DETECTION OF POLYMORPHISM IN PARENTAL GENOTYPES OF SELECTED MAPPING POPULATIONS IN GROUNDNUT
(*Arachis hypogaea* L.)

By

T.JAYAKUMAR B.Sc. (Ag.)

I.D.No. 08-612-009

Work done at

International Crops Research Institute for the Semi-Arid Tropics

(ICRISAT)

Patancheru, Greater Hyderabad - 502 324.

CENTER FOR PLANT BREEDING AND GENETICS

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE - 641003

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Thesis submitted in part fulfillment of the requirement for the Degree of Master of Science (Agriculture) in Plant Breeding and Genetics to the
Tamil Nadu Agricultural University,
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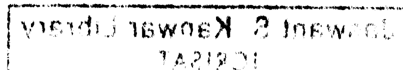

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CERTIFICATE

This is to certify that the thesis entitled **"DETECTION OF POLYMORPHISM IN PARENTAL GENOTYPES OF SELECTED MAPPING POPULATIONS IN GROUNDNUT (*Arachis hypogaea* L.)"** submitted in part fulfilment of the requirement for the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT BREEDING AND GENETICS** to the Tamil Nadu Agricultural University, Coimbatore is a record of ***bonafide*** research work carried out by **T. JAYAKUMAR,** under my supervision and guidance at **International Crops Research Institute for the Semi-Arid Tropics**, Patancheru and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles and that the work has not been published in part or full in any scientific or popular journal or magazine.

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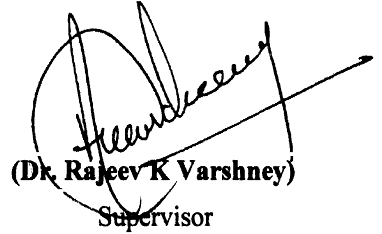

(Dr. Rajeev K Varshney)
Supervisor

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ABBREVIATIONS

%	:	Percent
°C	:	Degree Celsius
µg	:	Microgram
ABI	:	Applied Biosystems
AFLP	:	Amplified Fragment Length Polymorphism
BAC	:	Bacterial Artificial Chromosome
BIBAC	:	Binary Bacterial Artificial Chromosome
bp	:	Basepair
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DGR	:	Directorate of Groundnut Research, Junagadh.
DNA	:	Deoxyribo Nucleic Acid
dNTP	:	deoxy Nucleotide Tri Phosphate
EDTA	:	Ethylene Diamine Tetra Acetic Acid
FAO	:	Food and Agriculture Organization
g	:	Gram
ha	:	Hectare
ICRISAT	:	International Crops Research Institute for the Semi-Arid Tropics

ISSR	:	Inter Simple Sequence Repeat
kg	:	Kilogram
M	:	Molar
MAS	:	Marker Assisted Selection
Mbp	:	Mega basepair
mg	:	Milligram
Mha	:	Million Hectare
µl	:	Microlitre
ml	:	Millilitre
mM	:	Millimolar
Mt	:	Million tons
ng	:	Nanogram
PAGE	:	Poly Acrylamide Gel Electrophoresis
PCoA	:	Principal Coordinates Analysis
PCR	:	Polymerase Chain Reaction
PG	:	Parental Genotypes
PIC	:	Polymorphic Information Content
pmole	:	Picomoles

QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
sec	:	Seconds
SAT	:	Semi-Arid Tropics
SNP	:	Single Nucleotide Polymorphism
SSR	:	Simple Sequence Repeat
STMS	:	Sequence Tagged Microsatellite Site
TE	:	Tris- EDTA
UV	:	Ultraviolet
UAS-D	:	University of Agricultural Sciences, Dharwad.
V	:	Volt

ABSTRACT

DETECTION OF POLYMORPHISM IN PARENTAL GENOTYPES OF SELECTED MAPPING POPULATIONS IN GROUNDNUT (*Arachis hypogaea.* L.)

By

T. JAYAKUMAR

Degree : Master of Science (Agriculture) in Plant Breeding and Genetics.

Chairman : **Dr. A. KALAMANI,**
Professor and Head, Department of Forages,
Centre for Plant Breeding and Genetics,
Tamil Nadu Agricultural University,
Coimbatore - 641 003.

Co Chairman : **Dr. RAJEEV K. VARSHNEY**
Principal Scientist (Applied Genomics)
Applied Genomics Laboratory,
GT- Biotechnology, ICRISAT,
Patancheru, Greater Hyderabad -502 324.

Availability of polymorphic markers in a crop species is prerequisite to construct high density genetic linkage map and thus mapping of Quantitative Trait Loci (QTL) and genetic improvement through marker assisted breeding can be achieved. Lack of sufficient molecular markers hinder current genetic research in groundnut (*Arachis hypogaea.* L.), an allotetraploid species. It is necessary to identify more polymorphic molecular markers for potential use in groundnut genomics. The present study was carried out at M. S. Swaminathan Applied Genomics Laboratory, ICRISAT, Patancheru, India during 2009-2010, to screen the newly designed 1152 BAC-end SSRs among sixteen cultivated groundnut genotypes, which represents the

parents of twelve mapping populations. A set of 876 (76 per cent) primer pairs yielded scorable amplicons and 184 (16 per cent) primer pairs showed polymorphism among sixteen parental genotypes. The 184 SSR markers detected 2 to14 alleles with an average of 3.73 alleles per locus.

The Polymorphic Information Content (PIC) value for these 184 polymorphic markers varied form 0.04 to 0.87 with an average of 0.39 per marker. Among di, tri and compound-nucleotide repeats, compound SSRs showed higher PIC value (average 0.43 per marker) compared to di-nucleotide (average 0.40 per marker) and tri-nucleotide (average 0.30 per marker). Whereas the di-nucleotide repeats showed higher allele number (average 3.80 alleles per locus) followed by compound (average 3.70 alleles per locus) and tri-nucleotide repeats (average 3.60 alleles per locus). The investigation on relationship of repeat unit length with number of alleles and Polymorphic Information Content (PIC) value revealed that, no consistent relationship between number of repeat units and SSR polymorphism. Based on 686 alleles obtained from 184 polymorphic markers, a dendrogram was constructed to understand the relationship among the 16 genotypes. The UPGMA dendrogram based on genetic similarity revealed the existence of abundant SSR polymorphism among cultivated genotypes. It was concluded that these SSR markers can be effectively utilized for diversity studies, resolution of genetic map and marker assisted selection for cultivated groundnut.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.), the 'king' of oilseeds is commonly known as peanut or monkey nut. It belongs to Leguminosae or Fabaceae family. It is a self-pollinating crop with basic chromosome number ten ($2n = 4x = 40$) (Stebbins, 1957; Stalker and Dalmacio, 1986) and genome size 2800 Mb/1C (Guo *et al.*, 2009). Groundnut is widely used as a food and cash crop around the world. It is mainly grown by resource-poor farmers in Africa and Asia to produce edible oil (48–50 per cent), and for human consumption. In addition, groundnut haulms and cake (after oil extraction) are excellent animal feed. As an important oilseed crop, it is cultivated in more than 109 countries across Asia, Africa and America with around 24 million hectares (FAOSTAT, 2007) generating an annual groundnut (with shell) production of 37.8 million tonnes (FAOSTAT, 2007). India, China, Nigeria and Sudan are the top producers but more than 20 other countries, mainly in Asia and Africa.

1.1 Taxonomy

The genus *Arachis* (family Fabaceae) is native to South America, and contains 80 described species assembled into nine sections (*Arachis, Erectoides, Heteranthae, Caulorrhizae, Rhizomatosae, Extranervosae, Triseminatae, Procumbentes* and *Trierectoides*) based on their morphology, geographic distribution, and sexual compatibility relationship. Cultivated groundnut is a member of the family *Fabaceae*, tribe *Aeschynomeneae*, subtribe *Stylosanthinae* and genus *Arachis*. It is an allotetraploid with genome AABB (Krapovickas and Gregory, 1994). Botanically groundnut (*Arachis hypogaea* L. $2n = 4x = 40$, AABB) is classified into two subspecies which mainly differ in their branching pattern: subspecies *hypogaea* with alternate branching and subspecies *fastigiata* with sequential branching. Each subspecies is further divided into botanical varieties based on their morphological traits and growth habits; subsp. *hypogaea* into var. *hypogaea* (virginia) and var. *hirsuta*; and subsp. *fastigiata* into var. *fastigiata* (valencia), var. *vulgaris* (spanish), var. *peruviana*, and var. *aequatoriana* (Krapovickas and Gregory, 1994).

1.2 Origin and Distribution

The archaeological records support groundnut cultivation between 300 and 2500 BC in Peruvian desert oasis (Weiss, 2000; Smith, 2002). Groundnut originated in the southern Bolivia/north west Argentina region in South America and is presently cultivated in 109 countries of the world. Major groundnut producing countries are China, India, Indonesia, Myanmar, Thailand and Vietnam in Asia; Nigeria, Senegal, Sudan, Zaire, Chad, Uganda, Cote d'Ivory, Mali, Burkina Faso, Guinea, Mozambique, and Cameroon in Africa; Argentina and Brazil in South America and USA and Mexico in North America.

Arachis hypogaea was evolved probably from hybridization of two diploid wild species, *A. duranensis* (A-genome) and *A. ipaensis* (B-genome), followed by a rare spontaneous duplication of chromosomes (Halward *et al.,* 1991; Kochert *et al.,* 1996; Seijo *et al.,* 2004, 2007; Koppolu *et al.,* 2010). The resulting tetraploid plant would have been reproductively isolated from its wild diploid relatives. This isolation, coupled with the origin through a probably single hybridization event leads to a limited genetic diversity of groundnut, as observed in different studies using molecular markers (Kochert *et al.,* 1996; Subramaninan *et al.,* 2000; Herselman, 2003). In contrast, wild diploid *Arachis* species are genetically more diverse (Hilu and Staler, 1995; Moretzsohn *et al.,* 2004; Bravo *et al.,* 2006), providing a rich source of variation for agronomical traits, and DNA polymorphisms for genetic and genomic studies (Stalker and Simpson, 1995; Kameswara Rao *et al.,* 2003; Dwivedi *et al.,* 2007).

1.3 Economic Importance

Groundnut kernel contains high quality edible oil (50 per cent), easily digestible protein (25 per cent) and carbohydrates (20 per cent) on an average 40.1 per cent fat, 25.3 per cent protein and is fairly a rich source of calcium, iron, B complex vitamins like thiamine, riboflavin and niacin. It has multifarious usages. Groundnut oil is not only used as a major cooking medium for various food items but also utilized for manufacture of soap, cosmetics, shaving cream, lubricants, etc. In fact, groundnut plays a pivotal role in oilseed economy of India. It is estimated that the shell represents about 25 per cent of the dry weight of unshell groundnut, and the

kernel comprises about 75 per cent. Cotyledons are the main storage tissues and are concentrated source of protein, lipids, and dietary energy. Amino acid profile of raw groundnut is inferior to the profile of raw soybean in many respects. Comparatively, the protein content of raw groundnut is only about 70 per cent of that of raw soybean. Groundnuts and groundnut protein products are low in sulfur based aminoacids. Groundnuts are a reasonable source of dietary minerals especially potassium, phosphorus and magnesium. However, they are poor source of fat soluble vitamins like A, D, and K. Groundnut oil is an excellent source of mono- and poly-unsaturated fatty acids, exceeding the levels of these fatty acids in soybean and corn oil, but significantly lower than in sunflower and safflower oil. Groundnut oil contains about 1 per cent palmitic acid and 80 per cent oleic and linoleic acid (Nwokolo, 1996).

1.4 Major constraints in groundnut production

Groundnut breeders and physiologists have been working across the world to improve the yield of the crop under various biotic and abiotic stresses. Biotic stresses include diseases such as rust (*Puccinia arachidis*), early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaseoisariopsis personata*), crown rot (*Aspergillus niger*), stem and pod rot, stem necrosis, rosette disease and pests like termites (*Odontotermes sp.,*), whitegrubs (*Lachnosterna consanguiea*), jassids (*Empoasca kerri*), aphids (*Aphis craccivora)*, leaf miners (*Aproaerema modicella*), red hairy caterpillars (*Amsacta albistriga*), tobacco caterpillars and thrips etc, whereas among abiotic stresses, drought is predominant.

1.5 Molecular Markers

Recent advances in the area of crop genomics have offered tools to assist breeding (Varshney *et al.,* 2005, 2006). Molecular markers and genetic linkage maps are the pre-requisites for undertaking molecular breeding activities particularly identifying and localizing important genes controlling qualitatively and quantitatively inherited traits in wide range of species (Varshney *et al.,* 2006). Such tools would then simply speed up the process of introgression of agronomically desired traits such as yield, quality, biotic and abiotic stress resistance into preferred varieties, especially of complex traits such as drought.

In groundnut, several hundred of SSR markers have been developed by different research groups all over the world (Hopkins *et al.*, 1999; He *et al.*, 2003; Palmieri *et al.*, 2002, 2005; Ferguson *et al.*, 2004; Moretzsohn *et al.*, 2004, 2005; Nelson *et al.*, 2006; Mace *et al.*, 2007; Proite *et al.*, 2007; Gimenes *et al.*, 2007; Wang *et al.*, 2007; Cuc *et al.*, 2008; Gautami *et al.*, 2009; Knapp *et al.*, unpublished). Even though several hundred of microsatellite markers are available for groundnut, screening of these primers did not reveal much polymorphism in the parental genotypes of different mapping populations (Varshney *et al.*, 2007), because of the evolutionary genetic bottleneck in the form of polyploidy and self pollination. As a result, majority of the SSR markers have not been integrated into the groundnut genetic linkage maps. Hence there is a need to integrate novel SSR markers into genetic maps and enhance the marker density. Availability of information on marker polymorphism in parental genotypes of different mapping populations is essential to integrate and enhance the density of groundnut genetic maps.

1.6 Objectives

Keeping in view the importance of SSR markers in groundnut, a set of 1152 SSR markers were designed at ICRISAT from the BAC-end sequence data obtained from Dr. Doug Cook, University of California, Davis (UC-Davis), USA. The primer pairs for these markers were synthesized at University of California, Davis, USA. The present study was undertaken with the following objectives:

1. Validation of microsatellite markers on a panel of 16 groundnut genotypes.
2. Identification of informative set of SSRs for different mapping populations.
3. Studying the pattern of SSR genetic diversity among 16 groundnut genotypes.

2. REVIEW OF LITERATURE

Groundnut is a unique tetraploid species originated through recent polyploidization followed by subsequent chromosomal doubling (Kochert *et al.*, 1996). Even though extensive levels of morphological variation is observed in *Arachis hypogaea,* which is most probably due to the variation in few genes (Kochert *et al.*, 1991), molecular markers have showed little polymorphism in the germplasm of this species (Kochert *et al.*, 1991) Only few molecular markers have been identified linked with biotic resistance genes in groundnut (Garcia *et al.*, 1996; Burow *et al.*, 1996) and hence due to unavailability of tightly linked molecular markers, genetic improvement through marker assisted breeding is limited. Although a considerable number of AFLP markers have been identified (He and Prakash, 1997) still more DNA markers are needed to saturate the existing groundnut linkage map (Varshney *et al.*, 2009) and to initiate genetic studies for this plant species.

Tremendous progress has been made in recent years in the development of novel genetic tools such as molecular markers, genetic maps and whole-genome transcription profiling techniques to identify genomic regions and genes underlying plant stress responses in many crop species (Varshney *et al.*, 2005). The development of molecular techniques for genetic analysis has led to a great increase in our knowledge of crop genetics and genomics.

2.1 Molecular Markers Studies in Groundnut

2.1.1 Restriction Fragment Length Polymorphism (RFLP)

RFLPs represented the first generation molecular marker system that detected large number of polymorphisms in plants as well as animal genome mapping population. In *Arachis* RFLPs revealed very low level of polymorphism in cultivars (Kochert *et al.*, 1991) and in germplasm lines (Paik-Rao *et al.*, 1992) though considerable morphological and physiological variability exists among the lines (Halward *et al.*, 1991). RFLPs have been used to study diversity at molecular level in *Arachis* (Kochert *et al.*,, 1991) forming different clusters which corresponded closely with morphological groups (Stalker, 1990). Similarly Stalker *et al.* (1995) used RFLPs to study genetic diversity among eighteen

accessions of *A. duranensis* and found large amount of variation within the species. RFLP analysis clearly indicated the origin of cultivated groundnut from the cross between *A. duranensis* and *A. ipaënsis* (Kochert *et al.*, 1996). The genomic relationship between AA genome, BB genomes and AABB genome species were revealed by RFLPs (Gimenes *et al.*, 2002). The lowest genetic variation was detected within accessions of *A. duranensis* (17 accessions) followed by *A. batizocoi* (4 accessions) and *A. cardenasii* (9 plants of accession GKP 10017). Though RFLPs are codominant, robust, reliable and transferable across populations but it is time consuming, laborious, expensive and large amount of DNA is required.

