 40-50% reduction in chickpea yield globally (Ahmad et al. 2005). Considering the constraining issues and their relative affect on the global yield, it is very important and timely to work for the improvement of drought and salinity tolerance for stabilization of the yield in chickpea. Therefore, improving resistance to biotic and tolerance to abiotic stresses as well as a general increase in dry matter are major aims of chickpea breeders around the world.

In both Mediterranean and sub-tropical climates, seed filling in chickpea is subject to terminal drought, which limits seed yield (Turner et al. 2001). Many physiological processes associated with crop growth and development is reported to be influenced by water deficits (Turner and Begg 1978). Breeding for drought tolerance is generally considered slow due to the quantitative and temporal variability of available moisture across years, the low genotypic variance in yield under these conditions, inherent methodological difficulties in evaluating component traits (Ludlow and Muchow 1990), together with the highly complex genetic basis of this character (Turner et al. 2001). Genetic improvement of drought tolerance, therefore, is a challenge using conventional breeding approaches that rely on selection for yield in drought-stressed environments. However, the large genotype by environment (G × E) interactions for yield and the difficulties of controlling the level of water stress under natural conditions makes direct selection for yield ineffective. Thus, the

application of a holistic approach, combining genomics with breeding and physiology, termed as genomics-assisted breeding (Varshney et al. 2005b), provides strategies for improving component traits of drought tolerance that should prove more effective and efficient than the conventional selection methods. It is essential to identify quantitative trait loci (QTLs) or genes that confer drought tolerance so that these QTLs/genes can be deployed in breeding programmes. There are two main approaches in detecting the QTLs associated with the trait of interest- one being done by "linkage mapping" or "QTL interval mapping" and the other is based on linkage disequillibrium (LD) called as "linkage disequilibrium mapping" or "association mapping".

Due to relatively low levels of polymorphism between cultivated chickpea genotypes, inter-specific crosses between *C. arietinum* and *C. reticulatum* have been the primary focus for genetic studies of agronomic traits (Singh et al. 2008). A diverse array of technologies is available to identify and monitor DNA polymorphism and as a consequence molecular markers are now routinely used in the breeding programs of several crop species (Varshney et al. 2006, 2007). Various kinds of molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeat (SSR), sequence tagged site (STS) and single nucleotide polymorphism (SNP) markers have become available for characterization of plant genome structure and genetic improvement in several crops species through construction of genetic maps, gene tagging and marker-assisted selection (MAS) (Mohan et al. 1997; Gupta and Varshney 2000; Varshney et al. 2005b; Rafalski et al. 2002). Until recently, SSR markers have been proven as the markers of choice for plant breeding applications because of their wide genomic distribution, technical simplicity, amenability to high-throughput automation, co-dominant inheritance, high reproducibility, multi-allelic nature and chromosome-specific location. Very recently, SNP

markers have also started to emerge as informative and useful markers for chickpea genetics and breeding.

In case of chickpea, unlike other plant species, the progress in development of molecular markers has been very slow. Development of SSR markers was initiated only in late 90s. As a result, several hundred SSR markers were available at the time of undertaking this study (Choudhary et al. 2006; Hüttel et al. 1999; Lichtenzveig et al. 2005; Sethy et al. 2003, 2006a, 2006b; Winter et al. 1999). However, low level of polymorphism in chickpea germplasm has limited integration of SSR markers into chickpea genetic maps. Therefore, it is ascertained that there is need to increase the density of markers on the genetic maps and study marker-trait association using different approaches.

Keeping the above in view, the present study is proposed with the following objectives:

1) Development of novel set of microsatellite or simple sequence repeat (SSR) markers in chickpea
2) Integration of novel SSR markers into the reference genetic map using a mapping population derived from the cross *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777)
3) Construction of an intra-specific (*C. arietinum*) genetic map using a mapping population derived from the cross ICC 4958 × ICC 1882 and identification of QTLs for drought tolerance related traits by using linkage mapping approach
4) Identification of candidate genes for drought tolerance through comparative genomics and bioinformatics approaches and study of allelic sequence diversity (single nucleotide polymorphisms) in a reference set of chickpea
5) Identification of genes/markers associated with drought tolerance using candidate gene sequencing and genome-wide association approaches

2. REVIEW OF LITERATURE

2.1 Chickpea

The genus *Cicer* (family *Fabaceae*) consists of 43 species of which 9 are annual including cultivated chickpea, 33 are perennial and one is unclassified. The annual species have been classified into two sections, Monocicer and Chamaecicer, based on morphological characters, life cycle and geographical distribution (van der Maesen 1987). Eight of the annual species (*C. arietinum, C. reticulatum, C. echinospermum, C. bijugum, C. judaicum, C. pinnatifidum, C. yamashitae* and *C. cuneatum*) belong to the section Monocicer, while section Chamaecicer contains the annual species, *C. chorassanicum*.

Chickpea (*C. arietinum L.*) is one of the pulse crops domesticated in the Old World about 7000 years ago. Cytogenetic and seed protein analyses are consistent with *C. reticulatum* as the wild progenitor of domesticated *C. arietinum*, with south-eastern Turkey as the presumed centre of origin (Ladizinsky and Adler 1976). This claim was supported by van der Maesen (1987) based on the presence of the closely related annual species, *C. reticulatum* and *C. echinospermum* in southeastern Turkey. Three wild annual *Cicer* species, *C. bijugum, C. echinospermum* and *C. reticulatum,* closely related to cultivated chickpea, cohabit in this area. The cultivated species, *C. arietinum* is found only in cultivation and cannot colonize successfully without human intervention. On the other hand, the wild species (e.g. *C. reticulatum, C. bijugum*) occur in weedy habitats (fallow or disturbed habitats, roadsides, cultivated fields of wheat, and other places not touched by man or cattle), mountain slopes among rubble (e.g. *C. pungens, C. yamashitae*), and on forest soils, in broad-leaf or pine forests (e.g. *C. montbretii, C. floribundum*).

Cultivated chickpea (*C. arietinum*) is composed of two genetically distinct sub-types that are readily distinguished based on seed size and colour: *desi*, composed of small, angular, brown seeded varieties, and *kabuli*, composed of large, smooth, cream seeded varieties. Regarding the origin of *kabuli* and *desi* types of chickpea, it is reported that *desi* types originated first, followed by *kabuli* type which was developed by selection and mutation (Singh 1997). There is linguistic evidence that *kabuli* type reached India *via* Kabul, the capital of Afghan, about two centuries ago and acquired the name as '*kabuli chana*' in Hindi (van der Maesan 1972). In India, *desi* type of chickpea accounts for nearly 90% of total area under chickpea cultivation and remaining area being occupied by *kabuli* type. Across the world, the ratio of *desi* and *kabuli* chickpea production is 3:1. In addition `*gulabi'*, pea shaped forms of local importance are also recognized. *Kabuli* types are considered relatively more advanced because of their larger seed size and reduced pigmentation achieved through conscious selection. Study at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) revealed that *desi* and *kabuli* types differ in their dietary fiber components of seed both qualitatively and quantitatively (Singh 1985). For instance, *kabuli* types, as compared to *desi* types contain higher amount of dietary fiber, particularly cellulose and hemicellulose.

2.2 Constraints in Chickpea Production

Chickpea is a hardy, deep-rooted dryland crop and can grow to full maturity despite conditions that would prove fatal for most crops. It is grown on marginal land and rarely receives fertilizers or protection from diseases and insect pests (Singh and Reddy 1991). Despite of its economical importance chickpea productivity is low because of yield loses due to abiotic (drought, cold and salinity) and biotic stresses (*Helicoverpa*, *Ascochyta* blight, *Fusarium* wilt and *Botrytis* grey mold). Chickpea is grown mostly as a post-rainy season crop on soil moisture conserved from the preceding rainy season. The crop is therefore,

often exposed to terminal drought and heat stress. The estimated yield losses due to abiotic stress (6.4 mt) are much more than loss due to biotic stress (4.8 mt) (Ryan 1997). Among various factors effecting yield in chickpea, drought is a major constraint causing 40-50% reduction in chickpea yield globally (see Ahmad et al. 2005). With climate change and predictions of increased water scarcity in the future, water-stress is likely to remain as a primary constraint. As irrigation is often not available in the semi-arid tropics (SAT), especially for resource poor farmers, it is critical that genetic enhancement focus on maximizing the extraction of available soil moisture and improving the efficiency of water use in crop establishment, growth and yield.

2.3 Drought Stress in Chickpea

Drought, cold and salinity are the major abiotic stresses affecting chickpea in order of their importance (Croser et al. 2003). As drought is the major constraint in chickpea production, it is essential to develop varieties which can make use of available water resources and produce maximum yield. Drought is defined as a water stress due to lack or insufficient rainfall and/or inadequate water supply (Toker et al. 2007). The seriousness of drought stress depends on its timing, duration and intensity (Serraj et al. 2003). Often drought is accompanied by relatively high temperatures, which promote evapotranspiration and hence could accentuate the effects of drought and thereby further reduce crop yields.

Effect of drought stress depends on evapotranspiration, soil water holding capacity and the crop water requirements (Toker et al. 2007). Worldwide, 90% of chickpea is grown under rainfed conditions (Kumar and Abbo 2001) where terminal drought is one of the major constraints limiting crop productivity (Toker et al. 2007). Terminal drought is a usual feature in semi-arid tropics like south Asia and northern Australia where chickpea is grown in the post-rainy season on progressively receding soil moisture conditions (Leport et al.

1998; Siddique et al. 2000). The damage due to drought is compounded by heat stress in the warmer Mediterranean regions and regions like South Asia where temperature increases towards flowering (Singh et al. 1997) and it is difficult to differentiate between the damage caused by the individual stresses. As a result of drought stress, the growing season may be shortened affecting yield components, i.e., total biomass, pod number, seed number, seed weight and quality, and yield per plant (Toker et al. 2007). Flowering and seed set are the most critical growth stages affected by drought in chickpea (Khanna-Chopra and Sinha 1987).

2.3.1 Breeding for drought tolerance

Conventional breeding for drought tolerance is based on the selection for yield and its components under a water-limited environment (Blum 1985; Millan et al. 2006). The germplasm is usually screened for two important drought avoidance/tolerance traits; large root system and small leaf area (Turner et al. 2001; Saxena 2003). Previously, more than 1500 chickpea lines were screened for drought tolerance and the genotype ICC 4958 was the most promising for drought tolerance (Saxena et al. 1993). Subsequently, ICC 4958 was used in a three-way cross with *cv*. Annigeri and the *Fusarium* wilt resistant genotype ICC 12237. The progenies were selected for high yield and drought tolerance traits (Saxena 2003). Several lines combining the large root traits of ICC 4958 and the small leaf area traits of ICC 5680 were reported to be more drought tolerant and yielded similarly to the high yielding parent (Saxena 2003). In another study, a chickpea mini-core germplasm collection comprising 211 genotypes along with 12 popular cultivars and 10 annual wild chickpea genotypes was screened for root traits. Interestingly, several *C. arietinum* genotypes with more root depth than ICC 4958 were also identified, which could serve as an alternative source for the large root trait. An outstanding genotype, ICC 8261, which had the largest RLD and deepest root system, was identified in chickpea mini-core germplasm collection

(Kashiwagi et al. 2005; Krishnamurthy et al. 2003). Also, genotypic variation for osmotic adjustment in chickpea was studied across 120 mixed F_2 population obtained from crosses Kaniva × Tyson and Kaniva × CTS60543. The correlation of osmotic adjustment with yield under drought stress is unclear and the heritability was low (h^2 = 0.20- 0.33) (Morgan et al. 1991; Turner et al. 2001; Moinuddin and Khanna-Chopra 2004). In summary, breeding for drought tolerance has remained hampered due to limited knowledge about the genetic basis of drought tolerance and its negative correlations with productivity (Mitra 2001). Moreover, selection for yields in chickpea is not effective in early segregating generations because of its indeterminate growth habit. Therefore breeders have to select for crosses rather than plants in F_2 and F_3 generations (Ahmad et al. 2005).

2.3.2 Molecular breeding for drought tolerance

Recent years have witnessed tremendous progress in the development of novel genetic tools and genomics approaches including development of molecular markers, dense genetic maps and whole-genome transcription profiling techniques to identify genomic regions and genes/ quantitative trait loci (QTLs) underlying plant stress responses in many crop species (see Varshney et al. 2005a). The development of molecular techniques for genetic analysis has led to a great increase in our knowledge of crop genetics and genomics. Improvement in marker detection systems and the techniques used to identify markers-trait associations based on complete linkage maps and bulked segregant analysis has made great advances in recent years. Plant breeding has benefited from DNA marker technologies that were used to establish saturated genetic maps in major crop species including cereals (Varshney et al. 2006) and legumes (Varshney et al. 2010). Markers in a high density genetic map will allow the precise tagging of mono- and oligo-genic traits, with the dual goal of marker-assisted selection for traits and positional cloning of the underlying genes (Tanksley et al. 1995). Use of genomic tools like molecular markers and other tools in integrated approach for crop

improvement has also been referred as "genomics- assisted breeding" (Varshney et al. 2005b).

Mapping of the chickpea genome has been of interest to identify genomic locations of disease resistance genes and other yield related traits (Winter et al. 2000; Cho et al. 2002; Rajesh et al. 2002a, 2004; Abbo et al. 2005). However, due to very low polymorphisms in cultivated chickpea gene pool (Udupa et al. 1993; Labdi et al. 1996), progress in chickpea genomic research has been relatively slow compared with other legume species (see Varshney et al. 2010). Moreover for drought tolerance related traits, very limited efforts have been made in past in the area of molecular mapping. As mentioned above, root traits (e.g. root biomass, root volume, etc.) have been considered as target traits for breeding for drought tolerance. Since selection of root traits is very laborious, molecular tagging of major genes/ QTL for these traits may enable marker-assisted selection (MAS) and greatly improve the precision and efficiency of breeding (see Millan et al. 2006; Varshney et al. 2010). In this context, one RIL population from Annigeri (less root biomass) × ICC 4958 (high root biomass) was screened to identify molecular markers for root traits. Because of availability of only a few hundred SSR markers in public domain at the time of undertaking this study, genotyping data could be generated for only 14 polymorphic SSR markers. Therefore, a good genetic map couldn't be developed and single marker linear regression analysis showed association of SSR marker TAA170 with relatively high phenotypic variation for drought tolerance related traits like root length (R^2 = 33.1%), root weight (R^2 = 33.1%) and shoot weight (R^2 = 54.2%), where R^2 was the adjusted coefficient of determination (Chandra et al. 2004). With an objective to develop diverse mapping populations, contrasting parental genotypes were selected after screening the mini core collection for root traits (Kashiwagi et al. 2005). As a result, two RIL populations ICC 4958 ×ICC 1882 and ICC 283 × ICC 8261 have been developed and these showed good segregation for root traits.

2.4 Chickpea Genomics

2.4.1 Molecular markers

Molecular markers can be defined as the DNA segements that show differences in the nucleotide sequences of DNA of one or more individuals at corresponding sites on the homologous chromosomes that follow a simple Mendelian pattern of inheritance. Molecular markers offer numerous advantages over conventional morphological and biochemical markers as they are stable, virtually unlimited in number, detectable at all plant tissues regardless of growth, differentiation and development, not confounded by environment and having no pleiotropic and epistatic effects (Bennett 1994). These are the powerful tools for the fingerprinting, genetic diversity analysis, tagging of useful genes, construction of genetic and physical maps, identification of QTLs, marker-assisted selection (MAS), gene pyramiding, evolutionary studies and positional cloning of useful genes (Koebner et al. 2001; Gupta and Rustgi 2004; Varshney et al. 2005b; Agarwal et al. 2008). A number of molecular marker technologies have been utilized to visualize DNA polymorphism in several crop species (Staub et al. 1996; Gupta et al. 2002; Jones et al. 2009).

Depending on the method of detection of the sequence variation, the molecular markers have been classified as hybridization based (PCR-independent) molecular markers, PCR-dependent molecular markers and micro-array based molecular markers (Gupta et al. 2002). Hybridization based molecular markers include restriction fragment length polymorphism (RFLP). PCR-dependent molecular markers include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) or microsatellite, sequence tagged sites (STS) and cleaved amplified polymorphic sequence (CAPS) (Gupta et al. 2002, Semagn et al. 2006). Micro-array based molecular markers comprise of single nucleotide polymorphism (SNP), single feature polymorphism (SFP) and diversity array technology (DArT) (Gupta et al. 2008).

2.4.1.1 Hybridization based molecular markers

Hybridization based molecular markers were the first molecular markers used in genomics research in animal or plant systems. Restriction fragment length polymorphism (RFLP) was the first marker system of this category and was based on differences in the recognition sites for restriction enzymes in the genome of the species.

RFLP refers to difference between two or more samples of homologous DNA molecules arising from differing locations of restriction sites, and to a related laboratory technique by which these segments can be distinguished. In RFLP analysis, the DNA sample is digested by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. Although now largely obsolete, RFLP analysis was the first DNA profiling technique inexpensive enough to see widespread application. In addition to genetic fingerprinting, RFLP was an important tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing. RFLP is the first molecular marker to be developed and used for human (Botstein et al. 1980) and plant (Weber and Helentjaris 1989) genome mapping. RFLP markers are highly reproducible, co-dominant, locus specific markers, which make them ideal marker system for plant genome analysis. In fact, in chickpea also, the first genetic map constructed in chickpea constituted RFLP and RAPD markers along with isozyme markers (Simon and Muehlbauer 1997). Genetic diversity studies were also carried out using RFLP markers (Udupa et al. 1993) and microsatellite-derived RFLP markers (Sharma et al. 1995; Serret et al. 1997) that though showed the narrow genetic variability in chickpea cultivars.

2.4.1.2 PCR-based molecular markers

The invention of polymerase chain reaction (PCR) by K. Mullis in year 1983 revolutionized the molecular genetics research including molecular markers. As a result, a large number of

approaches for generation of molecular markers based on PCR have become available, primarily due to its apparent simplicity and high probability of success in giving amplification of large number of copies of target DNA. PCR-based marker systems have been further divided into two categories, (1) arbitrarily primed (non-sequence specific) which include RAPD, AFLP marker systems and (2) sequence tagged PCR based technique include STS, CAPS and SSRs.

2.4.1.2.1 Random amplified polymorphic DNA (RAPD)

RAPD is a dominant marker based PCR technique, which employs single decamer primer of arbitrary sequence sequences for differential amplification of genomic DNA from the genome (Williams et al. 1990). The polymorphism in the RAPD profile is produced by rearrangement or deletion at or between oligonucleotide primer binding sites in the genome, which causes absence or presence of a band in gel electrophoresis (Rafalski and Tingey 1993). This method is quick, simple and requires less amount of DNA per assay. Being based on random primers, it does not require prior knowledge of sequence information for its design. A large number of such primers are commercially available that work across organisms starting from bacteria to human. Due to these advantages, RAPD marker system has been used more frequently for genetic diversity studies, variety identification and understanding genetic relationships in crop species.

In case of chickpea also, several studies have been conducted by using RAPD markers. For instance, Ahmad (1999) and Sudupak et al. (2002) used RAPD markers to investigate genetic relationships among the annual *Cicer* species. The RAPD analysis placed *C. arietinum, C. reticulatum* and *C. echinospermum* in a single cluster, *C. yamashitae* and *C. chorassanicum* in the next cluster, *C. pinnatifidum, C. bijugum* and *C. judaicum* in third cluster and *C. cuneatum* in the fourth cluster.

Use of dominant RAPD markers can be enhanced with identification of coupling and repulsion phase markers linked to the gene of interest. The coupling and repulsion markers can be used together as a co-dominant pair and will be equally informative as co-dominant markers in detecting heterozygotes in segregating populations (Haley et al. 1994; Johnson et al. 1995; Singh et al. 2008). A major limitation of this marker system is dominant inheritance and non-reproducibility due to low annealing temperature. However, utility of desired RAPD marker can be increased by converting it into more reproducible informative marker (Paran and Michelmore 1993) termed as sequence characterized amplified region (SCAR).

2.4.1.2.2 Amplified fragment length polymorphism (AFLP)

In order to overcome the limitations of reproducibility associated with RAPD, AFLP marker system (Vos et al. 1995) was developed by selective amplification of DNA fragments obtained by restriction enzyme digestion. It combines the power of RFLP with the flexibility of PCR-based technology by ligating primer-recognition sequences (adaptors) to the restricted DNA and selective PCR amplification of these restriction fragments using a limited set of primers labelled either with radioisotope and/ or fluorescent dye. The amplified products thus can be resolved on sequencing gels/capillaries and visualized by autoradiography or by laser based scanning in an automated fragment analysis system. About 50 to 100 amplified fragments are obtained per assay in AFLP among all the DNA profiling systems, which increases its probability of detecting polymorphism many folds (Thomas et al. 1995; Joseph et al. 2004).

AFLP marker system has been used for genetic diversity estimation in cultivated chickpea and its wild relatives in order to discover the origin and history of chickpea (Nguyen et al. 2004; Talebi et al. 2008, 2009). The requirement of significant technical skills, laboratory facilities, financial resources and high quality genomic DNA for complete restriction

digestion and dominant inheritance has limited the use of AFLP markers in plant genetic analysis.

2.4.1.2.3 Cleaved amplified polymorphic sequence (CAPS)

The CAPS marker technique provides a way to utilize the DNA sequence of mapped RFLP markers by eliminating the tedious DNA blotting and thus is known as PCR-AFLP markers (Konieczny and Ausubel 1993). The CAPS assay is performed by digesting locus-specific PCR amplicons with one or more restriction enzymes followed by separation of digested DNA fragments on agarose or polyacrylamide gels. It deciphers the restriction fragment length polymorphism resulting from the single base substitution like SNPs and insertions/deletions, which modify the recognition sites of the restriction enzymes (Chelkowski and Stepien 2001). The primers are designed based on prior sequence information of genomic and cDNA sequences and cloned RAPD amplicons. The CAPS analysis is versatile and can be combined with single strand conformational polymorphism (SSCP), SCAR, AFLP and RAPD analysis to increase the possibility of finding DNA polymorphism. It is robust and cost-effective assay that can be implemented in laboratories lacking sufficient infrastructural facilities. CAPS markers are characterized by their co-dominant inheritance and locus specific nature and useful for genotyping applications (Parsons and Heflich 1997; Weiland and Yu 2003). However this technique works only when SNP site is present at the recognition site of restriction enzyme, while those changes outside the target site of enzyme cannot be assayed by CAPS analysis. In order to overcome this, a variant of the CAPS method called dCAPS (derived-CAPS) has been developed by Neff et al. (2002). In dCAPS analysis, the conversion of mutation sites into CAPS markers by the artificial introduction of restriction sites involves the creation of mismatched PCR primers. Its successful application is not always trivial depending on the number, positions and types of mismatches. A computer-based software tool ‘SNP2CAPS’ has also been

developed (Thiel et al. 2004) in order to deduce CAPS candidates from the sequences having SNPs.

In case of chickpea, CAPS and dCAPS markers have been developed from bacterial artificial chromosome (BAC)-end sequences (Rajesh et al. 2005) and EST sequences (Varshney et al. 2007c). The CAPS and dCAPS-RGA markers have also been integrated into composite genetic map of chickpea and their association with disease resistance was studied (Palomino et al. 2009).

2.4.1.2.5 Simple sequence repeats (SSRs) or Microsatellites

Microsatellite markers are also known as simple sequence repeats or SSRs or sequence-tagged microsatellite site (STMS) (Beckmann and Soller 1990) consisting tandem repeats of 1-6 bp in length (Gupta and Varshney 2000). They consist of head-to-tail tandem arrays of short DNA motifs and are often highly polymorphic due to variable numbers of repeat units. Microsatellites as DNA markers are advantageous over many other markers as they are highly polymorphic, highly abundant, analytically simple and readily transferable (Weber 1990). SSR loci show high levels of length polymorphism, on account of differences in the number of repeat units, and show co-dominance. Microsatellites are reported to be more variable than RFLPs and RAPDs, and have been widely utilized in plant genomic studies (Gupta and Varshney 2000). The advantages of microsatellite markers over other types of genetic markers will become more important, and more obvious, when they are used to track desirable traits in large-scale breeding programs and as anchor points for map-based gene cloning strategies (Brown et al. 1996). Because of its cost and time effectiveness, use of microsatellite markers in plant breeding has become a routine procedure in several crop species mainly cereals (Varshney et al. 2006) and also some legumes (Varshney et al. 2010). In late 90s, microsatellites or simple sequence repeats (SSR) have come to use in plant

genetics, and have proved as the molecular marker system of choice for many areas of genome analyses and breeding applications (Dib et al. 1996; Powell et al. 1996).

Gel hybridization of restriction-digested genomic DNA with microsatellite specific probes revealed polymorphic fingerprints in chickpea (Weising et al. 1992; Sharma et al. 1995a). However, the applicability of this technique for mapping was limited by a high incidence of non-parental bands in progenies, and by the finite number of polymorphisms detected between cultivars (~80 bands; Sharma et al. 1995a). Multi-locus banding patterns were also obtained when microsatellite-complementary oligonucleotides were used as PCR primers to amplify the chickpea genome, but intraspecific variation was again low (Sharma et al. 1995a; Ratnaparkhe et al. 1998). Another technique based on microsatellites employed sequence information of repeat-flanking regions to design locus-specific PCR primer pairs for amplifying the SSR locus and detecting the polymorphism (Weber 1990; Akkaya et al. 1992; Morgante and Olivieri 1993). This technique often referred as STMS/ SSR polymorphism. This technique has been used for polymorphism detection in several crop species (see Powell et al. 1996 for review), including soybean, bean, pea and chickpea (Akkaya et al. 1992; Akkaya et al. 1995; Maughan et al. 1995; Rongwen et al. 1995; Winter et al. 1999; Hüttel et al. 1999).

2.4.1.2.5.1 Microsatellite markers in chickpea

SSRs were found to be abundant in the chickpea genome and have moderately high level of intra-specific polymorphism compared to other marker systems (Sharma et al. 1995b). The study suggested that SSR markers are well suited for chickpea genome mapping and gene tagging. As a result, development of SSR markers started in case of chickpea. The first set of SSR markers in chickpea was developed by Hüttel et al. (1999). Infact, Winter et al. (1999) reported the first chickpea genetic map based on SSR markers and enlisted 174 primer pairs flanking such loci. Around 280 microsatellite markers were developed at

NIPGR (National Institute for Plant Genome Research) using hybridization based microsatellite enrichment (Sethy et al. 2003, 2006a, b; Choudhary et al. 2006; Bhatia group unpublished). Lichtenzveig et al. (2005) also developed 233 SSR markers from BAC and BIBAC libraries of chickpea. In total there are 778 microsatellite markers in chickpea (Table 1) and they have been extensively used to: (a) estimate genetic diversity among *Cicer* species (Udupa et al. 1999; Choumane et al. 2000), (b) construct genetic maps (Winter et al. 1999; Tekeoglu et al. 2002; Flandez-Galvez et al. 2003; Radhika et al. 2007), (c) locate QTLs of agronomic importance (Winter et al. 2000; Cho et al. 2002; Rajesh et al. 2002, 2004; Udupa and Baum 2003; Kottapalli et al. 2009).

Table 1: Microsatellite markers available in chickpea

References	Number of SSRs developed
Hüttel et al. 1999	28
Winter et al. 1999	174
Sethy et al. 2003, 2006a, b, Choudhary et al. 2006, NIPGR, unpublished	280
Lichtenzveig et al. 2005	233
Qadir et al. 2007	63
Total	**778**

2.4.1.3 Micro-array based molecular markers

Molecular markers based on micro-array based genotyping platform provide the means to simultaneously screen hundreds to thousands of markers per individual. These markers are particularly suited for applications related to whole-genome coverage, low costs and large population sizes. This category includes SNPs, SFPs and DArT markers.

2.4.1.3.1 Single nucleotide polymorphism (SNP)

Polymorphism derived between the individuals/ varieties may arise either due to insertion/ deletion (Indel) of multiple nucleotide bases or single nucleotide substitution. The detection of variation has led to the development of sequence based molecular marker called SNP (Wang et al. 1998). In case of plant genomes, SNPs are the most important basic unit of

genetic variation and represent commonest class of DNA based genetic markers (Cho et al. 1999; Rafalski 2002). In recent years, these markers have gained considerable importance in plant genetics and breeding because of their wide distribution, co-dominant inheritance, high reproducibility and chromosome specific location. The detection and assay of SNPs are highly amenable to automation (Gupta et al. 2001; Varshney 2008). SNPs are excellent genetic markers for various applications (Rafalski 2002) including assessment of genetic diversity (Nasu et al. 2002; Varshney et al. 2007b, 2008; Yang et al. 2004) and evolutionary studies (Novelli et al. 2004; Carlson et al. 2004, Varshney et al. 2007b), marker-assisted breeding (Anderson et al. 2005; Van et al. 2008; Varshney et al. 2007a), construction of high-density genetic map (Cho et al. 1999; Van et al. 2005), detection of genome wide linkage disequilibrium (Ching et al. 2002; Kim et al. 2006; Mather et al. 2007), population substructure (Schmid et al. 2006; Caicedo et al. 2007), association mapping of genes controlling complex traits (Jander et al. 2002; Li et al. 2008) in various plant species.

Due to progress in easy SNP genotyping and assay technologies, these markers are tend be the most preferred marker system in plant genomic studies in recent years. In chickpea, the SNPs were detected in coding and genomic regions of chickpea (Rajesh and Muehlbauer 2008; Varshney et al. 2009b) and provide a good source of information for further mapping and diversity studies in chickpea. Due to the introduction of next generation sequencing technologies (see Varshney et al. 2009c), SNP identification is expected to be a routine procedure in case of so called “orphan crops” species including chickpea (Varshney et al. 2009a).

2.4.1.3.2 Diversity array technology (DArT)

Diversity arrays technology (DArT) was developed as a hybridization-based alternative, which captures the value of the parallel nature of the microarray platform (Jaccoud et al.

2001). Subsequently, it was developed for different crops and used in linkage map construction and diversity analysis. The important plant species for which DArT has been developed include rice (Xie et al. 2006), barley (Wenzl et al. 2006), *Arabidopsis* (Wittenberg et al. 2005), eucalyptus (Lezar et al. 2004), wheat (Semagn et al. 2006b; Akbari et al. 2006), Cassava (Xia et al. 2005), pigeonpea (Yang et al. 2006) and sorghum (Mace et al. 2008). DArT simultaneously types several thousand loci in a single assay. DArT generates whole-genome fingerprints by scoring the presence versus absence of DNA fragments in genomic representations generated from samples of genomic DNA. DArT provides high quality markers that can be used for diversity analyses and to construct medium-density genetic linkage maps. The high number of DArT markers generated in a single assay not only provides a precise estimate of genetic relationships among genotypes, but also their even distribution over the genome offers real advantages for a range of molecular breeding and genomics applications. Of late in chickpea, DArT array with 15,360 features has been developed by ICRISAT in collaboration with DArT Pty Ltd, Australia. For developing DArT arrays, a set of 96 genotypes representing diverse genotypes from the reference set and parental genotypes of different mapping populations were used (unpublished data).

2.4.2 Genetic mapping

Genetic mapping is a procedure of locating the molecular markers or gene loci / QTLs in order, indicating the relative distances among them, and assigning them to their linkage groups on the basis of their recombination values from all pairwise combinations. A linkage map may be thought of as a 'road map' of the chromosomes derived from two different parents (Paterson 1996). The most important use for linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest; such maps may then be referred to as 'QTL' (or 'genetic') maps. 'QTL mapping' is based on the

principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Paterson 1996). Genes or markers that are close together or tightly-linked will be transmitted the closer they are situated on a chromosome (conversely, the higher the frequency of recombination between two markers, the further away they are situated on a chromosome). Markers that have a recombination frequency of 50% are described as 'unlinked' and assumed to be located far apart on the same chromosome or on different chromosomes (Hartl and Jones 2001; Kearsey and Pooni 1996). Mapping functions are used to convert recombination fractions into map units called centi- Morgans (cM). Linkage maps are constructed from the analysis of many segregating markers. The three main steps of linkage map construction are: (1) production of a mapping population; (2) identification of polymorphism between parental genotypes for molecular markers and (3) linkage analysis of markers. Linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio and is called a logarithm of odds (LOD) value or LOD score (Risch 1992). LOD >3 are typically used to construct linkage maps. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (null hypothesis). LOD values may be lowered in order to detect a greater level of linkage or to place additional markers within maps constructed at higher LOD values. Commonly used software programs include Mapmaker/ EXP (Lander et al. 1987; Lincoln et al. 1993) and MapManager QTX (Manly et al. 2001), GMendel (http://cropandsoil.oregonstate.edu/Gmendel), MSTMap (Wu et al. 2008). JoinMap is another commonly-used program which is generally used for constructing and combining the genetic maps developed for different mapping populations (Stam 1993). Various methods used to construct genetic maps are explained in Varshney et al. (2009c) and the basic principles of linkage map construction are reviewed by Collard et al. (2005).

2.4.2.1 Genetic mapping in chickpea

The beginnings of the linkage map development in chickpea were based on morphological and isozyme loci. However, their small number and the fact that expression of these markers is often influenced by the environment, makes them unsuitable for routine use. Furthermore, because of low level of polymorphisms among genotypes of cultivated chickpea (*C. arietinum* L.), inter-specific crosses (*C. arietinum* × *C. reticulatum*, *C. arietinum* × *C. echiniospermum*) were exploited for genetic mapping (Gaur and Slinkard 1990a,b). Subsequently, a number of genetic linkage maps of chickpea (*Cicer arietinum* L.) based on combination of different marker types have been published. A summary of these maps is available in Table 2.

2.4.3 Marker-trait association

Marker-trait association can be determined either by linkage-based approach or by linkage-disequilibrium (LD) based association mapping. In past, in several crops genetic mapping based approaches were used to identify the QTLs/genes for a trait (Gupta and Varshney 2004) and recently, use of LD-based association mapping also has been used for trait mapping (Ersoz et al. 2007; Varshney and Tuberosa 2007).

2.4.3.1 Linkage map based marker-trait association

For conducting marker–trait association by using linkage maps, three widely used methods have been used: single marker analysis (SMA), simple interval mapping (SIM), and composite interval mapping (CIM) (Tanksley 1993; Liu 1998).

Table 2: A list of some genetic maps in chickpea

Mapping population	Features of genetic map	Genome coverage	Reference
Inter-specific			
F_2 (*C. arietinum* × *C. reticulatum*)	7 linkage groups with 3 morphological and 26 isozymes	200 cM	Gaur and Slinkard 1990a, 1990b
F_2 (*C. arietinum* × *C. reticulatum*) and F_2 (*C. arietinum* × *C. echinospermum)*	8 linkage groups with 5 morphological and 23 isozymes	257 cM	Kazan et al. 1993
F_2 (*C. arietinum* × *C. reticulatum)* and F_2 (*C. arietinum* × *C. echinospermum)*	10 linkage groups with 9 morphological, 27 isozyme, 10 RFLP and 45 RAPD loci	527 cM	Simon and Muehlbauer 1997
RIL (*C. arietinum* × *C. reticulatum*)	11 linkage groups with 120 STMS loci	613 cM	Winter et al. 1999
RIL (*C. arietinum* × *C. reticulatum*)	16 linkage groups with 118 SSR, 96 DAF, 70 AFLP, 37 ISSR, 17 RAPD, 8 isozyme, 3 cDNA, 2 SCAR and 3 morphological marker	2078 cM	Winter et al. 2000
RIL (*C. arietinum* × *C. reticulatum)*	9 linkage groups with 89 RAPD, 17 ISSR, 9 isozyme, and 1 morphological marker	982 cM	Santra et al. 2000
RIL (*C. arietinum* × *C. reticulatum*)	23 linkage groups with RAPD, ISSR and 1 morphological marker	-	Hajj- Moussa et al. 2000
RIL (*C. arietinum* × *C. reticulatum)*	addition of RGA Potkin 1-2 171 to LG5 of Santra et al. (2000)	-	Rajesh et al. 2002b
RIL (*C. arietinum* × *C. reticulatum)*	Extended map of Santra et al. (2000) with 50 SSR and 1 RGA	1,175 cM	Tekeoglu et al. 2002
RIL (*C. arietinum* × *C. reticulatum*)	12 linkage groups with 47 R gene specific markers integrated to Winter et al. (2000)	2500 cM	Pfaff and Kahl 2003
F_2 (*C. arietinum* × *C. echinospermum*)	570 cM, 8 linkage groups, 14 SSR , 54 RAPD, 9 ISSR, 6 RGA	570 cM	Collard et al. 2003
RIL (*C. arietinum* × *C. reticulatum*)	10 linkage groups, 16 RAPD, 3 ISSR, 14 STMS	601.2 cM	Cobos et al. 2005
RIL (five wide crosses)	Consensus map, 8 linkage groups, 555 loci,135 SSRs,33 cross-species, rest others	652.67 cM	Millan et al. 2010
Intra-specific			
RIL (*C. arietinum*)	14 linkages groups with 68 SSR, 34 RAPD, 4 ISSR, and 5 morphological	297 cM	Cho et al. 2002
F_2 (*C. arietinum*)	8 linkage groups with 51 SSR, 3 ISSR, 12 RGA	535 cM	Flandez- Galvez et al. 2003
RIL (*C. arietinum*)	8 linkage groups with 53 STMS	318.2 cM	Cho et al. 2004
RIL (*C. arietinum*)	8 integrated linkage groups with 44 RAPD, 16 ISSR, 165 SSR, 2 RGA, 1 ASAP and two yield related traits	739.6 cM	Radhika et al. 2007
F_2 (*C. arietinum*)	10 linkage groups with 82 SSR, 2 EST	724.4 cM	Kottapalli et al. 2009
RIL (*C. arietinum,* fivecrosses)	Consensus map, 8 linkage groups, 229 loci, 99 SSR, rest others	426,99 cM	Millan et al. 2010

2.4.3.1.1 Mapping populations used for QTL interval mapping

The construction of a linkage map requires a segregating plant population (i.e. a population derived from sexual reproduction). The parents selected for the mapping population will differ for one or more traits of interest. Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals (Mohan et al. 1997), however larger populations are required for high-resolution mapping. Generally in self-pollinating species, mapping populations originate from parents that are both highly homozygous (inbred). In cross pollinating species, the situation is more complicated since most of these species do not tolerate inbreeding. Many cross pollinating plant species are also polyploid (contain several sets of chromosome pairs). Mapping populations used for mapping cross pollinating species may be derived from a cross between a heterozygous parent and a haploid or homozygous parent (Wu et al. 1992). Several different populations may be utilized for mapping within a given plant species, with each population type possessing advantages and disadvantages (McCouch and Doerge 1995; Paterson 1996). F_2 populations, derived from F_1 hybrids, and backcross (BC) populations, derived by crossing the F_1 hybrid to one of the parents, are the simplest types of mapping populations developed for self pollinating species. Their main advantages are that they are easy to construct and require only a short time to produce. Inbreeding from individual F2 plants allows the construction of recombinant inbred (RI) lines, which consist of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents. The length of time needed for producing RI populations is the major disadvantage, because usually six to eight generations are required. Double haploid (DH) populations may be produced by regenerating plants by the induction of chromosome doubling from pollen grains, however, the production of DH populations is only possible in species that are amenable to tissue culture (e.g. cereal species such as rice, barley and wheat). The major advantages of RI and DH populations are that they produce homozygous or ‘true-breeding’

lines that can be multiplied and reproduced without genetic change occurring. This allows for the conduct of replicated trials across different locations and years. Thus both RI and DH populations represent 'eternal' resources for QTL mapping. Furthermore, seed from individual RI or DH lines may be transferred between different laboratories for further linkage analysis and the addition of markers to existing maps, ensuring that all collaborators examine identical material (Paterson 1996; Young 1996).

2.4.3.1.2 Approaches for QTL interval mapping

Among different statistical analyses for linkage mapping based QTL mapping, SMA (single marker analysis- also called single-point analysis) is the simplest method for detecting QTLs associated with single markers. The statistical methods used for SMA include *t*- tests, ANOVA, and linear regression. Linear regression is most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker. In fact, this method is generally used in BSA approach for trait mapping. However, the main disadvantages of this method are: (1) the farther a QTL is from a marker, it is less likely to be detected as the recombination occurring between the marker and the QTL; (ii) this causes the magnitude of the effect of a QTL to be underestimated. The use of a large number of segregating markers covering the entire genome, usually at intervals less than 15 cM, may minimize both problems (Tanksley 1993). Linkage map-based trait mapping approach employs the SIM method that makes use of linkage maps and analyses intervals between adjacent pairs of linked markers along chromosomes simultaneously (Lander and Botstein 1989). The use of linked markers for analyses under SIM is considered statistically more powerful compared to single-point analysis as the recombination between the markers and the QTL is taken care of Liu (1998). The CIM approach, however, combines interval mapping with linear regression and includes additional molecular markers in the statistical model in addition to an adjacent pair

of linked markers for interval mapping (Jansen and Stam 1994). This method is more precise and effective at mapping QTLs as compared to single-point analysis (SMA) and SIM, especially when linked QTLs are involved.

2.4.3.1.3 QTL analysis for drought tolerance

Drought tolerance is the complex phenomenon involving many known and unknown pathways. The dissection of QTLs for such a complex trait is challenging and many attempts have been made in different crop species. In order to find the QTLs for drought tolerance, the traits like stomatal conductance (Sanguineti et al.1999; Juenger et al. 2005; Price et al. 1997), transpiration efficiency (Juenger et al. 2005; Specht et al. 2001; Kholova et al. 2010), osmotic adjustment (Diab et al. 2004; Saranga et al. 2004;Robin et al. 2003), relative water content (Diab et al. 2004; Sanguineti et al.1999), canopy temperature (Saranga et al. 2004), drought sensitivity index (Sanguineti et al.1999); leaf ABA (Kholova et al. 2010); chlorophyll content (Shen et al. 2007); water use efficiency (Quarrie et al. 2005), root traits (Chandra Babu et al. 2003; Li et al. 2005) and some yield related traits (Diab et al. 2004; Saranga et al. 2004; Moreau et al. 2004; Xu et al. 2005; Xiao et al. 2005; Specht et al. 2001; Quarrie et al. 2005; Dashti et al. 2007). Studies at ICRISAT had revealed the importance of root traits in drought tolerance in chickpea.

2.4.3.1.4 Importance of root traits in chickpea drought tolerance

The yields chickpea genotypes under rainfed and irrigated conditions were compared at ICRISAT in order to compare the yields under drought conditions and the potential yields (Saxena 2003). The study indicated that genotype ICC 4958 exhibited the best performance not only in field trials in ICRISAT but also in other Mediterranean environments, which had higher root biomass. Subsequent studies at ICRISAT on 12 diverse chickpea genotypes showed that the prolific root system upto the depth of 15-30 cm contributed positively

towards the seed yield under moderate to severe terminal drought conditions (Kashiwagi et al. 2006). The advantage of deep root systems towards drought tolerance mechanism was well substantiated in soybean (Kaspar et al. 1978), common beans (Sponchiado et al. 1989) and chickpea (Silim and Saxena 1993). Some major root attributes such as greater efficiency in water absorption per unit root length density, ability to change the rooting pattern across the soil depths for efficient access of soil moisture and the ability to produce larger root surface area per unit root biomass make chickpea the best choice for dryland cropping systems compared to other legumes and cereals (Thomas et al. 1995, Benjamin and Nielsen 2006). Chickpeas also found to have higher root surface to root weight ratio compared to soybean and field peas (Benjamin and Nielsen 2006). These results suggest that chickpeas are better equipped towards tolerance to drought stress and further improvement of root traits would be one of the promising approaches to improve drought avoidance in chickpea under terminal drought environments (Gaur et al. 2008).

2.4.3.1.5 QTL analysis for drought tolerance related traits

Compared to the conventional breeding approaches for improved productivity under water limited environments, genomics offers great opportunities for dissecting quantitative traits into their single genetic determinants (Young 1996; Dudley 1993; Tanksley 1993; Lee 1995; Beavis and Kein 1996; Quarrie 1996; Prioul et al. 1997; Tuberosa et al. 2002). Identification of QTLs is paving the way to MAS (Ribaut et al. 2002; Morgante and Salamini 2003) and assisted pyramiding of the beneficial QTL alleles. Marker-assisted breeding reduces the effect of environmental conditions during the selection process, which is a major hindrance in conventional breeding under drought. The increasing number of studies reporting QTLs for drought tolerance related traits in different crops under drought stress (Table 3) indicates a growing interest in this approach. With the invention of other genomic tools, sequencing and bioinformatics, new dimensions for deciphering and

manipulating the genetic basis of drought tolerance can be achieved (Tuberosa et al. 2002; Varshney et al. 2005b; Tuberosa et al. 2005).

Although considerable progress has been made in identifying QTLs in chickpea related to *Fusarium* wilt and *Ascochyta* blight disease resistance (Table 4), information on the genetic basis of traits related to drought tolerance in chickpea is limited. A deep root system capable of extracting additional soil moisture should positively impact yield under drought stress environments.

Table 3: Some QTL studies related to drought tolerance in selected crop species

Crop	Cross	Traits studied	Reference
Barley	Tadmor × Er/Apm	Osmotic adjustment, leaf relative water content, grain yield	Diab et al. 2004
Cotton	*G. hirsutum* × *G. barbadense*	Canopy temperature, osmotic potential, dry matter, seed yield	Saranga et al. 2004
Maize	F2 × F252	Silking date, grain yield, yield stability	Moreau et al. 2004
Maize	Os420 × IABO78	Stomatal conductance, drought sensitivity index, leaf temperature, leaf relative water content, anthesis-silking interval, grain yield	Sanguineti et al. 1999
Maize	X178 × B73	Yield traits measured in drought conditions-grain yield, 100 kernel weight, number of ears per plant	Xiao et al. 2005
Pearl millet	PRLT 2/89-33 × H 77/833-2	Leaf ABA, transpiration efficiency, transpiration at vapour pressure deficit	Kholova et al. 2010
Pearl millet	863B-P2 × ICMB 841-P3	Leaf ABA, transpiration efficiency, transpiration at vapour pressure deficit	Kholova et al. 2010
Rice	Zhenshan97B × Milyang 46	Chlorophyll content	Shen et al. 2007
Rice	CT9993 × IR62266	Root morphology, plant height, grain yield	Chandra Babu et al. 2003
Rice	IR62266 × IR60080.	Osmotic adjustment	Robin et al. 2003
Rice	IRAT109 × Yuefu	Root traits	Li et al. 2005
Rice	Teqing × Lemont	Phenology, yield components	Xu et al. 2005
Rice	Nipponbare × Kasalath	Stomatal frequency, leaf rolling	Ishimaru et al. 2001
Rice	Azucena × Bala	Stomatal conductance, leaf rolling, heading date	Price et al. 1997
Sorghum	B35 × Tx7000	Stay green, chlorophyll content	Xu et al. 2000
Soybean	Minsoy × Noir 1	Carbon isotope descrimination (CID), Transpiration efficiency, yield components	Specht et al. 2001
Wheat	SQ1 × Chinese spring	Water use efficiency, grain yield	Quarrie et al. 2005
Wheat	Beaver × Soissons	Flag leaf senescence	Verma et al. 2004
Wheat	Double haploid lines Chinese spring × SQ1	Stress susceptibilty index (SSI), mean productivity index (MP), tolerance index, stress tolerance index (STI) and yield components	Dashti et al. 2007

Table 4: QTL studies related to agronomic traits in chickpea

Cross	Type	Traits	Reference
ICCV96029 × CDC Frontier	Desi × Kabuli	*Ascochyta* blight	Tar'an et al. 2007
ILC72 × Cr5-10	*C. arietinum* × *C. reticulatum*	*Ascochyta* blight	Cobos et al. 2006
ILC3279 × WR315	Kabuli × Desi	*Ascochyta* blight	Iruela et al. 2006
Hadas × ICC 5810	Kabuli × Desi	*Ascochyta* blight, time of flowering	Lichtenzveig et al. 2006
PI359075 × FLIP84-92C	*C. arietinum*	*Ascochyta* blight	Cho et al. 2004
FLIP84-29C × PI599072	*C. arietinum* × *C. reticulatum*	*Ascochyta* blight	Tekeoglu et al. 2002
Lasseter × PI527930	*C. arietinum* × *C. echinospermum*	*Ascochyta* blight	Collard et al. 2003
ICC12004 × Lasseter	Desi	*Ascochyta* blight	Flandez-Galvez et al. 2003
ILC3279 × CA2156	*C. arietinum*	*Ascochyta* blight	Millan et al. 2003
ICC4958 × PI489777	*C. arietinum* × *C. reticulatum*	*Ascochyta* blight	Rakshit et al. 2003
FLIP84-92C × PI599072	*C. arietinum* × *C. reticulatum*	*Ascochyta* blight	Rakshit et al. 2003
ILC1272 × ILC3279	Kabuli	*Ascochyta* blight	Udupa and Baum 2003
FLIP84-92C × PI599072	*C. arietinum* × *C. reticulatum*	*Ascochyta* blight	Santra et al. 2000
PI359075 × FLIP84-92C(2)	*C. arietinum*	*Ascochyta* blight	Tekeoglu et al. 2000a
Blanco Lechoso × Dwelley	*C. arietinum*	*Ascochyta* blight	Tekeoglu et al. 2000a
FLIP84-92C × PI599072(3)	*C. arietinum* × *C. reticulatum*	*Ascochyta* blight	Tekeoglu et al. 2000a
CDC Frontier × ICCV 96029	*C. arietinum*	*Ascochyta* blight	Anbessa et al. 2009
CA2156 × JG62	Kabuli × Desi	*Fusarium* wilt	Cobos et al. 2005
CA2139 × JG62	Kabuli × Desi	*Fusarium* wilt	Cobos et al. 2006
C104 × WR315	*C. arietinum*	*Fusarium* wilt	Sharma et al. 2004
ICC4958 × PI489777	*C. arietinum* × *C. reticulatum*	*Fusarium* wilt	Tekeoglu et al. 2000b
ICC4958 × PI498777	*C. arietinum* × *C. reticulatum*	*Fusarium* wilt	Winter et al. 2000
C-104 × WR-315	*C. arietinum*	*Fusarium* wilt	Tullu et al. 1998
C104 × WR315	*C. arietinum*	*Fusarium* wilt	Mayer et al. 1997
FLIP84-92C × PI599072	*C. arietinum* × *C. reticulatum*	Beet armyworm	Clement et al. 2010

A preliminary study carried out at ICRISAT on drought tolerance using single marker linear regression analysis in chickpea (Annigeri × ICC 4958) indicated SSR marker "TAA170" associated with the drought tolerance related traits like root length, root length density and shoot dry weight. Apart, two mapping populations segregating for drought tolerance related traits- ICC 4958 × ICC 1882 and ICC 283 × ICC 8261- were developed based on the physiological studies (Kashiwagi et al. 2005, 2006), to dissect the QTLs for drought related root traits.

After identifying important QTLs, the next step involves the identification of candidate sequences, validate their role and proceed with the direct manipulation using the gene itself as marker for MAS (Tuberosa and Coraggio 2004). The recent progress in the profiling of transcriptome, proteome and metablome offers the possibility of investigating the response of genes to drought and other stresses. Current focus in chickpea functional genomics should be able to coordinate resources around the world and take full advantage of functional genomics for chickpea improvement (Coram and Pang 2007). Further development with respect to molecular breeding for agronomically important traits could lie in the integration of aspects of physiology and biotechnology towards plant breeding (Blum and Nguyen 2004). The characterization of key plant physiological mechanisms that restrain performance under drought, together with the associated regulatory genes, could therefore, facilitate the development of improved crop varieties showing water use efficiency and drought tolerance.

2.4.3.2 Association mapping

Association mapping, also known as linkage disequilibrium (LD) mapping, has emerged as a tool to resolve complex trait variation down to the sequence level by exploring historical and evolutionary recombination events at the population level (Nordborg and Tavare 2002;

Risch and Merikangas 1996). As a new alternative to traditional linkage analysis, association mapping offers three advantages, (i) increased mapping resolution, (ii) reduced research time, and (iii) greater allele number (Yu and Buckler 2006). Since its introduction to plants (Thornsberry et al. 2001), association mapping has continued to gain favourability in genetic research because of advances in high throughput genomic technologies, interests in identifying novel and superior alleles, and improvements in statistical methods (Zhu et al. 2008).

2.4.3.2.1 Strategies for association analysis

While using the association or LD mapping approach, the statistical power of associations is determined by the extent of LD with the causative polymorphism, as well as sample size used for the study (Wang and Rannala 2005). The decay of LD over physical distance in the study population determines the marker density required and the level of resolution that may be obtained in an association study. The most commonly used summary statistic for estimation of LD within the association study framework is known as r^2 (Hill and Robertson 1968; Lewontin 1988). The r is the Pearson's (product moment) *correlation coefficient* of the correlation that describes the predictive value of the allelic state at one polymorphic locus on the allelic state at another polymorphic locus, where r^2 is the squared value of correlation coefficient that is also called *coefficient of determination* and it explains the proportion of a sample variance of a response variable that is *explained* by the predictor variables when a linear regression is performed (Ersoz et al. 2007). *Lewontin's D* is another summary statistic for LD that is commonly used and describes the difference between the coupling gamete frequencies and repulsion gamete frequencies at two loci. From *D*, a second measure of LD, that is, normalized *D´* can also be estimated. It is important to estimate the rate of decay of LD with physical distance, to be able to extrapolate information gathered from a small collection of sampled loci to the whole genome

investigated. This extrapolation is essential for association mapping study design, since it may be used for determining the marker density required for scanning previously unexplored regions of the genome, as well as determining the maximum resolution that can be achieved for genotype–phenotype associations for the study population. Another important constraint for the use of association mapping for crop plants is unidentified population sub-structuring and admixture due to factors such as adaptation or domestication (Wright and Gaut 2005). Population structure creates genome-wide LD between unlinked loci. When the allele frequencies between sub-populations of a species are significantly different, due to factors such as genetic drift, domestication, or background selection, genetic loci that do not have any affect whatsoever on the trait may demonstrate statistical significance for their co-segregations with a trait of interest. In cases where the population structuring is mostly due to population stratification (Bamshad et al. 2004; Pritchard 2001), three methods are often proposed suitable for statistically controlling the effects of population stratification on association tests: (a) genomic control (GC) (Devlin et al. 2004) , (b) structured association (SA) method including two extensions that are modified for the type of association study as case-control (SA-model) (Pritchard et al. 2000) or quantitative trait association study (Q-model) (Thornsberry et al. 2001) , and (c) unified mixed model approach (Q + K) (Yu et al. 2006). After analyzing the LD decay, population structure, and appropriate genotyping of the population, marker–trait association studies are conducted. Whether the phenotype of interest has a binary or quantitative phenotype is also of interest for the association study design. When a binary trait is being investigated, case-control type populations are required for association analysis, where equivalent sized sub-populations of individuals that display the phenotype of interest (cases) and do not display the phenotype of interest (controls) are queried for allelic association of genetic loci with the case and control phenotypes in a statistically significant manner (see Ersoz et al. 2007). The statistical test performed is simply a hypothesis test that asks whether the allelic frequency

distribution of a locus is the same or different for a given locus between the two sub-populations. Most of the statistical methods aim to detect and correct for the effects of population stratification and ancestry differences between the case and control groups (Ersoz et al. 2007; Price et al. 2006).

The linkage mapping involves the screening of the mapping populations (F2s, RILs, DHs) which are products of only a few meioses. However, association mapping takes the advantage of the events that created association in the relatively distant past. Assuming many generations and therefore many meioses, the recombination will have removed the association between any markers, which is not tightly being linked to QTL. The LD refers to the non-random association of the alleles between gene loci. Many genetic and non-genetic factors, including recombination, drift, selection, mating pattern and admixture, affect the structure of LD. The decay of LD over physical distance in a population determines the density of the marker coverage needed to perform an association analysis (Rafalski and Morgante 2004, Gupta et al. 2005).

If LD declines rapidly, then a higher marker density is required to capture markers located close enough to functional site (Yu and Buckler 2006). Association approaches (2000 bp) have high resolution than the linkage approaches (10,000 kb). Thus association mapping allows much finer map than standard biparental cross approaches. The association studies can be carried out in two different approaches viz, whole genome scanning and candidate gene sequencing approach of association mapping.

2.4.3.2.3 Populations for association mapping

In a plant breeding program, three main types of populations could be considered for implementation of association mapping: germplasm bank collections, elite breeding materials and synthetic populations. In case of germplasm banks, core collections are

expected to represent most of the genetic variability with a manageable number of accessions (Zhong et al. 2009). In the case of elite materials, the sample could be composed by lines and checks evaluated in regional trials. For synthetic populations, the evaluation unit should be also the association unit (or closely related to it), whether it is an individual or a family.

2.4.3.2.4 Approaches for association mapping

The marker-trait association using association analysis can be either by candidate gene sequencing approach or by genome-wide association analysis.

2.4.3.2.4.1 Candidate gene sequencing based association mapping

A candidate gene is a gene, located in a chromosome region suspected of being involved in the expression of a trait such as drought, whose protein product suggests that it could be the gene in question. A candidate gene can also be identified by association with the phenotype and by linkage analysis to a region of the genome. The candidate genes are selected based on prior knowledge from mutational analysis, biochemical pathway or linkage analysis of the trait of interest. An independent set of random markers needs to be scored to infer genetic relationships. It is low cost, hypothesis-driven and trait-specific approach but will miss other unknown loci (Zhu et al. 2008).

The candidate gene sequencing approach is useful for quickly determining the association of a genetic variant with a trait and for identifying genes of modest effect. Association analysis has the potential to identify the single polymorphism within a gene that is responsible for the differences in the phenotype. In addition many plant species have high level of diversity for which association approaches are well suited to evaluate the numerous alleles available (Flint-Garcia et al. 2003). The first association study of quantitative trait based on a candidate gene was the analysis of flowering time and the *dwarf8* gene in maize

(Thornsberry et al. 2001). The *sugary1* gene in maize is responsible for the production of naturally occurring varieties of sweet corn. In an allelic diversity survey at this locus, association methods were used to map the mutation to a single nucleotide (Whitt et al. 2002).

Association analysis can also be used to prove a candidate gene as the gene underlying a QTL. In *Arabidopsis*, the *GLABROUS1(GL1)* locus was one of the six genes identified as candidates for the trichome initiation and density (Hauser et al. 2001). A similar type of study in maize shows that DNA sequence level comparison of maize inbred lines at the *bronze* locus demonstrated that two maize alleles could differ gene complement and in the content on non-genic insertions and deletions.

2.4.3.2.4.1.1 Candidate genes for drought tolerance

Although several genes were known to be involved in drought tolerance (Table 5), the association analysis based on candidate gene sequencing approach is meagerly reported. The drought tolerant candidate genes presented in the current research work are explained in detail. The candidate genes include Abscisic acid stress and ripening (*ASR*) gene, drought responsive element binding proteins (*DREB*), *ERECTA*, sucrose synthase (*SuSy*) and sucrose phosphate synthase (*SPS*).

Abscisic acid Stress and Ripening (ASR) gene- ASR is a stress-inducible gene that has been reported exclusively in plants. Iusem et al. (1993) reported the first *Asr* gene from cultivated tomato and since then *Asr* genes have been found in various species of dicotyledonous and monocotyledonous plants (Frankel et al. 2006), including *Cucumis melo* (Hong et al. 2002), lily (Wang et al. 1998), grape (Cakir et al. 2003), *Ginkgo biloba* (Shen et al. 2005), potato (Silhavy et al. 1995), maize (Riccardi et al. 1998), rice (Vaidyanathan et al. 1999), pummelo (Canel et al. 1995) and loblolly pine (Chang et al. 1996), but surprisingly these genes are

not present in *Arabidopsis* (Yang et al. 2005). The *Asr* genes in various species are presumed to act as part of a transcription-regulating complex involved in plant development processes such as senescence, fruit ripening, pollen maturation and glucose metabolism (Iusem et al. 1993; Silhavy et al. 1995; Wang et al. 1998; Hong et al. 2002; Cakir et al. 2003; Shen et al. 2005; Frankel et al. 2007) and also respond to different abiotic stress factors, including drought, salt, cold and limited light (Schneider et al. 1997; Huang et al. 2000; Maskin et al. 2001; Jeanneau et al. 2002; Kalifa et al. 2004).

Table 5: List of some candidate genes involved in drought tolerance

Gene	Function	Mechanism of Action	Reference
ABA Independent			
CBF/ DREB1D,DREB2A,DRE B2B/ABF3 (C-repeat Binding Factor/*DRE* Binding protein)	Stress induced transcription factors	Regulates expression of genes that encode RNA-binding proteins, sugar transport proteins, desaturase, carbohydrate metabolismrelated proteins, Lea proteins, osmo-protectant biosynthesis protein, and protease inhibitors, Kin (cold-inducible) proteins	Liu et al. 1998; Stockinger et al. 1997; Oh et al. 2005; Ito et al. 2006
C2H2 Zinc-finger-type protein, Stz	Transcription regulators	Regulation of gene expression downstream of the DRE/DREB regulon	Sakamoto et al. 2004
Erd1 (Early responsive to Dehydration1)	Caesinolytic ATP dependent protase	Under control of NAC1 transcriptional factors, encodes a ClpA (ATP binding subunit of the caseinolytic ATP-dependent protease) homologous protein	Tran et al. 2004
Rd29A	Drought resistant proteins	Under DREB2 transcriptional factor control	Liu et al. 1998
Arabidopsis *Rd26*	Transcription regulator	Encodes NAC Protein	Fujita et al. 2004
Mn-SOD	Mn-superoxide dismutase	Scavenges reactive oxygen species, prevents membrane damage	McKersie et al. 1996
Avp1	Vacuolar H+-pyrophosphatase	Increases root growth by increasing auxin fluxes	Gaxiola et al. 2001; Cattivelli et al. 2008
ERECTA	A putative leucine-rich repeat receptor-like kinase	transpiration efficiency regulator	Masle et al. 2005; Cattivelli et al. 2008
P5CS	δ-Pyrroline-5-carboxylate synthetase	Proline accumulation leads to increased Osmotolerance	Zhu et al. 1998; Kavi Kishor et al. 1995;
MtlD	Mannitol-1-phosphate dehydrogenase	Mannitol accumulation increases osmotolerance	Abebe et al. 2003; Cattivelli et al. 2008
GF14 Lambda	14-3-3 Protein	Produces a ‘‘stay green’’ phenotype with higher water stress tolerance and photosynthetic rates under drought stress	Yan et al. 2004; Cattivelli et al. 2008
NADP-Me	NADP-malic enzyme	Overexpression decreased stomatal conductance and improves water use efficiency	Laporte et al. 2002; Cattivelli et al. 2008

Contd…

…Contd

Sucrose synthase and Sucrose phosphate synthase (*SPS*)	Sucrose metabolism	Sucrose synthase and SPS transcript and protein levels are modulated by dehydration and rehydration	Yu et al. 1992; Fu and Park 1998; Ingram et al. 1997
ABA dependent			
SNAC1	Stress induced transcription factor SNAC1	expression reduces water loss by increasing stomatal sensitivity to ABA	Hu et al. 2006
Aba1/Aba2/ZEP,NCED,Los5/Aba3, and *Aao*	ABA Biosynthesis pathway	Aba1/2- Zeathanxin epoxidase NCED (9-cis-epoxycarotenoid dioxygenase), Los5 encodes sulfurylase generating molybdenum cofactor required by ABA aldehyde oxidase	Zhu 2002
Lea class/ *Dehydrin* type genes, (also designated as Rd / Cor/ Kin/LTIs in Arabidopsis), *Rab18* (maize), *Hva1* and *Hv22* (Barley, Rice) , *OsLEA3*	Lea proteins	Over-accumulation of Lea increases drought tolerance also in field conditions. Work under control of various transcription factors like bHLH, AREB, DREB, ATMyB2 ATMyC2	Zhu 2002; Bahieldin et al. 2005; Xiao et al. 2007; Xu et al. 1996; Choi et al. 2000; Uno et al. 2000; Abe et al. 1997; Zhu 2001
Farnesyl-transferase (*Era1*)	Negative-regulator of ABA sensing	Down-regulation of Era1 enhances plant's response to ABA and drought tolerance reducing stomatal conductance	Wang et al. 2005; Cattivelli et al. 2008
OsCDPK7	Ca dependent Protein Kinase	Positive regulator of cold and drought responsive genes in rice	Saijo et al. 2000; Cattivelli et al. 2008

Asr genes belong to a small gene family characterized by the presence of an ABA/WDS domain (Pfam family domain Pf02496). The number of plant *ASR* available in plant genome databases varies from one in grape (http://www.genoscope.cns.fr/ externe/GenomeBrowser/Vitis/), four in *Brachypodium distachyon* (http://www.brachypodium.org/), to up to seven in *Sorghum bicolor* (http://phyto3.phytozome.net/). Not much is known about the function of *ASR* proteins. In most studies, only one member of the family was studied. Because orthology was unclear (Frankel et al. 2006), attributing a function to each member of the family was difficult. Targeted experiments have revealed that members of the *ASR* gene family are induced by abscisic acid (ABA), various abiotic stresses including water stress and during the process of fruit ripening (Carrari et al. 2004). Therefore, the corresponding proteins were classified as hydrophilins, a wide protein group involved in adaptation to water deficits (Battaglia et al. 2008). Yeast one-hybrid experiments revealed that grape *ASR* protein bind to the promoter of a hexose transporter gene (Cakir et al. 2003). The above data were in close

agreement with the proposed role of *ASR* product as a transcription factor involved in sugar metabolism (Carrari et al. 2004; Kalifa et al. 2004). Furthermore, evidences suggested a dual role for an *ASR* from *Lilium longiflorum* (LLA23) uncovering an independent function as a protective molecule against water loss (Yang et al. 2005). Tomato *ASR1* proteins were localized to both the cytosol, as unstructured monomers, and to the nucleus, as structured DNA-bound homodimers (Kalifa et al. 2004; Konrad and Bar-Zvi 2008). The unstructured form of tomato *ASR1* proteins was shown to present a chaperone-like activity and could stabilize a number of proteins against denaturation caused by heat and freeze–thaw cycles. Several genetic experiments have implicated *Asr* in the response to water deficit. An increase in foliar senescence under drought conditions was shown in transgenic *ASR1* over-expressing lines in maize, whereas antisense lines showed the opposite effect (Jeanneau et al. 2002). Moreover, *ASR1* colocalized with a QTL for xylem sap ABA content, a QTL for leaf senescence and a QTL for anthesis–silking interval responsive to mild water deprivation in maize (de Vienne et al. 1999). Accelerated rates of amino acid substitutions were demonstrated in the water stress-induced *Asr2* gene of tomato species inhabiting dry environments, suggesting some positive selection during adaptation to arid conditions (Frankel et al. 2003). Over-expression of the *ASR* gene (e.g. lily *ASR* gene) in transgenic *Arabidopsis* can increase tolerance to drought and salt and decrease a plant's sensitivity to exogenous abscisic acid (ABA) (Yang et al. 2005). All known *ASR* genes contain two highly conserved regions. The first region contains a stretch of His residues at the N-terminus, possessing sequence specific Zn^{2+}-dependent DNA binding activity (Kalifa et al. 2004). The second region is a large part of the C-terminal sequence, often containing an NLS (Cakir et al. 2003). Recently, the study of the extent of nucleotide diversity in *ASR2* in two *Solanaceae* species provided some evidence of non-neutral evolution, potentially associated with unique climatic features or demographic events (Giombini et al. 2009). Very recently, the diversity studies in several members of *ASR* gene family and their co-evolution

in cultivated rice species with that of wild relatives were studied across 218 diverse rice genotypes. The *ASR* gene family in rice was characterized with expression pattern and sequence diversity in terms of intraspecific polymorphism and interspecific divergence. The study addressed questions about the molecular evolution of orthologs between a cultivated species and its wild relative, and that of paralogs within *O. sativa* and identified *ASR3* gene as the potential candidate for association studies related to drought tolerance (Philippe et al. 2010).

Drought responsive element binding protein (DREB)- The dehydration responsive element binding proteins (*DREB*) are important transcription factors that induce a set of abiotic stress-related genes and impart stress endurance to plants. The *DREB* transcription factors could be dichotomized as *DREB1* and *DREB2*, which are involved in two separate signal transduction pathways under low temperature and dehydration, respectively. They belong to the ERF (ethylene responsive element binding factors) family of transcription factors. ERF proteins are a sub-family of the *APETLA2* (AP2)/ethylene responsive element binding protein (*EREBP*) transcription factors that is distinctive to plants. There are ~124 ERF proteins in *Arabidopsis* (Riechmann et al. 2000). *ERF* proteins share a conserved 58–59 amino acid domain (the *ERF* domain) that binds to two *cis*-elements, the GCC box, found in many PR (pathogens related) gene promoters conferring ethylene responsiveness (Gu et al. 2000), and to the C repeat *CRT*/dehydration responsive element (*DRE*) motif involved in the expression of cold and dehydration responsive genes. The role DREB proteins in biotic and abiotic stress tolerance were reviewed in detail by Agarwal et al (2006). The first isolated cDNAs encoding DRE binding proteins were CBF1 (Stockinger et al. 1997), *DREB1A* and *DREB2A* (Liu et al. 1998) from *Arabidopsis*. Since then, DREB genes have been isolated from a wide variety of plants. Two *DREB1A* homologs (*DREB1B* and *DREB1C*) and one *DREB2A* homolog (*DREB2B*) were isolated from *Arabidopsis* (Liu et al. 1998). Two

homologs of *CBF1* (*CBF2* and *CBF3*) have also been identified from *Arabidopsis* (Gilmour et al. 1998; Medina et al. 1999). *CBF1* is identical to *DREB1B*, and its homologs, *CBF2* and *CBF3*, are identical to *DREB1C* and *DREB1A*, respectively. *CBF4*, a close homolog of *CBF*/*DREB1* has been reported from *Arabidopsis* (Haake et al. 2002). In wheat and barley, a number of *CBF* homologs have been mapped to low temperature QTLs, Fr-2 chromosomal region (V´ag´ujfalvi et al. 2005; Skinner et al. 2005; Miller et al. 2006). In wheat, a functional Fr-A1 allele reportedly plays a significant role in regulating the *CBF*-mediated *Cor*/*Lea* gene expression (Kobayashi et al. 2005). Also, a *PgDREB2A* (Accession no. AY829439) gene by cDNA library screening of *Pennisetum glaucum* seedlings has been isolated (Agarwal et al. 2007). While both *DREB1*/CBF and *DREB2* genes share a sequence similarity at AP2 domain and bind to the same DRE sequence, they are either up-regulated by low temperature (*DREB1*) or by drought/high salt concentration (*DREB2*). Transcription factors *DREB1A*/CBF3 and *DREB2A* specifically interact with cis-acting dehydration-responsive element/ C-repeat (DRE/CRT) involved in cold and drought stress–responsive gene expression in *Arabidopsis thaliana*. Intact *DREB2A* expression does not activate downstream genes under normal growth conditions, suggesting that *DREB2A* requires posttranslational modification for activation, but the activation mechanism has not been clarified. *DREB2A* domain analysis using *Arabidopsis* protoplasts identified a transcriptional activation domain between residues 254 and 335, and deletion of a region between residues 136 and 165 transforms *DREB2A* to a constitutive active form. Overexpression of constitutive active *DREB2A* resulted in significant drought stress tolerance but only slight freezing tolerance in transgenic *Arabidopsis* plants. Microarray and RNA gel blot analyses revealed that *DREB2A* regulates expression of many water stress–inducible genes. However, some genes downstream of *DREB2A* are not downstream of *DREB1A*, which also recognizes *DRE*/*CRT* but functions in cold stress–responsive gene expression (Sakuma et al. 2006). The *DREB2A* homolog in wheat known as *Wdreb2*,

expressed in wheat seedlings under abiotic stresses, such as cold, drought, and high salinity, and following treatment with exogenous ABA was used to generate transgenic tobacco plants expressing *Wdreb2* to clarify roles of *Wdreb2* in stress tolerance and the direct transactivation of *Cor/Lea* genes by *WDREB2* (Kobayashi et al. 2008). In case of rice, five cDNAs for *DREB* homologs: *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D*, and *OsDREB2A* were isolated and the expression of *OsDREB1A* and *OsDREB1B* was induced by cold, whereas expression of *OsDREB2A* was induced by dehydration and high-salt stresses (Dubouzet et al. 2003). Based on the physiological proofs regarding the involvement of *DREB2A* homologs in drought tolerance in several crop species, *DREB2A* transcription factor seems to be one of the most promising candidate genes for drought tolerance. As a part of GCP sponsored "Allelic diversity at orthologous candidate genes (ADOC) in GCP crops" project, *DREB2A* was considered as the candidate gene for drought tolerance in order to identify the sequence diversity across two legumes (chickpea and common bean) and three cereals (rice, sorghum, barley). The sequence analysis of *DREB2A* homologs across eight diverse genotypes of all the five crops indicated the conserved nature of *DREB2A* and low sequence diversity across the crop species studied (Nayak et al. 2009).

ERECTA gene- The *ERECTA* gene codes for a protein kinase receptor, one of a very large and complex family of signalling molecules called protein kinases, and their receptors, which mediate plants' responses to disease, predation and stress. *ERECTA* is involved in leaf organogenesis and reduces the density of stomata on the leaf under-surface, hence reduces the evapotranspiration. In *Arabidopsis*, the *ERECTA* gene has been shown to control organ growth and flower development by promoting cell proliferation (Shpak et al. 2004). In *Arabidopsis ERECTA* gene is known to be involved in inflorescence development and organ growth by promoting cell proliferation. Transgenic *Arabidopsis* plants that ectopically over express the *ERECTA* gene improve plant transpiration efficiency and drought tolerance by

affecting stomatal density, epidermal cell expansion, mesophyll cell proliferation and cell-cell contact. The *ERECTA* gene encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) and may controlling plant growth/organ size and biomass accumulation. In addition, Masle et al. (2005) isolated *Arabidopsis ERECTA* gene, a putative leucine-rich repeat receptor-like kinase that regulated transpiration efficiency located on *Arabidopsis* chromosome 2. The *ERECTA* gene can change both leaf stomatal number and leaf structure, and regulate the flowering time, and is proved to regulate plant transpiration efficiency and consequently to have a bright prospect in improving crop drought resistance and using water at high efficiency**.** The role of the *ERECTA* gene was identified by screening *Arabidopsis* inbred lines and mutant plants, thereby identifying the *ERECTA* homologs in both dicot and monocot crop species, including cereals. The contribution of *ERECTA* gene towards water use efficiency was confirmed using complementation assays on wilting mutant *Arabidopsis* plants (Masle et al. 2005). The transformation of *ERECTA* gene in the crop species would be major breakthrough in the area of agriculture, with respect to drought tolerance and agronomic performance. Recently, the *ZmERECTA* genes from maize are patented by Pioneer Hi-Bred International, Inc., that were involved in improving plant growth, transpiration efficiency and drought tolerance in crop plants (www.freepatentsonline.com/y2008/0078004.html). Several other genes for drought tolerance have been patented and summarized in Somvanshi (2009).

Sucrose synthase (SuSy) and sucrose phosphate synthase (SPS)- Sucrose synthase and the sucrose phosphate synthase are the key enzymes involved in the sugar metabolism pathway. *SuSy* enzyme belongs to the family of glycosyltransferases, especially, hexosyltransferases. It is also commonly known as UDP glucose-fructose glucosyltransferase, Sucrose synthatase, uridine diphosphoglucose-fructose glucosyltransferase. The enzyme sucrose synthase (UDP-D- glucose: D-fructose 2a-glucosyltransferase, EC 2.4.1.13) catalyses the

reversible conversion of sucrose uridine- diphosphate into fructose and UDP-glucose. Although the reaction is reversible, it is thought that the enzyme is mainly involved in the breakdown of sucrose (Huber and Huber 1996; Geigenberger and Stitt 1993; Geigen- berger et al. 1993). Hence the activity of sucrose synthase can be important in controlling either starch or cellulose biosynthesis by supplying UDP-glucose as a precursor or as an immediate substrate (Chourey et al. 1991; Delmer and Amor 1995). Two major isoforms of sucrose synthase have been described for many higher plants (e.g. Chourey et al. 1986; Marana et al. 1990; Chopra et al. 1992; Yu et al. 1992; Fu and Park 1995). Two classes of cDNA and genomic clones encoding sucrose synthase were characterized in *Craterostigma plantagineum* considered to have an important role in the unique phenomenon of surviving desiccation. Sucrose-synthase transcript and protein levels are modulated by dehydration and rehydration (Kleines et al. 1999). In case of *Arabidopsis*, induction of *AtSUS3* gene by drought and mannitol, an osmotic agent, is shown to be strong in stressed leaves and were found abundant in ESTs derived from dehydration treated rosette plants. *AtSUS3* was also desribed as a marker of dehydrating tissues and behaves as an osmoticum- but not sucrose-responsive gene (Baud et al. 2004).

Ingram et al. (1997) reported the isolation and characterization of cDNA clones encoding SPS from *Craterostigma plantagineum*, *a* resurrection plant in which the accumulation of sucrose is considered to play an important role in tolerance to severe protoplastic dehydration. Two distinct classes of cDNAs encoding *SPS* were isolated from *C. plantagineum*, and are represented by the clones Cpsps1 and Cpsps2. The transcripts corresponding to both cDNAs decrease to very low levels in dehydrating leaves of *C. plantagineum*. Only the *Cpspsl* transcript occurs in the roots, where it is present at a higher level than in leaves and increases upon dehydration of the plant. Higher enzymatic activities have been determined in protein extracts of dehydrated tissues compared with untreated

tissues, which correlates with an increase in protein levels. It is suggested that the overall regulation of *SPS* is strongly influenced by the changing composition of the cytoplasm in *C. plantagineum* leaves during the dehydration-rehydration cycle.

2.4.3.2.4.2 Genome-wide association mapping

Genome-wide association (GWA) mapping also known as whole genome scanning approach is a comprehensive approach to systematically search the genome for causal genetic variation. A large number of markers are tested for association with various complex traits, and prior information regarding candidate genes is not required. It works best for a research consortium with complementary expertise and adequate funding.

The whole genome scanning approach however requires a larger number of markers depending on the extent of LD decay in the germplasm. The first association study to attempt a genome scan in plants was conducted in wild beet (*Beta vulgaris* ssp. *maritima*) for the requirement of vernalization prior to bolting, is determined by the single gene (B) (Hansen et al. 2001). The appropriateness of a DNA sequencing platform for SNP discovery depends on the number of SNPs required for effective whole-genome scans in an association population. For example, the extensive LD in 95 *Arabidopsis* accessions and 102 elite barley inbred lines made it possible to association test a low number of evenly spaced SNPs discovered via capillary-based Sanger sequencing and still achieve a medium level of genome-wide mapping resolution (Aranzana et al. 2005; Rostoks et al. 2006). Alternatively, tens to hundreds of thousands of SNP markers are required for powerful whole-genome scans in crops with low LD and high haplotype diversity, such as maize and sunflower. In such a scenario, the 454-GS FLX (Margulies et al. 2005) and Illumina 1 G Genome Analyzer (www.illumina.com) are ideal platforms for identifying scores of SNPs through short read resequencing of allelic fragments from several genetically diverse individuals. After SNPs are identified, different array-based platforms can be used to

genotype thousands of tag SNPs in parallel. A high quality whole-genome reference sequence is extremely valuable in construction of a SNP haplotype map from short reads produced by the 454 and Illumina sequencing platforms. This is because short reads are more easily assembled by aligning to a preexisting genome reference sequence compared to de novo assembly. Also, a reference genome is useful in masking repetitive and paralogous sequences, as the orthology of high copy sequences is difficult to determine unless candidate SNPs are genetically mapped. Because the base calling accuracy of 454 and Illumina is presently lower than that of Sanger sequencing, emphasis should be placed on calling SNPs that have multiple read support (≥2× coverage/allele/ individual). The newness and expense of next-generation sequencing technologies have limited their wide-spread implementation for SNP discovery in crops. Recently, a 454-based transcriptome sequencing method was used in maize to identify more than 36,000 candidate SNPs between two maize inbred lines (Barbazuk et al. 2007). This 454-SNP study is a promising step toward development of numerous genome-wide SNP markers in a highly diverse crop species with a rapid breakdown of LD, but more importantly lays the framework for identifying SNPs based on sequencing of random genomic fragments.

