INHERITANCE OF SHOOT AND ROOT CHARACTERS AND MOLECULAR MARKERS IN CHICKPEA (*Cicer arietinum* L.)

By

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CERTIFICATE

Ms. K. Anupama has satisfactorily prosecuted the course of research and that the thesis entitled, **"Inheritance of shoot and root characters and molecular markers in chickpea (*Cicer arietinum* L.)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree in any University.

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CERTIFICATE

This is to certify that the thesis entitled, **"Inheritance of shoot and root characters and molecular markers in Chickpea (*Cicer arietinum* L.)"** submitted in partial fulfillment of the requirements for the degree of **'Doctor of Philosophy'** of the Acharya N.G.Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Ms. K. Anupama** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
BC_1P_1	F_1 x ICCV 2
BC_1P_2	F_1x JG 62
bp	Basepair
cc	Cubic centimeter
cDNA	Complementary DNA
cM	CentiMorgan
cm^2	Square centimeter
CTAB	Cetyl trimethyl ammonium bromide
°C	Degree celsius
DAF	DNA Amplification finger printing
df	Degrees of freedom
dATP	Deoxy adenosine 5'- triphosphate
dCTP	Deoxy cytosine 5'- triphosphate
dGTP	Deoxy guanidine 5'- triphosphate
DNA	Deoxyribose Nucleic Acid
dTTP	Deoxy thymidine 5'- triphosphate
EDTA	Ethylene diamine tetra acetic acid
et al.	And others
F_1	First filial generation
F_2	Second filial generation
F_3	Third filial generation
F_4	Fourth filial generation
F_7	Seventh filial generation
g	Gram
h	Hours
HCl	Hydrochloric acid
ISSR	Inter-Simple-Sequence-Repeat
>	Greater than

KCl	Potassium chloride
kg ha^{-1}	Kilograms per hectare
kg	Kilogram
LB	Luria-Bertani
m	Million
M	Molar
m ha	Million hectares
m	Meters
m.mho cm^{-1}	Milli mhos per centimeter
Mbp	Megabase pairs
Mg/mm	Milligram per millimeter
MgCl2	Magnesium chloride
$MgSO_4$	Magnesium sulphate
Min	Minute
ml	Milliliter
mM	Millimolar
µM	Micromolar
µg	Microgram
µl	Microliter
MP-PCR	Microsatellite Primed PCR
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanograms
NIL	Near Isogenic Lines
nM	Nanomolar
NS	Not significant
O.D.	Optical density
%	Per cent
P_1	Parent 1
P_2	Parent 2
PCR	Polymerase Chain Reaction

QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RILs	Random Recombinant Inbred Lines
RMMFP	Random Mixing of Microsatellite Flanking Primers
Rpm	Rotations per minute
s	Second
SAMPL	Selective Amplification of Microsatellite Polymorphic Length
SCAP	Sequence Characterised Amplified Regions
SE	Standard error
SSCP	Single Strand Confirmation Polymorphism
SSR	Simple Sequence Repeat
STMS	Sequence Tagged Microsatellite Sites
TE	Tris-EDTA
TBE	Tris Boriate-EDTA
TEMED	Tetramethyl ethylene diamine
U	Unit
UV	Ultra violet
V	Volts
v/v	Volume / volume
w/v	Weight / volume
χ^2	Chi-square

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Date: 1/05/02

(K.ANUPAMA)

DECLARATION

I, **K.ANUPAMA** hereby declare that the thesis entitled **" Inheritance of shoot and root characters and molecular markers in chickpea (*Cicer arietinum* L.)"**, submitted to Acharya N.G. Ranga Agricultural University for the degree of **'Doctor of Philosophy'** is a result of original research work done by me. It is further declared that thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

Gene action studies helps the plant breeder in selecting suitable breeding procedure for the improvement of the characters. Recently molecular breeding helped accelerating plant breeding in number of areas like disease resistance, insect resistance and quality characters. The objectives of this study were to investigate the inheritance of shoot and root characters and molecular markers and construction of linkage map in chickpea.

The material for investigation comprised of P_1, P_2, F_1, F_2, BC_1P_1, BC_1P_2, F_3, and 126 F_{10} generation random recombinant inbred lines (RILs) of the cross ICCV 2 and JG 62.The results of the present investigation are as follows.

- The mean values of F_1 for all the shoot and root characters studied were closer to the parent JG 62, indicating the presence of dominant alleles for all these characters in JG 62.
- There was sufficient variability between the parents for the characters studied for their effective utilization. All the shoot and root characters had high heritability estimates. Co-heritability estimates among most of the characters were significant.
- Days to first flower, days to first pod, days to maturity and number of nodes upto first flower are controlled by both additive and non-additive gene actions. Pod filling period and total reproductive period are governed by dominance gene action and epistatic gene action. Days to first flower, days to first pod, days to maturity, and podding duration are governed by duplicate epistasis and number of nodes up to first flower are governed by complimentary epistasis.
- Days to first flower days to first pod and days to maturity are governed by the same major gene (*efl-1*) through its pleoitropic effect and some other modifier genes.
- Number of nodes up to first flower is governed by complementary gene action. The two genes for number of nodes up to first flower were designated as *Nff-1* and

Nff-2. Therefore, the genotype for the parent ICCV 2 is *nff-1nff-1nff-2nff-2* and that for JG 62 is *Nff-1Nff-1Nff-2Nff-2*.

- Observations recorded on root length and root volume of the parents (ICCV 2 and JG 62) and 126 F_{10} RILs at the time of flowering of ICCV 2, JG 62 and at their own respective flowering time has shown that statistically significant variability for root traits exist at the flowering time and it is preferable to record measurements in chickpea at flowering time.

- Root length has additive and dominance x dominance gene action whereas only additive gene action was significant for root volume. Complementary epistasis operates for both the characters. Duplicate epistasis was found for leaf area, Shoot dry weight, total number of nodes upto flowering and complementary epistasis was found for root dry weight. Leaf let number was governed by a single gene and it is designated as L*ln*.

- Correlations between flowering and number of nodes up to first flower as well as between root length and root volume were high.

- Resistance to fusarium wilt is governed by two recessive genes under homozygous conditions. The digenic ratio of 9:6:1 obtained in F_2 generation was confirmed by the F_3 progeny and RIL population. The genotype of ICCV 2 is $h_1h_1h_2h_2h_3h_3$ and of JG 62 is $H_1H_1H_2H_2h_3h_3$.

- Seventy two percent of the molecular markers segregated in the expected Mendelian ratio (1: 1) and remaining twenty eight percent markers showed distorted segregation. Different marker classes exhibited varied segregation distortion. MP-PCRs had maximum segregation distortion (60%) and RAPDs had minimum segregation distortion (16%).

- Fifty-six markers out of 69 segregating markers formed nine linkage groups with seven morphological trait loci, four RAPD, one ISSR, 32 STMS, five MP-PCR and seven RMMFP loci covering 262.8 cM with an average distance of 4.7 cM between two consecutive markers.

- Linkage was observed for three important traits. Seed size locus was flanked by markers sa14 and TR 1, double podding was flanked by TR 1 and TA 14 and one of the two genes governing fusarium wilt resistance was linked to RMMFP marker ta36t146 at a distance of 18.3 cM.

- RMMFP, a new technique was found to be efficient in adding new markers. All the four sequenced polymorphic RMMFP loci had microsatellite. The microsatellite flanking sequences had high homology in three cases out of four sequenced markers.

In future for the saturation of chickpea intraspecific map either new STMS primers have to be designed or new techniques have to be developed which can detect the polymorphisms even at the intraspecific level and add more markers to get linkages with important genes.

INTRODUCTION

CHAPTER I

INTRODUCTION

Food legumes occupy a unique position in the Indian agriculture due to their high nutritive value and their ability to improve soil fertility. They form an indispensable constituent of the Indian diet. India is a major pulse growing country in the world sharing 28% area and 38% production (FAO, 2001).

Chickpea (*Cicer arietinum* L.), a self pollinated diploid (2n=2x=16), is the third most important pulse crop in the world after beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) with major production in South and West Asia and North Africa (Saxena, 1990 and Srivastava *et al.*, 1984). The Genus *Cicer* includes forty-three species (Vander Maesen, 1987), eight of which are annuals. *Cicer reticulatum* is considered as the probable ancestor of the cultivated chickpea. *Cicer* was first classified under *Viciae* but later considered as the tribe *Cicereae* (Kupicha, 1977 and Nozzolillo, 1985). It is used as a complement to cereal food and as snack food and sweets. Green fresh chickpeas are commonly consumed in Africa and Asia. Chickpea seeds have 21 to 31.5% protein and 2 to 5% oil. Protein digestibility of chickpea is high and it is low in anti nutritional factors (Saxena, 1990). The green plant parts and husk and broken bits of chickpea after harvest are used as a cattle feed. Germinating seeds are prophylactic against scurvy disease. In India chickpea was grown on 8.4 m hectares with a production of 6.6 m tons and with the productivity of 800 kg ha^{-1} in 1999/2000 (Economic Intelligence Service, 2000). Rajasthan ranks 1^{st} position in area with 2.8 m ha. Madhya Pradesh in production with 2.4 m tons and Himachal Pradesh in productivity with 1100 kg ha^{-1}. In Andhra Pradesh, chickpea is grown over an area of 0.13 m ha. that accounts to

around 0.9% of the total cropped area. The production is 84 thousand tons and productivity is 610 kg ha^{-1} (Economic Intelligence Service, 2000).

Kabuli and desi are the two main types of chickpeas. Desi or indigenous type is generally of small size, angular shaped, fibrous and variously coloured, while the kabuli is characterized by its large seed size, ram's head shape and cream coloured seeds with low percentage of fibre. Desi type accounts for 80-85% of the world chickpea production.

Low yield in major chickpea growing countries is probably due to biotic stresses (ascochyta blight and fusarium wilt), abiotic stresses (moisture stress, heat, cold) and also due to the use of marginal lands and poor nutritional status of the soils.

Fusarium wilt caused by *Fusarium oxysporum* Schlecht emd. : Fr. f. sp *.ciceris* (Padwick) Matuo & K. Sato is more important than aschochyta blight (Haware *et al.*, 1990 and van Rheenen, 1991). This disease causes 10% annual losses in India (Singh and Dahiya, 1973). Races 1, 2, 3 and 4 are reported from India (Haware and Nene, 1987) and 0, 5 and 6 from Spain (Jimenez-Diaz *et al.*, 1989b). Sources of resistance to Fusarium wilt were identified in chickpea germplasm. Development of wilt resistant varieties of chickpea is the most important method to control this disease since it has not been controlled under field conditions by chemicals or by crop rotation. The presence of different races further aggravates the problem.

Time of flowering is of great interest because it is related with crop adaptation. Reducing the crop duration can reduce most of the losses occurring during the flowering

and podding (Kumar *et al.*, 1996). Therefore genetic manipulation of flowering, podding and maturity times are fundamental to crop improvement.

Chickpea is one of the deep rooting species among the cool season food legumes. The rooting depth is about 1.20 to 1.35 m with large environmental variation. As chickpea is grown under residual soil moisture under rainfed conditions it is exposed to varying degrees of terminal drought stress and under this situation root mass and development pattern play a significant role in yield determination. The yield loss due to terminal stress is estimated to be 35 to 50% (Saxena *et al.*, 1993a). A deep root system seems to be related to yield under drought stress. Early developed prolific root system under water-limited environments is needed in chickpea. The development of chickpea with extensive root system is essential for a better adaptation for limited soil moisture. Singh *et al.* (1988) reported a large variation for root characters in chickpea. ICC 4958 which was registered as the most drought resistant germplasm has a large root system (Saxena *et al.*, 1993b).

The productivity of chickpea has not improved significantly by the use of conventional breeding methods. Recently molecular markers helped accelerating plant breeding in a number of areas like disease resistance, insect resistance and quality characters (Frisch and Melchinger, 2001 and Tullu, 1997). The DNA marker maps facilitate marker-assisted selection, cloning of resistance genes and mapping of quantitative trait loci (QTLs) of agronomic importance (Young, 1996 and Santra *et al.*, 2000).

Mapping and sequencing of plant genome helps in elucidating gene functions, gene regulation and their expression. Linkage maps based on molecular markers have

been constructed in many crops (O' Brien, 1993) and have been utilized to determine the gene number for particular traits and gene tagging (Paterson *et al.*, 1995).

There is a need to develop DNA marker map of chickpea in order to use in marker-assisted selection and cloning of important genes. Chickpea has moderate sized genome of around 750 Mbp (Arumuganathan and Earle, 1991). Linkage analysis and mapping genes in cultivated chickpea is limited. The reason being the less genetic variation within cultivated chickpea (Ahmad and Slinkard, 1992; Udupa *et al.*, 1993 and Labdi *et al.*, 1995) due to which researchers have mostly used interspecific crosses for the construction of the map with molecular and morphological loci (Simon and Muehlbauer, 1997; Tullu *et al.*, 1998 and Santra *et al.*, 2000). But Mayer *et al.* (1997) were able to map a gene for resistance to fusarium wilt 1 using an intra specific cross.

Most of the mapping studies with molecular markers have used either F_2 populations or backcrosses. But recombinant inbred lines (RILs) that are developed by single seed descent method from F_2 population have several advantages over other populations for genetic mapping. RILs are homozygous and, hence, they can be evaluated in different environments, which is useful in the analysis of quantitative traits as it helps in the accurate assessment of the genetic component of variance. For the RILs developed by selfing, there is a 2 fold or 4-fold increase in the recombination frequency between two linked markers (Taylor, 1978). High-resolution maps and more accurate map distances are obtained with RILs than with F_2 population (Burr and Burr, 1991). Both the dominant and co-dominant markers give the same information in RIL population in contrast to that in F_2 population which allows the use of both dominant such as RAPD, AFLP and co-dominant markers such as RFLP and STMS.

Knowledge of gene action helps in selection of the parents for the use in hybridization programme and also in the choice of appropriate breeding procedures for genetic improvement of various characters. Hence the knowledge of gene action is essential to the plant breeder for starting a judicious breeding programme. Construction of linkage maps based on intraspecific cross is also very important. This is because resistance traits to diseases like Ascochyta are found only in *C.arietinum* gene pool, but not in any of the crossable wild *Cicer* species (Huttel *et al.*, 1999).

Considering the aforesaid aspects, the present investigation was carried out with the following objectives to

1. study the inheritance of shoot and root characters.
2. estimate their heritability and co-heritability.
3. study the inheritance of molecular markers (RAPD and STMS).
4. determine linkages among the morphological characters and molecular markers and construct an intraspecific map for chickpea.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Review of literature pertaining to inheritance of flowering and maturity time, inheritance of nodes up to first flower, inheritance of resistance to fusarium wilt (race 1), inheritance of root characters, heritability of different characters in chickpea, correlations, inheritance of molecular markers and genetic linkage map are presented in this chapter.

2.1 Inheritance of flowering and maturity time

Knowledge of plant characteristics (both qualitative and quantitative) is essential for planning an effective breeding programme. This is useful in selection of individuals with adaptation to different agro-ecological zones. Measurement of genetic variability and understanding of inheritance of quantitative characters is of prime importance in chickpea to formulate a sound crop improvement programme.

Time of flowering plays an important role when the growing season is restricted by climatic factors like drought and high temperature. Losses in chickpea production either due to biotic or abiotic stresses mostly occur during flowering and podding i.e. the later part of the cropping season (Kumar *et al.*, 1996). Further, duration of the flowering period is a major yield determinant because of the indeterminate growth habit in chickpea (Bonfil and Pinthus, 1995). Therefore, the development of short duration varieties that avoid end-of-the-season drought will increase the chickpea productivity. For this reason, genetic analysis of flowering time, podding and maturity are very important for chickpea improvement.

Kidambi *et al.* (1988) reported additive and duplicate epistasis for days to first flower in chickpea and dominance and duplicate epistasis for days to maturity in three

crosses of chickpea. Malhotra *et al.* (1983) reported similar result and proposed pedigree and bulk methods for improving these characters.

Salimath and Bahl (1989) reported that additive and non-additive gene action are important, the former being predominant for days to first flower and the latter for days to maturity.

Jha *et al.* (1997) reported additive gene action for days to first flower and dominant gene action for days to maturity from line x tester analysis involving six lines and four testers in chickpea.

Or *et al.* (1999) obtained 3:1 ratio of late flowering: early flowering types in the F_2 population of the cross Hadas x ICC 5810. Hadas is a late flowering chickpea genotype and ICC 5810 is early flowering chickpea genotype. F_1's were intermediate between the parents. Based on this simple inheritance pattern, they suggested that the early flowering trait might be easily introduced in to popular late flowering genetic backgrounds.

Kumar and Rheenen (2000) studied the number of days taken for the appearance of first flower in 66 RILs developed from a cross of an extra short duration chickpea variety ICCV 2 and a medium duration variety JG 62. The time of flowering of recombinant inbred lines (RILs) showed two nearly equal peaks, one corresponding to ICCV 2 and other with JG 62. This indicated that the difference in the time of flowering between ICCV 2 and JG 62 was governed by a single major gene, late flowering being dominant to early flowering. They designated single recessive gene for the time of flowering as *efl-1*.

The literature on days to first flower, podding and maturity duration is meager in chickpea. Hence, information on other crops is presented hereunder.

Watts *et al.* (1970) reported additive gene action for flowering time in peas with dominance for late flowering characteristics.

Kumar and Das (1975) studied genetics of flowering in peas using diallel analysis and reported preponderance of additive action for both the traits along with the substantial dominance effect for maturity.

Gil and Martin (1988) studied the genetics of flowering and maturity times in *Vicia sativa*. In all the crosses the means of the F_1s were intermediate between the parental means and were similar to the corresponding F_2 and F_3 means and the means of the backcrosses were between their F_1's and corresponding recurrent parent. Additive gene action was found to be significant for the time of flowering.

Parmar and Godawat (1990) also reported additive gene action for flowering in nine crosses and maturity times in eight out of ten crosses in peas involving seven parents. But in some crosses dupicate type of epistasis was also reported. Crosses 6587-1 x R-1038, 6587-1 x R-177 for flowering and Bonneville x A.F, A.F x R-839, 6587-1 x r-1038 for maturity were identified as promising ones.

Sarker *et al.* (1999) reported monogenic inheritance for days to first flower based on the segregation pattern of four crosses and polygenic gene action due to continous distribution among F_2 segregants in other crosses of lentil.

2.2 Inheritance of nodes upto first flower

The number of nodes upto first flower is fixed for each genotype (Sachs, 1999). In *Pisum*, the number of nodes produced before flowering ranges from four in the early maturing varieties to hundred in the late maturing varieties. The node at which the first flower occur is influenced by photoperiod and stress. Under stress conditions, the flowering occurs at the lower nodes. The number of nodes to flowering is an indirect measure of earliness as it is positively correlated with the number of days to first flower (Sachs, 1999). Collins and Wilson (1974) studied the relationship between the node of

flowering and flowering time and reported that node of flowering is a useful index of development to estimate the time of flower initiation.

In *Pisum*, Murfet (1971a and 1975) reported multiple alleles governing the node number at which the first flower appears, thus having the basic role in determining the minimum length of the vegetative period. The multiple alleles identified are $Lf^{d}>Lf>lf>lf^{a}$. The expression of these multiple alleles is influenced by photoperiod and vernalization temperatures. The *'lf* locus is one among the other three (*E*, *Sn*, *hr*) flowering genes reported by Murfet (1975).

2.3 Inheritance of resistance to *Fusarium* wilt

Chickpea wilt was first reported from India by Butler (1918). Later, Narasimhan (1929), Mc Rae (1932) and Prasad and Padwick (1939) reported that wilt is caused by *Fusarium* species. Padwick (1940) identified the causal organism as *Fusarium orthoceras* var *ciceri*, which was renamed as *Fusarium oxysporium f.sp.ciceri* by Chatopadhyaya and Sen Gupta (1967) following the classification of Snyder and Hansen (1940). This disease is estimated to cause a 10% loss of annual yield (Singh and Dahiya, 1973).

Wilt is a typical vascular disease causing xylem browning or blackening, affecting the crop at all stages. Generally wilting at earlier stages causes greater loss than that at later stages. A highly susceptible cultivar, under favorable conditions, may wilt within 10 days of sowing in wilt infested fields where as a tolerant cultivar shows general yellowing and drying of lower leaves and wilting at a later stage, causing less yield damage. Around seven races of *Fusarium oxysporium* f.sp. *ciceri* have been reported in India, Spain and the United States, out of which four races (1-4) are prevalent in India (Haware and Nene, 1982). Over 150 resistant sources are identified, of which some are resistant to more than one race. (Nene *et al.*, 1981b and Haware *et al.*, 1992).

Dependable sources of resistance and genetics of resistance are necessary for the development of resistant varieties.

Ayyar and Iyer (1936) for the first time studied the mode of inheritance of reaction to wilt in *Cicer arietinum*. They indicated that a single gene with incomplete dominance conferred resistance to chickpea wilt. Pathak *et al.* (1975) reported recessive nature of resistance with a single gene difference from the crosses 315 with susceptible varieties T2 and T3.

Tiwari (1981) observed a simple monogenic control with susceptibility as dominant and resistance as recessive in six crosses of JG 315 as resistant parent. In three crosses where H 355 was used as resistant parent monogenic ratio could not find a good fit. They proposed backcross method for incorporation of wilt resistance in susceptible plants. Sindhu *et al.* (1983) reported single recessive gene inheritance and proposed the gene symbol (*rfo*) for the recessive gene and (*Rfo*) for its dominant allele. Haware *et al.* (1992) also reported that single recessive gene is responsible for resistance.

Kumar and Haware (1982) observed single gene inheritance in crosses involving WR 315 and CPS 1 (resistant) with C 104 (susceptible). However, in crosses when JG 62 was used as susceptible parent, the proportion of susceptible lines was greater than the expected for single recessive gene. They suggested the involvement of multiple genes.

The ratio obtained in F_2 population by Phillips (1983) indicated monogenic recessive inheritance but was not confirmed by the segregation in the F_3 and F_4 generations. Lopez (1974) suggested that resistance was governed by two pairs of recessive genes in crosses involving 19 different strains of chickpea.

Upadhyaya *et al.* (1983a and 1983b) reported two recessive gene control of resistance to fusarium wilt race-1. They proposed that two genes governed resistance in WR 315 x JG 62 and that both must be in the homozygous recessive state for complete resistance. These genes were named as h_1 and h_2. Homozygous recessive alleles at either

of the loci, conferred delayed wilting. Thus, when a late wilter (homozygous recessive at one locus) was crossed with the resistant line (homozygous recessive at both loci) the segregation pattern was monogenic but when an early wilter (homozygous dominant at both the loci) was crossed with the resistant line, segregation pattern was digenic.

Singh *et al.* (1987) obtained a monogenic ratio from the cross K 850 with JG 62 and digenic ratio from the cross K 850 with C 104. This revealed that K 850 carried a recessive allele for resistance at a locus different and independent of that carried by C 104 and recessive alleles at both loci together confer complete resistance.

Singh *et al.* (1988) did not observe any linkage between the locus controlling the number of flowers per node in JG 62 and locus delaying wilt in C 104. However, they reported linkage between these two loci in the cross of K 850 with JG 62 due to low frequency of double flowered wilt resistant segregants than expected from segregation from two or three loci.

Singh *et al.* (1991) studied F_1 and F_2 generations of the crosses H 208 x K 850 and H 208 x C 104. They indicated that cultivar H 208 carried a dominant allele for late wilting to race 1 of *Fusarium oxysporium* f.sp.*ciceri* at a locus different from the two reported earlier. Their results further indicated that the dominant allele in H 208 complemented the recessive alleles at either of the other two loci to confer complete resistance.

Dikshit and Singh (1994) obtained monogenic inheritance from the cross JG 74 x JG 62 and digenic inheritance from the cross PG 114 x JG 62 and suggested the use of bulk population breeding and backcross breeding for the development of resistant varieties.

The possible genetic constitution in respect to wilt (race 1) resistance and the wilting characteristics of the chickpea cultivars reported by Singh *et al.* (1987a) are as follows:

Table 1: The genetic constitution and wilt reactions of chickpea accessions

Cultivar	Genetic constitution	Wilt reaction
JG 62	$H_1H_1H_2H_2h_3h_3$	Early wilting
K 850	$h_1h_1H_2H_2h_3h_3$	Late wilting
C 104	$H_1H_1h_2h_2h_3h_3$	Late wilting
H 208	$H_1H_1H_2H_2H_3H_3$	Late wilting
WR 315	$h_1h_1h_2h_2h_3h_3$	Resistant
CPS 1	$h_1h_1h_2h_2h_3h_3$	Resistant
P 436 2	$h_1h_1h_2h_2h_3h_3$	Resistant
BG 212	$h_1h_1h_2h_2h_3h_3$	Resistant
JG 74	$h_1h_1h_2h_2h_3h_3$	Resistant

Gumber *et al.* (1995) studied the inheritance of resistance to fusarium wilt (race 2) in chickpea in the cross P 165 x C 104. The number of early wilting and late wilting plants fitted well to 13:3 ratio suggesting that two genes govern the resistance to race 2 of fusarium wilt. One of these must be homozygous recessive and the other dominant for complete resistance. Studies of Kumar (1998) with a set of three crosses i.e., WR 315 x C 104 (resistant x susceptible), WR 315x K 850 (resistant x tolerant) and K 850 x GW 5\7 (tolerant x tolerant) indicated the involvement of the three loci (two recessive and one dominant alleles), homozygous recessive forms at the first two loci confer resistance and a dominant allele at the third locus complements the dominant allele at the other two loci to confer tolerance.

2.4 Inheritance of root characters

The root system of a crop plays an important role in yield determination especially under residual soil moisture conditions. Chickpea is a deep rooting species. Its rooting

depth is normally about 1.20-1.35 m. However, environmental variation greatly affects its rooting depth. A large genotypic variability at the seedling stage was observed at ICRISAT in sand culture grown plants. Singh *et al.* (1988) observed significant variation for root volume, root dry mass, root-shoot ratio, shoot mass at 45 days after sowing among forty diverse genotypes. Even though great genetic variability for root characters is available in this species, the information on the inheritance is meager.

Waldia *et al.* (1992) reported additive, dominance and epistatic gene action for speed of radicle emergence in three crosses (H86-92 x Bhim, H 86-92 x Arjun, E100y'm' x ICCV 2) of chickpea, whereas only additive gene action was reported for root length excepting in one cross (H 86-92 x Bhim) where additive x dominance gene action was significant. They suggested the involvement of two genes with additive epistatic interaction for speed of the radicle emergence. Waldia *et al.* (1993) assigned gene symbols *sre1* and *sre2* to the two genes and reported preponderance of additive gene action for root length. High significant correlations were obtained between radicle emergence and root length in F_2 population of the crosses H 86-92 x Bhim, H 86-92 x Arjun and PROTEIN-3.L.N.2 x H 86-92.

Additive and dominance effects were reported for root length, root number, root thickness and root to shoot ratio in rice by Armenta-soto *et al.* (1983).

Saleh and Gritton (1994) studied in four crosses the genetic control of root weight, root volume, and root to shoot ratio in peas at flowering. Additive and dominance effects were important for root weight and volume in all the crosses. However, epistatic gene actions were important in two crosses. The nature of inheritance of root to shoot ratio was unclear. Root weight (0.41 to 0.81) and root volume (0.44 to 0.77) had moderate to high broad sense heritability. Similar results were also reported in cotton (Eirsa *et al.*, 1983) where additive, dominance and additive x additive epistatic gene actions were significant for root length and relative root weight (mg/mm length).

Equal contribution of additive and dominance gene actions for the expression of root length, root volume, root thickness, thick root number, root dry weight and root length density was reported by Ekanayake *et al.* (1995) in rice.

The results of all the above studies show that these characters (root characters) are greatly influenced by the environment and they are likely to be governed by multiple genes.

The review on the gene actions of various characters also indicates that the results varied with the material used for the investigation. The inferences drawn are valid for the concerned genetic material studied and hence, cannot be generalised.

2.5 Heritability of various characters

Heritability is the ratio of genetic variance to the phenotypic variance (Singh, 1997) expressed in percent. It is a good index of transmission of characters from parents to offspring (Falconer, 1989). The knowledge of heritability helps the plant breeder in predicting the behaviour of the succeeding generations and making desirable selections. It is dependent upon the variability present in the material and how it is affected by the environment. Depending on the component of variance used as the numerator in the calculation, the heritability is called narrow sense or broad sense heritability (Singh and Narayanan, 1997).

Athwal and Gill (1964) studied the heritability and coheritability estimates from crosses between improved variety PB 7 and three diverse varieties G.G.Bijapur, Bara chana and C 49. The broad sense heritability values were in general higher than their corresponding narrow sense heritability values. These were lowest for yield in all the crosses. Heritability values of seed/pod in PB 7 x Bara chana and flowering time in PB 7 x Bara chana and in PB 7 x G.G.Bijapur were high. Coheritability values of yield with characters like flowering time and seed/pod were almost double when compared to the

heritability value of yield alone. Chandra (1968) reported high heritability values for pod setting percentage, flowering duration, plant height, days to flower and primary branches.

Eser (1976) reported 84% heritability for pod length, 88 % for 100 seed weight, 30% for seeds/pod, 25% for pods/plant and 13% for seed yield. Gupta (1976) reported high heritability for ascorbic acid (75 to 91%), medium for 100 seed weight (59%) and low for harvest index (31%) and seed yield (30%).

Rastogi and Singh (1977) reported medium to high heritability value for days to first flower (50%-98%) and low to high for days to maturity (30% to 84%). Narrow sense heritability for days to first flower was high (96%) and for days to maturity was medium (33%)

Pandey and Tiwari (1983) studied the narrow sense heritability for various chartacters in five chickpea crosses. Heritability values for days to flowering, maturity and seed size were high in all the crosses.

Sharma and Maloo (1988) reported high heritability value with moderate genetic advance for days to flower.

High heritability (71%) with low genetic advance (8.7%) was reported for 50% flowering and also for time to maturity (heritability 65% and genetic advance 9.6%) by Mishra (1991) and Sharma *et al.* (1990) for flowering and maturity time.

Rao *et al.* (1994) reported high broad sense heritability value for days to first flower (71%) and days to maturity (66%) with medium to low genetic advance values. Samuel and Jagdev (1996) reported high heritability value (90.1%) for 50% flowering. Low narrow sense heritability (20%) for days to first flowering and high narrow sense heritability (70%) for days to maturity were reported by Kidambi *et al.* (1988).

In contrast to the low genetic advance values reported for days to flower, Govil and Jitendra (1989) reported high genetic advance with high heritability values for days to flower from the study of 45 varieties representing twenty countries.

Pundir *et al.* (1991) and Katiyar and Katiyar (1994) found high heritability with high genetic advance for leaf size and concluded additive genetic effect for the character.

Katiyar and Katiyar (1994) reported high heritability for leaf weight per plant and also for specific leaf weight.

Sabaghpour (2000) reported very high narrow sense heritability for 100 seed weight, leaf weight and specific leaf weight and moderate heritability for seed yield per plant.

Low heritability and genetic advance values were observed for root volume, root dry mass and root to shoot ratio in a study involving forty genotypes of chickpea under field conditions. (Singh *et al.*, 1988)

Saleh and Gritton (1994) studied the heritability of root characters in peas at flowering. Narrow sense heritability values ranged from 0.18 to 0.91 for root weight, 0.13-1.0 for root volume and 0.4 for root to shoot weight ratio. The broad sense heritability values were moderate to high for all the characters, thus indicating the presence of relatively high genetic variance for these traits.

Ekanayake *et al.* (1995) reported intermediate (0.55) narrow sense heritability for root volume in rice. Heritability estimates for high root length and number of thick roots were low to intermediate ranging from 0.33 to 0.55. They were relatively higher for root dry weight, root thickness and root length density (0.5 to 0.9).

2.6 Coheritability

Coheritability refers to the joint transmission of different character pairs. It helps in improving selection efficiency as it permits the study of simultaneous changes in

different characters (Srivastava and Jain, 1994). It deals with simultaneous inheritance of the characters (Singh and Narayanan, 1997) that takes both genotypic and phenotypic covariances in to account.

Pardhasaradhy and Medhi (1983) found high coheritability of root length in radish with all other characters including root diameter.

Misra *et al.* (1992) studied the coheritability of economic yield with different components. Number of pods per plant showed maximum coheritability with economic yield (0.73) followed by harvest index (0.70), number of secondary branches per plant (0.54) and biological yield per plant (0.43). Moderate estimates of coheritability were observed for number of primary branches per plant with seed yield (0.39) for plant spread (0.37) and pod bearing length (0.20).

Srivastava and Jain (1994) reported high coheritability estimates of biological yield, number of pods per plant, harvest index, and duration of reproductive phase in soybean with seed yield.

2.7 Heterosis

Heterosis is the superiority in the performance of the hybrid over both the parents. Commercial exploitation of heterosis in crop plants is regarded as a major break through in the realm of plant breeding. It has lead to considerable yield improvement of several cereals and other crops (Rai, 1979). In chickpea, the first report of hybrid was reported by Pal (1945) and later heterosis was demonstrated by Ramanujam *et al.* (1964).

Pal (1945) did not find heterosis for plant height, number of branches for plant, time of flowering and germination rate. But heterosis was reported by Ramanujam *et al.* (1964) for various characters. Since then heterosis was reported by various scientists (Deshmukh and Bhapkar, 1982; Bahl and Kumar, 1989; Shinde and Deshmukh, 1990;

Mandal, 1992; Katiyar and Katiyar, 1993 and Kamatar *et al.*, 1996) for various characters in chickpea.

2.8 Correlation coefficients

Eirsa *et al.* (1983) reported significant correlation of relative root weight with dry weight of root, percent dry weight of root and percent dry weight of plants in cotton. Their studies indicated significant negative correlation between relative root weight and root length and significant positive correlation between root length and dry root weight and there was no correlation between root length and seed weight.

Singh *et al.* (1988) found high positive correlation between root volume and dry root mass in chickpea and suggested that root volume can be used for screening purposes in lieu of dry root mass.

Waldia *et al.* (1993) observed positive correlation between speed of radicle emergence and root length in F_2 population i.e., the longer roots were associated with higher number of days taken for radicle emergence. They therefore suggested that the root development could be predicted on the basis of speed of radicle emergence.

Saleh and Gritton (1994) found a high correlation between root weight and root volume (0.83 to 0.96) in peas. Correlations of root weight and root volume with all the shoot characters measured were in general positive. Number of days to first flower had positive correlation with root weight and root volume in three crosses and negative correlation in one cross.

In rice Ekanayake *et al.* (1995) observed large positive correlation of dry root weight with root volume, root length, number of thick roots and root length density. They reported a strong negative correlation between root volume and root thickness. The shoot characters were positively correlated with the root characters.

2.9 Inheritance of molecular markers

Any characteristic that is useful to distinguish the plant varieties is known as a marker. These genetic markers are useful in identifying traits and in the construction of genetic maps. These linkage maps help the plant breeder to identify, manipulate and complement traits to their maximum advantage. In the genetic maps, the markers are arranged in a linear order in each linkage group based upon the recombination frequency between the markers. Greater the recombination frequency, greater is the relative distance between markers in the linkage groups.

The markers are broadly classified into morphological, biochemical and molecular markers.

2.9.1 Morphological markers

The morphological markers are visually observable characters, which differ between the selected parents. Characters such as colour, shape, size, height and number are some morphological markers. Simply inherited important morphological traits useful for genetic study in chickpea are for example type of leaf, flower colour, podding type (Muehlbauer and Singh, 1987).

2.9.2 Biochemical markers

Isozymes are the biochemical markers, which are various forms of the same enzyme differing in their electrophoretic mobility. Markert and Moller (1959) studied isozyme analysis for the first time. The alternate products known as allozymes encode these variant electrophoromorphs. Isozyme markers can be used to assess variability in plant population to develop isozyme fingerprints to identify cultivars and to locate and tag disease resistant genes (Weeden *et al.*, 1988). In general these markers have not been found very useful.

2.9.3 Molecular markers

DNA markers are ubiquitous, innumerable, discrete, nondeleterious, inherited by Mendelian laws, unaffected by environment and free from epistatic interactions (Beckman and Soller, 1986 and Tanksley *et al.*, 1989).

A major break through occurred when it was realised that genetic maps could be constructed using pieces of chromosomal DNA as direct markers for segregation pattern of chromosomal segments. (Botstein *et al.*, 1980)

Jeffreys *et al.* (1985) for the first time introduced the term DNA fingerprinting which refers to any approach of visualising DNA polymorphisms either by hybridization or polymerase chain reaction. In recent years various modifications of the basic term have appeared and other terms 'DNA profiling' and 'DNA typing' have been introduced.

A number of molecular markers have been designed and main types are mentioned briefly.

2.9.3.1 Restriction Fragment Length Polymorphism (RFLP)

It is a classical hybridization based finger printing and one of the first techniques used widely to detect variation (Botstein *et al.*, 1980). The variation in the restricted fragment length is due to the mutations in the DNA which could be a single base pair change or a DNA rearrangement. The RFLP analysis involves digestion of the DNA by a restriction enzyme, separation of the restriction fragments by agarose gel electrophoresis, transfer of the separated fragments from the gel to nitrocellulose filter by southern blotting, hybridization of the fragments with a radioactively labeled cloned probe and scoring of RFLPs using auto radiograms. Only hybridized fragments will be visible. Complementary DNA and random genomic libraries are used as probes in RFLP. cDNA clones recognize more polymorphism than random genomic clones (Havey and Muehlbauer, 1989). These RFLP markers are highly reproducible, are simple and have

codominance inheritance. However, the process is time consuming and requires radioactive labeled probes.

RFLPs have been used for the construction of linkage maps (Xu *et.al.*, 1991; Kiss *et al.*, 1993; Mc Couch *et al.*, 1988 and Helentjaris *et al.*, 1986) and gene tagging (Young *et al.*, 1988) in many crops. Besides this, RFLP can also be used for DNA finger printing, variety identification, and in determining levels of genotypic diversity and phylogenetic relationships.

2.9.3.2 Random Amplified Polymorphic DNA (RAPD)

RAPD assay, a PCR-based molecular marker technique, was developed simultaneously but independently by Williams *et al.* (1990) at Dupant Co and Welsh and Mc Clelland (1990) at the California Institute of Biological Research. The former used it for genomic mapping and the latter for genomic finger printing and as Arbitrarily Primed Polymerase Chain Reaction (AP-PCR).

This technique is based on the amplification of random segment with oligonucleotide primers or arbitrary nucleotide sequence. During the PCR reaction, the primer binds to the DNA at two different sites on opposite strands of the DNA template. A discrete DNA product is produced only when the priming sites are within the amplification distance of each other (200-2000 bp).

The amplification is dependent on the sequence of the primer and the DNA template. The primers are generated with > 50% G+C to ensure efficient annealing. DNA polymorphism among individuals of the same population could be detected due to mismatch at one or both priming sites or due to insertions or deletions within the amplified region (Williams *et al.*, 1990 and Rafalski *et al.*, 1994). RAPDs are dominant markers that can detect the presence of single allele at a locus.

The RAPDs are useful for cultivar identification, genetic mapping (Williams *et al.*, 1990) and phylogenetic pedigree and linkage analysis. RAPDs can also facilitate gene transfer via backcrossing to recurrent parent. A RAPD marker tightly linked to the gene of interest could facilitate the selection process by reducing the need to test the progeny.

The major drawback of RAPDs is their lack of reproducibility. This problem arises due to the lack of consistency in reaction conditions. Difficulty in assay reproducibility could be overcome by eliminating the variation in the DNA concentration, taking care to ensure consistent reaction conditions and thermal profile during amplification.

2.9.3.3 Amplified Fragment Length Polymorphism (AFLP)

It is a combination of RFLP and PCR amplification. This approach was developed by a private company Keygene in Netherlands led by Dr.Marc.Zabeau, which holds the patent for this technology. After the DNA is digested with restriction enzymes, a subset of fragments representing many loci are selected for PCR amplification and subsequent visualisation. This technique is also called as selective restriction fragment amplification (Zabeau, 1993). It is a highly sensitive method for detecting polymorphisms throughout the genome and is becoming increasingly popular.

2.9.3.4 Minisatellites or VNTRs

These hypervariable markers detects high levels of restriction fragment length polymorphisms due to tandem repeat arrays in the genome. Minisatellite markers were discovered in humans by Jeffreys *et al.* (1985). The repeat unit is 9-65 bp long, reiterated 10-300 times at each locus. There may be at least 1000 minisatellite loci in the human genome. The variation in the number of repeat units can be detected after DNA digestion with enzymes that do not cut within the repeat units but outside the minisatellite array.

Minisatellite markers have been used for a variety of purposes including mapping where they have been particularly useful because of their high polymorphic information content (Jeffreys *et al.*, 1985).

2.9.3.5 DNA markers based on microsatellites

DNA sequences with short repeated motifs (less than six bp) are termed as microsatellites. (Litt and Luty, 1989), simple sequence repeats (SSRs) by (Jacob *et al.*, 1995) or short tandom repeat (STRs) by Edwards *et al.* (1996). These microsatellites are abundant and randomlly distributed in all eukaryotic nuclear DNA examined (Tautz and Renz, 1984 and Gupta *et al.*, 1996). Frequency of microsatellites vary significantly among different organisms (Wang *et al.*, 1994; Gupta *et al.*, 1996 and Weising *et al.*, 1998). For instance, human genome is estimated to contain on an average ten fold more satellites than plant genome (Powell *et al.*, 1996a). The two most common dinucleotide repeats found in different crops are (AC)n and (GA)n. Trinucleaotide and tetranucleotides also occur in plant genomes, The most frequent of them being (AAG)n and (AAT)n (Gupta *et al.*, 1996). They are detected within and adjacent to the coding regions of the genes (Morgante and Oliver, 1993). Microsatellites are more useful than minisatellites as they are too long to allow amplification.