2.1.2 Random Amplified Polymorphic DNAs (RAPD)

RAPD is one of the simplest, inexpensive molecular markers method and requires only small amount of DNA. The advent of the RAPD assay (Williams *et al.*, 1990) provided an efficient method to detect DNA polymorphisms and generate a large number of molecular markers for genomic applications. RAPD markers for mapping and analysis of genetic diversity have been reported for a wide variety of plants including groundnut (Hilu and Stalker, 1995), tobacco (*Nicotiana spp.*) (Lin *et al.*, 2001), *Musa spp.* (Ude *et al.*, 2003), potato (*Solanum tuberosum L.*) (Sun *et al., 2003*) and barley (*Hordeum vulgare*) (Fernandez *et al.*, 2002). RAPD markers have been employed for identification of cultivars and to study the taxonomic relationships for management of plant genetic resources. To study variability in *Arachis* species germplasm, primers of arbitrary sequence have been utilized and reported very little variation in *Arachis* species. Dominant behavior of RAPD markers prevented differentiation of heterozygotes from homozygotes with certainly, limiting the usefulness of RAPD markers in the construction of a genetic linkage map in groundnut (Halward *et al.*, 1992). But RAPDs detected significant amount of variation (81.66 per cent) between *A. hypogaea* and synthetic amphidiploid (Lanham *et al.*, 1992). A maximum variation was observed among accessions of *A. cardenasii* and *A. glandulifera,* whereas in case of *A. hypogaea* and *A. monticola* least amount of variation was observed through RAPDs (Hilu and Stalker, 1995). Based on the above study, *Arachis duranensis* was found most closely related to the domesticated groundnut and is believed to be the donor of the A genome.

2.1.3 Amplified Fragment Length Polymorphism (AFLPs)

A variety of molecular marker techniques have been used to determine taxonomic relationships and genetic variation of crop species and their wild relatives. Among these methods, the amplified fragment length polymorphism (AFLP) method (Vos *et al.*, 1995) has been used successfully to analyze inter- and intraspecific genetic diversity in a wide range of crop species (Hill *et al.*, 1995; Powell *et al.*, 1996). The major advantage of the AFLP technique over other marker technologies is that it enables simultaneous analysis of a large number of marker loci throughout the genome (Powell *et al.*, 1996). Other benefits of the technique include high reproducibility, high levels of polymorphism detection, and no prior knowledge required of the genome being studied (Prabhu and Gresshoff, 1994; Lu *et al.*, 1996). AFLPs are good markers for establishing genetic relationship among *Arachis* species and can also detect high level of polymorphism than RAPDs and RFLPs (Gimenes *et al.*, 2002). Consequently, AFLPs are ideally suited for the study of genetic diversity within gene pools of species for which little information currently exists (Tomkins *et al.*, 2001), as is the case in genus *Arachis*. AFLP approach can detect considerable amount of genetic variation at molecular level in the cultivated groundnut germplasm to conduct evolutionary studies and to compare with other marker systems and also showed that the botanical varieties *aequatoriana* and *peruviana* were closer to subspecies *hypogaea* than subspecies *fastigiata* (He and Prakash, 2001). Though AFLPs detect multiple loci and generate high level of polymorphism, the major disadvantage is its dominant nature along with large amount of DNA requirement and complicated methodology (Vos *et al.*, 1995).

2.1.4 Simple Sequence Repeats (SSRs) or Microsatellites

Simple sequence repeats (SSRs) are also known as microsatellites or sequence-tagged microsatellite sites (STMS) (Beckmann and Soller, 1990). SSRs are multiallelic, co-dominant, reproducible and require small amounts of DNA, since they are PCR based markers. SSRs contain short, tandemly repeated DNA sequence motifs consisting of two to six nucleotide core units (Litt and Lutty, 1989). The high incidence of detectable polymorphism through changes in repeat numbers is due to an intramolecular mutation mechanism called DNA slippage (Gupta *et al.*, 1996). The regions flanking the

microsatellites are generally conserved and PCR primers relative to the flanking regions are used to amplify SSR containing DNA fragments.

Microsatellites are more variable than RFLPs and RAPDs, and have been widely utilized in plant genomic studies (Gupta and Varshney, 2000). Thus, it is believed that SSR markers will provide the molecular genetic differentiation to facilitate routine diversity analysis and molecular breeding applications (Dwivedi *et al.*, 2003). Transferability of SSR markers between the populations and between related genera is the major advantage over other marker system. Transferability is a consequence of the homology of flanking sequences of the microsatellites and size of the region between the primer pairs amenable to amplification by PCR. Interspecific transferability of SSR markers was observed in several studies (Peakall *et al.*, 1998; Wang *et al.*, 2004; Koppolu *et al.*, 2010) while inter generic transferability was also reported (Gautami *et al.*, 2009).

However, in general, as compared to other crop species, a low level of marker polymorphism was observed in groundnut (Stalker and Mozingo, 2001). The reasons for the low level of polymorphism in cultivated groundnut may be due to limited use of variability present in the germplasm, at least the level which can not be detected with the detection tools that are currently available (Varshney *et al.*, 2009).

Groundnut is thought to have evolved relatively recently through a single hybridization event, most likely between the unreduced gametes of two diploid species representing the A and B genomes (Kochert *et al.*, 1996). It is postulated that the resultant amphidiploid plant was then reproductively isolated from diploid wild relatives leading to a very narrow genetic base. Genetic maps have been reported for the genomes of both diploid (Halward *et al.*, 1993) and amphidiploid (Burow *et al.*, 2001) *Arachis*. However, as a consequence of the low level of genetic variation amongst cultivated groundnut, the first reported genetic linkage map of amphidiploid groundnut had to be based on an interspecific cross (Burow *et al.*, 2001). Although this map may be of limited value for molecular breeding due to the different recombination patterns compared with the intraspecific crosses that form the basis of groundnut breeding but an important milestone for groundnut genomics.

Considerable efforts have been made to develop SSR markers, which generally detect higher levels of polymorphism within species than other assays. As a result, the number of microsatellite markers published for groundnut has increased considerably in the last 10 years (Hopkins *et al.,* 1999; He *et al.,* 2003; Palmieri *et al.,* 2002, 2005; Fergusson *et al.,* 2004; Moretzsohn *et al.,* 2004, 2005; Nelson *et al.,* 2006; Mace *et al.,* 2007; Proite *et al.,* 2007; Gimenes *et al.,* 2007; Wang *et al.,* 2007; Cuc *et al.,* 2008; Gautami *et al.,* 2009; Knapp *et al.,* unpublished). Due to availability of large number of SSR markers, now it has been possible to develop genetic maps for A-genome (Moretzsohn *et al.,* 2005), B-genome (Moretzsohn *et al.,* 2009) as well as AB-genomes (Varshney *et al.,* 2009, Foncéka *et al.*, 2009; Hong *et al.*, 2008, 2010), Even with all these efforts by different laboratories, the number of mapped SSR loci on a single genetic map is still not satisfactory.

2.2 Molecular Genetic Diversity Studies in Groundnut

Genetic diversity refers the variations within the individual gene locus / among alleles of a gene, or gene combinations, between individual plants or between plant populations. Genetic diversity has several 'indicators', which are measured using various tools such as classical or Mendelian genetic analysis, that can be employed to evaluate variation in single known gene (controlling qualitative trait). The classical methods of diversity studies are based on morphological characters, which are influenced by various environmental factors. However, the molecular markers, which are unrestricted in number and not influenced by the environment, have the ability of sampling diversity directly at the genome level.

Molecular markers are powerful tools for germplasm screening and studies of genetic variability, and have been used successfully in different plant species (Jain *et al.*, 1994; Laurent *et al.*, 1994; Liu *et al.*, 1994; Lu *et al.*, 1996; Powell *et al.*, 1996). The first molecular study to evaluate phylogenetic relationships and genetic variability in the genus *Arachis* was based on isoenzymatic or seed protein analyses. Most of the further studies analyzed the variation within species (Cherry and Ory, 1973; Maass *et al.*, 1993) or sections (Singh *et al.*, 1991). A few studies compared accessions of different sections

(Lu and Pickersgill, 1993; Stalker *et al.*, 1994; Galgaro *et al.*, 1997) along with accessions and species relationships using RAPDs (Halward *et al.*, 1991, 1992; Galgaro *et al.*, 1998; Gimenes *et al.*, 2000).

SSR markers have been recently developed and proved to be useful for accession discrimination and assessment of genetic variation (Hopkins *et al.,* 1999; He *et al.,* 2003; Ferguson *et al.,* 2004). Since little genetic variability has been detected in cultivated groundnut, the use of a polymorphic marker, such as SSRs, in addition to distinguishing closely related genotypes, should also be useful for phylogenetic studies, as demonstrated in other crops, such as wheat (Lelley *et al.*, 2000), melon (Danin-Poleg, 2001), potato (Ashkenazi *et al.*, 2001), and coffee (Anthony *et al.*, 2002).

Microsatellite or SSR markers have been utilized for the detection of polymorphisms among cultivated groundnut accessions and for the genetic relationship analysis between *A. hypogaea* accessions and wild species of section *Arachis* (Moretzsohn *et al.,* 2004). Diversity studies in groundnut have generally revealed extensive phenotypic variation amongst varieties (Upadhyaya *et al.,* 2001, 2003) yet limited variation have been observed at molecular level (Halward *et al.*, 1991, 1992; Kochert *et al.*, 1991, Paik-Rao *et al.,* 1992; He and Prakash, 1997; Subramanian *et al.*, 2000; Moretzsohn *et al.*, 2004). It is hypothesized that this may be due to the selective neutrality of the molecular markers utilized, while phenotypic traits have been subjected to intense selection (He and Prakash, 1997). Although there is high level of morphological diversity among varieties of *A. hypogaea,* this has not been generally reflected in the level of detectable genetic diversity at molecular level (Grieshammer and Wynne, 1990; Halward *et al.,* 1991; Kochert *et al.,* 1991; Halward *et al.,* 1992; Paik-Rao *et al.,* 1992; Lacks and Stalker, 1993; Bianchi-Hall *et al.,* 1994; Lanham *et al.,* 1994).

3. MATERIAL AND METHODS

3.1 Plant Material

All 1152 BAC-end SSR markers were screened on 16 groundnut genotypes that constitute parental lines of twelve mapping population that are being used in various breeding programmes at ICRISAT, Patancheru, Directorate of Groundnut Research, Junagadh and University of Agricultural Sciences, Dharwad (Table 1). The detail of the parents of twelve mapping populations and their traits was provided in Table 2.

3.2 Methods

3.2.1 DNA extraction

DNA was extracted from the seedlings of sixteen genotypes by using a high-throughput mini- DNA extraction method as per Cuc *et al.* (2008).

3.2.2 High-throughput mini- DNA extraction

A. Sample preparation

- Leaves were harvested from 15 days old seedlings.
- Leaf tissue of 70-100 mg was placed in 12 × 8-well strip tube with strip cap (Marsh Biomarket, USA) in a 96 deep-well plate together with two 4 mm stainless steel grinding balls (Spex CertiPrep, USA).

B. CTAB extraction

- For each sample 450 µl of preheated (at 65°C 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 Geno Grinder 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 65°C for 10 min with shaking at periodical intervals.

C. Solvent extraction

- For each sample 450 µl of chloroform-isoamylalcohol (24:1) was added and mixed thoroughly by inverting twice.

- Plate was centrifuged at 5500 rpm for 10 min. The aqueous layer (300 µl) is transferred to fresh strip tubes (Marsh Biomarket, USA).

D. Initial DNA precipitation

- 0.7 vol (210µl) of isopropanol (stored at –20°C) was added to each sample and inverted gently to mix.

- Plate was centrifuged at 5000 rpm for 15 min.

- Supernatant was decanted from each sample and pellet was air dried for 20 min.

E. 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 37°C for 30 min.

F. Solvent extraction

- 200 µl of phenol-chloroform-isoamylalcohol (25:24:1) was added to each sample and inverted twice to mix.

- Plate was centrifuged at 5000 rpm for 5 min.

- Aqueous layer was transferred to a fresh 96 deep-well plate (Marsh Biomarket, USA).

- 200 µl chloroform-isoamylalcohol (24:1) was added to each sample and inverted twice to mix.

- 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 -20°C 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 per cent 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 resuspended in 100 µl low-salt TE and stored at 4°C.

3.2.3 DNA quantification

DNA was quantified by loading the samples on 0.8% agarose gel containing 0.5 µl/10 ml Ethidium bromide (10mg/ml) (Figure 1). The DNA was normalized to 5 ng/µl concentration by comparing visually. The diluted DNA samples with the standard λ DNA molecular weight markers (5 ng/µl and 10 ng/µl) on 0.8% agarose gel by running it in 0.5X TBE (Tris borate EDTA) buffer at a constant voltage (80 V) for 20 min. The images of gels were documented under UV illumination using Uvi Tech gel documentation system (DOL-008.XD, England) (Figure 2).

3.3 Assay of Microsatellite Markers

A set of 1152 SSR markers [developed at ICRISAT, India from Bacterial Artificial Chromosome (BAC) end sequences and synthesized at University of California, Davis, USA] were employed in the current study for marker validation, diversity assessment among the genotypes and for identification of informative SSR markers. The forward primers of these markers were synthesized with M13 tail for their ease in genotyping on ABI-3700/3130 automatic DNA sequencer (PE- Applied Biosystems, California, USA).

For marker validation a common PCR (Polymerase chain reaction) profile was used for the entire set of markers. All PCR reactions were performed in 5μl reaction volume consisting of 1 μl of 5 ng DNA template, 0.3 μl of 2 mM dNTPs, 0.5 μl of (1 pmole/μl M13 tailed forward primer : 2 pmole/μl reverse primer) and 1 μl of 2 pmole/μl of M13 labeled dye, 0.1 U (0.2 μl of 5U/μl) of *Taq* DNA polymerase (SibEnzymes, Russia), 0.5 μl of 10X PCR buffer (SibEnzymes, Russia), 0.3 μl of 25 mM $MgCl_2$ (SibEnzymes, Russia). In addition fluorescent dyes 6-FAM, VIC, NED, PET were used in the PCR reaction mixture for ease in detection on the ABI 3700/3130. PCR amplifications are performed on ABI thermal cycler (PE Applied biosystems, CA) using a common touchdown PCR amplification profile for the series of markers. A touch down PCR amplification profile with 3 min of initial denaturation cycle, followed by first 5 cycles of 94°C for 20 sec, 65°C for 20 sec and 72°C for 30 sec, with 1°C decrease in temperature per cycle, then 40 cycles of 94°C for 20 sec with constant annealing temperature (59°C) for 20 sec and 72°C for 30 sec, followed by a final extension at 72°C for 20 min.

The PCR products together with a 100 base pair ladder were tested for amplification on 1.2% agarose gel containing 0.5 μl/10ml ethidium bromide (10 mg/ml) by running it at a constant voltage of 80V for 30 min. The amplification was visualized under UV illumination using Uvi Tech gel documentation system (DOL-008.XD, England).

3.4 SSR Fragment Analysis

After confirming the PCR amplification on 1.2% agarose gel, five post-PCR multiplex sets were constructed based on the allele size range estimates and the type of forward primer label of the markers. Markers that had different labels and allele size ranges were considered for a set. For post PCR multiplexing, 1.5 μl PCR product of each of 6-FAM, VIC, NED and PET-labeled products were pooled (according to above mentioned criteria) and mixed with 7 μl of Hi-Di formamide (Applied Biosystems, USA), 0.25 μl of the LIZ-500 size standard (Applied Biosystems, USA) and 1.5 μl of sterile distilled water. The pooled PCR amplicons were denatured and size fractioned using capillary electrophoresis on an ABI 3700 automatic DNA sequencer (Applied Biosystems, USA). Allele sizing of the electrophoretic data thus

obtained was done using Genemapper® software version 4.0 (Applied Biosystems, USA).

3.5 Data Analysis

SSR loci were scored using Genemapper® software version 4.0, each allele was scored as present (1) or absent (0) at each SSR locus. Polymorphic information content (PIC) values for each of the SSR locus were calculated following Anderson *et al.* (1993).

$$\mathrm{PIC}_i = 1 - \sum p_{ij}^2$$

where p_{ij} is the frequency of the jth microsatellite allele for locus i

The data of microsatellite markers were analyzed using Numerical Taxonomy Multivariate Analysis System (NTSYS-pc), version 2.1 (Exeter software, Setauket, NY) (Rohlf, 2000). The genetic distance was calculated based on Jaccard's similarity coefficient using SIMQUAL procedure. The DNA data of SSR markers for 16 genotypes were clustered using an unweighted pair group method (UPGMA) with the module of SHAN in the NTSYS-pc package.

4. RESULTS

The present study aimed to validate newly developed markers, identify the polymorphic SSR markers for different mapping populations and understand the diversity features of newly developed SSR markers.

4.1 Quantification and Normalization of DNA

DNA from sixteen groundnut genotypes were checked for quality and quantity on 0.8% agarose along with 50 ng, 100 ng, 200 ng and 400 ng of uncut λ DNA as control/marker (Fig. 1). DNA was normalized to 5 ng/μl for performing PCR reactions (Fig. 2).

4.2 Validation and Polymorphism Assessment of SSR Markers

PCR components and PCR profiles for all the 1152 primer pairs were initially optimized on 2 genotypes (ICGV 86031 and TAG 24). Among 1152 primer pairs, a set of 876 (76 %) primer pairs provided scorable amplification with a touchdown PCR profile. Subsequently these 876 primer pairs were used for genotyping a set of 16 groundnut genotypes representing parents of twelve mapping population (Table 1 & 2). As a result, a total of 184 (16 %) markers showed polymorphism across 16 genotypes.

A total of 686 alleles were detected at 184 marker loci with an average of 3.7 alleles per marker. The number of alleles ranged from 2 (with 41 markers) to 14 (GNB 18 and GNB 515) per marker (Table 3). The PIC value for these 184 polymorphic markers ranged from 0.04 (GNB 786) to 0.87 (GNB 515) with an average of 0.39 (Fig. 3) (Table 3).

4.3 Relationship between Polymorphism and Repeat Units

Relationship of SSR polymorphism with respect to repeat unit length was evaluated by drawing two plots between: (i) repeat unit length and number of alleles and (ii) repeat unit length and PIC value. These analysis indicated that higher number of alleles were detected for the SSR markers with less than 12 repeats while SSR markers with higher number (>12) of repeats showed less number of alleles (Fig. 4).