Till date there are no reports of association studies in case of chickpea, however the association studies in other crop species especially in cereals such as rice (Wen et al. 2009), maize (Lu et al. 2009), barley (Malysheva-Otto et al. 2006; Cockram et al. 2008), sorghum (Shehzad et al. 2009) and wheat (Neumann et al. 2010) have revealed that the linkage based QTL analyses can be complemented by LD based association studies.

The association study in rice was carried out using 170 accessions from mini-core collection of Chinese germplasm with 84 SSRs markers on chromosome 7 and 48 markers on other chromosomes. The association of these markers on six morphological traits differing between *indica* and *japonica* cultivars was studied and physical position of the markers was

analysed (Wen et al. 2009). Genome-wide association mapping was carried out on 96 genotypes of wheat from winter core collection using 874 informative DArT markers, of which 215 markers were found to be associated with twenty agronomic traits studied across eight years (Neumann et al. 2010).

The association studies in legumes are limited to soybean and *Medicago*, where association map consisting of 150 markers was constructed on the basis of differences in allele frequency distributions between the two sub-populations of soybean for seed protein trait (Jun et al. 2008) and the genome-wide association studies started in *Medicago* as a part of HapMap (Haplotype Map) project on 384 inbred lines (http://www.medicagohapmap.org/about.php).

Association studies with high density marker coverage, large sample size and minimum population structure offer great promise in complex trait dissection. To date, candidate-gene association studies have searched only a tiny fraction of the genome. The debate of candidate genes versus genome scans is traced to the original milestone paper of Risch and Merikangas (1996). As genomic technologies continue to evolve, more genome-wide association analyses conducted in different plant species is expected. So far, there have been few successful results from candidate-gene association mapping. But for many research groups, starting with candidate-gene sequences and background markers will provide a firm understanding of population structure, familial relatedness, nucleotide diversity, LD decay, and many other aspects of association mapping. Afterward, this knowledge can be built on through comprehensive genome scans with intensive sequencing and high-density genotyping.

3. MATERIALS AND METHODS

3.1 Development of Microsatellite or SSR Markers

In order to increase the repertoire of marker resources in chickpea, SSR markers were developed by constructing the SSR-enriched library and mining bacterial artificial chromosome (BAC)- end sequences.

3.1.1 Construction of SSR-enriched library

The genomic library was constructed and screened with microsatellite probes in order to detect the clones containing microsatellites. SSR-enriched genomic DNA library was constructed in collaboration with University of Frankfurt, Germany. The size-fractioned genomic library of chickpea reference genotype ICC 4958 was constructed using 100 µg of DNA. The DNA was completely digested with *Mbo*I or *Sau*3AI in combination with *Taq*I enzyme. The fragmented genomic DNA was subjected to electrophoresis on 1.4 % low-melting agarose gel along with *Hin*dIII digested λ DNA ladder. The gel slices containing DNA fragments of size ranging from 800 to 1200 bp were excised using sharp blade and kept in 1.5 ml micro centrifuge tubes and purified as per the manufacturers instruction of Q1AEX II gel extraction kit (QIAGEN). Buffer QX1 was added; vortexed for 30 sec and 10 µl of Q1AEX II was added to each sample and mixed properly. This solution was incubated at 50 ºC for 10 min with vocational vortexing till agarose slice got dissolved. After complete dissolution of agarose the mixture was centrifuged at 13,000 rpm for 1 min and the supernatant was discarded. The pellet was washed with 500 µl of buffer QX I by vortexing followed by centrifugation. The pellet was washed with 500 µl of PE buffer, air dried for 15-30 min until the pellet become white and then dissolved in 20 µl of $T_1E_{0.1}$. The micro centrifuge tubes were incubated at room temperature for 5 min and stored at -20 ºC. The size, quantity and quality of the eluted DNA were checked on 0.8% agarose.

The purified DNA fragments were ligated into Promega pGEM 3Z(f) vector (Promega, Medison, USA) using manufacturer's instructions. The 5- 10 µg of eluted DNA is mixed with 2 mM dNTPs, 5U T_4 DNA ligase and 10 U of Klenow fragment. The mixture is incubated at room temperature for 30 min and the unused enzyme mixture is deactivated by incubating at 80 ºC for 15 min and cooled on ice. This mixture was finally ligated into pGEM 3Z(f) vector and incubated at 16ºC for 16 h. The transformed plasmids were recovered and electroporated into electro-competent *E. coli* Sure strain (DH10B, Stratagene) at 1.8 kV/cm using a Gene Pulser® (BIORAD, California, USA).

Approximately 400,000 clones were plated at a density of 20,000 colonies per plate on 24 X 24 cm Nunc petri dishes. Masterplates were generated and replicas were taken from these master plates and spotted in duplicate on positively charged PVDF-Macroarrays. Macroarrays were printed using fully automated systems with contact printing technology and solid pins. Masterplates, spotting and macroarray design was performed by RZPD GmbH, Germany. For enriching the genomic DNA library, synthetic oligos $(GA)_{10}$ and $(TAA)_{10}$ were enzymatically 3'end-labelled with digoxigenated oligonucleotides (DIG Oligonucleotide 3'-End Labeling Kit, Roche, Germany). Subsequently, macroarrays/filters were hybridized with above mentioned oligo-probes in Roti-Hybri-Quick buffer (Roth, Germany) including 10 µg/ml sheared, denatured *E. coli* DNA to minimize non-specific binding. Filters were hybridized at 55°C overnight and washed at 60°C. Washing was done for 3 times each for 10 min in 1:2, 1:5, and 1:10 dilutions of the hybridization buffer. The digoxigen was detected in a "direct detection assay" performed with the DIG Wash and Block buffer set, and DIG Luminescent detection Kit (Roche, Germany) for chemiluminescent detection with a monospecific antibody coupled to alkaline phosphatase in the presence of disodium (disodium 3-(4-methoxyspirol (l,2-dioxetane-3,2'- (5'-chloro) tricyclo[3.3.1.13~7] decan)-4-yl) phenyl phosphate) commercially known as CSPD. CSPD

is chemiluminiscent substrate, which produce a luminescent signal when acted upon by alkaline phosphatase, which dephosphorylates the substrates and yields anions that ultimately decompose, resulting in light emission that are recorded on the films. Filters were exposed to X-ray films (Amersham, Buckinghamshire, England) with intensifying screens for 4 h or overnight. Colonies giving rise to strong signals were scraped from the master plates. Colonies were re-grown and spotted on Hybond N membranes (Amersham, Buckinghamshire, England), lysed, and their DNA fixed on the membranes. Hybridization and chemiluminescent detection was done repeatedly to pick the clones with positive signals. These clones were grown on LB agar plates with ampicillin (100 µg/ml) overnight at 37°C. The presence of desired insert size in the recombinant plasmids was confirmed by colony PCR.

Colony PCR was performed by carrying out the lysis of randomly selected white colonies at 95°C for 10 min in 10 µl of sterile water. PCR reactions were performed in a 25 µl volume containing Taq buffer A (10 mM Tris-HCL, pH 8.3 with 15 mM $MgCl_2$), 0.25 µM of each dNTP, 0.1 U of Taq DNA polymerase, 10 µl of the cell lysate and 5 pmoles of each forward and reverse M13 universal primers. Amplification was carried out using PCR profile having initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50°C annealing temperature for 30 s, 72°C for 1 m and final extension of 72°C for 10 m. The amplified products were resolved on 1.2 % agarose gel, stained using ethidium bromide and visualized under UV light to confirm the presence and size of inserts in the positive colonies.

The SSR positive bacterial colonies were grown overnight at 37°C in 5 ml of Luria Broth medium with 100 µg/ ml Ampicillin in an orbital shaker (Thermo Electron Corporation, California, USA). The plasmid DNA from individual clones was isolated using standard

alkaline lysis method (Sambrook and Russell 2001). After checking the quality of the plasmid DNA on 0.8% agarose gel, the clones were sequenced using the BigDye Terminator cycle sequencing kit on an ABI3700/ABI3730XL (Applied Biosystems, California, USA). A total of 288 clones were sequenced in both directions using standard T7 promoter and SP6 primers and 19 clones were sequenced in only one direction by using M13-forward sequencing primer. The raw sequences were trimmed using Sequencher programme to remove the residues of the vector sequences following standard parameters. The cleaned sequences were checked for the quality of run, by inspecting the chromatogram of the sequence data. The sequences generated were subjected to CAP3, a contig assembly programme (http://pbil.univ-lyon1.fr/cap3.php) in order to define contigs. These contigs were subjected to *MI*cro*SA*tellite (*MISA)* tool to search microsatellites considering minimum 10 repeat units of mono- (N), and four repeats of di- (NN), tri- (NNN), tetra- (NNNN), penta- (NNNNN) and hexa- (NNNNNN) nucleotides. The *MISA* search tool is capable of identifying both perfect and compound SSRs. The microsatellites present within a distance of 100 bp are considered as compound SSRs.

3.1.2 Development of SSR markers from BAC end sequences of chickpea

The bacterial artificial chromosome (BAC) library (CAA1Ba) was developed from the chickpea accession ICC 4958 at University of California, Davis (UC-Davis). BAC end sequences (BESs) were obtained from ~25,000 clones. A total of 46,270 genome survey sequences (GSS) were submitted to NCBI by UC-Davis. These 46,270 sequences were screened for the presence of microsatellite sequences using *MI*cro*SA*tellite (*MISA)*.

3.1.3 Primer designing for novel SSRs

The sequences from SSR-enriched library and BESs were used for designing primer pairs for SSRs using Primer3 programme (http://frodo.wi.mit.edu/) in batch file. The SSR

markers developed from microsatellite enriched library were named as ICCM (ICRISAT Chickpea Microsatellite) markers and those developed from BESs were named as CaM (Cicer arietinum Microsatellite) markers.

3.2 Construction of Genetic Map

3.2.1 Plant material

Recombinant inbred lines (RILs) developed from crossing *C. arietinum* (ICC 4958) and *C. reticulatum* (PI 489777) were used to construct high density inter-specific map in order to identify the position of the newly developed microsatellite markers, while the RILs derived from drought tolerant *C. arietinum* (ICC 4958) and drought sensitive *C. arietinum* (ICC 1882) were used to construct intra-specific genetic map to identify quantitative trait loci (QTL) for root traits. Chickpea reference set comprising of 318 diverse genotypes was used for association analysis. The "reference set" was derived from microsatellite-based diversity studies of composite collection of chickpea (Upadhyaya et al. 2008). The details of the genotypes in the reference set of chickpea are tabulated in Appendix 1.

3.2.2 DNA isolation of chickpea genotypes

DNA from 131 RILs of ICC 4958 × PI 489777, 232 RILs of ICC 4958 × ICC 1882 and 318 chickpea genotypes of the reference set were isolated using high-throughput mini- DNA extraction method (Mace et al. 2003) as given in following section.

3.2.2.1 High-throughput DNA extraction

The steps involved in the DNA extraction protocol are explained below.

3.2.2.1.1 Sample preparation

- Leaves were harvested from 15 days old seedlings.

- Leaf tissues of 70-100mg was placed in 12 × 8-well strip tube with strip cap (Marsh Biomarket, New York, USA) in a 96 deep-well plate together with two 4 mm stainless steel grinding balls (Spex CertiPrep, USA).

3.2.2.1.2 CTAB extraction

- For each sample 450 µl of preheated (at 65ºC for half an hour) extraction buffer (100 mM Tris-HCl (pH-8, 1.4 M NaCl, 20 mM EDTA, CTAB (2-3% w/v), β-mercaptoethanol) was added to each sample and secured with eight strip caps.
- Samples were homogenized in a GenoGrinder 2000 (Spex CertiPrep, USA), following the manufacturers' instructions, at 500 strokes/min for 5 times at 2 min interval.
- Plate was fitted into locking device and incubated at 65ºC for 10 min with shaking at periodical intervals.

3.2.2.1.3 Solvent extraction

- For each sample 450 µl of chloroform-isoamyl alcohol (24:1) was added and mixed thoroughly by inverting.
- Plate was centrifuged at 5500 rpm for 10 min. The aqueous layer (300 µl) is transferred to fresh strip tubes (Marsh Biomarket, USA).

3.2.2.1.4 Initial DNA precipitation

- 0.7 vol (210µl) of isopropanol (stored at –20ºC) was added to each sample and mixed thoroughly by inverting.
- Plate was centrifuged at 5000 rpm for 15 min.
- Supernatant was decanted from each sample and pellet was air dried for 20 min.

3.2.2.1.5 RNase treatment

- 200 µl low salt TE (10 mM Tris EDTA (pH-8)) and 3 µl RNase was added to each sample and incubated at 37ºC for 30 min.

3.2.2.1.6 Solvent extraction

- 200 µl of phenol-chloroform-isoamyl alcohol (25:24:1) was added to each sample and mixed by inverting twice.
- Plate was centrifuged at 5000 rpm for 5 min.
- Aqueous layer was transferred to a fresh 96 deep-well plate.
- 200 µl chloroform-isoamylalcohol (24:1) was added to each sample and mixed by inverting twice.
- Plate was centrifuged at 5000 rpm for 5 min. Aqueous layer was transferred to fresh 96 deep- well plate
- A total of 315 µl ethanol-acetate solution [30 ml ethanol, 1.5 ml 3 M NaOAc (pH-5.2)] was then added to each sample and placed in 20ºC for 5 min.
- Plate was again centrifuged at 5000 rpm for 5 min.
- Supernatant was decanted from each sample and pellet was washed with 70% ethanol.
- Plate was centrifuged at 6000 rpm for 10 min.
- Supernatant was again decanted from each sample and samples were air dried for 1 hour.
- Pellet was re-suspended in 100 µl low-salt TE and stored at 4ºC.

3.2.2.2 DNA quantification

The extracted DNA was quantified by loading the samples on 0.8% agarose gel containing 0.5 µl/10 ml Ethidium bromide (10mg/ml). The DNA was normalized to 5 ng/µl concentration with visual comparison by loading DNA samples with the standard λ DNA molecular weight markers (2.5 ng/µl, 5 ng/µl, 10 ng/µl) on 0.8% agarose gel.

3.2.3 Screening of SSR markers

The 1,655 novel SSR markers developed from SSRs-enrichment library (ICCM) and BESs (CaM) were used to screen the parents of mapping populations namely ICC 4958, PI 489777 and ICC 1882. In addition, a set of 233 SSR markers developed by Lichtenzveig et al. (2005) named as "H-series" and 280 SSR markers (NIPGR-series) from published/unpublished markers obtained from Dr Sabhyata Bhatia (National Institute of Plant Genome Research, New Delhi, India) and 241 SSR markers from Winter-series procured from Dr. Peter Winter (GenXPro/ University of Frankfurt, Germany) were used to screen the polymorphism.

PCR reactions with final reaction mixture of 5 µl were conducted in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, California, USA**)** DNA thermocycler. The reaction mixture contained final concentration of 5 ng/µl of template DNA, 0.5 mM dNTPs, 0.5 µM of M13 tailed forward and 1 µM of reverse primer, 1 µM of M13 labeled primer (6FAM/NED/PET/VIC), 0.75 mM of $MgCl_2$, 0.1U of Taq DNA polymerase (AmpliTaq Gold) and 1X PCR buffer (AmpliTaq Gold).

For ICCM, CaM and NIPGR-series markers, a touch-down PCR profile (61-51°C) with an initial denaturation for 15 min at 94 °C, followed by 10 touch-down PCR cycles comprising of 94°C for 20 seconds (s), 61°C for 20 s and 72°C for 30 s were performed. These cycles were followed by 35 cycles of 94°C for 10 s with constant annealing temperature of 54°C for 20 s and 72°C for 30 s and a final extension was carried out at 72°C for 20 min. The amplified products were separated by capillary electrophoresis using ABI PRISM® 3700 DNA analyzer.

To amplify H-series markers, the two touch-down PCR profiles 60-55°C and 55-45°C were used. The PCR profile 60-55 °C was with an initial denaturation for 15 min at 94 °C, followed by 5 touch-down PCR cycles comprising of 94°C for 20 s, 60°C for 20 s and 72°C

for 30 s were performed. These cycles were followed by 35 cycles of 94°C for 10 s with constant annealing temperature of 56°C for 20 s and 72°C for 30 s and a final extension was carried out at 72°C for 20 min. PCR profile 55-45 °C was with an initial denaturation for 15 min at 94 °C, followed by 10 touch-down PCR cycles comprising of 94°C for 20 s, 55°C for 20 s and 72°C for 30 s were performed. These cycles were followed by 35 cycles of 94°C for 10 s with constant annealing temperature of 48°C for 20 s and 72°C for 30 s and a final extension was carried out at 72°C for 20 min. The products were stored at 4 °C until further use. PCR products were checked for amplification on 1.2% agarose gel. The amplified products were analyzed by capillary electrophoresis and the allele-calling was carried out by using GeneScan and Genotyper programs. The list of polymorphic markers was obtained based on the differences in base pair sizes in each pair of genotypes under study i.e. between ICC 4958 × PI 489777 and ICC 4958 × ICC 1882.

3.2.4 Genotyping on mapping populations

The markers polymorphic between ICC 4958 and PI 489777 were genotyped on 131 RILs of inter-specific mapping population and those polymorphic between ICC 4958 and ICC 1882 were genotyped on 232 RILs of intra-specific mapping population. After checking the amplification on 1.2% agarose, based on the amplicon sizes and florescent dyes used, the PCR products from each florescent dye (6FAM/NED/PET/VIC) were pooled to facilitate high-throughput multiplex capillary electrophoresis. While pooling, 1 µl from each PCR amplicon (with different dye or same dye with considerable difference in the amplicon sizes) were mixed with 0.1 µl of orange size standard LIZ, 7 µl of Hi-Di formamide and 2.9 µl of distilled water and denatured at 95˚C for 5 minutes. Capillary electrophoresis of denatured pooled products was performed using ABI3700 and ABI3130*xl* DNA genetic analyzer. After completion of electrophoresis run, the raw data files created by the ABI machine were processed through GeneScan version 3.7 software (Applied Biosystems, California, USA) for estimating the sizes of the PCR amplicons based on their relative

mobility compared to the internal size standard LIZ dye. Allele calling was done by Genotyper v 3.7 software (Applied Biosystems, California, USA). As the SSR markers developed at NIPGR were not labeled, genotyping was carried out using 6% polyacrylamide gel electrophoresis (PAGE).

Based on the amplicon sizes in the parents, data were scored for all optimized primers. The allele of the female parent was always scored as 'A' irrespective of the size of the amplicon. Similarly, the allele of the male parent was always scored as 'B' and the lines having alleles from both the parents were designated as 'H' and missing data was scored as '-'. In the present study two mapping populations (ICC 4958 × PI 489777 and IC 4958 × ICC 1882) were screened, with one common female parent (ICC 4958). Therefore, the allele scoring was as follows:

'A' – Allele of female parent (ICC 4958)

'B' – Allele of male parent (PI 489777 or ICC 1882)

'H '– Heterozygous (presence of both parental alleles)

'-' – Missing data (failed amplification)

3.2.5 Linkage map construction

Genotyping data obtained on the respective mapping population (RILs) were used for linkage analysis using JoinMap 4.0 (Stam 1993). The markers were classified into linkage groups (LGs) using linear regression of pairwise analysis with minimum LOD threshold of 3.0 and maximum recombination fraction of 0.4. Kosambi mapping function was used to estimate the map distances (Kosambi 1944).

3.3 QTL Interval Mapping

3.3.1 Phenotypic traits

The mapping population (comprising RILs) was developed at ICRISAT using two parents- *C. arietinum* (ICC 4958) and *C. arietinum* (ICC 1882) - which segregated for root traits. Phenotyping data was collected for drought tolerance related traits (especially root traits) *viz.,* shoot dry weight (SDW), stem dry weight (StDW), leaf dry weight (LDW), root dry weight (RDW), rooting depth (RD), ratio between RDW and total dry weight (R/T), root length (RL), root length density (RLD), root surface area (RSA) and root volume (RV) in years 2005 and 2007 with three replications by Crop Physiology Division at ICRISAT, Patancheru. For taking the root related observations, chickpea plants were grown in PVC cylinders (18 cm diameter, 120 cm height) with three replications. The PVC cylinders, except the top 15 cm, were filled with an equi-mixture (w/w) of vertisol and sand, mixed with di-ammonium phosphate at the rate of 0.07 g kg^{-1}. The soil water content of the mixture was equilibrated to 70% field capacity to create the conditions similar to those in the field at sowing time, where the soil is not fully saturated with water. A mixture of soil and sand was used to decrease the soil bulk density and facilitate root growth and extraction. Plants were sampled at 35 days after sowing (DAS) avoiding physically damaged plants, as previous studies showed that maximum variation in root dry weight and root length density among genotypes are best noticed in this environment at this stage, and that variation is reduced after 41 DAS (Krishnamurthy et al. 1996). The methods employed for recording the observations of the root traits are explained in brief in the following sections.

3.3.1.1 Shoot dry weight (SDW)

Total shoot which include stem and leaves of each plant were dried in a hot air oven at 80ºC for 72 h. The weight of this dry matter was measured as SDW. SDW is the sum of stem dry weight (StDW) and leaf dry weight (LDW).

3.3.1.2 Stem dry weight (StDW)

The weight of dried stem (without leaves) is measured after drying the stem in hot air oven at 80ºC for 72 h.

3.3.1.3 Leaf dry weight (LDW)

The weight of dried leaves (without stem) is measured after drying the leaves in hot air oven at 80ºC for 72 h.

3.3.1.4 Root dry weight (RDW)

The root dry weight (RDW) was recorded after drying the root in a hot air oven at 80ºC for 72 h.

3.3.1.5 Rooting depth (RD)

Rooting depth is measured after removing the soil particles from the root system under running water. The roots were straightened and depth to which the roots had been penetrated was measured.

3.3.1.6 Ratio of RDW and total dry weight (R/T)

It is ratio between the RDW and the total dry weight. Total dry weight is the collective dry weights of shoot and root system of each plant.

3.3.1.7 Root length (RL)

The soil from the roots is removed by rinsing the roots in running water. The root length was measured by using a digital image analysis system (WinRhizo, Regent Instruments INC., Canada). The image of the root is taken and the total length is obtained by measuring the length of tap and branch roots of the chickpea root system.

3.3.1.8 Root length density (RLD)

The root length density (RLD) was calculated by dividing the total root length by the cylinder volume at the maximum rooting depth.

3.3.1.9 Root surface area (RSA)

The root surface area was measured by using a digital image analysis system (WinRhizo, Regent Instruments Inc., Canada).

3.3.1.10 Root volume (RV)

The root volume is the volume of the soil in the PVC cylinder, at the maximum rooting depth.

3.3.2 Phenotypic data analyses

3.3.2.1 Analysis of variance (ANOVA)

The analysis of variance at for different root traits for two years- 2005 and 2007 for three replications was performed to test the significance of differences between genotypes and pooled analysis of the data to assess the contribution of different sources to the total variation by following Panse and Sukhatme (1961). GenStat (12^{th} edition) was used to calculate general ANOVA using phenotyping data from two years.

3.3.2.2 Correlation coefficient (r)

Correlation coefficient (r) among the different root traits was estimated by using software GenStat (12th edition).

3.3.2.3 Heritability (h^2)

Heritability in broad sense was computed as the ratio of genetic variance to the total phenotypic variance as suggested by Hanson et al. (1956) and expressed as percentage.

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where, σ^2_g = Genotypic variance and σ^2_p = Phenotypic variance

Heritability (broad sense) estimates were categorized into high, moderate and low by Robinson et al. (1966).

Heritability (%)	Classification
5-10	Low
10-30	Medium
30-60	High
>60	Very high

3.3.3 QTL analysis

Single-locus QTL analysis for each trait was carried out by composite interval mapping (CIM) using the Windows QTL Cartographer program version 2.5 (Basten et al. 1994). For each trait, the analysis was carried out using data from individual environment as well as pooled data averaged both the environments. The CIM performs first a multiple regression involving all the markers, then uses the markers explaining most of the genetic variation as co-factors when performing the classical single interval mapping. The standard model 6 of

Zmapqtl procedure (Basten et al. 1994) with forward regression and backward elimination module was used in the analysis by scanning the genome every 2 cM. The threshold levels to declare significant QTL were determined by performing 1000 permutations of the data by maintaining the chromosome-wise type I error rate of 0.05 (Churchill and Doerge 1994) and a LOD score of 3.0 was used for declaring presence of the QTLs. The relative contribution of a genetic component (R^2 or h^2) was calculated as the proportion of the phenotypic variation explained (PVE). The QTLs explaining more than 20% phenotypic variation were considered as major QTLs. The additive effects and R^2 of the detected QTL were estimated by the Zmapqtl procedure inbuilt in the QTL Cartographer.

3.4 Association Mapping

Two approaches were used for marker-trait association for drought tolerance in chickpea namely "candidate gene sequencing approach" and "genome-wide association".

3.4.1 Phenotypic data analysis

The phenotypic data on eight drought tolerance traits (SDW, RDW, RD, RT, RL, RLD, RSA and RV) was collected in the year 2007 on reference set of chickpea. The phenotypic analysis (includes mean, range, coefficient of variation and correlation) was carried out using GenStat (12^{th} edition).

3.4.2 Candidate gene sequencing approach

3.4.2.1 Identification of candidate genes for drought tolerance

The candidate genes responsible for drought tolerance were identified based on prior information of involvement of these genes in drought tolerance mechanism in other crop species. The following candidate genes were enlisted based on earlier reports in other crop species: abscisic acid stress and ripening gene (*ASR*), drought responsive element binding

protein (*DREB*) gene, *ERECTA*, sucrose synthase (*SuSy*) and sucrose phosphate synthase (*SPS*). The nucleotide and protein sequences for the candidate genes were downloaded from all the available databases such as National Centre for Biotechnology Information (NCBI-www.ncbi.nlm.nih.gov), The Institute for Genomic Research (TIGR-http://compbio.dfci.harvard.edu/tgi/plant.html) now called J. Craig Venter Institute (JCVI), SWISSPROT (www.expasy.ch/sprot/), The Arabidopsis Information Resource (TAIR-www.arabidopsis.org) and Legume Information System (LIS-www.comparative-legumes.org). The nucleotide sequences were transformed to amino acid sequences and sequences having right reading frame was used for designing the degenerate primers, which can work beyond the codon biasness across the seven GCP crops. Finally crop-specific primers were designed to amplify the candidate genes in respective crop species (This et al. 2010- http://www.intl-pag.org/18/abstracts/P05a_PAGXVIII_234.html). Different approaches were used to isolate candidate genes responsible for drought tolerance in these crops by using degenerate primers and reconciliated taxonomic trees (Nayak et al. 2009) and allelic series derived by specific PCR.

In order to identify the above mentioned candidate genes in chickpea, the sequences were downloaded either from chickpea or from related legume species. As the sequence information in chickpea is limited, sequence information on candidate genes was used from *Medicago*, which is phylogenetically related to chickpea. In this regards, DREB2 homolog which is called as Chickpea Apetala gene (*CAP2*) was already known in chickpea by the reports from Shukla et al. (2006), hence the primers were designed by using the sequence with GenBank accession DQ321719. For isolating *ASR*, *SuSy* and *SPS*, heterologous primers were designed from corresponding *Medicago* sequences. The details of the heterologous sequences from *Medicago* and primers used for isolation of candidate genes in chickpea are tabulated in Table 6.

ERECTA gene in chickpea was isolated using consensus primers designed at INRA-CNG, France. As *ERECTA* gene was of about 4 kb long, two pairs of consensus nested primers were used from protein sequences from *ERECTA* and *ERECTA* like sequences available in the databases. The primer sequences and expected sizes of the amplicon for the candidate genes are enlisted in the Table 6.

In order to amplify these candidate genes initially in two chickpea genotypes namely ICC 4958 and ICC 1882, PCR was set up with 10 µl reaction mixture comprising 5 ng of template DNA, 5 pmoles each of forward and reverse primers, 2 mM dNTP, 20 mM $MgCl_2$, 1X PCR buffer (AmpliTaq Gold) and 0.25 U of Taq polymerase (AmpliTaq Gold). PCR cycles comprising of denaturation of 94°C for 5 min, followed by 40 cycles of 94°C for 30 s with constant annealing temperature of 56°C for 50 s and 72°C for 1 min 30 s and a final extension was carried out at 72°C for 20 min. The PCR products were checked for amplification on 1.2 % agarose gel.

Table 6: Primers used to amplify candidate genes related to drought tolerance

Gene	Source sequence	GenBank/ TC ID	Primer sequences (5'-3')
Abscisic acid stress and ripening (*ASR*)	Medicago	AC152054	F: GGGAACTAATCCTTTCCAAACA R: CTGCAGCACCTAACTCACCA
CAP2 gene (*DREB2A*)	Chickpea	DQ321719	F: CGGCTTCCCTTCATTCGATCCA R: AGGCACAACACAAGAATCCA
CAP2 promoter	Chickpea	-	F: TGTGCTTCAAGTTGCACTCC R: CGGGGTCCTTATATACTGCAGA
ERECTA (fragment 7F-5R)	Degenerate	-	F: GTGTACAAACCTTAACAGCC R:CCA GTTAATTCGTTGTTTTC
ERECTA (fragment 8F-8R)	Degenerate	-	F: GGTCAGCTACAGAACATAGCA R:TCCATTTTCCATGTAGTCATAA
Sucrose synthase (*SUSY*)	Medicago	TC95820	F: GATACTGGCGGACAGGTTGT R: CATCCTTTGCTAGGGGAACA
Sucrose synthase (*SUSY*)	Medicago	AJ131964	F: GGGTCAGTCTCTGTTGATGC R: GACGTTCACCAAAGGTCAAAA
Sucrose phosphate synthase (*SPS*)	Medicago	BQ137986	F: TTTGGTCCACGCGATAAATA R: TGAATTGATATCCTCCCAAGA
Sucrose phosphate synthase (*SPS*)	Medicago	CB893717	F: TGCAGGACATGTCTCTTAGGC R: CTGCAAATCAAAGCATCAAAA

The above mentioned primers were used to amplify candidate genes.
GenBank accession IDs obtained from NCBI and TC (tentative consensus) sequence ID was obtained from JCVI/TIGR.
F- forward primer sequence; R- reverse primer sequence

3.4.2.2 Sequencing of candidate genes

A pilot experiment was set to sequence eight diverse genotypes of chickpea consisting of Annigeri, ICCV 2, ICC 4958, ICC 1882, ICC 283, ICC 8261, ICC 4411 and ICC 10029 genotypes. In order to sequence the candidate genes (*ASR*, *DREB2A*, *ERECTA*, *SuSy* and *SPS*) across eight diverse genotypes, 20 µl amplicons were obtained by setting PCR with same conditions. Wherever *Medicago* primers were used to amplify chickpea candidate genes, *Medicago* genotype- A17 was also used for amplification and sequencing along with eight chickpea genotypes. The amplified product (about 2 µl) was tested on 1.2 % agarose. The remaining PCR amplicons were purified using 1 unit of Exonuclease I and 1 unit of shrimp alkaline phosphatase (SAP) per 5 µl of PCR product. The Exo/SAP added PCR

products were incubated for 45 minutes at 37°C followed by denaturing at 80°C for 15 minutes in the thermal cycler for deactivating unused Exonuclease enzyme. The Exo/SAP treated amplicons were mixed with 1 µl of BigDye Terminator V3.1 (Applied Biosystems, California, USA), 2 µl of 5X sequencing dilution buffer and 3.2 *µ*M of primer (forward and reverse separately) and the volume was made to 10 µl. The sequencing PCR profile included an initial denaturation of 96°C for 30 seconds, followed by 60 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. The PCR products were stored at 4°C until further use. Before sequencing, the PCR products were treated with 2.5 µl of 125 mM EDTA and 25 µl of absolute ethanol and incubated for 15 minutes at room temperature to precipitate the DNA. The plate containing the PCR product was centrifuged at 4000 rpm for 30 min at 4°C. The Ethanol/ EDTA mix was poured off by inverting the plate, without losing the pellet. To each well, 60 µl of 70% ethanol was added and again spun at 4000 rpm for 20 minutes at 4°C. The ethanol was poured off as earlier. The plate was air-dried and 10 µl of HiDi formamide (Applied Biosystems, California, USA) was added and the products were denatured (94°C for 5 minutes, then immediately cooled to 4°C for 5 minutes) and sequenced using an ABI3700/ABI3130 automated sequencer (Applied Biosystems, California, USA). The large-scale sequencing of candidate genes across 318 genotypes of reference set was carried out at INRA-CNG, France using BigDye terminator cycle sequencing chemistry.

3.4.2.3 Sequence analysis

The raw sequences were used to obtain contigs by assembling the forward and reverse sequences of each genotype using DNA Baser V 2.9 tool. The sequences pertaining to each candidate gene were aligned using CLUSTALW (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The MSA files and multifasta files obtained from the MSA were further used identifying the sequence related parameters such

as number of genotypes sequenced; length of sequences; number of indels; indel frequency; number of SNPs and their types (transition or transversion); SNP frequency; nucleotide and haplotype diversity and polymorphic information content (PIC) of SNPs and haplotypes using a in-house tool developed at ICRISAT called "DIVersity ESTimator" module (DIVEST*)* (Jayashree et al. 2009). Further, in order to identify if any of the haplotypes can be associated with the country of origin of the genotypes under study, NETWORK programme version 4.516 was used to determine haplotype networks for each candidate genes studied.

3.4.2.4 Candidate gene based association analysis

The sequence variants (or SNPs) obtained in case of candidate genes along with the phenotypic data on drought tolerance related traits were used to analyse the association between the candidate genes and traits using standalone TASSEL version 2.1. The Q matrix obtained from STRUCTURE version 2.3.1 is used to define number of sub-populations in the dataset. General linear model was used to identify the candidate genes associated with drought tolerance related traits. Candidate genes associated with the drought tolerance related traits at significant P value of <0.01 were considered.

3.4.3 Genome-wide association approach

3.4.3.1 Structure analysis

Model-based cluster analysis was performed to infer genetic structure and define the number of clusters (gene pools) in the datasets using software STRUCTURE version 2.3.1 (Pritchard et al. 2000). The number of presumed populations (K) was set from 1 to 15, and each was repeated two times. For each run, burn-in and iterations were set to 1,00,000 and 2,00,000 respectively, and a model without admixture and correlated allele frequencies was used. The run with maximum likelihood was used to assign individual genotypes into

groups. Within a group, genotypes with affiliation probabilities (inferred ancestry) ≥80% were assigned to a distinct group, and those with <80% were treated as "admixture", i.e. these genotypes seem to have a mixed ancestry from parents belonging to different gene pools or geographical origin. Delta K (ΔK) value was computed according to Evanno's method (Evanno et al. 2005). The Q matrix obtained for the highest ΔK, was used for association analysis along with the genotypic and phenotypic data on the reference set of chickpea.

3.4.3.2 Genome-wide association analysis

In this approach 1,157 Diversity Array Technology (DArT) marker data was obtained on reference set of chickpea in collaboration with Andrzej Kilian from Diversity Arrays Technology Pty Ltd, Australia. These markers were presumed to be distributed across the whole genome and used as neutral markers for associating the phenotypic data obtained for root traits on reference set of chickpea. Association between markers and traits was worked out by both general linear model (GLM) and mixed linear model (MLM) as described by Yu et al. (2006) using software TASSEL version 2.1 (Bradbury et al. 2007). GLM function performs association analysis by a least squares fixed effects linear model. TASSEL utilizes a fixed effects linear model built by the user to test for association between segregating sites and phenotypes, while also accounting for population structure. In MLM, the markers being tested are considered as a fixed-effects factor, while as subpopulations are considered as a random effects factor. MLM can also reduce Type I and Type II errors. The unified mixed linear model simultaneously take into consideration both population structure (Q) and kinship (K). The population structure was inferred by programme STRUCTURE version 2.3.1, and kinship matrix was calculated from the marker data using standalone TASSEL version 2.1 programme. Significance of marker-trait associations were described at P-value (P≤0.01 for significant markers).

4. RESULTS

4.1 Development of Microsatellite (SSR) Markers

The present study involved the development of some novel microsatellite (SSR) markers, which, together with SSR markers from other sources were subsequently used for the study of genetic and QTL interval mapping. The markers were developed using following two different approaches:

4.1.1 Development of SSR markers from enriched genomic-DNA library

A genomic DNA library comprising of ca. 4,00,000 clones was constructed from the chickpea reference genotype "ICC 4958". Hybridization of this library with GA and TAA oligo probes leads to the identification of 359 positive clones. Of 359 positive clones, only 307 clones showed growth in the selection media. A total of 288 clones (forming three 96-well plates) from 307 clones were sequenced in both directions using standard T7 promoter and SP6 primers and 576 sequences were generated. Remaining nineteen (19) clones were sequenced in only one direction by using M13-forward sequencing primer. In total, 595 forward and/or reverse sequences were generated and after checking the quality of sequences and their length, 23 sequences which were either bad quality or sequence residues less than 100 bp and more than 700 bp were removed in order to ensure high quality sequence data. In total 572 cleaned sequences were processed using CAP3 programme to obtain non-redundant set of sequences (http://bioweb.pasteur.fr/seqanal/interfaces/cap3.html). The resulting 457 non-redundant set of genes comprising of a set of 115 contigs and 342 singleton sequences were referred to as genome survey sequences (GSSs). All these GSSs have been submitted to National Centre for Biotechnology Information (NCBI) GenBank accession number FI856537 to FI856993 (http://www.ncbi.nlm.nih.gov/).

Sequence analysis of 457 GSSs representing 28.67 kb sequence data, showed the presence of 643 SSRs in 299 GSSs. There was occurrence of one SSR for every 445 bp. A total of 165 GSSs contained more than one SSR while 221 GSSs were found to have compound SSRs. Sequence data for all SSR containing sequences (299), derived from microsatellite enrichment library were used for primer designing using the Primer3 programme. As a result, a total of 311 primer pairs were designed (Table 7). In some cases primer pairs were designed for more than one SSR from a single GSS with the goal of increasing the conversion of GSS into usable genetic markers and were named as 'a', 'b', etc. following their clone ID (eg. ICCM0001a, ICCM0001b). Although this increases the redundancy, it ensures not to miss any polymorphic SSR in a given sequence/ clone.

4.1.1.1 Frequency and distribution

Frequency of different SSRs identified during the present study revealed that di- and tri-nucleotide SSRs were the most abundant (39% and 40%, respectively) while, mono-nucleotide SSRs occurred in about 16% cases and tetra-nucleotide SSRs in about 3%. Other types of SSRs had <1% representation (Figure 1). In terms of repeat motifs, the tri-nucleotide repeat motif TAA/ATT was the most common, accounting 36.8% of all the repeat types, followed by di-nucleotide repeat GA/CT (19.2%). In majority (467, 73%) of the 643 SSR motifs, the microsatellites were of less than 10 repeat units followed by those having repeat units between 10 and 20 (137 cases, 21%). However, only a very few microsatellites were observed in other classes of SSR, whose repeat motifs were ranged more than 20 (Figure 2).

Table 7: Novel set of SSR markers (ICCM) developed from SSR-enriched library of chickpea

Marker	GenBank ID	SSR motif	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
ICCM0001a	FI856656	(AT)5	GAACTTGCTTGGGGACAGG	GAGGTGAGCTGAAGTGAGGC	246
ICCM0001b	FI856656	(CT)4tc(CT)4	TGCACAACGGCTATGTCTTC	GAGGTGAGCTGAAGTGAGGC	118
ICCM0002	FI856654	(TC)6	TCGTTCTCCGTTATGTGTGC	ATGCCTCCAATAGCATACGG	262
ICCM0003	FI856538	(CT)6	AATGGAAGAACGTCAGGGTG	TTCCACTGGGGCAAAATAAG	252
ICCM0004	FI856539	(AT)4	CACTCACACACGCACTTTCA	AAAAGAGAAGCCCACCAAAA	213
ICCM0005a	FI856657	(TC)4N(CT)4N(TC)4	TGCTGAGCGATCTGAGGATA	GCAGCAAAAATCAGCACAAA	277
ICCM0005b	FI856657	(AT)4	TTGGTGCAGATTTTTGTTGC	AGATTTGGGGGATAAAAGGG	241
ICCM0007a	FI856661	(CTC)4N(TC)4N(TC)4	TCTACATTCTCTCCGTGCCC	GAGGAGTGTAGGGGAGAGGG	242
ICCM0007b	FI856661	(TCCTC)4	CTCTCCCCACTCTCCCTTTC	TGAGTAGGATCGTAGTAGGGGG	202
ICCM0008a	FI856540	(A)10N(A)10	CTCATGGTGGCATTGAGAAA	TCCTTGAATTTTTGAGACACGA	230
ICCM0008b	FI856540	(GA)4N(AG)5t(GA)4	TCGTGTCTCAAAAATTCAAGGA	CACTCGACCACCACTGCTAA	235
ICCM0008c	FI856540	(CT)4	GGTGGTTTGTGGAGGTTGAT	AGGCAACCATTCATCCTTGT	245
ICCM0009a	FI856541	(GA)4	CACTTCAAAAGAGTGTTTGATTTGA	GGTTTGAAAGATGAGTGGTTTTG	189
ICCM0009b	FI856541	(A)11	AAAATATGGAAAGTCGGGCA	TGCATTTTCTTAGCGGTTTTT	257
ICCM0010a	FI856663	(CT)4	GACGGAAATACGGCTGGTTA	GCTGCAATATACTCCGCCTC	113
ICCM0010b	FI856662	(AT)4	ACGCCAATTCTTTTGAGCAC	TCAGCACTGGTGGAACCATA	142
ICCM0014a	FI856542	(GA)5	CGTGGTTGTGTTGTTTGAGG	TGTGTTCATCTCCCTCTCCC	134
ICCM0014b	FI856542	(TA)5	TTTGGGAACCTTTCTCATCAA	CCAAATCATTTTTGTGCACTG	254
ICCM0019a	FI856544	(AG)18	TTCCGGTTGTTGAGTAGAGAAA	ACTTCCACTTGTTCCATCCG	189
ICCM0019b	FI856544	(AG)4	CGGATGGAACAAGTGGAAGT	TCTCGTACCGGACCAGAGTC	153
ICCM0021a	FI856697	(CA)4N(AT)4N(ATT)5	AAACCGCATTGAGGAATGAC	TTTGCCACGATATGTTCAGG	232
ICCM0021b	FI856696	(AT)4	TCTTTCTAAGCAGTCTAGGATACGA	AGGAAGGGTGGGATAATTGG	229
ICCM0022	FI856699	(AT)4	TAAACCGCATTGACGAATGA	TGAATTCGCAAGAATCAAATG	123
ICCM0024	FI856545	(AT)4	CACTCACACACGCACTTTCA	AAAAGAGAAGCCCACCAAAAA	214
ICCM0026	FI856714	(AG)4	CCGGACATTGTTCTGAAGGT	TCTGTGCAGTGGAGCTATGG	216
ICCM0029	FI856721	(AG)4N(GA)4N(GA)4a(AG)5	CTTCCCCTTTCCAAAATTGA	TCGTTCACAGGTTTTCCCTC	244
ICCM0030a	FI856722	(TC)4N(CT)4N(TC)4	GGCAACGTACGGGATAAATG	GCAGCAAAAATCAGCACAAA	271
ICCM0030b	FI856722	(T)10	TTGCTGCTGATTTTTGTTGC	TCATCCATCATTCTAAATGTGTCA	257
ICCM0032	FI856546	(GA)4	TCTCTTGACACAAGTCTGCACAT	CCCACTCACATGTAGCGAAA	232
ICCM0034	FI856651	(GA)11	TTTGTTTGCGGAGGAATAGG	TCACCTCACCACACTTCTTTTC	259
ICCM0035	FI856547	(GT)4	TGAGGGTAAATAAATTGGTGGC	ATGATTTCCGAGGACAGTGG	101
ICCM0037	FI856548	(AG)4N(GA)4N(AG)4N(GA)5	CAAGCAATGGGAGACACCTT	CCACCACTTCAACGTCTCCT	212
ICCM0042	FI856740	(CA)4	TTCGTGATATTCATCAAGGTGG	TTTGACGTACTCTGTGTTTTGTTT	259
ICCM0043	FI856552	(GA)6	AAGATTGGATTCCACAACGC	ACAACCCACCCACACAAAC	274
ICCM0045	FI856553	(TA)4N(TC)12	TGAATCCCTCAATCCTGTGG	CTTATCTCCCCAGTTGCCAG	272
ICCM0052	FI856768	(TAT)6	CGTCGTGTCCATCTGTCTTG	CCAGGTGCAATAGGGAAATC	275
ICCM0053	FI856771	(GA)32t(AG)4	TGCGGTCGACTCTAGAGGAT	CTGAGAAGGAAGCCAACAGG	173
ICCM0059a	FI856779	(GAA)4	CGGTCGATCTGAGGATCACTA	GGGTTCAACTGTTCAGGTTTG	226

Contd…

ICCM0059b	FI856779	(AG)4N(GA)9N(GA)4	CAAACCTGAACAGTTGAACCC	AGGTTCTCCCTCGCTCTCTC	172
ICCM0060	FI856780	(AT)4N(CT)4	TGATGAACATGCTAACAAACTAACAA	CCAAGACAACTGGTGGAGGT	260
ICCM0061	FI856798	(AT)6N(TTA)4(TAA)4N(TTA)13	ATGCGGTAATCCTTGACTGG	TGAAATTGGGTTGGAAGTTTG	275
ICCM0062	FI856801	(AAT)4N(AAT)6	ATGCAACGCCTAAGTCTCGT	TAGGTACGTTTGGGGTCCTG	266
ICCM0063	FI856802	(TTA)6	TTGATTTATCCTGTGATGCTTTTT	GGCAGTCTGGTCATGTGAAA	194
ICCM0065a	FI856807	(TC)6	CATTCCCGCAAAGCTTGTAT	GGTGCCTTGGAGAAAATCAA	137
ICCM0065b	FI856807	(TC)4	CACGGTGTCTTCCACATGAC	TAAGCCAATACCAAGAGGCG	195
ICCM0066	FI856555	(TC)4	ACCATGCTCACCAACTCACA	TCTCTTGACACAAGTCTGCACAT	242
ICCM0067	FI856556	(TC)4	AACTGCAACCTCTCTCGCTC	TCTCTTGACACAAGTCTGCACAT	204
ICCM0068	FI856557	(ATT)22	TCTTCTTTGCTATCTGTCTCGC	TGCATGTCAAACATTAGACAACTTT	227
ICCM0069	FI856558	(ATT)22	TCTTCTTTGCTATCTGTCTCGC	TGCATGTCAAACATTAGACAACTTT	227
ICCM0072	FI856813	(GAA)4	CAAGACCTTGAACAGGAGGC	TCTTTCAATTTTCTAACAATTTCATC	200
ICCM0073a	FI856828	(TA)4N(A)11N(TA)4	TCGATCTGAGGATCTTTGGTG	TGGATACTATTAACGAAAAACTAGCG	219
ICCM0073b	FI856828	(TC)4	CTTGTCCACCCCACATTCTT	TAGAGAAATGGGGGAAGGGT	217
ICCM0074a	FI856830	(A)11N(TC)6	AAATCCCAAATTTAGAGCGG	AACCTTTGAAAAAGGCGGTT	183
ICCM0074b	FI856830	(T)15	AACCGCCTTTTTCAAAGGTT	CCTTCCAGGGGAAAAAGAAA	264
ICCM0075	FI856559	(AG)16	CTTTGTAACAATAAAATGCAAAGTAAA	GGAAGCACAGTCTGCACAAA	163
ICCM0076	FI856560	(ATA)17N(TAA)5	CTCATCGAATAGAACCTACCGA	CCGCTACACCTACAACGGTAA	270
ICCM0077a	FI856561	(AG)4N(T)10	CCACAAGAAGACAAAGGGGA	AAAAAGATGCTAAAACTAAACCAAAGA	217
ICCM0077b	FI856561	(A)10	GCCGAGAAAATAAATTTCACCA	GCCGCGACCATTAATTCTAA	124
ICCM0078a	FI856832	(TAT)4g(ATT)6ag(TAT)5N(AT)4	CTGAGGACGTTGGGAATACG	AAAAACTAATCTCGTGTTCAAATCC	280
ICCM0078b	FI856832	(TC)4	AATCCCAACGGTGAGAGATG	GGACAAGGAGTGGAAGGGA	279
ICCM0079	FI856562	(TTA)6	GAGCTAACGCCTTCGCTAGA	GAGAGGGATTAAAACAAATAGAGGAA	170
ICCM0080	FI856563	(T)10	CTGCGGTGACTCTGAGGAT	GAGAATCACGGGTGTTTCAAG	173
ICCM0081	FI856564	(TAA)6	GAGAGGGATTAAAACAAATAGAGGAA	GAGCTAACGCCTTCGCTAGA	170
ICCM0082	FI856833	(CT)19N(TC)4	TCACGATCTCACAGAGCCAC	TCCGTGATTCTGAGCAACAG	260
ICCM0083a	FI856835	(AT)4N(CT)7	CGCTCACACCATCTCACTTC	GAATGGAGGAAATACAGAGTGC	273
ICCM0083b	FI856837	(A)13	ATTGAAAAACCACGCACACA	AGCGACGACAGTGACCTTCT	140
ICCM0083c	FI856837	(T)11	TGTTTGTTGCCTAGCCACTG	TTGGACAGATTTTTGTTGTTTGTT	195
ICCM0084	FI856993	(GA)5	TTTTGATTGAGCATGCAATGT	GAACCTTTTGAGGTCTGTTGC	246
ICCM0085	FI856855	(T)10N(TAT)14	GTGGTCCATCTGTCTCGGTT	GGAAAAGGAGAAAGTTGTGGG	210
ICCM0086a	FI856856	(TA)4N(T)11	TGTGCATTGAGCCTATTGGT	GACAACAGCGGCATAATCAA	182
ICCM0086b	FI856856	(AC)4	CTTCCCCTTTACCCCGTTTA	AAAGAAATCGACAATAAAAGAGTGA	175
ICCM0088	FI856861	(T)10N(AT)5	AAAGGAAGGGAAGGAAATGC	GAGTTTGGGCAGGCAATAAA	240
ICCM0089a	FI856863	(A)12	AACACCGACTTTCCAAAACG	TTTGGGAAATACAACCTTTGAA	204
ICCM0089b	FI856862	(TAT)20	GGGATATCGCCAATATATTTTATACC	TTTGGCAACAAATCCTTTGA	126
ICCM0090a	FI856864	(TTA)6	ACGGGACTTGGATGACTTTC	AGACGCGTGCTTTCTTCCTA	257
ICCM0090b	FI856864	(TC)5	CTGCCTAGGAAGAAAGCACG	AAAATAAATGCGCCGTATGC	160
ICCM0093a	FI856871	(TAT)20	CTTCTGTTATTATCGCCGCC	AGCATCATGGAGCAGAGAGG	223
ICCM0093b	FI856871	(AT)4	TACCCTTTCTCTCCCCACCT	TCAGTAGTCGGGCAATAGATGA	129
ICCM0093c	FI856870	(AAAT)4	CCACTTTTAGGCGCACTTCT	CGACTCATTTTTCACGGACA	197
ICCM0094	FI856872	(AT)4	AGAGGCAAACAAGAACCGAA	AAGGGTTAGTGGAGGAATTATGAA	279
ICCM0095a	FI856875	(TC)4	CTCTCCATCCCATCCGACTA	GGAAGCCATATCCAGAGGGT	181

Contd…

ICCM0095b	FI856874	(ATTC)4	CGGGACATTTCCGTTAAAAA	CGAGTCGTTTTCTTGGCTTC	171
ICCM0096	FI856877	(AT)4N(CT)4N(TC)4	ACACCCCACCCTAATTACACT	GAGAGGTACGAAGCACGAGG	170
ICCM0097a	FI856669	(ATT)12N(TA)4	TGAGGACTGCCATACTCCAG	TCCCCTTTATGAGGGCTTTT	276
ICCM0097b	FI856668	(CTT)4	TCCAATTCCAAAACACACCA	CCTGAGGAGTAAAAGACGGG	130
ICCM0101a	FI856675	(A)13	TAACTGAGTTTCGGGTTCCG	CTTAACGGACGTTGTAGGGC	247
ICCM0101b	FI856674	(AG)4	GAAGACAAAAAGGGGCACAA	CCGATTGTTTCAAGACCAGA	105
ICCM0102	FI856676	(T)12	CACCAAAAAGGGAACTTTCG	AAAAATAGGGTGGGGAGGG	167
ICCM0103	FI856678	(A)11	ATGGGGGAATCGGAGACTAA	GGATAGGGAGGAGGGAACAG	110
ICCM0104	FI856566	(TTA)11	CCAAACCTCCAAAAATCTGC	TCATTTTTGATTCATTCTTGGG	278
ICCM0105	FI856567	(AT)4	TGCTTCCTTTTCAATCACCA	TGACAAAGGACAAATAAGTGTTTTA	280
ICCM0106	FI856681	(ATA)7	TGGAATTGCTACCGAATATGG	ACGATCGGAGAGAACGAGAA	267
ICCM0107a	FI856682	(TCG)4	GCAAAAAGTGTTGCTTCCGT	AAGCACATTGCCACTAGCAT	234
ICCM0107b	FI856682	(TA)4	GCAAAAAGTGTTGCTTCCGT	AAGCACATTGCCACTAGCAT	234
ICCM0110	FI856568	(AT)4N(TTA)7	AGAGGCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	183
ICCM0115	FI856706	(TC)4	ACCCTAAGGGCTCGTTTGAT	TAGGGATGGAGAGGAGAGCA	245
ICCM0116	FI856709	(T)11	TTTTGGTGCAAGAGAATGGG	GTCTTTCAAGAGGTCGCAGC	177
ICCM0117	FI856572	(TAA)4	AGTGACCAGGAAACACGGTC	GCAGAGATTGAATTTTGCCA	254
ICCM0118	FI856573	(T)11	AATTGGGAAGGAAAGCGAGT	TCGCCATTGCAATAATCAAA	279
ICCM0119	FI856710	(GA)4	TTATTGGATGAGTGGATGCG	ATTACGTAACCCAACGTCGC	192
ICCM0120a	FI856574	(TTA)12	TGTCTCGATAAGAGTTTGTTATTTTTC	CGTTTTGTTTCATATTCAAACTCG	220
ICCM0120b	FI856574	(ATT)13	CGAGTTTGAATATGAAACAAAACG	GCTTGTAGCTAGGCTCGACTC	166
ICCM0121a	FI856575	(TATT)4	CAAAAATTTGGATTCGGGAG	CTATTGCACCTGGGGATACG	238
ICCM0121b	FI856575	(A)10N(ATA)17	ACGTATCCCCAGGTGCAATA	AACAGATGTAGAAGGTATAATCCATGA	224
ICCM0122	FI856576	(AAT)4	GCAACCTGCCATCCATACTT	AGTGAATCAAATGATGAAAGCA	275
ICCM0123a	FI856577	(TTA)14	GGATGGTCTGCTGGAATCAT	AAAGACAACAAAAAGACAATCATGT	250
ICCM0123b	FI856577	(TAA)26	TTTTTACATGATTGTCTTTTTGTTG	TGAGGACTAAGATAATAGCAATCCAA	201
ICCM0124	FI856578	(AAT)4aaa(AAT)4	CCTCGGGAATTCAACTACCA	TCAAAAATCCACTTTCCACCA	279
ICCM0125a	FI856579	(AAAT)4	CGATCTGAGGATCAACTTGTGA	ACTAACCACCGTCGACCATC	253
ICCM0125b	FI856579	(TTA)5	TATTTATGGTCTGGTCCGGC	CGCTACCAAATATGGAACGACT	251
ICCM0127	FI856581	(TAA)27	TGTTGAACGAATTTACTCATCG	GGTGGGCTCCTATTGTTTGA	269
ICCM0128a	FI856731	(TA)4N(TAT)4	AACCCTAATTTATTTGCACTATTATCA	TCAAAATACGGTAGTAGGATAAGATGA	161
ICCM0128b	FI856730	(A)10	ATTTGGACGATGTGTCGCTT	TTATAGCCCCTGCTTTGCTG	266
ICCM0130a	FI856734	(AAT)22	GGATTTCGACTTTTATCCCTTTTT	CGGACTGGAATCAAAAGCTC	268
ICCM0130b	FI856734	(ATT)5	GAGCTTTTGATTCCAGTCCG	TGTAGGGGTGCATGGTGTAA	122
ICCM0131	FI856582	(AT)4N(TTA)8	AGAGCCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	192
ICCM0134	FI856748	(A)11	TTTTGGAGGCAGCTTTGAGT	TGAAGACAGAGACGGTGCAT	109
ICCM0138a	FI856753	(AG)4	ATAAATAGCCGGCCACAAGA	CCGATTGTTTCAAGACCAGA	119
ICCM0138b	FI856752	(ATA)11	CTGTTGCCGATTTTATATTATTTTT	AAATGTGTTGTTCTGGCCGT	168
ICCM0139	FI856755	(ATTC)4	TCACGATTTGAATGGTCGTG	CGTTTTCCCAGCTTCAACAT	151
ICCM0141	FI856757	(AAT)20	AAGGTATGATGCAGTTCCCG	GGGCGGAGGGTAATTATTGT	247
ICCM0142a	FI856759	(AC)4	GCATTGCCAATATCGAAGGT	AGTAGGTGCCAAATGCATCC	206
ICCM0142b	FI856759	(ATT)6	GGATGCATTTGGCACCTACT	GAGGTTGGTGTAGAATAGAATGGA	137
ICCM0142c	FI856758	(AC)7	GGAGGTCCCGAGTCTAAACC	TCTTCAAGTCTGCTTGACGTGT	105
ICCM0143	FI856761	(AC)5	CTGCGGTCGATCTAGAGGAT	AAGGTTGAAGGATGATTGCG	257

Contd…

ICCM0150a	FI856583	(ATTC)4	TGATTGAAATGGTCGTGTCC	AGTCGTTTTCTCGGCTTCAA	279
ICCM0150b	FI856583	(TAA)8N(AT)4	GTAAAGAGGGGCAGCTGTTG	AGAGGCAAACAAGAACCGAA	192
ICCM0152	FI856792	(T)13c(CTT)4N(AG)5	CGTCTCACGAAGGAGAAGTG	AAAAATTCACTTGCTAATATTTCACA	197
ICCM0154	FI856794	(A)11	AGCTTGTTCGACAGCAGGAT	TCGATGTGAATATGCCCTCTT	247
ICCM0155	FI856796	(A)11	GACGGCAGGATTAACTGCAT	TTCGATGTGAATATGCCCTCT	240
ICCM0156a	FI856797	(AT)7N(CA)4	TGCATTCCCCTTTAATTTGG	CAGTGGTGGGGAGACAAAAC	280
ICCM0156b	FI856797	(ATA)52N(AAT)6	GTTTCTCCGTCCGCACTTAT	AAACAGTGTAAATTGTTGTTGGAAA	276
ICCM0157	FI856584	(GA)8	TTTGTTTTGAGGCAACCACA	AAACAAACCCAGTGGGAGGT	258
ICCM0158	FI856585	(ATA)18N(TCT)16	CCAAAACTGACAAGTCCCGT	GGGAAAATATGAGGAAGTTTGC	275
ICCM0159	FI856814	(T)17N(T)10	TGAAAAATCGGAAACCCTACC	CCTTGTTTTCAGGCGATTGT	247
ICCM0160	FI856816	(AAC)4N(TAA)25	TTGCTTGAAACAACCTTTCG	CGGGTACAACCGTAGCAAAT	263
ICCM0161a	FI856818	(AT)4	GATGGTCATCGGTTCGATTC	AACGAGGCCCTCTGTAACG	267
ICCM0161b	FI856817	(TAA)4N(AAT)4	ACTGTCAGGAAGGAACGGTG	TCCGTAACAAAAATTGTGAAGAAA	279
ICCM0162a	FI856586	(ATT)12	TAGCGCAGTCGATCTGAGGA	ACGTATCCCCAGGTGCAATA	272
ICCM0162b	FI856586	(AAAT)4	GCAAGGTCTTTCCCTTGTCA	CGCCGCCAATTTTATTTTTA	278
ICCM0162c	FI856586	(T)11N(TA)4	TAAAAATAAAATTGGCGGCG	TCGTGTTAGGGTGTTTAGGGA	267
ICCM0162d	FI856586	(T)10	GACTCTGCTGGGGACAATTT	CGGGTTTAGAGACCCACTCA	225
ICCM0163	FI856820	(TC)4	CAACGAATTTCATGCTGTGG	TAGGGATGGAGAGGAGAGCA	274
ICCM0165	FI856821	(T)11	CGGACGTACACCTTTCGTTC	TGCTTCCGAATAACATAAAGCA	128
ICCM0166a	FI856824	(T)11	GCCTACTCGCGGATTTTTATC	CCAGGTGCAATAGGGAAATC	276
ICCM0166b	FI856824	(AAAT)7	TGGGGATACGTAGGAGCAAG	TTGGATTCGGGAGTCGATTA	233
ICCM0166c	FI856824	(AT)5	GCCTACTCGCGGATTTTTATC	CCAGGTGCAATAGGGAAATC	276
ICCM0166d	FI856823	(AT)4	GACTCCCCTACCACCTCACA	CGGACGCGACAAAAACTAC	275
ICCM0166e	FI856823	(TAT)48	AATAAAAATGCGGAAGTGGG	TGTGAGGTGGTAGGGGAGTC	276
ICCM0167	FI856588	(ATA)48	GAGTGTACGGGGATTATATGATGA	TCAAGAAAAGGAACCAAGGC	235
ICCM0169	FI856589	(TTA)30	AGAGGCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	251
ICCM0170a	FI856839	(TA)4	GCTTTGTTGCTTTCGTTCTTTT	AAAGTGTTTGGGGTGAGTGG	226
ICCM0170b	FI856839	(C)10	CTCGCATTCCTTTTCCACTC	GGGGGAAAGTATGGGATGAG	154
ICCM0171	FI856590	(ATTC)4	GACCGGGATCGTGTCATAAA	CGTTTTCCCAGCTTCAACAT	166
ICCM0172	FI856841	(AT)4N(AT)4	GCAGTCGATCTGAGGATCAAG	TTCACAAGATGTTTTCAGAACAAAG	278
ICCM0174	FI856591	(A)11	CAGCGACCTCCTACTGGGTA	CAAAAATGGAGGATTTTTCCTT	175
ICCM0176	FI856847	(TA)4	ATAGGCTAGACCGTCCGACA	TCTGAAATATGATGCAGCCG	273
ICCM0177a	FI856849	(AAT)28c(ATA)27c(TAA)4	CTTGAGTTCAAGCCAGAGAGG	GCGTTATTACTGTTACAAATGGCA	279
ICCM0177b	FI856848	(TAT)6N(TAT)11N(TAT)4N(TTA)6N(TAT)8	CCCTTCTTCCATTTCCGAAT	GGGGAGGAGAACGAAAAAGA	273
ICCM0178	FI856592	(AAT)13	AGTTTGGGTTTCACCGCCT	GAACGCGCTCTGTTCATAAT	280
ICCM0179	FI856850	(TAT)4	AAAGGCCAGTTTACCCGACT	ATTTGATGCAGCAAGCAGTG	214
ICCM0180	FI856852	(TAT)4	AGTCCCTGATCTCCCGAAGT	ATTTGATGCAGCAAGCAGTG	179
ICCM0181	FI856593	(ATA)5	CGGGTGTGGATAGCAAGTTT	TCTCTCCTTCCTAATAAAACAAACA	103
ICCM0183	FI856595	(TAT)15	TGAGGACTAAGATAATAGCAATCCAA	TTTTTACATGATTGTCTTTTTGTTG	168
ICCM0185	FI856597	(T)11	AAAGTTTGGCCTGGTCTGG	CATTCCATATTCAGTAGCATTCCA	250
ICCM0187	FI856599	(TAT)6	TGACCATCAATCCATTTCTTTTC	TGTTGACGTCTAATTTTGTCCG	280
ICCM0189	FI856601	(TAT)62	TCCAGTTCCAAATGGCATAA	CCCTTGAGTTCAAGCCAGAG	273
ICCM0190a	FI856602	(ATA)6N(ATA)9	GGGGGATTGTCTGAGTTTCA	AAAAGGCTGGAGACACCTCA	195

Contd…

ICCM0190b	FI856602	(TAT)5	TTTATTGCAGGAAGCGGTTT	CACCACTATCAAATGCCCCT	273
ICCM0191	FI856603	(T)10	CCTTACGCATAATCGACTCCA	AATTCAATTGAGTCGCCACC	130
ICCM0192a	FI856879	(TAT)15c(ATT)15	GCTGCCCAAATTTTGACATTA	CCGGGGATCAAATTCTTCTT	279
ICCM0192b	FI856878	(TAA)15tg(ATA)15	CGGACGGGGATAATTCTTCT	GCTGCCCAAATTTTGACATTA	279
ICCM0193a	FI856881	(TAA)5N(T)10	TGAACTTTCAAAACCAAACCAA	TTGTGACAATTTGAGGGTTCT	268
ICCM0193b	FI856881	(AAT)7	TCGATTATAGCTTTATCTTTACCCTTT	AAAAGTGTTGGGAGGGGTTC	242
ICCM0194	FI856883	(A)10N(T)13	CGATTGTCCTAGTTTTAAAAAGAAA	CGACTTCCTGAAGGAACGAA	200
ICCM0196	FI856605	(ATA)5N(AG)6	GTCGGGTGTGGATAGCAAGT	AACACAATTCCTCAAATAACAAACT	154
ICCM0197a	FI856885	(T)13	CGCGTCTAGCAAAACAAGAA	TTCTCGGCCTATAAACATCAA	280
ICCM0197b	FI856884	(TATT)7	GATTCCGGAGTCCATTACCA	CTATTGCACCTGGGGATACG	241
ICCM0198	FI856886	(A)15	CCATCCGAGAAAACTCGAAA	CAACGGTATCCATCGGAATC	163
ICCM0199a	FI856889	(A)10g(AGAA)4	ACCAAGCAGACCACAACAAT	GTTTTCCCGGCTTCAACAT	265
ICCM0199b	FI856889	(CATT)5	ACCAAGCAGACCACAACAAT	GTTTTCCCGGCTTCAACAT	265
ICCM0199c	FI856888	(TTA)15	TTAGAGGCAAACCAGAACCG	ATCTTGAAGTGGGCAAAACG	241
ICCM0200	FI856890	(TAA)4	ACGGAGTGACCAGGAAACAC	GCAGACCTACAGAAACAGAGGAA	231
ICCM0201	FI856892	(TAT)7(TTATTG)6*(GTTATT)4	ATAGAGAGACCCAAACCGCC	GCCAAAGGCAAAAGAGATTG	135
ICCM0202a	FI856894	(T)10N(T)10	CGCCGATCCATTATACTGAC	TTGCCTCTGATTCTGGTTCA	192
ICCM0202b	FI856894	(TTA)13	TGAACCAGAATCAGAGGCAA	CCAATTTGGTCCGGTTTTTA	207
ICCM0203	FI856606	(CT)6	TGGACGTAGGTTGTTGTGGA	TTGGTATCAGTGACCTCGCA	194
ICCM0204	FI856607	(TC)7	CACATACACTCCCCAATCCC	TGCAGACTGTTTGGTTCGAG	258
ICCM0205	FI856608	(TA)9	CGACCATGATTCTCTGATGTG	CACCTCTGCATTTCTTCAAACA	263
ICCM0207	FI856610	(TA)4	TCAACCATAAAGCACTCCCC	GGCCATTTGTGTTTTGTATGG	182
ICCM0210	FI856898	(TA)4N(TG)4N(CCA)4N(AG)16	GACCATTGCCCACTTCAACT	AGTTCTGCGAGAGGAATGGA	253
ICCM0212a	FI856902	(T)10N(AT)4N(TA)5N(TA)4	TCCTATACCGAAAACCCCATT	CAAAATGGATGGATTGTGGG	270
ICCM0212b	FI856901	(GA)4	ATTGCCGTTGAGAGAAGTCG	TCGGTCACCACACTACCAAA	183
ICCM0212c	FI856901	(AG)8	TTTGGTAGTGTGGTGACCGA	AACCCAAAAACGTGGACTCA	161
ICCM0214a	FI856612	(CT)6N(AC)4	CTCTTCAATAGCCCCATCCA	CGTTGGAGAGGCTGAAACAT	249
ICCM0214b	FI856612	(TTTA)5	ATGTTTCAGCCTCTCCAACG	TCGCACCTGAACTCTCTGTG	276
ICCM0215a	FI856613	(TC)5	CCTTCAGTGTTTGGCTCACA	CTCCAGGAATCCACAGCATT	253
ICCM0215b	FI856613	(T)11	AGAATGCTGTGGATTCCTGG	GCAAGCCCAAAACTTCAAGA	276
ICCM0216a	FI856614	(TG)4	CGGGACTTTCATCTGCTGTT	GTGGGACATCCTCCAAGAAA	200
ICCM0216b	FI856614	(AG)5	AAAGCTGGTGGTCGAGCTAA	GACCACCGAACCAGGATAAA	277
ICCM0219a	FI856906	(ACC)4	CCTTTTAAGGGCTGAAGGCT	TGAAAGAATGTGGGGGAGAG	203
ICCM0219b	FI856906	(CT)5	TCATCCTACCCAATTGCTCC	GTAGTGGGGTAGGGGATGGT	240
ICCM0219c	FI856905	(CT)4	CTCACCCACCACACCTATCC	GCGAAGGGAGAGAAGGAAGT	278
ICCM0219d	FI856905	(TC)5	CTCACCCACCACACCTATCC	GCGAAGGGAGAGAAGGAAGT	278
ICCM0220	FI856908	(TA)4N(CT)4	TCAACCATAAAGCACTCCCC	GGCCATTTGTGTTTTGTATGG	183
ICCM0222	FI856617	(CT)5	TCCGATTGGATTTTCAGGAC	GGTATCAGTGACCTCGCCAT	210
ICCM0223a	FI856912	(TCT)4	TACAACTTTTGACACCGCGA	AGTGGCAGTATGCGTTGAGA	224
ICCM0223b	FI856911	(TC)4	GCTCTGTCGGTCTTCCTGTC	ACAAAGCGCTCGAATAAGGA	208
ICCM0224	FI856914	(CT)4	ACCACCTTGCTCATCCTCAC	GAGTAGGAGGTGCGAAAACG	274
ICCM0225	FI856915	(CT)4	ACGTCCGGATTTGTTCTCAC	ATTATAGGAAGATGGCGGGG	252
ICCM0226a	FI856537	(A)12	CCAACGACGCGGATAAATA	AATGCCCACCCATAATTTCA	273
ICCM0226b	FI856537	(TA)4	GAAAAAGCGCTGTAAATGGC	CCTCGCATTTTGTTCTCAAAG	145

Contd…

ICCM0228	FI856619	(CT)6	TGGACGTAGGTTGTTGTGGA	GGACCGGGAGTCCCTTATTA	274
ICCM0229	FI856916	(C)10	TGTCTTATTCCTCCTCCCCC	AGGGGTTTTTGGGTTACCAG	158
ICCM0231a	FI856919	(TTA)21N(TC)4	CTGGGGATACGTAGGAGCAA	GGAGTGAGATAAGAAAGAGAGGAGG	278
ICCM0231b	FI856919	(TC)4	ATCCCACCTTACCACCCTTC	GGAATGAGGAGGGATTGAGA	279
ICCM0232	FI856920	(T)10	ACGGGAAGTTTCTGGGTCTT	TAGCGGAGAAACAGGACTGG	253
ICCM0233a	FI856922	(TA)4N(TTA)4t(TTA)4	CTCACCACTAGGGATGGGAA	AGACTCCCGAGGGATTGACT	242
ICCM0233b	FI856922	(A)10	AGTCAATCCCTCGGGAGTCT	TGTTGAGGGCCTTAGATTGG	256
ICCM0234a	FI856925	(TTA)4	GGGACTACTTTCGCGATTCA	GTGGGTAATCCGTGCGTAAT	229
ICCM0234b	FI856924	(TA)4	CAGGCTATGTCATCTCGTCG	CCTGACTGCCACAAGTTTCA	270
ICCM0234c	FI856924	(TCG)4	TGCTTCCGTACAGGCTATGTC	CCTGACTGCCACAAGTTTCA	280
ICCM0235a	FI856927	(TC)4	TCGCTCATGACAGACTCGAC	GTAGCAGGTGAGATGCACGA	173
ICCM0235b	FI856926	(GT)4c(GT)4	GCCGACCCGATTACCTTACT	GAGAGCTAAGGGGAAGGTGG	176
ICCM0236a	FI856621	(CGC)7	GCTTGTTGCCCTGTATTGGT	AAAACGCAAGCAAAGCAAGT	151
ICCM0236b	FI856621	(ATT)4N(A)10	ACTTGCTTTGCTTGCGTTTT	TTTGTGGGTTGGTTGATTTT	219
ICCM0236c	FI856621	(A)10N(TA)4	AAAATCAACCAACCCACAAA	ATCACTCTACCGTCAAAACGA	244
ICCM0237a	FI856622	(AT)4N(ATT)6	TCAACCATCCCTAAAAACATTTG	TTCCTTTGCCATTTATGTTTTG	184
ICCM0237b	FI856622	(A)13	CAAAACATAAATGGCAAAGGAA	TCGTGTTGTATTGTGCCGAT	155
ICCM0237c	FI856622	(AG)4N(GA)4	TGTGTTTCTCGATGGCAGAG	CTTTTTCTCCCTTTCCACCA	120
ICCM0238	FI856623	(TC)4N(TC)4	ACCATAGACGAACCACCACC	CCAAGGGGGTACAACTTGTGT	215
ICCM0240a	FI856650	(TA)12	ACCCGAACCCGCAAATAATA	GCAATGAGACTGGGGTTTTC	248
ICCM0240b	FI856650	(TA)4	ACCCGAACCCGCAAATAATA	GCAATGAGACTGGGGTTTTC	248
ICCM0242a	FI856929	(AAT)18	TGCATTCATCTGTTTCGCTC	GAAAATATTTGTGGTTATCCGATTTT	263
ICCM0242b	FI856928	(A)11	ATCCGCAACACAACAAAACA	CCCTACTCGTAATCGACTCTCG	252
ICCM0242c	FI856928	(TAT)5	CAAGTGCAATAGGGAAATCCA	ATAGGGCTTTCCACCGATTT	210
ICCM0243a	FI856931	(AT)5N(AT)4N(AT)8	TCAGGAACAGACGGAACTTTTT	GGGTTCAAATCCTATTGGGC	277
ICCM0243b	FI856931	(AT)4	ATTTGCGCCCAATAGGATTT	TTTTTCTATCGGAATATCTCATTTTCT	280
ICCM0243c	FI856930	(GA)41N(AG)10	ACGACGATTCTGGATTTTGG	AGTTTTGGTAGGGGGTCGAG	237
ICCM0244a	FI856933	(AT)8N(TTA)4(TAA)4N(TTA)6N(ATT)4	ATGCGGTAATCCTTGACTGG	TGCAGGGAGTGAATGTGTGT	252
ICCM0244b	FI856933	(TA)5	CACACATTCACTCCCTGCAA	GATGGAAGGGAGGGGTAAAA	268
ICCM0245	FI856935	(AG)5	GCGGCTGGTTTAAGAGTGAG	CCAACACGACCCAAATCAAT	182
ICCM0246a	FI856937	(AT)4	TCTGACAGCTCTTGCCTTGA	AACACCCAGACCCCTTTCAT	280
ICCM0246b	FI856937	(TA)4	TGAAGAGGAAGAGACGGGAG	AATCCATTTACGGGGGTAGC	268
ICCM0246c	FI856937	(TATTT)4	TGAAGAGGAAGAGACGGGAG	AATCCATTTACGGGGGTAGC	268
ICCM0246d	FI856936	(TC)4	GATCACGGTTACGAATGCAA	TAAGGTTCCCATTGGCTCTG	209
ICCM0247	FI856626	(TTA)8	CCTCAATTCATTTTTCTTCGG	TTTCCCGATAAACCATCTGTT	136
ICCM0249	FI856627	(T)12N(TAA)29	TTTCTTCGCATGGGCTTAAC	GGAGATTTGTTGGGTAGGCTC	193
ICCM0250	FI856940	(TAT)40	TTTCAAACACAATCTGAACGAGA	CCACCTTCGGGTAGGATACA	231
ICCM0251a	FI856943	(AC)4	TCCCTGCTATACACCCATCC	TGGGCATATATGGATCACGA	252
ICCM0251b	FI856943	(CG)4	CTACACCCGCCAACCTCTAC	AAGTGTATGTGACCGAGCCC	261
ICCM0251c	FI856943	(CA)4	AACCCATAATACGCGCTCAC	GGGGTGGTAAGGTAGGAGGA	206
ICCM0252a	FI856945	(CCT)4	TCTACCTCTCCGCTCTTCCA	TGGTGATAGGTGGTGGTTGA	203
ICCM0252b	FI856944	(T)11	TTGACGGTGGGGGTATACAT	TCCACACACTCCCACTACCA	267
ICCM0253	FI856946	(AT)5	TCCCTTACAAGCATTCCCTG	TGGGGACCGTTTTTCACTTA	110

Contd…

ICCM0254	FI856628	(TAA)4N(TAA)29	GCCAAGCCCATTAAAACACT	CGTTGTTAAAAACCGCGTTG	273
ICCM0255	FI856629	(ATA)8N(AAT)4N(AT)4	GGCTACCGAATATGGAATGC	TGGCCTGACCTACTTATGGC	272
ICCM0256a	FI856949	(AT)4	ACCGCTCATTTCCATACGTC	TGGATCAAGAGGGAGGATTG	195
ICCM0256b	FI856948	(CT)4	TTTCTCTTTTGGTGGTTGCC	TTAAGGTTTGCCACTCCTGG	247
ICCM0256c	FI856948	(TTA)19	TTAAGCCAGACGTGGGAAAC	AGAAAGAAGGGAAATGGGGA	236
ICCM0257	FI856630	(ATA)44N(AAT)11	TCGCTTCCAACATTCAAAAA	CAATTGCACTTATAGCACAAACA	255
ICCM0258	FI856950	(ATA)11	TGCATAGGGAAATCAAAACACA	TTATTTCACCGTCGTGTCCA	271
ICCM0259	FI856631	(TTA)15	AGAGGCAAACAAGAACCGAA	CGAAGCCGAGAAAATGACTC	261
ICCM0261	FI856633	(TAA)23	GTCCGGGGATTCAGTAGGAT	CAAGCCACGGAACTTGTTTT	232
ICCM0263a	FI856635	(TATT)7	CGGGGATAAATCAACACACC	GGGCAAGGTCTTACCCTTGT	265
ICCM0263b	FI856635	(ATA)19	GGTAGAAAATATTTATGTGTTGACCG	CTCGTTCACATACCGCCATA	260
ICCM0265a	FI856636	(AT)5	GGAACTCGGGATTGAAATAGTC	TTGCAAAGAAAACAATTTTAGGA	214
ICCM0265b	FI856636	(A)13	CGTTTAATCCTAAAATTGTTTTCTTTG	ACGGCGACAACCATTAATTC	190
ICCM0265c	FI856636	(TAT)9a(ATT)10N(ATT)11N(AAT)4	TACCGCCACGTTACGTTTTT	GAAAATATTTGTGTGTTGACCGA	248
ICCM0266	FI856955	(TAT)31	GAATCGTGAGGGGGAGATTT	GGGGGAATCAAAAGGCATAG	269
ICCM0267a	FI856637	(TATT)4	CAACGTCCGTTAAAACGGTTA	TGGGGATACGTAGGAGCAAG	179
ICCM0267b	FI856637	(TTA)4N(TAA)8N(ATA)12	CTTGCTCCTACGTATCCCCA	AGTCTCGTTCACATACCGCC	272
ICCM0268	FI856957	(CT)7N(TC)4	TTCATCTCTGCCCAAACTCC	TGGGTAGATGGAAGGAGTGG	220
ICCM0269a	FI856959	(AC)4	CCCTCTTTACACCCCACCTT	GTAGTGGAGTGGGGCAGGTA	129
ICCM0269b	FI856959	(TC)5	AACATCACTAACCCTCCCCC	AGGTGTGGGTGGTAGGAGTG	209
ICCM0270	FI856638	(TAT)16	TCACATACCGCCACAATACG	ACGTATCCCCAGGTGCAATA	276
ICCM0271	FI856639	(GT)4	ACCCGGGTATAAGGTTCCAC	TGCTTGTTTTTCATTTTCATTTTC	240
ICCM0272a	FI856961	(A)12	TTTCCACTTGGAACAGGCTC	AATGGACGATGGTTGGGTTA	280
ICCM0272b	FI856960	(GA)10N(AG)20	CGCGGTTGAGTTAGAGTGGT	CAAATCGGGGATTTTGTTTG	175
ICCM0272c	FI856960	(GA)4	CGCGATTATTACCCACGTTT	GGAAAGGAGGTACCGGAGTC	249
ICCM0273	FI856963	(TGA)4	TGTAACTCATCATCGCCAGC	AGACGTGTAGACAGATGCCC	108
ICCM0274	FI856640	(TC)9(TA)15	GACCCTACCCCGCAAGTAAT	TTTTTGTCCACACTCACACCT	265
ICCM0276	FI856965	(C)10	CTCCTACACTGCCTCCCCTC	TCATGCTTACTCCGTTGCAG	222
ICCM0277	FI856642	(TTA)11	GGCAAACAATAACCGAAAACA	GTAAAGAGGGCCAGCTGTTG	196
ICCM0278a	FI856643	(TTA)4	ATAGGGGACCAAAACTGCAA	GTGGGTAATCCGTGCGTAAT	203
ICCM0278b	FI856643	(AT)4	AAAATACACATCCTGACTGCCA	TTTTGCTTAGACTTGTAGGCATT	159
ICCM0280	FI856969	(T)11	ACTAGATGGTCGCATCCTGG	GGTGAAGGTGTGGATGAGGT	280
ICCM0281a	FI856644	(AC)9	TTCAACCCTCCCTACACGTT	GTTCTCTTCTTGCTGGTGCC	235
ICCM0281b	FI856644	(AAT)5N(A)11	TGGAACAACCAAGACCTTCA	GCTGCCACAAACACTGAGAA	264
ICCM0282a	FI856645	(CGC)7	CCTCGTTGTTGCCCTGTATT	AAAACGCAAGCAAAGCAAGT	154
ICCM0282b	FI856645	(ATT)4N(A)10	ACTTGCTTTGCTTGCGTTTT	TTTGTGGGTTGGTTGAATTTT	219
ICCM0282c	FI856645	(A)10N(TA)4	AAAATTCAACCAACCCACAAA	ATCACTCTACCGTCAAAACGA	250
ICCM0284a	FI856647	(AT)4	CGTATCTACACCCGCACTCA	TGGAAAATCCACTTTGATTGG	257
ICCM0284b	FI856647	(TA)4	CGTATCTACACCCGCACTCA	TGGAAAATCCACTTTGATTGG	257
ICCM0285	FI856971	(ATTC)5	TGAGGACAAGATTCCGTTCA	AACATGCGGGTTGTTTTCTC	267
ICCM0286a	FI856648	(GA)5	AGCATCACGCATACAGCTTG	ACATTGGCTCCATTGTTTGG	254
ICCM0286b	FI856648	(AG)4	ACCCCCAAAATGCTGTAGTG	ACGCCCCTTTACTGTTACGA	276
ICCM0288	FI856972	(TAA)4N(TTA)4N(TTA)4	TTATTTTTCGGATCCAACGC	GTGATTTTTGTTCGGCCATT	278

Contd…

…Contd

ICCM0289	FI856976	(T)13N(ACA)4	CAGCCTCCATGGCATAGATAA	TGCTTGAATGAGTGCAACAA	219
ICCM0290	FI856976	(A)15	TTGTTGCACTCATTCAAGCA	TTTTTATTGGGGCATTGAGC	244
ICCM0291	FI856978	(AT)4	AAGTATTCAATTATACGTGCACAAAA	TCATCCTTGTTAAGTCAACCACTT	249
ICCM0292	FI856978	(TAA)6	TGGTTGACTTAACAAGGATGAGTG	TCCTCAAGCAGAGGTGGTTC	267
ICCM0293	FI856982	(TAA)15tg(ATA)15	AGTGATGCCACGAGAATTGC	CTGGTTCGGAATTGTCATCC	250
ICCM0294	FI856986	(TTA)15	AGAGGCAAACAAGAACCGAA	CACCCAATTTTGTCCGATTT	185
ICCM0295	FI856987	(T)10	GAGGCACCAAATTCGTATCC	CAAAATTTTCTAATTCACCAAGACTTC	256
ICCM0296	FI856987	(TGATT)4	CGCCAAGTTTTACTATGTGCTG	TGTCTGGATGTTACATAAACACTCTT	227
ICCM0297	FI856987	(TAA)18	CATGATTTGATTTGATTTGATTTC	GGAGTGGGAAACCTTAAGCC	271
ICCM0298	FI856989	(TC)4N(AAT)4	GTGCACTTGTTCAGCGTTGT	CGCAAACACACATTCCTCTG	221
ICCM0299	FI856989	(CTT)7N(TCT)4	TTATGAAGCCGAAGCTCGTT	GAGCAGTAAACGTACCCCCA	272
ICCM0300	FI856990	(A)10	ATGGCCAAAATGAACTCCAG	AAAAGAGAAGGTTCCATCGG	173
ICCM0301	FI856992	(A)10	ATGGCCAAAATGAACTCCAG	AAAAGAGAAGGTTCCATCGG	173

SSR motifs having “N” nucleotide represent the interruption of few base pairs between two same/ different SSR motifs.
Marker names start with prefix ICCM, which represent ICRISAT Chickpea Microsatellites.
The primer names are followed by “a” or “b” represent their common origin from a single clone and are synthesized in order not to miss any informative markers.

