

**ESTIMATION OF GENE ACTION, COMBINING ABILITIES
AND HETEROSIS IN *Grewia optiva* Drummond**

Thesis

by

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*Submitted in partial fulfilment of the requirements
for the degree of*

DOCTOR OF PHILOSOPHY
in
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(Tree Improvement and Genetic Resources)



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This is to certify that the thesis entitled, “**Estimation of gene action, combining abilities and heterosis in *Grewia optiva* Drummond**”, submitted in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY in FORESTRY (Tree Improvement and Genetic Resources)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a record of bonafide research work carried out by **Mr. Saresh N. V (F-2009-13-D)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation has been fully acknowledged.

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ABBREVIATIONS

%	:	Per cent
°C	:	Degree centigrade
µm	:	micrometer
bp	:	Base pair
CD	:	Critical difference
cm	:	centimeter
cm ²	:	square centimeter
DNA	:	deoxyribonucleic acid
e.g.	:	for example
<i>et al</i>	:	co-workers
F ₁	:	First Filial generation
g	:	grams
i.e	:	that is
ISSR	:	Inter simple sequence repeats
Kb	:	Kilobase pair
km	:	kilo meters
m	:	meters
M	:	Molar
mA	:	milli ampere
mg	:	milli grams
ml	:	millilitres
mM	:	milli molar
mm	:	millimeter
msl	:	mean sea level
Ng	:	nanograms
No.	:	number
PCR	:	Polymerase chain reaction
RAPD	:	Random amplified polymorphic DNA
RFLP	:	Restriction fragment length polymorphism
SE	:	Standard error
UPGMA	:	Unweighted pair group mean average
v/cm	:	volt per centimeter
µ	:	micron
µl	:	micro litre

Chapter-1

INTRODUCTION

Livestock rearing is one of the major occupations in India that provides manure, draught power for agriculture and local transportation and forms important source of food and cash income to millions of households spread across various parts of the country. Closer scrutiny of the sector, however, reveals that the contribution to the GDP by livestock sector is far too low for such a large size of livestock population. This low productivity of the sector is as much attributable to underfeeding of the livestock and the poor livestock breeds. An idea of the meager fodder availability can be had from the fact that about 50 per cent of the cattle population, depending largely upon free rangeland grazing in forests, pastures, village commons and the like, end up getting only about 1.5 kg of dry fodder/ day/ ACU (Adult Cattle Unit) as against the healthy fodder requirement norm of 3 per cent body weight. In absolute terms, the country is, by 2020, facing an estimated shortage of 728 million tons of green fodder and 157 million tones of dry fodder (Anonymous, 2011).

Livestock plays an important role in mountain farming systems and Himachal Pradesh is one of the important livestock rearing states in India. In this state, mixed agricultural production system is practiced by the farmers with both crop and livestock husbandry as the important components. Availability of nutritious fodder is the biggest constraint in animal husbandry in this state. Except for rainy season, (July to September), there is scarcity of fodder throughout the year. Natural grasslands play an important role in supplying fodder to the animals. But, due to heavy grazing pressure, the productivity in these grasslands is very poor. In hilly and mountain regions, the demand of feeds and fodder for livestock is much higher than their availability (Singh and Bimal, 2004). In Himachal Pradesh, production of dry and green fodder was 2187,000 and 3230,000 tonnes respectively (Basic Animal Husbandry Statistics, 2004). The available fodder is not only insufficient but also poor in nutritive value. Whatever

fodder resources are available, their availability is also seasonal. The availability of green fodder is the most important single factor responsible for the success of animal husbandry. Leaf fodder is a very useful resource of green fodder, especially during winter months when all other fodder sources have been exhausted.

Less prone to periodic droughts, trees form an assured source of fodder year after year. The major sources of tree leaf fodder are the trees growing on forests and common lands. However, with high timber value plantations coming up on scrub forests, this resource is getting depleted from near habitations. Another source of tree leaf fodder is the trees growing on farm lands. *Grewia optiva* is one of the most important tree species used as fodder in Himachal Pradesh (Singh, 2005). The genus *Grewia* consists of some 150 species in world, 42 in India and 5 in Himachal Pradesh. It belongs to family Tiliaceae. It is naturally distributed in Indian subcontinent: Bhutan, Nepal, Pakistan and in India it is distributed in areas of Himachal Pradesh, Jammu and Kashmir, Punjab, Sikkim and Uttar Pradesh (Hooker, 1875). Several species of *Grewia* are used as a very important multipurpose or agroforestry tree, of which *Grewia optiva*, commonly known as 'Beul', having chromosome number $2n = 18$ (Coleman, 1982) and very popular agroforestry tree, which is grown in low and mid-hills regions in the western and central Himalaya on account of its utility as fodder, fuel and fiber. It occurs at elevations of 500-2100 meters, where the temperature ranges from 2° to 38°C and rainfall from 1, 200 to 2, 500 mm in the year (Joshie and Narain, 1992).

Grewia optiva is a small to medium-sized deciduous tree, 9-12 m in height; crown spreading; bole clear, 3-4 m, and about 1 m diameter. Branches smooth pale silvery-brown; bark dark brown, thick and roughish, exfoliating in small woody scales. Leaves opposite, ovate, acuminate, closely serrate, rough and hairy. Flowers 1-8, together; peduncles solitary, leaf opposed or exceptionally a few axillary. Sepals linear oblong green outside, white, pale yellow or red inside; petals white or pale yellow, shorter than the sepals, linear, claw distinct. Fruit is a drupe, 1-4 lobed, olive green and black when ripe (Troup, 1921). Old leaves are

shed in March and April and new ones appear in April-May. Flowers appear with the new flush of leaves. It flowers from the end of March to June; flowers are monoecious and lemon yellow in colour. Pollen grains exhibit sulphur yellow colour and having 75 per cent viability. The fruits are formed soon after and attain full size by September, ripening between October and December. The immature fruit is olive green, turning black on ripening. Fruits are much liked by birds, the major dispersal agent. The fruits are borne on previous year's shoots.

With the increase in demand for fodder, there is a need to develop new hybrids of *G. optiva* suited to different agro-climatic zones with improved yield. Therefore, it is essential to understand the genetic architecture of *G. optiva*, which provides useful guidelines to determine the source population and from which it is possible to derive appropriate genotypes with desired characters. The selection of parent is one of the important aspect in developing a potential hybrid, which is practiced after testing of parents for their combining ability [general combining ability (gca)] effects. It is also useful in understanding the type of gene action controlling various traits to develop suitable breeding strategy.

The main goal of breeding program is the genetic enhancement of yield. Effective tree breeding depends on an understanding of tree variation in nature and preserving such variation for future use. Information on the genetic variation and inter relationships of plant characters with tree volume end use and its component characters themselves is of great importance to a breeder in selecting a desirable genotype. Further breeding programmes will have to rely more on such crosses which assemble genotypes with high yield potential and a more stable performance after ascertaining the general and specific combining ability.

Combining ability studies reveal nature and magnitude of gene action involved in expression of quantitative traits which is important for successful development of crop varieties (Nazir *et al.*, 2005). Also, the correct choice of parents for hybridization is crucial for development of cultivars. Combining ability analysis provides such information so as to frame the breeding programme effectively. This analysis helps in identification of parents with high general

combining (GCA) and parental combinations with high specific combining (SCA). Significant GCA and SCA effects provide information to determine the efficacy of breeding for improvements in given traits and they can be used to identify the lines to be served as parents in a breeding program for improvement (Kearsey and Pooni, 1996). In addition, this technique enables the breeder to combine desirable genes that are found in two or more genotypes (Dabholkar, 1992).

Estimation of additive and non - additive gene action through this technique may be useful in determining the possibility of commercial exploitation of heterosis and isolation of pure line among the progenies of the good hybrids whereas, magnitude of heterosis provides basis and serves as guide for the choice of desirable F_1 hybrids so as to exploit hybrid vigour. The phenomenon of heterosis of F_1 hybrids can reflect special combining ability (SCA) and general combining ability (GCA) of parental lines. Combining ability concepts are the basic tools for improved production of crops in the form of F_1 hybrids. Identifying parental combinations with strong heterosis for yield and obtain genetic parameters are the most important steps in the development of new cultivars (Melchinger, 1999) and heterosis effects are generally more pronounced in crosses between genetically distinct materials.

Conventional breeding programmes reach this goal by generating an F_1 hybrid and F_2 segregating population and then screening the phenotypes of pooled or individual plants for presence of desirable traits, which is followed by a process of repeated backcrossing, selfing and testing. During this process breeder depends on accurate screening methods and availability of lines with clear-cut phenotypic characters, which is time consuming and difficult to achieve with classical methods. To optimize time and costs, it is necessary to verify the hybridity of new seedlings at an early stage before commercial plantation in order to assure uniformity and stability of the field performance and yield. The purity of F_1 hybrid seeds is traditionally assessed in a field grow-out trial (GOT), but these trials are time-consuming and labor-intensive and require large plots of land (Dongre and Parkhi, 2005).

Use of molecular markers facilitate these breeding processes, since it can provide means of detecting and resolving complications and accelerate the generation of new varieties and allow association of phenotypic traits with genomic loci. Molecular markers not only help in studying genetic diversity but also allow the easy and reliable identification of breeding lines, hybrids and cultivars. Because F₁ hybrids contain DNA from both parents, identification of male and female parent-specific markers will allow differentiation of true hybrids. In this regard, in recent years molecular techniques are providing a useful tool for the correct identification of plant species, included hybrid taxa. In particular, DNA markers have been often used for hybrid characterization in tree species (Dumolin *et al.*, 1995). Among these, RAPDs and ISSRS have been the preferred markers for fingerprinting tree species. RAPDs are fragments of genomic DNA amplified through PCR using a decamer primer of random sequence, where polymorphism depends upon the presence or absence of an amplification product. The use of RAPDs in different organisms began in the late 80's (Williams *et al.*, 1990), and due to their simplicity and speed they have become a very valuable tool for cultivar identification and genetic similarity studies in plants. On the other hand, ISSR markers share most of the advantageous features of RAPD markers with the addition of a potentially co-dominant pattern of inheritance (Zietckiewicz *et al.*, 1994) and have been considered a promising source of a large number of reliable, highly-polymorphic markers (Salimath *et al.*, 1995).

Wide diversity of edapho-climatic conditions under which *Grewia optiva* grows, makes it an ideal species for breeding purposes. Greater the diversity in material, more the chances of getting the desired types. There is collection of 60 genotypes of *G. optiva* from different districts of Himachal Pradesh, being maintained in the nursery is unique collection of germplasm. This variation, can be utilized for breeding purposes for some beneficial traits like fodder value, growth rate and for conservation of species. As *G. optiva* is an important agroforestry species in Himachal Pradesh and there is no previous attempt made to study heterosis, combining ability, gene action and use of molecular markers

for verification of hybrids. Keeping this in view, present investigation was carried out with the following objectives:

- i) To study the combining ability and gene action
- ii) To estimate heterosis effect in the species.
- iii) Verification (Paternity analysis) of F_1 hybrids as well as parents using molecular markers.

Chapter-2

REVIEW OF LITERATURE

The prime objective of any breeding programme is the improvement of desirable qualitative and quantitative characters like yield, fodder/timber quality etc. These characters having very complex trait and is the outcome of the effects of various components which determines there potential in a tree species. The main goal of most of the breeding programme is to increase the yielding ability of the tree species. Information on various quantitative traits, particularly of those that contribute to yield will be most useful in planning and successful implementation of the breeding programme. Hybridization is one of the means of obtaining increased yield and exploitation of heterosis.

The literature on combining abilities, gene action, heterosis and paternity analysis using molecular markers is reviewed in this chapter. Since the literature on combining abilities, gene action, heterosis and paternity analysis using molecular markers in *Grewia* species (Tiliaceae) is meager, reviews related to other crops is also included and has been reviewed and described under the following headings.

2.1 COMBINING ABILITY AND GENE ACTION

Combining ability of a line/ strain to produce superior progenies upon hybridization with other lines/strains is an important criteria to select parents for developing superior new hybrids. The combining ability analysis is thus useful in the testing the choice of desirable parents. It is also possible to know the magnitude of additive and dominance variance in the material being studied. Information on nature of gene action involved in inheritance of quantitative traits helps in deciding appropriate breeding method and efficient selection procedure.

Sprague and Tatum (1942) were the first to explain the concept of combining ability in terms of genetic variance. They defined the term general

combining ability as performance of a line or population in several hybrid combination and specific combining ability as those effects in certain combinations which significantly departed from what would have been expected on basis of average performance of the lines involved.

The concept of combining ability is important for the selection of desirable parental lines to be used in the hybridization programme. Davis (1927) for the first time developed a technique for testing general combining ability of inbred lines by means of an inbred X variety cross (to cross) which enabled the breeders to make rapid preliminary evaluation of inbreds. Diameter at breast height (dbh) and total height were assessed at 11.5 years from planting in a control pollinated progeny test of *Pinus radiata* in New South Wales. The material represented on 18 female X 4 male North Carolina II mating designs. General combining ability (GCA) and specific combining ability (SCA) variances were estimated as measures of additive genetic and dominance variances, respectively.

Tan (1977) during the estimates of general combining ability in *Hevea* parents for yield and girth reported that GCA estimates helps in choice of parents. A highly significant positive correlation ($r = 0.71$) is found between GCA estimates from seedling and clonal families.

Diallel crosses were made between clones of French and Swedish provenance of *Picea abies*. The F_1 hybrid seedlings and their French and Swedish intra-provenance half-sibs were tested over three growth periods at the Stockholm. In comparison with intra-provenance half-sibs, the hybrid progenies gave an intermediate response for the photoperiodic control over bud set and for the temperature requirements for bud-flushing. This indicates the prevalence of additive action of multiple factors in the determination of the photoperiodic and temperature response. By selection of suitable parents, inter-provenance crosses can be used to produce hybrids with desired photoperiodic characteristics and temperature requirements (Eriksson *et al.*, 1978).

King *et al.* (1992) estimated genetic parameters for crown form traits and bole taper in full sib progeny of Douglas fir (*Pseudotsuga menziesii*). Significant amounts of additive genetic variance were shown for all traits except branch thickness. Non additive genetic variance was statistically significant for three of the light crown form traits investigated. High individual tree heritability was expressed for branch angle, indicating that this trait could be improved through phenotypic selection. Progeny testing would be more effective for most other form traits. Branch thickness and branch length indicates stronger genetic differences when they are expressed as a proportion of stem size.

Ronnberg-Wastljung *et al.* (1994) estimated additive, dominance and epistatic variances of forty families of *Salix viminalis* produced by 8×8 factorial mating design. Additive variance was found with large standard deviations in many of the estimates. Dominance and epistatic variances of greater importance were found for weight character in the clay-sand experiment.

Fifty-five doubled haploids (DH) of *Coffea canephora* were crossed with either heterozygous genotypes or DH in order to study their combining ability.. Marked hybrid vigour was observed for all characters analyzed including yield. Large differences were evident among top-crosses involving different DH produced from the same parental clone reflecting the high level of heterozygosity of clones. Factorial mating design analysis indicated that all genetic variance was attributable to additive effects in estimates of yield as well as plant height and leaf characteristics. The general combining ability variance component was also predominant for stem girth and susceptibility to leaf rust, although effects due to interaction were detected. Some hybrid combinations had yield comparable to standard clonal varieties (Lashermes *et al.*, 1994).

Ronnberg-Wastljung and Gullberg (1996, 1999) evaluated the families of *Salix viminalis* developed by 8x8 factorial crossing at the age of 1, 4 and 6 years in two different environments. They observed that genetic relationship between growth components is the same over age and environments when the plants are grown without stress. The analysis showed high additive genetic variances for all

characters studied. The highest negative correlation was found between number of days to bud burst and weight indicating the importance of early bud burst for production.

General combining ability (GCA) and specific combining ability (SCA) were examined in 36 parent by Yanchuk (1996), 6-parent disconnected partial diallels across 4 different experimental series in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* DOUGL.) to examine the ratios of the 2 genetic variances, the distribution of GCA and SCA effects, and estimates of genetic gain from GCA and SCA for 3 growth traits. Height at age-7 and height and volume at age-12 were measured on approximately 150 trees per full-sib family in each diallel, across 11 different test sites within each series. The average percentage ratio of SCA variance to GCA variance was 36%, across all series and the 3 growth traits, with a range of 19% to 65%. GCA and SCA variances did not appreciably change for height growth from age 7 to age 12. While these results confirm current strategies in Douglas-fir breeding to select primarily on GCA, there are opportunities to utilize SCA variance in the production population. Gains for height growth from utilizing SCA are lower, which reflect either, i) lower SCA variances associated with height, or ii) competition has biased upward volume SCA variance by age 12 (relative to height).

The genetic causes of heterosis in tree growth were investigated by a comparative genetic analysis of intra- and inter-specific crosses derived from *Populus tremuloides* and *P. tremula*. During the first 3 years of growth, interspecific hybrids displayed strong heterosis in stem growth, especially volume index, over intraspecific hybrids. By a series of joint analyses on the combining ability and the genetic component, Li and Wu (1996) found that F_1 heterosis might be due to overdominant interaction between two alleles, one from the *P. tremuloides* parent and the other from the *P. tremula* parent, at the same loci. While additive effects appeared to influence seedling traits collectively across loci, a few major dominant loci had much larger effects on stem growth.

Lemos *et al.* (1997) in the study of importance of additive genetic and specific combining ability effects for rooting ability of stem cuttings which were

derived from clones obtained from seed of 24 crosses between 40 unrelated parents in *Eucalyptus globulus* reported that the SCA effects were low, explaining only 1.5 % of the total variance. Additive genetic-by-test and SCA-by-test interactions were also small, but significant, accounting for 8% and 6% of the variance, respectively. The study showed evidence of a high heritability and genetic variance, with low dominance effects and genotype-by-test interaction and should result in very high selection accuracy when predicting genetic merit for clones or families. This is important because it opens the possibility of successful bulk propagation of *E. globulus* based on untested progeny from high rooting parents.

A line $\times$ tester analysis was carried out in mulberry (*Morus* spp.) to determine the genetic interaction in the expression of various quantitative characters including leaf yield. Eight clonal varieties were selected, 3 of them were designated as lines (♀) and 5 of them were called as testers (♂). Combining ability studies were conducted on these parents along with their F_1 hybrids for the growth rate and other growth parameters. The ratio of General Combining Ability (GCA) and Specific Combining Ability (SCA) indicated the predominance of non-additive genes in mulberry. Results suggest that selective crossing followed by proper screening may be the best approach for breeding of high yielding varieties in mulberry (Vijayan *et al.*, 1997).

Zhang *et al.* (1998) studied the growth differences, combining ability effects, genetic variation, heritability and heterosis in 10.5 year old Chinese fir, (*Cunninghamia lanceolata*) progeny test in China, crossed in a 9 $\times$ 9 half-diallel design. The results indicated that GCA (general combining ability) played a primary role and SCA (specific combining ability) a secondary role in genetic effects and growth traits. It was recommended that during the different stages of Chinese fir genetic improvement, the various parents with different GCA and SCA should be selected as genetic material in breeding orchard and productive seeds orchard.

Liang-Yichi *et al.* (1999) reported from the studies (a progeny trial in Fujian Province) China, on 3 backward selection methods used for combining ability of Chinese fir (*Cunninghamia lanceolata*) with respect to growth rate selecting superior families for general combining ability (GCA), selecting superior families for specific combining ability (SCA), and correlated recurrent selection for GCA and SCA. The result indicated that there was significant benefit in using SCA selection based on GCA selection. The mean genetic gain for height was 9.65%, for diameter 8.95% and for volume 27.46%.

A diallel mating trial was carried out with 6 fast growing families of first generation Chinese fir (*Cunninghamia lanceolata*) China and the heterosis and combining ability of the progeny studied. At 7 year old there was significant divergence observed with respect to height, dbh and volume between the different combinations and the effects of general and specific combining abilities were significant. There were also different dominant or heterosis effects. The results indicate that parents for seed orchard establishment could be selected based on GCA selection (Li and Li, 1999).

A diallel analysis to identify general and specific combining ability (GCA and SCA) for chlorophyll contents was undertaken in a set of 7 tossa jute (*Corchorus olitorius*) varieties. GCA and SCA variances were significant at later stages of growth. Additive and non-additive genetic variance differed at the different growth stages. The cross combinations showing positive and significant SCA effects mainly involved one good and one poor general combining parent (Mitra *et al.*, 2000).

Singh (2002) carried out evaluation of full-sib progenies of selected clones of Poplar (*Populus deltoides* Bartr.) using 10 parents comprising of 5 lines (females) and 5 testers (males). The analysis showed that per cent contribution of line x tester interaction was higher than that of lines and testers for most of the traits. The variance due to SCA was higher than the variance due to GCA for most of the traits. Gene action study revealed non-additive genetic component had dominant role in most of the characters. The estimates of family heritability

with high to moderate expected genetic advance were found for several characters emphasizing the existence of both additive and non-additive genetic components.

Variances for general combining ability (GCA) and specific combining ability (SCA) effects were estimated for tree diameter (DBH) from a series of 20 sets of 6x6 half-diallel mating experiments in radiata pine, planted at ten sites across Australia. Significant SCA variance for DBH was almost equal to GCA variance for the combined analysis of all ten sites. About a quarter of extra genetic gain is achievable through use of SCA variance if selection is based on the best breeding values (Wu and Matheson, 2004).

Vijayan *et al.* (2008) carried out lines x tester analysis in five mulberry genotypes (3 females and 2 males, differing in salt tolerance). Clones of these parents along with F₁ hybrids were planted at different levels of salinity. The general combining ability (GCA) analysis has identified English black as the best combiner among the parents. The specific combining ability analysis (SCA) found that crosses English black × C776 and Rotndiloba × Mandalaya were good for plant height and leaf size whereas, English black x C776 and Rotundiloba x C776 were good for biochemical proline and chlorophyll.

Nine forage genotypes of fodder sorghum viz. M-48212, M-48273, IS-23980, IS-23960, IS-23963, SSG-59-3, SGL-87, AKFR-89 and IS-22450 were crossed in diallel fashion excluding reciprocals. The observations were recorded for 18 characters. The parents IS-23980 and AKFR-89 has good performance for yield and other characters. Among crosses IS-23980 x IS-23960 and M-48273 x AKFR-89 performed better for yield. Considering the *per se* performance, heterosis, heterobeltiosis and standard heterosis, gca effects of parents, sca effects of crosses six crosses viz. SSG-59-3 x IS-22450, IS-23980 x IS-23960, IS-23960 x IS- 22450, IS-23980 x IS-22450, IS-23980 x IS-23963 and IS-23960 x SGL-87 were identified as best crosses for further crop improvement programme (Kotgire, 2008).

Combining ability studies using line x tester mating design involving five females and 15 males were undertaken to identify good parents and best cross combinations for earliness and its related traits in castor. The estimated components of gca and sca variances showed the preponderance of additive gene action for number of nodes upto primary spike and non-additive gene action for plant height. Parents Kranthi, RG-1471, RG-178, DCS-9 and RG-2724 were the good general combiners for all the four traits. The crosses Kranthi x RG-1471, LRES-17 x RG-2724 and VP-1 x RG-1471 for most of the traits related to earliness in castor were the best specific combinations to exploit the non-fixable components. The study revealed that *per se* performance of crosses had significant and positive correlation with sca effects for most of the traits. (Sridhar *et al.*, 2010).

Choudhary (2011) reported that estimates of general combining ability variance were less than the specific combining ability variance for all the parameters in *Salix* spp. Moderately high (0.42 for per cent successful crosses) to low (0.15 for seed per capsule) narrow sense heritabilities and high heritability broad sense (0.74 to 0.99) for per cent successful crosses and seeds per catkin was estimated. Based on hybrid vigour over check cross, significant desirable SCA effects and mean performance of with respect to crossability parameters, combinations viz., *S. tetrasperma* (LNF) x *S. tetrasperma* (LNM), *S. tetrasperma* (LNF) x *S. tetrasperma* (TFB), *S. tetrasperma* (LG) x *S. tetrasperma* (TFB), *S. tetrasperma* (LN) x *S. tetrasperma* (TFB) and *S. matsudana* (PN-227) x *S. alba* (Kashmiri) were found promising.

With the aim of estimating genetic parameters and recognizing superior *Jatropha curcas* L. combinations Biabani *et al.* (2012) selected ten superior plants based on seed yield and oil content, and were crossed among them in a 10 x 10 half-diallel mating design to produce 45 F₁-hybrids. Analysis of variance for the combining ability indicated that GCA and SCA variance were significant at 1% probability for plant height, collar diameter and number of leaves in nursery stage. The low ratio of GCA/SCA exhibited the non-additive effects. Broad sense heritabilities were high for plant height, collar diameter and number of leaves.

Estimates for narrow sense heritability of the traits, plant height collar diameter and number of leaves were low. Percentages of heterosis and heterobeltiosis values for plant height, collar diameter and number of leaves ranged from negative to positive. This showed that the existence of dominance or non-additive gene actions might be present in the hybrids.

2.2 CONTROLLED CROSSING AND HETEROSIS

Hybrid vigour or heterosis was first reported by Koelreuter (1673). Subsequently many workers reported hybrid vigour in large number of plant species. However, the term heterosis was originally proposed by Shull as the "developmental stimulus resulting from the union of gametes and hybrid vigour as the manifest effect of heterosis" Presently, however, two terms are used as synonyms open and superiority of F_1 is estimated over the average of two parents, i.e. the mid parental value but Powers suggested that term heterosis should be used only when hybrid is either superior or inferior to both parents. Mather and Jinks (1971) defined heterosis as the amount by which the mean of F_1 family exceeds its better parent. For the economic point of view, a third term for heterosis is generally being used as "standard heterosis" for comparing performance of F_1 hybrid over the check which may be a released variety or standard parent.

Natural hybrids of *Alnus incana* X *A. glutinosa* showed hybrid vigour and is evident from its height and diameter growth which exceed those of *A. glutinosa* by 12 and 33% respectively, and those of *A. incana* by 16 and 45% respectively. Under good conditions, the hybrid can grow to 25-26 m high and 37-38 cm in diameter in 35-40 years (Kundzins and Pirags, 1960).

Critchfield (1964) successfully crossed *Pinus nigra* (Austrian pine) and *P. resinosa* (red pine), this was the first cross that involved Eastern and Western hemisphere hard pines and the first cross of red pine with any other species. The six hybrids was intermediate between their parents in most characteristics, but they greatly exceed either parent in early height growth. In one feature, the location of the principal resin canals in the leaves, some of the hybrids are unlike

either parent species but resemble other pines in the Lariciones group. The hybrids may prove useful in the transfer of genes between the two parent species, and particularly in the introduction into red pine of genes affecting insect resistance.

Besscketror (1972) studied the occurrence of heterosis in poplar hybrid and found highest number of heterotic hybrids (up to 2.5 per family). Blada (1993) reported the results of control crosses of *Pinus cembra* X *P. wallichiana* and *P. cembra* X *P. monticola*. The following important results were observed: (1) Significant genetic difference were found between hybrids and their parents for most traits, (2) both hybrid crosses resulted in individuals that have some important traits outside the range of the parents. (3) *P. cembra* X *P. wallichiana* was intermediate between parents in both blister rust resistance and growth traits, (4) *P. cembra* X *P. monticola* displayed heterosis in 6 of the 9 tested traits, including growth traits, it inherited outstanding form and growth from the male parents; (5) both hybrids represent a potentially important planting stock for middle to high altitude sites.

The greater uniformity of the F₁ generation progeny over the parental progenies has also been recognized as an advantage in favor of hybrids. This was the main conclusion drawn by Lacaze and Birot (1974) where the F₁ hybrid larch progeny tested was characterized by a remarkable homogeneity for most of the traits studied. Similar conclusions were drawn by Ferrand (1986) for height measured at the nursery stage.

Dhir and Mohn (1976) compared the crosses between and within two geographically diverse source of eastern cotton wood. Controlled pollinations were made within and between the Minnesota and Missouri provenances of *Populus deltoides*. The progeny were grown in green house and measured at 4 month old, inter-provenance crosses showed significantly higher performance in 6 of the 7 characters.

The hybrids of *Eucalyptus tereticornis* and *E. camaldulensis* expressed heterosis in growth rate at about two years of age, which surpassed the better *E. camaldulensis* parent, in both height and diameter growth. Further, within the

hybrid population as well as in that of the *E. tereticornis* parental progenies, there was indication of a positive association between average height and diameter on the one hand and earliness of flowering on the other. In other words, the taller and thicker stemmed individuals generally tended to be precocious in comparison with the relatively shorter and thinner stemmed individuals within the same population (Venkatesh and Sharma, 1976).

Dickmann and Stuart (1983) reported that each new generation from breeding and hybridization experiments offers possibilities for new clonal or family selections that can be propagated and, it tests confirm their superiority in poplar. Most breeding programmes has been aimed at producing first generation (F_1) hybrid combinations that show increased vigour over parents (heterosis) in terms of faster growth rate, better rooting of cuttings, improved adaptability in environmental factors, and greater resistance to disease and pests. Other objectives have been improvement in wood quality and stem strengthness. Most of these traits appear to depend on large number of different genes and generally involve a quantitative inheritance. If the F_1 are superior to both parents, additional gains in later generations are likely, and hybrid vigour may be fixed. Thus it is important to raise the offspring of a large number of crosses between individuals to provide as many as varied gene combinations as possible.

Paques (1989) in his critical review of larch and its incidence on breeding strategies revealed that hybrids generally ranks higher for several important characteristics such as stem form and wood mechanical properties. However, the hybrid ranks only intermediate for several wood physical properties.

Strauss (1986) carried out study on heterosis of 10-yr-old trees of knob cone pine (*Pinus attenuata* Lemm.) and revealed that inbreds, created by self pollination, had 46% of the heterozygosity of their mothers; the crossbreds, created by inter population crossing, had 155% of the heterozygosity of their mothers. Within the crossbreds, heterozygosity was positively correlated with trunk growth, but negatively correlated with cone production. Within the inbreds, heterozygosity was positively correlated with both trunk growth and cone production.

Matyssek and Schulze (1987) reported that the hybrid larch possessed three times greater above-ground biomass, although all larches displayed similar relative distributions of biomass. Relative growth rate was slightly lower than in the parent species but, hybrid larch achieved twice the annual carbon gain, increment in stem length and above-ground production, and its foliage-related stem growth was higher than in European (*Larix decidua*) but similar to Japanese (*L. leptolepis*) larch. The foliage biomass per crown length was about 30% higher in hybrid larch than in both of the parent species.

In artificial hybridization experiments of *Pinus brutia* and *P. halepensis* it was found that the production of F₁ interspecific hybrids can be obtained quite easily by conventional crossing methods. The evaluation of F₁ hybrids and other combinations (F₂ and BC's) 15 years after planting in four different sites in north Greece, showed that F₁ hybrids possess hybrid vigor in all habitats planted. Their superiority in growth over the pure species varied from 5-190%, related to planting site, and to particular trees of the parental species which were crossed to produce the hybrids (Panetsos, 1987).

Heterosis of wood quality characteristics among 18 *Populus* × *xiaozhuania* cv. 'popularis' improved clones has been studied and group-average method of clustering was done to cluster the *P.* × *xiaozhuania* cv. 'popularis' improved clones according to genetic distance. Significant heterosis was observed for wood quality characteristics among *P.* × *xiaozhuania* cv. 'popularis' improved clones in fiber length, fiber width, ratio of length to width, double wall thickness and wood specific gravity (Kesheng *et al.*, 1995).

Li *et al.* (1997) investigated the genetic causes of heterosis in tree growth by comparative genetic analysis of intra- and inter-specific crosses derived from *Populus tremuloides* and *P. tremula* growing under two contrasting sites. Juvenile growth traits, diameter and volume were measured to examine the heterosis of hybrid. During the first three years of growth, intra-specific hybrids displayed strong heterosis in stem growth especially volume index over inter-specific hybrids. The hybrids with higher heterozygosity were more stable across

sites than intra-specific crosses with lower heterozygosity. They found that strong heterosis of the hybrids in stem volume was largely due to heterotic gene action contributed from alleles of *P. tremula*.

The genetic causes of heterosis in tree growth were investigated by a comparative genetic analysis of intra- and inter-specific crosses derived from *Populus tremuloides* and *P. tremula*. A new analytical method was developed to estimate the effective number of loci affecting a quantitative trait and the magnitudes of their additive and dominant effects across loci. During the first 3 years of growth, interspecific hybrids displayed strong heterosis in stem growth, especially volume index, over intraspecific hybrids. By a series of joint analyses on the combining ability and the genetic component, it was found that F_1 heterosis might be due to overdominant interaction between two alleles, one from the *P. tremuloides* parent and the other from the *P. tremula* parent, at the same loci. Heterosis in aspen growth appeared to be under multi-genic control, with a slightly larger number of loci for stem diameter and volume (9–10) than for height (6–8). While additive effects appeared to influence seedling traits collectively across loci, a few major dominant loci had much larger effects on stem growth (Li and Wu, 1996).

Paramathma *et al.* (1997) studied heterosis in six *Eucalyptus* species (*E. alba*, *E. camaldulensis*, *E. microtheca*, *E. tereticornis*, *E. polycarpa* and *E. torelliana*) under complete diallel mating. The variance due to SCA was higher than the variance due to GCA for most of the characters. In Gene action study non-additive genetic component had dominant role in most of the characters suggesting that recurrent selection should be followed to capture both additive and non-additive genetic variances. An evaluation of the morphological and physiological characters of six month old seedlings of the parent and their hybrids for heterosis, among parents showed that *E. alba* and *E. tereticornis* gave higher than mean performance. Among the hybrids, *E. alba* x *E. tereticornis* registered the highest heterosis for most of the characters.

Larson (1998) reported that intra- and inter-specific crossing was carried between *S. viminalis*, *S. dasyclados* *Salix schwerinii*, *Salix triandra*, *Salix caprea*,

Salix daphnoides and *Salix eriocephala* with the objective of developing hybrids having high biomass production potential. Since it was difficult to predict the outcome of certain crosses, the breeding strategy was firstly to make different crosses. Secondly, the crosses included one parent known to perform well in biomass plantations in combination with a parent from the new collections. Further study revealed significant heterosis from hybrids of inter-specific crossings between Swedish and Russian willow.

Baltunis *et al.* (1998) compared five-year growth measurements among 25 intraspecific crosses of *Larix decidua* MILL. (E), *L. laricina* (DUROI) K. KOCH (T), *L. kaempferi* (SIEB. and ZUCC.) GORD. (J). Heights for intraspecific hybrid families ranged from 2.6 m to 3.8 m. By selecting the 5 best hybrid families in the test (4 of 5 were ExJ or JxE hybrids) gains of 37% and 104% in height and volume can be obtained in comparison with 2 commercial check lots of E and ExJ.

Juvenile growth and bud set phenology were analyzed to study the biological basis of heterosis (hybrid vigor) in interspecific hybrids of *Populus tremuloides* Michx. (T) and *P. tremula* L. (Ta). Growth, measured as seedling volume index, was significantly higher for each of the two reciprocal interspecific crosses, T x Ta and Ta x T, than for the T x T intraspecific cross. Broad-sense heritabilities were 2-6 times larger than narrow-sense heritabilities for growth and shoot components in the T x T intraspecific cross, suggesting an important role for dominance or overdominance in aspen growth. Internode length and leaf number contributed substantially to the heterosis of stem volume, but their effects on heterosis differed between the T x Ta and Ta x T hybrids (Li *et al.*, 1998).

Kopp *et al.* (1999) carried out controlled intra and inter-specific crosses in willows for development of clones with higher productivity. The total number of controlled crosses attempted was 121 with 64 inter-specific and 57 intra-specific crosses. Thirty-four full sib *S. eriocephala* families were produced using an 8 x 7 incomplete diallel mating design.

Performance in height, diameter, volume and stem straightness of some F₁ interspecific hybrids involving *Pinus caribaea* var. *hondurensis*, *P. elliottii*, *P. oocarpa*, *P. tecunumanii* established at two sites in Zimbabwe was reported. At 5 years of age, hybrid vigour (heterosis) in all the traits was exhibited in all the hybrids at both sites. The volume production of the hybrid between *P. caribaea* and *P. tecunumanii* was more than four times that of the commonly grown *P. elliottii* and 52% more than the best performing pure species (*P. tecunumanii*). This hybrid had less stem breakage than pure *P. tecunumanii* and better stem straightness than the parental species. The other hybrids also performed well relative to the pure species. The volume production of the hybrids between *P. caribaea* and *P. oocarpa*, and that between *P. caribaea* and *P. elliottii* were similar, and more than three times that of pure *P. elliottii* (Gwaze, 1999).

Survival, growth and stem straightness were assessed at 7 years of age for F₁ hybrids of *E. grandis* with *E. saligna*, *E. pellita* and *E. urophylla* and the same traits were assessed at 6 years for F₁ hybrids of *E. grandis* with both *E. camaldulensis* and *E. tereticornis* established at two low rainfall sites in Zimbabwe. Survival was moderate to high in all taxa. Both *E. grandis* x *E. saligna* and *E. grandis* x *E. urophylla* hybrids from controlled crosses outperformed the local control in growth and straightness with the former outperforming the local control by 41 %, and the latter by 16 % in volume. The natural hybrid *E. grandis* x *E. pellita* had similar growth to the local control, and the natural hybrid between *E. grandis* and *E. saligna* was outperformed by the control. Heterosis was exhibited in all the hybrids and was expressed differentially at different sites, in different traits and in crosses involving different species (Gwaze *et al.*, 2000).

Ten provenances of Maritime pine (*Pinus pinaster* Ait.) belonging to the three biosystematic groups of the species (Atlantic, Mediterranean, and North African) were crossed. Parental provenances and their hybrids were tested and measured for height growth at age 1, 2, 3, 8 and 13, as well as for insect resistance and stem crookedness in a trial in southwestern France. The results

show a decrease in the variance of heterosis for height growth with age. Heterosis measured as the relative superiority of the hybrid to the midparent varied from 10% to 42% at age 1 and between 2 and 20% at age 13. There was no age effect on the mean value of heterosis, which amounts to 9.7% at age 1 and 8.4% at age 13. No significant relationship between levels of heterosis and stem crookedness or insect resistance was detected (Harfouche, 2000).

Li *et al.* (2002) reviewed the advance in intrasection and intersection cross breeding of poplars in China over the past 50 years. Great progress has been made in Sections *Leuce* and *Aigeiros*, and satisfactory results of intersection hybridization have been achieved in the crossing between Sections *Tacamahaca* and *Aigeiros*. The modes of hybridization include single cross, double cross, triple cross, backcross, etc. It is known that using hybrids as parents to cross with other species or hybrids is an effective and easy way to obtain heterosis. Fast growth, cold and drought tolerance, pest and disease resistance, narrow crowns and rootage, etc. are breeding goals. The conventional artificial crossing is still a major breeding method, and a combination of the conventional artificial crossing with physical radiation and chemical induction can create new triploid individuals that possess higher yield potential. The super clones cultivated have already displayed enormous socioeconomic and ecological benefits in practice.

The measured performance of frost, growth and Pilodyn traits in the inter-specific F₁ hybrid *Eucalyptus nitens* X *E. globulus* was always intermediate or comparable with one or other of the parent species. In the frost trait, the inter-specific hybrid was no better than the frost sensitive *E. globulus*. Negative mid-parent heterosis was observed for early age growth traits in inter-specific *E. nitens* x *globulus* F₁ hybrids involving Taranna *E. globulus* male parents. The inter-specific F₁ hybrids demonstrated generally poor survival and a high proportion of abnormal and slow growing phenotypes, which eventually died (Volker, 2002).

In *Liriodendron tulipifera* Linn., the backcross and F₁ crossing were carried out by Jinshan and Zhangrong (2002) where in one year old hybrid

seedlings of direct crossing, reciprocal crossing, BC₁ and F₂ universally showed notable positive growth heterosis. Growth characters of the seedlings had extremely significant difference among families and expressed great variation among individuals of family. Growth performance of BC₁ families was particularly excellent. Thus family selection, individual selection and utilization of BC₁ and F₂ hybrids had tremendous potential.

Ten-year height and stem straightness data collected from over 43 progeny tests have been analyzed to enable the re-selection of the best Japanese and European larch (JL and EL) trees to create a HL breeding population. Genetic gains of 15–20% for 10-year height and 20–25% for 10-year stem straightness was predicted for 100% hybrid families relative to a JL seed stand control. The hybrid vigour obtained when controlled pollinations of the best EL parents with the best JL parents were crossbred (Lee, 2003).

Dias *et al.* (2003) found that the correlation values were high for traits which best reflect the commercial cacao yield such as wet seed weight/plant and wet seed weight/fruit, and for which high mid-parent heterosis was found (55.4% and 8.8%, respectively). The positive association between genetic distance and heterosis for a given trait depends on the presence of dominance effects and/or of differences in the frequency of the alleles controlling the trait considered. Dominance effects for the number of healthy fruits and the number of collected fruits/plant and, wet seed weight/plant were demonstrated, while additivity was held for wet seed weight/fruit. In addition, Arunachalam (1977) has also proven the role of additive x additive epistatic interactions in causing heterosis.

The study conducted by Mirjana and Vasilije (2004) showed that out of the 26 analyzed hybrid combinations of spruce, heterotic effect occurs in 13 combinations by some of the study characters, which is half of the total number of the combinations. For cone length, heterotic effect was identified in 8 combinations, which accounts for 30.77% of the total number of crossings. For cone width heterotic effect occurred in five hybrid combinations, which accounts for 19.23% of the total number. Seed number per cone is the character for which

heterotic effect was recorded in the highest number of combinations. Of 26 combinations, heterotic effect was recorded in 12 combinations, which amounts to 46.15%.

Sinha *et al.* (2004) obtained interspecific hybrids using mutant *Corchorus capsularis* female parent with a cultivated variety JRO-524 *C. oitorious*. The hybrid showed intermediate values between parents for plant height, fibre weight, stick weight and fibre percentage. It also showed intermediate banding pattern in stem like female parent. The interspecific cross CMU-011 x JRO-524 has low lignin and high cellulose contents than the cultivated *C. olitorious* var. JRO-524. The interspecific cross has better fibre fineness but lower strength value than JRO-524.

Thirteen parents (9 fertile females and 4 males) and thirty six F₁ hybrids were evaluated by Shivaji (2004) for twenty one traits by using line x tester mating design to study yield potential effect, fibre properties and reaction to pest and diseases on isogenic hybrids of *Gossypium hirsutum* L. GMS based hybrid exhibited highest significant heterosis in desirable direction for number of sympodia per plant followed by boll weight, number of seeds per boll, bundle strength, uniformity ratio, 50 % boll bursting, open boll damage at harvest and green boll damage. Whereas CMS based hybrids recorded highest significant heterosis in desirable direction for number of bolls per plant, plant height and fibre fineness.

Smart *et al.* (2005) carried out breeding in shrub willow (*Salix*) by controlled pollination at the State University of New York in USA to produce new high yielding cultivars for bioenergy plantations. Hybrids of *S. miyabeana* with *S. sacchalinensis*, *S. purpurea* and *S. viminalis* yielded progeny recording 120-140 per cent improvement in growth over the standard clone. Intra-specific hybrids of *S. purpurea* also showed improvement over parental means. Initial results indicated that clones that produced the maximum biomass were not necessarily the best selections for other applications.

Bison *et al.* (2006) carried out comparison experiment between open pollinated progenies (OP) and hybrids performance in *E. grandis* and *E.*

urophylla. They selected 15 OP progenies of *E. grandis*, 15 OP progenies of *E. urophylla*, and 15 hybrids between *E. grandis* x *E. urophylla*, plus four controls were evaluated. The hybrids performance for circumference at breast height (CBH) was higher, an average, 38,7% than the OP progenies for both species. Part of the heterosis in relation to parental means could be attributed to the inbreeding depression due to selfing that occurred in the OP progenies and the dominance controlling this character. For the basic wood density (BWD) the hybrids performance was the same of the OP progenies. Since there was divergence between the parents, it can be inferred that dominance has no importance for this trait.

The clonal and inter-clonal combining ability of fruit set in a *Hevea brasiliensis* four-parent diallel mating was evaluated using a randomized complete block experimental design with three replicates. Twelve main and reciprocal crosses were hand pollinated and percentage fruit set was recorded. There was significant variation of each of fruit set for the various crosses, general combining ability and reciprocal effect. The significant effect of clones and the interaction between clonal families and groups of crosses suggest the combination of gene action and environment on the incidence of fruit set in *H. brasiliensis* (Omokhame *et al.*, 2007).

Intra-specific hybrids within *Eucalyptus nitens* and *E. globulus* were compared directly with inter-specific *E. nitens* x *E. globulus* using common parents. Genetic parameters were estimated for all cross types and traits. A direct comparison of the general combining ability with the general hybridising ability was made. Heterosis was estimated for intra- and inter-specific hybrids. The intra-specific hybrids in both species exhibit intermediate heritabilities and levels of additive genetic variance. The mean performance of surviving inter-specific F₁ hybrids was generally intermediate for all traits, to varying degrees, between the parental intra-specific crosses, and there is little evidence for significant heterosis. Different provenances of *E. globulus* may exhibit different responses to hybridisation. There is evidence that specific hybrid families are produced that

outperform most of the pure species families for one or other of the traits examined (Volker *et al.*, 2008).

Growth, specific gravity, and wood fiber length of *Acacia mangium*, *A. auriculiformis*, artificial acacia hybrid clones, and combinations, which were planted in a trial forest in Bavi, Vietnam, in July 2001, were examined. Superiority of hybrids over their parents ranged from 36.3% to 41.6% for diameter, from 20.0% to 25.3% for height, from 6.9% to 20.7% for specific gravity, and from 6.1% to 12.8% for wood fiber length. The hybrid possessed heterosis in diameter, height, specific gravity, and wood fiber length regardless of whether the female parent was *A. mangium* or *A. auriculiformis*. Hybrids for which *A. auriculiformis* was the female parent and *A. mangium* was the male parent had a faster growth rate and longer wood fibers than the inverse hybrids. (Kim *et al.*, 2008).

Sophie *et al.* (2009) investigated the growth of two hybrid families viz., *Populus deltoides* 'S9-2' $\times$ *Populus nigra* 'Ghoy' (D $\times$ N family, 180 F₁) and *P. deltoides* 'S9-2' $\times$ *Populus trichocarpa* 'V24' (D $\times$ T family, 182 F₁) for 3-year period at two sites, i.e., in northern Italy and central France. Heterosis was considerably larger for the D $\times$ T family than for the D $\times$ N family during all growing seasons, especially for stem circumference at the end of the third growing season.

Eight newly developed mulberry (*Morus* sp.) hybrids viz., C-2036, S-1908, C-2037, C-2038, C-2039, C-2040, C-2041 and C-2042 were evaluated against S-1635 as check variety for growth, yield, physio-biochemical and bioassay parameters under irrigated conditions and zonal schedule of package of practices for cultivation in the alluvial soils of Gangetic plains of West Bengal. Among the test hybrids, C-2038 was recorded to have higher number of shoots per plant (10.93), total shoot length (1059.95 cm), unit leaf area (284.06 cm²), unit leaf weight (4.87 g), net photosynthetic rate (13.53 μ mol m² s⁻¹), physiological water use efficiency (1.028), carboxylation efficiency (0.0442), chlorophyll content (2.53 mg g⁻¹), total soluble protein (31.44 mg g⁻¹), total

soluble sugar (37.41 mg g^{-1}) and leaf yield (55.23 mt/ha/year) and better economic parameters of cocoon showing its superiority over check and other test hybrids. Heterosis was studied for leaf yield and its attributing characters in 8 hybrids developed. Significant positive heterosis ranging from 5.95 to 159.31% over better parent, 13.92 to 159.31% over mid parent and 1.50 to 27.79% over standard variety (S-1635) was observed among the crosses for leaf yield (Ghosh et al., 2009).

Butternuts can naturally hybridize with Japanese walnuts to produce vigorous F_1 hybrids, known as *Juglans bixbyi*, but commonly called “buarts”. Many F_1 interspecific hybrids of walnut species have hybrid vigor, among walnut hybrids, buarts are unusual in that they are fertile, self-compatibility and can produce a remarkable number of nuts. Besides hybrid vigor and high yielding potential, buarts and the more complex hybrids derived from them have inherited some resistance to the butternut canker fungus from Japanese walnut (*Farlee et al.*, 2010)

Soomro (2010) carried out heterosis studies in *Gossypium hirsutum* and the heterosis was manifested in different crosses for all the characters but the extent of heterosis varied between characters. It may be assumed that heterosis in one or more than one yield components conferred heterosis for seed cotton yield. The cross Reshmi x TH-3/83, Reshmi x Mc-Niar-3150, CIM-109 x Mc-Niar-3150, CIM-109 x TH-3/83 and Mc-Niar-3150 x CIM-109 displayed positive heterosis and heterobeltiosis for seed cotton yield/plant along with one or more than one yield component characters.

Khan et al. (2010) evaluate the potential of 6×6 F_1 and F_2 intra-*Gossypium hirsutum* diallel hybrids relative to heterosis, inbreeding depression, heritability and correlation. Average F_1 hybrids mean values were higher than those of F_2 s, while F_2 s exceeded the parents. Heterosis over better parents was more pronounced in F_1 s as compared to F_2 s. However, in F_1 s and F_2 s, the average positive heterosis was 11.54 and 4.50 % seeds boll; 3.40 and 2.41% seed index; 46.30 and 28.96% seed cotton yield and 6.04 and 1.52 %for cottonseed oil,

respectively, for the above traits. Even after segregation and inbreeding depression, the F_2 s expressed about 50% of F_1 s heterosis. The inbreeding depression in F_2 s was -6.51 to -16.92 with low to high heritability, and significant positive correlation of cottonseed oil with other traits.

The spontaneous inter-specific F_1 hybrids of *Eucalyptus torelliana* x *E. citriodora*, belonging to the same subgenus *Corymbia*, were identified using morpho-genetic markers. The hybrids along with parents were planted in the field to evaluate their comparative performance for growth and biomass production. Hybrid *E. torelliana* x *E. citriodora* showed standing volume of 0.18 m³, 0.53 m³ and 3.03 m³ and yield of 16.17 m³/ha/yr, 33.12 m³/ha/yr and 82.79 m³/ha/yr at three ages, respectively. The results of Parveen *et al.* (2010) revealed that hybrids had sustained hybrid vigour both for growth and standing volume production when compared with its parents even at the age of 23 years.

Heterosis was determined from a 6 X 6 half diallel cross of *Jatropha* genotypes for seed yield per plant and its components trait. Result showed that variation for days to flowering, days to fruit maturity, number of primary branches per plant, seed yield per plant and its component was significant. Both positive and negative heterosis was found for seed yield per plant. High mid parent heterosis (254.13%) and better parent heterosis (202.36%) were found for seed yield per plant in cross P_2 X P_5 and P_1 X P_3 respectively. Small heterosis over mid parent was also found for days of fruit maturity. The parent P_1 , P_2 , P_3 and P_5 were found to be superior for seed yield and its components when used in crosses. Consider earliness and seed yield per plant the hybrid combination P_1 X P_2 , P_1 X P_3 , P_2 X P_5 were promising (Islam *et al.*, 2011).

2.3 PATERNITY ANALYSIS USING RAPD AND ISSR MARKERS

Hybridization between plant species can induce speciation as well as phenotypic novelty and heterosis. Hybrids also can show genome rearrangements and gene expression changes compared with their parents. The speciation is possibly studied through the morphological characteristics or genetic traits and by using genetic markers. Genetic markers are applied to study the genetics of all

kinds of organisms. It can be used in wild and domesticated population of any animal or plant population. Genetic markers have been used to identify the genetic variation, mating systems, evaluating the impact of management, identifying the species, development of plant phylogeny, paternity and so on. There are several genetic markers frequently used in forest genetics according to the needs and objectives of study. For the wider use and application, the genetic marker should possess basic characteristics such as: inexpensive to develop and use, unaffected by environmental variation, polymorphic and highly reproducible. There are several genetic markers that have been developed and used in different chronological period. Among them biochemical markers and molecular markers has been tremendously effective and in application since their origin to date. There are only a small number of different marker loci therefore genetic information obtained from biochemical marker, such as Terpene or Allozyme, may not be sufficient representation of genes throughout the genome (White *et al.*, 2007). Thus, it may lead to erroneous conclusion about their protection and management (Szmidt and Wang, 1991). The limitation of biochemical marker insisted scientists to work for the development of molecular or DNA based genetic marker. Geneticist had developed this marker, so applied first in the beginning of 1980s (White *et al.*, 2007). Soon after the invention, molecular markers were used in plant for genetic analysis (Szmidt and Wang, 1991). The invention of PCR and other advanced technologies made the application of molecular marker easier and popular. With the advancement of technology and knowledge, various molecular markers and analyses have been developed and applied for many purposes.

DNA markers are very useful for confirming hybridity of artificial sexual hybrids or somatic fusion hybrids. Molecular markers are especially useful when hybridity is questioned by morphological reasons or for early screening of large putative hybrid populations (Rajendra, 2009).

Lin *et al.* (1994) used RAPD fingerprints to differentiate species and hybrids of the genera *Populus* and *Salix* as well as to identify individual clones. Fifty-five poplar clones and 81 willow clones were included in this study. Out of

the 17 random DNA primers tested, only 4 primers (Chl-1, and Deca-2, 7 and 10) differentiated 55 poplar clones into species and hybrids. Individual clones were further characterized by their DNA polymorphism obtained with primer Chl-1 or Deca-7. Species and hybrids of tree-type willow clones were differentiated with primers 2114, 2115 and 2116.

Durham and Korban (1994) used RAPDs to identify apple clones with introgressed genes from a wild apple species that was used to transfer apple scab resistance through many cycles of introgressive hybridization.

Significant specific combining ability effects (SCA) for two year growth were detected in a factorial of *Eucalyptus globulus* ssp. *globulus*. This factorial was established across 5 locations in Australia and involved crossing between and within two provenances (King Island and Taranna) in addition to crossing males from both provenances to a single female from south Flinders Island. Attempt was made to predict heterosis using genetic distance between parents calculated using RAPD markers. Sixty-six out of the 99 RAPD bands scored were polymorphic. A UPGMA dendrogram based on Nei's distance resulted in parents within provenance being clustered together in all but one case. The degree to which genetic distance predicted heterosis was statistically significant but accounted for less than 5% of the variation in SCA, and mainly reflected the greater genetic differentiation between parents involved in interprovenance crossing. SCA effects within provenances were unpredictable (Vaillancourt *et al.*, 1995).

Identification of 21 fig accessions (*Ficus carica* L.) representing different varieties was performed using the RAPD (Random Amplified Polymorphism DNA) technique. Out of 85 primers prescreened on four genotypes 12 primers revealed polymorphism and gave reproducible results. The 19 RAPD markers used provided 17 different banding patterns. The RAPD markers showed sufficient polymorphism for genotype discrimination, clonal stability, environmental stability and experimental reproducibility. The genetic variability observed in the fig genotypes was not structured into distinct subgroups probably

because an important gene flow occurred in the natural populations from which cultivars originated (Khadari *et al.*, 1995).

The Mendelian inheritance of RAPD and ISSR markers has been assessed using a progeny from a controlled cross in European beech (*Fagus sylvatica*). Out of a total of 165 amplification products 30 Mendelian markers with dominant mode of gene action were found. A subset of 11 markers has been used to estimate population parameters in a sample of 46 trees from a natural stand in the Northern Apennines (Italy). The same subset of markers was used for assessing paternity of 81 seedlings belonging to 9 open-pollinated sibships of 9 individuals each. Only 2 unambiguous paternity assignments out of 46 potential fathers was reported (Troggio *et al.*, 1996).

Chen and De Filippis (1996) applied the RAPD-PCR technique to analyze the relationship between four accessions of *Eucalyptus microcarys*, and their differences to *E. dalrympleana* and *Grevillea 'Elegance'*. The variability of the technique and in particular the origin and quality of the DNA extracted was analyzed. The results showed that RAPD technology is a quick, precise, sensitive and a relatively inexpensive for genomic analysis of trees.

RAPD variation was examined amongst ramets derived from 10 ortets which were either full-sibs, half-sibs, or unrelated, as well as their parents. Genetic similarity among samples was calculated from 117 RAPD bands. The pedigree of the material was successfully displayed by UPGMA clustering of the genetic similarity matrix, with parents generally clustering with their offspring. In a second experiment, 120 RAPD bands allowed the successful discrimination of selfs from their intra- and interprovenance half-sib outcrosses and again genetic similarity decreased with pedigree distance (Nesbitt *et al.*, 1997).

Random amplified polymorphic DNA (RAPD) markers of genomic DNA and restriction fragment length polymorphism (RFLP) of mitochondrial DNA using a mitochondrial genome specific probe, atp 6 (from maize), were used to identify the parentage of a *Rhizophora* hybrid. The combined RAPD profiles of two species, *Rhizophora apiculata* and *R. mucronata* had 96.5% similarity with

that of the hybrid, indicating that these two species are the probable parents. RFLPs for the mitochondrial genome were readily observed, and the RFLP profile of *R. apiculata* was exactly the same as that of the hybrid, establishing its maternal status for the hybrid (Parani *et al.*, 1997).

Shuangquan *et al.* (1998) used random amplified polymorphic DNA (RAPD) loci for paternity analysis to mark pollen dispersal in *Liriodendron chinense*. Observations on pollinator movements and flower emasculated experiments in natural populations showed that most of pollen grains deposited on stigmas in a flower are foreign but it is not certain whether cross pollination or long distance pollen transfer occurs in this insect-pollinated plant. The results indicated pollen via gene transportation reached at least 100 m away from the pollen source in a selected population. With its rapid and simple characters, RAPD methods can be a suitable technique for marking pollen flow and able to be widely used in various taxa.

Sanchez *et al.* (1998) were able to distinguish 25 poplar clones that included namely five clones of *Populus nigra*, *P. deltoides*, *P. alba* and *P. tremula*, three clones of *P. x canescens* and one clone of Platero (*P. tremula* x *P. alba* "Bolleana") and *P. trichocarpa* by RAPD markers. For hybrids such as *P. canescens* and Platero, the banding pattern was the same as either *P. alba* or *P. tremula*. On the other hand for hybrids such as *P. x euroamericana* (*P. canescens*) 1-214 and *P. deltoides* x *P. alba*(7/32 B) the banding pattern differed from both parents. The mentioned marker showed characteristic bands for every species and in the particular case of *P. alba* three different groups could be distinguished.