In the current study, tri-nucleotide repeats with the repeat unit ATT/TAA was highly polymorphic as compared to di-nucleotide repeats (TA and GA) and other tri-nucleotide repeats (GAA, CTT & CAA). Whereas TA repeat motifs had higher PIC value compared to other di-nucleotide repeats (GA, CA). Among di, tri and compound-nucleotide repeats, the SSR markers with compound repeat units showed higher PIC value (average 0.43 per marker) as compared to di-nucleotide (average 0.40 per marker) and tri-nucleotide (average 0.30 per marker) repeat units. Di-nucleotide repeats showed higher allele number (3.80 allele per locus) followed by compound (3.7 allele per locus) and tri-nucleotide (3.60 allele per locus) repeats units.

In total, 184 polymorphic markers had PIC values in the range of 0.04 to 0.87. Majority of these polymorphic markers (155/184, 84%) were derived for SSRs with 3 to 12 repeats while 29 (16%) markers were derived for SSRs with higher number of repeat units (>12) (Fig. 5). Though these trends were observed, a weak correlation or no relationship, however, was observed between the number of repeat units and the SSR polymorphism (Table 4).

4.4 SSR Polymorphism in Mapping Populations

As these SSR markers were screened on those genotypes that are parents of mapping populations, an effort was made to identify the polymorphic markers for twelve mapping populations. Out of 1152 markers, 876 primer pairs gave scorable amplification. The amplification of primer pairs between the parents of mapping populations ranged from minimum of 546 (TAG 24 × R 9227) to a maximum of 748 (JL 24 × ICGV 86590) markers (Table 5). In terms of polymorphic markers, the number of polymorphic markers ranged from 12 to 136 (Fig. 3). The percent polymorphism among the different mapping populations varied from 1.6 to 22.4 % (Fig. 6). Among polymorphic markers, six markers were found polymorphic for all 8 mapping populations (Appendix I). Comparative marker polymorphism among twelve mapping populations has been represented graphically in Figure 7 (Appendix I).

4.5 Genetic Relationships among 16 Groundnut Genotypes

Based on the scoring data (0 or 1) obtained for 184 SSR loci, a similarity matrix was generated by using NTSYS, version 2.1 software. The genetic distance was calculated based on Jaccard`s similarity coefficient (Table 6). Similarity index of

these 184 polymorphic marker loci ranged from 0.03 to 0.76 including synthetic amphidiploid (TxAG 6) and for cultivated genotypes alone similarity index ranged from 0.44 to 0.72. It was found that the two closely related genotypes were TG 26 and TG 49 with the highest similarity index (0.76). On the other hand two most distantly related cultivars were TxAG 6 and ICGS 76 with low similarity index (0.03). The present result depicted efficient use of SSR techniques to determine the genetic relationship between the genotypes.

Genetic similarity was used to prepare a unweighted pair group method (UPGMA) dendrogram, using the software NTSYS version 2.1. The UPGMA dendrogram grouped 16 groundnut genotypes into four major clusters Cluster A ('cl A'), Cluster B ('cl B'), Cluster C ('cl C') and Cluster D (`cl D`) (Fig. 8). Cluster 'cl A' and 'cl B' contained 1 genotype each; the 'cl C' contained only 8 genotypes and the cluster `cl D` contained 6 genotypes. These major clusters were further classified into sub clusters. The major cluster, 'cl C' was further divided into two sub clusters i.e. 'cl C I' (two genotypes) and 'cl C II' (six genotypes). Similarly sub clusters 'cl D I' and 'cl D II' of cluster 'cl D' contained 4 and 2 genotypes, respectively.

5. DISCUSSION

Groundnut is an important crop worldwide, distributed across the vast area in tropical, subtropical and also in temperate zones. It is widely cultivated for its valuable edible oil and protein. Even though groundnut is an economically and nutritionally important crop, narrow genetic diversity and unavailability of appropriate genomic resource hindered molecular mapping and molecular breeding applications. In the recent past, advent of next generation sequencing and genotyping technologies have given a boom to the research in several crop species.

Microsatellite or SSR markers have been proven as useful and advantageous molecular markers for genome mapping, genetic diversity studies, QTL mapping and marker assisted selection as they are codominant and multiallelic in nature (Gupta and Varshney, 2000). As a result several laboratories across the world developed a reasonable number and quality SSR markers for groundnut (Hopkins *et al.*, 1999; He *et al.*, 2003; Palmieri *et al.*, 2002, 2005; Ferguson *et al.*, 2004; Moretzsohn *et al.*, 2004, 2005; Nelson *et al.*, 2006; Mace *et al.*, 2007; Proite *et al.*, 2007; Gimenes *et al.*, 2007; Wang *et al.*, 2007; Cuc *et al.*, 2008; Gautami *et al.*, 2009; Knapp *et al.*, unpublished).

5.1. Validation of Novel Set of SSR Markers

Although there is an increased number of a microsatellite marker available in public domain, out of which only 135-146 SSR markers were mapped in cultivated groundnut genetic linkage map (Varshney *et al.*, 2009; Foncéka et al., 2009; Hong *et al.*, 2008, 2010). Several of these markers still need to be incorporated into genetic map to construct the high resolution map. Hence, the present study aimed at validation and polymorphism assessment of the newly developed 1152 BAC end genomic SSR markers on a panel of 16 groundnut genotypes and to study the genetic relationships among these 16 genotypes.

This study reports successful amplification of 876 out of 1152 SSR markers with the same PCR components and the same touchdown PCR cycle. Therefore, this study adds another set of about 900 markers to existing marker repertoire for groundnut.

5.2 Allelic Diversity and PIC value

The average number of alleles (3.70 alleles/ marker) in the present study was comparable with the earlier genetic diversity analysis (He *et al.*, 2003; Krishna *et al.*, 2004; Moretzsohn *et al.*, 2004; Gautami *et al.*, 2009) which reported 2 to 8 alleles per marker. A total of 184 markers provided 686 alleles with an average of 3.70 alleles per marker and had an average PIC value of 0.39. Similar kind of observations were made by earlier studies in groundnut (viz., Tang *et al.*, 2007; Barkley *et al.*, 2007; Cuc *et al.*, 2008; Gautami *et al.*, 2009). Number of alleles detected and the PIC value based on the frequencies of different alleles by a particular marker indicates the quality (discriminatory power) of the marker (Fregene *et al.*, 2003).

The present study reveals a positive correlation for number of alleles and PIC value (Fig. 9). For instance, GNB 18 and GNB 515 produced highest number of alleles (14) with highest PIC value 0.86 and 0.87 respectively, followed by GNB 682 with 10 alleles and PIC value of 0.76. Whereas 41 markers with two alleles each showed the minimum PIC value of 0.11 (Table 3).

In summary, like earlier studies (Hopkins *et al.*, 1999; He *et al.*, 2003; Barkley *et al.*, 2007; Tang 2007; Cuc *et al.*, 2008, Gautami *et al.*, 2009), the present study also underlines the importance of microsatellite markers for genetic diversity studies.

5.3 SSR Polymorphism and SSR Repeat Types

To understand the possible relationship between polymorphism of SSR markers with the repeat unit length of the corresponding SSRs, two scatter plots were made between repeat unit length and number of alleles detected (Fig. 4) and the PIC value calculated (Fig. 5). The scattered plot between repeat unit and the number of alleles, revealed maximum variation for 3 to 12 repeats with respect to allele number and a minimum variation was found in high number (>12) repeats. However, it does not provide any conclusive relationships between the number of alleles and repeat unit length as loci with longer repeats are much more likely to be more variable. Similar results were also reported by Ferguson *et al.* (2004) and Moretzsohn *et al.* (2005). Indeed, among polymorphic SSR markers, the GNB 981 marker containing highest number of repeat units (54) provided just 4 alleles while the GNB 18 marker with 27 repeat units revealed the highest number of alleles (14).

The scattered plot between number of repeat unit and the PIC value indicated that out of 184 polymorphic markers 155 (84 per cent) markers having PIC value range of (0.10 to 0.87) were between 3 to 12 repeats while other 29 (16 per cent) polymorphic markers were found in the high number (>12) repeats. The present study showed no significant relationship or association between polymorphism and repeat unit length similar to the previous reports (Love *et al.*, 1990, Yu *et al.*, 1999, He *et al.*, 2003, Ferguson *et al.*, 2004 and Cuc *et al.*, 2008). However few reports signified that the degree of polymorphism increases with the total length of the repeat units (Moretzshon *et al.*, 2005; Weber, 1990; Hüttel *et al.*, 1999; Burstin *et al.*, 2001).

5.4 Polymorphism among Parents of Mapping Population

Although 876 primer pairs resulted in scorable amplification, less number of markers were found to be polymorphic among parents of different mapping population. The number of polymorphic markers (136) was high for the cross TMV 2 / TxAG 6 which may be due to the distinct nature of the parents used for development of mapping population. Among parents, TMV 2 is a cultivated variety and TxAG 6 a synthetic amphidiploid. For remaining mapping population developed between cultivated genotypes, the number of polymorphic markers ranged from 12 to 35. This kind of less polymorphism among cultivated genotypes may be attributed to the complex nature of cultivated groundnut genome, their ploidy level and mode of reproduction. In the present study, the per cent polymorphism between parents of different mapping population ranged from 1.62 to 22.4 which were slightly lower as compared to the earlier reports (He *et al.*, 2003; Ferguson *et al.*, 2004 and Gautami *et al.*, 2009).

5.5 Polymorphism Assessment of Microsatellite Markers

The present investigation illustrates the genetic diversity for the novel set of SSR markers among 16 elite genotypes, which have been used as parents for development of 12 different mapping populations. The genetic relationship among each genotype indicated the possibilities to explore their utility in developing mapping population and possibilities of integrating these markers into available genetic linkage maps.

As groundnut genome size is estimated to be 2800 Mb/1C (Guo *et al.*, 2009), which is quite higher than any other crop species. A large number of polymorphic markers need to be developed and characterized as availability of large number of polymorphic markers will allow identifying tightly linked markers to economically important traits in groundnut. The identified polymorphic markers in the present study can be utilized for genotyping of mapping populations and integrate them to develop dense genetic linkage map of cultivated groundnut. Further these markers will also be useful in identifying important genes / QTLs governing different economically important traits, which can be potentially employed in Marker Assisted Selection (MAS).

5.6 Genetic Diversity and Relationships among 16 Diverse Genotypes

Knowledge of genetic diversity in a crop species is fundamental to its improvement. A variety of molecular, chemical and morphological descriptors are used to characterize the genetic diversity among and within crop species. Substantial diversity exists among cultivated groundnut both inter- and intra specific genotypes in morphological, physiological and agronomical traits. In this study, 184 SSR markers showed sufficiently high sensitivity to detect DNA polymorphism among the 16 cultivated genotypes. The dendrogram constructed, not only shows the extent of genetic relationship but also shows the level of similarity between the parents of twelve mapping population included in this study (Fig. 8).

All 16 genotypes used in study have revealed the genetic relationship among each other giving possibilities to explore their utility for further studies. The highest genetic diversity was detected between TxAG 6 and ICGS 76 with similarity coefficient of 0.03 and also found distantly related to all other genotypes, since it is a synthetic amphidiploid (Table 1). Among cultivated genotypes JL 24 and CSMG 84-1 were distantly related with similarity coefficient of 0.44 (Table 6) and the genotypes ICGS 44 and ICGS 76 were 72 % similar with similarity coefficient of 0.72. In cluster A, TxAG 6 was found distantly related to all other genotypes. Since it is a synthetic amphidiploid derived from the cross *A. batizocoi* × (*A. cardenasii* × *A. diogoi*). CSMG 84-1 alone forms a cluster B, since its pedigree is no way related to the other genotypes studied.

In cluster C, Trombay Groundnut (TG) series TG 26, TG 49, TAG 24 were grouped together with JL 24, GPBD 4 and GPBD 5 in the same sub cluster ('cl C II'). Reason behind this clustering of JL 24, GPBD 4 and GPBD 5 together with TG series is due to similar pedigree of these genotypes. Since female parent of TG 26 is a gamma ray mutant of JL 24 and the parents of GPBD 5 are TG 49 and GPBD 4. TMV 2 and ICGV 86031 formed separate sub cluster 'cl C I'.

Cluster D comprised of six genotypes *i.e.*, ICGS 44, ICGS 76, R 9227, TG 19, ICGV 86590 and ICGV 11337. The genotypes R 9227 and ICGV 86590 derived from common parents by two way and three way crosses respectively (Table 1). In addition, parents of mapping populations ICGS 44 and ICGS 76 comes under same cluster D. Even though the genotypes ICGS 44 and ICGS 76 clustered together based on genotypic data from these set of BAC-end genomic SSR markers, these genotypes were found to be diverse based on their pedigree.

The polymorphic markers reported in the present study may facilitate in estimating the genetic diversity in large set of genotypes or germplasms, development of dense linkage map, mapping of economically important traits, association studies and development of molecular Ids for germplasm registration. This information will also be used to remove duplicated accessions among groundnut genetic resources and selection of parents in breeding programmes.

6. SUMMARY

The present study was carried out at M.S. Swaminathan Applied Genomics Laboratory, ICRISAT, Patancheru, India which focused on screening of a set of 1152 BAC-end SSR markers across 16 genotypes, that are the parents of twelve mapping populations and assessment of genetic diversity among these 16 genotypes. The salient features of this study are following:

1) Screening of 1152 BAC-end SSR markers provided 876 SSR markers that yield scorable amplicon.

2) Screening of 876 SSR markers on a set of 16 parental genotypes yielded a total of 686 alleles with an average of 3.70 alleles per marker.

3) The polymorphic information content (PIC) value for polymorphic SSR markers ranged from 0.04 to 0.87 with an average of 0.39 per marker.

4) A positive correlation was found between number of alleles and PIC value. Two SSR markers namely GNB 18 and GNB 515 produced highest number of alleles (14) with highest PIC value 0.86 and 0.87 respectively.

5) Tri-nucleotide (ATT/TAA) repeats were found to possess higher polymorphism compared to di-nucleotide repeats (TA and GA) and also higher than other tri-nucleotide repeats (viz., GAA, CTT, CAA). Among di, tri and compound-nucleotide repeats, compound SSRs showed higher PIC value (average 0.43 per marker).

6) Number of polymorphic markers ranged from 12 (ICGS 44 × ICGS 76) to 136 (TMV 2 × *Tx*AG 6) with an average of 32.50 markers per population. Percent polymorphism ranged from 1.60 to 22.4 among twelve mapping populations.

7) Among the parental genotypes, the highest level of polymorphism (22.4 %) was between the parents TMV 2 and TxAG 6 and the low level of polymorphism (1.6 %) were between parents ICGS 44 and ICGS 76.

8) The dendrogram constructed based on the Jaccard`s similarity coefficient showed grouping of 16 genotypes into four major clusters. Trombay Groundnut (TG) series TG 26, TG 49, TAG 24 were grouped together with JL 24, GPBD 4 and GPBD 5, in the same sub cluster. Since female parent of TG 26 is a gamma ray mutant of JL 24 and the parents of GPBD 5 are TG 49 and GPBD 4. TMV 2 and ICGV 86031 forms separate sub cluster.

9) The genotypes R 9227 and ICGV 86590 grouped under same cluster, since derived from common parents by two way and three way cross respectively.