Flourescence insitu hybridisation (FISH) and in-gel hybridization in a variety of plant system suggested clustering of microsatellites around centromeric region (Schmidt and Heslop-Harrison, 1996). FISH insitu hybridization with $(A)_{16}$, $(CA)_8$, $(TA)_9$, $(AAC)_5$ and $(GATA)_4$ probes in chickpea revealed the presence of large arrays of SSRs in both eu- and heterochromatic fractions of the chickpea genome. CA repeats are largely confined to the centromeric regions while $(GATA)_4$ are unevenly distributed with major sites around centromere, $(A)_{16}$, $(AAC)_5$ and $(TA)_9$ are dispersed and predominantly euchromatic (Gortner *et al.*, 1998). while genetic and physical mapping suggested the

uniform distribution of microsatellites (Roder *et al.*, 1998a and 1998b). Therefore, more refined techniques are needed to study the organization of microsatellites.

Polymorphism at a given microsatellite locus in a species is due to variation in the length of SSR, and is believed to originate *in vivo* by the polymerase slippage during DNA replication (Levinson and Gutman, 1987). Thus slippage ultimately leads to the increase or decrease in the number of repeats. This slippage model is supported by *in vitro* experiments (Schlottere and Tautz, 1992) and by the studies on mutations (Weber and Wong, 1993 and Strand *et al.*, 1993). This change is shown to be directional (Rubinzstein *et al.*, 1995; Ellegren *et al.*, 1995; Amos *et al.*, 1996 and Primmer *et al.*, 1996).

Weising *et al.* (1992) reported the presence of repetitive motifs [$(GACA)_4$, $(GATA)_4$, $(GTG)_5$, $(CA)_8$, $(TCC)_5$, $(GGAT)_4$] in different accessions chickpea genome. Among all these repetitive motifs, $(GTG)_5$ has given species specific banding pattern.

Sharma *et al.* (1995) observed polymorphism with thirty-eight different di-, tri- and tetra nucleotide repeat motifs in the chickpea genome. They reported the presence of these simple tandom repeats in highly methylated regions of the chickpea genome, which are presumed to be transcriptionally silent.

2.9.3.5.1 Sequence Tagged Microsatellite Sites (STMS)

These are also known as Simple Sequence Repeat Polymorphisms. Hybridization based approach and PCR based approaches are used to exploit microsatellite sequences for the study of DNA polymorphism in eukaryotes.

STMS is a PCR based approach. The flanking sequences of the microsatellite regions are used to design primers to amplify microsatellite loci. The STMS markers reveal polymorphisms due to variation in the length of the microsatellites at specific individual loci.

STMS requires sequence information for DNA flanking the repeat itself, some of which may be available in DNA database for well-studied species. Otherwise it is necessary to produce genomic libraries enriched in microsatellites, from these to select potentially useful clones and then to sequence the DNA in order to design suitable primers that flank the repeat and amplify the genomic DNA.

The sequences flanking specific microsatellite loci in the genome are believed to be conserved within a particular species, across species within a genus, and rarely across the related genera. Choumane *et al.* (2000) studied the conservation of ninety microsatellite-flanking sequences in thirty-nine accessions of eight annual and one accession of a perennial species of chickpea. They found that all the primer sequences successfully amplified microsatellites in related species which indicates the conservation of microsatellite-flanking sequences in chickpea's relatives. But this conservation varied from 92.2 % in *C. reticulatum* to 50% in *C. cuneatum.*

Polyacrylamide gels are used which can detect even a single copy difference. Fragments with size differences >4bp can also be resolved on agarose gels. It is also possible to combine more than two primers in a PCR reaction, only when the products of the two primers do not overlap in size. This strategy is called multiplex PCR (Mitchell *et al.*, 1997 and Ribaut *et al.*, 1997). It leads to significant reduction in selection costs and screening time. Multiplex analysis of up to 24 different microsatellite loci per lane has been demonstrated (Schwengel *et al.*, 1994).

STMS markers require cloning and sequencing and thus initially it is very costly and labour intensive, and has tedious genotyping procedure. Another disadvantage of STMS technique is the commonly observed stuttering of bands. This means that instead of yielding one particular band, the enzymatic amplification of dinucleotide repeats commonly results in the cluster of "shadow bands" which are separated from each other by two base pair intervals. The additional bands are probably due to the result of slippage

events, that occur during the replication by Taq polymerase (Hange and Litt, 1993). Ordering of alleles according to size may help assigning allelic states correctly (Saghai-Maroof *et al.*, 1994). Slippage is less severe and amplimers are more clearly resolved if microsatellites with tri and tetrameric repeat units are amplified (Edwards *et al.*, 1991). However, as the locus specific primers become available, the approach becomes cost effective. STMS markers are locus specific, co-dominant, occur in large numbers and allow the unambiguous identification of alleles thus making them the markers of choice for a variety of purposes.

Huttel *et al.* (1999) screened 13,000 plasmid clones with a set of microsatellite-specific oligonucleotide probes. They designed flanking primer pairs for twenty-eight loci out of which twenty-two revealed single bands of expected size. Sixteen of these markers have shown polymorphism at an intraspecific level. Two markers CaSTMS 10 and CaSTMS 15 revealed twenty-five and sixteen alleles among sixty-three *Cicer arietinum* accessions reflecting gene diversity values of 0.937 and 0.922, respectively. The alleles of CaSTMS 15 were inherited in a Mendelian manner when tested on the F_7 RILs of C 104 x WR 315 cross.

Winter *et al.* (1999) designed primers for 218 loci in chickpea and used them for the detection of microsatellite length polymorphisms in six breeding lines and two wild species, *C. reticulatum* and *C. echinospermum*. Of these, 174 primer pairs gave interpretable banding patterns. Of the 174, 137 primer pairs produced at least two alleles. They mapped 120 STMS markers in ninety recombinant inbred lines from an interspecific cross to eleven linkage groups covering 613 cM.

2.9.3.5.2 Microsatellited - Primed PCR (MP-PCR) or Single Primer Amplification Reaction (SPAR)

MP-PCR combines some elements of alu-PCR (Sinnett *et al.*, 1990), STMS and RAPD analysis. Microsatellite complementry oligonucleotides are used as single PCR primers. If inversely repeated microsatellites are present within an amplifiable distance from each other, the inter repeat sequences are amplified. The resulting PCR products are separated on agarose gels and stained with ethidium bromide. MP-PCR was introduced by Meyer *et al.* (1993) in human fungal pathogen *Cryptococcus neaformans* and subsequently applied to other fungi (Meyer and Mitchell, 1995) as well as to animals (Perring *et al.*, 1993) and plants (Gupta *et al.*, 1994).

2.9.3.5.3 Anchored Microsatellites-Primed PCR (AMP-PCR) or Inter-SSR Amplification (ISA) or ISSR

This is the modification of MP-PCR where 5' or 3' anchored di or tri nucleotide repeats serve as single primers, the amplification products are separated on polyacrylamide gels and banding patterns revealed by autoradiography. This technique has several advantages over the unanchored variants of MP-PCR. First the primer anneals only to the ends of the microsatellite thus preventing internal priming and smear formation. Second the anchor allows the amplification of only a subset of the targeted inter repeat regions thereby reducing the high number PCR products expected from priming of dinucleotide inter repeat regions. Third the use of 5' anchors ensures that the targeted microsatellite is part of the product.

2.9.3.5.4 Random Amplified Microsatellite Polymorphism (RAMPO) or Random Amplified Hybridization Microsatellites (RAHM) or Random Amplified Microsatellites (RAM)

This approach is a combination of arbitrarily or microsatellite-primed PCR with microsatellite hybridization. In this approach, genomic DNA is amplified with a single arbitrary 10-mer primer (as in RAPD analysis) or with a microsatellite complimentary 15 or 16-mer primer (Gupta *et al.*, 1994) and the PCR products are electrophoresed, blotted and hybridized to a γ^{32} p or digoxigenin labeled mono-, di tri or tetra nucleotide repeat probe such as $(CA)_8$, $(GA)_8$, $(GTG)_5$, (GCGA). Subsequent autoradiography reveals reproducible probe dependent fingerprints that are polymorphic at the intraspecific level. This method was coined as RAMPO (Random Amplified Microstellite Polymorphism (Richardson *et al.*, 1995), RAHM (Random Amplified Hybridization Microsatellite, Cifarelli *et al.*, 1995) or as RAMS (Randomly Amplified microsatellite, Ender *et al.*, 1996).

2.9.3.6 Sequence Characterised Amplified Regions (SCARs)

This approach is based on RAPD technique. These markers are produced by cloning and sequencing RAPD fragments which are of particular interest. When the sequence is known, it is then possible to design primers which are longer than usual RAPD primers (24-mer oligonucleotides) that are exactly complimentary to the ends of the original RAPD fragments. When these primers are used in the PCR, single locus is amplified that corresponds to the original fragment. These loci are called SCAR's that are highly reproducible and are codominant markers.

2.9.3.7 Cleaved Amplified Polymorphic Sequence (CAPS)

These markers are also called as PCR-RFLP's. In this technique PCR primers are constructed for a particular locus. The PCR amplified product is digested with a restriction enzyme and visualised on an agarose gel using ethydium bromide staining. As in RFLP, polymorphism is detected by differences in restriction fragment size.

2.9.4 Inheritance of molecular markers in chickpea

Tuwafe *et al.* (1988) studied the inheritance of three isozymes in three F_2 families obtained by crossing six chickpea parental lines. Their results indicated that alcohol dehydrogenase is governed by a single locus (Adh1) with two codominant alleles, 6-phosphogluconate dehydrogenase by two loci (pgd1 and pgd2) each with two codominant alleles and peroxidase by a single locus designated as prx1. Linkage analysis showed that Adh1 is inherited independent of both loci pgd1 and pgd2.

Gaur and Slinkard (1990a) studied the genetics of 16 isozymes in chickpea based on F_2 segregation in an interspecific and an intraspecific cross. Each of these 16 isozymes exhibited monogenic inheritance. The isozymes AAT-3, GP1, PGM-2 and PGD-1 were found in plastids, ACO-2 and AAT in mitochondria and ACO-T, AAT-4, GP1-2, PGM-1, PGD2 in cytosol. Gaur and Slinkard further (1990b) studied the genetic control of other 9 isozymes. Alleles of all these isozyme loci expressed codominantly in heterozygotes and exhibited a single locus segregation ratio in F_2. The isozyme loci EST-2, MDH-2 and ME-1 expressed only in the flower. They observed the existence of several conserved linkage groups among *Cicer*, *Pisum* and *Lens*.

Kazan *et al.* (1993) studied the inheritance of several morphological and isozyme loci in crosses of cultivated lines and wild species of chickpea. Most of the isozymes showed single gene inheritance while esterase isozyme showed distorted segregation.

The linkage map proposed by them contained twenty three molecular markers and five morphological loci.

Mayer *et al.* (1997) found 1:1 segregation for the markers CS-27 and UBC-170 that were linked to the fusarium wilt resistance genes (race-1 and race-2).

Ratnaparkhe *et al.* (1998) studied the inheritance of Inter-Simple-Sequence-Repeats (ISSR) in a cross of cultivated chickpea and a closely related wild species (*Cicer reticulatum* Lad.). The 22 primers studied gave 31 segregating loci, all of which segregated in the expected Mendelian ratio of 1:1 (Presence: absence) in RILs. Primers based on (TG)n repeat gave the largest number of polymorphic loci. They also reported the linkage of an ISSR marker (UBC-855_{500}) to the gene for resistance to fusarium wilt race-4.

2.9.5 Inheritance of molecular markers in other crops

Mc Grath and Quiras (1991) studied the inheritance of isozymes and RFLP markers. They found codominant nature of isozyme and RFLP markers, which segregated in mendelian ratios. However six loci showed distorted segregation which were skewed towards their maternal alleles.

Bodenes *et al.* (1996) found the Mendelian segregation for the PCR based SSCP fragments in the F_2 progeny of pedunculated oak intraspecific crosses, 32P x A6, 34P x A3 and 33P x A3. Tsumura *et al.* (1996) found mendelian segregation for ISSR markers in Douglas-fir and sugi. Devely *et al.* (1991) and Fjellstrom and Parfil (1994) reported the inheritance of RFLP markers in loblolly pine and walnut respectively.

Chavarriaga *et al.* (1998) studied the inheritance of microsatellites in cassava. The microsatellites segregated in the expected 1:1, 1:2:1 or 1:1:1:1 ratio depending on the type of experimental material studied.

Similarly Fang and Roose (1999) studied the inheritance of the ISSR markers in citrus involving the progeny of *Sacaton citrumela* and *Trayes citrage*. Around 172 markers segregated in the expected Mendelian ratio, 22.9% deviated significantly from the expected Mendelian ratios. Most of the deviating markers over represented the poniers alleles. This distorted ratio may result from linkage to genes subjected to dirēctional selection either at gametic or zygotic stages.

2.10 Constructing genetic linkage maps

Location of genes and markers on chromosomes is called mapping of the genome of an organism. The genetic and molecular markers are mainly useful in the construction of maps. Linkage maps of many crops like maize, tomato, barley, common bean are already constructed (O' Brien, 1993). A high-density map with around 1000 markers is developed in tomato (Tanksley *et al.*, 1992).

2.10. 1 Prerequisites for the development of the linkage maps

- The parents developed for mapping should have highest degree of genomic polymorphism.
- Design of the test population. F_2 population or backcross populations are most commonly used for the mapping purpose. But the major drawbacks from using the F_2 population are:
    1. The population is ephemeral and repetition of experiment is not possible.
    2. The individuals tested for the linkage analysis should only be tested for the trait.
    3. The homozygous and heterozygous states of dominant markers like RAPD, mini and microsatellites cannot be determined.

Recently recombinant inbred lines (RILs) and near isogenic lines (NILs) are used for map construction. Recombinant inbred lines (RILs) developed by advancing F_2 population through single seed descent method are useful as permanent mapping populations. Due to several rounds of meiosis, RILs become homozygous and get fixed for different combinations of linkage blocks from original parents. These differing linkage blocks in individual RILs forms the basis for linkage analysis. A map based on RILs in peas was first developed by Domoney *et al.* (1986) to locate protein genes. Later Burr *et al.* (1988) in maize, Tahir (1990) in lentil, and Paran *et al.* (1995) in tomato used RILs to construct linkage maps. The near isogenic lines (NILs) which are similar to the recurrent parent used in the backcross programme except for the chromosomal segment carrying desired allele. They can be used for mapping, only when they have a marker genotype similar to the donor parent but different from the marker genotype of the recurrent parent (Melchinger, 1990). Young *et al.* (1988) and Hinze *et al.* (1991) used NILs for mapping in tomato and barley respectively. Only one trait can be mapped at a time using NIL progeny.

2.10.2 Characteristics of desirable markers

Any marker that can display polymorphism is suitable. For practical purpose, markers should be designed to:

(i) test large number of individual offspring quickly

(ii) require only a limited amount of DNA

(iii) produce reliable, clear cut results

(iv) should be evenly distribution through out the genome

(v) occur frequently in the genome

(vi) should have codominant behavior which allows to discriminate homozygous and heterozygotic states

(vii) display polymorphism between many cultivars

(viii) be inexpensive

2.10.3 Mapping strategies

Development of molecular marker technology and consecutive identification of markers tightly linked with many economically important genes renewed interest in genetic mapping. Construction of a genetic linkage map is a systematic step wise process. Initially polymorphic markers between the parents are identified which are then scored in the suitable mapping population developed between the cross between the same parental lines. Genetic maps require a) Calculation of pair wise recombination frequency of markers b) establishment of linkage groups and estimation of map distances, Finally c) determination of map order. All these analyses are performed using standard computer packages such as Linkage-1 (Suiter *et al.*, 1983), MAPMAKER (Lander *et al.*, 1987), Gmendal 2.0 (Echt *et al.*, 1992) and Join map (Stam, 1993). Once a linkage map has been constructed, it is correlated to chromosome karyotype or cytogenetic map (Young, 1994) by using aneuploids such as monosomic lines (Helentjaris *et al.*, 1986). Correlation of linkage groups to specific chromosomes allows efforts to concentrate on the defined linkage groups to detect markers linked to traits of interest.

2.11 Tagging of Quantitative Trait Loci (QTLs)

Most traits of economic importance (such as flowering, maturity time, yield and quality) are quantitative in nature. A number of methods to identify association between marker alleles and QTL have been proposed. They are of two types: one which considers one marker locus at a time and the other that considers all marker loci at once and attempts to estimate effects for each locus while adjusting for the other locus. Commonly

used computer programs for QTL mapping are MAPMAKER/QTL (Lander and Botstein, 1988), QTLSTAT (Knapp *et al.*, 1992), MAPMAKER/QT (Manly and Cudemore, 1996), Qgene (Nelson, 1997), and QTL cartographer (Basten *et al.*, 1994).

2.12 Mapping in Chickpea

Linkage maps of various crops have been constructed using molecular and morphological markers (O' Brien 1993). High-density genetic maps have also been constructed using RAPDs (Williams *et al.*, 1990) AP-PCR (Welsh *et al.*, 1990) and DAF (Caetano-Anolles *et al.*, 1991b). Linkages between molecular markers and traits help in indirect selection. Linkages between various mophological traits have been reported in genetic maps.

Bhat and Argikar (1951) for the first time detected genetic linkage in *Cicer arietinum.* They found 23.85% linkage between branching habit (u) and alternate leaflet arrangement (μ). It was also thought that early maturity is also linked with branching habit and leaflet arrangement.

Aziz *et al.* (1960) reported the linkage of corolla colour with seed coat surface and seed coat colour. Bhapkar and Patil (1963) reported linkage between P, a gene that effects flower colour and one of the two complementary genes that govern seed coat colouration. Ghatge *et al.* (1975) found linkage between three characters namely stem colour, seed coat colour and corolla colour. Patil and Deshmukh (1975) observed linkage between genes governing corolla colour and one of the three genes governing seed shape in chickpea. More and D'Cruz (1976) observed that corolla colour was linked with seed surface.

Reddy and Chopde (1977) reported linkage between one of the genes for corolla and leaf (Nls) shape which had a cross over frequency of about 28%. They also reported

the linkage of Lvco, a gene conditioning flower colour, with one of the two complimentary genes conditioning testa colour with a recombination value of 28.92%.

Pawar and Patil (1979) observed that gene for corolla was linked with the factor BSCa for seed coat colour with the recombination value of 40.65%. They also found that the same gene for corolla colour was linked with factor Rsa for seed surface with 22.97% recombination value and BSCa and Rsa had a linkage value of 26.53%. Thus their studies indicated that these three genes are present in the same linkage group.

None of the genes in chickpea were assigned to specific linkage groups except the genes described by Pawar and Patil (1979). However, the recombination frequencies were too close to independent assortment to be considered valid cases of linkage.

The first linkage map of chickpea based on interspecific cross (C. *arietinum* x C. *reticulatum*) with four linkage groups consisting of thirteen isozyme loci was reported by Gaur and Slinkard (1988). Later they added Est-10 to linkage group I (Aat-p, Enp, Pgm-p), four isozymes (Ald-pl, Glu-3, Gal-2, and Est-2) to linkage group II (Amy, Aat-m, Est-3) and Gal-3 and Prx-3 to linkage group IV (Gaur and Slinkard, 1990a). Besides the four linkage groups already reported, three additional linkage groups were constructed using morphological and isozyme loci (Gaur and Slinkard, 1990b).

Unfortunately, *C. arietinum* exhibits low or few polymorphisms at isozyme loci (Gaur and Slinkard, 1990b). This was also observed from the studies of Oram *et al.* (1987) and Tuwafe *et al.* (1988). This shows that the application of isozyme markers in chickpea mapping is limited.

The interspecific cross between the cultivated and wild germplasm was used to construct genetic linkage maps as very low polymorphism was exhibited with in the intraspecific cross. Simon and Muehlbauer (1997) started developing linkage map using interspecific cross of cultivated and wild germplasm. The cross was ICC 4958 x *C. reticulatum* (PI 498777). They used morphological, isozyme, RFLP and RAPD markers

in the F_2 population for developing the linkage map. They published the map in 1997 which consisted of nine morphological, forty-five RAPDs, ten RFLP markers covering 550 cM. They also compared the chickpea maps with the published maps of pea and lentil.

Tullu (1997) studied the cross WR 315 x C 104. WR 315 is resistant to races 1, 2 and 4 of fusarium wilt and C 104 is a late wilter. He found the amplification of DNA fragment linked to resistance to race 1 by UBC 170 and a fragment linked to susceptibility by CS27. There was 6 % recombination between the loci corresponding to the two RAPD markers (CS 27 and UBC 170) and 7% recombination between these loci and the locus that controls resistance to fusarium wilt race 1. He constructed allele specific associated primer (ASAP) for CS 27, the marker that was linked to susceptibility of fusarium wilt. He reported that the locus corresponding to resistance to race 4 was located nine map units from the loci corresponding to CS 27 and UBC 170_{500}. He found that the two loci which confer resistance to race 1 and race 4 were five map units apart with a standard error of 0.032.

Santra (1998) developed a linkage map from the RILs of the interspecific cross *C. arietinum* (FLIP 84-92c) and *C. reticulatum* (PI 498777). FLIP 84-92c is resistant to ascochyta blight and PI 498777 is susceptible. The resulting nine linkage groups consisted of one morphological trait locus, nine isozyme loci, seventeen ISSR and ninety RAPD loci. He detected two QTLs conferring resistance to blight. These two QTLs explained 56.88% of the total variation for ascochyta blight resistance in this cross.

Winter *et al.* (1999) developed a linkage map in chickpea using 120 STMS markers with ninety RILs derived from an intercross between *C. reticulatum* and the chickpea cultivar ICC 4958. These markers were assigned to eleven linkage groups covering 613 cM. Both clustering as well as random distribution was observed. About

39% of the markers deviated significantly from the expected 1:1 segregation ratio and 76% of the markers were located in three distinct regions in the genome.

Winter *et al.* (2000) further developed an integrated molecular map covering 2077.9 cM using 130 RILs from the same cross. A total of 303 markers including STMS, DAF, AFLP, ISSR, RAPD, isozymes, cDNAs, SCARS and three loci that confer resistance against different races of fusarium wilt were mapped covering 2077.9 cM with an average distance of 6.8 cM between the markers. The genes for resistance to races 4 and 5 were mapped to the linkage group that included an STMS and a SCAR marker linked to fusarium wilt race 2.

Rakshit *et al.* (2001) used bulk seggregant analysis to find the markers linked to aschochyta blight in the interspecific cross of cultivated chickpea and a susceptible accession of *C. reticulatum*. Out of 310 random 10-mers used three produced five polymorphic bands between the bulks. Two of them (opso6 and opso3) were transferred to the population that were mapped to linkage group 4 to markers UBC 733 B and UBC 181 flanking the major aschochyta resistance locus.

2.13 Cloning of the polymorphic fragments.

It is necessary to sequence the polymorphic fragments in order to know the molecular basis of the polymorphism. Davila *et al.* (1999) cloned and sequenced polymorphic randomly amplified microsatellite (RAMP) fragments produced by primers complimentary to microsatellites. All the sequences showed expected repeat motifs with the number of repeats varying from 5-10.

Most of the maps in chickpea were constructed based on interspecific crosses. After the availability of STMS markers, intraspecific maps are being developed. This is because STMS markers detects higher levels of polymorphism even at intraspecific level. The most extensive interspecific map available is 2000 cM long with some

markers linked to fusarium wilt resistance genes (Winter *et al.*, 2000). It is very important to develop an extensive intraspecific map that is highly useful for marker-assisted selection. New marker systems are required which can detect more polymorphisms even at intra specific level to saturate the linkage map and to obtain linkages with important traits.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried on the inheritance of shoot and root characters and molecular markers in chickpea. The experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru near Hyderabad, A.P, situated at an altitude of 545 m above the mean sea level at a latitude of 17° 32' N and longitude of 78° 16' E. The weather conditions during the crop growth period is presented in Fig1. The research material was provided by the chickpea breeding unit at ICRISAT. The experiment on shoot characters was conducted during the *Rabi* (post rainy season) 1999-2000 and the study of root characters was carried under controlled environmental conditions in a 'conviron'. Seven different generations and 126 random recombinant inbred lines (RILs) of F_{10} generation derived from the cross ICCV 2 and JG 62 were used for the inheritance studies on the morphological characters and the 126 RILs were used to conduct the molecular marker studies.

A part of the molecular marker work was conducted at Biocentre, Johann Wolfgang Goethe University, Frankfurt am Main, Germany.

3.1 MATERIALS

The experimental material consisted of seven generations P_1, P_2, F_1, F_2, F_3, BC_1P_1, BC_1P_2 and 126 RILs of a cross between two chickpea varieties ICCV 2 (P_1) and JG 62 (P_2). ICCV 2 is a medium bold seeded kabuli type and JG 62 is small seeded desi type variety. The characters of two parents are given in Table 2.

Table 2: Characteristic features of ICCV 2 and JG 62

CHARACTER	ICCV 2	JG 62
Varietal status	Released	Released
Flower	White	Pink
Seed type	Kabuli	Desi
Seed colour	White	Brown
Seed size	Medium (26g/100seeds)	Small (16g/100seeds)
Seed surface	Smooth	Rough
Seed fibre	Low	High
Anthocyanin pigment	Absent	Present
Fusarium wilt	Resistant	Susceptible
Pod borer	Susceptible	Tolerant
Collar rot	Susceptible	Resistant
Flowering	Very early	Medium
Maturity	Early	Medium
No.of pods/peduncle	One	Two
No.of pods/plant	Medium	High
Pod size	Bold	Small
No.of branches	Low	Medium
Branches	Long	Short
Canopy	Wide	Narrow
Drought	Escape	Tolerant
Internode length	Long	Short Small
Leaf size	Big	Low
Sugar content	High	Moderate
Plant height	Moderate	High
Seed yield	Medium	High
Malic acid	Low	High

Source: Chickpea Breeding, ICRISAT

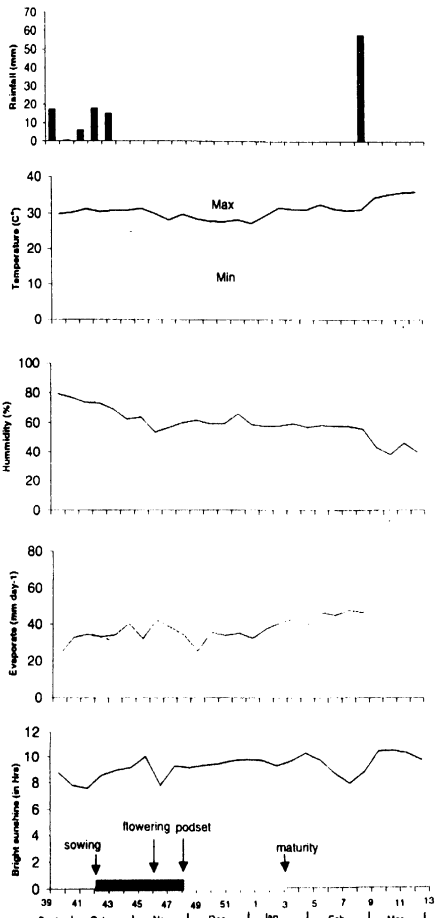


Fig 1: Weather conditions at ICRISAT during 1999-2000

3.2 METHODS

3.2.1 Experiment I Field studies

3.2.1.1 Inheritance of podding duration and growth habit

The parental, F_1, F_2, F_3, BC_1P_1, and BC_1P_2 generations of the cross ICCV 2 and JG 62 were planted without replication with a spacing of 60 cm between rows of 4m length and 20 cm between plants. The F_3 progeny were planted in rows of 4 m length with 60 cm between rows and 10 cm distance between the plants.

126 F_{10} RILs along with the parents, F_1 and three checks (Annigiri, ICCV 10 and ICCV 96029) were sown in three replications in Alpha design. Each replication consisted of 12 blocks and 11 treatments (lines) in each block. Each entry was planted in two rows of 4 m length with 60cm x 10cm spacing.

All the material was planted on 12th October 1999 in deep vertisol under conserved soil moisture conditions.

Table 3: Population size of P_1, P_2, F_1, F_2, F_3, BC_1P_1 and BC_1P_2 used in the experiment

P_1 (ICCV 2)	P_2 (JG 62)	F_1 (ICCV 2 x JG 62)	F_1 (JG 62 x ICCV 2)	F_2	F_3	BC_1P_1	BC_1P_2
15	16	20	15	306	202	39	37

3.2.1.1.1 Characters studied

Observations were recorded on three competitive random plants per plot in each of the RILs parents, checks, F_1 and also for each progeny in F_3. The characters in P_1, P_2, F_1, F_2, F_3, BC_1P_1 and BC_1P_2 were recorded on each individual plant. The particulars of the characters studied were as following.

3.3.1.1.1.1 Days to first flower (DFF)

It is the number of days from time of planting to the day on which the first flower appeared in the plant [(In case of P_1, P_2, F_1, F_2, F_3, BC_1P_1, BC_1P_2 and F_3) or in plot (Recombinant Inbred Lines)].

3.2.1.1.1.2 Days to first pod (DFP)

It is the number of days from sowing to the formation of first pod in plant or plot.

3.2.1.1.1.3 Days to maturity (DM)

It is the number of days taken from sowing to the time when more than 90 percent of pods have turned to light yellow or brown (Dry pod).

3.2.1.1.1.4 Podding duration

The podding duration (total reproductive period) was calculated from pod initiation period to pod filling period in days

3.2.1.1.1.4.1 Total reproductive period (TRP)

It is the number of days taken to mature after the appearance of the first flower.

TRP = DM – DFF

3.2.1.1.1.4.2 Pod initiation Period (PIP)

It is the number of the days from days to first flower to the formation of first pod.

PIP = DFP – DFF

3.2.1.1.1.4.3 Pod filling period (PFP)

It is the number of days taken for maturation after the formation of the first pod.

PFP = DM – DFP

3.2.1.1.1.5 Number of nodes to first flower

The number of nodes to first flower was determined by counting the number of nodes from the base of the plant to the first flower bearing node.

3.2.1.1.1.6 Growth habit

It was recorded by measuring the angle made by the primary branches with the vertical axis in both ICCV 2 and JG 62. As there was no difference between the parents for this character it was not recorded in any other progeny.

3.2.1.2 Inheritance of fusarium wilt resistance

ICCV 2 is resistant and JG 62 is susceptible to *Fusarium oxysporium* race 1. The two parents, F_1, F_2, and F_3 progenies were planted in wilt sick plots on 13th October 1999 with a spacing of 60 cm x 20 cm.

All the 126 F_{10} RILs along with the checks were planted in single rows in wilt sick plots on conserved soil moisture on 13th October 1999. After every 5th row, JG 62 was planted which is highly susceptible to fusarium wilt and after every 25 rows resistant cultivar WR 315 was planted

Table 4: Population sizes of P1, P_2, F_1, F_2, and F_3 used in the experiment

P_1 (ICCV 2)	P_2 (JG 62)	F_1 (ICCV 2 x JG 62)	F_2	F_3
15	15	10	206	100

The germination counts were taken. The first observation was recorded when JG 62 started wilting and subsequently every three days for one month and every 10 days until the end of the experiment. The dead plants were uprooted and examined. Only those showing characteristic typical vascular wilt were recorded as dead due to disease (Plate 1).

For the confirmation of the results, the 126 RILS were planted in pots inoculated with the pathogen *Fusarium oxysporium* race 1. The fungus culture was derived from a

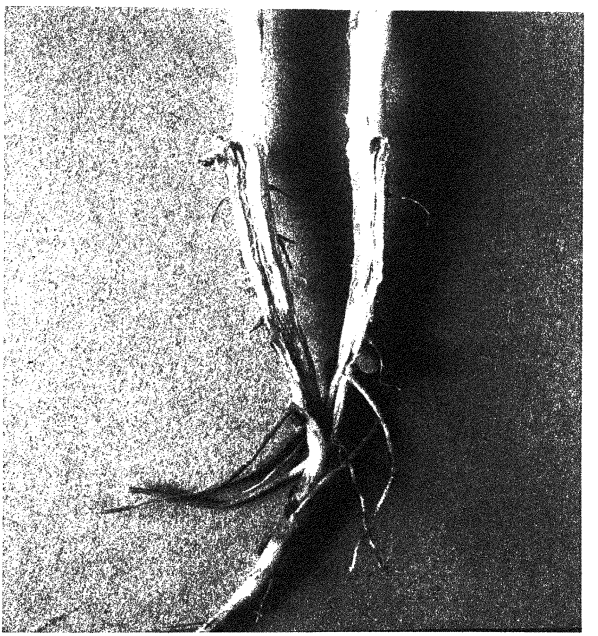


Plate 1: Typical symptoms (Xylem blackening) of wilt incidence

single spore multiplied in 100 g of sand-chickpea meal in a 250 ml flask incubated for 14 days at 25° C. One hundred grams of the inoculum were mixed thoroughly with 2 kg of a mixture of autoclaved soil (black soil) and riverbed sand (2:1, v/v) in 30 cm earthen pots. Two successive batches of the susceptible cultivar JG-62 were grown in pots (15 seeds per pot) and allowed to wilt. The temperature through out the study was maintained at 25-30° C. After the plants have wilted the plant debris was incorporated in to the soil.

In the center of the pot five seeds of JG 62 and five seeds of check (ICC 4958, C 104, K 850 and CPS 1 were used as check and each check was planted after every 3rd pot.) were planted. On the either side 15 seeds of each RILs were planted. Germination counts were taken and the first observation was recorded after JG 62 started wilting and subsequent observations were recorded twice a week for one month and once in 10 days until the end of the experiment.

During *Rabi* 2000 first JG 62 was planted in wilt sick plots. After the death of the JG 62, 126 RILs along with the parents were planted in the same row to be sure of the inoculum. Each row was 4 m long with the spacing of 60 cm x 20 cm. First observation was recorded after JG-62 started wilting and subsequent observations were recorded twice a week for one month and once in 10 days until end of the experiment.

3.2.2 EXPERIMENT II Pot culture studies

3.2.2.1 Inheritance of root length, root volume

Ten seeds of each parent were planted in pots at the rate of one plant per pot under controlled environmental conditions in the conviron: 22/15° C day and night temperatures with 45 % and 75 % relative humidity respectively. Root length and root volume were recorded at the time of flowering of ICCV 2 on five plants (around 30 DAS) and also at the time of flowering of JG 62 on remaining five plants (around 45

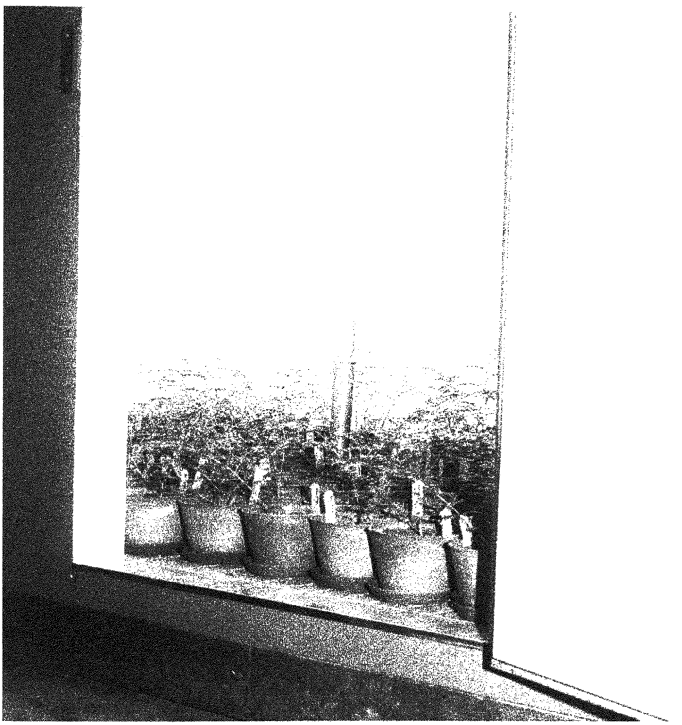


Plate 2: RILs of the cross ICCV 2 x JG 62 grown in the conviron for recording the root length and root volume

DAS). This preliminary experiment was conducted to know the root characteristics at the flowering time of both the parents.

Ten seeds of each parent and F_1s, 20 seeds of each backcross and 150 F_2 seeds were planted in 5" pots with the capacity of one kg soil. The evaluation within each population was conducted in a completely randomized design in which a plant was a replicate for the generation it represented. One plant was planted in each pot filled with autoclaved 1:1 soil sand mixture that had 1g of DAP and 400 g of FYM per kg of the mixture. The p^H of the mixture was 8.18 with 0.28 m.mho cm^{-1} E.C and 0.68% organic carbon. The pots were placed on the benches in the conviron. The pots were watered at regular intervals.

One hundred and twenty six F_{10} RILs along with the parents were planted in 12.5 cm pots filled with 1:1 sand soil mixture that had 1g of DAP and 400 g of FYM per kg of the mixture in three replications. One seed was planted in each pot. Each replication was kept in one conviron. The temperature, humidity, light and irrigation provided were the same for different generations (Plate 2).

3.2.2.2 Characters studied

3.2.2.2.1 Root length (m)

Each plant was harvested by carefully washing the pots to prevent the loss of roots. The roots were then carefully washed and the root length was measured using a root length scanner, (Comair, Common Wealth Aircraft Corporation Limited, Australia) (Plate 3a and Plate 3b).

3.2.2.2.2 Root volume (cc)

The root volume (cc) of each plant was measured by water displacement method (Pundir *et al.*, 1992) (Plate 4). The root portion of the plant was dipped in a cylinder

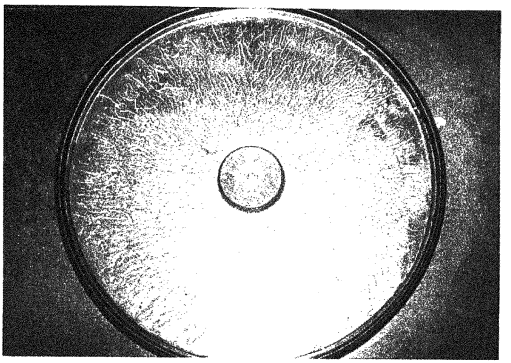


Plate 3a: Roots spread on the plate of the root length scanner

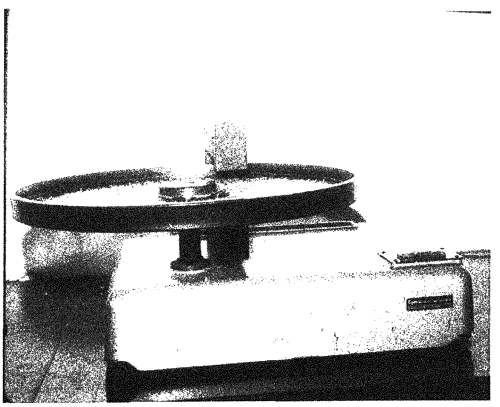


Plate 3b: General view of the root length scanner (Comair. Common Wealth Aircraft Corporation Limited. Australia)

Plate 4: Measurement of root volume by water displacement method

having water. The water displaced by the root was collected and weighed on a balance and reported in cc.

3.2.2.2.3 Leaflet number

The total number of leaflets of 3rd, 4th, and 5th pinnules from top of the primary branches were counted and taken as leaflet number.

3.2.2.2.4 Leaf area (cm^2)

Leaf area was measured on 3rd, 4th, and 5th pinnules from top of the primary branches with the help of a LI – COR LI-3100 Area meter, LI-COR Inc. Nebraska, USA.

3.2.2.2.5 Shoot dry weight (g)

The shoot dry weight was recorded after drying the shoots in oven at 80° C for 48 hours.

3.2.2.2.6 Dry root weight (g)

The root dry weight was recorded after drying the roots in oven at 80°C for 48 hours.

3.2.2.2.7 Leaf dry weight (g)

The dry weight of the leaves in the 3rd, 4th and 5th pinnules was recorded after drying the leaves in oven at 80°C for 48 hours.

3.2.2.2.8 Total number of nodes upto flowering

The total number of nodes produced by the plant up to flowering was counted to obtain this parameter.

3.2.2.2.9 Specific leaf weight

Specific leaf weight was calculated by following the formula suggested by Radford (1967).

$$SLW = \frac{\text{Leaf weight (g)}}{\text{Leaf area (cm}^2\text{)}}$$

3.2.2.2.10 Specific leaf area

Specific leaf weight was calculated using the following formula.

$$SLA = \frac{\text{Leaf area } (cm^2)}{\text{Leaf weight (g)}}$$

3.2.2.2.11 Leaf area/root length and leaf area/root volume

Area/root length and area/root volume are calculated using the following formula.

$$\text{Leaf area/root length} = \frac{\text{Leaf area } (cm^2)}{\text{Root length (m)}}$$

$$\text{Leaf area/root volume} = \frac{\text{Leaf area } (cm^2)}{\text{Root volume (cc)}}$$

3.2.2.2.12 Hundred seed weight

Weight of 100-seed in grams for RIL population was weighed and reported in grams.

All the above characters were recorded when the first flower appeared in all the seven generations studied and also for 126 F_{10} RILs.

A separate study was conducted with only 126 RILs and parents where all the RILs and parents were planted in three replications in two sets and observations for root volume were recorded in one set at the time of flowering of ICCV 2 and at the time of flowering of the other parent JG 62 in the second set.

3.2.3 EXPERIMENT III (Molecular marker studies)

3.2.3.1 Inheritance of molecular markers and construction of a linkage map.

The two parents ICCV 2 and JG 62 were screened by different molecular markers (RAPDs, ISSRs, STMS, SAMPL, MP-PCR and RMMFPs). The markers which exhibited polymorphism between the parents were used to genotype 126 F_{10} RILs.

3.2.3.2 DNA extraction

The DNA extraction was based on the modified CTAB extraction method (Saghai-Maroof *et al.*, 1984).