Triest *et al.* (1999) reported that molecular markers could represent a reliable tool for the rapid and precise identification system. Genetic variability was estimated by enzyme electrophoresis in 239 Belgian clones from the *Salix alba* - *S. fragilis* complex. This morphological complex suggested a high frequency of hybrids. The standard genetic variability measures showed higher values for the complex than for the separate species. However, the observed mean

heterozygosity was lower in the putative hybrid complex than for each of the species separately.

Parentage analysis was performed on the plum cultivars 'Cacaks Beauty', 'Cacaks Best', 'Cacaks Early' and 'Cacaks Fruitful' using the RAPD-technique with 26 decamer primers. The cross combination 'Stanley' 'Pozegaca' of the progeny 'Cacaks Fruitful' could be confirmed and the data were in agreement with those of the breeders Paunovic *et al.* (1974). For the cultivars 'Cacaks Beauty', 'Cacaks Best', and 'Cacaks Early' the analysis showed a high conformity with the mother 'Wangenheim' and the supposed father 'Stanley', whereas the questionable father 'Pozegaca' indicated a low similarity (Heinkel *et al.*, 2000).

Hardig *et al.* (2000) analysed molecular and morphological data from individuals in a hybrid zone between 2 species of willows (*Salix sericea* and *S. eriocephala*) in central New York State (USA), using randomly amplified polymorphic DNA (RAPD), chloroplast DNA (cpDNA), and ribosomal DNA (rDNA) markers as well as vegetative morphology and foliar chemistry data to identify individuals in terms of hybrid genealogy and to infer the direction and extent of backcrossing and introgression within the hybrid zone. Patterns of inheritance and expression within the hybrid zone suggest that morphological characters are often not expressed in a simple additive fashion.

Triest (2001) investigated morphology and molecular variation using RAPDs among 23 staminate and 14 pistillate individual of *S. fragilis* and *S. alba* from Belgium. Species-diagnostic RAPD markers were identified in progenies obtained from controlled crosses between *S. alba* and *S. fragilis* parents. A thorough screening of full-sib progenies of inter-specific controlled crosses was made to select homologous amplification products. The selected amplified products proved to be useful in a principal coordinate analysis for the estimation of variability of hybrid progenies. The RAPD analysis also revealed that the *S. fragilis* genotypes mainly consisted of staminate individuals whereas most of the pistillate trees belonged to the *S. alba* genotype cluster.

Aravanopoulos (2001) investigated genetic linkage in eleven isoenzymic loci in *S. eriocephala* progenies obtained by 2 x 2 balanced factorial mating design. Joint segregation was tested in 49 two-locus combinations over different families and in a total of 107 two-locus tests. When results were compared with those reported in other willow species, it was suggested that linkage conservation exists above the species level.

The potential for hybridization among three species of *Eucalyptus* L'Hér in the Series *Macrocarpae*, *E. macrocarpa* Hook (Mottlecuh), *E. pyriformis* Turcz. (pear-fruited mallee), and *E. youngiana* F. Muell. large-fruited mallee), was investigated using molecular data generated by randomly amplified polymorphic DNA (RAPD)–polymerase chain reaction (PCR) analysis. Samples of DNA from seedlings derived from controlled pollinations, and from different individuals from each species, were amplified with six different 10-mer RAPD primers. Two clusters were identified on the unweighted pair-group method arithmetic average dendrogram. From the 30 progeny investigated, 8 were assessed from the molecular data to be hybrids from controlled pollinations. The remaining two seedlings appeared to be derived from self-pollination. The parentage of two mature trees, thought to be natural hybrids involving the three species, was also investigated. One was confirmed as a cross between *E. youngiana* and *E. pyriformis*, but the second was less certain because of its low genetic similarity to all other individuals, and may be a hybrid involving species not included in this study (Neaylon *et al.*, 2001).

The genotype characteristic of cultivars was investigated, along with varieties of both of the jute species, *Corchorus olitorius* and *Corchorus capsularis*, in the germplasm collection at the Bangladesh Jute Research Institute (BJRI). DNA fingerprinting was generated for 9 different varieties and 12 accessions of jute cultivars by using random amplified polymorphic DNA (RAPD). A total of 29 arbitrary oligonucleotide primers were screened. Seven primers gave polymorphism within the varieties, and 6 primers detected polymorphism within the accessions that were tested. A dendrogram was engendered from these data, and this gave a distinct clustering of the cultivated

species of jute. Therefore, they generated RAPD markers, which were species-specific. These primers can distinguish between *C. olitorius* and *C. capsularis* (Hossain *et al.*, 2002).

Vijayan *et al.* (2003) studied species differentiation among the genotypes of the genus *Morus* attempts were made to elucidate the genetic relationships among 18 mulberry genotypes collected from India and Japan using 15 Inter Simple Sequence Repeat (ISSR) and 15 Random Amplified Polymorphic DNA (RAPD) primers. The ISSR primers generated 81.13% polymorphism while the RAPDs generated 71.78% polymorphism.

Gunter *et al.* (2003) discovered genetic markers linked to female sex determination locus in *Salix viminalis* L. through bulked segregant analysis of three full-sib families using approximately 1,000 arbitrary primers. Two RAPD markers that were present in the common female parent as well as in predominantly female progeny of these families were subsequently sequenced and converted to sequence characterized amplified region (SCAR) markers. The two SCAR markers are correlated with gender in the three full-sib families and are present in 96.4 per cent of the female progeny and 2.2 per cent of the males, providing evidence of linkage to a putative female-specific locus associated with gender determination in *S. viminalis*.

The fingerprints of 10 species including 27 accessions in genus *Corchorus* were investigated with the technique of RAPD. Twenty-five primers were screened from 119 random primers, and a total of 329 DNA fragments were amplified ranging from 0.3-3.0 kb, 253 (87.78%), which were polymorphic. The results showed that there were abundant genetic diversities among 15 wild species and 12 cultivated species in *Corchorus* with genetic similarity coefficients ranging from 0.49-0.98. The accessions could be clustered into three groups at cultivated species, and their close wild species were obviously different from wild species genetically (Qi *et al.*, 2003).

To identify the parentage of putative *Ornithogalum* hybrids using DNA fingerprinting techniques seeds were harvested from *Ornithogalum thyrsoides*

'Chesapeake Starlight', and 5 seedlings (SD1-SD5) were selected and evaluated for growth, flowering, and DNA fingerprinting. *Ornithogalum conicum*, Bokbaai type of *O. thyrsoides* 7050A, 7050B and a seedling from a cross between *O. conicum* x 7050C which were considered as a possible paternal parent of the seedlings were also analyzed. A total 169 random amplified polymorphic DNA (RAPD) bands were produced, of which 90 (53 %) were polymorphic. Based on the p-distance value, *O. conicum* was excluded as a potential source of pollen and Bokbaai type of *Ornithogalum* 7050 was considered as pollen source. However, the possibility that all seedlings could result from self-pollinated progenies of 'Chesapeake Starlight' was not excluded. Five randomly selected primer pairs for amplified fragment length polymorphism (AFLP) analysis generated total 298 fragments, of which 191 (63.4 %) were polymorphic bands. The presence of marker 109 in all four seedlings, SD1, SD 2, SD 4, and SD 5 and in *O. conicum* x 7050C concludes that marker 109 was inherited from 7050C. According to the p-distance and nonmaternal band analysis performed with RAPD and AFLP, it was concluded that the seedlings were not the result of self-pollination, and *O. thyrsoides* 7050C could be the paternal parent. *Ornithogalum thyrsoides*, instead of *O. conicum*, should be used in the future breeding efforts to breed a paper white flower with cup-shaped florets (Joung and Roh, 2004).

Eleven RAPD primers that had shown polymorphism were used for the study. Out of 65 fragments, 43 were found to be polymorphic, which indicated the presence of genetic diversity among F₂ plants. Out of 5.4 fragments per primer amplified, 3.58 fragments per primer were polymorphic. The cluster diagram and dice similarity coefficients indicated 60 to 82 per cent similarity between selected F₂ plants and the parents. The *Gossypium anomalum* formed separate cluster. The molecular markers indicated an average of 76.4 per cent of specific bands from DDhc-11 and only 42.3 per cent of specific bands from *G. anomalum*. Based on fibre strength values, 22 plants were categorized into nine groups representing the fibre strength from 17 to 25 g per tex. OPZ-19 amplified a 300 bp amplicon, which was present only in groups representing 23, 24 and 25 g per tex along with P₂ and F₁, but was absent in other groups indicating its association with fibre strength (Mohan, 2005).

Fossati *et al.* (2005) fingerprinted a collection of 66 poplar commercial clones widely cultivated in Italy, China, countries of southern Europe and belonging to various poplar species and hybrids using both amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) techniques. High level of variation was detected with both molecular techniques. A total of 201 AFLP bands were amplified out of which 96 per cent turned out to be polymorphic. Up to 15 SSR alleles were identified at a single locus with a mean of 9.3 alleles per locus in the case of *Populus x canadensis*.

Three controlled crosses were carried out involving *Mentha arvensis* and *Mentha spicata* [*M. spicata* CIMAP/C30 X *M. spicata* CIMAP/C33 (cv. Neera); *M. arvensis* CIMAP/C18 X CIMAP/C17 (cv. Kalka); and *M. arvensis* CIMAP/C17 X *M. spicata* CIMAP/C33]. The parents were subjected to random amplified polymorphic DNA (RAPD) analysis with 80 primers, and polymorphic primers were tested for detecting coinherited RAPD profiles among the progeny of these crosses. Of 50 seedlings tested from each intraspecific cross, all demonstrated dominant profiles with the selected RAPD primers except the detected hybrid from respective crosses. Coinherited markers could be detected with the primers OPJ 01, MAP 06, OPT 08, and OPO 20 for *M. arvensis*; OPJ 05, OPJ 14, OPO 19, and OPT 09 for *M. spicata*; and OPJ 07, OPJ 10, OPJ 11, OPJ 14, and OPO 02 for the cross *M. arvensis* X *M. spicata*. In our amplified fragment length polymorphism (AFLP) analysis, 40 coinherited marker fragments were identified for the cross involving *M. arvensis*, 32 for the cross involving *M. spicata*, and 41 for the interspecific cross between *M. arvensis* and *M. spicata*. In all crosses, similarity values between the parents were less than those between the parents and the hybrids. Although RAPD markers are generally considered dominant, it is possible to identify a few codominant markers that behave like restriction fragment length polymorphism (RFLP) markers. This molecular marker system may be helpful in rapidly screening out hybrids in crops where cross-pollination is a problem (Shasany *et al.*, 2005).

Roy *et al.* (2006) studied genetic diversity analysis of 20 exotic germplasm lines and 20 commercial varieties of the two cultivated species (*Corchorus*

olitorius and *C. capsularis*) and two wild relatives of jute (*C. aestuans* and *C. trilocularis*) was carried out using sequence tagged microsatellite site (STMS), inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. The first set of six STMS markers developed from the genomic sequence of *C. olitorius* was not fully transferable to the related species *C. capsularis*. The level of intraspecific polymorphism revealed by these markers was very low. The four ISSR and 22 RAPD primers employed in the study revealed 98.44% and 100% polymorphism, respectively, across all the species, while the level of polymorphism was significantly low within a species.

Roh *et al.* (2006) investigated the origin of seven *Ardisia crenata* Sims seedlings with non-variegated foliages (VSm) from a progeny of a mother plant with variegated foliage and red berries (VM), morphological and genetic characteristics of these seedlings were compared with mother plants of *A. crenata* with VM, plants with non-variegated leaves and white berries (WM), and plants with non-variegated leaves and red berries (RM). Genetic data include randomly amplified polymorphic DNA (RAPD) markers and sequence analysis of an unidentified locus that was obtained from seven VSm seedlings out of 261 seedlings of VM with variegated foliage (VS) seedlings, WM, and seedlings from WM (WS). RAPD analysis indicates that VM, WM, and progeny populations VSm and WS are more closely related to each other than to RM and RM progeny (RS). Based on the RAPD and the sequence analysis for the VSm and WM specific band, it is concluded that these seven VSm seedlings were resulted from cross-pollination between VM and WM. Hybrid origin of VSm seedlings between VM as a maternal source and WM as a paternal source is verified by polymerase chain reaction (PCR) utilizing sequence-characterized amplified region (SCAR) markers (forward primer, ARD-1-F and two reverse primers, ARD-2-R and ARD-3-R). These SCAR markers were successful to identify VM progenies with non-variegated leaves involving WM as a paternal source.

Sultana *et al.* (2006) constructed the first and very preliminary linkage map of the jute genome with eight ISSR (Inter Simple Sequence Repeat) markers and their different combinations using an F₂ population developed from a cross

between jute cultivars O-9897 and Acc. 1805 which differed in cold tolerance. The polymorphisms between the parents were detected and the progenies were scored. The screening of parents (O-9897 and Acc. 1805) with ISSR primers revealed, 3 single primers and 2 primer pairs were polymorphic between the parents, which collectively produced 10 clear and reproducible markers for mapping. Thus, a total of 10 segregating markers were used to construct the Jute linkage map. The size of the markers varied from approximately 500 bp to 1,400 bp.

Gao *et al.* (2006) used the Inter-Simple Sequence Repeat (ISSR) for genetic fingerprinting and identification of 28 important *Populus L.* (poplar) cultivars (varieties/clones), and determination of the genetic relationships among these cultivars. These 28 cultivars belonged to sections *Aigeiros*, *Tacahamaca*, *Leuce*, *Turanga*, and hybrids between sections *Aigeiros* and *Tacahamaca*. Out of 27 ISSR primers tested, eight primers generated clear multiplex profiles. The best three primers produced 154 easily detectable fragments, 129 (84%) of which were polymorphic among the cultivars. Each of these 3 primers produced fingerprint profiles unique to each of the accessions studied, and thus could be solely used for their identification. Twenty-five markers, unique to 10 of the cultivars studied, were detected. The results showed a clear-cut separation of cultivars among different sections of poplar, and were in agreement with the genealogy of the sampled cultivars.

Zhuang and Adams (2007) determined the allelic variation in gene expression in *Populus trichocarpa* X *P. deltoides* F₁ hybrids. The results suggest differential regulation of the two parental alleles in the hybrids. Thirteen of 19 genes showed allelic expression ratios in the hybrid that were significantly different from the ratios of the parental species. The *P. deltoides* allele of one gene was silenced in the hybrid. Modes of gene regulation were inferred from the hybrid–parent comparisons. The results from this study indicate that hybridization between plant species can have extensive effects on allelic expression patterns, some of which might lead to phenotypic changes.

Haque *et al.* (2007) observed the genetic diversity of 18 jute genotypes of the two cultivated species *Corchorus capsularis* L. and *C. olitorius* L. which include released varieties and collected races, referred to as accessions was evaluated. DNA profiling was generated using sequence independent RAPD markers. A total of 140 scorable loci were observed and a dendrogram was constructed with these markers. The relationship that is portrayed by this clustering also agreed with the available pedigree information on jute.

Amalendu and Kamble (2009) obtained polymorphism of genomic DNAs of nine parents of *Morus* and their seven hybrids from two wild and four cultivated species. Twelve arbitrary primers of Random Amplified Polymorphic DNA (RAPD) unraveled abundant polymorphism. Of the F₁ hybrids, the banding pattern indicates similar like their respective parents. However, a few hybrids showed unique bands, which are different from respective parents. The RAPD technique demonstrated that hereditary variability occurred in between wild and cultivated *Morus* species at inter and intra-specific levels.

The mating system and seed variation of *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) were studied using allozymes and random amplified polymorphic DNA (RAPD) markers, respectively. Multi-locus outcrossing rate estimations indicated that the hybrid was predominantly outcrossed (mean±s.e. $t_m = 0.86 \pm 0.01$). Seed variation was investigated using 35 polymorphic RAPD fragments. An analysis of molecular variance (AMOVA) revealed the highest genetic variation among seeds within a pod (66%–70%), followed by among pods within inflorescence (29%–37%), and the least variation among inflorescences within tree (<1%). In addition, two to four RAPD profiles could be detected among seeds within pod (Chin-Hong Ng *et al.*, 2009).

Thawaro and Te-chato (2009) reported the use of randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) primers to assess the hybrid verification and somaclonal variation of the cross 366 (D) × 72 (P) of *Elaeis quineensis* Jacq (Oil palm). After isolation of genomic DNA from mature zygotic embryo (MZE) RAPD and SSR analysis was carried out using seven

RAPD and eight SSR primers separately. RAPD and SSR analysis revealed that all the primers were able to amplify and provided polymorphic patterns of parents. But only one RAPD and SSR primer (OPT06 and EgCIR1772) provided a clear banding pattern which was used to distinguish hybrid between 366 (D) x 72 (P) of *Elaeis quineensis*. All hybrids showed the DNA patterns between the two parents.

Identifying fourteen mango hybrids along with their parents was designed using a DNA typing procedure employing RAPD markers. Ten of the 80 operon primers screened produced 139 usable bands. Cluster analysis based on Jaccard's coefficient of similarity using UPGMA revealed low to medium diversity among hybrids. Hybrids, which had "Neelum," "Alphonso," and "Mulgoa" as one of their parents, formed into distinct cluster. Hybrid "Neelgao" with "Mulgoa" as its male parent stood distinctly in the dendrogram comprising hybrids along with their parents (Vasanthaiah, 2009).

Dillon *et al.* (2009) used RAPD fingerprints to confirm the inter-specific hybrids of *Jatropha curcas* and *J. intergerrima*. RAPD analysis of *Jatropha curcas* and *J. intergerrima* that were used as parents and their 23 inter-specific hybrids was carried out using 61 random decamer primers. Out of 61 primers, the amplification was achieved in 51 primers producing 384 bands. The similarity value between parents (41 per cent) was less than those between parents and hybrids.

Ogunkanmi *et al.* (2010) studied, inter and intra genetic diversity analysis of 40 genotypes from *Corchorus olitorius* and 40 genotypes from *C. incisifolus* was carried out using sixteen RAPD markers. The molecular study of the two species classified *C. olitorius* into two main clusters with five genotypes unresolved while there are three clusters with all the genotypes clearly separated in *C. incisifolus*. The results of both inter and intra genetic diversities revealed a higher level of allelic diversity in *C. incisifolus* than in *C. olitorius*.

Genetic diversity and genetic distance of walnut samples were studied based on RAPD, which hybridized from Yunxin variety, Sichuan variety and

their hybrid (Yunxin variety (Female) x Sichuan variety). Then combine the dominant ratio of walnut quality to discuss how to predict heterosis by genetic distance based on RAPD. The results showed that different cross combination has different genetic similarity index and genetic distance. Genetic distance of parents has a negative relationship with thickness but has a positive relationship with kernel rate. Related coefficient between genetic distance and heterosis of thickness was significant ($P < 0.05$). While the related coefficient between genetic distance and heterosis of diameter and weight was very small. Analysis of variance showed that thickness variation was small between progeny and maternal parent, their difference was not obvious, which showed that character of thickness has a stable genetic ability. In a word, it is valuable to estimate the heterosis of main characters by RAPD genetic distance (Qianwen *et al.*, 2010).

The OPI16 RAPD profile as those generated by OPG10 revealed polymorphisms among the amplification products of different homo and heterozygote genotypes. About 73 percent of fragments showed absence/presence polymorphism, which could be particularly useful for discriminating most of the hybrids along with their parents. The clustering of hybrids and their parental lines into 3 clusters of genotypes: *CMS* lines (*except Drofa CMS*), *Rf* and F_1 genotypes. The closest relationships within the group was ascertained for paternal lines than maternal and hybrids. Such findings could help in distinguishing the hybrid pedigree. Considering these reasons it was compared the data of yield components for commercial and experimental F_1 sunflower hybrids to establish the relationship between these as indices of hybrid performance and genetic distances among parental genotypes of hybrid combinations (Maria *et al.*, 2010).

The hybrids obtained from *Salix* hybridization programme conducted Choudhary (2011) were tested for their paternity using four RAPD markers. RAPD banding pattern obtained after the amplification of genomic DNA of both the parents and their hybrid revealed that all the nine hybrids were true to type. The RAPD fragments obtained after PCR amplification was subjected to analysis for obtaining their similarity coefficient with respect to their parent. Results disclosed that six out of nine hybrids were highly similar to their male parents,

whereas, three hybrids showed more similarity towards female parents. Hybrid [(*S. tetrasperma* (LG) x *S. tetrasperma* (TWE))] recorded 100 per cent and 70 per cent similarity with male and female parent respectively suggesting dominant control of male parent. Similarly hybrids [(*S. tetrasperma* (LG) x *S. tetrasperma* (TFB) and *S. tetrasperma* (LN) x *S. tetrasperma* (TWE))] also recorded 100 per cent similarity with male parent. Maximum similarity (96 per cent) with female parent was recorded in *S. matsudana* (PN-227) x *S. matsudana* (PN-722) suggesting dominating control of female parent. Two other hybrids and [*S. alba* (SI-64-017) x *S. matsudana* (PN-722)] and [*S. matsudana* (PN-227) x NZ-1002] also recorded high similarity (87 per cent and 71 per cent) with their female parent.

In order to obtain F₁ hybrids with ornamental potential, four species of *Passiflora* (*P. alata*, *P. gardneri*, *P. gibertii*, and *P. watsoniana*) from the active germplasm Bank at UESC were hybridized. Flower buds, in pre-anthesis, of the genitors were previously protected, and the female buds were emasculated. To confirm hybridization, the genomic DNA of the genitor species and the supposed hybrids was extracted and RAPD primers were used to obtain molecular markers and select passion flower interspecific hybrids. Eight primers were used to confirm hybrids derived from *P. gardneri* with *P. alata*, *P. watsoniana* with *P. alata*, *P. watsoniana* with *P. gardneri*, and *P. gardneri* with *P. gibertii*; 75, 50, 45, and 46% of the informative bands, respectively, confirmed the hybrid nature of these plants (Conceicao *et al.*, 2011).

Sherry *et al.* (2011) evaluated the genetic relationship between *Prosopis* species from the section Algarobia, contained most species of economic importance, though *P. tamarugo* from section Strombocarpa was also included for comparison. In total, 12 *Prosopis* species and a putative *P. pallida* x *P. chilensis* hybrid were assessed for their genetic relationships based on RAPD markers and microsatellite transferability. The results showed that *P. pallida* and *P. juliflora* were not closely related despite some morphological similarity. Evidence also agreed with previous studies which suggested that the grouping of series in section Algarobia was artificial.

Samal *et al.* (2012) Studied the level and pattern of diversity in 65 mango (*Mangifera indica* L.) genotypes of India including 20 commercial cultivars, 18 hybrids, 25 local genotypes and two exotic cultivars based on qualitative and quantitative fruit characters as well as RAPD and ISSR profiles. Fifteen RAPD primers yielded 27 monomorphic and 129 polymorphic bands with percent polymorphism averaging 82.7%. Of a total 70 ISSR bands generated from eight ISSR primers, 60 bands (85.71%) were found to be polymorphic. Cumulative band data from these two methods precisely arranged accessions into eight clusters which correspond well with their pedigree relationship. UPGMA dendrograms had drawn using RAPD, ISSR and cumulative data showed highly similar grouping of genotypes on the basis of their parental origin.

2.4 FODDER AND FIBER PARAMETERS

Trees provide leaf fodder for livestock, the leaf fodder from trees and shrubs is as nutritious as that of leguminous fodder crops. Good quality tree fodder is important for higher productivity of livestock. Studies on chemical composition, nutritive value and palatability of some fodder tree species in India were carried out by Kehar *et al.* (1962). The work on this aspect in trees and crops is reviewed as under:

Lodhi *et al.* (1990) selected ten diverse lines of cow pea (HFC28, HFC136, HFC322, HFC354, HFC388, HFC617, HFC635, HFC637, HFC638 and HFC639) and crossed with 4 diverse testers (HFC42-1, FOS1, CO1 and Cowpea No. 10). Observations were recorded on a number of quantitative characters and heterosis was calculated over both the better parent and the better standard using line X tester analysis. Heterosis over the better standard for green fodder yield/plant ranged from -39.95 to 68.53% in environment I and from -43.82 to 181.48% in environment II; and for dry matter yield/plant from -51.1 to 132.98% in environment I and from -51.37 to 79.70% in environment II. High heterosis was noted for protein content. HFC388 X No. 10, HFC637 X No. 10, HFC28 X FOS1 in environment I and HFC354 X No. 10, HFC322 X No. 10 and HFC28 X FOS1 in environment II were considered the most promising for improving fodder yield and quality.

Aravindhan and Das (1996) in Tamil Nadu (India) evaluated 42 crosses of cowpea, derived from 14 lines and 3 testers, in terms of green fodder and seed yields, and heterosis and combining ability. Leaf area index, specific leaf weight, green fodder production, dry matter yield, leaf stem ratio, crude protein content, seed yield and total dry matter production were assessed. The hybrid UPC 9201 x CO 5 showed the highest heterotic expression for green fodder yield compared with the standard parent CO 5. The hybrid CS 55 x CO 4 showed the maximum heterotic effect for grain yield.

Castillo *et al.* (1998) reported that F₁ hybrid between *Leucaena leucocephala* K636 × *L. pallida* K748 (KX2 hybrid) in particular has demonstrated outstanding characteristics as forage plant combined with high palatability.

Msangi *et al.* (2004) in the evaluation of *Leucaena* species and its hybrid for fodder production reported significant differences among the species and its hybrid for fodder production. Crude protein content of *Leucaena* foliage was generally high, while the hybrid had the higher value for crude protein.

Lohan *et al.* (1983), while investigating on *Quercus incana* leaves found 11.64 per cent of crude protein and 2.50 per cent of condensed tannins in the dry matter. Eighty species along with variation in their nutritive values, like crude protein, digestible crude protein and crude fibre were reported by Gulati *et al.*, (1984). These species are not only easily digestible but also contain crude protein and digestible nutrients in good proportion. These species may be priority in selection of species in and around their natural habitat.

According to Negi (1986) chemical composition of thirty species of fodder tree leaves containing the average value of crude protein and crude fibre, in the leaves (in per cent on dry matter basis) ranged from 9.13-22.08 and 12.43-32.62 per cent, respectively. Seventeen cultivars of *Leucaena leucocephala* and *L. diversifolia* were evaluated and high degree of similarity in chemical characteristics was observed. *L. leucocephala* has higher dry matter of 20 t/ha/yr per annum and protein of more than 5500 kg/ha/ yr (Lahane *et al.*, 1987).

Sankhyan *et al.*, (2009), while evaluating *Grewia laevigata* from Shiwaliks found 100 leaf fresh weight, 100 leaf dry weight, crude protein, crude fibre, ether extract, ash content and nitrogen free extract ranging from 73.34-83.40 g, 32.82-40.13 g, 16.08-17.43 per cent, 13.17-14.47 per cent, 2.796-3.124 per cent, 8.226-10.04 per cent and 54.95-58.79 per cent respectively.

The percentage of proximate fodder analysis of 35 families *viz.*, crude protein, crude fibre ash content, ether extract, and nitrogen free extract ranged from 18.01-22.03 per cent, 18.33-21.75 per cent, 10.32-13.26 per cent, 4.30-5.74 per cent and 39.42-45.63 per cent respectively. For these characteristics, it is concluded that the families SI-8 (Dotliji), SI-13 (Sarpadol), HA-1 (Bharari), HA-2 (Patta Balakhar) and SI-11 (Adgu) were the best (Bhat, 2010).

Grewia optiva a natural cellulosic fiber, has distinctive characteristics and is widely used in India, for making low cost items such as ropes, bags, socks, boots, mats. etc. The bio-degradability and compatibility of *G. optiva* fiber in main sewage system enables it to use in the manufacture of disposable items. Cellulose in general is widely applied in many fields because of its renewability, biocompatibility and natural abundance. With the goal of using this fiber for various applications such as sanitary napkins, apparel, home furnishing, industrial uses, and as a component of soil for agriculture (Lie *et al.*, 2007; Zhang *et al.*, 2007).

The properties of compression molded *Grewia optiva* fiber reinforced Urea-Formaldehyde (UF) matrix based polymer composites, revealed that the mechanical properties such as: tensile strength, compressive strength and wear resistance of urea-formaldehyde resin increases to a significant extent and analysis of results shows that particle reinforcement is more effective as compared to short and long fiber reinforcement (Singha and Thakur, 2009).

Chapter-3

MATERIALS AND METHODS

The present investigation entitled “Estimation of gene action, combining abilities and heterosis in *Grewia optiva* Drummond” was carried out in the Department of Tree Improvement and Genetic Resources, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during 2010-2012. The details relating to material used, experimental site and methodology adopted for study are described under following headings:

- 3.1 EXPERIMENTAL SITE
- 3.2 EXPERIMENTAL MATERIAL
- 3.3 EXPERIMENTAL PROCEDURE
- 3.4 STATISTICAL ANALYSIS

3.1. EXPERIMENTAL SITE

3.1.1. Location

Nauni : Solan (H.P.)
Altitude : 1250 m msl
Latitude : 30° 51' N
Longitude : 76° 11' E.

(Source) : Survey of India, Toposheet No. 55F/1)

The region experiences subtropical climate with moderately hot summers and cold winters. Maximum temperature often exceeds 35°C in May-June and minimum temperature drops to as low as 2°C in January. Frost occurrence in winter is quiet common. Mean annual rainfall of the site is 1000-1300 mm/yr, with major during monsoon rainfall. Winter rains due to western disturbances are not uncommon.

Meteorological data for the year 2011-2012 is given as Appendix I. A seedling seed orchard of *Grewia optiva* Drummond was established in July 2000

(Plate 1) which consists of 60 families each under three replications at spacing of 2m x 2m in Department of Tree Improvement and Genetic Resources. The seed of different genotypes have been collected from various districts of Himachal Pradesh which include Sirmour, Solan, Chamba, Bilaspur, Mandi, Hamirpur, Kangra and Shimla. These half-sib families have been planted under Randomised Block Design. The hybridization experiment were conducted in the field whereas paternity analysis using molecular markers (Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR)) were carried out in the forest genetics laboratory.

3.2. EXPERIMENTAL MATERIAL

The plant material (flowering branches) of selected 6 females and 4 male genotypes were used for control crossing (hybridization) using Line X Tester (6X4 factorial) with 10 parents. All the female were crossed to each of male by hand pollination.

No of Lines (Females)	=	6
No of testers (Males)	=	4
Total no of crosses	=	24
Design	=	(6 X 4 factorial) with 10 parents.

Table 1. Source of parent material

Female Parents			
S. No.	District	Family	Code
1	Solan	Gaura	SO-1
2	Solan	Nauni	SO-2
3	Solan	Deog	SO-4
4	Solan	Kailar	SO-8
5	Chamba	Shahu	CH-2
6	Sirmour	Madhobag	SI-15
Male Parents			
1	Solan	Dharja	SO-3
2	Sirmour	Kalagat	SI-6
3	Bilaspur	Khutira	BI-4
4	Hamirpur	Hamirpur Kunal	HA-4



PLATE 1: View of established seed orchard of *Grewia optiva*.

3.3. EXPERIMENTAL PROCEDURE

3.3.1. Hybridization technique (control crosses)

The flowers were emasculated very carefully so as to avoid injury to the stigma 12 hours before opening. The emasculated buds were pollinated next day at the time of anthesis with the pollen of other plants. Pollen used in controlled hybridization was tested for *in-vitro* pollen viability. Every controlled cross involves single pollen and no pollen mixture was attempted. After pollination the flowers were bagged and tagged (Plate 2 and 3).

Seeds of controlled crosses were harvested when mature and stored at room temperature till sowing. Observations were recorded on following parameters.

3.3.2.1. Per cent successful crosses

Observations were recorded on per cent successful crosses as suggested by Jan and Pfeffer (1999).

$$\text{Per cent successful crosses (\%)} = \frac{\text{Successful crosses}}{\text{Total crosses}} \times 100$$

3.3.2.2. Average seed set per flower

The potential number of seeds produced per cross was counted for all the crosses. Fruits were collected when turned black in colour, depulped, dried, packed, labeled and stored in the laboratory of Department of Tree Improvement and Genetic Resources and for each cross total numbers of seeds were counted.

3.3.2.3. Seed size (mm)

Seeds of controlled crosses were harvested, after cleaning and drying for 48 hours in shady place. The length and width of the seeds was measured by using digital Vernier caliper.

3.3.3 Nursery growth characters

Sowing of seed was done on 18th April, 2012. The seeds were pre-treated with cooling boiled water for 24 hours. Seeds were sown in polybags containing potting mixture of sand, clay and FYM (2:1:1). The sown seeds were watered regularly till seedlings got good vigor. Weeding was done regularly to protect the juvenile seedlings. Experimental material obtained from the crosses (F₁ hybrids) was evaluated for various nursery characters, to determine the gene action, combining abilities and heterosis among *Grewia* genotypes observations were recorded on the following parameters:

3.3.3.1. Germination percentage

Germination count was made within 10 days after sowing. Per cent germination was calculated as the number of seeds that germinated out of number of seeds sown and expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{Germinated seeds}}{\text{Total no. of seed sown}} \times 100$$

3.3.3.2. Survival per cent

Survival percentage was calculated as the number of seedlings that survived after it reached 4 leaves stage out of number of germinated seedlings and expressed in percentage.

$$\text{Survival per cent (\%)} = \frac{\text{Survived seedling}}{\text{Total no. seeds germinated}} \times 100$$

3.3.3.3. Plant height (cm)

The height of the shoots was recorded from the ground level to the apex of the leading shoot by using measuring scale.

3.3.3.4. Collar diameter (mm)

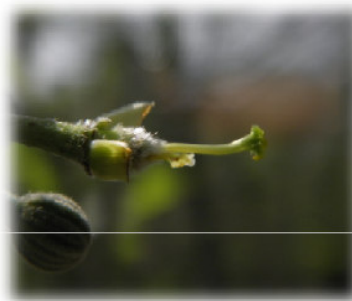
Collar diameter of the plant in the nursery was measured above the ground level at collar region with the help of digital calliper.



Pollen isolation and storage



Emasculation of flower



Completely emasculated flower



Control crossing in *Grewia optiva*



Flower bagged after control crossing

PLATE 2: Stages of control crossing in *Grewia optiva*



PLATE 3: Controlled crosses in the *G. optiva* genotypes

3.3.3.5. Number of leaves/plant

The average number of leaves per plant was recorded on the basis of twelve randomly selected plants of each progeny.

3.3.3.6. Internodel length (cm)

Measured as distance between two nodes, at mid of the plant height.

3.3.3.7. Leaf area (cm₂)

The full grown leaves were collected at randomly from the seedling and leaf area was measured with the help of leaf area meter.

3.3.4. Fodder quality parameters

Composite sample of leaves was taken from F₁ during early November. The sample was labeled properly for each crosses and replication. These samples were washed and treated with 0.1N HCl followed by washing with distilled water. These samples were dried till they attained constant weight and all moisture was evaporated. The dried samples were grinded and stored with proper labels and analysis for following nutritional parameters was done.

3.3.4.1. Ether extract (%)

Two grams of sample was extracted with petroleum ether (B.P.60-80⁰ C) in a Soxhlet Apparatus. Solvent got evaporated to constant dryness and the weight of the abstract was expressed as percentage of the weight of the sample taken.

$$\text{Ether extact (\%)} = \frac{\text{Weight of fat}}{\text{Original weight of sample}} \times 100$$

3.3.4.2. Crude fiber (%)

Crude fiber content was estimated by acid alkali digestion (ashing) as described by AOAC (1995) and Sankaram (1966).

2 g ether free samples was treated with 200 ml of 1.25 % H₂SO₄ for 30 minutes and then filtered through muslin cloth. Sample was washed with water

for no acidity and alkali, say 200 ml of 1.25 per cent NaOH was added to it and same process was repeated as for acid treatment. The sample was dried overnight and muffled at 500⁰C for 3-4 hr, then cooled and weight was recorded. The loss in weight before and after ashing was the weight of crude fiber.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of crude fibre}}{\text{Weight of sample}} \times 100$$

3.3.4.3. Crude protein (%)

Crude protein was estimated by Microkjeldhal method (Sankaram, 1966). For crude protein estimation, nitrogen content of leaves was estimated and multiplying it by the standard factor of 6.25.

Total nitrogen was estimated by taking 1g powdered sample to which 25 ml concentrated H₂SO₄ was added and then treating it with K₂SO₄ and CuSO₄ (20:1) to get the contents digested for 2-2½ hrs till blue colour appeared. The flask contents were cooled after which transferred to 100 ml volumetric flask and distilled water was added. 10 ml of digested material was added with 20 ml of 40% NaOH to make it alkaline and 2-3 drops of methyl red indicator were added in 20 ml of N/100 H₂SO₄ and back titration was done with N/100 NaOH to get pink to yellow colour. The volume of alkali used in titration to calculate the volume of N/100 H₂SO₄ used for NH₄ absorption was recorded and following calculation was done.

$$1 \text{ ml N/100 H}_2\text{SO}_4 = 0.00014 \text{ g N}$$

$$\text{N (\%)} = \frac{V \times 0.00014 \times D}{W \times A} \times 100$$

Where

- | | | |
|---|---|---|
| V | = | Volume of N/100 H ₂ SO ₄ taken or volume of N/100 NaOH used for titration |
| D | = | Dilution factor (volume medium volumetric flask) |
| W | = | Weight of proceed sample taken |
| A | = | Digested material (Aliquot taken) |

$$\text{Crude protein (\%)} = \text{N (\%)} \times 6.25.$$

3.3.4.4. Total ash (%)

Ash content was analyzed by taking some grams of oven dried sample, which was ignited till smoke ended up and then was taken to the muffle furnace at 600⁰C for 2 hrs. The sample was cooled and weighed, as outlined by AOAC (1995) and Sankaram (1966).

$$\text{Total ash (\%)} = \frac{\text{Weight of the ash}}{\text{Original sample weight}} \times 100$$

3.3.4.5. Nitrogen free extract (%)

Nitrogen free extract was determined by subtracting the sum of crude protein, crude fiber, ether extract and ash content from 100 as outlined by AOAC (1995) and Sankaram (1966).

$$\text{NFE (\%)} = 100 - (\text{CP\%} + \text{CF\%} + \text{EE\%} + \text{Total ash \%})$$

3.3.4.6. Leaf fresh weight

A composite sample of 10 leaves taken randomly from F₁ hybrid plants and weighed unbiasedly.

3.3.4.7. Leaf dry weight

Fresh leaves were dried at 60⁰C in hot oven till they loose moisture and attained a constant dry weight.

3.3.4.8. Leaf dry matter content:

The ratio of the weight of a leaf sample after drying at a temperature of (60± 2 °C) to its fresh weight and expressed in per cent.

$$\text{Dry matter content (\%)} = \frac{\text{Weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

3.3.5. Paternity analysis using molecular markers

The nuclear DNA of both the parents involved in the controlled crossing as well their F₁ was isolated for conformation of their hybridity through two

marker system viz., RAPD (Random amplified polymorphic DNA) and ISSR (Inter simple sequence repeat) markers (Table 2 & 3) were used.

3.3.5.1. Collection and storage of samples for DNA extraction

Fresh and disease free leaves were collected from F₁ hybrids and parents for paternity studies and carried to the laboratory in brown paper bags within 2-3 hours of collection. Use of polybags was avoided as it led to degradation of leaf samples and kept in deep freezer (– 20°C) for future DNA extraction. Total genomic DNA was isolated using the Dellaporta *et al.* (1983) method with slight modification made in buffer concentrations.

3.3.5.2. Solutions for DNA extraction and Purification

- 10 per cent (w/v) CTAB (Cetyltrimethylammonium bromide) -10g of CTAB was dissolved in 70ml of water and then final volume was made to 100 ml.
- 0.5M EDTA (pH 8.0) - 93.05 g of Ethylene Diamine Tetra Acetate was added to 400 ml of water. The pH of solution was adjusted to 8.0 by addition of NaOH (Sodium Hydroxide). The final volume was made to 500 ml and sterilized by autoclaving.
- 5M NaCl- 146.1 g of sodium chloride was dissolved in 400 ml of water and the final volume was made to 500 ml and sterilized by autoclave.
- PVP (polyvinyl pyrrolidone) - 0.75 g of PVP is added and dissolved in the 100 ml of extraction buffer.
- Phenol: Chloroform: Isoamyl alcohol (25:24:1) – 750 µl phenol, 720 µl Chloroform and 30 µl Isoamyl alcohol were mixed by vortexing. The resulted solution was kept at 40°C in coloured container.
- 1M Tris- 60.58 g Tris base was dissolved in 400 ml of water and the pH was adjusted to 8.0. The final volume was made to 500 ml and sterilized by autoclaving.
- Chloroform: Isoamyl (24:1) - 720 µl Chloroform and 30 µl Isoamyl alcohol were mixed by vortexing. The resulted solution was kept at 40°C in coloured container.

- DNA extraction buffer-- DNA extraction buffer contained 100 mM Tris-HCl, 20 mM EDTA (pH-8.0), 1.4 M NaCl, 2.0 (w/v) SDS, 0.2 per cent (v/v) 2-Mercaptoethanol and PVP.

3.3.5.3. DNA extraction and purification

- Leaf material (0.5 g) was grounded to fine paste in extraction buffer 7 ml in a pre-chilled pestle and mortar.
- The paste was transferred to a 15 ml centrifuge tube and the tube was incubated at 65°C for about 1½ hours.
- Following incubation, 7 ml of Phenol: chloroform: isoamyl alcohol (25:24:1) was added and the contents were mixed to form an emulsion.
- The emulsion was centrifuged at 13,500 rpm for 20 minutes at 4°C temperature.
- The supernatant was carefully transferred to fresh 15 ml centrifuge tubes without disturbing the interface.
- 5ml of Chloroform: Isomyl alcohol (24:1) was added to the supernatant and the contents were mixed by gentle inversion.
- The emulsion was then centrifuged at 13,500 rpm for 15 minutes at 4°C temperature.
- The upper aqueous phase was carefully transferred to 2ml eppendorf tubes.
- The nucleic acids were precipitated by addition of 2/3rd parts by volume of the chilled (20°C) ethanol and 1/10th parts by volume of 3 M sodium acetate and tubes were kept on ice for 1½ hours.
- The pellet was collected by centrifugation at 13,000 rpm for 10 minutes at 4°C and washed with cold (-20°C) 70 per cent ethanol.
- After the final centrifugation at 13,000 rpm for 15 minutes at 4°C the supernatant was drained and pellet was dried in vaccum drier approximately 20 minutes and resuspended in 400 µl of sterile distilled water.
- For removal of proteins 5 µl of RNase (10mg/ml) was added to each sample, mixed gently and incubated for 1 hour at 37°C.

- The sample was added with equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) and mixed thoroughly to emulsify.
- The resultant solution was centrifuged at 12000 rpm for 10 min and upper aqueous phase was transferred to new centrifuge tube.
- Added 1/10th volume of 3 M sodium acetate (pH 5.2) and two volumes of absolute ethanol (95 per cent) were added and mixed by gentle inversion and kept in ice for 30 min.
- Spun at 10000 rpm for 10 min to pellet the DNA.
- The DNA pellet was washed with 70 per cent ethanol.
- The pellet was dried in vacuum drier and dissolved in sterilized water/TBE buffer.

3.3.5.4. DNA Quantification

The DNA isolated by the above method was quantified by using Lambda 25 UV-vis spectrophotometer. First the absorbance was set to zero by using 50 µl of Tris EDTA buffer in the cuvette. The cuvette was properly washed with distilled water and to this cuvette 2 µl of the above isolated DNA and 48 µl of TE buffer was added, the ratio at 260/280 nm was recorded following the amount of DNA in terms of ng/µl. This quantification of isolated DNA provided with concentration of DNA present in a particular sample and also the 260/280 nm ratio was estimated to find out purity of DNA. This ratio is 1.8 for pure double stranded DNA. If the ratio is greater than 1.8 suggest RNA contamination, where as one less than 1.8 suggests protein in the sample. The sample deviating considerably from this value was subjected to another cycle of DNA purification. The quantified samples were brought to the final working concentration of DNA to 5ng/µl, which will then be used for the RAPD and ISSR analysis.

3.3.5.5. Random Amplified Polymorphic DNA Studies

3.3.5.5.1. DNA amplification

DNA amplification was carried out for RAPD analysis using decamer random primers. A total of twenty five decamer primers synthesized by M/S Bangalore Genei, India Limited were used in the present study (Table 2).

Table 2. Nucleotide sequences of 14 decamer random primers used to study polymorphism

Sr. No	Primer	Nucleotide sequences RAPD (5'-3')
1	OPC-13	AAGCCTCGTC
2	OPF-06	GGGAATTCGG
3	OPF-08	GGGATATCGG
4	OPF-12	ACGGTACCAG
5	OPA-01	CAGGCCCTTC
6	OPA-04	AATCGGGCTG
7	OPA-05	AGGGGTCTTG
8	OPO-20	ACACACGCTG
9	OPAE 10	CTCAAGCGCA
10	OPAC 15	TGCCGTGAGA
11	OPAE 02	CTCTCGGCGA
12	OPAE 15	CACGAACCTC
13	OPAC 07	GTGCCCCGATC
14	OPAC 10	AGTCCGCCTG

3.3.5.5.2. Standardization of concentration of DNA for RAPD

For standardizing the concentration of template DNA, PCR amplification was performed with different DNA concentrations using previously standardized master-mix concentrations. Five different concentrations of DNA were used which were 5 ng, 10 ng, 15 ng, 20 ng and 25 ng, for obtaining the maximum number of amplification products. It was found that maximum number of amplification products was observed at 20 ng of template DNA.

3.3.5.5.3. Standardization of PCR amplification conditions

Tests were performed for standardizing polymerase chain reaction amplification conditions mainly the annealing temperature. PCR amplification conducted at different annealing temperatures i.e. 36°C, 37°C, 38°C using standard concentrations of various components of reaction mixture.

Sr. No.	Reactions		Temperature and Time specifications	Number of cycles
1.	Initial Denaturation		94°C-for 3min	1
2.	A	Denaturation	92°C-for 45 sec	45
	B	Annealing of Primer	36°C-for 1min	
	C	Primer amplification	72°C-for 2min	
3.	Final amplification		72°C-for 10min	1
After completion			4°C- till electrophoresis	

3.3.5.5.4. Amplification of DNA by PCR

The amplification reactions were performed in a total volume of 25 μl containing.

Sr. No.	Constituents	Qty
1.	Sterile distilled water	15 μl
2.	Taq buffer (10X)	2.5 μl
3.	dNTPs (2.5 mM)	1.25 μl
4.	Primer (10 ng)	2.0 μl
5.	Taq DNA Polymerase (3U/ μl)	0.25 μl
6.	DNA (5 ng/ μl)	4 μl
	Total for 1 reaction	25 μl

The reagents were mixed thoroughly in a 2 ml microcentrifuge tube and vortexed for few seconds. 21 μl of mixture was distributed to each PCR tube and 4.0 μl of template DNA (5 ng/ μl) was added to each tube for each amplification reaction in thermal cycler (Corbett Thermal Cycler) programmed as above.

3.3.5.5.5. Electrophoresis in agarose gel

Amplification products were separated on 2 per cent agarose gel using 1X TBE buffer (Tris HCl pH 8.0, Boric Acid, Ethylene diamine-tetra acetic Acid) on horizontal gel electrophoresis apparatus (Banglore Genei, India Limited). Ethidium bromide at a concentration of (5 mg/ml) was used as intercalating agent. RAPD amplification products were mixed with 3 μl (10X orange dye) of loading dye and loaded onto the gel. Gel was run according to 5 V/cm of the length of gel till the bands separate. 1 kb DNA Ladder (100-10000 bp) and 100 bp DNA ladder markers (100-1500 bp) were used as a standard in the first well and last well of each gel respectively.

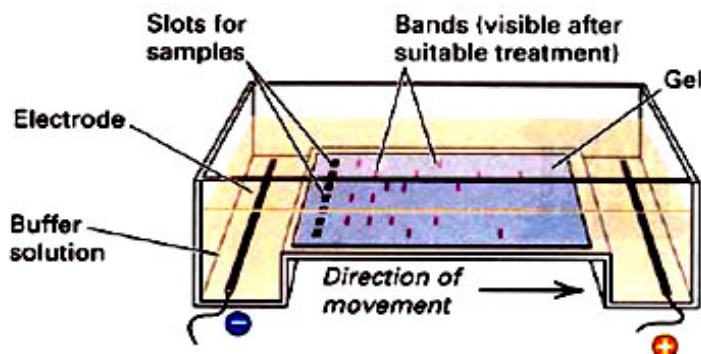


Figure 1: Apparatus used for gel electrophoresis

3.3.5.6. Inter Simple Sequence Repeat (ISSR) Studies

3.3.5.6.1. DNA amplification

DNA amplification was carried out for ISSR analysis. A total of eighteen ISSR primers synthesized by M/S Bangalore Genei, India Limited were used in the present study (Table 3).

Table 3. Nucleotide sequences of ISSR primers

S. No.	Primer	Sequences
1.	809	AGAGAGAGAGAGAGAGG
2.	810	GAGAGAGAGAGAGAGAT
3.	811	GAGAGAGAGAGAGAGAC
4.	825	ACACACACACACACACC
5.	834	AGAGAGAGAGAGAGAGYT
6.	835	AGAGAGAGAGAGAGAGYC
7.	864	ATGATGATGATGATGATG
8.	881	GGGGTGGGGTGGGGTC
9.	UBC-807	AGAGAGAGAGAGAGAGT
10.	UBC-826	ACACACACACACACACAC
11.	UBC-841	GAGAGAGAGAGAGAGAYC

3.3.5.6.2 Standardization of PCR amplification conditions

Tests were performed for standardizing polymerase chain reaction amplification conditions mainly the annealing temperature.

Sr. No.	Reactions		Temperature and Time specifications	Number of cycles
1.	Initial Denaturation		94°C-for 3min	1
2.	A	Denaturation	92°C-for 45 sec	45
	B	Annealing of Primer	55°C-for 1min	
	C	Primer amplification	72°C-for 2min	
3.	Final amplification		72°C-for 10min	1
	After completion		4°C- till electrophoresis	

3.3.5.6.3. Amplification of DNA by PCR Using ISSR primers

The amplification reactions were performed in a total volume of 24 µl containing

Sr. No.	Constituents	Qty
1.	Sterile distilled water	15 µl
2.	Taq buffer (10X)	2.5 µl
3.	dNTPs (2.5 mM)	1.25 µl
4.	Primer (10 ng)	1.0 µl
5.	Taq DNA Polymerase (3U/µl)	0.25 µl
6.	DNA (5 ng/µl)	4 µl
	Total for 1 reaction	24 µl

The reagents were mixed thoroughly in a 2 ml Eppendorf tube and vortexed for few seconds. 20 µl of mixture was distributed to each PCR tube and 4.0 µl of template DNA (5 ng/µl) was added to each tube for each amplification reaction in thermal cycler (Corbett Thermal Cycler) programmed as above.

3.3.5.6.4. Electrophoresis using agarose gel

Amplification products were separated on 2 per cent agarose gel using 1X TBE buffer (Tris HCl pH 8.0, Boric Acid, Ethylene diamine-tetra acetic Acid) on horizontal gel electrophoresis apparatus (Banglore Genei, India Limited). Ethidium bromide at a concentration of (5 mg/ml) was used as intercalating agent. ISSR amplification products were mixed with 3 µl (10X orange dye) of loading dye and loaded onto the gel. Gel was run according to 5 V/cm of the length of gel till the bands separate. 100 bp DNA ladder markers (100-1500 bp) were used as a standard in the first well and last well of each gel respectively.

3.3.6. ANALYSIS

3.3.6.1. RAPD and ISSR fingerprints

3.3.6.1.1. Viewing of amplified DNA

After the run was over, the gel was viewed under the UV light using Gel Documentation system and was photographed.

3.3.6.1.2. Scoring of bands

The amplified bands after separation were visualized using Gel Documentation system. Further the bands were scored for percentage polymorphism for each set of primer amplified product using NTSYS 2.2.

3.3.6.1.3. Analysis of RAPD and ISSR data

The scored bands were analyzed in the form of binary system to prepare the similarity index. The bands with same molecular weight and mobility were treated as identical fragments. Data matrices were prepared in which the presence of a band was coded as 1 whereas the absence as 0. The data matrices were analyzed by the SIMQUAL Program of NTSYS-PC (Version 2.2) and similarities

between genotypes were estimated using Jaccard similarity coefficient, calculated as $J = A / (N-D)$, where A is the number of positive matches (*i.e.* presence of band in both samples), D is the number of negative matches (*i.e.* absence of band in both samples) and N is the total sample size including both the number of matches and unmatches. Dendrogram was produced from the resultant similarity matrices using the UPGMA method.

3.4. STATISTICAL ANALYSIS

The data obtained for crosses (progenies) was subjected to statistical analysis as per the design. The statistical analysis for each parameter was carried out on mean values and the analysis of variance (ANOVA) table was set up as under:

3.4.1 Analysis of variance (ANOVA)

For working out the analysis of variance, the data were analysed by using the following model as suggested by Panse and Sukhatme (1967).

$$Y_{ij} = \mu + g_i + b_j + e_{ij}$$

$$i = 1, 2, \dots, g$$

$$j = 1, 2, \dots, r$$

where,

Y_{ij} = phenotypic observation of i^{th} entry and j^{th} replication

μ = general mean of the population

g_i = effect of i^{th} genotype

b_j = effect of j^{th} replication, and

e_{ij} = error component

Source of variation	d.f	Sum of Square	Mean of squares	Expectation
Replication	(r-1)	$\frac{1}{g} \sum j y_j^2 - C.F.$	M_r	-
Genotype	(g-1)	$\frac{1}{r} \sum i y_i^2 - C.F.$	M_g	$\sigma^2 e + r \sigma^2 g$
Error	(r-1)(g-1)	By subtraction	M_e	$\sigma^2 e$
Total	(rg-1)	-	-	-

Where,

r	=	No. of replications
g	=	No. of genotypes
M _r	=	Mean sum of squares due to replications
M _t	=	Mean sum of squares due to genotypes
M _e	=	Mean sum of squares due to error.
Genotypic variance (σ^2_g)	=	(M _g -M _e)/r
Phenotypic variance (σ^2_p)	=	$\sigma^2_g + \sigma^2_e$
Error variance (σ^2_e)	=	M _e

3.4.1.1 Critical Difference (CD)

The significance of differences among the treatment means was tested by 'F' test. Wherever 'F' test was found significant, (*) critical difference was calculated to test the significance between any two treatment means

The critical difference (CD) was calculated as under:

$$CD = SE_d \times t_{0.05} \text{ error degree of freedom}$$

Where;

SE_d = Standard error of difference calculated as:

$$SE_d = \sqrt{2M_e/r}$$

t_{0.05} error degree of freedom = T value at 5 per cent level of significance.

3.4.1.2 Variability and genetic parameters

Genotypic, Phenotypic and environmental variances were calculated as follows:

Coefficient of variability were worked out as suggested by Burton and De-vane (1953) and Pillai and Sinha (1968).

$$PCV (\%) = \frac{\sqrt{V_p}}{\bar{X}} \times 100 \quad V_p = \text{Phenotypic variance}$$

$$GCV (\%) = \frac{\sqrt{V_g}}{\bar{X}} \times 100 \quad V_g = \text{Genotypic variance}$$

$$ECV (\%) = \frac{\sqrt{V_e}}{\bar{X}} \times 100 \quad V_e = \text{Environmental variance}$$

Where,

PCV = Phenotypic Coefficient of Variability

GCV = Genotypic Coefficient of Variability

ECV = Environmental Coefficient of Variability

$\bar{X}$ = Population mean of character

$$CV (\%) = \left(\frac{SD}{\bar{X}} \right) \times 100$$

Where,

SD = Standard deviation

$\bar{X}$ = Population mean

3.4.1.3 Heritability (Broad sense)

Heritability in broad sense was calculated as suggested by Burton and De-Vane (1953) and Johnson *et al.* (1955).

$$h^2_{b.s} = \frac{V_g}{V_p} \times 100$$

Where,

$h^2_{b.s}$ = Heritability (broad sense)

V_g = Genotypic variance

V_p = Phenotypic variance

3.4.1.4 Genetic Advance

The expected genetic advance at 5 per cent selection intensity was calculated by the formula suggested by Lush (1940) and further used by Burton and De-Vane (1953) and Johnson *et al.* (1955).

$$\text{Genetic Advance (GA)} = \left[\frac{V_g}{V_p} \right] \times (\sqrt{V_p}) \times K$$

Where,

V_g = Genotypic variance

V_p = Phenotypic variance

K = Selection differential at 5 per cent selection intensity. The value of K = 2.06 (Allard, 1960).

3.4.1.5 Genetic gain

Genetic gain was worked out following the method suggested by Johnson *et al.*, (1955) as under:

$$\text{Genetic Gain (\%)} = \frac{\text{GA}}{\text{X}} \times 100$$

3.4.1.6. Combining ability analysis

Data pertaining to (6 x 4) factorial crosses was subjected to analysis. The estimates of variance for general and specific combining ability and their effects will be carried out as per the procedure given by Griffing (1956) methods IV (Direct crosses), Singh and Pawar (2005).