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Table: 1 List of parental genotypes and their pedigree

S.No	Genotypes	Pedigree
1	ICGS 44	Robut 33-1 × NCAC 28 21
2	ICGS 76	TMV10 × CHICO
3	CSMG 84-1	Selection from MA 10
4	ICGV 86031	F 334 A-B-14x NC Ac 2214
5	TAG 24	TGS2 (TG 18A × M 13) × TGE1 (Tall × TG 9)
6	GPBD 4	KRG 1 × CS 16 (ICGV 86855)
7	TG 26	BARCG1 (γ - ray mutant of JL 24) × TG 23 (TGS 2 × TGE 1)
8	TMV 2	Mass selection from Gudhiatham bunch
9	TxAG 6	*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)
10	R 9227	ICGS 7 × (NC Ac 2214)
11	ICGV 86590	(ICGS 7 × NC Ac 2214) × ICGV 86031
12	ICGV 11337	Not available
13	JL 24	Selection from EC 94943(Exotic collection)
14	TG 19	TG 17 × TG 1
15	TG 49	TG 28A × TG 26
16	GPBD 5	TG 49 × GPBD 4

Table: 2 Mapping population and their segregating traits

S.No	Mapping Populations	Source	Segregating traits
1	ICGS 44 × ICGS 76	ICRISAT	Drought tolerance traits viz., transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading (SCMR)
2	ICGS 76 × CSMG 84-1	ICRISAT	Drought tolerance traits viz., transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading (SCMR)
3	TAG 24 × ICGV 86031	ICRISAT	Drought tolerance traits viz., transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading (SCMR)
4	TAG 24 × GPBD 4	UAS-D	Rust and late leaf spot (LLS) resistance
	TMV 2 × TxAG 6	ICRISAT	Seed size and many others
	ICG 11337 × JL 24	ICRISAT	Late leaf spot (LLS) resistance
	TG 26 × GPBD 4	UAS-D	Rust and late leaf spot (LLS) resistance
8	TG 19 × GPBD 4	UAS-D	*Aspergillus* crown rot, rust and late leaf spot resistance
9	TG 49 × GPBD 4	UAS-D	*Aspergillus* crown rot, rust and late leaf spot resistance
10	GPBD 5 × GPBD 4	UAS-D	Rust and late leaf spot (LLS) resistance
11	TAG 24 × R 9227	UAS-D	*Sclerotium* rot resistance
12	JL 24 × ICGV 86590	DGR	Rust and *Sclerotium* rot resistance

Table 3. Number of alleles and PIC value of 184 polymorphic markers

Marker	No. of alleles	PIC value	Marker	No. of alleles	PIC value	Marker	No. of alleles	PIC value
GNB 02	4	0.39	GNB 174	3	0.35	GNB 303	3	0.21
GNB 09	4	0.46	GNB 177	3	0.21	GNB 304	7	0.75
GNB 18	14	0.86	GNB 178	5	0.68	GNB 309	3	0.21
GNB 38	4	0.56	GNB 181	5	0.67	GNB 310	4	0.44
GNB 39	3	0.29	GNB 190	4	0.31	GNB 311	3	0.29
GNB 40	3	0.29	GNB 200	2	0.11	GNB 312	3	0.21
GNB 41	2	0.11	GNB 206	3	0.45	GNB 317	5	0.68
GNB 50	4	0.31	GNB 211	3	0.29	GNB 336	2	0.11
GNB 51	3	0.29	GNB 216	3	0.29	GNB 344	4	0.66
GNB 58	8	0.63	GNB 218	3	0.50	GNB 349	3	0.43
GNB 59	3	0.21	GNB 226	4	0.44	GNB 357	5	0.72
GNB 63	4	0.31	GNB 232	3	0.43	GNB 366	2	0.11
GNB 66	3	0.21	GNB 235	2	0.19	GNB 368	3	0.21
GNB 70	3	0.29	GNB 236	4	0.31	GNB 374	5	0.47
GNB 73	4	0.61	GNB 241	4	0.48	GNB 378	6	0.62
GNB 74	3	0.21	GNB 246	2	0.11	GNB 385	2	0.11
GNB 87	2	0.11	GNB 249	3	0.29	GNB 387	5	0.54
GNB 98	6	0.68	GNB 253	4	0.44	GNB 392	4	0.51
GNB 100	5	0.64	GNB 256	3	0.40	GNB 396	3	0.29
GNB 107	5	0.69	GNB 260	3	0.21	GNB 397	4	0.55
GNB 122	2	0.11	GNB 261	3	0.21	GNB 417	5	0.68
GNB 126	5	0.56	GNB 262	5	0.66	GNB 428	5	0.71
GNB 136	7	0.70	GNB 264	3	0.29	GNB 432	2	0.11
GNB 138	3	0.29	GNB 267	2	0.11	GNB 448	3	0.40
GNB 142	2	0.11	GNB 174	3	0.35	GNB 303	3	0.21
GNB 143	2	0.26	GNB 177	3	0.21	GNB 304	7	0.75
GNB 145	6	0.73	GNB 178	5	0.68	GNB 309	3	0.21
GNB 152	4	0.46	GNB 181	5	0.67	GNB 310	4	0.44
GNB 155	6	0.69	GNB 190	4	0.31	GNB 311	3	0.29
GNB 159	4	0.62	GNB 200	2	0.11	GNB 312	3	0.21

Contd.,

Marker	No. of alleles	PIC value	Marker	No. of alleles	PIC value	Marker	No. of alleles	PIC value
GNB 513	2	0.11	GNB 775	4	0.58	GNB 1058	3	0.21
GNB 515	14	0.87	GNB 782	4	0.53	GNB 1060	2	0.11
GNB 554	2	0.11	GNB 786	3	0.40	GNB 1061	3	0.47
GNB 555	7	0.74	GNB 788	2	0.11	GNB 1063	4	0.48
GNB 569	6	0.68	GNB 804	2	0.11	GNB 1068	4	0.46
GNB 579	3	0.40	GNB 811	3	0.29	GNB 1069	5	0.63
GNB 588	3	0.21	GNB 816	4	0.39	GNB 1071	4	0.33
GNB 603	3	0.21	GNB 840	8	0.82	GNB 1072	9	0.64
GNB 608	3	0.53	GNB 841	2	0.11	GNB 1074	2	0.11
GNB 624	3	0.21	GNB 842	9	0.75	GNB 1075	2	0.11
GNB 629	3	0.21	GNB 844	2	0.11	GNB 1078	3	0.37
GNB 630	3	0.40	GNB 850	3	0.51	GNB 1086	3	0.21
GNB 632	2	0.11	GNB 853	5	0.64	GNB 1094	3	0.35
GNB 643	3	0.50	GNB 867	2	0.11	GNB 1095	3	0.29
GNB 649	2	0.36	GNB 880	2	0.11	GNB 1107	2	0.11
GNB 661	2	0.11	GNB 892	2	0.11	GNB 1112	4	0.53
GNB 666	3	0.29	GNB 895	2	0.11	GNB 1114	7	0.79
GNB 667	9	0.68	GNB 906	3	0.21	GNB 1120	2	0.11
GNB 668	3	0.29	GNB 920	3	0.29	GNB 1130	3	0.29
GNB 669	3	0.29	GNB 955	3	0.29	GNB 1131	2	0.11
GNB 670	3	0.35	GNB 980	4	0.44	GNB 1136	2	0.11
GNB 673	2	0.11	GNB 981	4	0.55	GNB 1148	5	0.53
GNB 679	5	0.70	GNB 989	3	0.29	GNB 1151	3	0.21
GNB 682	10	0.76	GNB 761	2	0.11	GNB 1055	5	0.68
GNB 692	3	0.35	GNB 775	4	0.58	GNB 1056	5	0.62
GNB 513	2	0.11	GNB 782	4	0.53	GNB 1058	3	0.21
GNB 515	14	0.87	GNB 786	3	0.40	GNB 1060	2	0.11
GNB 554	2	0.11	GNB 788	2	0.11	GNB 1061	3	0.47
GNB 555	7	0.74	GNB 804	2	0.11	GNB 1063	4	0.48
GNB 569	6	0.68	GNB 811	3	0.29	GNB 1068	4	0.46
GNB 579	3	0.40	GNB 1055	5	0.68			
GNB 761	2	0.11	GNB 1056	5	0.62			

Table 4. Correlation between number of repeat units and SSR polymorphism

Number of repeats/Alleles and PIC value	No. of alleles (r value)	PIC value (r value)	Significant values (0.01)
Mono-nucleotide repeats	-0.52	-0.52	0.99
Di-nucleotide repeats	0.44	0.44	0.30
Tri-nucleotide repeats	0.14	0.38	0.36
Compound nucleotide repeats	0.01	0.05	0.49

Table 5. Comparative marker polymorphism of BAC-end SSR markers on different parental combinations

Mapping Population	No. of markers tested	No. of markers Amplified	No. of Polymorphic markers	% polymorphism
ICGS 44 × ICGS 76	1152	741	12	1.62
ICGS 76 × CSMG 84-1	1152	655	20	3.05
TAG 24 × ICGV 86031	1152	697	16	2.30
TAG 24 × GPBD 4	1152	699	22	3.15
TMV 2 × TxAG 6	1152	606	136	22.44
ICG 11337 × JL 24	1152	741	37	4.99
TG 26 × GPBD 4	1152	720	27	3.75
TG 19 × GPBD 4	1152	715	26	3.64
TG 49 × GPBD 4	1152	685	27	3.94
GPBD 5 × GPBD 4	1152	673	16	2.38
TAG 24 × R 9227	1152	546	16	2.93
JL 24 × ICGV 86590	1152	748	35	4.68

Table 6. Jaccard`s similarity index among sixteen groundnut genotypes

	ICGS 44	ICGS 76	CSMG 84-1	ICGV 86031	TAG 24	GPBD 4	TG 26	TMV 2	TxAG 6	R 9227	ICGV 86590	ICGV 11337	JL 24	TG 19	TG 49	GPBD 5
ICGS 44	1															
ICGS 76	0.72	1														
CSMG 84-1	0.58	0.57	1													
ICGV 86031	0.62	0.61	0.54	1												
TAG 24	0.59	0.58	0.52	0.68	1											
GPBD 4	0.63	0.55	0.51	0.60	0.65	1										
TG 26	0.59	0.56	0.51	0.64	0.72	0.64	1									
TMV 2	0.57	0.54	0.47	0.70	0.63	0.62	0.67	1								
TxAG 6	0.04	**0.03**	0.04	0.04	0.04	0.05	0.04	0.05	1							
R 9227	0.65	0.63	0.56	0.61	0.65	0.60	0.60	0.55	0.08	1						
ICGV 86590	0.62	0.60	0.50	0.62	0.64	0.65	0.64	0.58	0.05	0.66	1					
ICGV 11337	0.61	0.56	0.51	0.54	0.54	0.59	0.52	0.58	0.05	0.56	0.67	1				
JL 24	0.57	0.54	**0.44**	0.64	0.64	0.70	0.67	0.63	0.04	0.55	0.60	0.56	1			
TG 19	0.63	0.61	0.54	0.57	0.58	0.64	0.63	0.54	0.04	0.60	0.61	0.59	0.59	1		
TG 49	0.66	0.59	0.55	0.61	0.67	0.63	**0.76**	0.59	0.05	0.64	0.62	0.58	0.61	0.61	1	
GPBD 5	0.61	0.55	0.50	0.61	0.64	0.70	0.64	0.58	0.04	0.56	0.62	0.56	0.63	0.62	0.70	1

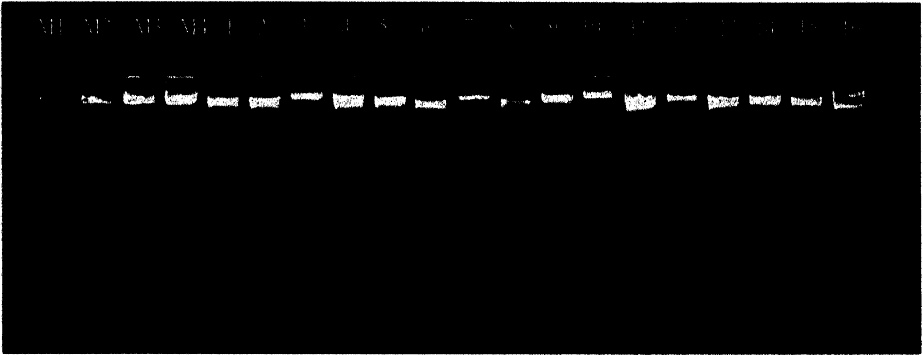

Figure1: Quantification of concentrated DNA samples in 0.8 % agarose gel

M1: 50 ng λ DNA, M2: 100 ng λ DNA, M3: 200 ng λ DNA and M4: 400 ng λ DNA

(1-16 corresponds to genotypes in Table-1)

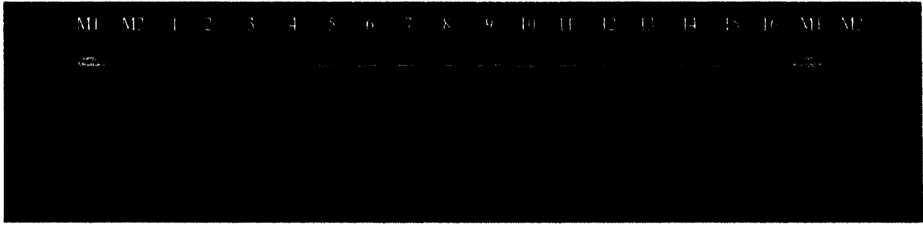

Figure 2: Quantification of diluted DNA samples in 0.8 % agarose gel

M1:10 ng λ DNA and M2:5 ng λ DNA (1-16 corresponds to genotypes in Table-1)