3.2.3.2.1 Chemicals used for DNA extraction

1. Liquid nitrogen.
2. CTAB extraction buffer: 100 mM Tris-HCl, p^H 8.0, 1.4 M NaCl, 20 mM EDTA, 0.25 % sodium sulphite (w/v) and 2-3% CTAB (Hexadecyl trimethyl ammonium bromide), 0.03% β- Mercaptoethanol.
3. Chloroform : Isoamyl alcohol (24:1)
4. Cold isoproponol
5. High salt $T_{10}E_1$: (10 mM Tris-HCl, 1 mM EDTA, 2M NaCl p^H 8)
6. RNase solution: 10 mM Tris-HCl, 15 mM NaCl and one percent RNase (w/v)
7. Phenol : Chloroform : isoamylalcohol (25:24:1)
8. Absolute alcohol
9. 7.5 mM Ammonium acetate solution
10. $T_{10}E_1$: 10 mM Tris-HCl, 1 mM EDTA, p^H 8.0
11. 70% ethanol.

3.2.3.2.2 DNA extraction procedure

1. 2 g of fresh young leaf was crushed to make fine powder in liquid nitrogen.
2. This powder was transferred into a sterile polypropelene tube containing 15 ml of CTAB solution that was preheated at 65° C in the water bath.
3. The samples were incubated at 65° C for 30 minutes, mixing gently every 15 minutes.
4. Tubes were removed from water bath and cooled to room temperature.
5. 15 ml of chloroform–isoamyl alcohol (24:1 v/v) was added to the tube containing the sample and extraction buffer.
6. The solution was shaken gently for 10 minutes at room temperature.
7. The solution was centrifuged for 10 minutes at room temperature at 6000rpm.
8. Step 2 was repeated if the upper layer does not look clear.
9. The top aqueous phase was pipetted in to a new sterile tube.
10. Eight ml of cold isopropanol was added to the solution in the tube. DNA was hooked out but if no DNA precipitate was seen, the solution was centrifuged for 15 minutes at 6000 rpm.
11. The pellet was washed once in 2 ml 70% ethanol and then air-dried for 10-15 minutes.
12. 500-700 µl high salt $T_{10}E_1$ was added to the eppendorf tube containing the DNA pellet and kept for 45 minutes at room temperature or incubated at 40-50° C for 10 minutes.
13. When DNA was fully suspended 5 µl of RNase was added and incubated at 37° C or at room temperature overnight.
14. An equal volume of cold phenol : chloroform : isoamylalcohol (25:24:1 v/v/v) was added and mixed well.

15. The solution was centrifuged for five minutes at 14000 rpm.
16. The top layer was pipetted in to another eppendorf tube and an equal amount of chloroform-isoamyl alcohol solution was added and centrifuged for five minutes at 14000 rpm.
17. The top layer was pippetted in to new eppendorf tube and 0.5 volume of 7.5 mM ammonium acetate solution and two volumes of absolute ethanol was added, mixed thoroughly and centrifuged at 14000 rpm for 5 minutes.
18. The DNA was removed with the glass hook and washed twice with 250 µl of 70% ethanol.
19. The supernatant solution was dispensed and the pellet was dried for 10 minutes.
20. The DNA was dissolved in appropriate volume of (150-250) of TE buffer.

3.2.3.3 Estimation of DNA quantity and quality

DNA concentration of the samples was estimated based on the spectrophotometer measurement at 260 nm. For this, five µl of DNA was diluted in 995 µl of sterile water and measured the absorbancy at 260 nm. The DNA concentration in the sample was calculated using the formula 1.0 O.D = 50 µg. The ratio of OD_{260} to OD_{280} was calculated to check the purity of the DNA. The DNA was considered to be pure when the ratio of OD_{260} to OD_{280} was between 1.8 and 2.0 (Maniatis *et al.*, 1982)

To test the quality of DNA, samples were run on 0.8% agarose gel in 0.5% TBE buffer and stained with ethidium bromide and checked for the contamination by RNA (which usually runs ahead) and the DNA was evaluated by comparing it with a standard undigested DNA sample.

3.2.3.4 Random Amplified Polymorphic DNA (RAPD)

Random decamer primers (*OPERON Tech. Inc.*, Alameda, USA) were used for RAPD analysis. PCR amplification was performed according to Williams *et al.* (1990). Amplification reaction was performed in 0.2 cm^3 thin walled PCR tubes containing 1 X reaction buffer (10 mM Tris-HCl p^H 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 2.5 mM $MgCl_2$, 250 uM each of dATP, dTTP, dCTP, dGTP, 0.2 uM Primer, 25 ng of the template DNA and 2 units of Taq DNA polymerase. The total reaction volume was 20 µl. Amplification was carried out in a thermal cycler (GeneAmp *PCR system 9600, Perkin Elmer*, Norwalk, USA) for 45 cycles. Each cycle consisted of a de-naturation step for one minute at 92° C, an annealing step for two minutes at 35° C and an extension step for two minutes at 72° C. The PCR products were electrophoresed in a 2.0 % agarose gel in 1 X TBE at 80 V for 3 h, stained with ethidium bromide and visualised by illumination against UV light and recorded on a Polaroid type film (*Polaroid*, Cambridge, USA).

3.2.3.5 Inter Simple Sequence Repeats (ISSR)

PCR reaction was performed with 25 ng of genomic DNA per 20 µl of the reaction volume containing 2.5 mM $MgCl_2$, 1 X reaction buffer, 0.24 µM primer 1 U of Taq DNA polymerase. In a thermocycler with denaturation of 94° C for 30 s, annealing at 50° C for 30 s and elongation at 72° C for 2 min for 35 cycles and a final elongation of 72° C for 10 min. PCR products were separated on 2 % agarose gel and stained with ethidium bromide.

3.2.3.6 Sequence Tagged Microsatellite Sites (STMS)

The microsatellite flanking primers designed by Winter *et al.* (1999) were tested using a thermal cycler (Perkin Elmer) in 20 µl reaction mix. The reaction mixture

contained 50 ng of genomic DNA, 2 µM of each primer, 1.5 mM $MgCl_2$ 200 µM of each dNTP, 1X reaction buffer (10 mM Tris-HCl p^H 9.0, 50 mM KCl, 1.5 mM $MgCl_2$) and 1 unit of Taq DNA polymerase. The temperature regime consisted of an initial denaturation step of 96° C for 2 min, followed by 35 cycles of denaturing at 96° C for 20 s, annealing at 55° C for 50 s and elongation at 60° C for 50 s.

After amplification an equal volume of formamide loading buffer [97.5% (v/v) formamide, 10 mM EDTA p^H 8.0, 0.05% (w/v) xylene cyanol, and 0.05% (w/v) bromophenol blue] was added and denatured at 95° C for 5 min. 3 µl of the sample were separated on denaturing 4% polyacrylamide gels (Maniatis *et al.* 1982) at 50 watts for two hours. The bands were visualized by staining the gel using silver staining technique.

3.2.3.6.1 Gel casting, preperation of 4% polyacrylamide gel and running of the gel

The long plate of the gel was treated with repel silane solution. This treatment was not needed each time if water beads on the surface of the plate. The notched glass plate was treated with binding solution to chemically cross link the gel to the glass plate. One surface of the glass plate was treated with bind silane solution. After 10 min the glass plate was washed with 95% ethyl alcohol for 2-3 times to remove excess binding binding solution which prevents the contamination of long glass plate with bind silane solution and thus preventing the tearing of the gel.

225 g of urea, 100 ml of TBE 5 X buffer (54g of Tris, 27.5 g of boric acid and 20 µl of 0.5M EDTA in a volume of one litre double distilled water) and 50 ml of 40% acrylamide solution were mixed and stirred until a homogenous solution was formed and the final volume was made to 500 ml with double distilled water .The 4% acrylamide solution was stored in dark glass bottles at 4° C.

100 ml of the above solution was taken to which 600 µl of 10% ammonium per sulphate and 100 µl of TEMED were added just before the preperation of the gel. This solution was poured in to casted gel according to the seque-gen manual. The gel was allowed to polymerize for one hour. 1X TBE buffer was used for running the gel. Pre-run was done at 100 watts for 20 minutes or until the temperature of the gel was around 45° C and 3 µl of the denatured sample was loaded per well. 2µl of 1KB ladder was used which served as molecular weight standard. The samples were run at 50 watts for two hours (Plate 5). After completion of gel run, the buffer was drained off, the two plates of the sequencing gels were separated and the glass plate containing the gel was used for developing by silver staining technique.

3.2.3.6.2 Silver staining technique

Solutions used

1. Fix / Stop solution: 10% glacial acetic acid. 200 ml of glacial acetic acid was dissolved in 1800 ml of distilled water.
2. Stain: 3 g of silver nitrate ($AgNO_3$) and 4.5 ml of 37% formaldehyde was dissolved in 3 l of double distilled water.
3. Developer: 30 g of sodium carbonate (Na_2CO_3) was dissolved per one liter of double distilled water. Immediately before use 1.5 ml of 37% formaldehyde and 20 µl of sodium thio sulphate (100 mg ml^{-1}) was added. For developing one gel four liters of developer solution was needed.

Procedure of silver staining

1. Solutions required for developing the gel were prepared.
2. After electrophoresis, the two glass plates were carefully separated.

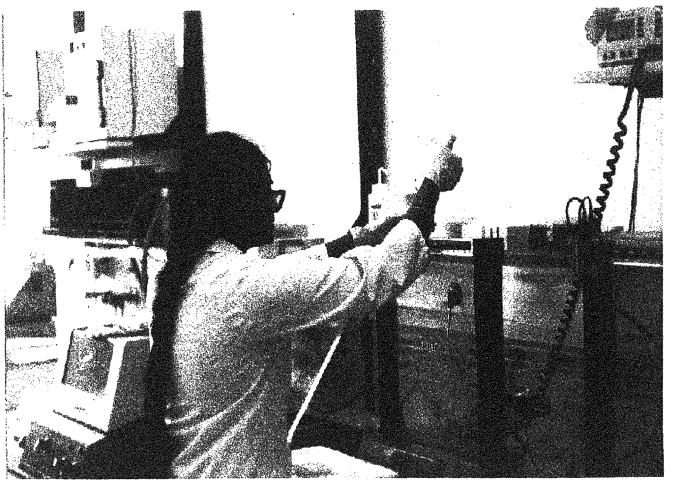


Plate 5: Photograph showing general view of sequencing gel

3. Fixing the gel: The glass plate with the gel was placed in a plastic tray containing 2 l fix solution. The tray was agitated until the tracking dyes were no longer visible. The Fix/Stop solution was saved and was used later.
4. The gel was rinsed in double distilled water for three times using agitation for two minutes each rinse.
5. Staining the gel: The gel was transferred to staining solution and agitated for 30 minutes.
6. The glass plate with the gel was rinsed with double distilled water for 5-10 seconds. Total time from the time when the gel was placed in water and to the time it was placed in the developing solution not exceeding 5-10 seconds, as longer rinses result in weak or no signal.
7. Developing the gel: The developing solution was chilled by keeping in cold room. Just before use, sodium thio sulphate and formaldehyde was added as described previously. The glass plate immediately after rinsing with water was transferred to the plastic tray containing two litres of developing solution and agitated until the template bands started to develop or until the first bands were visible. The glass plate was then transferred to the remaining two litres of chilled developing solution and agitated until the bands become clearly visible.
8. The developing reaction was terminated by placing the glass plate containing the gel to the Fix/stop solution and incubated for 2-3 minutes.
9. The gel was finally rinsed with double distilled water.
10. The gel was dried at room temperature.

The gel after drying was scored over the light box and then scanned as a TIF file for storage.

3.2.3.7 Selective Amplification of microsatellite Polymorphic Length (SAMPL)

This technique had different steps which were carried out as following.

1. Restriction of the DNA with the template: 200 ng of the genomic DNA was restricted with 6 U of EcoRI enzyme and incubated at 37° C for three hours.
2. Ligation of the adapters: The adapter mix consisted of EcoRI Oligo 1(5' CTCGTAGACTGCGTACC 3') and EcoRI Oligo 2 (5' AATTGGTACGCAGTC 3'). The two adapters are incubated at 98° C for 5 min along with 0.6 µl of 100 µM DDT and 6 µl of 10 X Tango buffer such that the final concentration of the adapter mix is 50 µM. After incubation the adapter mix was allowed to cool slowly to room temperature for annealing.

   A ligation mix was prepared with annealed adapter mix, 10µM of ATP, 1X Tango buffer in presence of the enzyme T4 Ligase (2 U). This ligation mix was added to the restricted sample and incubated for 37° C for 3 hours.

1. Preamplification: 1:5 diluted restricted-ligated template was preamplified in a r reaction volume of 20 µl containing 0.25 µM of EcoRI primer (Oligo 1), 250 µM each of dNTPs, 1 X PCR buffer, 1.5 mM MgCl2, and 1 U of Taq Polymerase in a thermocycler .The temperature regime consisted of an initial denaturation step of 94° C for 2 min, followed by 35 cycles of denaturing at 94° C for 30 s, annealing at 50° C for 30 s and elongation at 70° C for 60 s. 3 µl of the reaction product was checked on 1% agarose gel .
2. Selective amplification: The SAMPL selective primer was first labeled with γ^{32}P ATP (0.1 µl solution of 10 millicurie/ml, 3000ci/mmol) in presence of 0.5 U of PNK (Polynucleotide Kinase) and 1 X PNK buffer B. The final concentration of the labeled buffer was 10.0 µM.by incubating at 37° C for 30 min .The reaction was stopped by heating at 70° C for 5 min

Selective amplification was done with 2 µl of 1:10 diluted preamplified template in a reaction mix of 10 µl containing 0.5 µM of labeled primer, 0.5 µM of EcoRI primer (Oligo 1), 250 µM of each dNTPs, 1X buffer, 1.5 mM $MgCl_2$, and 4 U of Taq Polymerase. The thermocycler profile consisted of an initial denaturation at 94° C for 2 min, followed by 11 cycles of denaturing at 94° C for 30 s, annealing at 65° C for 30 s and elongation at 72° C for 60 s, followed by 25 cycles of denaturing at 94° C for 30 s, annealing at 56° C for 30 s and elongation at 72° C for 60 s and a final elongation of 72° C for 2 min.

3. After amplification the PCR products were mixed with 10 µl of the loading dye and denatured at 96° C for 3 min and aliquots of 2 µl were separated on 4% denaturing gel, dried on a gel drier at 80° C for 1 hour and exposed to X-ray film (Amersham) with intensifying screens overnight.

3.2.3.8 Microsatellite Primed - PCR (MP- PCR)

The selective primers which were used in the SAMPL technique were used as MP-PCR primers in this experiment. The amplification was done with 25 ng of genomic DNA in template in a reaction mix of 20 µl containing 2 µM of the primer, 250 µM of each dATP, dTTP, dCTP, dGTP, 1.5 mM MgCl2, 1X reaction buffer (10 mM Tris-HCl p^H 9.0, 50 mM KCl, 1.5 mM MgCl2) and 0.2 U of Taq DNA polymerase in a thermocycler with the temperature profile of 35 cycles of denaturing at 94° C for 2 min, annealing at 45° C for 1min30 s and elongation at 72° C for 1min30s and a final elongation of 72° C for 4 min. The PCR products were electrophoresed in a 2.0 % agarose gel in 1 X TBE at 80V for 3 h, stained with ethidium bromide and visualised by

illumination against UV light and recorded on a Polaroid film (*Polaroid*, Cambridge, USA).

3.2.3.9 Random Mixing of Microsatellite Flanking Primers (RMMFP)

It has been shown by Choumane *et al.* (2000) that STMS primer pairs from one species generate amplicons in another but these are aften non allelic. And also microsatellites are associated with repetitive elements in plants and animals. Based on these concepts Dr.Peter Winter, Plant Molecular Biology, Biocenter, Johann Wolfgang Goethe University, Frankfurt, Germany has developed a new technique known as Random mixing of Microsatellite Flanking Primers based on the assumption that this random mixing produces polymorphisms even across the species and genera.

Fifteen ng of the genomic DNA was amplified with 2 μM of each of the primers, 250 μM of each, dTTP, dCTP, dGTP, 25 μM of dATP, 0.03μM of α^{32} P dATP (0.1 μl solution of 10 millicurie/ml, 3000Ci/mmol), 1.5 mM MgCl2, 1Xreaction buffer (10 mM Tris-HCl p^{H} 9.0, 50 mM KCl, 1.5 mM MgCl2) and 0.2 U of Taq DNA polymerase in a thermocycler. Temperature profile consisted of initial denaturation at 96° C for 2 min, followed by 40 cycles of denaturing at 96° C for 20 s, annealing at 50° C for 23 s and elongation at 60° C for 40 s and a final elongation of 60° C for 5 min. After amplification the PCR products were mixed with 10 μl of the loading dye and denatured at 96° C for 3 min and aliquots of 2 μl were separated on 4% denaturing gel, dried on a gel drier at 80° C for 1 hour and exposed to X-ray film (Amersham) with intensifying screens overnight.

3.2.4 Sequencing of the amplicons

This helps to know the sequence information of the amplified products in which we are interested. The various steps in this are as follows.

1. **Agarose gel electrophoresis:**

The PCR reaction with the selected primer combinations (RMMFP) was done and run in 2% agarose gel. The same reaction was prepared twice in order to have more fragments for the sequencing reaction. The trays and combs used for gel electrophoresis were cleaned with 70% ethanol. The selected bands were cut from the Ethidium bromide stained gel, weighed and put in the eppendorf cup.

2. **DNA extraction:**

The DNA was extracted following the procedure given in QIA quick gel extraction kit protocol of Quiagen.

3. **Ligation of the extracted DNA in to vector pGemT:**

The DNA polymerase adds an overlapping A to the PCR-products. The vector pGemT has a linear structure with overlapping T-ends, which prevents the re-circulation of the vector and vector/vector ligation. As the A terminal in the DNA strand is complimentary to the T-ends of the vector the DNA fragment ligates to the vector at this position.

The concentration of DNA and the vector should be 1:1 in order to have the efficient ligation.

The extracted DNA was ligated to the pGemT vector in presence of 3U of T_4 DNA ligase, 1X reaction buffer, 10 mM of ATP and incubated at 4° C overnight. The reaction was stopped to inactivate the ligase by incubating at 65° C for 10 minutes.

4. **Transformation:**

The transformation was done in to the competent *E.coli* DH-5α-cells by electroporation. The bacteria containing the insert DNA can be selected by blue/white screening. The inserted fragment interrupts the gene sequence coding for β-galactosidase, which turns to blue colour by hydrolysation, thus remaining white in

colour. One of the inductors of the gene -galactosidase is IPTG (isopropylthiogalactosid) and X-Gal (50bromo-4-chloro3-indolyl-β-D-galactosid) is a substrate of β-galactosidase. The vector pGemT confers resistance to the antibiotic ampicillin.

Solutions required during transformation

SOC medium: SOC medium has the following substances.

2 % Bacto-Typton

0.5% Bacto-yeast-extract

0.05% NaCl

2.5 mM KCl

10 mM $MgCl_2$

20 mM Glucose

10 mM $MgSO_4$

p^H was adjusted to 7.0 with NaOH.

LB (Luria-Bertani)-Medium

1% (w/v) Bacto-Trypton

0.5% (w/v) Bacto-yeast-extract

1% (w/v) NaCl

p^H was adjusted to 7.0 with NaOH

LB-agar paltes with Ampicillin, X-Gal and IPTG:

1.5% Agar

100 $\mu g ml^{-1}$ Ampicillin

20 $\mu g ml^{-1}$ IPTG

20 $\mu g ml^{-1}$ X-Gal.

Preparation of LB-agar plates

Agar was added to the LB-medium and sterilized. After sterilization, the bottles containing the medium were kept in a water bath maintained at 65° C. At the time of preperation of the plates, Ampicillin, IPTG and X-Gal were added after LB-medium was around 60° C and poured in to the plastic plates.

The SOC medium was kept in a water bath at 37° C and the competent cells were taken out from the -80° C and kept in ice.

The ligation mixture was centrifuged and mixed slightly with the pipette. 2 µl of the ligation mix was pipetted in to 50 µl of the competent cells and then the ligation mix along with the competent cell are transferred to the electroporation cuvettes.

Electroporation was done with a BIORad Gene pulser at a voltage of 1.6 V and the resistance of 200 Ω.

750 µl of SOC-medium was added to the cuvettes immediately after electroporation and mixed slightly,which was then transferred to the eppendorf cup and incubated at 37° C for 60 – 90 minutes with mixing after every 15 min to prevent sinking of the cells to ground.

25-150 µl of the bacterial suspension was given to the agar plates and grown over night.

5. **Selection of white colonies:**

White colonies were selected and transferred with a sterile toothpick to a new plate and grown at 37° C over night.

6. **Colony-PCR1:**

A PCR was conducted to check the size of the cloned DNA. Each white single colony was picked with a new sterile tooth pick and transferred to the PCR cup containing 20 µl of sterile double distilled water and centrifuged shortly.

The colonies were incubated at 96° C for lysis of the cells and a PCR reaction was prepared with this cell suspension.

The primers used for the PCR reaction were derived from the flanking regions of the integretion site of the vector. The primers used were

SP 6: 5' – GAT TTA GGT GAC ACT ATA G – 3'

T7: 5' – TAA TAC GAC TCA CTA TAG GG – 3'

PCR was performed with 50 µl of the reaction mix containing 20 µl of the cell lysate, 1X reaction buffer (10 mM Tris-HCl p^H 9.0, 50 mM KCl, 1.5 mM MgCl2). 2.5 mM MgCl2, 150 µM of each dNTP, 0.2 µM of each primer and 0.4 U of Taq-polymerase. The thermocycler profile was an initial consisted of initial elongation of 96° C for 2 min, followed by 35 cycles of denaturing at 94° C for 20 s, annealing at 50° C for 23 s and elongation at 72° C for 1 min and a final elongation of 72° C for 5 min.

10 µl of the reaction was run on 1.2 % agarose gel and the rest of the solution was stored at 4° C or -20° C.

7. **Restriction of the amplicons:**

Twelve cloned from each transformation which had the correct insert size were selected and restriction was done with 10 µl out of 40 µl of the reaction of colony-PCR-1. The endonuclease used for restriction was HinfI (G↓ANTC). The restriction pattern shows whether the fragments of the same size contain the DNA of the same sequence.

Reaction mix:

10 µl colony-PCR 1

1 µl 10 X reaction buffer R (MBI Fermentas)

5 U Hinf 1

ddH_2O upto 20 µl

The reaction mix was incubated at 37° C for 2.5 hours and then the enzyme was inactivated by incubation at 72° C for 7 min. The entire reaction volume was used to run in the agarose gel.

8. **Colony-PCR-2:One clone of each restriction pattern were selected:**

The selected clones were grown in 3 ml of LB-mediun in a glass tube to which 3 µl of ampicillin was added. The clones were picked with a sterile toothpick and and transferred to the tube and incubated at 37° C over night by shaking them at a frequency of 140 revolutions /min.

1ml of each clone was taken in to the eppendorf cup and 100 µl out of 1 ml was incubated at 96° C for 5 minutes for cell lysis.

The cell lysate was centrifuged for 3 minutes at 4000rpm and the supernatant solution, which contains the DNA, was taken for colony-PCR 2.

The primers used in the reaction are also obtained from the integration site of the vector but farther away than the primers SP6 and T7.

The primers used were

M13 forward (VPCR1) : 5' – GTA AAA CGA CGG GCA GT – 3'

M13 reverse (VPCR2) : 5' – GGA AAC AGC TAT GAC CAT G – 3'.

The PCR conditions were same as that in colony PCR 1.

10 µl of the reaction was used to run in 1.2 % agarose gel to check the size of the insert.

9. **Sequencing reaction:**

Sequencing reaction was performed with a kit which had dideoxynucleotides. Each of the four bases was connected with a fluorescent molecule and the fluorescent DNA fragments were separated on a sequencing gel and detected by laser.

Template used for the sequencing reaction was the PCR product of colony-PCR 2. But before that, the PCR products were purified following a QIA quick PCR purification Kit protocol of Qiagen. Around 200 ng of DNA-Template was used for the sequencing reaction.

The primer used for the sequencing reation was T7.

10 µl of the reaction mix consisted of 200 ng of template DNA, 0.5 µM T7 primer and 4 µl of "Big dye Kit"(Has didieoxy nucleotides, reaction buffer, $MgCl_2$, Taq-polymerase). 10 mM Tris-HCl p^H 9.0, 50 mM KCl, 1.5 mM MgCl2) consisted of initial elongation of 96° C for 2 min, followed by 40 cycles of denaturing at 96° C for 20 s, annealing at 50° C for 23 s and elongation at 60° C for 40 s and a final elongation of 60° C for 5 min. After amplification the PCR products were mixed The temperature profile consisted of initial elongation of 96° C for 1 min, followed by 25 cycles of denaturing at 96° C for 10 s, annealing at 50° C for 5 s and elongation at 60° C for 4 min and a final elongation of 8° C for 5 min.

After the reaction the amplified fragments were precipitated and used for running in the sequencer (ABI 377 sequenator).

3.3 STATISTICAL ANALYSIS

The data were subjected to the statistical analysis. The procedures followed are described below.

3.3.1 Combined study of generations

The mean values foe each generation viz., P_1, P_2, F_1, F_2, BC_1P_1, BC_1P_2 and 126 F_{10} RILs were computed, and the variances were obtained from the deviations of the individual values from the pooled mean for each of the generation.

3.3.2 Generation mean analysis

Generation Mean Analysis was used to estimate the components of genetic variation. The testing of epistasis was necessary before estimation of the components of genetic variation because it helps in deciding the method of analysis for the components of variation.

3.3.2.1 Scaling test

Scaling test given by Mather (1949) was used to calculate the type of epistasis present in the expression of the characters under study.

$A = 2B_1 - P_1 - F_1$ $\quad VA = 4V(B_1) + V(P_1) + V(F_1)$

$B = 2B_2 - P_2 - F_1$ $\quad VB = 4V(B_2) + V(P_2) + V(F_1)$

$C = 4F_2 - 2F_1 - P_1 - P_2$ $\quad VC = 16V(F_2) + 4V(F_1) + V(P_1) + V(P_2)$

$D = 2F_2 - B_1 - B_2$ $\quad VD = 4VF_2 + VB_1 + VB_2$

The standard error of A,B, C, and D are calculated by taking the square root of respective variances and t values are calculated by dividing the effects of A,B,C and D by their respective standard error.

The calculated t values of these four tests were compared against 1.96 which is the table value of t at 5% level of significance. If the calculated value of these scales is higher than 1.96, it is considered significant and vice versa.

3.3.2.2 Six-parameter model

This method was used when non-allelic interactions were present as suggested by Hayman (1958).

The various gene effects and variances were estimated as following.

m = mean effect $=F_2$

d = additive effects = $B_1 - B_2$

h = dominance effects = $F_1 - 4F_2 - 1/2\ P_1 - 1/2P_2 + 2B_1 + 2B_2$

i = additive x additive gene interaction

$= 2B_1 - 2B_2 - 4F_2$

j = additive x dominance gene interaction

$= B_1 - 1/2\ P_1 - B_2 + 1/2\ P_2$

l = dominance x dominance gene interaction

$= P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2$

Where P_1, P_2, F_1, F_2, B_1, and B_2 are the mean values for the characters in P_1, P_2, F_1, F2, B_1, and B_2 populations.

The variances for the above gene effects are obtained as following.

$V_m = VF_2$

$V_d = VB_1 + VB_2$

$V_h = V\ F_1 + 4F_2 + 1/4VP_1 + 1/4VP_2 + 4VB_1 + 4VB_2$

$V_i = 4VB_1 + 4VB_2 + 16VF_2$

$V_j = VB_1 + 1/4V\ P_1 + VB_2 + 1/4V\ P_2$

$V_l = VP_1 + VP_2 + 4VF_1 + 16VF_2 + 16VB_1 + 16VB_2$

The standard errors, variances and t values were calculated. The calculated t values of these tests were compared against 1.96 which is the table value of t at 5% level of significance. If the calculated value of these scales is higher than 1.96, it is considered significant and *vice versa*.

3.3.3 Heritability

Heritability in RILs was calculated using the formula given by Falconer (1989)

$$h^2 = \frac{VG}{VP} \times 100$$

h^2 = Heritability

VG = Genotypic variance

VP = Phenotypic variance

Broad sense heritability of different generations was estimated according to Waldia *et al.* (1992).

$$h^2_{bs} = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100 = \frac{Vg}{VF_2} \times 100$$

$Vg = VF_2 - VE$

$$VE = \frac{VP_1 + VP_2 + VF_1}{3}$$

VE = variance of environment

VP_1=variance of parent one

VP_2=variance of parent two

VF_1=variance of F_1

VF_2=variance of F_2

The heritability in narrow sense was calculated utilizing the formula suggested by Warner (1952)

$$h^2_{ns} = \frac{1/2D}{VF_2} \times 100$$

D = additive variance

VF_2 = phenotypic variance of a trait in F_2 generation.

Additive genetic variance was calculated by the formula given by Fehr (1987)

$(1/2)\ VD = 2VF_2 - (VBC_1P_1+VBC_1P_2)$

VF_2 = variance in F_2 population

VBC_1P_1 = Variance in the back cross population with parent one

VBC_1P_2 = Variance in the back cross population with parent two

3.3.4 Coheritability

The coheritability of different characters was calculated by as per Janssens (1979).

$$\text{Coheritability } (X_1,X_2) = \frac{GCov(X_1X_2)}{PCov(X_1X_2)}$$

GCov = genotypic covariance of the character X_1 and X_2

PCov = phenotypic covariance of the character X_1 and X_2

3.3.5 Heterosis

Heterosis was calculated by using the following formula (Fehr, 1987)

$$\text{Mid parent heterosis (\%)} = \frac{F_1 - MP}{} \times 100$$

MP

$$\text{Better parent heterosis (\%)} = \frac{F_1 - BP}{BP} \times 100$$

F_1 = average performance of the Hybrid

MP = average performance of both the parents.

BP = average performance of the better parent.

3.3.6 Chi-square analysis

Chi-square test (χ^2) to find the goodness of fit was calculated as given by Panse and Sukhatme (1967)

$$\chi^2 = \Sigma \frac{(O-E)^2}{E} = \frac{d^2}{E}$$

Where Σ = summation

O = Observed frequencies

E = Expected frequencies

d = deviation of observed and expected frequencies.

Calculated Chi-square values are compared with table values given by Fisher and Yates (1963) for appropriate degrees of freedom (n-1).

3.3.7 Estimation of degree of dominance

The degree of dominance was calculated using the following procedure.

$$\tfrac{1}{2} D + \tfrac{1}{4} H = 2VF_2$$

$$\tfrac{1}{2} D + \tfrac{1}{2} H = VBC_1P_1 + VBC_1P_2$$

By solving the above two equations the value of H and D are obtained.

The degree of dominance is calculated as $\sqrt{H/D}$.

3.3.8 Test of significance of mean

Test of significance is a procedure for distinguishing whether the observed difference corresponds to any real difference among the genotypes or can be ascribed to mere sampling fluctuations.

The test of significance is a method of making due allowance for the sampling fluctuations affecting the results of experiments or observations.'t' and 'z' tests (based on the sample size) are used to test the significance of difference between two means. In the present study t test was used to test the significance of means for the quantitative characters studied among the parents (Kapur and Saxena, 1969).

$$t = \frac{| P_1 - P_2 |}{S\sqrt{1/n_1 + 1/n_2}}$$

If sample size is <30.

P_1 = mean of parent 1

P_2 = mean of parent 2

S = Standard deviation of the population.

n_1 and n_2 = the size of the samples for parent 1 and 2 repectively.

$$S = \frac{1}{n_1 + n_2 - 2} [(n_1 - 1) S_1^2 + (n_2 - 1) S_2^2]$$

S_1 and S_2 are the standard deviation for parent 1 and 2 respectively.

3.3.9 Correlation coefficient

Changes in one variable may be accompanied by in another and that a relation exists between the two, which indicates the correlation between the two variables. Correlation coefficient (r) is the measure of the direction and degree of closeness of the linear relationship between two variables.

Simple correlation coefficients among different characters studied were worked out using the formula suggested by Panse and Sukhatme (1967)

$$\text{Correlation coefficient } (r) = \frac{\sigma XY}{\sigma X \,.\, \sigma Y}$$

$$\sigma XY = \frac{\Sigma f(x-x)(y-y)}{N} = \frac{\Sigma f \,.\, dx \,.\, dy}{N}$$

σXY = Mean product moment or the co variance between X and Y

σX = standard deviation of X

σY = standard deviation of Y

dx and dy = deviations.

$$\sigma x = \frac{\Sigma fdx^2}{N}$$

$$\sigma y = \frac{\Sigma fdy^2}{N}$$

Significance of correlation coefficient

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

r is the estimate obtained from n pairs. The significance of correlation was tested by referring to the standard 't' table given by Snedecor and Cochran (1968) at 5% and 1% level of significance.

3.3.10 REML (Residual Error Maximum Likelihood) variance component analysis

The RILs were sown in an unbalanced incomplete block design (IBD). The variance components for all the characters of 126 F_{10} RILs and two parents were analyzed using REML which is also known as Restricted Maximum Likelihood model proposed by Patterson and Thompson (1971)

The model (using matrix and vector notation)

$Y = X\alpha + Z\beta + e$

Where

Y = vector of data (length n)

α = Vector of fixed effects (length p) with n x p design matrix x

β = vector of random effects (length q) with n x q design matrix Z

e = vector of random error (length n)

More generally, the random model $Z\beta$ is constructed from C model terms. Z and β can be partitioned as $Z = \{ Z_1 \mid Z_2 \mid \text{---} Z_c \}$ and $\beta = \{ \beta_1, \beta_2, ..., \beta_c \}$ where β_i is a vector of length q_i. The model in separate random terms can be written as

$$Y = X\alpha + {}^{c}\Sigma Z_i \beta_i + e.$$

It is assumed that random effects β_1 and e are mutually independent, normally distributed random variables with zero means. The REML algorithm estimates the variance components using residual maximum likelihood. The algorithm of likelihood function of y is given by

$$L = \text{const} - \tfrac{1}{2}\log|H| - \tfrac{1}{2}n\log T^2 - \tfrac{1}{2} T^2 (Y-X\alpha)^1 H^{-1} (Y-X\alpha)$$

Where H is essentially non singular matrix and T^2 is unknown scalar.

The REML then uses the variance parameter to form the generalized least square estimates of the treatment effects and the best linear unbiased predictors (BLUPs) of random effects.

The test of significance was done by dividing the REML estimated Variance components by twice of its respective standard error (SE) and referring to standard Z table values given by Fisher and Yates (1963) at 5% and 1% levels of probability.

3.3.11 Analysis of variance

To study the significant differences among the 126 RILs for the root characters, the data were analysed by using completely randomised desigh as described by Panse and Sukhatme (1967).

3.3.12 Linkage analysis

Data on certain morphological characters (double podding, seed size, stem colour, seed coat colour, flower colour, and seed type) which were recorded during early seasons for the same material was utilized along with the data on morphological characters and fusarium wilt resistance recorded during the present study in order to construct the linkage map.

MAPMAKER V2.0 (Lander *et al.* 1987) was used for linkage analysis. Loci were first divided into linkage groups at a LOD-score of 4 by two point analysis using the 'group' command. Marker order in linkage group was determined using the 'try' command of the program. Marker orders obtained were confirmed by multipoint analysis applying the 'ripple' function. Final map distances were calculated by applying the 'Kosambi' function (Kosambi, 1944) provided by the program.

RESULTS

CHAPTER IV

RESULTS

The results of the present investigation on "Inheritance of shoot and root characters and molecular markers in chickpea (*Cicer arietinum* L.)" are presented here under the following headings.

4.1 Experiment I (Field studies)

4.2 Experiment II (Pot culture studies)

4.3 Experiment lll (Molecular marker studies)

4.1 EXPERIMENT I (Field Studies)

An experiment was conducted during *Rabi* (post-rainy season) 1999/2000 to study the inheritance of growth habit and podding duration in chickpea (*Cicer arietinum* L.). The studies were conducted on parents, F_1, F_2, F_3, BC_1P_1, BC_1P_2 generations and 126 F_{10} random RILs of the cross ICCV 2 and JG 62 and data was recorded on days to first flower, days to first pod, days to maturity and number of nodes up to first flower. The results of this experiment are as follows.

4.1.1.1 Inheritance of growth habit

The difference between the parents for the growth habit was very negligible and it was not possible to differentiate them. Hence this character was not considered.

4.1.1.2 Inheritance of days to first flower

The mean number of days to first flower in parents ICCV 2 and JG 62 were 34.8±0.70 days and 44.5±0.29 days respectively. The days mean number to first flower in F_1 (42.1±0.31 days) was later than the mid-parental value (39.7 days) and was nearer

Table 5: Mean values of various characters in different generations of the cross ICCV 2 (P_1) x JG 62 (P_2)

Character	ICCV 2 (P_1)	JG 62 (P_2)	F_1	F_2	BC_1P_1	BC_1P_2	Mid-parent value
Days to first flower	34.8 ± 0.70	44.5 ± 0.29	42.1 ±0.31	41.95 ±0.40	40.0 ±1.04	46.0 ±0.65	39.7
Days to first pod	41.0 ±0.92	50.6 ±0.47	48.4 ±0.39	47.8 ±0.41	46.0 ±1.10	51.7 ±0.68	45.83
Days to maturity	84.5 ±0.218	98.7 ±0.58	93.9 ±0.66	92.6 ±0.38	89.3 ±1.11	95.6 ±0.37	91.67
Pod initiation period (days)	6.1 ±0.20	6.1 ±0.44	6.3 ±0.41	5.8 ± 0.26	6.0 ±1.03	5.7 ±0.67	6.1
Pod filling period (days)	43.5 ±0.82	48.1 ±0.98	45.5 ±0.69	45.1 ±0.21	43.5 ±0.62	44.0 ±0.64	45.84
Total reproductive period (days)	49.7 ±0.59	54.2 ±0.83	51.8 ±0.67	51.0 ±0.21	49.6 ±0.60	49.7 ±0.62	51.96
Number of nodes up to first flower	18.0	23.0	23.0	20.0	18.0	23.0	20.79

to that of the late parent (JG 62). The days to first flower in F_2 generation varied from 28 days to 58 days and the mean was 41.9±0.40 days. The mean number of days to first flowering in BC_1P_1 was 40±1 days while in BC_1P_2 it was 46±0.6 days (Table 5).

The gene effects and type of interactions are presented in Table 6 and Table 7. The A, B, C values calculated using Mather's formulae deviated significantly from zero. The estimates of six parameters along with their standard error are presented in Table 7. The estimates of three interaction parameters deviated significantly from zero. The most significant gene actions governing this character were additive gene action, dominance gene action and dominance x dominance gene action. Dominance gene action was expressed positively and dominance x dominance gene action was expressed negatively for this character.

The distribution pattern of days to first flower in F_2 population was near normal (Fig 2). The two major peaks coincided with the time of flowering of ICCV 2 and JG 62 suggesting the presence of one major gene and some minor genes. The range of F_2 population extended beyond both the parents.

4.1.1.3 Inheritance of days to first pod

The mean number of days to first pod in parents ICCV 2 and JG 62 were 41.0±0.92 days and 50.6±0.47 days respectively. In F_1 the first pod formed in 48.4±0.39 days and was closer to JG 62. The mean number of days to first pod of F_2, BC_1P_1 and BC_1P_2 generations were 47.8±0.41 days, 46.0±1.18 days and 51.7±0.68 days respectively (Table 5).

The A, B, and C values deviated significantly from zero indicating the inadequacy of additive-dominance model and the presence of non-allelic gene actions. The estimates of six-parameter model and their standard errors indicated the presence of all the three types of gene interactions (Table 7). Both additive and dominant gene actions were

Table 6: Estimates of A, B, C and D values obtained by Mather's formulae for various characters studied in chickpea

Character	A	B	C	D
Days to first flower	3.0 ±2.24	5.3* ±1.36	4.2* ±1.88	-1.4 ±1.47
Days to first pod	-3.1 ±2.40	-0.7 ±1.47	-8.0* ±2.06	-1.4 ±1.51
Days to maturity	-1.3 ±1.06	0.2 ±2.52	-0.6 ±2.07	0.2 ±1.89
Pod filling period	-1.8 ±1.46	-5.4* ±1.48	-2.2 ±2.00	2.9* ±0.93
Total reproductive period	-2.1 ±1.45	-6.4* ±1.63	-3.1* ±1.57	3.1* ±0.15
Number of nodes up to first flower	-4.5* ±1.48	0.4 ±0.95	-6.8* ±1.41	1.5 1.53

* significance at 5% level.

Table 7: Estimates of the additive, dominance and interaction parameters and type of epistasis for different characters in chickpea.

Character	m	d	h	i	j	l	Type of epistasis
Days to first flower	41.9* ±0.40	-6.0* ±1.23	6.5* ±3.00	4.1 ±2.96	-1.1 ±1.29	-12.5* ±5.30	Duplicate
Days to first pod	41.5* ±0.17	4.8* ±1.28	18.2* ±3.13	4.2 ±3.04	1.6 ±4.36	-11.2* ±5.54	Duplicate
Days to maturity	92.6* ±0.38	6.3* ±1.27	1.7 ±3.04	-0.5 ±2.90	-0.7 ±1.22	1.6 ±5.49	Duplicate
Pod filling period	45.1* ±0.04	0.4 ±0.84	-5.5* ±2.10	-5.1* ±1.90	-1.9 ±1.02	12.5* ±3.94	Duplicate
Total reproductive period	51.0* ±0.04	0.08 ±0.831	-5.2* ±2.03	-4.6* ±1.86	2.2* ±0.95	13.7* ±3.67	Duplicate
Number of nodes up to first flower	20.5* ±0.24	-5.1* ±0.77	5.6* ±1.90	-3.2 ±1.83	-2.5* ±0.82	1.2 ±3.41	Complementary

* significance at 5% level.