Analysis of variance for combining ability

The mathematical model for combining ability for ij^{th} cross grown in k^{th} block may be given as follows:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + b_k + e_{ijk}$$

where,

μ = population mean

g_i, g_j = gca effects of i^{th} and j^{th} parents

s_{ij} = sca effect of ij^{th} cross

b_k = effect of k^{th} block

g_i = effect of i^{th} genotype

e_{ijk} = random error

Source of variation	df	Sum of Square	Mean Sum of Square	Expected Mean Sum of Square
Crosses	$(lt-1)$	CSS		
Lines	$l-1$	LSS	MS_1	$\delta^2 + r(\text{Cov.FS} - 2\text{Cov.HS}) + rt(\text{Cov. HS})$
Tester	$t-1$	TSS	MS_2	$\delta^2 + r(\text{Cov.FS} - 2\text{Cov.HS}) + rl(\text{Cov. HS})$
Line $\times$ Tester interaction	$(l-1)(t-1)$	$L \times TSS$	MS_3	$\delta^2 + r(\text{Cov.FS} - 2\text{Cov. HS})$
Error	$(r-1)(lt-1)$	ESS	MS_4	$\delta^2 e$
Total	$(rlt-1)$			

where,

r = No. of replication

l = No. of lines

t = No. of tester

Combining ability Variances

Variance of GCA = Cov. HS (Covariance of half Sibs)

Variance of SCA = Cov. FS – 2 Cov. HS (Cov. FS= Covariance of Full Sibs)

$$\text{Cov. HS} = \frac{(\text{MS}_1 - \text{MS}_3) + (\text{MS}_2 - \text{MS}_3)}{r(l+t)}$$

$$\text{Cov. HS} = \frac{\text{MS}_1 + \text{MS}_2 + \text{MS}_3 - 3\text{MS}_4}{3r} + \frac{6r\text{Cov.HS} - r(l+t)\text{Cov. HS}}{3r}$$

$$\text{Cov. FS} - 2 \text{Cov. HS} = \left[\frac{\text{MS}_3 - \text{MS}_4}{r} \right]$$

The parents are non-inbreds or cross pollinated parents (inbreeding coefficient, $F=0$)

$$\text{Cov. HS} = \frac{1}{4} \delta_D^2 + \frac{1}{16} \delta_{DD}^2 + \text{Other forms of epistasis}$$

$$\text{Cov. FS} = \frac{1}{2} \delta_D^2 + \frac{1}{4} \delta_{H+}^2 + \frac{1}{4} \delta_{DD}^2 + \frac{1}{8} \delta_{DH}^2 + \frac{1}{16} \delta_{HH+}^2 + \text{Other forms of epistasis}$$

Assuming there is no epistasis.

$$\delta_D^2 (\text{Additive genetic variance}) = 4 \text{Cov. HS} \text{ or } 4 \delta^2 \text{ GCA}$$

$$\delta_H^2 (\text{Dominance genetic variance}) = 4 [\text{Cov. FS} - 2 \text{Cov. HS}] \text{ or } 4 \delta^2 \text{ SCA}$$

Combining ability effects

$$\text{gca effect of lines (g}_i\text{)} = \frac{x_{i..}}{rt} - \frac{x_{...}}{rlt}$$

$$\text{gca effect of testers (g}_j\text{)} = \frac{x_{.j.}}{rl} - \frac{x_{...}}{rlt}$$

$$\text{sca effect of testers (s}_{ij}\text{)} = \frac{x_{ij.}}{r} - \frac{x_{i..}}{rt} - \frac{x_{.j.}}{rl} + \frac{x_{...}}{rlt}$$

where,

$x_{...}$ - Total of all hybrids over replications

$x_{i..}$ - Total of i^{th} line over t testers and r replications

$x_{.j.}$ - Total of j^{th} testers over l lines and r replications

$x_{ij.}$ - Total of all hybrids between i^{th} line and j^{th} testers over r replications

Significance of effects by 't' test

$$\text{SE for gca effects of lines} = \sqrt{\frac{\text{EMS}}{rt}}$$

$$\text{SE for gca effects of testers} = \sqrt{\frac{\text{EMS}}{rl}}$$

$$\text{SE for gca effects of hybrids} = \sqrt{\frac{\text{EMS}}{rl}}$$

3.4.1.7. Estimation of Heterosis

Heterosis was calculated in terms of percentage increase or decrease of a hybrid against its mid parent value with respect to individual character, here after called relative heterosis or mid parent heterosis (Fehr 1987).

$$\text{Relative heterosis} = \frac{F_1 - \text{MP}}{\text{MP}} \times 100$$

MP is mean value Mid parent

3.4.1.8. Gene action

3.4.1.8.1. Graphical Analysis

Graphical analysis was based on the variance and co-variance values (V_r/W_r graph) following the procedure developed by Jinks and Hayman (1953). Here V_r is the variance of the progeny means in the array of the r th parent, which is composed of the parental mean and mean values of all crosses involving the parent. W_r is the covariance of the progeny means in the r th parent array with the mean values of non-recurrent parent. V_p is the variance of parental values.

V_r is always positive, while W_r may have both positive and negative values. Therefore, V_r and W_r are only a two quarter graph. V_r is taken in the X ordinate while W_r in the Y-ordinate of the graph.

Limits of the V_r/W_r graph are marked by a parabola curve. The limiting parabola is easily constructed on the basis of its formula.

$W_r = V_r \times V_p$ The corresponding values of W_r for all observed V_r values were calculated. Each V_r and $\sqrt{V_r \times V_p}$ point was plotted and parabola curve was drawn with a few intra and extrapolations. All the crosses were arranged into arrays in the form of diallel table. The information on gene action was inferred by plotting the covariance (W_r) of each array against its variance (V_r). The slope and the position of regression line fitted to the array points within the limited parabola ($W_r^2 = V_{lo} \cdot V_r$) indicated the degree of dominance and the presence or absence of gene interaction. The different arrays (varieties) were fitted within the limits of parabola using the individual variance and covariance as their limiting points. Array nearest to the point of origin possessed most of the dominant genes; while the array that laid farthest possessed most recessive genes, and the intermediate position signified the presence of both dominant and recessive genes in the array.

The population parameters estimates were E , D , F , H_1 and H_2 , E is the estimate of environmental variance, D is the additive genetic variance which may include a portion of the additive $\times$ additive epistatic variance as well as the additive genetic variance itself. H_1 is the dominance genetic variance parameter, which may include the dominance genetic variance proper, dominance $\times$ dominance epistatic variance and additive $\times$ dominance as well as the portion of the additive $\times$ additive variance not included within D . H_2 is the component of genetic variance due to non-additive (dominance) effects corrected for gene distribution. F is an indicator of the relative frequency of dominant and recessive alleles in the parents and may take a sign (positive or negative).

Estimates and ratios were calculated by using the above genetic parameters to provide the information about the genetic system operating for each trait. The standard error for each parameter was calculated. The estimates and ratios are; $(H_1/D)^{1/2}$ = Measures the overall average degree of dominance at each locus in the F_1 , $H_2/4H_1$ = is an estimate of average frequency of negative vs. positive alleles (at loci exhibiting dominance) in the parents and has a maximum value of 0.25. $[(4DH_1)^{1/2} + F] / [(4DH_1)^{1/2} - F]$ = is the ratio of the total number of dominant to recessive alleles in the parents.

Chapter-4

EXPERIMENTAL RESULTS

The present investigations were carried out to assess the nature of gene action, combining abilities and heterosis among different hybrids developed from selected genotypes of *G. optiva* in 6x4 (Line x Tester) cross and its paternity pattern were analysed by using RAPD and ISSR molecular markers. In order to suggest breeding procedure for improving the yield and other contributing characters, the collection and computation of data are done accordingly. The data collected on different characters were subjected to statistical and biometrical analysis covering all the proposed objectives. The results obtained are presented separately for laboratory and field studies under the following headings.

- 4.1 CONTROLLED POLLINATION (HYBRIDIZATION) UNDER LINE x TESTER MATING DESIGN
- 4.2 COMBINING ABILITY UNDER LINE x TESTER CROSSING SYSTEM
- 4.3 GENE ACTION USING GRAPHICAL (W_r/V_r) APPROACH AND GENETIC COMPONENTS OF VARIATION
- 4.4 HETEROSIS IN F_1 GENERATION
- 4.5 PATERNITY ANALYSIS USING RAPD AND ISSR MOLECULAR MARKERS

4.1 CONTROLLED POLLINATION (HYBRIDIZATION) UNDER LINE x TESTER MATING DESIGN

Intra-specific controlled crossing was carried out during March to mid may in 2011 and 2012. Twenty-four full-sib intra-specific families were produced using 6 × 4 Line X Tester mating design.

4.1.1 Crossability parameters

A cursory glance at the data presented in Table 4 revealed significant differences for crossability parameters viz., per cent successful crosses, average seed set per flower, germination percentage and survival percentage among the genotypes of *G. optiva*

4.1.1.1. Per cent successful crosses

The range of per cent successful crosses lied between 23.81 to 88.10 per cent for different crosses. Significant differences were observed among crosses. Per cent successful cross was highest in cross SO-2 X SO-3 that was 88.10 per cent followed by SO-2 X BI-4 (80.85 %) and CH-2 X BI-4 (76.47 %). The minimum percentage of successful cross was obtained for cross SO-8 X HA-4, which recorded 23.81 per cent and was followed by crosses SO-1 X HA-4 was 35.21 per cent (Table 4).

4.1.1.2. Average number of seeds per flower

Maximum number and statistically significant value for seeds per flower (3.80) was recorded for SO-8 X HA-4 and was followed by crosses SO-1 X SI-6, SO-1 X HA-4 and CH-2 X SI-6, which showed average seed set per flower value of 2.65, 2.56 and 2.47 respectively. However, cross SO-2 X HA-4, SO-2 X BI-4 and SO-2 X SO-3 registered minimum average seed set that was 1.12, 1.26 and 1.30 respectively (Table 4).

4.1.1.3. Germination percentage

Significant differences were observed among crosses for germination percentage (Table 4 and Fig. 2). Maximum germination percentage (72.72) was recorded for crosses involving SI-15 X BI-4 followed by SI-15 X SO-3 having 70.59, SI-15 X SI-6 and SO-1 X HA-4 70.31 per cent was recorded and were statistically similar. Minimum value for germination percentage was recorded for crosses SO-4 X SI-6, SO-4 X BI-4 and SO-4 X HA-4 *i.e.*, 34.21, 36.11 and 37.50 respectively.

4.1.1.4. Survival per cent

Survival percentage is one of the important factors in successful establishment of a species and is the best indicator of species with respect to adaptation and growth. The range for survival percentage was between 57.69 to 97.73 per cent among the crosses. Crosses *viz.*, SO-1 X BI-4 and SI-15 X SO-3 (97.73 and 97.22 per cent) recorded highest per cent survival among all other



Hybrid seeds obtained from 6 X 4
(Line X Tester) mating system



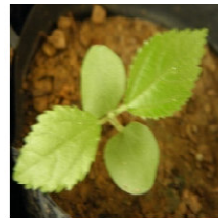
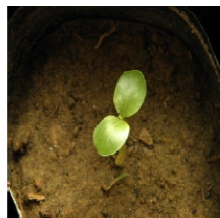
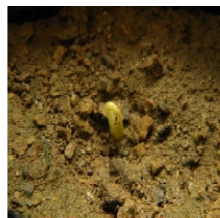
Measurement of seeds for seed
parameter studies



Pre sowing treatment of seeds



Sowing of seeds



Seedlings of *Grewia optiva* at different stages of growth under study

PLATE 4: Seeds obtained from controlled crosses , seed treatment, sowing of seeds and different stages of germination in *Grewia optiva*



Germinated seedlings from the seeds of controlled crosses in polybags



Survived seedlings of different crosses in polybags

PLATE 5: Germination and survival per cent of different crosses in *Grewia optiva* genotypes

crosses followed by 95.65 and 95.24 per cent survival of seedlings in crosses SO-2 X BI-4 and SO-2 X SI-6. Whereas, crosses CH-2 X BI-4 and SO-4 X SO-3 (57.69 and 66.67 per cent) recorded minimum survival per cent (Table 4, Fig.2, Plate 4 and 5).

Table 4. Per cent successful crosses, average seed set per flower, germination percentage and survival percentage of the crosses attempted among the selected genotypes of *Grewia optiva*

Crosses	Per cent successful cross	Average seed set per flower	Germination %	Survival %
SO-1 X SO-3	52.54	1.90	62.71	89.19
SO-1 X SI-6	40.00	2.65	67.92	94.44
SO-1 X BI-4	58.93	1.97	67.69	97.73
SO-1 X HA-4	35.21	2.56	70.31	82.22
SO-2 X SO-3	88.10	1.30	47.92	91.30
SO-2 X SI-6	54.55	1.63	53.85	95.24
SO-2 X BI-4	80.85	1.26	47.92	95.65
SO-2 X HA-4	75.56	1.12	39.47	80.00
SO-4 X SO-3	68.42	1.58	43.90	66.67
SO-4 X SI-6	53.85	1.81	34.21	92.31
SO-4 X BI-4	62.79	1.33	36.11	92.31
SO-4 X HA-4	76.19	1.50	37.50	88.89
SO-8 X SO-3	75.00	1.77	45.28	91.67
SO-8 X SI-6	69.77	1.67	46.00	86.96
SO-8 X BI-4	61.90	2.00	53.85	85.71
SO-8 X HA-4	23.81	3.80	65.79	92.00
CH-2 X SO-3	64.79	2.02	47.31	75.00
CH-2 X SI-6	61.22	2.47	62.16	93.48
CH-2 X BI-4	76.47	1.33	50.00	57.69
CH-2 X HA-4	68.89	1.97	57.38	91.43
SI-15 X SO-3	47.06	2.13	70.59	97.22
SI-15 X SI-6	45.76	2.37	70.31	93.33
SI-15 X BI-4	40.58	2.36	72.72	93.75
SI-15 X HA-4	41.67	2.34	69.51	87.72
Mean	59.33	2.34	55.02	88.00
Sem	3.30	0.12	2.56	1.98
CV	27.28	25.21	22.8	11.02

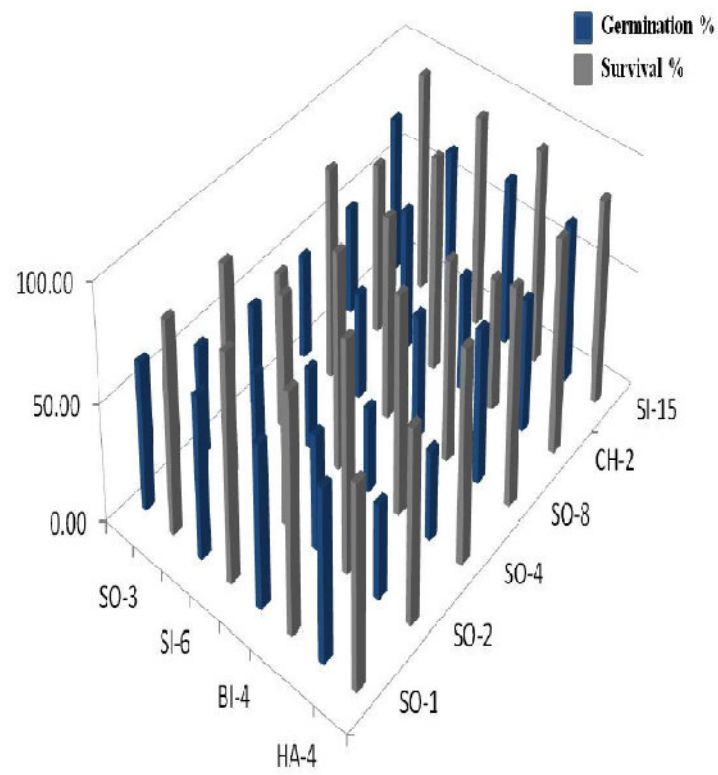


Figure 2. Germination and survival per cent of the crosses attempted among the selected genotypes of *Grewia optiva*

4.2 COMBINING ABILITY UNDER LINE x TESTER MATING DESIGN

Seamless of every breeding programme lies in the selection of suitable parents to be used in hybridization programme. The information about combining ability and relative magnitude of genetic variance for traits like yield and its components is essential for exploitation of the existing gene action in the population. Estimation of general and specific combining ability (Griffing 1956; Kempthorne, 1956) assesses the breeding value of parents for quantitative characters in both cross and self-pollinated plants. The combining ability analysis of variance for all the character is presented in Table 5 to 22.

The combining ability analysis was done for the each parameter separately. The analysis of variance for combining ability is presented in Appendix II to XIX. Highly significant variances were observed among crosses for all the characters. The estimates of general combining ability variance were more than the specific combining ability variance for all the character studied except for collar diameter (six months), and for fodder quality character where estimates of general combining ability variance were less than the specific combining ability variance. Therefore, the ratio GCA: SCA was less than one. The additive variance was more than the dominance variance was observed for germination and survival per cent, whereas, dominance variance was more than the additive variance was observed in seed diameter, seed length, plant height, collar diameter, number of leaves for two months observation and for fodder quality characters as such the ratio of additive genetic variance/ dominance genetic variance was less than unity in above mentioned parameters studied.

The estimates of general combining ability effects and specific combining ability effects for different parameters are appended in Tables 5 to 22 respectively and described below.

4.2.1. Seed parameters

4.2.1.1. Seed diameter (mm)

The perusal of the data appended in Table 5. The estimate of degree of dominance was 0.355. Moderate values of narrow sense (0.677) and high broad

sense (0.962) heritability were recorded for the parameter. Highly significant differences were observed among parents. However, one female parent (SI-15) exhibited positive value and rest of the female parents showed negative GCA effects (Table 6). General combining ability effects were significantly positive and high in male genotype BI-4 followed by HA-4, whereas, SI-6 showed significant negative GCA among parents.

Table 5. Estimation of variance components for seed characters in *G. optiva*

Sl No.	Variance components	Seed diameter (mm)	Seed length (mm)
1	Variances of gca (δ^2_g)	1.220	3.521
2	Variances of sca (δ^2_s)	0.308	0.263
3	Additive variance (D)	2.440	7.043
4	Dominance variance (H)	4.928	14.084
5	Degree of dominance	0.355	0.193
6	Heritability (ns)	0.677	0.627
7	Heritability (bs)	0.962	0.965

Table 6. General combining ability effects of different parents for seed characters in *G. optiva*

Parents	General combining ability effects	
Females	Seed diameter (mm)	Seed length (mm)
SO-1	-0.063	-0.079
SO-2	-0.308**	-0.190
SO-4	-0.094	-0.065
SO-8	-0.033	-0.216
CH-2	-0.165	-0.137
SI-15	0.663**	0.688**
Males		
SO-3	-0.075	-0.163
SI-6	-0.237*	-0.302**
BI-4	0.206*	0.352**
HA-4	0.106*	0.113
SEgi	0.109	0.109

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Close appraisal of the data presented in Table 7 revealed that twelve out of twenty four crosses revealed positive specific combining ability effects. Highest significantly positive specific combining ability effect was depicted by SO-2 X BI-4, which was followed by SO-4 X SO-3 exhibiting positively

significant specific combining ability effects. Crosses viz., CH-2 X HA-4, SI-15 X HA-4, SI-15 X SO-3, SO-1 X HA-4, SO-4 X BI-4, SO-2 X SI-6, CH-2 X SI-6, SO-8 X HA-4, SO-8 X SI-6 and SO-4 X SI-6 were among those exhibiting positive specific combining ability effects. However, cross SO-4 X SI-6 exhibited lowest positive SCA effects.

Table 7. Specific combining ability effects among different crosses for seed characters in *G. optiva*

Crosses	Specific combining ability effects	
	Seed diameter (mm)	Seed length (mm)
SO-1 X SO-3	-0.097	-0.142
SO-1 X SI-6	-0.140	0.132
SO-1 X BI-4	-0.008	-0.099
SO-1 X HA-4	0.245	0.110
SO-2 X SO-3	-0.517*	-0.511*
SO-2 X SI-6	0.107	0.009
SO-2 X BI-4	0.724**	0.806**
SO-2 X HA-4	-0.314	-0.304
SO-4 X SO-3	0.484*	0.310
SO-4 X SI-6	0.002	0.126
SO-4 X BI-4	0.165	0.065
SO-4 X HA-4	-0.676**	-0.501*
SO-8 X SO-3	-0.020	-0.011
SO-8 X SI-6	0.003	0.076
SO-8 X BI-4	-0.001	-0.059
SO-8 X HA-4	0.018	-0.006
CH-2 X SO-3	-0.105	0.128
CH-2 X SI-6	0.049	-0.175
CH-2 X BI-4	-0.309	-0.302
CH-2 X HA-4	0.365	0.349
SI-15 X SO-3	0.255	0.226
SI-15 X SI-6	-0.183	-0.167
SI-15 X BI-4	-0.435	-0.412
SI-15 X HA-4	0.363	0.353
SEsi	0.219	0.219

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.2.1.2. Seed length (mm)

Data presented in Table 5 revealed that estimate of degree of dominance was 0.193. Moderate narrow sense heritability (0.627) and high heritability broad

sense (0.965) was estimated for seed length. Highly significant differences were observed among parents. Among female parents SI-15 recorded significant and positive general combining ability effect (Table 6). General combining ability effects were significantly positive and high in BI-4 among male parents. HA-4 also recorded positively significant general combining ability effects. The data presented in Table 7. revealed that twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combination while twelve crosses exhibiting negative specific combining ability effects indicating poor cross combinations. Cross SO-2 X BI-4 exhibited highly significant and positive specific combining ability. Other crosses that exhibited positively significant specific combining ability effects were SI-15 X HA-4, CH-2 X HA-4, SO-4 X SO-3, SI-15 X SO-3, SO-1 X SI-6, CH-2 X SO-3, SO-4 X SI-6, SO-1 X HA-4, SO-8 X SI-6 and SO-4 X BI-4 while cross SO-2 X SI-6 exhibited lowest positive specific combining ability effect for seeds length. Female involved in the crosses were poor general combiners except SI-15, whereas, among males BI-4 showed good general combining ability.

4.2.2.1. Nursery studies of hybrids

4.2.2.1.1. Germination per cent

The data presented in Table 8, showed that estimate of degree of dominance was 0.336. High narrow sense and broad sense (0.976 and 0.982) heritability was estimated for germination per cent. Highly significant differences were observed among parents. Among female parents SI-15 and SO-1 recorded positive general combining ability effect where SO-4 showed highly significant negative general combining ability. General combining ability effects among male were positive and highly significant for HA-4 and SI-6 (Table 9). Thirteen out of twenty four cross exhibited positive specific combining ability effects. However, cross SO-8 X HA-4, SO-4 X SO-3 and CH-2 X SI-6 exhibited positively highly significant specific combining ability effects. Crosses viz., SO-2 X SI-6, SO-2 X SO-3, SI-15 X BI-4, SI-15 X SO-3, SO-1 X HA-4, CH-2 X HA-4, SO-8 X BI-4, SO-2 X BI-4, SO-1 X BI-4 and SO-1 X SI-6 exhibited positive specific combining ability effects (Table 10).

Table 8. Estimation of variance components for germination and survival per cent in *G. optiva*

SI No.	Variance components	Germination %	Survival %
1	Variances of gca (δ^2_g)	1479.287	11928.79
2	Variances of sca (δ^2_s)	111.052	6375.047
3	Additive variance (D)	2958.575	23857.59
4	Dominance variance (H)	333.157	6375.047
5	Degree of dominance	0.336	0.517
6	Heritability (ns)	0.976	0.885
7	Heritability (bs)	0.982	0.999

Table 9. General combining ability effects of different parents for germination and survival per cent in *G. optiva*

Parents	General combining ability effects	
Females	Germination %	Survival %
SO-1	12.121**	-8.831
SO-2	-7.754	-9.023
SO-4	-17.076	-14.697
SO-8	-2.286	-10.606
CH-2	-0.768	-20.252
SI-15	15.765**	63.409**
Males		
SO-3	-2.081	-14.507
SI-6	0.731**	39.643**
BI-4	-0.294	-12.602
HA-4	1.645**	-12.534
SEgi	0.136	23.377

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.2.2.1.2. Survival per cent

The estimated degree of dominance was 0.517 for survival per cent (Table 8). The higher narrow sense and broad sense (0.885 and 0.999) heritability was recorded. Among female parents SI-15 excelled with positive general combining ability effect where all other female parents expressed significant negative general combining ability where CH-2 recorded the highest. SI-6 showed positive general combining ability effects among male (Table 9). Out of twenty-four cross fourteen crosses exhibited positive specific combining ability. Hybrid SI-15 X SI-6 exhibited highly significant and positively specific combining ability effects. However, crosses CH-2 X HA-4, SO-4 X BI-4, SO-1 X BI-4, SO-8 X SO-3, SO-

4 X HA-4, SO-8 X HA-4, SO-2 X SO-3, SO-1 X SO-3, CH-2 X SO-3, SO-8 X BI-4, SO1 X HA-4 and SO-2 X HA-4 exhibited positive specific combining ability effects. Female involving the crosses SI-15 showed good general combiners (Table 10).

Table 10. Specific combining ability effects among different crosses for germination and survival per cent in *G. optiva*

Crosses	Specific combining ability effects	
	Germination %	Survival %
SO-1 X SO-3	-2.353**	12.780
SO-1 X SI-6	0.014	-36.095
SO-1 X BI-4	0.810**	19.411
SO-1 X HA-4	1.530**	3.904
SO-2 X SO-3	2.686**	15.073
SO-2 X SI-6	5.800**	-35.129
SO-2 X BI-4	0.919**	17.524
SO-2 X HA-4	-9.405	2.532
SO-4 X SO-3	8.038**	-3.858
SO-4 X SI-6	-4.424**	-32.374
SO-4 X BI-4	-1.535	19.884
SO-4 X HA-4	-2.079*	16.349
SO-8 X SO-3	-5.382**	17.010
SO-8 X SI-6	-7.381**	-41.823
SO-8 X BI-4	1.385**	9.194
SO-8 X HA-4	11.378**	15.618
CH-2 X SO-3	-4.863**	10.394
CH-2 X SI-6	7.194**	-25.667
CH-2 X BI-4	-3.830**	-9.207
CH-2 X HA-4	1.500**	24.480
SI-15 X SO-3	1.873**	-51.399
SI-15 X SI-6	-1.203	171.088**
SI-15 X BI-4	2.253**	-56.805
SI-15 X HA-4	-2.924**	-62.883
SEsi	0.027	57.263

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.2.2.1.3. Plant height (cm)

The cursory look at the data presented in (Table 11) showed that estimate of degree of dominance was 0.787. However, low narrow sense and broad sense

(0.081 and 0.105) heritability was estimated for plant height in two months old seedlings. Highly significant differences were observed among parents. Among female parents, SO-8 recorded positive general combining ability effect where SO-1 showed highly significant negative general combining ability. General combining ability effects among male were positive and significant in BI-4 (Table 12). Nine out of twenty four cross (37.5 %) exhibited positive specific combining ability effects. However, cross SO-4 X HA-4 and SO-1 X SO-3 exhibited positively significant specific combining ability effects. Crosses viz., SI- X BI-4, SO-2 X HA-4, SO-8 X BI-4, CH-2 X HA-4, SO-1 X SI-6, CH-2 X SO-3 and CH-2 X BI-4 (Table 13) exhibited positive specific combining ability effects. Female and male involved in the crosses were poor general combiners except SO-8 and BI-4.

The estimate of degree of dominance was 0.255 for six months seedling data (Table 14). The higher narrow sense and broad sense (0.965 and 0.996) heritability was estimated as plant developed. Highly significant differences were observed among parents. Among female parents again SO-8 excelled with positive general combining ability effect where SO-1 and SO-2 had highly significant negative general combining ability. BI-4 which showed positive and significant general combining ability effects among male in early stage of growth was again proved its consistence in six months also (Table 15). Out of twenty-four cross twelve (50 %) exhibited positive specific combining ability effects. Again, crosses SO-4 X HA-4, SO-2 X HA-4, SO-1 X SO-3 and SO-8 X SI-6 exhibited positively significant specific combining ability effects. However, crosses SI-15 X BI-4, SO-8 X BI-4, SI-15 X SO-3, CH-2 X HA-4, CH-2 X SI-6, CH-2 X BI-4, SO-2 and SI-15 X SI-6 exhibited positive specific combining ability effects. Female and male involved in the crosses SO-8 and BI-4 were showed good general combiners (Table 16).

4.2.2.1.4. Collar diameter (mm)

For collar diameter the degree of dominance estimated was 1.782 (Table 11) moderate narrow sense (0.608) and high heritability broad sense (0.910) was recorded.

Highly significant differences were observed among parents. Among female SO-8 and SO-4 recorded positively significant general combining ability effects (Table 12). Among males, general combining ability effects were positive and high significant in BI-4. Appraisal of the data presented in table 13 revealed that cross SO-4 X HA-4, SI-15 X BI-4 and SO-1 X SO-3 exhibited high positively significant specific combining ability effect. A total of thirteen crosses (54.16 %) exhibited positively significant specific combining ability effects indicating that these were good cross combinations while crosses exhibiting negative specific combining ability effects were poor cross combinations. Crosses SO-8 X SI-6, SO-2 X HA-4, SO-8 X HA-4, SI-15 X SI-6, CH-2 X BI-4, SI-15 X SO-3, CH-2 X SO-3, SO-2 X SO-3 and SO-8 X BI-4 also recorded positive specific combining ability effects. Females viz., SO-8 and SO-4 involved in the crosses were good general combiners, whereas, males were poor general combiners except BI-4 genotype.

Degree of dominance estimated was 0.543 (Table 14) for six months seedling. The narrow sense and broad sense heritability was recorded higher (0.941 and 0.998) for six months reading. Highly significant differences were observed among parents. Among females, SO-8 recorded positively significant general combining ability effects and SI-15, SO-1 and CH-2 showed negative general combining ability effects. Among males, general combining ability effects were positive and high significant in SI-6 and significant in SO-3 (Table 15). The data presented in table 16 revealed that cross SO-1 X SO-3, SO-8 X BI-4, SO-8 X SI-6, SO-4 X HA-4, CH-2 X HA-4 and SI-15 X BI-4 exhibited high positively significant specific combining ability effect. While, The crosses SO-2 X SO-3 and SO-1 X SI-6 also exhibited positive specific combining ability. The crosses exhibiting negative specific combining ability effects were poor cross combinations.

4.2.2.1.5. Number of leaves per plant

Highly significant differences were observed among parents for number of leaves and the degree of dominance estimated was 0.415 (Table 11) low narrow sense (0.550) and high broad sense (0.679) was recorded. Among female

SO-8 recorded positively significant general combining ability effects (Table 12). Among males, general combining ability effects were positive and significant in HA-4. Appraisal of the data presented in table 13 revealed that cross SO-1 X SO-3 exhibited high positively significant specific combining ability effect. A total of ten crosses exhibited specific combining ability effects indicating that these were good cross combinations while crosses exhibiting negative specific combining ability effects were poor cross combinations. Crosses SI-15 X BI-4, SO-4 X HA-4, SO-8 X SI-6, SO-2 X HA-4, CH-2 X BI-4, CH-2 X HA-4, SO-2 X SO-3, SI-15 X SI-6 and CH-2 X SO-3 were among those exhibited positively specific combining ability effects.

Table 11. Estimation of variance components for growth characters (two months) in *G. optiva*

SI No.	Variance components	Height (cm)	Diameter (mm)	No. of leaves	Internodel length (cm)
1	Variances of gca (δ^2g)	0.173	0.293	0.569	0.064
2	Variances of sca (δ^2s)	0.139	0.182	0.196	0.031
3	Additive variance (D)	0.279	0.388	0.786	0.182
4	Dominance variance (H)	0.692	0.728	1.138	0.128
5	Degree of dominance	0.787	1.782	0.415	0.492
6	Heritability (ns)	0.081	0.608	0.550	0.076
7	Heritability (bs)	0.105	0.910	0.679	0.087

Table 12. General combining ability effects of parents for growth characters (two months) in *G. optiva*

Parents	General combining ability effects			
Females	Height (cm)	Diameter (mm)	No. of leaves	Internodel length (cm)
SO-1	-0.249 ^{**}	-0.351 ^{**}	-0.309 ^{**}	-0.136
SO-2	-0.120 [*]	-0.179 ^{**}	-0.163	-0.059
SO-4	0.094	0.150 ^{**}	0.108	0.032
SO-8	0.363 ^{**}	0.333 ^{**}	0.503 ^{**}	0.166
CH-2	0.019	0.069	0.045	0.032
SI-15	-0.108	-0.022	-0.184 [*]	-0.034
Males				
SO-3	-0.081	-0.060	-0.038	-0.020
SI-6	-0.005	-0.003	-0.094	-0.007
BI-4	0.112 [*]	0.116 [*]	0.128	0.052
HA-4	-0.026	-0.054	0.003 [*]	-0.025
SEgi	0.058	0.037	0.087	0.016

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

For six months data, the degree of dominance estimated was 0.780 (Table 14), high narrow sense (0.982) and broad sense heritability (0.999) was recorded. Female SO-8 excelled with positively significant general combining ability effects (Table 15). In males, general combining ability effects were positive for BI-4 and SI-6 but non-significant. Eleven crosses exhibited positive specific combining ability effects indicating that these were good cross combinations while crosses exhibiting negative specific combining ability effects were poor cross combinations. Data presented in Table 16 revealed that cross SO-8 X SI-6 exhibited significant and positive specific combining ability effect. Crosses SO-4 X HA-4, SO-2 X HA-4, SO-1 X SO-3, SO-8 X BI-4, SO-2 X SO-3, CH-2 X SI-6, CH-2 X BI-4, SI-15 X BI-4, SI-15 X SO-3 and SO-1 X HA-4 were also exhibited positive specific combining ability.

Table 13. Specific combining ability effects among different crosses for growth characters (two months) in *G. optiva*

Crosses	Specific combining ability effects			
	Height (cm)	Diameter (mm)	No. of leaves	Internodal length (cm)
SO-1 X SO-3	0.385**	0.366**	0.476**	0.107**
SO-1 X SI-6	0.051	-0.007	-0.052	-0.022
SO-1 X BI-4	-0.299*	-0.260**	-0.274	0.018
SO-1 X HA-4	-0.137	-0.098	-0.149	-0.104**
SO-2 X SO-3	-0.002	0.020	0.080	0.014
SO-2 X SI-6	-0.037	0.002	-0.031	0.018
SO-2 X BI-4	-0.103	-0.120	-0.170	-0.075*
SO-2 X HA-4	0.142	0.099	0.122	0.044
SO-4 X SO-3	-0.167	-0.179*	-0.274	-0.061
SO-4 X SI-6	-0.020	-0.019	-0.052	-0.049
SO-4 X BI-4	-0.168	-0.191*	-0.024	-0.050
SO-4 X HA-4	0.386**	0.423**	0.351*	0.161**
SO-8 X SO-3	-0.227	-0.254**	-0.170	-0.111**
SO-8 X SI-6	0.313**	0.189*	0.302	0.084*
SO-8 X BI-4	0.072	0.019	-0.087	0.000
SO-8 X HA-4	-0.158	0.046	-0.045	0.027
CH-2 X SO-3	0.042	0.023	0.038	-0.011
CH-2 X SI-6	-0.126	-0.025	-0.240	-0.032
CH-2 X BI-4	0.015	0.030	0.122	-0.034
CH-2 X HA-4	0.069	-0.027	0.080	0.077*
SI-15 X SO-3	-0.031	0.025	-0.149	0.064
SI-15 X SI-6	-0.033	0.032	0.073	0.001
SI-15 X BI-4	0.367**	0.386**	0.434*	0.141**
SI-15 X HA-4	-0.303*	-0.443**	-0.358*	-0.206**
SEsi	0.117	0.075	0.174	0.032

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.2.2.1.6. Internode length (cm)

The table 11 revealed that estimate of degree of dominance was 0.492 with low narrow sense and broad sense (0.076 and 0.087) was estimated for internode length. Non-significant values was observed among the parents for two months growth data. Among females and males, none of the parents showed significant value whereas females SO-8, SO-4 and CH-2 and males parent BI-4 recorded higher positive general combining ability effect (Table 12). The data presented in (Table 13) revealed that twelve crosses (50 %) exhibited positive specific combining ability effects indicating that these were good cross combination. Cross SO-4 X HA-4, SI-15 X BI-4 and SO-1 X SO-3 exhibited highly significant and positive specific combining ability. Other crosses that exhibited positively significant specific combining ability effects were SO-8 X SI-6, CH-2 X HA-4, SI-15 X SO-3, SO-2 X HA-4, SO-8 X HA-4, SO-2 X SI-6 SO-2 X SO-3 and SI-15 X SI-6 exhibited positive specific combining ability effect for internode length.

For six months growth data table 14 revealed that estimate of degree of dominance was 0.345 with low narrow sense and broad sense (0.145 and 0.190) was estimated for internode length. Highly significant values was observed among parents, among females SO-8 and males parent SI-6 and BI-4 recorded highly significant and higher positive general combining ability effect (Table 15) The data presented in table 16 revealed that eleven crosses exhibited positive specific combining ability

Table 14. Estimation of variance components for growth characters (six months) in *G. optiva*

Sl No.	Variance components	Height (cm)	Diameter (mm)	No. of leaves	Internode length (cm)
1	Variances of gca (δ^2_g)	10.666	1.227	1.096	0.182
2	Variances of sca (δ^2_s)	5.544	2.896	0.225	0.172
3	Additive variance (D)	1.386	0.724	0.274	0.043
4	Dominance variance (H)	21.331	2.453	0.450	0.363
5	Degree of dominance	0.255	0.543	0.780	0.345
6	Heritability (ns)	0.965	0.941	0.982	0.145
7	Heritability (bs)	0.996	0.998	0.999	0.190

Table 15. General combining ability effects of parents for growth characters (six months) in *G. optiva*

Parents	General combining ability effects			
Females	Height (cm)	Diameter (mm)	No. of leaves	Internode length (cm)
SO-1	-0.946**	-0.214**	-0.142	-0.122
SO-2	-0.913**	0.115	-0.247	-0.053
SO-4	0.304	-0.097	0.087	0.020
SO-8	1.408**	0.674**	0.545**	0.235**
CH-2	0.070	-0.159**	-0.247	-0.036
SI-15	0.077	-0.318**	0.003	-0.044
Males				
SO-3	-0.440**	0.104*	-0.142	-0.049
SI-6	0.181	0.153**	0.052	0.051**
BI-4	0.402**	0.068	0.094	0.047**
HA-4	-0.143	-0.324**	-0.003	-0.048
SEgi	0.193	0.058	0.130	0.015

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Table 16. Specific combining ability effects among different crosses for growth characters (six months) in *G. optiva*

Crosses	Specific combining ability effects			
	Height (cm)	Diameter (mm)	No. of leaves	Internode length (cm)
SO-1 X SO-3	0.867*	0.738**	0.309	0.124**
SO-1 X SI-6	-0.045	0.042	-0.135	0.024
SO-1 X BI-4	-0.508	-0.486**	-0.177	-0.130**
SO-1 X HA-4	-0.314	-0.293*	0.003	-0.019
SO-2 X SO-3	0.142	0.269*	0.163	0.031
SO-2 X SI-6	-0.654	-0.305*	-0.365	-0.086**
SO-2 X BI-4	-0.391	-0.220	-0.156	-0.040
SO-2 X HA-4	0.903*	-2.680**	0.358	0.096**
SO-4 X SO-3	-0.316	-0.139	-0.087	-0.001
SO-4 X SI-6	-0.454	-0.177	-0.198	-0.084**
SO-4 X BI-4	-0.216	-0.201	-0.156	-0.030
SO-4 X HA-4	0.986*	0.517**	0.441	0.115**
SO-8 X SO-3	-0.362	-0.730**	-0.295	-0.190**
SO-8 X SI-6	0.809*	0.647**	0.594*	0.210**
SO-8 X BI-4	0.380	0.660**	0.302	0.139**
SO-8 X HA-4	-0.826*	-0.576**	-0.601*	-0.158**
CH-2 X SO-3	-0.666	-0.136	-0.170	-0.019
CH-2 X SI-6	0.205	-0.125	0.135	-0.053
CH-2 X BI-4	0.150	-0.139	0.094	0.010
CH-2 X HA-4	0.311	0.400**	-0.059	0.062*
SI-15 X SO-3	0.336	-0.002	0.080	0.056
SI-15 X SI-6	0.140	-0.081	-0.031	-0.011
SI-15 X BI-4	0.586	0.387**	0.094	0.051
SI-15 X HA-4	-1.061**	-0.304*	-0.142	-0.096**
SEsi	0.385	0.117	0.261	0.031

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

effects indicating that these were good cross combination while twelve crosses exhibiting negative specific combining ability effects indicating poor cross combinations. Cross SO-8 X SI-6, SO-8 X BI-4, SO-1 X SO-3, SO-4 X HA-4 and SO-2 X HA-4 exhibited highly significant and positive specific combining ability. Other crosses that exhibited positively significant specific combining ability effects were CH-2 X HA-4, SI-15 X SO-3, SI-15 X BI-4, SO-2 X SO-3, SO-1 X SI-6 and CH-2 X BI-4. while cross CH-2 X BI-4 exhibited lowest positive specific combining ability effect for internode length.

4.2.2.1.7. Leaf area (cm²)

The table 17 revealed that estimate of degree of dominance was 1.336 and values of broad sense heritability were high (0.999). The narrow sense heritability was high for leaf area and was 0.885. Analysis of variance for the combining ability (Appendix IX) indicated that GCA and SCA variance were significant at 5 and 1% probability level for leaf area. Female parents SO-8 and SI-15 presented the highest positive GCA with high significance. While, parent SO-2 presented the highest negative GCA effect (Table 18). For female parents all showed non-significant positive general combining ability except for SO-3 which had non-significant negative general combining ability. Estimates of SCA effects were positive and differed significantly in some hybrids. Higher SCA values suggested that parents for these hybrids were more diverse than the other parents. So hybrids having large SCA values may be developed as candidate hybrid varieties. Hybrids SO-4 X HA-4, SO-8 X SI-6 and CH-2 X SO-3 gave the highest positive significant SCA effects. Whereas, crosses SO-8 X SO-3 exhibited significant and negative specific combining ability (Table 19).

Table 17. Estimation of variance components for leaf area in F₁ generation in *G. optiva*

SI No.	Variance components	Leaf area
1	Variances of gca (δ^2_g)	270.154
2	Variances of sca (δ^2_s)	180.468
3	Additive variance (D)	540.301
4	Dominance variance (H)	721.871
5	Degree of dominance	1.336
6	Heritability (ns)	0.885
7	Heritability (bs)	0.999

Table 18. General combining ability effects of different parents for various leaf area (cm²) in *G. optiva*

Parents	Leaf area
Females	
SO-1	1.805
SO-2	-6.186
SO-4	-0.293
SO-8	5.878**
CH-2	-4.620
SI-15	3.416*
Males	
SO-3	-3.110
SI-6	1.502
BI-4	1.601
HA-4	0.006
SEgi	1.518

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Table 19. Specific combining ability effects of among different crosses for leaf area in *G. optiva*

Crosses	Specific combining ability effects
	Leaf area
SO-1 X SO-3	3.871
SO-1 X SI-6	-2.591
SO-1 X BI-4	-2.309
SO-1 X HA-4	1.030
SO-2 X SO-3	0.020
SO-2 X SI-6	-3.078
SO-2 X BI-4	5.668
SO-2 X HA-4	-2.610
SO-4 X SO-3	1.496
SO-4 X SI-6	-7.027
SO-4 X BI-4	-6.561
SO-4 X HA-4	12.092**
SO-8 X SO-3	-12.453
SO-8 X SI-6	15.091**
SO-8 X BI-4	7.209*
SO-8 X HA-4	-9.846
CH-2 X SO-3	7.173*
CH-2 X SI-6	-1.075
CH-2 X BI-4	-6.260
CH-2 X HA-4	0.163
SI-15 X SO-3	-0.107
SI-15 X SI-6	-1.319
SI-15 X BI-4	2.255
SI-15 X HA-4	-0.828
SEsi	3.036

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.2.3.1. Fodder quality characters

4.2.3.1.1. Crude protein (%)

The table 20 revealed that estimate of degree of dominance was 0.421 and values of broad sense heritability was high (0.969). The narrow sense heritability was moderate for crude protein content and was 0.630. Analysis of variance for the combining ability (Appendix XVI) indicated that GCA and SCA variance were significant at 1% probability level for crude protein in nursery stage. Female parents SO-1, SO-8, SO-4 and SO-2 presented the highest positive GCA with high significance. While, parent CH-2 presented the highest negative GCA effect (Table 21). For male parents SI-6 and BI-4 presented significant and positive general combining ability. Estimates of SCA effects for crude protein were positive and differed significantly in some hybrids as shown in the table 22. Higher SCA values suggested that parents for these hybrids were more diverse in crude protein production than the parents of other hybrids. So hybrids having large SCA values may be developed as candidate hybrid varieties. Hybrids SI-15 X BI-4, SO-8 X SI-6 and SO-4 X HA-4 gave the highest positive significant SCA effects. Whereas, crosses CH-2 X HA-4, SO-1 X SO-3, CH-2 X BI-4, SO-4 X SO-3, SO-8 X BI-4 and SO-2 X HA-4 also exhibited significant and positive specific combining ability.

4.2.3.1.2. Crude fibre (%)

The estimate of degree of dominance was 0.246 with low narrow sense and high broad sense (0.474 and 0.990) was estimated for crude fibre in *G. optiva* genotypes. Analysis of variance (ANOVA) indicated significant differences were existed among parents and hybrids at $P \leq 0.01$ (Appendix XVII). Variance of GCA and SCA were significant at 1% probability level (Table 20). SO-8 had the highest positive GCA effects among female parents, while parents SI-15 and SO-1 had the highest negative GCA effect and male parents BI-4 and SI-6 presented significant and positive general combining ability for same character (Table 21). Estimates of SCA effects were positive and differed significantly in some hybrids as shown in Table 22. Hybrids SO-4 X HA-4, SI-15 X BI-4 and SO-8 X SI-6 gave the highest positive significant SCA effects while hybrids SO-1 X SO-3,

SO-2 X SO-3, SO-8 X BI-4, SO-1 X BI-4, CH-2 X HA-4, SI-15 X SI-6, CH-2 XSI-6, CH-2 X SO-3, SO-2 X SI-6 and SO-2 X HA-4 also gave highest positive significant SCA effects. Whereas, SO-4 X SI-6 hybrid presented highly significant negative SCA effect.

4.2.3.1.3. Ether extract (%)

Data appended in Table 20 revealed that estimate of degree of dominance was 0.197. Low narrow sense heritability (0.430) and high heritability broad sense (0.991) was estimated. Highly significant differences were observed among parents. Among female parents CH-2 and SO-2 recorded significant and positive general combining ability effect (Table 21). General combining ability effects were significantly positive and high in SO-3 among male parents. The data presented in table 22 revealed that twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combinations. Cross SO-8 X HA-4 exhibited highly significant and positive specific combining ability. Other crosses that exhibited positively significant specific combining ability effects were CH-2 X BI-4, CH-2 X SO-3, SO-4 X SI-6, SO-1 X SI-6, SO-4 X BI-4, SO-2 X HA-4, SI-15 X SI-6 and SO-1 X BI-4.

4.2.3.1.4. Total ash (%)

The perusal of the data appended in Table 20. The estimate of degree of dominance was 0.191. moderate values of narrow sense (0.562) and broad sense (0.658) heritability were recorded for the parameter. The differences existing among parents were non-significant. However, female parent (SO-4 and SO-8) exhibited positive value and rest of the female parents showed negative GCA effects (Table 21). General combining ability effects were non-significantly in male genotype while HA-4 and BI-4 showed positive GCA whereas all other male parents showed negative GCA effect.

Close appraisal of the data presented in Table 22 revealed that twelve out of twenty four crosses revealed positive specific combining ability effects. Highest significantly positive specific combining ability effect was depicted by SO-4 X HA-4, which was followed by SO-8 X SI-6 exhibiting positively

significant specific combining ability effects. Crosses viz., SI-15 X B-4, SO-2 X SI-6, SO-1 X SO-3, CH-2 X SO-3, SO-2 X SI-6, CH-2 X SI-6, SO-1 X B-4, SO-2 X BI-4, SO-1 X BI-4 and SO-4 X HA-4 were among those exhibiting positive specific combining ability effects. However, cross SO-4 X SI-6 exhibited highest negative SCA effects. Female and male involved in the crosses were poor general combiners.

4.2.3.1.5. Nitrogen free extract (%)

Data appended in Table 20 revealed that estimate of degree of dominance was 0.347. High narrow sense heritability and heritability broad sense (0.914 and 0.991) was estimated. Highly significant differences were observed among parents. Among female parents SI-15 and CH-2 recorded significant and positive general combining ability effect (Table 21). General combining ability effects were non-significant and positive in all males except BI-4 that showed high negative GCA. The data presented in Table 22 revealed that twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combinations. Cross SO-4 X BI-4 exhibited highly significant and positive specific combining ability followed by SO-8 X SO-3 and SO-8 X HA-4. Other crosses that exhibited positively significant specific combining ability effects were SO-2 X BI-4, SO-4 X SI-6, SI-15 X SO-3, SI-15 X HA-4, CH-2 X SI-6, SO-1 X HA-4, SI-15 X SI-6, SO-1 X SI-6, SO-2 X HA-4, SO-1 X BI-4 and CH-2 X SO-3.

Table 20. Estimation of variance components for fodder characters in *G. optiva*

Sl No.	Variance components	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Total ash (%)	Nitrogen free extract (%)
1	Variances of gca (δ^2g)	8.148	3.219	0.411	3.228	24.381
2	Variances of sca (δ^2s)	23.029	6.549	5.277	8.461	35.119
3	Additive variance (D)	32.592	12.876	1.644	12.912	24.381
4	Dominance variance (H)	46.057	13.098	10.554	16.922	70.237
5	Degree of dominance	0.421	0.246	0.197	0.191	0.347
6	Heritability (ns)	0.630	0.474	0.430	0.562	0.914
7	Heritability (bs)	0.969	0.990	0.991	0.638	0.998

Table 21. General combining ability effects of parents for fodder characters in *G. optiva*

Parents	General combining ability effects				
Females	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Total ash (%)	Nitrogen free extract (%)
SO-1	1.356**	-0.461**	-0.038**	-0.074	-0.959**
SO-2	0.500**	-0.010	0.314**	-0.513	-0.446**
SO-4	0.573**	-0.233**	-0.280**	0.402	0.110**
SO-8	0.867**	1.239**	-0.523**	0.328	-1.967**
CH-2	-1.916**	-0.016	0.659**	-0.014	1.152**
SI-15	-1.380	-0.518**	-0.133**	-0.129	2.110**
Males					
SO-3	-0.850**	-0.214**	0.408**	-0.258	0.757
SI-6	0.154**	0.117**	-0.234**	-0.216	0.085
BI-4	1.011**	0.219**	-0.151**	0.081	-1.227
HA-4	-0.315**	-0.122**	-0.022**	0.393	0.385
SEgi	0.006	0.022	0.009	0.271	0.026

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Table 22. Specific combining ability effects among different crosses for fodder characters in *G. optiva*

Crosses	Specific combining ability effects				
	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Total ash (%)	Nitrogen free extract (%)
SO-1 X SO-3	1.111**	0.713**	-0.114**	0.454	-1.987**
SO-1 X SI-6	-0.045**	-0.721**	0.125**	0.176	0.557**
SO-1 X BI-4	-0.739**	0.377**	0.062**	0.074	0.287**
SO-1 X HA-4	-0.328**	-0.369**	-0.074**	-0.704	1.143**
SO-2 X SO-3	1.076**	0.538**	0.001	0.488	-1.955**
SO-2 X SI-6	-0.111**	0.051	0.200**	0.360	-0.464**
SO-2 X BI-4	-1.013**	-0.615**	-0.289**	0.104	2.036**
SO-2 X HA-4	0.047**	0.026	0.088**	-0.952	0.383**
SO-4 X SO-3	0.478**	0.381**	-0.140**	-0.389	-0.941**
SO-4 X SI-6	-0.247**	-0.912**	0.241**	-1.545**	1.903**
SO-4 X BI-4	-2.221**	-1.381**	0.124**	-0.767	3.769**
SO-4 X HA-4	1.990**	1.913**	-0.225**	2.701**	-4.731**
SO-8 X SO-3	-0.756**	-1.092**	-0.019	-0.965	2.881**
SO-8 X SI-6	2.537**	1.359**	-0.530**	1.212**	-4.237**
SO-8 X BI-4	0.472**	0.493**	-0.263**	0.647	-1.350**
SO-8 X HA-4	-2.254**	-0.760**	0.811**	-0.893	2.706**
CH-2 X SO-3	-0.684**	0.064	0.252**	0.424	0.110*
CH-2 X SI-6	-1.689**	0.068	-0.106**	0.260	1.548**
CH-2 X BI-4	0.958**	-0.338**	0.487**	-0.749	-0.310**
CH-2 X HA-4	1.415**	0.206**	-0.633**	0.065	-1.349**
SI-15 X SO-3	-1.225**	-0.604	0.019	-0.011	1.891**
SI-15 X SI-6	-0.446**	0.156**	0.071**	-0.463	0.694**
SI-15 X BI-4	2.542**	1.463**	-0.121**	0.691	-4.432**
SI-15 X HA-4	-0.871**	-1.016**	0.032	-0.217	1.848**
SEsi	0.012	0.045	0.018	0.543	0.052

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.3. GENE ACTION

The procedure outlined by Hayman (1954, 1958), Jinks (1954, 1956) and Mather and Jinks (1971) was followed to estimate the genetic components of variation and gene action. The means of genotypes arranged in Line X Tester tables for each trait in F₁ generation are presented in Tables 23 to 54 and the Wr/Vr graphs plotted in figures 3 to 18 and Wr and Vr (Appendix XX to XXX) are discussed character wise as under.

4.3.1. Nursery studies of hybrids

4.3.1.1. Germination per cent

High germination percentage from the viable seeds are desired for success of any breeding programme, as such the parents and crosses with positive general and specific combining ability effects, respectively, should be selected for breeding programme. Results regarding the germination per cent among parents and hybrids is presented in the Table 23 and 24. The array means of female parent (Line) SI-15 had the highest array mean (67.22) followed by genotype SO-1 (62.41) and among male parent (Tester) HA-4 had higher mean value (55.07) followed by SI-6 (54.62). While female genotype SO-4 (40.36) and male parents SO-3 (51.87) had the lowest array mean with poor germination capability (Table 23).

Among hybrids the cross SI-15 X HA-4 proved to be the best combination to achieve maximum germination (72.75) whereas, SO-4 X BI-4 showed the lowest germination capability (34.25) and exhibiting SCA for poor germination capability. Figure 3 reveals that the regression line passed Wr axis above the origin, indicating recessive genes are involved in controlling the character. From the position of array points on the regression line, it is observed that genotype SO-2, SO-4, SO-8 and CH-2 had maximum dominant genes as they were close to the origin. In the light of these results, we can say that combinations of these genotype will give lower germination percent. While SI-15, SO-1 HA-4, BI-4 and SO-3 had maximum recessive genes, as it was away from the origin and these combination will exhibit good germination percent. Positive values of

correlation (0.012) indicated that recessive genes controlled the trait. The genetic components of variance in F_1 generation revealed that the dominance component (H_1) and additive component (D) were non significant. It is observed that dominant components were greater than additive components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 14.120$ being more than a unity displayed over dominance type of gene action. The negative value of F (-169.990) the value of proportion of recessive and dominant alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (19.86) revealed that the dominant alleles were frequent than recessive alleles and that they were in increasing position as exhibited by h^2 (328.296). The value of H_1 was greater than H_2 indicating that positive and negative genes were asymmetrical at loci in the parents as confirmed by the value $H_2/4H_1$ (0.004), whereas the estimated ratio of h^2/H_2 (14.55) indicated that there were fourteen groups of genes controlling the character and that genes exhibited some degree of dominance. Further, the value of r^2 (0.150) showed the incomplete dominance of parents. Heritability in narrow sense (h^2 n.s), which is the ratio of additive or additive x additive variance to the total phenotypic variance, showed high narrow sense heritability (0.976) of additive genes (Table 24).

Table 23. Mean table of 6 X 4 parental crosses of *G. optiva* for germination per cent

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	43.48	62.71	67.89	67.66	70.32	312.06	62.41
SO-2	45.83	47.87	53.80	47.89	39.51	234.91	46.98
SO-4	50.00	43.90	34.25	36.12	37.51	201.79	40.36
SO-8	45.83	45.27	46.09	53.83	65.76	256.78	51.36
CH-2	41.18	47.31	62.18	50.13	57.40	258.20	51.64
SI-15	52.94	70.58	70.32	72.75	69.51	336.09	67.22
Selfing	45.45	47.82	41.66	45.45	---	180.38	
Total	363.10	382.35	370.03	385.46	1780.2 0	319.96	
Mean	51.87	54.62	52.86	55.07			

Table 24. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for germination per cent

E	0.001 ns
D	64.335 ns
H₁	12827.020 ns
H₂	22.570 ns
F	-169.990 ns
h²	328.296 ns
Heritability (b.s)	0.982
Heritability (n.s)	0.976
Average degree of dominance	14.120
Proportion of +ve & -ve effects in parents	0.004
Proportion of dominant & recessive alleles in parents	19.86
No. of genes controlling the character	14.55
r	0.012
r²	0.1501

ns- non-significant

4.3.1.2. Survival per cent

The array means for survival per cent of seedling is presented in the Table 25. Among female parent (Line) SO-2 had the highest array mean (90.78) followed by genotype SO-8 (89.51) and among male parent (Tester) SI-6 had higher mean value (89.81). It means that these genotypes may prove better general combiners and increases the survival capability in the progeny, while female genotype CH-2 (80.75), and male parents SO-3 (83.08) had the lowest array mean. Among hybrids the cross SO-1 X BI-4 proved to be the best combination showing highest value (97.74), CH-2 X BI-4 (57.70) showed the lowest value (Table 25). Figure 4 reveals that the regression line passed Wr axis below the origin, indicating over dominance type of gene action. From the position of array points on the regression line, it is observed that genotype HA-4 and SI-6 had maximum dominant genes as they were close to the origin which implies that combinations of these genotype will give more survival rate. While CH-2, SI-15 and SO-1 had maximum recessive genes, as it was away from the origin.