The SSR loci were categorized into two groups based on the length of their SSR tracts as Class I SSRs (≥20 nucleotides in length) and Class II containing perfect SSRs (>12 but <20 nucleotides in length) (Figure 3). Among Class I repeats, tri-nucleotide repeats (77% of all Class I repeats) are most abundant followed by di-nucleotide repeats (14% of all Class I repeats). Penta- nucleotide repeats (55%) followed by hexa- repeats (23%) contribute more to Class II repeats.

4.1.1.2 Functional annotation of GSSs

Annotation was performed for all the above 457 GSSs using BLASTN and BLASTX algorithms using significant similarity at threshold cut off value ≤1E-05. EST datasets of chickpea along with the ESTs of phylogenetically related legumes *viz.,* Medicago (*Medicago truncatula*), lotus (*Lotus japonicus*), common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*) and three model plant species *viz., Arabidopsis* (*Arabidopsis thaliana*), rice (*Oryza sativa*), poplar (*Populus alba*) were used for BLASTN analysis. The analysis revealed similarity with poplar (98.9%), chickpea (98.4%), Medicago (98.2%) and Lotus (98.2%) and common bean (98.2%) databases, followed by *Arabidopsis* (97.1%), soybean (96.2%), cowpea (96.2%), rice (62.3%) and peanut (49.6%). However percent significant similarity (>1E-05) ranged from 16% (Medicago) to 3% (peanut) (Table 8). Except one GSS (contig_ICCM0182 or FI856594), all the 456 GSSs showed similarity with sequence of at least one of the plant species, analyzed (for details see Nayak et al. 2010; supplementary material2,http://www.springerlink.com/content/f2n2663880630781/122_2010_Article_1265_ESM.html).

Table 8: Functional annotation of ICCM sequences with EST databases

BLAST algorithm	Database	Number of entries in database	Number of sequences showing similarity	No. of sequences with significant similarity (<1E-05)	% sequences with Expect values <1E-05	Median Expect values
BLASTN	Ca_EST	7,097	450	26	5.69	2E-60
	Mt_EST	249,625	449	76	16.63	5E-22
	Lj_EST	158,135	449	48	10.50	1E-16
	Pv_EST	83,448	449	35	7.66	4E-26
	Vu_EST	183,757	440	49	10.72	1E-14
	Gm_EST	880,561	440	73	15.97	3E-14
	Ah_EST	41,489	227	14	3.06	6E-15
	At_EST	1,527,298	444	44	9.63	1E-11
	Os_EST	1,220,877	285	20	4.38	2E-19
	Pa_EST	418,223	452	48	10.50	1E-12
BLASTX	Uniprot	385,721	409	137	29.98	3E-12

The database were downloaded from NCBI in May-June, 2008;
BLASTN- represents nucleotide BLAST and BLASTX- represents protein BLAST;
EST- Expressed Sequence Tags; Ca- *Cicer arietinum*; Mt- *Medicago truncatula*; Lj- *Lotus japonicus*;
Pv- *Phaseolus vulgaris*; Vu- *Vigna unguiculata*; Gm- *Glycine max*; Ah- *Arachis hypogaea*;
At- *Arabidopsis thaliana*; Os- *Oryza sativa*; Pa- *Populus alba*

Among these sequences, 40 were identified as related sequences among all the three analyzed cool season legumes, i.e., chickpea, *Medicago* and Lotus (Hologalegina clade) (tabulated in Table 9), while 29 sequences had similarity with all the three analyzed warm season legumes, i.e., soybean, common bean and cowpea (Phaseoleae clade; for details see Figure 4). Only 21 sequences were such which were identified as similar sequences in both Hologalegina and Phaseoleae species. Two of these GSSs (FI856609 and FI856659) showed significant similarity with sequences of all the plant species analyzed in the present study (Table 9).

Table 9: BLASTN results of forty ICCM sequences showing significant similarity with Hologalegina across nine plant databases

GenBank ID	MtEST	Status	LjEST	Status	PvEST	Status	GmEST	Status	VuEST	Status	AhEST	Status	AtEST	Status	OsEST	Status	PaEST	Status
FI856538	BF647794	S	DC593101	S	CV537219	NS	BE821868	NS	FG849962	NS	EE124305	NS	AU237357	NS	AU173398	NS	DB893303	NS
FI856553	AL370043	S	BW601148	S	CB543673	NS	BU546962	NS	FG872838	NS	ES711698	NS	EG528076	NS	AU069032	NS	CV269687	NS
FI856593	BG457233	S	AV415646	S	FE899220	S	BI785816	S	FG888230	NS	EG372491	S	AI996736	S	CI184358	NS	CV264502	S
FI856601	AJ500027	S	BP059613	S	FE701486	NS	BU080998	S	FF400051	S	ES753765	NS	BE662810	NS	EE332619	NS	BI121195	NS
FI856605	BG457233	S	AV415646	S	FE899220	S	BI785816	S	FG888230	NS	EG372491	S	AI996736	S	CI184358	NS	CV264502	S
FI856609	CB891132	S	AW720640	S	FE676952	S	FE676952	S	FF549647	S	ES709243	S	CK119439	S	CB665470	S	AJ778312	S
FI856610	AW694779	S	BW615729	S	CV544157	S	BE474541	S	FF394032	S	EG373533	S	BE523438	S	CA761436	NS	DT518947	S
FI856612	AL378288	S	BI417297	S	CV543800	S	BG839518	S	FF553773	S	EG028684	NS	AV786770	NS	DY639131	S	CK089137	NS
FI856615	BG645880	S	BW604002	S	FE701370	S	CF920550	S	BE431630	NS	-	NH	EH833512	S	CA761742	S	CX180709	S
FI856632	BF647270	S	AV773281	S	FE698995	S	EH219447	S	FG936150	S	CD037662	S	ES183866	S	CF310760	NS	CV235573	S
FI856649	BG457238	S	DC596338	S	FE694334	S	CA801552	S	FG888079	S	ES714416	NS	AI993538	NS	CT847281	NS	DT477130	NS
FI856659	AL375388	S	AV776549	S	FD789839	S	BF595294	S	FF541918	S	EG030524	S	BE039457	S	AU070441	S	AJ767301	S
FI856658	BF637754	S	AV427344	S	CV530824	NS	BG839887	S	FF385430	S	EG029288	S	EG463958	S	-	NH	CB239483	S
FI856688	BI309329	S	BW624821	S	CB540761	S	BE473461	S	FG812846	S	ES490874	NS	CB264452	S	-	NH	CX168848	S
FI856690	AW774014	S	AW720187	S	CB543190	S	BU090830	S	BM279570	S	CD038768	S	BU635030	S	-	NH	CN523788	S
FI856714	BF006502	S	BW609554	S	CV532091	NS	BI942808	NS	FG899673	S	EE125836	NS	AA597364	NS	-	NH	BI124703	NS
FI856763	BF637754	S	AV427344	S	CB539833	NS	BG839887	S	FF385430	S	EG029288	S	EG463958	S	AU163218	S	CB239483	S
FI856762	AW775940	S	AV776549	S	FD789839	S	BF595294	S	FF541918	S	EG030524	S	BE039457	S	-	NH	AJ767301	S
FI856813	BG648475	S	DC597701	S	CV541272	NS	BM523605	S	FF387748	NS	-	NH	BP642163	NS	CR288905	NS	DB886579	S
FI856833	BF646825	S	BI418738	S	FE897701	S	BM309058	S	FF541964	S	-	NH	EG476482	NS	-	NH	DB882029	NS
FI856863	CX525108	S	BP077976	S	FE899099	NS	EV263912	NS	FF539001	NS	-	NH	BP641788	NS	CI382607	NS	BU822317	NS
FI856759	BQ140817	S	DC598141	S	CB539108	NS	BM093062	S	FG841158	S	ES754139	NS	BP798054	NS	CB630581	NS	CX184353	S

Contd...

…Contd

FI856789	EX533262	S	AV416630	S	FD792127	S	BG839390	S	FF382415	S	-	NH	AV802419	S	EL342244	S	CV233353	S
FI856793	BF641857	S	BP053146	S	CV531334	NS	FK355208	S	FG871442	S	-	NH	AV825829	S	ES699123	NS	CF236488	S
FI856818	CA989991	S	DC598343	S	FD794992	S	BI972886	S	FG942899	S	-	NH	CB259302	S	CA767307.2	S	DT478308	S
FI856844	EY474674	S	AW719767	S	CV531103	S	-	NH	FF399093	S	-	NH	AI100469	NS	CI621991	NS	BU822292	NS
FI856849	EY475732	S	BP059613	S	FE701486	NS	BU080998	S	FG888504	S	-	NH	ES090893	NS	CB212843	NS	BI121195	NS
FI856848	AJ500027	S	BP059613	S	CX129681	NS	FK022100	NS	FF400051	S	-	NH	BP813310	NS		NH	BU865649	NS
FI856891	BG645078	S	BW607280	S	FE683245	S	BI945498	S	FF549647	S	-	NH	BU635798	S	CB665867	S	CX178800	S
FI856897	BG583849	S	BW629363	S	FD794289	NS	BU763648	S	FF544751	NS	-	NH	DR209259	S		NH	CK115567	NS
FI856908	AW694779	S	BW615729	S	CV536986	NS	FK015068	S	FG852864	S	-	NH	N95936	NS	CI190400	NS	CN519373	S
FI856907	BG648491	S	AV764780	S	CV544157	S	BE474541	S	FG812334	S	-	NH	AV520076	S	-	NH	CV254167	S
FI856915	BG457238	S	DC596338	S	FE694334	S	EV274987	S	FG888079	S	-	NH	AI993538	S	-	NH	CF237049	NS
FI856920	BG457238	S	DC596338	S	FE694334	S	EV274987	S	FG888079	S	-	NH	AI993538	S	-	NH	CF237049	NS
FI856931	AJ846730	S	AV421953	S	CV532363	S	BG839162	S	FC459095	S	-	NH	CB263699	S	CB619390	S	CV277789	S
FI856961	AL370164	S	AW720168	S	CV529604	S	AW164371	S	FC458407	S	-	NH	BX837635	S	DY256078	S	BI121486	S
FI856970	EY474979	S	BW619574	S	EX305156	S	CA851859	S	FF403252	S	-	NH	BX841347	S	-	NH	CV249294	S
FI856989	BI266681	S	BW622379	S	FE708429	NS	BM308571	S	FG939462	S	-	NH	DR282646	S	CA758794	NS	CV259125	NS
FI856990	BG456553	S	AV779916	S	CB556048	S	CO981905	S	FF538025	S	-	NH	CB261688	S	AU174024	S	AJ774391	S
FI856992	BG456553	S	AV779916	S	CB556048	S	CO981905	S	FF538025	S	-	NH	CB261688	S	AU174024	S	AJ774391	S

BLASTN analysis was carried out in order to check the similarity of the 457 ICCM sequences, across nine plant expressed sequence tags (EST) databases- include MtEST- EST database from *Medicago truncatula*; LjEST- from *Lotus japonica*; PvEST- from *Phaseolus vulgaris*; GmEST- from *Glycine max*; VuEST- *Vigna unguiculata*; AhEST- from *Arachis hypogaea*; AtEST- from *Arabidopsis thaliana*; OsEST- from *Oryza sativa*; PaEST- from *Populus alba*. The BLASTN analysis revealed 40 ICCM sequences (out of 457) showing significant similarity with species belonging to Hologalegina group of Papilionoideae family (*Medicago* and *Lotus*) and is tabulated in this table. The significant similarity was determined at an Expect value threshold of ≤1E–05, and indicated as S-significant similarity; NS-non significant similarity; NH-no hit in the database.

With an objective of deducing a putative function for newly isolated GSSs, all 457 GSSs were subjected to BLASTX analysis using UniProt database. Of 457 GSSs, 137 GSSs (29.9%) showed homology at ≤1E-05, while as 272 (59.5%) GSSs showed non-significant similarity (E-value = > 1E-05) and 48 (10.6%) GSSs did not provide any hit in UniProt database. All the 137 GSSs showing significant similarity to 84 unique protein sequences were used for deriving respective gene ontology (GO). The GO studies revealed that 64 of them were involved in biological, 64 in cellular and 67 sequences in molecular processes. According to the GO schema, single proteins typically have more than one ontology assignment as one protein might be involved in more than one process. Hence the number of sequences showing in each of the GO categories exceeded the number of unigenes analyzed.

4.1.2 Development of SSR markers from BAC-end sequences and their distribution

A set of 46,270 BAC-end sequences (BESs) representing, 33.2 Mbp genome coverage was used for mining microsatellite markers. In total 6,845 microsatellites were identified in 5,123 BESs, scanning one SSR per every 4.85 kb. About 1,245 BESs contained more than one SSR motifs, while 913 SSRs identified were in compound form. Among all SSRs surveyed, di-nucleotide motifs were the most abundant (37%), followed by tri-nucleotide motifs (~10% of total SSRs) (Figure 5). Most di- and tri-nucleotide sequences were AT-rich (i.e., TA and TAA), comprising ~2/3 of total SSRs. Majority of the SSR motifs occurred in the range of <10 to <20 repeat units category (Figure 6).

These SSRs were classified as Class I and Class II repeats according to the length of repeat motifs. Among Class I repeats, di-nucleotide repeats (42.7%) were more abundant followed by tri-nucleotide repeats (26%), while in case of Class II repeats, penta-nucleotides (65.3%) contributed highest, followed by hexa-nucleotide repeats (26.1%).

The distribution of Class I and Class II repeats among CaM microsatellites are depicted in Figure 7. In total 2,189 primers were designed for the flanking regions of microsatellites using Primer3, which were named as Cicer arietinum microsatellite (CaM) markers and 1,344 primer having higher repeat units were synthesized (Table 10).

Table 10: Novel set of SSR markers (CaM) derived from BAC-end sequences in chickpe

Primer Name	GenBank ID	SSR motif	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
CaM0001	EI846499	(T)12N(AT)6	CCACAACCAGATCCCCTAGA	TGCAACAGAGCAGTATGACAA	271
CaM0002	EI846523	(TA)32	TTGTAATGCAACAACCTTCCA	GGGTTTAAAGAGTGTTTGTTTCA	271
CaM0003	EI846602	(AC)13(AT)7	CACGGCCATTACATCAATCA	CGACATTGTAGCACCCCTTT	196
CaM0004	EI846639	(TA)5	ACGTTGAGGAGACACCTGCT	AACATAAGCACTGGGGGTTG	264
CaM0005	EI846649	(AT)5	CCACAACCAGATCCCCTAGA	TCAAACAAACAGATATGCAACAGA	274
CaM0006	EI846670	(AT)5	AGTCAGACGCTACTCGGCAT	TTTTAACCGTGGTTTGAAACTT	198
CaM0007	EI846678	(AC)6	AGGGAGGACGAAAATGACCT	AACCAATCTTGTGCATCCAA	271
CaM0008	EI846708	(AT)25g(TA)10N(AT)5	GGAACGAAGGAAACGTTTTTG	TCAATTGAGTTGTGGCGAGA	278
CaM0009	EI846725	(AT)5N(AT)19	TCAACAAGTTTCAAAAAGAAACCA	CTACGTACTGTGCGGCCAT	250
CaM0010	EI846739	(AC)5	CCTTCACTCTCCATCCCAAA	GAACCAAGTTGCGTGGACTT	203
CaM0011	EI846816	(TA)5	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	238
CaM0012	EI846821	(CTT)30	TTGATTGAAACCAAGACCAGC	CATCAGACGAAAATCCAGCA	267
CaM0013	EI846829	(TA)5	TTCCTGCTTCAAACTCCTTCA	ACAATCCATGTGGTCACTCA	127
CaM0014	EI846863	(AC)6	AGGGAGGACGAAAATGACCT	AACCAATCTTGTGCATCCAA	271
CaM0015	EI846874	(TTA)18(TAT)20	AAAGACTTTACGGCTACAGCTTAT	TTATATGATCGAGTGGTGGGC	218
CaM0016	EI846976	(TC)6	ATGTTTCCATGTGGGATGGT	AAGAGAGATTTCCCACTTATCTTGA	211
CaM0017	EI846976	(GA)6	CTTTATGGAGGGATGGAGGG	AAAGCGCTTCGTGTAAAACC	114
CaM0018	EI846982	(AT)29N(A)12	TCCAAGGAGATCACACGTACA	CACTCAGCATTTTCGAGTCGT	273
CaM0019	EI846994	(GCT)5	CCAAACTTGCAGCAACAAAA	CAAGACACGCCCCGTATTTA	105
CaM0020	EI847033	(GT)5	TTGTGGAGAATACTTGCCTGG	GGTTTCATCCAGTCCCTTCA	164
CaM0021	EI847115	(AT)12	TCATGTGGTATGCGTTTTTGA	CGATGGAAACCCTCATATCC	247
CaM0022	EI847127	(AT)5	ACAATTAAACTGTTCTCTAACCATTTC	TTTCTTTTTGGTAGGCCTTTTT	265
CaM0023	EI847135	(AT)27	CGGTGAATCCTTTACGGAGA	GCGCGTTAACAGAGGAAGAA	275
CaM0024	EI847179	(TTC)5	CTGCCCATTGAGGATTCATT	CCCTCAGATCATTGCCATCT	277
CaM0025	EI847184	(CT)5	TGCTTCCATCTGCTTTTCCT	TATACAGATTCCCATTGCCG	200
CaM0026	EI847201	(TA)5	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	237
CaM0027	EI847273	(AT)7N(AT)6	TTCAAAACATAACAGATCATGCG	GCGGCCATCTACGTTATCTC	137
CaM0028	EI847277	(ATG)5	CCCCTTGAAAGAGGACAAAA	TCACCAATCTTGGAGGGTTC	279
CaM0029	EI847301	(AG)5	TGGGATACAGTGGTTTCCAAAT	CGATTTCGGCCTTTTCAATA	114
CaM0030	EI847316	(AG)5	CTCTGGGATACAGTGGTCGC	CGTCCAGTCCCGAAATAAAA	136
CaM0031	EI847363	(CAT)12	CGCTGCTTGTAAATGAACCA	CCTACAATGACCTCTGTCCGA	242
CaM0032	EI847370	(AGA)5	GCTGACGCCATGAAGGATA	AGTCCGACCCTCATGGATTT	150
CaM0033	EI847384	(GAA)6	GCCTTGAATGCATCACAAAA	GAAGCCATCATGGAAAATTCA	241
CaM0034	EI847407	(AC)10	TGCAGCTGATGGTTTTGTTC	AACGGGTGAGGTTCTGATTG	255
CaM0035	EI847408	(TTA)7	TGTTCCGATGGAACCTTCTC	TTTTGCTTCCTAAAAGCGGA	149
CaM0036	EI847440	(TGA)7	CATTTTCCTGGAAGAACGGA	GCCCAACAACTTTATTCCCA	219
CaM0037	EI847459	(TA)7	TGATTATGGAGATGCGATTTTC	AAAGACTACCAATGATCAAAAGCA	145
CaM0038	EI847480	(TAA)43	CATGCTCGAATCTTATTTTGAGG	TCGATATAGCAAGGGAGAGGA	272
CaM0039	EI847556	(ATTA)5	GCGCGAAATATTCACCAACT	ACTTCATCCTCAAGCCCCTT	255

Contd…

CaM0040	EI847562	(TA)5	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	237
CaM0041	EI847578	(AT)5	TACAATGTCAAGCCATGCGT	GCAGTTGCAATGGCTTCTTT	280
CaM0042	EI847674	(TA)7	TTTTGGTGCTGTTGGTCAAG	TTGTGTTCCAAAAACTTCATACG	255
CaM0043	EI847726	(TAAC)7	CACTTGTTGGCATTTTACTTGC	AACGTTCTTGGTTTGTGACTGA	278
CaM0044	EI847754	(TA)17	CCACTAGCCTTGGGTTTCAA	TGTGTTACGGGTGAAATGAGA	273
CaM0045	EI847762	(AT)8	CTCTCGTCGCAACTCAAATG	TGCTTAGGCTTTTAGCTCGAAT	224
CaM0046	EI847762	(T)12N(AT)17	AGGATGGGTTCTCTGGCTTT	TCATTTGAGTTGCGACGAGA	127
CaM0047	EI847786	(AT)5g(TAA)5	TTGGCCACTAGCTTCATTCA	GGAAAACATCTTTTGCTTTGG	101
CaM0048	EI847831	(TA)8	TTTTCGAACCCGAGAAAGAA	TTGAGTTGCGACGAGAGTTG	191
CaM0049	EI847878	(AT)9c(TA)25	AATGCGTTACGGGTGAAATG	TTCCTTTCTTTGTTTTTCCTTCC	252
CaM0050	EI847890	(TA)6	TTGTGTATGAAAGGAGGATCG	GGTGCCTTGATAACCTTGGA	268
CaM0051	EI847897	(AAT)11	TTTTCGTCCTCCCTAGGCTT	TCTGAACAAGAGTTAGAGGCAAAA	262
CaM0052	EI847941	(AT)12	GGGGGAAGATGGAAAATCTG	CGTTATGGGTGAAATGGGAA	239
CaM0053	EI847952	(TG)5	TGCAGCTTCTGATGGTATGC	GCCGATTCAGATGATACACG	262
CaM0054	EI847994	(GCT)5	CCAAACTTGCAGCAACAAAA	CATGTGCTCCTTTTCGAGGT	215
CaM0055	EI848006	(AT)5	ATTGAACCGACACTCAACCC	GGAGGAACCCTGCTCTGATA	203
CaM0056	EI848147	(TTGATT)5N(TTC)8	ATCGTATCCTGTTCCGATGC	GGACAGTTTGAGTCACGCAG	167
CaM0057	EI848152	(AG)5	ATCAGTATCAAGCGCACAGC	TCCGATTGGATTTTCAGGAC	156
CaM0058	EI848226	(TG)5	TAATCATCCTCGGGTTGAGG	CTCGAGGATGGGATTTTTCA	181
CaM0059	EI848266	(TA)5	AAGAACAAGCCCACATGGAG	CGCATCCATGAATCAACAAG	228
CaM0060	EI848275	(AT)5	TGGTCGACAAGCAACTGAAG	GCAACAGAGCAATATGGCAA	280
CaM0061	EI848284	(AT)5	ATGCATTACGGGTGAAATGG	GGAGAAGGAGGGAGAAATGG	263
CaM0062	EI848286	(AT)10	TTTGTTTCATTGCTGCTTCG	TTTGTAGTTTCTAGTCGTGTCCC	128
CaM0063	EI848286	(AAT)5	ATGTCTCCACCACACTCCGT	TGCGGATTCTTTTCAGATGAT	254
CaM0064	EI848387	(TA)5	GCATACGGCTTTCTGGATGT	GCCAGATGAAGGAACAGGAA	194
CaM0065	EI848411	(AG)5	AGCGAGACTCAATCCAGCAT	CCAAATGGGGTTTCCTTTT	128
CaM0066	EI848437	(CAA)5	TTCATCCTCATCAACTCCCA	GCCCATTTCACTTGTCATTTG	168
CaM0067	EI848438	(TG)5	CGATGAAGATGGGGATGATT	TATGCGGTAAAATGATGCGA	250
CaM0068	EI848465	(AAATA)5	CGAGGTGAAAGGAAAGAACG	TCCATCCCATTCCAACTCAT	264
CaM0069	EI848482	(TA)5	GCATACGGCTTTCTGGATGT	GCCAGATGAAGGAACAGGAA	194
CaM0070	EI848486	(AG)5	CTCTGGGATACAGTGGTCGC	CCAGTCCCGAAATTAAACGA	133
CaM0071	EI848557	(TA)5	GCATACGGCTTTCTGGATGT	GCCAGATGAAGGAACAGGAA	194
CaM0072	EI848574	(TA)7	TCAAGAGTTCGGTTCAATCTG	TGAGATAAAATCACGTAGCCCC	242
CaM0073	EI848575	(AT)6	TGGTGTCAATTGATCCTCTTTC	TTCTCCACCTACCATCGCTT	138
CaM0074	EI848578	(TA)8	CGGACAGAGGAAGAAGACGA	TATTTTGGATTTTGGCTCGG	272
CaM0075	EI848586	(TA)16	TCAGAGGAGGAGGATGGAAA	AATGGGTTACGGGTGAAATG	270
CaM0076	EI848620	(TTTG)5	GCTGAGAAAGCTAAGGTCCG	TCCTACTGGCAATTTGCATTT	239
CaM0077	EI848675	(TA)5	CATGCTGGAGTATGAATTAAGAACA	AAAAACATTCAAATCACTCATATTGT	272
CaM0078	EI848756	(TA)10	AATGAAACACCCTGAACCCA	AGCGTGAATGTGACGTCTTG	143
CaM0079	EI848807	(TG)8	TTGAAGGTGAGTCAACAAATCA	TCAAATTCATCGCAGACCAA	221
CaM0080	EI848820	(TA)5	TCCTTTTCTTTTCTCCCCAAA	ATATTGGCTCGGAGGCTTTT	263
CaM0081	EI848879	(TTA)11N(TTA)22	TCATGTCTTAGCCTTGTTGCTT	TAGTGACGTCGAATGGTGGA	183
CaM0082	EI848920	(AT)5	TCAAAACCATATTGGTAATGCAA	AATTCGGTGGTTCGTTTAACA	254
CaM0083	EI848952	(TA)5	GCATACGGCTTTCTGGATGT	GCCAGATGAAGGAACAGGAA	194

Contd…

CaM0084	EI848986	(CT)5	AACCATCCTAATTTGACACCAA	TGGTTGCTTTTTACTTTTCATTTTT	250
CaM0085	EI849035	(AT)7	TGTTTGATTATTTTTCGGGCA	CCAATTCCATTCCCAAACAC	122
CaM0088	EI849101	(AT)10	CGGTGAATCCTTTACGGAGA	AGGAGGAAGGGGGTCTTTCT	272
CaM0089	EI849127	(AT)5N(AT)8	GCTATTCAGGAACAGACGGAA	CCTATTGGGCGCAAATTTAT	272
CaM0090	EI849128	(AT)6	TTTCTTTTCCTTTTCCTTCCTTC	TTTAGATTTTGGCTCGGAGG	275
CaM0091	EI849142	(TTA)5	CCTGAATTGATTGAAGCCAAA	TTCATTAAAAATATGTTCATTCCG	255
CaM0092	EI849144	(AT)6	TCCTTTCTGTTTCCCTCCAA	CGGTGAATCCTTTACGGAGA	139
CaM0093	EI849190	(TTG)5	TCGAATTTTGAGATGAGACCAA	GGCAACAACAGCAGTAACGA	263
CaM0095	EI849195	(AT)7	ACTCCCTCCGTCGCTTAGTA	TGTCGTGAGAAGAATCTTTGGA	280
CaM0096	EI849226	(TA)9	CATGTTCAAATTTGCTACACAAAA	TCTTTGCTCACAACAAAACCA	276
CaM0097	EI849226	(ATT)5	AACGTTTAGACCACATGGCTG	TTTCTACAATTTAGCCGTACATGC	256
CaM0099	EI849254	(AT)5N(AT)8	GCTATTCAGGAACAGACGGAA	TATGGGGCGCAAATTTATTG	270
CaM0100	EI849260	(TTAT)6	CGCATAATATGGGTATGGGC	TTTGCAAACATTTATCTTTCGATT	248
CaM0102	EI849280	(TA)16	CCTGATGTTCCTCCAATTTAGC	ATTATGTTGGTTTATCGAACAATG	275
CaM0104	EI849313	(AT)31	CGAGGCAGAGGAAGAAGATG	GGAATGAAATGATGGGGGTA	271
CaM0107	EI849366	(TTG)7	ACTGAGTTGCACCTGGTGGT	CAACAACTTCAGCAACAACATC	121
CaM0108	EI849370	(TA)5N(A)11	ATTGGGCAATAAGCAAAGGA	TTCTTTATGTTTTAGATGGAGATGC	245
CaM0109	EI849376	(ATA)5	GCTGTTGGCATTTAACTGTTTC	TTTCAAACTTTTCAGATCTGGACTT	248
CaM0111	EI849398	(AAT)13	CCTCTCCTAGAACACCCCAA	GGTGTCAACAACCTAACTGTTTATTTT	280
CaM0113	EI849479	(TTA)52	AATGGAGAATGATGGGTTGC	GCCCCGTGTCCCTTATAAAT	272
CaM0115	EI849508	(T)12N(AT)6	CCACAACCAGATCCCCTAGA	TGCAACAGAGCAGTATGACAA	271
CaM0118	EI849551	(TA)19	CAATTGAGTTGCGACGAGAG	AGGCAGGGAGAGGAATTGAT	213
CaM0120	EI849587	(TTC)5N(T)10	TTTCGTTTTTCCTCTTCCTCTG	ACACAAAAATAAAGGGGTAATCAT	147
CaM0121	EI849588	(ATG)6	CCCCTTGAAAGAGGACAAAA	TCATCTTGCTTTTCAATGTTGACT	126
CaM0122	EI849604	(TA)6	ACTCAAGCTTGTCCCAAAGG	CATTGTGCTGCCCCTAATTC	191
CaM0123	EI849604	(AT)7(GT)10	AATCGGGGGATCATAACACA	CCTCGCGTTCTACGTTTCTC	278
CaM0125	EI849617	(AG)6	TGCTTCAGTGTGTATCCGTGA	TTTATGTAACATCGGTGCCC	156
CaM0126	EI849634	(TA)6	AGTGAGGACCAGCCAAGTTT	CCCCTTTGTCAGTTAAGGCA	262
CaM0127	EI849661	(A)15N(T)10	GAGGAGGGCTATCATCACCA	GCAATTGAGTGGAAAAAGGC	198
CaM0128	EI849667	(TTA)5	CCAAATGAAACTGAATCGCA	TGCCTTGTCAATTAGCACCA	270
CaM0129	EI849681	(AAT)7	TTCTCATTGGAAAAGCCCAC	AAGCATGTATTGCAATGTCCA	214
CaM0130	EI849693	(AAG)6	CCAGGATAAAACAGCCCAGA	GCTTTTCTCACTTCTCCCCC	197
CaM0135	EI849776	(GCT)5	AAGGCAGCAAACCTCAAAAA	GTATTTACCAAGGCGTGGCT	164
CaM0137	EI849786	(TA)21	AACAAGGCATCATCAAAATCG	AGTCCGCATTTTATTGGCAC	204
CaM0139	EI849803	(AC)6	TTGGATGCACAAGATTGGTT	AGGTCATTTTCGTCCTCCCT	169
CaM0140	EI849865	(AT)19	ATGAACCCAATGCCACTTCT	CAGGATATTATATTGGTTGTTTGCT	218
CaM0141	EI849899	(TA)9	CGTGTAGCTGTTTGGGTGTG	TAGGGCAATTGTGCACATGA	229
CaM0142	EI849925	(T)11N(TTA)32	ATGGAAAATATGCCATTGCC	TGGAAAGATAGGCTCAAAACC	276
CaM0143	EI849950	(T)12N(AT)6	CCACAACCAGATCCCCTAGA	TGCAACAGAGCAGTATGACAA	271
CaM0144	EI849960	(ATT)22	TCGATTACGTTTTGAGTTTTGTTT	CACTTTTCGCACTAAAGTGTATGAA	280
CaM0147	EI850009	(AT)6	TACCTCAAAACCCCTTGAGC	CCCAAGTCCACCTTGTGTAA	117
CaM0148	EI850017	(ATA)8(ATT)9N(TTA)17ttt(TTA)19	TTTCTGAGTGTTCTTTTCTTAGAGTG	AAAGAAGGAACCAAACCACTATT	280
CaM0149	EI850075	(AT)6	CCTTTGTTTTTCCTTCCTTCC	CCCTAAAACCTAAAACCTAAACCC	255
CaM0151	EI850097	(A)10N(AG)10	TGACATGTTAGGTTTTGTTTGTTTG	CTCTCTCGCTCTGTGTGTGC	191

Contd…

CaM0152	EI850098	(ATTA)5	AGTTGCATTGGAATTATGTATTGA	TGCTTGCAACAAAGAAGTAGGA	273
CaM0154	EI850115	(AT)10N(AT)14t(TA)6	CAGATGCATGCTCCCCTAAT	TCGTACATCCAACTACGGTCT	192
CaM0157	EI850153	(TA)12	TTATTATTGTTATCGTCGTCTTTCA	GGTAGCGATCAGCAAACACA	236
CaM0158	EI850153	(ATA)5	AAAATGTGTTTGCTGATCGCT	ATAAAGACGGTTGTTGCGTG	127
CaM0160	EI850252	(TA)21	TATTTTGGATTTTGGCTCGG	TTTCCTTTCTTTTTCCCTCCA	252
CaM0163	EI850427	(GAA)5	CACCAAAACGATTTCCCTTC	CTTCTTCCTCTGTTTTTGCCA	123
CaM0165	EI850443	(AT)10	CTTGGGAACTGGAGAGCAAG	CCTTTCCCCTCAAACAAACA	272
CaM0166	EI850453	(GAA)5	CCAAAACGATTTCCCTTCAA	TCTGTTTTTGCCATCAAGCA	116
CaM0167	EI850482	(AT)8	GTGTTGTCAACTGGTGTGGC	AGAAACATCCACAACAGCCC	260
CaM0168	EI850495	(TTA)7	TGGCCTGACCTGACCTATTT	CCAAATATGGAATGCTACCGA	272
CaM0169	EI850512	(AT)9	GAGAAAGGAGGGATTTTGGC	TGCGTTACGGGTAAAATGGT	268
CaM0172	EI850554	(TAT)7	TCACATACCGCCACATTACG	TTGCTCCTACGTATCCCCAG	266
CaM0173	EI850588	(AT)26	CAGAAGCCTTAGCAAAACACA	TCACAAAACTCATGTGGTAGGA	196
CaM0174	EI850596	(TTC)5	CTGCCCATTGAGGATTCATT	CCCTCAGATCATTGCCATCT	277
CaM0175	EI850617	(AT)15	CGGGTAGCAGAGGAAGAAGA	TTTTCGGAGAATTTAATGCG	279
CaM0176	EI850635	(TA)10	TGGCTTTTCAAAGAACAATGG	TCACAATGAAACAAAGGGCA	226
CaM0177	EI850685	(AT)5g(TA)7	TTTCATGACCAAACAATTCCA	GGCTTTTCCTTGATCAACCC	277
CaM0181	EI850898	(AGA)5	GGGAAAACAGGCCACATAAA	CTGTGTTGGCCATCAGACAT	190
CaM0182	EI850929	(AT)9	AACATGTAATTTAAGTGTGGGGG	CAATCATGCCAATCCAAACA	141
CaM0183	EI850929	(TTA)26	GCTTGAGGGGGAGTGTTAGA	GAGAAGAGAAAATAATAAGGGTTTGAA	280
CaM0186	EI850992	(CTT)9	AAAGTGGTCAGTGGTCAATCA	ATTTCCCGGACCATGAATTT	224
CaM0187	EI851048	(TA)18N(TA)28	ACCCAATTTTGGGGGATAAA	TCTTTGCTCACAACACAACCA	237
CaM0191	EI851133	(TA)21	CAGAGGAGGAGGATGGAAAA	AATGGGTTACGGGTGAAATG	271
CaM0193	EI851159	(GT)6	AACCAATCTTGTGCATCCAA	GGGAGGACGAACATGACCTA	170
CaM0194	EI851174	(AT)16	ATTTTCCCTCCACACAAACA	TATTTTGGATTTTGGCTCGG	274
CaM0195	EI851194	(AC)6	TTGGATGCACAAGAATGGTT	TGAGGTCATTTTCATCCTTCC	221
CaM0196	EI851212	(ATT)5	CAGAAAGGAGCTTGGAAGGA	TGCAAACACTCAACCCAAAA	258
CaM0197	EI851234	(CTT)5	TGATCCTCCATCCTTCTTGG	GCAACATGGGATGATTCAGA	201
CaM0198	EI851246	(TA)6	TTTGATTAATTTCCGTAGTTGATTTG	TCATCCTAAAGAATTTGCCATT	280
CaM0199	EI851254	(TG)8	GAACATTTTCTCCATGGCCT	TCCACCACAAATTTGATTCCT	270
CaM0200	EI851254	(AGA)7	GTCGTGCCAGCACAAGAGTA	CCATTCACACACACACACACA	260
CaM0203	EI851282	(TAT)7	GCGTTAGCAAACAATGCCTA	TGGTTAGGTTCCCAATCTTCA	179
CaM0204	EI851302	(AT)15(GT)26	GAAGACAAAGTAATTACACATCCTCA	TGCACACATTCTTTCACGCT	280
CaM0205	EI851310	(AGA)6	GCTGACGCCATGAAGGATA	TAGGCAAAATCCAGAGTCCG	167
CaM0206	EI851353	(AT)14	AATGCGTTACGGGTGAAATG	AGAAGGAGGGAAATTGGTCG	280
CaM0211	EI851482	(AT)7aa(AT)18	TTCCTTTCTTTTTCCCTCCAA	ATATTGGCTCGGAGGCTTTT	280
CaM0213	EI851489	(AT)12	GACATTGTTGTTAAATTGGTGTTGA	TCGCATTCCATCTCTGTTGA	222
CaM0214	EI851512	(GT)6	AGGTGTGCTTGTGTCATTGG	AAGCCTAGGGAGGACGAAAA	256
CaM0215	EI851542	(T)10N(TA)7	CCATTCATTCGTTAGTGCATTT	TTTTCCATCAAAGTGTGGCA	246
CaM0216	EI851629	(AT)6ac(AT)38	CCTTTTCCTTTCCCCTCAAA	ATTTTGGTTCGGAGGCTTTT	181
CaM0220	EI851757	(TA)26N(T)10	GTTTACCCATGTGTTTGCGA	CCTTTTGATTTTTGAGAAACGTG	237
CaM0221	EI851775	(AC)16(AT)11	CCTTCTCTTTTCCCTCCACA	TGCCTTATGGGTGAAATGGT	162
CaM0227	EI851879	(ATG)5	CCCCTTGAAAGAGGACAAAA	TCACCAATCTTGGAGGGTTC	279
CaM0229	EI851949	(TGA)7	CGTGTTTTTCCTCGACACCT	CATTTTGAACTGCTTTCTCGC	205

Contd…

CaM0231	EI852088	(AT)22	GAAAGGAGAAGGCAAGGAGC	CAGATTGAATTGCAGCGAGA	244
CaM0232	EI852150	(AT)6	TTTACCGCTGTTCCATTTCC	GAAGGGACACTTCCAACAGG	228
CaM0233	EI852173	(T)12N(AT)5	GTGAGGCATGGAGAGGAAGA	TGAGAAGTGATGGGAGGTGA	210
CaM0235	EI852212	(AAT)6	TGAAAAAGCCCACCAACACT	TCCAATCATGCTAGTCCCAA	255
CaM0236	EI852222	(AT)9t(ATA)9	AGCTTATGGTGATGCGAATG	AACAAAAATGAAGGAGGGGG	271
CaM0237	EI852228	(TGT)5	ATGACGACGTTGATGACGAA	CCCCCAAATGTTTGTGAGAA	193
CaM0239	EI852252	(TC)10	TTTTGAAAGATGCTACCACCA	GACGTGGAAAATCCGAAAGA	203
CaM0240	EI852279	(AAT)10	AAGTTAACATGTGAAGGAACCTAGA	TTTTATGCCTATAAAGTTGGCCT	278
CaM0244	EI852407	(TCTCT)6	TTTCCCCTTCTTTCTCAACA	TTCAGAGATTGGATGAGAAGGTT	223
CaM0245	EI852415	(TA)5N(TA)5N(AT)7	AGTGAAGGGATGGGTGTGAG	CGAGCATGGAGGGAAACATA	192
CaM0246	EI852421	(AATA)6	CACGCATCCTTCTTCTCCTC	TGAATGTGTGTGTCCATGAGAG	189
CaM0247	EI852425	(TA)20	CGTGGGTTCAGAGGAAGAAG	TGCCTTATGGGTGAAATGGT	263
CaM0249	EI852448	(AT)6N(AT)6	TCAAAACAGAACAGATCATGTGG	CCTCTACGTACTGTGCAACCA	199
CaM0251	EI852496	(T)16N(TA)13	GGCTTTTCCTTTTGACATCTT	AAATCATCACCATATAATCCAATCAA	277
CaM0253	EI852522	(TA)6	GGGAGGAGGAACAAGAGTGA	GGAATTTGTGGGTGAAATGG	145
CaM0256	EI852561	(AT)18	ATCTACTCTCGCCGCAACTC	TGGTGAATTAAGTCCCAAAAACA	226
CaM0257	EI852563	(AT)39	AGGTACTTGCCCAAATGACG	CCATCTCATTGTAAAACTCCTTCC	240
CaM0258	EI852603	(AT)25	TGGGATTTTCCTTCCTTCCT	GCATTACGGGTAAAAAGGCA	251
CaM0259	EI852655	(AT)12	GAACCAGCCCCTTTAGGAGA	AGGCAAAAAGGCAGAAAACA	276
CaM0260	EI852656	(TA)6	ACTCCGCATAAAATGAAACAA	TTGTCTTACCCGTTGGAGAAT	274
CaM0263	EI852728	(TCA)6	GGCTTTTTCAGCAGACTTGG	TGGCACTGGTAGTGTGAATGA	253
CaM0265	EI852771	(TG)10N(AG)5	ACGTGGCCTCACACAATACA	TTGGTAATCCGGTCCTCTTG	226
CaM0268	EI852844	(AG)7	TGCAAATGAAGTACACAAGCC	TCCAACGTCATTTCCAACAA	275
CaM0269	EI852859	(AT)9	TCAAAACAGAACAGGTCAACAAA	TGTGCAGCCATCTACGTTGT	152
CaM0272	EI852888	(TAA)5	TTGTGGGACAATTTCATCTTG	AACAAACAAGAACTTTTAATTTGAACA	183
CaM0273	EI852905	(AT)6	TATTGGTCGGACACCCCTTA	TGAGAAATGGTCGTCTACATGC	198
CaM0276	EI852955	(ATA)5	CGGATCCACTAGCCGTAGAG	GTTCGAGAAAGCAAATCCGA	267
CaM0277	EI852999	(GT)6	GCCTTGGTAAATTTTGGGGT	TGAGATTTCAAGGGGAAATGTT	108
CaM0278	EI853015	(GA)11	TTGTACTCACCTGCAACCCA	TGCAGTAGAAGTTTGGGCTCT	251
CaM0280	EI853052	(TTC)9	TGGGAAGCGTTGTGAATGTA	AAGAAAAGGCCTGGTTTGGT	256
CaM0283	EI853127	(ATT)5	TCAATTACGATCGACACCCA	TCTAGCAAGCTGTACAATCTAGGAA	219
CaM0284	EI853142	(CT)6	GCATTGCAACTTTGTGGTGT	CAATACCAGGGTGATGGTATGA	242
CaM0285	EI853199	(AT)6N(AT)9(AC)6	TCGAGAAGCAACTCAAGTCGT	AGGGGTGTGTTGGTAAAGAA	268
CaM0286	EI853206	(AT)15	CAAGCGCTATGTGACCTCCT	TCACCCCAATATGATCTGATG	279
CaM0291	EI853280	(AAT)7	GGCCGCCGTACTTTTTATTT	TACCGTATGTTGGATGGGCT	114
CaM0292	EI853285	(TA)5N(A)11	ATTGGGCAATAAGCAAAGGA	TTCTTTATGTTTTAGATGGAGATGC	245
CaM0293	EI853320	(AAT)13	ATGGCTGGCTTCTTTCTTCA	AGTCGTGAGGGGGAGATTTT	237
CaM0294	EI853330	(AT)6	TCATGTGGTATGCGTTTTTGA	CGTTGGAAACCCTCATATCC	243
CaM0297	EI853465	(AT)23	CGCGACAGAGGAAGAAGAAG	ATGCGTTATGGGTGAAATGG	237
CaM0300	EI853621	(CA)9(TA)34	TGCGTGTTTCTTTTCTCGATT	AAACCCTCTGCATATGTGGG	185
CaM0305	EI853724	(TTC)5	TGCTGAATATTTAAGTGGTGACG	ACATTGAAGGATTCGGACCA	126
CaM0308	EI853813	(TAA)5	TTGTGAGAGTGAGTGGCTGG	TGATTAATTTGGACCCGTCG	270
CaM0309	EI853865	(A)10N(AAAT)7	AAGCCCACGTCACTCAAGAT	TTCGGGAGTCGATTACGAGT	238
CaM0310	EI853879	(TA)6	CAACGGTTGCATCATAATCTG	TGAGAAACAAATTCTTCCACCA	225

Contd…

CaM0311	EI853879	(ATC)5N(CAT)7N(TTC)5	GCGCACACCTTCAATCAACT	GATTCTCCAAAAAGCTAACGGA	278
CaM0314	EI853933	(ATA)7	ATTGGTTCAACCGACCAATC	GAGCAACGGAGTTTCCATTC	185
CaM0315	EI853943	(AT)26	CGCAGGGAGAGGAAGAAGAT	AATGCGTTACGGGTGAAATG	272
CaM0316	EI854030	(AT)21	ATAAGTGATTGCGAGGCAGG	TTGCTGAGACATTTAGGCCA	222
CaM0317	EI854030	(AAT)13	TGGCCTAAATGTCTCAGCAA	AGAGGCAAACAAGAACCGAA	261
CaM0318	EI854044	(TA)6	AAGGAGACACATGCTGAGGG	CTGGGGGTTGAAATCAGAAA	251
CaM0319	EI854046	(TA)7	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	235
CaM0320	EI854055	(TAAA)5	GAGGCAATGTTGGAAACTCG	GCCTTTGTTAAATTTGGATGATG	267
CaM0322	EI854120	(ATA)5	ACCCAAAGAAGTCATCCGTG	TGAGAGTGAGTGGGGTTGAG	228
CaM0325	EI854205	(ATA)13N(ATA)14	CGAACAATTGGTTAAAACATGC	GGGGAGATAATGTAACATCCTAAA	279
CaM0326	EI854206	(ATA)5atc(ATA)7N(ATA)14	TAATTTTATTAAGTATCTTGCGCATT	CGAAATTTCTTTAAGGAGGGG	239
CaM0327	EI854249	(TAA)5	CCCCAAATTGAAGAAGGAAA	CACTCAAGGCAGAAGGAGAAA	246
CaM0328	EI854262	(TG)6	AGGTCGTTTTCATCCTCCCT	TGGATGCACGAGATTGGTTA	268
CaM0332	EI854297	(AT)6	TTGTTTTGATGACGGTCAGG	ATAATTTAGAATTAGGCCAACAATTT	228
CaM0334	EI854308	(TC)10	AAAGGAGGCTGGAAGGTCAT	TGCAGCTGTGATGTTGTTCA	209
CaM0335	EI854329	(AT)11	TACGGGAGGAAGAAGATGGA	TGCGTTACGGTGAAATGGTA	217
CaM0336	EI854339	(TA)5N(AT)5	TCATCTGCCAATTGAGTTGC	AGCAACAGGGAGAGGAAACA	252
CaM0338	EI854354	(TA)8	CAACAAGCGCTTTAAATGACC	TGCCAGAACTGATTTTCTGC	238
CaM0339	EI854362	(AT)6	GGAGAAAGAGGGAATTTGGC	GCGTTACGGGTGAAATGGTA	267
CaM0340	EI854366	(AT)12	AATTGCGGCGAGAGAAGATA	GCGTGAAAACAAGCAACGTA	224
CaM0341	EI854393	(TA)5N(TA)5	CATGCATATGGATCTGAATGTAGTT	TCACAATCAGTGCACACAACA	251
CaM0343	EI854462	(GT)6	TCATTTTCGTCCTTCCTTGG	CCACACCTATTGGATGCACA	275
CaM0344	EI854469	(AT)15g(TA)7	GCGGGTCAGAGGTAGAAGAT	AATGCGTTACGGGTGAAATG	276
CaM0345	EI854527	(CTT)5	TCTACTTGTGGGTTGCCTCC	CAAGCAACGTCATTTAGTTCTCA	166
CaM0346	EI854531	(GT)6	AAGCAATCTTGTGCATCCAA	AGGGAGGACGAAAATGACCT	271
CaM0348	EI854570	(TTG)5	ACATCCGCTACTGGACCAAG	CAACTCACATGTTGATTTCATGC	280
CaM0352	EI854629	(AT)31	TTTTGGTCGATGTTGGTTCA	TGGATGATTTGATGTCACATAGG	140
CaM0353	EI854650	(TTA)5	AATCCAATCAAAGAAACCGAA	AATCCCTGGCAAGGACTTTT	253
CaM0356	EI854713	(TA)20	AGGGAAGAGGAAGAAGAGCG	TCAATTGAGTTGCGACTAGGG	252
CaM0357	EI854732	(GA)37	AATCGAGTTGCTTCCTCGAA	CGCAGTTCTTCTCTTGTCCC	162
CaM0358	EI854746	(TA)5N(TA)7	TCATCAGTTTCCTTTCATTCCTT	TGGGAAGTGATGGGTGAAAT	190
CaM0360	EI854793	(AT)5N(T)12N(T)12	AGAAGACAAAGGGGAGCACA	TGACGAAATTTGTTTTCTTGATCT	263
CaM0361	EI854802	(AT)16	GCGCGTTAACAGAGGAAGAA	TTAAGGCGTTACGGGTGAAA	272
CaM0362	EI854803	(GT)7	CCAATTTTGTGCATCCATGT	ATGGAGAACGGATGTGACCA	120
CaM0366	EI854951	(A)10N(TA)5	GCTTGTATTTTAATTCATGAAACTTGG	GCAAATCTTTGAACTGGTGTTTT	205
CaM0367	EI854954	(TAT)17	TTTGTCGACTGAGACCTTGC	TTGACGAAATCGAGATAAAATTCA	241
CaM0368	EI854954	(A)10(AAT)6N(CT)6	GTTTGGTGGCCAAAGGGTAT	AAAAATGGAGGAGGGGGAG	217
CaM0369	EI854959	(CTCAAC)7	TGCTGCCTTATCCACAACTG	CTCAGGAGGGGCTGTATCTG	239
CaM0371	EI854998	(AT)16N(AT)5	TTGCAAGCATCGTACGTGAG	CCAATATATTTTAACTGTGGTTTGAA	259
CaM0372	EI855026	(AT)6	TCCTTTCTGTTTCCCTCCAA	CGGTGAATCCTTTACGGAGA	139
CaM0375	EI855040	(ATT)5	CAACTCGGCTTGAGTTCACA	GAAAGCGATACTGGCACCAT	171
CaM0377	EI855130	(AT)8	TTTGTATGATATTCGCTTGTTAATGA	TTTCCTCAAATTCCAAAAATTCA	251
CaM0378	EI855181	(AGA)5	GAGAAGCTTATGGCTGACGC	AAGCAAAATCCAGAGTCCGA	175
CaM0379	EI855218	(TC)19	ATAGGTGGGCTGTGATGAGC	TTCCAATTGCTCCCAATTTC	254

Contd…

CaM0382	EI855269	(GT)6	AACCAATCTTGTGCATCCAA	AGGGAGGACGAAAATGACCT	171
CaM0383	EI855282	(AT)7	CGATTGACATACATCCATTTCC	TGTGCAGCCATCTACGTTGT	278
CaM0384	EI855355	(TA)7	ATCCCCAAAATCCACATTCA	TCAATGGTGTGGTTTCTTTCA	166
CaM0385	EI855355	(AT)6	TTTTGGGGACATTTGGAGAG	TAAATGGTGTGCCCCTTGTT	185
CaM0387	EI855399	(AT)6	TGATGCTATCTGACCATAGCCTT	TCACATGGAACCCACATGAT	274
CaM0388	EI855446	(AT)8	GCAACGAAAATGAAAATGGG	AATGCGTTACGGGTGAAATG	274
CaM0389	EI855460	(AT)12	TGCTTTCGCAAAAACAACTT	TCATACCCAAAAACATTGTCCA	197
CaM0391	EI855489	(AG)40	CGAGTAAAAACCTCATGTACCG	CAAACTACTCACACCCACGG	242
CaM0392	EI855492	(TA)10	GAACCGTTCGTATTTCTGCG	CCATTTGAGCTCGGAAAACT	220
CaM0393	EI855520	(ATA)17	CGTGAGTTTGGATTTGCTCA	TTTTAATGGATGATTGAATGCAC	170
CaM0394	EI855531	(T)13N(TA)9N(TA)8	TTTCTCTCCCCCTGCTTTTT	TTCTCGTAACATATCCGCTATCTG	223
CaM0396	EI855537	(TAT)26	CTGTCGTGTCCATCTGTCTCA	TGTGTTGACCGATTTTATTATTATTTT	280
CaM0397	EI855540	(AT)8	GCTTGTGTCGACAATCAGGA	CAGTCCACATGAATGGTTGC	214
CaM0398	EI855569	(CTC)5	CAAACCCTAAACCTGCTCCA	GATTTTCCCTGGTTGTCGAA	245
CaM0399	EI855580	(TC)10N(TC)7	ATAGCTTCGCAGTCCACACC	AATATGACAGCATGAGTGAGACA	226
CaM0400	EI855581	(TA)7	GAAGCAAAGGGAAGCAAAGA	AGTTTGGGATGTGGGTGAAA	218
CaM0403	EI855604	(TAA)8	TTTTCTCATCCCAAATTCTCAAA	TGCAATCACTGCCTCAAAAC	254
CaM0407	EI855677	(CGC)8	CTGAACCGAACCAAACCACT	CGTTGTGCGAAGAGAGAAGA	277
CaM0410	EI855715	(AG)16	AAAGAGCCAAAAGGCATCAA	TCATGTCTCTCTCCTCCATCTCT	175
CaM0411	EI855725	(AT)8	GCAACGAAAATGAAAATGGG	AATGCGTTACGGGTGAAATG	274
CaM0413	EI855747	(AT)12	CTGCACGTCATTGCAAAACT	GCTGCATTCCACTTTCTGCT	152
CaM0414	EI855782	(AT)5N(A)10N(AG)5	GGAACCACGCTAGAGAACTCC	AGGATCAATCGCTGCTTTCT	269
CaM0416	EI855812	(ATT)5	TTCAGGATCTGTTCAAATCAGC	TGCTCACCCAAACTTTGTCTT	275
CaM0417	EI855814	(TA)6	ATTTTGGTTCGGAGGCTTTT	TTTCTTTTTCCCTCCAAGCA	249
CaM0420	EI855887	(AT)10	TCAAAACAGAACAGATCATGTGG	GTACTGTGCGGCCATCTACG	198
CaM0421	EI855939	(T)10aa(GT)9(AT)18	GTTGGCGTGGAAGATAAGGA	AATTGCGGCGAGAGAAAATA	221
CaM0422	EI855976	(AT)6ag(AC)8	AAATGCTCCCCTGATTGACA	TCGTACATCCAAATACGGTCT	131
CaM0423	EI856010	(TAT)7	TATCCGACAACGTACGAGCA	TGGGTAGAAAATATTTATGTATTGACA	278
CaM0425	EI856031	(TA)12	GTCAATTGAGTTGCGACGAA	CACTTTTGAAGCCGAGGAAG	225
CaM0428	EI856175	(AT)6	TATTTTGGATTTTGGCTCGG	CCCCCAAACAAACACATTTT	125
CaM0429	EI856185	(TCA)9N(ATC)11N(TCA)7N(ATT)14	TCTCATCATCATAATCATCACCTTT	GGACCTAAATTGAATTCGCC	270
CaM0430	EI856189	(TAA)27N(TAA)6	TGAGATAATTATTCTATAAATGGTCGG	CGTGGTATTAAACGGAGAAACTG	255
CaM0431	EI856196	(TA)6	CAGCTCGGTTCACCATTTTT	AAAAGCTGCTGCTGTTTGAT	279
CaM0432	EI856223	(TA)41	CATGATAATGAATGTGGGGAGA	CCTCAAAGTGCGATCAACAA	206
CaM0434	EI856279	(AT)21	CGAGCCAAAATCCAAAACAT	CAACACAACATTCAACAATCAAA	196
CaM0435	EI856279	(AT)23	CGCGTAGGGAGGAAGAAGAT	AATGCGTTACGGGTGAAATG	276
CaM0436	EI856290	(ATT)20ta(ATT)5	TGAAAATGGAGAAGGATGGG	AAGGGGAGGAGATTGAAACG	218
CaM0437	EI856317	(AG)6	TGATCTCCGGTAAAACGAGG	CCAACCACAACGAGACAATG	260
CaM0438	EI856337	(AAACA)5	CGTATCCATGACCAACACCA	TGAACTAGGCATGATGTGTGC	223
CaM0440	EI856347	(AT)6	TTTCTTTTTCCCTCCAACCA	TATTTTGGATTTTGGCTCGG	253
CaM0441	EI856373	(AT)21	GGAGAAGAGGAATGTTGCGA	CGGTGAATCCTTTACGGAGA	262
CaM0443	EI856442	(TAA)37	TCGTTTGCATAAGATGGAACA	GTACAACCGCCGCAAATATC	279
CaM0446	EI856503	(T)10N(AT)6	TGTGCATCTTCGTAGGGAAA	TGAAATTAAGTTAAGGAACTCCTAGTG	215
CaM0451	EI856579	(AATA)5	CAAGCCTTTGGGACAATGTT	TAAAGCCCCACTTGATGTCG	144

Contd…

CaM0452	EI856591	(GTA)5	CTTCCGCACAACCAATTTTT	CTTTGGCAGTTTTGCAATGA	219
CaM0453	EI856635	(AT)40	TCTTTGCTCACAACACAACCA	TGTTTAAGCCCATTTGAGCC	276
CaM0454	EI856710	(AT)21	CGGTGAATCCTTTACGGAGA	TTTCCTTTCTTCCTTTTTCCTTC	253
CaM0456	EI856726	(TTA)42	GCGGCTAGGGTTTCAGTATTC	TGGTAATACGTTATTAGTGAAATTGTG	273
CaM0458	EI856764	(AT)18	TTTCCTTTCTTTTTCCCTCCA	TTGAGTTGCAACGAGAGTGG	190
CaM0460	EI856821	(GA)8	TTACTTTCGGGAGACATGGG	TTCTTTTCATCACCATACTTGAATAA	239
CaM0461	EI856866	(A)12g(TA)7	ATGGCCCATCTATCCAATGA	TTGTCGTCCAATCCTACCAAG	159
CaM0462	EI856942	(GT)6	TTTGGTCATTTTCGTCCTCC	TTGGATGCACAAGATTGGTT	271
CaM0463	EI856964	(AAT)9N(AT)5	TGGCATGGTGGACTCATTTA	GGGCTTATGGCCTGACCTAC	270
CaM0464	EI856986	(TA)9	GGGGACCACTCTATCTTTTCG	TCACGTCCCAGTTCTCCAAT	258
CaM0465	EI856989	(TG)6	GCCCCTTACAAGTGAGTGTGA	CCAGTTCAAGTTTGTGTCTGATTT	274
CaM0466	EI856989	(CA)11	TCCTGAATTTGCTATGTGTCTGA	TCACACTCACTTGTAAGGGGC	245
CaM0468	EI857016	(AT)6	ATGTGGAACAAACTACCGGC	CCGTTACCACAACCACAAAG	196
CaM0469	EI857093	(GT)6	AACCAATCTTGTGCATCCAA	GAATAAGCCTCGGGAGGAAG	101
CaM0471	EI857115	(AT)48	TGAGCAAGAGGGAGAGGAAA	GATATATAGATATAGGGGTGGGGTAA	224
CaM0472	EI857135	(TA)7	GGCGAAAGCCACTAATTGAA	TACCTCCTGTTTCAGCCGTC	276
CaM0473	EI857159	(AGA)5	CTGACGCCATGAAGGAAATC	AGTGGTCTTGGAAAGCGAAA	198
CaM0474	EI857186	(T)10N(TA)5	CTTCCCCTTGTCAAACCAAA	TGGATTTGGAAGTCGATTACG	215
CaM0475	EI857186	(TA)12	TGTGTCGTATATTGATTTGTGTATCG	TGGTTTGACAAGGGGAAGAC	209
CaM0476	EI857206	(GT)6	AACCAATCTTGTGCATCCAA	AAGCCTAGGGAGGACGAAAA	277
CaM0480	EI857283	(ATT)7	TTGATGCCGAAAAAGAGGAG	TTTCGGTAAACGTTGGTTTT	238
CaM0484	EI857392	(AT)7	AAAAATTGGATAAAATCAAACAATG	TTGTTTTCTTTCCCTTTTTGTTC	233
CaM0485	EI857392	(AT)8N(A)13	CAAAAAGGGAAAGAAAACAATG	CCTTTTTGTTCGGATGTTGTT	206
CaM0486	EI857434	(AT)7	CATCACCGTTCTCTCACGAA	TTCAAACCGTGGAATGTGAA	240
CaM0487	EI857438	(AT)15t(TA)20	AAGTCGCCATTTGCAAAAAC	TGGACAATAGTAAACCTGATCGAA	257
CaM0488	EI857456	(ATT)5	GGAGGGGGAGCAATAATAGG	TGATTTGTCTCATGCCGCTA	150
CaM0489	EI857470	(ATT)5	GGAGGGGGAGCAATAATAGG	TGATTTGTCTCATGCCGCTA	150
CaM0491	EI857496	(A)13(AT)10	CAGCAAAATGGGAGTCCTTC	ACACGTGAGAGGCACAAATG	182
CaM0492	EI857532	(TTG)5	GTTCTTGCCATTGCGAATTT	CCCCTTCTTACCCTTTTCGT	235
CaM0493	EI857539	(TTG)5	GCGAATTTGGCAAGAAGAAG	CCTCCAATAAACCAACCCCT	238
CaM0494	EI857553	(AT)18	CTTTTTCCCTCCAACCAACC	TTTGGATTTTGACTCGGAGG	274
CaM0499	EI857748	(TCT)13	AAATGGATTTCAGCCAATCG	CAAAAATCGACCACAGCAAA	265
CaM0500	EI857756	(ATA)27	AAGTTTGAATTGAGGTTAGATTTGA	CCCAGTACGTAAGGTTTGGGT	252
CaM0501	EI857804	(AT)5N(AT)7	CAACAAGTTTCCAAAAGAAACCA	CTACGTACTGTGCGGCCAT	225
CaM0502	EI857831	(AGC)5	TTCATTTGTACAATGGGCGA	GCAAAACTTGCAGCAACAAA	164
CaM0503	EI857831	(TA)9a(AT)5	GCTTTTAAGGCACTATCGAATGA	TCAATGCGGTCTCTGAAGTG	195
CaM0504	EI857871	(TA)7	TCGAGATTGCACTATTTTAATGGA	TGTGACACCCTGAATACCACA	199
CaM0505	EI857911	(AAC)5	ACACCAGGCACAACCCTAAG	AATTTGCTTTTGTTGTTTCGAT	271
CaM0506	EI857914	(TTC)5	ATGTGAGGGGATTGGACGTA	TTGAGACCGGGCTTTGATAC	230
CaM0507	EI857918	(ATT)21a(ATT)9N(A)15	GAAGGAGAGAAGAAGGGGGA	AATTAGGTTTTGACACGTCCG	225
CaM0514	EI858141	(TCT)5	CGATGGTTGATGCGAGTAGA	CGGACAAGGAAGAGCAAGAG	179
CaM0515	EI858141	(AAAG)5	AATCCTGCTCCTGAACCAAA	TCTACTCGCATCAACCATCG	153
CaM0516	EI858153	(TA)9	CGTAATTTTTGCAACATTTGTGA	TTTTTGCAAACCCAAGTTCG	279
CaM0517	EI858159	(TA)10	GGAGAGAGGGAGGATTTTGG	AATGGGTTACGGGTGAAATG	280

Contd…

CaM0519	EI858190	(TATTT)5	GTTCAAGAGCCACCCACTGT	TCGAAAGATCTAATACAAAATTGCC	280
CaM0520	EI858211	(TTA)26N(TAT)11	AATGTAGCCCACTAAGCCGA	CAAATTACAAACATAGGCAACCC	236
CaM0522	EI858217	(TGT)5N(GTT)6	CTTCCACCTCCTTTTTCCAA	TCATTACGTAACTCAAACACATCAAA	187
CaM0523	EI858295	(CT)9g(AC)6	GCGGGGTCATCCTGTACTTA	GGCAGAAAATGAACAGGCATA	270
CaM0525	EI858386	(AAT)5N(TTA)18(TA)16	TGAGAAACTTGATATCCTAAGCGA	ATGTGACACCCTAAACCCCA	241
CaM0526	EI858413	(TA)10	TTTTGACCACACAACCATACA	AGCAATTTCGGTTGCTTGAG	260
CaM0527	EI858424	(TA)10	TTTCCTTTCTTTTTCCCTCCA	TTTGGCTCGGAAGCTTTTTA	261
CaM0529	EI858454	(GA)6	CTTGCATAATGAATGCGACA	TTTTCAACCTCTCCGACTTCA	100
CaM0533	EI858545	(TA)7	TTGTAGCCACCCCATTTGAT	CGACATGGCAAAATTCACAC	144
CaM0539	EI858666	(AT)26g(TA)6tg(TA)5ttaa(AAT)14	GATGGAGGGAGAACGTGAAA	TGTGACGTGATGTGAGGTGA	265
CaM0541	EI858916	(AT)7	TGAGAGATTTTGAGTGCTCTATGC	TGTCGGTATTACATATCAAAGTTAGCA	272
CaM0543	EI858929	(AAG)5	AAAGCAGGATGTCCCTCAAA	TCTGTTTTTGCCATCAAGCA	143
CaM0547	EI859063	(TA)6N(A)12	TTTGATTCCCCTTTTAAGTTTG	TTGATTTTGGATGGAGGGAC	280
CaM0549	EI859119	(TTA)5	AGAGGCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	182
CaM0550	EI859121	(AT)13	TCATGTGGTATGAGTTTTTGACA	CTACGTACTGTGCGGCCAT	193
CaM0551	EI859212	(TTTTGT)5	ATTTTTGGAGGACTTTCGGG	TGAAAGAGAAAGAAGAAAGAGAAAGA	272
CaM0552	EI859230	(AT)14	GCGCGTTAACAGAGGAAGAA	CGGTGAATCCTTTACGGAGA	279
CaM0553	EI859244	(TG)7(TA)6N(AT)5	CAATTTTCTGCACTCTCGCA	GCAAATGCTCCCCTAATTGA	280
CaM0555	EI859299	(TA)8	CAGCTTTCCTCCGTTTTCTG	TGGTTTGAGAGGTTATGGGTG	192
CaM0558	EI859387	(AT)26	CGTAGGGAGGAAGAAGGTGA	AATGCGTTACGGGTGAAATG	280
CaM0565	EI859533	(AC)6	AGGGAGGACGAAAATGACCT	GGGTTTTTAACCAATCTTGTGC	179
CaM0566	EI859687	(AT)12N(AT)5	CCTTTCAATGTAGTTGCGACG	CGTTTCCACAGCGCTTATTT	265
CaM0567	EI859707	(AT)18	TCGGGTTTTCCTTCCTTTCT	CGGTGAATCCTTTACGGAGA	268
CaM0568	EI859756	(AT)6N(A)10	AAGAAGCTGAAACACGAGGC	GAGTTGCCGCGAGAGTAGAT	200
CaM0571	EI859902	(AAG)5	CCAGGATAAAACAGCCCAGA	GCTTTTCTCACTTCTCCCCC	175
CaM0572	EI859969	(TA)6N(AT)23	TCAACAAGTTTCAAAAAGAAACCA	TGTGCGACCATCTACGTTGT	262
CaM0573	EI860023	(TA)8	TTTTCGAACCCGAGAAAGAA	TTGAGTTGCGACGAGAGTTG	191
CaM0574	EI860034	(CCT)5	ACTCATGCTACCGTATCCGC	ACATAAGCAGAAGGAGCCGA	240
CaM0575	EI860051	(TA)10	AAATGGAGGAAGGAGGGAGA	AATGCGTTACGGGTGAAATG	260
CaM0576	EI860079	(TA)9	TGTGCAGCCATCTACGTTGT	TCAAAACAGAACAGGTCAACAAA	152
CaM0577	EI860088	(A)10(AAT)5	ATGATTCCCCAACATCCAAA	CGGGGAAACAGCTTTATTGA	138
CaM0578	EI860147	(TA)6	TTGAATGATGTGACGAAATTTTA	TTGGTGTAAGTCCTAGAACCAATC	280
CaM0579	EI860167	(TA)13	CGCTTATAGACAAAAGCGCTATAAA	AGTGTACGTTTGACCTGCCA	264
CaM0581	EI860187	(TA)5tc(TA)7	AGGCAATGCAAAGAAGAGGA	AGGAGCTGATTGTGCAACCT	170
CaM0582	EI860208	(TC)9(TA)18	GCGAGCAGAGGAAGAAGAAG	TGTGTTATGGGGGAAATGGT	232
CaM0583	EI860226	(TA)12	CCCTTCAACAAAACACACCC	TCCATGTTATTACCGCTACCA	245
CaM0589	EI860305	(TAT)11	CCAGCATGAGTTGTTCATAACC	GAAAAATGGACGCCAAAGAA	237
CaM0590	EI860308	(AT)11	ATGGAGGGAACGTGTTTCTG	TGCCTTATGGGTGAAATGGT	232
CaM0591	EI860359	(TAT)8	TGTGAGGTGTAACATCCGAAA	TTAATATTGGCAAATGAATCATAATC	255
CaM0594	EI860404	(AT)16taa(AAT)7	GGCTTCACGGGAAAAATGT	TGAGGTGACAGGCGTAATGA	169
CaM0595	EI860419	(AT)9	GCGACAGAGGAAGAAGATGG	AGGGGAATGAAATTATGGGG	237
CaM0596	EI860427	(AT)9N(AT)17	AACAAACTCCTTCGCTCCAA	CCACATGATCTGTTATGTTTTGAA	275
CaM0597	EI860447	(ACC)7	CAAACCACCATTCACACCTG	TGTTGGTGGGTTTGATGAGA	214
CaM0598	EI860454	(GA)9N(AG)15	ATTGGAGTTGGAGACGGTTG	CCATTGCACTCCCATTCTTT	213

Contd…

CaM0599	EI860464	(TA)6	TTGTGGAGCTTTGAGAACGA	TGGATGTCTTGCTTTTGCAG	224
CaM0600	EI860465	(TC)11N(GT)5	GAAATGTGGGGGATTCAAGA	TTGAGGGCATAAAGAGGTGG	210
CaM0601	EI860467	(AT)11	AAATTTTCAGCAACCCGAGA	TTGTGTTGAATTGCTGAGGC	231
CaM0602	EI860467	(CA)5tN(AT)19	AGTCGAGCAGCATGGAAAAT	CAATTGAGTTGCGACGAGAG	246
CaM0603	EI860470	(TAT)14	ATGTTCCTCCCGTTGCTAAA	GATTTGAGGTCATGCATATGTTTT	264
CaM0604	EI860501	(AC)7	TTTGCCTCATCATTTATGTACTCTG	CAGCACCAAAAATGTCCTGA	278
CaM0606	EI860531	(AT)5N(AT)8	GCTATTCAGGAACAGACGGAA	TTGGGCGCAAATTTATTG	268
CaM0607	EI860539	(TTA)5	CCTGAATTGATTGAAGCCAAA	TTCATTAAAAATATGTTCATTCCG	255
CaM0610	EI860570	(TA)8	ATTTCTGGTGCAGGATTTGC	TGATGCCTTATGCAAGCTTTT	251
CaM0613	EI860702	(ATT)52	AAACCCTTTTTATTTTCAATTTTCA	CCAATTTGATTTGATGTCGC	280
CaM0615	EI860705	(TTA)32(TTG)6	AAATCATCGTCCAAAGGGAA	TCACTGAAATTTATCTGGATTGG	243
CaM0617	EI860777	(AT)5ac(AT)6	CGGGTGACTCCAAATATGCT	AAACCTTTAGATGCTTAACCTTTTT	191
CaM0618	EI860785	(GT)6	GTTTGGGTCATTGGGTGAAC	GCCTAGGGAGGAGGAAAATG	150
CaM0620	EI860849	(TA)22	ATTCCCAAACATTGGCAAAA	GTTTGGGGTTACTCATGGGA	209
CaM0621	EI860865	(AAC)5	TCTCGAGTTCATGCCATCAT	TGTTGATCATGTCAAAAAGGGA	102
CaM0622	EI860872	(AG)14	GTAAGAAAATGGGGGAGGCT	GAAGCCGTGGCTAGAGATTG	195
CaM0624	EI860886	(ATA)5	GGAATCGCTACCGAATATGG	CTATGGTCTGGCCTGGAATG	279
CaM0627	EI860972	(T)11N(AT)9	GGGTTTAAAGGGTGTTTTGTTTC	TGCACTGCAGAGAATTCCAA	263
CaM0629	EI860976	(ATA)5	GGAATCGCTACCGAATATGG	CTATGGTCTGGCCTGGAATG	279
CaM0631	EI860997	(TG)6	AGGTCGTTTTCATCCTCCCT	TGGACGCACAAGATTGGTTA	268
CaM0632	EI861006	(AT)15	GCATTGCAGTTGTCAAGTCG	AATTATTTACGCTACTAAAAAGCAACA	225
CaM0633	EI861034	(TTG)5	TGAGGTTCAATATTCGCCGT	TGAAAACCTGAAAGGGCAAC	255
CaM0636	EI861078	(GT)5N(T)15	TAATGCATCCGTTCTGCTCA	AGCCACATTACCAGCCAAAG	218
CaM0638	EI861146	(AT)10	TCAAAACAGAACAGATCATGTGG	GCGGCCATCTACGTTGTCTA	191
CaM0639	EI861153	(ATA)5N(AAT)22	CACACCAAGACAACGCATAGA	CACCATGTTCTCGTCCTTTCT	232
CaM0640	EI861153	(AT)16	GGTGATTTCTATGATGTGCTAATTT	AGTCACGCGCACCTAAAAGT	225
CaM0641	EI861155	(TA)17	TTGACAAAAGCGCTGTAAAA	TTGAACTTTAACTGATCATCCCAA	261
CaM0643	EI861191	(AT)10	CTCGTGCTCACAATACTCGG	TCGTCCATGTTAGTTGCTGC	223
CaM0644	EI861216	(AT)13	GGATGGACCCTAGCTCAACA	TGATTGGACAAAATGAACCTACC	234
CaM0645	EI861227	(TA)12	AAAAGCCCCCAAAATACACC	CTCCCACAATAAGACAGTGCTC	150
CaM0647	EI861257	(TA)7	TGTGAAAGAGTCCCTACTGGG	CATCAATAAATTCCCCTGTTTCA	267
CaM0653	EI861344	(TTC)6	TGGAAAGAAAAACCGTCTGC	TTGCCAAGGATAAAGGCAAG	277
CaM0654	EI861347	(TTA)28	GCGCAAAACTTGCATCTCTA	TGAATTCATTCATTTTAGTTTGTTCA	279
CaM0655	EI861377	(AG)8	CCATAGTTAGGCCAGGCTGA	GAGAGCGTGGAGGTTCAACT	237
CaM0656	EI861395	(TC)6at(TC)5	TCACTCTCTCGCAAAACCCT	GAGTGAAACCGAGAGCGAAC	208
CaM0657	EI861425	(TA)19	GCCCAATTTCTTCTTGCACT	TCCAAAATAGACGTTCCCTCC	276
CaM0658	EI861468	(ATC)11	TGTTTGGGCTTTTTGCTAAC	CATTCAGCCCCAAAACCTAA	219
CaM0661	EI861555	(TAA)9tag(TAA)43	TCGTTTGCATAAGATGGAACA	TGCTATTAAGTGTGACCAGCAA	271
CaM0664	EI861622	(AT)9N(AT)11	GGCGGTTCTCTTCCTCTGTT	CAATTGAGTTGCGACGAGAG	260
CaM0668	EI861750	(AT)16	TCATGTGGTATGCGTTTTTGA	GCGGCCATCTACGTTGTCTA	178
CaM0673	EI861772	(AG)8	TTTCGTGGGAAAATTTTGGA	TTCTTTCTGCCCCAAACATC	279
CaM0674	EI861772	(AG)7	GTGGGGTTTGAGGATTTGAA	TCCCTCTTTCCTTCACCCTT	226
CaM0675	EI861779	(AT)7	ACGGGAGGAAGAAGATGGAA	GCGGTAAATCCTTTACGGAG	265
CaM0677	EI861783	(TCTT)8N(T)11	GGAGCTTGAGAAGGACATGC	TCCCCCTTACTTAAAACCCAA	246