Fig 2: Distribution of days to first flower expressed as percentage of individuals in the F population during *rabi* 1999/2000

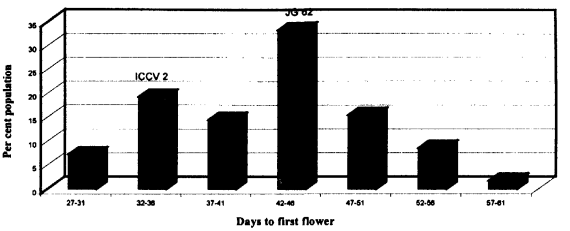
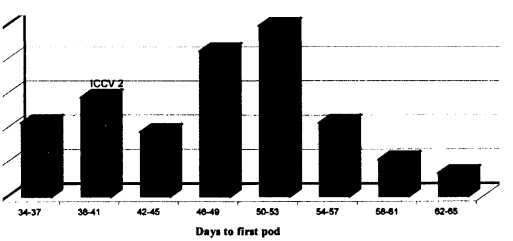


Fig 3: Distribution of days to first pod expressed as percentage of individuals in the F population during *rabi* 1999/2000



present but dominant gene action was more significant. Among the three interactions additive x additive interaction and dominance x dominance interaction were significant. Dominance gene action expressed positively and dominance x dominance expressed negatively.

The distribution pattern in F_2 population was continuous and near normal (Fig 3).

4.1.1.4 Inheritance of days to maturity

The mean number of days to maturity in ICCV 2 and JG 62 were 84.5 ± 0.21 days and 98.7 ± 0.58 days respectively. F_1 matured in 93.9± 0.66 days. F_2, BC_1P_1 and BC_1P2 generations matured in 92.6 ± 0.38 days, 89.3 ± 1.11 days and 95.6 ± 0.37 days respectively (Table 5).

The deviations of B and C values from zero were not significant (Table 6). Even though the A value deviated from zero, the results of six-parameter model gave non-significant estimates of all interaction effects. Both additive and dominance gene actions were present but the most significant gene action was additive gene action (Table 7). The distribution pattern of days to maturity indicated a continuous variation in F_2 population (Fig 4).

When the individuals in F_2 population which matured in more than 90 days were grouped into one class and those that matured in less than 90 days were grouped into another class, they fitted well to 3: 1 ratio of late maturing individuals: early maturing individuals. This was further confirmed by 1: 1 segregation of backcross progeny (BC_1P_1) into early and late maturing individuals (Table 12).

4.1.1.5 Inheritance of podding duration

4.1.1.5.1 Pod initiation period

The pod initiation period was similar in ICCV 2 (6.14±0.20 days) and JG 62 (6.11±0.44 days). It was 6.3±0.41 days in F_1 and 5.8±0.26 days in F_2 (Table 5). As there was no statistical difference between the parents for this character, further analysis was not carried out.

4.1.1.5.2 Pod filling period

The pod filling period in ICCV 2 was 43.5± 0.82 days and in JG 62 it was 48.1±0.96 days. It was 45.5±0.69 days F_1, 45.5±0.62 days in BC_1P_1 and 44.0±0.60 days in BC_1P_2 (Table 5).

The A, B, C and D values showed significant deviations from zero revealing that additive-dominance model was not sufficient to explain the genetic variation for this character (Table 6). The estimates obtained from six-parameter model indicate that all the three types of interactions were present. The most significant gene actions that govern this character were dominance main effect, additive x additive gene interaction and dominance x dominance gene interaction. Dominance gene action expressed negatively and dominance x dominance gene action expressed positively for this character (Table 7).

4.1.1.5.3 Total reproductive period

The total reproductive period for each of the six generations were 49.7±0.59, 54.2±0.83, 51.8±0.67, 51±0.2, 49.6±0.60 and 49.7±0.62 days respectively (Table 5).

The significant deviation of A, B, C and D values from zero indicate the presence of interaction gene effects (Table 6). The estimates of six-parameter model indicate the presence of all the three types of interactions. The significant gene actions were

Fig 4: Distribution of days to maturity expressed as percentage of individuals in the F population during *rabi* 1999/2000

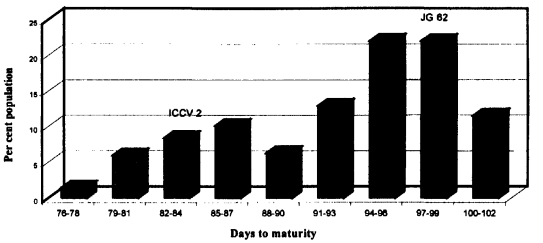
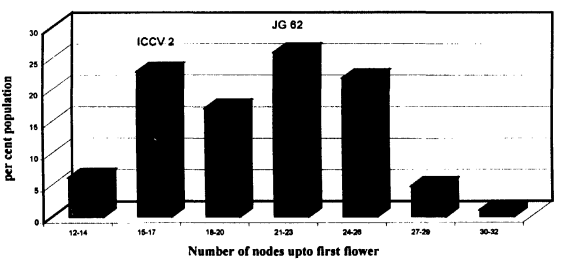


Fig 5: Distribution of number of nodes upto first flower as percentage of individuals in the F population during *rabi* 1999/2000



dominance main effect and all the three interaction effects. Dominance gene action expressed negatively and dominance x dominance gene action expressed positively for total reproductive period (Table 7).

4.1.1.6 Inheritance of number of nodes up to first flower

In parent ICCV 2, the first flower formed at 18th node and in JG 62 at 23rd node. The first flower in F_1 formed at 23rd node which was similar to that of parent JG 62. The node number at which the first flower formed in F_2, BC_1P_1 and BC_1P_2 was 20th node, 18th node and 23rd node respectively (Table 5).

The deviation of A and C values from zero revealed the inadequacy of additive-dominance model to explain the genetic variation for this character (Table 6). All the three interaction estimates deviated from zero indicating all the three types of interactions. The most significant gene actions that govern this character were additive, dominance, and additive x dominance gene actions. Both dominance and dominance x dominance gene actions expressed positively for number of nodes up to first flower (Table 7).

The individuals in F_2 population, which had flower at 21st node and above were classified into one group and that had flower at 20th node and lower into another group, gave a good fit of 9: 7 ratio. The segregation of F_2 was further confirmed by the segregation of the BC_1P_1 which gave a good fit to 1: 3 segregation of the individuals with flower at higher node to the individuals with flower at lower node with a χ^2 value of 2.026 (Table 12). The continuous variation found in F_2 population supported the polygenic control of number of nodes up to first flower (Fig 5).

4.1.1.7 Components of variation in F_2 and backcrosses

4.1.1.7.1 Days to first flower

The variance in ICCV 2, JG 62 and F_1 was 3.47, 0.77 and 0.98.The variance in the F_2 population was higher (49.66) than the variance in both the parents. The variance in BC_1P_1 was 41.07 and was higher than the variance in BC_1P_2 (14.84). The H and D values and degree of dominance are presented in Table 8.The degree of dominance was 0.45. The value of $\sqrt{HD}$ was not equal to the differences in the variances of the backcrosses (Table 9).

4.1.1.7.2 Days to first pod

The variance in ICCV 2, JG 62 and F_1 was 6, 2 and 1.6 respectively. The variance in the F_2 population (52.27) was more than the variances in both the parents (Table 8). The variance in BC_1P_1 was 44.17 and in BC_1P_2 was 15.28. The degree of dominance was 0.40 and the value of $\sqrt{HD}$ deviated from the differences between the variances of backcrosses (Table 9).

4.1.1.7.3 Days to maturity

The variance observed in ICCV 2, JG 62 and F_1 was 0.61,0.69 and 4.48. High variance in the F_2 population (41.32) over both the parents was observed for this character. The variance in the backcross to JG 62 was lower than the variance in the backcross to ICCV 2 (Table 8). The degree of dominance was 0.92 and the $\sqrt{HD}$ value was not equal to the differences between the variances of two backcrosses (Table 9).

4.1.1.7.4 Podding duration

The variances for the characters pod filling period and total reproductive period in ICCV 2, JG 62 and F_1 were 2.95, 8.36, 4.84 and 1.23, 6.25 and 4.50 respectively. The

Table 8: Variances observed for various characters in different generations of the cross ICCV 2 x JG 62

Character	ICCV 2 (P_1)	JG 62 (P_2)	F_1	F_2	BC_1P_1	BC_1P_2
Days to first flower	3.47	0.77	0.98	49.66	41.07	14.84
Days to first pod	6.00	2.00	1.60	52.27	44.17	15.28
Days to maturity	0.61	0.69	4.48	41.32	49.82	4.07
Pod filling period (days)	2.95	8.36	4.84	13.21	10.55	14.00
Total reproductive period (days)	1.23	6.25	4.50	12.65	10.80	13.00
Number of nodes up to first flower	1.14	1.52	1.78	17.50	16.29	4.86

Table 9: The estimates of D, H, $\sqrt{h/d}$, $\sqrt{HD}$ and VBC_1P_1-VBC_1P_2 for different characters

Character	H	D	$\sqrt{h/d}$	$\sqrt{HD}$	VBC_1P_1-VBC_1P_2
Days to first flower	18.08	93.78	0.45	39.00	26.22
Days to first pod	15.90	96.59	0.40	18.47	28.89
Days to maturity	47.09	55.22	1.92	51.91	44.62
Pod filling period (days)	23.78	3.77	2.50	9.40	3.45
Total reproductive period (days)	25.38	4.62	2.33	10.78	3.00
Number of nodes up to first flower	8.76	27.68	1.56	15.53	11.43

variance in the F_2 population for both characters was 13.21 and 12.65 and higher than the variances in the parents (Table 8). The variances in both the backcross generations were found to be almost equal. The degree of dominance for both the characters was 2.50 and 2.51 respectively. The √HD value was not equal to the difference of variance of two backcrosses (Table 9).

4.1.1.7.5 Number of nodes up to first flower

The variance in ICCV 2 and JG 62 and F_1 for number of nodes up to first flower was 1.14,1.52,1.78 respectively. F2 (17.50) variance was higher than the variances in both the parents. The variance in the backcross to JG 62 was lower (4.86) than the variance in the backcross to ICCV2 (16.29) (Table 8). The degree of dominance was 0.56. And the √HD value was not equal to the difference in the variance of two backcrosses (Table 9).

4.1.1.8 Heterosis

The magnitude of mid-parent and better parent heteroses for days to first flower, days to first pod, days to maturity, podding duration and number of nodes up to first flower are presented in Table 10.

4.1.1.8.1 Days to first flower

The F_1 value of days to first flower was in between mid-parental value and JG 62 value. This shows partial dominance, which was also reflected by the low mid-parental heterosis (6.03%). The better parent heterosis was 20.80%.

Table 10: Mid parent and better parent heterosis for different characters studied in F_1 of the cross ICCV 2 x JG 62

Character	Mean of ICCV 2 (BP)	Mean of JG 62	Mean of mid parent	Mean of F_1	Mid parent heterosis (%)	Better parent heterosis (%)
Days to first flower	34.8	44.5	39.7	42.1	6.03	20.90
Days to first pod	41.0	50.6	45.8	48.4	5.60	18.04
Days to maturity	84.5	98.7	91.6	93.9	2.51	11.12
Pod filling period	43.5	48.1	45.8	45.5	-0.65	4.59
Total reproductive period	49.7	54.2	51.9	51.8	-0.19	4.22
Number of nodes up to first flower	18.1	23.4	20.7	23.7	14.49	30.63

4.1.1.8.2 Days to first pod

The F_1 value of days to first pod was present between the mid-parent value and the value of JG 62 indicating partial dominance for this character. Mid-parental heterosis was 5.6% and better parental heterosis was 18.04%.

4.1.1.8.3 Days to maturity

The average value of F_1 was in between mid parental value and the value of JG 62. The mid-parental heterosis was low (2.4%) and better parental value was positive and 11.03%.

4.1.1.8.4 Podding duration

The mid-parental heterosis for pod filling period and total reproductive period was very low and negative (-0.74% and -0.32%). The better parental heterosis value for both the characters was 4.42% and 4.19%.

4.1.1.8.5 Number of nodes up to first flower

The mean number of nodes up to first flower in F_1 was equal to the mean number of nodes up to first flower in JG 62.This indicated the presence of complete dominance for this character. Both mid-parent (13.99%) and better parent (30.63%) heteroses were positive.

4.1.1.9 Studies based on recombinant inbred lines

4.1.1.9.1 Days to first flower

The residual maximum likelihood (REML) variance component for the RILs and parents showed major differences among the RILs with respect to days to first flower. Replication variance was non-significant (Table 11).

Table 12: Chi-square values for different characters in F_2, backcross and RIL population

Character	Expected Ratio	χ^2	Probability
Days to first flower (RIL)	1(E) : 1(L)	2.56	0.1-0.2
Days to first pod (RIL)	1(EP) : 1(LP)	0.80	0.3-0.5
Days to maturity (RIL)	1(EM) : 1(LM)	1.54	0.2-0.3
Days to maturity (F_2)	1(EM) : 3(LM)	3.64	0.05-0.1
Days to maturity (BC_1P_1)	1(EM) : 1(LM)	0.24	0.5-0.7
Number of nodes up to first flower (RIL)	1(HN) : 2(LN)	1.27	0.2-0.3
Number of nodes up to first flower (F_2)	9(HN) : 7(LN)	1.86	0.2-0.3
Number of nodes up to first flower(BC_1P_1)	1(HN) : 3(LN)	2.03	0.1-0.2
Leaflet number	3(H) : 1(L)	1.58	0.2-0.3
Leaflet number	1(H) : 1(L)	2.23	0.1-0.2

E - early flowering
L - late flowering
EP - early podding
LP - late podding
EM - early maturing
LM - late maturing
LN - flower initiation at lower node
HN - flower initiation at higher node
H - high leaflet number
L - low leaflet number

Fig 6: Distribution of days to first flower expressed in percentage of individuals in RIL population during *rabi* 1999-2000

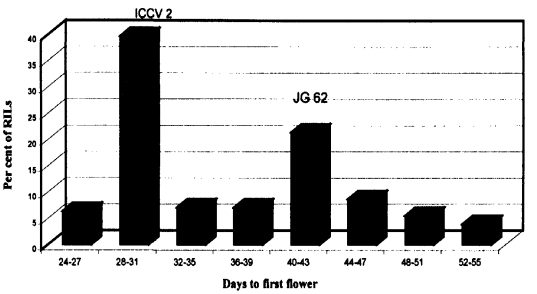
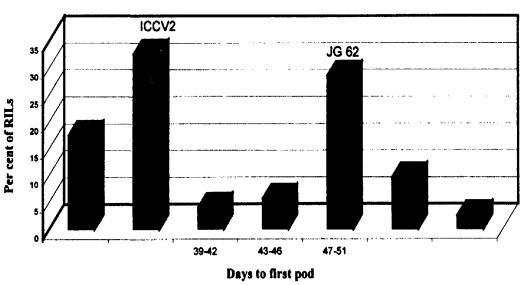


Fig 7: Distribution od days to first pod expressed in percentage of individuals in RILs during *rabi* 1999-2000



The distribution for days to first flower for RIL population was continuous and the two major peaks corresponded to the time of flowering of ICCV 2 and JG 62 (Fig 6). When the RILs were classified in to two groups, one that flowered earlier than 38 days and the other after 39 days, a 1: 1 ratio was obtained with a χ^2 value of 2.56 which was non-significant at 5% level of significance (Table 12).

4.1.1.9.2 Days to first pod

The residual maximum likelihood (REML) variance component for the RILs and parents was highly significant and replication variance was non-significant (Table 11).

There was bimodal distribution for days to first pod. 52% of RILs belonged to the group which podded earlier and 44% of RILs which podded later and 4% were in between both of them (Fig 7). The χ^2 value (0.8) for the segregation of RILs in 1: 1 ratio for early podding and late podding was non-significant (Table 12).

4.1.1.9.3 Days to maturity

The residual maximum likelihood (REML) variance component for RIL and parents was highly non-significant and replication variance was non-significant (Table 11).

The distribution of days to maturity for RILs was found to be bimodal with 55.55% of RILs maturing along with with ICCV 2 and 44.44% maturing along with JG 62 (Fig 8). The χ^2 value for 1: 1 segregation of RILs into early maturing and late maturing (1.54) was non- significant at 5% and 1% level indicating the role of a major gene in regulating maturity time (Table 12).

Table 11: Table showing differences among the RILs for various characters in chickpea

S.No	Days to first flower	Days to first pod	Days to maturity	Pod filling period	Total reproductive period	Number of nodes up to first flower
1	41.0	48.0	90.0	42.0	49.0	20
2	30.0	35.0	81.0	46.0	51.0	16
3	31.0	37.0	85.0	48.0	54.0	17
4	33.5	40.0	79.0	39.0	45.5	18
5	31.0	37.0	80.0	43.0	49.0	18
6	46.0	51.0	97.0	46.0	51.0	25
7	33.6	41.0	84.0	43.0	50.4	17
8	39.0	45.0	81.0	36.0	42.0	15
9	28.0	34.7	79.0	44.3	51.0	15
10	34.0	40.0	87.0	47.0	53.0	22
11	45.5	51.0	101.0	50.0	55.5	24
12	28.0	34.0	79.0	45.0	51.0	16
13	42.0	48.0	96.0	48.0	54.0	22
14	31.5	36.7	82.0	45.3	50.5	16
15	32.0	37.0	89.0	52.0	57.0	17
16	41.0	48.0	96.0	48.0	55.0	22
17	45.0	51.0	96.0	45.0	51.0	24
18	43.0	49.0	99.0	50.0	56.0	22
19	43.0	49.0	97.0	48.0	54.0	25
20	43.0	49.0	99.0	50.0	56.0	24
21	45.0	50.0	99.0	49.0	54.0	24
22	25.0	30.0	77.0	47.0	52.0	13
23	27.0	33.6	77.0	43.4	50.0	16
24	30.0	35.7	85.0	49.3	55.0	16
25	38.5	34.8	76.0	41.2	37.5	16
26	28.0	34.0	79.0	45.0	51.0	15
27	45.3	50.0	99.0	49.0	53.7	24
28	45.0	52.6	99.0	46.4	54.0	25
29	41.5	48.0	101.0	53.0	59.5	24
30	41.0	47.0	98.0	51.0	57.0	23
31	28.5	34.0	76.0	42.0	47.5	15
32	28.0	34.7	80.0	45.3	52.0	15
33	29.0	34.0	80.0	46.0	51.0	16
34	30.0	36.0	79.0	43.0	49.0	17
35	41.0	48.0	96.0	48.0	55.0	23
36	39.0	46.5	91.0	44.5	52.0	20
37	44.3	49.6	100.0	50.4	55.7	24
38	43.0	49.0	100.0	51.0	57.0	24
39	41.0	49.0	96.0	47.0	55.0	19

Cont..

Table 11 Cont..

40	29.0	33.0	76.0	43.0	47.0	15
41	28.0	32.0	79.0	47.0	51.0	15
42	28.8	35.0	82.0	47.0	53.2	15
43	48.3	54.6	100.0	45.4	51.7	26
44	48.0	54.0	101.0	47.0	53.0	25
45	24.0	30.0	77.0	47.0	53.0	13
46	30.0	36.0	83.0	47.0	53.0	15
47	28.0	35.0	76.0	41.0	48.0	15
48	31.0	37.7	81.0	43.3	50.0	17
49	30.0	36.0	82.0	46.0	52.0	16
50	31.0	37.0	85.0	48.0	54.0	17
51	32.0	36.5	83.0	46.5	51.0	17
52	29.0	34.0	80.0	46.0	51.0	15
53	45.5	51.0	98.0	47.0	52.5	24
54	48.0	54.6	100.0	45.4	52.0	25
55	43.0	49.0	98.0	49.0	55.0	23
56	41.0	47.0	97.0	50.0	56.0	22
57	55.0	60.0	105.0	45.0	50.0	30
58	25.0	30.3	80.0	49.7	55.0	12
59	38.0	44.5	96.0	51.5	58.0	20
60	51.0	56.0	101.0	45.0	50.0	28
61	42.0	48.5	99.0	50.5	57.0	21
62	48.0	54.0	98.0	44.0	50.0	27
63	48.0	54.0	99.0	45.0	51.0	25
64	27.8	33.6	80.0	46.4	52.2	14
65	45.3	53.0	101.0	48.0	55.7	27
66	41.7	49.0	97.0	48.0	55.3	23
67	31.0	36.0	87.0	51.0	56.0	17
68	31.0	35.5	85.0	49.5	54.0	17
69	32.0	36.0	83.0	47.0	51.0	19
70	48.0	54.0	99.0	45.0	51.0	26
71	41.0	48.7	95.0	46.3	54.0	20
72	53.5	59.0	103.0	44.0	49.5	26
73	34.0	40.0	93.0	53.0	59.0	18
74	24.0	30.6	79.0	48.4	55.0	13
75	28.0	33.0	78.0	45.0	50.0	15
76	29.0	35.5	80.0	44.5	51.0	17
77	31.0	38.0	80.0	42.0	49.0	17
78	28.9	35.0	79.0	44.0	50.1	15
79	32.0	38.0	83.0	45.0	51.0	18
80	38.0	43.5	91.0	47.5	53.0	18

Cont..

Table 11 Cont..

81	29.0	35.0	77.0	42.0	48.0	14
82	41.0	48.5	97.0	48.5	56.0	24
83	30.0	35.0	96.0	61.0	66.0	16
84	27.0	33.0	76.0	43.0	49.0	15
85	41.0	48.0	97.0	49.0	56.0	23
86	31.0	36.0	79.0	43.0	48.0	17
87	29.0	35.5	78.0	42.5	49.0	17
88	31.0	36.3	81.0	44.7	50.0	18
89	43.0	49.0	97.0	48.0	54.0	23
90	28.0	36.0	97.0	61.0	69.0	15
91	43.5	49.5	101.0	51.5	57.5	22
92	45.0	51.0	100.0	49.0	55.0	25
93	38.0	46.0	92.0	46.0	54.0	20
94	45.5	51.0	96.0	45.0	50.5	25
95	38.0	46.5	90.0	43.5	52.0	19
96	41.0	48.0	89.0	41.0	48.0	20
97	27.0	35.0	79.0	44.0	52.0	15
98	35.0	41.0	89.0	48.0	54.0	19
99	25.0	30.0	77.0	47.0	52.0	13
100	54.0	59.0	89.0	30.0	35.0	27
101	31.0	37.0	83.0	46.0	52.0	18
102	43.0	49.0	96.0	47.0	53.0	24
103	27.6	34.3	80.0	45.7	52.4	15
104	31.3	37.7	81.0	43.3	49.7	16
105	29.5	34.0	79.0	45.0	49.5	15
106	30.3	35.0	81.0	46.0	50.7	17
107	30.7	38.0	81.0	43.0	50.3	17
108	31.0	36.7	80.0	43.3	49.0	17
109	41.0	47.3	95.0	47.7	54.0	22
110	41.0	48.0	96.0	48.0	55.0	20
111	49.0	55.0	99.0	44.0	50.0	25
112	43.0	49.0	96.0	47.0	53.0	23
113	29.0	35.0	79.0	44.0	50.0	18
114	31.0	37.0	81.0	44.0	50.0	16
115	38.6	45.0	94.0	49.0	55.4	19
116	29.0	36.0	81.0	45.0	52.0	16
117	28.5	35.5	77.0	41.5	48.5	17
118	30.0	36.0	79.0	43.0	49.0	17
119	31.0	36.0	81.0	45.0	50.0	18
120	28.0	33.6	80.0	46.4	52.0	25

Cont..

Table 11 Cont..

121	48.0	54.0	100.0	46.0	52.0	25
122	32.0	36.0	80.0	44.0	48.0	18
123	30.0	36.0	80.0	44.0	50.0	15
124	41.0	47.0	96.0	49.0	55.0	22
125	41.0	48.0	95.0	47.0	54.0	22
126	43.3	49.3	96.0	46.7	52.7	22
127	30.6	36.5	75.0	38.5	44.3	16
128	42.5	49.0	100.0	51.0	57.5	23
129	38.5	44.5	93.5	49	55	20
130	45.1	50.5	96.5	46	51.3	23.33
131	24	29.55	77	47.45	53	11.15
132	37.2	42	83	42	46.5	20.08
S.E.D	1.14	1.40	1.90	1.98	1.95	0.90
L.S.D	2.25	2.74	3.73	3.88	3.82	1.78

Fig 8: Distribution of days to maturity expressed in percentage of individuals in RIL population during *rabi* 1999-2000

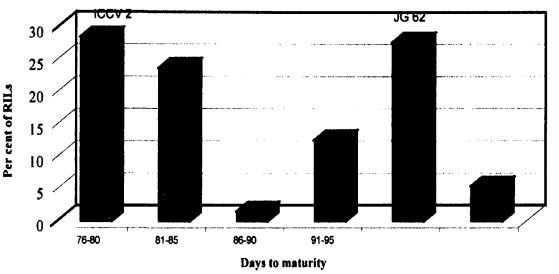
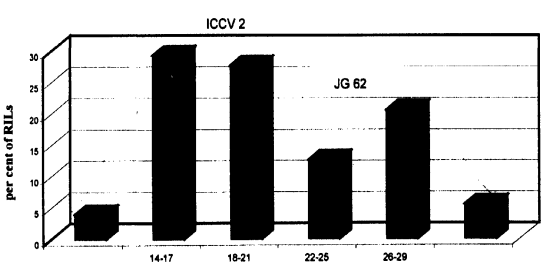


Fig 9: Distribution of numberof nodes up to first flower expressed in percentage of individuals in RIL population during *rabi* 1999-2000



4.1.1.9.4 Podding duration

The REML variance component for RILs and parents was highly significant and replication variance was non-significant for both the characters (Table 11).

4.1.1.9.5 Number of nodes up to first flower

The residual maximum likelihood (REML) variance component for RIL and parents for number of nodes up to first flower was highly significant and replication variance was non-significant (Table 11).

The distribution of number of nodes upto first flower was near normal indicating the multigenic control of this character (Fig 9). The χ^2 value (1.27) for 2: 1 segregation of RILs for low and high node number (1.27) was non-significant (Table 12). This revealed the probable role of two genes in controlling this character.

4.1.1.10 Correlation coefficients

Correlation coefficients which indicate the degree and direction of association between the characters under study were computed for F_2 generation and F_{10} RILs and are presented in Table 13 And Table 14.

4.1.1.10.1 Days to first flower

In F_2, Days to first flower had significant positive correlation with days to first pod (0.991**), days to maturity (0.864**) and number of nodes upto first flower (0.749**). Negative correlation was observed with pod filling period and total reproductive period.

In RILs, days to first flower had significantly high and positive correlation with days to first pod (0.994**), days to maturity (0.980**) and number of nodes upto first flower(0.941**). Positive non-significant correlation was observed with pod initiation period (0.023) and total reproductive period (0.077).

Table 13: The phenotypic correlation coefficients between different characters in F_2 generation

	DFF	DFP	DM	NNFF	PFP	TRP
DFP	0.991**					
DM	0.864**	0.863**				
NNFF	0.749**	0.744**	0.762**			
PFP	-0.405*	-0.425*	0.090	-0.102		
TRP	-0.378*	-0.360*	0.140	-0.723**	0.962**	
PIP	0.136	0.270	0.171	0.12	-0.226	0.048

* - Significant at 5% level ** - Significant at 1% level

Table 14: The phenotypic correlation coefficients between different characters in RIL population

	DFF	DFP	DM	PFP	TRP	PIP
DFP	0.994**					
DM	0.980**	0.913**				
PFP	0.023	0.022	0.428*			
TRP	0.077	0.103	0.489*	0.968**		
PIP	0.22	0.291	0.295	-0.006	0.244	
NNFF	0.941**	0.931**	0.857**	0.036	0.080	0.179

* - Significant at 5% level ** - Significant at 1% level

4.1.1.10.2 Days to first pod

In both F_2 and RILs days to first pod had highly significant and positive correlation with days to first flower (0.991**, 0.994** respectively), days to maturity (0.863**. 0.913** respectively) and number of nodes up to first flower (0.744**, 0.931** respectively). In F_2 negative correlation was observed with pod initaition period (-0.425*) and total reproductive period (-0.36*). In RILs non-significant positive correlation was observed with pod initiation period (0.022) and total reproductive period (0.103).

4.1.1.10.3 Days to maturity

In both F_2 generation and F_{10} RIL population, days to maturity had significant positive correlation with days to first flower (0.864**, 0.980**), days to first pod (0.863**, 0.913**) and number of nodes upto first flower (0.762**, 0.857**). Positive but low correlation was observed with pod initiation period (0.090) and total reproductive period (0.140) in F_2 but in RILs significant positive correlation was observed with both these characters (0.428*, 0.489*).

4.1.1.10.4 Podding duration

In F_2 generation both pod filling period and total reproductive period had negative correlation with days to first flower (-0.405*, -0.378*) and days to first pod (-0.435*, -0.360*) and number of nodes upto first flower (-0.102, -0.70). Positive non-significant correlation was observed with days to maturity (0.090, 0.140).

But in RILs, both pod filling period and total reproductive period had non-significant positive correlation with days to first flower (0.023, 0.077), days to first pod (0.022,0.103) and number of nodes upto first flower (0.036, 0.080). Positive significant correlation was observed with days to maturity (0.428*, 0.489*).

Table 15: Estimates of heritability for different characters in F_2 and RILs of chickpea cross ICCV 2 x JG 62

Character	F_2 population		F_{10} RIL
	h^2(b)(%)	h^2(n)(%)	h^2(b)(%)
Days to first flower	98	87	97
Days to first pod	99	86	96
Days to maturity	97	66	95
Pod filling period	84	14	59
Total reproductive period	91	18	66
Number of nodes up to first flower	89	79	95

Both in F_2 and RILs, pod initiation period did not have significant correlation with any character. However the correlation was negative with pod filling period.

4.1.1.10.5 Number of nodes up to first flower

In both F_2 generation and RILs, number of nodes up to first flower had significant positive correlation with days to first flower (0.749**, 0.941** respectively), days to first pod (0.744**, 0.931** respectively) and days to maturity (0.762**, 0.857**). In F_2 number of nodes up to first flower had negative correlation with podding duration and non-significant positive correlation in RILs.

4.1.1.11 Heritability

Broad sense and narrow sense heritability estimated from F_2 generation and broad sense heritability estimated from RIL population are presented in Table 15.

4.1.1.11.1 Days to first flower

The broad sense heritability was 98% and narrow sense heritability was 87% for days to first flower. The heritability estimated in F_{10} RIL population was 97%.

4.1.1.11.2 Days to first pod

High broad sense heritability (99%) and narrow sense heritability (86%) were observed for this character. The heritability estimate obtained in f_{10} RIL population was also high (97%).

4.1.1.11.3 Days to maturity

The broad sense and narrow sense heritability estimates were 97% and 66% respectively. The broad sense heritability obtained in F_{10} RILs was 96%.

Table 16: Estimates of co-heritability for different characters in RIL population

	DFP	DM	PFP	TRP	NNFF
DFF	0.97	0.99	2.5	1.50	0.98
DFP		0.99	2.85	1.35	0.98
DM			0.73	0.77	0.99
PFP				0.64	1.25
TRP					1.09

DFF - days to first flower
DFP - days to first pod
DM - days to maturity
PFP - pod filling period
TRP - total reproductive period
NNFF - number of nodes up to first flower

4.1.1.11.4 Podding duration

Both pod filling period and total reproductive period had high broad sense heritability (84% and 92% respectively) and had very low narrow sense heritability (14% and 18% respectively). The heritability estimates in RILs for both the characters were 60% and 66% respectively.

4.1.1.11.5 Number of nodes up to first flower

This character exhibited high broad sense (90%) and high narrow sense heritabilities (79%). The broad sense heritability obtained from RIL population was 95%.

4.1.1.12 Co-heritability

The characters which had significant correlation with each other were analyzed for the co-heritability estimates and presented in Table 16.

Days to first flower had high coheritability with days to first flower (0.97), days to maturity (0.99) and with number of nodes upto first flower. (0.98)

Days to first pod had high coheritability with days to maturity (0.99), days to first flower (0.97) and with number of nodes upto first flower (0.98).

Days to maturity had high coheritability value with days to first flower (0.99), days to first pod (0.99) and number of nodes up to first flower (0.99). Days to maturity had also shown high coheritability with pod filling period (0.73) and with total reproductive period (0.77).

Number of nodes up to first flower had high coheritable value with days to first flower, days to first pod and days to maturity (0.98, 0.98, 0.99 respectively).

Table 17: Reaction of the parents F_1 and F_2 generation to fusarium wilt in wilt sick plot

Genotypes	Total	Reaction	Mean percent wilting at days after sowing							
			16	23	27	34	41	48	80	At Maturity
ICCV 2	16	Resistant							8.93	
JG 62	40	Susceptible	50	42	8					
F_1	15	Susceptible		33	50	17				
F_2	206	Segregating in to EW, LW, and resistant	20	16	18	14	13	11		8

Table 18: Segregation for wilt resistance in F_2 generation of cross ICCV 2 X JG 62

Class	Number of F_2 plants		χ^2	Ratio
	Observed	Expected		
Susceptible	189	193.13	0.09	15
Resistant	17	12.88	1.32	1
Total	206	206.00	1.41	

$\chi^2 = 1.41$ P = 0.2-0.3

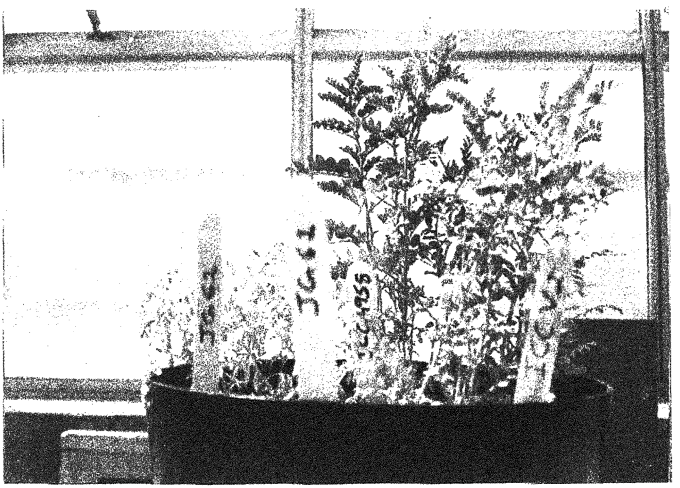


Plate 6: Susceptible (JG 62) and resistant (ICCV 2) chickpea parents used to study the inheritance of Fusarium wilt resistance

Pod filling period had slightly high correlation with total reproductive period (0.64) and they both had high coheritable value with days to maturity (0.73 and 0.77 respectively).

4.1.2 Inheritance of *Fusarium* wilt resistance

4.1.2.1 Reaction of parents, F_1 and F_2 to *Fusarium* wilt in wilt sick plots

The resistant parent, ICCV 2 was free from wilting upto 80 days, 11% of plants wilted after 80 DAP. 50% plants of the susceptible parent JG-62 wilted 16 DAP, 42% wilted 23 DAP and remaining 8% wilted 27 DAP. 33% of F_1 individuals of this cross wilted 23 DAP, 50% wilted 27 DAP and remaining 17% wilted 34 DAP (Table 17).

In F_2 generation, 20% wilted 16 DAP, 16% wilted 23 DAP and 18%wilted 27 DAP, 14% wilted 34 DAP, 13% 41 DAP and 10% 48 DAP. Remaining 8% of F_2 plants did not show any wilting symptoms and were resistant (Plate 6).

4.1.2.2 Segregation for wilt resistance in F_2 and F_3 generations

The resistant parent ICCV 2 showed only 11% wilting 80 DAP and all the plants of the susceptible parent wilted by 27 DAP. The F_1 plants wilted by 34 DAP and hence it was also considered as susceptible.

The F_2 segregation of susceptible and resistant plants gave a good fit to the expected 15(S): 1(R) ratio indicating that resistance was controlled by two recessive genes (Table 18). The susceptible plants were further classified into early wilters and late wilters depending on the time of wilting. The susceptible F_2 individuals those wilted along with JG 62 plants were grouped as early wilters (EW) and the F_2 individuals that wilted later than JG 62 were classified as late wilters (LW). Thus classified F_2 individuals gave a good fit to the expected 9(EW): 6(LW): 1(R) ratio (Table 19).

Table 19: Segregation of the F_2 population of the cross ICCV 2 X JG 62 in to early wilters, late wilters and resistant plants

Class	Number of F_2 plants		χ^2	Ratio
	Observed	Expected		
Early wilter	111	115.87	0.204	9
Late wilters	78	77.25	0.0072	6
Resistant	17	12.87	1.32	1
Total	206	206.00	1.531	

$\chi^2 = 1.53$ P = 0.3-0.5

Table 20: Segregation for wilt resistance in F_3 generation

Class	Number of F_3 progenies		Ratio	χ^2
	Observed	Expected		
All Susceptible	47	43.75	7	0.24
Segregating	50	50.00	8	0
All resistant	3	6.25	1	1.69
Total	100	100		1.93

$\chi^2 = 1.93$ P = 0.3-0.5

Table 21: Segregation for wilt resistance in F_3 generation

Class	Number of F_3 progenies		Ratio	χ^2
	Observed	Expected		
All Susceptible	47	43.75	7	0.24
Segregating				
1. in to 15:1	25	25.00	4	0
2. in to 3:1	25	25.00	4	0
All resistant	3	6.25	1	1.69
Total	100	100		1.93

$\chi^2 = 1.93$ P = 0.5-0.7

Table 22: Number of plants susceptible and resistant to race 1 of *Fusarium oxysporium* f. sp. *ciceri* in F_3 progenies of the cross ICCV 2 X JG 62 segregating 3:1

Family No.	Observed		Expected		Total	χ^2	P (n-1)
2	14	6	15.00	5.00	20	0.266	0.5-0.7
5	11	3	10.50	3.50	14	0.095	0.7-0.8
7	26	8	25.50	8.50	34	0.039	0.8-0.9
19	4	2	4.50	1.50	6	0.222	0.5-0.7
22	8	1	6.75	2.25	9	0.925	0.3-0.5
31	17	8	18.75	6.25	25	0.653	0.3-0.5
39	9	1	7.50	2.50	10	1.200	0.2-0.3
40	7	2	6.75	2.25	9	0.037	0.8-0.9
43	8	1	6.75	2.25	9	0.925	0.3-0.5
46	16	5	15.75	5.25	21	0.015	0.9
50	8	2	7.50	2.50	10	0.133	0.2-0.3
51	4	2	4.50	1.50	6	0.222	0.5-0.7
54	13	3	12.00	4.00	16	0.333	0.5-0.7
55	15	4	14.25	4.75	19	0.157	0.5-0.7
62	9	2	8.25	2.75	11	0.272	0.5-0.7
64	7	1	6.00	2.00	8	0.666	0.3-0.5
71	13	4	12.75	4.25	17	0.019	0.8-0.9
73	8	2	7.50	2.50	10	0.133	0.7-0.8
74	15	6	15.75	5.25	21	0.142	0.7-0.8
77	30	8	28.50	9.50	38	0.315	0.5-0.7
82	15	6	15.75	5.25	21	0.142	0.7-0.8
87	20	6	19.50	6.50	26	0.051	0.8-0.9
86	12	4	12.00	4.00	16	0.000	1
89	13	3	12.00	4.00	16	0.333	0.5-0.7
100	20	6	19.50	6.55	26	0.059	0.8-0.9
Total	322	96	313.50	104.55	418	0.929	0.3-0.5

Heterogenity $\chi^2 = 6.425$ P = Above 0.99

Table 23: Segregation of 25 F_3 families for wilt resistance in the ratio of 15:

Progeny number	Observed		Expected		Total	χ^2	P (n-1)
1	30	4	31.87	2.13	34	1.764	0.1-0.2
3	16	3	17.81	1.18	19	2.991	0.05-0.1
6	10	1	10.40	0.60	11	0.282	0.5-0.7
8	24	2	24.37	1.62	26	0.092	0.7-0.8
11	12	1	12.18	0.81	13	0.045	0.8-0.9
13	23	1	22.50	1.50	24	0.177	0.5-0.7
14	14	1	14.06	0.93	15	0.005	0.9-0.95
16	26	1	25.31	1.68	27	0.298	0.5-0.7
18	8	1	8.43	0.56	9	0.367	0.5-0.7
28	11	1	11.25	0.75	12	0.088	0.7-0.8
33	24	3	25.31	1.68	27	1.088	0.2-0.3
37	17	1	16.87	1.12	18	0.014	0.9-0.95
38	45	2	44.06	2.9	47	0.319	0.5-0.7
47	8	1	8.43	0.56	9	0.367	0.5-0.7
48	36	3	36.56	2.43	39	0.138	0.7-0.8
56	31	3	31.87	2.12	34	0.384	0.5-0.7
59	20	1	19.68	1.31	21	0.079	0.7-0.8
61	32	2	31.87	2.12	34	0.007	0.9-0.95
63	11	1	11.25	0.75	12	0.088	0.7-0.8
65	32	3	32.81	2.18	35	0.328	0.5-0.7
76	25	2	25.31	1.68	27	0.061	0.8-0.9
79	14	1	14.06	0.93	15	0.005	0.9-0.95
80	31	1	30.00	2.00	32	0.533	0.3-0.5
88	31	1	30.00	2.00	32	0.533	0.3-0.5
93	13	1	15.12	0.87	14	0.316	0.5-0.7
Total	544	42	547.29	38.62	586	0.317	0.05-0.1

Heterogenity $\chi^2 = 10.063$ P = Above 0.99

Table 24: Segregation of $126F_{10}$ RILs in to early wilters, late wilters and resistant lines

Class	Number of 126 F_{10} RILs		χ^2	Ratio
	Observed	Expected		
Early wilter	31.0	31.5	0.0079	1
Late wilter 1	38.0	31.5	1.3400	1
Late wilters 2	26.0	31.5	0.9600	1
Resistant	31.0	31.5	0.0079	1
Total	126.0	126.0	0.008	

$\chi^2 = 0.008$ P = 0.99

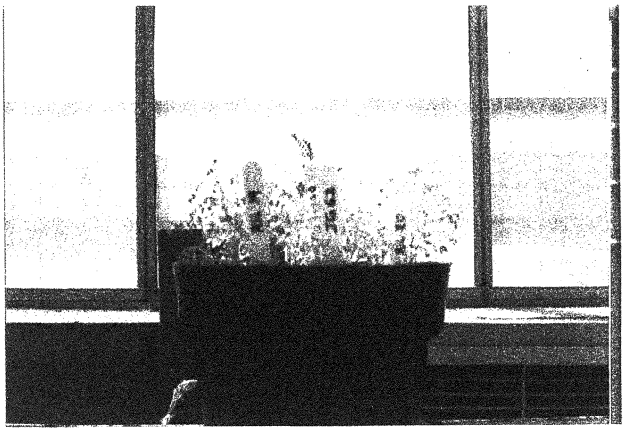


Plate 7a:

Plate 7b:

Plate 7a and 7b: RILs of the cross ICCV 2 x JG 62 differing in resistance to Fusarium wilt

The segregation of F_2 generation was further confirmed by screening 100 F_3 families to the incidence of wilt disease in wilt sick plot. Out of the 100 F_3 families, 47 families were susceptible, 50 families segregated in to susceptible and resistant plants and three families had all resistant plants, which fitted well to the expected 7:8: 1 ratio (χ^2 = 1.9) (Table 20). Among the segregating progenies, 25 progenies segregated in to 3 susceptible: 1 resistant ratio and remaining 25 progenies segregated into 15 susceptible: 1 resistant ratio. The progenies were thus classified into all susceptible, segregating susceptibles and resistant in the ratio of 15: 1 and 3: 1 and all resistant which gave a good fit to 7:4:4:1 ratio (χ^2=1.9) expected from the segregation at two loci (Table 21).