Positive values of correlation (0.011) indicated that recessive genes controlled the trait. The genetic components of variance in F₁ generation revealed that the dominance component (H₁) and additive component (D) were

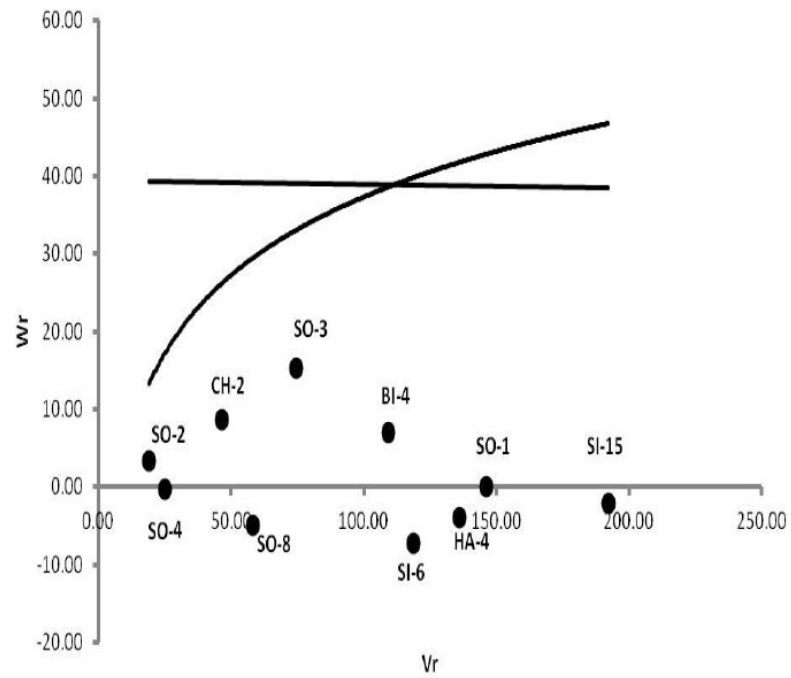


Figure 3. W_r/V_r graph for germination per cent

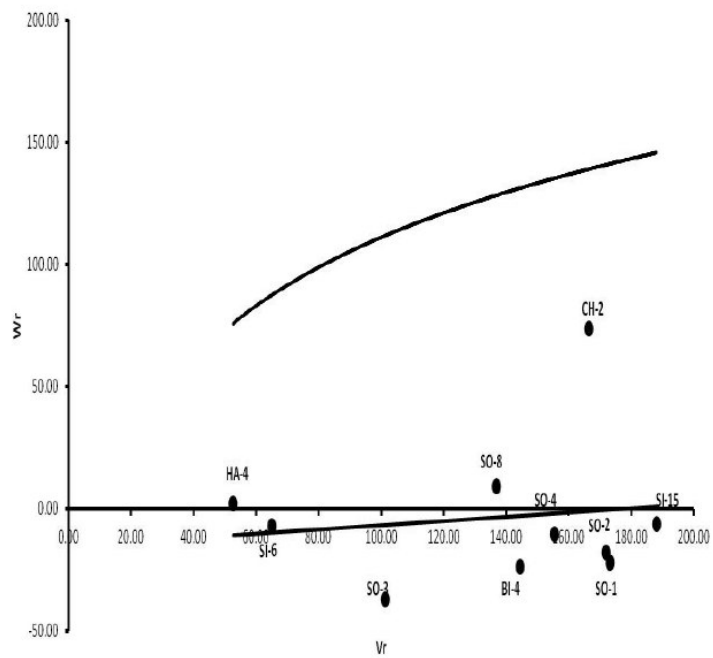


Figure 4. W_r/V_r graph for survival per cent

non significant. It is observed that dominant components were more than additive components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 8.574$ being more than a unity displayed over dominance type of gene action, which was also confirmed in W_r/V_r graph. The negative and non significant value of F (-3453.410) the value of proportion of recessive and dominant alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (11.703) revealed that the dominant alleles were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (424.470). The value of H_1 was greater than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ (0.02), whereas the estimated ratio of h^2/H_2 (0.860) indicated that there were one groups of genes controlling the character and that genes exhibited higher degree of dominance. Further, the value of r^2 (0.017) showed the incomplete dominance of parents. Heritability in narrow sense (h^2 n.s), which is the ratio of additive or additive x additive variance to the total phenotypic variance, showed high heritability (0.885) of additive genes (Table 26).

Table 25. Mean table of 6 X 4 parental crosses of *G. optiva* for survival per cent

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	89.21	94.48	97.74	82.30	80.00	443.73	88.75
SO-2	91.31	95.26	95.66	80.74	90.91	453.88	90.78
SO-4	66.70	92.34	92.35	88.88	87.50	427.77	85.55
SO-8	91.66	86.98	85.75	92.24	90.91	447.54	89.51
CH-2	75.40	93.49	57.70	91.46	85.71	403.77	80.75
SI-15	97.27	93.40	93.77	87.76	66.67	438.86	87.77
Selfing	70.00	72.73	60.00	73.33	---	276.06	
Total	581.55	628.67	582.98	596.72	581.55	2891.62	
Mean	83.08	89.81	83.28	85.25			

Table 26. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for survival per cent

E	0.008ns
D	72.190 ns
H₁	5307.611 ns
H₂	489.951 ns
F	-3453.410 ns
h²	424.470 ns
Heritability (b.s)	0.999
Heritability (n.s)	0.885
Average degree of dominance	8.574
Proportion of +ve & -ve effects in parents	0.020
Proportion of dominant & recessive alleles in parents	11.703
No. of genes controlling the character	0.866
r	0.011
r²	0.017

ns- non-significant

4.3.1.3. Plant height

Results regarding to plant height among parents and hybrids is presented in the Table 27 to 30 for two and six months seedling. The array means of plant height for two and six months seedling, female parent (Line) SO-8 had the highest array mean (6.60 cm and 15.75 cm) followed by genotype SO-4 (6.45 cm and 14.86 cm) and among male parent (Tester) BI-4 had higher mean value (6.61 cm and 15.79 cm) followed by SI-6 (6.45 cm and 15.60 cm). It means that these genotypes may prove better general combiners and served as a better parents for tallness, while female genotype SO-1 (6.03 cm and 13.76) and SI-15 (6.09 cm and 14.23 cm), and male parents SO-3 (6.48 and 15.08 cm) had the lowest array mean indicating better general combining ability for dwarfness (Table 27 and 29).

Among hybrids the cross SO-8 X SI-6 proved to be the best combination both at two and six months seedling showing highest value (7.24 cm and 18.05 cm) for tallness whereas, SO-1 X BI-4 and SI-15 X HA-4 showed the lowest value (6.13 cm) in two months observation and SO-1 X HA-4 (14.25 cm) exhibiting SCA for dwarfness (Table 27 and 29). Figure 5 reveals that the regression line passed Wr axis below the origin, indicating over dominance type of gene action. From the position of array points on the regression line, it is

observed that genotype SO-1, SO-2, SI-15, CH-2 and SO-4 had maximum dominant genes as they were close to the origin. In the light of these results we can say that combinations of these genotype will produce tallest plants. While BI-4, SI-6 and HA-4 had maximum recessive genes, as it was away from the origin. These results exhibited that BI-4, SI-6 and HA-4 combination will produce dwarf plants. Positive values of correlation (0.185) indicated that recessive genes controlled the trait.

The genetic components of variance for plant height (two months observation) in F_1 generation revealed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components were greater than additive components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 1.664$ being more than a unity displayed over dominance type of gene action, which was also confirmed in W_r/V_r graph (Figure 5). Positive values of correlation (0.185) indicated that recessive genes controlled the trait. The positive and highly significant value of F (0.901) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.998) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (3.203). The value of H_1 was less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ (0.180), whereas the estimated ratio of h^2/H_2 (3.180) indicated that there were at least three groups of genes controlling plant height and that genes exhibited some degree of dominance. Further, the value of r^2 (0.864) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), which is the ratio of additive or additive x additive variance to the total phenotypic variance, showed low heritability (0.081) at two months of growth (Table 28).

The regression line passed W_r axis below the origin, indicating over dominance type of gene action (Figure 6) for six month observation. From the position of array points on the regression line, it is observed that genotype SO-1, SO-2, SO-4 and CH-2 had maximum dominant genes as they were close to the origin. In the light of these results we can say that including these genotype and

the combinations of these will produce tallest plants. While BI-4 had maximum recessive genes, as it was away from the origin. These results exhibited that combination of BI-4 will produce dwarf plants. Negative values of correlation (-0.504) indicated that dominant genes controlled the trait. The genetic components of variance for plant height in F₁ generation revealed that the dominance component (H₁) and additive component (D) were non-significant. Dominant components were greater than additive components. H₁ value being not equal to H₂ showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 5.189$ being more than a unity displayed over dominance type of gene action, which was also confirmed in Wr/Vr graph. The positive and non-significant value of F (10.556) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (24.907) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h² (60.488). The value of H₁ was greater than H₂ indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents (Table 30). As confirmed by the value H₂/4H₁ (0.181), whereas the estimated ratio of h² /H₂ (3.810) indicated that there were at least three to four groups of genes controlling plant height and that genes exhibited some degree of dominance. Further, the value of r² (0.771) showed the complete dominance of parents. Heritability in narrow sense (h² n.s), which is the ratio of additive or additive x additive variance to the total phenotypic variance, showed high heritability (0.965) of additive genes.

Table 27. Mean table of 6 X 4 parental crosses of *G. optiva* for plant height (two months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	6.63	6.37	6.13	6.16	4.88	30.16	6.03
SO-2	6.37	6.41	6.46	6.57	6.31	32.11	6.42
SO-4	6.42	6.49	6.73	7.03	5.60	32.26	6.45
SO-8	6.63	7.24	7.12	6.75	5.25	32.98	6.60
CH-2	6.55	6.46	6.72	6.63	5.07	31.43	6.29
SI-15	6.35	6.43	6.94	6.13	4.60	30.45	6.09
Selfing	6.43	6.29	6.18	6.14	---	25.05	
Total	45.37	45.68	46.28	45.41	37.88	214.43	
Mean	6.48	6.53	6.61	6.49			

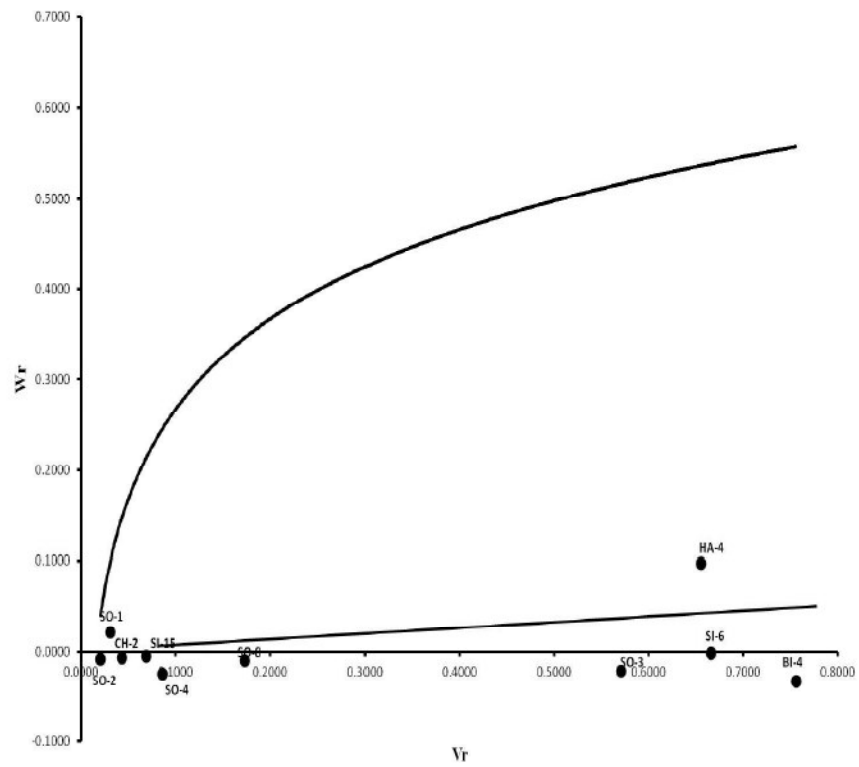


Figure 5. W_r/V_r graph for plant height (two months)

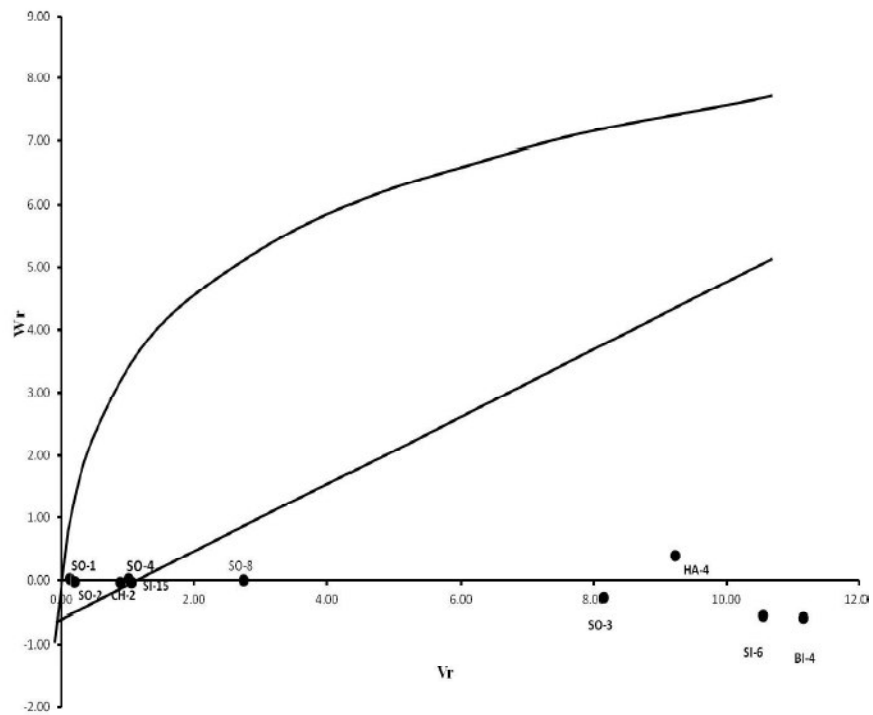


Figure 6. W_r/V_r graph for plant height (six months)

Table 28. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for plant height (two months)

E	0.014**
D	0.294**
H ₁	0.824**
H ₂	1.007**
F	0.901**
h ²	3.203**
Heritability (b.s)	0.105
Heritability (n.s)	0.081
Average degree of dominance	1.664
Proportion of +ve & -ve effects in parents	0.180
Proportion of dominant & recessive alleles in parents	0.998
No. of genes controlling the character	3.180
r	0.185
r ²	0.864

** Significant at 1 per cent level of significance

Table 29. Mean table of 6 X 4 parental crosses of *G. optiva* for plant height (six months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	15.13	14.84	14.60	14.25	9.98	68.81	13.76
SO-2	14.44	14.27	14.75	15.50	12.33	71.29	14.26
SO-4	15.20	15.68	16.14	16.80	10.49	74.31	14.86
SO-8	16.26	18.05	17.84	16.09	10.47	78.71	15.74
CH-2	14.62	16.11	16.28	15.89	9.23	72.13	14.43
SI-15	15.63	16.05	16.72	14.53	8.21	71.13	14.23
Selfing	14.31	14.23	14.22	14.15	---	56.90	
Total	105.59	109.23	110.54	107.21	31.71	493.28	
Mean	15.08	15.60	15.79	15.32			

Table 30. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for plant height (six months)

E	0.148 ns
D	3.388 ns
H ₁	91.908 ns
H ₂	15.875 ns
F	10.556 ns
h ²	60.486 ns
Heritability (n.s)	0.965
Heritability (b.s)	0.996
Average degree of dominance	5.189
Proportion of +ve & -ve effects in parents	0.043
Proportion of dominant & recessive alleles in parents	24.907
No. of genes controlling the character	3.810
r	-0.504
r ²	0.771

ns- non-significant

4.3.1.4. Collar diameter (mm)

Data presented in Table 31 and 33 show that array means for collar diameter of female parent (Line) SO-8 had the highest array mean (2.60 mm and 3.31 mm) for two and six month data and among male parent (Tester) BI-4 had higher mean value (2.47 mm) followed by SI-6 (2.39 mm) for two months collar diameter. Parent SO-3 and SI-6 showed 3.09 mm and 3.02 mm for six months seedling observation. It means that these genotypes can serve as a better general combiners for higher collar diameter growth. Whereas for two months observation female genotype SO-1 (2.04 mm) and SO-2 (2.22 mm), male parents HA-4 (2.33 mm) had the lowest array mean indicating better general combining ability for lower diameter production. In six months observation line SI-15 (2.50 mm) and tester HA-4 (2.54 mm) showed lesser array mean which implies as a good general combiner for poor diameter growth (Table 33).

The cross SO-8 X SI-6 (2.97 mm and 4.41 mm) proved to be the best combination showing higher value for collar diameter both in two and six months seedling data. Cross SI-15 X HA-4 and SO-1 X HA-4 showed the lowest value (1.93 mm and 1.94 mm) for two months observation while for six months data SO-8 X HA-4 (2.11 mm) exhibiting specific combining ability for less diameter growth (Table 31 and 33).

The W_r/V_r graph (Figure 7) reveals that the regression line passed W_r axis below the origin, indicating over dominance type of gene action for both two and six months seedling observation. In the early stage of growth the position of array points on the regression line, genotype SO-2, CH-2 and SO-3 had maximum dominant genes as they were close to the origin. These results indicate that including these genotype and the combinations of these will produce desired diameter in plants. While SO-8, BI-4, SO-1, and HA-4 had maximum recessive genes, as it was away from the origin. These results exhibited that including these genotype will produce thin plants. Negative correlation (-0.417) indicated that dominant genes were controlled the trait. The genetic components of variance for diameter in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It further showed that dominant

components were lesser than additive components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 0.793$ being less than a unity displayed recessive type of gene action. The positive and highly significant value of F (0.030) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.627) revealed that the recessive alleles were frequent than dominant alleles and that they were in increasing position as exhibited by h^2 (0.971). The value of H_1 was less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ (0.177), whereas the estimated ratio of h^2/H_2 (3.45) indicated that there were at least four groups of genes controlling diameter growth and that genes exhibited some degree of dominance. Further, the value of r^2 (0.976) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed moderate heritability (0.608) of additive genes (Table 32).

The position of array points on the regression line (Figure 8) in six months seedling data, it showed that genotype HA-4, CH-2 and SO-4 had maximum dominant genes as they were close to the origin. These results indicate that include these genotype and the combinations to produce desired diameter in plants. While SO-1 and SO-8 had maximum recessive genes, as it was away from the origin and results exhibited that including these genotype (SO-1 and SO-8) will produce dwarf plants. Negative correlation (-0.238) indicated that dominant genes were controlled the trait. The genetic components of variance for diameter in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It is further suggested that additive components were greater than dominant components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 1.224$ being more than a unity displayed over dominance type of gene action. The positive and highly significant value of F (0.311) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.077) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (1.726). The value of H_1 was less than H_2 indicating that positive

and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ (0.199), whereas, the estimated ratio of h^2/H_2 (1.810) indicated that there were at least one to two groups of genes controlling diameter growth and that genes exhibited some degree of dominance. Further, the value of r^2 (0.0015) showed partial dominance of parents. Heritability in narrow sense (h^2 n.s), showed high heritability (0.941) of additive genes (Table 34).

Table 31. Mean table of 6 X 4 parental crosses of *G. optiva* for diameter (two months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	2.40	2.08	1.95	1.94	1.83	10.21	2.04
SO-2	2.23	2.27	2.26	2.31	2.02	11.09	2.22
SO-4	2.36	2.40	2.66	2.96	1.94	12.32	2.46
SO-8	2.47	2.97	2.91	2.77	1.87	12.99	2.60
CH-2	2.48	2.49	2.66	2.43	1.87	11.93	2.39
SI-15	2.39	2.45	2.93	1.93	1.78	11.47	2.29
Selfing	2.27	2.07	1.95	1.94	---	8.22	
Total	16.58	16.73	17.32	16.30	78.22	14.00	
Mean	2.37	2.39	2.47	2.33			

Table 32. Estimation of genetic component of variation in 6 X 4 parental crosse of *G. optiva* for diameter (two months)

E	0.006 ^{**}
D	0.031 ^{**}
H1	0.021 ^{**}
H2	0.282 ^{**}
F	0.030 ^{**}
h^2	0.971 ^{**}
Heritability (b.s)	0.910
Heritability (n.s)	0.608
Average degree of dominance	0.793
Proportion of +ve & -ve effects in parents	0.177
Proportion of dominant & recessive alleles in parents	0.627
No. of genes controlling the character	3.450
r	-0.417
r^2	0.976

^{**} Significant at 1 per cent level of significance

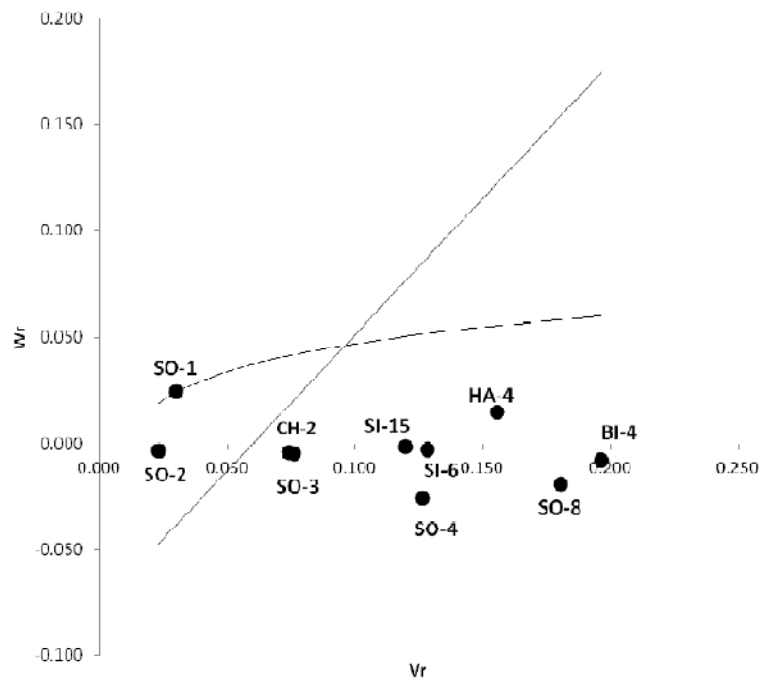


Figure 7. W_r/V_r graph for collar diameter (two months)

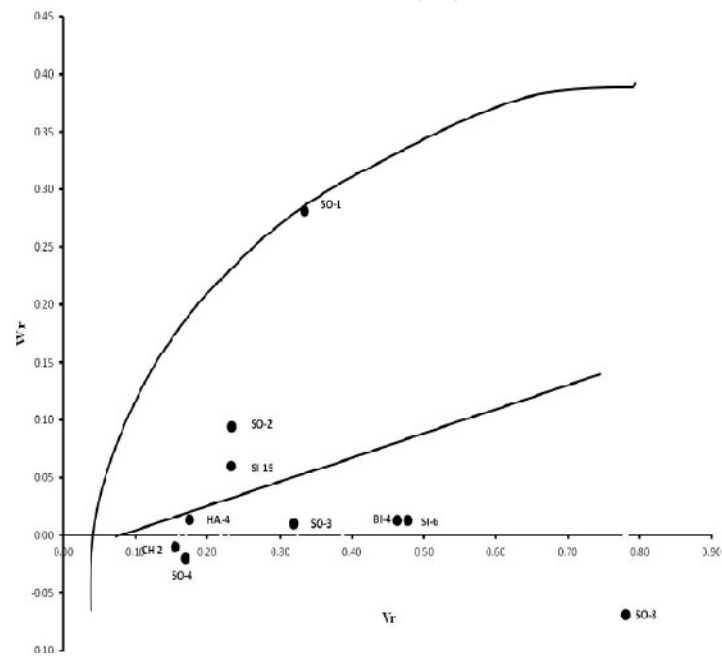


Figure 8. W_r/V_r graph for collar diameter (six months)

Table 33. Mean table of 6 X 4 parental crosses of *G. optiva* for collar diameter (six months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	selfing	Total	Mean
SO-1	3.56	2.92	2.30	2.11	2.04	12.93	2.59
SO-2	3.42	2.90	2.90	2.98	2.19	14.40	2.88
SO-4	2.80	2.82	2.71	3.03	2.07	13.42	2.68
SO-8	2.98	4.41	4.34	2.71	2.11	16.55	3.31
CH-2	2.75	2.80	2.71	2.85	2.01	13.12	2.62
SI-15	2.72	2.69	3.07	1.99	2.01	12.48	2.50
Selfing	3.39	2.62	2.25	2.09	---	10.35	
Total	21.63	21.15	20.28	17.77	12.43	93.26	
Mean	3.09	3.02	2.90	2.54			

Table 34. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for collar diameter (six months)

E	0.014 ^{**}
D	0.178 ^{**}
H ₁	0.341 ^{**}
H ₂	0.954 ^{**}
F	0.311 ^{**}
h ²	1.726 ^{**}
Heritability (b.s)	0.998
Heritability (n.s)	0.941
Average degree of dominance	1.224
Proportion of +ve & -ve effects in parents	0.199
Proportion of dominant & recessive alleles in parents	0.837
No. of genes controlling the character	1.810
r	-0.238
r ²	0.002

^{**} Significant at 1 per cent level of significance

4.3.1.5. Number of leaves

The perusal of the data appended in Table 35 for early stages of seedlings show that array means for number of leaves in female parent (Line) SO-8 had the higher (6.37) followed by genotype CH-2 (6.33) and in male parent (Tester) BI-4 showed higher mean value (6.43) followed by SO-3 (6.40). It means that these genotypes can serve as better general combiners for higher production of leaves. Whereas female genotype SO-1 (5.92) and male parent SI-6 (8.07) had the lowest array mean indicating better general combining ability for less number of leaves production. The cross SO-8 X SI-6 proved to be the best combination showing

highest leaf number (7.08) whereas, cross SI-15 X HA-4 (5.83) exhibited specific combining ability for less number of leaf emergence.

The W_r/V_r graph (Figure 9) showed that the regression line passed W_r axis below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-2, CH-2, SI-15, SO-4 and SO-1 had maximum dominant genes as they were close to the origin. These results indicate that including these genotype and the combinations of these will produce more number of leaves in plants. While SI-6, BI-4, HA-4 and SO-3 had maximum recessive genes, as it was away from the origin. These outcome exhibited that including these genotype (SI-6, BI-4, HA-4 and SO-3) will produce minimum number of leaves. Negative correlation (-0.568) indicated that dominant genes were controlled the trait. The genetic components of variance for number of leaf production in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components were more than additive components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 1.591$ being more than a unity displayed over dominance type of gene action. The positive and highly significant value of F (1.380) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.990) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (2.120). The value of H_1 was less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ (0.193), whereas, the estimated ratio of h^2/H_2 (1.690) indicated that there were at least two groups of genes controlling the production of leaves and that genes exhibited some degree of dominance. Further, the value of r^2 (0.835) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed low heritability (0.550) of additive genes (Table 36).

Table 37 show that array means for number of leaves for later stages (six month old seedling) in female parent (Line) SO-8 had the higher (8.45) followed

by genotype SO-4 (8.18) and in male parent (Tester) SI-6 showed higher mean value (8.26) followed by BI-4 (8.24). It means that the genotypes can serve as better general combiners for higher production of leaves. Whereas female genotype SO-1 (7.73) and male parent SO-3 (8.07) had the lowest array mean indicating better general combining ability for less number of leaves production. The hybrid SO-8 X SI-6 proved to be the good combiner showing highest leaf number (9.42) and the hybrid CH-2 X SO-3 (7.67) exhibited specific combining ability for less leaf production. The Figure 10 showed that the regression line passed Wr axis below the origin, indicating over dominance type of gene action. It indicates that the genotype SO-1, CH-2, SO-2, SI-15 and SO-4 had maximum dominant genes as they were close to the origin. These results indicate that including these genotype and the combinations of these will produce more number of leaves in plants. While SI-6, BI-4 and HA-4 had maximum recessive genes, as it was away from the origin. These results exhibited that including these genotype (SO-1 and SO-8) will produce minimum number of leaves. Negative correlation (-0.311) indicated that dominant genes were controlled the trait. The genetic components of variance for number of leaf production in F₁ generation showed that the dominance component (H₁) and additive component (D) were highly significant. It is observed that dominant components were more than additive components. H₁ value being not equal to H₂ showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 1.388$ being more than a unity displayed over dominance type of gene action. The positive and highly significant value of F (0.666) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.985) revealed that the recessive alleles were frequent than dominant alleles and that they were in increasing position as exhibited by h² (1.932). The value of H₁ was less than H₂ indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents (Table 38) as confirmed by the value H₂/4H₁ (0.153), whereas, the estimated ratio of h² /H₂ (2.744) indicated that there were at least two to three groups of genes controlling the production of leaves and that genes exhibited some degree of dominance. Further, the value of r² (0.647) showed the complete dominance of parents. Heritability in narrow sense (h² n.s), showed high heritability (0.982) of additive genes.

Table 35. Mean table of 6 X 4 parental crosses of *G. optiva* for number of leaves (two months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	6.50	5.92	5.92	5.92	5.33	29.58	5.92
SO-2	6.25	6.08	6.17	6.33	6.58	31.42	6.28
SO-4	6.17	6.33	6.58	6.83	5.00	30.92	6.18
SO-8	6.67	7.08	6.92	6.83	4.33	31.83	6.37
CH-2	6.42	6.08	6.67	6.50	6.00	31.67	6.33
SI-15	6.00	6.17	6.75	5.83	4.38	29.13	5.83
Selfing	6.79	6.00	6.00	6.00	---	24.79	
Total	44.79	43.67	45.00	44.25	31.69	209.33	
Mean	6.40	6.24	6.43	6.32			

Table 36. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for number of leaves (two months)

E	0.030**
D	0.422**
H1	1.069**
H2	1.254**
F	1.380**
h ²	2.120**
Heritability (b.s)	0.679
Heritability (n.s)	0.550
Average degree of dominance	1.591
Proportion of +ve & -ve effects in parents	0.193
Proportion of dominant & recessive alleles in parents	0.990
No. of genes controlling the character	1.690
r	0.568
r ²	0.835

** Significant at 1 per cent level of significance

Table 37. Mean table of 6 X 4 parental crosses of *G. optiva* for number of leaves (six months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	8.25	8.00	8.00	8.08	6.33	38.67	7.73
SO-2	8.00	7.67	7.92	8.33	8.00	39.92	7.98
SO-4	8.08	8.17	8.25	8.75	7.63	40.88	8.18
SO-8	8.33	9.42	9.17	8.17	7.17	42.25	8.45
CH-2	7.67	8.17	8.17	7.92	7.58	39.50	7.90
SI-15	8.17	8.25	8.42	8.08	6.63	39.54	7.91
Selfing	8.00	8.13	7.75	8.06	---	24.79	
Total	56.50	57.79	57.67	57.39	31.93	272.68	
Mean	8.07	8.26	8.24	8.20			

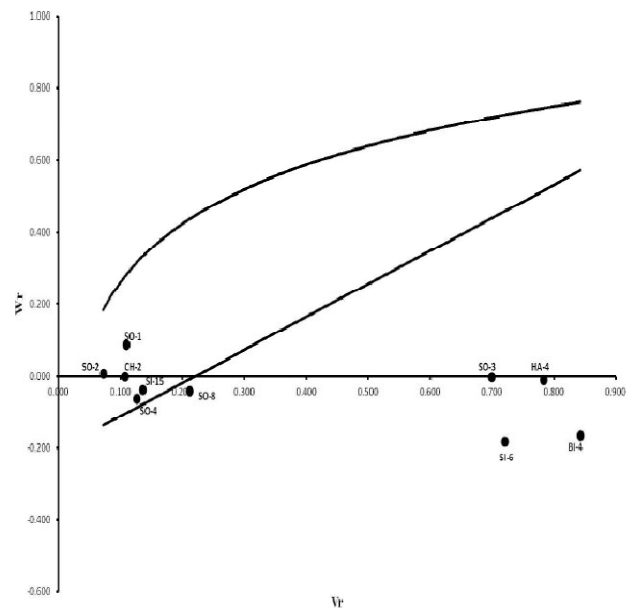


Figure 9. W_v/V_r graph for number of leaves (two months)

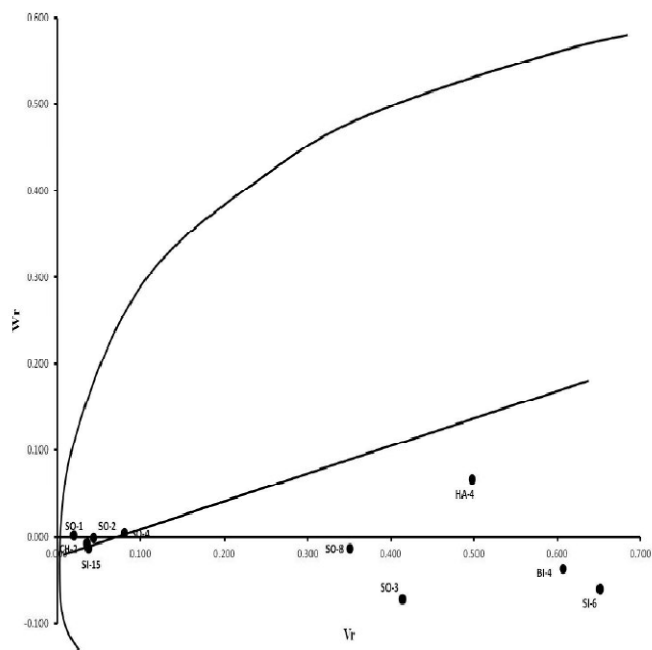


Figure 10. W_v/V_r graph for number of leaves (six months)

Table 38. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for number of leaves (six months)

E	0.068 ^{**}
D	0.252 ^{**}
H₁	0.498 ^{**}
H₂	0.704 ^{**}
F	0.666 ^{**}
h²	1.932 ^{**}
Heritability (b.s)	0.999
Heritability (n.s)	0.982
Average degree of dominance	1.388
Proportion of +ve & -ve effects in parents	0.362
Proportion of dominant & recessive alleles in parents	0.985
No. of genes controlling the character	2.744
r	-0.311
r²	0.647

^{**}Significant at 1 per cent level of significance

4.3.1.6. Internodel length (cm)

Cursory glance at the data presented in Table 39 and 41 shows that for early and later stages (two and six months) the array means for internodel length in female parent (Line) SO-8 had the higher (1.34 cm and 1.96 cm). For male parent (Tester) BI-4 showed higher mean value (1.34 cm) followed by SO-3 and SI-6 (1.24 cm) for two months reading, genotype SI-6 showed 1.88 cm for six month data. It means that the genotypes can serves as better general combiners for more internodel length. Whereas for both the stages of growth, female genotype SO-1 (1.09 cm and 1.68 cm) and male parent HA-4 (1.21 cm and 1.78 cm) had the lowest internodel length indicating better general combining ability for low internodel length. The hybrid SO-8 X SI-6 and SO-8 X BI-4 proved to be the best combination showing higher internodel length (1.51cm and 1.48 cm) for two months data whereas, the same hybrids prove to be a good combiner for six months observation showing 2.34 cm and 2.27cm for increased internodel length. SO-1 X HA-4 and SI-15 X HA-4 (1.00 cm) (two months data) while SO-X BI-4 and SO-1 X HA-4 (1.64 cm and 1.66 cm) is proved to be good specific combiner for less internodel growth (Table 39 and 41).

In the Figure 11 (two months) where the regression line is completely below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-2 and SO-1 had maximum dominant genes as they were close to the origin. These indicate that including these genotype and the combinations of these will produce increased internodel length in plants. While SI-6, SO-8, HA-4 and BI-4 had maximum recessive genes, as it was away from the origin, including these genotype will produce lesser internodel length in the plants. Negative correlation (-0.085) indicated that dominant genes were controlled the trait. The genetic components of variance for internodel length in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components was more than additive components. H_1 value being at par to H_2 showed almost equal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 0.864$ being less than a unity displayed partial dominance type of gene action. The positive and highly significant value of F (0.017), the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F / [1/4(4DH_1)]^{1/2} - 1/2F$ (0.994) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (0.199). The value of H_1 was more than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents and confirmed by the value $H_2/4H_1$ (0.029). The estimated ratio of h^2/H_2 (3.395) indicated that there were at least three groups of genes controlling the production of internodel length and that genes exhibited some degree of dominance. Further, the value of r^2 (0.646) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed low heritability (0.076) (Table 40).

The W_r/V_r graph (Figure 12) showed that the regression line passed W_r axis below the origin, indicating over dominance type of gene action. The position of array points on the regression line, the genotype SO-2, CH-2, SO-4 and SI-15 had maximum dominant genes as they were close to the origin. These indicated that including these genotype and the combinations of these will

produce increased internode length in plants. While SI-6, BI-4, SO-8, SO-1 and HA-4 had maximum recessive genes, as it was away from the origin, including these genotype will produce lesser internode length in the plants. Negative correlation (-0.421) indicated that dominant genes controlled the trait. The genetic components of variance for internode length in F_1 generation showed that the dominance component (H_1) and additive component (D) were non-significant. It is observed that dominant components were more than additive components. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 1.121$ being more than a unity displayed over dominance type of gene action. The positive and highly significant value of F (0.037) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (1.052) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (0.238). The value of H_1 was less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents (Table 42). Confirmation by the value $H_2/4H_1$ (0.100), whereas the estimated ratio of h^2/H_2 (2.957) indicated that there were at least three groups of genes controlling the production of leaves and that genes exhibited some degree of dominance. Further, the value of r^2 (0.246) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed low heritability (0.145).

Table 39. Mean table of 6 X 4 parental crosses of *G. optiva* for internode length (cm) (two months)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	1.22	1.10	1.20	1.00	0.93	5.44	1.09
SO-2	1.20	1.22	1.18	1.23	1.08	5.91	1.18
SO-4	1.22	1.24	1.30	1.43	1.00	6.19	1.24
SO-8	1.30	1.51	1.48	1.43	0.97	6.69	1.34
CH-2	1.27	1.26	1.32	1.35	1.00	6.19	1.24
SI-15	1.28	1.23	1.43	1.00	0.95	5.88	1.18
Selfing	1.18	1.11	1.20	1.00	---	4.50	
Total	8.66	8.66	9.11	8.44	5.93	40.80	
Mean	1.24	1.24	1.30	1.21			

Table 40. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for internodal length (cm) (two months)

E	0.001**
D	0.010**
H1	0.059**
H2	0.008**
F	0.017**
h ²	0.199**
Heritability (b.s)	0.087
Heritability (n.s)	0.076
Average degree of dominance	0.864
Proportion of +ve & -ve effects in parents	0.029
Proportion of dominant & recessive alleles in parents	0.994
No. of genes controlling the character	3.395
r	-0.085
r ²	0.646

** Significant at 1 per cent level of significance

Table 41. Mean table of 6 X 4 parental crosses of *G. optiva* for internodal length (cm) (six months)

Testers	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
Lines							
SO-1	1.80	1.80	1.64	1.66	1.48	8.38	1.68
SO-2	1.78	1.76	1.80	1.84	1.75	8.93	1.79
SO-4	1.82	1.83	1.88	1.93	1.50	8.97	1.79
SO-8	1.84	2.34	2.27	1.88	1.48	9.80	1.96
CH-2	1.74	1.81	1.87	1.83	1.50	8.74	1.75
SI-15	1.81	1.84	1.90	1.66	1.46	8.67	1.73
Selfing	1.80	1.80	1.62	1.65	---	6.86	
Total	12.58	13.18	12.98	12.44	10.70	60.34	
Mean	1.80	1.88	1.85	1.78			

Table 42. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for internodal length (cm) (six months)

E	0.001ns
D	0.016ns
H1	0.020ns
H2	0.080ns
F	0.037ns
h ²	0.238ns
Heritability (b.s)	0.087
Heritability (n.s)	0.076
Average degree of dominance	1.121
Proportion of +ve & -ve effects in parents	0.100
Proportion of dominant & recessive alleles in parents	1.052
No. of genes controlling the character	2.957
r	-0.421
r ²	0.246

ns- non-significant

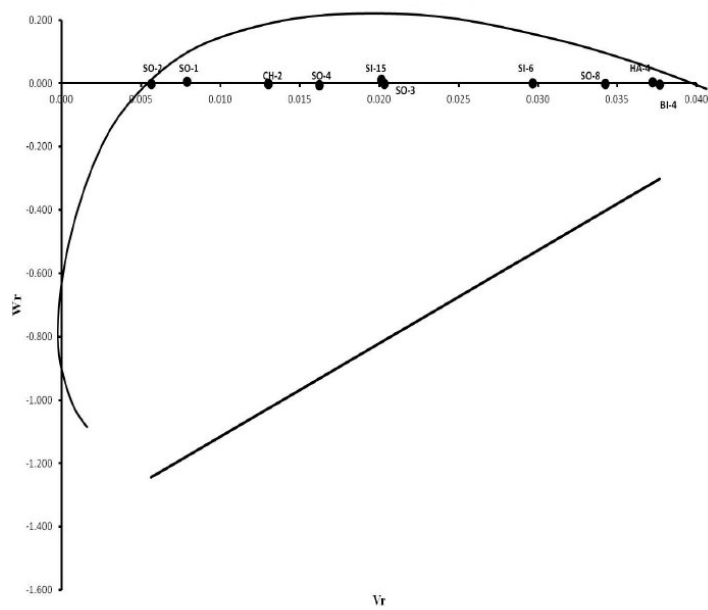


Figure 11. W_r/V_r graph for internode length (two months)

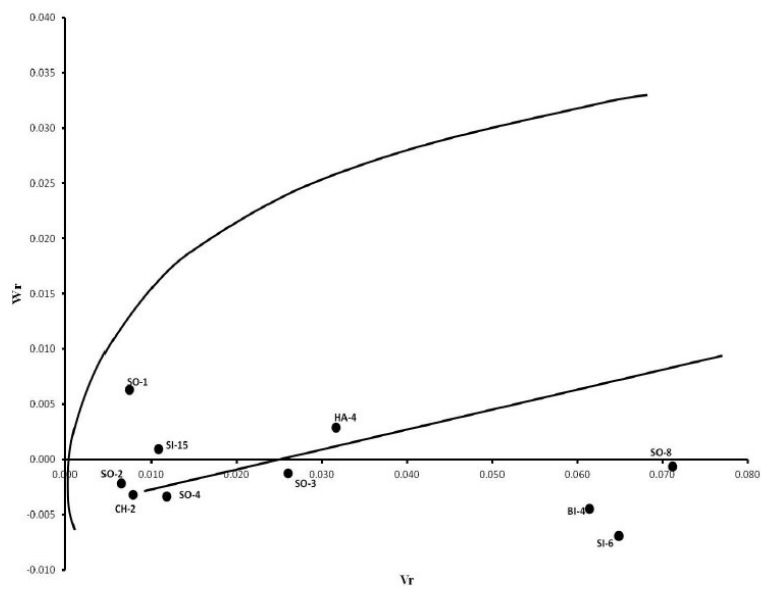


Figure 12. W_r/V_r graph for internode length (six months)

4.3.1.7. Leaf area (cm²)

The data in Table 43 shows that array means for leaf area (cm²) in female parent (Line) SO-8 and SI-15 had the higher value (34.28 and 33.03) and in male parent (Tester) BI-4 and SI-6 showed higher mean value (33.17 and 31.98). It means that the genotypes can serve as better general combiners. Whereas female genotype SO-2 (25.59) and male parent SO-3 (28.16) had the lowest value indicating better general combining ability for smaller leaf area production.

The hybrid SO-8 X SI-6 and SO-8 X BI-4 proved to be the best combination showing more leaf area (53.90 and 46.12). The Wr/Vr graph (Figure 13) showed that the regression line is below the origin, indicating over dominance type of gene action. The position of array points on the regression line, describes that genotype SO-1 SO-3 and SI-15 had maximum dominant genes as they were above the regression line and close to the origin. Whereas SO-8, SI-6, BI-4 and SO-4 had maximum recessive genes, as it was away from the origin. These genotype will result in less leaf development in plants. Positive correlation (0.564) suggest that recessive genes were controlled the trait. The genetic components of variance in F₁ generation showed that the dominance component (H₁) and additive component (D) were non-significant. It is observed that dominant components were higher magnitude than additive components. H₁ value being more than H₂ showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 20.20$ being more than a unity displayed over dominance type of gene action. The negative value of F (-340.105) the value of proportion of recessive and dominant alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (80.97) revealed that the recessive alleles were frequent than dominant alleles and that they were in increasing position as exhibited by h² (80.677). The value of H₁ is more than H₂ indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value H₂/4H₁ (0.005), whereas the estimated ratio of h² /H₂ (14.55) indicated that there is fourteen set of gene controlling the production which exhibited complete degree of dominance. Further, the value of r² (0.020)

showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed moderate heritability (0.566) (Table 44).

Table 43. Mean table of 6 X 4 parental crosses of *G. optiva* for leaf area (cm²)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	33.99	32.14	32.53	34.27	26.38	159.32	31.86
SO-2	22.15	23.67	32.51	22.64	27.00	127.97	25.59
SO-4	29.52	25.61	26.18	43.23	26.47	151.01	30.20
SO-8	21.74	53.90	46.12	27.47	22.15	171.38	34.28
CH-2	30.87	27.24	22.15	26.98	25.83	133.07	26.61
SI-15	31.63	35.03	38.70	34.02	25.79	165.17	33.03
Selfing	27.19	26.28	34.02	27.33	---	114.82	
Total	197.11	223.87	232.20	215.94	153.62	1022.75	
Mean	28.16	31.98	33.17	30.85			

Table 44. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for leaf area

E	9.220ns
D	11.641ns
H ₁	5435.724ns
H ₂	91.225ns
F	-340.105ns
h^2	80.677ns
Heritability (b.s)	0.996
Heritability (n.s)	0.982
Average degree of dominance	20.20
Proportion of +ve & -ve effects in parents	0.005
Proportion of dominant & recessive alleles in parents	80.970
No. of genes controlling the character	0.880
r	0.564
r^2	0.020

ns- non-significant

4.3.2. Fodder Quality parameter

4.3.2.1. Crude fat (ether extract) (%)

The data in Table 45 shows that array means for crude fat (ether extract) (%) in female parent (Line) CH-2 had the higher value (5.94 %) and in male parent (Tester), SO-3 showed higher mean value (5.74 %). It means that the genotypes can serves as better general combiners for more crude fat (ether extract) per cent. Whereas female genotype SO-8 (4.88 %) and male parent SI-6

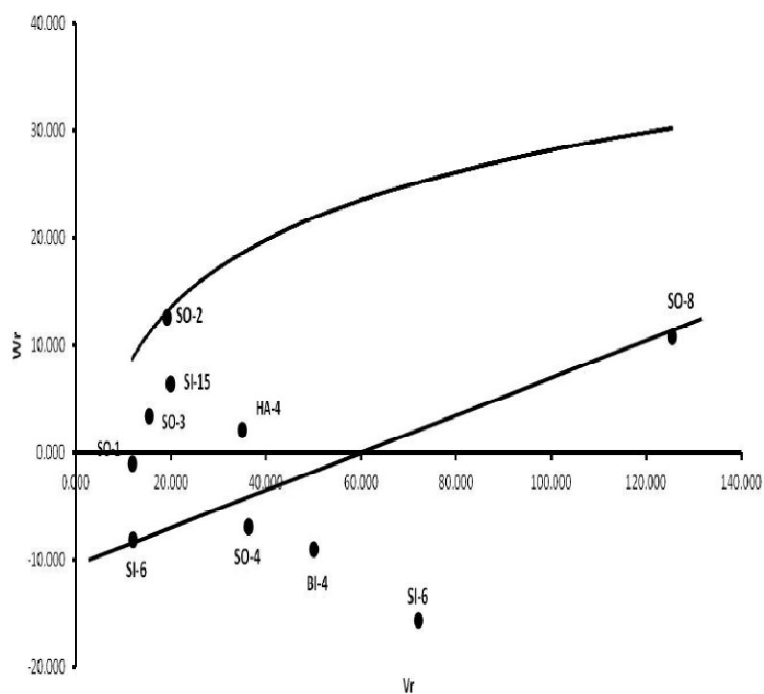


Figure 13. W_r/V_r graph for leaf area (cm^2)

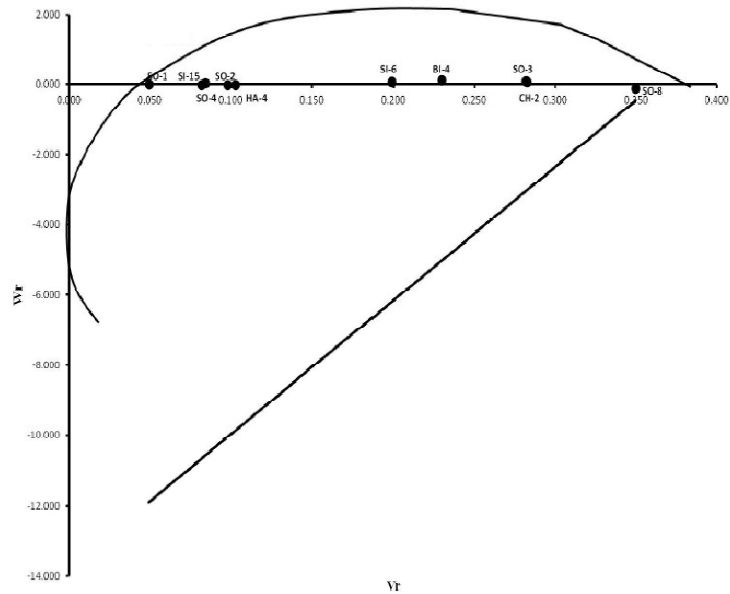


Figure 14. W_r/V_r graph for crude fat (ether extract) (%)

(5.21 %) had the lowest value indicating better general combining ability for low crude fat (ether extract) per cent.

The hybrid CH-2 X SO-3 and CH-2 X BI-4 proved to be the best combination showing higher crude fat (ether extract) per cent (6.69 and 6.36) whereas, cross SO-4 X BI-4 and SI-15 X SI-16 (5.06 and 5.07 %) exhibited specific combining ability for less per cent of crude fat (ether extract). The W_r/V_r graph (Figure 14) showed that the regression line is completely below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-1, SI-15, SO-2 and HA-4 had maximum dominant genes as they were above the regression line and close to the origin. These indicate that including these genotype and the combinations of these will produce increased crude fat (ether extract) per cent in plants. Whereas SO-3, CH-2, SO-8 and BI-4 had maximum recessive genes, as it was away from the origin. These genotype will have lesser crude fat (ether extract (%)) in the plants. Positive correlation (0.012) suggest that recessive genes were controlled the trait. The genetic components of variance in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components were higher magnitude than additive components. H_1 value being more than H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 0.917$ being less than unity displayed partial dominance. The positive and highly significant value of F (0.182) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.753) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (0.040). The value of H_1 is more than H_2 indicating that positive and negative genes were symmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ (0.253), whereas the estimated ratio of h^2/H_2 (0.312) indicated that there is one set of gene controlling the production which exhibited complete degree of dominance. Further, the value of r^2 (0.937) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed low heritability (0.430) (Table 46).

Table 45. Mean table of 6 X 4 parental crosses of *G. optiva* for crude fat (ether extract (%))

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	5.62	5.22	5.24	5.23	5.33	26.65	5.33
SO-2	6.09	5.65	5.24	5.75	5.07	27.79	5.56
SO-4	5.35	5.09	5.06	4.84	5.20	25.55	5.11
SO-8	5.23	4.08	4.43	5.63	5.03	24.41	4.88
CH-2	6.69	5.69	6.36	5.37	5.60	29.70	5.94
SI-15	5.66	5.07	4.96	5.24	4.67	25.60	5.12
Selfing	5.50	5.67	5.53	5.07	---	21.77	
Total	40.15	36.46	36.83	37.13	30.90	181.47	
Mean	5.74	5.21	5.26	5.30			

Table 46. Estimation of genetic component of variation in 6 X 4 parental Line X Tester of *G. optiva* for crude fat (ether extract) (%) in F₁ generation

E	0.001**
D	0.151**
H1	0.162**
H2	0.128**
F	0.182**
h ²	0.040**
Heritability (b.s)	0.991
Heritability (n.s)	0.430
Average degree of dominance	0.917
Proportion of +ve & -ve effects in parents	0.253
Proportion of dominant & recessive alleles in parents	0.753
No. of genes controlling the character	0.312
r	0.012
r ²	0.937

** Significant at 1 per cent level of significance

4.3.2.2. Crude protein (%)

The data in Table 47 shows that array means for crude protein (%) in female parent (Line) SO-8 had the higher value (17.01 %) and in male parent (Tester) BI-4 showed higher mean value (16.12 %). It means that the genotypes can serve as better general combiners for higher crude protein (%) production. Whereas female genotype SO-1 (14.95 %) and male parent SO-3 (15.40 %) had the lowest value indicating better general combining ability for lesser crude protein (%) production.

The cross SO-8 X SI-6 and SO-8 X BI-4 proved to be the best combination showing higher crude protein per cent production (18.05 % and 17.84 %) whereas, cross SO-1 X HA-4 and SO-2 X SI-6 (14.25 % and 14.27 %)

exhibited specific combining ability for less per cent production of crude protein. The Wr/Vr graph (Figure 15) showed that the regression line is completely below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-4, CH-2 and SO-8 had maximum dominant genes as they were above the regression line and close to the origin. These indicate that including these genotype and their combinations will produce increased crude protein per cent in plants. Whereas SO-1, SO-2 and SI-6 had maximum recessive genes, as it is away from the origin. These genotype will have lesser crude protein in the plants. Positive correlation (0.123) suggest that recessive genes were controlled the trait. The genetic components of variance in F₁ generation showed that the dominance component (H₁) and additive component (D) were highly significant. It is observed that dominant components were lower than additive components. H₁ value is less than H₂ showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 0.490$ being less than unity displayed partial dominance. The negative and highly significant value of F (-0.040) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.396) revealed that the recessive genes were frequent than dominant genes and that they were in increasing position as exhibited by h² (2.220). The value of H₁ is less than H₂ indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value H₂/4H₁ (0.458),

Table 47. Mean table of 6 X 4 parental crosses of *G. optiva* for crude protein (%) in F₁ generation

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	15.13	14.84	14.60	14.25	15.93	74.75	14.95
SO-2	14.44	14.27	14.75	15.50	16.10	75.06	15.01
SO-4	15.20	15.68	16.14	16.80	16.63	80.45	16.09
SO-8	16.26	18.05	17.84	16.09	16.20	85.04	17.01
CH-2	14.62	16.11	16.28	15.89	15.75	78.64	15.73
SI-15	15.63	16.05	16.72	14.53	16.45	79.37	15.87
Selfing	16.54	16.71	16.59	16.62	---	66.46	
Total	107.81	111.71	112.86	109.68	97.06	539.72	
Mean	15.40	15.96	16.12	15.67			

Whereas, the estimated ratio of h^2/H_2 (11.077) indicated that there were eleven group of genes controlling the production which exhibited complete degree of dominance. Further, the value of r^2 (0.875) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed moderate heritability (0.630) (Table 48).

Table 48. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for crude protein (%)

E	0.148 ^{**}
D	0.455 ^{**}
H1	0.109 ^{**}
H2	0.200 ^{**}
F	-0.040 ^{**}
h^2	2.220 ^{**}
Heritability (b.s)	0.969
Heritability (n.s)	0.630
Average degree of dominance	0.490
Proportion of +ve & -ve effects in parents	0.458
Proportion of dominant & recessive alleles in parents	0.396
No. of genes controlling the character	11.077
r	0.123
r^2	0.875

^{**} Significant at 1 per cent level of significance

4.3.2.3. Crude fibre (%)

The data in Table 49 shows that array means for crude protein (%) in female parent (Line) SO-8 had the higher value (20.35 %) and in male parent (Tester) BI-4 showed higher mean value (19.70 %). It means that the genotypes can serves as better general combiners for higher crude fiber (%) production. Whereas, female genotype SI-15 (18.89 %) and male parent SO-3 (19.37 %) had the lowest value indicating better general combining ability for lesser crude protein (%) production.

The cross SO-8 X SI-6 and SO-8 X BI-4 proved to be the best combination showing higher crude fibre content (22.32 % and 21.56 %) whereas, cross SI-15 X HA-4 (17.95 %) exhibited specific combining ability for less per cent production of crude fiber. The W_r/V_r graph showed that the regression line is completely below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype CH-2, SO-2 and SO-1 had maximum dominant genes as they were above the

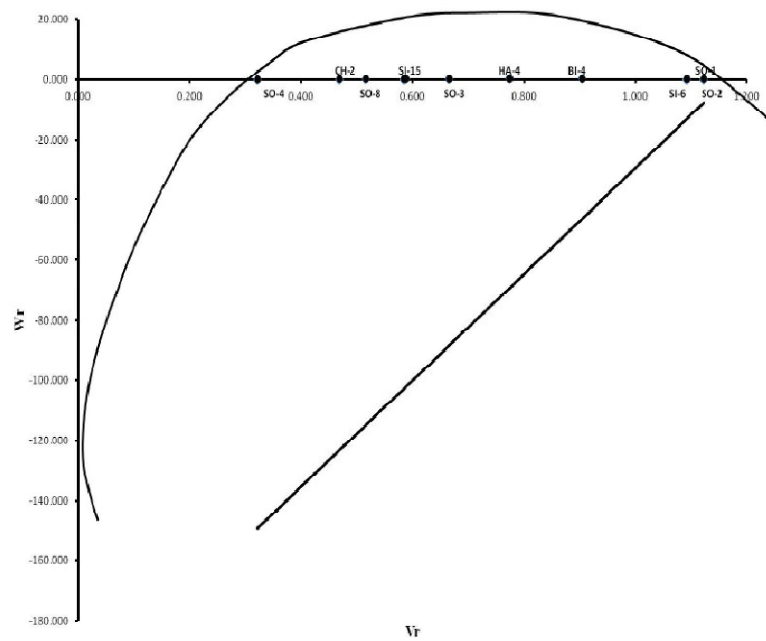


Figure 15. W_r/V_r graph for crude protein (%)

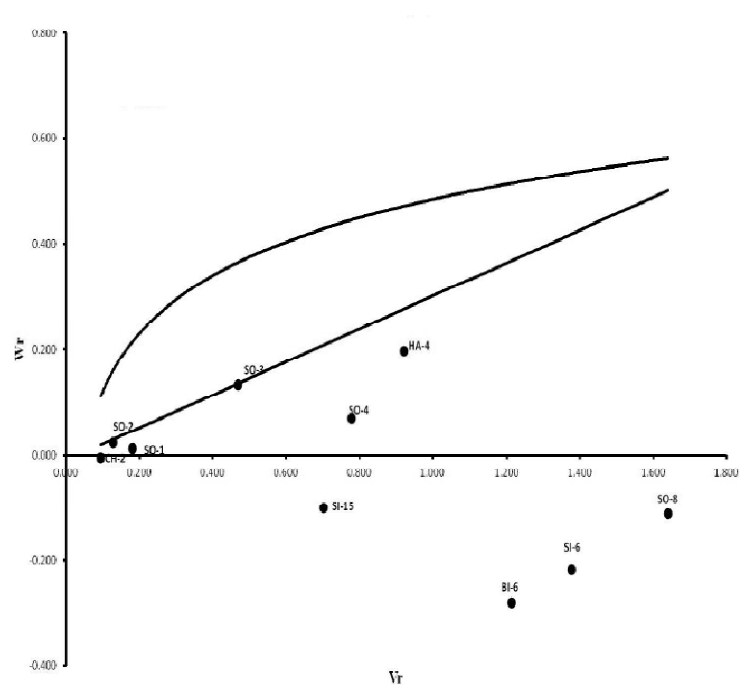


Figure 16. W_r/V_r graph for crude fiber (%)

regression line and close to the origin. These indicate that including these genotype and the combinations of these will produce increased crude protein per cent in plants. Whereas SO-8, SI-6, BI-4 and HA-4 had maximum recessive genes, as it is away from the origin (Figure 16). These genotype combination will produce lesser crude protein in the plants. Negative correlation (-0.518) suggest that dominant genes were controlled the trait. The genetic components of variance in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that additive components were greater than dominant components. H_1 value is less than H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 2.239$ being more than a unity displayed over dominance type of gene action. The positive and highly significant value of F (0.360) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (1.117) revealed that the dominant genes were frequent than recessive genes and that they were in increasing position as exhibited by h^2 (2.960). The value of H_1 is less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents (Table 50) as confirmed by the value $H_2/4H_1$ (0.174), whereas the estimated ratio of h^2/H_2 (1.510) indicated that there were two group of genes controlling the production which exhibited complete degree of dominance. Further, the value of r^2 (0.263) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed low heritability (0.474).