Contd…

CaM0678	EI861826	(AT)8	ATTCGCGAGAATGGAGTGAA	GAGGGGAATGAAATGATGGA	234
CaM0681	EI861873	(TA)7	AGAAGCAAAGCTCTCGCAAC	GACCATTGGGTTTCTTGCAT	249
CaM0683	EI861973	(AT)20	TTTTCCTTTCTTTTTCCCCC	TATTTTGGATTTTGGCTCGG	157
CaM0684	EI862050	(AC)6	AGGGAGGACGAAAATGACCT	AACCAATCTTGTGCATCCAA	271
CaM0685	EI862059	(TG)7	TTTTGCTTTCCTTGTGAATTTCT	CGCAATTCGAAGTTTTGTTG	151
CaM0686	EI862076	(TTTA)6	ATCAGCATCAGCTGCATCAC	GCAATCACACAAACACTGCC	223
CaM0688	EI862151	(AT)6	CCTTTCCTTTCCTTTCTTTTCC	CAATTGAGTTGCGACGAAAG	196
CaM0689	EI862155	(AT)8	GAGTTGCGGCGCTAGTAGAT	GCGAGGCAATGTTTTCATTA	182
CaM0691	EI862180	(AT)5N(TA)22	CAGGGAACCTTTTTGGTGTT	TTGAGTTGCGACGAGAGTTG	266
CaM0692	EI862181	(TAT)16	CCGTCACGTTACGTTGTTATTT	CCAGGTGCAATAGGGAAATC	269
CaM0693	EI862227	(AT)6	TGGGTCACGGTTAGAGGAAG	TATTTTGGATTTTGGCTCGG	274
CaM0694	EI862295	(AT)7	CCTTTCTTTTCCCCTCCAAA	TTGGTATTGAACTCGGAGGC	244
CaM0695	EI862302	(AG)7N(GA)7	TCAGGGAGATTCATTTTGCC	CCCAAACCCTCACCCTATTC	273
CaM0698	EI862402	(AT)12	AATGCGTTACGGGTGAAATG	GAGAAGGAGGGAAAAATGGC	264
CaM0699	EI862407	(AT)28	GCAGAGGAAGAAGATGGAAAA	GCGTTACGGGTGAAATGGTA	279
CaM0702	EI862457	(GT)6	AACGAATCTTGTGCATCCAA	AAGCTAAGGGAGGACGACAA	277
CaM0703	EI862508	(AT)10	AGAAGGAGGAAAATGGGTCG	AATGCGTTACGGGTGAAATG	274
CaM0705	EI862538	(AT)5g(TA)14	GGTCACACGTAGGGAGGAAG	AATGCGTTACGGGTGAAATG	275
CaM0707	EI862590	(TA)5N(A)10	AAAGATGAGAAGTTAGACTTGAACACA	TGGGTAAAATGGGTGAGTGC	229
CaM0709	EI862628	(AT)20	CGCAGGTAGCAGAGAGGAAG	TGGCTCGGAGGCTTTTTAG	279
CaM0711	EI862681	(CT)6	CACTCGCAACAAACTCTCCA	TTCAACCCCCTTTATCCCTC	212
CaM0713	EI862793	(TAT)50	AAAAGGTTTAATTGTAGTTTTGATTCC	TTCAAAATAAGAGAGTGAGACAAAAA	248
CaM0714	EI862800	(TTA)8N(AT)18(GT)10	ATTGAATTGCGGCGAAAGTA	CCTCCGAGTCAAAATCCAAA	261
CaM0717	EI862873	(AT)8	AAAATACGAGTACGCACCGC	AAAATCAATTGCCATCTTTTATGA	261
CaM0719	EI862946	(AT)9	ACGAAACCAATTTTAGGGGG	TTTTCCTTTTCCTTCCTTCCA	236
CaM0720	EI863044	(TTC)6N(T)10	TTTTCAATTATTTATTTTGGAGGTCA	GGAAGAATCACTATGATTGTGTGG	242
CaM0722	EI863083	(CTT)5	TGCAATTCATTGAAAGCATCA	GGCAAGGATGAACCATCTTC	203
CaM0723	EI863098	(AT)6	TGCTGTCCTGGAAGAGGTTT	TGCTCAAAGATTAAAAAGATGTCA	280
CaM0725	EI863137	(TA)9	CAGTGGAGCAGGTGACCTTT	TTGCTCCACCTCTTGGAATC	168
CaM0726	EI863145	(AT)6	TTGCGTGAGTTTTTGTTTTGA	CACAGTGGTTGAATGTTTTACG	141
CaM0727	EI863165	(AT)6	CCTAGATCGGTCTGCGACTC	GCAACAGAGCAGTATGGCAA	246
CaM0731	EI863236	(AT)16	TTTCTTTCTTTTTCCTCCACACA	GAGGCTTTTTCGCCAAAGTT	268
CaM0733	EI863248	(TC)11	GCGGCGAAGCTATATTGAAG	CACCCATGGATTTGAACCAC	238
CaM0734	EI863268	(AT)24	TTTCCTTTCTTTTTCCCTCCA	CGGTGAATCCTTTACGGAGA	181
CaM0735	EI863280	(AT)10	TCAAAACAGAACAAATCATGTGG	CTTTATGTACTGTGCGGCCA	204
CaM0736	EI863337	(AT)20	AGAACCCTTGCACAACCATT	CCCTTTGAGCATGGAAAACT	198
CaM0737	EI863345	(T)11N(TA)6	GCTGACATGAAGCCATCAGA	ATCCGCCCAAATTACCTTTC	165
CaM0740	EI863370	(TA)14	TTTTCCTTTCTTTTTCCCCC	TATGAACTTTTCGGGCGATT	226
CaM0743	EI863386	(AT)8N(TG)8(AG)6	GGTTGATGAGCTTGACCCTT	TGACTGTCCCACTACAACCA	219
CaM0744	EI863387	(AT)6	GTGAGGACCAGCCCACTTTA	CAGATCAAACAACATAATATGCAACA	202
CaM0745	EI863428	(TG)6	AATCAAGAACAACTTTATTTTCCAG	AACATGCCAAGGGACTTGAG	137
CaM0746	EI863441	(GCA)5	TCAGAATCCTCCACCCAAAC	TGGACTTGCGTGTTTACGAG	122
CaM0747	EI863468	(AT)6	GTCCATCTTTTGTTGCCCAC	CATTTTGGTCCCCGATAGAA	233
CaM0750	EI863528	(GA)6	TCCATGATCGTTTGCATTGT	CCTATTTTTGTTTCGCCCAT	141

Contd…

CaM0751	EI863561	(TTA)5	ATTATGCCTGACAGATCGGC	CCAAATATGGAATGCTACCGA	172
CaM0753	EI863632	(TTA)23	AATTGCGGCGAGAGAAGATA	TCAGTTTCTCTTTTCGATTCTTTC	221
CaM0760	EI863753	(AT)5N(AT)6	TCAAAACAGAACAGATCATGTGG	GTACTGTGCGGCCATCTACA	190
CaM0761	EI863771	(AT)23	CAATTGAGTTGCGACGAGAG	AGCAAAAGGAAGCAACGAAA	279
CaM0763	EI863853	(TA)7	TATTTTGGATTTTGGCTCGG	CCTTTCCCCTCAATCAAACA	263
CaM0764	EI863856	(AT)6	TGTTTGTTTTGCTGCTTTGC	AGCATTGGGGTTGAAATCAT	256
CaM0769	EI863941	(AT)6	TGGAATGACTACCGAGTGTTTG	CGTCTATTAGGGCATGGTTTG	261
CaM0771	EI863971	(AT)6	AGGGAGAGGAAGGAGATGGA	TGCGGTGAATCCTTTACAGA	226
CaM0772	EI863992	(AG)7at(AG)22	ATCCTGCGTCCATTGTTAGG	TCTCTCTTTCATTTCTCTCATCATTC	260
CaM0773	EI864000	(TA)9	TCTTTTCTCCCCCAAACAAA	TTTAGTGCGTTACGGGTGAA	130
CaM0774	EI864047	(AAG)6	CCAGGATAAAACAGCCCAGA	GCTTTTCTCACTTCTCCCCC	196
CaM0777	EI864109	(AG)6	GGATACAGTGGCTGCGAAAT	CCAGTCCCGAAATTAAACGA	132
CaM0779	EI864137	(AG)6	TGATCTCCGGTAAAACGAGG	AATCACACACCCACGTGAAA	203
CaM0782	EI864185	(AT)5N(AT)5	TCAAAACAGAACAGATCATGTGG	GCGGCTATCTACGTTGTCTCA	181
CaM0786	EI864267	(TTA)8	AATTAATCACCACAAGCCTTTTT	CAAATTTATGAACTTTGCTTGCTT	219
CaM0787	EI864268	(GTG)6	TGGAAATTTCAAGAGTGGTGG	AGCATCGCCTTTGTTTCCTA	128
CaM0788	EI864274	(TTA)5	AATCCAATCAAAGAAACCGAA	AATCCCTGGCAAGGACTTTT	253
CaM0789	EI864291	(TG)9	TTCAGACACACGGTTTCACC	ATGGAGCCTGTACGGATGAG	225
CaM0790	EI864296	(TTC)7	CAATCTCGGTTTTCTGGGAA	CATGATAAAGAAAGCACGGGA	247
CaM0791	EI864321	(TA)6	TTGGTTTATTTGGGTTGGGA	CATGAAATGAACTGAACTGAATAGAGA	195
CaM0795	EI864439	(TA)5N(T)11	CGCGTCACATATTAAATGCAAA	GTTCTTCAGGTGGTCGCTTC	275
CaM0796	EI864502	(CAT)8	ACCGTCACCTCAAACACAAA	AAAAGATGGATGCGTTGTGA	272
CaM0797	EI864564	(AT)9	TGACCTCTCTTTGATTGGGG	AGAGAGGGAGCCATTCATCC	257
CaM0798	EI864568	(AT)6	CGGTCTCTACCTCTCCCCTT	TGCAACAGATGTAGTATGGCAA	210
CaM0799	EI864569	(T)10N(ATT)6	TGGAGCATTGTCACTTAAGCC	ACGGTGTCGAACACACCATA	210
CaM0800	EI864573	(CT)7	CCCCTTTGCATATTCCCATT	TGATGTTTGAACAAGTGTTAGGG	257
CaM0801	EI864578	(TCG)5	CAGAATCCTCATCGTCGGAT	TCGCAACATTTAGCAGCATC	132
CaM0803	EI864590	(TCG)5	CAGAATCCTCATCGTCGGAT	TCGCAACATTTAGCAGCATC	132
CaM0805	EI864627	(TAA)24N(T)11	TGGTTAAAACATGCTCAAATCCT	TGGCGTTAATTTTAGGGACG	277
CaM0806	EI864628	(TA)9	GCACAAATTTGGTTGGCAC	TGCCCTTCACATTTACTCCC	255
CaM0807	EI864637	(AT)28	CATACCATGCCTCAAACCCT	TTGCACATTCCCACTACCAA	233
CaM0810	EI864668	(AGA)5	CTGACGCCATGAAGGAAATC	TGAGGAGAGAAAGCCCTCAA	233
CaM0811	EI864673	(GAT)7	AGATATCGCGAATTTCACCG	GCATTTTCAATTCCGAAACC	198
CaM0812	EI864718	(TA)14(T)13	TTCAATGATGGATTTTGGTTCA	CAAGAGACCCGAAAGAGATAAAA	280
CaM0814	EI864799	(TAT)6(T)10	TTCCTTAACGAGACCAATTTTCA	TGACAATTTTTGTGTGGCTGA	268
CaM0815	EI864803	(AT)20(GT)37	TTGAAAATGGTGGGGGATT	AAGGCAGAAGAGCAAATCAAA	255
CaM0819	EI864854	(AT)23	ATGGGCTTGGTTTTGTTTCA	ATACATCTTGTTATCTAATGTGCACTC	225
CaM0820	EI864879	(AAC)6	GATGAAGCAATGTGATACGCA	CGTTGTGTAATTGAAGCAGGA	237
CaM0821	EI864888	(AT)6	TCTGGTTACCACTTTGCTCTTTT	TCCAAGAGTTATTGGATGATGA	253
CaM0822	EI864901	(TA)6	TTACCTGCGAAACGGTAATTT	TTTCACGGTTAACAGAGGGG	242
CaM0827	EI865106	(AT)6	TTGAAATCCATTTCCTATCCA	CTACGTACTGTGCGGCCAT	258
CaM0828	EI865130	(AT)6	TCAACAAGTTTCAAAAAGAAACCA	CTACGTACTGTGCGGCCAT	234
CaM0829	EI865205	(TA)12tg(TA)12N(AT)5	GGTACACCAAGCCACAAACC	GAAGGAAGTGTAACATCCTCTACATT	275
CaM0830	EI865324	(TA)8	CAGCACAATGCTTGCTTGAT	TCAATGCTTTCCAACATATTACAGA	246

Contd…

CaM0831	EI865356	(AT)6	TCAACAAGTTTCAAAAAGAAACCA	CTACGTACTGTGCGGCCAT	234
CaM0832	EI865429	(TTC)5	CCAGATTGGTGGTGAATCAG	ATTGGGCAATGACAAAGGAA	211
CaM0833	EI865486	(AT)6	TTTGGACCAATTTTGTGGCT	CCAATTTATTTTCTCAAAACTCCC	254
CaM0834	EI865499	(ATT)6N(TA)29	TCATGTTCCTCCAGCTGCTA	TTGAGGTCATGCATATGTTGTTC	277
CaM0836	EI865537	(TA)7N(AT)20	GTGAGAGGATGGGGACAGAA	ATGCGTTATGGGTGAAATGG	249
CaM0840	EI865666	(AT)38	TTTCCTTTCTTTTTCCCTCCA	AATGGGTTACGGGTGAAATG	170
CaM0841	EI865676	(AT)5N(T)N(T)12	AGAAGACAAAGGGGAGCACA	TGACGAAATTTGTTTTCTTGATCT	263
CaM0842	EI865793	(GT)6	TCATTTTCGTCCTTCCTTGG	CCACACCTATTGGATGCACA	275
CaM0845	EI865873	(TCT)9	AATGGTAAATGCGATGGGAA	ATCATGCATCCATCACCTCA	255
CaM0847	EI865910	(AT)5N(AAT)20	AGGGAAAGGGAAAGGGAAAT	CAATTATACATGGTATGGAAAACATCA	268
CaM0848	EI865930	(AGA)15	CATCGAGCTGCAAAGAAAGA	AGACGAAGGTGAAAGTTGGG	199
CaM0849	EI865939	(CAT)5	TTCTCCCATCGTTGGTATATGA	TGGTGATGTGATAAATTTGTGATG	208
CaM0850	EI865940	(ATT)5	TCAATTACGATCGACACCCA	CAAGCTGTACAATCTAGGAATATTGA	214
CaM0851	EI865941	(AT)14	GCGTTACGGGTGAAATGGTA	AGAAGGAGGGAAATTGGTCG	278
CaM0853	EI865988	(CT)6	TTTTCACTGATCCCCCAAAG	TGGAAATCAAACCTGACCAAG	170
CaM0854	EI866003	(AT)7	TGATAGTGAGAACCAGCCCA	CAGAGCAATTACTGAGCAAGTCA	255
CaM0855	EI866006	(TTA)7	TCAGCATTGCATCGATTTTT	TGACACCTAATTTTATCCGATTTTT	273
CaM0856	EI866026	(TTA)16	AGGCAAACAAGAACCGAAAA	CGTCTGTTTCTTCGATTTCCA	214
CaM0857	EI866034	(TC)6	TTTTGGATCTGTTCTTGCCC	GACGTTGAAACGTTGAAGGAG	278
CaM0861	EI866100	(T)11N(TA)18	TGGAAGCTTATATGGCCTCG	AAAAACGTGGTTTTATATGGTTTTT	252
CaM0862	EI866127	(AT)6	TGTGTCCAATTGTCAATGCC	TGGAAATAAAATGGTTGACATCAT	215
CaM0863	EI866130	(AC)6	TCAAATAGCCTGTCAACCCC	GCCTTTGTATTCAAACCCGA	139
CaM0864	EI866131	(CT)9	TGGATGTGACGTCATAATAAGCA	TAGTAGCGCCTCCATACCGT	180
CaM0869	EI866235	(CAA)6	CATCTGCACAACTGCAACCT	GACATTGGGAGCACTTGGAT	257
CaM0870	EI866242	(AT)7	CATCACCGTTCTCTCACGAA	TTCAAACCGTGGAATGTGAA	240
CaM0872	EI866324	(AT)6N(AT)5	CCAATATCAACTAACAATTTCAAAACA	TGTGCAGCCATCTACGTTGT	154
CaM0873	EI866341	(TA)6	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	238
CaM0874	EI866358	(AGC)5	ACACGCCCCATATTTACCAA	CCAAACTTGCAGCAACAAAA	101
CaM0876	EI866440	(AT)15	ATGTGTGGTGGAACCTTTGA	AAACCAACCACAAATGCAAA	257
CaM0877	EI866441	(ACA)6	AACTCAAACACATCAACAACAAAAA	TTTGAGGTGAGACACTTACTTCC	114
CaM0878	EI866456	(GA)5N(GA)5	CACCAACACTTGTTTTCCCC	CCCCAAATATTTCATTTTGCC	243
CaM0879	EI866461	(TA)5N(TA)23	TTGACAAAAGCGCTGTAAAA	TTGAACTTTAACTGATCATCCCAA	270
CaM0880	EI866471	(GA)12	AAGGGTGTTTTGGGGATTCT	TCAACATCCATACAACAAACACA	272
CaM0881	EI866471	(TTG)7	AAGGGTGTTTTGGGGATTCT	TCACAACACCTTTCCATCTCTC	246
CaM0882	EI866480	(AT)6N(AT)5	TCAAAACAGAACAGATCATGTGG	GGAACCCTAATATCCTCCACG	210
CaM0884	EI866523	(AT)10ac(AT)27	TGCCTTATGGGTGAAATGGT	TTTCCCTCCACACAAACACA	184
CaM0886	EI866534	(AT)17	TGAGAATTTTTCTTGAACTTGAACTG	AAGTCTTTTCCAGCACTTTTGC	256
CaM0887	EI866553	(AC)6	TCAAATAGCCTGTCAACCCC	GCCTTTGTATTCAAACCCGA	139
CaM0889	EI866609	(AT)13ag(AT)5N(AT)7	GGGCGGTTCTCTTCCTCTAT	CAATTGAGTTGCGACGAGAG	266
CaM0890	EI866639	(GT)6	CGTCCTCCCTTGGCTTATTT	TTGGATGCACAAGATTGGTT	259
CaM0894	EI866776	(AT)8	TCCCTTGCACGACAATTACA	GCTTCGACACTGTAGCACCTC	190
CaM0896	EI866855	(AT)15	TCATGTGGTATGCGTTTTTGA	TGGAAACCCCCATATCCTCT	250
CaM0898	EI866942	(AT)6	TCACTTTACCAACTGCTCGC	TTTGCAGTTGATGGCATTGT	238
CaM0899	EI866951	(GA)5t(AG)14	CTCAGTCCTTGCCATTGTGA	TCTCATGGAGAGGAAGTTTCAA	221

Contd…

CaM0900	EI866974	(T)11N(AT)5	TGGTGCTTTGATGTTTCACTG	AGCATTGGGGTTGAAATCAT	273
CaM0903	EI867068	(CT)5(CA)9	TGTGAATTTTACAATCAGCTATGAA	CTTCCATCATCACTTCCGCT	275
CaM0906	EI867102	(AT)21	TGCAACAAAAACTTCTTTCCC	CATGGTTTTTGTGTTCATCCA	256
CaM0909	EI867184	(TAA)5(AT)14	TCGTCAATTGAGTTGCGACA	AGAAAGGAGAAGGCGAGGAG	215
CaM0910	EI867191	(AT)6	GTCACGCGAGGTAGAGGAAG	TGCCTTATGGGTGAAATGGT	211
CaM0911	EI867202	(CA)7	TGTTGGTCATCGGACACAGT	TGTGGTGAAGCATACCGAGA	181
CaM0913	EI867259	(TG)8	TCAAATCACTATTTTGGTTCTCACA	AACAAGACCTGCGAAAATCG	269
CaM0914	EI867264	(AT)6	TCAAAACAAAACAGATCATGTGG	TCCTCTACGTATTGTGCGGC	198
CaM0917	EI867319	(AT)6	TCAAAACAGAACAGATCATGTGG	GCGGCCATCTACGTTGTCTA	183
CaM0919	EI867343	(AG)7	GTCTCCCCTCATTGAGACCA	AGTGTTGCAGTTGGACTCCC	253
CaM0922	EI867364	(TA)6	TCGCGGGTAGTAGAGAGGAA	TATTTTGGATTTTGGCTCGG	274
CaM0923	EI867368	(TA)6	TTTTACGATGAAAAAGTGAGAGGA	CGCAAGAAAAGAAGTTTTCCA	249
CaM0924	EI867368	(AT)11N(TA)5N(TA)5	GTTGAATTATTGAAATTCATCGTTT	TCTCACTTTTTCATCGTAAAACTCC	153
CaM0925	EI867385	(AT)6	TCATGTGGTATGCGTTTTTGA	CTCTACATACTGTGCGGCCA	179
CaM0926	EI867395	(AT)5N(AT)13	TCAAAACAGAACAGATCATGTGG	TCCTTTACGTATTGTGCGGC	212
CaM0927	EI867399	(ATA)5	CTTAAGGCTCGTTTGATGCC	TTTCATATTGTTTGTTATCATGTGC	142
CaM0928	EI867415	(AT)5N(A)10	TTCAACAACGCCTCAGTACA	CTTCGAGTGCATTGGTATCG	218
CaM0929	EI867440	(CTT)5	TCTGTTTTTGCCATCAAGCA	CCAAAACGATTTCCCTTCAA	113
CaM0933	EI867542	(AT)14	TTTCTTTTTCCCTCCAACCA	TATTTTGGATTTTGGCTCGG	269
CaM0934	EI867548	(CT)6	GTCTCCCAAGAAAACCACGA	CGTACAGTCGGGGAAATTGT	136
CaM0936	EI867562	(TTC)5	CACCAACAAGAGCAAGTGGA	CCAAAACAACCATAAAGTTCACC	267
CaM0937	EI867572	(TA)8	TTTTCCTTTCTTTTTCCCCC	TTTTGGATTTTGGCTTGGAG	266
CaM0938	EI867658	(AT)5N(AT)8	TCAAAACAGAACAGATCATGTGG	TGTGCGACCATCTACGTTGT	190
CaM0943	EI867838	(TC)6	ATGTTTCCATGTGGGATGGT	GGAACCTTTAATGTGTGATTGG	276
CaM0944	EI867838	(GA)6	CTTTATGGAGGGATGGAGGG	AAAGCGCTTCGTGTAAAACC	114
CaM0945	EI867894	(AT)6	TCAAAACAGAACAGATCATGTGG	GCGGCCATCTACGTTGTCTA	183
CaM0947	EI867923	(AT)31	AGGCTGTTTTCACTAGCCCA	TCTCTACCTCAAAACCCCTTGA	114
CaM0948	EI868019	(AT)39	CCTTCCTTGCTTTTTCTTTCC	TGCGTTACGTGTGAAATGGT	271
CaM0949	EI868032	(AAT)18(TAT)6	AAGCAAGACGAGATTTTGGG	CTCCAACCCTCCAAATTCAG	271
CaM0950	EI868040	(T)11N(AT)5	TCAATTCCTTCTTTCTCATTGAA	TGAAATTTGAAAAGCACCCA	194
CaM0952	EI868046	(AT)31aa(AT)5N(AT)15	CAGATGCATGCTCCCCTAAT	GCAAAACGTTGTTTAAGACCC	278
CaM0953	EI868077	(AT)5N(TA)9	GGGGAGGAAGATGGAAAATC	ATGCGTTACGGGTAAAATGG	255
CaM0955	EI868108	(AC)6	CCTAAAACAACAGAACAAAAGTGC	GCAGCTGCTATGTCACTCAAA	217
CaM0957	EI868140	(CAGTT)5	TGGTTTATTTGGGTTTGGATG	TTCATGCATGTAATCTGAATTGTG	223
CaM0958	EI868152	(TTA)7	TCGTATATGAAGCCAATGTTGC	AAATTTTGTTGTCCTTTTCATCA	118
CaM0959	EI868177	(GTG)5	AGCTCCATCTTCAATGGGTG	AGCACTACCCAAACCACCAG	190
CaM0960	EI868190	(TA)27	CCGAGCCAAAATCCAAAATA	CAGTCATTCACAATAATCACCAGA	179
CaM0961	EI868191	(TC)6	TAAAAGTTTCAGCATCCGCC	TGATTCAACAGTGACATGCAA	109
CaM0963	EI868214	(GT)7	GGGATGATTGGCTCTTTTCTC	TTTTTGCTCCATCAAGAGGC	279
CaM0964	EI868215	(TA)8	TTTCTTTTCTTGTCCTCTGTACATTC	TGCAAATCCTATTTGTAATTTTTCG	249
CaM0966	EI868231	(GAA)6	CCAAAACGATTTCCCTTCAA	TCTGTTTTTGCCATCAAGCA	116
CaM0968	EI868302	(TTA)5	AACCATTTACACGGCCATTC	GAATAGGAAGCAACAGGCCA	167
CaM0974	EI868400	(AAAT)6	GGAGTAGGGGGTCGTTCAAT	TGGTGCACTGGGTATCTTGA	267
CaM0977	EI868434	(TA)9	TTTATGATTTACGCCCCTCG	ATGCATGGCGTGTGTACG	142

Contd…

CaM0979	EI868476	(AT)8	TCAAAACAGAACAGATCATGTGG	CTCTATGTACTGTGCGGCCA	200
CaM0980	EI868481	(TTC)6	TAGGCAAAATCCAGAGTCCG	GAGAAGCTTATGGCTGACGC	179
CaM0981	EI868526	(CAA)5	CAACCACCAAATGGGTATCA	ATGTTGAGCCTGTCCCTGAC	249
CaM0982	EI868558	(CA)10	TTTGCATCTCACGCATCATA	CCAAAGGTGCGATGAAAATC	217
CaM0983	EI868566	(AT)26	CGTTCGTGAAAGCTTCAAGTC	TGTTTTAGATGTGTGTGAGACTTTT	272
CaM0985	EI868604	(CA)10	CAATCCATGCATATTTGTTGGT	ATACCAAAGGTGCGAGGAAA	140
CaM0992	EI868710	(AT)8	TCAAAACAGAACAGATCATGTGG	GCGGCCATCTACGTTGTCTA	188
CaM0996	EI868777	(AT)6	AACCTTCATTTTGGGCCTTT	CACTTCACGATACACCCACCT	222
CaM0997	EI868780	(TA)7	AATATGCCGTTTCCATTCCA	TATGGAAGTGGTGGATGGTG	100
CaM0998	EI868800	(TA)7N(AT)19	GAAGAAGATGAAGAGCGTGGA	TGGGAAGTGATGGGTGAAAT	211
CaM0999	EI868805	(TC)6	ACCGAGTCGGCTCTCTATCA	CACCAAAAGAAAGAAGAAGGAAA	109
CaM1001	EI868871	(GTTA)5	TCAAATCCTCCCCTTGAATC	ACCCCTAACTGCCTCCAACT	275
CaM1007	EI869042	(TA)6N(A)12	TTTGATTCCCCTTTTAAGTTTG	TTGATTTTGGATGGAGGGAC	280
CaM1008	EI869042	(GA)6	AAAGCCCAATATGTGCAAGC	TGGGTTTTGCTCTTCCTCTC	131
CaM1010	EI869062	(ATGA)5N(T)10N(T)14	CGGGTCGTTTTCTCGACTT	CAACGACATGAGATCCAACTT	276
CaM1011	EI869086	(ATGA)5N(T)10N(T)14	CGGGTCGTTTTCTCGACTT	CAACGACATGAGATCCAACTT	276
CaM1012	EI869090	(TA)7N(AT)20	GTGAGAGGATGGGGACAGAA	ATGCGTTATGGGTGAAATGG	249
CaM1013	EI869095	(AT)8	GAGATGGCAGCAGATGTTGA	TTTCCAACCAAACATGCAAA	252
CaM1014	EI869114	(TA)6	ACGTTGAGGAGACACCTGCT	AACATAAGCACTGGGGGTTG	269
CaM1016	EI869152	(ATT)16	AATGCAAAACAATAAGCGGG	GGAAAATTAAGGAAACAAACGG	238
CaM1017	EI869165	(TAT)5	TAAAAAGAAGGCCGACATGG	TTCTTCACGTGGGACAAACA	173
CaM1019	EI869214	(TA)6	CCGATTTACACAATGCTATCCA	GGGTTAAAAGGGTATTTGTTTCA	123
CaM1020	EI869214	(AT)14	CCGATTTACACAATGCTATCCA	TCAGTATGGGTAGAGCATGTAGG	273
CaM1022	EI869243	(AC)5att(TA)5	CATTTTTCTACTTTATGCGTTTTTG	TGTGAAGAGAAGCATAATCCGA	126
CaM1024	EI869285	(A)10N(GA)5N(TTA)9(ATT)7	GAAGCAAGGAGAAGAGAAGGAA	ATCAGGTATGACACGTCCCG	240
CaM1025	EI869378	(AT)32	TGTTGATGAGACTTTTTGATTGTTT	TCTTCCTGTTGGCTTTCCAT	245
CaM1026	EI869383	(TAA)13	CCGTCCAAATCAAATGAAAA	TGCATTGCTAATAAATACGTGTCTC	249
CaM1027	EI869436	(TA)5N(TA)7	CAAGCGCTGTAAACTGCATC	TGGTATGAGTTTTTGACAATTTTTG	280
CaM1028	EI869455	(AG)7	TGGAACCAAAGTGTGCAAAA	CTCTTGTCTTTCTTTCCCGC	178
CaM1029	EI869459	(TA)9	GCAACGATGCACCTTGTAAA	CTCTCACGCGTTTCAACAAA	192
CaM1030	EI869479	(TA)9	TGCCTCTCGGTAGGCTCTAA	GGGTAATTATGGCTGCTTCTG	204
CaM1032	EI869556	(CT)7	CCAGTCCCGAAAATAAACGA	TGGAATACAGTGGCTGCTTG	140
CaM1035	EI869663	(AC)8	AGAAAGGTGTGTGTGGGGTC	TCATGGTATTGCTTTCGCAG	153
CaM1036	EI869671	(TAT)5N(TTA)10	GCAATGATGCATACTCATGTAAGTC	TTGACAATGTGTTATATGTACCTCAGA	192
CaM1037	EI869694	(AT)6	AATCGATTGGCTGAAATCCA	GCGGCCATCTACGTTGTCTA	257
CaM1039	EI869800	(AT)6	TTTACCGCTGTTCCATTTCC	GAAGGGACACTTCCAACAGG	228
CaM1042	EI869844	(AT)26	CTTATGGCCTGGCCTGTTTA	TGATGTGCAATAGGACAATGG	235
CaM1047	EI869937	(A)12(CA)5	GGGTACAAAGATAAAAGACAGCG	GAAAGCGCTGTAGAAGGTGC	193
CaM1048	EI869961	(T)13N(TA)9N(TA)8	CCCCCTGCTTTTTGGTAGAT	TTCTCGTAACATATCCGCTATCTG	216
CaM1050	EI869993	(GAC)5N(GAT)5N(GAC)5N(T)10	TCAAGGTCAAGTTTGAAGACGA	TGTTGTCGAATTGGTACATTCTTT	198
CaM1051	EI870000	(CA)7	TGAGAATCCATTCCTTCGCT	TCAATGGGCCAAAAAGAAAG	137
CaM1053	EI870048	(TTA)49	AGGAAGAAGGGGGAAGAGTG	GGCAGAAGGGGATATTGGTT	269
CaM1054	EI870153	(AT)5ttaa(AT)16ac(AT)5	TTCCTTTCCCCTCAAACAAA	TGCCTTATGGGTGAAATGGT	160
CaM1056	EI870170	(AC)6	GCTTAGGGAGGACGAAAATG	AACCAATCTTGTGCATCCAA	175

Contd…

CaM1059	EI870200	(AT)7	GGTGAATAGCATGTTGATTTGG	AAGAAGGTATTTGTACCACGCA	255
CaM1061	EI870206	(CA)6	GCCAAATTGACCTAGCTTGC	GGGGAGGCGTTATTAGAAGG	234
CaM1064	EI870250	(AG)8	ACCCCCGGATAAGATAATGC	GTTCGAGGAAACAGGTGGAG	238
CaM1065	EI870341	(AT)11	GCTACTCCAAGGAGATTACACG	CGTTTTGGGTCGTTGTTACC	259
CaM1068	EI870413	(ATA)34	TGGATGCAAAAGATTTGAGC	TTCAAAGAAAGAAACACTTTTTCAA	217
CaM1070	EI870431	(A)11N(ATT)17	GATTTTCATTTAACTACTCCACGAA	AATCAAAATCAATAAAATCAAGGAG	235
CaM1072	EI870534	(TA)10	TGCGCGTTATAATGCAATTC	TGGCAGCTCAAAAAGTTCCT	181
CaM1076	EI870620	(AGA)5	CTGACGCCATGAAGGAAATC	AGTGGTCTTGGAAAGCGAAA	198
CaM1077	EI870622	(TATT)13	TGACCTGGCCTGACCTATTC	CAAACAATTGCTATTCCTTCTAGTCA	234
CaM1079	EI870699	(A)10N(TA)5N(TG)7	TGGCTTTTCTAGCTGCATCA	TGGAATGCCAATAAAGGGAA	278
CaM1081	EI870764	(TA)8	TTGAAGGGTTTTTGTTGTTGG	CAAAACCAGAATAGCCGAGG	267
CaM1083	EI870782	(TG)6	TTAAGCGGTGCCTATACCGT	TCGTTGTCACAAGTACGCAA	209
CaM1084	EI870809	(AT)6N(TA)42c(AT)8N(TA)5	AACCGTTTTGGTGTTTCTTGA	TGCATTATGGGTGAAATGGT	280
CaM1085	EI870815	(TTA)5	AATCCAATCAAAGAAACCGAA	AATCCCTGGCAAGGACTTTT	253
CaM1086	EI870828	(ATA)7	AAACCCCAGACTTTTGAGGC	TGAAGCCCAAATTTTCTCAA	274
CaM1087	EI870860	(AAG)5	GCGGACTAGGTCGCTCTCTT	CCAAAAGCTCTCCGAATCTG	197
CaM1088	EI870893	(TTC)5	CCAAAAGCTCTCCGAATCTG	CACGAAGTCCCCAGGATAAA	207
CaM1089	EI870908	(TA)5c(AT)10g(CA)7	GGGTTGAATCTTGATCACACAA	CCTCCTGCTTCCGATACTGA	159
CaM1090	EI870909	(AT)11	GCGTGACAGAGGAAGGAGAA	CGGTGAATCCTTTACGGAGA	234
CaM1094	EI871002	(AT)21	CTACTAACCATCTTTGCTTCTAAATTC	CCCACTTTGCAGAGGAACAT	149
CaM1095	EI871010	(AATA)6	TTTTTCCCATCAAGGACAGG	CATCAATGGTGCACTGGGTA	194
CaM1098	EI871052	(AAAT)5	AGACACGGATGCCAAACATT	CACACACTCACTCGCCATTT	236
CaM1101	EI871128	(TAA)23	CGGGTAGAATGTAACACCCAG	TTAAATGGACGTGGGTAACG	263
CaM1104	EI871232	(TC)7	GACAGCTGTGCGTTTGAAGA	TTAGTTGCTAATGCGATTGGA	224
CaM1105	EI871246	(TAT)5	AGGCATCCTAGTGTTGGGTG	TTTATTTCCTTCACGTGGGC	139
CaM1108	EI871317	(AT)8	GCGGTGAATCGTTTACGAAG	GGGAGGAGGAGATGGTTTTC	204
CaM1109	EI871322	(TA)26	AAGCACCATATGGAAGCCAA	ATCCTCGGGCACCTATTCTT	206
CaM1110	EI871327	(CTT)5	CAATGACAAACCAAACCACG	ATCGAGACTCACCGACTGCT	199
CaM1111	EI871369	(AT)11	AGGAGGAAGAAGTGGTCACG	TGCCTTATGGGTGAAATGGT	231
CaM1112	EI871378	(AT)8	TCAAAACAGAACAGATCATGTGG	CTACGTACTGTGCGGCCAT	198
CaM1114	EI871383	(TAA)5	AAAATAAACTGAATAATGCCTCCTT	GGTGGTTGCCTTTTCTATCG	280
CaM1115	EI871396	(TA)9	ATATGATGCGTGTGCCAAAA	AGGCAAGATCATTCCTCCAT	104
CaM1116	EI871447	(TC)6	TTTTGGATCTGTTCTTGCCC	GACGTTGAAACGTTGAAGGAG	278
CaM1117	EI871452	(CAGTT)5	GGGTTGGGATGACAATGAGA	GCTATGCATGTAATCTGAACTGTG	158
CaM1121	EI871570	(TA)6a(AT)26	TTTCTTTTTCCCTCCAACCA	GCGGTGAATCTTTTACGAGG	173
CaM1122	EI871582	(AG)21	CCAAAGGGGTGAGTTTTTGA	CCCCCTTAATTTCTTTCTCCA	249
CaM1123	EI871582	(AAG)8	TTGTTTGGAAGGGTGATTCC	TCAAAAACTCACCCCTTTGG	140
CaM1125	EI871652	(T)N(TC)12	CACCCATTTTGATGGTCTGA	CAACAATTCCACTGCCTCTG	280
CaM1126	EI871667	(AT)16	CTTCGCATGACAGAGGAAGG	GCGTTACGGGTGAAATGGTA	256
CaM1129	EI871710	(AT)6N(AT)10N(AT)8N(AT)11N(AT)10	TTCCTCTGTTTCGTTTCCTTTT	CAAAATAGTTTGAGAAGTGATGATTTT	280
CaM1131	EI871738	(TC)6	TTTGGTTTTTGGTTCAAAGGA	TCAGACTTCAAATATGATGATGGTG	267
CaM1132	EI871804	(GT)5(AT)11	GTTGAATCGCTTTTGCATCA	TAGCACAAACCATCCACTGC	267
CaM1133	EI871844	(ATT)8N(TC)7N(CT)6	AGATTGTGGTTTTTCCGGTG	TCGAATCATCATCAACTAAAGAGG	255
CaM1134	EI871858	(AT)30	TGCGGTGAATCTTTTACGAA	TTTCCTTTCTTTTTCCCTCCA	185

Contd…

CaM1135	EI871868	(TTC)7	ATAATCGACGGGAAAAGGCT	CACTCCACACTCCACCAATG	200
CaM1136	EI871935	(GA)5N(ATT)7ta(ATT)5	TGAAAATGGAGAAGGATGGG	TGGAATCATTTGCTTTTGGC	187
CaM1139	EI871986	(TAT)5	TGGTTGAGCAGTACCTTCCC	TCTTCTCTGGCAACCGTTCT	235
CaM1141	EI872043	(TA)6	TCTCGCGAGGAAATGTTTTT	GGAAGTGATGGGTGAAATGG	173
CaM1142	EI872111	(AAAT)5	CGCAAGCATTACATAAGCCA	GGGGACAGATAAGACAGGCA	255
CaM1143	EI872136	(AC)9(AT)26	TCGGGTTTTCCTTCCTTTCT	AATGCGTTACGGGTGAAATG	272
CaM1149	EI872207	(TG)5N(AG)7	TTGGAGATTGGTGATAAAGGG	TCCAACTCATGATTCTCTTCTCTG	280
CaM1150	EI872283	(AT)6	CCTAGATCGGTCTGCGACTC	TGCAACAGAGCAGTATGACAA	247
CaM1151	EI872291	(AT)13	GCTTCTTATAATGGCGGCAA	GCGCTATAAAGAGATGCGCT	174
CaM1153	EI872343	(TTA)5	AGAGGCAAACAAGAACCGAA	GTAAAGAGGGGCAGCTGTTG	148
CaM1154	EI872351	(AC)6	AGGGAGGACGAAAATGACCT	GGGTTTTTAACCAATCTTGTGC	179
CaM1155	EI872366	(ATA)5	TTCAGCTTAAAAAGCTATGATACCAA	TTGAGATGTATTGTTCAGTTAAAGACA	271
CaM1156	EI872370	(TAT)7	CTCGTTCACATACCGCCATA	TTGCTCCTACGTATCCCCAG	275
CaM1157	EI872397	(AT)16	ATGGCTATATGACAAGCCCG	ATCACGACAACAACAACCGA	220
CaM1158	EI872397	(AT)15	GGTTACCCTACGTGTTTGCC	GCCTGAATATAAAATACGGGCTT	229
CaM1159	EI872401	(TAT)8	GCAGCTCTAAATGCTTTATTCCTC	ACCAAACAACTTCCCCCAA	279
CaM1160	EI872495	(AT)17	AGCAACAGGGAGAGGAAACA	TGCCTTATGGGTGAAATGGT	226
CaM1161	EI872513	(TA)5N(AC)6N(AT)10	GCGCTTATTGACAAAAGTGTT	TGAGTACTGGGGATTTTTCTTGA	280
CaM1162	EI872521	(AT)8N(TA)5	TCAAAACAGAACAGATCATGTGG	CTACGTACTGTGCGGCCAT	198
CaM1163	EI872538	(TC)7	GACAGCTGTGCGTTTGAAGA	TTAGTTGCTAATGCGATTGGA	224
CaM1166	EI872648	(TA)7	AATATGCCGTTTCCATTCCA	TATGGAAGTGGTGGATGGTG	100
CaM1167	EI872648	(TA)6	CTAAGAGCCGAAACTCGTGG	TTTGTGCTTCAATCTCTGGC	268
CaM1168	EI872661	(AT)34	CGAGGCAGAGGAAGAAGATG	TGCCTTATGGGTGAAATGGT	271
CaM1169	EI872674	(TTC)9	CCAAAAGCTCTCCGAATCTG	CCACGAAGTCCCCAAGATAA	220
CaM1170	EI872676	(AT)27	TTCTTTTTCCCTCCAACCAA	CCCATAACTGCGGTGAATCT	160
CaM1172	EI872737	(TA)19	AGCTTTCCTCCGTTTTTGGT	AATTGAGTTGCGACGAGAGG	231
CaM1175	EI872802	(AT)7(AG)7	GGTAGCTAGTTGATCAAGTTCTTTCTT	ATCTCGCCAATAACCACCAC	196
CaM1176	EI872818	(TA)9	TGTCGTATCACCGTCGACAT	TCAAAACTCAACTTCTAAATACGCTC	266
CaM1178	EI872897	(GT)7	AAGCAATCTTGTGCATCCAA	CCTAGGGAGGAAGAAATGACC	275
CaM1179	EI872914	(AT)16aa(AT)17	CGAGGCAGAGGAAGAAGATG	AATGCCTTTTGGGTGAAATG	273
CaM1180	EI872919	(AT)6	CGAAAGACGAAAATGGAGGA	GCGTTACGGGTGAAATGGTA	279
CaM1181	EI873020	(AT)5N(A)10	TTCAACAACGCCTCAGTACA	CTTCGAGTGCATTGGTATCG	218
CaM1182	EI873027	(AT)8	TTTTCCTTTCTTTTTCCCCC	TATTTTGGATTTTGGCTCGG	267
CaM1184	EI873037	(ATT)28(TAAA)5N(TC)5N(T)10	ATTTCCTTGTGAAACGCACC	TTGTGGAGTGAGAAGAAGCAA	262
CaM1186	EI873062	(TC)7	ATTTTCAGGGGATGCACAAT	AAATTAGCAAAACGCATGGC	123
CaM1190	EI873144	(TA)6	TCGCGGGTAGTAGAGAGGAA	TATTTTGGATTTTGGCTCGG	274
CaM1191	EI873153	(AAC)6	AATATGGAGGCGATCACTGC	ATCTGCAGCATCTGTGGTTG	135
CaM1192	EI873168	(GT)6	TGTGCGTCCAATAGGTGTGT	AAGGAGGACGAAAATGACCT	262
CaM1193	EI873181	(AT)7	TGGTCATATTGATCAAAGGTTG	GGGGTGATTATGGAGCAAGA	266
CaM1195	EI873225	(AG)5N(AG)5	GTTGGGGAAAATGATTCGTG	TTGTCCTGTTGGGCCATATT	183
CaM1197	EI873236	(AT)39	TCCTTTTCCTTTTCCTTCCTTC	TGCGGTGAATCTTTTACGAA	231
CaM1199	EI873326	(ATA)38	AAAATTGTAACATCCCTCTTTAGCA	GTTTTCGTTCTCTCCTCCCC	262
CaM1200	EI873351	(TA)6	TCCAAACATCATCAAAGCCA	CTCACCAACTTGAAAGAGTGTCA	253
CaM1201	EI873380	(TA)8	TTTTCCTTTCTTTTTCCCCC	TTTTGGATTTTGGCTTGGAG	266

Contd…

CaM1202	EI873429	(GA)6	CAACCCAAATCCCTTTTCTTC	AGCACTGCATAATGGGAAGG	226
CaM1204	EI873439	(CA)7	AACGATCATGGACCCAAAAG	TCGTGAGTTTTGGTGAGGTG	133
CaM1207	EI873555	(TAA)7	AAAAGTAGTCCTAAGGAGCATAAAAA	CCGATCGAATCAGTTAGTCCA	232
CaM1209	EI873564	(AT)6N(AT)5	TCAAAACAGAACAGATCATGTGG	TCCTCTACGTATTGTGCGGC	198
CaM1210	EI873570	(ATT)5	TCAATTACGATCGACACCCA	TCTAGCAAGCTGTACAATCTAGGAA	219
CaM1211	EI873588	(GT)7	TTCTTGGTGCAGAAAAGGCT	ATGCCCTGAATAAAGCAGGA	227
CaM1212	EI873593	(TA)6	TTTGTATGTTTTCTCAAACTTTATGC	AAAAGAATGAAATGTCCGTCAAA	188
CaM1214	EI873621	(AT)7	ATTCACGGTGAGGAGACACC	CAACAAGTGCAGTGTATCAAATCA	267
CaM1216	EI873648	(AAG)5	TTCATTTGGCAATGCAATTT	TGCGGAATAATCCATTTGCT	238
CaM1218	EI873716	(AT)5N(AT)21aa(AT)18	TTTCCTTTCCCCTCAAACAA	TGCCTTATGGGTGAAATGGT	193
CaM1222	EI873848	(AT)21	AATTGCTGAGGCATTTAGCC	TTGCTGCAACTCAATTTACAGAT	255
CaM1224	EI873862	(T)13N(TA)9N(TA)8	CTTTTCTCTCCCCCTGCTTT	TTCTCGTAACATATCCGCTATCTG	225
CaM1228	EI873918	(ATA)23	TTTTCTTTTTACGATCAAGATCAAAC	CAATTGAATGTGTGGTTAATGGA	246
CaM1230	EI873963	(G)10(AG)8	TCACTCATTTGTCAAAGCGG	ATCCCCTTCTTCTCCTCCAA	102
CaM1231	EI873986	(TTA)30tt(TTG)6	TTTGCACCCACAAAATCTGA	TGTGTAATTTAAGTGTGGGGG	211
CaM1232	EI874002	(GA)8N(AG)5	TCAAGGTTTTACAAACATCGTCA	GCCTTGCACAAAAATTGAGA	280
CaM1235	EI874066	(TGA)5	TCATGATGACACAGTTGGCA	GAGCCTCAAACTCCTTCCCT	151
CaM1238	EI874129	(AT)5(A)10	AACGTTAAAATCGTTTGATTGC	TTTTATTGATTTCGTTGTTTGGG	166
CaM1239	EI874140	(AT)6	GCTTAACCAAGGCAGTGGAG	TGAAGGTCCCTGTTGTCTGA	104
CaM1240	EI874143	(AT)9N(TTA)28	TCCCAATTGAGTGACATCTTTC	GCAACAACATAATTTTGCCA	277
CaM1242	EI874160	(TAT)7	GCGTTAGCAAACAATGCCTA	TTTGCTAGATTGGTTAGGTTCACA	189
CaM1243	EI874192	(TA)14N(TA)6	AATCCTCTCGTGCAACATCA	AGGGTTTAAAAGGTATTTGTTTCA	235
CaM1244	EI874212	(AT)6	GAGTCTTCAACGTTACAGATCCAG	TGCGAACAAATTCTCTGCATAA	137
CaM1247	EI874264	(AAG)6aac(AAG)5	AATACCCGAGCCTTGTCAAA	AATCATTCGGAGGTGCAAGT	225
CaM1251	EI874329	(AT)5N(TA)9	TCTTTTCTTTTCCTTTTCTTTCCA	GCGGTGAATCTTTTACGAGG	158
CaM1256	EI874425	(AT)18	TTTGCTGCATCAACCTATGC	CCGACAATTCCATTGCTTTT	234
CaM1257	EI874432	(T)14N(AT)5	CACGAGTAGGGAGGGAAACA	CGAAGAATTTATTACCGGACAA	211
CaM1258	EI874464	(TTC)5	CCAAAAGCTCTCCGAATCTG	CACGAAGTCCCCAGGATAAA	207
CaM1259	EI874468	(TTA)7	TTTACTCAGAGACACCTTAGAGGAAT	ACGTGTGTGGCTAACATGGA	262
CaM1260	EI874478	(AC)6	GATGGACGAAATTGACCTAAATG	CATCCTCCCTAGGCATATTCA	207
CaM1262	EI874565	(TA)6	GCGAGCAGAGGAAGAAGAAG	TGTGTTATGGGGGAAATGGT	196
CaM1263	EI874576	(TA)21	ATGGAAGAAGAAGACGGGGT	GGTTTGGAAAGTGATGGATGA	240
CaM1264	EI874598	(AT)6N(TA)5	CACAAGTTAATCACACATAGCAACA	TTGCTGCACCTTCATACTGG	253
CaM1265	EI874598	(TA)6	CACAAGTTAATCACACATAGCAACA	ACCTTTTTCGTTTGCTGCAC	264
CaM1266	EI874621	(TA)19	AATCGATTGGCTGAAATCCA	GCGGCCATCTACGTTGTCTA	277
CaM1274	EI874745	(AT)15	GTCATCCGTGCAAACTACCC	TGAGGTGGAAAGAAAGAAAAAGA	194
CaM1275	EI874747	(TTA)10	AAGAGCGGTGGCTTTCAATA	ACCCACCAAACCACATGACT	230
CaM1277	EI874791	(AT)6	TCAAAACAGAACAGATCATGTGG	GCGGCCATCTACGTTTCTAA	182
CaM1278	EI874810	(TA)5N(TTA)9	GGGCTTATGGCCTGACCTAC	TGGCATGGTGGACTCATTTA	270
CaM1279	EI874829	(TA)7	TGGAAAGTGTACCAGATCATCTCT	GGAATTAGGGGTGGTTATTCC	277
CaM1282	EI874856	(ATT)7	AGCTTGGAATCTGGCTAATGA	GGGTGCATGTTGGGAATAAC	177
CaM1283	EI874883	(TTA)7	AGAGGCAAACAAGAACCGAA	TGCTATTCGAAATGAGTCAAAAA	267
CaM1284	EI874901	(TA)6	GCGAGCAGAGGAAGAAGAAG	TGTGTTATGGGGGAAATGGT	196
CaM1286	EI874974	(AT)17	TCTTTTCCGTTTCCTTCCTTC	TGCGTTACGGTGAAATGGTA	157

Contd…

CaM1287	EI874979	(AT)6	TATTTTGGATTTTGGCTCGG	CCCCCAAACAAACACATTTT	125
CaM1290	EI875016	(TTA)6	AGAGGCAAACAAGAACCGAA	TTGAGCCTACCTGTTGACACC	182
CaM1293	EI875059	(AAG)6	CACGAAGTCCCCAGGATAAA	GCTTTTCTCACTTCTCCCCC	207
CaM1294	EI875088	(GAT)6(AAT)41(TAT)22	TGCCTTAATTGTTAGATGTTTGGA	AACAACAAGACCTTAATCAAGTAAAAT	269
CaM1295	EI875093	(ATA)18	GGATTAAATTTGTTCCACCATTT	TCAACCGGTTATCACCAAAA	274
CaM1296	EI875113	(AT)6N(A)10	CAAATTGAAAGATTGATTGCATGT	TGCATTCCATCTCTGTTGAGAC	279
CaM1298	EI875136	(ATTTTT)8	GCTAAGCTAAAACTCTGCTTTTTAAT	AGTTGGCCATGACCATCCTA	254
CaM1301	EI875257	(AT)20N(AT)11	GGTCAACAGTTCAAGGTGCAT	TTCGAGAAATGTGGATATTGGA	267
CaM1304	EI875339	(AT)12	TGGGTGCTATTTGCAATCAA	GCTTCGACACTGTAGCACCTC	245
CaM1306	EI875390	(TA)18	AACCCTGTGGTGGATTTACG	CAACTCATGAAACCAACCGA	216
CaM1307	EI875424	(AG)16	GAACGGTAAAACTGGGCGTA	TCGGCTTCAAAGCTATGTTGT	186
CaM1311	EI875512	(CATA)5	TTGGTCAAAGATGTTTGGATTC	TCATCTGAGAAATGGAATACGG	226
CaM1312	EI875515	(AT)7	CAATCAATGCGGTCTCTGAA	TTGTTTGACGAGGGCACTTT	279
CaM1313	EI875529	(AG)7	TTTGCATCGCATTTGTTCTT	GCGTGAGTCACCTTGAACAC	187
CaM1314	EI875543	(TA)5N(TA)24N(AT)11	CCTCCTTGCTTGCTTTCTTG	GGGAAGTGATGGGTGAAATG	217
CaM1316	EI875593	(TAT)9	CTCGTTCACATACCGCCATA	CCCTAGGTGCAATAGGGAAA	266
CaM1320	EI875660	(AT)6	CCAACATCACCCAGAATGAG	AACTACTGCAGCATCAGCCA	188
CaM1324	EI875710	(AT)18	TTTCCTTTCTTTTTCCCTCCA	TTGAGTTGCAACGAGAGTGG	190
CaM1326	EI875725	(TAT)6	TGGGAACGTTGAAAAATATTGA	TGGCTTTAAATAAATACGTGCAAA	257
CaM1327	EI875791	(AT)18	CAGATGCATGCTCCCCTAAT	TCCTATCGTACATCCAACTACGG	159
CaM1328	EI875795	(TA)7	TGTTACACAGATTAAAGGTGATGTG	CAACCGCTATAAAAAGGTCTCG	173
CaM1330	EI875887	(TA)8	AAGAAAGACGAGGTTCGCAA	TTCTGGGCTGTTTGGTTAAAA	266
CaM1331	EI875922	(AAC)5	GACACCCCTTCACCTCAAAA	TTGGACATGTGGAGAGGAGA	237
CaM1333	EI875945	(GT)7	TAACGGAAACGCCTTCAATC	CCACCCTTATTCGTTGGAGA	198
CaM1334	EI875961	(AC)8	GGAAGTTGCTCCTCTCCACA	GAGGTTTGTGTTTTGCGGTT	236
CaM1336	EI875996	(AT)18	CAGATGCATGCTCCCCTAAT	TGCACTCTCGCAAAATTCAG	234
CaM1337	EI875998	(GA)6	TTGAAGGGAAAGGCTGAAAA	ATTTTCCCACACCAACCCTT	183
CaM1340	EI876037	(CTT)12	TCTGTTTTTGCCATCAAGCA	CCAAAACGATTTCCCTTCAA	134
CaM1341	EI876044	(TA)40	CCCTTGATGTTGAGAAGTTTGA	TTCTTCCCCTCTTTGTCATCA	279
CaM1342	EI876076	(AT)5N(AT)8	TCGTTTTCATAATTTGTCTCTTTCA	AGAAAAGCGCCCATTGTCTA	260
CaM1343	EI876107	(AT)23	TCAAAACAGAACAGATCATGTGG	TTGGGAACCCTAACATCCTC	247
CaM1344	EI876115	(AT)9	ACCGAAAAAGAAGGGAGAGG	AGAAGTGATGGGAGGTGACG	242
CaM1346	EI876147	(TA)38	CTCGTCCAACAATAGCCCAT	GCTTTTGTGTGGTTCCAAATC	257
CaM1347	EI876154	(TTG)5	TTTTTCAAATTAAGCCTTTGGC	TCTGAAACAGAAAGGCAGCA	101
CaM1348	EI876162	(AC)7	AGGACAACATCAATGGCTAGA	TGTGTGTTATATGGATATTTGTCCG	262
CaM1349	EI876162	(TA)6	TTTTACGATGAAAAAGTGAGAGGA	CGCAAGAAAAGAAGTTTTCCA	249
CaM1350	EI876162	(AT)11N(TA)5N(TA)5	TTCGATGGCTAGATTACATACTGC	TCTCACTTTTTCATCGTAAAACTCC	210
CaM1351	EI876162	(AC)8	AACAATTAACGGACAAATATCCA	GCAGTATGTAATCTAGCCATCGAA	140
CaM1353	EI876223	(GAT)6	TCAAAACCACTCCTTGGTCA	AAATTTGATCTTGACCGATTCA	258
CaM1354	EI876285	(AT)11	GCATACGATGTTACTCTGGAGG	TGAACGTTTAGGGAAGAGAAGG	206
CaM1355	EI876288	(AT)22	TGTCATCCTCTAATATTGCCTTCA	CCCATATTTTGGGACGTTTC	211
CaM1356	EI876298	(AT)26	GGAGACACCTGCTGAGGGTA	TGCAACAGATGTAGTATGGCAG	261
CaM1358	EI876349	(TA)5N(TA)15N(AT)5	GATGTGAGTGAAGTGACGTGG	AGAAAAGGAAGACGTTCGCA	226
CaM1359	EI876349	(AT)10	TTGTGTTGAATTGCTGAGGC	TCGTCGCAACTCTATTGACAG	242

Contd…

CaM1360	EI876390	(TA)18	TTCCTTTCCCCTCAAACAAA	TGCCTTATGGGTGAAATGGT	148
CaM1361	EI876398	(AT)6	CGGGTAGCAGAGGAAGAAGA	CGGTGAATCCTTTACGGAGA	273
CaM1362	EI876399	(TA)8	AAGAAAGACGAGGTTCGCAA	TTCTGGGCTGTTTGGTTAAAA	266
CaM1363	EI876440	(TA)7N(A)14	AACACTGTGCCATTTTGCAG	CATACCCATCCTCTCCTCCA	243
CaM1364	EI876452	(AT)6	TATTTTGGATTTTGGCTCGG	CTTTTTCCCTCCAAACAAACA	130
CaM1365	EI876457	(AC)8	GGAAGTTGCTCCTCTCCACA	GAGGTTTGTGTTTTGCGGTT	236
CaM1366	EI876481	(ATT)5	GGAGGGGGAGCAATAATAGG	ATGATTTGTCTCATGCCGGT	151
CaM1371	EI876534	(GA)6	AAAGGCCCAATATGTGCAAG	TGAGTCACGAACCTGCTGTC	226
CaM1372	EI876550	(AT)21	CCTGCAACACTTGCTTTTGA	AAATGGGCTGTTACAACTTGC	264
CaM1375	EI876650	(AG)7	TTTGCATCGCATTTGTTCTT	AAGGCCTGAGTCACCTTGAA	190
CaM1376	EI876669	(TTA)41	TGTAAAAATGCTAAATGTGGAACAA	CAGAGAGAGTAATTTTCGTCTTCAAA	219
CaM1377	EI876701	(AT)9	AGGGGAGGAAGAAGATGGAA	GGAATGAAATGATGGGGGTA	212
CaM1379	EI876924	(T)16N(TA)13	ACCTCACCCTGTTGTAAATCAT	TCAAAAATTTGATGCATTAGGAAA	263
CaM1380	EI876940	(TA)6	TCAAGTCAGTTCACCCTGGC	CAACCTTGGCCGATAATTTG	182
CaM1381	EI876941	(AT)16	CAGGGAGGGAGAGGAAGAAG	GGAATGAAATGATGGGGGTA	232
CaM1382	EI876955	(AT)30	GGAATGGCTACCGAATATGG	TAAAAAGGCTAGGCTCAGGC	269
CaM1383	EI876980	(CTT)7	CCTCTATTTTTGCCATCAAGC	CCAAAACGATTTCCCTTCAA	124
CaM1385	EI877006	(ATA)42t(TAA)5	AAAACTCATGCTTTGGTGAAGTC	GCAAGAAACGAAGAAAGAAGGA	257
CaM1388	EI877123	(AT)19	CTCCGAGCCAATATCCAAAA	AAGATTTAAAGGGTGTTTGTTTCG	236
CaM1389	EI877137	(AT)13	ATAATGAATTCGCGAGGCAG	CAATTGAGTTGCGACGAGAG	272
CaM1395	EI877245	(AT)28N(T)10	TCCTTTTCTCCTATTTCCCTCC	TGAGAAACTTTTCCGGTGCT	275
CaM1396	EI877256	(TA)32	AGGCAGGGAGAGGAATTGAT	TTGTCAATTGAGTTGCGAGG	258
CaM1399	EI877361	(AT)11	GAAAGATGGAGAAACGCGAG	TGCCTTATGGGTGAAATGGT	246
CaM1402	EI877412	(ATA)35	CACCCAAATCCCCAAAATAA	TGCCTTTTGTATTTGAAAAATGTG	247
CaM1403	EI877417	(AT)11	ATTGGAGGAAGGAGGGAGAA	CGGTGAATCCTTTACGGAGA	259
CaM1405	EI877431	(AT)7aa(AT)6	AGAAGGAGGGAAAATGGGTC	GCGTTACGGGTGAAATGGTA	280
CaM1410	EI877556	(AT)9ac(AT)23	CGTCGCAACTCAATTGACAG	GCGTATGCATTTAGCTCAATTTT	274
CaM1411	EI877556	(AT)13	TGTTTTCAAACCCAGCAACA	CTGTCAATTGAGTTGCGACG	241
CaM1412	EI877600	(AT)6	GCAGATCCTTCCCTCTAAACA	GCCTGATCTTGCTGATAGTGG	143
CaM1413	EI877605	(AG)6	TGATGAGAGGAAAATGGGGA	CTCCCACATTCTCAAAATCCA	170
CaM1415	EI877631	(CT)6	ACAACATCCCATGCCAATCT	TGACCCCGATATTCTTCGAG	260
CaM1416	EI877652	(AAAT)5	CACAGAAACATGGTTAGGTGGA	TTGGCTTGTTTGTTACCATCTG	154
CaM1417	EI877674	(CAC)6	CTCCTCCGAAACCAAAAACA	GTTTTGGGGAATTTGAGGGT	146
CaM1418	EI877697	(TTA)5	CGAAACATTTGTTCGAATGAGA	CGTGACAACCCCAGACTTTT	229
CaM1419	EI877719	(AT)10	CGGTGAATCCTTTACGGAGA	CCTTCTCTTTTCCCTCCACA	146
CaM1420	EI877722	(AAAT)5	CTCTGGTGCTCAGCCCTTAC	AGAGCTTCAGCTTGAGGTGC	180
CaM1421	EI877745	(AT)9	ATTTTGGCTCGGAGGTTTTT	TTTCCTTTCTTTTTCCCTCCA	135
CaM1422	EI877762	(TTG)7	ATTCAGGCAAAAGGGGAGTT	GCATAAATCGTGCTCGACAA	273
CaM1423	EI877827	(AT)41	CCTTTTCTTTTCCTTTTCCTTCC	TGCGGTGAGTCTTTTATGAAG	243
CaM1424	EI877833	(AT)35	GTGGCTGATGGGAGAAAGAA	TGACCAATGACCATCCATGT	278
CaM1425	EI877877	(AT)8	ATGCGTTACGGGTGATATGG	AATCTTGGGCGTGAAGTGTC	260
CaM1426	EI877890	(AT)6	TTTCTTTTTCCCTCCAACCA	ATGTTTTGGATTTTGGCTCG	257
CaM1427	EI877901	(AT)26	TCTCCAACCCAACTCCAAAC	TTGAGAAATGTGATATCCGAAA	234
CaM1429	EI877963	(T)11(TA)8	TCGTCACCACTATTCCTACCA	CGCATTGATTCCATTCCATT	244

Contd…

CaM1431	EI877994	(ATA)15	TGTGCAAGATTCCTGTTCCA	AAAAATTTCAATTGTTTCCATTACA	138
CaM1432	EI877997	(AC)7	TTTGCCTCATCATTTATGTACTCTG	CAGCACCAAAAATGTCCTGA	278
CaM1436	EI878034	(AT)16ac(AT)14	GAGGGCGAGGAAGAAGTTTT	GGAATGAAATGATGGGGGTA	250
CaM1439	EI878053	(AC)6	ACATGATCCATTTTCGGTGC	TTTGTGTTTCAGAGTGTTCTGG	194
CaM1443	EI878211	(ATG)5	CCCCTTGAAAGAGGACAAAA	CGAATGCCATATTCAAGCAA	245
CaM1446	EI878290	(AT)15	ACGGGAGGAAGAAGATGGAA	TGCGTTACGGTGAAATGGTA	254
CaM1448	EI878411	(AT)9	GGTTATTAAAAGAATGAAAACGCA	ATCCTTGCTTCCGAGTTCAC	163
CaM1449	EI878422	(TTA)6	CTATTAAGCCTGATTGGCCG	TTGCCGAATTTGGAACAACT	150
CaM1451	EI878481	(TTAT)7	AGACGTGGTCAACCACAAAA	CACAACTTAATTATGCCCCCA	248
CaM1453	EI878532	(A)10tt(A)12N(GAA)9	CATGATGCAACATCTCACCA	GTTTTGGTGGCTCTGGAAAT	239
CaM1454	EI878565	(TA)8	TTGGTTTATTTGGGTTGGGA	TTTACATGAACTGAACTGAAACTGAA	204
CaM1455	EI878567	(AAT)20	TTGCAAAACATTTTTGGGCT	TTGACAAAATTAGGTGTCAACAA	220
CaM1458	EI878617	(TTAT)8	TGGATTAAGTCATCGACTGTGC	GTTTGCCATTTTTGAAGGCT	263
CaM1461	EI878656	(AT)24	CCTTAGCCCAATTTCTTTTCC	TCATTAAATCCATGGGGAGTC	277
CaM1462	EI878656	(TA)12	CCTTAGCCCAATTTCTTTTCC	TCATTAAATCCATGGGGAGTC	277
CaM1465	EI878684	(TA)11(CA)5	TTCATCATGCTTCATTGTTGC	TGGCGAGATTGTGTGAAGAG	105
CaM1466	EI878711	(TA)15	TTTGTATTTATCCGCTGCAAA	TGTGACACCCTGAATACCACA	110
CaM1467	EI878723	(AT)7	AAACGAAAAATGGAGGAGGG	AATGCGTTACGGGAGAAATG	278
CaM1469	EI878861	(GA)9N(AG)5	CAAACATCGTCATTTTATGTCTGA	ACACCAGCCTTGCACAAAA	277
CaM1470	EI878867	(AAT)13aaa(AAT)5	GGGGCAGCTAATGACACCTA	AGAGGCAAACAAGAACCGAA	211
CaM1472	EI878890	(AT)11(TA)9t(TA)11t(TA)8	TTTCTTTTCCTTTTCCTTCCTTC	GTGTTACGGGTGAAATGGGA	239
CaM1473	EI878910	(TTA)7	TCACATACCGCCACATTACG	CATACGTATCCCTAGGTGCAA	280
CaM1477	EI878939	(AT)6	TGGTGTTGGATACCCTGTCA	CACAAAAATTTGAATCCATGAAA	148
CaM1478	EI878969	(AT)6	AGGGAGAAAGAGGTGTTCGC	TGCGCTGAATCTTTTACGAA	267
CaM1481	EI878988	(AGA)5N(AAG)6	GGGAATGTATACCATTTTCATAGG	TCGAAAGAAGATTACTTTTTAGCAA	187
CaM1483	EI879047	(TA)7	GAAAGCGGTGCTTTTGAGAG	TGAATGGGAGTACTGGTAGGG	218
CaM1484	EI879051	(ATT)5	CCCACGGGAAGACATAAAAA	TTTGCGATTAAATTGTGTGGA	277
CaM1487	EI879088	(TAAA)7	GCTTAGTAGATTTGACCAATTTGAA	ACAAAGGTTGAGTGATGGGG	190
CaM1490	EI879185	(AC)6	AACACACCTATTGGTTGCACA	AGGTCATTTTCGTCCCCTTT	260
CaM1491	EI879190	(ATA)6	AAAGACAACAAAAAGACACGTAAAAA	TGTAGCAAGCAATGAAAGCA	197
CaM1492	EI879190	(TTA)5	ACGAATATTTATGGCACGGC	TTTTTACGTGTCTTTTTGTTGTCTTT	270
CaM1493	EI879209	(AT)6	GCCGGCAATCTAGCACTAAG	AACTGAATTGACCATTTCAACAAA	147
CaM1496	EI879269	(ACA)5	GGTGAATCAATCTATGAAAATTGG	GAGGGCTGCACATCCTTTTA	120
CaM1497	EI879270	(ACA)5	GGTGAATCAATCTATGAAAATTGG	GAGGGCTGCACATCCTTTTA	120
CaM1501	EI879355	(AT)17N(TA)6	AATCCTCTCGTGCAACATCA	AGGGTTTAAAAGGTATTTGTTTCA	230
CaM1502	EI879364	(AAT)10ata(AAT)7N(TAA)8	TCAGAATGTCAAATTCAATTGTTG	TTGACTGCCACCAGTTACCA	175
CaM1503	EI879367	(AT)6	TATTTTGGATTTTGGCTCGG	TGGTTCACGGTTGATCAGAG	277
CaM1504	EI879371	(AT)7	AGTTTGGGATGTGGGTGAAA	GAAGCAAAGGGAAGCAAAGA	218
CaM1505	EI879462	(AT)23	ATGAAAGAAGGAGGGAGGGA	TGCGGTGAATCTTTTACGAA	279
CaM1506	EI879466	(GA)10	TGTGAGTGGAGAAGGGTGGT	CTTTCACCCCCACAAACACT	100
CaM1507	EI879502	(TA)5N(TA)6	CCATCTCCGTTGTCTCAGGT	CAAAACATAACAGATCATGTGCAA	127
CaM1508	EI879531	(TA)6	CCCAACCTAACCCGATTTTT	ATTGAACTGTTGGGACTGCC	191
CaM1509	EI879537	(GT)6	TTTTCGTCCTCCCTAGGCTT	CATCCAATGACCCAAACACA	187
CaM1510	EI879575	(AT)21	CGGGTAGCAGAGGAAGAAGA	TGCGTTACGGGTAAAATGGT	270

Contd…

CaM1511	EI879576	(AT)6	CGGTGAATCCTTTACGGAGA	TTTCCTTTCTTTTTCCCTCCA	135
CaM1512	EI879588	(CAT)5	ATTAAATGCGCAAACGGCTA	TGATGTGGGAAGAAAGGAGG	182
CaM1515	EI879617	(AG)5N(TA)18	GCAATGAGAAGGGAAGGAAA	GCGGAAAACCAATTTACCAA	247
CaM1517	EI879655	(AATG)5	CGTTTTCCCGACTTCAACAT	CAAGGCAGACAACAACATGAG	262
CaM1518	EI879679	(AC)6	AACACACCTATTGGTTGCACA	AGGTCATTTTCGTCCCCTTT	260
CaM1519	EI879698	(TA)12	TTGCTTTGTGACTTACTGCCA	AGAGGATCAGAGGCGAGGTT	251
CaM1520	EI879735	(AT)7	TTGTTGAAAGAATTTAGCTTGTCA	GCAGTGGCATAGGATATGAGG	272
CaM1521	EI879737	(AT)14	CGACATGCCTTATCCCTACG	CCCCTCCAGTATAGGGATTCA	251
CaM1522	EI879738	(ATTT)5	ATGTAATATGCGTGTTTTGATGA	GTCTTCGTCGATCGTCGTCT	173
CaM1523	EI879746	(TAT)33	TCACATATTCCTTCCACACTTCA	TTTCCCTCTGTCATCTATTTTATGTG	203
CaM1524	EI879750	(AAAT)5	TTGGAAAAGCCCACCTACAC	CCAATCATGCTAGTCCAAACAA	261
CaM1527	EI879884	(AG)9	AGTTGAATTGTGTTTCGCCA	GGGAGGACTAGATGTAACAGCA	221
CaM1528	EI879901	(CTT)5	TGTAGGGTTCCTCAAGTGGTG	GTCAACCAAGCATCTGGACA	193
CaM1529	EI879902	(AT)26	TGAGTTGTGAGTTTGATGCCA	TTGAAAAATTCAATCCAAATCAA	178
CaM1530	EI879913	(AT)17	GGTTAGGAGGCAACAAAACG	GGCGATTTTCTGCATGTATGT	266
CaM1531	EI879979	(CT)9	GATTTAGCTTCCTGCACCTGTT	AGCCTCAATTGGCTCAACAC	243
CaM1532	EI880002	(TA)7	ACAAAAGGAAGCAGCGAAAA	TGGTTTGGAGAGGTTATGGG	280
CaM1533	EI880061	(CCA)7	ACATCACATGGTGGTGGAAG	GAGAAACGGCGAAGAGATTG	209
CaM1536	EI880145	(AT)6	TGATTTGGAGCATCGTCAAC	TTGGAGTAGTTTTTGGGGGA	260
CaM1537	EI880164	(T)10(TTC)7	GCTGCAAAACATAGAAAACCG	AGCTGCACAAACAACAAACG	216
CaM1539	EI880279	(A)13c(AG)5	GCCATGCTCAGGTTTTTGAG	TTTCAATCATGGAGGAAATAACA	240
CaM1540	EI880290	(AT)35	TTCCTTTCTTTGTTTTTCCTTCC	CGGTAAATCCTTTTCGGAGA	279
CaM1541	EI880303	(AGA)5	CTGACGCCATGAAGGAAATC	TACGCGAAATCCAGAGTCCT	163
CaM1542	EI880315	(AT)10N(TA)10	CTGGTAAACAACCATCAAAGC	ATCCAAGCGAATCAATGACA	279
CaM1543	EI880360	(A)13N(T)10N(AT)5tact(TA)5	TTTTCTAACCCACCAAGCCT	GAAATTTGTAGATATTGGATTGGTTT	271
CaM1544	EI880379	(AG)7	TCATGTTAAATGGGTGTGACC	CTATCCAAAAATCAGCCCGA	252
CaM1545	EI880390	(AT)24	TTCATTTTCCTTTTTCCCTCC	CAATTGAGTTGCGACGAGAG	182
CaM1546	EI880412	(AT)9	CAGGTCAACAAATTTCCAAAAA	TGTGCAGCCATCTACGTTGT	143
CaM1548	EI880471	(AT)30gtt(TA)16	TTCTTTTTCCCTCCAACCAA	AATGCGTTACGGGTGAAATG	189
CaM1549	EI880476	(AAAT)6	ATGGCAATGCAATCAACATC	CCTGAGGTAGTGATGCACTTGTA	277
CaM1551	EI880500	(AT)5(TG)10	TCAAGTCATACGCAACTCGG	AACCGTATTTGAAACTTTTATTTATCA	198
CaM1552	EI880546	(TG)9	GATCTATATCGGCAACCCCA	GGTTTTGCTAACTTGCACCC	179
CaM1556	EI880568	(TA)8	AGCGCAAACTTACAAGGACG	AGCTTTCGATGCACAAACAA	263
CaM1557	EI880578	(TTG)6	ATTGGGAGGGAAAGTTGCTC	TTTCAATTTCTGTTCAAAATCGTT	277
CaM1558	EI880628	(AGA)5	CTGACGCCATGAAGGAAATC	AGTGGTCTTGGAAAGCGAAA	165
CaM1559	EI880652	(AT)18	TGTGTTACGGGTGAAATGGT	GAGGAGGGAGGGATTTTGAG	280
CaM1563	EI880700	(AGA)5	CTGACGCCATGAAGGAAATC	AGTGGTCTTGGAAAGCGAAA	165
CaM1566	EI880785	(AT)17	TGACACCCTAAACCCCAAAA	TCTTTTGATTTTTGAGAAACGTGA	178
CaM1567	EI880807	(TAA)9	CGGAATGCTGGTTGTAACAC	TGAGGACTAAGATAATAGCAATCCAA	276
CaM1568	EI880807	(TTA)6	CGGAATGCTGGTTGTAACAC	TGAGGACTAAGATAATAGCAATCCAA	276
CaM1569	EI880852	(AT)10N(A)10	TTGAACTTTAACTGATCATCCCAA	GAAAGAGCGCTGTAAAAGACC	241
CaM1571	EI880894	(TTCAG)5	TTCAGTTCAGTTCAGTTCAGAAAA	TGCATGTAATCTGAATTGAGTGTTT	183
CaM1573	EI880966	(TA)13	CCTGCATCCATGAATCAACA	CACATGGAGCATTCCCACTA	241
CaM1574	EI880980	(AT)6	TGGTGTTTTGCTGTTTTTGC	AGCATTGGGGTTGAAATCAT	245