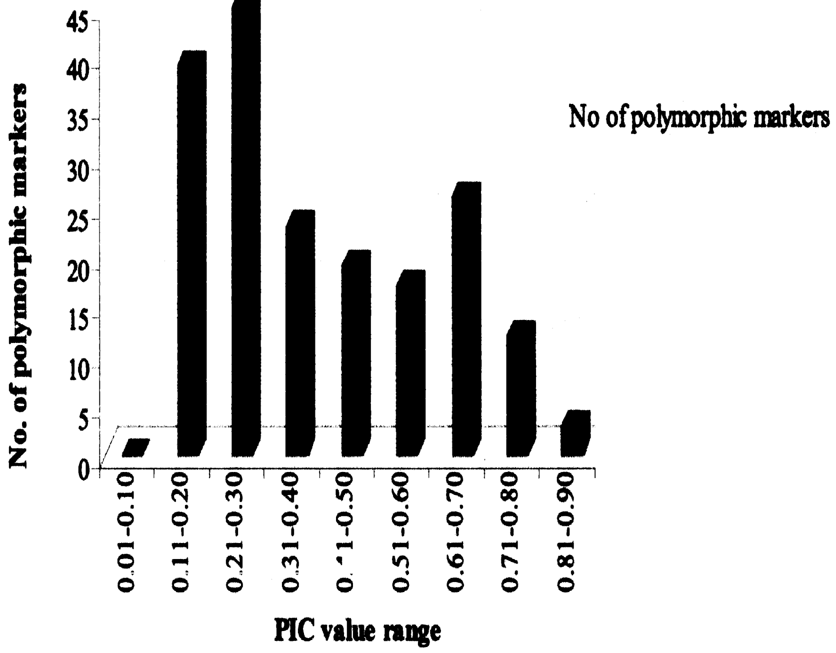

Figure 3: Graphical representation of PIC value range of polymorphic markers

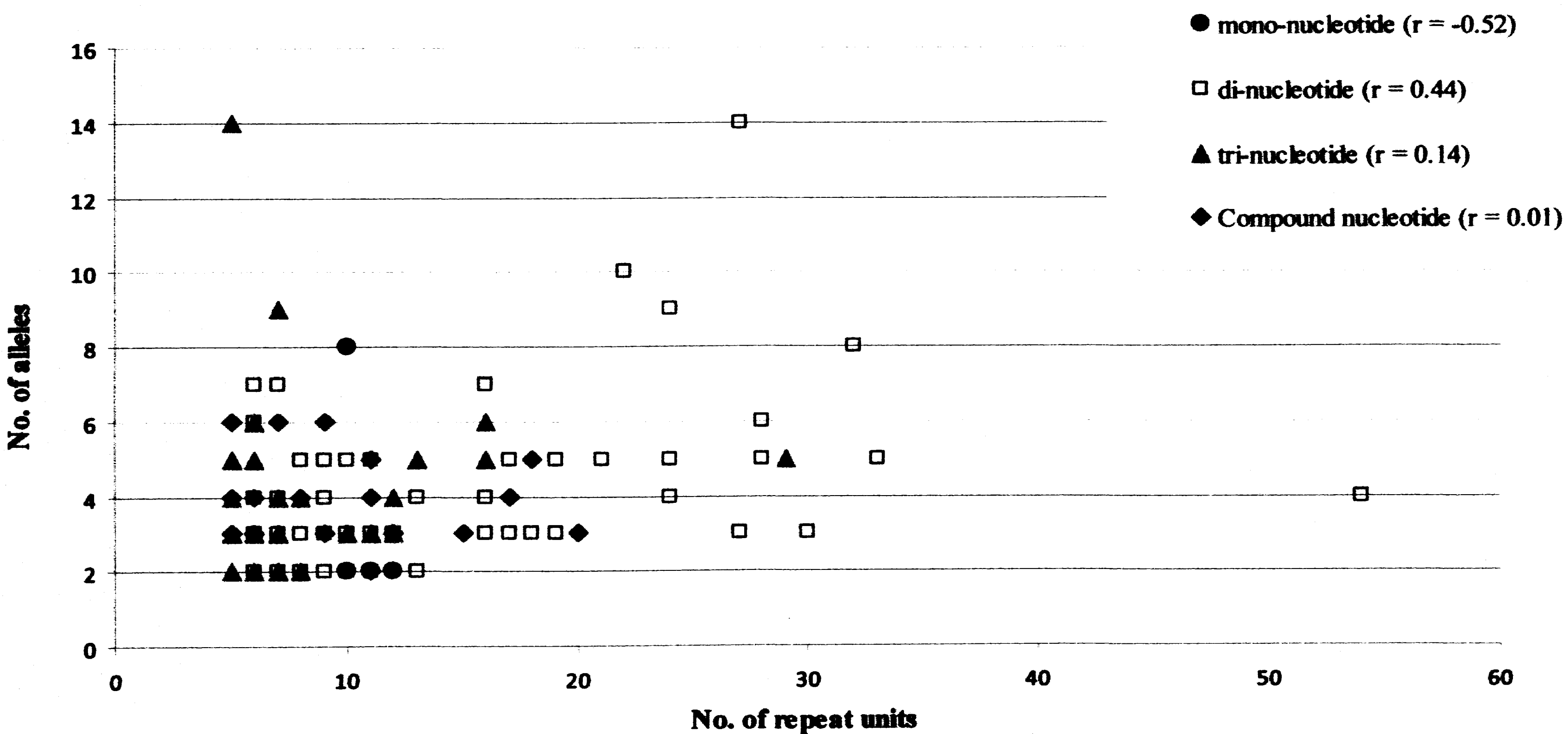

Figure 4: Scattered plot between number repeat units and number of alleles

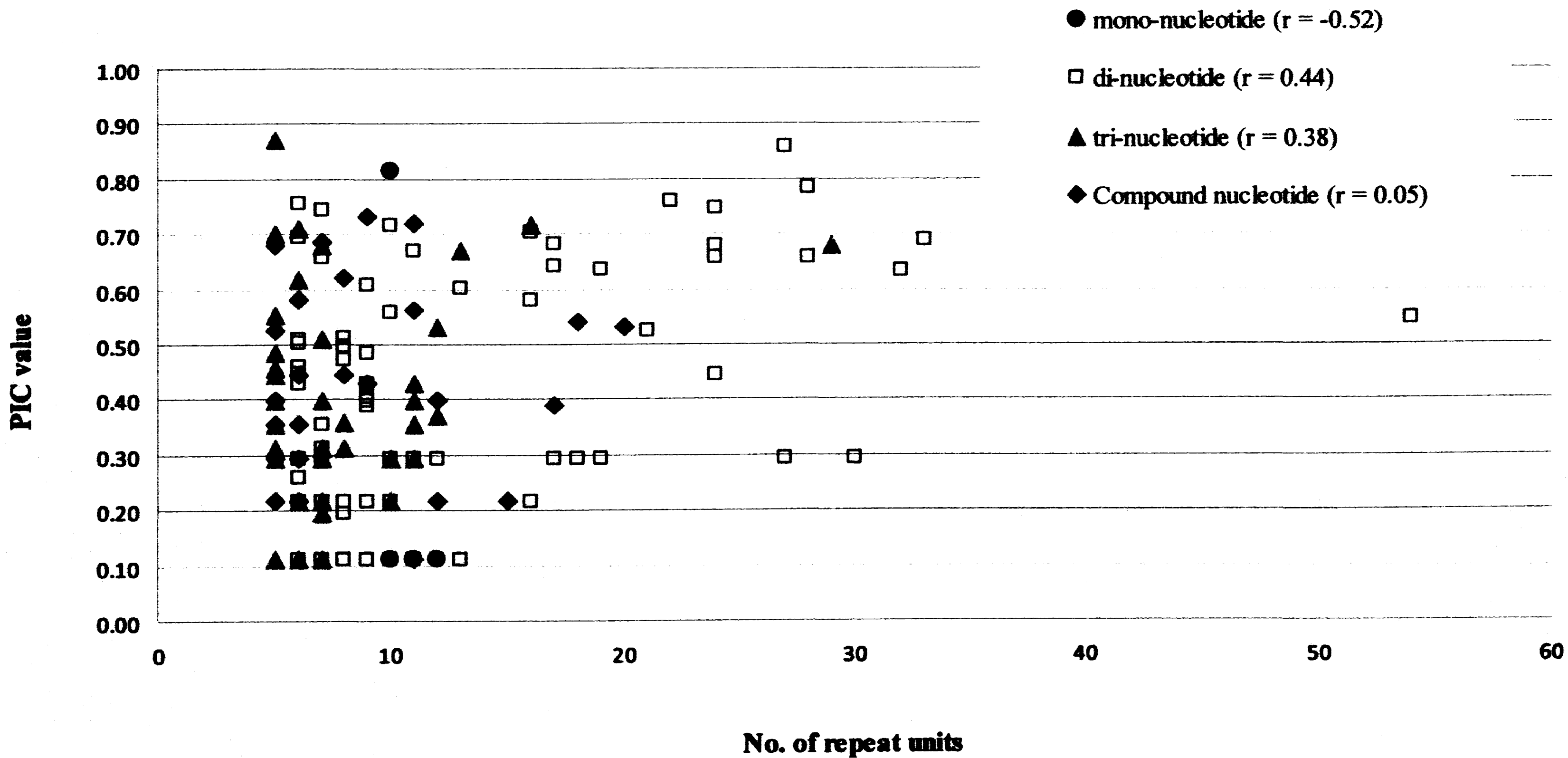

Figure 5: Scattered plot between number repeat units and polymorphic information content value

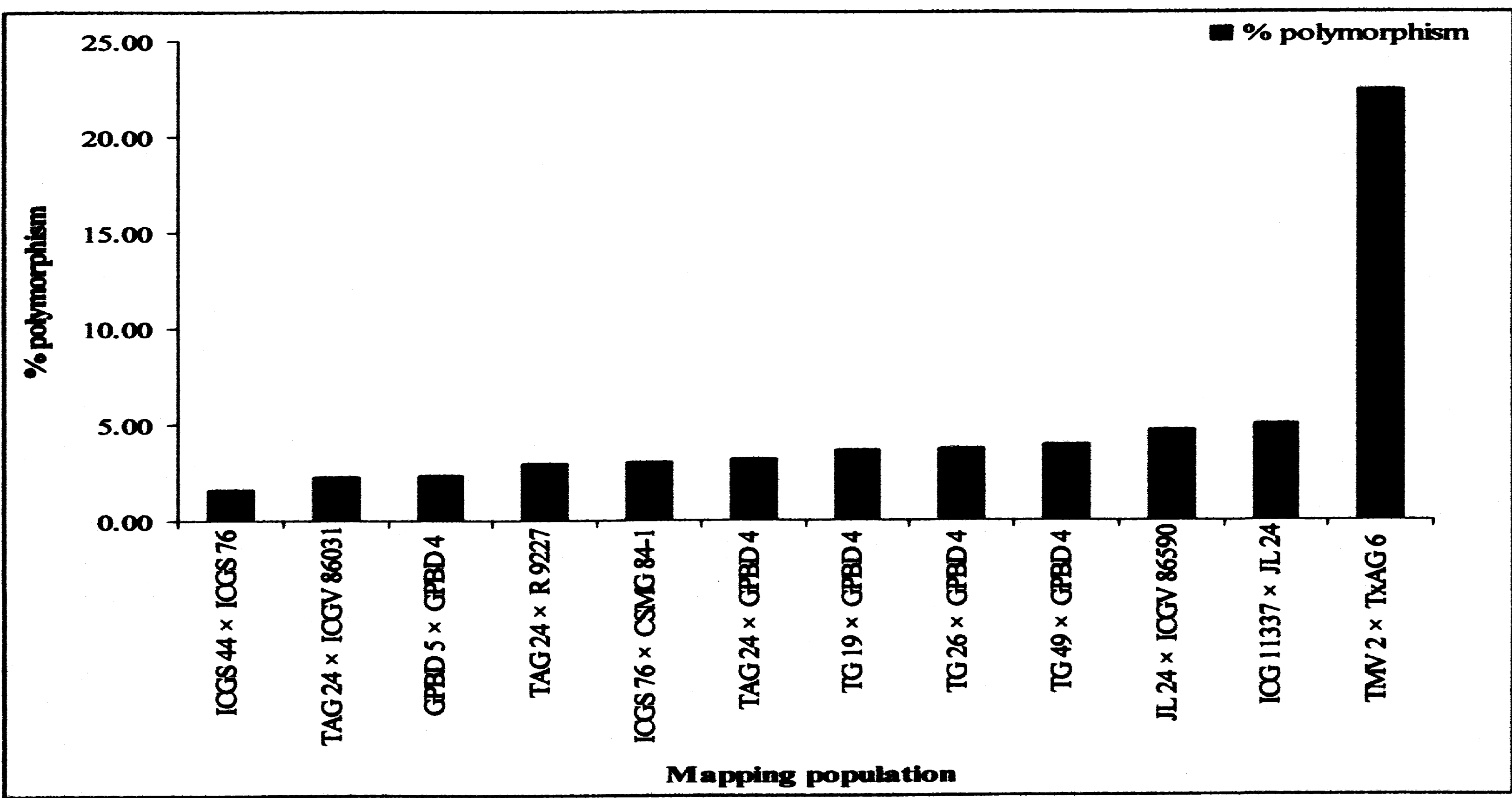

Figure 6: Percent polymorphism of BAC-end SSRs for different mapping population

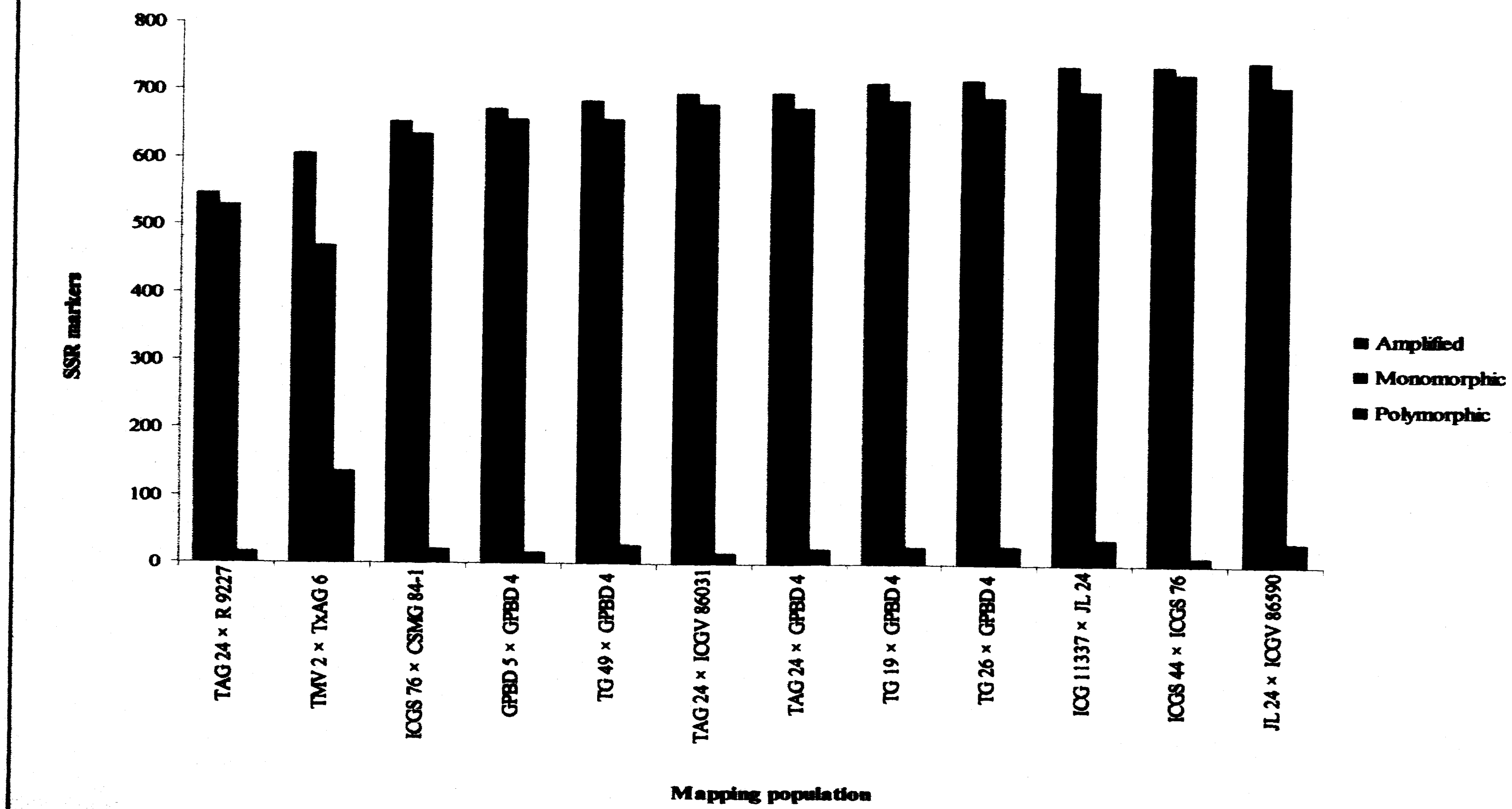

Figure 7: Polymorphic status of BAC-end SSR for different mapping population

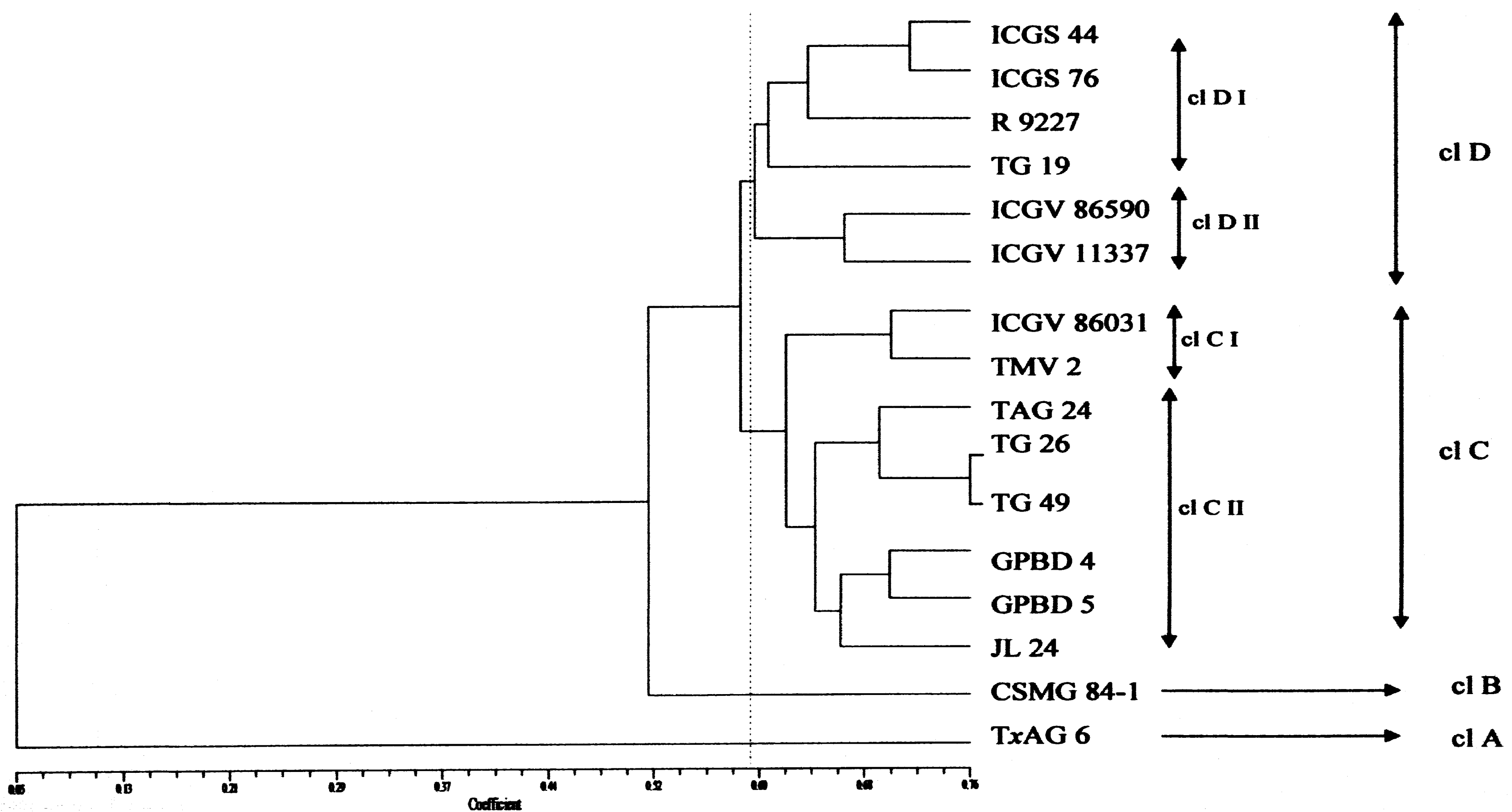

Figure 8: Dendrogram indicating genetic relationship among the 16 groundnut genotypes generated using UPGMA method

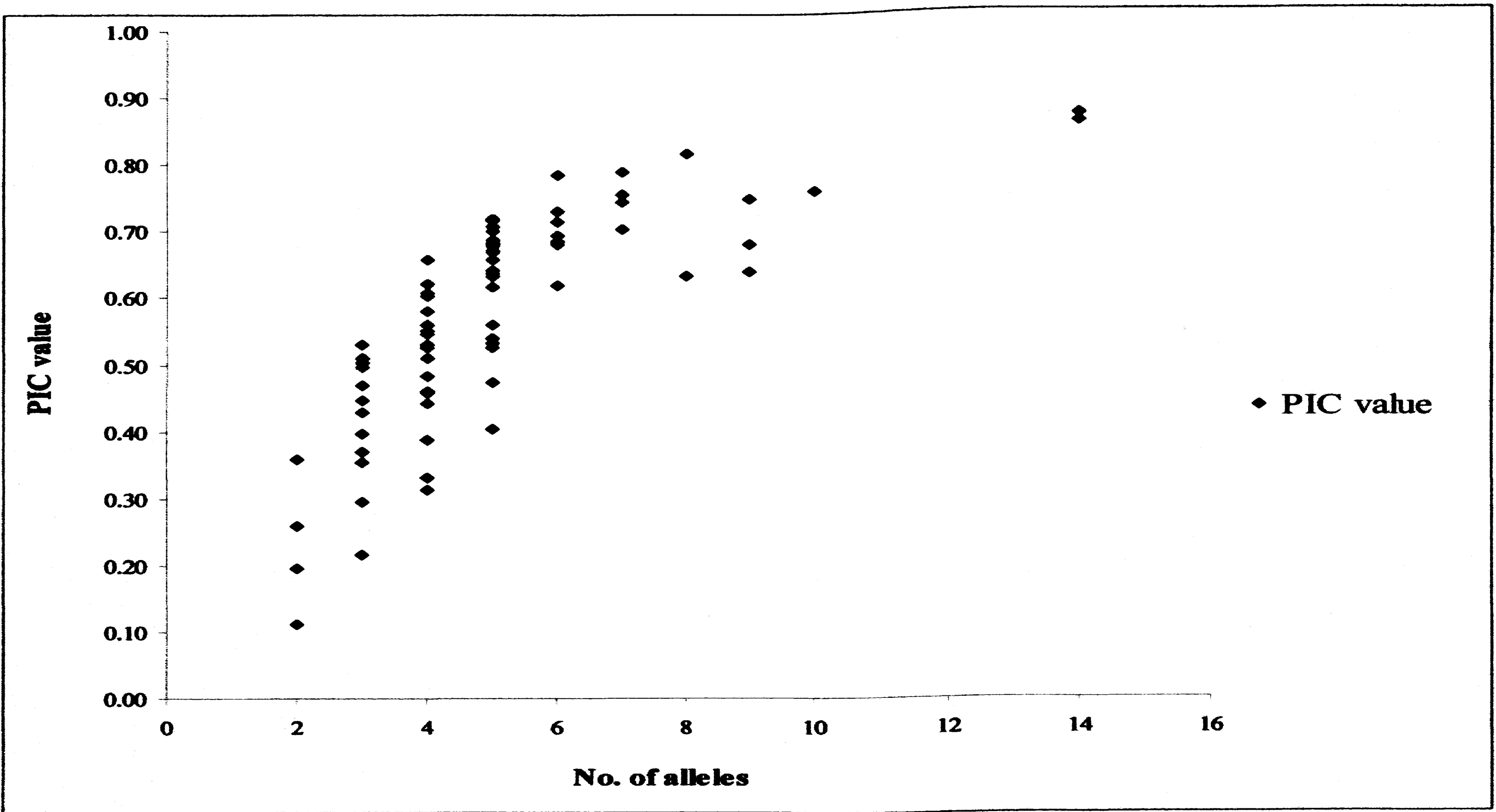

Figure 9: Graphical representation of relationship between PIC value and number of alleles