Table 49. Mean table of 6 X 4 parental crosses of *G. optiva* for crude fibre (%)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	19.64	18.54	19.74	18.65	18.80	95.38	19.08
SO-2	19.92	19.76	19.20	19.50	18.95	97.33	19.47
SO-4	19.54	18.58	18.21	21.16	19.10	96.59	19.32
SO-8	19.54	22.32	21.56	19.96	18.35	101.73	20.35
CH-2	19.44	19.77	19.47	19.67	17.85	96.21	19.24
SI-15	18.27	19.36	20.77	17.95	18.10	94.45	18.89
Selfing	19.25	19.00	18.95	19.10	---	76.30	
Total	135.60	137.33	137.90	136.00	111.15	657.98	
Mean	19.37	19.62	19.70	19.43			

Table 50. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for crude fiber (%)

E	0.002 ^{**}
D	0.260 ^{**}
H1	1.490 ^{**}
H2	1.960 ^{**}
F	0.360 ^{**}
h²	2.960 ^{**}
Heritability (b.s)	0.990
Heritability (n.s)	0.474
Average degree of dominance	2.239
Proportion of +ve & -ve effects in parents	0.174
Proportion of dominant & recessive alleles in parents	1.117
No. of genes controlling the character	1.510
r	-0.518
r²	0.263

^{**}Significant at 1 per cent level of significance

4.3.2.4. Total ash (%)

The data in Table 51 shows that array means for total ash (%) in female parent (Line) SO-4 had the higher value (10.23 %) and in male parent (Tester) HA-4 showed higher mean value (10.26 %). It means that the genotypes can serves as better general combiners for higher ash production. Whereas, female genotype SO-2 (9.43 %) and male parent SI-6 (9.71 %) had the lowest value indicating better general combining ability for lower ash production.

The cross SO-4 X HA-4 and SO-8 X SI-6 proved to be the best combination showing higher ash content (13.55 % and 11.37 %) whereas, cross SO-4 X SI-6 and SO-2 X HA-4 (17.95 % and 8.98 %) exhibited specific combining ability for less per cent of ash. The Wr/Vr graph showed that the regression line is completely above the origin, indicating partial dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-2, SO-3, SO-1 and CH-2 had maximum dominant genes as they were close to the origin. These indicate that including these genotype and the combinations of these will produce more ash content. Whereas SO-8, SO-4,

SI-15, BI-4 and HA-4 had maximum recessive genes, as it is away from the origin (Figure 17). These genotype combination will produce lesser ash content in the future generation. Positive correlation (0.269) suggest that recessive genes were controlled the trait. The genetic components of variance in F₁ generation showed that the dominance component (H₁) and additive component (D) were highly significant. It is observed that dominant components were greater than additive components. H₁ value is less than H₂ showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 4.208$ being more than a unity displayed over dominance type of gene action. The negative and highly significant value of F (-0.329) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (0.686) revealed that the recessive genes were frequent than dominant genes and that they were in increasing position as exhibited by h² (3.787). The value of H₁ is less than H₂ indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value H₂/4H₁ (0.151), whereas, the estimated ratio of h² /H₂ (1.644) indicated that there were two group of genes controlling the production which exhibited complete degree of dominance. Further, the value of r² (0.013) showed the complete dominance of parents. Heritability in narrow sense (h² n.s), showed moderate heritability (0.562) (Table 52).

Table 51. Mean table of 6 X 4 parental crosses of *G. optiva* for total ash (%)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	10.17	9.93	10.13	9.66	9.33	49.23	9.85
SO-2	9.77	9.68	9.72	8.98	9.00	47.15	9.43
SO-4	9.80	8.69	9.76	13.55	9.33	51.14	10.23
SO-8	9.15	11.37	11.11	9.88	8.77	50.28	10.06
CH-2	10.20	10.08	9.37	10.49	8.68	48.81	9.76
SI-15	9.65	9.24	10.69	10.10	9.03	48.71	9.74
Selfing	9.67	9.00	8.67	9.17	---	36.50	
Total	68.42	68.00	69.45	71.82	54.14	331.83	
Mean	9.77	9.71	9.92	10.26			

Table 52. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for total ash (%)

E	0.295 ^{**}
D	0.093 ^{**}
H1	1.639 ^{**}
H2	2.303 ^{**}
F	-0.329 ^{**}
h²	3.787 ^{**}
Heritability (b.s)	0.638
Heritability (n.s)	0.562
Average degree of dominance	4.208
Proportion of +ve & -ve effects in parents	0.151
Proportion of dominant & recessive alleles in parents	0.686
No. of genes controlling the character	1.644
r	0.269
r²	0.013

^{**} Significant at 1 per cent level of significance

4.3.2.5. Nitrogen free extract (%)

The data in Table 53 shows that array means for nitrogen free extract (%) in female parent (Line) SI-15 and CH-2 had the higher value (48.23 % and 47.24 %) and in male parent (Tester) HA-4 and SO-3 showed higher mean value (46.74 % and 46.73 %). It means that the genotypes can serves as better general combiners for higher nitrogen free extract (%). Whereas female genotype SO-8 (44.81 %) and male parent BI-4 (45.27 %) had the lowest value indicating better general combining ability for lower nitrogen free extract (%).

The cross SI-15 X SO-3 and SI-15 X HA-4 proved to be the best combination showing higher nitrogen free extract (50.85 % and 50.44 %) whereas, cross SO-8 X SI-6 and SO-8 X BI-4 (17.95 % and 8.98 %) exhibited specific combining ability for less per cent of nitrogen free extract. In Wr/Vr graph the regression line passed Wr axis is below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-2, SO-1 and CH-2 had maximum dominant genes as they were close to the origin. These indicate that including these

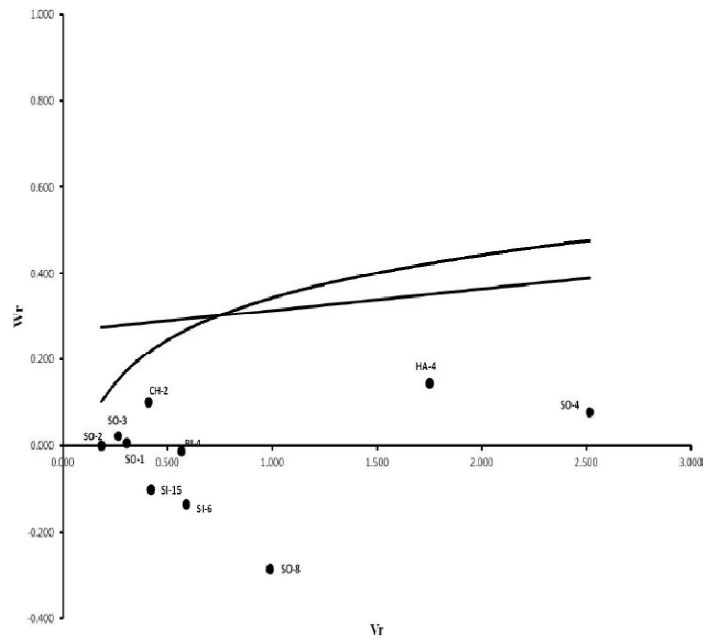


Figure 17. W_r/V_r graph for total ash (%)

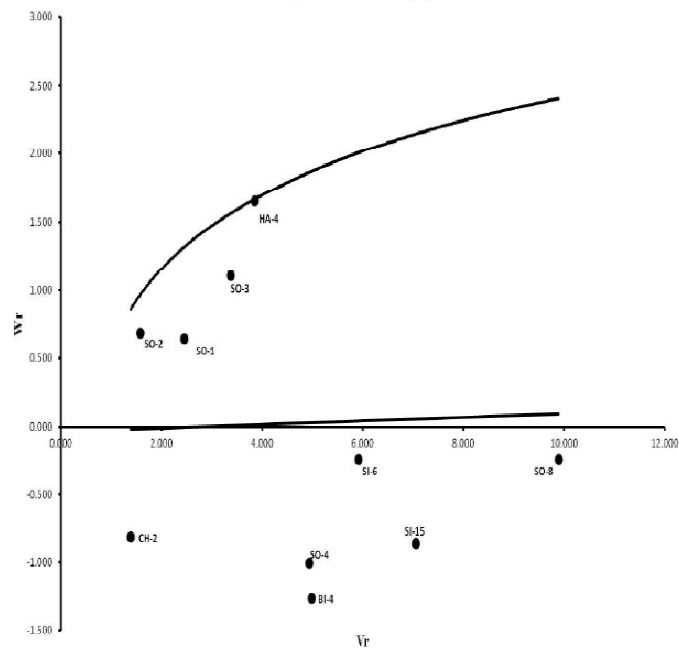


Figure 18. W_r/V_r graph for nitrogen free extract (%)

genotype and their combinations will produce more nitrogen free extract. Whereas SO-8, BI-4 and HA-4 had maximum recessive genes, as it is away from the origin (Figure 18). These genotype (SO-8, BI-4 and HA-4) combination will produce lesser nitrogen free content generation. Negative correlation (-0.339) suggest that dominant genes were controlling the trait. The genetic components of variance in F_1 generation showed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components were greater than additive components. H_1 value is more than H_2 showed unequal gene frequencies at all loci. The average degree of dominance $[1/4(H_1/D)]^{1/2} = 4.443$ being more than a unity displayed over dominance type of gene action. The negative and highly significant value of F (-2.666) the value of proportion of dominant and recessive alleles in parents $[1/4(4DH_1)]^{1/2} + 1/2F/[1/4(4DH_1)]^{1/2} - 1/2F$ (13.372) revealed that the recessive genes were frequent than dominant genes and that they were in increasing position as exhibited by h^2 (5.219). The value of H_1 is more than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents (Table 54) as confirmed by the value $H_2/4H_1$ (0.124), whereas the estimated ratio of h^2/H_2 (0.434) indicated that there were single genes controlling the production which exhibited complete degree of dominance. Further, the value of r^2 (0.037) showed the complete dominance of parents. Heritability in narrow sense (h^2 n.s), showed high heritability (0.914).

Table 53. Mean table of 6 X 4 parental crosses of *G. optiva* for nitrogen free extract (%)

Testers Lines	SO-3	SI-6	BI-4	HA-4	Selfing	Total	Mean
SO-1	43.90	45.77	44.19	46.66	46.72	227.26	45.45
SO-2	44.45	45.27	46.46	46.41	47.44	230.03	46.01
SO-4	46.02	48.19	48.74	41.86	46.04	230.85	46.17
SO-8	47.76	39.97	41.55	47.22	47.57	224.07	44.81
CH-2	48.11	48.88	45.71	46.28	47.22	236.20	47.24
SI-15	50.85	48.98	42.54	50.44	48.32	241.13	48.23
Selfing	46.03	46.96	47.68	48.35	---	189.02	
Total	327.13	324.03	316.87	327.21	283.31	1578.57	
Mean	46.73	46.29	45.27	46.74			

Table 54. Estimation of genetic component of variation in 6 X 4 parental crosses of *G. optiva* for nitrogen free extract (%)

E	0.003*
D	1.232**
H1	24.327**
H2	12.039**
F	-2.666**
h²	5.219**
Heritability (b.s)	0.999
Heritability (n.s)	0.914
Average degree of dominance	4.443
Proportion of +ve & -ve effects in parents	0.124
Proportion of dominant & recessive alleles in parents	13.372
No. of genes controlling the character	0.434
r	-0.339
r²	0.037

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

4.4. HETEROSIS

The heterosis values expressed as the percentage increase or decrease over mid parent (relative heterosis or mid parent heterosis) are presented in Table 55 to 71.

4.4.1. Seed parameters

4.4.1.1. Seed diameter and length (mm)

Data presented in Table 55 revealed relative heterosis for seed diameter and length (mm). F₁ hybrids displayed 58.33 % negative heterosis and 41.66 % showed positive heterosis for seed diameter and length over their mid parents. Maximum positive mid parent heterosis for seed diameter (13.54 and 8.75 %) was revealed in F₁ SI-15 X HA-4 and SI-15 X SO-3, whereas maximum negative heterosis (-29.67 and -17.86 %) was expressed by the cross SO-1 X HA-4 and SO-2 X SO-3 respectively. Whereas for seed length crosses SI-15 X HA-4 and SO-2 X BI-4 (14.94 and 13.44 %) have maximum positive heterosis while in SO-1 X HA-4 and SO-2 X SO-3 having (-33.03 and -14.03 %) maximum negative mid parent heterosis.

4.4.2. Nursery trait

4.4.2.1. Plant height

Results regarding to plant height is presented in the Tables 56 and 57. Among F₁ hybrids, the highest plant height at two and six month seedling were recorded and showed that there was a general tendency for increase in plant height over mid parent in Line X Tester (6 X 4) crosses. For two month seedling data, cross SI-15 X BI-4, SO-8 X SI-6 and SO-8 X BI-4 displayed maximum relative heterosis (+28.75, 25.53 and 24.49 %). Out of 24 crosses only one (SO-2 X SO-3) showed maximum negative relative heterosis (−0.07 %), while in six months seedling all the crosses expressed positive heterosis. Maximum positive heterosis (+49.06, 46.20 and 44.56 %) was shown by cross SI-15 X BI-4, SO-8 X SI-6 and SO-8 X BI-4. The lowest positive mid parent heterosis

Table 55. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for seed parameters

Crosses	F ₁ hybrids		Mid parent value		% increase (+) or decrease (-) over MP	
	Diameter (mm)	Length (mm)	Diameter (mm)	Length (mm)	Diameter	Length
SO-1 X SO-3	4.75	5.55	4.92	5.80	-3.48	-4.26
SO-1 X SI-6	4.60	5.65	4.83	5.89	-4.87	-4.00
SO-1 X BI-4	5.03	5.97	5.00	5.89	0.63	1.30
SO-1 X HA-4	5.14	5.94	7.31	8.88	-29.67	-33.03
SO-2 X SO-3	4.25	5.19	5.18	6.04	-17.86	-14.03
SO-2 X SI-6	4.60	5.47	5.09	6.13	-9.60	-10.63
SO-2 X BI-4	5.39	6.56	5.04	5.79	6.98**	13.44**
SO-2 X HA-4	4.54	5.55	5.15	6.15	-11.88	-9.75
SO-4 X SO-3	5.16	5.90	5.02	5.77	2.81**	2.29*
SO-4 X SI-6	4.80	5.66	4.93	5.86	-2.63	-3.41
SO-4 X BI-4	5.03	6.10	5.10	5.86	-1.28	4.10**
SO-4 X HA-4	4.43	5.50	5.00	5.88	-11.38	-6.53
SO-8 X SO-3	4.83	5.54	4.97	5.69	-2.73	-2.57
SO-8 X SI-6	4.73	5.50	4.88	5.78	-3.08	-4.77
SO-8 X BI-4	5.05	5.89	5.04	5.79	0.28	1.88*
SO-8 X HA-4	4.99	5.75	4.94	5.81	1.07	-0.89
CH-2 X SO-3	4.67	5.71	5.11	5.78	-8.59	-1.27
CH-2 X SI-6	4.66	5.38	5.02	5.87	-7.06	-8.42
CH-2 X BI-4	4.72	5.77	5.18	5.88	-8.80	-1.77
CH-2 X HA-4	5.15	6.08	5.08	5.90	1.47	3.12**
SI-15 X SO-3	5.56	6.40	5.11	5.72	8.75**	11.97**
SI-15 X SI-6	5.11	6.00	5.02	5.81	1.73	3.37**
SI-15 X BI-4	5.25	6.31	5.19	5.81	1.26	8.56**
SI-15 X HA-4	5.77	6.70	5.09	5.83	13.54**	14.94**

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

was observed in SO-2 X SI-6 and SO-2 X SO-3 (+7.44 and 8.40 %) respectively (Tables 57 and Plate 6). The presence of positive significant heterosis over mid parent and better parent indicates significant increase in the height of F₁ hybrids and the negative heterosis over MP indicates significant decrease in the height of F₁ hybrids as compared to their mid parent. The analysis of variance for plant height (Appendix XXXI and XXXII-) revealed highly significant differences among parents and their F₁ hybrids. This implies that there was significant genetic variability existing among parents and their F₁ hybrids for plant height.

Table 56. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for plant height (cm) (two months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	4.88	6.43	6.63	5.65	17.17**
2	SO-1 X SI-6	4.88	6.29	6.37	5.58	14.07**
3	SO-1 X BI-4	4.88	6.18	6.13	5.53	10.93**
4	SO-1 X HA-4	4.88	6.14	6.16	5.51	11.80**
5	SO-2 X SO-3	6.31	6.43	6.37	6.37	-0.07
6	SO-2 X SI-6	6.31	6.29	6.41	6.30	1.75*
7	SO-2 X BI-4	6.31	6.18	6.46	6.25	3.40**
8	SO-2 X HA-4	6.31	6.14	6.57	6.23	5.49**
9	SO-4 X SO-3	5.60	6.43	6.42	6.02	6.65**
10	SO-4 X SI-6	5.60	6.29	6.49	5.94	9.22**
11	SO-4 X BI-4	5.60	6.18	6.73	5.89	14.14**
12	SO-4 X HA-4	5.60	6.14	7.03	5.87	19.66**
13	SO-8 X SO-3	5.25	6.43	6.63	5.84	13.41**
14	SO-8 X SI-6	5.25	6.29	7.24	5.77	25.53**
15	SO-8 X BI-4	5.25	6.18	7.12	5.72	24.49**
16	SO-8 X HA-4	5.25	6.14	6.75	5.70	18.51**
17	CH-2 X SO-3	5.07	6.43	6.55	5.75	13.91**
18	CH-2 X SI-6	5.07	6.29	6.46	5.68	13.76**
19	CH-2 X BI-4	5.07	6.18	6.72	5.63	19.41**
20	CH-2 X HA-4	5.07	6.14	6.63	5.60	18.36**
21	SI-15 X SO-3	4.60	6.43	6.35	5.52	15.11**
22	SI-15 X SI-6	4.60	6.29	6.43	5.44	18.03**
23	SI-15 X BI-4	4.60	6.18	6.94	5.39	28.75**
24	SI-15 X HA-4	4.60	6.14	6.13	5.37	14.20**

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance



PLATE 6: F_1 's showing heterosis for plant height character over its parents

Table 57. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for plant height (cm) (six months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	9.98	14.31	15.13	12.15	24.58 ^{**}
2	SO-1 X SI-6	9.98	14.23	14.84	12.10	22.62 ^{**}
3	SO-1 X BI-4	9.98	14.22	14.60	12.10	20.66 ^{**}
4	SO-1 X HA-4	9.98	14.15	14.25	12.07	18.09 ^{**}
5	SO-2 X SO-3	12.33	14.31	14.44	13.32	8.40 ^{**}
6	SO-2 X SI-6	12.33	14.23	14.27	13.28	7.44 ^{**}
7	SO-2 X BI-4	12.33	14.22	14.75	13.28	11.11 ^{**}
8	SO-2 X HA-4	12.33	14.15	15.50	13.24	17.05 ^{**}
9	SO-4 X SO-3	10.49	14.31	15.20	12.40	22.58 ^{**}
10	SO-4 X SI-6	10.49	14.23	15.68	12.36	26.93 ^{**}
11	SO-4 X BI-4	10.49	14.22	16.14	12.35	30.68 ^{**}
12	SO-4 X HA-4	10.49	14.15	16.80	12.32	36.38 ^{**}
13	SO-8 X SO-3	10.47	14.31	16.26	12.39	31.23 ^{**}
14	SO-8 X SI-6	10.47	14.23	18.05	12.35	46.20 ^{**}
15	SO-8 X BI-4	10.47	14.22	17.84	12.34	44.56 ^{**}
16	SO-8 X HA-4	10.47	14.15	16.09	12.31	30.74 ^{**}
17	CH-2 X SO-3	9.23	14.31	14.62	11.77	24.16 ^{**}
18	CH-2 X SI-6	9.23	14.23	16.11	11.73	37.34 ^{**}
19	CH-2 X BI-4	9.23	14.22	16.28	11.73	38.81 ^{**}
20	CH-2 X HA-4	9.23	14.15	15.89	11.69	35.92 ^{**}
21	SI-15 X SO-3	8.21	14.31	15.63	11.26	38.73 ^{**}
22	SI-15 X SI-6	8.21	14.23	16.05	11.22	43.06 ^{**}
23	SI-15 X BI-4	8.21	14.22	16.72	11.21	49.06 ^{**}
24	SI-15 X HA-4	8.21	14.15	14.53	11.18	29.90 ^{**}

4.4.2.2. Collar diameter (mm)

The highest collar diameter was recorded for two months seedling is given in Table 58. Results revealed all crosses showed positive mid parent heterosis in two months seedling. SI-15 X BI-4, SO-4 X HA-4 and SO-8 X BI-4 (+57.30, 52.87 and 52.81 %) and expressed maximum positive MP heterosis, the lower positive mid parent heterosis was observed in crosses SO-1 X HA-4, SO-1 X BI-4 and SO-2 X SO-3 (3.02, 3.36 and 3.86 %) respectively. Whereas maximum positive significant heterosis was shown by the cross SO-8 X BI-4 and

SO-8 X SI-6 (+98.63 and 86.37 %) respectively. One cross out of twenty-four F₁ hybrids displayed negative heterosis with maximum values of -2.91 % over mid parent was SI-15 X HA-4 in six months seedling (Tables 59).

Analysis of variance for collar diameter (mm) (Appendix XXXIII and XXXIV) displayed highly significant ($P \leq 0.01$) differences among parents and their F₁ hybrids. This means that genotypes were significantly different in their diameter growth and could be exploited for further improvement of this character.

Table 58. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for collar diameter (mm) (two months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	1.83	2.27	2.40	2.05	17.13 ^{**}
2	SO-1 X SI-6	1.83	2.07	2.08	1.95	6.86 ^{**}
3	SO-1 X BI-4	1.83	1.95	1.95	1.89	3.36 ^{**}
4	SO-1 X HA-4	1.83	1.94	1.94	1.89	3.02 ^{**}
5	SO-2 X SO-3	2.02	2.27	2.23	2.14	3.86 ^{**}
6	SO-2 X SI-6	2.02	2.07	2.27	2.05	10.78 ^{**}
7	SO-2 X BI-4	2.02	1.95	2.26	1.98	14.17 ^{**}
8	SO-2 X HA-4	2.02	1.94	2.31	1.98	16.72 ^{**}
9	SO-4 X SO-3	1.94	2.27	2.36	2.10	12.07 ^{**}
10	SO-4 X SI-6	1.94	2.07	2.40	2.00	19.82 ^{**}
11	SO-4 X BI-4	1.94	1.95	2.66	1.94	36.98 ^{**}
12	SO-4 X HA-4	1.94	1.94	2.97	1.94	52.87 ^{**}
13	SO-8 X SO-3	1.87	2.27	2.47	2.07	19.12 ^{**}
14	SO-8 X SI-6	1.87	2.07	2.97	1.97	50.44 ^{**}
15	SO-8 X BI-4	1.87	1.95	2.91	1.91	52.81 ^{**}
16	SO-8 X HA-4	1.87	1.94	2.77	1.91	45.42 ^{**}
17	CH-2 X SO-3	1.87	2.27	2.48	2.07	19.75 ^{**}
18	CH-2 X SI-6	1.87	2.07	2.49	1.97	26.20 ^{**}
19	CH-2 X BI-4	1.87	1.95	2.66	1.91	39.55 ^{**}
20	CH-2 X HA-4	1.87	1.94	2.43	1.91	27.74 ^{**}
21	SI-15 X SO-3	1.78	2.27	2.39	2.02	18.14 ^{**}
22	SI-15 X SI-6	1.78	2.07	2.45	1.92	27.53 ^{**}
23	SI-15 X BI-4	1.78	1.95	2.93	1.86	57.30 ^{**}
24	SI-15 X HA-4	1.78	1.94	1.93	1.86	3.69 ^{**}

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Table 59. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for diameter (mm) (six months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	2.04	3.39	3.56	2.71	31.26 ^{**}
2	SO-1 X SI-6	2.04	2.62	2.92	2.33	25.22 ^{**}
3	SO-1 X BI-4	2.04	2.25	2.30	2.15	7.28 ^{**}
4	SO-1 X HA-4	2.04	2.09	2.11	2.07	1.93 ^{**}
5	SO-2 X SO-3	2.19	3.39	3.42	2.79	22.75 ^{**}
6	SO-2 X SI-6	2.19	2.62	2.90	2.40	20.57 ^{**}
7	SO-2 X BI-4	2.19	2.25	2.90	2.22	30.45 ^{**}
8	SO-2 X HA-4	2.19	2.09	2.98	2.14	39.40 ^{**}
9	SO-4 X SO-3	2.07	3.39	2.80	2.73	2.83 ^{**}
10	SO-4 X SI-6	2.07	2.62	2.82	2.34	20.23 ^{**}
11	SO-4 X BI-4	2.07	2.25	2.71	2.16	25.32 ^{**}
12	SO-4 X HA-4	2.07	2.09	3.03	2.08	45.93 ^{**}
13	SO-8 X SO-3	2.11	3.39	2.98	2.75	8.43 ^{**}
14	SO-8 X SI-6	2.11	2.62	4.41	2.37	86.37 ^{**}
15	SO-8 X BI-4	2.11	2.25	4.34	2.18	98.63 ^{**}
16	SO-8 X HA-4	2.11	2.09	2.71	2.10	28.89 ^{**}
17	CH-2 X SO-3	2.01	3.39	2.75	2.70	1.61
18	CH-2 X SI-6	2.01	2.62	2.80	2.32	21.09 ^{**}
19	CH-2 X BI-4	2.01	2.25	2.71	2.13	26.81 ^{**}
20	CH-2 X HA-4	2.01	2.09	2.85	2.05	39.01 ^{**}
21	SI-15 X SO-3	2.01	3.39	2.72	2.70	0.73
22	SI-15 X SI-6	2.01	2.62	2.69	2.31	16.26 ^{**}
23	SI-15 X BI-4	2.01	2.25	3.07	2.13	44.14 ^{**}
24	SI-15 X HA-4	2.01	2.09	1.99	2.05	-2.91

^{**} Significant at 1 per cent level of significance

4.4.2.3. Number of leaves per plant

The data for number of leaves per plant for two and six months seedling is given in Table 60 and 61. Results revealed that twenty one out of twenty four crosses showed positive mid parent heterosis and only three crosses displayed negative mid parent heterosis. SO-8 X SI-6, SO-8 X BI-4 and SO-8 X HA-4 expressed maximum positive significant mid parent heterosis (+37.10, 33.87 and 32.26 %). Maximum negative relative heterosis (-6.54,-3.31 and -1.99%) was expressed by the crosses SO-2 X SO-3, SO-2 X SI-6 and SO-2 X BI-4 for two

months seedling. For six months seedling it was observed that there is a general tendency for increase in number of leaves per plant. Maximum hybrids expressed positive heterosis over mid parent and two among them showed negative mid parent heterosis. Maximum significant positive heterosis (+23.16 and 22.91 %) was shown by the cross SO-8 X SI-6 and SO-8 X BI-4 respectively. Maximum negative mid parent heterosis (-4.91 and -1.60 %) was exhibited by SO-2 X SI-6 and CH-2 X SO-3 respectively. Cross SO-2 X SO-3 maintained its consistence without showing any percent increase or decrease for the character.

The analysis of variance (ANOVA) for number of leaves/plant (Appendix XXXV and XXXVI) revealed that there existed significant genetic differences among parents and their F₁ hybrids, which manifested that there was significant genetic variability among parents and their F₁ hybrids.

Table 60. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for number leaves (two months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	5.33	6.79	6.50	6.06	7.22 ^{**}
2	SO-1 X SI-6	5.33	6.00	5.92	5.67	4.41 ^{**}
3	SO-1 X BI-4	5.33	6.00	5.92	5.67	4.41 ^{**}
4	SO-1 X HA-4	5.33	6.00	5.92	5.67	4.41 ^{**}
5	SO-2 X SO-3	6.58	6.79	6.25	6.69	-6.54
6	SO-2 X SI-6	6.58	6.00	6.08	6.29	-3.31
7	SO-2 X BI-4	6.58	6.00	6.17	6.29	-1.99
8	SO-2 X HA-4	6.58	6.00	6.33	6.29	0.66
9	SO-4 X SO-3	5.00	6.79	6.17	5.90	4.59 ^{**}
10	SO-4 X SI-6	5.00	6.00	6.33	5.50	15.15 ^{**}
11	SO-4 X BI-4	5.00	6.00	6.58	5.50	19.70 ^{**}
12	SO-4 X HA-4	5.00	6.00	6.83	5.50	24.24 ^{**}
13	SO-8 X SO-3	4.33	6.79	6.67	5.56	19.85 ^{**}
14	SO-8 X SI-6	4.33	6.00	7.08	5.17	37.10 ^{**}
15	SO-8 X BI-4	4.33	6.00	6.92	5.17	33.87 ^{**}
16	SO-8 X HA-4	4.33	6.00	6.83	5.17	32.26 ^{**}
17	CH-2 X SO-3	6.00	6.79	6.42	6.40	0.33
18	CH-2 X SI-6	6.00	6.00	6.08	6.00	1.39
19	CH-2 X BI-4	6.00	6.00	6.67	6.00	11.11 ^{**}
20	CH-2 X HA-4	6.00	6.00	6.50	6.00	8.33 ^{**}
21	SI-15 X SO-3	4.38	6.79	6.00	5.58	7.46 ^{**}
22	SI-15 X SI-6	4.38	6.00	6.17	5.19	18.88 ^{**}
23	SI-15 X BI-4	4.38	6.00	6.75	5.19	30.12 ^{**}
24	SI-15 X HA-4	4.38	6.00	5.83	5.19	12.45 ^{**}

Table 61. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for number of leaves (six months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	6.33	8.00	8.25	7.17	15.12 ^{**}
2	SO-1 X SI-6	6.33	8.13	8.00	7.23	10.66 ^{**}
3	SO-1 X BI-4	6.33	7.75	8.00	7.04	13.61 ^{**}
4	SO-1 X HA-4	6.33	8.06	8.08	7.19	12.36 ^{**}
5	SO-2 X SO-3	8.00	8.00	8.00	8.00	0.00
6	SO-2 X SI-6	8.00	8.13	7.67	8.06	-4.91
7	SO-2 X BI-4	8.00	7.75	7.92	7.88	0.53
8	SO-2 X HA-4	8.00	8.06	8.33	8.03	3.81 ^{**}
9	SO-4 X SO-3	7.63	8.00	8.08	7.81	3.47 ^{**}
10	SO-4 X SI-6	7.63	8.13	8.17	7.88	3.70 ^{**}
11	SO-4 X BI-4	7.63	7.75	8.25	7.69	7.32 ^{**}
12	SO-4 X HA-4	7.63	8.06	8.75	7.84	11.60 ^{**}
13	SO-8 X SO-3	7.17	8.00	8.33	7.58	9.89 ^{**}
14	SO-8 X SI-6	7.17	8.13	9.42	7.65	23.16 ^{**}
15	SO-8 X BI-4	7.17	7.75	9.17	7.46	22.91 ^{**}
16	SO-8 X HA-4	7.17	8.06	8.17	7.61	7.30 ^{**}
17	CH-2 X SO-3	7.58	8.00	7.67	7.79	-1.60
18	CH-2 X SI-6	7.58	8.13	8.17	7.85	3.98 ^{**}
19	CH-2 X BI-4	7.58	7.75	8.17	7.67	6.52 ^{**}
20	CH-2 X HA-4	7.58	8.06	7.92	7.82	1.24
21	SI-15 X SO-3	6.63	8.00	8.17	7.31	11.68 ^{**}
22	SI-15 X SI-6	6.63	8.13	8.25	7.38	11.86 ^{**}
23	SI-15 X BI-4	6.63	7.75	8.42	7.19	17.10 ^{**}
24	SI-15 X HA-4	6.63	8.06	8.08	7.34	10.12 ^{**}

4.4.2.4. Internodel length (cm)

The performance of F₁ hybrids for internodel length (cm) for both two and six months seedling is presented in Table 62 and 63. All the hybrids showed positive mid parent heterosis. Maximum positive heterosis was exhibited in SO-8 X HA-4 and SO-8 X SO-3 (+45.76 and 45.09 %), whereas lowest positive mid parent heterosis was observed in SI-15 X HA-4 and SO-2 X BI-4 (+2.56 and 3.65 %) respectively. In case of six months observation revealed that there was a general tendency of increase in internodel length of F₁ hybrids over their mid parent. Out of 24 crosses only one (SO-2 X SI-6) showed maximum negative

relative heterosis (−0.94 %). Cross SO-2 X SO-3 again maintained its consistence with respect to internode length character without showing any percent increase or decrease over mid parent. Maximum significant positive heterosis (+46.63 and 43.00 %) was shown by the cross SO-8 X BI-4 and SO-8 X SI-6 respectively. The presence of positive significant heterosis over mid parent indicates significant increase in the internode length of F₁ hybrids and the negative heterosis over MP indicates significant decrease in the internode length of F₁ hybrids.

The analysis of variance (Appendix XXXVII and XXXVIII) showed highly significant differences among parents and their F₁ hybrids. Significant mean squares for genotypes imply that parental entries and their F₁ hybrids differed significantly for internode length and this variability could be exploited in further breeding programmes.

Table 62. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease(-) over mid parent (relative heterosis) for internode length(cm) (two months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	0.93	1.18	1.22	1.05	15.42 ^{**}
2	SO-1 X SI-6	0.93	1.11	1.10	1.02	7.98 ^{**}
3	SO-1 X BI-4	0.93	1.20	1.20	1.06	12.94 ^{**}
4	SO-1 X HA-4	0.93	1.00	1.00	0.96	3.90 ^{**}
5	SO-2 X SO-3	1.08	1.18	1.20	1.13	5.88 ^{**}
6	SO-2 X SI-6	1.08	1.11	1.22	1.10	10.82 ^{**}
7	SO-2 X BI-4	1.08	1.20	1.18	1.14	3.65 ^{**}
8	SO-2 X HA-4	1.08	1.00	1.23	1.04	17.60 ^{**}
9	SO-4 X SO-3	1.00	1.18	1.22	1.09	11.45 ^{**}
10	SO-4 X SI-6	1.00	1.11	1.24	1.06	17.55 ^{**}
11	SO-4 X BI-4	1.00	1.20	1.30	1.10	18.18 ^{**}
12	SO-4 X HA-4	1.00	1.00	1.43	1.00	43.33 ^{**}
13	SO-8 X SO-3	0.97	1.18	1.30	1.08	20.93 ^{**}
14	SO-8 X SI-6	0.97	1.11	1.51	1.04	45.09 ^{**}
15	SO-8 X BI-4	0.97	1.20	1.48	1.08	36.92 ^{**}
16	SO-8 X HA-4	0.97	1.00	1.43	0.98	45.76 ^{**}
17	CH-2 X SO-3	1.00	1.18	1.27	1.09	16.03 ^{**}
18	CH-2 X SI-6	1.00	1.11	1.26	1.06	19.13 ^{**}
19	CH-2 X BI-4	1.00	1.20	1.32	1.10	19.70 ^{**}
20	CH-2 X HA-4	1.00	1.00	1.35	1.00	35.00 ^{**}
21	SI-15 X SO-3	0.95	1.18	1.28	1.07	19.53 ^{**}
22	SI-15 X SI-6	0.95	1.11	1.23	1.03	18.79 ^{**}
23	SI-15 X BI-4	0.95	1.20	1.43	1.08	32.56 ^{**}
24	SI-15 X HA-4	0.95	1.00	1.00	0.98	2.56 ^{**}

Table 63. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for internodel length (cm) (six months)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	1.48	1.80	1.80	1.64	9.92 ^{**}
2	SO-1 X SI-6	1.48	1.80	1.80	1.64	9.92 ^{**}
3	SO-1 X BI-4	1.48	1.62	1.64	1.55	6.20 ^{**}
4	SO-1 X HA-4	1.48	1.65	1.66	1.56	6.23 ^{**}
5	SO-2 X SO-3	1.75	1.80	1.78	1.78	0.00
6	SO-2 X SI-6	1.75	1.80	1.76	1.78	-0.94
7	SO-2 X BI-4	1.75	1.62	1.80	1.68	6.93 ^{**}
8	SO-2 X HA-4	1.75	1.65	1.84	1.70	8.42 ^{**}
9	SO-4 X SO-3	1.50	1.80	1.82	1.65	10.10 ^{**}
10	SO-4 X SI-6	1.50	1.80	1.83	1.65	11.11 ^{**}
11	SO-4 X BI-4	1.50	1.62	1.88	1.56	20.86 ^{**}
12	SO-4 X HA-4	1.50	1.65	1.93	1.57	22.86 ^{**}
13	SO-8 X SO-3	1.48	1.80	1.84	1.64	12.47 ^{**}
14	SO-8 X SI-6	1.48	1.80	2.34	1.64	43.00 ^{**}
15	SO-8 X BI-4	1.48	1.62	2.27	1.55	46.63 ^{**}
16	SO-8 X HA-4	1.48	1.65	1.88	1.56	20.11 ^{**}
17	CH-2 X SO-3	1.50	1.80	1.74	1.65	5.56 ^{**}
18	CH-2 X SI-6	1.50	1.80	1.81	1.65	9.60 ^{**}
19	CH-2 X BI-4	1.50	1.62	1.87	1.56	19.79 ^{**}
20	CH-2 X HA-4	1.50	1.65	1.83	1.57	15.98 ^{**}
21	SI-15 X SO-3	1.46	1.80	1.81	1.63	10.86 ^{**}
22	SI-15 X SI-6	1.46	1.80	1.84	1.63	12.90 ^{**}
23	SI-15 X BI-4	1.46	1.62	1.90	1.54	23.41 ^{**}
24	SI-15 X HA-4	1.46	1.65	1.66	1.55	6.65 ^{**}

4.4.2.5. Leaf area (cm²)

Results presented in Table 64 revealed relative heterosis for leaf area (cm²). 70.00 % of the F₁ hybrids displayed positive heterosis and 30.00 % showed negative heterosis over their mid parents. Maximum positive mid parent (122.57 %) was revealed in F₁ SO-8 X SI-6 followed by SO-8 X BI-4 (64.20 %), whereas maximum negative heterosis (-25.99 and -18.25 %) was expressed by the cross CH-2 X BI-4 and SO-2 X SO-3 respectively.

Analysis of variance (ANOVA) for leaf area displayed highly significant differences ($P \leq 0.01$) among parents and their F₁ hybrids. Thus, such a variability can be utilized in further breeding programmes.

Table 64. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for leaf area (cm²)

Sl No.	Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	26.38	27.19	33.99	26.79	26.90 ^{**}
2	SO-1 X SI-6	26.38	26.28	32.14	26.33	22.07 ^{**}
3	SO-1 X BI-4	26.38	34.02	32.53	30.20	7.69 ^{**}
4	SO-1 X HA-4	26.38	27.33	34.27	26.86	27.60 ^{**}
5	SO-2 X SO-3	27.00	27.19	22.15	27.10	-18.25
6	SO-2 X SI-6	27.00	26.28	23.67	26.64	-11.17
7	SO-2 X BI-4	27.00	34.02	32.51	30.51	6.55 ^{**}
8	SO-2 X HA-4	27.00	27.33	22.64	27.17	-16.67
9	SO-4 X SO-3	26.47	27.19	29.52	26.83	10.03 ^{**}
10	SO-4 X SI-6	26.47	26.28	25.61	26.38	-2.90
11	SO-4 X BI-4	26.47	34.02	26.18	30.24	-13.46
12	SO-4 X HA-4	26.47	27.33	43.23	26.90	60.73 ^{**}
13	SO-8 X SO-3	22.15	27.19	21.74	24.67	-11.87
14	SO-8 X SI-6	22.15	26.28	53.90	24.22	122.57 ^{**}
15	SO-8 X BI-4	22.15	34.02	46.12	28.09	64.20 ^{**}
16	SO-8 X HA-4	22.15	27.33	27.47	24.74	11.02 ^{**}
17	CH-2 X SO-3	25.83	27.19	30.87	26.51	16.44 ^{**}
18	CH-2 X SI-6	25.83	26.28	27.24	26.06	4.52 ^{**}
19	CH-2 X BI-4	25.83	34.02	22.15	29.93	-25.99
20	CH-2 X HA-4	25.83	27.33	26.98	26.58	1.49
21	SI-15 X SO-3	25.79	27.19	31.63	26.56	19.07 ^{**}
22	SI-15 X SI-6	25.79	26.28	35.03	26.04	34.52 ^{**}
23	SI-15 X BI-4	25.79	34.02	38.70	29.91	29.40 ^{**}
24	SI-15 X HA-4	25.79	27.33	34.02	26.56	28.08 ^{**}

4.4.2.6. Leaf weight (g)

Results presented in Table 65 revealed relative heterosis for leaf fresh and dry weight (g). 54.16 % of the F₁ hybrids displayed negative heterosis and 45.83 % showed positive heterosis for fresh leaf weight and 41.66 % of crosses exhibited negative heterosis and 59.33 % with positive heterosis for leaf dry weight over their mid parents. Maximum positive mid parent heterosis for fresh leaf weight (169.29 and 103.62 %) was revealed in F₁ SO-8 X SI-6 and SO-8 X BI-4, whereas maximum negative heterosis (-41.32 and -37.95 %) was expressed by the cross SO-2 X HA-4 and SO-2 X SO-3 respectively. Whereas for leaf dry weight, crosses SO-8 X SI-6 and SO-8 X BI-4 (171.56 and 121.28 %) have maximum positive heterosis while in SO-2 X SO-3 and SO-2 X SI-6 having (-45.21 and -36.84 %) maximum negative mid parent heterosis.

4.4.2.7. Leaf dry matter production

Results presented in Table 66 revealed relative heterosis for leaf dry matter production. 66.66 % of the F₁ hybrids exhibited positive heterosis and 33.33 % showed negative heterosis for leaf dry matter production over their mid parents. Maximum positive mid parent heterosis for leaf dry matter production (61.20 and 40.14 %) was revealed in F₁ SO-1 X BI-4 and SO-2 X HA-4, whereas maximum negative heterosis (-13.13 and -11.68 %) was expressed by the cross CH-2 X SI-6 and SO-2 X SO-3 respectively.

Table 65. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for 10 leaf weight (g)

Crosses	F ₁ hybrids		Mid parent value		% increase (+) or decrease (-) over MP	
	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)	Fresh wt	Dry wt
SO-1 X SO-3	1.96	0.91	1.59	0.59	23.27**	54.24**
SO-1 X SI-6	1.11	0.57	1.40	0.53	-20.71	8.57**
SO-1 X BI-4	1.18	0.62	1.38	0.45	-14.49	37.78**
SO-1 X HA-4	1.95	0.67	1.46	0.46	34.02**	45.65**
SO-2 X SO-3	1.12	0.40	1.81	0.73	-37.95	-45.21
SO-2 X SI-6	1.08	0.42	1.62	0.67	-33.13	-36.84
SO-2 X BI-4	1.87	0.76	1.60	0.59	17.24**	28.81**
SO-2 X HA-4	0.98	0.49	1.67	0.60	-41.32	-18.33
SO-4 X SO-3	1.43	0.65	1.64	0.65	-12.54	0.78
SO-4 X SI-6	1.39	0.54	1.45	0.58	-3.81	-6.90
SO-4 X BI-4	1.19	0.50	1.43	0.51	-16.49	-0.99
SO-4 X HA-4	2.43	1.00	1.50	0.52	62.00**	94.17**
SO-8 X SO-3	1.00	0.49	1.59	0.61	-37.11	-19.67
SO-8 X SI-6	3.77	1.48	1.40	0.55	169.29**	171.56**
SO-8 X BI-4	2.81	1.04	1.38	0.47	103.62**	121.28**
SO-8 X HA-4	1.27	0.42	1.46	0.48	-12.71	-12.50
CH-2 X SO-3	1.79	0.70	1.54	0.67	16.23**	4.48**
CH-2 X SI-6	1.36	0.53	1.35	0.61	0.74	-12.40
CH-2 X BI-4	1.27	0.46	1.33	0.53	-4.51	-13.21
CH-2 X HA-4	1.24	0.58	1.41	0.54	-11.74	7.41**
SI-15 X SO-3	1.61	0.63	1.71	0.70	-5.85	-9.35
SI-15 X SI-6	2.30	0.86	1.52	0.63	51.32**	36.51**
SI-15 X BI-4	2.40	0.93	1.50	0.56	60.00**	67.57**
SI-15 X HA-4	2.19	0.88	1.58	0.57	39.05**	55.75**

Table 66. Mean performance of parents, F₁ hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for leaf dry matter production

Crosses	Female parents	Male parents	F ₁ hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
SO-1 X SO-3	1.37	1.81	46.43	36.32	27.82 ^{**}
SO-1 X SI-6	1.37	1.43	51.35	37.36	37.46 ^{**}
SO-1 X BI-4	1.37	1.39	52.54	32.59	61.20 ^{**}
SO-1 X HA-4	1.37	1.54	34.36	31.56	8.86 ^{**}
SO-2 X SO-3	1.80	1.81	35.71	40.44	-11.68
SO-2 X SI-6	1.80	1.43	38.89	41.47	-6.23
SO-2 X BI-4	1.80	1.39	40.64	36.71	10.71 ^{**}
SO-2 X HA-4	1.80	1.54	50.00	35.68	40.14 ^{**}
SO-4 X SO-3	1.46	1.81	45.45	39.15	16.12 ^{**}
SO-4 X SI-6	1.46	1.43	38.85	40.18	-3.31
SO-4 X BI-4	1.46	1.39	42.02	35.42	18.64 ^{**}
SO-4 X HA-4	1.46	1.54	41.15	34.38	19.68 ^{**}
SO-8 X SO-3	1.37	1.81	49.00	37.78	29.69 ^{**}
SO-8 X SI-6	1.37	1.43	39.26	38.82	1.14
SO-8 X BI-4	1.37	1.39	37.01	34.05	8.68 ^{**}
SO-8 X HA-4	1.37	1.54	33.07	33.02	0.15
CH-2 X SO-3	1.27	1.81	39.11	43.83	-10.78
CH-2 X SI-6	1.27	1.43	38.97	44.86	-13.13
CH-2 X BI-4	1.27	1.39	36.22	40.10	-9.68
CH-2 X HA-4	1.27	1.54	46.77	39.07	19.72 ^{**}
SI-15 X SO-3	1.61	1.81	39.13	40.56	-3.52
SI-15 X SI-6	1.61	1.43	37.39	41.59	-10.10
SI-15 X BI-4	1.61	1.39	38.75	36.83	5.21 ^{**}
SI-15 X HA-4	1.61	1.54	40.18	35.80	12.25 ^{**}

4.4.3. Fodder characters

4.4.3.1. Crude protein (%)

Results presented in Table 67 reveal relative heterosis for crude protein. There was a general tendency for increase in crude protein content in hybrids. 87.5 % of the F₁ hybrids manifested positive heterotic effects over mid parents. Maximum positive heterosis (+35.77, 29.50 and 29.13 %) was shown by the crosses SO-8 X SI-6, SI-15 X BI-4 and SO-8 X BI-4 respectively. Maximum negative heterotic effects over MP (-4.51, -2.96 and -2.44%) was exhibited by SI-15 X SO-3, CH-2 X SI-6 and CH-2 X SO-3.

Analysis of variance (ANOVA) for crude protein (Appendix XXXIX) displayed highly significant differences ($P \leq 0.01$) among parents and their F_1 hybrids. Thus these entries can further be exploited in breeding programme.

Table 67. Mean performance of parents, F_1 hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for crude protein (%)

Sl No.	Crosses	Female parents	Male parents	F_1 hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	15.93	16.54	20.83	16.23	28.30**
2	SO-1 X SI-6	15.93	16.71	20.65	16.32	26.54**
3	SO-1 X BI-4	15.93	16.54	20.81	16.23	28.19**
4	SO-1 X HA-4	15.93	16.63	19.91	16.28	22.31**
5	SO-2 X SO-3	16.10	16.54	19.95	16.32	22.25**
6	SO-2 X SI-6	16.10	16.71	19.78	16.41	20.53**
7	SO-2 X BI-4	16.10	16.54	19.69	16.32	20.64**
8	SO-2 X HA-4	16.10	16.63	19.43	16.36	18.72**
9	SO-4 X SO-3	16.63	16.54	19.41	16.58	17.04**
10	SO-4 X SI-6	16.63	16.71	19.69	16.67	18.11**
11	SO-4 X BI-4	16.63	16.54	18.55	16.58	11.87**
12	SO-4 X HA-4	16.63	16.63	21.45	16.63	29.00**
13	SO-8 X SO-3	16.80	16.54	18.46	16.67	10.76**
14	SO-8 X SI-6	16.80	16.71	22.75	16.76	35.77**
15	SO-8 X BI-4	16.80	16.54	21.53	16.67	29.13**
16	SO-8 X HA-4	16.80	16.63	17.50	16.71	4.71**
17	CH-2 X SO-3	15.75	16.54	15.75	16.14	-2.44
18	CH-2 X SI-6	15.75	16.71	15.75	16.23	-2.96
19	CH-2 X BI-4	15.75	16.54	19.25	16.14	19.24**
20	CH-2 X HA-4	15.75	16.63	18.38	16.19	13.51**
21	SI-15 X SO-3	16.45	16.54	15.75	16.49	-4.51
22	SI-15 X SI-6	16.45	16.71	17.50	16.58	5.54**
23	SI-15 X BI-4	16.45	16.54	21.36	16.49	29.50**
24	SI-15 X HA-4	16.45	16.63	16.63	16.54	0.53

4.4.3.2. Crude fat (ether extract %)

Results presented in Table 68, revealed relative heterosis for crude fat (ether extract) (%) 54 % of the F_1 hybrids showed positive heterotic effects over their mid parents. Maximum positive heterosis (+20.46 %) was exhibited by the cross CH-2 X SO-3, whereas maximum negative heterotic effects over mid parent (-23.74 %) was manifested by the cross SO-8 X SI-6. Analysis of variance (ANOVA) for crude fat (ether extract) (%) (Appendix XL) displayed highly

significant differences ($P \leq 0.01$) among parents and their F_1 hybrids. Thus these entries can further be exploited in breeding programme.

Table 68. Mean performance of parents, F_1 hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for crude fat (ether extract) (%) (%)

Sl No.	Crosses	Female parents	Male parents	F_1 hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	5.33	5.50	5.62	5.42	3.82**
2	SO-1 X SI-6	5.33	5.67	5.22	5.50	-5.09
3	SO-1 X BI-4	5.33	5.53	5.24	5.43	-3.56
4	SO-1 X HA-4	5.33	5.07	5.23	5.20	0.64
5	SO-2 X SO-3	5.07	5.50	6.09	5.28	15.27**
6	SO-2 X SI-6	5.07	5.67	5.65	5.37	5.22**
7	SO-2 X BI-4	5.07	5.53	5.24	5.30	-1.13
8	SO-2 X HA-4	5.07	5.07	5.75	5.07	13.42**
9	SO-4 X SO-3	5.20	5.50	5.35	5.35	0.08
10	SO-4 X SI-6	5.20	5.67	5.09	5.43	-6.26
11	SO-4 X BI-4	5.20	5.53	5.06	5.37	-5.71
12	SO-4 X HA-4	5.20	5.07	4.84	5.13	-5.71
13	SO-8 X SO-3	5.03	5.50	5.23	5.27	-0.63
14	SO-8 X SI-6	5.03	5.67	4.08	5.35	-23.74
15	SO-8 X BI-4	5.03	5.53	4.43	5.28	-16.15
16	SO-8 X HA-4	5.03	5.07	5.63	5.05	11.55**
17	CH-2 X SO-3	5.60	5.50	6.69	5.55	20.46**
18	CH-2 X SI-6	5.60	5.67	5.69	5.63	0.93
19	CH-2 X BI-4	5.60	5.53	6.36	5.57	14.27**
20	CH-2 X HA-4	5.60	5.07	5.37	5.37	0.08
21	SI-15 X SO-3	4.67	5.50	5.66	5.08	11.34**
22	SI-15 X SI-6	4.67	5.67	5.07	5.17	-1.87
23	SI-15 X BI-4	4.67	5.53	4.96	5.10	-2.72
24	SI-15 X HA-4	4.67	5.07	5.24	4.87	7.74**

4.4.3.3. Crude fibre (%)

Results presented in Table 69, on relative heterosis for crude fibre (%) revealed that 75 % of the F_1 hybrids showed positive heterotic effects. Maximum positive heterosis of 19.52 and 15.59 % was exhibited by the cross SO-8 X SI-6 and SO-8 X BI-4, whereas maximum negative heterotic effects over mid parent (-4.28 and -3.49 %) was manifested by the cross SO-4 X BI-4 and SI-15 X HA-4 respectively. Analysis of variance (ANOVA) for crude fibre (Appendix XLI)

displayed highly significant differences ($P \leq 0.01$) among parents and their F_1 hybrids. Thus these entries can further be exploited in breeding programme.

Table 69. Mean performance of parents, F_1 hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for crude fiber (%)

Sl No.	Crosses	Female parents	Male parents	F_1 hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	18.80	19.25	19.64	19.03	3.25**
2	SO-1 X SI-6	18.80	19.00	18.54	18.90	-1.90
3	SO-1 X BI-4	18.80	18.95	19.74	18.88	4.58**
4	SO-1 X HA-4	18.80	19.10	18.65	18.95	-1.57
5	SO-2 X SO-3	18.95	19.25	19.92	19.10	4.29**
6	SO-2 X SI-6	18.95	19.00	19.76	18.98	4.15**
7	SO-2 X BI-4	18.95	18.95	19.20	18.95	1.32
8	SO-2 X HA-4	18.95	19.10	19.50	19.03	2.50**
9	SO-4 X SO-3	19.10	19.25	19.54	19.18	1.90*
10	SO-4 X SI-6	19.10	19.00	18.58	19.05	-2.48
11	SO-4 X BI-4	19.10	18.95	18.21	19.03	-4.28
12	SO-4 X HA-4	19.10	19.10	21.16	19.10	10.80**
13	SO-8 X SO-3	18.35	19.25	19.54	18.80	3.93**
14	SO-8 X SI-6	18.35	19.00	22.32	18.68	19.52**
15	SO-8 X BI-4	18.35	18.95	21.56	18.65	15.59**
16	SO-8 X HA-4	18.35	19.10	19.96	18.73	6.61**
17	CH-2 X SO-3	17.85	19.25	19.44	18.55	4.80**
18	CH-2 X SI-6	17.85	19.00	19.77	18.43	7.32**
19	CH-2 X BI-4	17.85	18.95	19.47	18.40	5.82**
20	CH-2 X HA-4	17.85	19.10	19.67	18.48	6.49**
21	SI-15 X SO-3	18.10	19.25	18.27	18.68	-2.17
22	SI-15 X SI-6	18.10	19.00	19.36	18.55	4.37**
23	SI-15 X BI-4	18.10	18.95	20.77	18.53	12.11**
24	SI-15 X HA-4	18.10	19.10	17.95	18.60	-3.49

4.4.3.4. Total ash (%)

Results presented in Table 70, revealed relative heterosis for total ash (%) content. 87.50 % of the F_1 hybrids displayed positive heterosis over their mid parents. Maximum positive mid parent (46.44 %) was revealed in F_1 SO-4 X HA-4, whereas maximum negative heterosis (-5.19 %) was expressed by the cross SO-4 X SI-6.

Analysis of variance (ANOVA) for total ash (Appendix XLII) displayed highly significant differences ($P \leq 0.01$) among parents and their F_1 hybrids. Thus, these entries can further be exploited in breeding programme.