4.1.2.3 Segregation for wilt resistance in F_{10} recombinant inbred lines (RIL) population

The 126 F_{10} generation RILs gave a good fit to the expected 1(EW): 2(LW): 1(R) ratio for the segregation of two genes during *Rabi* 1998-1999. However it was not possible to distinguish the late wilters into two groups. From the pot-culture experiment only susceptible RILs were identified but there was no late wilting. Hence the experiment was repeated during *Rabi* 1999-2000 in a wilt sick plot. The parents and 126 F_{10} RILs were planted in the row where previously JG 62 was planted and wilted. The 126 F_{10} RILs gave a good fit to 1(EW): 1(LW1): 1(LW2): 1(R) ratio expected from the segregation at two loci (χ^2=2.31) (Table 24). The F_{10} 126 RILs classified in to different groups based on their wilting are presented in Table 25 (Plate 7a and 7b).

4.2 EXPERIMENT II (Pot culture studies)

An experiment to study the inheritance of root length and root volume was conducted in chickpea in a glass house at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). The study was conducted on P_1, P_2, F_1, F_2, BC_1P_1, and

Table 25: Classification of 126 F_{10} RILs in to early wilters, late wilters 1, late wilters 2 and resistants

Serial number	Early wilter	Late wilter 1	Late wilter 2	Resistant
1	2	1	10	4
2	3	6	22	7
3	5	8	23	16
4	9	12	25	21
5	11	17	26	27
6	13	34	33	35
7	14	36	41	42
8	15	38	46	44
9	18	40	49	47
10	19	48	55	54
11	20	50	56	57
12	24	51	68	58
13	28	52	73	61
14	29	53	75	62
15	30	59	76	63
16	31	66	77	64
17	32	67	78	65
18	37	74	79	69
19	39	81	83	70
20	43	82	93	71
21	45	84	100	72
22	60	85	102	80
23	86	87	106	90
24	88	89	107	92
25	97	91	118	95
26	101	94	123	96
27	108	99		98
28	111	103		112
29	116	104		117
30	119	105		120
31	125	109		121
32		110		
33		113		
34		114		
35		115		
36		122		
37		124		
38		126		
Total	31	38	26	31

Table 26: Root length and root volume of ICCV 2 and JG 62 at the time of flowering of ICCV 2 and at the time of flowering of JG 62

Genotype	Flowering of ICCV 2		Flowering of JG 62	
	Root length (m)	Root volume (cc)	Root length (m)	Root volume (cc)
ICCV 2 1	11.1	3.9	13.5	4.1
2	12.1	4.1	13.4	4.5
3	12.5	3.9	14.1	4.1
4	10.0	3.8	13.5	4.5
5	10.5	4.0	14.0	4.2
Mean	11.2	4.0	13.7	4.3
JG 62 1	10.1	3.5	16.2	6.9
2	10.5	3.6	17.5	6.9
3	11.2	4.0	17.8	6.5
4	10.3	3.2	16.5	5.9
5	9.8	3.0	16.3	5.8
Mean	10.4	3.5	16.9	6.2

Table 27: Test of significance for root length and root volume at different times of flowering

Time of flowering of	Root length	Root volume
ICCV 2	2.09^{NS}	1.59^{NS}
JG 62	6.94*	9.94*
Respective flowering time	11.70*	13.47*

NS - Non-significant
* - Significant at 5% level

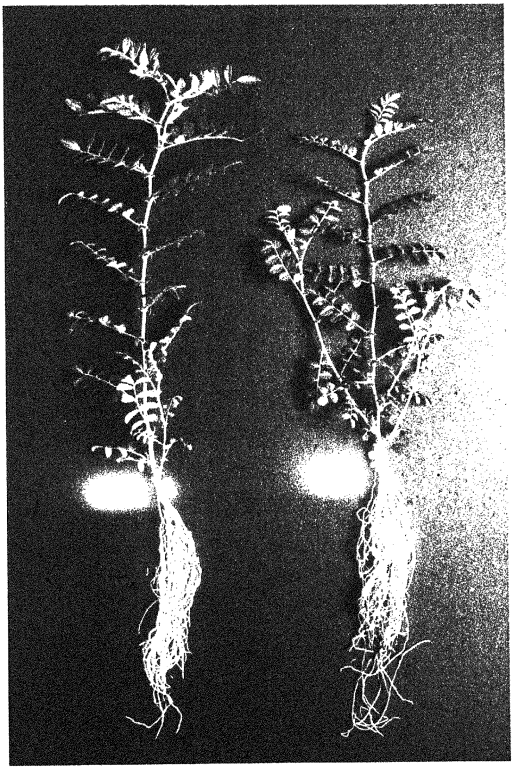


Plate 8: Root characteristics of the parents (ICCV 2 and JG 62) at the flowering time of ICCV 2

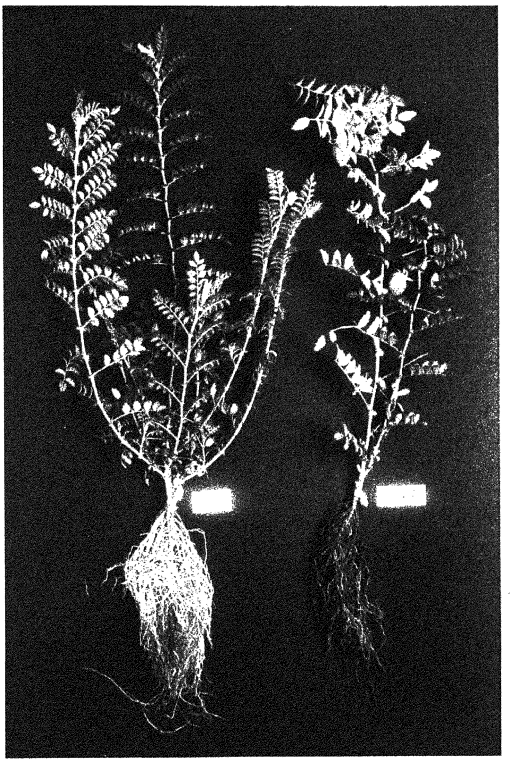


Plate 9: Differences in the root characteristics of the parents (ICCV 2 and JG 62) at the flowering time of JG 62

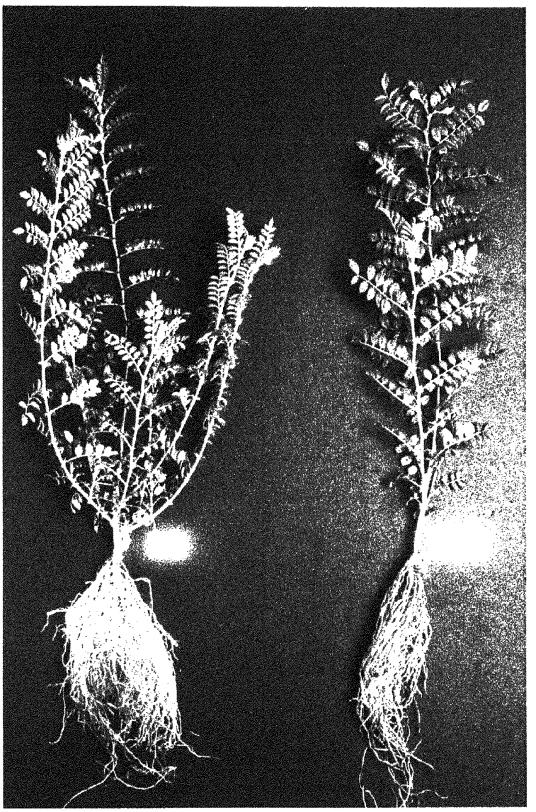


Plate 10: Differences in the root characteristics of the parents (ICCV 2 and JG 62) at their respective flowering times

BC_1P_2, generations and 126 F_{10} RILs. A preliminary experiment was conducted on the parents to know the variation for root characters at the flowering time of each parent. The RILs and parents were planted in three replications during May 2000 in a 'Conviron'. Data were recorded at the time of flowering of each individual plant on root length, root volume, number of leaflets in 3rd, 4th and 5th pinnules from top, total leaf area, and total number of nodes up to flowering. The samples were dried in an incubator and dry weights were recorded for leaf, root and shoot. The results of this experiment are as follows.

Ten seeds of each parent were planted and observations on root length and root volume were recorded at the time of flowering of ICCV 2 and at the time of flowering of JG 62. The root length and root volume values are presented in Table 26.

The mean root length of ICCV 2 and JG 62 at the time of flowering of ICCV 2 was 11.24 m and 10.38 m and root volume was 3.94 cc and 3.46 cc (plate 8). The root length of ICCV 2 was 13.68 m and JG 62 was 16.86 m and root volume was 4.28 cc and 6.2 cc at the time of flowering of JG 62. These results indicated significant differences between the parents for both the root length and root volume at the time of flowering of JG 62 (Table 27) (Plate 9). When the root length and root volume of parents were compared at their respective flowering times, these showed relatively wider difference than those at the time of flowering of JG 62 (Plate 10). Hence in the inheritance study of root characters, observations in F_1, F_2, BC_1P_1, BC_1P_2 and 126 F_{10} RILs were recorded at their respective flowering times i.e., as and when they flowered. Observations for all the RILs were also recorded for root volume at the time of flowering of ICCV 2 and of JG 62 to confirm the above results.

Table 28: Mean values along with their standard errors for various characters studied in the cross ICCV 2 (P_1) and JG 62 (P_2)

Character	P_1 (ICCV 2)	P_2 (JG 62)	F_1	F_2	BC_1P_1	BC_1P_2
Root length (m)	11.7 ±0.59	17.6 ±0.65	22.7 ±1.12	16.9 ±0.38	14.3 ±1.07	17.5 ±0.97
Root volume (cc)	4.1 ±0.13	6.5 ±0.23	7.1 ±0.41	5.5 ±0.13	5.0 ±0.39	6.2 ±0.34
Leaflet number	35.7 ±0.25	40.4 ±0.24	39.7 ±0.75	38.4 ±0.19	37.3 ±0.63	39.3 ±0.49
Leaf area (cm^2)	10.5 ±0.35	6.9 ±0.44	8.8 ±0.63	8.6 ±0.20	9.7 ±0.67	8.0 ±0.51
Root dry weight (g)	0.28 ±0.013	0.44 ±0.014	0.54 ±0.048	0.33 ±0.010	0.27 ±0.032	0.37 ±0.029
Shoot dry weight (g)	1.0 ±0.04	1.5 ±0.04	1.3 ±0.07	1.3 ±0.04	1.0 ±0.10	1.1 ±0.09
Total number of nodes	44	89	58	65	52	61

Table 29: Estimates of A, B, C and D values obtained by Mather's formulae for various characters studied in chickpea

Character	A	B	C	D
Root length	-5.9* ±2.60	-5.3* ±2.33	-7.1* ±2.84	3.9* ±1.88
Root volume	-1.3 ±0.90	-1.3 ±0.83	-2.9* ±0.98	1.7 ±085
Leaf area	0.05 ±0.035	0.6 ±0.42	-0.4 ±0.25	1.3 ±1.006
Root dry weight	-0.3* ±0.08	-0.3* ±0.07	-0.5* ±0.10	0.1 ±0.12
Shoot dry weight	-0.2 ±0.22	-0.6* ±0.21	0.1 ±0.21	1.8* ±0.212
Total number of nodes	2.4 ±12.30	-25.5* ±11.80	12.4 ±8.99	13.4 ±9.99

* - Significant at 5% level

4.2.1 Inheritance of root length

The mean root length of ICCV 2 was 11.7±0.59 m and of JG 62 was 17.6±0.65 m. The mean root length of F_1 was 22.7±1.12 m. The root length of F_2, BC_1P_1, and BC_1P_2, were 16.9±0.38 m, 14.3±1.07 m and 17.5±0.97 m, respectively (Table 28).

The A, B, and D values obtained deviated significantly from zero indicating the inadequacy of additive-dominance model to explain the variation for this character and the presence of interaction effects (Table 29). The results of six-parameter model (Table 30) indicated that the estimates of two parameters (i and l), deviated from zero but the most significant interaction effect was dominance × dominance (l) gene action along with additive main effect. Dominance (h) and dominance × dominance (l) gene actions expressed positively for root length.

The frequency distribution for root length is presented in Fig 10. The F_2 distribution pattern was near normal. The range for root length in the F_2 population (9.1 m to 31.3 m) extended beyond the limits of either of the parents indicating recombination between the genomes of the two parents.

4.2.2 Inheritance of root volume

The mean root volume of ICCV 2 was 4.1±0.13 cc and of JG 62 was 6.5±0.23 cc. The F_1 of this cross had a mean root volume of 7.1±0.41 cc. The mean root volume of F_2 generation was 5.5±0.13 cc and the backcrosses had the root volume of 5.0±0.39 cc and 6.2±0.34 cc (Table 28).

The deviations of A, B, C and D values from zero indicated the inadequacy of additive-dominance model. Among the three interaction effects only dominance × dominance (l) effect deviated significantly from zero but this action was non-significant.

Table 30: Estimates of the additive, dominance and interaction parameters and type of epistasis for different characters in chickpea

Character	m	d	h	i	j	l	Type of epistasis
Root length	16.9* ±0.38	3.2* ±1.49	3.9 ±3.57	-4.1 ±3.36	-0.3 ±1.56	15.2* ±6.62	Complementary
Root volume	5.5* ±0.11	-1.2* ±0.52	2.2 ±1.22	0.4 ±1.15	0.005 ±0.542	2.2 ±2.32	Complementary
Leaf area	8.6* ±0.20	1.7* ±0.85	1.4 ±2.03	1.1 ±1.89	-0.3 ±0.93	-1.7 3.80	Duplicate
Root dry weight	0.33* ±0.009	0.09* ±0.04	0.1 ±0.10	-0.04 ±0.094	-0.01 ±0.044	0.5* ±0.20	Complementary
Shoot dry weight	1.3* ±.03	0.002 ±0.1396	-0.9* ±0.10	-0.9* ±0.30	0.2 ±0.14	1.7* ±0.59	Duplicate
Total number of nodes	65.9* ±1.86	8.7 ±8.30	-43.7* ±18.37	-35.5* ±17.20	13.9 ±8.38	58.7 ±34.40	Duplicate

* Significant at 5% level

Fig 10: Distribution of root length expressed in percentage individuals in F population

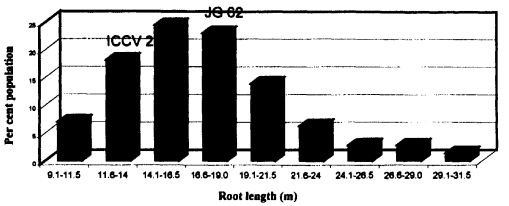


Fig 11: Distribution of root volume expressed in percentage of individuals in F population

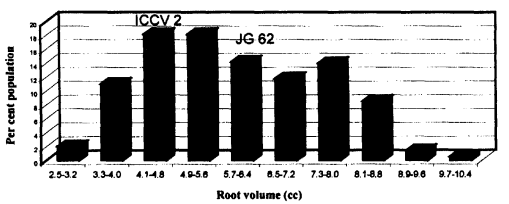
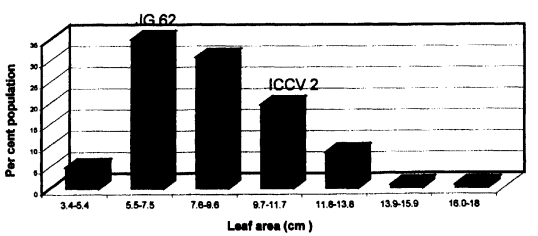


Fig 12: Distribution of leaf area expressed in percentage of individuals in F population



Therefore additive (d) gene action was the most significant gene action governing root volume (Table 30).

The frequency distribution of F_2 generation was closer to normal curve. The range of F_2 extended beyond either parents (Fig 11).

4.2.3 Inheritance of leaflet number in 3rd, 4th, and 5th pinnules from top and total leaf area

The number of leaflets in 3rd, 4th, and 5th pinnules taken together in ICCV 2, JG 62, F_1, F_2, BC_1P_1, BC_1P_2 were 35.7±0.25, 40.4±0.24, 39.7±0.75, 38.4±0.19, 37.3±0.63, and 39.3±0.49 respectively (Table 28).

The area of the leaflets in 3rd, 4th, and 5th pinnules in all generations was 10.5±0.35cm^2, 6.9±0.44 cm^2, 8.8±0.63cm^2, 8.6±0.20 cm^2, 9.7±0.67 cm^2 and 8.0±0.51 cm^2 respectively. The value of F_1 for leaf area indicated intermediate expression of this character.

The ratio of F_2 individuals with number of leaflets similar to ICCV 2 to the individuals with number of pinnules similar to JG 62 (39-42) gave a good fit to 1:3 indicating single gene inheritance of this character. This ratio was further confirmed by the 1:1 ratio obtained in backcross generation (Table 12).

The A, B C and D values obtained for leaf area suggested the adequacy of additive-dominance model to explain the variation present for this character. Out of the two main effects, estimate of additive (d) was significant (Table 30).

The frequency distribution for leaf area was presented in Fig 12. Near normal curve was observed for leaf area. The extension of F_2 population over both the parents indicated the presence of transgressive segregation.

Fig 13:Distribution of root dry weight expressed in percentage of individuals in F population

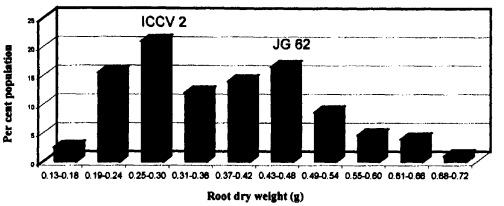


Fig 14: Distribution of dry shoot weight expressed in percentage of individuals in F population

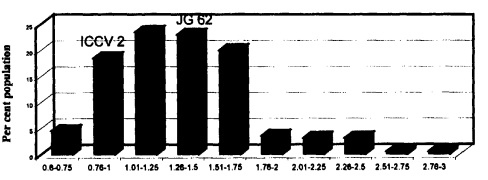
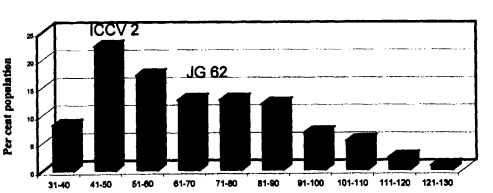


Fig 15: Distribution of total number of nodes upto flowering expressed in percentage of individuals in F population



4.2.4 Inheritance of root dry weight

The mean root dry weight of ICCV 2 and JG 62 were 0.28±0.013 g and 0.44±0.014 g. Dry root weight of F_1, F_2, BC_1P_1 and BC_1P_2 generations were 0.54±0.048 g, 0.33±0.010 g, 0.27±0.032 g and 0.37±0.029 g respectively (Table 28).

The deviations of A, B, and C values from zero were not significant but they were significant as their standard error values are low. Results of six-parameter model suggested the presence of significant additive (d) and dominance × dominance (l) gene effect (Table 30).

The frequency distribution for root dry weight is presented in Fig 13. Continuous variation observed in F_2 population was closer to normal curve. The range of F_2 population extended beyond the parental values.

4.2.5 Inheritance of shoot dry weight

The mean shoot dry weight of ICCV 2 was 1.01±0.039 g and JG 62 was 1.50±0.043 g. The mean shoot dry weight of F_1 was 1.33±0.076 g and of F_2 and of backcross generations was 1.33±0.039 g, 1.09±0.101 g and 1.10±0.095 g, respectively (Table 28).

The A, B and C values obtained did not deviate significantly from zero, but the effect of B was significant as its standard error was low. The results of six-parameter model indicated the presence of dominance, additive × additive (i) gene action and dominance × dominance (l) gene action. Dominance (h) gene action expressed negatively and dominance × dominance (l) gene action expressed positively for this character.

The distribution of shoot dry weight is depicted in Fig 14. The distribution of F_2 population for shoot dry weight was continuous and near normal. F_2 population having shoot dry weight beyond both the parents was present.

4.2.6 Inheritance of total number of nodes upto flowering

The total number of nodes upto flowering in different generations were 44, 89, 58, 65, 52 and 61. The value of F_1 was present between both the parents i.e. exhibiting intermediate expression (Table 28).

The A, B, C and D values provided the inadequacy of additive-dominance model to explain the variation for this character. The estimates of i, j and l deviated from zero indicating the presence of all three interaction effects. The most significant gene actions governing this character were additive × additive (i) gene action and dominance (h) gene action. Both dominance × dominance (l) gene action and dominance gene action expressed positively for total number of nodes up to flowering (Table 30).

The F_2 distribution for this character is presented in Fig 15. The distribution pattern is closer to normal curve, and transgressive segregants were present.

4.2.7 Component of variation in F_2 and backcrosses

4.2.7.1 Root length

The variances for root length in ICCV 2, JG 62 and F_1 were 1.435, 2.123 and 4.909 respectively. The variance in the F_2 population was higher (21.244) than that in either of the parents (Table 31). The variances of BC_1P_1 and BC_1P_2 were 12.936 and 9.467. The H and D values and degree of dominance are presented in Table 32. The degree of dominance was 0.36.The value of $\sqrt{HD}$ was not equal to the difference in the variances of two backcrosses.

Table 31: Variances observed for various characters in different generations of the cross ICCV 2 x JG 62

Character	ICCV 2 (P_1)	JG 62 (P_2)	F_1	F_2	BC_1P_1	BC_1P_2
Root length	1.435	2.123	4.909	21.244	12.936	9.467
Root volume	0.071	0.276	0.681	2.007	1.569	1.190
Dry root weight	0.007	0.001	0.009	0.117	0.011	0.008
Leaf area	0.505	2.393	1.601	6.208	4.602	2.632
Total number of nodes	0.916	25.800	19.583	471.550	365.820	323.380
Dry shoot weight	0.006	0.009	0.023	0.164	0.103	0.091
Population size	5	5	5	145	10	10

4.2.7.2 Root volume

The variances for root volume in ICCV 2, JG 62 and F_1 were 0.071, 0.276 and 0.681 respectively. The variance in the F_2 population (2.007) was higher than the variance in both the parents. Variance in BC_1P_1 (1.569) was higher than the variance in BC_1P_2 (1.190). The degree of dominance was 0.87 and the value of $\sqrt{HD}$ was not equal to the difference between the variances of backcrosses (Table 32).

4.2.7.3 Leaf area

The variances for leaf area in ICCV 2, JG 62 and F_1 was 0.505, 2.393, 1.601 respectively (Table 31). The variance in the F_2 population (6.208) was higher than the variances in both the parents. The variance in BC_1P_1 was less than the variance in BC_1P_2. The degree of dominance for both the characters was 0.49 and 0.5 respectively. The $\sqrt{HD}$ value was not equal to the difference in variances of two backcrosses for both the characters (Table 32).

4.2.7.4 Root dry weight

The variances for root dry weight in ICCV 2, JG 62 and F_1 were 0.007, 0.001, and 0.009 respectively (Table 31). High variance in the F_2 population (0.117) over either of the parents was observed for this character. The variance in the BC_1P_1 (0.008) was lower than the variance BC_1P_2 (0.011). The degree of dominance was 1.36 and the $\sqrt{HD}$ value was not equal to the difference between the variances of two backcrosses.

4.2.7.5 Shoot dry weight

The variances for shoot dry weight in P_1, P_2 and F_1 for dry root weight was 0.006, 0.009, and 0.023 respectively (Table 31). The variance in the F_2 was (0.164) higher than the variance in both the parents. The variance in BC_1P_1 was lower than the variance in

Table 32: The estimates of D, H, √h/d, √HD and VBC_1-VBC_2 for different characters

Character	H	D	√h/d	√HD	VBC_1-VBC_2
Root length	4.001	11.586	0.368	10.8	3.468
Root volume	1.635	2.51	0.807	2.026	0.379
Dry root weight	0.0156	0.0084	1.36	0.014	0.0016
Leaf let number	1.7168	7.115	0.49	3.495	1.555
Leaf area	1.8212	6.6863	0.52	3.489	2.125
Total number of nodes	808.852	507.808	1.26	640.89	42.44
Dry shoot weight	0.075	0.266	0.53	0.141	0.012

Table 31: Mid parent and better parent heterosis for various characters studied in F_1 of the cross ICCV 2 × JG 62

Character	P_1 (ICCV 2)	P_2 (JG 62)	Mean of F_1	Mean of mid parent	Mid-parent heterosis (%)	Better parent heterosis (%)
Root length	11.7	17.6	22.77	14.71	54.85	29.10
Root volume	4.1	6.5	7.18	5.33	34.60	9.97
Leaf let number	35.7	40.4	39.75	38.07	4.39	-1.60
Leaf area	10.5	6.9	8.89	8.77	1.31	27.18
Root dry weight	0.28	0.44	0.54	0.36	59.15	22.47
Shoot dry weight	1.0	1.5	1.30	1.25	5.81	11.49
Total number of nodes	44	89	58.75	66.92	-12.21	-34.43

BC_1P_2.The degree of dominance was 0.53.The $\sqrt{HD}$ value was not equal to the difference in the variance of two backcrosses (Table 32).

4.2.8.6 Total number of nodes

Variances for total number of nodes in ICCV 2, JG 62 and F_1 was 0.916, 25.800 and 19.583 respectively (Table 31). F_2 variance (471.550) was higher than the variances in both the parents. The variance in BC_1P_1 (323.380) was lower than the variance in BC_1P_1 (365.820). The degree of dominance was 1.26 and the $\sqrt{HD}$ value was not equal to the difference in the variance of two backcrosses (Table 32).

4.2.8 Heterosis

The magnitude of mid-parent and better parent heterosis for root length, root volume, number of leaflets in 3rd, 4th and 5th pinnules, their area, root dry weight, shoot dry weight, specific leaf area and total number of nodes are presented in Table 33.

4.2.8.1 Root length

The mid-parent heterosis for root length was positive and high (54.85%) and better parent heterosis was positive (29.10%).

4.2.8.2 Root volume

High and positive mid-parent heterosis (34.60%) and positive better parent heterosis (9.97%) were observed.

Table 34: Table showing differences among the RILs for various characters in chickpea

S.No	Root length (m)	Root volume (cc)	Leaf let number	Total leaf area (cm^2)	Total number of nodes	Shoot dry weight (g)	Root dry weight (g)
1	9.90	4.46	36.0	222	44	0.926	0.160
2	9.50	2.93	36.5	151	26	0.750	0.155
3	4.25	2.89	36.5	153	38	0.665	0.110
4	11.60	3.97	36.0	157	41	0.925	0.205
5	3.95	2.10	39.0	110	24	0.560	0.105
6	11.80	6.40	38.0	182	74	1.555	0.345
7	18.35	5.60	36.0	213	45	1.075	0.285
8	7.25	2.90	36.0	131	24	0.625	0.110
9	6.85	2.90	35.5	98	21	0.475	0.150
10	8.90	3.80	40.5	197	31	1.125	0.190
11	12.10	4.20	42.0	256	73	2.265	0.320
12	7.75	3.63	36.5	147	20	0.705	0.150
13	16.25	5.59	38.5	231	64	1.585	0.355
14	8.90	2.56	37.5	168	31	0.830	0.115
15	7.25	3.03	35.5	152	33	0.975	0.155
16	9.15	5.31	36.5	133	60	1.105	0.220
17	17.35	7.42	40.0	178	79	1.900	0.490
18	15.25	6.69	41.5	250	81	2.505	0.505
19	22.45	8.15	42.0	180	91	2.195	0.485
20	23.75	9.09	42.0	183	69	1.690	0.580
21	14.20	7.12	38.5	225	81	2.670	0.515
22	8.40	3.57	36.0	128	17	0.705	0.135
23	6.25	2.99	35.5	131	23	0.550	0.140
24	10.70	4.09	36.5	183	38	0.915	0.170
25	7.00	2.96	36.0	146	25	0.685	0.120
26	6.65	1.82	37.5	91	28	0.520	0.085
27	16.15	8.02	41.5	326	85	2.620	0.440
28	17.25	6.71	42.0	235	85	2.375	0.350
29	15.95	7.66	41.0	247	71	2.155	0.420
30	28.75	12.15	42.0	323	86	2.820	0.865
31	6.25	2.37	36.0	92	19	0.415	0.120
32	8.85	3.37	37.0	140	30	0.655	0.140
33	7.70	3.00	37.0	144	27	0.585	0.150
34	6.50	3.31	37.0	165	32	0.990	0.160
35	22.15	8.40	38.0	238	77	1.570	0.470
36	12.75	5.90	36.0	184	61	1.105	0.270
37	27.05	9.52	42.0	287	86	2.405	0.630
38	12.30	8.85	42.0	320	87	1.955	0.505
39	12.75	5.73	39.5	174	61	1.245	0.270

Cont..

Table 34 Cont..

40	8.50	2.65	37.0	157	28	0.840	0.175
41	15.20	5.71	37.0	192	26	1.155	0.230
42	8.55	3.34	36.5	166	33	0.775	0.140
43	25.65	11.22	42.0	313	117	2.690	0.565
44	23.20	12.50	42.0	318	107	3.035	0.685
45	7.50	3.50	36.0	156	23	0.695	0.165
46	10.95	3.60	35.5	147	22	0.625	0.180
47	4.20	2.50	37.0	88	26	0.500	0.145
48	4.20	2.14	36.0	151	22	0.805	0.165
49	5.80	2.50	37.0	158	23	0.760	0.140
50	10.10	3.58	36.5	211	28	0.785	0.230
51	8.55	3.72	39.0	196	33	1.170	0.190
52	5.90	2.29	36.0	103	25	0.425	0.105
53	13.65	9.92	42.0	256	119	2.905	0.630
54	15.45	7.75	36.0	166	89	1.665	0.505
55	21.80	10.58	39.0	294	61	2.115	0.650
56	15.85	7.66	37.5	176	55	1.535	0.470
57	10.95	8.14	37.0	167	25	2.530	0.410
58	4.85	2.14	36.0	92.	25	0.395	0.115
59	17.65	7.57	38.5	228	62	1.610	0.405
60	22.75	9.58	41.5	234	105	2.390	0.500
61	16.25	8.11	36.0	209	80	1.520	0.510
62	15.95	10.75	42.0	357	87	2.645	0.625
63	17.25	10.07	40.5	209	91	2.575	0.475
64	4.40	2.31	36.0	107	28	0.545	0.125
65	12.75	9.59	42.0	280	59	2.480	0.510
66	16.60	7.36	40.0	263	79	2.100	0.480
67	11.35	5.18	36.0	199	33	0.995	0.260
68	6.10	2.85	40.5	178	24	0.850	0.215
69	7.65	3.28	41.0	159	21	1.120	0.170
70	21.00	10.82	42.0	254	90	2.440	0.630
71	7.15	3.90	41.0	132	34	0.650	0.190
72	29.35	15.35	41.0	513	117	2.595	0.885
73	8.25	3.77	36.0	237	38	0.770	0.210
74	7.20	2.83	37.0	127	30	0.655	0.135
75	7.55	3.35	36.5	111	31	0.720	0.150
76	3.75	1.68	36.0	92	21	0.610	0.080
77	4.90	2.13	36.0	144	25	0.815	0.115
78	5.15	2.37	36.0	124	22	0.680	0.135
79	4.05	2.20	36.0	136	26	0.655	0.105
80	13.00	3.35	36.0	117	40	0.595	0.240

Cont..

Table 34 Cont..

81	5.50	2.13	35.5	114	36	0.575	0.125
82	8.05	3.50	40.5	162	78	1.490	0.315
83	2.70	1.31	37.0	87	19	0.635	0.090
84	6.50	2.65	36.0	107	21	0.595	0.135
85	19.20	6.70	39.0	183	64	1.605	0.330
86	7.60	3.12	39.5	171	39	0.570	0.155
87	6.65	2.42	36.0	78	27	0.520	0.115
88	9.70	3.64	37.0	169	42	0.700	0.250
89	20.50	7.73	38.0	240	76	1.480	0.405
90	20.15	5.73	40.5	160	41	0.840	0.365
91	18.25	9.94	40.5	318	105	3.025	0.630
92	21.95	9.10	42.0	257	68	1.705	0.455
93	14.60	6.61	36.0	154	65	1.021	0.305
94	13.65	7.17	37.0	242	83	1.750	0.390
95	14.55	4.95	36.5	165	63	1.035	0.285
96	10.80	5.16	36.0	191	42	0.850	0.315
97	5.40	2.43	36.0	86	30	0.660	0.125
98	6.75	3.48	36.5	195	37	0.955	0.130
99	5.85	1.94	36.0	111	26	0.455	0.105
100	16.40	7.06	36.5	235	72	1.805	0.515
101	9.50	4.64	37.0	170	30	1.045	0.220
102	10.90	5.41	36.0	122	70	1.350	0.335
103	6.35	3.02	35.0	112	25	0.565	0.155
104	4.75	1.95	37.5	117	27	0.615	0.115
105	6.35	2.33	36.5	130	25	0.790	0.125
106	3.90	2.57	39.0	116	20	0.925	0.135
107	5.10	2.21	40.0	120	26	0.585	0.105
108	5.30	2.14	36.0	116	25	0.640	0.115
109	22.30	9.52	39.5	306	81	0.995	0.405
110	12.05	4.59	36.0	177	59	1.120	0.315
111	23.20	9.80	42.5	259	111	3.205	0.505
112	13.15	5.43	36.0	177	68	1.450	0.370
113	6.80	2.75	37.5	126	27	0.740	0.135
114	8.40	2.99	36.0	195	37	0.875	0.145
115	9.00	4.61	37.0	166	34	0.825	0.185
116	7.05	2.63	40.0	162	29	0.785	0.130
117	6.85	2.64	36.0	138	25	0.710	0.155
118	4.10	2.18	37.5	123	33	0.785	0.120
119	7.70	3.16	39.0	192	32	0.865	0.185
120	7.30	3.19	36.0	130	21	0.820	0.135
121	23.95	11.85	37.0	193	96	2.595	0.820
122	3.95	2.38	36.5	175	32	0.760	0.160

Cont..

Table 34 Cont..

123	4.75	2.41	37.0	107	32	0.710	0.135
124	12.30	6.47	37.0	164	68	1.470	0.380
125	17.70	8.77	37.0	360	76	1.670	0.480
126	18.55	6.70	42.0	155	67	1.710	0.440
127	10.5	3.46	36	150	36	0.5	0.21
128	18.5	7.52	42.0	190	85	1.4	0.45
S.E.D	2.551	1.016	1.059	71.6	10.69	0.42	0.06
L.S.D	5.048	2.011	2.096	142	21.167	0.82	0.12

4.2.8.3 Number of leaflets and leaflets area in 3rd, 4th and 5th pinnules

Mid-parent heterosis was positive (4.39%) and better parent heterosis was negative (-1.6%) for number of leaflets and mid parental heterosis was 1.31% for leaf area and better parent heterosis was 27.2%.

4.2.8.4 Root dry weight

High and positive mid-parent (59.15%) and better parent (22.47%) heteroses were observed for root dry weight.

4.2.8.5 Shoot dry weight

The mid-parent heterosis for shoot dry weight was 5.81% and better parent heterosis was 11.49%.

4.2.8.6 Total number of nodes

Both the mid-parent heterosis (-12.21%) and better parent heterosis (-34.43) were negative for total number of nodes

4.2.9 Studies based on Recombinant Inbred lines (RILs)

The CRD analysis has shown that the RILs were highly significant for all the characters studied viz., root length, root volume, leaflet number leaflet area, dry root weight, dry shoot weight, total number of nodes (Table 34). These results revealed significant differences among the RILs with respect to the characters listed above. Replications variance was highly non-significant for all the characters indicating that the replication effects were negligible and the variation present in the treatments was precisely estimated.

Fig 16: Distribution of root length expressed in percentage of individuals in RIL population

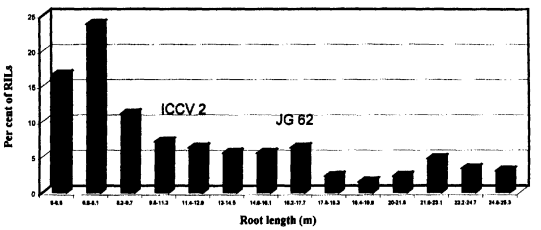


Fig 17: Distribution of root volume expressed in percentage of individuals in RIL population

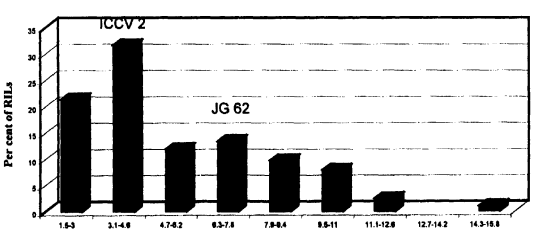


Fig 18: Distribution of shoot dry weight expressed in percentage of individuals in RIL population

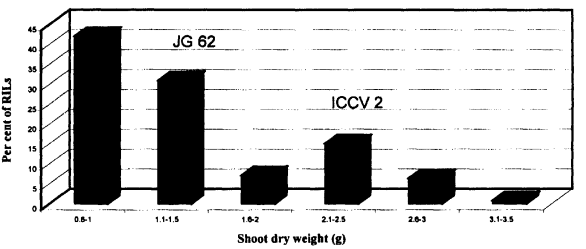


Fig 19: Distrubution of root dry weight expressed in percent of individuals in RIL population

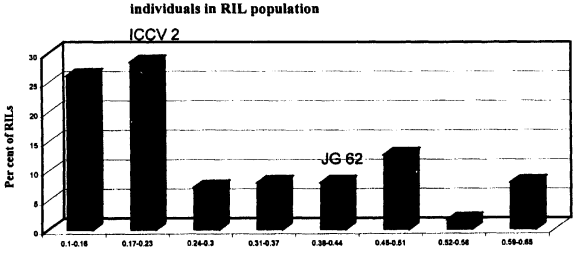


Table 35: Root volume of 126 F_{10} RILs at the time of flowering of ICCV2, JG 62 and at their respective flowering times

RIL number	At the flowering of ICCV 2 (cc)	At the flowering of JG 62 (cc)	Respective flowering time (cc)
1	1.94	6.29	4.46
2	2.53	3.30	2.93
3	2.36	3.26	2.89
4	3.08	4.50	3.97
5	1.85	3.00	2.10
6	2.59	5.79	6.4
7	2.40	5.80	5.60
8	2.05	3.09	2.90
9	2.06	2.81	2.90
10	3.06	4.85	3.80
11	2.21	4.37	4.20
12	2.30	3.27	3.63
13	2.08	7.22	5.59
14	1.89	2.50	2.56
15	1.78	3.95	3.03
16	1.98	5.55	5.31
17	2.25	7.40	7.42
18	2.46	3.92	6.69
19	3.13	8.42	8.15
20	3.03	9.93	9.09
21	3.24	6.50	7.12
22	2.15	2.97	3.57
23	2.29	2.74	2.99
24	3.18	4.21	4.09
25	2.05	3.26	2.96
26	1.97	3.30	1.82
27	3.35	8.03	8.02
28	1.92	5.11	6.71
29	2.87	7.77	7.66
30	2.94	5.72	12.15
31	2.48	3.11	2.37
32	2.28	3.63	3.37
33	2.67	4.07	3.00
34	2.48	5.56	3.31
35	3.06	4.90	8.40
36	1.47	4.25	5.90

Cont..

Table 35 Cont..