Appendix I. Amplification and polymorphic status of 876 BAC-end SSR markers on different mapping populations

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 01	M	NA	M	M	M	M	M	M	M	M	NA	M
GNB 02	P	M	M	M	P	M	M	M	M	M	NA	M
GNB 03	M	M	M	M	M	M	M	M	M	M	M	M
GNB 04	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 05	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 06	M	M	M	M	M	M	M	M	M	M	M	M
GNB 07	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 08	M	M	NA	NA	NA	M	M	M	M	M	NA	M
GNB 09	M	M	M	M	NA	M	M	P	M	M	M	M
GNB 10	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 12	M	M	M	M	M	M	M	M	M	M	M	M
GNB 13	M	M	M	M	M	M	M	M	M	M	M	M
GNB 14	M	M	NA	NA	M	M	M	NA	M	M	NA	M
GNB 15	M	M	M	NA	M	M	NA	NA	NA	NA	M	M
GNB 17	M	M	M	M	NA	M	M	M	M	NA	NA	M
GNB 18	P	NA	NA	NA	P	P	NA	NA	NA	NA	NA	P
GNB 20	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 21	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 22	M	M	M	M	M	M	M	M	M	M	M	M
GNB 24	M	NA	NA	M	NA	M	M	M	M	M	M	M
GNB 27	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 28	M	NA	NA	NA	M	NA	NA	NA	NA	NA	NA	NA
GNB 29	M	M	M	M	M	M	M	M	M	M	M	M
GNB 30	M	M	M	M	M	M	M	M	M	M	M	M
GNB 31	M	M	M	M	M	M	M	M	M	M	M	M
GNB 32	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 33	M	M	M	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 34	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 35	M	M	M	M	M	M	M	M	M	M	M	M
GNB 36	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 37	M	M	M	M	NA	NA	M	M	NA	M	NA	NA
GNB 38	M	P	M	P	P	M	P	P	P	P	M	P
GNB 39	NA	NA	NA	M	P	M	M	M	M	M	M	M
GNB 40	M	M	NA	M	P	NA	M	M	M	M	NA	NA
GNB 41	M	M	M	M	P	M	M	M	M	M	M	M
GNB 42	NA	NA	M	M	NA	M	M	M	M	M	M	M
GNB 43	M	M	M	M	M	M	M	M	M	M	M	M
GNB 46	M	NA	NA	M	M	M	M	M	M	M	M	M
GNB 47	M	M	M	M	M	M	M	M	M	M	M	M
GNB 48	M	M	M	M	M	M	M	M	M	NA	M	NA
GNB 49	M	M	M	M	M	M	M	NA	M	M	M	M
GNB 50	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 51	M	P	M	M	M	M	M	P	M	M	M	M
GNB 52	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 53	M	M	M	M	NA	M	M	M	NA	M	M	NA
GNB 54	NA	NA	NA	M	NA	M	M	NA	M	M	NA	M
GNB 56	M	M	M	M	NA	NA	M	M	M	M	M	NA
GNB 57	M	NA	NA	NA	M	NA	M	NA	NA	M	M	NA
GNB 58	P	NA	NA	NA	NA	M	P	M	M	M	M	P
GNB 59	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 60	M	M	M	M	M	M	M	M	M	M	M	M
GNB 62	M	M	M	M	M	M	M	M	M	M	M	M
GNB 63	M	M	M	M	P	NA	M	M	M	M	NA	NA
GNB 63	M	M	M	M	M	M	M	M	M	M	M	M
GNB 64	M	M	M	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 66	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 67	M	M	M	M	M	M	M	M	M	M	M	NA
GNB 70	M	P	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 71	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 72	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 73	M	NA	M	M	P	M	M	M	NA	M	M	M
GNB 74	M	M	M	M	P	M	M	M	M	M	M	M
GNB 75	NA	NA	M	M	M	NA	M	M	NA	M	NA	M
GNB 77	M	M	M	M	M	M	M	M	M	M	M	M
GNB 79	M	M	M	M	M	M	M	M	M	M	M	M
GNB 80	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 81	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 82	NA	M	NA	M	M	M	M	M	M	M	NA	M
GNB 83	NA	NA	M	M	NA	M	M	M	M	M	M	M
GNB 84	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 85	NA	NA	M	M	M	M	M	M	M	M	NA	M
GNB 87	M	M	M	M	P	M	M	M	M	M	M	M
GNB 88	M	M	M	M	M	NA	M	M	M	M	M	NA
GNB 89	NA	NA	M	M	M	NA	M	M	M	M	M	NA
GNB 91	M	M	M	M	M	M	M	M	M	M	M	M
GNB 92	NA	NA	M	M	NA	NA	M	M	M	M	M	NA
GNB 94	M	M	M	M	M	M	M	M	M	M	M	M
GNB 95	M	NA	NA	NA	NA	M	NA	NA	NA	NA	M	M
GNB 96	M	M	M	M	M	M	M	M	M	M	M	M
GNB 98	P	NA	M	P	P	P	P	P	P	M	M	P
GNB 99	NA	NA	M	M	NA	NA	M	M	M	M	M	M
GNB 100	M	M	M	M	M	M	P	M	P	M	M	P
GNB 101	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 102	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 103	NA	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 104	M	M	M	NA	M	M	NA	NA	NA	NA	NA	M
GNB 105	M	NA	NA	M	M	M	M	M	M	M	NA	M
GNB 106	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	NA
GNB 107	P	P	M	M	P	M	P	M	M	M	M	P
GNB 108	M	M	M	M	M	M	M	M	M	M	M	M
GNB 109	M	NA	NA	M	NA	M	M	M	M	M	NA	M
GNB 110	M	NA	NA	M	NA	M	M	M	M	M	NA	M
GNB 111	NA	M	M	M	M	NA	M	M	M	M	M	NA
GNB 112	M	M	M	NA	NA	NA	NA	NA	NA	NA	NA	M
GNB 113	M	NA	NA	NA	NA	M	NA	M	M	M	NA	M
GNB 114	M	M	M	M	M	NA	M	M	M	M	M	NA
GNB 115	NA	M	NA	NA	M	NA	NA	NA	NA	NA	NA	NA
GNB 116	NA	NA	M	M	NA	NA	M	NA	M	NA	NA	NA
GNB 118	M	NA	NA	NA	NA	NA	M	NA	M	M	NA	NA
GNB 119	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 120	M	NA	M	M	NA	NA	M	NA	NA	M	M	NA
GNB 121	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 122	M	M	M	M	P	M	M	M	M	M	M	M
GNB 123	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 124	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	NA	NA
GNB 125	NA	M	M	M	M	NA	M	M	M	M	NA	M
GNB 126	M	NA	M	M	P	M	NA	M	M	M	M	NA
GNB 127	NA	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 128	M	M	M	M	M	M	M	M	M	M	M	M
GNB 130	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 131	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 132	M	NA	M	M	M	NA	M	M	M	M	M	NA
GNB 133	M	M	NA	M	M	M	NA	M	NA	M	M	M
GNB 134	M	M	M	M	M	M	M	M	M	M	M	M
GNB 136	P	NA	NA	NA	NA	P	M	P	P	M	M	M
GNB 137	M	M	M	M	M	M	NA	M	M	M	M	NA
GNB 138	M	NA	M	NA	P	M	M	M	M	M	M	M
GNB 139	NA	M	M	M	NA	M	M	M	M	M	M	M
GNB 140	M	M	NA	NA	NA	M	NA	NA	NA	NA	M	M
GNB 141	M	M	M	M	M	M	M	M	M	M	M	M
GNB 142	M	M	M	M	P	M	M	M	M	M	M	M
GNB 143	M	P	M	M	P	M	M	M	M	M	M	M
GNB 144	M	M	M	M	M	M	M	M	M	M	M	M
GNB 145	M	NA	NA	P	P	P	P	P	P	P	P	P
GNB 147	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 148	M	M	NA	NA	NA	M	NA	NA	NA	NA	M	NA
GNB 150	M	M	M	M	M	M	M	M	M	M	M	M
GNB 151	NA	NA	M	M	M	NA	M	M	M	M	M	M
GNB 152	M	M	NA	NA	P	M	NA	NA	NA	NA	NA	P
GNB 153	M	M	M	M	M	M	M	M	M	M	M	M
GNB 154	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 155	M	NA	P	P	P	P	P	P	P	P	P	P
GNB 156	NA	NA	M	M	NA	NA	M	M	NA	M	NA	NA
GNB 157	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 159	M	M	M	M	P	M	M	M	M	M	M	M
GNB 160	P	M	NA	M	NA	NA	M	M	NA	M	NA	M
GNB 161	M	M	M	M	P	M	NA	M	M	M	NA	M
GNB 164	M	M	M	M	M	M	M	M	M	M	M	NA
GNB 166	M	M	M	NA	P	M	NA	M	M	M	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 167	M	NA	P	P	M	P	P	M	P	M	P	P
GNB 168	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 169	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 170	M	NA	M	P	NA	M	P	P	P	P	NA	M
GNB 172	M	M	M	M	M	M	M	M	M	M	M	M
GNB 173	NA	NA	M	M	NA	M	NA	NA	M	NA	M	M
GNB 174	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 175	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 176	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 177	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 178	M	NA	M	P	NA	NA	P	M	P	M	NA	NA
GNB 179	M	M	M	NA	M	NA	NA	NA	NA	NA	M	NA
GNB 180	M	M	M	M	M	M	M	M	M	M	NA	NA
GNB 181	M	M	M	P	M	M	P	M	P	P	M	M
GNB 183	M	M	M	NA	M	M	NA	NA	NA	NA	M	M
GNB 184	M	NA	M	M	NA	M	NA	M	M	M	M	M
GNB 185	NA	NA	M	M	NA	NA	M	M	M	NA	NA	NA
GNB 187	M	M	M	M	M	M	M	M	M	M	M	M
GNB 188	M	M	NA	M	M	M	M	NA	M	M	M	M
GNB 189	M	M	NA	M	M	M	M	M	M	M	M	M
GNB 190	M	P	M	M	M	M	NA	M	M	M	M	M
GNB 191	M	NA	M	M	M	M	M	NA	M	M	M	M
GNB 193	M	NA	M	M	M	NA	M	M	M	M	M	M
GNB 194	M	NA	NA	NA	NA	NA	M	M	M	M	NA	M
GNB 195	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 196	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 197	M	NA	M	M	NA	NA	NA	M	NA	M	NA	NA
GNB 198	M	NA	M	M	M	M	M	M	M	M	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 199	NA	NA	M	M	M	M	M	M	M	M	NA	NA
GNB 200	M	M	M	M	P	M	M	M	M	M	M	M
GNB 201	M	M	M	M	M	M	M	M	M	NA	NA	M
GNB 203	M	NA	M	NA	NA	NA	NA	NA	NA	NA	NA	M
GNB 205	M	M	M	M	M	M	M	M	M	M	M	M
GNB 206	M	NA	NA	M	P	M	NA	NA	NA	NA	NA	NA
GNB 207	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 208	M	NA	M	M	M	M	M	M	NA	M	M	NA
GNB 211	NA	M	M	M	P	M	M	M	M	M	M	M
GNB 212	M	M	M	M	NA	M	M	M	NA	M	M	NA
GNB 213	M	M	M	M	M	M	M	M	M	M	M	M
GNB 214	M	NA	M	M	NA	M	NA	M	M	M	NA	M
GNB 215	M	M	M	NA	M	M	NA	NA	NA	NA	NA	M
GNB 216	M	M	NA	P	M	M	P	M	P	M	M	M
GNB 218	M	M	P	M	P	P	M	M	M	M	NA	P
GNB 219	M	M	M	M	M	NA	M	M	M	M	M	NA
GNB 220	M	M	M	M	M	M	M	M	M	M	M	M
GNB 222	M	NA	M	M	M	NA	M	M	M	M	M	NA
GNB 224	NA	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 225	M	M	NA	NA	M	M	M	M	M	M	NA	M
GNB 226	M	P	M	M	NA	P	P	M	M	M	P	P
GNB 228	M	NA	M	M	M	M	M	M	M	M	NA	M
GNB 230	M	M	NA	NA	M	NA	M	M	M	NA	NA	NA
GNB 231	M	M	M	M	M	M	M	M	M	M	M	NA
GNB 232	M	NA	M	M	P	M	NA	NA	NA	NA	NA	M
GNB 233	NA	NA	M	M	M	M	M	M	M	M	NA	M
GNB 234	NA	M	M	M	M	M	M	M	M	M	NA	M
GNB 235	M	M	M	M	P	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 236	M	M	M	M	P	M	M	M	M	M	M	M
GNB 239	M	M	M	M	M	M	M	M	M	M	M	M
GNB 241	M	NA	M	M	NA	NA	M	P	M	P	M	NA
GNB 244	NA	NA	NA	NA	NA	M	NA	M	M	M	NA	M
GNB 245	M	M	M	M	M	M	M	M	M	M	M	M
GNB 246	M	M	M	M	P	M	M	M	M	M	M	M
GNB 247	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 248	M	M	M	M	M	M	M	M	M	M	M	M
GNB 249	M	M	M	NA	P	M	NA	NA	NA	NA	NA	M
GNB 250	M	M	M	M	M	NA	M	M	NA	NA	NA	M
GNB 251	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 253	M	M	NA	M	P	M	M	M	M	NA	NA	M
GNB 254	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 256	NA	NA	NA	NA	P	M	NA	NA	NA	NA	M	M
GNB 257	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 259	M	M	M	M	M	M	M	M	M	M	M	M
GNB 260	M	M	M	M	P	M	M	M	M	M	M	P
GNB 261	M	M	M	M	P	M	M	NA	M	M	M	M
GNB 262	P	P	P	M	P	M	P	P	P	P	NA	P
GNB 264	M	M	M	M	M	NA	M	M	M	M	P	M
GNB 265	NA	NA	M	NA	M	M	NA	NA	NA	NA	NA	M
GNB 266	M	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 267	M	M	M	M	P	M	M	M	M	M	M	M
GNB 268	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 269	M	NA	NA	NA	M	M	M	M	M	M	NA	M
GNB 270	M	NA	M	NA	M	M	NA	NA	NA	NA	NA	M
GNB 271	M	NA	M	M	M	M	NA	M	M	M	M	M
GNB 272	M	M	M	NA	M	M	NA	NA	NA	NA	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 274	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 275	M	M	M	M	M	NA	M	M	M	M	M	NA
GNB 276	M	M	M	M	P	M	M	M	M	M	M	M
GNB 277	M	M	M	M	M	M	M	M	M	M	M	M
GNB 279	M	M	M	M	M	M	M	M	M	M	M	M
GNB 280	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 282	M	M	M	M	M	M	M	M	M	M	M	M
GNB 283	M	M	M	M	P	M	M	M	M	M	M	M
GNB 284	NA	M	M	NA	P	P	NA	NA	NA	NA	P	M
GNB 285	M	M	M	M	M	M	M	M	M	M	M	M
GNB 286	M	M	M	M	P	M	M	M	M	M	M	M
GNB 287	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 288	M	M	M	M	NA	M	M	M	NA	M	M	M
GNB 289	M	NA	M	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 290	NA	NA	M	M	M	NA	M	M	NA	NA	M	NA
GNB 291	M	M	M	M	M	M	NA	NA	NA	NA	NA	M
GNB 292	M	M	M	M	M	M	M	M	NA	M	NA	M
GNB 293	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 294	NA	M	M	M	M	M	M	M	M	M	M	M
GNB 295	M	NA	M	NA	NA	M	NA	NA	NA	NA	M	M
GNB 296	NA	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 297	M	M	M	M	NA	M	M	M	M	M	M	NA
GNB 298	M	M	NA	NA	P	M	NA	NA	NA	NA	NA	M
GNB 299	M	M	M	M	P	NA	NA	M	NA	M	M	M
GNB 301	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 302	M	M	M	M	M	M	M	NA	M	M	NA	M
GNB 303	M	P	M	M	M	M	M	M	M	M	M	M
GNB 304	M	P	P	M	P	M	M	P	P	M	P	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 306	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 307	NA	M	M	M	NA	M	M	M	M	M	NA	M
GNB 309	M	M	NA	M	P	M	M	M	M	M	M	M
GNB 310	M	NA	M	M	P	M	M	M	NA	M	NA	M
GNB 311	M	NA	NA	NA	P	M	M	M	NA	M	NA	M
GNB 312	M	M	M	M	P	M	M	M	M	NA	M	M
GNB 313	NA	M	NA	M	M	NA	M	M	M	M	NA	NA
GNB 314	M	M	M	M	M	M	M	M	M	M	M	M
GNB 315	M	M	M	M	NA	M	M	M	M	NA	M	M
GNB 316	M	M	M	M	M	M	M	M	M	M	M	M
GNB 317	M	NA	M	M	NA	P	M	M	M	M	NA	NA
GNB 318	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 319	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 320	M	NA	M	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 321	M	M	M	M	M	M	M	M	M	M	M	M
GNB 322	M	M	M	M	NA	M	NA	M	M	M	NA	M
GNB 323	M	M	M	M	M	M	M	M	M	M	M	M
GNB 327	NA	NA	M	M	M	M	M	M	M	NA	M	M
GNB 328	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 330	M	NA	M	M	M	NA	M	M	NA	NA	M	NA
GNB 331	M	M	M	M	M	M	M	M	M	M	M	NA
GNB 333	M	M	M	M	M	M	M	M	NA	NA	M	M
GNB 334	M	M	M	M	NA	M	M	M	M	M	NA	NA
GNB 335	M	M	M	M	M	M	M	M	M	M	M	M
GNB 336	M	M	M	M	P	M	M	M	M	M	M	M
GNB 340	M	M	M	M	M	M	M	M	M	M	M	NA
GNB 341	NA	M	M	M	NA	M	NA	M	M	M	NA	NA
GNB 342	M	NA	M	M	M	M	M	M	M	M	NA	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 344	NA	NA	NA	NA	M	P	NA	P	M	M	NA	P
GNB 346	M	M	M	M	M	M	M	M	M	M	M	M
GNB 349	M	NA	M	NA	P	NA	NA	NA	NA	NA	NA	NA
GNB 351	M	M	M	M	NA	M	M	NA	NA	M	NA	NA
GNB 353	M	M	M	M	M	M	M	M	M	M	M	M
GNB 354	M	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 355	NA	NA	M	M	M	NA	M	M	M	M	M	M
GNB 356	M	NA	M	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 357	P	M	P	P	P	P	P	P	P	P	M	M
GNB 358	NA	M	M	M	M	M	M	M	M	M	M	M
GNB 360	M	M	M	M	M	M	M	M	M	M	M	M
GNB 361	M	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	M
GNB 362	M	M	M	M	M	M	M	NA	M	M	NA	M
GNB 363	M	NA	M	M	NA	NA	NA	NA	NA	NA	NA	NA
GNB 364	M	M	NA	NA	NA	M	M	M	M	M	M	M
GNB 366	M	M	M	M	P	M	M	M	M	M	M	M
GNB 367	M	M	M	M	M	M	M	M	M	M	M	M
GNB 368	M	M	M	M	P	M	M	M	M	M	M	NA
GNB 369	M	M	M	M	M	M	M	M	M	M	M	M
GNB 373	M	M	M	M	NA	NA	M	M	M	M	NA	NA
GNB 374	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 375	M	M	M	M	M	M	M	M	M	M	M	M
GNB 376	M	M	M	M	NA	M	M	M	M	M	M	NA
GNB 378	M	M	M	NA	P	M	M	M	M	M	NA	M
GNB 380	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 381	NA	M	M	M	M	M	M	M	M	M	M	M
GNB 382	M	M	M	M	M	M	NA	M	M	M	NA	NA
GNB 383	M	M	M	M	NA	M	M	M	M	M	M	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 384	M	M	M	M	M	M	M	M	M	M	NA	NA
GNB 385	M	M	M	M	P	M	M	M	M	M	M	M
GNB 386	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 387	M	M	NA	NA	P	P	M	M	M	M	M	M
GNB 389	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 390	M	M	M	M	M	NA	M	M	M	M	M	M
GNB 391	NA	M	M	M	NA	NA	M	M	NA	M	NA	NA
GNB 392	M	M	M	P	P	P	P	M	P	M	M	P
GNB 393	M	M	M	M	M	M	M	M	M	M	M	M
GNB 394	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 395	M	M	M	M	M	M	M	M	M	M	M	M
GNB 396	M	NA	M	M	P	M	M	M	M	M	NA	M
GNB 397	M	M	M	M	P	M	M	M	M	M	M	NA
GNB 399	M	M	M	M	M	M	M	M	M	M	M	M
GNB 400	M	NA	M	M	M	M	M	M	M	M	NA	M
GNB 401	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 402	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	NA
GNB 404	M	M	M	M	M	M	M	M	M	M	M	M
GNB 405	NA	NA	NA	NA	NA	M	M	M	NA	M	NA	NA
GNB 406	M	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 407	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 410	M	M	M	M	M	M	M	M	M	M	M	M
GNB 413	M	M	NA	M	NA	M	M	M	M	M	NA	NA
GNB 414	M	M	M	M	NA	M	M	M	M	M	NA	NA
GNB 416	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 417	M	M	M	P	M	P	P	M	P	P	M	P
GNB 417	M	M	M	M	M	M	M	M	M	M	M	M
GNB 418	NA	NA	NA	NA	M	M	M	NA	M	NA	NA	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 420	M	M	M	M	M	M	M	M	M	M	M	M
GNB 422	NA	NA	M	NA	NA	NA	NA	NA	M	NA	NA	NA
GNB 423	M	M	M	M	NA	NA	M	M	M	M	NA	M
GNB 426	M	M	M	M	M	M	M	M	M	M	M	M
GNB 427	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 428	M	M	M	M	P	M	P	M	M	M	M	M
GNB 432	M	M	M	M	P	M	M	M	M	M	M	M
GNB 435	NA	NA	M	M	M	M	M	M	M	NA	NA	M
GNB 442	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 443	NA	M	NA	NA	M	M	NA	M	M	NA	NA	NA
GNB 445	M	M	M	M	M	M	M	M	M	M	M	M
GNB 446	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 447	M	NA	NA	M	M	M	M	M	M	M	M	NA
GNB 448	M	M	NA	NA	P	M	NA	NA	NA	NA	NA	M
GNB 449	NA	NA	NA	NA	NA	M	NA	M	NA	M	NA	NA
GNB 450	M	M	NA	M	M	M	M	M	M	M	M	M
GNB 452	M	M	M	M	M	NA	NA	M	M	NA	M	NA
GNB 453	M	M	M	M	M	M	M	M	M	M	M	M
GNB 454	M	M	M	M	M	M	NA	NA	NA	NA	NA	M
GNB 455	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 456	NA	M	M	NA	M	M	NA	NA	NA	NA	M	M
GNB 457	M	M	M	M	M	M	NA	M	M	M	M	M
GNB 458	M	M	M	M	M	M	M	M	M	M	M	M
GNB 459	M	M	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 460	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 461	M	M	M	P	NA	P	M	P	P	M	M	P
GNB 462	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 464	M	NA	M	M	P	M	P	M	M	M	P	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 466	NA	M	NA	M	M	M	M	M	NA	NA	NA	M
GNB 467	M	M	M	M	P	P	M	P	P	P	P	P
GNB 467	NA	NA	M	M	M	M	NA	M	M	M	NA	M
GNB 469	M	M	M	M	M	M	M	M	M	M	M	M
GNB 470	M	M	M	M	M	M	M	M	M	M	M	M
GNB 472	M	M	M	M	M	M	M	M	M	M	M	M
GNB 473	M	M	M	M	M	M	M	M	M	M	M	M
GNB 474	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 475	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 476	M	M	NA	M	M	M	M	M	M	M	M	M
GNB 477	M	M	M	M	M	M	M	M	M	M	M	M
GNB 479	M	M	M	M	M	M	M	M	M	M	M	M
GNB 480	M	M	M	NA	M	M	NA	NA	NA	NA	M	M
GNB 481	M	M	NA	M	M	NA	NA	NA	NA	NA	M	NA
GNB 482	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	NA
GNB 483	M	M	M	M	M	M	M	M	M	M	M	M
GNB 485	M	M	M	M	NA	NA	M	M	M	M	M	M