Table 70. Mean performance of parents, F_1 hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for total ash (%)

Sl No.	Crosses	Female parents	Male parents	F_1 hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	9.33	9.67	10.17	9.50	7.07 ^{**}
2	SO-1 X SI-6	9.33	9.00	9.94	9.17	8.38 ^{**}
3	SO-1 X BI-4	9.33	8.67	10.13	9.00	12.56 ^{**}
4	SO-1 X HA-4	9.33	9.17	9.66	9.25	4.48 ^{**}
5	SO-2 X SO-3	9.00	9.67	9.77	9.33	4.66 ^{**}
6	SO-2 X SI-6	9.00	9.00	9.68	9.00	7.57 ^{**}
7	SO-2 X BI-4	9.00	8.67	9.72	8.83	10.06 ^{**}
8	SO-2 X HA-4	9.00	9.17	8.98	9.08	-1.17
9	SO-4 X SO-3	9.33	9.67	9.80	9.50	3.21 ^{**}
10	SO-4 X SI-6	9.33	9.00	8.69	9.17	-5.19
11	SO-4 X BI-4	9.33	8.67	9.77	9.00	8.50 ^{**}
12	SO-4 X HA-4	9.33	9.17	13.55	9.25	46.44 ^{**}
13	SO-8 X SO-3	8.77	9.67	9.15	9.22	-0.67
14	SO-8 X SI-6	8.77	9.00	11.37	8.88	28.04 ^{**}
15	SO-8 X BI-4	8.77	8.67	11.11	8.72	27.41 ^{**}
16	SO-8 X HA-4	8.77	9.17	9.88	8.97	10.16 ^{**}
17	CH-2 X SO-3	8.67	9.67	10.20	9.17	11.30 ^{**}
18	CH-2 X SI-6	8.67	9.00	10.08	8.83	14.11 ^{**}
19	CH-2 X BI-4	8.67	8.67	9.37	8.67	8.09 ^{**}
20	CH-2 X HA-4	8.67	9.17	10.49	8.92	17.68 ^{**}
21	SI-15 X SO-3	9.03	9.67	9.65	9.35	3.23 ^{**}
22	SI-15 X SI-6	9.03	9.00	9.24	9.02	2.50 ^{**}
23	SI-15 X BI-4	9.03	8.67	10.69	8.85	20.82 ^{**}
24	SI-15 X HA-4	9.03	9.17	10.10	9.10	10.96 ^{**}

4.4.3.5. Nitrogen free extract (%)

Results presented in Table 71 revealed relative heterosis for nitrogen free extract (%). 66.66 % of the F_1 hybrids displayed negative heterosis and 33.33 % showed positive heterosis over their mid parents. Maximum positive mid parent (7.79 %) was revealed in F_1 SI-15 X SO-3, whereas maximum negative heterosis (-15.43 and 12.76 %) was expressed by the cross SO-8 X SI-6 and SO-8 X BI-4 respectively. Analysis of variance (ANOVA) for nitrogen free extract (Appendix

XLIII) displayed highly significant differences ($P \leq 0.01$) among parents and their F_1 hybrids. Thus, such a variability can be utilized in further breeding programmes.

Table 71. Mean performance of parents, F_1 hybrids and percentage increase (+) or decrease (-) over mid parent (relative heterosis) for Nitrogen free extract (%)

Sl No.	Crosses	Female parents	Male parents	F_1 hybrids	Mid parent (MP)	% increase (+) or decrease (-) over MP
1	SO-1 X SO-3	46.72	46.03	43.90	46.38	-5.34
2	SO-1 X SI-6	46.72	46.96	45.77	46.84	-2.28
3	SO-1 X BI-4	46.72	47.68	44.19	47.20	-6.37
4	SO-1 X HA-4	46.72	48.35	46.66	47.54	-1.84
5	SO-2 X SO-3	47.44	46.03	44.45	46.74	-4.90
6	SO-2 X SI-6	47.44	46.96	45.27	47.20	-4.10
7	SO-2 X BI-4	47.44	47.68	46.46	47.56	-2.33
8	SO-2 X HA-4	47.44	48.35	46.41	47.90	-3.09
9	SO-4 X SO-3	46.04	46.03	46.02	46.04	-0.04
10	SO-4 X SI-6	46.04	46.96	48.19	46.50	3.63 ^{**}
11	SO-4 X BI-4	46.04	47.68	48.74	46.86	4.02 ^{**}
12	SO-4 X HA-4	46.04	48.35	41.86	47.96	-12.73
13	SO-8 X SO-3	47.57	46.03	47.76	46.80	2.06 [*]
14	SO-8 X SI-6	47.57	46.96	39.97	47.27	-15.43
15	SO-8 X BI-4	47.57	47.68	41.55	47.63	-12.76
16	SO-8 X HA-4	47.57	48.35	47.22	47.79	-1.19
17	CH-2 X SO-3	47.22	46.03	48.11	46.63	3.18 ^{**}
18	CH-2 X SI-6	47.22	46.96	48.88	47.09	3.79 ^{**}
19	CH-2 X BI-4	47.22	47.68	45.71	47.45	-3.68
20	CH-2 X HA-4	47.22	48.35	46.28	47.79	-3.15
21	SI-15 X SO-3	48.32	46.03	50.85	47.18	7.79 ^{**}
22	SI-15 X SI-6	48.32	46.96	48.98	47.64	2.81 ^{**}
23	SI-15 X BI-4	48.32	47.68	42.54	48.00	-11.37
24	SI-15 X HA-4	48.32	48.35	50.44	48.33	4.35 ^{**}

4.4.4. Variability and genetic parameters

Critical appraisal of Table 72 revealed that mean value for collar diameter, plant height and number of leaves was 2.94 mm, 15.65 cm and 8.23 respectively. The genotypic and phenotypic variances were maximum for collar diameter (34.81 and 34.84), while the minimum was exhibited by number of leaves (3.58 and 3.72) respectively. As regards to the genotypic coefficient of variation, collar diameter exhibited the maximum (344.32 per cent), followed by plant height (85.06 per cent) while the minimum was noticed for number of

leaves (66.01 per cent). Similarly, maximum phenotypic coefficient of variation (344.45 per cent) was exhibited by collar diameter while the minimum (67.23 per cent) was estimated for number of leaves per plant. Heritability percentage (broad-sense) was the high for all the trait *i.e.*, 0.99, 0.97 and 0.96 for collar diameter, plant height and number of leaves. Collar diameter had the maximum genetic advance (12.15) followed by plant height (6.84), while the minimum genetic advance was exhibited for number of leaves (3.83). Maximum genetic gain was observed in collar diameter (413.77 per cent) while the minimum was recorded in plant height (43.73 per cent).

Table 72. Estimation of variability and genetic parameters for heterosis in nursery traits among crosses of *G. optiva* genotypes

Sl. No.	Genetic Parameter	Nursery trait		
		Plant height	Collar diameter	No. of leaves
1	Mean	15.65	2.94	8.23
2	Environmental variance	0.29	0.03	0.13
3	Genotypic variance	11.32	34.81	3.58
4	Phenotypic variance	11.62	34.84	3.72
5	Genotypic coefficient of variation (per cent)	85.06	344.32	66.01
6	Phenotypic coefficient of variation (per cent)	86.15	344.45	67.23
7	Heritability broad sense	0.97	0.99	0.96
8	Genetic advance	6.84	12.15	3.83
9	Genetic gain	43.73	413.77	46.55

4.5. PATERNITY ANALYSIS USING RAPD AND ISSR MARKERS

The hybrids obtained after the completion of hybridization programme were tested for their paternity using RAPD and ISSR markers. A total of fourteen initially screened RAPD markers and eleven ISSR markers were used to confirm the hybrids on the basis of banding pattern. A close appraisal of the RAPD and ISSR banding pattern [Plate 7-18] obtained after the amplification of genomic DNA of both the parents and their hybrid revealed that all the hybrids were true to type. The RAPD and ISSR fragments obtained after PCR amplification was subjected to analysis for obtaining their similarity coefficient with respect to their parent. The scoring of bands for presence as 1 (band present) and 0 (band absent) for each hybrid combination was done separately by using Alpha Imager

software. The data matrix so obtained was analyzed with NTSYS-2.2 software to obtain the Jaccard's similarity correlation coefficient.

4.5.1. RAPD studies

4.5.1.1. Line X Tester crosses of SO-1 X SO-3, SI-6, BI-4 and HA-4

A close appraisal of the data appended in Table. 73. disclosed that all the hybrids (SO-1 X SO-3, SO-1 X SI-6, SO-1 X BI-4 and SO-1 X HA-4) were highly similar to their male parents suggesting dominant control of male parent in 6 X 4 (Line X Tester) matting design. Among fourteen primers, every band observed in the hybrid was present either in female or male or in both [Plate 7]. Similarity correlation coefficient of hybrid SO-1 X SO-3 between the female (SO-1) and male (SO-3) genotypes for individual primers varied from 20 to 100 %. In female parent (SO-1) the similarity varied from 20 to 100 %. respectively. OPA 04, OPA 05, OPA 07, OPAC 10 and OPAE 10 showed 100 % similarity whereas, OPAE 15 showed 20 % followed by OPF 08 (33 %). While in male, (SO-3) the similarity ranged from 20 to 100 %. The primer OPF 08 exhibited 20 % similarity whereas OPA 07, OPAC 10, OPAC 15, OPAE 10, OPC 13, OPF and OPO 20 expressed cent percent of hybrid similarity with male parent. The average similarity was 75 % towards the female and highly similar to the male *i.e.*, 83 % suggesting dominant control of male parent in 6 X 4 (Line X Tester) design.

In SO-1 X SI-6 hybrid similarity was compared with female (SO-1) and male (SO-3) genotypes for individual primers varied from 20 to 100 %. In SO-1 the similarity ranged from 20 to 100 % respectively. OPA 04, OPAC 15, OPAE 10, OPF 06, OPF 12 and OPO 20 showed 100 % similarity. OPAE 15 showed lesser similarity of 20 % followed by OPF 08 (33 %). Where in male (SI-6) the similarity also ranged from 20 to 100 %. The primer OPF 08 exhibited 20 % similarity followed by OPA 01 and OPA 04 (67 %) while OPA 05, OPAC 10, OPAC 15, OPAE 10, OPAE 15, OPC 13 and OPF 06 expressed cent per cent similarity between hybrid and male parent. The average similarity for female was 82 % and 84 % similar to the male parents showing dominant control of male parent. The similarity of individual primers varied from 20 to 100 % for SO-1 X

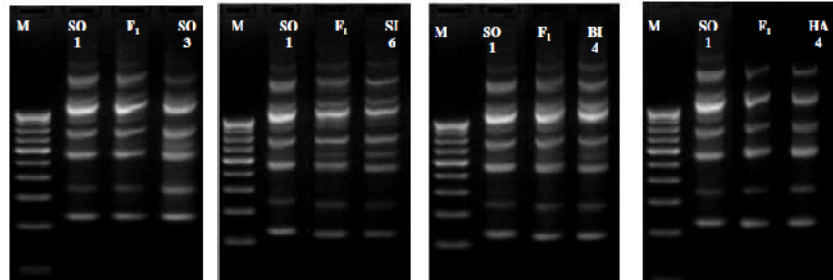
BI-4 hybrid and between its parents. For SO-1 the similarity ranged from 20 to 100 % respectively. Primers OPA 07, OPAC 15, OPAE 10 and OPF 06 showed 100 % similarity. OPAE 15 showed lesser similarity of 20 % and 50 % similarity was observed in OPA 04 primer. The primer OPF 08 exhibited 40 % while OPA 07, OPAC 10, OPAE 10, OPAE 15, OPF 06 and OPO 20 expressed cent percent similarity with male parent. The average similarity for male was 92 % and 75 % similar to female parents again showing the dominance of male parent.

The similarity of individual primers for SO-1 X HA-4 hybrid and between its parents varied from 17 to 100 %. For SO-1, the similarity ranged from 17 to 100 % respectively. Primers OPAE 10 showed 100 % similarity. OPA 04 showed lowest similarity of 17 % followed by OPAE (20 %). For HA-4 the range varied from 25 to 100 %. The primer OPA 04, OPAE 15, OPO 20, OPAC 10 and OPAE 02 exhibited 25, 60, 63 and 88 % respectively. while all other primers exhibited cent percent similarity with male parent. The average similarity for male was 89 % and 67 % similar to female parents the dominance of male was completely expressed among the hybrid.

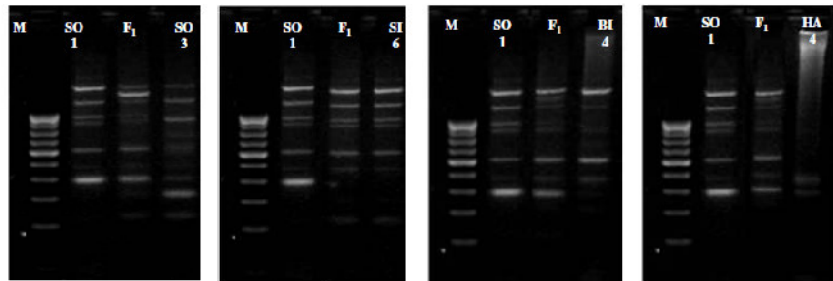
Table 73: Similarity coefficient values of hybrids revealed by RAPD primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-1 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-1 X SO-3		SO-1 X SI-6		SO-1 X BI-4		SO-1 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	OPA 01	0.50	0.67	0.50	0.67	0.75	0.80	0.60	1.00
2	OPA 04	1.00	0.67	1.00	0.67	0.50	1.00	0.17	0.25
3	OPA 05	1.00	0.60	0.80	1.00	0.60	0.67	0.60	1.00
4	OPA 07	1.00	1.00	0.89	0.89	1.00	1.00	0.67	1.00
5	OPAC 10	1.00	1.00	0.80	1.00	0.75	1.00	0.88	0.88
6	OPAC 15	0.67	1.00	1.00	1.00	1.00	0.67	0.67	1.00
7	OPAE 02	0.89	0.88	0.89	0.89	0.89	0.89	0.89	0.88
8	OPAE 10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9	OPAE 15	0.20	0.80	0.20	1.00	0.20	1.00	0.20	0.60
10	OPC 13	0.83	1.00	1.00	1.00	0.71	0.88	0.83	1.00
11	OPF 06	0.67	1.00	1.00	1.00	1.00	1.00	0.67	1.00
12	OPF 08	0.33	0.20	0.33	0.20	0.60	0.40	0.60	1.00
13	OPF 12	0.71	0.86	1.00	0.71	0.71	0.88	0.71	1.00
14	OPO 20	0.75	1.00	1.00	0.75	0.75	1.00	0.83	0.63
	Average	0.75	0.83	0.82	0.84	0.75	0.92	0.67	0.89

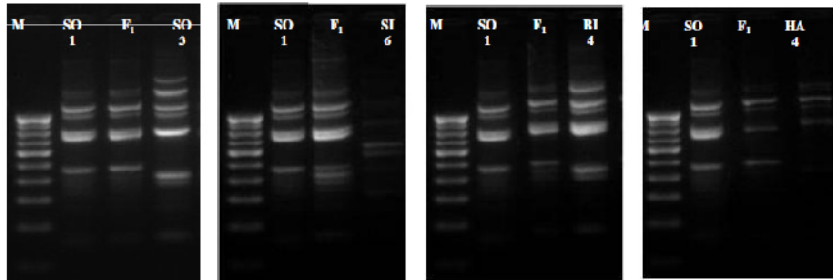
OPA 07



OPAE 15



OPF 12



OPAE 02

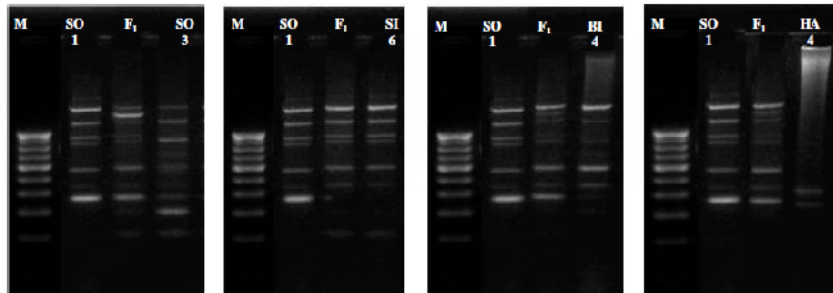


PLATE 7: Paternity analysis of Line X Tester crosses (SO-1 X SO-3, SI-6, BI-4 and HA-4) using RAPD primers

4.5.1.2. Line X Tester crosses of SO-2 X SO-3, SI-6, BI-4 and HA-4

Among the fourteen primers, every band observed in the hybrid was present either in female or male or in both [Plate 8]. The data appended in Table. 77. disclosed that all the hybrids (SO-2 X SO-3, SO-2 X SI-6, SO-2 X BI-4 and SO-2 X HA-4) were highly similar to their male parents suggesting which ultimately depleting the dominance of male parent in 6 X 4 (Line X Tester) matting design. Hybrid [SO-2 X SI-6] recorded mean maximum similarity with male (99 %) and female parent (74 %) followed by SO-2 X HA-4 in which 95 % contribution from male and 76 % from female. The hybrid SO-2 X SO-3 showed 80 % contribution of female parent and while male dominated by contributing 87 % to the progeny. In SO-2 X BI-4, the contribution of female was 72 per cent and 84 percent by male parent, which showed lesser contribution of female among the group of hybrids. Similarity correlation coefficient of hybrid SO-2 X SO-3 between the female (SO-2) and male (SO-3) genotypes for individual primers varied from 50 to 100 %. In female parent (SO-2) OPAC 10, OPAC 15, OPF 06 and OPO 20 showed 100 % similarity whereas, OPC 13 showed 50 % similarity. While in male, (SO-3) the similarity ranged from 50 to 100 %. The primer OPC 13 exhibited 50 % similarity whereas OPA 04, OPAE 02, OPAE 15, OPF 08, OPF 12 and OPO 20 expressed cent percent of hybrid similarity with male parent (Table 74).

For SO-2 X SI-6 hybrid similarity was compared for individual primers and it varies from 50 to 100 % with female (SO-2) and 83 to 100 % for male (SO-3) genotypes. OPAC 10 and OPO 20 exhibited 100 % similarity. OPC 13 showed lesser similarity of 50 % followed by OPA 01, OPAE 10 and OPAE 15 (60 %) respectively. For male (SI-6) OPAE 02, OPO-20 showed 83 % similarity followed by OPA 01, OPF 12 (89 and 88 %) while ten primer expressed cent percent similarity between hybrid and male parent.

For individual primers similarity varied from 20 to 100 % between SO-2 (female) and SO-2 X BI-4 (hybrid), it was 25 to 100 % between BI-4 (male) and SO-2 X BI-4. Primers that showed 100 % similarity with the SO-2 are OPAC 10, OPAC 15, OPF 06 and OPO 20. OPC 13 showed similarity of 50 % and 60 %

similarity were observed in OPAE 10 and OPAE 15 primers. While similarity of cent percent was noticed in primers OPA 04, OPAE 02, OPAE 15, OPF 08, OPF 12 and OPO 20 between BI-4 and hybrid. The primer OPC 13 showed lowest similarity of 50 % with BI-4 parent.

The coefficient of similarity of individual primers between SO-1 X HA-4 hybrid and SO-1 varied from 25 to 100 % and the similarity varied from 60 to 100 % for HA-4 respectively. Primers OPF 08 showed 25 % similarity followed by OPAE 15 (40 %) whereas, OPAC 10, OPAE 10, OPC 13 and OPF 12 showed highest similarity of 100 % between hybrid and SO-1. For HA-4 and hybrid primer OPAC 15 and OPC 13 exhibited 60 and 75 % similarity respectively. while all other primers exhibited cent percent similarity with male parent (Table 74).

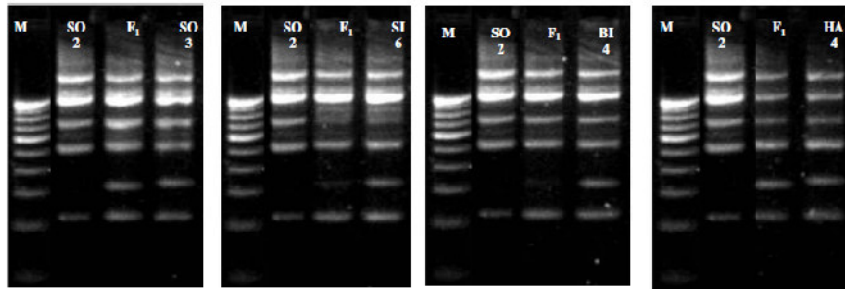
Table74: Similarity coefficient values of hybrids revealed by RAPD primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-2 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-2 X SO-3		SO-2 X SI-6		SO-2 X BI-4		SO-2 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	OPA 01	0.80	0.75	0.60	0.88	0.80	1.00	0.80	1.00
2	OPA 04	0.67	1.00	0.67	1.00	0.67	1.00	0.50	1.00
3	OPA 05	0.75	0.88	0.75	1.00	0.60	1.00	0.75	1.00
4	OPA 07	0.83	0.86	0.83	1.00	1.00	0.83	0.83	1.00
5	OPAC 10	1.00	0.75	1.00	1.00	1.00	1.00	1.00	1.00
6	OPAC 15	1.00	0.75	0.75	1.00	0.60	0.83	0.60	0.60
7	OPAE 02	0.75	1.00	0.63	0.83	0.63	1.00	0.88	1.00
8	OPAE 10	0.60	0.60	0.60	1.00	0.60	0.60	1.00	1.00
9	OPAE 15	0.60	1.00	0.60	1.00	0.20	0.25	0.40	1.00
10	OPC 13	0.50	0.50	0.50	1.00	0.80	0.60	1.00	0.75
11	OPF 06	1.00	0.75	0.75	1.00	0.50	0.67	0.75	1.00
12	OPF 08	0.89	1.00	0.75	1.00	0.88	1.00	0.25	1.00
13	OPF 12	0.88	1.00	0.88	0.89	0.88	1.00	1.00	1.00
14	OPO 20	1.00	1.00	1.00	0.83	1.00	1.00	0.83	1.00
	Average	0.80	0.87	0.74	0.99	0.72	0.84	0.76	0.95

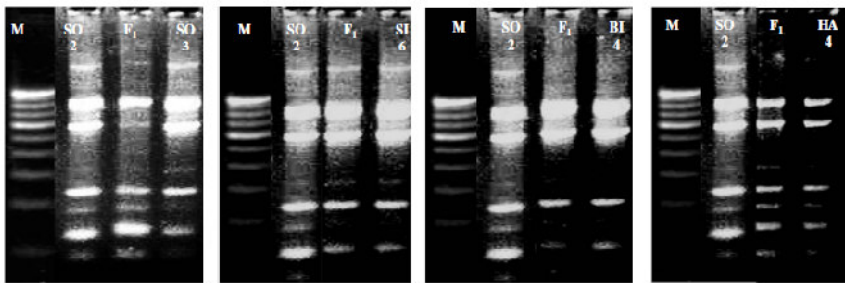
4.5.1.3. Line X Tester crosses of SO-4 X SO-3, SI-6, BI-4 and HA-4

The investigation of data presented in Table. 75. disclosed that three out of four hybrids (SO-4 X SO-3, SO-4 X BI-4 and SO-4 X HA-4) were highly similar to their male parents suggesting dominant control of male parent and

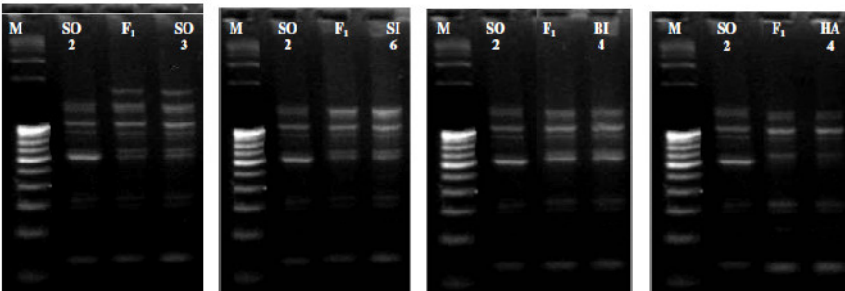
OPA 07



OPAE 15



OPF 12



OPAE 02

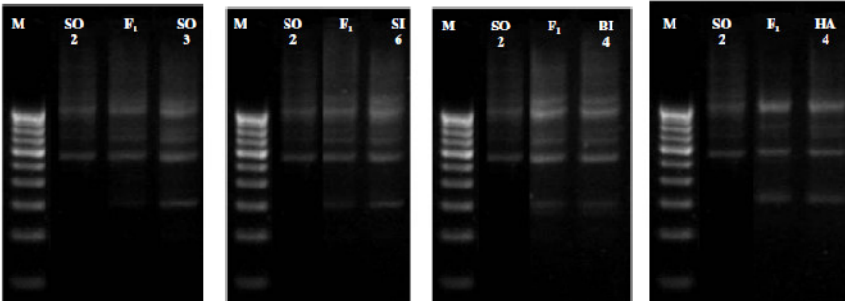


PLATE 8: Paternity analysis of Line X Tester crosses (SO-2 X SO-3, SI-6, BI-4 and HA-4) using RAPD primers

SO-4 X SI-6 was more similar to their female parents in 6 X 4 (Line X Tester) matting design. Hybrid [SO-4 X SO-3] recorded maximum similarity with male (97 per cent) and female parent (84 per cent) followed by SO-2 X HA-4 in which 96 per cent contribution from male and 66 percent from female where the contribution of female to hybrid was less among the group. The hybrid SO-2 X BI-4 showed 77 percent contribution of female parent and while male dominated again by contributing 89 per cent to the progeny. Maximum similarity (91 per cent) with female parent was recorded in SO-4 X SI-6 suggesting dominating control of female parent. These data were obtained from fourteen primers, which explored that the band observed in the hybrid was present either from female or male or from both [Plate 9].

The similarity of hybrid SO-4 X SO-3 between the female (SO-4) genotypes for individual primers varied from 40 to 100 % and it is 80 to 100 % between male (SO-3). In female parent (SO-4) OPA 01, OPAC 15, OPAE 10, OPC 13 and OPO 20 showed 100 % similarity whereas, OPAC 10 showed 40 % similarity followed by OPAE 15 (60 %). While in SO-3 the primer OPA 05 exhibited 80 % similarity which is followed by OPC 13 and OPF 08 (86 and 88 %) whereas ten out of fourteen primer showed cent per cent similarity which showed that male parent contribution is maximum to hybrid (Table 78).

For SO-4 X SI-6 hybrid similarity was compared for individual primers and it varies from 50 to 100 % with female (SO-4) and 60 to 100 % for male (SI-6) genotypes. Nine out of fourteen primer showed 100 per cent similarity, while OPAC 10 and OPF 06 exhibited 50 and 75 % similarity between SO-4 and hybrid. For male (SI-6) OPAE 15 showed lesser similarity of 60 % followed by OPA 07 and OPAC 10 (80 %) respectively. Whereas, OPA 01, OPAC 15 and OPF 06 expressed cent percent similarity between hybrid and male parent.

Similarity for individual primers varied from 40 to 100 % between female (SO-4) and SO-2 X BI-4 (hybrid), it was 25 to 100 % between BI-4 (male) and SO-2 X BI-4. Three primers that showed 100 % similarity with the SO-4 are OPA 04, OPAE 02 and OPO 20. OPAC 15 showed similarity of 40 % and 60 % similarity were observed in OPAE 15 primers. While nine primers

showed cent percent similarity whereas the primer OPF 08 showed lowest similarity of 25 % followed by OPAE 02 (75 %) with BI-4 parent.

The coefficient of similarity of individual primers between SO-4 X HA-4 hybrid and SO-1 varied from 25 to 100 % and the similarity varied from 50 to 100 % for HA-4 respectively. Primers OPAE 02 showed 25 % similarity followed by OPAC 10 (40 %) whereas, OPA 01, OPA 04 and OPAE 02 showed highest similarity (100 %) between hybrid and SO-1. For HA-4 and hybrid primer OPAC 15 exhibited 50 % similarity. While all other primers exhibited cent percent similarity with male parent (Table 75).

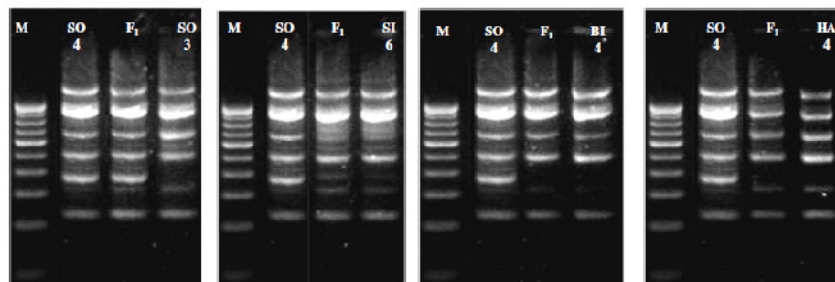
Table 75: Similarity coefficient values of hybrids revealed by RAPD primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-4 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-4 X SO-3		SO-4 X SI-6		SO-4 X BI-4		SO-4 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	OPA 01	1.00	1.00	0.86	1.00	0.75	1.00	1.00	1.00
2	OPA 04	0.83	1.00	1.00	0.83	1.00	1.00	1.00	1.00
3	OPA 05	0.83	0.80	1.00	0.83	0.50	1.00	0.50	1.00
4	OPA 07	0.70	1.00	1.00	0.80	1.00	1.00	0.70	1.00
5	OPAC 10	0.40	1.00	0.50	0.80	0.70	1.00	0.40	1.00
6	OPAC 15	1.00	1.00	1.00	1.00	0.40	1.00	0.50	0.50
7	OPAE 02	0.88	1.00	0.88	1.00	1.00	0.75	0.25	1.00
8	OPAE 10	1.00	1.00	1.00	0.80	0.80	0.80	1.00	1.00
9	OPAE 15	0.60	1.00	1.00	0.60	0.60	1.00	0.75	1.00
10	OPC 13	1.00	0.86	1.00	0.83	0.86	0.86	0.67	1.00
11	OPF 06	0.75	1.00	0.75	1.00	0.75	1.00	0.75	1.00
12	OPF 08	0.86	0.88	1.00	0.83	0.75	0.25	0.17	1.00
13	OPF 12	0.88	1.00	0.78	0.89	0.71	0.83	0.86	1.00
14	OPO 20	1.00	1.00	1.00	0.86	1.00	1.00	0.67	1.00
	Average	0.84	0.97	0.91	0.86	0.77	0.89	0.66	0.96

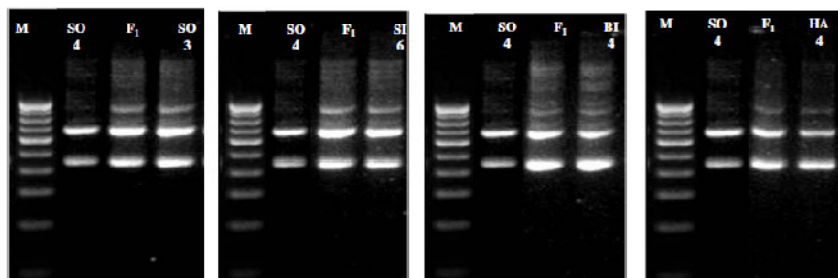
4.5.1.4. Line X Tester crosses of SO-8 X SO-3, SI-6, BI-4 and HA-4

The data presented in Table 76 explains that the hybrid [SO-8 X HA-4] recorded maximum similarity with male (89 per cent) and female parent (61 per cent) followed by SO-8 X SO-3, SO-8 X BI-4 and SO-8 X SI-6 in which 87, 83 and 79 per cent contribution from male and 62, 61 and 75 percent from female respectively. Information from all the hybrids (SO-8 X SO-3, SO-8 X SI-6,

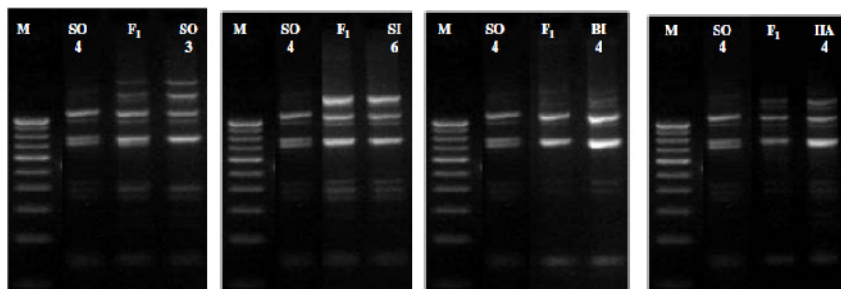
OPA 07



OPAE 15



OPF 12



OPAE 02

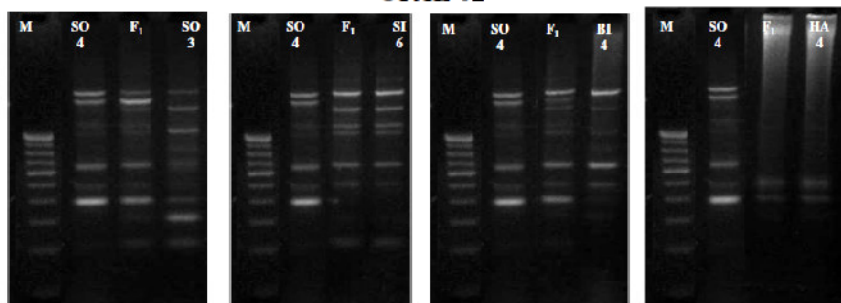


PLATE 9: Paternity analysis of Line X Tester crosses (SO-4 X SO-3, SI-6, BI-4 and HA-4) using RAPD primers

SO-8 X BI-4 and SO-8 X HA-4) it is clear that the hybrids were highly similar to their male parents suggesting dominant control of male parent (Plate 10).

The similarity of hybrid SO-8 X SO-3 between the female (SO-8) genotypes for individual primers varied from 25 to 100 % and it is 50 to 100 % between male (SO-3). In female parent (SO-8) lower similarity exhibited by OPF 06 (25 %) followed by OPF 08 (29 %) and OPAE 02 (33 %) while two primer OPAC 15 and OPAE 10 showed 100 % similarity. While for SO-3 the primer OPAC 15 exhibited 50 % similarity which is followed by OPF 06 and OPO 20 (80 %) respectively, whereas OPA 07, OPAC 10, OPAE 02, OPAE 10 and OPC 13 showed cent per cent similarity which showed that male parent contribution is maximum to hybrid (Table 79).

For SO-8 X SI-6 hybrid similarity was compared for individual primers and it varies from 25 to 100 % with female (SO-8) and 50 to 100 % for male (SI-6) genotypes. Four out of fourteen primer showed 100 per cent similarity, while OPF 06 and OPO 20 exhibited 25 and 33 % similarity between SO-8 and hybrid. For male (SI-6) OPAE 02 showed similarity of 50 % followed by OPAC 15, OPAE 15, OPF 06 and OPF 08 (67 %) respectively. Where OPA 01, OPA 05 and OPA 07 showed cent percent similarity between hybrid and male parent.

Similarity for individual primers varied from 25 to 100 % between female (SO-8) and SO-8 X BI-4 (hybrid), it was 50 to 100 % between BI-4 (male) and SO-8 X BI-4. Out of fourteen primer only OPAE 10 showed 100 % similarity with the SO-4 and OPF 06 and OPF 08 showed 25 % similarity respectively. While seven primers showed cent percent similarity whereas the primer OPA 04 showed similarity of 50 % and is followed by OPF 12 (57 %) with BI-4 parent (Table 79).

The individual primers for similarity estimation was varied between 17 to 100 % for SO-4 X HA-4 hybrid and SO-1 and the similarity varied from 40 to 100 % for HA-4 respectively. Primers OPF 06 showed 17 % similarity followed by OPAE 02 (25 %) whereas, OPF 12 showed highest similarity (100 %) between hybrid and SO-1. For HA-4 and hybrid primer OPC 13 exhibited 40 %

similarity. While OPA 01, OPA 07, OPAC 15, OPAE 10 and OPF 12 primers exhibited cent percent similarity with male parent (Table 76).

Table 76: Similarity coefficient values of hybrids revealed by RAPD primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-8 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-8 X SO-3		SO-8 X SI-6		SO-8 X BI-4		SO-8 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	OPA 01	0.86	1.00	0.86	1.00	0.88	1.00	0.86	1.00
2	OPA 04	0.50	0.88	0.67	0.83	0.50	0.50	0.67	0.67
3	OPA 05	0.50	0.67	0.50	1.00	0.75	1.00	0.50	0.67
4	OPA 07	0.83	1.00	0.83	1.00	0.83	1.00	0.83	1.00
5	OPAC 10	0.75	1.00	0.75	0.75	0.75	1.00	0.75	0.75
6	OPAC 15	1.00	0.50	1.00	0.67	0.40	0.60	0.50	1.00
7	OPAE 02	0.33	1.00	1.00	0.50	0.33	1.00	0.25	0.75
8	OPAE 10	1.00	1.00	0.75	0.75	1.00	1.00	0.80	1.00
9	OPAE 15	0.50	0.83	1.00	0.67	0.60	1.00	0.60	0.60
10	OPC 13	0.83	1.00	0.83	0.83	0.83	0.80	0.83	0.40
11	OPF 06	0.25	0.80	0.25	0.67	0.25	0.80	0.17	0.86
12	OPF 08	0.29	0.88	1.00	0.67	0.25	0.63	0.33	0.86
13	OPF 12	0.67	0.89	0.75	0.89	0.67	0.57	1.00	1.00
14	OPO 20	0.40	0.80	0.33	0.83	0.50	0.67	0.50	0.80
	Average	0.62	0.87	0.75	0.79	0.61	0.83	0.61	0.89

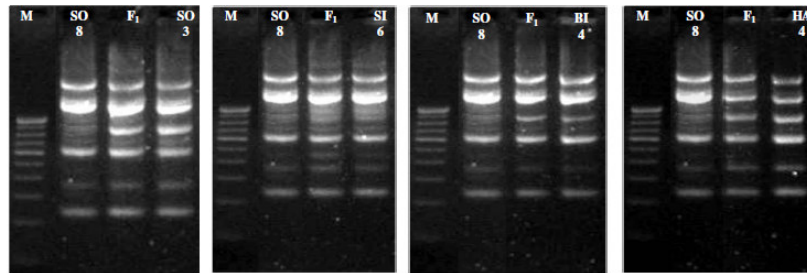
4.5.1.5. Line X Tester crosses of CH-2 X SO-3, SI-6, BI-4 and HA-4

The data presented in Table 77 reveals that the hybrid [CH-2 X SI-6] recorded maximum similarity with male (89 per cent) and female parent (62 per cent) followed by CH-2 X BI-4, CH-2 X SO-3 and CH-2 X HA-4 in which 85, 83 and 79 per cent contribution from male and 62, 61 and 68 percent from female respectively. These results highlights that the male parent contribution was higher than the females (Plate 11).

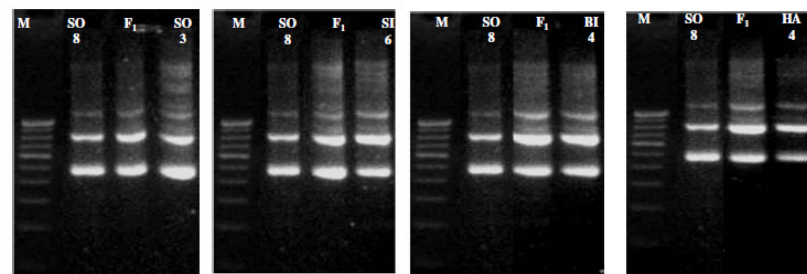
The similarity of hybrid CH-2 X SO-3 between the female (SO-8) genotypes for individual primers varied from 25 to 100 % and it is 33 to 100 % between male (SO-3). In female parent (CH-2) lower similarity exhibited by OPF 12 and OPO 20 (25 %) followed by OPF 08 (33 %) while two primer OPAC 15 and OPAE 02 showed 100 % similarity. While for SO-3 the primer OPF 08 exhibited 33 % similarity. Primer OPA 01, OPA 07, OPAC 10, OPAC 15, OPAE 02, OPAE 15 and OPF 06 showed cent per cent similarity (Table 79).

Hybrid

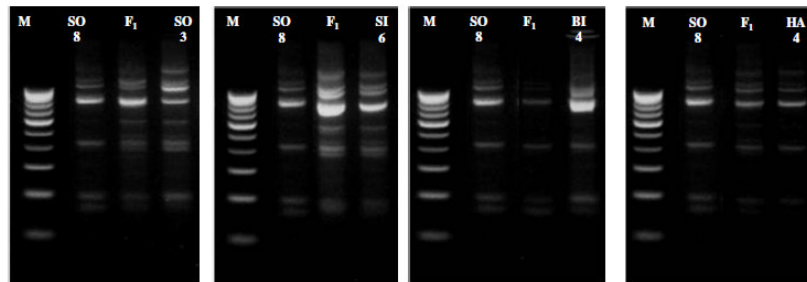
OPA 07



OPAE 15



OPF 12



OPAE 02

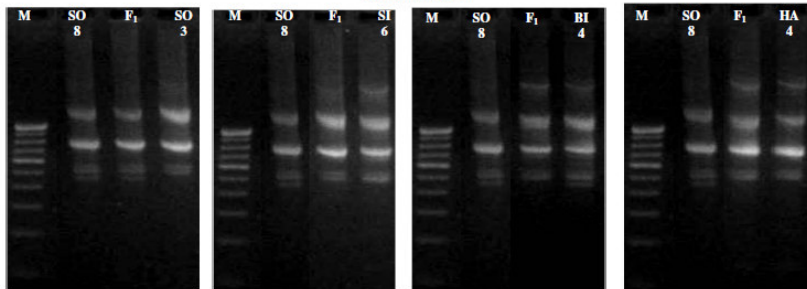
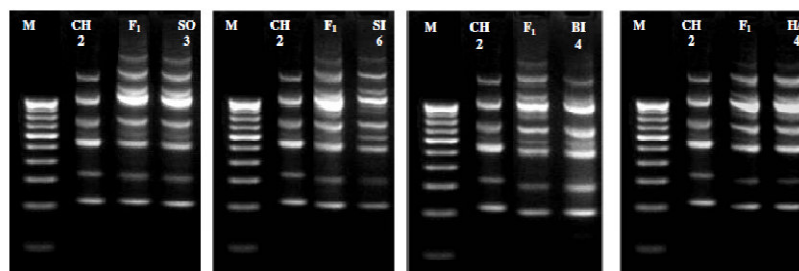
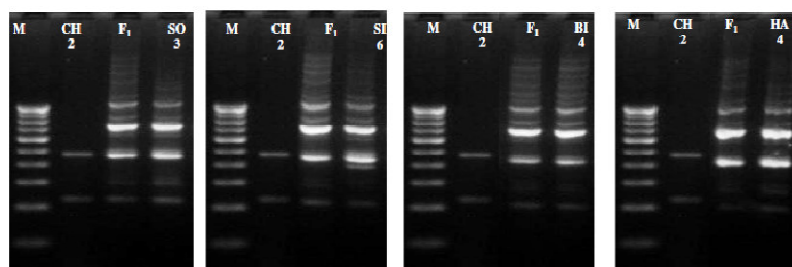


PLATE 10: Paternity analysis of Line X Tester crosses (SO-8 X SO-3, SI-6, BI-4 and HA-4) using RAPD primers

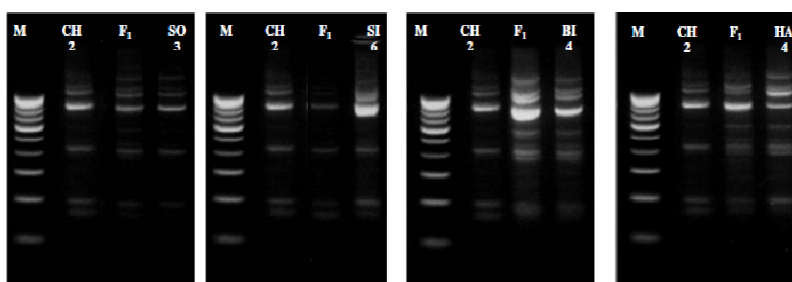
OPA 07



OPAE 15



OPF 12



OPAE 02

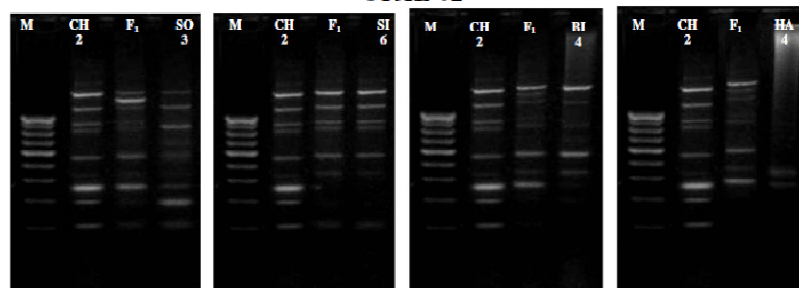


PLATE 11: Paternity analysis of Line X Tester crosses (CH-2 X SO-3, SI-6, BI-4 and HA-4) using RAPD primers

CH-2 X SI-6 similarity varies from 33 to 100 % with female (SO-8) and 20 to 100 % for male (SI-6) genotypes. Two primer OPAC 10 and OPAE 10 showed 100 per cent similarity, while OPA 05, OPAE 15, OPF 08 and OPF 12 exhibited 33 % similarity between CH-2 and hybrid. OPF 08 showed 20 % similarity between male (SI-6), where six out of fourteen primers exhibited cent percent similarity between hybrid and male parent (Table 77).

Similarity for primers varied from 20 to 100 % between female (CH-2) and CH-2 X BI-4 (hybrid), it was 17 to 100 % between BI-4 (male) and SO-8 X BI-4. Out of fourteen primers only OPAE 10 and OPC 13 showed 100 % similarity with the CH-2 and OPF 12 and OPO 20 showed 20 and 25 % similarity respectively. While, five primers (OPAC 10, OPAE 02, OPAE 10, OPAE 15 and OPF 06) showed cent percent similarity whereas the primer OPF 08 showed similarity of 17 % followed by OPA 05 (50 %) with BI-4 parent (Table 77).

Similarity estimation was between 25 to 100 % for CH-2 X HA-4 hybrid and CH-2 and the similarity varied from 25 to 100 % for HA-4 respectively. Primers OPF 12 showed 25 % similarity followed by OPF 08 (33 %) while, OPAE 10 and OPC 13 exhibited highest similarity (100 %) between hybrid and CH-2. For HA-4 and hybrid, primer OPF 08 exhibited 25 % similarity. While seven primers exhibited cent percent similarity with male parent (Table 77).

4.5.1.6. Line X Tester crosses of SI-15 X SO-3, SI-6, BI-4 and HA-4

The Table 78 data reveals that the hybrid [SI-15 X HA-4] recorded maximum similarity with male (91 per cent) and female parent (71 per cent) followed by SI-15 X BI-4, SI-15 X SO-3 and SI-15 X HA-4 in which 86, 85 and 82 per cent contribution from male and 78, 72 and 77 per cent from female respectively. These results shows that the male parent contribution was higher than the females (Plate 12).

The similarity of hybrid SI-15 X SO-3 between the female (SI-15) genotypes for individual primers varied from 20 to 100 % and it is 50 to 100 % between male (SO-3). In female parent (SI-15) lower similarity exhibited by OPF 06 and OPAC 10 (20 and 40 %) where three primer OPA 04, OPA 05 and OPF

12 showed 100 % similarity. While for SO-3 the OPAE 10 exhibited 50 % similarity. Six primers (OPA 01, OPA 04, OPA 05, OPAC 10, OPAC 15 and OPAE 15) showed cent percent similarity (Table 78).

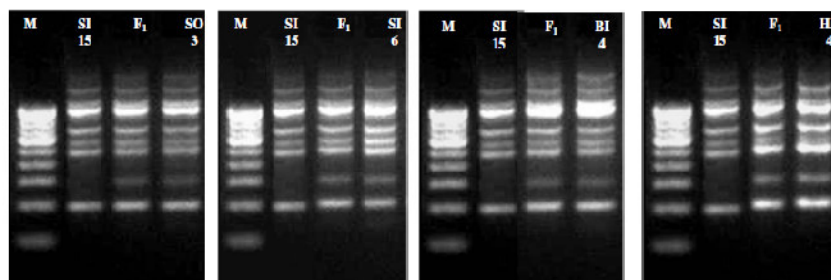
Hybrid SI-15 X SI-6 similarity varies from 33 to 100 % with female (SI-15) and 50 to 100 % for male (SI-6) genotypes. Five primers OPA 04, OPA 05, OPAE 02, OPAE 10 and OPO 20 showed 100 per cent similarity, while OPF 06 exhibited 33 % similarity between CH-2 and hybrid. OPAE 02 showed 50 % similarity between male (SI-6), where three primers exhibited cent percent similarity between hybrid and male parent. Four primers (OPA 05, OPAC 15, OPAE 02 and OPC 13) showing 100 percent similarity and the OPF 06 depleted 33 % between female (SI-15) and SI-15 X BI-4 (hybrid), it was 57 with primer OPF 08 and eight primers exhibited 100 % similarity between BI-4 (male) and SI-15 X BI-4 (Table 78).

Table 77: Similarity coefficient values of hybrids revealed by RAPD primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of CH-2 X SO-3, SI-6, BI-4 and HA-4

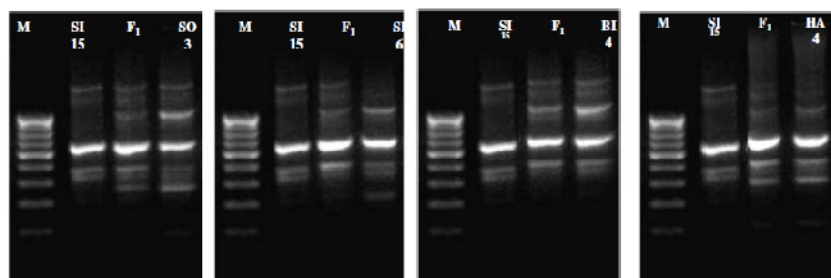
Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		CH-2 X SO-3		CH-2 X SI-6		CH-2 X BI-4		CH-2 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	OPA 01	0.80	1.00	0.83	0.67	0.83	0.67	0.80	1.00
2	OPA 04	0.75	0.67	0.60	1.00	0.50	0.67	0.75	1.00
3	OPA 05	0.40	0.80	0.33	1.00	0.50	0.50	0.67	0.75
4	OPA 07	0.60	1.00	0.75	1.00	0.60	0.90	0.67	1.00
5	OPAC 10	0.80	1.00	1.00	0.40	0.80	1.00	0.80	1.00
6	OPAC 15	1.00	1.00	0.25	1.00	0.75	0.60	0.80	0.60
7	OPAE 02	1.00	1.00	0.90	0.80	0.89	1.00	0.89	0.75
8	OPAE 10	0.75	0.75	1.00	0.75	1.00	1.00	1.00	1.00
9	OPAE 15	0.40	1.00	0.33	1.00	0.40	1.00	0.40	1.00
10	OPC 13	0.60	0.60	0.80	0.86	1.00	0.60	1.00	0.60
11	OPF 06	0.67	1.00	0.67	1.00	0.67	1.00	0.67	0.67
12	OPF 08	0.33	0.33	0.33	0.20	0.33	0.17	0.33	0.25
13	OPF 12	0.25	0.67	0.33	0.80	0.20	0.80	0.25	1.00
14	OPO 20	0.25	0.75	0.50	1.00	0.25	2.00	0.50	0.50
	Average	0.61	0.83	0.62	0.89	0.62	0.85	0.68	0.79

Similarity was varied from 25 to 100 % for SI-15 X HA-4 hybrid and SI-15 and the similarity varied from 40 to 100 % for HA-4 respectively. Primers OPF 06 showed 25 % similarity followed by OPAC 10 and OPAE 15 (40 %)

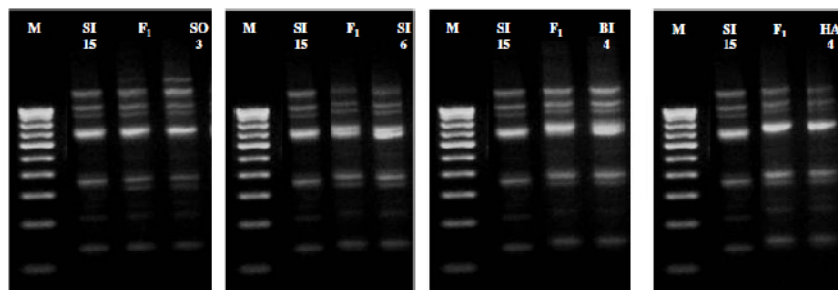
OPA 07



OPAE 15



OPF 12



OPAE 02

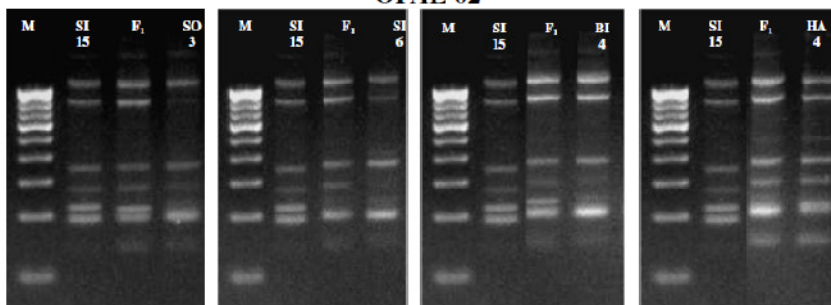


PLATE 12: Paternity analysis of Line X Tester crosses (SI-15 X SO-3, SI-6, BI-4 and HA-4) using RAPD primers

while, OPA 07, OPAE 02 and OPAE 10 exhibited highest similarity (100 %) between hybrid and SI-15. For HA-4 and hybrid, primer OPC 13 exhibited 40 % similarity. While nine primers exhibited cent per cent similarity with male parent (Table 78).

Table 78: Similarity coefficient values of hybrids revealed by RAPD primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-2 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SI-15 X SO-3		SI-15 X SI-6		SI-15 X BI-4		SI-15 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	OPA 01	0.75	1.00	0.75	1.00	0.75	1.00	0.75	1.00
2	OPA 04	1.00	1.00	1.00	1.00	0.86	1.00	0.75	0.88
3	OPA 05	1.00	1.00	1.00	0.75	1.00	1.00	0.75	1.00
4	OPA 07	0.88	0.89	0.88	0.89	0.78	1.00	1.00	1.00
5	OPAC 10	0.40	1.00	0.50	0.80	0.50	0.75	0.40	1.00
6	OPAC 15	0.50	1.00	0.50	1.00	1.00	1.00	0.43	1.00
7	OPAE 02	0.83	0.60	1.00	0.50	1.00	0.67	1.00	1.00
8	OPAE 10	0.75	0.50	1.00	0.75	0.75	1.00	1.00	1.00
9	OPAE 15	0.50	1.00	0.67	0.75	0.50	0.75	0.40	1.00
10	OPC 13	0.75	0.80	0.60	0.60	1.00	0.75	0.60	0.40
11	OPF 06	0.20	0.60	0.33	0.75	0.33	0.60	0.25	0.75
12	OPF 08	0.71	0.88	0.71	0.86	0.71	0.57	0.83	1.00
13	OPF 12	1.00	0.88	0.88	0.89	0.88	1.00	0.88	0.88
14	OPO 20	0.88	0.75	1.00	1.00	0.88	1.00	0.86	0.86
	Average	0.72	0.85	0.77	0.82	0.78	0.86	0.71	0.91

4.5.2. ISSR studies

4.5.2.1. Line X Tester crosses of SO-1 X SO-3, SI-6, BI-4 and HA-4

The data presented in Table. 79. disclosed that the hybrids SO-1 X SO-3 and SO-1 X HA-4 were highly similar to their male parents suggesting dominant control of male parent. The contribution of male and female were equal for SO-1 X BI-4 hybrid *i.e.*, 80 percent respectively. Maximum similarity (83 per cent) with female parent was recorded in SO-1 X SI-6. Among eleven primers, every band observed in the hybrid was present either in female or male or in both [Plate 13]. Similarity correlation coefficient of hybrid SO-1 X SO-3 between the female (SO-1) and male (SO-3) genotypes for individual primers varied from 50 to 100 %. Primer 825 and 881 showed 50 % similarity whereas, 864 UBC-826 and UBC-841 showed 100 % similarity. While in male (SO-3) the primer 864 exhibited 50 % similarity, whereas, 825, 881 and UBC-826 expressed cent per

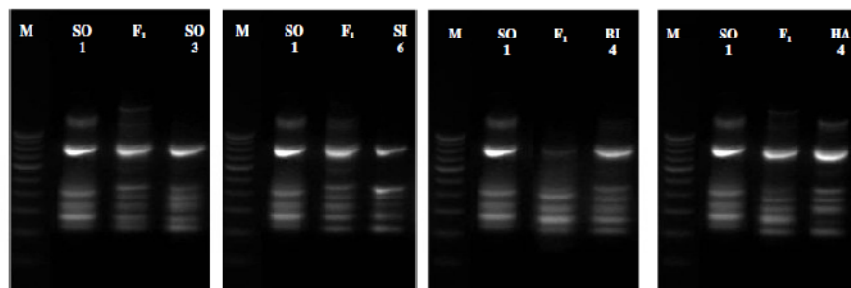
cent similarity of hybrid with male parent. The average similarity was 77 % towards the female and highly similar to the male *i.e.*, 79 % parents suggesting dominant control of male parent in 6 X 4 (Line X Tester) design.

The cross SO-1 X SI-6 similarity was varied from 67 to 100 % with female (SO-1) parent. In SI-6 the similarity ranged from 25 to 100 % respectively. Primers 810 and UBC-807 showed 67 % similarity and 864, 881, UBC-826 and UBC-841 showed higher similarity of 100 % with female parent. Whereas in male (SI-6) the primer UBC-807 exhibited 25 % similarity while 864 and UBC-841 expressed cent percent similarity between hybrid and male parent. The average similarity for female was 83 % and 78 % similar to the male parents showing dominant control of female parent. The similarity of individual primers varied from 50 to 100 % and 40 to 100 % for SO-1 X BI-4 hybrid and between female and male parents. For SO-1 primers 825, 881 and UBC-807 showed 50 % similarity. Five out of eleven primers showed cent per cent similarity. The average similarity for male and female was 80 % respectively showing same percent of contribution of both the parent (Table 79).