37	3.12	6.60	9.52
38	1.79	7.02	8.85
39	1.98	4.88	5.73
40	2.49	3.63	2.65
41	1.79	2.71	5.71
42	1.79	2.49	3.34
43	2.22	5.41	11.22
44	2.99	6.51	12.50
45	1.93	2.11	3.50
46	2.45	3.11	3.60
47	2.15	3.43	2.50
48	1.76	2.56	2.14
49	1.18	2.45	2.50
50	2.48	3.17	3.58
51	2.25	4.27	3.72
52	2.05	2.50	2.29
53	1.91	6.66	9.92
54	1.61	6.52	7.75
55	2.81	5.50	10.58
56	2.47	4.29	7.66
57	3.79	8.11	8.14
58	2.29	2.36	2.14
59	3.31	7.34	7.57
60	2.50	6.15	9.58
61	2.53	6.00	8.11
62	2.81	5.79	10.75
63	3.68	6.03	10.07
64	2.04	3.42	2.31
65	4.61	7.84	9.59
66	3.24	4.63	7.36
67	2.71	4.24	5.18
68	2.07	4.18	2.85
69	2.05	5.03	3.28
70	2.36	6.20	10.82
71	2.60	8.06	3.90
72	2.81	7.26	15.35
73	2.43	4.34	3.77
74	2.25	2.82	2.83
75	2.21	2.55	3.35
76	1.36	3.05	1.68
77	2.06	2.45	2.13
78	2.09	3.46	2.37
79	2.32	2.95	2.20
80	2.35	3.89	3.35

Cont..

Table 35 Cont..

81	1.51	1.74	2.13
82	2.10	5.86	3.50
83	2.00	5.89	1.31
84	3.00	2.84	2.65
85	2.39	5.55	6.70
86	2.06	3.13	3.12
87	2.08	2.25	2.42
88	2.14	3.73	3.64
89	2.73	5.49	7.73
90	3.30	3.37	5.73
91	2.01	5.13	9.94
92	2.28	9.20	9.10
93	1.82	4.18	6.61
94	3.05	7.60	7.17
95	2.65	6.14	4.95
96	2.59	6.20	5.16
97	1.36	2.91	2.43
98	2.73	4.15	3.48
99	1.96	2.27	1.94
100	3.32	7.11	7.06
101	1.65	4.19	4.64
102	2.29	6.45	5.41
103	1.72	2.21	3.02
104	2.70	4.19	1.95
105	2.13	3.84	2.33
106	2.16	4.90	2.57
107	3.04	6.96	2.21
108	2.17	5.38	2.14
109	3.05	4.99	9.52
110	1.76	8.40	4.59
111	2.51	5.33	9.80
112	2.59	5.98	5.43
113	2.90	3.85	2.75
114	2.55	5.16	2.99
115	2.55	5.54	4.61
116	2.73	2.54	2.63
117	2.01	2.75	2.64
118	3.19	4.82	2.18
119	2.85	4.50	3.16
120	2.31	4.00	3.19
121	2.12	6.65	11.85
122	2.40	3.80	2.38
123	1.90	3.82	2.41

Cont..

Table 35 Cont..

124	2.23	5.61	6.41
125	2.61	5.82	8.77
126	2.22	5.60	6.70
S.E.D	1.10	1.20	1.01
L.S.D	2.20	2.34	2.01

Distribution for all the characters studied showed a continuous variation (Fig 16, Fig 17, Fig 18 and Fig 19). The graphs were not similar to normal curves for any of the characters. RILs showing expression beyond the ranges of both the parents were present for all the characters.

Root volume observations were recorded at the time of flowering of ICCV 2 and JG 62. There was no significant difference among the RILs for root volume at the time of flowering of ICCV 2 but statistically significant differences were observed at the time of flowering of JG 62.The root volume of the RILs that flowered along with ICCV 2 was almost same at both the flowering times. The RILs that flowered late had significantly more root volume at the flowering time of JG 62 than at ICCV 2. The root volume of the RILs at the time of flowering of ICCV 2, JG 62 and at their respective flowering time are presented in Table 35.

4.2.10 Correlation coefficients

Correlation coefficients indicate the association between pairs of the characters under study. These were computed for F_2 generation and RILs and are presented in Table 36 and 37 respectively.

4.2.10.1 Root length

In F_2 population, root length had significant positive correlation with root volume (0.905**), root dry weight (0.912**), no of leaflets per pinnule (0.403*), leaf area (0.544**), total number of nodes (0.631**), root shoot ratio (0.338**), leaf dry weight (0.615**) and shoot dry weight (0.719**). Root length had shown significant negative correlation with leaf area/root length (-0.386*).

Table 36: The phenotypic correlation coefficients between different characters in F_2 generation of the cross ICCV 2 x JG 62

	Rl	Rv	Rdw	Lno	La	Sla	Tnn	Arl	Rsr	Ldw	Sdw	Slw
Rv	0.91**											
Rdw	0.91**	0.88**										
Lno	0.41*	0.38*	0.40*									
La	0.54**	0.62**	0.50**	0.54**								
Sla	-0.22	-0.21	-0.21	-0.13	0.05							
Tnn	0.63**	0.62**	0.56**	0.54**	0.59**	-0.52						
Arl	-0.39*	-0.16	-0.34*	0.26	0.59**	0.24	0.04					
Rsr	0.34*	0.32*	0.55**	-0.07	0.10	0.22	0.27	-0.13				
Ldw	0.62**	0.68**	0.56**	0.61**	0.84**	-0.47*	0.82**	0.31*	0.03			
Sdw	0.72**	0.71**	0.63**	0.51**	0.76**	-0.37	0.90**	0.12	-0.28	0.89**		
Slw	0.20	0.22	0.20	0.14	-0.05	-0.42*	0.51**	-0.22	-0.25	0.46	0.37*	
Arv	-0.23	-0.30*	-0.23	0.20	0.54**	0.25	0.02	0.83**	-0.10	0.31*	-0.27	-0.27

* Significant at 5% level ** Significant at 1% level

In the RIL population similar results were obtained. In RIL population Root length had shown significant negative correlation with leaf area/root volume (-0.35*), which was non-significant in F_2 population.

4.2.10.2 Root volume

In F_2 population, root volume had positive significant correlation with root length (0.905**), root dry weight (0.877**), number of leaflets (0.380*), leaf area (0.616**), total number of nodes (0.621**), root/shootratio (0.322*), leaf dry weight (0.677**) and shoot dry weight (0.705**). It had significant negative correlation with leaf area/root volume. There was no correlation with specific leaf area, specific leaf weight and leaf area/root length.

Similar results are obtained for root volume in RIL population. Root length did not have significant correlation with 100 seed weight in RIL population.

4.2.10.3 Root dry weight

Root dry weight had significant positive correlation with root length (0.912**). Root volume (0.877**), number of leaflets (0.400**), leaf area (0.494*), total number of nodes (0.564**), root shoot ratio (0.536**), leaf dry weight (0.559**) and shoot dry weight (0.632**). It had significant negative correlation with leaf area/root length (-0.343*).

In RIL population significant negative correlation was obtained with area/root volume (-0.33*) but not with leaf area/root length. Correlations with 100 seed weight, specific leaf area and specific leaf weight were non-significant.

4.2.10.4 Number of leaflets

Number of leaflets had positive significant correlation with root length (0.403*), root volume (0.380*), root dry weight (0.400*), leaf area (0.543**), total number of nodes (0.537**), leaf dry weight (0.608**) and shoot dry weight (0.511**). The correlations with remaining characters were non-significant.

Similar results were obtained in RIL population.

4.2.10.5 Leaf area

Leaf area had significant positive correlation with root length (0.544**), root volume (0.616**), root dry weight (0.494*), number of leaflets (0.543**), total number of nodes (0.598**), leaf area/root length (0.593**), leaf dry weight (0.837**), shoot dry weight (0.761**), and leaf area/root volume (0.542**). The correlations with specific leaf area, specific leaf weight and root/shootwere non-significant.

In RIL population leaf area had significant positive correlation with seed weight (0.48*). The relationship with remaining characters were same as in F_2 population.

4.2.10.6 Specific leaf area

In F_2 population specific leaf area had significant correlation with total number of nodes (-0.523**), Leaf dry weight (-0.468*), shoot dry weight (-0.370*) and specific leaf weight (-0.421*).

In RIL population specific leaf area had significant correlation with leaf area/root length (0.45*), leaf area/root volume (0.35*), specific leaf weight (-0.39*) and total number of nodes (-0.49*). With all other characters the correlations were found to be non-significant.

4.2.10.7 Total number of nodes per plant

Total number of nodes per plant had positive significant correlation with root length (0.631**), root volume (0.621**), leaflet number (0.537**), root dry weight (0.564**), leaf area (0.598**), leaf dry weight (0.822**), shoot dry weight (0.902**), root/shoot ratio (0.181**), specific leaf weight (0.311**). It had significant negative correlations with all other characters.

Similar results were obtained in RIL population for total number of nodes.

4.2.10.8 Leaf area /root length and leaf area /root volume

Both these characters had negative correlation with root length (-0.386*, -0.230), root volume (-0.159, -0.301*), root dry weight (-0.343*, -0.228), root/shoot ratio (-0.126, -0.103) and specific leaf weight (-0.216, -0.267). With remaining other characters positive correlation was observed for both the characters. Positive significant correlation was observed with other characters.

Similar results were observed for both the characters in RIL population with all the characters.

4.2.10.9 Root/shoot ratio

Root/shoot ratio had significant positive correlation with root length (0.338*), root volume (0.322*) and root dry weight (0.536**). With all the other characters the correlation was non-significant.

In RIL population the root/shoot ratio had significant correlation with leaf area/root length (-0.36*) and leaf area/root volume (-0.37*) in addition with root length (0.40*), root volume (0.33*) and root dry weight (0.39*).

Table 37: The phenotypic correlation coefficients between different characters in F_{10} RIL population of the cross ICCV 2 x JG 62

	Rl	Rv	Lno	La	Sw	Sla	Arl	Arv	Rsr	Slw	Ldw	Rdw	Sdw
Rv	0.91**												
Lno	0.54**	0.57**											
La	0.55**	0.63**	0.46*										
Sw	-0.13	0.05	0.07	0.48*									
Sla	-0.19	-0.19	-0.08	0.16	-0.04								
Arl	-0.39*	-0.31*	0.17	0.48*	0.04	0.45*							
Arv	-0.35*	-0.36*	0.13	0.39*	-0.02	0.35*	0.63**						
Rsr	0.40*	0.33*	0.01	-0.17	0.14	0.10	-0.36*	-0.37*					
Slw	0.10	0.20	0.16	0.08	0.54**	-0.39*	-0.08	-0.06	-0.12				
Ldw	0.59**	0.56**	0.48*	0.79**	-0.05	-0.29	-0.04	-0.06	-0.03	0.23			
Rdw	0.81**	0.94**	0.57**	0.58**	-0.03	-0.24	-0.29	-0.33*	0.39*	0.14	0.46*		
Sdw	0.76**	0.87**	0.66**	0.69**	-0.06	-0.28	0.06	0.07	-0.03	0.29	0.79**	0.87**	
Tnn	0.80**	0.89**	0.67**	0.59**	-0.19	-0.49*	-0.18	-0.19	-0.18	0.49*	0.69**	0.87**	0.90**

* Significant at 5% level ** Significant at 1% level

4.2.10.10 Leaf dry weight

Leaf dry weight had significant positive correlation with root length (0.615**), root volume (0.677**), root dry weight (0.559**), number of leaflets (0.608**), leaf area (0.837**), total number of nodes (0.822**), leaf area/root length (0.312*), shoot dry weight (0.889**), specific leaf weight (0.460*) and leaf area/root volume (0.306*). Significant negative correlation was observed with specific leaf area (-0.468*).

In RIL population similar results were obtained.

4.2.10.11 Shoot dry weight

Shoot dry weight had shown significant positive correlation with root length (0.719**), root volume (0.705**), leaflet number (0.511**), root dry weight (0.632**), leaf area (0.761**), total number of nodes (0.902**) and specific leaf weight (0.371*). It had significant negative correlation with specific leaf area (-0.370*).

Similar results were obtained in RIL population. However, the correlation was not significant with specific leaf area and specific leaf weight.

4.2.10.12 Specific leaf weight

Specific leaf weight had significant negative correlation with total number of nodes (-0.523**), leaf dry weight (-0.468*), shoot dry weight (-0.370*), and specific leaf weight (-0.421*). Positive correlation was observed with leaf area (0.049), leaf area/root length (0.238), leaf area/root volume (0.252) and root/shoot ratio (0.224). With remaining all characters non-significant negative correlations were observed.

In RIL population the relationship of specific leaf area remained the same as in F_2 population with all characters

Table 38: Estimates of heritability for different characters in F_2 and RILs of the cross ICCV 2 x JG 62

Character	F_2 population		RIL
	h^2(b)(%)	h^2(n)(%)	h^2(b)(%)
Root length	77	93	85
Root volume	66	63	89
Leaf let number	58	76	80
Leaf area	74	84	80
Dry root weight	22	42	89
Dry shoot weight	86	81	89
Total number of nodes	96	54	86

4.2.10.13 Hundred seed weight

The 100 seed weight had significant correlation with leaf area (0.48*) and specific leaf weight (0.54**). With all other characters correlations were non-significant.

4.2.11 Heritability

Broad sense and narrow sense heritability values estimated from F_2 generation and broad sense heritability estimated from RIL population are presented in Table 38.

4.2.11.1 Root length

Root length had broad sense heritability of 77% and narrow sense heritability of 93% for root length. The heritibility estimated from RIL population was 85%.

4.2.11.2 Root volume

Moderate broad sense heritability (66%) and narrow sense heritability (63%) were observed for this character. The heritability estimate obtained from RIL population was high (89%).

4.2.11.3 Leaf let number and leaf area

Broad sense heritability and narrow sense heritability for leaflet number were of 58% and 76% respectively and of leaf area were 74%and 84% respectively. The heritability estimates from RILs for both the characters were 80% and 80% respectively.

4.2.11.4 Root dry weight

The broad sense and narrow sense heritability estimates for root dry weight were 22% and 42% respectively. The broad sense heritability obtained from RILs was 89%.

Table 39: Estimates of co-heritability for different characters in F_{10} RIL population of the cross ICCV 2 x JG 62

	Rl	Rv	Pno	La	Tnn	Sdw	Rdw	Ldw	Sla	Slw	Rsr	Arl	Arv	Sw
Rl		0.90	0.98	0.99	0.95	0.94	0.89	0.62	0.92	0.98	0.58	0.91	0.93	1.00
Rv			0.96	1.00	0.91	0.92	0.91	270.7	0.97	1.00	0.80	0.94	0.91	1.01
Pno				1.10	0.96	0.98	0.98	4.06	1.07	1.02	7.44	1.06	0.96	1.03
La					0.95	1.0	1.01	1.09	0.73	18.7	1.32	0.82	0.84	1.00
Tnn						0.89	1.06	1.07	0.98	1.14	1.55	0.86	0.88	1.01
Sdw							0.94	0.75	0.94	0.74	3.29	0.97	0.95	1.14
Rdw								1.01	0.22	1.02	0.66	0.95	0.95	1.01
Ldw									0.38	0.12	0.60	0.77	0.66	0.97
Sla										0.28	0.04	0.79	0.70	1.09
Slw											0.46	0.82	0.58	0.97
Rsr												0.90	0.97	1.05
Arl													0.86	1.00
Arv														1.02

Rl	Root length	Ldw	Leaf dry weight
Rv	Root volume	Sla	Specific leaf area
Pno	Number of leaflets	Slw	Specific leaf weight
La	Leaf area	Rsr	Root shoot ratio
Tnn	Total number of nodes	Arl	Area/root length
Sdw	Shoot dry weight	Arv	Area/root volume
Rdw	Root dry weight	Sw	Seed weight

4.2.11.5 Shoot dry weight

The broad sense heritability for shoot weight was 86% and narrow sense heritability was 81%. The heritability estimate from RIL population was 89%.

4.2.11.6 Total number of nodes

This character had high broad sense (96%) and moderate narrow sense heritabilities (54%). The broad sense heritability obtained from RIL population was also high (86%).

4.2.12 Co-heritability

The co-heritabilty estimates are presented in Table 39.

All the characters had high co-heritable estimates with one another except with some exceptions. The co-heritable values between some characters were more than one that might be due to negative correlation coefficients or no correlations between them. This shows that most of the characters are co-heritable.

4.3 EXPERIMENT III (Molecular marker studies)

The parents ICCV 2 and JG 62 were screened with different molecular markers and the polymorphic markers were used to genotype the 126 F_{10} RIL population. The data were analyzed using MAPMAKER V2.0 (Lander *et al.*, 1987) programme and the linkage map constructed are presented as follows.

4.3.1 Primer selection and DNA polymorphism

Initially, the two parents, ICCV 2 and JG 62 were screened with 200 RAPDs, 85 STMS, 36 SAMPL, 23 MP-PCR, 50 ISSRs and 765 combinations of RMMFPs. Out of these primers 6 RAPDs, 1 ISSR, 37 STMS, 5 MP-PCR and 12 RMMFPs were

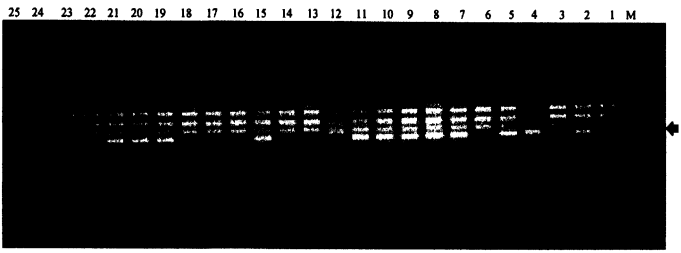


Plate 11: RAPD profile of the RILs of the cross ICCV 2 x JG 62 with primer F9. M indicates molecular weight marker, Lane 1 represents parent ICCV 2 and lane 2 represents JG 62. Arrow indicates the segregating marker.

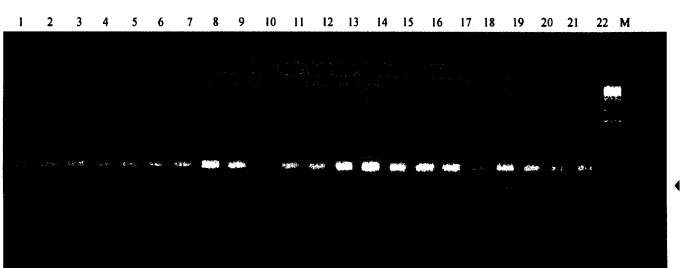


Plate 12: ISSR profile of the RILs of the cross ICCV 2 x JG 62 with primer UBC 858. M indicates molecular weight marker, Lane 1 represents parent ICCV 2 and lane 2 represents JG 62. Arrow indicates the segregating marker.

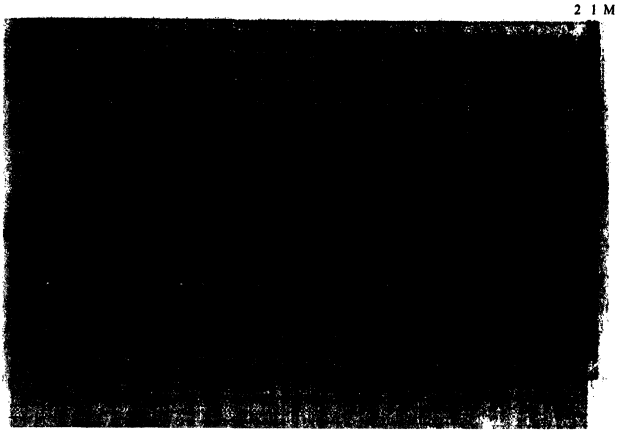


Plate 13: STMS profile of the RILs of the cross ICCV 2 X JG 62 with the primer GA137. M indicates molecular weight marker, Lane 1 represents parent ICCV 2 and lane 2 represents JG 62

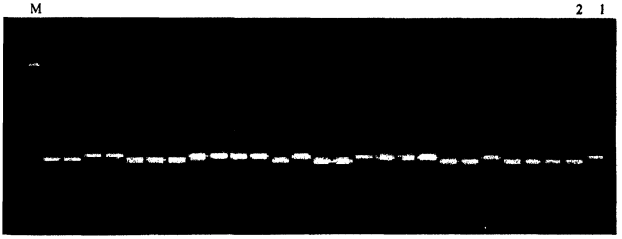


Plate 14: STMS profile of the RILs of the cross ICCV 2 X JG 62 with the primer Ca STMS 5.M indicates molecular weight marker, Lane 1 represents parent ICCV 2 and lane 2 represents JG 62

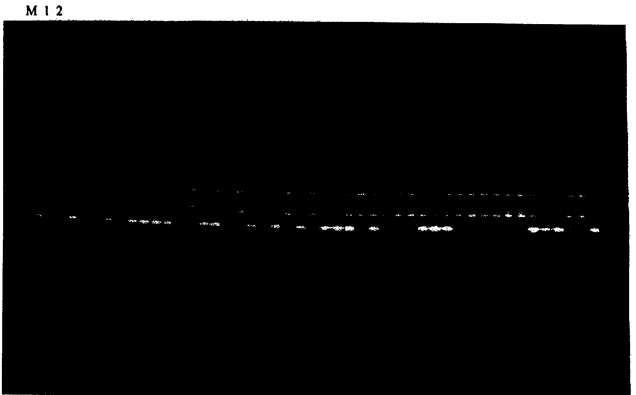


Plate 15: MP-PCR profile of the RILs of the cross ICCV 2 x JG 62 with primer SA 18. M indicates molecular weight marker, Lane 1 represents parent ICCV 2 and lane 2 represents JG 62. Arrow indicates the segregating marker.

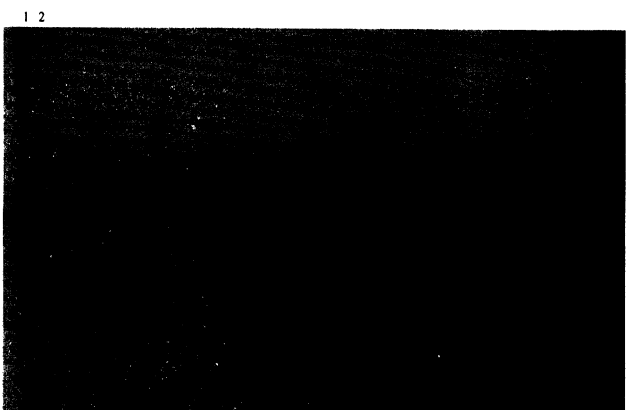


Plate 16: RMMFP profile of the RILs of the cross ICCV 2 x JG 62 with the primer Combination TS 71rTS36r . Lane 1 represents ICCV 2 and lane 2 represents JG 62

polymorphic between the parents. None of the SAMPL primers were polymorphic between the parents. The primers that produced unambiguous fragments and gave repeatable patterns were selected and used to amplify the genomic DNA from the RILs of this cross.

4.3.2 Inheritance of molecular markers

Segregation of all RAPD and ISSR markers was evaluated in 126 RIL population and that of 6 STMS markers in 126 RIL population and remaining 31 STMS markers in 84 RIL population. Segregation of MP-PCR markers and RMMFP markers was evaluated using 94 RIL population. The details of the DNA markers and the segregation in RIL population are presented in the Table 40.

Seventy two percent of the markers segregated in the ratio of 1:1 as expected for the recombinant inbred lines. Remaining twenty-eight percent of the markers had distorted segregation due to the preferential inheritance of the alleles of a particular parent.

Different marker classes exhibited varied segregation distortion. It was relatively more pronounced for MP-PCR (60%) and less for RAPDs (16%) (Table 41). The STMS marker TA-103 had a χ^2 value of 34.7 and was deleted from MAPMAKER analysis. One of the RMMFP GA2GA8R1 had a χ^2 value more than 10. Most of the markers that had segregation distortion were grouped together, while some were not linked to any group. For example Linkage group 1 and Linkage group 4 mostly have the markers with segregation distortion i.e., around χ^2 value of 7. The pattern of DNA fragments amplified by RAPD ISSR STMS, MP-PCR and RMMFP are shown in Plate11, Plate 12, Plate 13, Plate 14, Plate 15 and Plate 16.

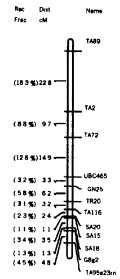
4.3.3 Linkage map construction and mapping of the agronomically important genes

Based on the data obtained using DNA and morphological markers a skeleton linkage map of the intra specific cross was constructed using MAPMAKER V2.0 (Lander *et al.*, 1987)) and is depicted in Fig 20 and a few important features of each linkage group are described in Table 42. Out of the 69 segregating markers 56 formed 9 linkage groups while 13 markers remained unlinked. The present map consisted of 9 linkage groups with seven morphological trait loci, 4 RAPD, 1 ISSR, 32 STMS, 5 MP-PCR and 7 RMMFP loci covering 262.8 cM with an average distance of 4.7 cM between two consecutive markers. The longest linkage group included 12 markers covering 73.2 cM distance with an average of 6.1 cM between the consecutive markers while three markers (TR 2, TA 3 and TA 127) formed the smallest linkage group covering only 2.9 cM length and average distance between two markers in each group ranged from 2.9 cM to 73.2 cM and 0.96 cM to 7.58 cM respectively. The longest gap between two markers corresponded to 24 cM in linkage group 3 (Fig 20).

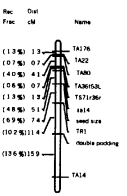
The percentage of unlinked markers varied for different marker types. As summarized in Table 41, at Lod score 3 only 14% of STMS markers were unlinked whereas 42% of RMMFPs were unlinked. The distribution of markers was not random. Linkage group 4 and linkage group 9 had only STMS markers whereas in linkage group 1 intermingling of different marker types was observed.

4.3.4 Linkage of agronomically important traits

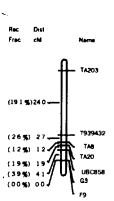
Linkage for agronomically important characters was observed for double podding, seed size and fusarium wilt resistance gene. The seed size locus was flanked by MP-PCR marker sa-14 on one side at a distance of 5.1cM and by TR1 loci on the other side at a distance of 7.4 cM in linkage group 2. Similarly TR 1 and TA 14 flanked the gene for



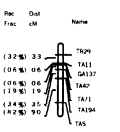
Linkage group 1



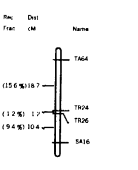
Linkage group 2



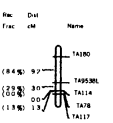
Linkage group 3



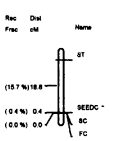
Linkage group 4



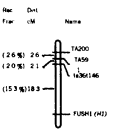
Linkage group 5



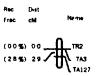
Linkage group 6



Linkage group 7



Linkage group 8



Linkage group 9

Fig 20: Linkage map of chickpea cross ICCV 2 X JG 62 based on morphological traits and molecular markers

Table 41: Number of markers analysed and segregation distortion of different marker types

Marker type	Number of Markers	Unlinked markers (%)	Distorted segregation
RAPD	6	2(33%)	1 (16%)
ISSR	1	0(0%)	0(0%)
STMS	37	5(14%)	10(27%)
AMP-PCR	5	0(0%)	3(60%)
RMMFP	12	5(42%)	4(33%)

Table 42: Linkage groups of chickpea (*Cicer arietinum* L.)

Linkage group	Length (cM)	Number of markers	Average spacing[a] (cM)	Largest length [b] (cM)
1	73.2	12	6.1	22.8
2	47.9	10	5.3	15.9
4	33.9	7	4.8	24.0
3	18.9	7	2.7	9.0
5	30.3	4	7.6	18.7
6	13.5	5	2.7	9.2
7	19.2	4	4.8	18.8
8	23.0	4	5.7	18.3
9	2.9	3	1.0	2.9
	262.8[c]	56[c]	4.7[d]	

a Average spacing between two consecutive markers
b Largest interval between two consecutive markers
c Total of all linkage groups
d Average of all linkage groups

double podding on either side at a distance of 11.4 cM and 15.9 cM, respectively in linkage group 2 (Fig 20).

Two genes govern fusarium wilt resistance in this cross. One of the two genes (H_1) governing the expression of this character was linked in linkage group 8 to the RMMFP Ta36t146 at a distance of 18.3 cM (Fig 20).

When the present linkage map was compared to the recent linkage map of the interspecific cross in chickpea (Winter *et al.*, 2000), a total of 17 STMS loci showed synteny in 6 linkage groups.

4.3.5 Residual heterozygosity

The F_{10} RILs are expected to be completely homozygous at all loci. But in the present experiment 0.8% of the RILs i.e., one RIL out of 126 RILs had heterozygous condition for 40% of the co-dominant STMS markers. Heterozygous loci were considered as missing data and were not considered for mapping.

4.3.6 Random mixing of microsatellite flanking primers (RMMFPs)

The microsatellite flanking primers were used by mixing randomly instead of using the particular right and left primers to produce the amplification products. This technique was developed by Dr. Peter Winter, Plant Molecular Biology, Biocenter, Johann Wolfgang Goethe University, Frankfurt am Main, Germany. Out of 765 primer combinations tested 20 were polymorphic between the parents, but only 12 polymorphic markers were used to genotype the RIL population and others were not utilized because of lack of reproducibility. The segregation pattern of these molecular markers was similar to other markers (Plate 17). Out of 12 markers, 7 markers were linked to different linkage groups and 5 were unassigned. Out of these 7 linked markers Ta36t146 was

[illegible] of 18.3 cM. Out of

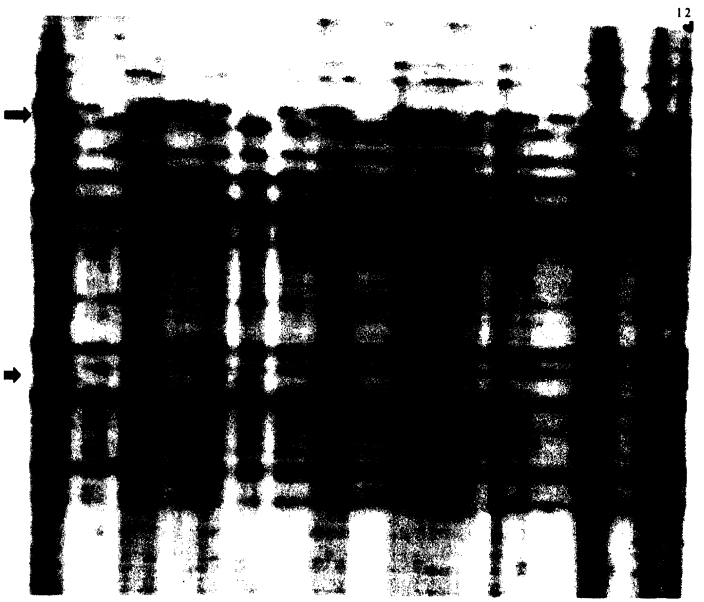


Plate 17: RMMFP profile of the RILs of the cross ICCV 2 x JG 62 with primer combination TA11rTA45sr. Lane 1 represents ICCV 2 and lane 2 represents JG 62. Arrow indicates the segregating marker.

theses 12 segregating markers 75 % were co-dominant markers. On an average every combination tested produced 10-15 amplicons.

4.3.7 Sequencing of the polymorphic loci produced by RMMFPs

Four co-dominant polymorphic loci produced by RMMFPs were cloned in vector pGemT and sequenced. The sequence information of these four loci is presented in the Table 43.

In the first locus amplified by Ta 36r and Ta 146l the polymorphism was due to the length of the microsatellite region. The repeat (TTA) is 39 times in ICCV 2 and 28 times in JG-62. The flanking region of the microsatellite was same in both the parents.

In the second locus amplified by TA 36r and TS 53l, the polymorphism was also due to the difference in the length of the microsatellite region. The repeats (TTA) was 39 times in ICCV2 and 27 times in JG 62. The flanking region of the microsatellite was same in both the parents in this case also.

In the third locus, the microsatellite repeat (TTA) is 27 times in JG 62. The other allele from ICCV 2 was not sequenced.

When the three loci amplified by three different primer combinations were compared for homology in their sequences, a high homology was observed in the flanking sequences of the microsatellite region.

When the first and second loci were compared where one primer was common, the primer-binding site for this common primer was same in both the cases except for the substitution of C in place of G in the second loci. The region flanking the microsatellite had homology upto a certain extent and then there was no homology in the sequence. The primer-binding site for the other primer was entirely different in both the loci. The segregation pattern of these loci in the RILs was also different. When the third locus was compared to the first and second loci, there was homology in the microsatellite flanking

Table 43: Sequences of the regions amplified by different combinations of microsatellite flanking primers

Primer combination 1: TA36rTA146l

ICCV 2

(TA36r)**TTTCAACTTAAGACATGAAATTTGTTTTT**AACGGTTCCTT(**TTA)39**AAA
ATTAAAATAACACATTACAATAGTAACCGACAATTTTTTTTGAATAATTATCTC
CCTTCCTAAGGTAAAATATTAAAATAAAATAAAATGTCTGGATGTTACAGATTA
GATCTTAAAATCTCAACACAGTAATTTGAGTTAATTTTTGAACTTAAAGTACCA
CATGCATTAAATAAT**ATAATTTAAGGACTAACATATTAAACTTAG**(TA146l)

JG 62

(TA36r)**TTTCAACTTAAGACATGAAATTTGTTTTT**AACGGTTCCTT(**TTA)28**AAA
ATTAAAATAACACATTACAATAGTAACCGACAATTTTTTTTGAATAATTATCTC
CCTTCCTAAGGTAAAATATTAAAATAAAATAAAATGTCTGGATGTTACAGATTA
GATCTTAAAATCTCAACACAGTAATTTGAGTTAATTTTTGAACTTAAAGTACCA
CATGCATTAAATAAT**ATAATTTAAGGACTAACATATTAAACTTAG**(TA146)

Primer combination 2: TA36rTS53l

ICCV2

(TA36r)**TTTCAACTTAAGACATGAAATTTGTTTTT**AACCGTTCCTT(**TTA)39**AAA
ATTAAAATATCACATTACAATAATAACCGACATTAGAATAATTATCTCAATTCC
TAATAAGGTAAAATATTAAAATAAAATAAAATGTCTGGATGTTACAAAAGACA
CATTCAACCTAGGAAAAAAAAACTCGGTTTCTATGCAATAATATTGATATATGA
TATTGAACAATAATTGAC**ATTATACAAATGAACTTTTGGAACGATC**(TS53l)

JG 62

(TA36r)**TTTCAACTTAAGACATGAAATTTGTTTTT**AACCGTTCCTT(**TTA)27**AAA
ATTAAAATATCACATTACAATAATAACCGACATTAGAATAATTATCTCAATTCC
TAATAAGGTAAAATATTAAAATAAAATAAAATGTCTGGATGTTACAAAAGACA
CATTCAACCTAGGAAAAAAAAACTCGGTTTCTATGCAATAATATTGATATATGA
TATTGAACAATAATTGAC**ATTATACAAATGAACTTTTGGAACGATC**(TS53l)

Primer combination 3: TA95rTS38l

JG 62

(TA95r)**GGAAAGTGATATTTGAACATAAATC**AAGTTAAGAACCTAAGCCAACT
TTAAGCCAGCCAGAAGCAGTGGGGAACGTAGTATTAAATTGAGAAAATGATAT
TTTAACCTNAACCATGAAATTTGTTTTTAACCGTTCCTT(**TTA)27**ATATTATCAA
AAATTAAAATAATACATTACAGTAGTAACTATTTAGAATAATTATTTCAACTTC

ACTACTAATAAGATAAAATATAAAAATAAAATAAAATAAAATATTTAGATGNC
CCATAGTTTTGTCTTCGAAATTTGACTGTAATNAAAAGAAAATGTGAGTCTTAC
ATTGAGTTTGTGAATTTAAAATGTC(TS38I)

Primer combination 4: TS71rTS36r

ICCV 2

(TS36R)**TAATGTAGATTTATTTCTGGGCAAT**CCAACTTTTGGACTTTTCCGTTG
GATCCGTTTTTAATCCCATGTTTTTTTATATGGGCGATCCAACTTTTGGACTTTC
CCGCTGGATCCGCTTTTGATACCATTTTTTTTTTTTTTTTTTGGCGATCTTTTGCT
GGGTGTACAACTTCGACCCTTTTGCTGAACGTGGGCTTCGGCTCTTTTACTGAGC
GTAGAACTTCAGCCTTTTTCGTTTGCTTAAGAAGTATTTTAATAAAAAGCCTGTT
TTAATCTTAAACAATAGAAATGAATAAAAATGAAACTAAGAGTCATTTAAGAT
TCATTTCTCCCCGTCGACAACTGTGGATTAAAACAGGCTGGAAATGAAAAATAA
TTTTTCCTTTATTACTA**(TTA)13**TTTATATACTAATATACACTTCTTTATATTTTTA
TTTTTATTTATT**AATCCCTATATTCGAAAATTGTTAG**(TS71R)

JG 62

(TS36R)**TAATGTAGATTTATTTCTGGGCAAT**CCAACTTTTGGACTTTTCCGTTG
GATCCGTTTTTAATCCCATGTTTTTTTATATGGGCGATCCAACTTTTGGACTTTC
CCGCTGGATCCGCTTTTGATACCATTTTTTTTTTTTTTTTTTGGCGATCTTTTGCT
GGGTGTACAACTTCGACCCTTTTGCTGAACGTGGGCTTCGGCTCTTTTACTGAGC
GTAGAACTTCAGCCTTTTTCGTTTGCTTAAGAAGTATTTTAATAAAAAGCCTGTT
TTAATCTTAAACAATAGAAATGAATAAAAATGAAACTAAGAGTCATTTAAGAT
TCATTTCTCCCCGTCGACAACTGTGGATTAAAACAGGCTGGAAATGAAAAATAA
TTTTTCCTTTATTACTA**(TTA)16**TTTATATACTAATATACACTTCTTTATATTTTTA
TTTTTATTTATT**AATCCCTATATTCGAAAATTGTTAG**(TS71R)

Note: The red colour indicates the flanking homologous sequences.

sequence. The sequence of the primer TA36r was found in one of the flanking side of the microsatellite in the loci amplified by the third combination. The homology of this sequence of the primer to the sequence found in the amplicon is 75%. The segregation of all these three loci was different in the RIL progeny.

The fourth loci amplified by Ts 71r and Ts 36 r also had the microsatellite repeat (TTA). The polymorphism due to this primer combination was also due to the difference in the length of the microsatellite region. The repeat (ATT) was 13 times in ICCV 2 and 16 times in JG-62. The flanking region of the microsatellite was same in both the parents. When the flanking sequence of microsatellite of this locus was compared to the flanking microsatellite sequence of the other three loci, there was no homology in the sequence.

DISCUSSION

CHAPTER V

DISCUSSION

The results of the investigation on the inheritance of shoot and root characters, and molecular markers in chickpea (*Cicer arietinum* L.) are discussed in this chapter. The data were recorded on days to first flower, days to first pod, days to maturity, number of nodes up to first flower, fusarium wilt resistance, root length, root volume, leaf let number, leaf area, root dry weight, shoot dry weight, leaf dry weight, and total number of nodes up to first flower on P_1, P_2, F_1, F_2, BC_1P_1, BC_1P_2, F_3, and 126 RILs of the cross ICCV 2 and JG 62.

Flowering time has an immense bearing on the agronomic performance of a crop and is fundamental for its improvement. It is a major component of adaptation, particularly when the growing season is restricted by climatic factors such as terminal drought and high temperatures (Subbarao *et al.*, 1993). The time to flowering is determined by the genotype, moisture availability, temperature profile, and photoperiod response of the variety. The duration of the reproductive phase of the crop in most of the chickpea-growing areas is limited by the initiation of flowering and the end-of-season drought that terminates seed set. Because of the indeterminate growth habit of the chickpea, duration of its flowering period is a major yield determinant (Bonfil and Pinthus, 1995). Early flowering combined with other desirable plant traits might make it possible to prolong the reproductive phase in various target environments.

Root volume and root length have been identified as important traits contributing to drought tolerance in chickpea. Chickpea is one of the deep rooting species among cool season food legumes and its rooting depth varies from 1.20 m to 1.35 m. Chickpea is usually grown on the residual soil moisture and on marginal lands. As a result, chickpea is generally exposed to varying degrees of terminal drought stress as determined by the

previous rainfall, atmospheric evaporative demand and soil characteristics. The yield loss due to terminal stress is estimated to be 35 to 50% (Saxena *et al.*, 1993a). Optimizing root systems to minimize soil related stress is needed. Early prolific root system under limited water environments is needed in chickpea. Genetic improvement of crop species requires knowledge of intraspecific variability which is reported in chickpea by Singh *et al.*, (1988). But the information on the inheritance of root characters in chickpea is meagre. Waldia *et al.* (1993) studied the inheritance of the speed of radicle emergence and reported two independent genes with additive epistatic interaction.

In the present study, inheritance of flowering time, maturity and podding duration and root characters were studied and the results obtained are discussed here.

The mean values of F_1 for all the characters studied (days to first flower, days to first pod, days to maturity, podding duration and number of nodes up to first flower) were closer to the parent JG 62, indicating the presence of dominant alleles for all these characters in JG 62. Thus late flowering was dominant over early flowering, late maturity over early maturity, higher pod filling period and total reproductive period over low pod filling period and total reproductive period and initiation of flower at higher node to that at lower node. Dominance of late flowering over early flowering was also reported for chickpea by Kumar and van Rheenen (2000) and Orr *et al.* (1999) and in lentil by Sarker *et al.* (1999).

Parents differed significantly from each other for both root length and root volume. The mean values of F_1 for all the characters except total number of nodes up to first flower were closer to the mean of JG 62. This indicated that the dominant genes for all the characters are present in JG 62.