GNB 489	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 490	M	M	M	M	M	M	M	M	M	M	M	M
GNB 491	NA	M	M	M	NA	M	NA	M	M	M	M	M
GNB 494	M	M	M	M	NA	M	NA	M	M	M	NA	M
GNB 496	M	M	M	M	M	M	M	M	M	M	M	M
GNB 498	NA	NA	M	M	NA	M	NA	M	M	M	NA	M
GNB 500	NA	NA	M	M	P	M	M	M	M	M	NA	P
GNB 501	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 503	M	M	M	M	P	M	M	M	M	M	M	P
GNB 504	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 505	M	M	M	NA	NA	NA	NA	NA	NA	NA	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 506	NA	M	M	M	NA	M	M	M	NA	M	NA	M
GNB 507	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 508	M	M	M	M	M	M	M	M	M	M	M	M
GNB 509	M	M	M	M	M	M	M	M	M	M	M	M
GNB 510	M	M	M	M	M	M	M	M	M	M	M	M
GNB 511	M	M	M	M	P	M	M	M	M	M	NA	M
GNB 513	M	M	M	M	P	M	M	M	M	M	M	M
GNB 513	M	M	M	M	M	M	M	M	M	M	M	M
GNB 515	M	P	M	NA	NA	P	NA	NA	NA	NA	M	P
GNB 516	M	M	M	M	M	M	M	M	M	M	M	M
GNB 517	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 518	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 520	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 521	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 522	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 523	M	M	NA	NA	NA	NA	NA	M	M	M	NA	NA
GNB 524	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 525	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 526	M	M	M	M	M	M	M	M	M	M	M	M
GNB 527	M	M	M	M	M	M	M	M	M	M	M	M
GNB 528	M	M	M	M	M	M	M	M	M	M	M	M
GNB 529	M	M	M	M	M	M	M	M	M	M	M	M
GNB 530	M	M	M	M	M	M	M	M	M	M	M	M
GNB 530	M	P	NA	NA	NA	M	NA	NA	NA	NA	M	M
GNB 531	M	M	M	M	M	M	M	M	M	M	M	M
GNB 532	M	M	M	M	M	M	M	M	M	M	M	M
GNB 533	NA	M	NA	M	M	NA	M	M	M	M	M	M
GNB 534	M	M	M	M	NA	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 535	M	M	M	M	M	M	M	M	M	M	M	M
GNB 536	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 537	M	M	M	M	M	M	M	M	M	M	M	M
GNB 538	M	M	M	M	M	M	M	M	M	M	M	M
GNB 539	M	M	M	M	M	M	M	M	M	M	M	M
GNB 540	M	M	M	M	M	M	M	M	M	M	M	M
GNB 541	M	M	M	M	M	M	M	M	M	M	M	M
GNB 542	M	M	M	M	M	M	M	M	M	M	M	M
GNB 543	M	M	M	M	M	M	M	M	M	M	M	M
GNB 544	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 545	M	M	M	M	M	M	M	M	M	M	M	M
GNB 546	M	M	M	M	M	M	M	M	M	M	M	M
GNB 547	M	M	M	M	M	M	M	M	M	M	M	M
GNB 549	M	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 550	M	M	M	M	M	M	M	M	M	M	M	M
GNB 551	M	M	M	M	M	M	M	M	M	M	M	M
GNB 552	M	M	M	M	M	M	M	M	M	M	M	M
GNB 553	NA	NA	NA	NA	M	NA	M	M	M	M	NA	NA
GNB 554	M	M	M	M	P	M	M	M	M	M	M	M
GNB 555	M	M	M	NA	NA	NA	NA	NA	NA	NA	NA	M
GNB 557	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 558	M	M	M	M	M	M	M	M	M	M	M	M
GNB 559	M	M	M	M	M	M	M	M	M	M	M	M
GNB 560	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 561	M	M	M	M	M	M	M	M	M	M	M	M
GNB 562	M	M	M	M	M	M	M	M	M	M	M	M
GNB 563	M	M	M	M	NA	M	M	M	NA	M	NA	M
GNB 564	M	M	M	M	M	M	M	M	M	M	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 565	M	M	M	M	M	M	M	M	M	M	M	M
GNB 566	M	M	M	M	M	M	M	M	M	M	M	M
GNB 567	M	M	M	M	NA	M	M	NA	NA	NA	M	NA
GNB 568	M	M	M	M	M	M	M	M	M	M	M	M
GNB 569	M	M	M	P	P	P	M	M	M	M	M	M
GNB 570	M	M	M	M	M	M	M	M	M	M	M	M
GNB 571	M	M	M	M	M	M	M	M	M	M	M	M
GNB 573	M	M	NA	NA	P	M	M	M	M	M	NA	M
GNB 574	M	M	M	M	M	M	M	M	M	M	M	M
GNB 575	M	M	M	M	M	M	M	M	M	M	M	M
GNB 576	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 577	M	NA	M	M	M	NA	M	M	M	NA	NA	NA
GNB 578	M	M	M	M	M	M	M	M	M	M	M	M
GNB 579	NA	NA	M	M	P	NA	M	M	M	M	NA	M
GNB 580	NA	NA	NA	NA	NA	M	M	M	M	M	NA	M
GNB 582	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 583	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 585	M	M	M	M	M	M	M	M	M	M	M	M
GNB 586	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 587	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 588	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 590	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 591	M	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 593	M	NA	M	M	NA	M	M	NA	M	NA	M	M
GNB 594	M	M	M	M	M	M	M	M	M	M	M	M
GNB 596	M	M	M	M	M	M	M	M	M	M	M	M
GNB 598	M	M	M	M	M	M	M	M	M	M	M	M
GNB 599	M	M	NA	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 600	M	M	M	M	M	M	M	M	M	M	M	M
GNB 603	M	NA	M	M	P	M	M	M	M	M	M	M
GNB 606	M	NA	NA	M	M	M	M	M	M	M	NA	M
GNB 607	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 608	NA	NA	NA	NA	P	M	NA	NA	NA	NA	M	M
GNB 610	M	M	M	M	M	M	M	M	M	NA	NA	M
GNB 612	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 614	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 615	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 616	M	M	M	M	M	M	M	M	M	M	M	M
GNB 617	NA	NA	M	M	M	M	M	M	M	M	M	NA
GNB 622	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 623	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 624	NA	NA	M	M	P	M	M	M	M	M	M	M
GNB 625	NA	NA	M	M	NA	NA	M	M	M	M	M	M
GNB 626	NA	NA	M	M	M	NA	M	M	M	M	NA	M
GNB 627	NA	NA	M	M	NA	NA	M	M	M	M	NA	NA
GNB 629	M	M	M	M	P	M	M	M	M	M	M	M
GNB 630	M	M	NA	NA	P	M	NA	NA	NA	NA	NA	M
GNB 631	NA	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 632	M	M	M	M	P	M	M	M	M	M	M	M
GNB 633	M	M	M	M	M	M	M	M	M	M	M	M
GNB 634	M	M	M	M	M	M	M	M	M	M	M	M
GNB 635	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 636	M	M	NA	NA	M	M	M	M	M	M	NA	M
GNB 637	NA	NA	NA	NA	NA	NA	NA	M	NA	M	NA	NA
GNB 638	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 639	NA	NA	NA	NA	NA	M	NA	M	M	NA	NA	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 640	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 642	M	M	NA	NA	NA	M	M	M	M	M	NA	M
GNB 643	NA	NA	NA	P	M	P	P	M	P	M	NA	P
GNB 644	NA	NA	M	M	M	NA	M	NA	NA	NA	M	NA
GNB 646	M	NA	NA	NA	M	M	M	M	M	M	NA	M
GNB 649	M	M	M	P	P	P	P	M	P	M	P	P
GNB 650	M	M	M	M	M	M	M	M	M	M	M	M
GNB 651	M	M	M	M	M	M	M	M	M	M	M	M
GNB 654	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 655	M	M	M	M	NA	NA	M	M	M	M	NA	NA
GNB 656	M	M	M	M	M	M	M	M	M	M	M	M
GNB 657	M	M	M	M	M	M	M	M	M	M	M	M
GNB 658	M	M	M	M	M	M	M	M	M	M	M	M
GNB 660	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 661	M	M	M	M	P	M	M	M	M	M	M	M
GNB 662	NA	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 664	NA	M	M	M	NA	M	M	M	M	NA	M	M
GNB 665	NA	NA	M	M	M	NA	M	M	M	NA	M	M
GNB 666	M	NA	M	M	P	P	M	M	M	M	M	M
GNB 667	NA	NA	M	M	P	M	M	M	M	M	M	M
GNB 668	M	M	M	NA	P	M	NA	NA	NA	NA	M	M
GNB 669	NA	NA	M	M	P	M	M	M	M	M	M	M
GNB 670	M	M	NA	M	NA	P	M	M	M	M	M	M
GNB 671	M	M	M	M	M	M	M	M	M	M	M	M
GNB 672	M	M	NA	M	M	M	M	M	M	M	NA	M
GNB 673	M	M	M	M	P	M	M	M	M	M	M	M
GNB 674	M	M	M	M	M	M	M	M	M	M	M	M
GNB 675	M	M	M	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 676	M	M	M	M	M	M	M	M	M	M	M	M
GNB 677	NA	M	M	M	NA	M	M	M	M	NA	M	M
GNB 678	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 679	M	M	M	M	M	M	M	M	M	P	M	M
GNB 680	M	M	M	M	M	M	M	M	M	M	M	M
GNB 681	M	M	M	M	M	M	M	M	M	M	M	M
GNB 682	NA	NA	M	M	M	P	P	P	M	P	P	P
GNB 683	NA	NA	M	NA	M	M	NA	NA	NA	NA	NA	M
GNB 684	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 687	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 688	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 689	M	M	NA	M	M	M	M	M	M	M	NA	M
GNB 691	M	M	M	M	M	M	M	M	M	M	M	M
GNB 692	NA	NA	M	M	NA	M	M	M	M	M	NA	P
GNB 693	M	M	M	M	M	M	M	M	M	M	M	M
GNB 694	NA	NA	M	M	P	M	M	M	M	M	M	M
GNB 695	M	M	M	M	M	M	M	M	M	M	M	M
GNB 696	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 698	M	M	M	M	M	M	M	M	M	M	M	M
GNB 699	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 700	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 703	M	M	M	M	M	NA	M	M	M	M	M	M
GNB 704	M	M	M	M	M	M	M	M	M	M	M	M
GNB 706	M	M	M	M	M	M	M	M	M	M	M	M
GNB 707	M	NA	NA	M	M	M	M	M	M	M	M	M
GNB 709	M	M	M	M	M	M	M	M	M	M	M	M
GNB 710	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 712	M	M	M	M	P	M	M	P	P	P	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 713	M	NA	M	M	M	M	M	M	M	NA	NA	M
GNB 714	NA	NA	M	M	NA	M	NA	M	NA	M	NA	M
GNB 716	M	M	M	M	P	M	M	M	M	M	M	M
GNB 717	M	M	M	M	M	M	NA	M	M	NA	NA	M
GNB 718	NA	M	NA	NA	M	M	NA	M	NA	M	NA	M
GNB 720	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 722	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 728	NA	NA	NA	NA	NA	M	M	M	M	M	NA	M
GNB 729	M	M	M	M	P	M	M	M	M	M	M	M
GNB 732	M	M	M	M	M	M	M	M	M	M	M	M
GNB 733	M	NA	M	NA	P	NA	NA	NA	NA	NA	M	M
GNB 734	NA	NA	NA	NA	M	NA	M	M	M	M	NA	NA
GNB 735	NA	NA	NA	M	NA	NA	NA	NA	NA	NA	NA	NA
GNB 737	M	NA	NA	NA	NA	M	M	M	M	M	NA	M
GNB 738	M	M	M	M	P	M	M	M	M	M	M	M
GNB 741	NA	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 742	M	M	M	M	M	M	M	M	M	M	M	M
GNB 743	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 745	M	M	M	M	M	M	M	M	M	M	M	M
GNB 746	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 747	M	NA	NA	M	M	M	M	M	M	M	M	NA
GNB 748	M	M	M	M	M	M	M	M	M	M	M	M
GNB 749	M	NA	M	M	NA	P	M	M	M	M	M	M
GNB 750	M	NA	M	M	M	M	M	M	M	M	NA	M
GNB 752	M	M	NA	NA	NA	M	M	M	M	M	NA	M
GNB 753	M	M	NA	NA	NA	M	M	M	M	M	NA	M
GNB 755	M	M	M	M	M	M	M	M	M	M	M	M
GNB 756	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 757	M	M	M	M	M	NA	M	M	M	M	M	M
GNB 758	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	M
GNB 759	M	M	M	M	M	M	M	M	M	M	M	M
GNB 760	M	M	M	M	NA	NA	M	M	M	M	NA	M
GNB 761	M	M	M	M	P	M	M	M	M	M	M	M
GNB 762	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 764	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 765	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 766	M	M	M	M	M	M	M	M	M	M	M	M
GNB 767	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 768	M	M	NA	NA	M	NA	M	M	M	M	NA	M
GNB 770	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 772	M	M	M	M	M	NA	M	M	M	M	M	NA
GNB 773	M	M	M	M	M	M	M	M	NA	NA	M	M
GNB 774	M	M	M	M	M	M	M	M	M	M	M	M
GNB 775	M	M	M	M	P	M	M	M	M	M	M	M
GNB 776	M	M	M	M	M	NA	M	M	M	M	NA	M
GNB 778	M	M	M	M	M	M	M	M	M	M	M	M
GNB 780	M	M	M	M	M	M	M	M	M	M	M	M
GNB 781	M	M	M	NA	M	M	NA	NA	NA	NA	M	M
GNB 782	P	NA	NA	M	NA	NA	NA	NA	M	NA	NA	NA
GNB 785	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 786	NA	M	M	M	P	M	M	M	NA	NA	NA	M
GNB 788	M	M	M	M	P	M	M	M	M	M	M	M
GNB 790	M	M	M	M	M	M	M	M	M	M	M	M
GNB 791	M	M	M	M	M	M	M	M	M	M	M	M
GNB 792	NA	M	NA	NA	NA	M	M	M	M	NA	NA	M
GNB 793	M	M	M	M	M	M	M	M	M	M	NA	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 794	M	M	M	M	M	M	M	M	M	M	M	M
GNB 795	M	M	M	M	M	M	M	M	M	M	M	M
GNB 796	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 797	M	M	M	M	M	M	M	M	M	M	M	M
GNB 798	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 800	M	M	M	M	M	M	M	M	M	M	M	M
GNB 801	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 802	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 804	M	M	M	M	P	M	M	M	M	M	M	M
GNB 806	M	M	M	M	M	M	M	M	M	M	M	M
GNB 807	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 808	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	NA
GNB 809	M	M	NA	M	M	NA	M	M	NA	NA	M	M
GNB 810	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 811	M	M	M	M	P	M	M	M	NA	M	NA	M
GNB 812	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 813	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 814	M	M	M	M	NA	M	NA	M	M	M	M	M
GNB 815	M	M	M	M	M	M	M	M	M	M	M	M
GNB 816	M	M	P	M	NA	M	M	M	M	M	NA	M
GNB 817	M	M	M	M	M	M	M	M	M	M	M	M
GNB 818	M	M	M	M	M	M	M	M	M	M	M	M
GNB 819	M	M	M	M	M	M	M	M	M	M	M	M
GNB 820	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	M
GNB 821	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 824	M	M	M	M	M	M	M	M	M	M	M	M
GNB 826	M	M	M	M	M	M	M	M	M	M	M	M
GNB 829	M	M	M	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 833	M	M	M	M	M	M	M	M	M	M	M	M
GNB 834	NA	NA	M	M	M	M	M	M	M	M	NA	M
GNB 835	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 836	M	M	M	M	M	M	M	M	M	M	M	M
GNB 839	M	M	M	M	M	M	M	M	M	NA	NA	M
GNB 840	P	M	P	P	NA	P	M	P	P	M	NA	P
GNB 841	M	M	M	M	P	M	M	M	M	M	M	M
GNB 842	M	P	P	M	NA	P	M	P	M	M	NA	P
GNB 843	M	M	M	M	NA	M	M	M	NA	M	M	M
GNB 844	M	M	M	M	P	M	M	M	M	M	M	M
GNB 845	M	M	M	M	M	M	M	M	M	M	M	M
GNB 846	M	M	M	M	M	M	M	M	M	M	M	M
GNB 847	M	M	M	M	M	M	M	M	M	M	M	M
GNB 850	M	M	M	M	NA	NA	P	P	P	M	P	P
GNB 851	M	M	M	M	M	M	M	M	M	M	M	M
GNB 852	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 853	M	M	NA	NA	M	NA	NA	NA	NA	NA	NA	P
GNB 854	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 855	NA	M	M	M	NA	M	M	M	M	M	M	M
GNB 856	M	M	M	M	M	M	M	M	M	M	M	M
GNB 857	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 858	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 859	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 861	M	M	M	M	M	M	M	M	M	M	M	M
GNB 862	M	M	M	M	M	M	M	M	M	M	M	M
GNB 863	M	M	M	M	M	M	M	M	M	M	M	M
GNB 864	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 865	M	M	M	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 865	M	M	M	M	M	M	M	M	M	M	M	M
GNB 869	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 870	M	M	M	M	P	M	M	M	M	M	M	M
GNB 871	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 872	M	M	M	M	M	M	M	M	M	M	M	M
GNB 873	M	M	M	M	P	M	M	M	M	M	M	M
GNB 874	M	NA	M	M	NA	M	M	M	M	M	NA	M
GNB 877	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 879	M	M	M	M	M	M	M	M	M	NA	NA	M
GNB 881	M	M	M	M	M	M	M	M	M	M	M	M
GNB 882	M	M	M	M	M	NA	M	NA	M	M	NA	NA
GNB 883	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 884	M	M	M	M	P	M	M	M	M	M	M	M
GNB 885	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 888	M	M	M	M	M	M	M	M	M	M	M	M
GNB 891	M	M	M	M	M	M	M	M	M	M	M	M
GNB 894	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 897	NA	M	M	M	M	M	M	M	M	M	NA	M
GNB 898	M	M	M	M	P	M	M	M	M	M	M	M
GNB 899	M	M	M	M	M	M	M	M	M	M	M	M
GNB 900	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 904	M	M	M	M	M	M	M	M	M	M	M	M
GNB 905	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 906	M	M	M	M	M	NA	M	M	NA	M	NA	NA
GNB 908	M	M	M	M	M	M	M	M	M	M	M	M
GNB 909	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	NA
GNB 910	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 911	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	M	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 913	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 915	M	M	M	M	M	M	M	M	M	M	M	M
GNB 916	M	M	M	M	M	M	M	M	M	M	M	M
GNB 917	M	M	M	M	M	M	M	M	M	M	M	M
GNB 918	M	M	M	M	M	M	M	M	M	M	M	M
GNB 921	M	M	M	M	M	M	M	M	M	M	M	M
GNB 922	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 924	M	M	M	M	M	M	M	M	M	M	M	M
GNB 927	NA	M	NA	NA	NA	M	M	M	M	M	NA	M
GNB 933	M	M	NA	NA	P	M	NA	NA	NA	NA	NA	M
GNB 934	M	M	M	M	M	M	M	M	M	NA	M	M
GNB 936	M	M	M	M	M	M	M	M	M	M	M	M
GNB 939	M	M	M	M	M	M	M	M	M	M	M	M
GNB 941	M	M	M	M	P	M	M	M	M	M	M	M
GNB 942	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 943	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 946	M	M	M	M	M	M	M	M	M	M	M	M
GNB 947	M	M	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 948	NA	M	M	M	M	M	M	M	M	NA	M	M
GNB 949	M	M	M	M	M	M	M	M	M	M	M	M
GNB 950	M	NA	M	M	M	M	M	M	M	M	M	M
GNB 951	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 953	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 954	M	M	M	M	P	M	M	M	M	M	M	M
GNB 956	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	M	NA
GNB 959	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 960	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	M	NA
GNB 962	M	M	M	M	M	M	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 964	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 965	M	M	M	M	M	M	M	M	M	M	M	M
GNB 968	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 973	M	M	M	M	M	M	M	M	M	M	M	M
GNB 974	M	P	P	NA	NA	M	NA	NA	NA	NA	NA	NA
GNB 975	NA	M	P	P	P	P	M	M	M	M	M	M
GNB 976	M	M	M	M	M	M	M	M	M	M	M	M
GNB 977	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 978	M	M	M	M	M	M	M	M	M	M	M	M
GNB 979	M	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 984	M	M	M	M	M	NA	M	NA	M	M	NA	NA
GNB 985	M	M	M	M	NA	NA	M	M	M	M	NA	M
GNB 986	M	M	M	M	M	NA	NA	M	M	NA	NA	NA
GNB 988	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 990	M	M	M	M	NA	NA	M	M	M	M	M	NA
GNB 993	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 994	M	M	M	M	P	M	M	M	M	M	M	M
GNB 995	M	M	M	M	M	M	M	M	M	M	M	M
GNB 996	M	M	M	M	P	P	M	M	M	P	M	M
GNB 997	M	M	M	M	M	M	M	M	NA	M	M	M
GNB 998	M	M	M	M	M	M	M	NA	M	M	M	M
GNB 1000	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1008	NA	M	M	M	NA	M	M	M	M	M	NA	M
GNB 1010	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1011	NA	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 1012	M	M	M	M	P	P	P	P	M	M	P	P
GNB 1013	M	M	M	M	NA	NA	M	NA	NA	NA	NA	NA
GNB 1014	M	M	M	M	NA	M	M	M	M	M	M	NA