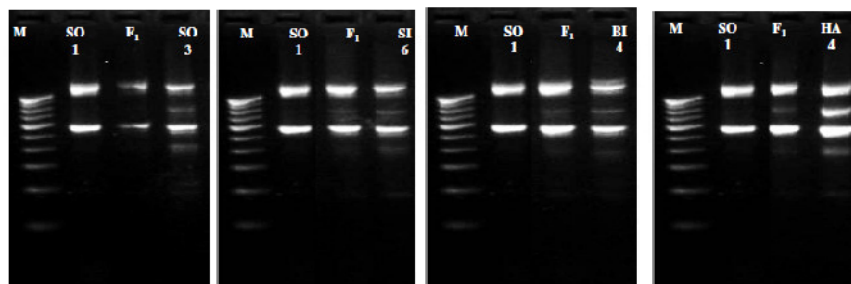
Table 79: Similarity coefficient values of hybrids revealed by ISSR primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-1 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-1 X SO-3		SO-1 X SI-6		SO-1 X BI-4		SO-1 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	809	0.67	0.86	0.80	0.80	0.80	0.60	0.75	1.00
2	810	0.67	0.60	0.67	0.75	1.00	0.40	0.67	1.00
3	811	0.80	0.80	0.80	1.00	0.80	0.60	1.00	1.00
4	825	0.50	1.00	0.50	0.75	0.50	1.00	0.80	1.00
5	834	0.88	0.88	0.88	0.88	1.00	0.68	0.88	1.00
6	835	0.83	0.83	0.83	0.83	0.67	0.57	1.00	1.00
7	864	1.00	0.50	1.00	1.00	1.00	1.00	0.89	1.00
8	881	0.50	1.00	1.00	0.50	0.50	0.50	0.50	1.00
9	UBC-807	0.60	0.71	0.67	0.25	0.50	1.00	0.75	0.67
10	UBC-826	1.00	1.00	1.00	0.83	1.00	1.00	0.60	1.00
11	UBC-841	1.00	0.50	1.00	1.00	1.00	1.00	0.89	1.00
	Average	0.77	0.79	0.83	0.78	0.80	0.80	0.79	0.97

For SO-1 the similarity ranged from 17 to 100 % respectively. Two primers expressed 100 % similarity was 811 and 835 where 881 showed lowest similarity of 50 % followed by UBC-826 (60 %). For HA-4, the range varied



UBC 807



UBC 841

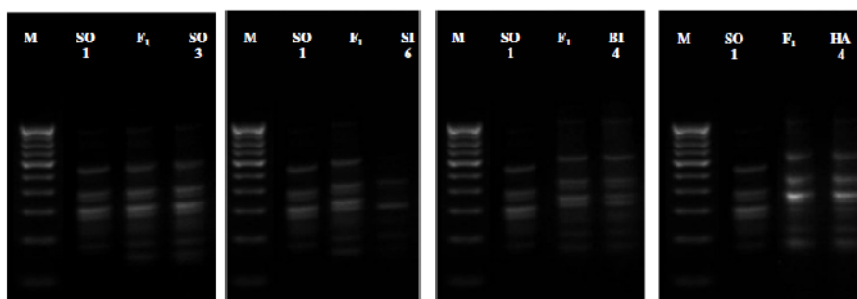


PLATE 13: Paternity analysis of Line X Tester crosses (SO-1 X SO-3, SI-6, BI-4 and HA-4) using ISSR primers

from 67 to 100 %. The primer UBC-807 exhibited 67 %. while all other primers exhibited cent percent similarity between hybrid and male parent. The average similarity for male was 97 % and 79 % similar to female parents the dominance of male was completely expressed among the hybrid.

4.5.2.2. Line X Tester crosses of SO-2 X SO-3, SI-6, BI-4 and HA-4

The data in Table 80 showed that the hybrids SO-2 X SI-6, SO-2 X HA-4 and SO-2 X HA-4 were highly similar to their male parents suggesting dominant control of male parent. Maximum similarity (82 per cent) with female parent was recorded in SO-2 X SO-3 [Plate 14]. Similarity correlation coefficient of hybrid SO-1 X SO-3 between the female (SO-2) and male (SO-3) genotypes for individual primers varied from 33 to 100 %. Primer 881 showed 33 % similarity whereas, 809, 810 and UBC-826 showed 100 % similarity. While in male (SO-3) the primer 834 exhibited 33 % similarity, whereas 810, 825 and UBC-807 expressed cent per cent similarity of hybrid with male parent. The average similarity was 82 % towards the female and 79 % similar to the male parents suggesting dominant control of female parent.

The cross SO-2 X SI-6 similarity was varied from 50 to 100 % with female (SO-2) parent. In SI-6 the similarity ranged from 40 to 100 % respectively. Primers 834 and 809 showed 40 and 60 % similarity and six out of eleven primers showed 100 % similarity between female parent. Whereas in male (SI-6) the primer UBC-807 exhibited 40 % similarity while eight primer expressed cent percent similarity between hybrid and male parent. The average similarity for female was 84 % and 96 % similar to the male parents showing dominant control of male parent. The similarity of individual primers varied from 33 to 100 % and 50 to 100 % for SO-2 X BI-4 hybrid and between female and male parents. For SO-2 primers 881 exhibited 33 % similarity and three out of eleven primers showed cent per cent similarity (825, 864 and UBC-841). In BI-4 maximum similarity (100 %) was observed in primers 809, 834, 881, UBC-807 and UBC-826. The average similarity for female and male was 81 and 85 % respectively showing dominance of male parent.

The similarity ranged from 33 to 100 % respectively between SO-2 and SO-2 X HA-4. Three primers expressed 100 % similarity was 864, UBC-826 and UBC-841, where 881 showed lowest similarity of 33 %. For HA-4 the range varied from 67 to 100 %. The primer 809 exhibited 67 % followed by UBC-807 (80 %) while seven out of eleven primers exhibited cent percent similarity between hybrid and male parent. The average similarity for female was 78 % and 91 % similar to male parents the dominance of male was completely expressed among the hybrid.

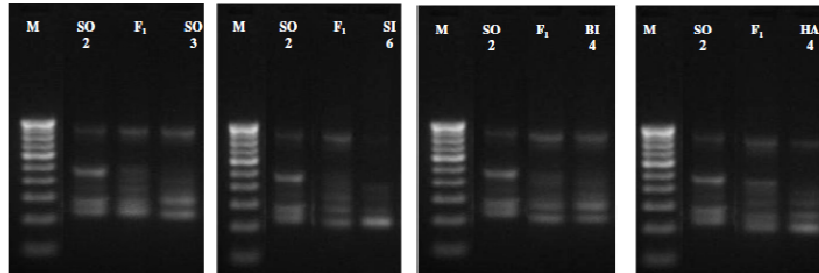
4.5.2.3. Line X Tester crosses of SO-4 X SO-3, SI-6, BI-4 and HA-4

The Table 81 explains that the hybrids SO-4 X BI-4, SO-4 X SO-3 and SO-4 X HA-4 were highly similar to their male parents suggesting dominant control of male parent. The contribution of male and female was same to its progeny in SO-4 X SI-6 and the similarity showed was 75 per cent respectively [Plate 15].

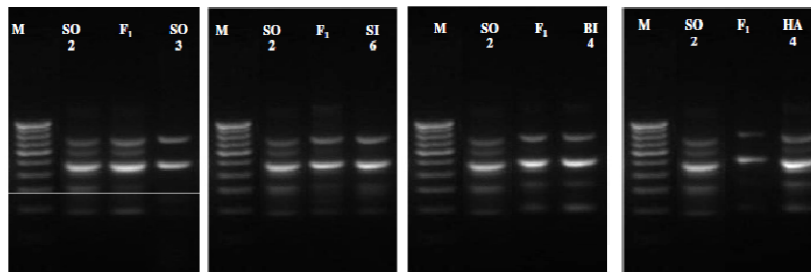
Table 80: Similarity coefficient values of hybrids revealed by ISSR primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-2 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-2 X SO-3		SO-2X SI-6		SO-2 X BI-4		SO-2 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	809	1.00	0.50	0.60	1.00	0.75	1.00	0.75	0.67
2	810	1.00	1.00	0.83	1.00	0.80	0.67	0.83	1.00
3	811	0.88	0.63	1.00	1.00	0.86	0.86	0.86	1.00
4	825	0.67	1.00	0.67	1.00	1.00	0.57	0.67	0.83
5	834	0.75	0.33	0.50	0.78	0.75	1.00	0.75	1.00
6	835	0.60	0.50	1.00	0.71	0.80	0.50	0.60	0.75
7	864	0.86	0.86	1.00	1.00	1.00	0.86	1.00	1.00
8	881	0.33	0.50	0.67	1.00	0.33	1.00	0.33	1.00
9	UBC-807	0.80	1.00	1.00	0.40	0.80	1.00	0.80	0.80
10	UBC-826	1.00	1.25	1.00	1.00	0.80	1.00	1.00	1.00
11	UBC-841	1.17	1.17	1.00	1.00	1.00	0.86	1.00	1.00
	Average	0.82	0.79	0.84	0.96	0.81	0.85	0.78	0.91

The correlation coefficient of similarity of hybrid SO-4 X SO-3 between the female (SO-4) and male (SO-3) genotypes for individual primers varied from 33 to 100 % and 50 to 100 %. Primer 809 showed 33 % similarity followed by 810 whereas, 811, 881 and UBC-826 showed 100 % similarity. While in male



UBC 807



UBC 841

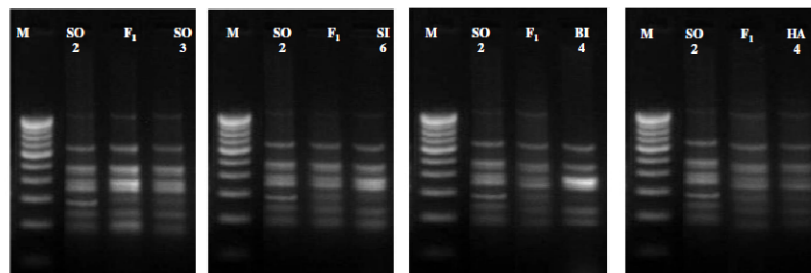
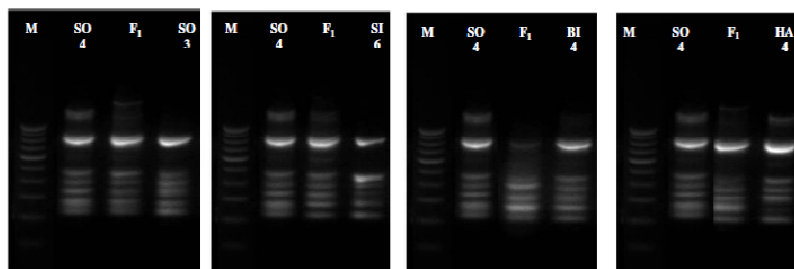
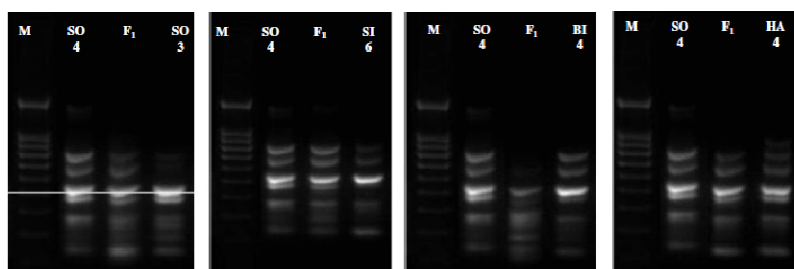


PLATE 14: Paternity analysis of Line X Tester crosses (SO-2 X SO-3, SI-6, BI-4 and HA-4) using ISSR primers



UBC 807



UBC 841

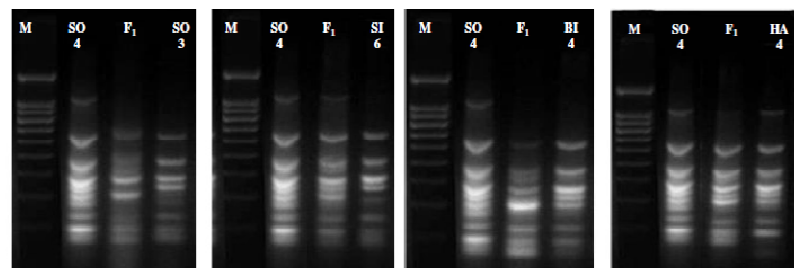


PLATE 15: Paternity analysis of Line X Tester crosses (SO-4 X SO-3, SI-6, BI-4 and HA-4) using ISSR primers

(SO-3) the primer 881 exhibited 50 % similarity followed by 809 and UBC-807 (67 %) whereas primer 811 exhibited cent per cent similarity of hybrid with male parent. The average similarity was 84 % towards the female and 85 % similar to the male parents suggesting control of male parent (Table 81).

The cross SO-4 X SI-6 similarity was varied from 50 to 100 % with female (SO-4) parent. In SI-6 the similarity ranged from 55 to 100 % respectively. Primers 811 showed 100 % similarity and ten out of eleven primers showed varying similarity ranging between 50 to 91 % between hybrid and female parent. Whereas in male (SI-6) the primer 881 exhibited 50 % similarity which is followed by 810 (57 %) similarity between hybrid and male parent. The average similarity between hybrid, female and male was 75 % similar showing control of both female and male parent. The similarity of individual primers varied from 50 to 100 % and 67 to 100 % for SO-4 X BI-4 hybrid and between female and male parents. For SO-4 six out of eleven primer exhibited 50 % similarity (809, 834, 835, 865, 881 and UBC-841) and three out of eleven primers showed cent percent similarity (811, 825 and UBC-807). In BI-4 maximum similarity (100 %) was observed in five primers 810, 811, 825, 835 and 864. The average similarity for male and female was 95 and 67 % respectively showing dominance of male parent.

Table 81: Similarity coefficient values of hybrids revealed by ISSR primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-4 X SO-3, SI-6, BI-4 and HA-4

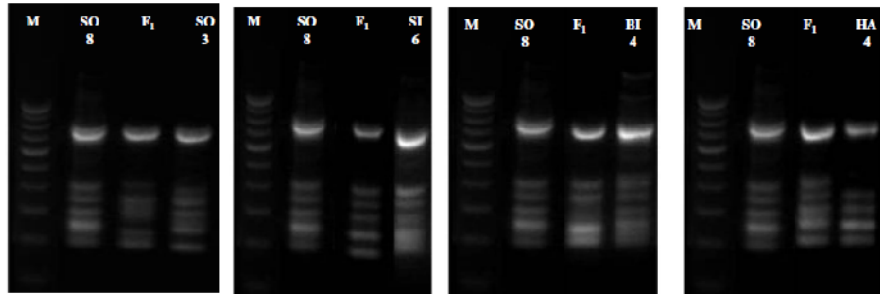
Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-4 X SO-3		SO-4 X SI-6		SO-4X BI-4		SO-4 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	809	0.33	0.67	0.83	0.40	0.50	0.88	0.67	0.80
2	810	0.67	0.83	0.57	0.57	0.57	1.00	0.57	0.57
3	811	1.00	1.00	1.00	0.75	1.00	1.00	1.00	0.75
4	825	0.83	0.83	0.83	0.83	1.00	1.00	1.00	0.83
5	834	0.88	0.86	0.88	0.86	0.50	0.67	0.75	0.75
6	835	0.90	0.90	0.91	0.55	0.50	1.00	1.00	0.90
7	864	0.89	0.75	0.67	0.86	0.50	1.00	0.78	0.78
8	881	1.00	0.50	0.50	1.00	0.50	0.80	0.50	0.50
9	UBC-807	0.83	0.67	0.83	0.83	1.00	0.83	0.83	1.00
10	UBC-826	1.00	0.75	0.60	0.80	0.75	0.80	0.60	1.00
11	UBC-841	0.89	0.75	0.67	0.86	0.50	1.00	0.78	0.78
	Average	0.84	0.85	0.75	0.75	0.67	0.95	0.77	0.79

In SO-4 X HA-4 and SO-4 similarity ranged between 50 to 100 %. Three primers expressed 100 % similarity (811, 825 and 835) whereas 881 showed lowest similarity of 50 % followed by 810 and UBC-826). For HA-4 the range varied from 50 to 100 %. The primer 881 exhibited 50 % followed by 810 (57 %) while two primers exhibited cent percent similarity between hybrid and male parent. The average similarity for female was 77 % and 79 % similar to male parents were expressing its dominance (Table 81).

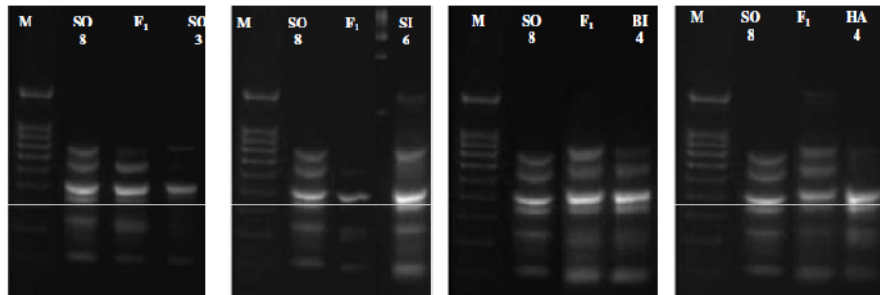
4.5.2.4. Line X Tester crosses of SO-8 X SO-3, SI-6, BI-4 and HA-4

The data presented in Table 82. reveled that the hybrids SO-8 X HA-4, SO-8 X BI-4 and SO-8 X SI-6 were highly similar to their male parents suggesting dominant control of male parent. Maximum similarity (85 per cent) with female parent was recorded in SO-8 X SO-3 suggesting dominating control of female parent [Plate 16]. The correlation coefficient of Similarity of hybrid SO-8 X SO-3 between the female (SO-8) and male (SO-3) genotypes for individual primers varied from 57 to 100 per cent and 50 to 100 per cent. Primer 825 showed 57 % similarity followed by UBC-826 (60 per cent) whereas, six primers viz., 809, 810, 834, 864, 881 and UBC-841 showed 100 per cent similarity. While in male (SO-3) the primer 881 exhibited 50 % similarity followed by UBC-841 (63 %) whereas primer 810 and UBC-826 exhibited cent percent similarity of hybrid with male parent. The average similarity was 85 % towards the female and 77 % similar to the male parents suggesting control of female parent (Table 82).

The cross SO-8 X SI-6 similarity was varied from 50 to 100 per cent with female (SO-8) parent. In SI-6 the similarity ranged from 50 to 100 per cent respectively. Primers 809, 811 and UBC-807 showed 100 % similarity and eight out of eleven primers showed varying similarity ranging between 50 to 88 per cent between hybrid and female parent. Whereas in male (SI-6) the primer 881 and UBC-826 exhibited 50 % similarity which is followed by 834 and 835 (57 per cent) similarity between hybrid and male parent. The average similarity between hybrid, female and male was 78 and 83 per cent similar showing control of male parent. The similarity of individual primers varied from 44 to 100 per



UBC 807



UBC 841

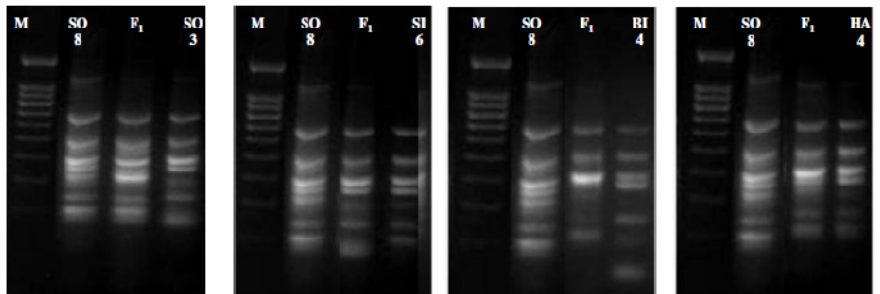


PLATE 16: Paternity analysis of Line X Tester crosses (SO-8 X SO-3, SI-6, BI-4 and IIA-4) using ISSR primers

cent and 50 to 100 % for SO-8 X BI-4 hybrid and between female and male parents. For SO-8 primer 835 exhibited 44 per cent similarity and two primers exhibited cent percent similarity (811 and 825). In BI-4 maximum similarity (100 %) was observed in six primers 809, 811, 825, 834, UBC-807 and UBC-826. The average similarity for male and female was 84 and 73 per cent respectively showing dominance of male parent (Table 82).

Whereas in SO-8 X HA-4 and SO-8 similarity ranged between 50 to 100 per cent. Two primers expressed 100 % similarity (811 and 834), whereas, 881 showed lowest similarity of 50 % followed by UBC-826. For HA-4 the range varied from 60 to 100 %. The primer UBC-807 exhibited 60 per cent followed by 835 (67 %) while four primers exhibited cent per cent similarity between hybrid and male parent. The average similarity for female was 80 per cent and 84 per cent similar to male parent (Table 82).

Table 82: Similarity coefficient values of hybrids revealed by ISSR primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SO-8 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SO-8 X SO-3		SO-8 X SI-6		SO-8 X BI-4		SO-8 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	809	1.00	0.83	1.00	0.83	0.83	1.00	0.83	1.00
2	810	1.00	1.00	0.80	1.00	0.80	0.75	0.80	0.75
3	811	0.75	0.75	1.00	0.75	1.00	1.00	1.00	1.00
4	825	0.57	0.67	0.71	1.00	0.71	1.00	0.86	0.67
5	834	1.00	0.83	0.67	0.80	0.83	1.00	1.00	0.83
6	835	0.78	0.86	0.67	0.67	0.44	0.50	0.67	0.67
7	864	1.00	0.63	0.88	0.86	0.75	0.75	0.88	0.86
8	881	1.00	0.50	0.50	1.00	0.50	0.50	0.50	1.00
9	UBC-807	0.67	0.80	1.00	0.50	0.83	1.00	0.83	0.60
10	UBC-826	0.60	1.00	0.50	0.83	0.60	1.00	0.60	1.00
11	UBC-841	1.00	0.63	0.88	0.86	0.75	0.75	0.88	0.86
	Average	0.85	0.77	0.78	0.83	0.73	0.84	0.80	0.84

4.5.2.5. Line X Tester crosses of CH-2 X SO-3, SI-6, BI-4 and HA-4

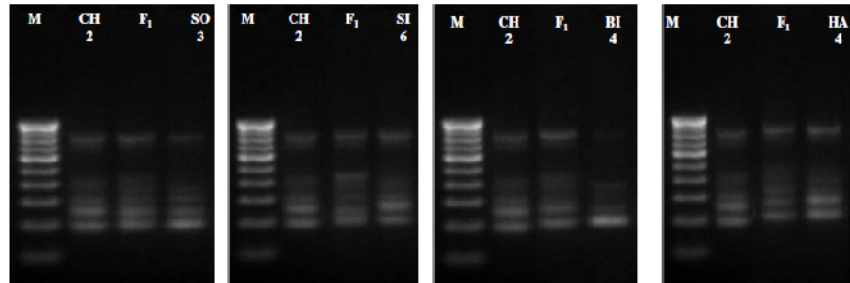
Data appended in Table 83. revealed that the hybrids CH-2 X SO-3, CH-2 X SI-6, CH-2 X BI-4 and CH-2 X HA-4 were highly similar to their male parents suggesting dominant control of male parent [Plate 17]. The similarity of hybrid CH-2 X SO-3 between the female (CH-2) and male (SO-3) genotypes for

individual primers varied from 40 to 100 per cent and 50 to 100 per cent. Primer 809 and UBC-807 showed 40 per cent similarity whereas, primers 834 and 864 showed 100 per cent similarity. While in SO-3 the primer 809 and UBC-807 exhibited 50 % similarity and primer 825, UBC-826 and UBC-841 exhibited cent percent similarity of hybrid with male parent. The average similarity was 71 % towards the female and 92 % similar to the male parents (Table 83).

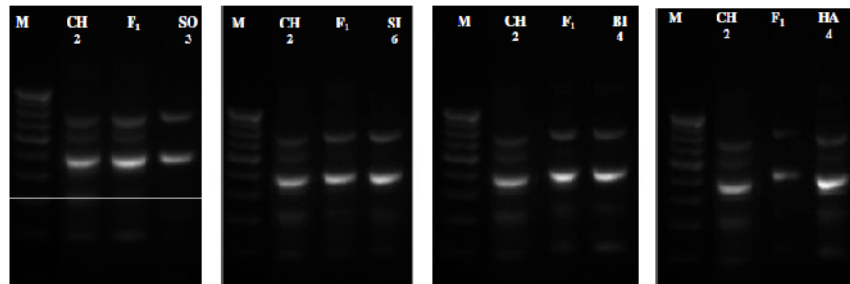
The cross CH-2 X SI-6 similarity was varied from 44 to 100 per cent with female (CH-2) parent. In SI-6 the similarity ranged from 33 to 100 per cent respectively. Primers 810 showed 44 per cent similarity and 811 primers showed cent per cent similarity between hybrid and female parent. Whereas in male (SI-6) the primer UBC-807 exhibited 33 % similarity which is followed by 811 (63 per cent) similarity between hybrid and male parent. The average similarity between hybrid, female and male was 75 and 84 per cent similar showing control of male parent. The similarity varied from 40 to 100 per cent and 20 to 100 % for CH-2 X BI-4 hybrid and between female and male parents. For CH-2 primer 881 exhibited 40 per cent similarity and 809 primers exhibited cent percent similarity. In BI-4 maximum similarity (100 %) was observed in five primers 809, 864, 881, UBC-807 and UBC-826. The average similarity for male and female was 84 and 74 per cent respectively showing dominance of male parent (Table 83).

Table 83: Similarity coefficient values of hybrids revealed by ISSR primer data using Jaccard's Similarity correlation coefficient

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		CH-2 X SO-3		CH-2 X SI-6		CH-2 X BI-4		CH-2 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	809	0.40	0.50	0.60	1.00	1.00	1.00	0.60	0.60
2	810	0.67	0.60	0.44	0.89	0.67	0.86	0.57	0.86
3	811	0.75	0.60	1.00	0.63	0.75	0.86	0.25	0.40
4	825	0.67	1.00	0.80	0.83	0.57	0.71	0.67	1.00
5	834	1.00	0.67	0.83	0.80	0.83	0.20	0.67	0.80
6	835	0.71	0.71	0.86	1.00	0.86	0.86	0.57	0.80
7	864	1.00	0.86	0.86	0.83	0.71	1.00	0.71	0.71
8	881	0.60	0.71	0.60	1.00	0.40	1.00	0.40	1.00
9	UBC-807	0.40	0.50	0.83	0.33	0.80	1.00	0.40	0.40
10	UBC-826	0.75	1.00	0.60	1.00	0.75	1.00	0.60	1.00
11	UBC-841	0.86	1.00	0.86	0.88	0.75	0.75	0.86	0.88
	Average	0.71	0.92	0.75	0.84	0.74	0.84	0.57	0.77



UBC 807



UBC 841

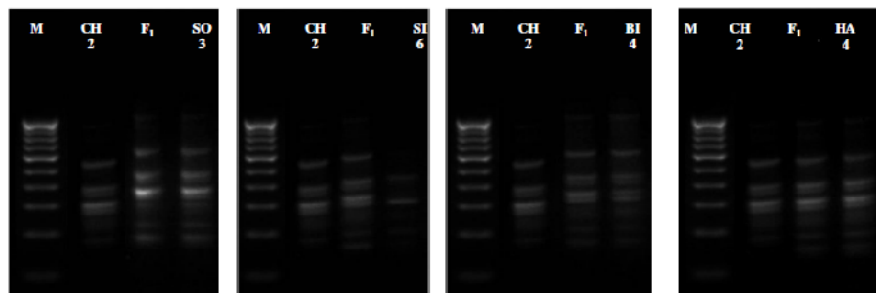
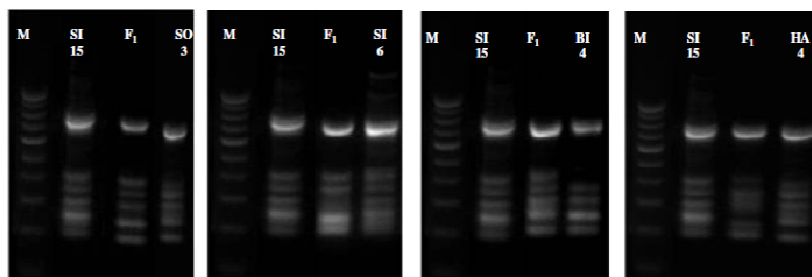
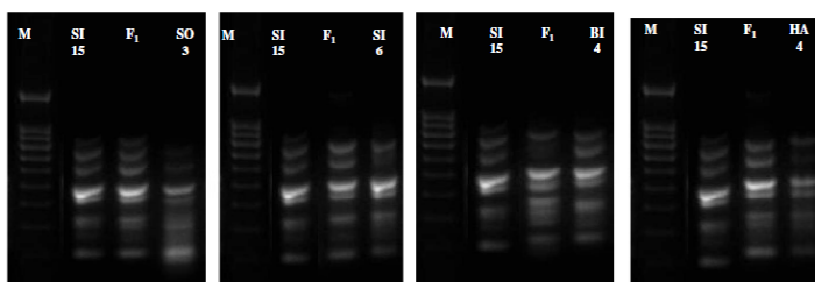


PLATE 17: Paternity analysis of Line X Tester crosses (CH-2 X SO-3, SI-6, BI-4 and HA-4) using ISSR primers



UBC 807



UBC 841

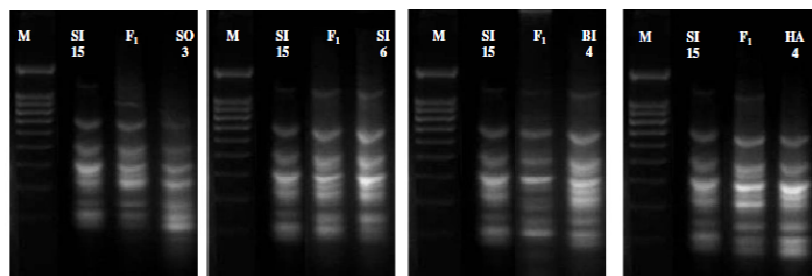


PLATE 18: Paternity analysis of Line X Tester crosses (SI-15 X SO-3, SI-6, BI-4 and HA-4) using ISSR primers

Whereas in CH-2 X HA-4 and CH-2 similarity ranged between 40 to 86 per cent. Primers expressed maximum similarity was UBC-841 (86 per cent) whereas 881 and UBC-807 showed lowest similarity of 40 per cent. For HA-4 the range varied from 40 to 100 %. The 811 and UBC-807 primer exhibited 40 per cent similarity while 825 primers exhibited cent percent similarity between hybrid and male parent. The average similarity for female was 57 per cent and 77 per cent similar to male parent (Table 83).

4.5.2.6. Line X Tester crosses of SI-15 X SO-3, SI-6, BI-4 and HA-4

In Table 84 data appended revealed that the hybrids SI-15 X SO-3, SI-15 X BI-4 and SI-15 X HA-4 were highly similar to their male parents suggesting dominant control of male parent, whereas in SI-15 X SI-6 the contribution of male and female was 82 per cent respectively [Plate 18]. The similarity of hybrid SI-15 X SO-3 between the female (SI-15) and male (SO-3) genotypes varied from 33 to 100 per cent and 78 to 100 per cent. Primer 825 and 809 showed 33 and 40 per cent similarity whereas, primers 834, 835, UBC-826 and UBC-841 showed 100 per cent similarity. While in SO-3 the primer 810 exhibited 78 % similarity and primer 809, 811, 835, 864, 881 and UBC-826 exhibited cent percent similarity of hybrid with male parent. The average similarity was 74 per cent towards the female and 93 per cent similar to the male parents.

The cross SI-15 X SI-6 similarity was between 40 to 100 per cent with female (SI-15) parent. In SI-6 the similarity ranged from 50 to 100 per cent respectively. Primers 881 showed 40 per cent similarity and 4 primers (834, 864, 881 and UBC-826) showed cent percent similarity between hybrid and female parent. Whereas in male (SI-6) the primer 881 exhibited 50 per cent similarity which is followed by 864 (63 per cent) similarity between hybrid and male parent. The average similarity between hybrid, female and male was 82 per cent. The similarity varied from 33 to 100 per cent and 50 to 100 per cent for SI-15 X BI-4 hybrid and between female and male parents. For CH-2, primer 809 exhibited 33 per cent similarity and four out of eleven primers exhibited cent percent similarity. In BI-4 maximum similarity (100 %) was observed in three

primers 825, 834 and UBC-807. The average similarity for male and female was 84 and 78 per cent respectively showing dominance of male parent (Table 83).

Whereas in SI-15 X HA-4 and CH-2 similarity ranged between 33 to 86 per cent. Primers expressed maximum similarity was UBC-807 (100 per cent) whereas 809 and 825 showed lowest similarity of 33 and 40 per cent respectively. For HA-4 the range varied from 83 to 100 %. The 834 primer exhibited 83 per cent similarity while eight primers exhibited cent percent similarity between hybrid and male parent. The average similarity for female was 75 per cent and 99 per cent similar to male parent (Table 84).

Table 84: Similarity coefficient values of hybrids revealed by ISSR primer data using Jaccard's Similarity correlation coefficient for Line X Tester crosses of SI-15 X SO-3, SI-6, BI-4 and HA-4

Sl. No.	Primers	Jaccard's Similarity correlation coefficient							
		SI-15 X SO-3		SI-15 X SI-6		SI-15 X BI-4		SI-15 X HA-4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	809	0.40	1.00	0.50	1.00	0.33	0.83	0.33	1.00
2	810	0.78	0.78	0.78	1.00	0.88	0.89	0.78	1.00
3	811	0.43	1.00	0.75	0.80	0.60	0.80	0.75	1.00
4	825	0.33	0.86	0.40	0.83	0.40	1.00	0.40	1.00
5	834	1.00	0.83	1.00	1.00	1.00	1.00	1.00	0.83
6	835	1.00	1.00	0.88	0.75	0.71	0.71	0.86	1.20
7	864	0.89	1.00	1.00	0.63	0.75	0.75	0.89	1.00
8	881	0.50	1.00	1.00	0.50	1.00	0.50	0.50	1.00
9	UBC-807	0.86	0.83	0.88	0.75	1.00	1.00	1.00	1.00
10	UBC-826	1.00	1.00	1.00	0.83	1.00	0.83	0.83	1.00
11	UBC-841	1.00	0.88	0.89	0.89	0.88	0.88	0.89	0.89
	Average	0.74	0.93	0.82	0.82	0.78	0.84	0.75	0.99

Chapter-5

DISCUSSION

Genetic improvement of any crop depends upon the nature and extent of genetic variability available and the magnitude of interrelationships of heritable and non-heritable variation in yield and its major contributing characters. Because the extent of genetic variability within a population reflects the intensity of interactions among various evolutionary processes such as mutation, genetic drift, and natural selection (Wu *et al.*, 1999). If such genetic variability is not available locally, plant breeder should have an idea of the availability of genetic diversity of the breeding material. This would ensure organized and systematic hybridization program for creating genetic variability to be exploited for genetic improvement of the character under consideration. The information about heterotic potential among *Grewia optiva* gene pool and early filial populations is essential to optimize the possible use of hybrid and production of hybrid seed through establishing bicalonal seed orchards. In the present study entitled “Estimation of gene action, combining abilities and heterosis in *Grewia optiva* Drummond”, an attempt has been made to understand the genetic system controlling inheritance of yield, its components, and the combining ability analysis from a Line X Tester cross. The studies further guided to provide information on heterosis, dominance estimates, genetic selection indices *i.e.*, heritability, genetic advance and genetic gain using 6 X 4 (Line X Tester) matting design for important nursery growth and fodder quality characters of crosses. The results obtained have already been described in the foregoing chapter. Different facts of the results obtained were discussed in the light of available literature and figures under the following broad headings:

- 5.1 CONTROLLED POLLINATION (HYBRIDIZATION)**
- 5.2 COMBINING ABILITY ANALYSIS**
- 5.3 GENE ACTION USING GRAPHICAL (W_r/V_r) APPROACHES AND GENETIC COMPONENTS OF VARIATION**

5.4 HETEROSIS IN F₁ GENERATION

5.5 PATERNITY ANALYSIS USING RAPD AND ISSR MOLECULAR MARKERS

5.1 CONTROLLED POLLINATION (HYBRIDIZATION) UNDER LINE x TESTER MATING DESIGN

Controlled pollination (hybridization) is a vital means of combining genes of two or more varieties to produce progenies, the evaluation and selection of which leads to the recommendation of new varieties that have gene combinations superior to the parent population. The objective of hybridization is to capture a sufficiently inclusive and unbiased genetic representation and to generate large hybrid families. There are three main motives for hybridization *viz.*, i) combine desirable characters from different species ii) capture heterosis or hybrid vigor and iii) to obtain increased developmental homeostasis i.e. greater phenotypic stability in varied environment (Stettler *et al.*, 1996). Intra-specific crosses among different *Grewia optiva* genotypes were conducted in Line X Tester (6 X 4) matting design to obtain appropriate and most promising combination for improvement of some beneficial characters like fodder value, growth rate and also for conservation of species.

Controlled hybridization carried between different *G. optiva* genotypes using 6 x 4 in Line X tester matting design was successful. Out of twenty four controlled crosses attempted all the crosses successfully produced viable seeds. A cursory glance at the data presented in Table 4 revealed significant differences for crossability parameters studied *viz.*, per cent successful crosses, average seed set per flower, germination percentage and survival percentage among selected genotypes of *Grewia optiva*.

The range of per cent successful crosses lied between 23.81 to 88.10 per cent for different crosses. Significant differences were observed among crosses for percentage of successful crosses. Per cent successful cross was highest in cross SO-2 X SO-3 that was 88.10 per cent and minimum was obtained for cross SO-8 X HA-4, which recorded 23.81 per cent. Maximum number and statistically significant value for seeds per flower (3.80) was recorded for SO-8 X HA-4.

However, cross SO-2 X HA-4 (1.12) registered minimum average seed set (Table 4).

Maximum germination percentage (72.72) was recorded for crosses involving SI-15 X BI-4 (70.59). Minimum value for germination was recorded for crosses SO-4 X SI-6 was 34.21. The range for survival percentage was between 57.69 to 97.73 per cent among the crosses. Crosses *viz.*, SO-1 X BI-4 (97.73 per cent) recorded highest per cent survival among all other crosses. Whereas, crosses CH-2 X BI-4 (57.69 per cent) recorded minimum survival per cent (Table 4 and Figure 2).

The above findings are in agreement with the findings of Verma (2012) who reported several intra-specific combinations of *Grewia optiva* in which the range of per cent successful crosses varied between 20 to 70 per cent. The per cent successful cross was highest in cross HA-4 (Hamirpur Kanal) × SI-10 (Seenaghat) that was 70 per cent and minimum of 20 per cent was observed in SO-8 (Kailar) × KA-1 (Dharamshala). Maximum number of seeds per flower (4.00) was for SO-3 (Dharja) × SO-1 (Gaura) cross, whereas CH-6 (Saru) × SI-11 (Adgu) and SO-2 (Nauni) × HA-4 (Hamirpur Kanal) crosses registered minimum average seed set (1.00). Maximum germination percentage (100 per cent) was recorded for crosses involving HA-4 (Hamirpur Kanal) × BI-4 (Kuthira) and SO-8 (Kailar) × KA-1 (Dharamshala) and germination was nil in cross SO-2 (Nauni) × HA-4 (Hamirpur Kanal), SO-7 (Oyali) × SO-11 (Mishuar), SO-12 (Kasauli) × SO-11 (Mishuar) and MA-4 (Tatahar) × HA-4 (Hamirpur Kanal). The survival percentage was between zero per cent to 66.66 per cent and for crosses *viz.*, CH-6 (Saru) × SI-11 (Adgu) and SO-8 (Kailar) × KA-1 (Dharamshala) recorded highest per cent survival.

In *Salix*, intra-specific crosses exhibited high crossability as compared to inter-specific crosses. The per cent successful crosses was ranged between 7.30 to 69.80 per cent [(*Salix tetrasperma* (LNF) × *S. alba* (SE63-007) and *S. tetrasperma* (LP) × *S. tetrasperma* (TFB)]. High percentages of successful crosses, average seed per catkin, seed per capsule (fruit) and germination percentage were obtained when *S. tetrasperma* (TFB) was used as male parent in

crossing with other females of *S. tetrasperma* and maximum survival percentage (33.80 per cent) was recorded in intra-specific cross between *S. matsudana* (PN-227) × *S. matsudana* (PN-722) (Choudhary, 2011). Another experiment conducted by Khanduri and Sharma (2002) performed intra-specific hybridization in *Pinus roxburghii* and results revealed that controlled pollination resulted in enormous fertilization success, with no signs of incompatibility.

Similarly in another study conducted by Papanastasi and Aravanopoulos (2008) reported that totally 33 pollinations were made that resulted in the formation of 84 pods, of which 24 developed fully and produced 65 seeds. Fifteen seeds germinated, resulting in the production of two putative fertile hybrids when they crossed *Robinia pseudoacacia* L. and *R. pseudoacacia* var. *monophylla* Carr.

Pichot and Teissier (1988) in 4 x 6 factorial mating between clones of *Populus nigra* producing twenty one full sib families and Pichot and Teissier (1989) during 6 x 6 factorial crossing scheme between clones of *P. deltoides* resulting thirty three full sib families. The present investigation is also in agreement with the findings of Bastien *et al.* (1996) and Kadam (2002) in Poplar.

5.2 COMBINING ABILITY ANALYSIS

The quantitative characters, which are desirable to the plant breeders are polygenically controlled. Phenotypic expression of these characters is influenced to a larger extent by environment. Therefore, analysis of genes controlling these characters at individual level becomes difficult. Breeding for qualitative characters has been successful since their genetic background can be easily analyzed. In case of quantitative characters, knowledge of such understanding of inheritance is correlated with the degree of success in their improvement during selection.

Genetic variation can be divided into three components: *viz*, additive or fixable gene action arising from average effects of genes; dominance arising from interallelic interaction of genes and epistatic arising from non-allelic interaction of genes. Additive gene action is the result of additive genetic variance while

non-additive variance is due to dominance and epistasis. In the present investigation, the genetic architecture for yield and its components was analyzed through analysis of combining ability (Griffing, 1956; Kempthorne, 1956). The concept of combining ability has greater importance in plant breeding as it helps to predict the efficiency of parents *viz a viz* the type of gene action involved in the expression of any character based on early generation performance. Beside, it also enables to study the comparative performance of lines in hybrid combinations. It was with such a system that the terms general and specific combining ability were introduced by Sprague and Tatum (1942). According to them general combining ability is used to designate the average performance of a parent in general combinations while specific combining ability is used to designate hybrid performance in those cases where certain combinations do relatively better or worse than would be expected on the basis of the average performance of their parents involved. The general combining ability is recognized as a measure of additive gene action while specific combining ability is regarded as an estimate of non additive gene action. The combining ability analyses are useful in identifying better parents and desirable cross combinations in both the cross and self-pollinated plants. Line X Tester analysis is, therefore, of great value to plant breeders who attempt to select parents to be used in hybrid production and also for those whose primary objective is to transfer specific character as it assists them in identifying desirable parents for producing potential segregating populations for selection.

Early evidences indicated that all inbred parents do not perform similarly when crossed together, but certain inbred parents perform comparatively better than others in transmitting favorable genes to their offsprings. Plant breeders are very much interested in determining the genetic potential of their inbred parents in hybrid combinations for two reasons. First, that inbred parents may be identified which produce good progeny in specific combinations, second purpose is to identify those inbred lines, which form good combinations with series of other inbred parents. The former types of inbreds are useful for hybrid development, whereas later types are desirable for hybridization and selecting from segregating populations (Baloch and Bhutto, 2003).

In the present study, mean sum of squares due to parents and crosses were highly significant for all the characters. GCA variances were highly significant for seed diameter, seed length, germination per cent, survival per cent, nursery characters like height, collar diameter, number of leaves, internode length and leaf area for both two and six months data except collar diameter (six months) (Table 5, 8, 11, 14 and 17). The GCA variances were higher than the SCA variances which phenomenon suggested the additive and additive x additive gene actions involved in controlling the character in present material. Bharad *et al.*, (2000), Gul Hassan *et al.* (2000), Baloch (2002), Deshpande and Baig (2003) and Mert and Boyaci (2003) showed that the proportion of GCA variances were higher than SCA variances and suggested that these characters were predominantly controlled by additive genes. There was higher magnitude of SCA variances as compared to GCA variances for collar diameter (six months) and fodder quality characters (crude protein, crude fiber, crude fat (ether extract), total ash and nitrogen free extract) and as such ratio of general combining ability to specific combining ability was found less than unity. All the characters exhibited high dominance variances (Table 14 and 21). Moderately high to low narrow sense heritability and fairly high value of broad sense heritabilities were estimated for all the characters. It shows the preponderance of non-additive gene action governing these characters. The present investigation is supported by the findings of Choudhary (2011) in evaluation of full sib progenies of selected *Salix* species who concluded that dominance variance increased at a faster rate than additive variance in early stage. It is an added worth for species like *G. optiva* which can be easily manipulated through vegetative means like grafting and being maintained under field conditions so can utilize dominance variance more effectively. While Kadam (2002) also came with same outcome in full sib progenies of selected clones of Poplar (*P. deltoides*). Li and Wu (1996) found that F_1 heterosis might be due to overdominant interaction between two alleles, one from the *P. tremuloides* parent and the other from the *P. tremula* parent, at the same loci. While additive effects appeared to influence seedling characters collectively across loci, a few major dominant loci had much larger effects on stem growth.

5.2.1. General combining ability

Estimates of general combining ability revealed that female parent SI-15 (highly significant) and male parent BI-4 and HA-4 (significant) exhibited positive value showing that they are good general combiners for seed parameters (seed diameter and seed length) (Table 6). Female genotype SI-15 and SO-1 and male parent SI-6 and HA-4 having positive GCA effects and were best general combiners for germination and survival per cent (Table 9). SO-8 the only female parent genotype proved to be good general combiners for all nursery characters *viz.*, plant height, collar diameter, number of leaves per plant and internodel length at all the interval of study. Whereas for male parents BI-4 expressed its positive general combining ability for plant height, collar diameter (for two months data) and number of leaves per plant (non significant positive general combining ability). SI-6 and SO-3 showed positive general combining ability for collar diameter while SI-6 and BI-4 for internodel length (Table 12 and 15). The female parents SO-8 and SI-15 displayed positive GCA effects and proved to be good general combiners for leaf area, whereas, same SI-6, BI-4 and HA-4 parent also exhibit positive but non-significant general combining ability effect for the same parameter (Table 18). For fodder quality parameter, four female parent SO-8, SO-4, SO-2 and SO-1, while two male parents SI-6 and BI-4 were having highly significantly positive and good general combining ability with respect to crude protein content. The female parent SO-8, which proved to be as a good general combiner for nursery characters again proved its combining ability for crude fiber content and BI-4 and SI-6 as good male parent for the same parameter. CH-2 and SO-2 among female parents and SO-3 among males showed as a better general combiner for ether extract. There is non-significant positive combining ability effect for total ash between males (HA-4 and BI-4) and females (SO-4 and SO-8). Males SO-3, SI-6 and HA-4 exhibited non-significant positive general combining ability however female genotypes SI-15 and CH-2 were highly significant and good general combiners for nitrogen free extract (Table 21).

The above results are in parallel with the findings of Biabani *et al.* (2012) made crosses among the ten superior *Jatropha curcas* L in a 10 x 10 half-diallel mating design indicated that GCA and SCA variance were highly significant for plant height, collar diameter and number of leaves in nursery stage. The findings of Choudhary (2011) also supports the present study by exhibited low ratio of GCA/SCA with non-additive effects between intra- and inter-specific GCA for both the female parents of *Salix tetrasperma* (LP) and *S. tetrasperma* (LNF) exhibited significantly positive general combining ability effects for number of seed per catkin and for germination per cent. Among the male parents *S. tetrasperma* (TFB), *S. tetrasperma* (TWE) and *S. tetrasperma* (LNM) showed highest positively significant general combining ability effect for all the characters. Whereas *S. alba* (SI-63-007) showed positively significant general combining ability effect for number of seed per catkin. Earlier Bastien *et al.* (1996) reported significant positive link between intra- and inter-specific GCA for both *Populus deltoides* and *P. trichocarpa* parent clones. Results further demonstrated that there was significant positive relationship between the estimated GCA and the clonal values for six *P. trichocarpa* parents used as control revealing that selection on mother tree breeding values could be performed using their clonal values. The above results are in agreement with the findings of Li *et al.* (1997), Pichot and Teissier (1988 and 1989), Ronnberg and Gulberg (1999) on Poplars and Willows. Irfanullah *et al.* (1993) reported positive GCA effects for plant height, bolls per plant and staple length in *Gossypium hirsutum* L.

Thus improvement of above mentioned characters should be done based on simultaneous exploitation of both additive and non-additive components of genetic variance. Conventional breeding methods can exploit only additive or additive x additive type of gene effects. In this situation, biparental mating or reciprocal recurrent selection may increase the frequencies of genetic recombination and hasten the rate of genetic improvement, whereas SCA effects being lower than GCA effects suggest the importance of additive type of gene action and in that case transgressive segregates could be selected in early generations. The estimates of GCAs revealed that it was difficult to select a good

combiner for all the characters together because combining ability effects of the parents were not consistent for all the characters. However, out of six female parents, genotype SO-8 was a good general combiner for the characters (Plant height, collar diameter, number of leaves per plant, internode length, crude protein and crude fibre content). Among four males, genotype BI-4 and SI-6 was the good general combiner for plant height, collar diameter, internode length, crude protein and crude fibre. This suggests that genotype SO-8, BI-4 and SI-6 has greater concentration of positive genes with additive or additive x additive effects for these characters. Therefore, for the improvement of yield and its components, genotypes SO-8, BI-4 and SI-6 could be used as cross combinations in *G. optiva* improvement programs.

5.2.2 Specific combining ability

For the seed parameters Table 7 revealed that twelve out of twenty four crosses revealed positive specific combining ability effects for seed diameter. Highest significantly positive specific combining ability effect was in SO-2 X BI-4, which was followed by SO-4 X SO-3 exhibiting positively significant specific combining ability effects. For seed length twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combination. Cross SO-2 X BI-4 exhibited highly significant and positive specific combining ability. Other crosses that exhibited positively significant specific combining ability effects were SI-15 X HA-4, CH-2 X HA-4, SO-4 X SO-3, SI-15 X SO-3, SO-1 X SI-6, CH-2 X SO-3, SO-4 X SI-6, SO-1 X HA-4, SO-8 X SI-6 and SO-4 X BI-4.

In nursery, studies cross SO-8 X HA-4, SO-4 X SO-3 and CH-2 X SI-6 exhibited positively highly significant specific combining ability effects for germination per cent while out of twenty-four cross fourteen crosses exhibited positive specific combining ability. Hybrid SI-15 X SI-6 exhibited highly significant and positively specific combining ability effects with respect to survival per cent (Table 10). Plant height for two months data showed nine out of twenty-four crosses exhibited positive specific combining ability effects. However, cross SO-4 X HA-4 and SO-1 X SO-3 exhibited positively significant

specific combining ability effects (Table 13), while twelve crosses exhibited positive specific combining ability effects. Again, crosses SO-4 X HA-4, SO-2 X HA-4, SO-1 X SO-3 and SO-8 X SI-6 exhibited positively significant specific combining ability effects for six months data on plant height (Table 16). Information obtained from the table 13 revealed that cross SO-4 X HA-4, SI-15 X BI-4 and SO-1 X SO-3 exhibited high positively significant specific combining ability effect *i.e.*, total of thirteen crosses exhibited positively significant specific combining ability effects indicating that these were good cross combinations for collar diameter character. For six months observation on collar diameter cross SO-1 X SO-3, SO-8 X BI-4, SO-8 X SI-6, SO-4 X HA-4, CH-2 X HA-4 and SI-15 X BI-4 exhibited high positively significant specific combining ability effect. SO-1 X SO-3 exhibited high positively significant specific combining ability effect in two months and total ten crosses exhibited specific combining ability effects for two months reading and cross SO-8 X SI-6 exhibited significant and positive specific combining ability effect for six months observation on number of leaves (Table 16). Coming to internode length in the different crosses the result revealed that twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combination. Cross SO-4 X HA-4, SI-15 X BI-4 and SO-1 X SO-3 exhibited highly significant and positive specific combining ability for two months observation (Table 13). Whereas, from table 16 it was noted that eleven crosses exhibited positive specific combining ability in which cross SO-8 X SI-6, SO-8 X BI-4, SO-1 X SO-3, SO-4 X HA-4 and SO-2 X HA-4 exhibited highly significant and positive specific combining ability with respect to internode length. Hybrids SO-4 X HA-4, SO-8 X SI-6 and CH-2 X SO-3 having highest positive significant SCA effects for leaf area character (Table 19).

While for fodder quality characters which are appended in table 22, hybrids SI-15 X BI-4, SO-8 X SI-6 and SO-4 X HA-4 gave the highest positive significant SCA effects for crude protein content. For crude fiber content hybrids SO-4 X HA-4, SI-15 X BI-4 and SO-8 X SI-6 gave the highest positive significant SCA effects. Twelve out of twenty four crosses exhibited positive specific combining ability effects for crude fat content indicating that these were

good cross combinations among these cross SO-8 X HA-4 exhibited highly significant and positive specific combining ability. In terms of total ash content, the data in table 22 revealed that twelve out of twenty four crosses showing positive specific combining ability effects. Highly significant positive specific combining ability effect was depicted in SO-4 X HA-4 hybrid. However for nitrogen free extract twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combinations. Cross SO-4 X BI-4 exhibited highly significant and positive specific combining ability followed by SO-8 X SO-3 and SO-8 X HA-4.

The information, which we discussed formerly, indicates that it was not necessary that parents having higher estimates of general combining ability effects would always give higher estimates of specific combining ability effects. Usually the highest estimates of specific combining ability effects are obtained from crosses involving diverse parents. Sometimes specific interaction effects, most likely complementary of poor x poor crosses, may perform better than high x high and high x low general combining ability combinations. The resultant performance of traits from poor x poor cross indicates that a high magnitude of non-additive component was responsible for confirming the highest rank to the pertinent cross combination. However, the present cause is strongly supported by the findings of Choudhary (2011), Li and Wu (1996), Bisoffi (1993) and Kadam 2002.

Biabani *et al.* (2012) analysis of specific combining ability in his study that some hybrids of *Jatropha curcas* L presented significant SCA effects for each trait. Hybrids My2.1 × My2.2, In1.1 × My2.2, Ph1.2× In1.2 and Ph1.2× In1.2 gave the higher positive significant SCA effects for plant height. Soomro (2010) in his study on *Gossypium hirsutum* L. come up with the finding that none of the cross combinations had desirable effects for all the characters together. The cross NIAB-78 x TH-3/83 performed better in seed cotton yield along with nine yield components from rest of the crosses and displayed positive SCA effects in 10 out of 12 characters (plant height, sympodia/plant, bolls/plant, boll weight,

seed index, lint index, GOT%, staple length, fibre fineness and seed cotton yield/plant).

The hybrids viz., SO-4 X HA-4, SO-8 X SI-6, SO-1 X SO-3 and SI-15 X BI-4, which recorded positively significant SCA effects for most of the parameters are recommended for heterosis breeding.

5.3. GENE ACTION

In quantitative genetics, genotypic value of an individual is determined by various types of gene actions such as additive, dominance and their interactions (Falconer, 1989). Theoretically, inbreds that show good SCA are predominantly having dominant genes. Whereas, inbreds which perform well in general terms, referred to as having good general combining ability, are the inbreds with predominant additive genes. Additive and dominance genetic variances are important to breeders in that, they are attributable as how far a particular trait is amenable to selection in segregating generations, or is useful for hybrid development. For these purposes the present work was carried out to assess the gene action for certain quantitative and quality characters in twenty four hybrids developed by crossing ten parents viz. SO-1, SO-2, SO-4, SO-8, CH-2 and SI-15 as females (Lines) and SO-3, SI-6, BI-4 and HA-4 as males in 6 X 4 (Line X Tester) matting design.

. For the germination percentage female parent (Line) genotype SI-15 and among male parent (Tester) HA-4 were the best general combiner and could be used as a parent to get better germination (Table 23) as these parents had the preponderant additive and nonadditive genes controlling these characters. However, additive genetic effects were more important than non-additive genetic effects in F_1 because of greater value of GCA variances than SCA. Among hybrids the cross SI-15 X SI-6 proved to be the best specific combiner combination to achieve maximum germination (70.58) being predominantly controlled by dominant genes. Figure 3 indicating recessive genes are involved in controlling the character. Positive values of correlation (0.012) indicated that recessive genes controlled the trait (Table 24). Survival percent among female

parent (Line) SO-2 had the highest array mean (90.78) and among male parent (Tester) SI-6 had higher value (89.81) which means that these genotypes may prove to be better general combiners and increases the survival capability in the progeny (Table 25). Among F_1 the cross SO-1 X BI-4 for early observation and proved to be the best combination showing highest value (97.74) from Figure 4 it is clear that over dominance type of gene action is present. Positive values of correlation (0.011) indicated that recessive genes controlled the trait (Table 26).

For the characters plant height at two and six months observation female parent (Line) SO-8 had the highest array mean (6.60 cm and 15.75 cm) among male parent (Tester) BI-4 had higher mean value (6.61 cm and 15.79 cm). It implies that these genotypes are better general combiners and served as a better parents for tallness (Table 27 and 29). The character is having over dominance type of gene action. For two months observation it indicated that recessive genes controlled the trait ($r = 0.185$) while as growth proceeds the data showed that dominant gene controls the trait ($r = -0.504$). As regards the character collar diameter data presented in Table 31 and 33 show that female parent (Line) SO-8 had the highest value of 2.60 mm and 3.31 mm for two and six month data and among male parent (Tester) BI-4 had higher mean value (2.47 mm) for two months and SI-6 showed 3.09 mm and 3.02 mm for six months seedling observation. It means that these genotypes can serves as a better general combiners for higher collar diameter growth. The cross SO-8 X SI-6 (2.97 mm and 4.41 mm) proved to be the best combination showing higher value for collar diameter both in two and six months seedling data (Table 32 and 34). The W_r/V_r graph (Figure 7) indicating over dominance type of gene action for both two and six months seedling observation. Positive correlation (0.417) indicated that recessive genes were controlled the trait in early stage of growth and in the later stage of growth it was controlled by dominant gene ($r = -0.238$). These results are in line with those of Li and Wu (1996) showed that F_1 heterosis might be due to overdominant interaction between two alleles, one from the *P. tremuloides* parent and the other from the *P. tremula* parent, at the same loci. While additive effects appeared to influence seedling traits collectively across loci, a few major dominant loci had much larger effects on stem growth. Similarly, findings of

Laxman and Ganesh (2003) who also obtained significant GCA and SCA variances for plant height.