Contd…

CaM1575	EI880995	(AAATA)8	TGGCTCAAGAAAGAATGCCT	CACCCCGAAATCTGTTTTGT	202
CaM1577	EI881071	(AT)6g(TA)23	TCCTTTGTTTTTCCTTTCTTCCT	AATGCGTTACGGGTGAAATG	237
CaM1578	EI881073	(GT)6	TCGACCACATTTGTCTGCAT	CCAATGTGCTGCTGTGAAGT	248
CaM1579	EI881088	(AT)6N(TTA)10	TGCTTAAGAGTCAATGACCACC	GCAACAACATAATTTTGCCA	271
CaM1581	EI881112	(TTTA)9	AACGGTGTGAAAGGGAAATG	CCAAGTCACTCGTTACAAAACC	142
CaM1582	EI881173	(AT)7N(T)13	TTTGATTTGGGTTCTCAATCTT	GTTCGAAATTAGGATCAACAGTG	268
CaM1583	EI881180	(ATG)5	TGTCACTGTGTTCAGGTGGG	GGAAACAATTCCCCCTTTGT	258
CaM1584	EI881181	(GAA)6	AGAAAGGGGAAAAACCCTCA	GCCTCTCGTTCGTTTTATGC	242
CaM1585	EI881204	(AGC)5	CCAATAAAGAGGCCAAACCA	CACCACCACCATTCAGTTTG	170
CaM1586	EI881213	(ATG)5	TGTCACTGTGTTCAGGTGGG	GGAAACAATTCCCCCTTTGT	258
CaM1588	EI881226	(A)10N(AT)5	TGCACTCCAATAACAAAGGAA	TGAGTGAACCTCTAGTTTTCCG	227
CaM1589	EI881234	(CT)7	TCGCGAAGTAACTTGATCCC	GTGATTGTGCTTTCCCTCGT	186
CaM1590	EI881234	(CAG)5	CAGCAGCTAGGTCATCCACA	TCCTTTTGGCAACACAAACC	228
CaM1591	EI881258	(AAC)5	TCAAAGGCCAAAAACCCTAA	GGCTTCATTTCAAAGGGGAT	229
CaM1592	EI881259	(AT)6	TGGTGTTTTGCTGTTTTGTTG	CATATGCATTGGGGTTGAAA	280
CaM1593	EI881274	(CTT)7	CTGCTTGTTTGCCTGAAATG	GCAACATGGGATGATTCAGA	278
CaM1594	EI881343	(AT)19N(TA)7N(AT)10	ACCGAGTGTCGAACTCAAGG	GTTTGGGGTGGTGATTCTCA	252
CaM1597	EI881415	(AT)27gtg(TA)6N(TA)9	GCAAAACGTTGTTTAAGACCC	CAGATGCATGCTCCCCTAAT	260
CaM1599	EI881466	(AC)6	TTGGATGCACAAGAATGGTT	TGAGGTCATTTTCATCCTTCC	221
CaM1600	EI881473	(AT)6	GTGAGGACCAGCCCACTTTA	TCAAATTAAACAACATGTGCAGTG	226
CaM1606	EI881629	(AT)5N(TA)9	GCGTGACAGAGGAAGGAGAA	GCGGTGAATCTTTTACGAGG	262
CaM1607	EI881648	(TC)8N(TC)11	GTCAGTGGCAGGTTCTGTGA	TTGTGCCTCATTCATAAAAGGA	269
CaM1608	EI881730	(AT)11	CCTTCCTTCTAGTTTTCCCCC	GCTCAGAGGCTTTTTAGCCA	260
CaM1610	EI881807	(AT)5N(T)15	TGGAAACACAAATTGAAAGCA	TTGCAAAGAAAACAATTTTAGGA	279
CaM1611	EI881820	(AT)21	TGACACCCTAAACCCCAAAA	CCTTTTGATTTTTGAGAAACGTG	178
CaM1612	EI881832	(GAA)6N(AT)49	ACCAACACAAGCTTTTTCGC	TGAGGGATGTGATGTGGGTA	274
CaM1613	EI881875	(GA)5N(TTA)5	AAACAACAAAAAGGGGGAGG	GGCATTCATCAGCTCAAACA	247
CaM1614	EI881884	(CGG)5	TGAAGCAGTAAGAAGGGGGA	AGCAAATTGACCACCACACA	176
CaM1616	EI881994	(AT)22	GGGGAGGAAGATGGAAAATC	AATGCGTTACGGGTGAAATG	267
CaM1617	EI882022	(AT)22	GCGTGACAGAGGAAGGAGAA	CGGTGAATCCTTTACGGAGA	260
CaM1618	EI882033	(TA)8	CGGGTAGCAGAGAGGAAGAA	TATTTTGGATTTTGGCTCGG	277
CaM1619	EI882058	(AT)7g(TA)5	AAATGGGAGGAGGAGGAAAA	GCGTTACGGGTGAATTGGTA	272
CaM1620	EI882086	(CTT)5	GTTAGTTGAGGGGGCATTCA	CCTTACCGCTGCTTGAGTTC	260
CaM1623	EI882153	(GCT)5	AAGGCAGCAAACCTCAAAAA	GTATTTACCAAGGCGTGGCT	164
CaM1625	EI882231	(AT)7	TGATAGTGAGAACCAGCCCC	TGCAACAGATGTAGTATGGCAG	174
CaM1626	EI882291	(TA)21tc(TA)10tc(TA)27	CCTTTTGATTTTTGAGAAACGTG	GTGACACCCTAAACCCCAAA	259
CaM1629	EI882338	(CAT)5	TTCTCCCATCGTTGGTATATGA	TGGTGATGTGATAAATTTGTGATG	208
CaM1630	EI882339	(TAT)7N(A)10	TCACTCCTCAAGTGGAACCC	TCTCTGGCAACCGTTCTTCT	169
CaM1632	EI882367	(AT)27	CGGTGAATCCTTTACGGAGA	GCGCGTTAACAGAGGAAGAA	275
CaM1633	EI882374	(AT)7	GAAAAATGGAGGAAGGAGGG	TGCGTTACGGGTAAAATGGT	278
CaM1636	EI882436	(TAAA)6	TGACATTTTTCGCGTAGTGA	ATGACATCGAGGTTGGGACT	210
CaM1637	EI882442	(AG)6	TGGTTGTAATAGCAAGAGCTGAA	TGATGAGACCTACGTCACGG	233
CaM1638	EI882456	(TA)8	GGTGGGTGGATTTTGCTAGA	GGTATTGCCATGTGTTGTGG	247
CaM1640	EI882470	(AG)19	GTCCACGACCTCGTTTGACT	TCATCACAAAGCACTCGACC	269

Contd…

CaM1642	EI882492	(TA)24	GAGGGAGAGGAAGATGGAAAA	GCGTTACGGGTGAAATGATAA	280
CaM1644	EI882587	(CAT)5	GGGTTCACAAAAACTGGCTC	GTGGAAGAGGAACCGGAGTT	278
CaM1645	EI882601	(TAT)7N(A)10	CTTGCCTGTGTCGAAGATGA	TCTTCTCTGGCAACCGTTCT	271
CaM1646	EI882634	(AT)8	CCTTTCTTTTTCCCTCCCAC	TATTTTGGATTTTGGCTCGG	259
CaM1647	EI882651	(TA)6	CGACTCACGTTGAGGAGACA	GCACTGGGGATTGAAATCAT	256
CaM1648	EI882653	(TAA)20N(TAA)5	AAATGGTCGGTTACTATGGTAGTT	TGGGGAACGTTAAAGTTGGA	275
CaM1649	EI882681	(AT)11	TTGCTTAGGCATTTAGCACAAA	CGACGAATAATTTTAATCGGGT	149
CaM1650	EI882710	(TA)11	AAAAGGAGCTAAAAACTCAAGCC	GGATGAGAGAATTAAATTTTGACCA	273
CaM1651	EI882711	(TA)8	TGCAAGTAGCTGAGTAGGGTTC	TGCTTTTCTTAAAACAGAAAAAGG	118
CaM1656	EI882738	(CTT)5	TGCAATTCATTGAAAGCATCA	GGCAAGGATGAACCATCTTC	203
CaM1657	EI882800	(AT)7	TTCTTTTTCCCTCCAACCAA	GCTCAGAGGCTTTTTAGCCA	240
CaM1658	EI882831	(AG)5N(AG)11N(AG)9	GGGGAGTTGAATATGGTTTTACC	AGCAACAATGAGTGGTGAACT	279
CaM1660	EI882850	(AT)39	AGGCCGTATTGCTTTTGAGA	TTTTTGAATTCTGGGGGAAA	164
CaM1662	EI882857	(TTA)13	GTGTTGGGCCTAAATGAGGA	TGGTAATACGTTAGTAATGAAATTGTG	154
CaM1664	EI882889	(AT)21N(A)10	TTTTTAAGTTCGGGGTGGTG	GCTTTACCACTTGGAGTTAGGC	276
CaM1666	EI882956	(GAA)5	AATGAGGATGATGCAGAGGG	CACATTGACGTGGTTTCAGG	144
CaM1667	EI882965	(GAA)5	TGCGATATTTTAAGACAACGGT	GTTTATCAGCAACCCCTCCA	166
CaM1668	EI883028	(T)14(AT)5	GCGAAATTCAGCACAAGGAT	AATCGCTCCACCAATTCAAC	249
CaM1673	EI883105	(GAA)6	TCCACCAAAATGATTTCCCT	TCTGTTTTTGCCATCAAGCA	120
CaM1674	EI883108	(AC)18(AT)7	TTGAGGAAGGAGGGAGATGA	TGCCTTATGGGTGAAATGGT	237
CaM1676	EI883121	(AT)20	GCAGAGGAAGAGGATGGAAA	TTAGTGCGTTACGGGTGAAA	238
CaM1677	EI883131	(TA)14	ATGCGAGCAGAGGAAGAAGA	CGGTGAATCCTTTACGGAGA	236
CaM1678	EI883140	(TA)7	GTCCTTTGATCCAGCTTTCG	GGTTACAGTGATGAGACCACCA	213
CaM1679	EI883145	(ATAAA)5	GCAGCAAGCTTTGACACTCA	AATTTCTATATCGGCAACTACTTAAAA	117
CaM1680	EI883146	(ATA)16	CCTATTTTGAGGTCATATAGGAGGA	TTCCAGAATGAACCTTTTTGC	247
CaM1682	EI883150	(TA)6	AAGGAGACACATGCTGAGGG	CTGGGGGTTGAAATCAGAAA	251
CaM1683	EI883157	(TA)6	CGACTCACGTTGAGGAGACA	TGGGGATTGAAATCATAAAACA	252
CaM1684	EI883208	(TA)11	AACTCAAGCCTTATAAGTTCGTCAT	GGATGAGAGAATTAAATTTTGACCA	260
CaM1685	EI883237	(TA)14N(T)11	CCCGTACGTGTGAGAAAACC	CGAAATGTCAATTTTCCAAAGG	279
CaM1687	EI883262	(AT)20N(CAA)6	TGTATGTCTTGTTTGTTTGCCTT	TGACAACTATGCGCTCCTCT	280
CaM1692	EI883346	(AT)8	TCATGTGGTATGCGTTTTTGA	GCGGCCATCTACGTTGTCTA	162
CaM1693	EI883410	(TA)9	GGGGACCACTCTATCTTTTCG	TCACGTCCCAGTTCTCCAAT	258
CaM1696	EI883443	(AT)31	TCGAATTTTCTTTCCTTCCTTTT	TGTGTTACGGGTGAAATGGT	264
CaM1702	EI883530	(TAT)23tactac(TAT)5	GCTTCCACCCACTTCAAAAC	TTGTTTGGTTTTGTTTGACTCC	264
CaM1704	EI883551	(TAT)6	CTTGCCTGTGTCGAAGATGA	AGTGGGTCATGCCCATTAAA	197
CaM1706	EI883598	(TA)7	AATGGAGGAAGGAGGGAAAA	ATGCGTTATGGGTGAAATGG	255
CaM1707	EI883644	(CA)8a(AT)12N(TA)5	ACGAGAATGGATTTTGCGAG	TATCGGTCAATTGAGTTGCG	280
CaM1708	EI883645	(TA)7	TTCCCTCCAACCAAACACAT	ATGTTTTGGATTTTGGCTCG	258
CaM1712	EI883739	(AT)6	AAAATGGGAGAAAGGAGGGA	TTCGAGCGAAACGGTAATTT	264
CaM1713	EI883757	(TA)24	GGAGGGGAACGTGTTTTTCT	CGGTGAATCCTTTACGGAGA	235
CaM1714	EI883772	(GA)6	CAAGCACAGAATGACCCAAA	TTCCCACACCAACCCTTCTA	271
CaM1720	EI883814	(GT)6	AACCAATCTTGTGCATCCAA	AGGGAGGACGAAAATCACCT	171
CaM1722	EI883829	(ATT)36	TGCAAAACAATAAGCGAGGTC	GGAAAATTAAGGAAACAAACGG	263
CaM1723	EI883857	(TA)7	TTTTCCTTTCTTTTTCCCCC	TATTTTGGATTTTGGCTCGG	266

Contd…

CaM1724	EI883870	(ATA)16(ATT)14	AGGGGTGGAGGGGTTTAGTA	TTCTCCAACCTTCCAGATTCA	270
CaM1725	EI883898	(AT)18	CATTGTGAGTGGTGTGGACC	CGACTGTAACCCTCCACGAT	251
CaM1726	EI883905	(AT)16	AATGGGTTACGGGTGAAATG	GCGTGACAGAGGAAGAGGAT	261
CaM1730	EI884010	(GTG)5	AACCGTTACGAGGGTCAGTG	GCTTCACCTTCTGATTCCCA	242
CaM1732	EI884048	(TA)6	ACGTTGAGGAGACACCTGCT	AACATAAGCACTGGGGGTTG	265
CaM1736	EI884092	(A)10gaaaatt(TTA)5	AGCACCCTACGACATCCTTT	GCACCGGGAGATACGTAGAA	232
CaM1737	EI884112	(TTA)7	TGTTCCGATGGAACCTTCTC	CGCTTCATGCCTTTTGATTT	127
CaM1738	EI884114	(AT)7	CATAAAACCAATAGATGAAATACCGA	CACGTGAGATTTGGTTCTGTG	205
CaM1739	EI884130	(AT)17ag(AT)9	GGAGGAAGGAGGGAGATGAG	AGCTGCGGTGAATCTTTACG	278
CaM1741	EI884145	(ATA)5	GGCAGTGTTTGCAACAAGAA	TTCATGGATTTTGTCAACTCAAA	254
CaM1742	EI884153	(AT)16	TCCTTTTCATAGAGATACGAACAAAA	TCAATACGAGGATTGGAATATGA	244
CaM1743	EI884155	(TTTCCT)5N(TA)27	TCCTTTGTTTTTCGTTTCTTCC	GGGTTACGGGTGAAATGGTA	252
CaM1745	EI884184	(GT)6	AACCAATCTTGTGCATCCAA	AGGGAGGACGAAAATCACCT	171
CaM1746	EI884275	(TA)8	TATTTTGGATTTTGGCTCGG	TCCTTTCTTTTTCCCTCCAAA	251
CaM1747	EI884283	(TA)16	TCAACAAATGTTGGGGTTTG	TCCATTTAAACAATATTTGCGTG	252
CaM1748	EI884291	(AT)17	GGGTGAAGAGGAAGAAGATGG	GCGTTACGGGTAAAAAGGTG	265
CaM1749	EI884294	(ATA)6	TGGTTTGGCTTGAACACAAC	GATAGTTCATGTGCCGTCCC	125
CaM1750	EI884351	(TA)18	ATTTTTAACGCGTCCCACTG	TTGGTTGCATTGTACACCGT	223
CaM1751	EI884351	(AT)5N(AT)9	GCACGTTCATCATTCCTCCT	ACAACACCGCAATGTTTTGA	217
CaM1753	EI884410	(ATA)5	ATGGGAATTTTGAGCATCCA	TTTGCTGTAAACTCACGACCA	194
CaM1754	EI884414	(T)12N(ATT)6	AATGAAGTTGCGGAAGTTGG	CAGGGACTAAAACTGCTCGC	203
CaM1756	EI884456	(AT)6	ACACCCAATTGGTCCTGTTC	CATGAACCAGAAAATGGGAC	233
CaM1758	EI884473	(AT)9	AATTGCTCTTGTTAAGTCTGAAAAT	TCTTTAGTAGCCCGCCAAGA	241
CaM1759	EI884482	(AT)6	TCAAAACAGAACAGATCATGTGG	CGGCCATCTACGTTGTCTCT	182
CaM1760	EI884491	(TA)9	AAATGTCAAAATTACCTTCTTTAATTG	TTTTTAAATTTGACGTGTTAAGTGG	249
CaM1761	EI884507	(TA)6	TTCATCATGGCAAAATCGTG	CACCGTCTTCATCCCTGATT	180
CaM1762	EI884521	(AT)17	TATTCTCACCCAGCAAACCC	GCGTTACGGGTAAAAAGGTG	124
CaM1763	EI884523	(TTTA)5	GACTTTCTCAATGTGCGGCT	TGTATTTTAATGGTCACACCCA	147
CaM1764	EI884532	(TTA)7	AGAGGCAAACAAGAACCGAA	TGTTGACACATAATTTTGTCCG	174
CaM1765	EI884535	(TAA)44N(TAA)5	TGAGATAATTATTCTATAAATGGTCGG	TTTCAACCTAAGACATTTTTAACCG	278
CaM1766	EI884544	(AT)5N(AT)6	TCAAAACAGAACAAATCATGTGG	CGTTGGAAACCCTCATATCC	260
CaM1767	EI884583	(AT)13	TGAATTCGATGCAATTATGC	ACATTTGTTAGTATATGGAGTGTCTCA	248
CaM1770	EI884591	(GT)6	AAAAGCCATTTAATCACACGA	TGCAAAATTAAAGCTCGCAA	241
CaM1771	EI884592	(GT)6	TGTGCGTCCAATAGGTGTGT	AGGGAGGACGAAAATGACCT	162
CaM1773	EI884612	(TAAA)5	CACGAGTCCGATAAGGGTGT	ATGATTCCCCAACATCCAAA	164
CaM1775	EI884680	(AT)22	AATCGATTGGCTGAAATCCA	TTTTTACACATGATCTGTTATGTTTTG	263
CaM1777	EI884730	(AT)8	CTCCGAGCCAATATCCAAAA	TTGGGGTTAAGGGTTAAGGG	253
CaM1778	EI884731	(T)11N(AT)5N(T)10	CCACAAGAAGACAAAGGGGA	TTGCGAAGAAAACAATTCTAGG	274
CaM1780	EI884751	(AG)8	TGAATTCATGGGGTTTGGTT	GCCCTCTTTTTGTGACCAAT	162
CaM1781	EI884797	(GT)7(AG)6	TCACACACACACACACACACA	TGTGTTCTTGGCTTTTTCAGA	226
CaM1782	EI884797	(CA)12	GGTCAACACCCAAAGAAGACA	ACACACACACACGCTCATCA	278
CaM1783	EI884806	(AT)6	TTGTCTTGCTGTTTTGCTGC	CCCCTTTGTCAATTAAGGCA	234
CaM1784	EI884825	(TG)6	GCCCCTTACAAGTGAGTGTGA	TCACTCATTCTTTTAGTTTGATGTGTT	116
CaM1786	EI884862	(ATA)5	TGAAATGGAATGCTACCGAA	GGCCTGACAGACCGACCTAT	166

Contd…

CaM1788	EI884868	(TC)9	TTTCGGTGCTTCCTAGTGCT	GCGAAAATGTGGGTGAAAGT	194
CaM1790	EI884897	(GT)5N(T)15	TAATGCATCCGTTCTGCTCA	AGCCACATTACCAGCCAAAG	218
CaM1791	EI884912	(AT)10	TCAAAACAGAACAGATCATGTGG	CTACGTACTGTGCGGCCAT	202
CaM1794	EI884971	(AG)8	TGAATTCATGGGGTTTGGTT	GCCCTCTTTTTGTGACCAAT	162
CaM1795	EI884974	(AAT)6	GGCAAATATGCTTAAAACCTTATCA	TGATATCACACAACGATGGGA	278
CaM1796	EI885000	(T)10N(AT)5	CAGAATTTGTCATTGTTCGATTG	GGTCTCTCAAGGTTTCTCTCG	268
CaM1797	EI885000	(TA)8	TGCCTTTACCTTTGACTTCG	AAAACAGAAGTAAAATTAACACCCG	116
CaM1800	EI885031	(AT)5N(AT)6	TCAAAACAGAACAGATCATGTGG	GTACTGTGCGGCCATCTACA	190
CaM1803	EI885095	(AT)19	ACCACACTACGGTGCTAGGG	CCTTCTCTTTTCCCTCCACA	235
CaM1805	EI885133	(GCT)5	CCCGAAAAGGTTTGTCACAT	ATTTACCAAGGCATGGCTGA	280
CaM1806	EI885134	(AG)5N(TAT)32	GGGAAGAAAAGAAGGAAGGAAA	TACCCTACCACTCCGCATGT	275
CaM1807	EI885145	(AT)13	CAGTGAGAGGAAGGAGACGG	TTAACTGCGGCGAATCTTTT	256
CaM1808	EI885151	(AT)17tacttt(TA)22	TCCTTTTCCTTTTCCTTCCTTC	AATGCGTTACGGGTGAAATG	220
CaM1809	EI885181	(CA)10(TA)9	AACGACTCGATTGAATGATGG	AACGTGATTTTATCGAAATTAAAAA	108
CaM1810	EI885194	(ATT)7	TGGAGCTGGCACAATATCAG	ATTTCAAGGGGAACTGCTCA	276
CaM1811	EI885211	(AT)6	CGGTCTCTACCTCTCCCCTT	TGCAACAGATGTAGTATGGCAA	210
CaM1812	EI885224	(ATA)8	CTCCAGGTGCAATAGGGAAA	TCACATACCGCCACATTACG	255
CaM1818	EI885284	(GT)7	AGGTCATTTTCGTCCTCCCT	CACACATATTGGATGCACAAGA	133
CaM1821	EI885377	(AAT)5	TGACACATTATCTCAAATTCCTCAA	TGGCTGATATTCCTCTTGGC	157
CaM1822	EI885421	(AT)8	GACCGATTCCTTGGTAGGTG	TCTTGGTTTTTGGATGGAAGA	220
CaM1824	EI885437	(TG)8	CCACAACCTACCTTTGCTTTC	AACACCTTGATCCCAAGTGC	117
CaM1825	EI885448	(AGAA)7	TCCTCTCCTAGAACACCCCA	TGTTAATTGCTTCAGCCCAA	143
CaM1826	EI885456	(AT)14N(AT)5	TGTGGTATGAGGCTTTCACAA	CGGCCATCTACGTTGTCTTA	180
CaM1827	EI885508	(TA)5aaatat(TA)11	CTTCTTTTCCAAATAAACCCCT	CATGTGATACTCCATTTGCATCT	234
CaM1829	EI885569	(TTA)6	AGCTTTAGCTTGAGGGGGAG	AGCTTAATGCTGACTTGATTACAA	246
CaM1834	EI885614	(AAT)5	CCAAAATTCCTGCAGAATAACC	GGAATCCAAAGATTGGCTCA	277
CaM1835	EI885651	(AC)18(AT)11	GGCAAGGAGAGGAAGAAAGG	TGTCAATTGAGTTGCGACAAG	266
CaM1836	EI885658	(TA)5N(TA)5	CGTTCTTCTACATCAACACATGC	TTCTTCATCTTGTGCTTCCAAA	276
CaM1838	EI885678	(GAA)5	CAAAGCAGAATGTCCAGCAA	TCCTCTGTGTTTGCCATCAA	147
CaM1839	EI885687	(AT)8	GGAGACACCTGCTGAGGGTA	TGCAACAGATGTAGTATGGCAG	225
CaM1841	EI885721	(AT)6	CGAAAATGAAAACGGGAGAA	GCGTTACGGGTGAAATGGTA	279
CaM1842	EI885735	(TTTA)5	TTCTTTTCAATCAATCTTTTTCTTCA	CCTCTCTCTTTCATGGATGTACC	157
CaM1843	EI885751	(AT)27	TGTTTGTAAATGCTCTCCTAATTGA	CCAACTGCGATCTATTTCCA	207
CaM1846	EI885803	(GAA)5	TGGTTGTTGGTGTTGCAGAT	CGTTTGTTTCTTCCTCTCATAACA	268
CaM1847	EI885809	(AAT)12at(TAA)11	TGTTGACACCTAATTTTGTCCG	GGCAAACAAGAACTGAAAGCA	214
CaM1848	EI885824	(AG)7	CACAAATTACTTGCAGCATAAGAAA	GTATGCTTTGTCGAGCCTCC	218
CaM1849	EI885845	(AT)10	CGGGTAGCAGAGGAAGAAGA	GCGGTAAATCCTTTACGGAG	276
CaM1850	EI885871	(A)10t(A)11(AT)20*	GGGGTGCTCAACTTGTTTGT	GGGCTGAAAAATCAAAGAGG	257
CaM1851	EI885874	(AT)35	TTTCCTTCCTTCCCTTTTCC	GCGTTACGGGTAAAAAGGTG	269
CaM1852	EI885894	(CAA)5	TGAGAATCTTTCACTTCCAGCA	GCCATAGATCCCATTTGTTGA	234
CaM1853	EI885911	(AT)6	ACGGGAGGAAGAAGATGGAA	TGCGTTACGGTGAAATGGTA	240
CaM1855	EI885969	(AT)18	TGCAGCTAGGTTAAAACAAGAGG	ACTTGAGGGGGTGTTGACAT	280
CaM1858	EI885988	(AT)8	TCGTGGTGATTCCCAATACA	TGTTTTGGGTGGTGGTTCTC	157
CaM1859	EI885994	(AAT)5	CCAAAATTCCTGCAGAATAACC	GGAATCCAAAGATTGGCTCA	277

Contd…

CaM1861	EI886046	(TTA)9	GCAACAAAACAAATTGGAGGA	CAAATTATTGCGTGATGTGACTG	256
CaM1862	EI886084	(TG)6	AGGTCGTTTTCATCCTCCCT	TGGATGCACGAGATTGGTTA	268
CaM1863	EI886180	(TA)6N(AT)30	TCAAAACAGAACAGATCATGTGG	CTATACGTACTGTGCGGCCA	236
CaM1864	EI886188	(AT)8	GTGAGGACCAGCCCACTTTA	TCAAATTAAACAACATGTGCAGTG	230
CaM1865	EI886223	(TAA)9tac(TAA)13N(T)12	CAAACCTCTTTTCTTTGCCG	TTCCATCACGTCACCCACTA	198
CaM1868	EI886327	(TA)6	TCACATTCTATTGAACAAACCATTT	TGCCAAACACAAACAACAAA	208
CaM1869	EI886336	(TA)5N(AT)7	TGGACGTCAGGAAGTGACAA	GGCCAGACTCAGGCTCTCTA	239
CaM1871	EI886381	(AT)12	CGAAACCGAAAATGGAAGAA	TGCCTTATGGGTGAAATGGT	268
CaM1872	EI886412	(AT)11ag(AT)14	CTTTTCCTTTCCCCTCCAAC	ATTTTGGCTCGGAGGTTTTT	154
CaM1874	EI886426	(GAA)5	CAAAGCAGAATGTCCAGCAA	TCCTCTGTGTTTGCCATCAA	147
CaM1876	EI886447	(AGC)5	ACACGCCCCATATTTACCAA	CCAAACTTGCAGCAACAAAA	101
CaM1881	EI886505	(AC)18(AT)12	TGACGCTTTATTTCTTGTGTTAATTT	ATTTTACACCTTTCTGATACTTTGAAT	279
CaM1882	EI886546	(TCT)6	TGAAGGCAATCTCCTCTTCC	TGACAAGCAAGTGGAGACCTAA	255
CaM1883	EI886547	(AT)10ag(AT)14	CGCGTAGGGAGGAAGAAGAT	TGTGTTACGGGTGAAATGGT	278
CaM1884	EI886589	(TC)7	GACAGCTGTGCGTTTGAAGA	TTAGTTGCTAATGCGATTGGA	224
CaM1885	EI886643	(GT)6	TTTTCATCCTCACTGGGCTT	GGATGCACAAGATTGGTTGA	160
CaM1887	EI886681	(AC)9	TGGGATTCTTTTGGATCAGC	CAAGTTGGATGTATGCAAGCC	206
CaM1889	EI886697	(TA)6	GACACAGTGGCGACTTCTTG	CTATTGGAGGCCACCTTTGA	240
CaM1890	EI886697	(TTG)5(TTC)8	TCACACGTACGGGATTTTGA	CTTCTGCAGCACTTCCTCCT	135
CaM1892	EI886723	(TA)5N(TA)5	TCATCTCCAGTTGATGCTAACAG	TCATTGGTATCACCATCATTCA	272
CaM1895	EI886799	(AGA)5	CTGACGCCATGAAGGAAATC	AGTGGTCTTGGAAAGCGAAA	198
CaM1896	EI886853	(TTG)5	TCGAATTTTGAGATGAGACCAA	GCATAAATCGTGCTCGACAA	243
CaM1900	EI886879	(AT)5aaag(AT)18	GCGGTCAGAGGAAGAAGAAG	ATGCGTTATGGGTGAAATGG	266
CaM1901	EI886926	(TA)21	TCCTTTTCCTTTTCCTTCCTTC	AATGGGTTACGGGTGAAATG	181
CaM1902	EI886983	(CAT)5	GGGAACCAACTTGGAGCTAA	TGTTGCTGCATGATTGATGA	277
CaM1903	EI887062	(TA)13	TGTGATGCAACCTAACAGTCA	CCATGTACACTTACACGGTAGAAGA	278
CaM1904	EI887108	(AT)6N(AT)5N(AT)5	GGATTCAATTTGTAATTTGGGAA	CCAAATAAGTTGATTTCAAGGATTG	236
CaM1905	EI887129	(AT)18	CCACTCTCGTTGCAACTCAAT	AGGCACTTTGCCCAATTTTA	261
CaM1906	EI887166	(GCT)5	AATCTGGATCGGGGAAAATC	GCTGCCCATGTTCCTTTTTA	114
CaM1907	EI887228	(AG)7	GCTGGATTGAGAGTGTGGGT	ATACATAAGGCCCAACACGG	161
CaM1908	EI887228	(AG)10	GGCTGGATTGAGAGTGTGGT	TGAGTGGGTACAAGGGAAAA	153
CaM1910	EI887237	(TA)9	GAGAAAGGAGGGAAATTGGC	ATTTTGGCTCGGAGGATTTT	273
CaM1911	EI887242	(TA)10	CCTTCAAAGAAAATGGTGCG	CGTCAAGGAAGCACTCTCAG	273
CaM1915	EI887300	(TA)17t(TA)5t(TA)5t(TA)5t(TA)8	TTTTCCTTTCTTTTTCCCCC	TTGCGGAGAATTTAATGCGT	202
CaM1918	EI887393	(AAT)5N(ATT)6	CCAAAAAGAAAATTGCAATCCA	TTCGTGCTCTCCTCTTCGAT	242
CaM1920	EI887404	(TTA)17	GCGCAAAACTTGCATCTCTA	TGAATTCATTCATTTTAGTTTGTTCA	246
CaM1922	EI887420	(AG)6	GGATACAGTGGCTGCGAAAT	CCAGTCCCGAAATTAAACGA	134
CaM1927	EI887483	(TTA)7	GCGCAAAACTTGCATCTCTA	TGAATTCATTCATTTTAGTTTGTTCA	219
CaM1931	EI887587	(AT)37	GCTTGCATCCAATTAATTTTCA	AGATCTCATTGGAAGTGGATGA	269
CaM1932	EI887593	(TATT)6	TCAAAAATTTGGATTCGGGA	TGGGGATACGTAGGAGCAAG	238
CaM1933	EI887642	(TA)8	CAGCTTTCCTCCGTTTTCTG	TGGTTTGAGAGGTTATGGGTG	192
CaM1934	EI887643	(TAAA)5	TTGGTGTGAAATGGCGATAA	TTTCAATCCAACTTTTTGCTCA	240
CaM1936	EI887654	(TTA)29	AACTGATGTTGATTCTAATGGGG	TGAATTCATTCATTTTAGTTTGTTCA	251
CaM1938	EI887702	(TA)37	ACTTCCACCTGCTTCAATGG	ACCCAGAAGTTAACACCCAA	214

Contd…

CaM1939	EI887707	(TAAT)5	TGCCAAAATTGCAGGTTACA	CCATGTTTTTGGGCTTTTGT	212
CaM1941	EI887740	(AT)10(AG)6N(AG)5	TGATTTTGTTTTCCATGGTTTTT	TGCAATTTGTTAGGCCTTAATGT	273
CaM1942	EI887759	(AAT)31	TGGGAGGTTTAGGGTCTACG	AAAAATCCCTCCAACGGTAAA	277
CaM1943	EI887765	(TTA)24	ATACATTCCCACTTGAGCCG	TACTCTCAAATGGGCCAAGC	208
CaM1944	EI887770	(TCA)7	ACCACCACTGCCTCTTGTTC	CTCCAACAACTTCCCACGTT	149
CaM1945	EI887771	(TA)6	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	233
CaM1947	EI887784	(AT)6	TTCTTTTTCCCTCCAACCAA	GCTCAGAGGCTTTTTAGCCA	238
CaM1948	EI887831	(TAT)6	TAATTCCACCTCACCGATGC	TCACCTTGAAAATTGTAAGCGAT	236
CaM1953	EI887896	(AT)7	GTGAAAGTGGGGTAGGGTCA	GTAATATGACGTGCAAGCGG	260
CaM1954	EI887972	(AT)8	GGAAATGGAAGAAGGAGGGA	GAACGAAATGATGGGGGTAA	268
CaM1955	EI887978	(ATA)5	GCTTGAACGATTTATATGCAATTT	CTCCTCCACCATGCTTGTTT	168
CaM1956	EI887995	(TAG)5	ACAGAACTAGGGGGAGGGTG	TCGGGTGTCGCATAATCATA	279
CaM1958	EI888021	(AC)6	TCCGATGACACAAACACACC	GGGATTTTAACCAATCTTGTGC	252
CaM1959	EI888032	(TAG)5	CATAACCAGAGGGAGGGTGA	TCCGGTGTCGCATAATCATA	278
CaM1960	EI888044	(CT)7	CCAGTCCCGAAAATAAACGA	TGGAATACAGTGGCTGCTTG	140
CaM1961	EI888046	(TG)9	TGGGAAGACCTATTGAGAGCA	CATCCGAATACCTGGCACTT	205
CaM1963	EI888103	(AT)5tattttta(AT)22gtata(TG)6	AGTCAGACGCTACTCGGCAT	CCGTGTTTGAAACTTTTATTTATCA	216
CaM1965	EI888112	(AT)23	AAAGGAACAGGCGAGAATCA	CTGTCAATTGAGTTGCGACG	233
CaM1966	EI888143	(ATT)5	TTCATGGATCACTTCGTGGA	TGGATGATTTATTTTATGAAGGAGT	266
CaM1967	EI888147	(AT)6	GAAAGGGATAGCGAGAGGCT	TGAGTTGCGACGAGAAAAGA	199
CaM1969	EI888175	(TA)19	TGAAACACAAAGCTTCTGAATATACC	TCTGCATGTCAGCCTACCAG	262
CaM1971	EI888230	(AT)22	GGCAGAGGAAGAAGATGGAA	GGAGAATTTAAGCGTTACGGG	279
CaM1973	EI888255	(AT)8N(TA)20	AACGGTTGAATTGAAAACTCATT	TGGCCATTTATCTGCACAAG	196
CaM1975	EI888263	(AG)5g(GA)8	CAAGCACATCAAATAGTGGGA	TGAATCATTATCCCTCCTCCA	112
CaM1977	EI888347	(TAA)10(CAA)18	AACAAAATTAGTTCTGGTAATAGTTGG	GGAGGTGATGGTGGTTAGGA	271
CaM1978	EI888350	(TA)5N(TA)27N(AT)7	TGCCTGTTTCCTTGCTTTCT	GGTTTGGAAAGTGATGGGTG	203
CaM1982	EI888413	(AT)9	GGCAGAAGTGGGACAATGAT	TGTGCATCTGGTGTTGTTTG	280
CaM1983	EI888423	(T)13N(TA)9N(TA)8	CTTTTCTCTCCCCCTGCTTT	TTCTCGTAACATATCCGCTATCTG	225
CaM1984	EI888430	(AT)6	TTCTGTTTCATTCCTTTTGTGTT	TGATGCCTTGTGAAGACTCG	268
CaM1986	EI888470	(TA)6	CATGCACCAACAGATCCAAC	TTCAATTACCATTGCAGAAGC	201
CaM1987	EI888483	(T)10N(TTA)6	GAGCTCAGCGTATTCAAACAA	TTGCCCACTTTGAGCCTTAC	278
CaM1988	EI888563	(AT)25	ACGGAACACGGATTTGGATA	TGACTAAAATTGTTATGTTCGTGAAA	163
CaM1991	EI888604	(AT)17	AACTAATACTCCCTCTTTTTCACAAT	AAATGAGTGTCATGTAAAATTTTTGT	266
CaM1992	EI888627	(A)11N(AT)5	TTTCAAAACGGAAAAGATTTCAA	TGTGCAGCCATCTACGTTGT	166
CaM1993	EI888681	(AT)8	GTGAGGACCAGCCCACTTTA	TCAAATTAAACAACATGTGCAGTG	230
CaM1994	EI888697	(AC)6	AGCCAAGGGAAGACGAAAAT	TCGTCCTCCCCAGACTTATG	116
CaM1999	EI888747	(TAA)7N(AAT)6	AGTGCAAACGTTAGATGGGG	TGACAAAACTTGACGAAAATTAGG	235
CaM2000	EI888751	(GAA)5	GACTCCCATGAAACCAAGGA	AAGAACTCTCCAGGCAACGA	158
CaM2001	EI888772	(CA)7	AAGAAAGGAGAAGGCGAAGC	GCTGTTAAAAATCGGGGTGT	148
CaM2003	EI888804	(AT)6	TGGTTCAAAATCAATCTGCG	TGTATGATTAATGTGTGATTGTGTGA	262
CaM2004	EI888807	(AT)6	ATCAAGTGGTCATGGTGGTG	TTGGTTTATACCGGTCTTTTTCTC	118
CaM2006	EI888815	(TTA)28ttt(TTA)9	CCGGCTAGGGTTTCAGTATTC	AAAATTAATTCTGGTAACAGTTGGTTT	247
CaM2007	EI888823	(AT)9	TGCATTTGACCCACACTCAT	AATCATGGCGTAGGATCTTGA	195
CaM2010	EI888853	(AT)15	ATCCTTTCCTTTTCTCCCCA	AGTGCGGTGAATCCTTTACG	165

Contd…

CaM2011	EI888881	(A)12g(TA)N(A)10	TGAAGACAAGAAAATGGCCC	TCCAAATGTGGAGTGTAATTTCT	248
CaM2012	EI888903	(ATA)5	GCCTCGGTATTCACTTTTCAG	TGTTGTCTCACACCACTAATTTCA	248
CaM2013	EI888976	(AG)7	GAAGAAAAGTGAAAAACGGAGG	GTTCATTGATGGCTGCCTCT	274
CaM2015	EI889048	(TTA)13(TAA)22	AACTGATGTTGATTCTAATGGGG	TGAATTCATTCATTTTAGTTTGTTCA	266
CaM2016	EI889073	(TCC)5	ACGCATATGGATTGGTGGAT	TGGACGATTTTGACCATGAA	152
CaM2017	EI889080	(GT)10	TTTTCTTGGAAGGTTGATGCT	TCGTTTTTGGTAAGATTGGAGAA	108
CaM2020	EI889141	(AAT)5	TGGAAAACTAAATGGTGTTTGG	AGGTTTCTGGAACATCACCG	253
CaM2021	EI889172	(CT)12	GCTGATTCACTTGTGCAGGA	GTGTTTGAACTGTGGATGCC	266
CaM2022	EI889179	(TA)6	ATTTAAAGCGTTTCGGGTGA	CCTTTCTTTTCCCTCCAAGC	111
CaM2023	EI889207	(CAA)5	TGGCCAATTAAAGGAACAATG	TTGTTGCTACAAGCACTGGG	220
CaM2026	EI889218	(TAT)10N(TTA)16	CCTTTTATAATTTTTGGATTTGATCG	AAATGGAAAGCTTCATTCATACAA	246
CaM2029	EI889255	(AT)25	CACACAGGGAGAGGAAGAAGA	TGTGTTACGGGTGAAATGGT	280
CaM2031	EI889290	(AAT)11	TCCACCTTTTTGACACTTATACACA	TTCCAGCAATCATAAAGTTCAGA	280
CaM2032	EI889344	(ATAA)5	GCCCTCCAACATATTAACCTT	CCTTGTAACATCCCACTTTTTCA	205
CaM2035	EI889384	(TC)5N(T)12	ACGCCTGTGTCATTGCTTTT	TGAGAAATTATGCTTGTGAGGA	271
CaM2036	EI889403	(GAGG)5	TGTGCGACCAATTTTGTTGT	CTGATAGGAACCCGGATTGA	149
CaM2038	EI889467	(AT)15	TCAAACTTACAAATCTACGCTAATGAA	GCTGAGGTGGGAAACATTTG	250
CaM2039	EI889487	(AG)7N(T)11	GGGGTAAAACGAATGGGAAT	ACCTAGGCAAAGCAAAGCTG	186
CaM2040	EI889518	(T)10N(TA)15	TTCACAAAATTCATGGTCCG	CAGAAAATGAACGCAACAGC	257
CaM2041	EI889572	(AT)24(AC)5N(AT)14	CAAATGCTCCCCTAATTGACA	CGTTGTTTAAGACCCTCCTTC	280
CaM2043	EI889615	(ATA)22	TGTGTGCAAAAGTAATTGATTGG	GGGGAAACGTGTTATTTTAGCC	280
CaM2045	EI889642	(TAA)5	TCAGTGTCGTGTTTGGTGGT	TGATGGAACTGAGTTGGTGC	230
CaM2048	EI889683	(AT)33	TGCTGGCAATCAAATAACTCC	GAACGAGATTTCATCACACCAA	267
CaM2049	EI889687	(AGAT)15	CCCTTTGGAAAGAGAGGAGG	AAGCCGATTCTTGGGACTTT	214
CaM2050	EI889730	(TTA)10	GCGCAAAACTTGCATCTCTA	TGATTTCATTCATTTTAGTTTGTTCA	234
CaM2051	EI889758	(AC)8(AT)13	TTTCCTTTCTTTTTCCCTCCA	CGGTGAATCCTTTACGGAGA	165
CaM2052	EI889780	(AT)9	TTGCAGGCAAAGAGTCAAGA	AGGGAGTGAATCAACAACGG	223
CaM2053	EI889782	(TA)6	TTGGTTTATTTGGGTTGGGA	TGCATGTAATCTGAATTGAGTGTTT	236
CaM2054	EI889805	(TA)37	ATGTTGCATTTATCCCCCAA	GGACGGATAAGGCATTTGAA	276
CaM2055	EI889806	(AGG)5	GCTTTGCAAGCTTCTTCTTCA	AAAGAAGCATTAACGCCCAA	251
CaM2059	EI889921	(TA)7	ACAAAAGGAAGCAGCGAAAA	TGGTTTGGAGAGGTTATGGG	280
CaM2060	EI889933	(CTT)5	GTGGATGCTGGAGGGAATTA	CAATTGCTATGGTGGTTATGGA	261
CaM2061	EI889944	(AT)8	ATTTAAATGTCGTGACCGGC	TGGGAATGTTGAAGTTACAGAGG	169
CaM2062	EI889982	(GAA)5N(TA)7	AGGAATGCTGTTTGGTGTCC	TGAGGTAGAAGTTCTCTTGCGTC	277
CaM2063	EI889995	(TA)17	CCGATGTGCATTATGTTGTTG	CCGTCTCTTTCACATGGGTT	159
CaM2064	EI890022	(TA)8N(TA)5	AGATCACCAATTGGGAACAAA	CACCTTCTCGACTTTCCTATGTG	221
CaM2066	EI890053	(AT)19	CTATTGGATGTCGTGGCAAA	ATGACATGTCGTGCGATAAA	202
CaM2067	EI890058	(AAT)5	TTGAGAAACATGGTAGAGTCCAA	TTTGTTTGGTGCATTTGCTT	268
CaM2068	EI890070	(AAC)7N(AAC)7	TTTGAACTTTTTCACAACTTTAACAA	ACAAGGTGGTTGTTGTGAGC	266
CaM2071	EI890164	(AC)6	CTATGCCATGGGGACAAACT	AAATACTTCCTCCGTTCCAAA	195
CaM2074	EI890217	(AT)19ac(AT)7	TTCTTTCCAAGCAAACACACA	AATTTAATGCGCTACGGGTG	154
CaM2075	EI890223	(AT)6	GGAGAAAGAGGGAATTTGGC	GCGTTACGGGTGAAATGGTA	267
CaM2077	EI890291	(TA)8	AATGAGATCTTGCCCGAATG	TCGCCGTGTTTATGAGACTG	217
CaM2078	EI890332	(TA)6	CGAAAGCAAAGAAAAGGTGG	GGAATTTGTGGGTGAAATGG	190

Contd…

CaM2079	EI890439	(GT)6	CAAAGGAATTGTCGGGTCAC	TGGGATTCTTTTGGATCAACTT	103
CaM2080	EI890440	(TTG)8(TTA)44	TCGTAGTAGCAGCTAGGGTTCA	CAAAATTAGTTCTGGTAATAGTTGGTG	276
CaM2084	EI890534	(AC)8(AT)20	TCCGAGGAAGAAGATGGAAA	GCGTTACGGGTGAAATGGTA	278
CaM2085	EI890549	(TA)5catac(AT)6N(TA)17(AT)5	TGGACAAAAGCGCTGTAAAA	AAGCATTGTTTGAGTACTGGGAA	258
CaM2086	EI890578	(ATA)5	CGAACCAATGAGTAATTAATGGAA	AACCTCACAATCGGAATACACA	256
CaM2087	EI890596	(GA)5N(GA)5	CACCAACACTTGTTTTCCCC	TGGTGCTACAGCAACGCTTA	224
CaM2088	EI890618	(AT)27	GAAGAAAGAAGAAGGGTTCGC	CAATTGAGTTGCGACGAGAG	250
CaM2090	EI890649	(AT)6	GCTTTGCAGTATCTCTTCCACA	AACATTCAGCAGCTGTTCCC	266
CaM2091	EI890661	(AG)8	TGAATTCATGGGGTTTGGTT	GCCCTCTTTTTGTGACCAAT	162
CaM2092	EI890683	(TA)6	GTGAGGACCAGCCCACTTTA	TCCTCCCCCTATGTTAAACAAC	232
CaM2093	EI890698	(TA)12	AAAAGCCCCCAAAATACACC	TTCTTCCTCTTCTCTCCCACA	163
CaM2094	EI890711	(AT)23N(AT)9	TGGAAATGTTTTCCCTTTGC	GGAATGAAATGATGGGGGTA	225
CaM2095	EI890753	(AT)5N(AT)13N(GT)5	TAGATTTGATCGCGAGGTGC	CCTAAAACCCTAAAACCTAAACTAAAA	275
CaM2096	EI890771	(AT)6N(AT)5	TCAAAACAGAACAGATCATGTGG	TCCTCTACGTATTGTGCGGC	198
CaM2098	EI890787	(AT)24	ATACTACCACGCAAGTGGGC	TGGAGGTATTCAAATGGGGA	201
CaM2099	EI890802	(TA)18	GAACCGTTCGTATTTCTGCG	CTTCGACACTGTAGCACCCA	246
CaM2100	EI890826	(AC)6	TTGAGGAAGAAAATGACCTAAATG	TCCTCCCGAGGCTTATTCTT	107
CaM2101	EI890830	(TA)5(CA)7	CTCCAACGGGTTCGAAGATA	TTGCAATGATTTATAGACAAAAAGA	201
CaM2102	EI890831	(AT)29	GAGAGAGTTCACGAGGCAGG	ATTCGTTTGGGTTTGGTTTT	176
CaM2103	EI890842	(GA)5N(ATT)35ta(ATT)5	TGAAAATGGAGAAGGATGGG	GCCCCGTGTCCCTTATAAAT	241
CaM2105	EI890906	(AT)7	TCAAAACAGAACAGATCATGTGG	TTACGTACTGTGCGGCCAT	200
CaM2106	EI890916	(TA)27	CGGCAGAGGAAGAAGAAGG	TGCGGTGAATCTTTTACGAA	276
CaM2107	EI890987	(AAT)24N(ATTT)5	TCACACGGTAGTTGTTAAGGATG	CCGTCTCTATTCATCCGTGG	277
CaM2109	EI891044	(AT)45	ACGCGACAGAGGAAGAAGAT	TGCCTTATGGGTGAAATGGT	269
CaM2110	EI891047	(TA)8	TTTTCAAATGCACCATAAAAATC	CCACTTACATGAAAAATTGACGA	266
CaM2112	EI891152	(TA)33	TTTCCTTTCTTTTTCCCTCCA	TATTTTGGATTTTGGCTCGG	191
CaM2113	EI891156	(AT)23	TATTTTGGATTTTGGCTCGG	TCCCTCCAAACAAACATATACA	159
CaM2114	EI891197	(AT)41	ATGAGAATGGGTTTTGCGAG	CGGTGAATCCTTTACGAAGAA	249
CaM2117	EI891242	(GA)12	CCACAAGGAAGTTCGGAAGA	CTCCCAAACGCTCTCGTAAG	188
CaM2118	EI891318	(CAT)5	CATTTACTATCAAGTCACCAATATTCA	TCAAATCATCAAGAAAAGTTTAGGAA	195
CaM2119	EI891370	(AT)22	CGGTGAATCCTTTACGGAGA	GGAGAAGAGGAATGTTGCGA	264
CaM2121	EI891410	(AT)9	TGTGCATCCAATAAGTGCGT	TTCTCTCCAAACAAACATATATAAACA	234
CaM2122	EI891421	(TAT)8	TCACATACCGCCACATTACG	CCAGGTGCAATAGGGAAATC	253
CaM2124	EI891462	(AT)8	GAGAAGGGAGGGAGGTTCAC	AATGCGTTACGGGTGAAATG	240
CaM2125	EI891469	(TA)6	TTGGTTTATTTGGGTTGGGA	GCTTGATTTACATGAACTGAACTGA	233
CaM2126	EI891478	(GT)6	CATTTTCGTCCTCCATAGGC	GGATGCACATGATTGGTTGA	163
CaM2128	EI891516	(C)17N(AT)43	TCGAGGTAGCCATCCTCATC	GGGGGATAAAGGAGTCTTTCC	277
CaM2131	EI891567	(AAT)37	AGCATCGTGAATATTGAAGGG	AAAAATCCCTCCAACGGTAAA	242
CaM2133	EI891600	(AT)6	TCAAAACAGAACAGATCATGTGG	GCGGCCATCTACGTTGTCTA	183
CaM2134	EI891635	(AT)8	TCAAAACAGAACAGATCATGTGG	CTTTATGTACTGTGCGGCCA	200
CaM2136	EI891697	(TTA)6	CTGCGCAAAACTTGCATCTA	TGAATTCATTCATTTTAGTTTGTTCA	218
CaM2139	EI891709	(AT)28	CGTAGGGAGGAAGAAGATGG	CGTTACGGGTGAAATGGTAA	280
CaM2142	EI891730	(TA)8	GACGTGAAAAGGGAATTGGA	ATCTGCCCATTGAATTACGG	229
CaM2143	EI891751	(A)10N(TAAA)5	ACAGGGAGATCCCTTTCCTT	CCAATCATGTCAATCCAAACA	262

Contd…

…Contd

CaM2144	EI891760	(AT)7	TGATAGTGAGAACCAGCCCC	AGGCAAAAAGGCAGAAAACA	275
CaM2145	EI891796	(ATA)6	ACTTGGGCTGAAGGTTTCTG	AAAAGATAGGAAAATATTGCCGC	210
CaM2148	EI891849	(AT)6	TTTCTTTTCCTTTTCCTTCCTTC	TTTAGATTTTGGCTCGGAGG	275
CaM2149	EI891878	(TA)8	GCGGGTAACAGAGAGGAAGA	TATTTTGGATTTTGGCTCGG	276
CaM2150	EI891908	(AT)8	GGCCTTATATGGCGAGGAAT	CAGAAGCCTTAGCAAAACACA	232
CaM2151	EI891964	(AT)6	CGGTCTCTACCTCTCCCCTT	TGCAACAGATGTAGTATGGCAA	210
CaM2152	EI891979	(TG)7	CCCATCACAAAATCACACCA	AATGAAGTAAGTGGGCCGC	125
CaM2153	EI892018	(AT)6	CGGTCTCTACCTCTCCCCTT	TGCAACAGATGTAGTATGGCAA	210
CaM2154	EI892037	(AT)6	TTGTTTTGATGACGGTCAGG	ATAATTTAGAATTAGGCCAACAATTT	228
CaM2155	EI892153	(TA)28	CAAAGAAAAGGATTCCCTGC	AGCCCTGAGTTTCATCATGC	162
CaM2156	EI892178	(GT)6	GCAGTAAGTTTGTGGCGGAT	CAGCATTTTGTGAGGCAGAA	115
CaM2157	EI892178	(TA)39N(TAA)5	TTGGTTTGGGGTGAAAACAT	GGTGACATGCGTAATGGTGA	196
CaM2158	EI892187	(AT)8N(AT)7	CTCCCCGCCTAATTGACAT	CATCCAACTACGGTCTATATCCA	188
CaM2160	EI892247	(AT)7	TGATAGTGAGAACCAGCCCC	TGCAACAGATGTAGTATGGCAG	174
CaM2162	EI892252	(TTC)8	GCTTTCACACGCTCAAACAA	TTTGGGAAGAATGGAAGTGG	165
CaM2164	EI892301	(TA)17	GTCATGACCGACACACAAGC	TGGGAATGTTGAAGTTACAGAGG	176
CaM2165	EI892305	(TAA)5	TTCATTTTTGAAATTTCCGGT	CAACTTATTTTCTTAACAACTCTCTCG	280
CaM2166	EI892309	(AT)7	AAATGGAGGAGAAGGAGGGA	GCGTTATGGGTGAAAGGGTA	268
CaM2168	EI892368	(AT)9ac(AT)20	CCTTTCCTTTTTCCTCCACA	ACTGCGGTGGATCTTTTACG	191
CaM2169	EI892394	(AC)8	CCCTATCGTGAAAAACGGAG	AGTCCCCCAGACGTAGGAGT	253
CaM2174	EI892426	(ATA)5N(AAT)23	TTTTGAGGTCATACAGGAGGA	TGACATAAATTTTGGGGACGA	278
CaM2175	EI892430	(AAC)5	AAAGGGAGGCCTGAGAGAAG	AATGGGTGGAAGATCCGACT	161
CaM2178	EI892544	(AAAT)5	AAATTGGTGGCCAGATGTGT	CATCCTAGGACTGCTCTGCC	247
CaM2181	EI892588	(AT)6N(AT)19	AATTAATATACAATCCCCTAATTGACA	TCCTATCGTACATCCAACTACGG	278
CaM2182	EI892608	(AT)5N(AT)5	TCAAAACAGAACAGATCATGTGG	GCGGCTATCTACGTTGTCTCA	181
CaM2186	EI892642	(TAT)5	GCGTTAGCAAACAATGCCTA	TTTGCTAGATTGGTTAGGTTCACA	182
CaM2187	EI892699	(AT)14ag(AT)9	TCCCTCCACACAAACACAAA	TTGGTATTGAACTCGGAGGC	277
CaM2188	EI892735	(AT)8	ACGGTCTAACCTTTTGTGCC	TGCAAGACAATTAGTCAAAAGGA	168
CaM2189	EI892739	(TA)5N(TA)39	CAAAGTTTATGTTCTGATCAGTTTTTG	GATCTGATCATGTAATTTCGCA	255

SSR motifs having "N" nucleotide represent the interruption of few base pairs between two same/different SSR motifs.
Marker names start with prefix CaM, which represent Cicer arietinum Microsatellites.

4.2 Preparation of Genetic Linkage Maps

4.2.1 Screening of SSR markers on the parental genotypes of mapping populations

The parental genotypes of two mapping populations derived from the crosses "ICC 4958 × PI 489777" and "ICC 4958× ICC 1882" were used for testing the polymorphism with following set of SSR markers:

4.2.1.1. ICCM-series

All the 311 ICCM markers were screened on three chickpea genotypes i.e. ICC 4958, PI 489777 and ICC 1882, which are the parents of two mapping populations (inter-specific: ICC 4958 × PI 489777 and intra-specific: ICC 4958 × ICC 1882) pertaining to the present study. Of the 311 ICCM markers, a set of 234 (75%) markers produced scorable amplicons. While 52 (22.2%) SSR markers showed polymorphism on parents of the inter-specific mapping population, only 23 (9.8%) SSR markers were polymorphic between parents of the intra-specific mapping population.

4.2.1.2 CaM-series

Similar to ICCM markers, 1,344 CaM markers were tried for amplification and only 1,214 (90.3%) markers gave scorable amplicons. Among them, 253 (20.8%) makers showed polymorphism between ICC 4958 and PI 489777, whereas only 109 (8.9%) showed polymorphism between ICC 4958 and ICC 1882.

4.2.1.3 H-series

Another set of 233 H-series markers earlier developed from screening BAC and BIBAC libraries of chickpea (Lichtenzveig et al. 2005) were also screened on the three parental genotypes of two mapping populations that provided only 153 scorable markers. Furthermore, only 52 (33.9%) markers detected polymorphism between the parental

genotypes of the inter-specific mapping population, while 33 (21.5%) markers detected polymorphism between the two parental genotypes of ICC 4958 and ICC 1882 mapping population.

4.2.1.4 Winter-series

Furthermore, another set of 241 published markers from Dr. Peter Winter group (Hüttel et al. 1999; Winter et al. 1999; Pfaff and Kahl 2003) was screened on only parents of intra-specific mapping population. Of 241 Winter- series markers, 183 markers gave scorable amplicons, and 77 markers showed polymorphism. In case of the inter-specific mapping population, genotyping data for 359 Winter-series markers (including 130 SSR markers) were made available from Dr. Peter Winter (GenXPro/University of Frankfurt, Germany).

4.2.1.5 NIPGR-series

Another set of 280 published/unpublished markers from NIPGR, New Delhi (Sethy et al. 2003, 2006a, 2006b; Choudhary et al. 2006) were screened on the parental genotypes of the intra-specific mapping population (ICC 4958 × ICC 1882). As a result, 203 markers gave scorable amplicons and 56 markers showed polymorphism between the parental genotypes. These markers were not screened on the inter-specific mapping population in this study, as NIPGR, the developers of these markers were already involved in mapping of these markers onto inter-specific map.

4.2.2 Integration of novel SSR loci in the inter-specific genetic map

The genotyping data were generated for all 357 (52 ICCM, 253 CaM and 52 H-series) polymorphic SSR markers identified on 131 RILs of the inter-specific mapping population. In addition, the genotyping data for 359 published markers by Dr. Peter Winter's group

(Winter et al. 1999, 2000; Pfaff and Kahl 2003) were kindly provided by Dr. Peter Winter (University of Frankfurt/ GenXPro, Germany).

In summary, genotyping data were compiled for a total of 716 markers (357 SSRs in the present study and 359 markers from published studies). These data were used to construct the genetic map using JoinMap v 4.0 programme. Briefly, 47 (90.3%) of 52 ICCM marker loci, 186 (73.5%) of CaM marker loci, 45 (86.5%) of 52 H–series SSR loci and 343 (95.5%) of 359 reported marker loci could be mapped onto the eight linkage groups (LGs). The inter-specific map constructed in the present study contained a total of 621 marker loci spanning a genetic distance of 984.11 cM (average distance = 123.0 cM/linkage group), with inter-marker distance of 1.58cM (Figure 8).

Mapped marker loci were equally distributed across all the 8 linkage groups (LGs) except for linkage group "LG5" and the smallest group "LG8". The number of marker loci per linkage group ranged from 22 (LG8) to 144 (LG5) with an average of ~78 markers per linkage group (Table 11). LG1 represented the largest linkage group in terms of size with map distance 187.9 cM and LG8 was the shortest with map distance 79.9 cM among eight linkage groups. Average inter-marker distance ranged from 0.97 cM (LG5) to 3.63 cM (LG8). The number of marker loci mapped along with the inter-marker distances for each linkage group is tabulated in Table 12. Considering the physical size of the chickpea genome as 740 Mbp (Arumuganathan and Earle 1991), 1 cM distance in the present map approximately equals 751 kbp.

The earlier published map (Winter et al. 2000) had sixteen linkage groups comprising of eight major and eight minor linkage groups. The markers of minor group LG15 of Winter et al. (2000) map were integrated in the LG5 of the present map; marker loci from LG13 were

integrated into the LG6 and while that of LG9 were integrated into LG7 of the present map. This clearly showed that the novel markers identified during the present study provided bridge points to establish eight major linkage groups in chickpea. The nomenclature of different linkage groups (LGs) for inter-specific map during the present study was followed as per published map of Winter et al. (2000).

Table 11: Distribution of different types of mapped marker loci on different linkage groups (LGs) of the inter-specific map of chickpea

Markers	CaM	ICCM	H-series	Winter-series	Total
Markers screened	1344	311	233	-	-
Scorable markers	1214	234	153	-	-
Polymorphic markers	253	52	52	359	716
LG1	20	3	4	44	71
LG2	7	4	6	51	68
LG3	20	9	7	48	84
LG4	17	11	5	47	80
LG5	80	8	8	48	144
LG6	22	6	6	48	82
LG7	17	4	6	43	70
LG8	3	2	3	14	22
Total mapped	186	47	45	343	621

CaM (Cicer arietinum Microsatellite) and ICCM (ICRISAT Chickpea Microsatellite) markers were developed in the present study while, H-series and Winter-series markers were developed by Lichtenzveig et al. (2005) and Winter et al. (1999) respectively.

Table 12: Distribution of mapped marker loci on different linkage groups of the inter-specific map of chickpea

Linkage group	No. of markers mapped	Map distance (cM)	Inter marker distance (cM)
LG1	71	187.99	2.64
LG2	68	90.67	1.33
LG3	84	130.48	1.55
LG4	80	146.72	1.83
LG5	144	141.03	0.97
LG6	82	111.68	1.36
LG7	70	95.56	1.36
LG8	22	79.96	3.63
Total	621	984.11	1.58

The linkage groups are written as 'LG'.
The map distances and inter-marker distances were calculated in centi-Morgan (cM) map units.

4.2.3 Construction of the intra-specific genetic map

In case of the intra-specific mapping population, screening of a total of 2,409 SSR markers including 311 ICCM; 1,344 CaM; 233 H-series; 280 NIPGR-series and 241 Winter-series markers (Winter et al.1999; Hüttel et al. 1999; Pfaff and Kahl 2003) yielded 1,987 scorable amplicons and 307 (23 ICCM; 109 CaM; 33 H-series; 57 NIPGR and 85 Winter-series) polymorphic markers. All these polymorphic markers were used to generate the genotyping data on 232 RILs of the mapping population. Subsequently these data were used to construct

the intra-specific genetic map with help of JoinMap v 4.0. While developing the map, only 16 (69.5%) of 23 ICCM marker loci, 83 (76.1%) of 109 CaM marker loci, 29 (87.87%) of 33 H–series, 43 (75.43%) of 57 NIPGR marker loci and 59 (69.4%) of 85 Winter-series markers loci could be mapped onto the eight linkage groups.

The developed intra-specific genetic map contains 230 marker loci spanning a total of 466.95 cM genetic distance (Figure 9). Number of marker loci mapped ranged from 9 (LG5) to 44 (LG6) with an average of approximately 29 markers per linkage group. The LG5 of the intra-specific map spanned the highest genetic map distance (76.03 cM) followed by the LG2 (2A and 2B together) with 73.72 cM map distance. The LG8 with 27.7 cM distance covered the least map distance among all others. Average inter-marker distance ranged from 0.86 (LG4) to 8.44 cM (LG5). The number of marker loci mapped along with the respective map distances and inter-marker distances in case of intra-specific map are given in Table 13.

Table 13: Distribution of mapped SSR marker loci on different linkage groups of the intra-specific map of chickpea

Linkage group	No. of SSRs mapped	Map distance (cM)	Inter marker distance (cM)
LG1	21	66.52	3.16
LG2A	18	54.44	3.02
LG2B	7	19.28	2.75
LG3	23	58.47	2.54
LG4	61	52.51	0.86
LG5	9	76.03	8.44
LG6	44	82.30	1.87
LG7	28	29.67	1.05
LG8	19	27.69	1.45
Total	230	466.95	2.03

LG-linkage groups; cM- centi Morgan

4.3 QTL Interval Mapping for Drought-tolerance Traits

4.3.1 Phenotyping data analyses

In order to identify quantitative trait loci (QTLs) for drought tolerance in the intra-specific mapping population, phenotyping data for drought tolerance traits for two years (2005 and 2007) with three replications were obtained from the Crop Physiology Division of ICRISAT. The phenotypic data was obtained for ten root traits *viz.*, shoot dry weight (SDW), stem dry weight (StDW), leaf dry weight (LDW), root dry weight (RDW), rooting depth (RD), ratio of root dry weight and total dry weight (RT), root length (RL), root length density (RLD), root surface area (RSA) and root volume (RV) in year 2005 while as for eight traits (all traits except StDW and LDW) in year 2007. The pooled analysis of variance (ANOVA) for all the traits measured showed highly significant differences among genotypes and environments (Table 14). The mean square values due to two environments (2005 and 2007) differed significantly from one another for all the traits. Highly significant differences (P value <0.001) were found in genotypes (RILs) for all traits except for the trait RD.

Table 14: Analysis of variance (ANOVA) for drought tolerance related root traits in chickpea

		Mean sum of squares							
Source of variation	df	SDW	RDW	RD	RT	RL	RLD	RSA	RV
Environment	1	87.7487***	10.21073***	3885.7***	301.45***	594600000***	1.737003***	8728493***	538.83***
Replication (Environment)	4	7.0542***	1.98612***	15388.8***	752.4***	93920000***	0.278948***	5423246***	1860.91***
Genotypes or RILs	231	0.3895***	0.04191***	212.2^{NS}	35.31***	3499000***	0.010745***	98740***	23.26***
RILs × Environment	231	0.1339*	0.03304*	243.1^{NS}	17.73^{NS}	1684000^{NS}	0.005222*	61126^{NS}	16.84^{NS}
Pooled error	883 to 911	0.1109	0.02778	219	15.98	1.45E+06	0.004322	56079	15.82

		mean sum of squares	
Source of variation	df	LDW	StDW
Replication	2	3.91222***	0.4163***
Genotypes or RILs	231	0.14792***	0.04169***
Error	461	0.07378	0.01358

Pooled analysis of variance (ANOVA) was calculated using GenStat 12th Edition for eight root traits- namely SDW (shoot dry weight), RDW (root dry weight), RD (rooting depth), RT (ratio of RDW and total dry weight), RL (root length), RLD (root length density), RSA (root surface area) and RV (root volume).
The significance was calculated at F probability value and shown as asterisks.
* <0.05 (at 5% probability level); ** <0.01 (at 1% probability level); *** <0.001 (at 0.1% probability level).
As LDW (leaf dry weight) and StDW(stem dry weight) data is for one year 2005, ANOVA was calculated using single environment data.

Among the interactions, environment × replication interaction revealed high significance among all the traits; and genotype (or RILs) × environment interactions showed non-significant variation for RD, RT, RL, RSA and RV traits under study, indicating no significant effect of environment on genotypes. The phenotypic data for LDW and StDW were available for only one environment (2005) and single environment ANOVA was conducted. The ANOVA results showed that for LDW and StDW traits, both replication mean sum of squares (mss) and genotype mss were found have high significant variation.

The correlation between the traits under study was computed using GenStat (12^{th} edition) programme. The two-sided test of correlation showed that among the ten traits, there was significant correlation (P value < 0.05) between all the traits except for the following pairs of traits: "RL and RD","RL and RT", "RLD and RD" and "RLD and RT" (Table 15). Furthermore, heritability (h^2) of the traits was estimated from the components of variance obtained using GenStat (Table 16). The heritability of the traits varied from 5.46 (for RDW) to 40.89% (for StDW). High heritability (classified as heritability range of 30-60%) was observed in case of StDW, while as low heritability (classified as the h^2 range of 5-10%) was observed in RV and RDW. However, most of the traits (SDW, LDW, RT, RL, RLD and RSA) showed medium heritability (classified as the h^2 range of 10-30%).

Table 15: Two-sided test of correlation studies across ten drought tolerance related root traits using GenStat

	SDW	StDW	LDW	RDW	RD	RT	RL	RLD	RSA	RV
SDW	-									
StDW	0.8638***	-								
LDW	0.9711***	0.7186***	-							
RDW	0.6972***	0.5401***	0.7065***	-						
RD	0.313***	0.1936***	0.3403***	0.5907***	-					
RT	-0.3513***	-0.3835***	-0.3032***	0.4065***	0.3849***	-				
RL	0.5591***	0.5475***	0.5124***	0.544***	0.0283^{NS}	0.017^{NS}	-			
RLD	0.5568***	0.545***	0.5104***	0.5427***	0.0258^{NS}	0.0191^{NS}	0.9984***	-		
RSA	0.6722***	0.599***	0.644***	0.7204***	0.1684***	0.1026**	0.899***	0.8972***	-	
RV	0.6641***	0.553***	0.6546***	0.7564***	0.2484***	0.156***	0.6924***	0.6906***	0.9379***	-

The correlation coefficients are calculated using two-sided test of correlation using GenStat 12th Edition.
The significance of correlation was calculated at F probability value and shown as asterisks
* <0.05 (at 5% probability level); ** <0.01 (at 1% probability level); *** <0.001 (at 0.1% probability level).

Table 16: Heritability of the drought tolerance related root traits

Traits	**Genotypic variance**	**Phenotypic variance**	**h^2 (percent)**	**Classification**
SDW	0.043	0.154	27.96	Medium
StDW	0.009	0.023	40.89	High
LDW	0.023	0.098	24.29	Medium
RDW	0.001	0.029	5.469	Low
RT	2.86	18.41	15.53	Medium
RL	326985	1753415	18.64	Medium
RLD	0.0009	0.005	18.90	Medium
RSA	6695	61923	10.81	Medium
RV	1.12	16.84	6.65	Low

Heritability (h^2) in percent was calculated and classified as high, medium or low according to Robinson et al. (1966).
The abbreviation of the traits: SDW-shoot dry weight; StDW- stem dry weight; LDW-leaf dry weight; RDW-root dry weight; RT- RDW/total dry weight ration; RL-root length; RLD-root length density; RSA- root surface area; RV-root volume.

4.3.2 QTL analyses for drought tolerance related traits

Genotypic and phenotypic data obtained on 232 RILs, as presented above, were analysed for identification of the main effect QTLs (M-QTLs) using QTL Cartographer version 2.5 (Basten et al. 1994, 2002) following composite interval mapping (CIM) approach. QTL analysis was conducted using data of individual replications as well as mean data over replications (R) collected during year 2005 for ten traits and during year 2007 for eight traits. Besides this, the analysis was also conducted using the data pooled over the means of the two seasons for eight common traits. LOD score of 3.0 was used for declaring the presence of a putative QTL. In total 47 significant QTLs (LOD $\geq$ 3) were identified for the ten root traits, which included seven major and stable QTL, showing more than 20 % phenotypic variation (Table 17). The number of significant QTLs ranged from one (RD) to eight (RSA). Two QTLs were detected for RV; three QTLs each for LDW and RT; four QTLs were detected for StDW; six QTLs were detected for SDW and RL and seven QTLs were detected for RDW and RLD. Two adjacent genomic regions on LG6 containing two important QTL region defined by marker interval "NI21-TAA170" and "ICCM0249-H1G20" for all traits except for rooting depth (RD). The QTL study indicated presence of 18 discrete genomic regions comprising a total of 47 QTLs. While nine regions had single QTL, the remaining nine regions constituted more than one QTL. In the present study, only 18 discrete genomic regions were contributing to the ten phenotypic traits studied. Linkage group "LG6" was found to have maximum number of QTLs (30 of 47) followed by 7 QTLs on LG1. The respective QTL positions on respective LGs for root traits are presented in Figure 10. The details of QTLs for drought tolerance related traits are presented in Table 18 and are also explained in the following sections.

Table 17: List of major QTLs explaining >20% phenotypic variation

Trait name	Name of QTL	LG	Marker interval	Position (cM)	LOD	R^2 (%)	Additive effect
SDW05_R1	*QTLSDW4*	6	NI21-TAA170	37.30	11.51	24.39	0.20
SDW05_R3		6	NI21-TAA170	37.30	9.31	21.41	0.19
SDW07_R3		6	NI21-TAA170	39.30	10.24	20.30	0.12
SDW05_Mean		6	NI21-TAA170	37.30	21.34	41.34	0.20
SDW07_Mean		6	NI21-TAA170	37.30	18.03	34.98	0.15
SDW_Pooled Mean		6	NI21-TAA170	37.30	28.66	49.91	0.18
SDW_Pooled Mean	*QTLSDW6*	6	ICCM0249-H1G20	52.06	14.04	28.25	0.13
StDW05_R1	*QTLStDW2*	6	NI21-TAA170	37.30	11.58	24.78	0.07
StDW05_R3		6	NI21-TAA170	39.30	13.60	26.42	0.06
StDW05_Mean		6	NI21-TAA170	37.30	23.80	47.75	0.08
LDW05_R3	*QTLLDW2*	6	NI21-TAA170	37.30	9.56	22.81	0.14
LDW05_Mean		6	NI21-TAA170	37.30	16.10	31.81	0.12
RT05_Mean	*QTLRT2*	6	NI21-TAA170	37.30	11.03	24.56	-1.34
RT_Pooled Mean		6	NI21-TAA170	39.30	16.18	28.57	-1.30
RL_Pooled Mean	*QTLRL3*	6	NI21-TAA170	39.30	11.97	21.33	360.2
RLD_Pooled Mean	*QTLRLD4*	6	NI21-TAA170	39.30	12.46	21.36	0.01

Seven major QTLs were detected by QTL analysis.
SDW-shoot dry weight; StDW- stem dry weight; LDW-leaf dry weight;
RT- RDW/total dry weight ration; RLD-root length density; RSA- root surface area.
The trait names are suffixed by '05'- data from year 2005 and '07'-trait data from year 2007.
R1, R2, R3- represent the replications. cM- centi Morgan; LOD- logarithm of odds;
R^2- phenotypic variation explained in percent
.

Table 18. Main effect QTLs for drought tolerance related root traits using single locus analysis using QTL cartographer

Trait/QTLs	Marker interval (LG)	Position (cM)/LOD								Pooled mean			
		2005				2007				Position	LOD	PV%	A
		RI	RII	RIII	Mean	RI	RII	RIII	Mean				
Shoot Dry Weight (SDW)													
QTLSDW1	GA26-NI4 (LG5)	-	-	-	-	-	-	-	-	12.64	3.25	5.78	0.06
QTLSDW2	NI4-NI202 (LG5)	-	-	-	-	-	23.72/ 3.84	-	-	-	-	-	-
QTLSDW3	STMS11-NI21 (LG6)	-	-	28.23/ 5.44	-	-	30.23/ 3.20	-	-	-	-	-	-
QTLSDW4	NI21-TAA170 (LG6)	37.30/ 11.51	37.30/ 6.3	37.30/ 9.31	37.30/ 21.34	37.30/ 6.6	39.30/ 9.3	39.30/ 10.24	37.30/ 18.03	37.30	28.66	49.9	0.18
QTLSDW5	TA46-ICCM0249 (LG6)	-	-	-	-	-	-	49.71/ 4.53	-	-	-	-	-
QTLSDW6	ICCM0249-H1G20 (LG6)	50.06/ 8.74	52.06/ 5.50	-	52.06/ 7.76	-	-	-	52.06/ 7.51	52.06	14.04	28.25	0.13
Stem Dry Weight (StDW)													
QTLStDW1	STMS11-NI21 (LG6)	-	-	28.23/ 7.6	-	Data not available				NA			
QTLStDW2	NI21-TAA170 (LG6)	37.30/ 11.5	39.30/ 10.75	39.30/ 13.6	37.30/ 23.80								
QTLStDW3	TA46-ICCM0249 (LG6)	-	49.71/ 6.08	-	-								
QTLStDW4	ICCM0249-H1G20 (LG6)	50.06/ 6.64	-	52.06/ 3.59	52.06/ 9.02								
Leaf Dry Weight (LDW)													
QTLLDW1	STMS11-NI21 (LG6)	-	-	28.23/ 5.84	-	Data not available				NA			
QTLLDW2	NI21-TAA170 (LG6)	39.30/ 9.24	39.30/ 5.91	37.30/ 9.56	37.30/ 16.1								

Contd…

…Contd

QTLLDW3	ICCM0249-H1G20 (LG6)	50.06/ 7.31	-	-	52.06/ 8.74								
Root Dry Weight (RDW)													
QTLRDW1	ICCM0009a-STMS21 (LG1)	-	16.44/ 3.86	-	-	-	-	-	-	-	-	-	-
QTLRDW2	H1N12-CaM0111 (LG2B)	-	-	-	-	-	-	-	-	11.79	3.08	5.15	-0.02
QTLRDW3	GA26-NI4 (LG5)	-	-	18.64/ 3.9	-	-	-	-	-	-	-	-	-
QTLRDW4	NI21-TAA170 (LG6)	-	-	39.30/ 4.19	-	-	-	-	-	-	-	-	-
QTLRDW5	TAA170-NI127 (LG6)	-	-	-	40.21/ 4.28	-	-	-	-	40.21	6.66	11.02	0.02
QTLRDW6	ICCM0249-H1G20 (LG6)	-	-	-	52.06/ 4.18	-	-	-	-	52.06	5.65	11.5	0.02
QTLRDW7	TA25-CaM1274 (LG8)	-	-	-	-	-	-	13.18/ 3.31	-	-	-	-	-
Rooting depth (RD)													
QTLRD1	NI189-TA71 (LG7)	-	-	-	-	10.33/ 3.28	-	-	-	-	-	-	-
RDW / Total Dry Weight Ratio (RT)													
QTLRT1	NI184-ICCM0009b (LG1)	-	-	6.01/ 3.04	8.01/ 3.38	-	-	-	-	-	-	-	-
QTLRT2	NI21-TAA170 (LG6)	37.30/ 7.27	37.30/ 5.67	37.30/ 3.53	37.30/ 11.03	39.30/ 3.72	-	39.30/ 4.77	39.30/ 9.14	39.30	16.18	28.57	-1.30
QTLRT3	ICCM0249-H1G20 (LG6)	50.06/ 3.98	-	-	-	-	52.06/ 3.87	-	-	-	-	-	-

Contd…

Root Length (RL)													*…Contd*
QTLRL1	NI136-NI263 (LG1)	-	-	57.83/ 5.23	57.83/ 4.38	-	-	-	-	-	-	-	-
QTLRL2	GA34-CaM0500 (LG3)	-	20.37/ 3.12	-	-	-	-	-	-	-	-	-	-
QTLRL3	NI21-TAA170 (LG6)	39.30/ 4.62	39.30/ 9.16	-	-	-	-	37.30/ 3.08	37.30/ 6.16	39.30	11.97	21.33	360.27
QTLRL4	TAA170-NI127 (LG6)	-	-	40.21/ 7.65	40.21/ 9.35	-	-	-	-	-	-	-	-
QTLRL5	TA46-ICCM0249 (LG6)	-	-	-	-	-	-	47.71/ 3.28	-	-	-	-	-
QTLRL6	ICCM0249-H1G20 (LG6)	52.06/ 4.51	-	52.06/ 4.19	52.06/ 8.44	54.06/ 3.01	-	-	-	50.06	6.34	10.89	256.38
Root Length Density (RLD)													
QTLRLD1	NI136-NI263 (LG1)	-	-	-	57.83/ 4.01	-	-	-	-	-	-	-	-
QTLRLD2	GA34-CaM0500 (LG3)	-	20.37/ 3.10	-	-	-	-	-	-	-	-	-	-
QTLRLD3	NI91-GA24 (LG6)	-	-	2.01/ 4.18	-	-	-	-	-	-	-	-	-
QTLRLD4	NI21-TAA170 (LG6)	39.3/ 4.52	39.3/ 9.15	-	-	-	-	-	37.3/ 5.01	39.3	12.46	21.36	0.019
QTLRLD5	TAA170-NI127 (LG6)	-	-	40.21/ 7.63	40.21/ 9.43	-	-	-	-	-	-	-	-
QTLRLD6	TA46-ICCM0249 (LG6)	-	-	-	-	-	-	47.71/ 4.10	-	-	-	-	-
QTLRLD7	ICCM0249-H1G20 (LG6)	52.06/ 4.36	52.06/ 3.83	52.06/ 4.17	52.06/ 8.40	-	-	-	-	50.06	7.02	11.34	0.014
Root Surface Area (RSA)													
QTLRSA1	CaM1742-CaM0403 (LG1)	-	-	-	56.56/ 3.23	-	-	-	-	-	-	-	-
QTLRSA2	NI136-NI263 (LG1)	-	-	57.83/ 4.65	-	-	-	-	-	-	-	-	-

Contd…

…*Contd*

QTLRSA3	NI4-NI202 (LG5)	-	-	21.72/ 3.90	-	-	-	-	-	-	-	-	-
QTLRSA4	NI21-TAA170 (LG6)	39.30/ 3.92	37.30/ 5.82	-	39.30/ 9.52	-	-	-	37.30/ 3.39	39.3	8.79	15.71	52.31
QTLRSA5	TAA170-NI127 (LG6)	-	-	40.21/ 5.70	-	-	-	-	-	-	-	-	-
QTLRSA6	TA46-ICCM0249 (LG6)	49.71/ 3.03	-	-	-	-	-	-	-	-	-	-	-
QTLRSA7	ICCM0249-H1G20 (LG6)	-	-	-	52.06/ 5.81	56.06/ 3.39	-	-	-	52.06	3.31	7.10	34.88
QTLRSA8	NI170-CaM1918 (LG8)	23.55/ 3.65	-	-	-	-	-	-	-	-	-	-	-
Root Volume (RV)													
QTLRV1	CaM0144-ICCM0297 (LG1)	-	-	60.57/ 3.56	-	-	-	-	-	-	-	-	-
QTLRV2	NI21-TAA170 (LG6)	37.30/ 3.10	35.30/ 3.69	-	39.30/ 4.72	-	-	-	-	37.30	5.59	12.82	0.70

LG-linkage group; respective LGs are written in parenthesis. cM- centi Morgan genetic distance; R- replication;
There are three replications (RI, RII, RIII) each for years 2005 and 2007. PV%- phenotypic variation explained in percent;
LOD- logarithm of odds; A- additive effect; NA-not applicable

4.3.2.1 QTLs for shoot dry weight (SDW)

A total of six QTLs (*QTLSDW1*, *QTLSDW2*, *QTLSDW3, QTLSDW4, QTLSDW5* and *QTLSDW6)* were identified for shoot dry weight (SDW). The QTL "*QTLSDW1"* flanked by markers GA26 and NI4 was identified at LOD value of 3.25 on LG5 using phenotypic data pooled over means of the years 2005 and 2007. Further, adjacent to markers flanking"*QTLSDW1",* another QTL "*QTLSDW2"* flanked by markers NI4 and NI202 was observed in RII of year 2007. The remaining four QTLs (*QTLSDW3, QTLSDW4, QTLSDW5* and *QTLSDW6)* were detected on LG6 defined by marker intervals - STMS11-NI21, NI21-TAA170, TA46-ICCM0249 and ICCM0249-H1G20 respectively. Among the six QTLs detected for SDW, *QTLSDW4* (with marker interval NI21-TAA170) and *QTLSDW6* (marker interval ICCM0249-H1G20) were found to be stable (with >20% phenotypic variation) and consistent across the years. The QTL "*QTLSDW4*" explained the highest phenotypic variation (49.9%) at LOD value of 28.66. Based on the above results, the QTL "*QTLSDW4"* was considered as the major (showing > 20% phenotypic variation) consistent QTL for shoot dry weight that can be used further for marker-assisted selection (MAS).