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 1015	M	M	NA	NA	NA	NA	NA	NA	NA	M	NA	NA
GNB 1016	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1017	NA	M	M	M	M	M	M	M	M	M	M	M
GNB 1018	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 1019	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 1020	M	P	P	M	P	M	M	M	M	M	M	M
GNB 1021	M	P	P	M	P	M	M	M	M	M	P	M
GNB 1022	M	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GNB 1025	M	M	M	M	P	NA	M	M	M	M	NA	NA
GNB 1026	M	P	M	P	P	P	P	P	P	M	M	NA
GNB 1028	M	M	M	M	NA	NA	M	NA	NA	NA	NA	NA
GNB 1030	M	M	NA	NA	M	M	M	M	M	M	NA	M
GNB 1031	NA	NA	NA	M	NA	NA	M	P	M	M	P	P
GNB 1032	M	M	M	M	NA	M	M	M	M	P	M	M
GNB 1032	M	M	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 1033	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1036	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1038	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1039	M	M	M	M	NA	M	M	M	M	M	NA	M
GNB 1041	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1043	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1047	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1048	M	NA	M	M	NA	M	M	M	M	M	M	M
GNB 1049	NA	NA	NA	NA	NA	M	NA	NA	NA	NA	NA	NA
GNB 1051	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1054	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1057	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1058	NA	M	M	M	M	P	M	M	M	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 1059	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1060	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1061	M	M	M	P	P	P	M	M	M	M	M	M
GNB 1062	M	M	M	M	M	M	NA	M	M	NA	NA	M
GNB 1063	M	M	M	NA	P	M	NA	NA	NA	NA	M	M
GNB 1064	NA	M	M	M	M	M	M	M	M	NA	NA	M
GNB 1065	M	M	M	M	M	M	NA	M	M	M	M	M
GNB 1066	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1067	M	M	NA	M	M	M	M	M	M	M	M	M
GNB 1068	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1069	M	M	M	M	P	NA	M	M	NA	NA	M	NA
GNB 1070	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1071	M	P	M	P	P	M	M	P	P	P	M	M
GNB 1072	P	P	P	NA	NA	P	NA	NA	NA	NA	NA	P
GNB 1073	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1074	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1075	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1076	NA	M	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 1077	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1078	M	M	NA	NA	P	M	NA	NA	NA	NA	NA	P
GNB 1079	M	M	M	M	M	NA	M	M	NA	NA	NA	NA
GNB 1080	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1081	M	M	M	M	M	M	M	M	M	M	NA	M
GNB 1082	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1083	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1084	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1085	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1086	M	M	M	M	P	M	M	M	NA	M	M	M

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 1087	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1088	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1089	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1090	M	M	M	M	NA	M	M	M	NA	M	NA	M
GNB 1091	NA	NA	M	M	NA	M	M	M	M	M	M	M
GNB 1092	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1093	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1094	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1095	M	M	P	M	NA	M	P	M	M	M	M	M
GNB 1096	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1097	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1098	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1099	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1100	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1101	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1102	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 1103	M	M	M	M	M	M	M	NA	M	M	M	M
GNB 1104	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1105	M	M	NA	NA	M	M	M	M	M	M	NA	M
GNB 1106	M	M	NA	NA	M	M	M	M	M	M	NA	M
GNB 1107	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1108	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1109	NA	NA	M	NA	M	M	NA	NA	NA	NA	M	M
GNB 1110	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1111	M	M	NA	M	M	M	M	M	M	M	M	M
GNB 1112	M	M	M	NA	P	P	M	P	P	M	NA	M
GNB 1113	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1114	M	P	P	P	P	P	P	P	P	M	P	P

Marker ID	ICGS 44 × ICGS 76	ICGS 76 × CSMG 84-1	TAG 24 × ICGV 86031	TAG 24 × GPBD 4	TMV 2 × TxAG 6	ICG 1337 × JL 24	TG 26 × GPBD 4	TG 19 × GPBD 4	TG 49 × GPBD 4	GPBD 5 × GPBD 4	TAG 24 × R 9227	JL 24 × ICGV 86590
GNB 1115	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1116	NA	NA	M	M	M	M	M	M	M	M	M	M
GNB 1117	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1118	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1120	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1121	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1122	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1123	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1124	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1125	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 1126	M	M	NA	NA	NA	M	NA	NA	NA	NA	NA	M
GNB 1127	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1128	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1129	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1130	NA	M	M	M	M	P	M	M	M	NA	M	M
GNB 1131	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1132	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1133	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1134	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1135	NA	M	NA	NA	NA	M	M	M	M	M	M	M
GNB 1136	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1137	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1138	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1139	NA	M	M	M	NA	M	M	M	M	M	M	M
GNB 1140	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1141	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1142	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1143	NA	M	M	M	NA	M	M	M	M	M	M	M

Marker ID	ICGS 44 x ICGS 76	ICGS 76 x CSMG 84-1	TAG 24 x ICGV 86031	TAG 24 x GPBD 4	TMV 2 x TxAG 6	ICG 1337 x JL 24	TG 26 x GPBD 4	TG 19 x GPBD 4	TG 49 x GPBD 4	GPBD 5 x GPBD 4	TAG 24 x R 9227	JL 24 x ICGV 86590
GNB 1144	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1145	M	M	M	M	NA	M	M	M	M	M	M	M
GNB 1146	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1147	M	M	M	M	M	M	M	M	M	M	M	M
GNB 1148	M	M	M	M	P	M	M	M	M	M	NA	M
GNB 1149	M	NA	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 1150	M	M	NA	NA	M	M	NA	NA	NA	NA	NA	M
GNB 1151	M	M	M	M	P	M	M	M	M	M	M	M
GNB 1152	M	M	M	M	M	M	M	M	M	M	NA	NA

* M –Monomorphic
P – Polymorphic
NA – Not amplified

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