5.1 Heritability of shoot characters

On an average ICCV 2 flowered in 34.8±0.70 days and JG 62 in 44.5±0.29 days. The F_1 of this cross flowered in 42.1±0.31 days. The days to first pod in ICCV 2 was 41±0.92 days and that of JG 62 was 50.6±0.47 days and F_1 was 48.4±0.39 days. ICCV 2 matured in 84.5 ± 0.21 days, JG 62 in 98.7±0.58 days and F_1 in 93.9±0.66 days. The pod filling period and total reproductive period in ICCV 2 were 43.5±0.82 days and 49.7±0.59 days respectively. These were 48.1±0.98 days and 54.2±0.83 days for JG 62. First flower initiation in ICCV 2 was at 18^{th} node, in JG 62 was at 23.4^{th} node and in F_1 was at 23^{rd} node. The results indicate the existence of sufficient variability between the parents for the characters studied for their effective utilization. More diverse the parents, the greater the chances of recovering desirable recombinations. Thus, crop improvement depends on the magnitude of genetic variability in the base population. This variability can be easily utilized if the heritability of these characters is high.

Heritability is a good index of the transmission of characters from parents to their offspring (Falconer, 1981). The estimates of heritability help the plant breeder to make decisions for selection of elite genotypes from the target population.

The broad and narrow sense heritability estimates for days to first flower and days to first pod were high in segregating generations as well as in RIL population. High narrow sense heritabilities for these characters indicate that the genotypic variation for these characters was due to additive gene action. Therefore, selection for these traits can be effective. Rao *et al.* (1994) reported high broad sense heritability for days to flowering. High narrow sense heritability for days to first flower in chickpea was also reported by Pandey *et al.* (1990). Sabaghpour (2000) also reported high broad sense heritability and narrow sense heritability for days to first flower in chickpea.

Days to maturity had high broad sense heritability in RIL population as well as in segregating generations but had medium narrow sense heritability. Low estimate of narrow sense heritability for days to maturity was reported by Rastogi and Singh (1977) in chickpea. Moderate narrow sense heritability for days to maturity indicate the role of non-additive gene action. Predominance of dominant gene action over additive gene action for days to maturity was reported by Salimath and Bahl (1989), Kidambi *et al.* (1988) and Jha *et al.*(1997).

Both pod filling period and total reproductive period had high broad sense heritability but low narrow sense heritability. The heritability estimate for RIL population was also moderate. High broad sense heritability estimate and low narrow sense heritability estimate indicate that the expression of these characters were mostly influenced by the environment.

Number of nodes up to first flower had high broad sense heritability in F_2 population and also F_{10} RIL population. The narrow sense heritability was also high. This indicate the significant role of the additive gene action in the expression of this character.

5.2 Heritability of root characters

In the present studies, the root length and root volume of ICCV 2 and JG 62 were same at the time of flowering of ICCV 2. At the time of flowering of JG 62, ICCV 2 and JG 62 significantly differed in root length. However, the difference was more significant when the root length and root volume were measured at their respective flowering time. Hence, the observations were recorded in all the generations at their respective flowering times i.e. as and when they flowered.

The mean root length of ICCV 2 was 11.7±0.59 m and that of JG 62 was 17.6±0.65 m. The mean root length of F_1 was 22.7±1.12 m. The root volume of ICCV 2,

JG 62 and F_1 were 4.1±0.13 cc, 6.5±0.23 cc and 7.1±0.41 cc respectively. The average number of leaflets in ICCV 2 was 35.7±0.25, in JG 62 was 40.4±0.24 and in F_1 was 39.7±0.75. The mean leaf area of ICCV 2 and JG 62 were 10.5±0.35 cm^2 and 6.9±0.44 cm^2 respectively. The leaf area of F_1 was 8.8±0.63 cm^2. The mean root dry weight and mean shoot dry weight of ICCV 2 were 0.28±0.013 g, 1.0±0.04 g respectively, of JG 62 were 0.44±0.014 g, 1.5±0.04 g and that of F_1 was 0.54±0.048 g and 1.3±0.07 g respectively. On an average, the total number of nodes up to flowering in ICCV 2 were 44, in JG 62 were 89 and in F_1 were 58. The results indicate the existence of sufficient variability between the parents for the characters studied for their effective utilization. And this variability can be utilized if the heritability of these characters is high.

The broad sense heritability and narrow sense heritability of root length were high. The heritability estimate of RIL population was also high. High narrow sense heritability indicated predominance of additive gene action.

Root volume had moderate broad sense heritability and narrow sense heritability. The heritability estimate of RIL population was high.

Both leaflet number and leaf area had high broad sense and narrow sense heritability. The heritability estimate from RIL population was also high. Katiyar and Katiyar (1994) reported high broad sense heritability but low narrow sense heritability for leaf area.

Root dry weight had low broad sense and narrow sense heritability. But the heritability estimate from RIL population was high. This showed high environmental influence in the expression of this character.

Shoot dry weight had high broad sense and narrow sense heritability indicating the importance of additive gene action in governing this character.

Total number of nodes up to first flower had high broad sense heritability but narrow sense heritability indicating the importance of non-additive gene action for this character. The estimate from RIL population was high.

5.3 Components of variation in F_2 and backcrosses

The variances in parents (ICCV 2 and JG 62) and F_1 were lower for all the characters. The variances in F_2 population for all the characters were higher than both the parents indicating the segregation of all the characters studied. The variance in BC_1P_2 was lower than the variance in BC_1P_1. This indicated the preponderance of dominant alleles for all the characters in JG 62. The value of $\sqrt{HD}$ was not equal to the difference in the variances of the backcrosses. This showed that the degree of dominance varied from one gene pair to the other. The difference being large for some characters indicating the presence of some recessive alleles in JG 62 even though it mostly had dominant alleles for these characters.

5.4 Heterosis

Heterosis, which is defined as the superiority of F_1 hybrid over its both parents and its commercial exploitation in crop plants is regarded as a major break through in the realm of plant breeding. It occurs in self and cross pollinating crops and has lead to considerable yield improvement of several cereals and other crops (Rai, 1979). In chickpea, the first report of hybrid was by Pal (1945) and later heterosis was demonstrated by Ramanujam *et al.* (1964).

The hybrid of present cross was not earlier in flowering than the early parent, ICCV 2 but was similar to that of late flowering parent JG 62. This shows that late flowering is dominant over early flowering. The mid-parent and better parent heterosis

was positive for days to first flower. Pal (1945) also found similar results and Katiyar and Katiyar (1993) found significant heterosis for days to flowering.

Days to first pod of the F_1 was almost the same as that of late parent JG62 indicating dominance of late podding over early podding. Days to first pod had positive mid-parent heterosis and better parent heterosis.

Days to maturity had positive mid-parent and better parent heteroses. The maturity time of hybrid was nearer to the late parent, indicating dominance of late maturity over earlyness. Similar results were reported by Deshmukh and Bhapkar (1982) and Shinde and Deshmukh (1990) in chickpea.

Both pod filling period and total reproductive period had very low negative mid-parent heterosis and positive better parent heterosis. But Katiyar and Katiyar (1993) reported significant heterosis for days of reproduction period.

Number of nodes up to first flower of F_1 was almost same as that of JG 62, but was more than the mean of the parents and that of ICCV 2. It had positive mid-parent heterosis and better parent heterosis.

The mean root length of F_1 was longer than both the parental means. The mid-parent and better parent heteroses were positive and high indicating the dominance of longer root length over the shorter ones. The root volume had high mid-parent and better parent heteroses revealing the presence of dominant alleles in parent JG 62. High mid-parent heterosis for root volume in peas was reported by Saleh and Gritton (1994)

Leaflet number had low positive mid-parent heterosis and low negative better parent heterosis. Leaf area had low positive mid-parent heterosis and medium better parent heterosis.

Both root dry weight and shoot dry weight of F_1 were higher than the mid-parent value. Root dry weight had high mid-parent heterosis and better parent heterosis. Saleh and Gritton (1994) reported high mid-parent heterosis for root weight in peas. Shoot dry

weight had positive mid-parent and better parent heteroses. Total number of nodes per plant had negative mid-parent and better parent heteroses.

5.5 Gene interactions

To understand the major gene effects and different digenic interactions for various characters, additive-dominance (three parameter model) and digenic interactions (six-parameter model) models were applied to six generations of this cross to estimate different genetic parameters that account for the variation for different characters under study.

The inadequacy of simple additive-dominance model indicated the presence of non-allelic gene interactions governing the expression of days to first flower. Additive gene action, dominance gene action and additive x additive gene actions were significant. Additive gene action was more significant than dominance gene action. Opposite signs of dominance and dominance x dominance gene action indicate duplicate type of epistasis. Hence, simple recurrent selection can be resorted for the improvement of this character. Both additive and non-additive gene actions were reported by Salimath and Bahl (1989); Kidambi *et al.*(1988); Malhotra *et al.* (1993) and Jha *et al.* (1997).

For days to first pod, the inadequacy of additive-dominance model revealed the presence of inter-allelic gene actions. Additive, dominance, additive x additive and dominant x dominant gene actions were significant and opposite signs of dominance and dominance x dominance gene action indicate duplicate type of epistasis. Hence, simple recurrent selection can be resorted for genetic improvement of this trait in this cross.

In case of days to maturity, additive-dominance model was satisfied to explain the genetic variation present in this cross. Both additive and dominance gene actions were present but additive gene action was more significant. Hence, pedigree method of breeding could be followed for the isolation of superior lines from segregating

Table 44: List of RILs maturing earlier and having flower formation at lower node than ICCV 2

S.No	RIL Number	Days to maturity	RIL Number	Number of nodes up to first flower
1	22	77	8	15
2	23	77	9	15
3	25	76	22	13
4	31	76	26	15
5	40	76	31	15
6	45	77	32	15
7	47	76	40	15
8	75	78	41	15
9	81	77	42	15
10	84	76	45	13
11	87	78	46	15
12	99	77	47	15
13	117	77	52	15
14			58	12
15			64	14
16			74	13
17			78	15
18			81	14
19			84	15
20			90	15
21			97	15
22			99	13
23			103	15
24			105	15
25			123	15
ICCV 2		80		17
JG 62		97		23

generations in this cross. Thirteen RILs out of 126 RILs developed in this cross were found to mature earlier than the early maturing parent ICCV 2 (Table 44). Both additive and dominance gene actions were reported for maturity by Salimath and Bahl (1989); Kidambi *et al.* (1988) and Jha *et al.* (1997).

For podding duration additive-dominance model was not sufficient to explain the genetic variation. Dominant gene action, additive x additive gene action and dominance x dominance gene action were significant for pod filling period and whereas dominant gene action and all the three types of epistatic gene actions were significant for total reproductive period. Opposite signs of dominance and dominance x dominance gene action indicate duplicate gene action and biparental crosses could be practiced for the improvement of these characters.

With reference to number of nodes up to first flower, the assumption of additve-dominance model was not satisfied indicating the presence of interallelic interactions for governing the expression of this trait. Additive, dominance and additive x dominance gene actions were significant and same sign of dominance and dominance x dominance gene action indicate complementary gene action. Hence, for this trait in simple pedigree breeding will be rewarding for bringing improvement. Twenty-five of the 126 RILs developed first flower at lower a node than the parent ICCV 2 (Table 44).

The distribution pattern for all the characters in F_2 population was found to be near normal and hence suggests polygenic control. Both normal distribution and bimodal distributions for days to flowering were observed by Sarker *et al.* (2000) in lentil, a close relative of chickpea. In the present study the two major peaks coincided with the times of flowering of ICCV 2 and JG 62 indicating the presence of major gene (*efl-1*) and some modifier genes. The presence of a major gene with some modifier genes were reported by Or *et al.* (1999) and Kumar and Rheenen (2000) in chickpea. Transgressive segregants as a result of recombination between the genomes of the parents were

observed. Segregants that flowered beyond the parents were reported by Kumar and van Rheenen (2000). Thus if the segregants were considered as superior to the parents, they can be evaluated for the particular character and used as a new variety.

A 3:1 segregation of F_2 population into late maturing and early maturing individuals was observed which was confirmed by the segregation of the BC_1P_1. This suggests days to flowering and days to maturity is governed by a major gene (*efl*-1) with some modifier genes, as reported by Kumar and van Rheenen (2000).

Segregation of F_2 population into a 9 (flowering at a higher node): 7 (flowering at a lower node) ratio was observed and further confirmed by 1:3 segregation in the BC_1P_1. This clearly indicated that two genes with complementary gene action governed the number of nodes upto first flower. ICCV 2 has two genes in the recessive condition and JG 62 has their dominant alleles. Genes for this trait named earlier in chickpea. Therefore the two genes for number of nodes up to first flower were designated as *Nff-1* and *Nff-2*. Thus the genotype for the parent ICCV 2 will be: *nff-1nff-1nff-2nff-2* and that for JG 62 is *Nff-1Nff-1Nff-2Nff-2*. In peas Murfet (1971) reported multiple alleles governing these characters.

For root length additive dominance was not adequate to explain the genetic variance for this character. The most significant gene actions governing root length are additive and dominance x dominance gene action. Preponderance of additive component for the inheritance of root length was reported by Waldia *et al.* (1988 and 1993). The same sign of dominance and dominance x dominance gene actions indicated complementary epistasis. Hence, the pedigree method can be adopted for the improvement of this character.

The additive dominance model was not adequate to explain the genetic variance for root volume, but none of the interactive effects were found to be significant even from six-parameter model. Hence the most significant gene action was additive gene

Table 45: List of RILs having root volume and root dry weight higher than JG 62

S.No	RIL Number	Root volume (cc)	RIL Number	Root dry weight (g)
1	20	9.09	18	0.505
2	30	12.15	21	0.515
3	37	9.52	20	0.580
4	43	11.22	30	0.865
5	44	12.50	37	0.630
6	53	9.92	38	0.505
7	55	10.58	43	0.565
8	60	9.58	44	0.685
9	62	10.75	53	0.630
10	63	10.07	54	0.505
11	65	9.59	55	0.650
12	70	10.82	60	0.500
13	72	15.35	61	0.510
14	91	9.94	62	0.625
15	92	9.10	65	0.510
16	111	9.80	70	0.630
17	121	11.85	72	0.885
18			91	0.630
19			100	0.515
20			111	0.505
21			121	0.820
ICCV 2		4.01		2.010
JG 62		6.92		4.440

action for the expression of this trait. Pedigree method can be followed to improve the root volume. Significant additive genetic variance over dominance variance for root volume was reported by Saleh and Gritton (1994) in *Pisum*. Seventeen RILs had root volume higher than JG 62 and these can be selected for higher root volume (Table 45). These can be a valuable material for study of and evaluation as drought resistant elite germplasm.

The F_2 population gave a good fit to 3:1 ratio of individuals with high leaf let number to low leaflet number. This ratio was further confirmed by 1:1 segregation in the BC_1P_1. This indicates that the leaflet number is under single gene control. We propose a gene *Lln* for high leaf let number and *lln* for low leaf let number. Thus the genotype for ICCV 2 is *llnlln* and that for JG 62 is *LlnLln*.

Additive dominance model was sufficient to explain the genetic variance for leaf area. Additive gene effect was significant gene action governing this character.

Inadequacy of additive dominance model indicates the role of inter-allelic gene action for root dry weight. Additive and dominance x dominance gene actions were significant in governing this character. Same sign of dominance and dominance x dominance gene action indicate complementary gene action. Hence the pedigree method can be practiced for the improvement of this trait. Twenty-one of the 126 RILs developed had root dry weight higher than the parent JG 62 (Table 45).

Inadequacy of additive dominance model indicated the presence of epistasis for shoot dry weight. Six-parameter model indicated the presence of dominance, additive x additive and dominance x dominance gene actions. Opposite signs of dominance and dominance x dominance gene action indicated duplicate gene action. Hence, biparental crosses can be adopted for the improvement of this trait.

With reference to total number of nodes up to flowering, the assumption of additive dominance model did not explain the genetic variation. Six-parameter model

indicated the presence of all interaction effects. The most significant gene action governing this character was dominance and additive x additive gene action.

The F_2 plants exhibited continuous distribution for root length and root volume. The distribution was indicative of quantitative gene action, that is, all these characters are governed by multiple genes. Transgressive segregants were observed for all the characters, which indicated substantial recombination between the genomes of two parents.

5.6 Correlation coefficients

Correlations coefficients indicate degree and direction of association between different traits. They help in deciding a suitable selection criterion for the genetic improvement of complex but associated characters. In the present investigation correlation coefficients were worked out for different characters in F_2 and F_{10} RIL population and are discussed below.

The days to first flower, days to first pod and days to maturity were positively correlated among themselves in both F_2 and RIL population indicating that late maturing genotypes were also late in flowering and have longer pod filling period.

The maturity time was positively correlated with the pod filling period and total reproductive period but was non-significant in F_2 and significant in F_{10} RIL population. The positive correlation indicate that late maturing genotypes have longer pod filling period and total reproductive period.

The pod initiation period was negatively associated with pod filling period which indicated that genotypes with longer pod initiation period will have short pod filling period.

The positive association of pod filling period with total reproductive period suggest that selection should be done for longer pod filling period to obtain high yielding

genotypes. Hanson (1985) reported that genotypes with higher pod filling period had higher seed yield in soybean. Similar results were reported by Gumber *et al.* (1996) in pigeonpea.

Significant positive association of days to first flower, days to first pod and days to maturity with number of nodes up to first flower indicated that genotypes which flower early had first flower formed at lower node. A strong correlation of flowering time and number of nodes up to first flower was reported in peas by Tedin (1942) and Rowlands (1964).

Root length had significant positive correlation with root volume, root dry weight, leaf area, number of leaflets, leaf dry weight, shoot dry weight and total number of nodes both in F_2 and RIL population. Similarly root volume also had significant positive correlation with all the above characters. Saleh and Gritton (1994) observed significant positive correlation of root volume with root dry weight, shoot weight, leaf area, and number of nodes on the main stem in *Pisum.*

Root dry weight had significant positive correlations with root length, root volume, number of leaflets, leaf area, and total number of nodes, root/shoot ratio, leaf dry weight and shoot dry weight. Singh *et al.* (1988) observed high correlation of root weight with shoot mass and also root/shoot ratio in chickpea. Armenta-soto *et al.* (1983) observed significant positive correlation with shoot weight, tiller number and root-shoot ratio.

Root length, root volume and root dry weight had negative correlation with specific leaf area and leaf area/root length and leaf area/root volume. Root length and root volume had no correlation with 100 seed weight. Eissa *et al* (1983) observed no correlation between root length and seed weight.

The results indicate that in general, plants with larger and heavier root systems had larger and heavier shoots and higher dry total leaf area than those with smaller and lighter root systems.

5.7 Studies on recombinant inbred lines

Results obtained from REML variance component analysis for the shoot characters indicated significant difference among the 126 F_{10} RILs for the characters studied. There was sufficient variability for days to first flower, days to first pod, days to maturity, podding duration and number of nodes up to first flower among the RILs.

The 126 F_{10} RILs gave a good fit to 1:1 ratio of early flowering to late flowering, early podding to late podding and early maturing to late maturing individuals. This indicate that a major gene might be governing all these characters through its pleiotropic effect. This gene was reported as *efl-1* by Kumar and van Rheenen (2000). For the number of nodes up to first flower a 2:1 ratio of individuals with flower at lower node to that at higher node was observed indicating that two genes (*Nff-1* and *Nff-2*) govern the expression of this character. These genes can be considered as flowering genes determining the position of the first formed flower.

The CRD analysis for the root characters indicated significant difference among the 126 F_{10} RILs for root length and root volume. For F_{10} RILs, the observations on root length were recorded as and when the RILs flowered and root volume was recorded for all the RILs at the time of flowering of ICCV 2, JG 62 and as when they flower. The results obtained were same as that with parents i.e. no significant difference among the RILs at the time of flowering of ICCV 2 and significant difference at the time of flowering of JG 62. Hence, in chickpea, the observations on root traits should be preferably recorded at the time of flowering to observe critical differences. Veitenheimer (1981) reported pronounced variability for various root characters among pea genotypes

at flowering. Saleh and Gritton (1994) also reported the similar results. This might be because the plant diverts all the resources to the sink that is fruit (pod) after flowering and hence further development of the root system is stopped. The distribution of RIL population for root length and root volume was found to be continuous but were not similar to normal curve. This indicate that more number of RILs should be used

The results of generation mean analysis had shown that both additive and non-additive gene interactions were important for the inheritance of different characters in chickpea. The improvement of the characters where only additive component was significant (days to maturity, root volume, leaf area) would be easier by practicing simple recurrent selection in the segregating populations. But for the other characters where both additive and non-additive (dominance and epistatic interactions) were present, recurrent selection, biparental mating could be suggested to obtain desirable recombinants. In case of characters like pod filling period, total reproductive period, and shoot dry weight which were governed mostly by non-additive gene actions, delayed selection will be useful in isolating desirable segregants, after effecting biparental crosses. Hence the knowledge on gene number and gene actions governing different characters will enhance the chickpea improvement.

5.8 Inheritance of *Fusarium* wilt

Chickpea wilt, caused by *Fusarium oxysporium f.*sp.*ciceri* is a serious soil-borne disease in many countries. This disease causes 10% annual losses in India (Singh and Dahiya, 1973). This is a typical vascular disease causing xylem browning or blackening affecting the crop at all stages. Wilting at earlier stages causes greater loss than at the later stage i.e after flowering. Development of *Fusarium* wilt resistant genotypes is

essential as it is the best method to control the disease. This requires the information about the number of genes governing fusarium wilt resistance.

Single recessive gene inheritance for *Fusarium* wilt resistance was reported by Pathak *et al.* (1975); Ayyar and Iyer (1936); Sindhu et al (1983) and Phillips (1983). Digenic nature of wilt resistance was reported by Kumar and Haware (1982); Upadhyaya *et al.* (1983a, 1983b) and Lopez (1974).

In the present study, from the reaction of the parents to *Fusarium* wilt, indicate that ICCV 2 is resistant and JG 62 is susceptible to fusarium wilt. The time of wilting in F_1 generation was closer to that of susceptible parent JG 62, revealing the dominance of susceptibility over resistance.

The segregation of F_2 population in to 15 susceptible : 1 resistant individuals revealed digenic control of wilt resistance in this cross. This was in agreement with the report of Lopez (1974); Upadhyaya *et al.* (1983a , 1983b); Dikshit and Singh (1992) for resistance to race 1 and Gumber *et al* (1995) for resistance to race 2. Further classification of the susceptible F_2 individuals in to early wilters and late wilters gave a good fit to 9:6:1 ratio of early wilters, late wilters and resistant individuals. This was in agreement with the results of Singh *et al.* (1987). The segregation of F_2 individuals was further confirmed by the F_3 families which gave a good fit of 7:4:4:1 ratio of all susceptible, segregating susceptibles and resistants and all resistants expected from the segregation at the two loci. These results revealed that the two parents ICCV2 and JG 62 differed in two genes. Resistance to race 1 of the pathogen was reported to be controlled by three genes H_1, H_2, and H_3 (Singh *et al.* 1987a). The genotype of JG 62 was reported as $H_1H_1H_2H_2h_3h_3$. Hence the genotype of ICCV 2 would be $h_1h_1h_2h_2h_3h_3$. The h_1 and h_2 are the two late wilting genes reported by Singh *et al.* (1987).

These results were also confirmed by the 1:2: 1 segregation obtained in the F_{10} RIL population grown in wilt sick plot during *rabi* 1998-1999 and 1:1:1: 1 ratio of early

wilters: late wilter 1: late wilter 2: resistant ratio obtained in wilt sick plot during *rabi,* 1999-2000.The genotype of early wilters is $H_1H_1H_2H_2h_3h_3$, of late wilter 1 which wilt along with K 850 is $h_1h_1H_2H_2h_3h_3$, of late wilter 2 which wilt along with C 104 is $H_1H_1h_2h_2h_3h_3$ and the genotype of resistant RILs is $h_1h_1h_2h_2h_3h_3$. This confirmed the digenic control of resistance to fusarium wilt in the material studied.

5.9 Inheritance of molecular markers and construction of a linkage map

A large number of DNA marker strategies have been developed and are being used in genome analysis and molecular linkage map construction in plants, animals and fungi (O'Brien 1993). Initially RFLPs were used for linkage map construction. In recent years, polymerase chain reaction (PCR) based DNA markers are more frequently used because of their high sensitivity, and requirement of only small amounts of template DNA.

Low levels of polymorphism were reported in cultivated chickpea by isozyme analysis as well as by RFLP analysis (Ahmad and Slinkard, 1992; Udupa *et al.*, 1993 and Labdi *et al.*, 1996) and by RAPD analysis (Huttel *et al.*, 1999). However, relatively high level of polymorphism was detected using RAPDs by Banerjee *et al.* (1999). But RAPDs are dominant non-reproducible markers. Recently STMS markers are more frequently used because of their co-dominant nature and high reproducibility. In chickpea, 240 STMS markers are available designed by Winter *et al* (1990) and Huttel *et al.* (1999).

Recombinant inbred lines (RILs) produced by single seed descent method from F_2 to F_{10} and beyond are homozygous and can be evaluated in different environments. Hence, they are useful for the analysis of quantitative traits as the error due to the environmental component is less. Another advantage of RILs over F_2 population is that both dominant and co-dominant markers give similar information which allows the use of dominant markers like RAPDs, ISSRs and co-dominant markers like RFLP, STMS.

5.10 Segregation of molecular markers

Seventy two per cent of the markers segregated in the Mendelian 1: 1 ratio. Such Mendelian segregation of markers in RILs in chickpea was reported by Huttel *et al.* (1999), Winter *et al.* (1999), Mayer *et al.* (1997) and Santra (2000). Remaining 27% of the markers had segregation distortion due to preferential inheritance of the alleles of a particular parent. Similar high distorted segregation for markers in RIL population was reported by Wang *et al.* (1994) and Xu *et al.* (1997) in rice; Paran *et al.* (1995) in tomato and Winter *et al.* (2000) in chickpea. Paran *et al.* (1995) suggested that high segregation distortion in RIL population resulted from a cumulative effect of selection against alleles of one of the parents during the propagation of the RILs. Segregation distortion has severe drawbacks for the map-based cloning of the genes as it reflects recombination suppression at specific genome region and leads to under estimation of the physical distance between the gene of interest and markers located next to it (Winter *et al.* 2000).

Segregation distortion differed in different marker classes. MP-PCRs had maximum (60%) and RAPDs had minimum segregation distortion (16%). Moreover, the distorted markers were grouped together as in linkage group 4 and linkage group 9. Similar results were also reported by Winter *et al.* (2000).

Some of the RILs (0.8%) were heterozygous for 40 per cent of the STMS markers. Such heterozygosity in RIL population was also reported by Paran *et al.* (1995), Winter *et al.* (2000) and Burr *et al.* (1988). There was a synteny between 17 STMS loci in the present map with the interspecific map reported by Winter *et al.* (2000). This showed the transferability of the STMS markers.

5.11 Construction of a linkage map

Based on the data obtained using DNA and morphological markers a skeleton linkage map of this intra specific cross was constructed using MAPMAKER V2.0 (Lander *et al.*, 1987). Out of the 69 segregating markers, 56 formed 9 linkage groups while 13 markers remained unlinked. The present map consisted of 9 linkage groups with seven morphological trait genes, 4 RAPD, 1 ISSR, 32 STMS, 5 MP-PCR and 7 RMMFP loci covering 262.8 cM with an average distance of 4.7 cM between two consecutive markers. The longest linkage group included 12 markers covering 73.2 cM distance with an average of 6.1 cM between the consecutive markers while three markers (TR 2, TA 3 and TA 127) formed the smallest linkage group covering only 2.9cM length and average distance between two markers in each group ranged from 2.9 cM to 73.2 cM and 0.96 to 7.58 respectively. The longest gap between two markers corresponded to 24 cM in linkage group 5. The first linkage map of chickpea based on interspecific cross (C. *arietinum* x C. *reticulatum*) with four linkage groups consisting of 13 isozyme loci was reported by Gaur and Slinkard (1988). Later Gaur and Slinkard (1990b) added three linkage groups to the existing four linkage groups. Simon and Muehlbauer (1997) published the map which consisted of nine morphological, 45 RAPDs and 10 RFLP markers covering 550 cM. Santra (1998) developed a linkage map from the RILs of the interspecific cross *C. arietinum* (Flip 84-92c) and *C. reticulatum* (PI 498777) which had nine linkage groups with one morphological trait locus, nine isozyme loci, 17 ISSR and 90 RAPD loci. Winter *et al.* (1999) developed a map which consisted of 11 linkage groups covering 613 cM. Later, Winter *et al.* (2000) further developed an integrated molecular map using 130 RILs in the same cross. A total of 303 markers including STMS, DAF, AFLP, ISSR, RAPD, isozymes, cDNAs, SCARS and three loci that confer resistance against different races of fusarium wilt were mapped covering 2077.9 cM with an average distance of 6.8 cM between the markers.

5.12 Linkage of agronomically important traits

One of the important goals of genetic mapping in any crop is the tagging of important genes. In chickpea, the most important emphasis is on tagging genes resistant to important fungal pathogens such as *Aschochyta rabiei* and *Fusarium oxysporium f* sp *ciceri.*

Seed size locus was flanked by sa14 at a distance of 5.1cM and by TR1 on the other side at a distance of 7.4 cM. Double podding gene was flanked by TR1 and TA 11 on either side at a distance of 11.4 cM and 15.9 cM respectively. These two genes were linked to linkage group 2.

Resistance to race 1 of fusarium is controlled by two recessive genes in this cross. One of these two genes (H_1) was linked to group 8 to the RMMFP ta36t46 at a distance of 18.3 cM. Linkage of different genes in chickpea was reported by Tullu (1997), Santra (1998) and Winter *et al.* (1997). Many more number of markers are needed to saturate the linkage map and also to reduce the gaps between linked gene and the markers.

5.13 Random mixing of microsatellite flanking primers (RMMFP)

As the polymorphism found at the intra specific level in chickpea is very low, new techniques are needed to saturate the genome map and to find the linkages with important genes.

Random mixing of microsatellite flanking primers was developed by Dr. Peter Winter, Biocenter, Germany (Unpublished). In this technique, microsatellite flanking primers were used by mixing two primers randomly instead of using the particular right and left primers. Amplification was done when the complementary sequences of the

primers were found in the genome at an amplification distance in both the strands of the genome.

In the present experiment, out of 765 combinations tested, 20 were found to be polymorphic and 12 were genotyped on the RIL population. Out of these 12, seven were linked to different linkage groups. Marker Ta36t146 was linked to one of the two genes governing fusarium wilt resistance revealing the importance of these markers. 75% of the markers were co-dominant markers, a condition that is highly useful.

5.14 Sequencing of the amplicons

Four primer combinations which produced polymorphic markers were cloned and sequenced to know the reason for polymorphism. The polymorphism due to all four primer combinations was due to the difference in the repeat length of the microsatellites. This indicated that the new technique RMMFPs is highly useful as it gives co-dominant markers. The microsatellite repeat in all the four loci was (TTA). This is because of high number of dinucleotide repeats and also AT rich trinucleotide repeats in plant DNA (Weising *et al.,* 1998).

When the microsatellite flanking sequences of all the four markers were compared, a high percent of homology was observed in the first three cases. This showed that the microsatellites have conserved flanking sequences which are also reported in chickpea by Choumane *et al.* (2000). In the first and second primer combinations, one primer was common. The primer binding site for this primer in both the cases was the same except for the substitution of C in place of G in the second locus. The primer binding site of the other primer was entirely different in both the cases. The segregation pattern of these two markers was also different, thus indicating that the region amplified by both the primers were entirely different.

Hence the new technique RMMFP is highly useful to detect the polymorphism at the intraspecific level. And most of the markers are co-dominant in nature which can be used in any population type, hence can be used to saturate the chickpea genome map. This sequencing experiment has shown that most of the microsatellites have conserved flanking sequences and hence if the suitable primers are designed, they can be used across the species and the genera.

Linkage maps are highly useful in marker-assisted selection particularly for fusarium wilt resistance and achochyta blight in chickpea. The intra-specific linkage map is more useful than inter-specific map as the resistance traits to diseases found only in *C.arietinum* gene pool, but not in any of the crossable wild *Cicer* species (Huttel *et al.*, 1999). The newly developed DNA marker technique 'RMMFP' will be highly useful in adding more markers to saturate the linkage map and find linkages with agronomically important traits.

More research is required on the number of nodes up to first flower under different photoperiod and temperature conditions. Root length studies should be conducted on more number of RILs for this population. I used only 126 RILs. Increased number of molecular markers are needed to saturate the linkage map and to link important agronomic traits. For this, new techniques need to be designed for obtaining higher level of polymorphism in chickpea than is presently available. Use of additional markers and RILs in the ICCV 2 x JG 62 cross could make this map much more useful for marker assisted selection.

5.15 Conclusions:

Based on the above studies the following conclusions can be drawn.

1. Days to first flower, days to first pod and days to maturity are governed by the major gene (*efl-1*) and some minor genes.
2. Number of nodes up to first flower is governed by two complementary genes named as *Nff-1* and *Nff-2*.
3. Number of leaflets is governed by a single gene that was named as *Lln*.
4. Resistance to fusarium wilt is governed by two recessive genes.
5. Root length and root volume variability was maximum at flowering stage and, hence, observations should be taken at the flowering time.
6. Root volume and root length are governed by multiple genes.
7. Most of the quantitative characters are governed by additive genetic variance.
8. Parent JG 62 had dominant alleles for almost all the characters.
9. The chickpea linkage map had 9 linkage groups with seven morphological trait loci and 49 molecular markers covering 262.8 cM with an average distance of 4.7 cM between two markers.
10. Agronomically important traits such as seed size, double podding, and fusarium wilt resistance (H_1) were linked to different linkage groups. These linkages will be useful in marker assisted selection.
11. RMMFPs was found to be an efficient method for adding new markers and therefore, for saturating the linkage map.
12. All the sequenced polymorphic loci had microsatellite repeat motif (TTA).
13. Three of the microsatellite loci amplified had conserved flanking sequences. Hence when suitable primers are designed, they can be used across the species and also across genera.

SUMMARY

CHAPTER VI

SUMMARY

Chickpea, the third most important pulse crop, includes forty-three species, eight of which are annuals. It is used as a complement to cereal food and as snack food and sweets. Major loss in yield of chickpea is due to biotic stress (Ascochyta blight and fusarium wilt), abiotic stress (moisture stress, heat, cold) and also due to poor nutritional status of the soil and use of marginal lands. Early harvesting of chickpea as a result of reduced crop duration helps in avoiding most of the biotic and abiotic stresses which usually occur at flowering and podding time. Early developed prolific root system under water-limited environments is needed in chickpea. As molecular markers helped accelerating plant breeding in many crops, construction of intraspecific linkage map is very important in chickpea. Hence, the present investigation was carried out with the following objectives to:

1. study the inheritance of shoot and root characters
2. estimate their heritability and co-heritability
3. study the inheritance of molecular markers (RAPD and STMS) and
4. determine linkages among the morphological characters and molecular markers and construct an intraspecific map for chickpea.

The investigation was conducted in three experiments. They are 1. Field studies to study the inheritance of podding duration and fusarium wilt resistance, 2. Pot culture studies to study the inheritance of root length and root volume and 3. Molecular marker studies to study the inheritance of molecular markers and to construction of linkage map. The experiment material consisted of P_1, P_2, F_1, F_2, BC_1P_1, BC_1P_2, F_3, and 126 F_{10} generation random recombinant inbred lines (RILs) of the cross ICCV 2 and JG 62. The inheritance of

podding duration was conducted during *Rabi,* 1999-2000 under conserved soil moisture conditions. Inheritance of fusarium wilt was studied in wilt sick plot during *Rabi,* 1999-2000, 2000-2001 and also in glass house. Inheritance of root characters was studied in conviron under controlled environmental conditions and inheritance of molecular markers was carried out in Applied Genomic Laboratory, ICRISAT. RMMFPs and sequencing of polymorphic loci was conducted at Biocentre, Johann-wolfgang Goethe University, Frankfurt am Main, Germany.

The mean values of F_1 for all the characters studied were closer to the parent JG 62, indicating the presence of dominant alleles for all these characters in JG 62. Thus late flowering was dominant over early flowering, late maturity over early maturity, higher pod filling period and total reproductive period over low pod filling period and total reproductive period and initiation of flower at higher node to that at lower node. Similarly, high root length was dominant over low root length, high root volume over low root volume, less leaf are over more leaf area, more leaflet number over less leaflet number and high dry root weight over low dry root weight.

There was variability between the parents for all the characters studied, which can be better utilized if the heritability of these characters is high. The heritability (both broad sense and narrow sense) estimates values were high for all the shoot and root characters. Mid-parent and better parent heteroses was found to be positive for all the shoot characters except negative mid-parent heterosis for pod filling period and total reproductive period. Both mid-parent and better parent heteroses were higher for root length, root volume and dry root weight. Mid-parent and better parent heteroses were negative for total number of nodes up to first flower. The variances in parents and F_1 were low for all the characters and high in F_2 population indicating the segregation of all the characters. The variance in BC_1P_2

was less than the variance in BC_1P_1 for all the characters indicating the preponderance of dominant alleles for all the characters in parent JG 62.

The most significant gene action governing days to first flower was additive gene action, dominance and dominance x dominance gene action. Days to first pod was governed by additive, dominance, additive x additive and dominance x dominance gene actions. The most significant gene action for days to maturity was additive. Number of nodes up to first flower was governed by additive, dominance and additive x dominance gene action. Both pod filling period and total reproductive period were governed by additive and non-additive gene actions. Days to first flower, days to first pod, days to maturity and podding duration were governed by duplicate epistasis and number of nodes up to first flower by complementary epistasis.

The frequency distribution of F_2 for days to first flower was normal. But the two major peaks coincided with the time of flowering of ICCV 2 and JG 62 indicating the presence of one major gene and some minor genes. This was also confirmed by the distribution of RIL population. For days to first pod the distribution of F_2 was continuous and near normal and in RIL population it was bimodal indicating multigenic control and the presence of the major gene in governing this character. Distribution of F_2 population for days to maturity was continuous and gave a good fit to 3(LM): 1 (EM) ratio which was confirmed by 1: 1 segregation of BC_1P_1 and 1: 1 segregation of RIL population. This showed that days to maturity was also governed by a major gene and some minor genes. Hence it can be expected from the above results that days to first flower, days to first pod and days to maturity might be controlled by the same major gene through its pleiotropic effect. This gene was reported as *efl-1* gene by Kumar and van Rheenen (2000).

The distribution of F_2 population was found to be near normal for number of nodes up to first flower indicating polygenic control of this character. Segregation of F_2 population

into 9 flowering at higher node : 7 flowering at lower node was observed which was confirmed by 1: 3 segregation in BC_1P_1. This clearly indicated that two genes with complementary gene action governed the number of nodes up to first flower. The two genes for number of nodes up to first flower were designed as *Nff1* and *Nff2*. Therefore, the genotype for the parent ICCV 2 is *nff1nff1nff2nff2* and that for JG 62 is *Nff1Nff1Nff2Nff2*. This was also confirmed by 1: 2 segregation in the RIL population.

For both root length and root volume, additive genetic variance was more significant. Complimentary epistasis was found to govern both the characters. Leaf area and dry root weight were also governed by additive genetic variance. Shoot dry weight and total number of nodes up to first flower were governed by non-additive gene action. Leaf area, shoot dry weight and total number of nodes up to first flower had duplicate epistasis and complementary epistasis governed root dry weight. The F_2 population gave a good fit to 3:1 ratio of individuals with high leaflet number to low leaflet number which was further confirmed by 1:1 segregation in BC_1P_1. This indicated that the leaf let number is under single gene control. The gene symbols L*ln* (high leaf let number) and *lln* (low leaf let number) were assigned for leaflet number.

The distribution of F_2 population for all the characters were continuous and near normal indicating multigenic control for all the characters. The distribution of RIL population for root length and root volume was found to be continuous but were not similar to normal curve. This indicate that more number of RILs should be used. Root volume was recorded on RILs at the time of flowering of ICCV 2, JG 62 and their respective flowering times. The results obtained here were similar to those obtained with the parents i.e, significant variation in root traits was observed at their respective flowering times.

The improvement of the characters where only additive component was significant would be easier by practicing simple recurrent selection in the segregating generations. But

for other characters where both additive and non-additive (dominance and epistatic interactions) were present recurrent selection, biparental mating could be practiced to obtain desirable recombinants.

The REML (residual maximum likelihood) variance component for the RILs and parents showed major differences among the RILs for all the shoot characters studied and replication variance was found to be non-significant. There were significant differences among the RILs for root length and root volume.

Significant correlations were observed among various characters. This indicated that late maturing genotypes are late in flowering and have longer pod filling period and the genotypes with high pod initiation period have short pod filling period. The results indicated that the selection should be done for longer pod filling period to obtain high yielding genotypes. A high correlation of flowering time with number of nodes up to first flower was observed. There was a high correlation between root length and root volume. The selection should be done for the individuals with high root length and root volume so that they can be used under limited water environments.

The parent ICCV 2 was resistant to fusarium wilt and JG 62 was susceptible. The F_1 of this cross wilted 34 DAP that was almost along with JG 62. Hence F_1 was also considered susceptible and therefore susceptibility was dominant over resistance. The F_2 individuals gave a good fit to 15(S): 1(R), indicating the digenic control of resistance. The susceptible F_2 individuals were again classified to early wilters and late wilters and thus classified F_2 individuals gave a good fit to 9(EW): 6(LW): 1(R). The segregation of F_2 was confirmed by 7(S):8(segregating):1(R) ratio and 1(EW): 1(LW1) : 1(LW2) : 1(R) ratio in 126 F_{10} RIL population. The genotype of JG 62 was reported as $H_1H_1H_2H_2h_3h_3$ and hence the genotype of ICCV 2 should be $h_1h_1h_2h_2h_3h_3$ as the segregation is for two genes.

Segregation of the markers was evaluated in 126 F_{10} RIL population. Seventy two percent of the markers segregated in 1:1 ratio and remaining twenty eight percent had segregation distortion. Different marker classes exhibited varied segregation distortion. MP-PCRs had maximum segregation distortion (60%) and RAPDs had minimum segregation distortion (16%).