4.3.2.2 QTLs for stem dry weight (StDW)

A total of four QTLs (*QTLStDW1, QTLStDW2, QTLStDW3* and *QTLStDW4*) were identified for StDW present on LG6. The QTL "*QTLStDW1"* was detected at marker interval STMS11-NI21 in case of RIII of year 2005 data. A major and consistent QTL "*QTLStDW2"* was detected in case of all the three replications and mean data calculated over replications from year 2005. This QTL is flanked by markers NI21 and TAA170 with 47.75% phenotypic variation. The third QTL "*QTLStDW3"* was detected only in case of RII of year 2005 flanked by markers TA46 and ICCM0249. The QTL "*QTLStDW4"* was

detected in case of RI, RIII and mean calculated over replications of year 2005. As the phenotypic data for StDW for the year 2007 was not available, consistency of the QTLs identified could not be tested across the years. Nevertheless, based on the above results, the QTL "*QTLStDW2"* was found to be major (showing >20% phenotypic variation) and consistent across the replications for stem dry weight.

4.3.2.3 QTLs for leaf dry weight (LDW)

The phenotypic data of leaf dry weight available only for the year 2005 was used to identify QTLs. Three QTLs namely *QTLLDW1, QTLLDW2* and *QTLLDW3* were identified for the trait LDW. The QTL "*QTLLDW1"* was identified on LG6 flanked by marker loci STMS11 and NI21 was observed in case of RIII of year 2005. QTL "*QTLLDW2"* was detected in case of all replications and mean calculated over replications on LG6 flanked by markers NI21 and TAA170.The phenotypic variation explained by this QTL was 31.8% based on mean data. Similarly, *"QTLLDW3"* was identified on LG6 at marker interval "ICCM0249-H1G20" with phenotypic variation of 18.16% for the mean calculated over replications. Therefore, the QTL "*QTLLDW2"* was considered as the major QTL contributing 31.8% phenotypic variation at LOD value of 16.10.

4.3.2.4 QTLs for root dry weight (RDW)

A total of seven QTLs namely (*QTLRDW1, QTLRDW2, QTLRDW3, QTLRDW4, QTLRDW5, QTLRDW6* and *QTLRDW7*) were identified for root dry weight based on QTL analysis for phenotyping data for two years. The QTL "*QTLRDW1"* was detected on LG1 flanked by markers ICCM0009a/b and STMS21. No QTL was identified either in the year 2005 or the year 2007 on LG2B, but interestingly the QTL"*QTLRDW2"*was detected on LG2B using data pooled over the years. The QTL "*QTLRDW3"* was detected on LG5 defined by marker interval GA26-NI4 in RIII of year 2005. Three other QTLs (*QTLRDW4,*

QTLRDW5, QTLRDW6) were detected on LG6 defined by marker intervals NI21-TAA170, TAA170-NI127 and ICCM0249-H1G20 respectively. The QTL"*QTLRDW4"*was detected in case of only mean data calculated over replications of year 2005, while as the other two QTLs-"*QTLRDW5*and *QTLRDW6"* were detected in case of mean data calculated over replications of 2005 and the pooled mean calculated across years 2005 and 2007. The QTL "*QTLRDW7"* was observed on LG8 for RIII of year 2007 flanked by markers TA25 and CaM1274. In summary, the QTL "*QTLRDW6",* contributed the highest phenotypic variation (11.53%) for RDW at LOD of 5.65.

4.3.2.5 QTLs for rooting depth (RD)

A single QTL (*QTLRD1)* was observed on LG7 flanked by markers NI189 and TA71 for RI of year 2007 data of rooting depth. The phenotypic variation explained by this single QTL was 6.3% at LOD value of 3.2.

4.3.2.6 QTLs for the ratio of RDW to total dry weight (RT)

QTL analysis identified three QTLs (*QTLRT1, QTLRT2* and *QTLRT3)* for RT. The QTL "*QTLRT1"*flanked by markers NI184 and ICCM0009a/b was identified in RIII and mean of year 2005. The remaining two QTLs*QTLRT2* and *QTLRT3* were detected on LG6 defined by marker intervals "NI21-TAA170" and "ICCM0249-H1G20" respectively. While the QTL "*QTLRT2*" was detected across all replications, means and pooled mean except for RII of 2007, the QTL *"QTLRT3"* was detected in RI of year 2005 and RII of year 2007. The highest phenotypic variation (28.57%) at LOD value of 16.18 was observed in pooled mean calculated across years.

4.3.2.7 QTLs for root length (RL)

A total of six QTLs (*QTLRL1, QTLRL2, QTLRL3, QTLRL4, QTLRL5* and *QTLRL6)* were identified for root length across different replications of the years 2005 and 2007 and the averages of the replications taken across the years and their pooled means. The QTL "*QTLRL1"* flanked by markers NI136 and NI263 was detected on LG1 while the QTL *"QTLRL3"* was observed on LG3 flanked by GA34 and CaM0500. The remaining four QTLs were detected on LG6 defined by marker intervals NI21-TAA170, TAA170-NI127, TA46-ICCM0249 and ICCM0249-H1G20 respectively. Among all QTLs observed for RL, two QTLs "*QTLRL3"* and *"QTLRL6"* were detected in more than four replications of two years and also in the pooled mean. A major and stable QTL "*QTLRL3"* contributing to the phenotypic variation of 21.33% was observed at LOD value of 11.97.

4.3.2.8 QTLs for root length density (RLD)

In total, seven QTLs (*QTLRLD1, QTLRLD2, QTLRLD3, QTLRLD4, QTLRLD5, QTLRLD6* and *QTLRLD7*) were detected for RLD. The QTL *"QTLRLD1"* was identified in the mean of year 2005 defined by marker interval NI136-NI263 on LG1; the QTL "*QTLRLD2"* was observed in RII (of year 2005) defined by marker interval "GA34-CaM0500" on LG3; the QTL "*QTLRLD3"* in RIII (of year 2005) defined by marker interval "NI91-GA24" on LG6; the QTL "*QTLRLD4"* was observed in case of RI, RII (of year 2005), mean (of year 2007) and pooled mean on LG6 defined by marker interval "NI21-TAA170"; the QTL "*QTLRLD5"* was detected in RIII and mean of year 2005; the QTL "*QTLRLD*6" was observed on LG6 in RIII of year 2007; and the QTL "*QTLRLD*7" was detected on LG6 for all replications of year 2005 and pooled mean computed across the years. Based on the above results, two QTLs "*QTLRLD4"* and *"QTLRLD7"*were considered as the consistent QTLs detected across the replications. However, the QTL "*QTLRLD*4" was considered as

the major QTL explaining the highest phenotypic variation (21.36%) at 12.46 LOD value in pooled mean calculated across the years.

4.3.2.9 QTLs for root surface area (RSA)

As many as eight QTLs (*QTLRSA1, QTLRSA2, QTLRSA3, QTLRSA4, QTLRSA5, QTLRSA6, QTLRSA7* and *QTLRSA8)* were identified for RSA. Two QTLs "*QTLRSA1"* and*"QTLRSA2"*were detected on LG1 defined by marker intervals "CaM1742-CaM0403" and "NI136-NI263" respectively. The QTL"*QTLRSA3"* was detected on LG5 flanked by markers NI4 and NI202 identified in RIII of year 2005. The four QTLs "*QTLRSA4, QTLRSA5, QTLRSA6, QTLRSA7"* were detected on LG6 with the marker intervals NI21-TAA170, TAA170-NI127, TA46-ICCM0249, ICCM0249-H1G20 respectively. The remaining QTL"*QTLRSA8"* was detected on LG8 flanked by markers NI170 and CaM1918. Two QTLs "*QTLRSA4"* and *"QTLRSA7"* were detected in more than three replications and mean data. Among these, "*QTLRSA4"* explained highest phenotypic variation of 15.71% at LOD value of 8.79.

4.3.2.10 QTLs for root volume (RV)

A total of two QTLs (*QTLRV1* and *QTLRV2)* were detected for root volume. The QTL "*QTLRV1"* was detected in RIII of year 2005 defined by marker interval "CaM0144-ICCM0297" on LG1. And the other QTL "*QTLRV2"* identified in RI, RII, mean of year 2005 and pooled mean calculated across years was defined by marker interval "NI21-TAA170" on LG6. The QTL "*QTLRV2"* explained the phenotypic variation of 12.82% at LOD value of 5.59.

In summary, seven major QTLs (*QTLSDW4, QTLSDW6, QTLStDW2, QTLLDW2, QTLRT2, QTLRL3* and *QTLRLD4)* were detected in the present study, which contributed more than

20% phenotypic variation. Highest phenotypic variation (49.9%) was explained by the QTL "*QTLSDW4"* at LOD value of 28.66 at the marker interval "NI21-TAA170" on LG6. Six of the seven major QTLs were spanned by markers NI21 and TAA170 on LG6, and one QTL "*QTLSDW6"* was defined by marker interval "ICCM0249-H1G20". Considering this into account, the genomic region defined by marker interval "NI21-TAA170" was considered as the "QTL hot-spot" region for drought tolerance traits" (Figure 11). This genomic region is being recommended as the prime genomic region for introgression drought tolerance through marker-assisted backcrossing (MABC) strategy.

4.4 Association Mapping

During the present study, two approaches were used to associate the genes/markers to drought tolerance in chickpea. One being "candidate gene sequencing approach", where the genes known to be involved in drought tolerance were used and in other approach, "genome-wide association" was studied using DArT markers.

4.4.1 Phenotypic data analyses

The statistical analysis is carried out for drought tolerance related traits obtained on reference set of chickpea in the year 2007. The values of mean, range and coefficient of variation (CV%) for all the eight traits are presented in Table 19. The CV% for genotypes included in the present study varied for different traits, with the highest CV for RV (24.42%) and lowest for RD (10.05%). Correlation coefficients were also worked out and presented in Table 20.The two-sided test of correlation showed that among the eight traits under study, there was significant correlation (P value < 0.05) between all the traits except RL-RT and RT-RLD. In the present study, significantly high, positive correlation was found with the following pairs: RDW-RD, RDW-RL, RDW-RLD, RDW-RSA, RDW-RT, RDW-RV, RDW-SDW, RD-RL, RD-RLD, RD-RSA, RD-RT, RD-RV, RD-SDW, RL-RLD, RL-

RSA, RL-RV, RL-SDW, RLD-RSA, RLD-RV, RLD-SDW, RSA-RT, RSA-RV, RSA-SDW, RT-RV, RT-SDW, RV-SDW. The correlation studies also showed the negative (significant) correlation between SDW and RT. The significant positive correlations between most of the above traits indicated their suitability for the study of marker-trait associations using common set of markers.

Table 19: Descriptive statistics for drought tolerance related root traits in the chickpea reference set

Trait	Mean	Coefficient of variation (CV%)	Range
SDW	1.87	21.36	0.78-3.2
RDW	0.68	19.27	0.29-0.9
RD	106.28	10.06	60-134.33
RT	24.10	12.06	13.78-38.42
RL	4938.16	17.21	2469.49-7867.23
RLD	0.17	14.99	0.1-0.26
RSA	738.06	19.66	332.83-1198.6
RV	9.06	24.42	3.49-16.03

The abbreviation of the traits: SDW-shoot dry weight; RDW-root dry weight; RD-rooting depth; RT- RDW/total dry weight ration; RL-root length; RLD-root length density; RSA- root surface area; RV-root volume.

Table 20: Correlation coefficients among drought tolerance related root traits in chickpea

	RDW	RD	RL	RLD	RSA	RT	RV	SDW
RDW	-							
RD	0.6867***	-						
RL	0.8321***	0.646***	-					
RLD	0.7231***	0.3248***	0.8803***	-				
RSA	0.8916***	0.6331***	0.9068***	0.7837***	-			
RT	0.2393***	0.2091***	0.0668^{NS}	-0.0336^{NS}	0.2119***	-		
RV	0.8439***	0.667***	0.7423***	0.6314***	0.9662***	0.2936***	-	
SDW	0.7169***	0.3612***	0.6924***	0.6811***	0.6396***	-0.4694***	0.6344***	-

The abbreviation of the traits: SDW-shoot dry weight; RDW-root dry weight; RD-rooting depth; RT- RDW/total dry weight ration; RL-root length; RLD-root length density; RSA- root surface area; RV-root volume. The significance of correlation was calculated at F probability value and shown as asterisks * <0.06 (at 6% probability level); ** <0.01 (at 1% probability level);
*** <0.001 (at 0.1% probability level).

4.4.2 Candidate gene based association analysis

4.4.2.1 Identification of genes involved in drought tolerance

Candidate genes involved in conferring drought tolerance in different crop plant species were identified through literature search. An attempt was made to isolate some of these genes in chickpea either using specific primers designed from chickpea sequences or using heterologous primers designed from *Medicago* sequences and the details of sequence analysis are mentioned in Table 21. The primer pairs for isolated genes were initially

screened on a set of 8 diverse chickpea genotypes (Annigeri, ICCV2, ICC 4958, ICC 1882, ICC 283, ICC 8261, ICC 4411 and ICC 10029) to identify the SNPs.

4.4.2.1.1 Isolation and sequence analysis of *CAP2* (*DREB2A* homologue)

DREB2A homologue in chickpea (also known as *CAP2* gene) and its promoter (*CAP2* promoter) were amplified using primer pairs for sequences DQ321719 and its promoter as per the reports of Shukla et al. (2006) and personal communication with Dr. Debasis Chattopadhyay (NIPGR, New Delhi).

Approximate amplicon size of the *CAP2* gene was 1 kbp while *CAP2* promoter was approximately 700 bp. PCR amplicons for the *CAP2* and its promoter were obtained on eight diverse genotypes of chickpea. The sequence analysis showed single transition nucleotide variant (C/G) at the 166th position of the reported chickpea promoter region, which is equivalent to the 124th position in the chickpea *CAP2* promoter region of the sequence data obtained in the present study (Figure 12). But no SNP was observed in *CAP2* gene.

Table 21: Summary of sequence diversity of candidate genes across eight diverse chickpea genotypes

Gene	Amplicon size	BLASTN results	e-value	SNP/Indel
CAP2 gene (*DREB2A*)	1000	1. DQ321719 (*CAP2* gene *C. arietinum*)	0.00	0 SNP
CAP2 promoter	700	-		1 SNP
Abscisic acid stress and ripening (*ASR*)	680	1. TC10668 similar to *ASR* protein homolog	2.80E-18	6 SNP, 1Indel
		2. *Medicago truncatula* clone (AC126014.6)	3.00E-29	
		3. *Prunus armeniaca* (apricot) *ASR* (U93164.1)	0.003	
Sucrose synthase (*SUSY*)	1600	1. TC96820 homologue to *SUS2*-Pea	4.00E-76	1 SNP
		2. TC94447 homologue to *SUS3* of Pea (Q9AVR8)		
		3. *Lotus japonicus* genomic DNA clone (AP009336)	4.00E-148	
		4. *Pisum sativum SUS2* (AJ001071)	2.00E-88	
Sucrose synthase (*SUSY*)	900	1. *M.truncatula SusS1* gene (AJ131964)	2.00E-20	2 SNP
		2. *Lotus japonicus* genomic DNA clone (AP009336.1)	3.00E-18	
		3. *Vigna radiata* mRNA for *SUSY*(D10266.1)	3.00E-06	
Sucrose phosphate synthase (*SPS*)	400	1. *M. truncatula* (BQ137986) *SPS* like protein	7.90E-60	6 SNP
		2. TC103232 homolog to *Medicago sativa* SPS (Q9AXK3)	9.60E-21	
Sucrose phosphate synthase (*SPS*)	400	1. CB893717 similar to *SPS2*-CRAPL	6.10E-49	0 SNP
		2. *Arabidopsis thaliana* Surose phosphate synthase (NM_100370)	4.00E-06	

4.4.2.1.2 Isolation and sequence analysis of ASR gene

Abscisic acid stress and ripening (*ASR*) gene was isolated using the heterologous primers derived from *Medicago* sequence AC152054. Amplification was carried out across eight chickpea genotypes along with one *Medicago* genotype (A17). As a result, a single amplicon of 700 bp was obtained for the chickpea genotypes used. The BLASTN analysis of the sequences obtained in chickpea showed significant similarity with TC10658 (similar to *ASR* protein homolog) in *Medicago* database of TIGR with e-value 2.8E-18. The sequences also showed similarity with *ASR* genes of *Prunus armeniaca* (apricot) *ASR* (U93164.1) and *Glycine max ASR*-like protein mRNA (AY382827). Multiple sequence

alignment (MSA) of these sequences for 8 genotypes showed presence of 5 SNPs, while one deletion of 46 bp was observed in ICCV2 genotype (Figure 13).

4.4.2.1.3 Isolation and sequence analysis of SuSy gene

For isolating the sucrose synthase (*SuSy*) gene in chickpea, heterologous primers were designed from *Medicago* sequences TC95820 (homolog to *SUS2* Pea) and AJ131964 (*Medicago truncatulaSUS1* gene). Amplification was carried out across eight chickpea genotypes along with one *Medicago* genotype (A17) and around 1500 bp amplicon was obtained in case of TC95820- derived sequences, while 900 bp amplicon was obtained in case of AJ131964- derived sequences. BLASTN analysis of TC95820-derived chickpea sequences showed significant similarity with *Medicago* sequence TC95820 (homologue to *SUS2*-Pea) with e-value 4E-75, AP009335 (*Lotus japonicus* genomic DNA clone) with e-value 4E-148 and AJ001071 (*Pisum sativum SUS2*) with e-value 2E-88. Multiple sequence alignment of these sequences showed presence of one SNP (Figure 14).

4.4.2.1.4 Isolation and sequence analysis of SPS gene

Heterologous primers designed using *Medicago* sequence BQ137986 and CB893717 were used to isolate sucrose phosphate synthase (*SPS*) in chickpea. Amplification across eight genotypes in chickpea yielded products of 400 bp in both cases. BLASTN analysis of BQ137986-derived chickpea sequences showed significant similarity with AY851389 (*Medicago sativa-SPS*) with e-value 4E-101 and BQ137986 (*M. truncatula SPS* like protein) with e-value 7.9E-50 whereas CB893717-derived sequences showed significant similarity with *Craterostigma plantagineum SPS2* with 6.1E-49 e-value. Six SNPs were observed in case of BQ137986-derived chickpea sequences while no SNP was observed in case of AY851389 derived sequences (Figure 15).

4.4.2.1.5 Isolation and sequence analysis of ERECTA gene fragments

About 4300 bp long *ERECTA* gene fragment was isolated from eight chickpea genotypes using consensus primers. In this case, five pairs of consensus nested (overlapping) primers were used from protein sequences from *ERECTA* and *ERECTA* like sequences available in the databases. The contig sequences were obtained for each genotype and BLAST analysis of the sequences showed similarity with AC1511806.21 (*M. trucatula* clone mth2-57p22) with 0.0 e-value and 91% coverage, FJ014802.1 (*Glycine max* clone cw119 *ERECTA* mRNA) with 7E-125 e-value and XM_002516098.1 (*Ricinus communis ERECTA* putative gene) with e-value 2E-99. The contig sequences obtained were used for multiple sequence alignment. The MSA showed the presence of 20 SNPs and 3 Indels across eight chickpea genotypes.

4.4.2.2 Sequence diversity analysis of candidate genes in the reference set of chickpea

A reference set (comprising 318 chickpea genotypes) derived from composite collection of chickpea based on SSR diversity studies (Upadhyaya et al. 2008), was used for anlysing the sequence diversity. A set of five candidate genes (*ASR*, *CAP2* gene and its promoter, *ERECTA*, *SPS* and *SuSy*) isolated in eight diverse chickpea genotypes were used for sequencing all 318 genotypes of the reference set. Good quality sequence data were obtained in case of *CAP2* gene, *CAP2* promoter, two fragments of *ERECTA*, *ASR* gene and *SPS* gene. The details of the sequence analysis of the candidate genes analysed using DIVersityESTimator (*DIVEST*) tool is presented in Table 22.

Table 22: Estimates on sequence diversity in the chickpea reference set for five candidate genes

Candidate gene	*ASR*	*CAP2*	*CAP2* promoter	*ERECTA*_7f_5r	*ERECTA*_8f_8r	*SPS*
Genotypes with successful sequences	193	227	137	79	147	236
Sequence length (bp)	621	367	629	921	1189	312
No. of Indels	2	0	0	1	0	1
Indel frequency	1/310.60	0	0	1/921.00	0	1/312.00
No. of SNPs	34*	0	1	13	20	3
Transition	22	0	0	9	10	2
Transversion	13	0	1	4	10	1
SNP frequency	1/18.26	0	1/629.00	1/70.86	1/69.46	1/104.00
Nucleotide Diversity (Pi)	0.0014	0	0	0.0029	0.0029	0.0011
Average PIC of SNP	0.1	0	0.43	0.27	0.1	0.01
No. of Haplotypes	4	1	2	4	3	4
Haplotype Diversity	0.833	0	0.438	0.372	0.324	0.034
PIC of Haplotypes	0.829	0	0.436	0.367	0.322	0.034

The sequence diversity was computed using '*DIVEST*' tool.
ASR-Abscisic acid stress and ripening gene; *CAP2*-Chickpea Apetala 2 gene (homolog of *DREB2A*); 7f-5r and 8f-8r are the primer combinations used to amplifying *ERECTA* genes;
SPS- sucrose phosphate synthase;
PIC-polymorphic information content;
SNP-single nucleotide polymorphism.

Number of genotypes for which good quality sequences were obtained varied from 79 (*ERECTA* fragment obtained from 7f-5r primer pairs) to 235 genotypes (*SPS* gene) out of 318 genotypes attempted The longest sequence obtained was for *ERECTA* (fragment obtained from 8f-8r primer pairs) which was about 1,189 bp and the shortest sequence was obtained in case of *SPS* gene with 312 bp. Sequence diversity analysis of these sequence data showed a varying number of SNPs. Highest number of SNPs (34) were obtained for ASR gene, amongst which 22 were of transition type variants, whereas 13 were of transversion type and one was tri-allelic. Apart from SNPs, two Indels were also detected. The *CAP2* gene was found to be conservative across all 227 genotypes with no SNPs and Indels. In case of *CAP2* promoter, one SNP was found (which was the same observed when eight chickpea genotypes were sequenced as a pilot experiment).

For *ERECTA* gene sequencing, two fragments obtained from 7f-5r and 8f-8r primer pairs were sequenced in order to find the diversity among reference chickpea genotypes. About 13 SNPs (9 transitions and 4 transversions) were obtained in case of *ERECTA* (7f-5r) while 20 SNPs (10 transitions and 10 transversions) were observed in case of *ERECTA* (8f-8r) gene fragment. Only one Indel was observed in case of *ERECTA* (7f-5r) gene fragment. One Indel and three SNPs were observed in case of *SPS* gene sequence. No nucleotide diversity was observed for *CAP2* gene and promoter while as in case of *SPS* it was 0.0011 and 0.0029 for both *ERECTA* fragments. Average polymorphic information content (PIC) value of SNPs ranged from 0 (*CAP2* gene) to 0.43 (*CAP2* promoter). One haplotype was observed in case of *CAP2* gene because of no occurrence of SNP, while single SNP in case of *CAP2* promoter yielded two haplotypes. Three haplotypes were found in case of *ERECTA* (8f-8r) and four haplotypes were found in case of *ASR*, *ERECTA* (7f-5r) and *SPS* genes. Haplotype diversity ranged from 0.324 (*ERECTA* 8f-8r) to 0.833 (*ASR*). Average (PIC) of haplotypes value ranged from 0.322 (*ERECTA* 8f-8r) to 0.829 (*ASR*).

4.4.2.3 Haplotype networks for candidate genes

Based on the sequence information, haplotype networks were drawn using program NETWORK. The network figures showed number of haplotypes observed in each gene and the SNP position which separates one haplotype from the other. The network diagrams can be drawn with presence of more than two haplotype blocks. The size of the haplotypes depends on the number of individuals forming the haplotype blocks. For example, larger the size of the haplotype (depicted as circles), number of individuals representing that haplotype are more. The colour code is given as per the origin of the genotypes. *CAP2* gene represented single haplotype with all the genotypes sequenced while *CAP2* promoter had only one SNP, forming two haplotype blocks. Hence haplotype network graphs could not be

drawn for *CAP2* gene and its promoter. In this study although we could find more than two haplotype blocks in some of the candidate genes like *ASR*, *SPS, ERECTA* (7f-5r) and *ERECTA* (8f-8r), there was no clear distinction between the origin of the genotypes and the haplotype information. There were three minor haplotypes (H1, H2, H4) derived from a major haplotype (H3) was observed in *ASR* haplotype networks with SNPs ranging from one to four (Figure 16). SPS gene haplotype network showed presence of three minor haplotypes (H1, H2 and H4) derived from H3 with single nucleotide variation (Figure 17). Similarly, in *ERECTA*- 7f-5r gene fragment, one major haplotype (H1) defined by 10 SNPs and two minor haplotypes (H3, H4) defined by single SNP were derived from major haplotype H2 [Figure 18(A)].In case of other *ERECTA* fragment (8f-8r) two haplotypes (H1 and H2) derived from H3 with 6 and 13 SNPs respectively [Figure 18(B)].

4.4.2.4 Candidate genes association with drought tolerance related traits

The candidate genes for drought tolerance defined by sequence variants and the phenotypic data on drought tolerance related traits, both obtained on reference set of chickpea were employed to find the association between trait and genes. Although during the present study five candidate genes were identified, the association with the phenotypic data was detected with only two cases namely ASR and CAP2 promoter. Five SNPs of ASR gene were found to be associated with shoot dry weight (SDW) with phenotypic variation ranging from 8.5 to 12%. Interestingly, the sole SNP found in case of CAP2 promoter was detected be associated with shoot dry weight (SDW) and root dry weight (RDW) with phenotypic variation of 19.2% and 13.48% respectively (Table 23)

.

Table 23: Association of candidate genes with drought tolerance related traits

Locus	Trait	P-value	R^2 (%)
ASR290(G/A)	SDW	0.00099	12.07
ASR291(G/C)	SDW	0.00099	12.07
ASR391(C/T)	SDW	0.00099	11.47
ASR447(C/T)	SDW	0.00099	11.43
ASR615(C/T)	SDW	0.00099	8.59
CAP2prom124(C/G)	SDW	0.00099	19.21
CAP2prom124(C/G)	RDW	0.00099	13.48

The association of candidate genes with the drought tolerance related traits was computed using TASSEL v 2.1 ASR- Abscisic acid stress and ripening gene; CAP2promo-Chickpea Apetala 2 promoter; SDW-shoot dry weight; RDW-root dry weight.
The locus name (eg. ASR290(G/A) is named as gene name (eg. ASR) is followed by position (290 bp) and type of SNP(G/A). The candidate genes associated with the trait at significant P value <0.01 were selected.

4.4.3 Genome-wide association (GWA)

Phenotypic data on eight drought tolerance related traits including shoot dry weight (SDW), root dry weight (RDW), root length (RL), root length density (RLD), RT ratio (RDW/TDW), rooting depth (RD), root surface area (RSA) and root volume (RV) were recorded in year 2007 on reference set comprising of 288 genotypes of chickpea. In order to undertake genome-wide association studies in chickpea reference set, genotyping data on 1,157 DArT markers were generated at Diversity Arrays Technology, Pty Ltd, Australia on 287 genotypes. However, phenotypic data was available for only 270/287 genotypes and were therefore, used for the study of population structure. Initially population structure was tested by checking the number of sub-populations (K) from one to fifteen (K = 1 to K =15). However, no clear-cut peak was observed when LnPD (natural log of probability of data) values were plotted against the K values, therefore, a modified method proposed by Evanno et al. (2005), was followed. In this case ΔK (delta K) was plotted against the number of sub-populations (K) (Figure 19). The analysis showed the presence of the highest ΔK value at K = 11, indicates a deep partitioning of the genetic population structure into eleven clusters. The population structure obtained at K=11 is depicted in Figure 20. The Q-matrix of the run with the highest similarity over all runs i.e., at K=11 was used for association analysis along

with the genotypic and phenotypic data using programme TASSEL version 2.1 in order to find out marker-trait associations.

Both general linear model (GLM) and mixed linear model (MLM) with 0.001% minor allele frequency were used to identify markers associated with drought related root traits. The genotyping data of only 724 DArT markers could be finally used for association studies after filtering the minor alleles. The two model approaches were compared for all traits. In general the number of significant marker-trait associations identified using GLM were much higher than associations identified using MLM at P value <0.01 (Table 24). In total 26 markers showed significant association with the eight drought-related root traits studied in the present study. Among them, 18 markers showed associations with only one trait, while as 8 markers showed associations with more than one trait. These eight markers were considered to be associated with co-localized/pleiotropic QTLs controlling more than one trait. The marker "cpPb-675943" was found associated with six traits (SDW, RDW, RL, RLD, RSA and RV), while as the marker "cpPb-677783" was associated with five traits (SDW, RDW, RL, RLD and RSA). The marker "cpPb-681914" was associated with five traits (SDW, RL, RLD, RSA and RV), while as marker "cpPb-172290" was found associated with four traits (RL, RLD, RSA and RV). Marker "cpPb-324158" was associated with three traits (RL, RSA and RV) where as marker "cpPb-491616" showed association with three traits (RD, RDW and RL). Similarly, the marker "cpPb-490159" showed association with RL and RLD where as, marker "cpPb-677787" showed association with RSA and RV (see Table 24). The phenotypic variation explained (PVE) by an individual marker was more while using GLM than for MLM. The PVE% varied from 4.66 (cpPb-675943- RSA) to 2.24 (cpPb-491143-RD) using GLM and it varied from 4.06% (cpPb-675943- RSA) to 2.11% (cpPb-677783- RLD) using MLM. The details of marker-trait associations identified through genome-wide association analysis are tabulated in Table 24.

Table 24: Association of DArT markers with drought tolerance related traits based on genome-wide association approach

Trait	Marker	GLM		MLM	
		P value (<0.01)	R^2 (%)	P value (<0.01)	R^2 (%)
Shoot Dry Weight (SDW)	cpPb-677783	0.000999	3.14	0.0068	2.46
	cpPb-676943	0.007	2.74	0.0089	2.47
	cpPb-681914	0.007	2.69	0.0087	2.39
	cpPb-490406	0.002	2.72	-	-
	cpPb-489699	0.006	2.64	-	-
Rooting Depth (RD)	cpPb-491616	0.002	3.79	0.0012	3.21
	cpPb-491143	0.01	2.24	-	-
Root Dry Weight (RDW)	cpPb-491616	0.002	3.48	0.0016	3.49
	cpPb-677692	0.003	3.06	0.0041	2.73
	cpPb-676943	0.003	3.14	0.0061	2.76
	cpPb-489261	0.006	2.73	0.0061	2.6
	cpPb-326886	0.007	2.77	0.0068	2.49
	cpPb-677783	0.007	2.61	-	-
Root Length (RL)	cpPb-676943	0.003	3.83	0.0022	3.63
	cpPb-681914	0.003	3.62	0.0024	3.3
	cpPb-324168	0.004	2.66	0.0086	2.41
	cpPb-677783	0.004	2.97	0.0069	2.66
	cpPb-172290	0.004	2.64	0.0086	2.4
	cpPb-491616	0.009	2.74	0.0067	2.74
	cpPb-490169	-	-	0.0092	2.66
	cpPb-677883	-	-	0.0096	2.32
Root length Density (RLD)	cpPb-677783	0.000999	3.84	0.0063	2.11
	cpPb-172924	-	-	0.0029	2.63
	cpPb-490169	0.003	2.98	0.0017	3.02
	cpPb-681914	0.002	3.48	-	-
	cpPb-172290	0.006	2.47	-	-
	cpPb-172160	0.006	2.93	-	-
	cpPb-676943	0.006	3.16	-	-
	cpPb-680078	0.008	2.81	-	-
	cpPb-676268	0.009	2.6	-	-
	cpPb-490017	0.01	2.61	-	-
	cpPb-491632	0.01	2.61	-	-
	cpPb-682364	0.01	2.63	-	-
Root Surface Area (RSA)	cpPb-681914	0.000999	3.82	0.0021	3.24
	cpPb-172290	0.002	3.67	0.0016	3.28
	cpPb-324168	0.003	3.63	0.0016	3.32
	cpPb-676943	0.003	4.66	7.61E-04	4.06
	cpPb-677787	0.007	2.63	0.0081	2.36
	cpPb-677783	0.01	2.71	0.0099	2.24

Contd…

…Contd

RDW/TDW ratio (RT)	cpPb-489973	0.000999	3.8	-	-
	cpPb-677200	0.003	3.78	-	-
	cpPb-488740	0.01	2.69	-	-
Root Volume (RV)	cpPb-172290	0.000999	3.64	0.0016	3.29
	cpPb-676943	0.000999	4.22	0.0013	3.67
	cpPb-681914	0.000999	3.12	0.0066	2.64
	cpPb-324168	0.003	3.64	0.0014	3.37
	cpPb-677787	0.006	2.93	0.006	2.66
	cpPb-680739	0.009	2.48	-	-

GLM-general linear model (based on Q-matrix);
MLM-mixed linear model (based on Q-matrix + kinship);
Markers used for analysis are DArT and are named as chickpea probe (cpPb);
markers associated with the trait at significant P value <0.01 were selected from both GLM and MLM;
R^2 represents the phenotypic variation explained in percent.

5. DISCUSSION

This study deals with development of SSR markers from enriched genomic DNA library as well as BAC-end sequences, integration of these markers in genetic maps, identification of QTL and genes by using linkage mapping and association genetics approaches. These results have been discussed in context of available studies.

5.1 Development of SSR Markers

SSR markers have become the markers of choice for plant genetics and breeding applications. Despite the fact that hundreds of SSR markers have been isolated in chickpea using SSR- enriched library (Hüttel et al. 1999; Sethy et al. 2006a, 2006b; Winter et al. 1999) or BAC end sequence (Lichtenzveig et al. 2005) approaches, the narrow genetic background of cultivated chickpea germplasm demands the development of SSR markers in large number so that these can be used in chickpea genetics and breeding. With an objective of enriching the SSR marker repertoire, a SSR enriched library for GA and TAA repeat motifs was constructed from *C. arietinum* ICC 4958 genotype, which is being used as a reference genotype for developing genomic and genetic resource by the chickpea community.

Sequencing of 307 putative SSR positive clones yielded 457 non-redundant genome survey sequences (GSSs) of which 299 (65.4%) GSSs provided 643 SSRs. While comparing the SSR-enrichment efficiency with other studies like groundnut where 490 SSRs were found in 720 SSR positive clones (68%) (Cuc et al. 2008), the present study also showed SSR isolation efficiency at the rate of 65%. In terms of different classes of SSRs, tri-nucleotide (40%) and di-nucleotide (39%) motifs constituted the major proportion of SSRs followed by mono-nucleotides (16%) and tetra-nucleotide (3%). Similar kind of distribution of different

SSR classes was observed in different SSR isolation studies in chickpea (Hüttel et al. 1999; Winter et al. 1999; Lichtenzveig et al. 2005). While comparing the abundance of different SSR motifs, TAA/ATT repeats were found more abundant followed by GA/CT. These observations are not surprising because: (1) the library was enriched for TAA and GA repeat motifs, and (2) TAA repeat motifs have been reported abundant in earlier studies in chickpea (Hüttel et al. 1999; Winter et al. 1999; Lichtenzveig et al. 2005) as well as other legume or plant species such as, soybean (Akkaya et al. 1992; Cregan et al. 1994), *Medicago* (Mun et al. 2006), tomato (Smulders et al. 1997) and pine (Echt and May-Marquardt 1997). This was also illustrated in a comparative study on SSRs in ESTs of different legume and cereal species (Jayashree et al. 2006) and *in silico* sequence analysis among some cereal species (Varshney et al. 2002).

In case of BAC-end sequences (BESs), only 5,123 of 46,270 BESs were detected to have 6,845 SSRs scanning one SSR per every 4.85 kb. Unlike the SSRs derived from microsatellite enriched library, the di-nucleotide SSRs (mainly "AT" rich) were most abundant compared to tri-nucleotide repeats in identified BES-SSRs. This fact corroborates the fact that AT repeat motifs are abundant in chickpea genome. BAC libraries have been targeted for isolation of SSRs in chickpea earlier (Rajesh et al. 2004; Lichtenzveig et al. 2005). BES-derived SSR markers have been developed in several other legume species like Medicago (Nam et al. 1999), soybean (Cregan et al. 1999), common bean (Vanhouten and MacKenzie 1999), etc. One of the most important advantages of the BES-SSR markers over genomic or EST-derived markers is that they serve as anchor points between genetic and physical maps. Such linkages have been shown in several crops like rice (Tao et al. 2001; Chen et al. 2002), maize (Coe et al. 2002), cotton (Xu et al. 2008), melon **(**González et al. 2010), etc. As at the time of initiation of this study, no physical map was available for

chickpea, the developed BES-SSR marker resources is an useful resource for linking the genetic map of chickpea with future physical map.

Identified SSRs through both enriched library (ICCM series) as well as BAC-end sequences (CaM series) were analyzed in terms of the length of SSR tracts as Class I SSRs (≥20 nucleotides in length) and Class II containing perfect SSRs (>12 but <20 nucleotides in length) (Temnykh et al. 2001). Among ICCM SSRs, Class I SSRs have abundance of tri-nucleotide repeats (77%) followed by di-nucleotide repeats (14%) while as Class II repeats have more penta- nucleotide repeats (55%) followed by hexa- repeats (23%). Similarly Class I CaM SSRs include higher proportion of di-nucleotide repeats (42.7%) followed by tri-nucleotide repeats (26%), while as Class II CaM SSRs include highest contribution from penta-nucleotides (65.3%) followed by hexa-nucleotides (26.1%). Availability of information on this aspect of SSRs is important for the selection of potential polymorphic SSR markers. In a parallel study carried out at ICRISAT, the ICCM and CaM markers were screened on 48 genotypes of chickpea (for detailed results, see Nayak et al. 2010). The study revealed that in case of ICCM markers, average PIC value of Class I SSRs was higher (0.38) than that of Class II SSRs (PIC = 0.22), thus demonstrated the potentiality of Class I SSRs over Class II SSRs. Similarly, in case of CaM markers, average PIC value of Class I SSRs was higher (0.21) compared to Class II SSRs (0.11). Similar results were also reported in an earlier study in rice by (Temnykh et al. 2001). Majority of the Class I SSRs contains tri-nucleotide repeats, thus indicating the importance of tri-nucleotide repeat motifs over others. In general, tri-nucleotide repeats were considered as the most polymorphic sites (Varshney et al. 2005a). In addition to tri-nucleotide repeats, compound SSRs constituted the majority of polymorphic markers during the present study. PIC values of compound SSRs (average PIC of ICCM = 0.29 and CaM = 0.27) were comparable to that of tri-nucleotide repeats. This can be attributed to the fact that the markers with compound SSRs

have more than one SSR motif, which increases their chances to be polymorphic (Ghislain et al. 2004).

While integrating newly developed SSR ICCM and CaM series markers into the inter-specific genetic map, the H-series markers, derived from BAC libraries (Lichtenzveig et al. 2005) were also attempted. During the testing of pairs of newly isolated SSR markers, a large number of them were lost because of several reasons like clone duplication, chimera formation, lack of flanking sequences for primer designing. This loss of primer pairs during marker development was termed as "attrition" by Squirrell et al. (2003). Total attrition rate of ICCM markers was 77.8% (only 22.2% were polymorphic) in inter-specific mapping population and 90.2% (only 9.8% were polymorphic) in intra-specific mapping population. The loss of primer pairs attributing to the attrition rate in CaM markers was about 79.2% (20.8% were polymorphic) in inter-specific mapping population and 91.1% (8.9% were polymorphic) in case of intra-specific mapping populations. This clearly showed that attrition rates of primer pairs in case of microsatellite enriched library and that derived from BESs were very similar to each other. Similar observations were made in case of rye while comparing microsatellite enriched library and BESs showed minor differences in the attrition rates and very high rates of attrition rate was obtained (about 90%) when chromosome specific microsatellites were studied (Kofler et al. 2008), while in case of cucurbits it was found to be 80% (Gong et al. 2008). When large number of SSR markers is required, difficulties and wastage due to clone duplication, chimera formation, lack of flanking sequence and poor amplification of PCR primers are all encountered, and can lead to massive attrition rates relative to the initial numbers of clones sequenced (up to 90%, Squirrell et al. 2003). However, with the accurate reporting of attrition rates at each step, the SSR development process can be further refined and improved to give greater efficiency of marker production.

5.1.1 Functional annotation of GSSs developed from the SSR enriched library

Similarity analysis was performed for 457 GSSs obtained from SSR enriched library using BLASTN and BLASTX algorithms, and significant similarity was determined at an Expect value threshold of ≤1E–05. Relatively few of the GSS sequences had E values that surpassed this score, irrespective of the species data set under analysis. This is consistent with the expectation that randomly selected short genomic sequences only occasionally correspond to gene coding regions that will match EST data sets. Nevertheless, in cases where BLAST hits with e-value lower than 1E–05 threshold were recorded, the degree of similarity, expressed as either nucleotide identity of deduced protein similarity, was highest for phylogenetically related species, decreasing in rank order of phylogenetic distance (i.e., *Medicago* > lotus > soybean = cowpea = common bean > poplar > Arabidopsis > rice). Among these sequences, 40 were identified as related sequences in all three analyzed cool season legumes, i.e., chickpea, *Medicago*, and Lotus (Hologalegina clade), while 29 sequences had similarity with all three analyzed warm season legumes, i.e., soybean, common bean, and cowpea (Phaseoleae clade). Only 21 sequences were identified as similar sequences in both Hologalegina and Phaseoleae species. Two of these GSSs (FI856609 and FI856659) showed significant similarity with sequences of all the plant species analyzed in the present study. This observation is evident from the evolutionary taxonomy of family Leguminosae (subfamily Papilionoideae) that crops like *Medicago* and *Lotus* are taxonomically related to chickpea and therefore, higher sequence similarity was observed with these crop species. The phylogenetic relationships in Leguminosae based on evolutionary taxonomy (Doyle 1994) and recent molecular analysis (Kajita et al. 2001), showed that the Hologalegina clade of leguminosae phylogenetic tree (Doyle and Lucknow 2003; Wojciechowski et al. 2004) consists of legume subfamilies Loteae (Lotus), Robinieae (Sesbania) and Inverted Repeat Lacking Clade (IRLC), that are characterized by loss of 25

kb chloroplast inverted repeat (*Medicago*, *Pisum*, *Trifolium*, *Cicer*), which represent economically important cool season legumes. Though, less than one-third of the SSR-associated GSS sequences had significant hits in these databases, the derived annotations add a potentially useful data type to the marker metadata. Not surprisingly, chickpea GSS (from which the SSRs were developed) had higher similarity with ESTs of other legume species, and to dicot out groups (i.e. poplar and *Arabidopsis*) than to monocot (i.e. rice) data sets.

5.2 Genetic Mapping in Chickpea

5.2.1 Comprehensive inter-specific genetic map of chickpea

Despite the availability of few hundred microsatellite markers in chickpea, surveying the genomic regions in chickpea has been considered challenging task due to the low level of polymorphism in cultivated chickpea germplasm (Buhariwalla et al. 2005; Radhika et al. 2007). Hence an inter-specific mapping population derived from ICC 4958 (*C. arietinum*) and PI 489777 (*C. reticulatum*) was used to integrate novel microsatellite (ICCM and CaM) and published H-series markers. This mapping population has been widely used in past by chickpea community in order to incorporate several hundred microsatellite markers (Winter et al. 1999, 2000; Tekeoglu et al. 2002) and gene based markers (Pfaff and Kahl 2003). The diverse genetic background of the parents showed higher degree of polymorphism not only at the genetic level but also at phenotypic levels such as resistance to *Fusarium* wilt (Winter et al. 2000) and *Ascochyta* blight (Rakshit et al. 2003), thus, facilitating their trait mapping. Therefore, this population is generally considered as the "International reference" mapping population.

The present genetic map of chickpea represents 621 marker loci that spanned 984.11 cM genetic distance. The previous chickpea genetic maps on this population represented 2,077.9

cM (Winter et al. 2000) and 2,483 cM (Pfaff and Kahl 2003). Before undertaking this study, the most accepted genetic map among inter-specific genetic map of chickpea was that of Peter Winter (Winter et al. 2000) with inter-marker distance of 7.0 cM, followed by that published by Pfaff and Kahl (2003) with inter-marker distance 8.3 cM. This study represents the most comprehensive and moderately dense genetic map spanning 984.11 cM with an average inter-marker distance of 1.58 cM. As per the estimated physical size of the chickpea genome (740 Mbp; Arumuganathan and Earle 1991), 1 cM distance in the present map equals 751 kbp, as compared to 360 kbp in case of Winter et al. (2000). It is also important to mention that genetic distance of the map is also a function of the compute programme used for development of the genetic map. For instance while publishing the part of results present in this study in Nayak et al. (2010), the most commonly used mapping programme – MAPMAKER was used. The genetic distance calculated using MAPMAKER was higher compared to JoinMap programme. Hence the map distances obtained earlier (eg. Winter et al. 2000, Nayak et al. 2010) were higher as compared to the map presented in this study, though marker order remained more or less same across the maps. The reason attributing towards this difference is kind of algorithm used in both the mapping programmes. MAPMAKER determines the mapping distance based on maximum likelihood multipoint estimates, while JoinMap uses linear regression of pair-wise distances (Lander et al. 1987; Stam 1993; Van't Hof et al. 2008).

5.2.2 Intra-specific genetic map of chickpea

The mapping population segregating for root traits was used to construct intra-specific genetic map in order to locate QTLs responsible for drought tolerance related traits. The mapping population using ICC 4958 (drought tolerant genotype) and ICC 1882 (drought sensitive genotype) was developed at ICRISAT based on previous physiological studies segregating for root traits (Kashiwagi et al. 2006, 2008; Gaur et al. 2008). The genotypes

with contrasting character for root traits were used to develop mapping population in order to dissect QTL(s) responsible for drought tolerance. The developments of SSR markers revolutionized the genetic analysis (Gupta and Varshney 2000) and opened new possibilities in the study of complex traits in the crop plant species especially crops like chickpea with narrow genetic background. Although use of inter-specific crosses segregating for important agronomic traits can be alternative option for the dissecting genes/QTLs for complex traits, it is limited due to difficulty in crossing and probable linkage drags associated with the inter-specific crosses. Nevertheless in past, inter-specific crosses have been used for analysing QTLs for simple traits like resistance to *Fusarium* wilt (Winter et al. 2000), *Ascochyta* blight (Rakshit et al. 2003) and carotenoid concentration (Abbo et al. 2005). Intra-specific crosses have been used widely in chickpea for identifying QTL(s) responsible for important agronomic traits like double podding (Cho et al. 2002; Radhika et al. 2007), *Ascochyta* blight resistance (Udupa and Baum 2003; Cho et al. 2004; Taran et al. 2007; Kottapalli et al. 2009), *Fusarium* wilt resistance (Cobos et al. 2005) and some yield related traits like seed weight (Radhika et al. 2007).

In the present study, a total of 2,409 microsatellite markers were screened on the parents of the intra-specific mapping population, of which only 307 markers were detected as polymorphic. As a result, an intra-specific genetic map was constructed with 230 SSR loci spanning 466.95 cM with an average inter-marker distance of 2.03 cM. This map is comprised of SSR markers exclusively, thus making it the first of its kind among chickpea intra-specific maps (*C. arietinum* × *C. arietinum*). Earlier reports on intra-specific maps used other types of marker systems like RAPD, RFLP, RGA and some gene-specific markers along with the SSR marker type. For instance, Cho et al. (2002) mapped 80 marker loci which included 55 SSRs, 20 RAPDs, 3 ISSRs and 2 morphological markers on linkage map derived from RILs of ICCV 2 × JG 62, while 138 marker loci including 118 RAPDs,

13 SSRs, 4 morphological markers and 3 ISSRs were mapped on the joint map derived from CA 2139 × JG 62 and CA 2156 × JG 62 RILs (Cobos et al. 2005). More recently, Kottapalli et al. (2009) mapped 84 marker loci which included 82 SSRs and 2 EST markers on F_2 population derived from cross ICC 4991 × ICCV 04516 at ICRISAT, which was considered as the highest number of SSRs mapped on any intra-specific map derived from single mapping population before this study. However the map developed in this study has surpassed any intra-specific genetic map in terms of SSR marker loci integrated so far.

When the presence of markers were compared with the inter-specific map, the LG1 of the intra-specific map corresponds to the LG1 of the inter-specific map; LG2 of intra-specific map corresponds to LG2 of inter-specific map; LG3 (intra-specific) ≡ LG6 (inter-specific); LG4 (intra-specific) ≡ LG3 (inter-specific); LG5 (intra-specific) ≡ LG7 (inter-specific); LG6 (intra-specific) ≡ LG4 (inter-specific); LG7 (intra-specific) ≡ LG5 (inter-specific) and LG8 (intra-specific) ≡ LG8 (inter-specific). However, the order of marker loci on both inter- and intra-specific maps differed in several instances. Similar observations were also made by Radhika et al. (2007) when they compared their map with the inter-specific map of Winter et al. (2000). For example, LG1 (from Radhika et al. 2007) contained the marker loci from linkage groups- III, V and XIII. This may be due to larger number of markers available recently, which may act as bridging markers to join two or more LGs from earlier published maps like Winter et al. (2000). Similar observations were also made by Flandez-Galvez et al. (2003). The difference in the genetic background can also attribute to the difference in the distribution of marker loci. By developing separate intra-specific maps for *C. arietinum* and *C. reticulatum* using common SSR markers and comparing them might provide the molecular insight of the likely chromosomal rearrangements that led to the evolution of *C. arietinum* from *C. reticulatum*.

5.3 Dissecting QTLs for Drought Tolerance

In the present study, the phenotyping data of year 2005 and year 2007 with three replications was used with the genetic mapping data for the intra-specific mapping population for QTL analysis of drought tolerance related root traits. In the year 2005 phenotypic data were available on ten drought tolerance traits while as in the year 2007, data were available for eight traits. Although, several QTL studies had been carried out in intra-specific chickpea crosses (*C. arietinum*), majority of them focussed on disease resistance like *Fusarium* wilt (Cobos et al. 2005, Sharma et al. 2004) and *Ascochyta* blight (Iruela et al. 2006; Lichtenzveig et al. 2006; Tar'an et al. 2007). This study seems to be the first study to identify the QTLs for drought tolerance traits in chickpea.

Altogether, a total of 47 significant QTLs (LOD>3) were identified, which included one QTL for RD, two QTLs for RV; three QTLs each for LDW and RT; four QTLs were detected for StDW; six QTLs were detected for SDW and RL; seven QTLs were detected for RDW and RLD and eight QTLs were detected for RSA. Interestingly, the QTL flanked by 6.9 cM marker interval "NI21-TAA170" detected on LG6 was common for all traits except for rooting depth (RD) which contributed maximum phenotypic variation upto 49.91%. Apart from this QTL, adjacent region on LG6, a genomic region of 7.7 cM distance defined by marker interval "ICCM0249-H1G20" is also contributing phenotypic variation of about 28.25% for SDW. In terms of phenotypic variation contributed by different QTLs, the QTL - *QTLSDW4* showed highest (49.9%) phenotypic variation (PV) for shoot dry weight (SDW) on pooled mean computed across all the replications of both years at a LOD score of 28.66 on LG6 flanked by marker interval "NI21-TAA170". The QTL- *QTLStDW3* for stem dry weight (StDW) explained the highest PV (47.75%) at LOD score of 23.80 at the same position on LG6. In total seven major QTLs (contributing >20% PV) were identified in this study and six of them were flanked by markers NI21 and TAA170. As

highest phenotypic variation was detected for all traits except for RD in this genomic region ("NI21-TAA170" spanning 6.9 cM map), this region has been considered as the "QTL hot-spot" for drought tolerance related traits.

One candidate marker TAA170 present in QTL hot-spot was also found associated with days to first flowering (DFF) under drought condition in an earlier study (Rehman 2009) and resistance to *Ascochyta* blight in chickpea (Aryamanesh et al. 2009). Similarly based on single marker linear regression analysis for 14 SSR markers on a set of 257 RILs (ICC 4958 × Annigeri) detected "TAA170" marker that is associated with high phenotypic variation for drought tolerance related traits like root length (R^2 = 33.1%), root weight (R^2 = 33.1%) and shoot weight (R^2 = 54.2%) (Chandra et al. 2004). The above information available from these studies supports the observation about presence of the QTL hot-spot in the region "NI21-TAA170" on LG6 of chickpea genome. It is speculated that this QTL hot-spot region may contain the presence of several transcription factors, as it was the case of sub-mergence tolerance QTL in rice (Xu et al. 2006), that may be responsible for expression of genes, conferring drought tolerance and present on other location in genome. However, this needs to be confirmed after cloning and characterization of the QTL hot-spot in coming future. Presence of transcription factors in the QTL regions for such major QTLs for complex traits like flowering time, plant architecture, sugar content, fruit weight etc. in plant species like *Arabidopsis*, rice, maize and tomato (see Paran and Zamir 2003; Salvi and Tuberosa 2005). In short-term, the QTL hot-spot can be deployed for introgressing drought tolerance in elite chickpea lines through marker-assisted selection (MAS).

5.4 Association Analysis for Drought Tolerance

In the present study association analysis is carried out using "candidate-gene approach" and "genome-wide association" approaches.

5.4.1 Candidate gene sequencing approach

Due to lack of genome sequence information of chickpea genome till recently, identification of genes responsible for complex traits like drought tolerance was a daunting task in chickpea at the time of initiation study. Drought tolerance is complex phenomena involving many tolerance mechanisms inter-related with each other. Identification of candidate genes responsible for drought tolerance was a part of an international collaborative project funded by Generation Challenge Programme (GCP) entitled "Allelic Diversity at Orthologous Candidate genes (ADOC) in seven GCP crops"- one among them was *chickpea*. An extensive survey of literature was carried out in order to enlist some of the genes which might have role in drought tolerance mechanism in crop species. Subsequently, most promising candidate genes were selected and these this included abscisic acid stress and ripening gene (*ASR*), drought responsive element binding protein (*DREB2A*) gene, *ERECTA*, sucrose synthase (*SuSy*), sucrose phosphate synthase (*SPS*) and vacuolar invertases (*VI*). However, sequence information for majority of these genes was not available in chickpea. Therefore systematic efforts by using comparative genomics and bioinformatics approaches were made to isolate the gene sequences in chickpea. For instance, *DREB2A* homologue of chickpea was isolated from sequence information available from chickpea. As *Medicago truncatula* is the known taxonomic ally of chickpea, the genomic information about *Medicago* was searched from different database including NCBI, TIGR and *Medicago* sequence repository (www.medicago.org). Putative candidate genes namely *ASR*, *SuSy* and *SPS* in chickpea were isolated using respective sequence information obtained from *Medicago* candidate gene sequences.

CAP2 gene (DREB2A homolog): Chickpea *CAP2* (homolog of DREB2A) and its promoter, known to enhance tolerance to dehydration and salt stress, were isolated, characterized and

the expression studies were carried in transgenic tobacco (Shukla et al. 2006). The sequence information was used to design nested primers in order to isolate full-length *CAP2* gene during present study. In order to identify orthologous *DREB2A* from five crop species - including three cereals (rice, barley and sorghum) and two legume species (chickpea and common bean) specific and degenerate primers derived from reconciled phylogenetic analysis approach was carried out in the extended study of the project. The study also showed extreme conservation of *DREB2* genes among different crops and *AP2* domain of the *DREB2* genes remained conserved across five species studied (see Nayak et al. 2009). *DREB* transcription factors bind to dehydration responsive element (DRE) of the genes at the promoter region and regulate the expression of downstream genes. The DRE containing core sequence A/GCCGAC was identified as a cis-acting promoter element, which regulates gene expression in response to drought, high salinity and cold stresses in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki 1994). The *DREB* proteins induce a set of abiotic stress responsive genes and maintain water balance in plant systems thus imparting abiotic stress tolerance. *DREB* proteins are transcription factors that bind to the promoter of genes such as *rd29A*, thereby inducing expression in response to drought, salt and cold (Shinozaki and Yamaguchi-Shinozaki 1997; Agarwal et al. 2006). The homolog of *DREB2A* gene (known as *CAP2* gene) and its promoter were sequenced on 318 diverse chickpea genotypes. No sequence variant, however, was observed in case of *CAP2* gene and a single SNP was observed in case of *CAP2* promoter. This shows the conservation of *CAP2* gene across chickpea genotypes. Comparative study of occurrence of SNPs at gene and promoter regions revealed that there are more SNPs in close proximity to transcriptional start sites than in regions further upstream, and the number of SNPs found in the predicted transcription factor binding sites and UTR regions is higher than in non-binding site sequences in eukaryotes including humans (Guo and Jamison 2005). Occurrence of SNP at regulatory region, accounting for the loss of function of seed shattering gene has been

already shown in case of rice, which indicates that a single sequence variant can cause a major effect on the function of a gene(s) (Konishi et al. 2006). The conservation *AP2* domain of the *DREB2A* gene was observed not only within chickpea sequences, but also across other legumes like common bean and cereals like rice, sorghum and barley (Nayak et al. 2009). Due to conserved nature of the gene, it is difficult to directly relate the genotypic variation to the phenotypic variation. However, the conformational association of these transcription factors with other proteins and/ or DNA sequences certainly affect the drought response mechanism.

Abscisic acid stress and ripening (ASR) gene: This was one of the candidate genes selected for studying sequence diversity among chickpea genotypes. The *ASR* gene family is present in Spermatophyta. Its members are generally activated under water stress. The *ASR* protein is a low molecular weight plant-specific protein encoded by an abiotic stress-regulated gene. Its expression is regulated by water stress, salt stress and plant hormone Abscisic acid (ABA). Over-expression of *ASR* gene in transgenic plants is known to induce water- and salt- stress tolerance (Kalifa et al. 2004). There is experimental evidence that tomato *ASR1*, one of the proteins of the family, accumulates in seed during late stages of embryogenesis, a physiological process characterized by water loss. *In vitro* electrophoretic assays and direct visualization of single molecules by atomic force microscopy in tomato confirmed that *ASR1* forms homodimers that bind to double stranded DNA (Maskin et al. 2007; Goldgur et al. 2007). Although *ASR* gene function is not published in case of *Medicago*, *ASR* like sequences which were similar to some of the reported *ASR* sequences in other crops were used to design primers and amplified in case of chickpea. The sequence diversity across chickpea genotypes (193 sequences) showed 34 SNPs and two indels, which found to be the highest among the candidate genes studied in the present study. The nucleotide diversity was found be 0.0014 while haplotype diversity was 0.833. The results could not be

compared with any other studies in chickpea, as there is no information available on *ASR* prior to this study. However, similar study in case of rice was carried out, where the polymorphism of four members of *ASR* gene family was studied in a worldwide collection of 204 accessions of *Oryza sativa* L. and 14 accessions of wild relatives (*O. rufipogon* and *O. nivara*). The nucleotide diversity of the *ASR* genes was globally low, but contrasted for the different genes, leading to different shapes of haplotype networks. Statistical tests for neutrality were used and compared to their distribution in a set of 111 reference genes spread across the genome, derived from another published study (Caicedo et al. 2007). *ASR3* diversity exhibited a pattern concordant with a balancing selection at the species level and with a directional selection in the tropical japonica sub-group. This study provided a thorough description of the organization of the *ASR* family, and the nucleotide and haplotype diversity of four *ASR* in *O. sativa* species. *ASR3* stood out as the best potential candidate for drought tolerance studies and the polymorphism detected represented as the first step towards an association study between genetic polymorphisms of ASR gene family and variation in drought tolerance traits (Philippe et al. 2010).

Sucrose synthase (SuSy) and Sucrose phosphate synthase (SPS): These are the enzymes involved in sugar metabolism. They are known to be up-regulated in dehydration stress (Elster 1994). *SuSy* gene in chickpea is known to be involved in increasing the seed size (Kumar and Turner 2009). Although partial *SuSy* gene was isolated using heterologous primers from *Medicago* in case of eight diverse genotypes, sequencing was failed in case of reference set; hence it is abandoned from further analysis. Since *SuSy* was proved to be a potential candidate gene for drought tolerance in many plant species like *Arabidopsis* (Baud et al. 2004), rice (Gorantla et al. 2005; Fu et al. 2007), soybean (Gonzalez et al.1995), it can be used as a potential candidate gene for drought tolerance. *SPS* gene is also known to be involved with drought tolerance in maize (Abdel-latif 2007), wheat (Fresneau et al. 2007)

and C4 resurrection plant *Sporobolus* (Whittakar et al. 2007). Putative *SPS* gene was identified in chickpea using heterologous primers from *Medicago* in the present study. The sequence analysis of this gene on the reference set of chickpea showed the presence of three SNPs and one indel represented as four haplotypes across 235 chickpea genotypes. This shows the conservation of this gene across chickpea genotypes. The study on sequence diversity on *SPS* gene is very limited. In case of sugarcane, the sequence diversity of *SPS* gene was studied between two cultivars and ten SNPs were identified in 400 bp sequenced region. These SNPs were screened on the mapping population derived from the two cultivars. The SNP frequency did not vary in the two bulked DNA samples, suggesting that SNPs from this *SPS* gene family are not associated with variation in sucrose content. Using an ecotilling approach, two of the *SPS* Gene Family III haplotypes were mapped to two different linkage groups in sugarcane (McIntyre et al. 2006).

ERECTA gene: The role of *ERECTA* genes in case of drought tolerance pertains to its involvement in stomatal density and evapotranspiration (Shpak et al. 2004; Masle et al. 2005). Two fragments of *ERECTA* genes were isolated in the present study. The sequence diversity of *ERECTA* gene is not published. As a part of GCP sponsored ADOC project, sequence diversity of ERECTA gene is identified in rice, sorghum, barley, common bean, chickpea, potato and cassava. Overall, *ERECTA* gene was found to be diverse in case of cereals as well as legumes [Masle et al (unpublished)]. In case of chickpea, in total 33 SNPs (13 from fragment obtained from *ERECTA*-7f-5r and 20 from fragment obtained from *ERECTA*-8f-8r) making 7 haplotypes (4 in *ERECTA*-7f-5r and 3 in *ERECTA*-8f-8r) were observed. Nucleotide diversity was found to be 0.0029 which was high compared all other candidate genes under study. The sequence diversity studies across reference set of chickpea, provides the insights regarding the appropriate haplotypes which could be involved in drought tolerance mechanism.

The phenotypic data on obtained on drought tolerance traits indicated the association of ASR genes with shoot dry weight (SDW) and CAP2 promoter with SDW and root dry weight (RDW). The involvement of ASR genes in sugar accumulation during stress is detected in several studies (Cakir et al. 2003; Carrari et al. 2004; Frankel et al. 2007). This fact is also proven during the present study where the association between ASR gene and shoot dry weight is detected. This might be due to the fact that accumulation of sugar increases the dry weight during stress conditions. This indicates the involvement of ASR genes in ABA-dependent pathways involved in the drought tolerance mechanisms during stress condition. Similarly, the association of CAP2 promoter (homolog of DREB2A) with SDW and RDW indicates the involvement of CAP2 gene with drought tolerance mechanism. Unlike other DREB2 genes, CAP2 gene in chickpea is known to be involved in ABA-dependent pathways for drought tolerance (Shukla et al. 2006). Single SNP at promoter region of CAP2 gene, can regulate the CAP2 gene products (i.e. transcription factor), so that it regulates drought tolerance traits.

During the present study shoot dry weight was showing the highest phenotypic variation (19.2%) for the CAP2 promoter-SDW association. Interestingly, the QTL studies based on linkage mapping approaches also showed highest phenotypic variation (49.9%) for shoot dry weight. Precise association of these genes with respect to drought tolerance can be obtained if the expression and physiological studies of these genes can be undertaken on the reference set or mapping population of chickpea.

5.4.2 Genome-wide association mapping

In total 270 genotypes (from chickpea reference set) were used in the marker-trait association analysis. The extent of variability (in terms of CV%) available for different traits

indicated suitability of reference set of chickpea for the study of marker-trait associations. The correlation studies revealed the presence of significant positive correlations between most of the root traits under study. This suggests their suitability for the study of marker-trait associations using common set of markers.

Association mapping is an innovative linkage disequilibrium based methodology to dissect quantitative traits. Although large number of markers are necessary for detecting association of complex traits using GWA, but this method does not require any prior information about genes for the traits of interest. Advantage of GWA over candidate gene sequencing approach, involves the detection of unknown loci associated with the trait. As an alternative to traditional linkage analysis, association mapping offers three advantages- i) increased mapping resolution, ii) reduced research time and iii) greater allele numbers (Yu and Buckler 2006). Since its introduction to plants (Thornsberry et al. 2001), association mapping has continued to gain favourability in genetic research. There are limited studies of association mapping in case of plant species.

Application of association-mapping approaches in plants is complicated by the population structure present in most germplasm sets (Flint-Garcia et al. 2003) to overcome this problem, linear models with fixed effects for sub-populations (Breseghello and Sorrells 2006) or a logistic regression-ratio test (Pritchard et al. 2000; Thornsberry et al. 2001) can be employed. Owing to the large germplasm sets required for dissecting complex traits, the probability increases that partially related individuals are included. This applies in particular when genotypes selected from plant-breeding populations are used for association mapping (Thornsberry et al. 2001; Kraakman et al. 2004). Association mapping identifies QTLs by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse

germplasm (Zhu et al. 2008). Association analysis was applied using structure (Q)-kinship (K) mixed-model approach (Yu et al. 2006) that promises to correct for linkage disequilibrium (LD) caused by population structure and relatedness relationship.

In the present study, an attempt was made to associate neutral DArT markers to drought related root traits like SDW, RDW, RL, RLD, RT, RD and RV using a reference set of chickpea. The likely number of sub-populations was obtained based on the delta K value derived from Evanno's method (Evanno et al. 2005). In the present study, at K=11 there was deep portioning of population into eleven sub-populations, which might be due to the selection pressure due to domestication and breeding. At K=11, delta K value was found to be maximum and this information was further used in association analysis to avoid false positives. Association analysis identified 26 significant markers for ten root traits. Among them, 18 markers showed significant association with single trait and 8 markers showed associations with more than one trait. Hence, these eight were believed to be associated with co-localized/pleiotropic QTLs. The co-localization of specific genes/QTLs/markers could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response. The presence of several co-localized/pleiotropic QTLs verified the complex quantitative nature of drought tolerance in chickpea and allowed the identification of some important genomic regions for traits related to drought tolerance. The markers associated with more than one trait may be efficiently utilized in improvement of more than one trait simultaneously through marker assisted selection (MAS). Till date there are no reports of association studies in case of chickpea, however the association studies in other crop species especially in cereals such as maize (Lu et al. 2009), barley (Malysheva-Otto et al. 2006; Cockram et al. 2008), sorghum (Shehzad et al. 2009) and wheat (Neumann et al. 2010) have revealed that the linkage based QTL analyses can be complemented by LD based association studies. Association mapping studies in legumes are limited to soybean

and *Medicago*, where association map consisting of 150 markers was constructed on the basis of differences in allele frequency distributions between the two sub-populations of soybean for seed protein trait (Jun et al. 2008) and the genome-wide association studies has been started in *Medicago* as a part of HapMap (Haplotype Map) project on 384 inbred lines (http://www.medicagohapmap.org/about.php). The phenotypic variation explained using GLM was found to be comparatively higher compared to that computed from MLM in the present study. This was also evident from studies of association mapping in case of wheat (Neumann et al. 2010) where, the GLM and MLM models were compared to give marker-trait associations. The association studies in crop species are taking advantage of development of high-throughput marker technologies like DArT, GoldenGate SNPs and advanced statistical tools.

6. SUMMARY

The present study entitled "Identification of QTLs and genes for drought tolerance using linkage mapping and association mapping approaches in chickpea (*Cicer arietinum*)" was conducted with the following objectives: (1) Development of novel set of microsatellite or simple sequence repeat (SSR) markers in chickpea, (2) Integration of novel SSR markers into the reference genetic map using a mapping population derived from the cross *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777), (3) Construction of an intra-specific (*C. arietinum*) genetic map using a mapping population derived from the cross ICC 4958 × ICC 1882 and identification of QTLs for drought associated traits by using linkage mapping approach, (4) Identification of candidate genes for drought tolerance through comparative genomics and bioinformatics approaches and study of allelic sequence diversity (single nucleotide polymorphisms) in a reference set of chickpea and (6) Identification of genes/markers associated with drought tolerance using candidate gene sequencing and genome-wide association approaches.

The conclusions from the research work are briefly summarized below.

1. A total of 1,655 novel SSR markers were developed in chickpea by using two different approaches: (a) using SSR-enriched library (311), and (b) mining the BAC-end sequences (BESs) (1,344). Development of SSR markers from SSR-enriched library has already been published in Nayak et al. 2010 (Theor Appl Genet 120:1415-1441).
2. The parental genotypes of inter-specific mapping population (ICC 4958 × PI 489777) was screened with 1,888 SSR markers including 1,655 markers developed during the present study and 233 H-series markers (Lichtenzveig et al. 2005). In total 357 SSR markers were found polymorphic and genotyping data were generated

for these markers. In addition, genotyping data were obtained for 359 markers (which include 130 SSR markers) from University of Frankfurt (Germany) and used for constructing the genetic map.

3. A comprehensive inter-specific genetic map was prepared consisting of 621 loci spanning a genetic distance of 984.11 cM (average distance = 123 cM/linkage group), with average inter-marker distance of 1.58 cM was constructed. This is probably the most comprehensive genetic map of chickpea developed so far. Part of this map has already been published by Nayak et al. 2010 (Theor Appl Genet 120:1415-1441).

4. For identification of QTLs for drought tolerance traits, the parental genotypes of the intra-specific mapping population (ICC 4958 × ICC 1882) were screened with a total of 2,409 SSR markers including 1,655 developed in this study, 280 developed at National Institute of Plant Genome Research (NIPGR), 233 H-series, 241 Winter-series markers. As a result, 307 marker showed polymorphism between parental genotyped and the genotyping data were obtained on the mapping population. Genotyping data generated for 307 SSR markers were used for constructing the genetic map.
5. An intra-specific map consisting of 230 SSR loci, spanning 466.95 cM genetic distance was constructed. This is the most dense SSR genetic map based on a single intra-specific mapping population of chickpea.
6. Genotyping data for the intra-specific mapping population was analyzed together with phenotyping data for drought tolerance traits obtained for two years i.e. 2005 (ten traits) and 2007 (eight traits). The QTL analysis detected 47 significant QTLs (LOD ≥ 3) for the ten root traits using single-locus analysis, of which seven were consistent and showed more than 20% phenotypic variation.

7. The QTL analysis revealed the presence of a "QTL hot-spot" region on LG6 that contained QTLs for several drought tolerance traits including shoot dry weight (SDW) explaining upto 49.9% phenotypic variation. This genomic region was found to contain the QTLs for all but rooting depth (RD) traits.
8. For undertaking association mapping for drought tolerance, two approaches namely candidate gene sequencing and genome-wide scanning approaches were used on the reference set comprising of 318 genotypes which was defined based on the molecular diversity of 2,915 accessions at 48 SSR loci (Upadhyaya et al. 2008).
9. In case of the candidate gene sequencing approach, five candidate genes associated with drought tolerance were selected based on their prior sequence information and published reports in different crop species. The candidate genes included chickpea Apetala2 (*CAP2*-which is the homolog of *DREB2A*), abscisic acid stress and ripening hormone (*ASR*), sucrose synthase (*SuSy*), sucrose phosphate synthase (*SPS*) and *ERECTA* genes. For isolating candidate genes, primers were designed using gene specific, degenerate and heterologous primers from *Medicago*. The part of present study on DREB2A is already published in Nayak et al. 2009 (Plant Sci 117: 460-467).
10. The sequence diversity analyses showed the conservation of *CAP2* across the reference set with no SNPs, while *CAP2* promoter sequences showed only one SNP. In case of *ASR* gene 34 SNPs were found while 3 SNPs were found in *SPS* gene. Thirteen SNPs were found in *ERECTA* gene fragment (7f-5r), while 20 SNPs were found in case of *ERECTA* (8f-8r) fragment. Nucleotide diversity was highest in case of *ERECTA* gene fragments (0.0029), followed by *ASR* (0.0014) and *SPS* (0.0011). The analysis showed the presence of four haplotypes in *ASR*, *ERECTA* (7f-5r) and *SPS* genes, while three haplotypes were detected in case of *ERECTA* (8f-8r) gene fragment and only two haplotypes were found in case *CAP2* promoter.

11. Association analysis based on candidate gene sequencing showed the association of two genes (ASR and CAP2 promoter) with drought tolerance related traits. The ASR gene was found to be associated with shoot dry weight (SDW) with 12% phenotypic variation and CAP2 promoter was found to be associated with shoot dry weight (SDW) and root dry weight (RDW) with phenotypic variation 19.21% and 13.48% respectively.
12. Genome-wide association studies using 1,157 DArT markers showed the significant association of 26 markers with eight drought tolerance related traits.

In summary, developed genomic resources such as SSR markers and genetic maps will be useful for chickpea genetics and breeding applications. Moreover, markers and genes associated with QTLs for drought tolerance related traits will be useful for molecular breeding for drought tolerance in chickpea improvement.

7. LITERATURE

Abbo S, Molina C, Jungmann R, Grusak MA, Berkovitch Z, Reifen R et al (2005) Quantitative trait loci governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). Theor Appl Genet 111:185–195

Abdel-latif A (2007) Response of maize leaf sucrose phosphate synthase to salinity research. J Agri Biol Sci 3:930-933

Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K (1997) Role of *Arabidopsis* MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. Plant Cell 9:1859-1868

Abebe T, Guenzi AC, Martin B, Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol 131:1748-1755

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their application in plant sciences. Plant Cell Rep 27:617-631

Agarwal P, Agarwal PK, Nair S, Sopory SK, Reddy MK (2007) Stress-inducible DREB2A transcription factor from *Pennisetum glaucum* is a phosphoprotein and its phosphorylation negatively regulates its DNA-binding activity. Mol Genet Genomics 277:189–198

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 21:1263–1274

Ahmad F (1999) Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual Cicer species. Theor Appl Genet 98:657–663

Ahmad F, Gaur P, Croser J (2005) Chickpea (*Cicer arietinum* L.). In: Singh R, Jauhar P (eds) Genetic Resources, Chromosome Engineering and Crop Improvement – Grain Legumes, CRC Press USA, pp. 185-214

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409-1420

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphism of simple sequence repeat DNA in soybean. Genetics 132:1131-1139

Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB (1995) Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci 35: 1439–1445

Anbessa Y, Taran B, Warkentin TD, Tullu A, Vandenberg A (2009) Genetic analyses and conservation of QTL for Ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 119:757-765

Andersen JR, Schrag T, Melchinger AE, Zein I, Lübberstedt T (2005) Validation of *Dwarf8* polymorphisms associated with flowering time in elite European inbred lines of maize (*Zea mays* L.). Theor Appl Genet 111: 206–217

Aranzana, MJ, Kim S, Zhao K, Bakker E, Horton M, Jakob K, Lister C, Molitor J, Shindo C, Tang C, Toomajian C, Traw B, Zheng H, Bergelson J, Dean C, Marjoram P, Nordborg M (2005) Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. PLoS Genet 1:e60

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9: 208-219

Aryamanesh N, Nelson MN, Yan G, Clarke HJ, Siddique KHM (2009) Mapping a major gene for growth habit and QTLs for ascochyta blight resistance and flowering time in a population between chickpea and *Cicer reticulatum*. Euphytica 173:307-319

Bahieldin A, Hesham HT, Eissa HF, Saleh OM, Ramadan AM, Ahmed IA, Dyer WE, EI-Itriby H, Modkour MA (2005) Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. Plant Physiol 123: 421-427

Bamshad M, Wooding S, Salisbury BA, Stephens JC (2004) Deconstructing the relationship between genetics and race. Nat Rev Genet 5:598–609

Barbazuk WB, Emrich SJ, Chen HD, Li L, Schnable PS (2007) SNP discovery via 454 transcriptome sequencing. Plant J 51:910–918

Basten CJ, Weir BS, Zeng ZB (1994) Zmap – a QTL cartographer. Computing Strategies and Software. Proc 5th Congr on Genetics Applied to Livestock Production: Guelph, Ontario, Canada

Battaglia M, Olvera-Carrillo Y, Garciarrubio A, Campos F, Covarrubias AA (2008) The enigmatic LEA proteins and other hydrophilins. Plant Physiol 148:6–24

Baud S, Vaultier M-N, Rochat C (2004) Structure and expression profile of the sucrose synthase multigene family in *Arabidopsis*. J Exp Bot 55:397–409

Beavis WD, Kein P (1996) Genotype-by-Environment Interaction. Kang MS, Gauch HJ (eds) CRC Press, Boca Raton, USA pp 123-149

Beckmann JS, Soller M (1990) Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. Nat Biotechnol 8:930–932

Benjamin JG, Nielsen DC (2006) Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Res 97: 248-253

Bennet J (1994) DNA-based techniques for the control of rice insects and diseases: transformation, gene tagging and DNA fingerprinting. In: Teng PS, Heong KL, Moody K (eds), Rice Pest Science and Management, IRRI, Philippines pp. 148-172

Blum A (1985) Breeding crop varieties for stress environments. Critical Rev Plant Sci 2: 199-238

Blum A, Nguyen HT (2004) Physiology and biotechnology integration for plant breeding: Epilogue. In: Nguyen HT, Blum A (eds) Physiology and Biotechnology Integration for Plant Breeding, Marcel Dekker, Inc. New York, USA

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ram-Doss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635

Breseghello F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat (Triticum aestivum L.) cultivars. Genetics 172:1165-1177

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzaez-Candelas F, Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench). Theor Appl Genet 93:190–198

Buhariwalla HK, Jayashree B, Eshwar K, Crouch JH (2005) Development of ESTs from chickpea roots and their use in diversity analysis of the *Cicer* genus. BMC Plant Biol 5:16

Caicedo AL, Williamson SH, Hernandez RD, Boyko A, Fledel-Alon A, York TL, Polato NR, Olsen KM, Nielsen R, McCouch SR, Bustamante CD, Purugganan MD (2007) Genome-wide patterns of nucleotide polymorphism in domesticated rice. PLoS Genet 3:e163

Cakir B, Agasse A, Gaillard C, Saumonneau A, Delrot S, Atanassova R (2003) A grape ASR protein involved in sugar and abscisic acid signaling. Plant Cell 15:2165-2180

Canel C, Bailey-Serres JN, Roose ML (1995) Pummelo fruit transcript homologous to ripening-induced genes. Plant Physiol 108:1323-1324

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA (2004) Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 74:106–120

Carrari F, Fernie AR, Iusem ND (2004) Heard it through the grapevine? ABA and sugar cross-talk: the ASR story. Trends Plant Sci 9: 57–59

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Res 105:1-14

Chandra Babu R, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh SK, Palchamy A, Sadasivam S, Sarkarung S, Wade LJ, Nguyen HT (2003) Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Sci 43:1457–1469

Chandra S, Buhariwalla HK, Kashiwagi J, Harikrishna S, Sridevi KR, Krishnamurthy L, Serraj R, Crouch JH (2004) Identifying QTL-linked markers in marker-deficient crops. International Crop Science Congress, Brisbane, Australia.

Chang SJ, Puryear JD, Dias MADL, Funkhouser EA, Newton RJ, Cairney J (1996) Gene expression under water deficit in loblolly pine (*Pinus taeda*): Isolation and characterization of cDNA clones. Physiol Plantarum 97:139-148

Chelkowski J, Stepien L (2001) Molecular markers for leaf rust resistance genes in wheat. J Appl Genet 42:117–126

Chen M, Presting G, Barbazuk W, Goicoechea J, Blackmon B, Fang G, Kim H, Frisch D, Yu Y, Higingbottom S, Phimphilai J, Phimphilai D, Thurmond S, Gaudette B, Li P, Liu J, Hatfield J, Sun S, Farrar K, Henderson C, Barnett L, Costa R, Williams B, Walser S, Atkins M, Hall C, Bancroft I, Salse J, Regad F, Mohapatra T, Singh, N, Tyagi A, Soderlund C, Dean R, Wing R (2002) An integrated physical and genetic map of the rice genome. Plant Cell 14:537–545

Ching A, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, Morgante M, Rafalski AJ (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet 3:19

Cho R, Mindrinos M, Richards DR, Sapolsky RJ, Anderson M, Drenkard E, Dewdney J, Reuber TL, Stammers M, Federspiel N, Theologis A, Yang WH, Hubbell E, Au M, Chung EY, Lashkari D, Lemieux B, Dean C, Lipshutz RJ, Ausubel FM, Davis RW, Oefner PJ (1999) Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. Nat Genet 23:203–207

Cho S, Chen W, Muehlbauer FJ (2004) Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to ascochyta blight. Theor Appl Genet 109:733-739

Choi H, Hong J, Ha J, Kang J, Kim SY (2000) ABFs, a family of ABA responsive element binding factors. J Biol Chem 275:1723-1730

Choi HK, Mun JH, Kim DJ, Zhu H, Baek JM, Mudge J, Roe B, Ellis N, Doyle J, Kiss GB, Young ND, Cook DR (2004b) Estimating genome conservation between crop and model legume species. Proc Natl Acad Sci USA 101:15289-15294

Chopra S, Del-Favero J, Dolferus R, Jacobs M (1992) Sucrose synthase of *Arabidopsis*: Genomic cloning and sequence characterization. Plant Mol Biol 18:131-134

Choudhary S, Sethy NK, Shokeen B, Bhatia S (2006) Development of sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Mol Ecol Notes 6:3–95

Choumane W, Winter P, Weigand F, Kahl G (2000) Conservation and variability of sequence-tagged microsatellite sites (STMSs) from chickpea (*Cicer aerietinum* L.) within the genus *Cicer*. Theor Appl Genet 101:269–278

Chourey PS, Latham MD, Still PE (1986) Expression of two sucrose synthetase genes in endosperm and seedling cells of maize: evidence of tissue-specific polymerization of protomers. Mol Gen Genet 203: 251-255

Chourey PS, Taliercio EW, Kane EJ (1991) Tissue-specific expression and anaerobically induced post-transcriptional modulation of sucrose synthase genes in *Sorghum bicolor* M. Plant Physiol 96:485-490

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963−971

Clement SL, Sharma HC, Muehlbauer FJ, Elberson LR, Mattinson DS, Fellman JK (2010) Resistance to beet armyworm in a chickpea recombinant inbred line population. J Appl Entomol 134:1-8

Cobos MJ, Fern´andez MJ, Rubio J, Kharrat M, Moreno MT, Gil J, Mill´an T (2005) Alinkagemapin chickpea (*Cicer arietinum* L.) in two populations from Kabuli × Desi crosses: Location of a resistance gene for fusarium wilt race 0. Theor Appl Genet 110:1347–1353

Cobos MJ, Rubio J, Strange RN, Moreno MT, Gil J, Millan T (2006) A new QTL for Ascochyta blight resistance in an RIL population derived from an interspecific cross in chickpea. Euphytica 149:105-111

Cockram J, White J, Leigh FJ, Lea VJ, Chiapparino E, Laurie DA, Mackay IJ, Powell W, O'Sullivan DM (2008) Association mapping of partitioning loci in barley. BMC Genetics 9:16

Coe E, Cone K, McMullen M, Chen S-S, Davis G, Gardiner J, Liscum E, Polacco M, Paterson A, Sanchez-Villeda H, Soderlund C, Wing R (2002) Access to the maize genome: an integrated physical and genetic map. Plant Physiol 128:9-12

Collard BCY, Jahufer MZZ, Brouwer JB, Pang, ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169–196

Collard BCY, Pang ECK, Ades PK, Taylor PWJ (2003) Preliminary investigation of QTLs associated with seedling resistance to Ascochyta blight from *Cicer echinospermum*, a wild relative of chickpea. Theor Appl Genet 107:719–729

Coram T, Pang E (2007) Transpirational profiling of chickpea genes differentially regulated by salicylic acid, methyl jasmonate, and aminocyclopropane carboxylic acid to reveal pathways of defence-related gene regulation. Funct Plant Biol 34:52-64

Cregan PB, Bhagwat AA, Akkaya MS, Rongwen J (1994) Microsatellite fingerprinting and mapping of soybean. Meth Mol Cell Biol 5:49–61

Cregan PB, Mudge J, Fickus EW, Marek LF, Danesh D, Denny R, Shoemaker RC, Matthews BF, Jarvik T, Young ND (1999) Targeted isolation of simple sequence repeat markers through the use of bacterial artificial chromosomes. Theor Appl Genet 98:919–928

Croser JS, Ahmad F, Clarke HJ, Siddique KHM (2003) Utilization of wild *Cicer* in chickpea improvement- Progress, constraints, and prospects. Aust J Agr Res 54: 429-444

Cuc LM, Mace E, Crouch J, Quang VD, Long TD, Varshney RK (2008) Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC Plant Biol 8:55

Dashti H, Yazdi-Samadi B, Ghannadha M, Naghavi MR, Quarri S (2007) QTL analysis for drought resistance in wheat using doubled haploid lines. Int J Agr Biol 9:98–101

de Vienne D, Leonardi A, Damerval C, Zivy M (1999) Genetics of proteome variation for QTL characterization: application to drought stress responses in maize. J Exp Bot 50:303–309

Delmer DP, Amor Y (1995) Cellulose biosynthesis. Plant Cell 7:987-1000

Devlin B, Bacanu SA, Roder K (2004) Genomic control to the extreme. Nat Genet 36: 1129 – 1130

Diab AA, Teulat-Merah B, This D, Ozturk NZ, Benscher D, Sorrells ME (2004) Identification of drought-inducible genes and differentially expressed sequence tags in barley. Theor Appl Genet 109:1417–1425

Dib C, Fause S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996). A

comprehensive genetic map of the human genome based on 5,264 microsatellties. Nature 380:152-154

Doyle JJ (1994) Phylogeny of the legume family: an approach to understanding the origins of nodulation. Annu Rev Ecol Syst 25:325-334

Doyle JJ, Lucknow M (2003) The rest of the iceberg: legume diversity and evolution in a phylogenetic context. Plant Physiol 131:900–910

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 33:751-763

Dudley JW (1993) Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. Crop Sci 33:660-668

Echt CS, May-Marquardt P (1997) Survey of microsatellite DNA in pine. Genome 40:9-17

Elster R (1994) Physiologische und molekulare charakterisierung des saccharosestoffwechsels der trockentoleranten wiederauferstehungspflanze *Craterostigma plantagineum* Hochst. PhD Thesis, Univ. Köln.

Ersoz ES, Yu J, Buckler ES (2007) Applications of linkage disequilibrium and association mapping in crop plants. In: Varshney RK, Tuberosa RT (eds) Genomics Assisted Crop Improvement. Springer, The Netherlands (Vol I) pp. 97-120

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol Ecol 14:2611-2620

Flandez-Galvez H, Ford R, Pang ECK, Taylor PWJ (2003) An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence-tagged microsatellite site and resistance gene analogue markers. Theor Appl Genet 106:1447–1456

Flint-Garcia SA, Thornsberry JM, Edward S, Buckler IV (2003) Structure of linkage disequilibrium in plants. Annu Rev Plant Biol 54:357-374

Frankel N, Carrari F, Hasson E, Iusem ND (2006) Evolutionary history of the *Asr* gene family. Gene 378:74-83

Frankel N, Hasson E, Iusem ND, Rossi MS (2003) Adaptive evolution of the water stress-induced gene Asr2 in *Lycopersicon* species dwelling in arid habitats. Mol Biol Evol 20:1955–1962

Frankel N, Nunes-Nesi A, Balbo I, Mazuch J, Centeno D, Iusem ND, Fernie AR, Carrari F (2007). *ci21A/Asr1* expression influences glucose accumulation in potato tubers. Plant Mol Biol 63:719-730

Fresneau C, Ghashghaie J, Cornic G (2007) Drought effect on nitrate reductase and sucrose-phosphate synthase activities in wheat (*Triticum durum* L.): role of leaf internal CO_2. J Exp Bot 58:2983-2992

Fu BY, Xiong JH, Zhu LH, Zhao XQ, Xu HX, Gao YM, Li YS, Xu JL, Li ZK (2007) Identification of functional candidate genes for drought tolerance in rice. Mol Genet Genomics 278:599-609

Fu H, Park WD (1995) Sink- and vascular-associated sucrose synthase functions are encoded by different gene classes in potato. Plant Cell 7:1369-1385

Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Takagi MO, Tran LSP, Yamaguchi-Shinozaki K, Shinozaki K (2004) A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress signalling pathway. Plant J 39:863-876

Gaur PM, Krishnamurthy L, Kashiwagi J (2008) Improving drought-avoidance root traits in chickpea (*Cicer arietinum* L.): Current status of research at ICRISAT. Plant Prod Sci 11:3-11

Gaur PM, Krishnamurthy M, Kashiwagi J (2008) Improving drought-avoidance root traits in chickpea (*C. arietinum*)- current status of research at ICRISAT. Plant Prod Sci 11: 3-11

Gaur PM, Slinkard AE (1990a) Inheritance and linkage of isozyme coding genes in chickpea. J Hered 81:455-461

Gaur PM, Slinkard AE (1990b) Genetic control and linkage relations of isozyme markers in chickpea. Theor Appl Genet 80: 648-656

Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR (2001) Drought- and salt-tolerant plants result from overexpression of the AVP1 H+-pump. Proc Natl Acad Sci USA 98:11444-11449

Geigenberger P, Langenberger S, Wilke I, Heineke D, Heldt HW, Stitt M (1993) Sucrose is metabolized by sucrose synthase and glycolysis within the phloem of *Ricinus communis* L. seedlings. Planta 190:446-452

Geigenberger P, Stitt M (1993) Sucrose synthase catalyzes a readily reversible reaction in vivo in developing potato tubers and other plant tissues. Planta 189: 329-339

Ghislain M, Spooner DM, Rodroguez F, Villamon F, Nffloez J, Vosquez C, Waugh R, Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor Appl Genet 108:881–890

Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF (1998) Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. Plant J 16:433–442

Giombini MI, Frankel N, Iusem ND, Hasson E (2009) Nucleotide polymorphism in the drought responsive gene Asr2 in wild populations of tomato. Genetica 136:13–25

Goldgur Y, Rom S, Ghirlando R, Shkolnik D, Shadrin N, Konrad Z, Bar-Zvi D (2007) Desiccation and Zinc binding induce transition of tomato Abscisic Acid Stress Ripening 1, a water stress- and salt stress-regulated plant-specific protein, from unfolded to folded state. Plant Physiol 143:617–628

Gong L, Stift G, Kofler R, Pachner M, Lelley T (2008) Microsatellites for the genus Cucurbita and an SSR-based genetic linkage map of Cucurbita pepo L. Theor Appl Genet 117:37–48

González EM, Gordon AJ, James CL, Arrese-Igor C (1995) The role of sucrose synthase in the response of soybean nodules to drought. J Exp Bot 46:1515-1523

González VM, Garcia-Mas J, Arús P, Puigdomènech P (2010) Generation of a BAC-based physical map of the melon genome. BMC Genomics 11:339

Gorantla M, Babu PR, Lachagari VBR, Feltus FA, Paterson AH, Reddy AR (2005) Functional genomics of drought stress response in rice: transcript mapping of annotated unigenes of an indica rice (*Oryza sativa* L cv Nagina 22). Curr Sci 89:496–514

Gu YQ, Yang C, Thara VK, Zhou J, Martin GB (2000) *Pti4* is induced by ethylene and salicylic acid, and its product is phosphorylated by Pto kinase. Plant Cell 12:771–786

Guo Y, Jamison CD (2005) The distribution of SNPs in human gene regulatory regions. BMC Genomics 6:140

Gupta PK, Roy JK, Prasad M (2001) Single nucleotide polymorphism: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. Curr Sci 80:524–535

Gupta PK, Rustgi S (2004) Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct Integr Genom 4:139-162

Gupta PK, Rustgi S, Kulwal PL (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol Biol 57:461–485

Gupta PK, Rustgi S, Mir RR (2008) Array-based high-throughput DNA markers for crop improvement. Heredity 101:5–18

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Gupta PK, Varshney RK (2004) Cereal genomics: an overview. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer Academic Publishers, The Netherlands pp. 1-18

Gupta PK, Varshney RK, Prasad M (2002) Molecular markers: principles and methodology. In Jain SM, Ahloowalia BS, Brar DS (eds) Molecular Techniques in Crop Improvement. Kluwer Academic Publishers, The Netherlands pp. 9-54

Haake V, Cook D, Riechmann JL, Pineda O, Thomashow MF, Zhang JZ (2002) Transcription factor *CBF4* is a regulator of drought adaptation in *Arabidopsis*. Plant Physiol 130:639–648

Hajj Moussa E, Millán T, Moreno MT, Gil J (2000) Genetic analysis of seed size, plant height, days to flower and seeds/plant by using both morphological and molecular markers in chickpea. J Genet Breed 54:101–107

Haley SD, Afanador L, Kelly JD (1994). Selection for monogenic pest resistance traits with coupling- and repulsion phase RAPD markers. Crop Sci 34:1061-1066

Hansen M, Kraft T, Ganestam S, Sall T, Nilsson N (2001) Linkage disequilibrium mapping of the bolting gene in sea beet using AFLP markers. Genet Res 77:61–66

Hanson CH, Robinson HG, Comstock RE (1956) Biometrical studies of yield in segregating populations of Korean Lespediza. Agron J 48:268-272

Hartl D, Jones E (2001) Genetics: Analysis of Genes and Genomes, Jones and Bartlett Publishers, Sudbury, MA, USA

Hauser MT, Harr B, Schlottere C (2001) Trichome distribution in *Arabidopsis thaliana* and its close relative. Mol Biol Evol 18: 1754-63

Hill WG, Robertson A (1968) Linkage disequilibrium in finite populations. Theor Appl Genet 38:226 – 231

Hong SH, Kim IJ, Yang DC, Chung WI (2002) Characterization of an abscisic acid responsive gene homologue from *Cucumis melo*. J Exp Bot 53:2271-2272

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci 103:12987-12992

Huang JC, Lin SM, Wang CS (2000) A pollen-specific and desiccation-associated transcript in *Lilium longiflorum* during development and stress. Plant Cell Physiol 41:477-485

Huber SC, Huber JL (1996) Role and regulation of sucrose-phosphate synthase in higher plants. Annu Rev Plant Physiol Plant Mol Biol 47: 431-444

Hüttel B, Winter P, Weising K, Choumane W, Weigand F (1999) Seguence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217

Ingram J, Chandler J, Gallagher L, Salamini F, Bartels D (1997) Analysis of cDNA clones encoding sucrose-phosphate synthase in relation to sugar interconversions associated

with dehydration in the resurrection plant *Craterostigma plantagineum* Hochst. Plant Physiol 115:113–121

Iruela M, Rubio J, Barro F, Cubero JI, Millán T, Gil J (2006) Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance. Theor Appl Genet 112:278–287

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of rice DREB1/ CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol 47:1–13

Iusem ND, Bartholomew DM, Hitz WD, Scolnik PA (1993) Tomato (*Lycopersicon esculentum*) transcript induced by water deficit and ripening. Plant Physiol 102:1353-1354

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:e25

Jander G, Norris SR, Rounsley SD, Bush DF, Levin IM, Last RL (2002) Arabidopsis map-based cloning in the post-genome era. Plant Physiol 129: 440–450

Jansen R, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Jayashree B, Bhanuprakash A, Jami A, Reddy SP, Nayak S, Varshney RK (2009) Perl module and PISE wrappers for the integrated analysis of sequence data and SNP features. BMC Res Notes 2:92

Jayashree B, Punna R, Prasad P, Bantte K, Hash CT, Chandra S, Hoisington DA, Varshney RK (2006) A database of simple sequence repeats from cereal and legume expressed sequence tags mined *in silico*: survey and evaluation. *In Silico* Biol 6:0054

Jeanneau M, Gerentes D, Foueillassar X, Zivy M, Vidal J, Toppan A, Perez P (2002) Improvement of drought tolerance in maize: towards the functional validation of the *Zm-Asr1* gene and increase of water use efficiency by over-expressing C4-PEPC. Biochimie 84:1127-1135

Johnson E, Miklas PN, Stavely JR, Martinez-Crusado JC (1995) Coupling-and repulsion phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean. Theor Appl Genet 90:659-664

Jones N, Ougham H, Thomas H, Pasakinskiene I (2009) Markers and mapping revisited: finding your gene. New Phytol 183:935–966

Joseph M, Gopalakrishnan S, Sharma RK, Singh VP, Singh AK, Singh NK, Mohapatro T (2004) Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Mol Breed 13: 377-387

Jun T-H, Van K, Kim MY, Lee HS, Walker DR (2008) Association analysis using SSR markers to find QTL for seed protein content in soybean. Euphytica 162:179-191

Kajita T, Ohashi H, Tateishi Y, Bailey CD, Doyle JJ (2001) rbcL and legume phylogeny, with particular reference to phaseoleae, millettieae, and allies. Syst Botany 26:515-536

Kalifa Y, Perlson E, Gilad A, Konrad Z, Scolnik PA, Bar-Zvi D (2004) Over-expression of the water and salt stress-regulated *Asr1* gene confers an increased salt tolerance. Plant Cell Environ 27:1459–1468

Kashiwagi J, Krishnamurthy L, Crouch, JH and Serraj R (2006) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Field Crops Res 95:171-181

Kashiwagi J, Krishnamurthy L, Gaur PM, Chandra S, Upadhyaya HD (2008) Estimation of gene effects of the drought avoidance root characteristics in chickpea (*C. arietinum* L.). Field Crops Res 105:64-69

Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Krishna H, Chandra S, Vadez V, Serraj R (2005) Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146:213–222

Kaspar TC, Stanley CD, Taylor HM (1978) Soybean root growth during the reproductive stages of development. Agron J 70:1105-1107

Kavi Kishor PB, Hong Z, Miao GH, Hu CAA, Verma DPS (1995) Overexpression of d-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol 25:1387-1394

Kazan K, Muehlbauer FJ, Weeden NF, Ladizinsky G (1993) Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theor Appl Genet 86:417-426

Kearsey M, Pooni H (1996) The genetical analysis of quantitative traits. Chapman and Hall, London.

Khanna-Chopra R, Sinha SK (1987) Chickpea: physiological aspects of growth and yield. In Saxena MC, Singh KB (eds) The Chickpea Wallingford, CAB International, UK pp. 163–189

Kholova J, Hash CT, Lava Kumar P, Yadav RS, Kocova M, Vadez V (2010) Terminal drought-tolerant pearl millet [Pennisetum glaucum (L.) R. Br.] have high leaf ABA and limit transpiration at high vapour pressure deficit. J Exp Bot 61:1431–1440

Kim S, Zhao K, Jiang R, Molitor J, Borevitz JO, Nordborg M, Marjoram P (2006) Association mapping with single-feature polymorphisms. Genetics 173:1125–1133

Kleines M, Elster R-C, Rodrigo M-J, Blervacq A-S, Salamini F, Bartels D (1999) Isolation and expression analysis of two stress-responsive sucrose-synthase genes from the resurrection plant *Craterostigma plantagineum* Hochst. Planta 209:13–24

Kobayashi F, Ishibashi M, Takumi S (2008) Transcriptional activation of Cor/Lea genes and increase in abiotic stress tolerance through expression of a wheat DREB2 homolog in transgenic tobacco. Transgenic Res 17:755–767

Kobayashi F, Takumi S, Kume S, Ishibashi M, Ohno R, Murai K, Nakamura C (2005) Regulation by *Vrn-1*/*Fr-1* chromosomal intervals of CBF- mediated *Cor*/*Lea* gene expression and freezing tolerance in common wheat. J Exp Bot 56:887– 895

Koebner RMD, Powell W, Donini P (2001) The contribution of current and forthcoming DNA molecular marker technologies to wheat and barley genetics and breeding. Plant Breed Rev 21:181-220

Kofler R, Bartos J, Gong L, Stift G, Suchánkova P , Simkova H, Berenyi M, Burg K, Dolezel J, Lelley T (2008) Development of microsatellite markers specific for the short arm of rye (*Secale cereale* L.) chromosome 1. Theo Appl Gen 117:915-926

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403-410

Konishi S, Izawa T, Lin SY, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. Science 312:1392–1396

Konrad Z, Bar-Zvi D (2008) Synergism between the chaperone-like activity of the stress regulated ASR1 protein and the osmolyte glycine-betaine. Planta 227:1213–1219

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Kottapalli P, Gaur PM, Katiyar SK, Crouch JH, Buhariwalla HK, Pande S, Gali KK (2009) Mapping and validation of QTLs for resistance to an Indian isolate of Ascochyta blight pathogen in chickpea. Euphytica 165:79–88

Kraakman ATW, Niks RE, van den Berg PM, Stam P, Van Eeuwijk FA (2004) Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168:435–446

Krishnamurthy L, Johansen C, Ito O (1996) Genetic variation in root system development and its implications for drought resistance in chickpea. In: Ito O, Johansen C, Adu-Gyamfi JJ, Katayama K, Rao K, JVDK, Rego TJ (eds), Root and Nitrogen in Cropping Systems of the Semi-arid Tropics. Culio Corporation, Tsukuba, Japan, pp. 234– 250

Krishnamurthy L, Kashiwagi J, Upadhyaya HD, Serraj R (2003) Genetic diversity of drought-avoidance root traits in the mini-core germplasm collection of chickpea. Intl Chickpea and Pigeonpea Newsletter 10:21-24

Kumar A, Turner NC (2009) Growth and sucrose synthase activity of developing chickpea (*Cicer arietinum* L.) seeds uder field conditions. Austr J Crop Sci 3:20-27

Kumar J, Abbo S (2001) Genetics of flowering time in chickpea and its bearing on productivity in semi arid environments. Adv Agron 72:107-138

Labdi M, Robertson LD, Singh KB, Charrier A (1996) Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphisms. Euphytica 88:181–188

Ladizinsky G, Adler A (1976) The origin of chickpea (*Cicer arietinum* L.). Euphytica 25:211-217

Lander E, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Green P (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Laporte MM, Shen B, Tarczynski MC (2002) Engineering for drought avoidance: Expression of maize NADP-malic enzyme in tobacco results in altered stomatal function. J Exp Bot 53:699-705

Lee M (1995) DNA markers and plant breeding programs. Adv Agron 55:265-344

Leport L, Turner NC, French RJ, Tennant D, Thomson BD, Siddique KHM (1998) Water relations, gas exchange and growth of cool-season grain legumes in a Mediterranean-type environment. Euro J Agron 9:295-303

Lewontin RC (1988) On measures of gametic disequilibrium. Genetics 120:849 – 852

Lezar S, Myburg AA, Berger DK, Wingfield MJ, Wingfield BD (2004) Assessment of microarray-based DNA fingerprinting in *Eucalyptus grandis*. Theor Appl Genet 109:1329-1336

Li L, Paulo M-J, Strahwald J, Lübeck J, Hofferbert H-R, Tacke E, Junghans H, Wunder J, Draffehn A, van Eeuwijk F, Gebhardt C (2008) Natural DNA variation at candidate loci is associated with potato chip color, tuber starch content, yield and starch yield. Theor Appl Genet 116:1167–1181

Li Z, Mu P, Li C, Zhang H, Li Z, Gao Y, Wang X (2005) QTL mapping of root traits in a doubled haploid population from a cross between upland and lowland japonica rice in three environments. Theor Appl Genet 110:1244–1252

Lichtenzveig J, Bonfil DJ, Zhang HB, Shtienberg D, Abbo S (2006) Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to *Didymella rabiei* the causal agent of Ascochyta blight. Theor Appl Genet 113:1357–1369

Lichtenzveig J, Scheuring C, Dodge J, Abbo S, Zhang HB (2005) Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea, *Cicer arietinum* L. Theor Appl Genet 110:492–510

Lincoln S, Daly M, Lander E (1993) Constructing genetic linkage maps with MAPMAKER/EXP. Version 3.0. Whitehead Institute for Biomedical Research Technical Report, 3rd Edn

Liu B (1998) Statistical Genomics: Linkage, Mapping and QTL Analysis. CRC Press, Boca Raton, USA

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 10:1391–1406

Lu Y, Yan J, Guimara˜es CT, Taba S, Hao Z, Gao S, Chen S, Li J, Zhang S, Vivek BS, Magorokosho C, Mugo S, Makumbi D, Parentoni SN, Shah T, Rong T, Crouch JH, Xu Y (2009) Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. Theor Appl Genet 120:93–115

Lu Y, Yan J, Guimarães CT, Taba S, Hao Z, Gao S, Chen S, Li J, Zhang S, Vivek BS (2009) Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. Theor Appl Genet 120:93-115

Ludlow MM, Muchow RC (1990) A critical evaluation of the traits for improving crop yields in water limited environments. Adv Agron 43:107-153

Mace ES, Xia L, Jordan DR, Halloran K, Parh DK, Huttner E, Wenzl P, Kilian A (2008) DArT markers: diversity analyses and mapping in *Sorghum bicolor*. BMC Genomics 9:26

Mace ES, Buhariwalla HK, Crouch JH (2003) A high throughput DNA extraction protocol for tropical molecular breeding programs. Plant Mol Biol Rep 21:459a–459h

Malysheva-Otto LV, Ganal MW, Röder MS (2006) Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (Hordeum vulgare L.). BMC Genetics 7:6

Manly KF, Cudmore RH, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 12:930–932

Mantri NL, Ford R, Coram TE, Pang CK (2007) Transcriptional profiling of chickpea genes differentially regulated in response to high-salinity, cold and drought. BMC Genomics 8:303

Marana C, Garcia-Olmedo F, Carbonero P (1990) Differential expression of two types of sucrose synthase-encoding genes in wheat in response to anaerobiosis, cold shock and light. Gene 88:167-172

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie TP, Jirage KB, Kim J-B, Knight JR, Lanza JR, Leamon JH, Lefk owitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376

Maskin L, Frankel N, Gudesblat G, Demergasso MJ, Pietrasanta LI, Iusem ND (2007) Dimerization and DNA-binding of ASR1, a small hydrophilic protein abundant in plant tissues suffering from water loss. Biochem Biophys Res Commun 352:831–835

Maskin L, Gubesblat GE, Moreno JE, Carrari FO, Frankel N, Sambade A, Rossi M, Iusem ND (2001) Differential expression of the members of the *Asr* gene family in tomato (*Lycopersicon esculentum*). Plant Sci 161:739–746

Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. Nature 436:866-870

Mather KA, Caicedo AL, Polato NR, Olsen KM, McCouch S, Purugganan MD (2007) The extent of linkage disequilibrium in rice (*Oryza sativa* L.). Genetics 177:223–2232

Maughan PJ, Maroof MAS, Buss GR (1995) Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. Genome 38:715-723

Mayer MS, Tullu A, Simon CJ, Kumar J, Kaiser WJ, Kraft JM, Muehlbauer FJ (1997) Development of a DNA marker for fusarium wilt resistance in chickpea. Crop Sci 37:1625-1629

McCouch SR, Doerge RW (1995) QTL mapping in rice. Trends Genet 11:482-486

McIntyre CL, Jackson M, Cordeiro GM, Amouyal O, Hermann S, Aitken KS, Eliott F, Henry RJ, Casu RE, Bonnett GD (2006) The identification and characterisation of alleles of sucrose phosphate synthase gene family III in sugarcane. Mol Breed 18:39-50

McKersie BD, Bowley SR, Harjanto E, Leprice O (1996) Water-deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiol 111:1177-1181

Medina J, Bargues M, Terol J, P´erez-Alonso M, Salinas J (1999) The *Arabidopsis CBF* gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. Plant Physiol 119:463–469

Millan T, Clarke HJ, Siddique KHM, Buhariwalla HK, Gaur PM, Kumar J, Gil J, Kahl G, Winter P (2006) Chickpea molecular breeding: New tools and concepts. Euphytica 147, 81-103

Millan T, Rubio J, Iruela M, Daly K, Cubero JI, Gil J (2003) Markers associated with *Ascochyta* blight resistance in chickpea and their potential in marker-assisted selection. Field Crops Res 84:373-384

Millan T, Winter P, Jüngling R, Gil J, Rubio J, Cho S, Cobos MJ, Iruela M, Rajesh PN, Tekeoglu M, Kahl G, Muehlbauer FJ (2010) A consensus genetic map of chickpea (*Cicer arietinum* L.) based on 10 mapping populations. Euphytica 175:175-189

Miller AK, Galiba G, Dubcovsky J (2006) A cluster of 11 *CBF* transcription factors is located at the frost tolerance locus *Fr- Am2* in *Triticum monococcum*. Mol Genet Genomics 275:193– 203

Mitra J (2001) Genetics and genetic improvement of drought resistance in crop plants. Curr Sci 80:758-763

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997). Genome mapping, molecular markers and marker assisted selection in crop plants. Mol Breed 3:87-93

Moinuddin and Khanna-Chopra R (2004) Osmotic adjustment in chickpea in relation to seed yield and yield parameters. Crop Sci 44:449–455

Moreau L, Charcosset A, Gallais A (2004) Use of trial clustering to study QTL x environment effects for grain yield and related traits in maize. Theor Appl Genet 110:92–105

Morgan JM, Rodriguez-Maribona B, Knights EJ (1991) Adaptation to water-deficit in chickpea breeding lines by osmoregulation: Relationship to rain yields in the field. Field Crops Res 27:61-70

Morgante M, Oliveri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3: 175–182

Morgante M, Salamini F (2003) From plant genomics to breeding practice. Curr Opin Biotechnol 14:214-219

Mun JH, Kim DJ, Choi HK, Gish J, Debellé F, Mudge J, Denny R, Endré G, Dénarié J, Kiss GB, Roe B, Young ND, Cook DR (2006) Distribution of microsatellites in the genome of *Medicago truncatula*: A resource of genetic markers that integrate genetic and physical maps. Genetics 172:2541-2555

Nam YW, Penmetsa RV, Endre G, Uribe P, Kim D, Cook DR (1999) Construction of a bacterial artificial chromosome library of *Medicago truncatula* and identification of clones containing ethylene-response genes. Theor Appl Genet 98:3-4

Nasu S, Suzuki J, Ohta R, Hasegawa K, Yui R, Kitazawa N, Monna L, Minobe Y (2002) Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza sativa, Oryza rufipogon) and establishment of SNP markers. DNA Res 31:163-71

Nayak SN, Jayashree B, Upadhyaya HD, Hash CT, Kavi Kishor PB, Chattopadhyay D, Rodriquez LM, Blair MW, Baum M, McNally KL, This D, Hoisington DA, Varshney RK (2009) Isolation and sequence analysis of DREB2A homologues in three cereals and two legume species. Plant Sci 117: 460-467

Nayak SN, Zhu H, Varghese N , Choi HK, Datta S, Horres R, Jüngling R, Singh J, Kavi Kishor PB, Kahl G, Winter P, Cook DR, Varshney RK (2010) Integration of novel SSR and gene-based marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. Theor Appl Genet 120:1415-1441

Neff MM, Turkand E and Kalishman M (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet 18:613-615

Neumann K, Kobiljski B, Denčić S, Varshney RK, Börner A (2010) Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). Mol Breed DOI: 10.1007/s11032-010-9411-7

Nguyen TT, Taylor PWJ, Redden RJ, Ford R (2004) Genetic diversity estimates in Cicer using AFLP analysis. Plant Breed123:173-79

Nordborg M, Tavare S (2002) Linkage disequilibrium: What history has to tell us. Trends Genet 18:83–90

Novelli VM, Takita MA, Machado MA (2004) Identification and analysis of single nucleotide polymorphisms (SNPs) in citrus. Euphytica 138:227–237

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim Y-K, Kim NY-K, Nahm BH, Kim J-K (2005) Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138:341–351

Palomino C, Fernández-Romero MD, Rubio J, Torres A, Moreno MT, Millán T (2009) Integration of new CAPS and dCAPS-RGA markers into a composite chickpea genetic map and their association with disease resistance. Theor Appl Genet 118:671–682

Panse VG, Sukhatme PV (1961) Statistical methods for agricultural workers. ICAR, New Delhi, India

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985-93

Paran I, Zamir D (2003) Quantitative traits in plants: beyond the QTL. Trends Genet 19: 303-306

Parsons BL, Heflich RH (1997) Genotypic selection methods for the direct analysis of point mutations. Mutation Res 387:97-121

Paterson AH (1996) Making genetic maps. In: Paterson AH (eds) Genome Mapping in Plants, R. G. Landes Company, Austin, Texas, USA pp. 23–39

Pfaff T, Kahl G (2003) Mapping of gene-specific markers on the genetic map of chickpea (*Cicer arietinum* L.). Mol Genet Genomics 269:243–251

Philippe R, Courtois B, McNally KL, Mournet P, El-Malki R, Paslier MCL, Fabre D, Billot C, Brunel D, Glaszmann JC, This D (2010) Structure, allelic diversity and selection of *Asr* genes, candidate for drought tolerance, in *Oryza sativa* L. and wild relatives. Theor Appl Genet DOI 10.1007/s00122-010-1348-z

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1: 215–222

Price AH, Young EM, Tomos AD (1997) Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa*). New Phytol 137:83-91

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38:904–909

Prioul JL, Quarrie S, Causse M, de Vienne D (1997) Dissecting complex physiological functions through the use of molecular quantitative genetics. J Exp Bot 48:1151-1163

Pritchard JK (2001) Deconstructing maize population structure. Nat Genet 28:203–204

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association mapping in structured populations. Am J Hum Genet 67:170–181

Pundir RPS, Rao NK, van der Maesen LJG (1985) Distribution of qualitative traits in the world germplasm of chickpea (*Cicer arietinum* L.). Euphytica 34:697-673.

Qadir SA, Datta S, Singh NP, Kumar S (2007) Development of highly polymorphic SSR markers for chickpea (*Cicer arietinum* L.) and their use in parental polymorphism. Indian J Genet 67:329-333

Quarrie SA (1996) New molecular tools to improve the efficiency of breeding for increased drought resistance. Plant Growth Regul 20:167-178

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tubersosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Doding D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring _ SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Radhika P, Gowda S, Kadoo N, Mhase L, Jamadagni B, Sainani M, Chandra S, Gupta V (2007) Development of an integrated intraspecific map of chickpea (*Cicer arietinum* L) using two recombinant inbred line populations. Theor Appl Genet 115:209–216

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94-100

Rafalski A, Morgante M (2004) Corn and humans: recombination and linkage disequilibrium in two genomes of similar size. Trends Genet 2:103-111

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet 9:275-80

Rajesh PN, Coyne C, Meksem K, DerSharma K, Gupta V, Muehlbauer FJ (2004) Construction of a *Hin*dIII bacterial artificial chromosome library and its use in identification of clones associated with disease resistance in chickpea. Theor Appl Genet 108:663–669

Rajesh PN, McPhee K, Muehlbauer FJ (2005) Detection of polymorphism using CAPS and dCAPS markers in two chickpea genotypes. Intl Chickpea and Pigeonpea Newsletter 12:4–6

Rajesh PN, Muehlbauer FJ (2008) Discovery and detection of single nucleotide polymorphism (SNP) in coding and genomic sequences in chickpea (*Cicer arietinum* L.). Euphytica 162:291-300

Rajesh PN, Tullu A, Gil J, Gupta VS, Ranjekar PK, Muehlbauer FJ (2002) Identification of an STMS marker for the doublepodding gene in chickpea. Theor Appl Genet 105:604–607

Rakshit S, Winter P, Tekeoglu M, Juarez Muñoz J, Pfaff T, Benko-Iseppon AM, Muehlbauer FJ, Kahl G (2003) DAF marker tightly linked to a major locus for *Ascochyta* blight resistance in chickpea (*Cicer arietinum L.*). Euphytica 132:23-30

Ratnaparkhe MB, Santra DK, Tullu A, Muehlbauer FJ (1998) Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theor Appl Genet 96: 348–353

Rehman AU (2009) Characterization and Molecular Mapping of Drought Tolerance in Kabuli Chickpea (*Cicer arietinum* L.) PhD thesis, University of Saskatchewan, Saskatoon, Canada

Ribaut JM, Banziger M, Betran J, Jiang C, Edmeades GO, Dreher K and Hoisington D (2002) Use of molecular markers in plant breeding: drought tolerance improvement in tropical maize. In: Kang MS(eds) Quantitative Genetics, Genomics, and Plant Breeding, CABI Publishing, Wallingford, USA pp. 85-99

Riccardi F, Gazeau P, de Vienne D, Zivy M (1998) Protein changes in response to progressive water deficit in maize. Plant Physiol 117:1253-1263

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, PilgrimM, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) *Arabidopsis* transcription factor: genome wide comparative analysis among eukaryotes. Science 290:2105–2110

Risch N (1992) Genetic linkage: Interpreting LOD scores. Science 255: 803–804.

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517

Robin S, Pathan MS, Courtois B, Lafitte R, Carandang S, Lnceraas S, Amante M, Nguyen HT, Li Z (2003) Mapping osmotic adjustment in an advanced back-cross inbred population of rice. Theor Appl Genet 107:1288–1296

Robinson HF (1966) Quantitative genetics in relation to breeding on the centennial of Mendelism. Indian J Genet 26:171-187

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90:43-48

Rostoks N, Ramsay L, Mackenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney RK, Marshall DF, Graner A, Close TJ, Waugh R (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc Natl Acad Sci USA 103:18656-18661

Ryan J (1997) A global perspective on pigeonpea and chickpea sustainable production systems: present status and future potential. In: Asthana A, Ali M (eds) Recent

Advances in Pulses Research, Indian Society for Pulses Research and Development, India pp. 1–31

Saijo Y, Hata S, Kyozuka J, Shimamoto K, Izui K (2000) Over-expression of a single Ca2+-dependent protein kinase confers both cold and salt/ drought tolerance on rice plants. Plant J 23:319-327

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004) *Arabidopsis* Cys2/His2-type zinc-finger proteins function as transcription repressors under drought-, cold-, and high-salinity-stress conditions. Plant Physiol 136: 2734-2746

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006a) Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. Plant Cell 18:1292–1309

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10:297-304

Sambrook J, Russell DR (2001) Molecular Cloning: A Laboratory Manual (Third Edition), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA

Sanguineti MC, Tuberosa R, Landi P, Salvi S, Macaferri M, Casarini E, Conti S (1999) QTL analysis of drought-related traits and grain yield in relation to genetic variation for leaf abscisic acid concentration in field-grown maize. J Exp Bot 50:1289-1297

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ, Muehlbauer FJ (2000) Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. Crop Sci 40:1606–1612

Saranga Y, Jiang C-X, Wright RJ, Yakir D, Paterson AH (2004) Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. Plant Cell Environ 27:263–277

Saxena NP (2003) Management of drought in chickpea-holistic approach. In: Saxena NP (eds) Management of Agricultural Drought-Agronomic and Genetic options. Oxford and IBH publishing Co.Pvt Ltd. New Delhi, India pp. 103-122

Saxena NP, Johansen C, Saxena MC and Silim SN (1993) Selection for drought and salinity tolerance in cool-season food legumes. In: Singh KB, Saxena MC (eds) Breeding for Stress Tolerance in Cool Season Food Legumes. John Wiley and Sons, Chichester, UK pp. 245–270

Schmid KJ, Torjek O, Meyer R, Schmuths H, Hoffmann MH, Altmann T (2006) Evidence for a large-scale population structure of *Arabidopsis thaliana* from genome-wide single nucleotide polymorphism markers. Theor Appl Genet 112:1104-1114

Schneider A, Salamini F, Gebhardt C (1997) Expression patterns and promoter activity 406 of the cold-regulated gene *ci21A* of potato. Plant Physiol 113:335-345

Semagn K, Bjornstad A, Ndjiondjop MN (2006a) An overview of molecular marker methods for plants. African J Biotech 5:2540-2568

Semagn K, Bjornstad A, Skinnes H, Maroy AG, Tarkegne Y, William M (2006b) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome 49:545-555

Serraj R, Bidinger FR, Chauhan YS, Seetharama N, Nigam SN, Saxena NP (2003) Management of drought in ICRISAT cereal and legume mandate crops. In: Kijne JW, Barker R, Molden D (eds) Water productivity in agriculture: Limits and opportunities for improvement. CAB International, Wallingford, UK pp. 127-144

Serret MD, Udupa SM, Weigand F (1997) Assessment of genetic diversity of cultivated chickpea using microsatellite derived RFLP markers: implications for origin. Plant Breed 116:573–578

Sethy NK, Choudhary S, Shokeen B, Bhatia S (2006a) Identification of microsatellite markers from *Cicer reticulatum*: molecular variation and phylogenetic analysis. Theor Appl Genet 112:347– 357

Sethy NK, Shokeen B, Bhatia S (2003) Isolation and characterization of sequence-tagged microsatellite sites markers in chickpea (*Cicer arietinum* L.). Mol Ecol Notes 3:428–430

Sethy NK, Shokeen B, Edwards KJ, Bhatia S (2006b) Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). Theor Appl Genet 112:1416-1428

Sharma KD, Winter P, Kahl G, Muehlbauer FJ (2004) Molecular mapping of *Fusarium oxysporum* f. sp. *ciceris* race 3 resistance gene in chickpea. Theor Appl Genet 108:1243-1248

Sharma PC, Hüttel B, Winter P, Kahl G, Gardner RC, Weising K (1995) The potential of microsatellites for hybridization-and polymerase chain reaction-based DNA fingerprinting of chickpea (*Cicer arietinum* L.) and related species. Electrophoresis 16:1755–1761

Sharma PC, Winter P, Bünger T, Hüttel B, Weigand F, Weising K, Kahl G (1995) Abundance and polymorphism of di-, tri-, and tetra- nucleotide tandem repeats in chickpea (*Cicer arietinum* L.). Theor Appl Genet 90:90–96

Shehzad T, Okuizumi H, Kawase M, Okuno K (2009) Development of SSR-based sorghum (*Sorghum bicolor* (L.) Moench) diversity research set of germplasm and its evaluation by morphological traits. Gen Res Crop Evol 56: 809-827

Shen B, Zhang JY, Zhang KQ, Dai WM, Lu Y, Fu LQ, Ding JM, Zheng KL (2007) QTL mapping of chlorophyll contents in rice. Agric Sci China 6:17-24

Shen G, Pang Y, Wu W, Deng Z, Liu X, Lin J, Zhao L, Sun X, Tang K (2005) Molecular cloning, characterization and expression of a novel *Asr* gene from *Ginkgo biloba*. Plant Physiol Biochem 43:836-843

Shinozaki K, Yamaguchi-Shinozaki K (1997) Gene expression and signal transduction in water-stress response. Plant Physiol 115:327–334

Shpak ED, Berthiaume CT, Hill EJ, Torii KU (2004) Synergistic interaction of three ERECTA-family receptor-like kinases controls *Arabidopsis* organ growth and flower development by promoting cell proliferation. Development 131:1491-1501

Shukla RK, Raha S, Tripathi V, Chattopadhyay D (2006) Expression of CAP2, an AP2-family transcription factor from chickpea enhances growth and tolerance to dehydration and salt tress in transgenic tobacco. Plant Physiol 142:113–123

Siddique KHM, Brinsmead RB, Knight R, Knights EJ, Paull JG, Rose IA (2000) Adaptation of chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) to Australia. In: Knight R (eds) Linking Research And Marketing Opportunities for Pulses in the 21st Century. Kluwer Academic Publishers, The Netherlands pp. 289–303

Silhavy D, Hutvagner G, Barta E, Banfalvi Z (1995) Isolation and characterization of a water-stress-inducible cDNA clone from *Solanum chacoense*. Plant Mol Biol 27:587-595

Silim SN, Saxena MC (1993) Adaptation of spring-sown chickpea to the Mediterranean basin: Response to moisture supply. Field Crops Res 34: 121-136

Simon CJ, Muehlbauer FJ (1997) Construction of a chickpea linkage map and its comparison with maps of pea and lentil. J Hered 88:115-119

Singh KB (1997) Chickpea (*Cicer arietinum* L.). Field Crops Res 53:161-170

Singh KB, Omar M, Saxena MC, Johansen C (1997) Screening for drought resistance in spring chickpea in the Mediterranean region. J Agron Crop Sci 178:227-235

Singh KB, Reddy MV (1991) Advances in disease resistance breeding in chickpea. Adv Agron 45:191

Singh R, Sharma P, Varshney RK, Sharma SK, Singh NK (2008) Chickpea improvement: role of wild species and genetic markers. Biotechnol Genet Eng Rev 25: 267-314

Singh U (1985) Nutritional quality of chickpea (*Cicer arietinum* L.): current status and future research needs. Plant Foods Human Nutrition 35:339-351

Skinner JS, Zitzewitz J, Sz˝ucs P, Marquez-Cedillo L, Filichkin T, Amundsen K, Stockinger EJ, Thomashow MF, Chen THH, Hayes PM (2005) Structural, functional, and phylogenetic characterization of a large *CBF* gene family in barley. Plant Mol Biol 59:533–551

Smulders MJM, Bredemeijer G, Rus-Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 97:264–272

Somvanshi VS (2009) Patenting drought tolerance in organisms. Recent Pat DNA Gene Seq 3:16-25

Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Germann M, Orf JH, Lark KG (2001) Soybean response to water: A QTL analysis of drought tolerance. Crop Sci 41:493-509

Sponchiado BN, White JW, Castillo JA, Jones PG (1989) Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp Agric 25: 249-257

Squirrell J, Hollingsworth PM, Woodhead M, Russell J, Lowe AJ, Gibby M, Powell W (2003) How much effort is required to isolate nuclear microsatellites from plants? Mol Ecol 12:1339–1348

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. Hort Sci 31:729–741

Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA 94:1035–1040

Sudupak MA, Akkaya MS, Kence A (2002) Analysis of genetic relationships among perennial and annual Cicer species growing in Turkey using RAPD markers. Theor Appl Genet 105:1220–1228

Talebi R, Jelodar NB, Fayyaz F, Mardi M (2009) AFLP-based fingerprinting of diverse chickpea genotypes: origin, history of chickpea. New Biotechnol 25: S302

Talebi R, Naji AM, Fayaz F (2008) Geographical patterns of genetic diversity in cultivated chickpea (*Cicer arietinum* L.) characterized by amplified fragment length polymorphism. Plant Soil Environ 54:447–452

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11:63-68

Tao Q, Chang Y-L, Wang J, Chen H, Islam-Faridi MN, Scheuring C, Wang B, Stelly DM, Zhang H-B (2001) Bacterial artificial chromosome-based physical map of the rice genome constructed by restriction fingerprint analysis. Genetics 158:1711–1724

Tar'ran B, Warkentin TD, Tullu A, Vandenberg A (2007) Genetic mapping of Ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using a simple sequence repeat linkage map. Genome 50:26-34

Taylor PWJ, Ford R (2007) Chickpea genome mapping. In: Kole C (eds) Genome Mapping and Molecular Breeding in Plants Vol III. Pulses, Sugar and Tuber Crops. Springer, Tokyo, Japan pp. 109-122

Tekeoglu M, Rajesh PN, Muehlbauer FJ (2002) Integration of sequence tagged microsatellite sites to the chickpea genetic map. Theor Appl Genet 105:847–854

Tekeoglu M, Santra DK, Kaiser WJ, Muehlbauer FJ (2000a) Ascochyta blight resistance inheritance in three chickpea recombinant inbred line populations. Crop Sci 40:1251–1256

Tekeoglu M, Tullu A, Kaiser WJ, Muehlbauer FJ (2000) Inheritance and linkage of two genes that confer resistance to fusarium wilt in chickpea. Crop Sci 40:1247–1251

Temnykh S, Declerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441-1452

Thiel T, Kota R, Grosse I, Stein N, Graner A (2004) SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development. Nucleic Acids Res 32:e5

This D, Courtois B, Philippe R, Mournet P, Billot C, Glaszmann JC, Schaftleitner R, Simon R, Rojas P, Bonierbale M, Varshney R, Hash CT, Upadhyaya H, Nayak S, Brunel D, Malki RE, Paslier MCL, McNally K, Baum M, Choumane W, Korff MV, Blair M, Fregene M (2010) Some data on allele diversity at orthologous candidate genes in GCP crops. Plant & Animal Genomes XVIII Conference, January 9-13, Town & Country Convention Center San Diego, CA, USA poster P234

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995a) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly

linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8:785-794

Thomas S, Fukai A and Hammer GL (1995b) Growth and yield responses of barley and chickpea to water stress under three environments in South Queensland. II Root growth and soil water extraction pattern. Aust J Agric Res 46:17-33

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D (2001) *Dwarf8* polymorphisms associate with variation in flowering time. Nat Genet 28:286–289

Toker C, Lluch C, Tejera NA, Serraj R, Siddique KHM (2007) Abiotic Stresses. In: Yadav SS, Redden R, Chen W, Sharma B (eds) Chickpea Breeding and Management. CAB International, UK pp. 474-496

Tran LSP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Functional analysis of *Arabidopsis* NAC transcription factors controlling expression of Erd1 gene under drought stress. Plant Cell 16:2481-2498

Tuberosa R, Coraggio I (2004) In: Klee P, Chrisou P (eds) Handbook of Plant Biotechnology. John Wiley & Sons, UK pp. 413-468

Tuberosa R, Frascaroli E, Salvi S, Sanguineti MC, Conti S, Landi P (2005) QTLs for tolerance to abiotic stresses in maize: Present status and prospects. Maydica 50:559–570

Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S., S. 2002. Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. Annals Bot 89:941-963

Tullu A, Muehlbauer FJ, Kaiser WJ, Simon CJ, Mayer MS, Kumar J, Kraft JM (1998) Inheritance and linkage of a gene for resistance to race 4 of Fusarium wilt and RAPD markers in chickpea. Euphytica 102:227–232

Turner NC, Begg JE (1978) Responses of pasture plants to water deficits. In: Wilson JR (eds) Plant Relations in Pastures, CSIRO, Melbourne, Australia pp. 50-66

Turner, N.C., Wright, G.C. and Siddique, K.H.M. (2001) Adaptation of grain legumes (pulses) to water-limited environments. Adv Agron 71:193-231

Udupa SM, Baum M (2003) Genetic dissection of pathotypespecific resistance to ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor Appl Genet 106:1196–1202

Udupa SM, Robertson LD, Weigand F, Baum M, Kahl G (1999) Allelic variation at (TAA)*n* microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. Mol Gen Genet 261:354–363

Udupa SM, Sharma A, Sharma AP, Pai RA (1993) Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. J Plant Biochem Biotechnol 2:83–86

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) *Arabidopsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci USA 97:11632-11637

Upadhyaya HD, Dwivedi SL, Baum M, Varshney RK, Udupa SM, Gowda CLL, Hoisington D, Singh S (2008) Genetic structure, diversity and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). BMC Plant Biol 8:106

V´ag´ujfalvi A, Aprile A, Miller A, Dubcovsky J, Delugu G, Galiba G, Cattivelli L (2005) The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost resistance in wheat. Mol Genet Genomics 274:506–514

Vaidyanathan R, Kuruvilla S, Thomas G (1999) Characterization and expression pattern of an abscisic acid and osmotic stress responsive gene from rice. Plant Sci 140:21-30

van der Maesen LJG (1972) '*Cicer* L.' A Monograph of the Genus with Special Reference to Chickpea (*Cicer arietinum* L.), its Ecology and Cultivation. (Mededelingen Landbouwhoge school: Wageningen)

van der Maesen LJG (1987) Origin, history and taxonomy of chickpea. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, UK pp. 11–34

Van K, Onoda S, Kim MY, Kim KD, Lee SH (2008) Allelic variation of the Waxy gene in foxtail millet [*Setaria italica* (L.) P. Beauv.] by single nucleotide polymorphisms. Mol Genet Genom 279:255–266

Van K, Hwang EY, Kim MY, Park HJ, Lee S-H, Cregan PB (2005) Discovery of SNPs in soybean genotypes frequently used as the parents of mapping populations in the United States and Korea. J Hered 96:529-535

Van't Hof AE, Marec F, Saccheri IJ, Brakefield PM, Zwaan BJ (2008) Cytogenetic characterization and AFLP-based genetic linkage mapping for the butterfly *Bicyclus anynana*, covering all 28 karyotyped chromosomes. PLoS ONE 3: e3882

Vanhouten W and MacKenzie S (1999) Construction and characterization of a common bean bacterial artificial chromosome library. Plant Mol Biol 40:977–983

Varshney RK (2009) Gene-based marker systems in plants: high throughput approaches for discovery and genotyping. In: Jain SM, Brar DS (eds) Molecular Techniques in Crop Improvement. Vol II, Springer, The Netherlands pp. 119-142

Varshney RK and Tuberosa R (2007a) Genomics-assisted crop improvement: an overview. In: Varshney RK, Tuberosa R (eds) Genomic Assisted Crop Improvement: Genomics Approaches and Platforms. Volume I, Springer, The Netherlands pp. 1-12

Varshney RK, Beier U, Khlestkina E, Kota R, Korzun V, Röder M, Graner A, Börner A (2007b) Single nucleotide polymorphisms in rye: discovery, frequency and applications for genome mapping and diversity studies. Theor Appl Genet 114:1105-1116

Varshney RK, Close TJ, Singh NK, Hoisington DA, Cook DR (2009a) Orphan legume crops enter the genomics era! Curr Opin Plant Biol 12: 202-210

Varshney RK, Graner A, Sorrells ME (2005a) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48-55

Varshney RK, Graner A, Sorrells ME (2005b) Genomics-assisted breeding for crop improvement. Trends Plant Sci 10:621–630

Varshney RK, Hiremath PJ, Lekha P, Kashiwagi J, Jayashree B, Deokar AA, Vadez V, Xiao Y, Srinivasan R, Gaur PM, Siddique KHM, Town CD, Hoisington DA (2009b) A comprehensive resource of drought- and salinity responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.) BMC Genomics 10:523

Varshney RK, Hoisington DA, Tyagi AK (2006) Advances in cereal genomics and applications in crop breeding. Trends Biotechnol 24:490-499

Varshney RK, Nayak SN, Jayashree B, Eshwar K, Upadhyaya HD, Hoisington DA (2007c) Development of cost-effective SNP assays for chickpea genome analysis and breeding. SAT eJournal 3 (1)

Varshney RK, Nayak SN, May GD and Jackson SA (2009c) Next generation sequencing technologies and their implications for crop genetics and breeding. Trends Biotechnol 27:522-530

Varshney RK, Thiel T, Sretenovic-Rajicic T, Baum M, Valkoun J, Guo P, Grando S, Ceccarelli S, Graner A (2008) Identification and validation of a core set of informative genic SSR and SNP markers for assaying functional diversity in barley. Mol Breed 22: 1-13

Varshney RK, Thiel T, Stein N, Langridge P, Graner A (2002) *In silico* analysis on frequency and distribution of microsatellites in ESTs of some cereal species. Cell Mol Biol Lett 7:537-546

Varshney RK, Thudi M, May GD, Jackson SA (2010) Legume genomics and breeding. Plant Breed Rev 33:257-304

Verma V, Foulkes M, Worland A, Sylvester-Bradley R, Caligari P, Snape J (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255–263

Vos P, Hogers R, Bleeker M, Reijans M, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kulper M, Zabeau M (1995) AFLP; a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407-4414

Wang CS, Liau YE, Huang JC, Wu TD, Su CC, Lin CH (1998) Characterization of a desiccation-related protein in lily pollen during development and stress. Plant Cell Physiol 39:1307-1314

Wang DG, Fan J-B, Siao C-J, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES (1998) Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280:1077–1082

Wang Y, Rannala B (2005) *In silico* analysis of disease-association mapping strategies using the coalescent process and incorporating ascertainment and selection. Am J Hum Genet 76:1066–1073

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT, McCourt P, Huang Y (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J 43:413-424

Weber JL (1990) The informativeness of human (dC-dA)n, (dGdT)n polymorphisms. Genomics 7:524–539

Weber D, Helentjaris T (1989) Mapping RFLP loci in maize using B-A translocations. Genetics 121:583–590

Weiland JJ, Yu MH (2003) A cleaved amplified polymorphic sequence (CAPS) marker associated with root-knot nematode resistance in sugarbeet. Crop Sci 43:1814-1818

Weising K, Kaemmer D, Weigand F, Epplen JT, Kahl G (1992) Oligonucleotide fingerprinting reveals various probe dependent levels of informativeness in chickpea (*Cicer arietinum*). Genome 35: 436–442

Wen W, Mei H, Feng F, Yu S, Huang Z, Wu J, Chen L, Xu X, Luo L (2009) Population structure and association mapping on chromosome 7 using a diverse panel of Chinese germplasm of rice (*Oryza sativa* L.). Theor Appl Genet 119: 459-470

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Jaroslava O, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ,

Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206–228

Whitt SR, Wilson LM, Tenaillon MI, Gaut BS and Buckler ES (2002) Genetic diversity and selection in maize starch pathway. Proc Natl Acad Sci USA 99:12959-62

Whittaker A, Martinelli T, Farrant JM, Bochicchio A, Vazzana C (2007) Sucrose phosphate synthase activity and the co-ordination of carbon partitioning during sucrose and amino acid accumulation in desiccation-tolerant leaf material of the C4 resurrection plant *Sporobolus stapfianus* during dehydration. J Exp Bot 58:3775-3787

Williams PC, Singh U (1987) The chickpea–nutritional quality and evaluation of quality in breeding programs. In: Saxena MC, Singh KB (eds) The Chickpea. Wallingford, CAB International, UK pp. 329–356

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531-6535

Winter P, Benko-Iseppon AM, Hüttel B, Ratnaparkhe M, Tullu A, Sonnante G, Pfaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum* L.) genome based on the recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for *Fusarium* races 4 and 5. Theor Appl Genet 101:1155–1163

Winter P, Pfaff T, Udupa SM, Hüttel B, Sharma PC, Sahi S, Arrequin-Espinoza R, Weigand F, Muehlbauer FJ, Kahl G (1999) Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*C. arietinum* L.). Mol Gen Genet 262:90–101

Wittenberg AHJ, van der Lee T, Cayla C, Kilian A, Visser RGF, Schouten HJ (2005) Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana*. Mol Gen Genomics 274:30-39

Wojciechowski MF, Lavin M, Sanderson MJ (2004) A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. Am J Bot 91:1846–1862

Wright SI, Gaut BS (2005) Molecular population genetics and the search for adaptive evolution in plants. Mol Biol Evol 22:506–519

Wu C, Sun S, Nimmakayala P, Santos F, Meksem K, Springman R, Ding K, Lightfoot DA, Zhang H-B (2004a) A BAC- and BIBAC-based physical map of the soybean genome. Genome Res 14:319–326

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor Appl Genet 83:294-300

Wu Y, Bhat PR, Close TJ, Lonardi S (2008) Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. PLoS Genet 4: e1000212

Xia L, Peng K, Yang S, Wenzl P, Carmen de Vicente M, Fregene M, Kilian A (2005) DArT for high-throughput genotyping of cassava (*Manihot esculenta*) and its wild relatives. Theor Appl Genet 110:1092–1098

Xiao B, Huang Y, Tang N, Xiong L (2007) Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theor Appl Genet 115: 35-46.

Xiao YN, Li XH, George ML, Li MS, Zhang SH, Zheng YL (2005) Quantitative trait locus analysis of drought tolerance and yield in maize in china. Plant Mol Biol Rep 23:155–165

Xie Y, McNally K, Li C-Y, Leung H, Zhu Y-Y (2006) A high-throughput genomic tool: Diversity array technology complementary for rice genotyping. J Integr Plant Biol 48:1069-1076

Xu D, Duan X, Wang B, Hong B, Ho T, Wu R (1996) Expression of a late embryogenic abundant protein gene HVA1 from barley confers tolerance to water deficits and salt stress in rice. Plant Physiol 110:249-257

Xu JL, Lafitte HR, Gao YM, Fu BY, Torres R, Li ZK (2005) QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. Theor Appl Genet 111:642–1650

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice Nature 442:705-708

Xu Z, Kohel RJ, Song G, Cho J, Yu J, Yu S, Tomkins J, Yu JZ (2008) An integrated genetic and physical map of homoeologous chromosomes 12 and 26 in Upland cotton (*G. hirsutum* L.) BMC Genomics 9:108

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low temperature, or high salt stress, Plant Cell 6:251–264

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6:251-264

Yan J, He C, Wang J, Mao Z, Holaday SA, Allen RD, Zhang H (2004) Overexpression of the *Arabidopsis* 14- 3-3 protein GF14 lambda in cotton leads to a ‘‘stay-green’’ phenotype and improves stress tolerance under moderate drought conditions. Plant Cell Physiol 45:1007-1014

Yang CY, Chen YC, Jauh GY, Wang CS (2005) A Lily ASR protein involves abscisic acid signaling and confers drought and salt resistance in *Arabidopsis*. Plant Physiol 139:836-846

Yang S, Pang W, Ash G, Harper J, Carling J, Wenzl P, Huttner E, Zong X, Kilian A (2006) Low level of genetic diversity in cultivated Pigeonpea compared to its wild relatives is revealed by diversity arrays technology. Theor Appl Genet 113: 585-595

Yang W, Bai X, Kabelka E, Eaton C, Kamoun S, van der Knaap E, Francis D (2004). Discovery of single nucleotide polymorphisms in *Lycopersicon esculentum* by computer aided analysis of expressed sequence. Mol Breed 14: 21-34

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479-501

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17:155–160

Yu J, Pressoir G, Briggs WH, Vroh BI, Yamasaki M, Doebley J, Mcmullen M, Gaut B, Holland JJ, Kresovich S, Buckler E (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38: 203–208

Yu WP, Wang AY, Juang RH, Sung HY, Su JC (1992) Isolation of rice sucrose synthase cDNA and genomic DNA. Plant Mol Biol 18:139-142

Zhong S, Dekkers JCM, Fernando RL, Jannink J-L (2009) Factors affecting accuracy from genomic selection in populations derived from multiple inbred lines: a barley case study. Genetics 182:355-364

Zhu BC, Su J, Chan MC, Verma DPS, Fan YL, Wu R (1998) Overexpression of a d-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water-stress and salt-stress in transgenic rice. Plant Sci 139:41-48

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. The Plant Genome 1:5–20

Zhu H, Cannon SB, Young ND, Cook DR (2002) Phylogeny and genomic organization of the TIR and non-TIR NBS-LRR resistance gene family in *Medicago truncatula*. Mol Plant Microbe Interact 15:529-539

Zhu JK (2001) Cell signaling under salt, water and cold stresses. Curr Opin Plant Biol 4:401-406

Appendix 1: List of genotypes of reference set of chickpea

Names	Source country	ISO codes for countries	Continent	Seed shape	Species
Annigeri	India	IND	Asia	Desi	*C. arietinum*
G130	India	IND	Asia	Angular	*C. arietinum*
ICC10018	India	IND	Asia	Desi	*C. arietinum*
ICC10301	Mexico	MEX	North America	Angular	*C. arietinum*
ICC10341	Turkey	TUR	Euro-Asian	Pea	*C. arietinum*
ICC10393	India	IND	Asia	Desi	*C. arietinum*
ICC10399	India	IND	Asia	Desi	*C. arietinum*
ICC10466	India	IND	Asia	Kabuli	*C. arietinum*
ICC10516	India	IND	Asia	Angular	*C. arietinum*
ICC1052	Pakistan	PAK	Asia	Desi	*C. arietinum*
ICC10673	Turkey	TUR	Euro-Asian	Desi	*C. arietinum*
ICC10685	Turkey	TUR	Euro-Asian	Desi	*C. arietinum*
ICC10755	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*
ICC1083	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC10885	Ethiopia	ETH	East Africa	Kabuli	*C. arietinum*
ICC10939	India	IND	Asia	Desi	*C. arietinum*
ICC10945	India	IND	Asia	Desi	*C. arietinum*
ICC1098	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC11121	India	IND	Asia	Desi	*C. arietinum*
ICC11198	India	IND	Asia	Desi	*C. arietinum*
ICC11244	AFG	AFG	Asia	Angular	*C. arietinum*
ICC11279	Pakistan	PAK	Asia	Desi	*C. arietinum*
ICC11284	Union of Soviet Socialist Republics	USSR	Asia	Desi	*C. arietinum*
ICC11303	Chile	CHL	South America	Kabuli	*C. arietinum*
ICC11372	India	IND	Asia	Angular	*C. arietinum*
ICC11378	India	IND	Asia	Desi	*C. arietinum*
ICC11498	India	IND	Asia	Desi	*C. arietinum*
ICC11524	ICRISAT	IND	Asia	Angular	*C. arietinum*
ICC11584	India	IND	Asia	Desi	*C. arietinum*
ICC1161	Pakistan	PAK	Asia	Desi	*C. arietinum*
ICC11627	India	IND	Asia	Desi	*C. arietinum*
ICC1164	Nigeria	NGA	Western Africa	Desi	*C. arietinum*
ICC11664	India	IND	Asia	Desi	*C. arietinum*
ICC11764	Chile	CHL	South America	Kabuli	*C. arietinum*
ICC11781	CHL	CHL	South America	Owl's head	*C. arietinum*
ICC1180	India	IND	Asia	Desi	*C. arietinum*
ICC11819	Chile	CHL	South America	Kabuli	*C. arietinum*
ICC11857	Unknown			Angular	*C. arietinum*
ICC11879	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*
ICC11903	Germany	DEU	Europe	Desi	*C. arietinum*
ICC1194	India	IND	Asia	Desi	*C. arietinum*

Contd…

ICC11944	Nepal	NPL	Asia	Desi	*C. arietinum*
ICC12021	LBN	LBN	Asia-middle east	Angular	*C. arietinum*
ICC12028	Mexico	MEX	North America	Desi	*C. arietinum*
ICC12037	Mexico	MEX	North America	Kabuli	*C. arietinum*
ICC1205	India	IND	Asia	Desi	*C. arietinum*
ICC12155	Bangladesh	BGD	Asia	Desi	*C. arietinum*
ICC12299	Nepal	NPL	Asia	Desi	*C. arietinum*
ICC1230	India	IND	Asia	Desi	*C. arietinum*
ICC12321	Unknown			Desi	*C. arietinum*
ICC12324	Unknown			Kabuli	*C. arietinum*
ICC12328	Cyprus	CYP	Europe	Kabuli	*C. arietinum*
ICC12379	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC12492	ICRISAT	IND	Asia	Kabuli	*C. arietinum*
ICC12537	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC12654	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC12726	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC12851	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC12866	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC12916	India	IND	Asia	Desi	*C. arietinum*
ICC12928	India	IND	Asia	Desi	*C. arietinum*
ICC12947	India	IND	Asia	Desi	*C. arietinum*
ICC13077	India	IND	Asia	Kabuli	*C. arietinum*
ICC13124	India	IND	Asia	Desi	*C. arietinum*
ICC13187	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13219	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC13283	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13357	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13380	IRN	IRN	Asia-middle east	Owl's head	*C. arietinum*
ICC13441	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13461	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13523	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC1356	India	IND	Asia	Desi	*C. arietinum*
ICC13599	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC13628	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13719	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13764	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC13816	Union of Soviet Socialist Republics	USSR	Asia	Kabuli	*C. arietinum*
ICC13863	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC13892	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC1392	India	IND	Asia	Desi	*C. arietinum*
ICC1397	India	IND	Asia	Desi	*C. arietinum*
ICC1398	India	IND	Asia	Desi	*C. arietinum*
ICC14051	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC14077	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC14098	Ethiopia	ETH	East Africa	Desi	*C. arietinum*

Contd…

ICC14199	Mexico	MEX	North America	Kabuli	*C. arietinum*
ICC1422	India	IND	Asia	Desi	*C. arietinum*
ICC1431	India	IND	Asia	Desi	*C. arietinum*
ICC14402	ICRISAT	IND	Asia	Desi	*C. arietinum*
ICC14446	Italy	ITA	Europe	Kabuli	*C. arietinum*
ICC14669	India	IND	Asia	Desi	*C. arietinum*
ICC14778	India	IND	Asia	Desi	*C. arietinum*
ICC14799	India	IND	Asia	Desi	*C. arietinum*
ICC14815	India	IND	Asia	Desi	*C. arietinum*
ICC14831	India	IND	Asia	Desi	*C. arietinum*
ICC1510	India	IND	Asia	Desi	*C. arietinum*
ICC15248	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC15264	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC15294	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC15333	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC15406	Morocco	MAR	North-Western Africa	Kabuli	*C. arietinum*
ICC15435	Morocco	MAR	North-Western Africa	Kabuli	*C. arietinum*
ICC15510	Morocco	MAR	North-Western Africa	Desi	*C. arietinum*
ICC15518	Morocco	MAR	North-Western Africa	Kabuli	*C. arietinum*
ICC15567	India	IND	Asia	Desi	*C. arietinum*
ICC15606	India	IND	Asia	Desi	*C. arietinum*
ICC15610	India	IND	Asia	Desi	*C. arietinum*
ICC15612	Tanzania	TZA	East Africa	Desi	*C. arietinum*
ICC15614	Tanzania	TZA	East Africa	Desi	*C. arietinum*
ICC15618	India	IND	Asia	Desi	*C. arietinum*
ICC15697	Syria	SYR	Asia	Kabuli	*C. arietinum*
ICC15762	Syria	SYR	Asia	Desi	*C. arietinum*
ICC15785	Syria	SYR	Asia	Desi	*C. arietinum*
ICC15802	Syria	SYR	Asia	Kabuli	*C. arietinum*
ICC15868	India	IND	Asia	Desi	*C. arietinum*
ICC15888	India	IND	Asia	Pea	*C. arietinum*
ICC16171	MYA	MMR	Asia	Angular	*C. arietinum*
ICC16207	Myanmar	MMR	Asia	Desi	*C. arietinum*
ICC16230	MYA	MMR	Asia	Angular	*C. arietinum*
ICC16261	Malawi	MWI	South-Eastern Africa	Desi	*C. arietinum*
ICC16269	Malawi	MWI	South-Eastern Africa	Desi	*C. arietinum*
ICC16374	Malawi	MWI	South-Eastern Africa	Desi	*C. arietinum*
ICC16487	Pakistan	PAK	Asia	Desi	*C. arietinum*
ICC16524	Pakistan	PAK	Asia	Desi	*C. arietinum*
ICC16654	China	CHN	Asia	Kabuli	*C. arietinum*
ICC16796	Portugal	PRT	Europe	Kabuli	*C. arietinum*
ICC16903	India	IND	Asia	Desi	*C. arietinum*

Contd…

ICC16915	India	IND	Asia	Desi	*C. arietinum*
ICC1710	India	IND	Asia	Desi	*C. arietinum*
ICC1715	India	IND	Asia	Desi	*C. arietinum*
ICC18679	Iraq	IRQ	Asia-middle east	Kabuli	*C. arietinum*
ICC18719	IRN	IRN	Asia-middle east	Owl's head	*C. arietinum*
ICC18720	Morocco	MAR	North-Western Africa	Kabuli	*C. arietinum*
ICC1882	India	IND	Asia	Desi	*C. arietinum*
ICC18836	Syria	SYR	Asia	Kabuli	*C. arietinum*
ICC19011	Syria	SYR	Asia	Kabuli	*C. arietinum*
ICC19122	France	FRA	Europe	Kabuli	*C. arietinum*
ICC1915	India	IND	Asia	Desi	*C. arietinum*
ICC1923	India	IND	Asia	Desi	*C. arietinum*
ICC2065	India	IND	Asia	Desi	*C. arietinum*
ICC2072	India	IND	Asia	Desi	*C. arietinum*
ICC2210	Algeria	DZA	North Africa	Desi	*C. arietinum*
ICC2242	India	IND	Asia	Desi	*C. arietinum*
ICC2263	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2277	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC2482	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC2507	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2580	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2593	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC2629	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2679	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2720	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2737	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC283	India	IND	Asia	Desi	*C. arietinum*
ICC2884	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2919	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2969	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC2990	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3025	Iran	IRN	Asia-middle east	Angular	*C. arietinum*
ICC3218	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3230	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3239	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3325	Cyprus	CYP	Europe	Desi	*C. arietinum*
ICC3362	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3378	Iran	IRN	Asia-middle east	Pea	*C. arietinum*
ICC3391	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3410	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC3421	Isreal	ISR	Asia	Kabuli	*C. arietinum*
ICC3512	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3582	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3631	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3658	Iran	IRN	Asia-middle east	Angular	*C. arietinum*

Contd…

ICC3761	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3776	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3892	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC3946	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC4093	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC4182	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC4363	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC440	India	IND	Asia	Desi	*C. arietinum*
ICC4418	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC4463	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC4495	Turkey	TUR	Euro-Asian	Desi	*C. arietinum*
ICC4533	India	IND	Asia	Desi	*C. arietinum*
ICC456	India	IND	Asia	Desi	*C. arietinum*
ICC4567	India	IND	Asia	Desi	*C. arietinum*
ICC4593	India	IND	Asia	Desi	*C. arietinum*
ICC4639	India	IND	Asia	Desi	*C. arietinum*
ICC4657	India	IND	Asia	Desi	*C. arietinum*
ICC4814	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC4841	Morocco	MAR	North-Western Africa	Kabuli	*C. arietinum*
ICC4853	Unknown			Kabuli	*C. arietinum*
ICC4872	India	IND	Asia	Pea	*C. arietinum*
ICC4918	India	IND	Asia	Desi	*C. arietinum*
ICC4991	India	IND	Asia	Desi	*C. arietinum*
ICC506	India	IND	Asia	Desi	*C. arietinum*
ICC5135	India	IND	Asia	Desi	*C. arietinum*
ICC5201	India	IND	Asia	Angular	*C. arietinum*
ICC5221	India	IND	Asia	Desi	*C. arietinum*
ICC5337	India	IND	Asia	Kabuli	*C. arietinum*
ICC5383	India	IND	Asia	Desi	*C. arietinum*
ICC5434	India	IND	Asia	Desi	*C. arietinum*
ICC5504	Mexico	MEX	North America	Desi	*C. arietinum*
ICC5613	India	IND	Asia	Desi	*C. arietinum*
ICC5639	India	IND	Asia	Desi	*C. arietinum*
ICC5845	India	IND	Asia	Desi	*C. arietinum*
ICC5878	India	IND	Asia	Desi	*C. arietinum*
ICC5879	India	IND	Asia	Pea	*C. arietinum*
ICC6263	Union of Soviet Socialist Republics	USSR	Asia	Kabuli	*C. arietinum*
ICC6279	India	IND	Asia	Desi	*C. arietinum*
ICC6293	Italy	ITA	Europe	Desi	*C. arietinum*
ICC6294	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6306	Union of Soviet Socialist Republics	USSR	Asia	Desi	*C. arietinum*
ICC637	India	IND	Asia	Desi	*C. arietinum*
ICC6571	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6579	Iran	IRN	Asia-middle east	Desi	*C. arietinum*

Contd…

ICC67	India	IND	Asia	Desi	*C. arietinum*
ICC6802	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6811	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6816	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6874	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6875	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC6877	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC7052	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC708	India	IND	Asia	Desi	*C. arietinum*
ICC7184	Turkey	TUR	Euro-Asian	Desi	*C. arietinum*
ICC7255	India	IND	Asia	Kabuli	*C. arietinum*
ICC7272	Algeria	DZA	North Africa	Kabuli	*C. arietinum*
ICC7305	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC7308	Peru	PER	South America	Kabuli	*C. arietinum*
ICC7315	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC7323	Union of Soviet Socialist Republics	USSR	Asia	Pea	*C. arietinum*
ICC7326	Unknown			Desi	*C. arietinum*
ICC7413	India	IND	Asia	Pea	*C. arietinum*
ICC7441	India	IND	Asia	Desi	*C. arietinum*
ICC7554	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC7571	Isreal	ISR	Asia	Kabuli	*C. arietinum*
ICC762	India	IND	Asia	Desi	*C. arietinum*
ICC7668	Union of Soviet Socialist Republics	USSR	Asia	Kabuli	*C. arietinum*
ICC7819	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC7867	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC791	India	IND	Asia	Desi	*C. arietinum*
ICC8058	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC8195	Pakistan	PAK	Asia	Desi	*C. arietinum*
ICC8200	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC8261	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*
ICC8318	India	IND	Asia	Desi	*C. arietinum*
ICC8350	India	IND	Asia	Pea	*C. arietinum*
ICC8384	India	IND	Asia	Desi	*C. arietinum*
ICC8515	Greece	GRC	Europe	Desi	*C. arietinum*
ICC8521	Italy	ITA	Europe	Desi	*C. arietinum*
ICC8522	Italy	ITA	Europe	Desi	*C. arietinum*
ICC8607	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC8621	Ethiopia	ETH	East Africa	Desi	*C. arietinum*
ICC867	India	IND	Asia	Desi	*C. arietinum*
ICC8718	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC8740	Afghanistan	AFG	Asia	Kabuli	*C. arietinum*
ICC8752	Afghanistan	AFG	Asia	Kabuli	*C. arietinum*
ICC8855	Afghanistan	AFG	Asia	Kabuli	*C. arietinum*
ICC8950	India	IND	Asia	Desi	*C. arietinum*

Contd…

ICC9002	Iran	IRN	Asia-middle east	Desi	*C. arietinum*
ICC9053	Iran	IRN	Asia-middle east	Angular	*C. arietinum*
ICC9137	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC9402	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC9418	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC9434	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
ICC9483	Iran	IRN	Asia-middle east	Owl's head	*C. arietinum*
ICC95	India	IND	Asia	Desi	*C. arietinum*
ICC9586	India	IND	Asia	Desi	*C. arietinum*
ICC9590	Egypt	EGY	North-East Africa	Desi	*C. arietinum*
ICC9636	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC9643	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC9702	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC9712	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC9755	Afghanistan	AFG	Asia	Desi	*C. arietinum*
ICC9816	AFG	AFG	Asia	Owl's head	*C. arietinum*
ICC9848	Afghanistan	AFG	Asia	Pea	*C. arietinum*
ICC9862	Afghanistan	AFG	Asia	Pea	*C. arietinum*
ICC9872	Afghanistan	AFG	Asia	Kabuli	*C. arietinum*
ICC9895	Afghanistan	AFG	Asia	Pea	*C. arietinum*
ICC9942	India	IND	Asia	Desi	*C. arietinum*
ICCV10	ICRISAT	IND	Asia	Angular	*C. arietinum*
ICCV95311	ICRISAT	IND	Asia	Kabuli	*C. arietinum*
IG10309	Syria	SYR	Asia	Kabuli	*C. arietinum*
IG10500	Syria	SYR	Asia	Kabuli	*C. arietinum*
IG10569	Syria	SYR	Asia	Kabuli	*C. arietinum*
IG10701	Syria	SYR	Asia	Kabuli	*C. arietinum*
IG11045	Syria	SYR	Asia	Kabuli	*C. arietinum*
IG5503	Syrian Arab Republic	SYR	Asia		*C. arietinum*
IG5949	Unknown			Kabuli	*C. arietinum*
IG6044	Sudan	SDN	North-East Africa	Kabuli	*C. arietinum*
IG6047	Afghanistan	AFG	Asia	Kabuli	*C. arietinum*
IG6055	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
IG6067	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*
IG6154	Iran	IRN	Asia-middle east	Kabuli	*C. arietinum*
IG6343	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*
IG6443	Algeria	DZA	North Africa	Owl's head	*C. arietinum*
IG69438	Cyprus	CYP	Europe	Kabuli	*C. arietinum*
IG69761	Uzbekistan	UZB	Asia	Kabuli	*C. arietinum*
IG70445	TUR	TUR	Euro-Asian	Owl's head	*C. arietinum*
IG70826	Greece	GRC	Europe	Kabuli	*C. arietinum*
IG7087	USA	USA	North America	Kabuli	*C. arietinum*
IG71005	France	FRA	Europe	Pea	*C. arietinum*
IG7148	Algeria	DZA	North Africa	Kabuli	*C. arietinum*
IG72070	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*

Contd…

					…*Contd*
IG72109	Turkey	TUR	Euro-Asian	Kabuli	*C. arietinum*
IG7296	Afghanistan	AFG	Asia	Kabuli	*C. arietinum*
IG72970	Turkey	TUR	Euro-Asian	Desi	*C. reticulatum*
IG73074	Turkey	TUR	Euro-Asian	Desi	*C. echinospermum*
IG73082	Turkey	TUR	Euro-Asian	Desi	*C. reticulatum*
IG73086	Turkey	TUR	Euro-Asian	Desi	*C. reticulatum*
IG73247	Syrian Arab Republic	SYR	Asia		*C. arietinum*
IG73458	Syria	SYR	Asia	Kabuli	*C. arietinum*
IG74036	Moldova	MDA	Europe	Pea	*C. arietinum*
IG74052	Italy	ITA	Europe	Kabuli	*C. arietinum*
KAK2	ICRISAT	IND	Asia	Kabuli	*C. arietinum*
L550	India	IND	Asia	Owl's head	*C. arietinum*

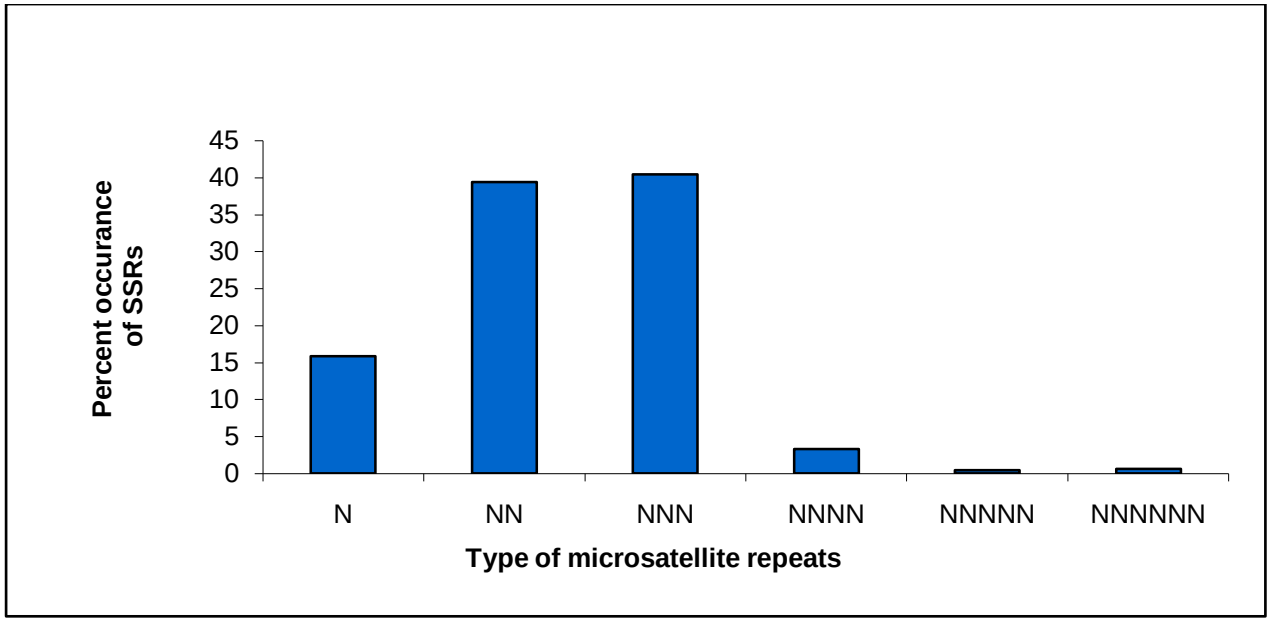


Figure 1. Frequency of different SSR classes in sequences obtained from SSR enriched library

N: mono-nucleotide repeats; NN: di-nucleotide repeats; NNN: tri-nucleotide repeats; NNNN: tetra-nucleotide repeats; NNNNN: penta-nucleotide repeats and NNNNNN: hexa-nucleotide repeats.