A skeleton linkage map was constructed using MAPMAKER V2.0 (Lander *et al.* , 1987). At LOD score 3.0, fifty-six out of sixty nine markers formed nine linkage groups with seven morphological trait loci, four RAPD, one ISSR, thirty-two STMS, five MP-PCR and seven RMMFP loci covering 262.8 cM with an average distance of 4.7 cM between two consecutive markers.

Linkages were observed for three important traits. Seed size locus was flanked by marker sa 14 on one side at a distance of 5.1 cM and by TR 1 on the side at a distance of 7.4 cM. Double podding gene was flanked by TR 1 and TA 14 on either side at a distance of 1.4 cM and 15.9 C respectively. One of the two genes governing fusarium wilt resistance was linked to RMMFP marker ta36t146 at a distance of 18.3 cM.

RMMFP (Random mixing of microsatellite flanking primers), a new technique developed by Dr. Peter Winter, was found to be efficient in adding more markers to the chickpea map. Out of 765 primer combinations tested, 20 were polymorphic and 12 were reproducible. Out of the 12 markers, seven were linked to different linkage groups. Marker ta36t146 was linked to one of the loci for fusarium wilt resistance at a distance of 18.3 cM. Seventy five percent of these markers were co-dominant markers.

Four co-dominant polymorphic loci produced by RMMFPs were cloned and sequenced. The polymorphism in all the loci was due to the length of the microsatellite region. In all the cases the microsatelite motif present was (TTA). There was high

homology in the flanking sequences of the microsatellites. Hence if the suitable primers are designed, they can be used across species and also genera.

Further, more emphasis should be given on the number of nodes up to first flower. The effect of photoperiod and temperature on the node of flower initiation should be studied similar to the work done in peas and root character studies should be done on more number of RIL progeny. New STMS primers have to be designed or new techniques have to be developed to find polymorphisms in chickpea where intraspecific polymorphism is very low in order to get linkages with other important traits.

LITERATURE CITED

LITERATURE CITED

Ahmad F and Slinkard A E 1992 Genetic relationships in the genus *Cicer* L as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theoretical and Applied Genetics 84:688-692.

*Amos W Sawcer S J Feakes R W and Rubinzstein D C 1996 Microsatellites show mutational bias and heterozygote instability. Nature Genetics 13: 390-391.

Armenta-soto J Chang T T Loresto G C and O' Toole J C 1983 Genetic analysis of root characters in rice. SABRAO Journal 15(2):103-116.

Arumuganathan K and Earle E D 1991 Nuclear DNA content of some important plant species. Plant Molecular Biology Report 9:208-218.

Athwal D S and Gill G S 1964 Genetic variation, heritability and correlations of various yield components in crosses of gram (*Cicer arietinum* L.). Journal of Research, Punjab Agricultural University 1:116-128.

Ayyar R V and Iyer B R 1936 A preliminary note on the mode of inheritance to *Fusarium* wilt in *Cicer arietinum*. Proceedings of Indian Academy of Sciences 3:438-443.

*Aziz M A Shah S and Asghar M 1960 Association of qualitative characters in gram (*Cicer arietinum* L.). Proceedings of Pakistan Science Conference 12:74.

Bahl P N and Kumar J 1989 Evaluation and utilization of high yielding hybrids of chickpea. Indian Journal of Genetics and Plant Breeding 49(1):53-58.

Banerjee H Pai R A and Sharma R P 1999 Restriction fragment length polymorphism and random amplified polymorphic DNA analysis of chickpea accessions. Biologia Plantarum 42(2):197-208.

*Basten C J Zeng S B and Weir B S 1994 ZMAP- A QTL cartographer. Proceeding of the 5th world congress on genetics applied to livestock production 22:65-66.

Beckman C H and Soller M 1986 Restriction fragment length polymorphisms in genetics improvement of agricultural species. Euphytica 35:111-124.

Bhapkar D G and Patil J A 1963 Inheritance of foliage, flower and seed coat colour in gram. Crop Science 3:361.

Bhat N R and Argikar 1951 A genetic linkage in *Cicer arietinum* L. Heredity 5:143-146.

Bodenes C Laigret F and Kremer A 1996 Inheritance and molecular variations of PCR-SSCP fragments in pedunculate oak. (*Quercus robur* L.). Theoretical and Applied Genetics 93:348-354.

*Bonfil D J and Pinthus M.J 1995. Response of chickpea to nitrogen and the comparison of the factors affecting chickpea seed yield to those affecting wheat grain yield. Experimental Agriculture 31:39-47.

*Botstein D White R L Skolnil M and Davis R W 1980 Construction of genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314 -331.

Burr B and Burr F A 1991 Recombinant inbreds for molecular mapping in Maize. Trends in Genetics 7:55-60.

Burr B Burr F A Thompson K H Albertson M C and Stuber C W 1988 Gene mapping with recombinant inbred in maize. Genetics 118:519-526.

*Butler E J 1918 Fungi and diseases in plants. Thacker Spink and Co, Calcutta, India. pp:244-251.

Caetano-Anolles G Bassam B J and Gresshoff P M 1991b DNA amplification fingerprinting: a strategy for genome analysis. Plant Molecular Biology Reporter 9:295-305.

Chandra S 1968 Variability in gram. Indian Journal of Genetics and Plant Breeding 28:205-210.

*Chatopadhyaya S B and Sen Gupta P K 1967 Studies on wilt disease of pulses. I. Variation and taxonomy of fusarium species associated with the wilt diseases of pulses. Indian Journal of Mycological Research 5:45-53.

Chavarriaga A P Maya M M Bonierbala M W Kresovich S Fregene MA Tohme J and Kochert G 1998 Microsatellites in cassava (*Manihot esculentacrantz*): discovery, inheritance and variability. Theoretical and Applied Genetics 97: 93-501.

Choumane W Winter P Weigand F and Kahl G 2000 Conservation and variability of sequence tagged microsatellites (STMS) from chickpea. (*Cicer arietinum* L.) with in the Genus *Cicer*. Theoretical and Applied Genetics 101:269-278.

*Cifarelli R A Gallitelli M and Cellini F 1995 Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellites containing DNA clones. Nucleic Acids Research 23:3802-3803.

Collins W J and Wilson J H 1974 Node of flowering as a index of plant development. Annals of Botany 38:175-180.

Davila J A Loarse Y and Ferrer E 1999 molecular characterization and genetic mapping of random amplified microsatellite polymorphism in barley. Theoretical and Applied Genetics 98:265-273.

DeScenzo R A and Harrington T C 1994 use of $(CAT)_5$ as a DNA finger printing probe for fungi. Phytopathology 84:534-540.

Deshmukh R B and Bhapkar D G 1982 Heterosis and inbreeding depression in chickpea. Indian Journal of Genetics and Plant Breeding 42:208-212.

Devely M E Jermstad K D Tauer C G and Neak P N 1991 Inheritance of RFLP loci in loblolly pine tree generation pedigree. Theoretical and Applied Genetics 83:238-242.

Dikshit H K and Singh I S 1994 Inheritance of resistance to fusarium wilt (*Fusarium oxysporium* f sp *ciceri*) in gram (*Cicer arietinum*). Indian Journal of Agricultural Sciences 64(8):579-582.

Domoney C Ellis T H N and Davies D R 1986 Organization and mapping of legumin genes in *Pisum*. Molecular General Genetics 202:280-285.

Economic intelligence service 2000 Agriculture September pp: 97

Echt C Knapp S and Liu B H 1992 Genome mapping with non-inbred crosses using Gmendal 2.0. Maize Genetics Co-operative News Letter 66:27-29.

*Edwards A A Civitello H A Hammond and Caskey C T 1991 DNA typing and genetic mapping with trimeric and tetrameric tandom repeats. American Journal of Human Genetics 49:746-756.

*Edwards K J Barker K H A Daly A Jones C and Karp A 1996 Microsatellite libraries enriched for several microsatellite sequences in plants. Biotechniques 20:758-760.

Eirsa A M Jenkins J N and Vaughan 1983 Inheritance of seedling root length and relative root weight in rice. Crop Science 23:1107-1111.

Ekanayake I J OTool J C Carrity D P and Masajo T M 1995 Inheritance of root characters and their relations to drought resistance in rice. Crop Science 25:927-933.

*Ellegren H Primmer C R and Sheldon B C 1995 Microsatellite 'evolution': directionality or bias? Nature Genetics 11:360-362.

*Ender A Schwen K Stadler T Streit B and Schierwater B 1996 RAPD identification of microsatellites in Daphina. Molecular Ecology 5:437-441.

*Eser 1976 Heritability of some important plant characters, their relationship with plant yield and inheritance of *Aschochyta* blight resistance in chickpea (*Cicer arietinum* L.). Ankara University. Ziraat Fak.Yayinlari 620:40.

Falconer D S 1989 Introduction to Quantitative Genetics. John Wiley and sons, Inc., New york.

Fang D Q and Roose M L 1999 Inheritance of Inter-Simple sequence repeat markers in citrus. The Journal of Heredity 90(1):247-248.

FAO 2001 Quaterly bulletin of statistics. Food and Agricultural Organization of the United Natior,,Rome. 10:16.

*Fehr W R 1987 Principles of cultivar development. Macmillan, New York pp:115-125.

Fisher R A and Yates F 1963 Statistical tables for Biological Agricultural and Medical Research. Oliver and Boyd.

Fjellstrom R G and Parfil D E 1994 RFLP inheritance and linkage in walnut. Theoretical and Applied Genetics 89:665-670.

Frisch M and Melchinger A E 2001 Marker assisted backcrossing for introgression of a recessive gene. Crop Science 41:1485-1494.

Gaur P M and Slinkard A E 1988 Inheritance and linkage relationships of some isozyme loci in *Cicer* Abstract 32. 41.1 Genome 30 (suppl. 1):243.

Gaur P M and Slinkard A E 1990a Inheritance and linkage of isozyme coding genes in chickpea. Journal of Heredity 80:455-461.

Gaur P M and Slinkard A E 1990b Genetic control and linkage relations of additional isozyme markers in chickpea. Theoretical and Applied Genetics 80:648-656.

*Ghatge R D Kohle A K and D'Cruz R 1975 Inheritance of certain colour characters in gram (*Cicer arietinum* L.). VII.Botanique 6:117-124.

Gil J and Martin L M 1988 genetics of days to flowering in *Vicia sativa* L.. Legumes Research 11(2):59-65.

*Gortner G Nerino M Weising K Zink D Nagl W Kahl G 1998 Chromosomal localization and distribution of simple sequence repeat and the arabidopsis type telomere sequences in the genome of *Cicer arietinum* L. Chromosome Research 6:97-104.

Govil J N and Jitendra K 1989 Variation in genetic parameters with change in environment in chickpea. Indian Journal of Agricultural Research 23(1):1-8.

Gumber R K Kumar J and Haware M P 1995 Inheritance of resistance to fusarium wilt in chickpea. Plant Breeding 114:277-279.

Gumber R K Sarvjeet S and Sandhu T S 1996 Association of seed yield with different phases of the reproductive period in pigeon pea. Indian Journal of Genetics and Plant Breeding 56(3):318-322.

Gupta M Chyi Y S Romero-Severson J and Owen J L 1994 Amplification of DNA markers for evolutionary diverse genomes using single primers of simple sequence repeats. Theoretical and Applied Genetics 89:998-1006.

Gupta P K Balyan H S Sharma P C and Ramesh B 1996 Microsatellites in plants: A new class of molecular markers. Current Science 70:45-54.

Gupta S C 1976 Heritability and interrelationships of some agronomic and quality characters in Bengal Gram (*Cicer arietinum* L.). M,Sc Thesis, JNKVV Jabalpur, India.

*Hange XY and Litt M 1993 A study of the origin of shadow bands seen when typing dinucleotide repeat polymorphism by the PCR. Human Molecular Genetics 2:411-415.

Hanson W D 1985 Association of seed yield with partitioning length of the reproductive period in soybean genotypes. Crop Science 25:525-529.

Havey M J and Muehlbauer F J 1989 Linkages between restriction fragment length, isozyme and morphological markers in lentil. Theoretical and Applied Genetics 77:395-401.

Haware M P Jimenez-Diaz R M Amin K S and Halila H 1990 Integrated management of wilt and root-rots of chickpea. In: Van Rheenen H A and Saxena M C (Eds), Chickpea in the nineties: Proceedings of the second international workshop on chickpea improvement. December 1985,ICRISAT center, Patancheru, India.pp:129-137.

*Haware M P and Nene N L 1982 Races of *Fusarium oxysporium f.*sp.*ciceri*. Plant Disease 66:809-810.

*Haware M P Nene Y L Pundir R P S and J N Rao 1992 Screening of world Chickpea germplasm for resistance to fusarium wilt. Field Crops Research 30:147-154.

Hayman B I 1958 The separation of epistatic from additive and dominance variation in generation means. Heredity 12:371-390.

Helentjaris T Slocum M Wright S Schaefer A and Nienhuis J 1986 Constructions of genetic linkage maps in maize and tomato using restriction fragment length polymorphism. Theoretical and Applied Genetics 72:761-769.

Helentjaris T Weber D F Wright S 1986 Use of monosomics to map cloned DNA fragments in maize. Proceedings of National Academy of Sciences, USA 83:6035-6039.

Hinze K Thopson R D Ritter E Salamani F Schulzelefert P 1991 Restriction length polymorphism mediated targeting of the ml-o resistance locus in barley (*Hordeum vulgare*). Proceedings of the National Academy of Sciences, USA 88:3691-3695.

Huttel B Winter P Weising K Choumane W weigand W and Kahl G 1999 Sequence tagged microsatellite markers for chickpea (*Cicer arietinum* L.). Genome 42:210-217.

*Jacob H J Brown D M Bunker R K Daly M J Dzau V J Good man A Kolike G Kren V Kurtz T Lernmark A Levan G Mao Y Petterson A Pravence M Simon J S Szprirer C Szpirer J Trolliet M R Winer E S and Lander E S 1995 A genetic linkage map of laboratory rat, *Rattus norvegicus*. Nature Genetics 9:63-69.

Janssens M J 979 Coheritability: its relation to correlation response, linkage, and pleiotropy in cases of polygenic inheritance. Euphytica 28: 601-608.

Kumar J and Haware M P 1982 Inheritance of resistance to Fusarium wilt in chickpea. Phytopathology 72:1035-1036.

Kumar J Sethi S C Johansen C Kellet T G Rahman M M and Van Rheenen H A 1996 Potential of short-duration chickpea varieties. Indian Journal of Dryland Agriculture Research and Development 11:28-32.

Kumar J and van Rheenen H A 2000 A major gene for time of flowering in chickpea. The Journal of Heredity 91(1):67-68.

Kumar S 1998 Inheritance of resistance to fusarium wilt (race 2) in chickpea. Plant Breeding 117:139-142.

*Kupicha F K 1977 The delimitation of the tribe *Vicieae* and the relationships of *Cicer* L. Botanical Journal of the Linnean Society 74:131-162.

Labdi M Robertson L D Singh K B and Charrier A 1995 Genetic diversity and phylogenetic relationships among the annual *cicer* species as revealed by isozyme polymorphism. Euphytica 88:181-188.

Lander E S and Botstein D 1988 Mapping Mendelian factors underlying Quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Lander E S Green P Abrahamson J Barlow A Daly M Lincoln S and Newburg L 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174 -181.

*Levinson G and Gutman G A 1987 Slipped strand misspairing: a major mechanism for DNA sequence evolution. Molecular Biology Evolution 4:203-221.

*Litt M and Luty J A 1989 A hyper variable microsatellite revealed by in vitro amplification of a dinucleatide repeat with in cardiac muscle actin gene. American Journal of Human Genetics 44:397-401.

*Lopez G H 1974 Inheritance of the character resistant to wilt (*Fusarium* sp) in chickpea (*Cicer arietinum* L.) under field conditions. Agr.Tec.Mex.,3:286-289.

Malhotra R S Singh K B and Lal B 1983. Combining ability for yield and its components in chickpea. Indian Journal of Genetics 43:49-151.

Mandal A K 1992 Pattern of variation of harvest index in chickpea crosses. Indian Journal of Genetics and Plant Breeding 52(2):164-168.

Maniatis T Fritsh E R and sambrook J 1982 Quantification of DNA and RNA. In: Molecular cloning: A laboratory manual . Cold Spring harbor laboratory press. pp: 448-469.

Manly N F and Cudemore E H 1996 New version of map manager genetic mapping software. Plant Genome IV (abstract). pp:105.

Markert L M and Moller F 1959 Multiple forms of isozymes: Tissues, oncogenetics and species specific patterns. Proceedings of the National Academy of Sciences, USA 45:753-763.

Mather K 1949 Biometrical Genetics Methuen and Co Ltd, London.

Mayer M S Tullu A Simon C J Kumar J Kaiser W J Kraft J M and Muehlbauer F J 1997 Development of a DNA marker for Fusarium wilt resistance in chickpea. Crop science 37:1625-1629.

Mc Rae W 1932 Report of imperial mycologist.Sci.Agri.Res.Inst, Pusa 1930-1931.pp:73-86.

Mc Couch S R Kochert G Yu Z H Khush G S Coffman W R and Tanksley S D 1988 Molecular mapping of rice chromosomes. Theoretical and Applied Genetics 76:815-829.

Mc Grath J M and Quiras C F 1991 Inheritance of isozyme and RFLP markers in *Brassica campestris* and comparison with *B.oleracea*. Theoretical and Applied Genetics 82:668-673.

Melchinger A E 1990 Use of molecular markers in breeding oligogenic disease resistance. Plant Breeding 104:1-19.

*Meyer W And Mitchell P G 1995 polymerase chain reaction finger printing in fungi using single primers specific to minisatellites and simple repetitive DNA sequences. Strain variation in *Cryptococcus neoformans*. Electrophoresis 16:1648-1656.

*Meyer W Mitchell T G Freedman E Z and Vilgaly R 1993 Hybridization probes for conventional DNA fingerprinting used as single primers in the PCR to distinguish strains of *Cryptococcus neoformans*. Journal of Clinical Microbiology 31:2274-2280.

Mishra R C 1991 Stability of heritability genetic advance and character association estimates in chickpea. International Chickpea Newsletter 25:10-11.

Misra R Rao S K and Koutu G K 1992 correlated response in chickpea (*Cicer arietinum* L.). Agricultural Science Digest 12(2):69-72.

Mitchell S E Kresovich S Jester C A Hernandez C J and Szewe-Fadden A K 1997 Application of multipolex PCR and Flouresence-based, semi-automated allele sizing technology for genotyping plant genetic resources. Crop Science 37:617-624.

More D C and D'Cruz R 1976 Genetic studies in Bengal Gram (*Cicer arietinum* L.), V.D-70-10 crossed with white flowered white grained-II. Journal of Maharastra Agricultural University 1:11-14.

Morgante M and Oliver A M 1993 PCR-amplified microsatellites as markers in plant genetics. Plant Journal 3:175-182.

Muehlbauer F J and Singh K B 1987 Genetics of chickpea. In: M C Saxena and K B Singh (eds), The Chickpea. CAB International, UK. pp: 99-125.

Murfet I C 1971a Flowering in *Pisum*: Three distinct phenotypic classes determined by the interaction of a dominant early and dominant late gene. Heredity 26:243-257.

Murfet I C 1975 Flowering in *Pisum* : Multiple alleles at the *lf* locus. Heredity 35(1):85-98.

*Narasimhan R 1929 A preliminary note on fusarium parasite on Bengal Gram. Madras Agricultural Department Year Book.pp:5-11.

Nelson, J C 1997 QGENE: software for marker based genome analysis and breeding. Molecular Breeding 3:239-245.

Nene Y L Haware M P Reddy M N and Pundhir R P S 1981b Sources of resistance to selected chickpea diseases. Pulse Pathology Progress Report, ICRSAT, Hyderabad, India,No:8.

*Nozzolillo C 1985 Seedling morphology and anatomy of eight *Cicer* species and their taxonomic value. Canadian Journal of Botany 63: 1-6.

*O' Brien S J 1993 Genetic maps: Locus maps of complex genomes, sixth edition. Cold spring harbor laboratory press, plainview, Newyork. 6(1-6):261.

Oram R N Shaik M A Q Zaman K M S and Brown A D H 1987 Isozyme similarity and genetic differences in morphology between Hypersola, a high yielding, a high protein mutant of chickpea (*Cicer arietinum* L.) and its parental cultivar. Environmental Experimental Botany 27:455-462.

Or E Hovav R and Abbo S 1999 A major gene for flowering time in chickpea. Crop science 39:315-322.

Padwick G W 1940 The genus Fusarium. II A critical study of the fungus causing wilt of gram (Cicer arietinum L.) and of related species of the subsection orthocera, with special relation to the variability of key characteristics. Indian Journal Agricultural Sciences 10:241-284.

Pal B P 1945 Studies in hybrid vigour-notes on manifestation of hybrid vigour in gram, sesame, chillies and maize. Indian journal of Genetics and Plant Breeding 24:122-129.

Pandey R L and Tiwari A S 1983 Heritability and Genetic gain in chickpea. International Chickpea Newsletter 9:5-6.

Panse V G and Sukhatme P V 1967 Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research, New Delhi.

Paran I Goldman I Tanksley S D and Zamir D 1995 Recombinant inbred lines for genetic mapping in tomato. Theoretical and Applied Genetics 90:542-548.

Pardhasaradhy V A and Medhi P R 1983 Coheritability, path coefficient and discriminate functions in radish. Indian Journal of Agricultural Science 3(2):111-114.

Parmar B S and Godawat S L 1990 Inheritance of flowering and maturity times in pea (*Pisum sativum* L.). International Journal of Tropical Agriculture 8(1):23-26.

Paterson A H Tanksley S D and Sorrells M E 1995 DNA markers in plant improvement. Advances in Agronomy 46:40-90.

Pathak M M .Singh K P and Lal S B 1975 Inheritance of resistance to wilt (*Fusarium oxysporium* f.*ciceri*) in gram. Indian Journal of Farm Sciences 3:10-11.

Patil J A and Deshmukh R B 1975 Inheritance and linkage studies in Bengal Gram (*Cicer arietinum* L.) T-54-A X D-70-10 chickpeas. Research Journal of MPKV 6:88-95.

Patterson H D and Thompson R 1971 Recovery of inter-block information when block sizes are unequal. Biometrica 58:545-554.

Pawar A M and Patil J A 1979 Genetic studies in Bengal Gram (*Cicer arietinum* L.). Journal of Maharashtra Agricultural University 4:61-64.

Perring T M Cooper AD Rodrigne C A Farrar C A and Bellows T S 1993 Identification of white fly species by genomic and behavioural studies. Science 259:74-77.

*Phillips J C 1983 Inheritance of fusarium wilt resistance in chickpea. Agronomy abstracts. American Society of Agronomy: 76.

Powell W Machray G C and Provan J 1996a Polymorphism revealed by simple sequence repeats. Trends in Plant Science 1:215-222.

Prasad N and Padwick G W 1939 The genus *Fusarium* : II. A species of Fusarium as a cause of wilt in gram (*Cicer arietinum* L.). Indian Journal of Agricultural Science 9:371-380.

*Primmer C R Ellegren H Saino N and Moller A P 1996 Directional evolution in germline microsatellite mutations. Nature Genetics 13:391-393.

Pundir R P S Reddy K N and Mengesha M H 1991 Genetics of some physio-morphic and yield traits of chickpea (*Cicer arietinum* L.) Legume Research 14(4):157-161.

Pundir R P S Reddy K N and Mengesha M H 1992 Pod volume and pod filling as useful traits of chickpeas. International Chickpea Newsletter 27:27-28.

Radford P J 1967 Growth analysis formulae-their use and abuse. Crop Science 8:171-175.

Rafalski J A Hanafey M K Tingey S V and Williams J G K 1994 Technology for molecular breeding RAPD markers, Microsatellites and Machines. Plant Genome analysis. Ed P M gresshoff CRC press. Boca Raton pp:19-27.

Rai B 1979 Heterosis Breeding. Agro-Biological Publications, New Delhi,36.

*Rakshit S Winter P Tekeoglu M Juarez J M Pfaff T Benko-Iseppon A M Muehlbauer F J Kahl G 2001 A major locus for *Aschochyta* blight resistance in chickpea is located on linkage group 4 of the genetic map. Theortical and Applied Genetics. In press.

Ramanujam S Rohewal S S and Singh S P 1964 Potentialities of heterosis breeding in *Cicer*. Indian Journal of Genetics and Plant Breeding 24:122-129.

Rao S S Sinha R and Das G K 1994 Genetic variability Heritability, expected genetic advance and correlation studies in chickpea. Indian Journal of Pulses Research 7(1):25-27.

Rastogi K B and Singh L 1977 Heritability Phenotypic correlation coefficients of some of the characters in the F_2 populations of Gram (*Cicer arietinum* L.). Crop Improvement 4(2):191-197.

Ratnaparkhe M B Santra D K Tullu A and Muehlbauer F J 1998 Inheritance of inter-simple-sequence-polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theoretical and Applied Genetics 96:348-353.

Reddy V G and Chopde P R 1977 Genetic studies in Bengal Gram (*Cicer arietinum* L.) chikkodi V.V. X Chrysanthefolia Journal of Maharashtra Agricultural University 2:224-226.

*Ribaut J M Hu X Hoisington D and Gonzalez-de-Leon D 1997 Use of STSs and SSRs as rapid and reliable preselection tools in a marker assisted selection–backcross scheme. Plant Molecular Biology Report 15:154-162.

*Richardson T Cato S Ramser J Kahl G and Weising K 1995 Hybridization of microsatellites to RAPD: A new source of polymorphic markers. Nucleic Acids Research 23:3798-3799.

Roder M S Korzun V Gill B s and Ganal M W 1998a The physical mapping of microsatellite markers in wheat. Genome 41:278-283.

Roder M S Korzun V Wendehake K Plachke J Tixer M H Leroy P and Ganal M W 1998b A microsatellite map of wheat. Genetics 149:2007-2023.

Rowlands D G 1964 Genetic control of flowering in *Pisum sativum* L. Genetica 35:75-94.

*Rubinzstein D C Amos W Leggo J Goodburn S Jain S Li S H Margolis R L Ross C A and Ferguson-Smith 1995 Microsatellite evolution –evidenc for directionality and variation in rate between species. Nature Genetics 10:337-343.

Sabaghpour S H 2000 Genetic studies of qualitative and quantitative traits in chickpea (*Cicer arietinum* L.) Ph.D Thesis, Submitted to A.N.G.R.A.U, Rajendranagar, Hyderabad-30.

Sachs T 1999 Node counting : an internal control of balanced vegetative and reproductive development. Plant Cell Environment 22:757-766.

Saghai-Maroof M A Biyaahev R M Yang G P Zhang Q and Allard RW 1994 Extrordinarilly polymorphic microsatellite DNA in barley. Species diversity, Chromosomal locations and population dynamics. Proceedings of National Academy of Sciences, USA 91:5466-5470.

Saghai-Maroof M A Soliman K M Jargensen R A and Allard R W 1984 Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of National Academy of Sciences, USA 81:8014-8018.

Saleh G and Gritton E 1994 Heritability and gene effects for root characteristics in peas measured at flowering. Euphytica 78:185-191.

Salimath P M and Bahl P N 1989 combining ability studies in crosses involving tall and dwarf types in chickpea. Indian Journal of Genetics 49:29-34.

Samuel K M and Jagdev P N 1996 Indian Journal of Pulses Research 8(2):123-127.

Santra D K 1998 Application of Biotechnology for the improvement of Ascochyta blight resistance in chickpea (*Cicer arietinum* L.). Ph.D Thesis. Division of Biological Sciences, National Chemical laboratory, Pune.

Santra D K, Tekeoglu M, Ratnaparkhe M, Kaiser W J and Muehlbauer F J 2000 Identification and mapping of QTLs conferring resistance to Ascochyta Blight in Chickpea. Crop Science 40:1606-1612.

Sarker A Erskine W Sharma B and Tyagi M C 1999 Inheritance and linkage relationships of days to flower and morphological loci in lentil (*Lens culinis* Medikus subsp. *Culinaris*). Journal of Heredity 90:270-275.

Saxena M C 1990 Problems and potential of chickpea production in the nineties. In: Chickpea in the nineties. Van Rheenen H A and Saxena M C (Eds), Proceedings of the second international workshop on the chickpea improvement, ICRISAT, Patancheru, India. pp:13-28.

Saxena N P Johansen C Saxena M G and Silim S N 1993a Selection for drought and Salinity tolerance in cool season food legumes. In: Breeding for stress tolerance in cool season food legumes. (Singh K B and Sabena M C (eds)), Chi Chester, John Wiley.pp: 245-270.

Saxena N P Krishnamurthy L and Johansen C 1993b Registration of a drought resistant chickpea germplasm. Crop Science 33:1424.

*Schlottere C and Tautz D 1992 Slippage synthesis of simple sequence DNA. Nucleic Acids Research 20:211-215.

✓Schmidt T and Heslop-Harrison J S 1996 The physical and Genome organization of microsatellites in sugar beets. Proceeding of National Academic Science 93:8761-8765.

Schwengel DA Jedlicka AE Nantha Kumar E J Weber J L and Levitt R C1994 Comparision of Flourescence-based semi-automated genotyping of multiple microsatellite loci with auto-radiographic techniques. Genomics 22:46-54.

Sharma B D Sood B C and Malhotra V V 1990 Studies on variability heritability and Genetic advance in chickpea. Indian Journal of Pulses Research 3(1):1-6.

Sharma P C Winter P Bunger T Huttel B Weigand F Weising K and Kahl G 1995 Abundance of dt-, tri-, and tetra nucleotide tandom repeats in chickpea (*Cicer arietinum* L.). Theoretical and Applied Genetics 90:90-96

Sharma P P and Maloo S R 1988 Variability parameters in chickpea (*Cicer arietinum* L.). Madras Agricultural Journal 75(9-10):330-333.

Shinde N V and Deshmukh R B 1990 Heterosis and inbreeding depression for yield and its components in chickpea. Indian Journal of Pulses Research 3(2):121-126.

Simon C J and Muehlbauer F J 1997 Construction of chickpea linkage map and its comparison with the maps of pea and lentil. Journal of Heredity 88:115-119.

Sindhu J S Singh K P and Slinkard A E 1983 Inheritance of resistance to Fusarium wilt in chickpeas. Indian Journal of Farm Sciences 74:68.

Singh B D 1997 Plant Breeding. Kalyani publishers, New Delhi.pp:702.

Singh H Haware M P Kumar J and Smithson J B 1991 Resistance to wilt in chickpea (*Cicer arietinum* L.): Third locus for late wilting to race 1.Proceedings of satellite symposium on pulses, IARI, New Delhi. pp:170.

Singh H Kumar J Haware M P and Smithson J B 1987a Genetics of resistance to fusarium wilt in chickpea. In: Genetics. Plant Pathogenesis. Day P R and Jellis J G (eds). Blackwell Science Publications, Oxford, England (Poster abstract). pp:339-341.

Singh H Kumar J Smithson B Haware M P 1987 Complementation between genes for resistance to race 1 of *Fusarium oxysporum* f.sp.*ciceri* in chickpea. Plant pathology 36:539-543.

Singh H Kumar J Smithson B Haware M P 1988 Associations among *Fusarium* wilt resistance, flower colour and number of flowers per fruiting node in chickpea (*Cicer arietinum* L.). Journal of Agricultural Science 110:407-409.

Singh K B and Dahiya B S 1973 Breeding for wilt resistance in Bengal Gram (Abstract). Proceedings of Symposium on the wilt problem and breeding for wilt resistance in Bengal Gram, Indian Agricultural Research Institute New Delhi, India, September 1973. pp:13-14.

Singh N B Dube P S and Singh A K 1988 Genetic variability in root characteristics in chickpea. International Chickpea Newsletter 18:35-37.

Singh O Gowda C L L Seethi S C DasGupta T and Smithson J B 1992 Genetic analysis of agronomic characters in chickpea. I.Estimates of genetic variances from diallele mating system. Theoretical and Applied Genetics 83:956-962.

Singh P and Narayanan S S 1997 Biometrical Techniques in Plant Breeding. Kalyani Publishers, New Delhi.

Sinnett D Deragon J M Simar L R and Labnda D 1990 Alumorphs-Human DNA polymorphisms detected by polymerase chain reaction using Alu-specific primers. Genomics 7:331-334.

Snedecor G N and Cochran N G 1968 Statistical Methods. Oxford and IBM publishing company private limited, New Delhi.

*Snyder W C and Hansen 1940 The species concept in *Fusarium*. American Journal of Botany 27:64-67.

Srivastava A N and Jain J K 1994 Variability and coheritability estimates of physiological and economic attributes in soybean. Indian Journal of Genetics and Plant Breeding 54(2):179-183.

Srivastava S K Singh S N and Kahsi M N 1984 Assessment of yield losses in some promising gram cultivars due to fusarium wilt. Indian Journal of Plant Protection 12:125-126.

Stam P 1993 Construction of integrated genetic linkage maps by means of a new computer package Join map. Plant Journal 3:739-744.

Strand M Prolla T Liskay R and Petes T 1993 Establishment of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch pair. Nature 365:274-276.

Subbarao G V Johansen C Slinkard A E Nageswara Rao R C Saxena N P and Chauhan Y S 1993 Strategies for improving drought resistance in grain legumes. Critical Review Plant Science 14:469-523

Suiter K A Wendel J K and Case J S 1983 Linkage-1: A Pascal computers program for the detection and analysis of genetic linkage. Journal of Heredity 74:203-204.

Tahir M 1990 Use of isozyme polymorphisms in lentil (*Lens culinaris* Medik.) for gene mapping and definition of quantitative trait loci. Ph.D Thesis, Washington State University, Pullman, WA, USA. 115p.

Tanksley S D Ganal M W Prince J P deVincente M C Bonierbale M W Broun P Fulton T M Giovanonni J J Grandillo S Martin G B Mesenger R Miller J C Miller L Paterson A H Pineda O Roder M Wing R A Wu W And Young N D 1992 High density molecular linkage maps of the tomato and potato genomes; Biological inferences and practical applications. Genetics 132:1141-1160.

*Tanksley S D Young N D Paterson A H and Bonierbale M W 1989 RFLP mapping in plant breeding: new tools for old science. Bio/Technology 7:257-264.

Taylor B A 1978 Recombinant inbred strains: Use in gene mapping.In: Origin of inbred mice. Edited by Morse H C, Academic Press,Newyork. pp:423-438.

*Tautz D and Renz M 1984 Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12:4127-4138.

*Tedin H 1987 Nagra synpunkter vid foragling avartee. Sveriges utsades foris Tidskrift 7:111.

Tiwari A.S 1981 Studies on wilt inheritance in gram. JNKVV Research Journal 15:133-134.

Tsumura Y Ohba K and Strauss S H 1996 Diversity and inheritance of Inter-Simple-Repeat-Polymorphisms in Douglas-Fir (*Psuedotsuga menzierii*) and Sugi (*Cryptomeria japonica*). Theoretical and Applied Genetics 92:40-45.

Tullu A 1997 Genetics of Fusarium wilt resistance in chickpea Ph.D Thesis.Washington State University.

Tullu A Muehlbauer F J Simon C J Mayer M S Kumar J Kaiser W J Kraft J M 1998 Inheritance and linkage of a gene for resistance to race 4 of fusarium wilt and RAPD markers in chickpea. Euphytica 102:227-232.

Tuwafe S Kahler A L Boe A and Ferguson M 1988 Inheritance and geographical distribution of allozyme polymorphisms in chickpea (*Cicer arietinum* L.). Journal of Heredity 79:170-174.

*Udupa S M Sharma A Sharma R P and Rai R A 1993 Narrow Genetic Variability in Cicer arietinum L as revealed by RFLP analysis. Journal of Plant Biochemistry and Biotechnology 2:83-86.

Upadhyaya H D Haware M P Kumar J and Smithson J B 1983a Resistance to wilt in chickpea I. Inheritance of late-wilting in response to race 1. Euphytica 32:447-452.

Upadhyaya H D Smithson J B Haware M P and Kumar J 1983b Resistance to wilt in chickpea. II. Further evidence for two genes for resistance to race 1. Euphytica 32:749-755.

Vander Maesen L J G 1987 Origin, history and taxonomy of chickpea. In:Saxena M C and Singh K B (Eds),The Chickpea CAB. International, Wellington, UK.pp:11-34.

van Rheenen R H 1991 Production aspects and prospects of chickpea.In: Uses of tropical grain legumes. Proceedings of a consultants meeting, March 1989, ICRISAT center, Patancheru, India.pp:31-35.

Veitenheimer E E 1981 Pea (*Pisum sativum* L.) root growth and development as affected by genotype media and *Aphanomyces euteiches* Drechs. M.S. Thesis University of Wiscousin, Madison.

Waldia R S Chhabra A K Tomar R P S and Solanki I S 1992 Inheritance of speed of radicle emergence and root length in chickpea (*Cicer arietinum* L). Indian Journal of Genetics and plant Breeding 52(4):376-378.

Waldia R S Chhabra A K Tomar R P S and Solanki I S 1993 Inheritance of speed of radicle emergence and root length in chickpea (*Cicer arietinum* L). Journal of Genetics and Breeding 47:1-4.

Wang G Mackill D J Bonmam J M McCouch S R Champoux M C and Nelson R J 1994 RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434

Warner J N 1952 A method for estimating heritability. Agronomy Journal 44: 427-430.

Watts L E Stevenson E and Crampton M J 1970 Inheritance of flowering time in six pea cultivars (*Pisum sativum* L). Euphytica 19:405-410.

*Weber J L and Wong C 1993 Mutation of human short tandem repeats. Human Molecular Genetics 2:1123-1128.

*Weeden N F Zamir D and Tadmore Y 1988 Applications of isozyme analysis in pulse crops. Pp. 979-987. In: R.J. Summerfield (ed.), World crops: Cool Season Food Legumes. Kluwer Academic Publishers, the Netherlands.

Weising K Kaemmer D Weigand F Epplen J T and Kahl G 1992 Oligonucleotide finger printing reveals various probe dependent levels of informativeness in chickpea (*Cicer arietinum* L.) Genome 35:436-442.

Weising K Winter P Huttel B and Kahl G 1998 Microsatellite markers for molecular breeding. Journal of Crop Production 1:113-143.

*Welsh J and Mc Clelland M 1990 Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18:7213-7218.

Williams J G, K Kubelik A R Livak K J Rafalski J A and Tingey S V 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Winter P Benko-Iseppon m Huttel B Ratnaparkhe M Tullu A Sonnante G Pfaff T Teleoglu M Santra D Sant V J Rajesh P N Kahl G and Muehlbauer F J 2000 A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C.arietinum* x *C.reticulatum* cross: Localization of resistance

genes for Fusarium wilt races 4 and 5. Theoretical Applied Genetics 101(7):1155-1162.

Winter P Pfaff T Udapa S M Huttel B Sharma P C Sahi S Arreguin-Espinoza, Weigand F Muehlbauer F J and Kahl G 1999 Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L) genome. Molecular General Genetics 262:90-101.

*Xu Y Zhu L Xiao J Huang N McCouch S R 1991 Chromosomal regions associated with segregation distortion of molecular markers in F_2 backcross, double haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Molecular General Genetics 253:535-545

Young N D 1994 Constructing a plant genetic linkage map with DNA markers .In: Phillips R L and Vasil I K (Eds), DNA based markers in plants. Kluwer Academic Publishers, The Netherlands .pp:39-57.

*Young N D 1996 QTL mapping and quatitative disease resistance in plants. Annual Review of Phytopathology 34:479-501

Young N D Zamir D Ganal MW Tanksley S D 1988 Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. Genetics 120:579-585.

Zabeau M 1993 Selective Restriction Fragment Amplification: a general method for DNA finger printing. Publication number 0534858 A1. European Patent Application. EP 534858 A1, Munich, Germany.

* - Original not seen

APPENDICES

Appendix 1: Residual Maximum Likelihood (REML) variance component analysis for the characters studied in parents and RILs of the cross ICCV 2 x JG 62

S.No.	Response variate	Source of variation	Effective d.f	Variance	S.E ±
1	Days to first flower	Parents and 126 RILs	125	60.00*	7.50
		Replications	2	0.02^{NS}	0.04
		Blocks within replication	21.27	0.00^{NS}	0.08
		Error	225.8	1.33	0.18
2	Days to first pod	Parents and 126 RILs	125	63.02*	7.92
		Replications	2	0.00^{NS}	0.02
		Blocks within replication	21.08	0.00^{NS}	0.11
		Error	227.3	1.99	0.27
3	Days to maturity	Parents and 126 RILs	125	75.95*	9.62
		Replications	2	0.00^{NS}	0.06
		Blocks within replication	20.83	0.15^{NS}	0.25
		Error	226.3	3.39	0.47
4	Pod filling period	Parents and 126 RILs	125	8.70*	1.41
		Replications	2	0.00^{NS}	0.06
		Blocks within replication	14.41	0.01^{NS}	0.27
		Error	228	4.05	0.61
5	Total reproductive period	Parents and 126 RILs	125	0.22*	0.07
		Replications	2	0.02^{NS}	0.02
		Blocks within replication	15.78	0.16^{NS}	0.27
		Error	225	0.58	0.07
6	Number of nodes upto first flower	Parents and 126 RILs	126	16.04*	2.03
		Replications	1	0.09^{NS}	0.13
		Blocks within replication	20.68	0.003^{NS}	0.05
		Error	228.4	0.84	0.11

* Significant at 5% level of significance
NS Not Significant

Appendix 2: Analysis of variance (mean squares) of different charters in chickpea

Source	df	Root length	Root volume	Leaflet number	Leaf area	Shoot dry weight	Root dry weight	Total number of nodes
126 RILS	125	79.86*	17.99*	10.06*	150.12*	17.59	0.069*	1518.70*
Replications	2	8.59	1.35	1.95	10.43	6.54	0.006	96.03
Error	251	6.57	1.03	1.12	8.29	4.58	0.0042	114.30

* Significant at 5% level of significance