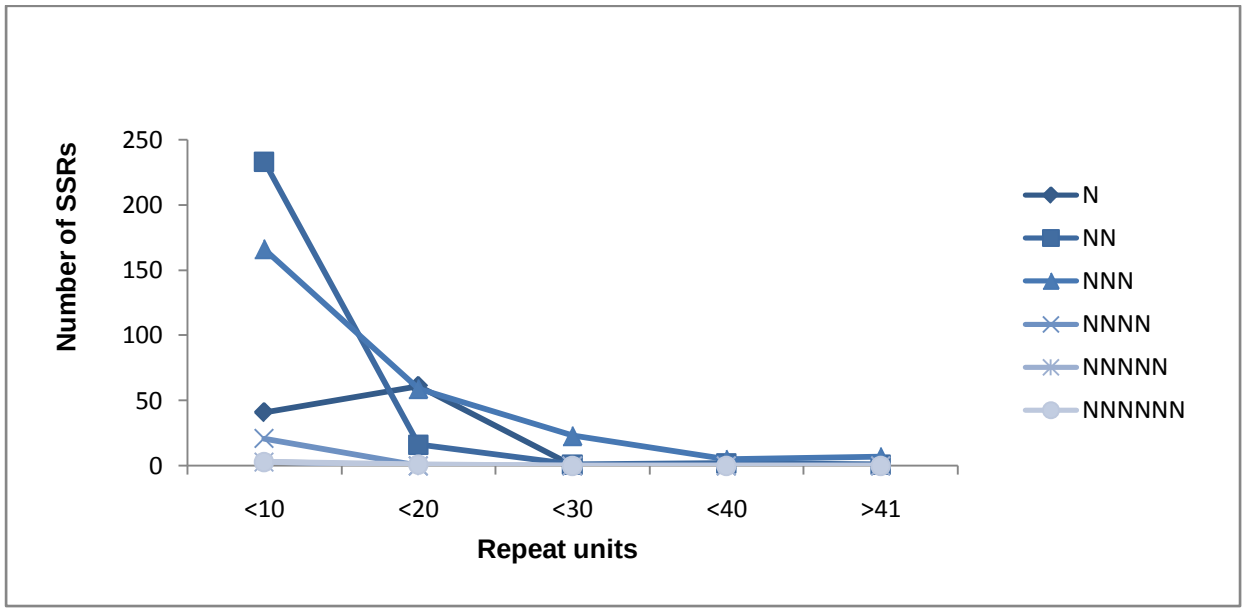


Figure 2. Distribution of microsatellites with varying repeat units in SSR enrichment library

N: mono-nucleotide repeats; NN: di-nucleotide repeats; NNN: tri-nucleotide repeats; NNNN: tetra-nucleotide repeats; NNNNN: penta-nucleotide repeats and NNNNNN: hexa-nucleotide repeats.

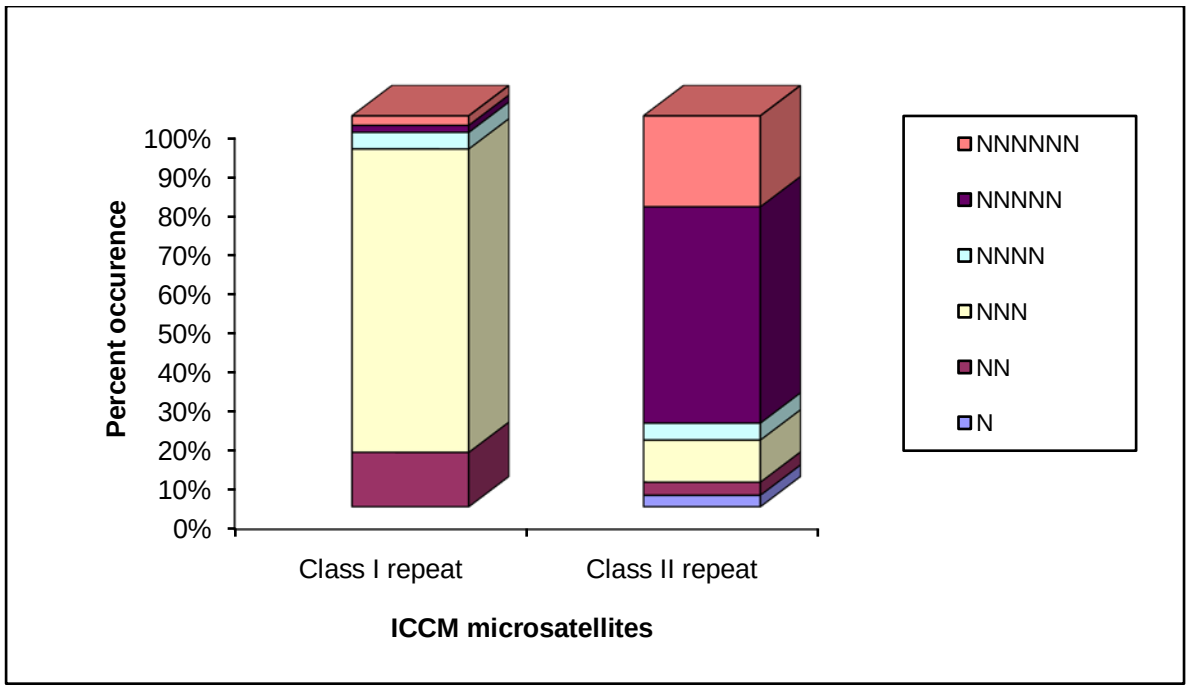


Figure 3. Distribution of Class I and Class II repeats in newly isolated ICCM microsatellites

N: mono-nucleotide repeats; NN: di-nucleotide repeats; NNN: tri-nucleotide repeats; NNNN: tetra-nucleotide repeats; NNNNN: penta-nucleotide repeats and NNNNNN: hexa-nucleotide repeats.

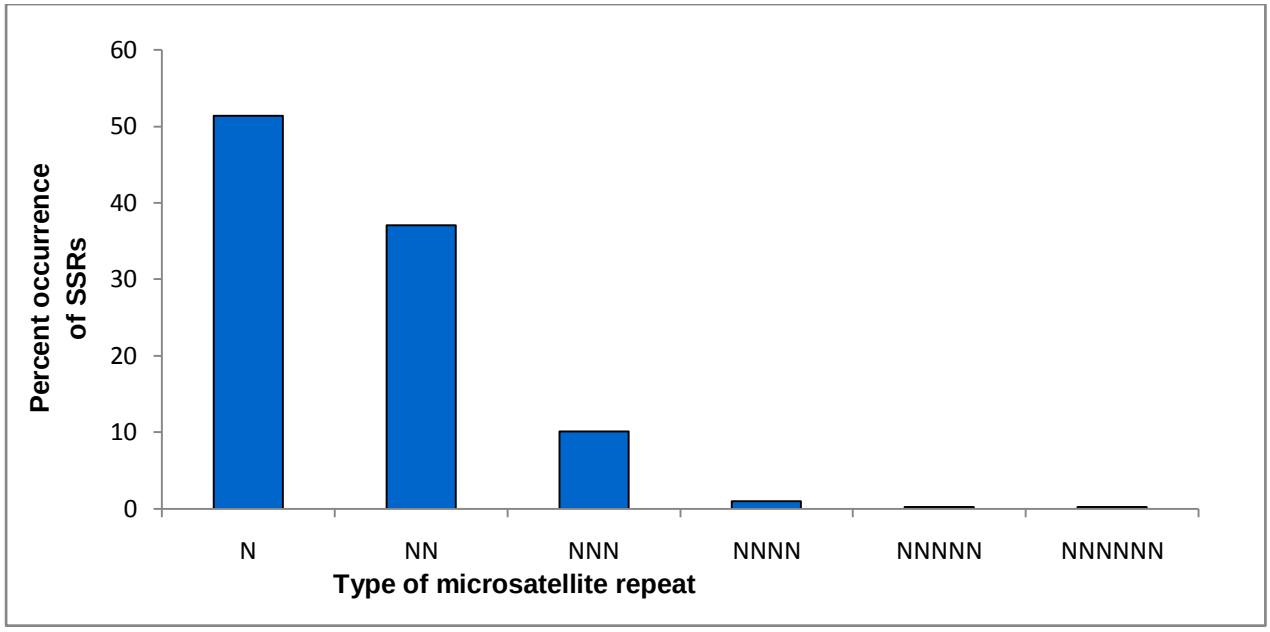


Figure 5. Frequency of different SSR classes in BAC end sequences

N: mono-nucleotide repeats; NN: di-nucleotide repeats; NNN: tri-nucleotide repeats; NNNN: tetra-nucleotide repeats; NNNNN: penta-nucleotide repeats and NNNNNN: hexa-nucleotide repeats.

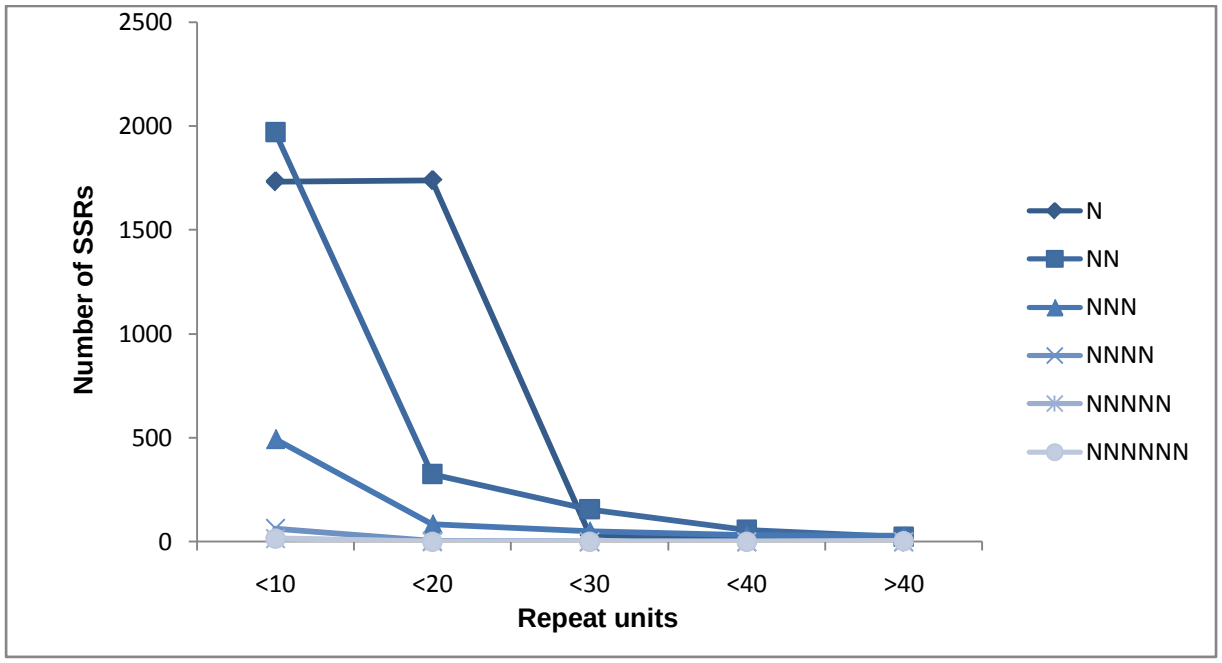


Figure 6. Distribution of microsatellites with varying repeat units in BAC-end sequences

N: mono-nucleotide repeats; NN: di-nucleotide repeats; NNN: tri-nucleotide repeats; NNNN: tetra-nucleotide repeats; NNNNN: penta-nucleotide repeats and NNNNNN: hexa-nucleotide repeats.

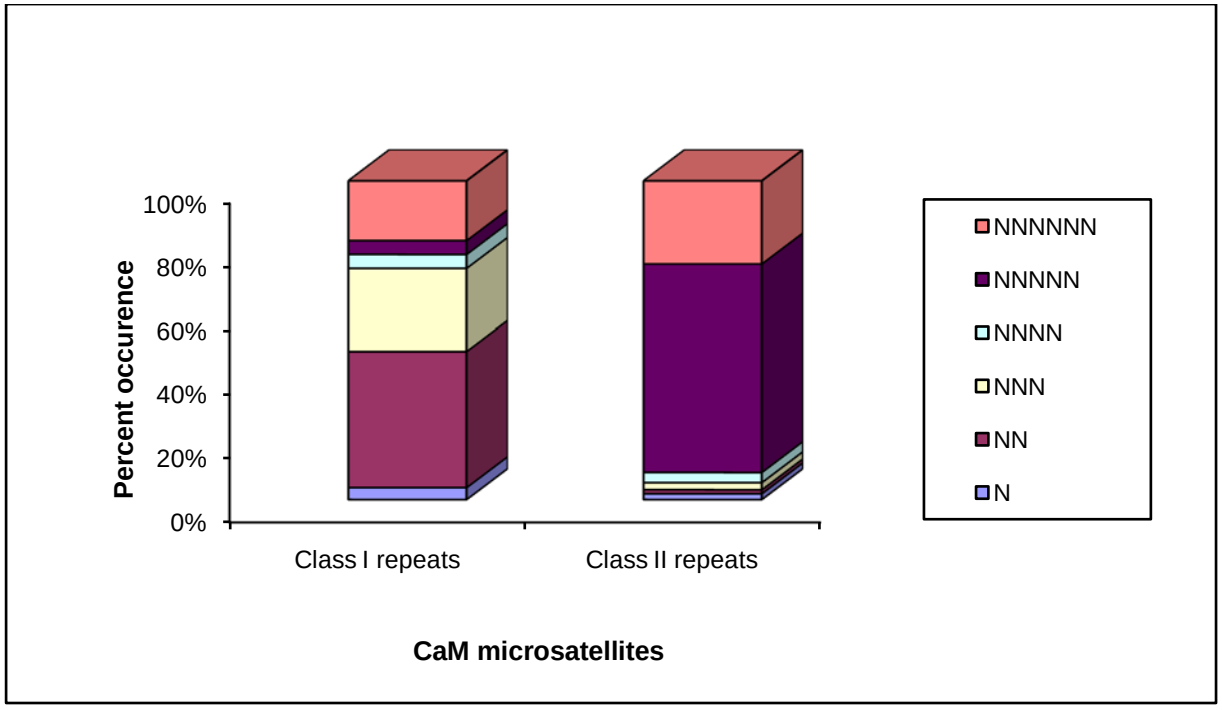


Figure 7. Distribution of Class I and Class II repeats in newly isolated CaM microsatellites

N: mono-nucleotide repeats; NN: di-nucleotide repeats; NNN: tri-nucleotide repeats; NNNN: tetra-nucleotide repeats; NNNNN: penta-nucleotide repeats and NNNNNN: hexa-nucleotide repeats.

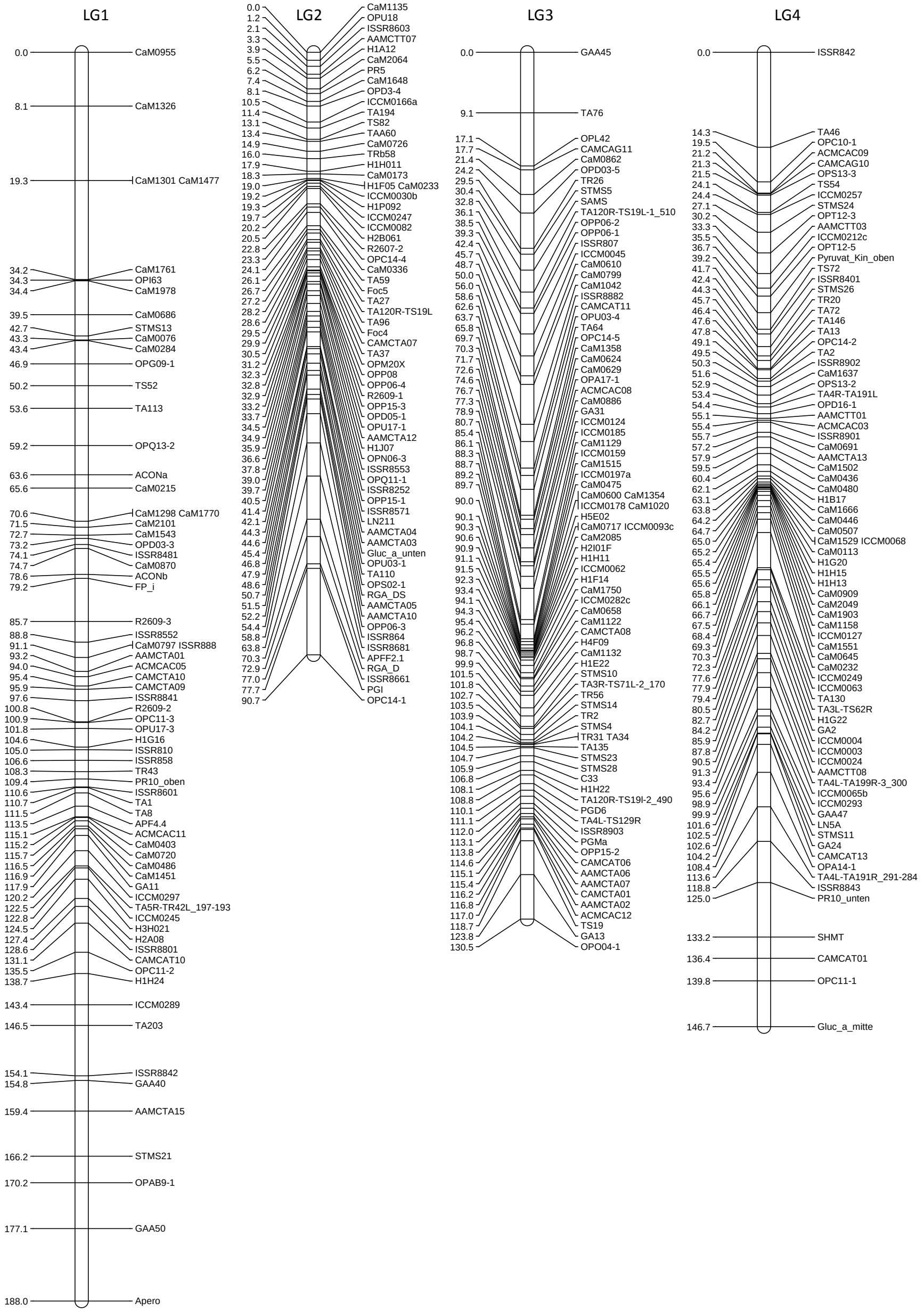
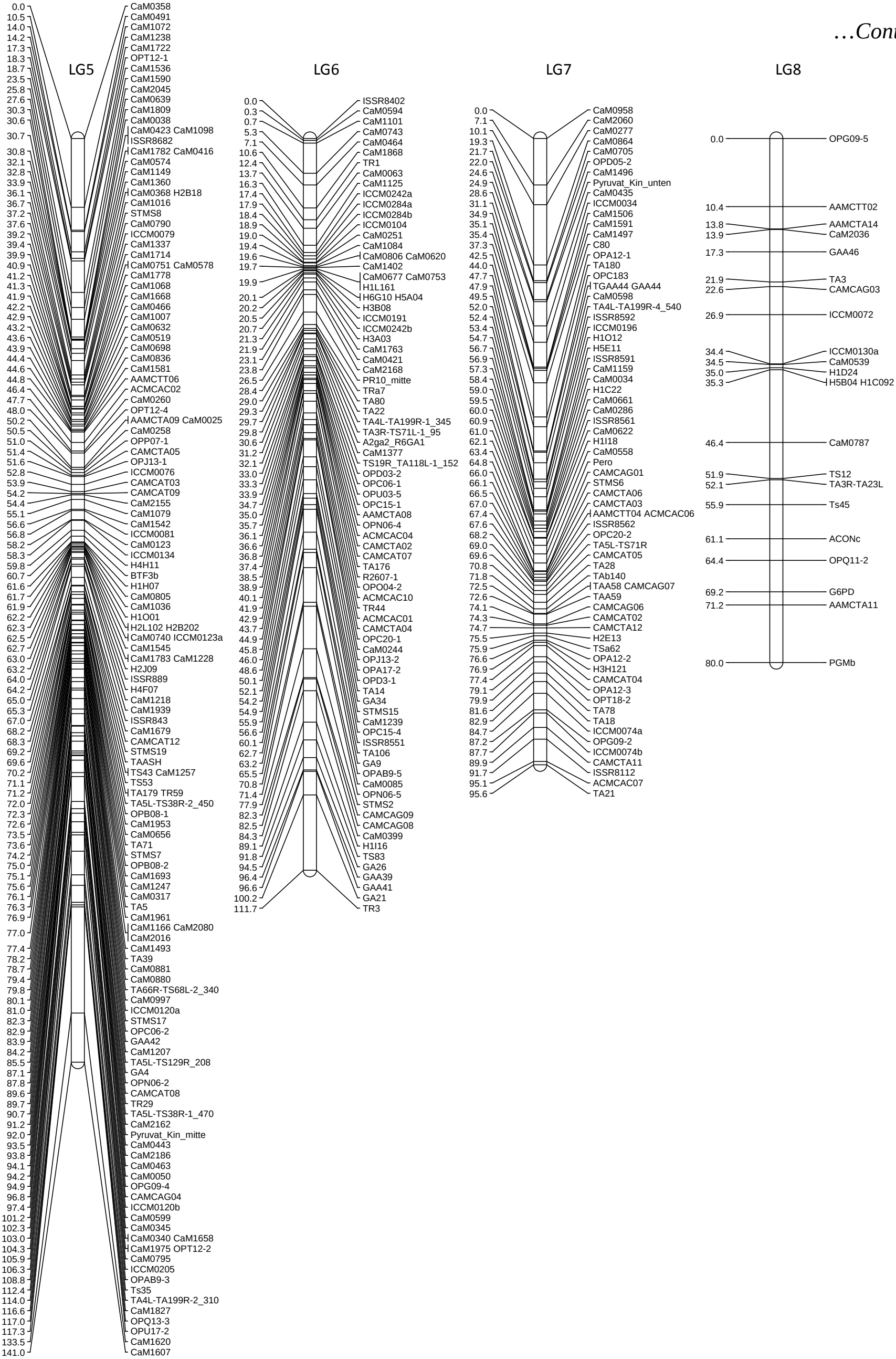


Figure 8. Inter-specific genetic map of chickpea derived based on mapping populations- *C. arietinum* ICC 4958 *C. reticulatum* PI 489777 *Contd…*

…Contd


LG5
0.0 CaM0358
10.5 CaM0491
14.0 CaM1072
14.2 CaM1238
17.3 CaM1722
18.3 OPT12-1
18.7 CaM1536
23.5 CaM1590
25.8 CaM2045
27.6 CaM0639
30.3 CaM1809
30.6 CaM0038
30.7 CaM0423 CaM1098
ISSR8682
30.8 CaM1782 CaM0416
32.1 CaM0574
32.8 CaM1149
33.9 CaM1360
36.1 CaM0368 H2B18
36.7 CaM1016
37.2 STMS8
37.6 CaM0790
39.2 ICCM0079
39.4 CaM1337
39.9 CaM1714
40.9 CaM0751 CaM0578
41.2 CaM1778
41.3 CaM1068
41.9 CaM1668
42.2 CaM0466
42.9 CaM1007
43.2 CaM0632
43.6 CaM0519
43.9 CaM0698
44.4 CaM0836
44.6 CaM1581
44.8 AAMCTT06
46.4 ACMCAC02
47.7 CaM0260
48.0 OPT12-4
50.2 AAMCTA09 CaM0025
50.5 CaM0258
51.0 OPP07-1
51.4 CAMCTA05
51.6 OPJ13-1
52.8 ICCM0076
53.9 CAMCAT03
54.2 CAMCAT09
54.4 CaM2155
55.1 CaM1079
56.6 CaM1542
56.8 ICCM0081
58.2 CaM0123
58.3 ICCM0134
59.8 H4H11
60.7 BTF3b
61.6 H1H07
61.7 CaM0805
61.9 CaM1036
62.2 H1O01
62.3 H2L102 H2B202
62.5 CaM0740 ICCM0123a
62.7 CaM1545
63.0 CaM1783 CaM1228
63.2 H2J09
64.0 ISSR889
64.2 H4F07
65.0 CaM1218
65.3 CaM1939
67.0 ISSR843
68.2 CaM1679
68.3 CAMCAT12
69.2 STMS19
69.6 TAASH
70.2 TS43 CaM1257
71.1 TS53
71.2 TA179 TR59
72.0 TA5L-TS38R-2_450
72.3 OPB08-1
72.6 CaM1953
73.5 CaM0656
73.6 TA71
74.2 STMS7
75.0 OPB08-2
75.1 CaM1693
75.6 CaM1247
76.1 CaM0317
76.3 TA5
76.9 CaM1961
77.0 CaM1166 CaM2080
CaM2016
77.4 CaM1493
78.2 TA39
78.7 CaM0881
79.4 CaM0880
79.8 TA66R-TS68L-2_340
80.1 CaM0997
81.0 ICCM0120a
82.3 STMS17
82.9 OPC06-2
83.9 GAA42
84.2 CaM1207
85.5 TA5L-TS129R_208
87.1 GA4
87.8 OPN06-2
89.6 CAMCAT08
89.7 TR29
90.7 TA5L-TS38R-1_470
91.2 CaM2162
92.0 Pyruvat_Kin_mitte
93.5 CaM0443
93.8 CaM2186
94.1 CaM0463
94.2 CaM0050
94.9 OPG09-4
96.8 CAMCAG04
97.4 ICCM0120b
101.2 CaM0599
102.3 CaM0345
103.0 CaM0340 CaM1658
104.3 CaM1975 OPT12-2
105.9 CaM0795
106.3 ICCM0205
108.8 OPAB9-3
112.4 Ts35
114.0 TA4L-TA199R-2_310
116.6 CaM1827
117.0 OPQ13-3
117.3 OPU17-2
133.5 CaM1620
141.0 CaM1607
LG6
0.0 ISSR8402
0.3 CaM0594
0.7 CaM1101
5.3 CaM0743
7.1 CaM0464
10.6 CaM1868
12.4 TR1
13.7 CaM0063
16.3 CaM1125
17.4 ICCM0242a
17.9 ICCM0284a
18.4 ICCM0284b
18.9 ICCM0104
19.0 CaM0251
19.4 CaM1084
19.6 CaM0806 CaM0620
19.7 CaM1402
19.9 CaM0677 CaM0753
H1L161
20.1 H6G10 H5A04
20.2 H3B08
20.5 ICCM0191
20.7 ICCM0242b
21.3 H3A03
21.9 CaM1763
23.1 CaM0421
23.8 CaM2168
26.5 PR10_mitte
28.4 TRa7
29.0 TA80
29.3 TA22
29.7 TA4L-TA199R-1_345
29.8 TA3R-TS71L-1_95
30.6 A2ga2_R6GA1
31.2 CaM1377
32.1 TS19R_TA118L-1_152
33.0 OPD03-2
33.3 OPC06-1
33.9 OPU03-5
34.7 OPC15-1
35.0 AAMCTA08
35.7 OPN06-4
36.1 ACMCAC04
36.6 CAMCTA02
36.8 CAMCAT07
37.4 TA176
38.5 R2607-1
38.9 OPO04-2
40.1 ACMCAC10
41.9 TR44
42.9 ACMCAC01
43.7 CAMCTA04
44.9 OPC20-1
45.8 CaM0244
46.0 OPJ13-2
48.6 OPA17-2
50.1 OPD3-1
52.1 TA14
54.2 GA34
54.9 STMS15
55.9 CaM1239
56.6 OPC15-4
60.1 ISSR8551
62.7 TA106
63.2 GA9
65.5 OPAB9-5
70.8 CaM0085
71.4 OPN06-5
77.9 STMS2
82.3 CAMCAG09
82.5 CAMCAG08
84.3 CaM0399
89.1 H1I16
91.8 TS83
94.5 GA26
96.4 GAA39
96.6 GAA41
100.2 GA21
111.7 TR3
LG7
0.0 CaM0958
7.1 CaM2060
10.1 CaM0277
19.3 CaM0864
21.7 CaM0705
22.0 OPD05-2
24.6 CaM1496
24.9 Pyruvat_Kin_unten
28.6 CaM0435
31.1 ICCM0034
34.9 CaM1506
35.1 CaM1591
35.4 CaM1497
37.3 C80
42.5 OPA12-1
44.0 TA180
47.7 OPC183
47.9 TGAA44 GAA44
49.5 CaM0598
52.0 TA4L-TA199R-4_540
52.4 ISSR8592
53.4 ICCM0196
54.7 H1O12
56.7 H5E11
56.9 ISSR8591
57.3 CaM1159
58.4 CaM0034
59.0 H1C22
59.5 CaM0661
60.0 CaM0286
60.9 ISSR8561
61.0 CaM0622
62.1 H1I18
63.4 CaM0558
64.8 Pero
66.0 CAMCAG01
66.1 STMS6
66.5 CAMCTA06
67.0 CAMCTA03
67.4 AAMCTT04 ACMCAC06
67.6 ISSR8562
68.2 OPC20-2
69.0 TA5L-TS71R
69.6 CAMCAT05
70.8 TA28
71.8 TAb140
72.5 TAA58 CAMCAG07
72.6 TAA59
74.1 CAMCAG06
74.3 CAMCAT02
74.7 CAMCTA12
75.5 H2E13
75.9 TSa62
76.6 OPA12-2
76.9 H3H121
77.4 CAMCAT04
79.1 OPA12-3
79.9 OPT18-2
81.6 TA78
82.9 TA18
84.7 ICCM0074a
87.2 OPG09-2
87.7 ICCM0074b
89.9 CAMCTA11
91.7 ISSR8112
95.1 ACMCAC07
95.6 TA21
LG8
0.0 OPG09-5
10.4 AAMCTT02
13.8 AAMCTA14
13.9 CaM2036
17.3 GAA46
21.9 TA3
22.6 CAMCAG03
26.9 ICCM0072
34.4 ICCM0130a
34.5 CaM0539
35.0 H1D24
35.3 H5B04 H1C092
46.4 CaM0787
51.9 TS12
52.1 TA3R-TA23L
55.9 Ts45
61.1 ACONc
64.4 OPQ11-2
69.2 G6PD
71.2 AAMCTA11
80.0 PGMb

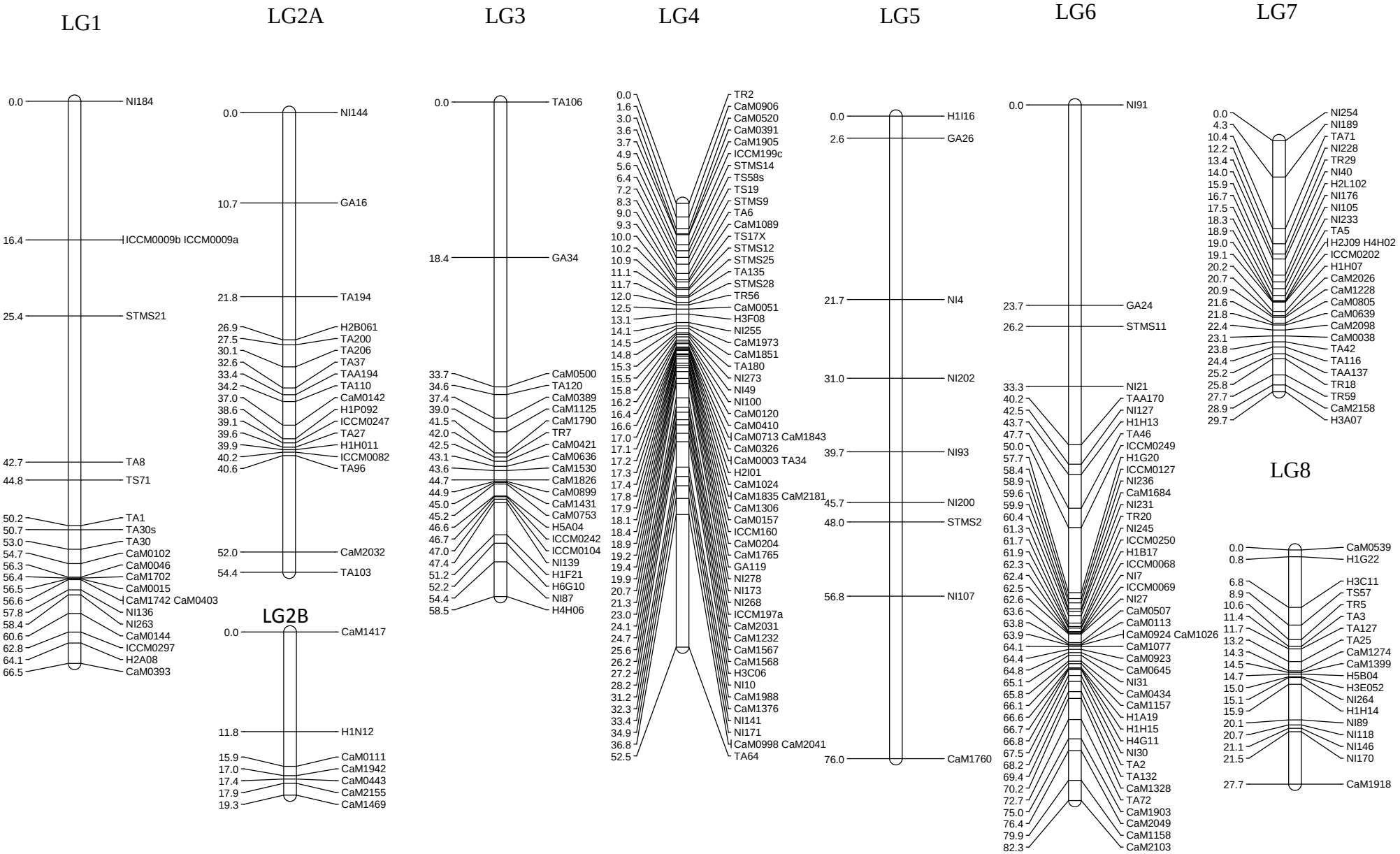


Figure 9. Intra-specific map of chickpea derived based on mapping population- *C. arietinum* ICC 4958 *C. arietinum* ICC 1882

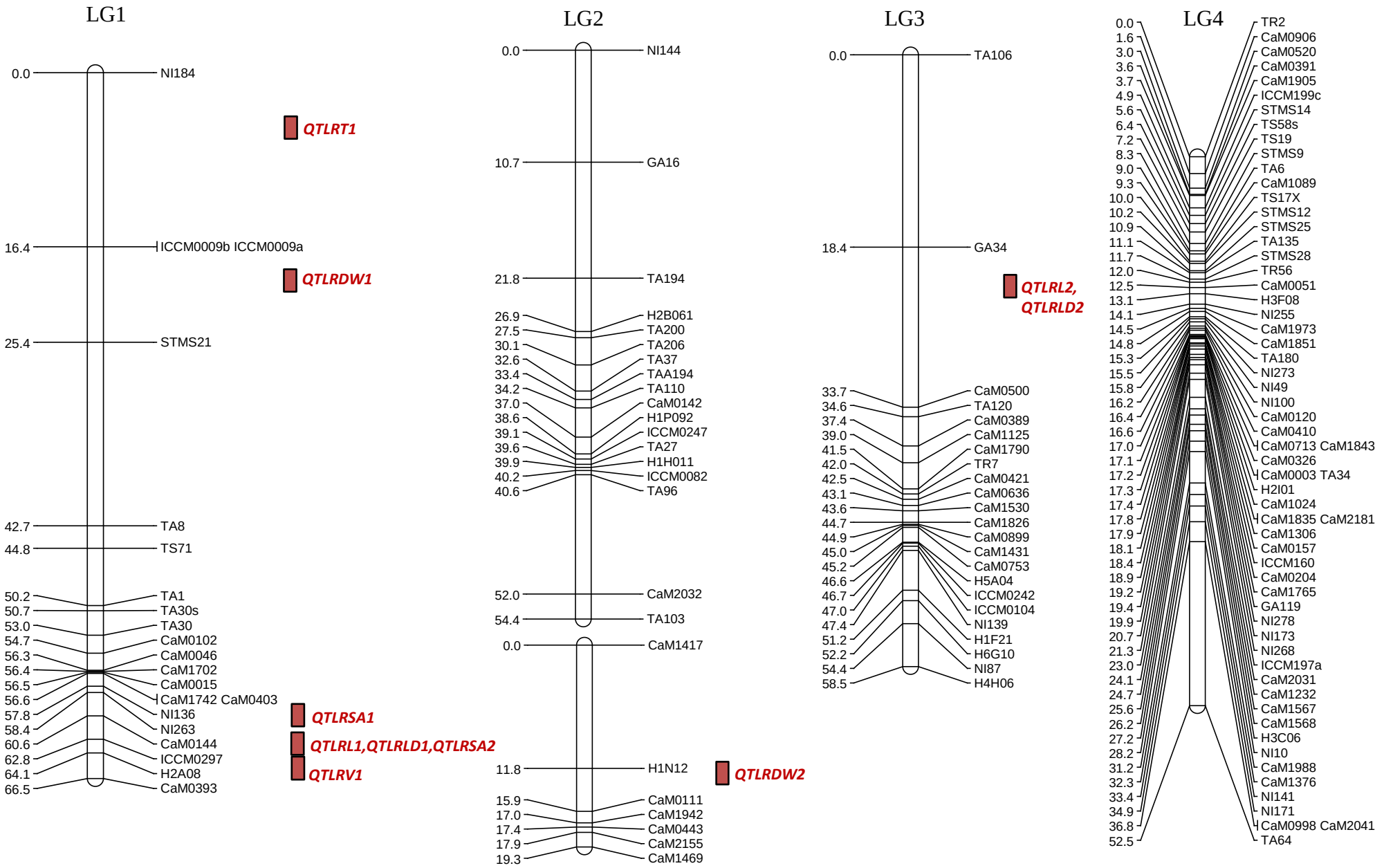
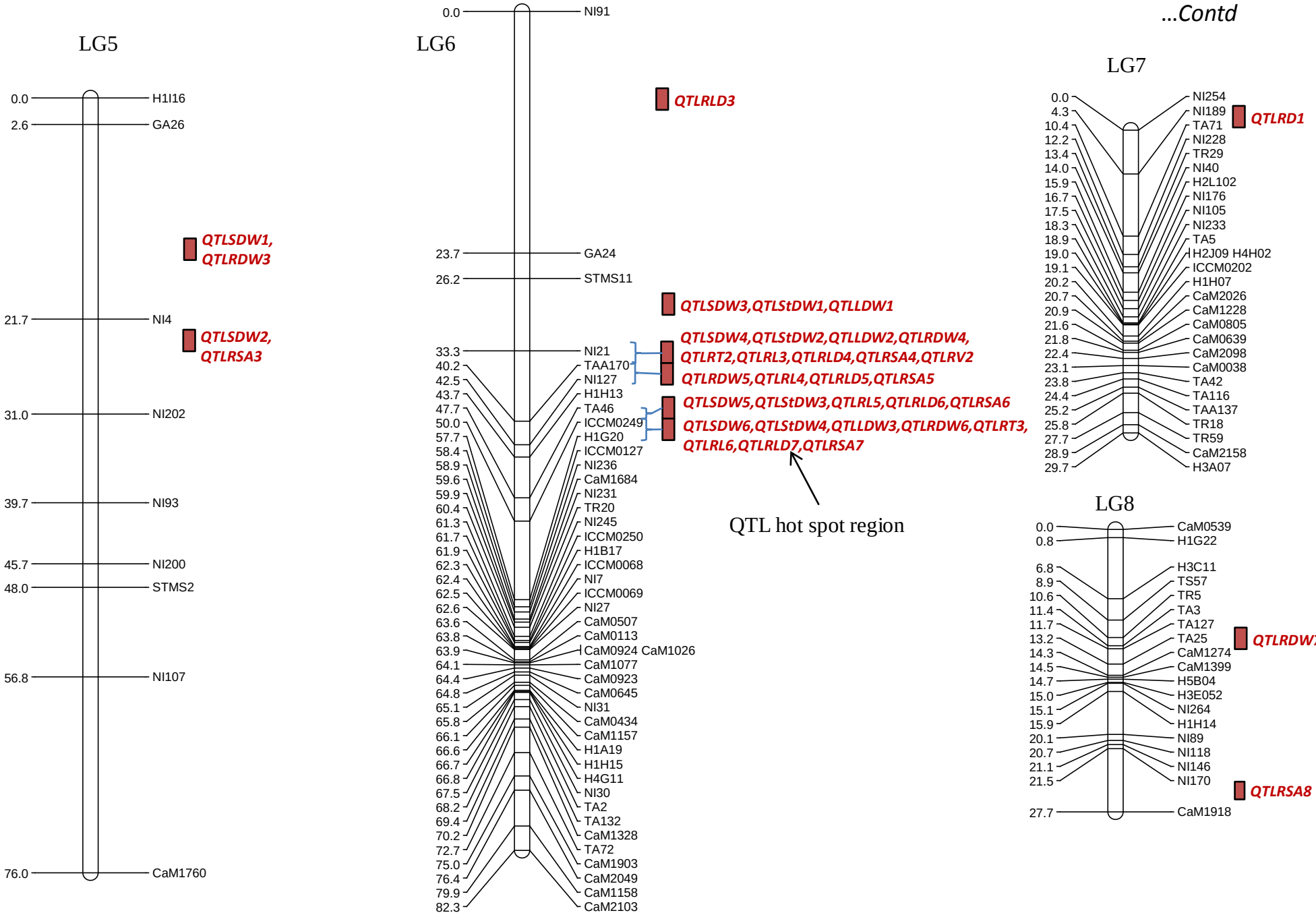


Figure 10. QTL map for drought tolerance related traits based on intra-specific mapping population- ICC 4958 ICC 1882 *Contd…*


LG5
0.0 H1I16
2.6 GA26
QTLSDW1, QTLRDW3
21.7 NI4
QTLSDW2, QTLRSA3
31.0 NI202
39.7 NI93
45.7 NI200
48.0 STMS2
56.8 NI107
76.0 CaM1760
LG6
0.0 NI91
QTLRLD3
23.7 GA24
26.2 STMS11
QTLSDW3,QTLStDW1,QTLLDW1
QTLSDW4,QTLStDW2,QTLLDW2,QTLRDW4, QTLRT2,QTLRL3,QTLRLD4,QTLRSA4,QTLRV2
QTLRDW5,QTLRL4,QTLRLD5,QTLRSA5
QTLSDW5,QTLStDW3,QTLRL5,QTLRLD6,QTLRSA6
QTLSDW6,QTLStDW4,QTLLDW3,QTLRDW6,QTLRT3, QTLRL6,QTLRLD7,QTLRSA7
33.3 NI21
40.2 TAA170
42.5 NI127
43.7 H1H13
47.7 TA46
50.0 ICCM0249
57.7 H1G20
58.4 ICCM0127
58.9 NI236
59.6 CaM1684
59.9 NI231
60.4 TR20
61.3 NI245
61.7 ICCM0250
61.9 H1B17
62.3 ICCM0068
62.4 NI7
62.5 ICCM0069
62.6 NI27
63.6 CaM0507
63.8 CaM0113
63.9 CaM0924 CaM1026
64.1 CaM1077
64.4 CaM0923
64.8 CaM0645
65.1 NI31
65.8 CaM0434
66.1 CaM1157
66.6 H1A19
66.7 H1H15
66.8 H4G11
67.5 NI30
68.2 TA2
69.4 TA132
70.2 CaM1328
72.7 TA72
75.0 CaM1903
76.4 CaM2049
79.9 CaM1158
82.3 CaM2103
QTL hot spot region
LG7
0.0 NI254
4.3 NI189
QTLRD1
10.4 TA71
12.2 NI228
13.4 TR29
14.0 NI40
15.9 H2L102
16.7 NI176
17.5 NI105
18.3 NI233
18.9 TA5
19.0 H2J09 H4H02
19.1 ICCM0202
20.2 H1H07
20.7 CaM2026
20.9 CaM1228
21.6 CaM0805
21.8 CaM0639
22.4 CaM2098
23.1 CaM0038
23.8 TA42
24.4 TA116
25.2 TAA137
25.8 TR18
27.7 TR59
28.9 CaM2158
29.7 H3A07
LG8
0.0 CaM0539
0.8 H1G22
6.8 H3C11
8.9 TS57
10.6 TR5
11.4 TA3
11.7 TA127
13.2 TA25
QTLRDW7
14.3 CaM1274
14.5 CaM1399
14.7 H5B04
15.0 H3E052
15.1 NI264
15.9 H1H14
20.1 NI89
20.7 NI118
21.1 NI146
21.5 NI170
QTLRSA8
27.7 CaM1918

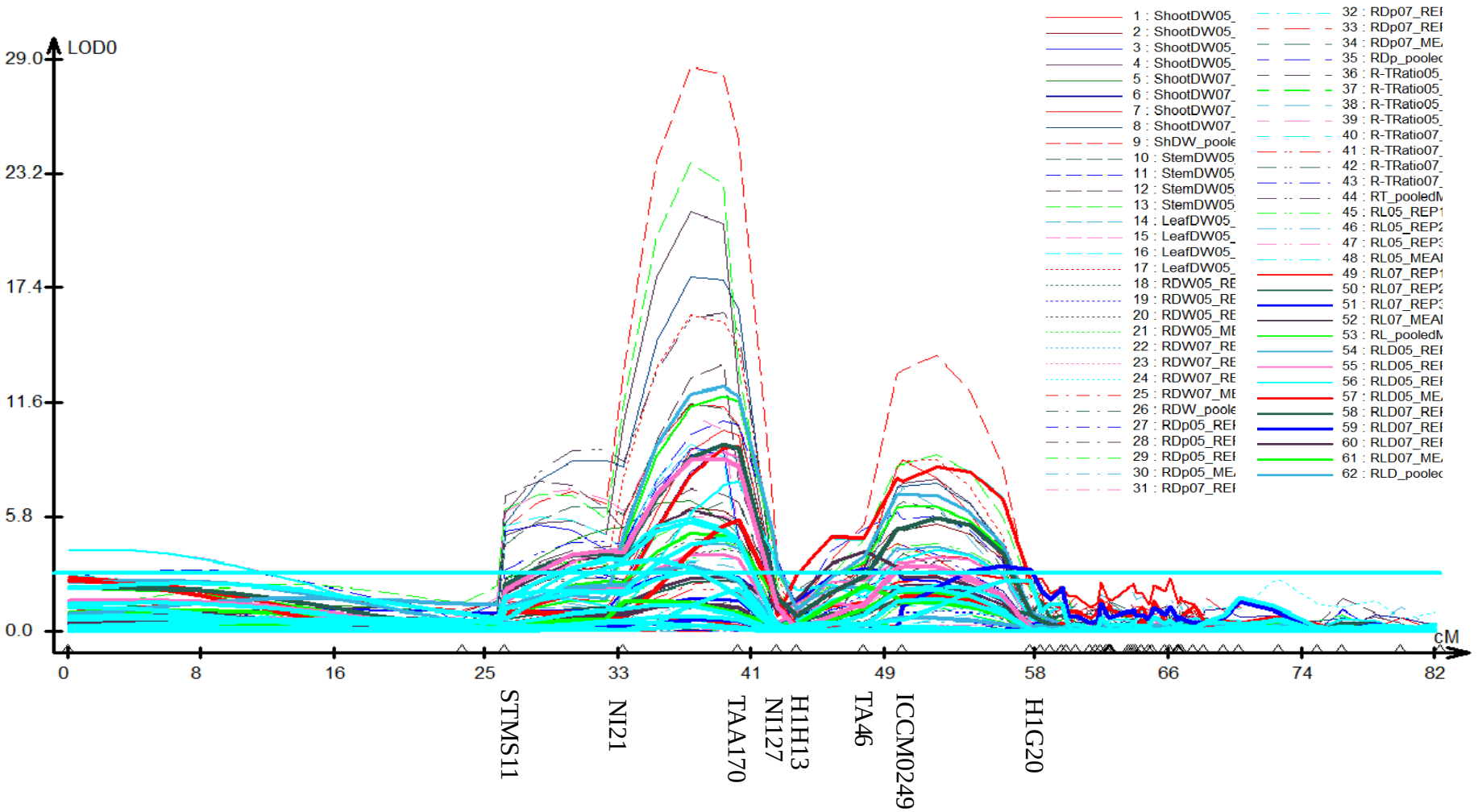


Figure 11. A snapshot of “QTL hot spot region” located on LG6 of intra-specific mapping population- ICC 4958 ICC 1882

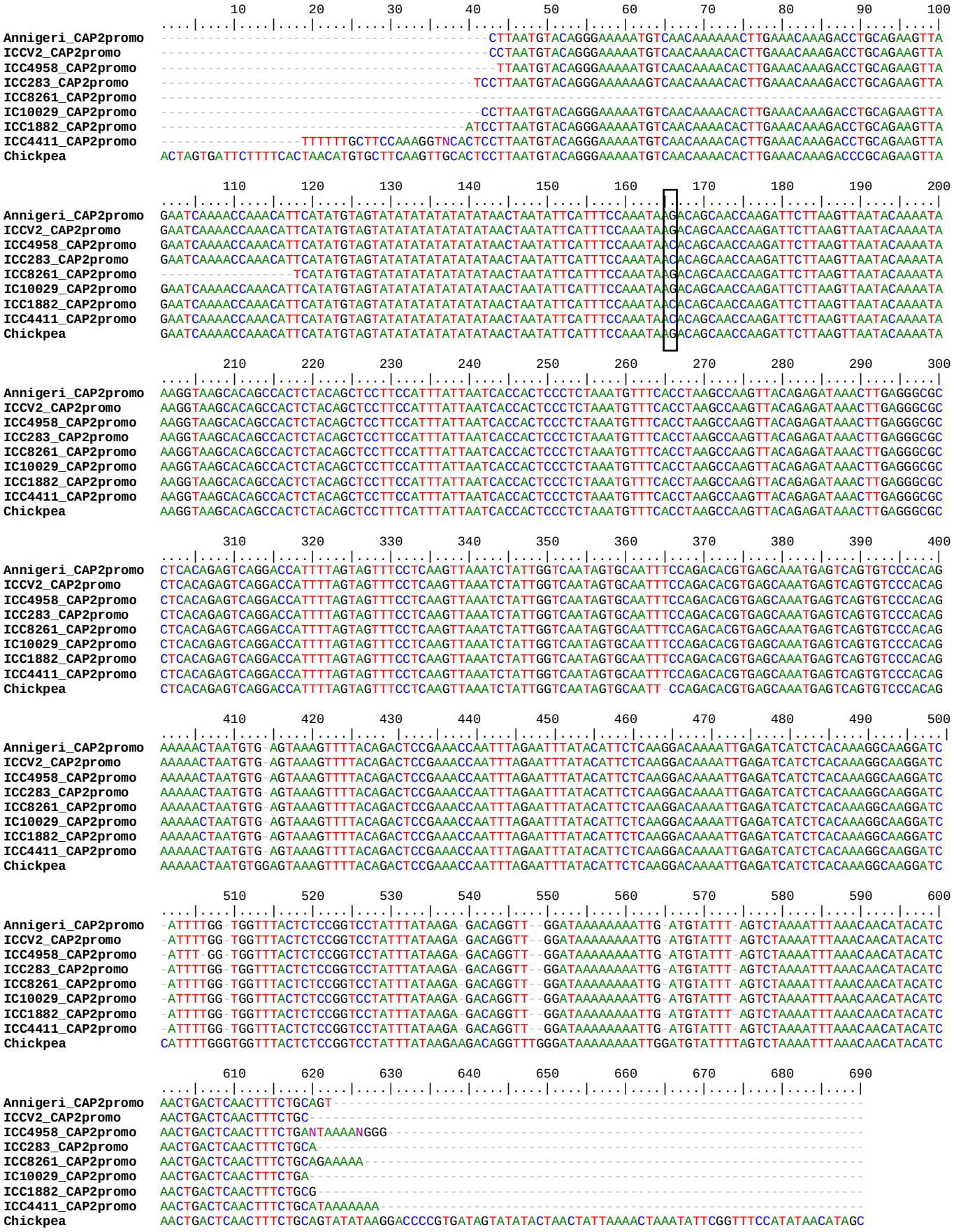


Figure 12. Multiple sequence alignment (MSA) of *CAP2* promoter across eight chickpea genotypes along *CAP2* promoter sequence

Occuerence of SNPs in eight genotypes is shown in the box.

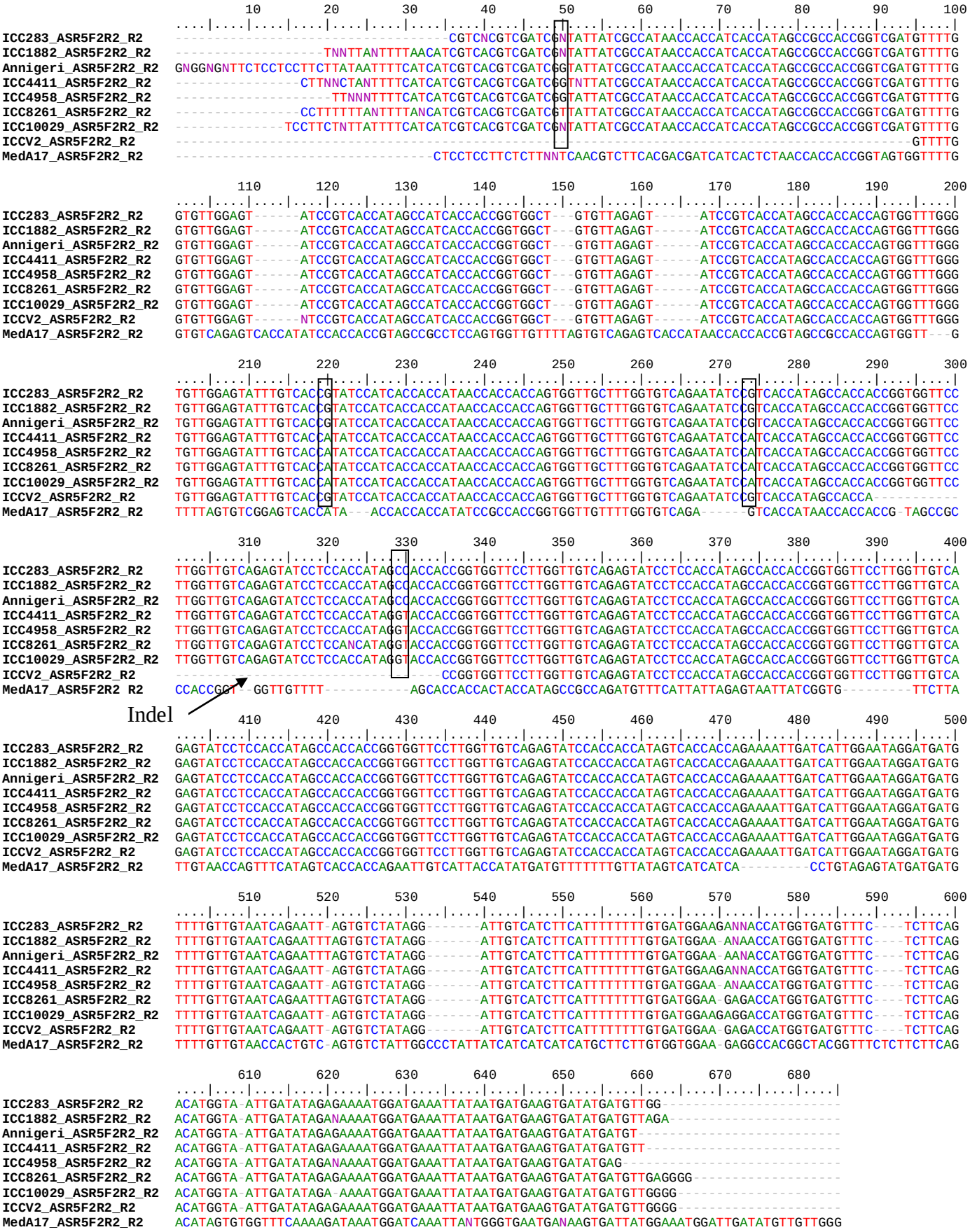


Figure 13. MSA of abscisic acid stress and ripening (*ASR)* gene across eight chickpea genotypes along with one Medicago genotype (A17)

Occurrence of SNPs and Indels across eight genotypes has been shown in box and arrow respectively.

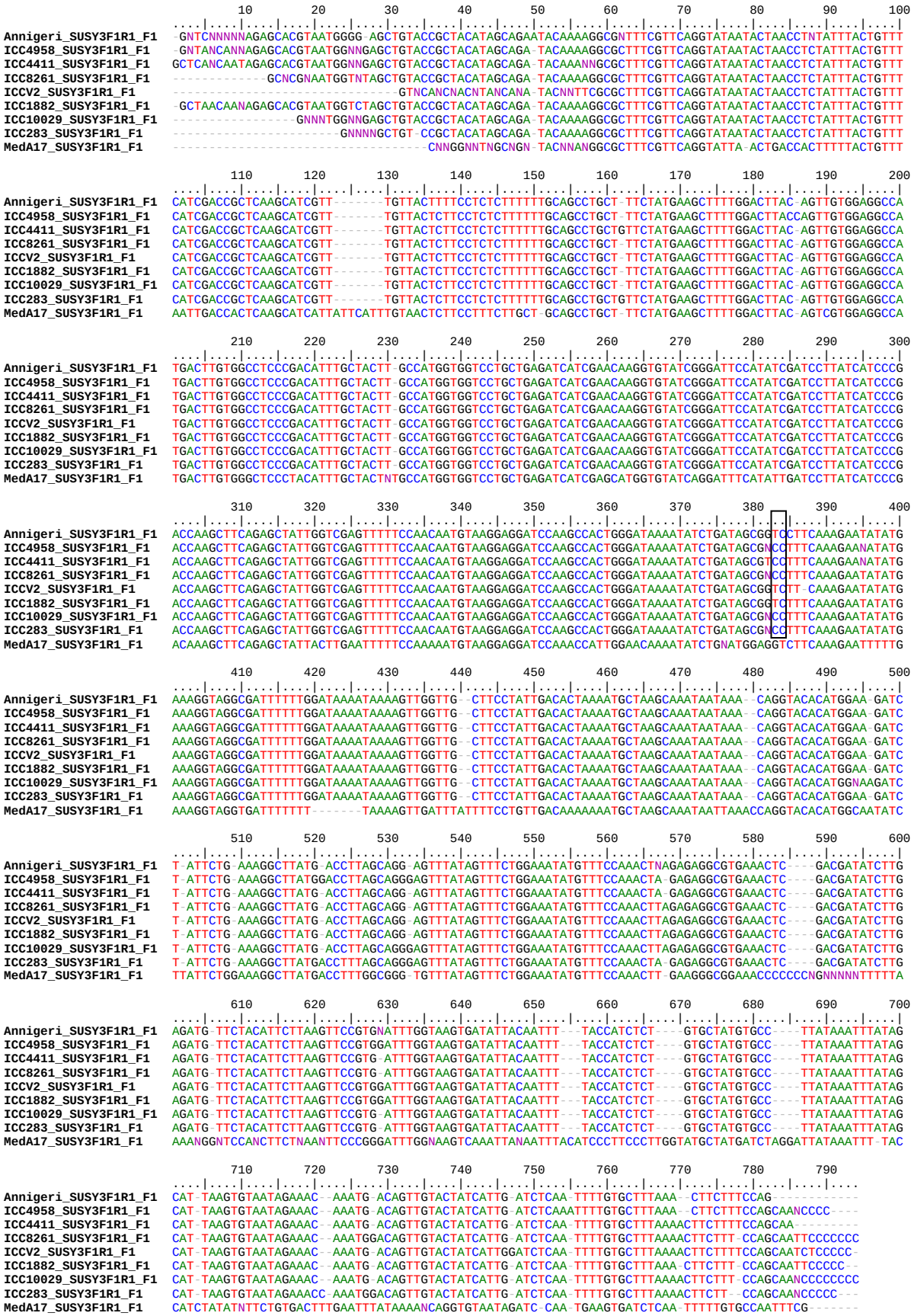


Figure 14. MSA of sucrose synthase (*SuSy)* gene across eight chickpea genotypes along with one Medicago genotype (A17)

Occurrence of SNPs in eight genotypes is shown in the box.

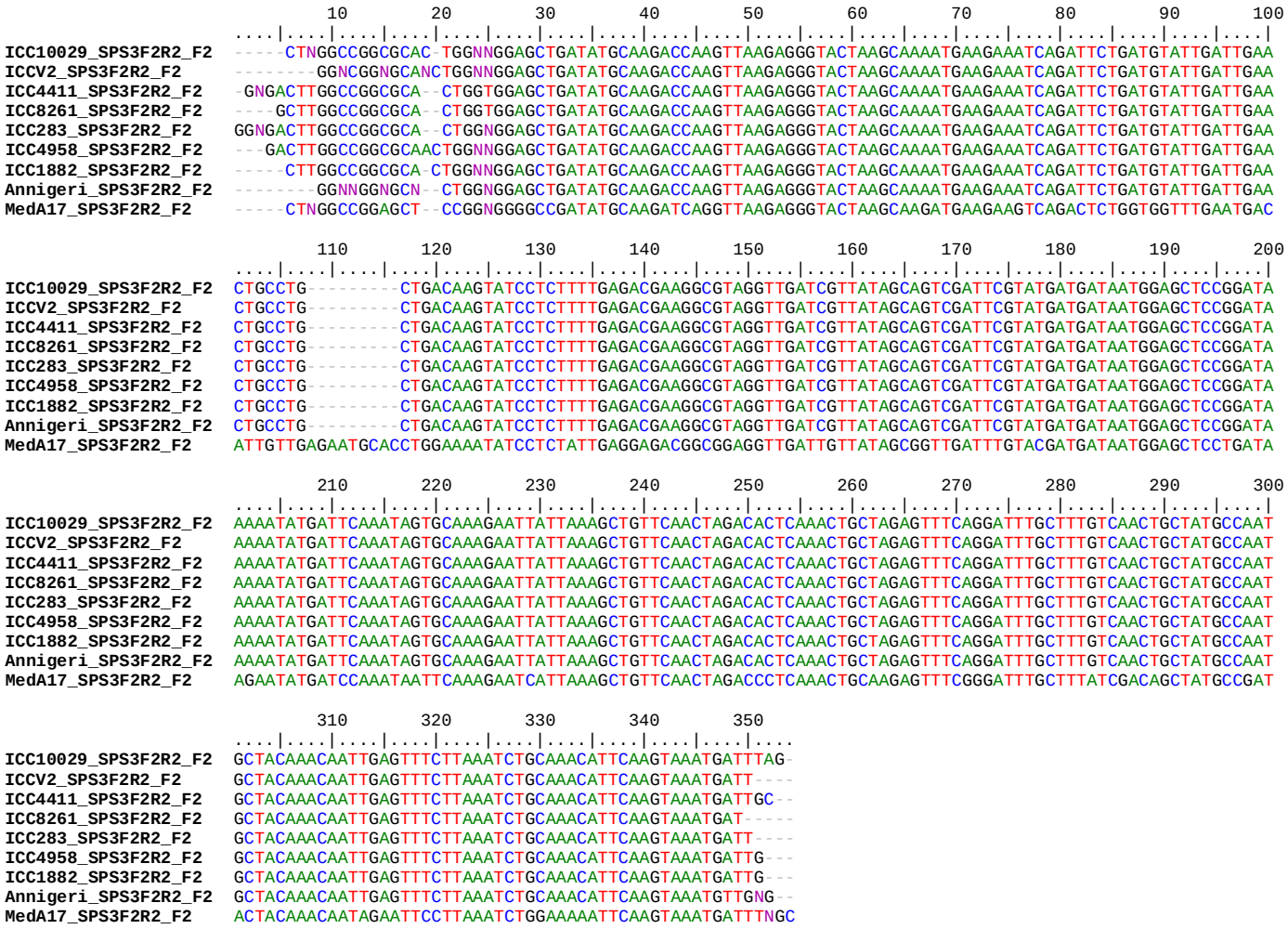


Figure 15. MSA of sucrose phosphate synthase (*SPS)* gene across eight chickpea genotypes along with one Medicago genotype (A17)

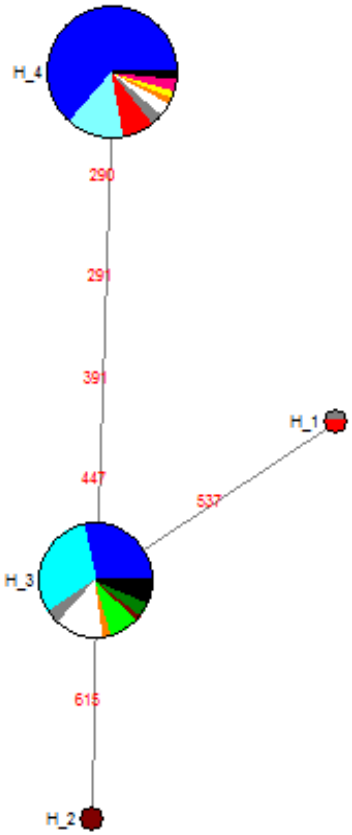


Figure 16. Haplotype network of *ASR* gene developed based on country of origin of genotypes of chickpea reference set

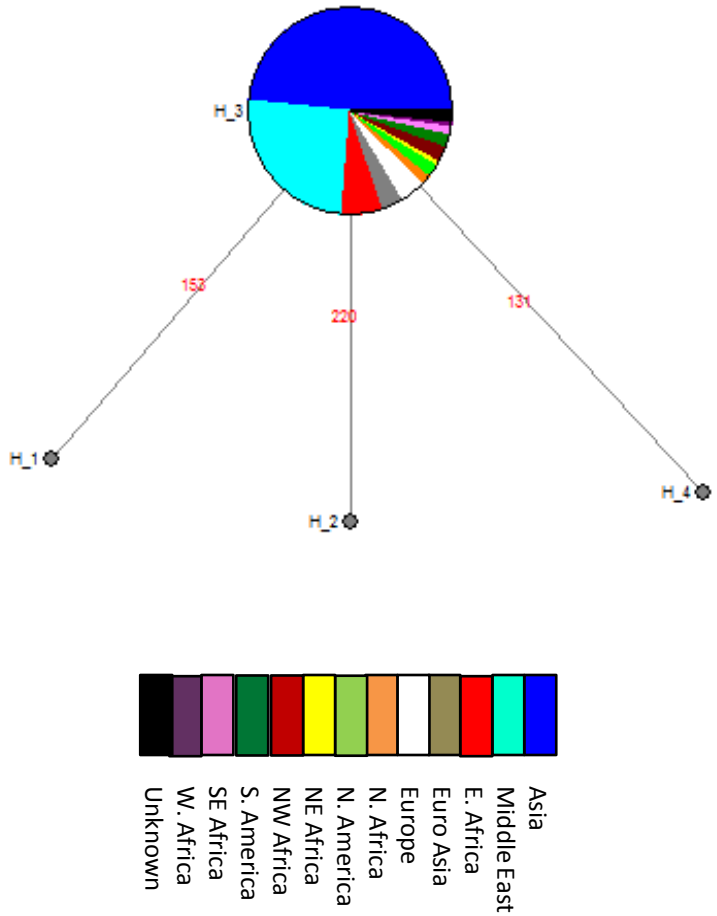


Figure 17. Haplotype network of *SPS* gene developed based on country of origin of genotypes of chickpea reference set

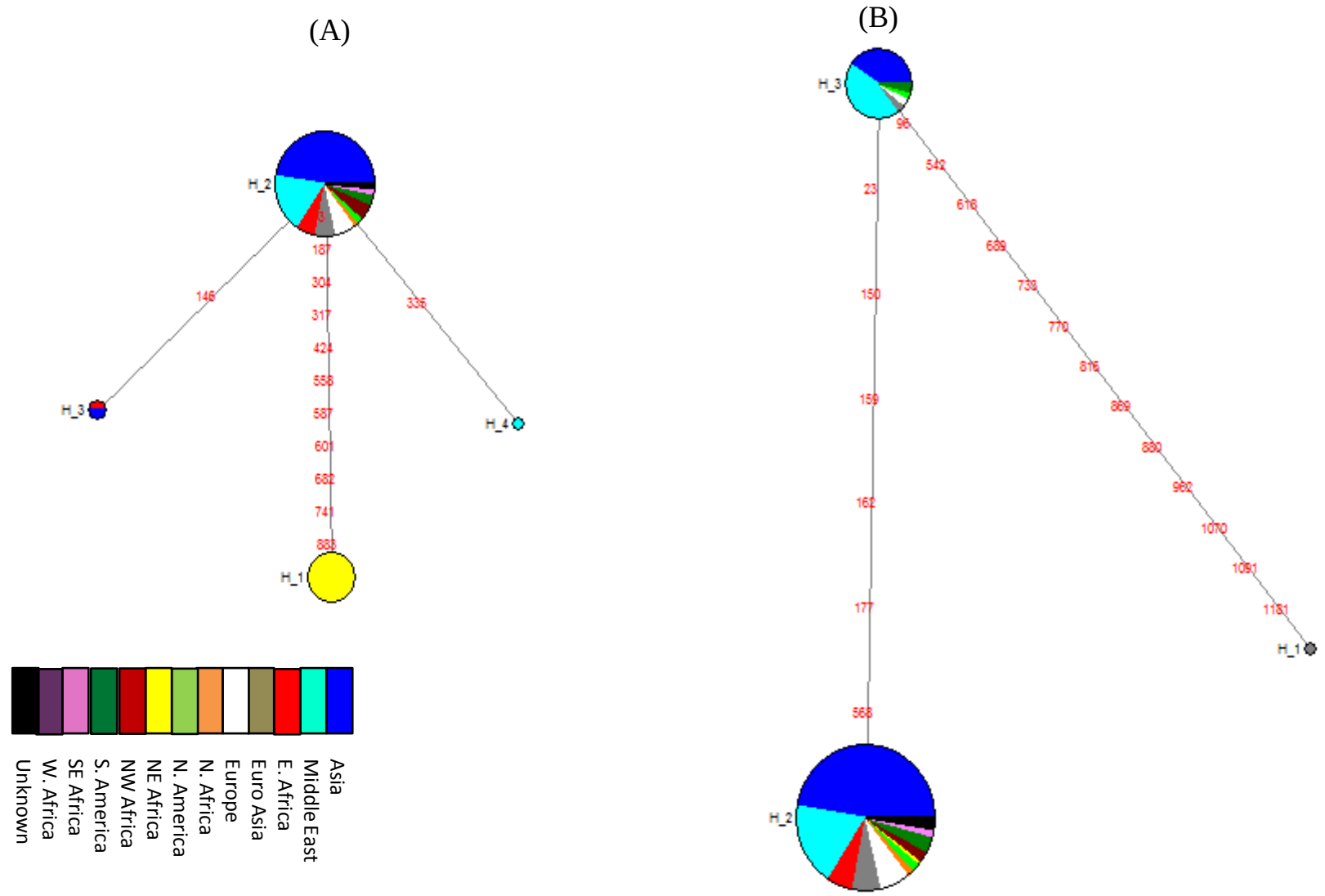


Figure 18. Haplotype network of fragments of *ERECTA* gene developed based on country of origin of genotypes of chickpea reference set The fragment *ERECTA(7f-5r)* is represented in (A) *and ERECTA(8f-8r)* is represented in (B)

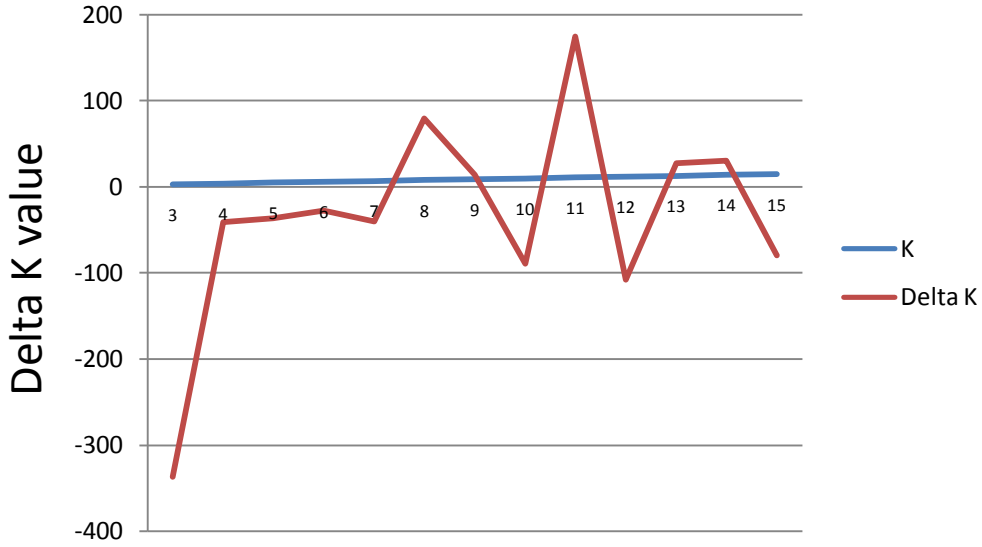


Figure 19. Estimation of delta K value using Evanno's method

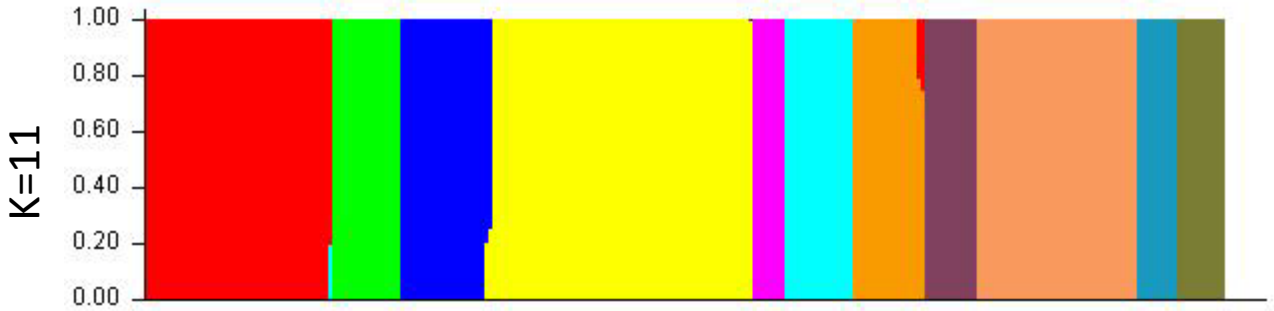


Figure 20. Structure plot of reference set of chickpea at K=11