With respect to the character number of leaves per plant female parent (Line) SO-8 (6.37) and male parent (Tester) BI-4 showed higher mean value (6.43) for better general combiners (Table 35). Whereas cross SO-8 X SI-6 proved to be the best combination showing highest leaf number (7.08). The W_r/V_r graph (Figure 9) showed that the regression line passed W_r axis below the origin, indicating over dominance type of gene action with 'r' (-0.568) value indicated that dominant genes were controlled the trait. Table 37 Number of leaves for later stages (six month old seedling) female parent (Line) SO-8 had 8.45 while male parent (Tester) SI-6 showed higher value (8.26) showing better general combining ability. The hybrid SO-8 X SI-6 proved to be the good specific combiner showing highest leaf number (9.42). The Figure 10 showed over dominance type of gene action supported by 'r' (-0.311) value indicated that dominant genes were controlled the trait. cursory glance at the data presented in Table 39 and 41 shows that for early and later stages (two and six months) the array means for internode length in female parent (Line) SO-8 had the higher (1.34 cm and 1.96 cm). For male parent (Tester) BI-4 showed higher mean value (1.34 cm) for two months, genotype SI-6 showed 1.88 cm for six month data exposed its general combining ability. The hybrid SO-8 X SI-6 proved to be the best combination showing higher internode length (1.51cm and 2.34 cm) for two and six months observation. These results are supported by those of Hassan *et al.* (2000), Laxman and Ghanesh (2003) who also reported preponderance of dominant genes in the control of this character in *Gossypium hirsutum*. The Figure 11 and 12 shows over dominance type of gene action which was supported by the positive correlation (0.085 and 0.145) value indicating that recessive genes were controlling the trait in early stage and later stages of study.

As for as for leaf area (cm^2), female parent (Line) SO-8 (34.28) and in male parent (Tester) BI-4 showed higher mean value (33.17) which can serves as better general combiners (Table 43). Whereas hybrid SO-8 X SI-6 combination showing more leaf area (53.90). The W_r/V_r graph (Figure 13) showed that the

regression line is below the origin, indicating over dominance type of gene action. The positive correlation (0.564) suggest that recessive genes were controlled the trait (Table 44). Similar studies conducted by Vijayan *et al.* (2008) in mulberry (*Morus* spp.) to determine the genetic interaction in the expression of various quantitative characters including leaf yield. Combining ability studies were conducted on these parents along with their F₁ hybrids for the growth rate and other growth parameters. The ratio of General Combining Ability (GCA) and Specific Combining Ability (SCA) results indicated the predominance of dominant genes in mulberry. Singh (2002) in his study on Poplar (*Populus deltoides* Bartr.) using 10 parents comprising of 5 lines (females) and 5 testers (males). The variance due to SCA was higher than the variance due to GCA for most of the traits. Gene action study revealed non-additive genetic component had dominant role in most of the characters.

The data in Table 45 shows that crude fat (ether extract) (%) in female parent (Line) CH-2 had the higher value (5.94 %) and in male parent (Tester) SO-3 showed higher mean value (5.74 %) and these genotypes can serves as better general combiners for more crude fat (ether extract) (%) per cent. The hybrid CH-2 X SO-3 proved to be the best combination showing higher crude fat (ether extract) (%) per cent (6.69 %). The Wr/Vr graph (Figure 14) indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-1, SI-15, SO-2 and HA-4 had maximum dominant genes as they were above the regression line and close to the origin. Positive correlation (0.012) suggest that recessive genes were controlled the trait. For crude protein (%) the female parent (Line) SO-8 had the higher value (17.01 %) and in male parent (Tester) BI-4 showed higher mean value (16.12 %) which can serves as better general combiners for higher crude protein (%) production. The cross SO-8 X SI-6 proved to be the best combination showing higher crude protein per cent production (18.05 %) whereas, from Wr/Vr graph (Figure 15) showed that the regression line is completely below the origin, indicating over dominance type of gene action. The position of array points on the regression line, it is observed that genotype SO-4, CH-2 and SO-8 had maximum dominant genes as they were above the regression line and close to

the origin. These indicate that including these genotype and the combinations of these will produce increased crude protein per cent in plants. Positivity of 'r' (0.123) suggests that recessive genes were controlling the trait. Table 50 shows that array means for crude protein (%) in female parent (Line) SO-8 had the higher value (20.35 %) and in male parent (Tester) BI-4 showed higher mean value (19.70 %) indicating that the genotypes can serves as better general combiners. SO-8 X SI-6 is the best combination showing higher crude fibre content (22.32 %) The Wr/Vr graph showed that the regression line is completely below the origin, indicating over dominance type of gene action and negative correlation (-0.518) suggest that dominant genes were controlled the trait. (Figure 16). For female parent (Line) SO-4 (10.23 %) and in male parent (Tester) HA-4 (10.26 %) showing higher mean value. It means that the genotypes can serves as better general combiners for higher ash (%) production. SO-4 X HA-4 is proved to be the best combination showing higher ash content (13.55 %) The Wr/Vr graph (Figure 17) showed that the regression line is completely above the origin, indicating partial dominance type of gene action whereas positive correlation (0.269) suggest that dominant genes were controlled the trait (Table 52 and 53). The data in Table 54 shows that array means for nitrogen free extract (%) in female parent (Line) SI-15 (48.23 %) and in male parent (Tester) HA-4 showed higher mean value (46.74 %). It means that the genotypes can serves as better general combiners for higher nitrogen free extract (%). The cross SI-15 X SO-3 proved to be the best combination showing higher nitrogen free extract (50.85 %). In Wr/Vr graph the regression line passed Wr axis is below the origin, indicating complete dominance type of gene action (Figure 18). Negative correlation (-0.339) suggest that dominant genes were controlling the trait (Table 55). The finding are in conformity with Aravindhan and Das (1996) who evaluated 42 crosses of cowpea, derived from 14 lines and 3 testers, in terms of green fodder and seed yields, he found the hybrid UPC 9201 x CO 5 showed the highest heterotic and dominant gene action for green fodder yield compared with the standard parent CO 5. The hybrid CS 55 x CO 4 showed the maximum heterotic effect and exhibiting over dominant gene action for grain yield showing negative correlation suggesting that dominant genes were controlling the trait. Ronnberg-Wastljung *et al.* (1994) estimated additive, dominance and epistatic

variances of forty families of *Salix viminalis* produced by 8×8 factorial mating design. Additive variance was found with large standard deviations in many of the estimates. Dominance and epistatic variances of greater importance were found for weight character in the clay-sand experiment.

Genetic component of variation

The genetic components of variance in F_1 generation revealed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components were greater than additive components in germination per cent, survival per cent, plant height (six months), internode length (at both intervals) and for nitrogen free extract. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci for all the character studied. The average degree of dominance ranged from 0.490 to 20.200. The value being more than a unity displayed over dominance type of gene action in case of germination per cent, survival per cent and for all nursery characters like plant height, number of leaves per plant (at both the interval) except for collar diameter and internode length (two months). Whereas, for fodder quality characters (crude fibre, total ash and nitrogen free extract) dominant gene action was found, while, crude fat (ether extract %) and crude protein which expressed recessive gene action. The value of F ranged between -3453.410 to 10.556. Positive and highly significant value of F implies that the dominant genes were frequent than recessive genes and that they were in increasing position as supported by h^2 values for all nursery characters (plant height, collar diameter, number of leaves per plant and internode length), ether extract (%), and crude fibre (%). The negative and highly significant value of F implies that the recessive genes were frequent than dominant genes for germination per cent, survival per cent, leaf area, crude protein (%), total ash (%) and nitrogen free extract (%). Value of H_1 was less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ for all character except for ether extract (%) and crude protein (%) in which positive and negative genes were symmetrical at loci showing partial dominance in the parents. Further, the value of r^2 showed the incomplete, complete and no dominance of parents for the character observed which is confirmed from W_r/V_r graphs (Figure 3 to 18).

Incomplete dominance were expressed in germination per cent, survival per cent and leaf area as these characters are controlled by recessive genes, while, complete dominance were there for plant height, number of leaves, internode length, ether extract and crude protein due to the reason that the recessive alleles which controls the character is having higher frequency than the dominant allele. Partial dominance was observed for crude fibre, total ash and nitrogen free extract, whereas these characters are strongly controlled by dominant gene and its allelic frequency was more than the recessive allelic frequency.

Heritability and other genetic parameters signify the utility of variability in advanced breeding programmes. Heritability provide a measure of genetic variation and its magnitude gives an idea about the scope for affecting genetic improvement through selection while genetic gain gives the relative measurement of change produced by selection in mean genetic level of the species. Heritability in narrow sense (h^2 n.s), which is the ratio of additive or additive x additive variance to the total phenotypic variance, in the present investigation characters showed low to high narrow sense and broad sense heritability was observed.

High broad sense and narrow sense heritability estimates were recorded for germination percent (0.982 and 0.976), survival percent (0.999 and 0.885), plant height (0.996 and 0.965), collar diameter (0.998 and 0.941), number of leaves (0.999 and 0.982), leaf area (0.996 and 0.982), crude protein (0.969 and 0.630) and nitrogen free extract (0.999 and 0.914). This indicated the occurrence of additive genetic variation among hybrids. This shows the scope for further improvement through clonal selection. The results of the present investigation are similar with Verma (2012) findings where the heritability percentage (broad-sense) was the maximum for collar diameter and number of leaves (46.0 per cent), whereas, minimum heritability percentage (broad sense) was exhibited by plant height (45.0 per cent) coupled with moderate genetic gain were recorded for all other characters studied in *Grewia optiva* hybrids. Similarly findings were encountered by Pichot and Teissier (1988, 1989), Wu (2000), Kadam (2002), Singh and Singh (2004), Sophie *et al.* (2009) and Ozel *et al.* (2010) in poplar and Choudhary (2011) in *salix* species.

5.4. HETEROSIS

The selection potential of any cross combination on the basis of heterosis estimates may be effectively used for improvement in a particular trait. The genetic basis of heterosis has been proposed as (a) simple gene action, (b) heterozygosity *per se*, (c) dominant and partially dominant growth factors, (d) physiological aspects, (e) multiple alleles, (f) over or super dominance, (g) cytoplasm, and (h) additive effects. A large number of experiments have shown that heterosis is based on directional dominance and epistasis, but there is little evidence of real over dominance (Jinks, 1956). Manifestation of heterosis usually depends on genetic diversity of parental lines. The lines are considered diverse if they manifest relatively higher heterosis than those that express little (Hallauer and Miranda, 1988). Results of the present investigations suggested that heterosis over mid parent value was prevalent for all the characters studied.

With regard to seed parameter heterosis over their mid parents was highly significant and maximum positive mid parent heterosis for seed diameter was revealed in F₁ SI-15 X HA-4 (13.54 %), whereas maximum negative heterosis was expressed by the cross SO-1 X HA-4 (-29.67 %). For seed length crosses SI-15 X HA-4 and SO-2 X BI-4 (14.94 and 13.44 %) have maximum positive heterosis, while in SO-1 X HA-4 having (-33.03 %) maximum negative mid parent heterosis (Tables 55). The highest plant height at two and six month seedling were recorded and showed that there was a general tendency for increase in plant height over mid parent. For two months seedling data, cross SI-15 X BI-4 displayed maximum relative heterosis (28.75 %). Only one cross (SO-2 X SO-3) showed maximum negative relative heterosis (-0.07 %), while in six months seedling maximum positive heterosis was shown by cross SI-15 X BI-4 (49.06 %). The lowest positive mid parent heterosis of 7.44 % was observed in SO-2 X SI-6 (Tables 56 and 57). With respect to collar diameter for two months data SI-15 X BI-4 (+57.30 %) expressed maximum positive MP heterosis, the lower positive mid parent heterosis was observed in crosses SO-1 X HA-4 (3.02 %) (Table 58). Maximum positive significant heterosis was shown by the cross SO-8 X BI-4 (+98.63 %) and SI-15 X HA-4 displayed negative heterosis

values of -2.91 % over mid parent in six months seedling data (Tables 59). For two and six months seedling observation on number of leaves F₁ SO-8 X SI-6 exhibited maximum positive significant mid parent heterosis (+37.10 and 23.16 %) at both interval. Maximum negative relative heterosis (-6.54 %) was expressed by the crosses SO-2 X SO-3 and SO-2 X SI-6 exhibited maximum negative mid parent heterosis (-4.91 %) for six months data (Table 60 & 61). In two and six months observations for intermodal length, maximum positive heterosis was exhibited by SO-8 X HA-4 and SO-8 X BI-4 (+45.76 and 46.63 %), whereas lowest positive mid parent heterosis was observed in SI-15 X HA-4 (+2.56 %) for two months observation and SO-2 X SI-6 showed maximum negative relative heterosis (-0.94 %) at six months data (Table 62 & 63). For leaf area (cm²) maximum positive mid parent value of 122.57 % was found in SO-8 X SI-6, whereas maximum negative heterosis of -25.99 % was expressed by the cross CH-2 X BI-4 (Table 64). Heterosis for leaf fresh and dry weight (g) F₁ hybrids displayed highly significant positive and negative heterosis over their mid parents. Maximum positive mid parent heterosis for fresh leaf weight (169.29 %) was revealed in F₁ SO-8 X SI-6, whereas maximum negative heterosis (-41.32 %) was expressed by the cross SO-2 X HA-4. For leaf dry weight, crosses SO-8 X SI-6 (171.56 %) have maximum positive heterosis while in SO-2 X SO-3 having (-45.21 and -36.84 %) maximum negative mid parent heterosis (Table 65). In case of leaf dry matter production maximum positive mid parent heterosis of 61.20 % was revealed in F₁ SO-1 X BI-4 and maximum negative heterosis (-13.13 %) was expressed by the cross CH-2 X SI-6 (Table 66).

There was highly significant and a general tendency for increase in fodder quality character in hybrids. For crude protein maximum positive heterosis, 29.50 and 29.13 %) was shown by the crosses SO-8 X SI-6 (+35.77 %) and maximum negative heterotic effects over MP was exhibited by SI-15 X SO-3 (-4.51 %) (Table 67). Maximum positive heterosis (+20.46 %) was exhibited by the cross CH-2 X SO-3 and maximum negative heterotic effects over mid parent (-23.74 %) was manifested by the cross SO-8 X SI-6 for crude fat (ether extract %) (Table 68). Maximum negative heterotic effects over mid parent (-4.28 %) was

manifested by the cross SO-4 X BI-4 and maximum positive heterosis of 19.52^{**} % was exhibited by the cross SO-8 X SI-6 for crude fiber (%) (Table 69). For total ash (%) content the maximum positive mid parent (46.44^{**} %) was revealed by F₁ SO-4 X HA-4, whereas maximum negative heterosis (-5.19 %) was expressed by the cross SO-4 X SI-6 (Table 70). However, maximum positive mid parent (7.79 %) was revealed in F₁ SI-15 X SO-3, whereas maximum negative heterosis (-15.43 and 12.76 %) was expressed by the cross SO-8 X SI-6 for nitrogen free extract (%) (Table 71).

The above results are in conformity with the findings of Castillo *et al.* (1998) reported that F₁ hybrid between *Leucaena leucocephala* K636 × *L. pallida* K748 (KX2 hybrid) in particular has demonstrated outstanding characteristics as forage plant combined with high palatability. Msangi *et al.* (2004) in the evaluation of *Leucaena* species and its hybrid for fodder production reported significant differences among the species and its hybrid for fodder production. Crude protein content of *Leucaena* foliage was generally high, while the hybrid had the higher value for crude protein. Ghosh *et al.* (2009) tested eight mulberry (*Morus* sp.) hybrids viz., C-2036, S-1908, C-2037, C-2038, C-2039, C-2040, C-2041 and C-2042 were evaluated against S-1635. Among the test hybrids, C-2038 was recorded to have higher number of shoots per plant (10.93), total shoot length (1059.95 cm), unit leaf area (284.06 cm²), unit leaf weight (4.87 g), net photosynthetic rate (13.53 μ mol m² s⁻¹), physiological water use efficiency (1.028), carboxylation efficiency (0.0442), chlorophyll content (2.53 mg g⁻¹), total soluble protein (31.44 mg g⁻¹), total soluble sugar (37.41 mg g⁻¹) and leaf yield (55.23 mt/ha/year) showing its superiority over check and other test hybrids. Heterosis for leaf yield and its attributing characters in 8 hybrids were significant positive heterosis was ranging from 5.95 to 159.31% over better parent, 13.92 to 159.31% over mid parent and 1.50 to 27.79% over standard variety (S-1635) was observed among the crosses for leaf yield.

Heterosis was determined for cross of *Jatropha* genotypes for seed yield per plant and its components trait. Both positive and negative heterosis was found for seed yield per plant. High mid parent heterosis (254.13%) and better parent

heterosis (202.36%) were found for seed yield per plant in cross $P_2 \times P_5$ and $P_1 \times P_3$ respectively. Small heterosis over mid parent was also found for days of fruit maturity. The parent P_1 , P_2 , P_3 and P_5 were found to be superior for seed yield and its components when used in crosses. Consider earliness and seed yield per plant the hybrid combination $P_1 \times P_2$, $P_1 \times P_3$, $P_2 \times P_5$ were promising (Islam *et al.*, 2011). Similarly superiority of intra-specific hybrids has been already demonstrated by earlier workers (Hathaway, 1977; Ronnberg and Gulberg, 1999; Kopp *et al.*, 2002; Smart *et al.*, 2005; Choudhary, 2011) in willows, Dongsen *et al.* (1992), Li and Wu (1996), Kadam (2002), Singh and Singh (2004) and Ozel *et al.* (2010) in Poplars.

5.5. VERIFICATION (PATERNITY ANALYSIS) USING RAPD AND ISSR MARKERS

DNA markers are very useful for confirming hybridity of artificial sexual hybrids or somatic fusion hybrids. Molecular markers are especially useful when hybridity is questioned by morphological reasons or for early screening of large putative hybrid populations (Rajendra, 2009). RAPD and ISSR markers have been successfully used for genetic fingerprinting including verification of controlled crosses (hybrids) in tree species (White *et al.*, 2007). RAPD markers based on the presence or absence of polymorphism among group of individuals was employed for hybrid verification among selected genotypes of *G. optiva*.

A close appraisal of the Plate 7-18 revealed that banding pattern of parent and hybrid generated by fourteen different RAPD and eleven ISSR primers matched with each other. All the hybrids showed the DNA pattern between the two involved parents.

Fourteen RAPD primers *viz.*, OPA 01, OPA 04, OPA 05, OPA 07, OPAC 10, OPAC 15, OPAE 02, OPAE 10, OPAE 15, OPC 13, OPF 06, OPF 08, OPF 12 and OPO-20 were successful in confirming hybridity among twenty-four intra-specific hybrids of *G. optiva* which disclose that 23 hybrids were highly similar to their male parents. Similarity correlation coefficient of hybrid between the female and male genotypes for individual primers varied from 17 to 100 %. Whereas overall similarity correlation coefficient of hybrid between the female

and male genotypes for all primers varied from 61 % to 91 % similar to female parents and for male it is 79 % to 99 % expressing its dominance. Crosses between SO-1 X SO-3, SO-1 X SI-6, SO-1 X BI-4, SO-1 X HA-4, SO-2 X SO-3, SO-2 X SI-6, SO-2 X BI-4, SO-2 X HA-4, SO-4 X SO-3, SO-4 X BI-4, SO-4 X HA-4, SO-8 X SO-3, SO-8 X SI-6, SO-8 X BI-4, SO-8 X HA-4, CH-2 X SO-3, CH-2 X SI-6, CH-2 X BI-4, CH-2 X HA-4, SI-15 X HA-4, SI-15 X BI-4, SI-15 X SO-3 and SI-15 X HA-4 were highly similar to their male parents suggesting dominant control of male parents. While SO-4 X SI-6 was more similar to their female parents (91 %) than the male (86 %) (Table 73 to 78).

The present findings are consisted with the results of Triest *et al.* (1999 and 2001) and Thawaro and Te-Chato (2009) on application of molecular markers in the hybrid verification in oil palm. Amalendu and Kamble (2009) obtained polymorphism of genomic DNAs of nine parents of *Morus* and their seven hybrids from two wild and four cultivated species. Twelve arbitrary primers of Random Amplified Polymorphic DNA (RAPD) unraveled abundant polymorphism. Of the F₁ hybrids, the banding pattern indicates similar like their respective parents. However, a few hybrids showed unique bands, which are different from respective parents. The RAPD technique demonstrated that hereditary variability occurred in between wild and cultivated *Morus* species at inter and intra-specific levels. Choudhary (2011) tested paternity of *salix* hybrids using four RAPD markers. Results disclosed that six out of nine hybrids were highly similar to their male parents, whereas, three hybrids showed more similarity towards female parents. Hybrid *S. tetrasperma* (LG) x *S. tetrasperma* (TWE), *S. tetrasperma* (LG) x *S. tetrasperma* (TFB), *S. tetrasperma* (LN) x *S. tetrasperma* (TWE) recorded 100 per cent similarity with male parent. Maximum similarity (96 per cent) with female parent was recorded in *S. matsudana* (PN-227) x *S. matsudana* (PN-722), *S. alba* (SI-64-017) x *S. matsudana* (PN-722) and *S. matsudana* (PN-227) x NZ-1002 recorded high similarity (87 per cent and 71 per cent) suggesting dominating control of female parent. In order to obtain F₁ hybrids with ornamental potential, four species of *Passiflora* (*P. alata*, *P. gardneri*, *P. gibertii*, and *P. watsoniana*) from the active germplasm Bank at UESC were hybridized. Flower buds, in pre-anthesis, of the genitors were

previously protected, and the female buds were emasculated. To confirm hybridization, the genomic DNA of the genitor species and the supposed hybrids was extracted and RAPD primers were used to obtain molecular markers and select passion flower interspecific hybrids. Eight primers were used to confirm hybrids derived from *P. gardneri* with *P. alata*, *P. watsoniana* with *P. alata*, *P. watsoniana* with *P. gardneri*, and *P. gardneri* with *P. gibertii*; 75, 50, 45, and 46% of the informative bands, respectively, confirmed the hybrid nature of these plants (Conceicao *et al.*, 2011). Similarly study was carried by Samal *et al.* (2012) in 65 mango (*Mangifera indica* L.) genotypes of India including 20 commercial cultivars, 18 hybrids, 25 local genotypes and two exotic cultivars based on qualitative and quantitative fruit characters as well as RAPD profiles. Fifteen RAPD primers yielded 27 monomorphic and 129 polymorphic bands with percent polymorphism averaging 82.7% with its parents.

Eleven ISSR primers viz., 809, 810, 811, 825, 834, 835, 864, 881, UBC-807, UBC-826 and UBC-841 were successful in confirming hybridity among twenty-four intra-specific hybrids of *G. optiva*. Paternity analysis disclosed that crosses between SO-1 X SO-3, SO-1 X HA-4, SO-2 X SI-6, SO-2 X BI-4, SO-2 X HA-4, SO-4 X SO-3, SO-4 X BI-4, SO-4 X HA-4, SO-8 X SI-6, SO-8 X BI-4, SO-8 X HA-4, CH-2 X SO-3, CH-2 X SI-6, CH-2 X BI-4, CH-2 X HA-4, SI-15 X SO-3, SI-15 X BI-4 and SI-15 X HA-4 were highly similar to their male parents indicating dominant control of male parents. SO-1 X SI-6 (83 and 78 %), SO-2 X SO-3 (82 and 79 %) and SO-8 X SO-3 (85 and 77 %) hybrids showed more similarity towards female parents, whereas SO-4 X SI-6 (75 and 75 %), SO-1 X BI-4 (80 and 80 %) and SI-15 X SI-6 (82 and 82%) were showing equal contribution from both parents. Similarity correlation coefficient of hybrid between the female and male genotypes for individual primers varied from 25 to 100 % and 20 to 100 %. Whereas overall similarity correlation coefficient of hybrid between the female and male genotypes for all primers varied from 57 % to 85 % similar to female parents and for male, it is 77 % to 97 % expressing its dominance (Table 79 to 84). Similar paternity analysis was done by Gao *et al.* (2006) used the Inter-Simple Sequence Repeat (ISSR) for genetic fingerprinting and identification of 28 important *Populus L.* (poplar) cultivars (varieties/clones),

and determination of the genetic relationships among these cultivars. Out of 27 ISSR primers tested, eight primers generated clear multiplex profiles. The best three primers produced 154 easily detectable fragments, 129 (84%) of which were polymorphic among the cultivars. Each of these 3 primers produced fingerprint profiles unique to each of the accessions studied, and thus could be solely used for their identification. Samal *et al.* (2012) in mango (*Mangifera indica* L.) genotypes based on qualitative and quantitative fruit characters as well as ISSR profiles. Of a total 70 ISSR bands generated from eight ISSR primers, 60 bands (85.71%) were found to be polymorphic. Cumulative band data from these two methods precisely arranged accessions into eight clusters which correspond well with their pedigree relationship. UPGMA dendrograms had drawn using RAPD, ISSR and cumulative data showed highly similar grouping of genotypes on the basis of their parental origin.

Chapter-6

SUMMARY AND CONCLUSION

The present investigation entitled “**Estimation of gene action, combining abilities and heterosis in *Grewia optiva* Drummond**” was carried out in the Department of Tree Improvement and Genetic Resources, Dr Y.S. Parmar University of Horticulture & Forestry Nauni, Solan (HP) during 2010-2012. There is collection of 60 genotypes of *Grewia optiva* from different districts of Himachal Pradesh at being maintained in the nursery is unique collection of germplasm from which ten parent were selected on the basis of previous study on morphological traits carried out in the department. The investigations included assessing the nature of gene action, combining abilities and heterosis among different hybrids developed from selected genotypes of *G. optiva* in 6x4 (Line x Tester) cross and its paternity pattern were analysed by using RAPD and ISSR molecular markers.

Grewia optiva genotypes included in the study were ten genotypes of five districts, Solan (SO), Sirmour (SI), Chamba (CH), Bilaspur (BI) and Hamirpur (HA). The plant material (flowering branches) of selected 6 females (SO-1, SO-2, SO-4, SO-8, CH-2 and SI-15) and 4 male (SO-3, SI-6, BI-4 and HA-4) genotypes were control crossed (hybridization) using Line X Tester (6X4 factorial) was successful.

6.1 CONTROLLED POLLINATION (HYBRIDIZATION)

Out of twenty four controlled crosses attempted all the crosses successfully produced viable seeds. The range of per cent successful crosses lied between 23.81 to 88.10 per cent for different crosses and was highest in cross SO-2 X SO-3 (88.10 per cent) and minimum was obtained for cross SO-8 X HA-4, which recorded 23.81 per cent. Maximum number and statistically significant value for seeds per flower (3.80) was recorded for SO-8 X HA-4. However, cross SO-2 X HA-4 (1.12) registered minimum average seed set.

Maximum germination percentage (72.72) was recorded for crosses involving SI-15 X BI-4 (70.59). Minimum value for germination was recorded for crosses SO-4 X SI-6 was 34.21. The range for survival percentage was between 57.69 to 97.73 per cent among the crosses. Crosses *viz.*, SO-1 X BI-4 (97.73 per cent) recorded highest per cent survival among all other crosses. Whereas, crosses CH-2 X BI-4 (57.69 per cent) recorded minimum survival per cent.

6.2 COMBINING ABILITY ANALYSIS

The mean sum of squares due to parents and crosses were highly significant for all the characters. GCA variances were highly significant for seed diameter, seed length, germination per cent, survival per cent, nursery characters like plant height, collar diameter, number of leaves, internodel length and leaf area for both two and six months data except collar diameter (six months). The GCA variance was higher than the SCA variances. Higher magnitude of SCA variances as compared to GCA variances for collar diameter (six months) and fodder quality characters {crude protein, crude fiber, crude fat (ether extract), total ash and nitrogen free extract}.

6.2.1 General Combining Ability

Estimates of general combining ability revealed that female parent SI-15 (highly significant) and male parent BI-4 and HA-4 (significant) exhibited positive value showing that they are good general combiners for seed parameters (seed diameter and seed length). Female genotype SI-15 and SO-1 and male parent SI-6 and HA-4 having positive GCA effects and were best general combiners for germination and survival per cent.

SO-8 the only female parent genotype was proved to be good general combiners for all nursery characters *viz.*, plant height, collar diameter, number of leaves per plant and internodel length at all the interval of study. Whereas for male parents BI-4 expressed its positive general combining ability for plant height, collar diameter (for two months data) and number of leaves per plant (non significant positive general combining ability). SI-6 and SO-3 showed positive

general combining ability for collar diameter while SI-6 and BI-4 for internode length. The female parents SO-8 and SI-15 displayed positive GCA effects and proved to be good general combiners for leaf area whereas Same SI-6, BI-4 and HA-4 parent also exhibit positive but non-significant general combining ability effect for the same parameter.

For fodder quality parameter, four female parent SO-8, SO-4, SO-2 and SO-1, while two male parents SI-6 and BI-4 were having highly significantly positive and good general combining ability with respect to crude protein content. The female parent SO-8 which proved to be as a good general combiner for nursery characters and crude fiber content and BI-4 and SI-6 as good male parent for the same parameter. CH-2 and SO-2 among female parents and SO-3 among males showed as a better general combiner for ether extract. There is non-significant positive combining ability effect for total ash between males (HA-4 and BI-4) and females (SO-4 and SO-8). Males SO-3, SI-6 and HA-4 exhibited non-significant positive general combining ability however female genotypes SI-15 and CH-2 were highly significant and good general combiners for nitrogen free extract.

6.2.2 Specific Combining Ability

Twelve out of twenty four crosses revealed positive specific combining ability effects for seed diameter. Highest significantly positive specific combining ability effect was in SO-2 X BI-4. For seed length twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combination.

In nursery, studies cross SO-8 X HA-4, SO-4 X SO-3 and CH-2 X SI-6 exhibited positively highly significant specific combining ability effects for germination per cent while out of twenty-four cross fourteen crosses exhibited positive specific combining ability. Hybrid SI-15 X SI-6 exhibited highly significant and positively specific combining ability effects with respect to survival per cent.

Plant height for two months data showed nine out of twenty-four crosses exhibited positive specific combining ability effects. However, cross SO-4 X HA-4 and SO-1 X SO-3 exhibited positively significant specific combining ability effects, while twelve crosses exhibited positive specific combining ability effects crosses SO-4 X HA-4, SO-2 X HA-4, SO-1 X SO-3 and SO-8 X SI-6 exhibited positively significant specific combining ability effects for six months. Cross SO-4 X HA-4, SI-15 X BI-4 and SO-1 X SO-3 exhibited high positively significant specific combining ability effect *i.e.*, Total of thirteen crosses exhibited positively significant specific combining ability effects indicating that these were good cross combinations for collar diameter character. For six months observation on collar diameter cross SO-1 X SO-3, SO-8 X BI-4, SO-8 X SI-6, SO-4 X HA-4, CH-2 X HA-4 and SI-15 X BI-4 exhibited high positively significant specific combining ability effect. SO-1 X SO-3 exhibited high positively significant specific combining ability effect in two months and total ten crosses exhibited specific combining ability effects for two months reading and cross SO-8 X SI-6 exhibited significant and positive specific combining ability effect for six months observation on number of leaves.

For internode length twelve crosses exhibited positive specific combining ability effects indicating that these were good cross combination. Cross SO-4 X HA-4, SI-15 X BI-4 and SO-1 X SO-3 exhibited highly significant and positive specific combining ability for two months observation. Whereas, for six months observation eleven crosses exhibited positive specific combining ability in which cross SO-8 X SI-6, SO-8 X BI-4, SO-1 X SO-3, SO-4 X HA-4 and SO-2 X HA-4 exhibited highly significant and positive specific combining ability with respect to internode length. Hybrids SO-4 X HA-4, SO-8 X SI-6 and CH-2 X SO-3 having highest positive significant SCA effects for leaf area character (Table 19).

While for fodder quality characters hybrids SI-15 X BI-4, SO-8 X SI-6 and SO-4 X HA-4 gave the highest positive significant SCA effects for crude protein content. For crude fiber content, hybrids SO-4 X HA-4, SI-15 X BI-4 and SO-8 X SI-6 gave the highest positive significant SCA effects. Twelve out of twenty four crosses exhibited positive specific combining ability effects for crude

fat content indicating that these were good cross combinations among these cross SO-8 X HA-4 exhibited highly significant and positive specific combining ability. In terms of total ash content, the data revealed that twelve out of twenty four crosses showing positive specific combining ability effects. Highly significant positive specific combining ability effect was depicted in SO-4 X HA-4 hybrid. However for nitrogen free extract twelve crosses exhibited positive specific combining ability effects was SO-4 X BI-4.

The hybrids viz., SO-4 X HA-4, SO-8 X SI-6, SO-1 X SO-3 and SI-15 X BI-4, which recorded positively significant SCA effects for most of the parameters are recommended for heterosis breeding.

6.3. Gene action

For the germination percentage female parent (Line) genotype SI-15 and among male parent (Tester) HA-4 were the best general combiner as these parents had the preponderant additive and nonadditive genes controlling these characters. Among hybrids the cross SI-15 X SI-6 proved to be the best specific combiner combination to achieve maximum germination (70.58) being predominantly controlled by dominant genes. W_r/V_r indicating recessive genes are involved in controlling the character. Positive values of correlation (0.012) indicated that recessive genes controlled the trait.

Survival per cent among female parent (Line) SO-2 had the highest array mean (90.78) and among male parent (Tester) SI-6 had higher value (89.81) which means that these genotypes may prove to be better general combiners and increases the survival capability in the progeny. Cross SO-1 X BI-4 for early observation and proved to be the best combination showing highest value (97.74) W_r/V_r graph clear indicating over dominance type of gene action. Positive values of correlation (0.011) indicated that recessive genes controlled the trait.

Plant height at two and six months observation female parent (Line) SO-8 had the highest array mean (6.60 cm and 15.75 cm) among male parent (Tester) BI-4 had higher mean value (6.61 cm and 15.79 cm). The character showed over dominance type of gene action. For two months observation it indicated that

recessive genes controlled the trait ($r = 0.185$) while as growth proceeds the data showed that dominant gene controls the trait ($r = -0.504$).

As regards the character collar diameter, female parent (Line) SO-8 had the highest value of 2.60 mm and 3.31 mm for two and six month data and among male parent (Tester) BI-4 had higher mean value (2.47 mm) for two months and SI-6 showed 3.09 mm and 3.02 mm for six months seedling observation. SO-8 X SI-6 (2.97 mm and 4.41 mm) proved to be the best combination showing higher value for collar diameter both in two and six months seedling data. The W_r/V_r graph indicating over dominance type of gene action for both intervals. Recessive genes ($r = 0.417$) indicated that were controlled the trait in early stage of growth and in the later stage of growth it was controlled by dominant gene ($r = -0.238$).

With respect to number of leaves per plant female parent (Line) SO-8 (6.37) and male parent (Tester) BI-4 showed higher mean value (6.43) as better general combiners. Whereas cross SO-8 X SI-6 proved to be the best combination showing highest leaf number (7.08). The W_r/V_r graph showed over dominance type of gene action with $r = -0.568$. For six month old seedling female parent (Line) SO-8 had 8.45 while male parent (Tester) SI-6 showed higher value (8.26) showing better general combining ability. The hybrid SO-8 X SI-6 proved to be the good specific combiner showing highest leaf number (9.42). Over dominance type of gene action supported by $r = -0.311$ value indicated that dominant genes were controlled the trait.

For early and later stages (two and six months) the array means for internode length in female parent (Line) SO-8 had the higher (1.34 cm and 1.96 cm). For male parent (Tester) BI-4 showed higher mean value (1.34 cm) for two months, genotype SI-6 showed 1.88 cm for six month data exposed its general combining ability. The hybrid SO-8 X SI-6 proved to be the best combination showing higher internode length (1.51 cm and 2.34 cm) for two and six months observation. The graph showed over dominance type of gene action, which was supported by the positive correlation (0.085 and 0.145) value indicating that recessive genes were controlling the trait in early stage and later stages of study.

Leaf area (cm^2) female parent (Line) SO-8 (34.28) and in male parent (Tester) BI-4 showed higher mean value (33.17) whereas, hybrid SO-8 X SI-6 combination showing more leaf area (53.90). The Wr/Vr graph indicating over dominance type of gene action. The positive correlation (0.564) suggest that recessive genes were controlled the trait.

Crude fat (ether extract) (%) in female parent (Line) CH-2 had the higher value (5.94 %) and in male parent (Tester) SO-3 showed higher mean value (5.74 %) and these genotypes can serves as better general combiners for more crude fat (ether extract) (%) per cent. The hybrid CH-2 X SO-3 proved to be the best combination showing higher crude fat (ether extract (%)) per cent (6.69 %). The Wr/Vr graph indicating over dominance type of gene action. Positive correlation (0.012) suggest that recessive genes were controlled the trait.

For crude protein (%) the female parent (Line) SO-8 had the higher value (17.01 %) and in male parent (Tester) BI-4 showed higher mean value (16.12 %) which can serves as better general combiners for higher crude protein (%) production. The cross SO-8 X SI-6 proved to be the best combination showing higher crude protein per cent production (18.05 %) whereas, from Wr/Vr graph showed over dominance type of gene action. The Positivity of 'r' (0.123) suggests that recessive genes were controlling the trait.

The means for crude fibre (%) in female parent (Line) SO-8 had the higher value (20.35 %) and in male parent (Tester) BI-4 showed higher mean value (19.70 %) indicating that the genotypes can serves as better general combiners. SO-8 X SI-6 is the best combination showing higher crude fibre content (22.32 %). The Wr/Vr graph indicating over dominance type of gene action and negative correlation (-0.518) suggest that dominant genes were controlled the trait.

For female parent (Line) SO-4 (10.23 %) and in male parent (Tester) HA-4 (10.26 %) showing higher value. SO-4 X HA-4 is proved to be the best combination showing higher ash content (13.55 %) The graph showing

completely above the origin, indicating partial dominance type of gene action whereas positive correlation (0.269) suggests that dominant genes were controlled the trait.

Female parent (Line) SI-15 (48.23 %) and in male parent (Tester) HA-4 showed higher mean value (46.74 %). It means that the genotypes can serve as better general combiners for higher nitrogen free extract (%). The cross SI-15 X SO-3 proved to be the best combination showing higher nitrogen free extract (50.85 %). The regression line passed Wr axis is below the origin, indicating complete dominance type of gene action and it is supported by negative correlation (-0.339) indicating dominant genes were controlling the trait.

6.3.1. Genetic component of variation

In F_1 generation revealed that the dominance component (H_1) and additive component (D) were highly significant. It is observed that dominant components were greater than additive components in germination percent, survival percent, plant height (six months), internode length (at both intervals) and for nitrogen free extract. H_1 value being not equal to H_2 showed unequal gene frequencies at all loci for all the character studied. The average degree of dominance ranged from 0.490 to 20.200. The value being more than a unity displayed over dominance type of gene action in case of germination per cent, survival per cent and for all nursery characters like plant height, number of leaves per plant (at both the interval) except for collar diameter and internode length (two months).

Whereas, for fodder quality characters (crude fibre, total ash and nitrogen free extract) dominant gene action was found, while, crude fat (ether extract %) and crude protein which expressed recessive gene action. The value of F ranged between -3453.410 to 10.556. Positive and highly significant value of F implies that the dominant genes were frequent than recessive genes and that they were in increasing position as supported by h^2 values for all nursery characters (plant height, collar diameter, number of leaves per plant and internode length), ether extract (%), and crude fibre (%).

The negative and highly significant value of F implies that the recessive genes were frequent than dominant genes for germination per cent, survival per cent, leaf area, crude protein (%), total ash (%) and nitrogen free extract (%). Value of H_1 was less than H_2 indicating that positive and negative genes were asymmetrical at loci showing dominance in the parents as confirmed by the value $H_2/4H_1$ for all character except for ether extract (%) and crude protein (%) in which positive and negative genes were symmetrical at loci showing partial dominance in the parents. Further, the value of r^2 showed the incomplete, complete and no dominance of parents for the character observed which is confirmed from W_r/V_r graphs.

Incomplete dominance were expressed in germination percent, survival percent and leaf area as this characters are controlled by recessive genes, while, complete dominance were there for plant height, number of leaves, internode length, ether extract and crude protein due to the reason that the recessive alleles which controls the character is having higher frequency than the dominant allele. Partial dominance was observed for crude fibre, total ash and nitrogen free extract, whereas these characters are strongly controlled by dominant gene and its allelic frequency was more than the recessive allelic frequency.

High broad sense and narrow sense heritability estimates were recorded for germination per cent (0.982 and 0.976), survival per cent (0.999 and 0.885), plant height (0.996 and 0.965), collar diameter (0.998 and 0.941), number of leaves (0.999 and 0.982), leaf area (0.996 and 0.982), crude protein (0.969 and 0.630) and nitrogen free extract (0.999 and 0.914).

6.4. Heterosis

For seed parameter heterosis over their mid parents was highly significant and maximum positive mid parent heterosis for seed diameter and seed length was revealed in F_1 SI-15 X HA-4 (13.54 %), whereas maximum negative heterosis was expressed by the cross SO-1 X HA-4 and SO-1 X HA-4 having (-33.03 and -29.67 %).

The highest plant height at two and six month seedling were recorded for cross SI-15 X BI-4 displayed maximum relative heterosis (28.75 %). Only one cross (SO-2 X SO-3) showed maximum negative relative heterosis (−0.07 %), while in six months seedling maximum positive heterosis was shown by cross SI-15 X BI-4 (49.06 %). The lowest positive mid parent heterosis of 7.44 % was observed in SO-2 X SI-6.

With respect to collar diameter for two months data SI-15 X BI-4 (+57.30 %) expressed maximum positive MP heterosis, the lower positive mid parent heterosis was observed in crosses SO-1 X HA-4 (3.02 %). Maximum positive significant heterosis was shown by the cross SO-8 X BI-4 (+98.63 %) and SI-15 X HA-4 displayed negative heterosis values of -2.91 % over mid parent in six months seedling data.

For two and six months seedling observation on number of leaves F₁ SO-8 X SI-6 exhibited maximum positive significant mid parent heterosis (+37.10 and 23.16 %) at both interval. Maximum negative relative heterosis (−6.54 %) was expressed by the crosses SO-2 X SO-3 and SO-2 X SI-6 exhibited maximum negative mid parent heterosis (−4.91 %) for six months data

Observations for intermodal length (two and six months), maximum positive heterosis was exhibited by SO-8 X HA-4 and SO-8 X BI-4 (+45.76 and 46.63 %), whereas lowest positive mid parent heterosis was observed in SI-15 X HA-4 (+2.56 %) and SO-2 X SI-6 (−0.94 %) showed maximum negative relative heterosis.

Leaf area (cm²) exhibited maximum positive mid parent value of 122.57 % was found in SO-8 X SI-6, whereas maximum negative heterosis of -25.99 % was expressed by the cross CH-2 X BI-4. Heterosis for leaf fresh and dry weight (g) F₁ hybrids displayed highly significant positive and negative heterosis over their mid parents. Maximum positive mid parent heterosis for fresh leaf weight (169.29 %) was revealed in F₁ SO-8 X SI-6, whereas maximum negative heterosis (−41.32 %) was expressed by the cross SO-2 X HA-4. For leaf dry weight, crosses SO-8 X SI-6 (171.56 %) have maximum positive heterosis

while in SO-2 X SO-3 having (-45.21 and -36.84 %) maximum negative mid parent heterosis. In case of leaf dry matter production maximum positive mid parent heterosis of 61.20 % was revealed in F₁ SO-1 X BI-4 and maximum negative heterosis (-13.13 %) was expressed by the cross CH-2 X SI-6.

There was highly significant and a general tendency for increase in fodder quality character was observed in hybrids. For crude protein maximum positive heterosis, 29.50 and 29.13 %) was shown by the crosses SO-8 X SI-6 (+35.77 %) and maximum negative heterotic effects over MP was exhibited by SI-15 X SO-3 (-4.51 %).

Maximum positive heterosis (+20.46 %) was exhibited by the cross CH-2 X SO-3 and maximum negative heterotic effects over mid parent (-23.74 %) was manifested by the cross SO-8 X SI-6 for crude fat (ether extract %). Maximum negative heterotic effects over mid parent (-4.28 %) was manifested by the cross SO-4 X BI-4 and maximum positive heterosis of 19.52 % was exhibited by the cross SO-8 X SI-6 for crude fiber (%).

The maximum positive mid parent (46.44 %) was revealed by F₁ SO-4 X HA-4, whereas maximum negative heterosis (-5.19 %) was expressed by the cross SO-4 X SI-6 for total ash. However, maximum positive mid parent (7.79 %) was revealed in F₁ SI-15 X SO-3, whereas maximum negative heterosis (-15.43 and 12.76 %) was expressed by the cross SO-8 X SI-6 for nitrogen free extract (%).

6.5. Verification (paternity analysis) Using RAPD and ISSR Markers

Fourteen RAPD primers viz., OPA 01, OPA 04, OPA 05, OPA 07, OPAC 10, OPAC 15, OPAE 02, OPAE 10, OPAE 15, OPC 13, OPF 06, OPF 08, OPF 12 and OPO-20 were successful in confirming hybridity among twenty-four intra-specific hybrids of *G. optiva* which disclose that 23 hybrids were highly similar to their male parents.

Similarity correlation coefficient of hybrid between the female and male genotypes for individual primers varied from 17 to 100 %. Whereas overall similarity correlation coefficient of hybrid between the female and male

genotypes for all primers varied from 61 % to 91 % similar to female parents and for male it is 79 % to 99 % expressing its dominance.

Crosses between SO-1 X SO-3, SO-1 X SI-6, SO-1 X BI-4, SO-1 X HA-4, SO-2 X SO-3, SO-2 X SI-6, SO-2 X BI-4, SO-2 X HA-4, SO-4 X SO-3, SO-4 X BI-4, SO-4 X HA-4, SO-8 X SO-3, SO-8 X SI-6, SO-8 X BI-4, SO-8 X HA-4, CH-2 X SO-3, CH-2 X SI-6, CH-2 X BI-4, CH-2 X HA-4, SI-15 X HA-4, SI-15 X BI-4, SI-15 X SO-3 and SI-15 X HA-4 were highly similar to their male parents suggesting dominant control of male parents. SO-4 X SI-6 was more similar to their female parents (91 %) than the male (86 %).

Eleven ISSR primers viz., 809, 810, 811, 825, 834, 835, 864, 881, UBC-807, UBC-826 and UBC-841 were successful in confirming hybridity among twenty-four intra-specific hybrids of *G. optiva*.

Paternity analysis disclosed that crosses between SO-1 X SO-3, SO-1 X HA-4, SO-2 X SI-6, SO-2 X BI-4, SO-2 X HA-4, SO-4 X SO-3, SO-4 X BI-4, SO-4 X HA-4, SO-8 X SI-6, SO-8 X BI-4, SO-8 X HA-4, CH-2 X SO-3, CH-2 X SI-6, CH-2 X BI-4, CH-2 X HA-4, SI-15 X SO-3, SI-15 X BI-4 and SI-15 X HA-4 were highly similar to their male parents indicating dominant control of male parents.

SO-1 X SI-6 (83 and 78 %), SO-2 X SO-3 (82 and 79 %) and SO-8 X SO-3 (85 and 77 %) hybrids showed more similarity towards female parents, whereas SO-4 X SI-6 (75 and 75 %), SO-1 X BI-4 (80 and 80 %) and SI-15 X SI-6 (82 and 82%) were showing equal contribution from both parents. Similarity correlation coefficient of hybrid between the female and male genotypes for individual primers varied from 25 to 100 % and 20 to 100 %. Whereas overall similarity correlation coefficient of hybrid between the female and male genotypes for all primers varied from 57 % to 85 % similar to female parents and for male, it is 77 % to 97 % expressing its dominance.

CONCLUSION

- ◎ For achieving maximum crossability SO-2 X SO-3 combination can be used, while, for seed set per flower SO-8 X HA-4 was a good combination.

- Cross combination between SO-1 X BI-4 can achieve maximum survival of seedling which is very important for the success of any breeding programme.
- SO-8 female and BI-4, SI-6 and SO-3 male parent genotype was proved to be good general combiners for all nursery characters viz., plant height, collar diameter, number of leaves per plant and internode length.
- Fodder quality parameter, female parent SO-8 and SO-4, while SI-6 and BI-4 were good general combining ability with respect to crude protein content.
- SO-8 X HA-4 and SO-4 X SO-3 exhibited positive and highly significant specific combining ability effects for germination per cent and SI-15 X SI-6 exhibited highly significant and positively specific combining ability effects with respect to survival per cent. SO-4 X HA-4 exhibited positively significant specific combining ability effects for plant height. For collar diameter cross SO-1 X SO-3, SO-8 X BI-4 and SO-8 X SI-6 exhibited high positively significant specific combining ability effect was found easier and genotype may be used for objective specific breeding. SO-8 X SI-6 exhibited significant and positive specific combining ability effect for number of leaves and internode length.
- The genetic components of variance in F_1 generation revealed that dominance components (H_1 and H_2) and additive component (D) were highly significant for all the characters. The regression line exhibited that most of the characters were controlled by dominance and over dominance type of gene action.
- Over dominant gene action with low to high narrow sense and broad sense heritability was observed for many character studied except for germination % and total ash content in which recessive gene action was observed with moderate to high narrow sense heritability.
- For nursery characters female parent SO-8 showed over dominant gene action for plant height, collar diameter. Male genotype SI-6 exhibited over dominant gene action for plant height, number of leaves and internode length with high broad and narrow sense heritability

- Cross SO-8 X SI-6 showed over dominant gene action for plant height, collar diameter, number of leaves with high broad and narrow sense heritability. SO-4 X HA-4, SO-8 X SI-6 and CH-2 X SO-3 having highest positive significant SCA effects for leaf area character
- For fodder quality characters SI-15 X BI-4 and SO-8 X SI-6, SO-4 X HA-4 gave the highest positive significant SCA effects for crude protein content. SO-4 X HA-4 having good SCA for crude fiber and total ash content.
- From Wr/Vr graph maximum dominant gene for nursery character were observed in SO-1, SO-2, SI-15, CH-2 and SO-4 which can be further used for heterosis breeding. For fodder quality character, female parent CH-2, SO-8 and SI-15 showed over dominant gene action for crude protein, crude fat and nitrogen free extract. Male genotype SO-3, SO-8 and HA-4 exhibited over dominant gene action for crude protein, crude fat and nitrogen free extract. Cross CH-2 X SO-3, SO-8 X SI-6 and SI-15 X SO-3 showed over dominant gene action for crude protein, crude fat and nitrogen free extract.
- SI-15 X BI-4 showed 49.06 % higher plant height growth and nitrogen free extract (7.79 %). SO-8 X BI-4 cross showed higher heterosis for collar diameter (98.63 %) and Internode length (46.63 %). SO-8 X SI-6 cross showed higher heterosis for number of leaves (23.16), leaf area (122.57 %), leaf weight (171.56), crude protein content (35.77 %) and crude fiber (19.52 %). CH-2 X SO-3 exhibited 20.46 % of heterosis for ether extract and SO-4 X HA-4 (46.44 %) for total ash content.
- The 14 RAPD markers data disclosed that 23 out of 24 hybrids were highly similar to their male parents, whereas, SO-4 X SI-6 hybrids showed more similarity towards female parents.
- The 11 ISSR markers disclosed that 20 out of 24 hybrids were highly similar to their male parents, whereas, SO-1 X SI-6 hybrids showed more similarity towards female parents. While SO-1 X BI-4, SO-4 X SI-6 and SI-15 X SI-6 got equal contribution from both parents.

Chapter-7

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Title of the Thesis	:	“Estimation of gene action, combining abilities and heterosis in <i>Grewia optiva</i> Drummond”
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ABSTRACT

The present investigation entitled “Estimation of gene action, combining abilities and heterosis in *Grewia optiva* Drummond” was carried out in the Department of Tree Improvement and Genetic Resources, Dr Y. S. Parmar University of Horticulture & Forestry Nauni, Solan (HP) during 2010-2012. There is collection of *Grewia optiva* genotypes from different districts of Himachal Pradesh and were maintained in the nursery from which ten genotypes was included in the study and the plant material of 6 females (SO-1, SO-2, SO-4, SO-8, CH-2 and SI-15) and 4 male (SO-3, SI-6, BI-4 and HA-4) genotypes were control crossed using Line X Tester (6X4 factorial) mating design. Out of twenty four controlled crosses attempted all the crosses successfully produced viable seeds. The per cent successful crosses was highest in SO-2 X SO-3 (88.10 %) and minimum was for SO-8 X HA-4 (23.81 %). Maximum seeds per flower (3.80) was recorded for SO-8 X HA-4. Maximum germination percentage (72.72) was recorded for crosses involving SI-15 X BI-4 (70.59), survival percentage was in SO-1 X BI-4 (97.73 per cent) recorded highest per cent survival, while, CH-2 X BI-4 (57.69 per cent) recorded lower survival per cent. GCA variances were highly significant for seed diameter, seed length, germination per cent, survival per cent, nursery characters like plant height, collar diameter, number of leaves, internode length and leaf area. Higher magnitude of SCA variances as compared to GCA variances for collar diameter (six months) and fodder quality characters. SO-8 the female parent genotype was proved to be good general combiners for all nursery characters, male parents BI-4 expressed its positive general combining ability for plant height, collar diameter (for two months data) and number of leaves per plant. SI-6 and SO-3 showed positive general combining ability for collar diameter while SI-6 and BI-4 for internode length. For fodder quality parameter, SO-8 and SO-4, while SI-6 and BI-4 were good general combining ability with respect to crude protein content. The F_1 SO-4 X HA-4 recorded positively significant SCA effects for most of the parameters. The genetic components of variance in F_1 generation revealed that dominance components (H1 and H2) and additive component (D) were highly significant for all the characters. The regression line exhibited that most of the characters were controlled by dominance and over dominance type of gene action. Over dominant gene action with low to high narrow sense and broad sense heritability was observed for many character studied. For nursery characters, SO-8 and SI-6 showed over dominant gene action for plant height, collar diameter with high broad and narrow sense heritability. In SO-8 X SI-6 over dominant gene action was observed for plant height, collar diameter and number of leaves. For fodder quality characters SI-15 X BI-4 gave the highest positive significant SCA effects for crude protein content. From W_r/V_r graph maximum dominant gene action for nursery character were observed in SO-1, SO-2, SI-15, CH-2 and SO-4 and for fodder quality character, female parent CH-2, SO-8 and SI-15 and male genotype SO-3, SO-8 and HA-4 exhibited over dominant gene action for crude protein, crude fat and nitrogen free extract. SI-15 X BI-4 showed higher mid parent heterosis for plant height (49.06 %), SO-8 X BI-4 expressed its heterotic ability for collar diameter (98.63 %) and Internode length (46.63 %). Whereas, SO-8 X SI-6 cross showed higher heterosis for number of leaves (23.16), leaf area (122.57 %), leaf weight (171.56), crude protein content (35.77 %) and crude fiber (19.52 %). The paternity analysis using molecular markers disclosed the fact that F_1 's are highly similar to their male parents. The 14 RAPD markers data showed that 23 out of 24 hybrids were highly similar to their male parents, whereas, eleven ISSR markers disclosed that 20 out of 24 hybrids were highly similar to their male parents.

Signature of Major Advisor

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APPENDIX I

Mean monthly meteorological data of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) for the year 2009-10

Months	2011				
	Temperature (°C)			Relative humidity (%)	Rainfall (mm)
	Maximum	Minimum	Mean		
January	18.10	0.40	9.30	54.00	23.20
February	19.00	0.30	9.70	60.00	61.50
March	24.40	8.30	16.40	48.00	18.20
April	26.50	10.70	18.60	51.00	33.70
May	32.30	16.50	24.40	46.00	31.70
June	29.10	17.70	23.40	66.00	178.20
July	27.40	19.20	23.30	80.00	263.60
August	27.9	19.2	23.6	79.9	2.9
September	28.4	16.4	22.4	74.4	2.3
October	27.0	9.8	18.4	67.3	3.1
November	24.5	5.6	15.1	49.9	3.2
December	20.5	0.9	10.7	48.5	3.1
2012					
January	14.9	0.7	7.8	62.0	3.4
February	18.9	3.6	11.3	56.0	3.7
March	23.9	8.2	16.0	57	4.1
April	29.2	12.2	20.7	38.5	4.2
May	32.1	17.3	24.7	50.5	4.0
June	31.4	17.9	24.6	21.9	3.8
July	14.9	0.7	7.8	62.0	3.4

APPENDIX – II

Analysis of variance for combining ability (seed diameter)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	3	11.88	3.96	27.56	2.74
Crosses	23	12.07	0.52	3.65	1.69
GCA	9	764.47	84.94	591.22	2.02
SCA	45	11969.69	265.99	1851.40	1.55
Error	69	9.91	0.14		

APPENDIX – III

Analysis of variance for combining ability (seed length)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	3	13.59	4.53	31.66	2.74
Crosses	23	13.36	0.58	4.06	1.69
GCA	9	1075.71	119.52	835.26	2.02
SCA	45	16794.30	373.21	2608.07	1.55
Error	69	9.87	0.14		

APPENDIX – IV

Analysis of variance for combining ability (germination %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.032478	0.016239	0.000682	3.127676
Crosses	23	10840.07	471.3072	19.80524	1.684628
GCA	9	57074.45	6341.605	266.4865	2.016601
SCA	45	633554.1	14078.98	591.6261	1.546281
Error	70	1665.796	23.79709		

APPENDIX – V

Analysis of variance for combining ability (survival %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2.00	19734.37	9867.18	4.77	3.13
Crosses	23.00	241598.53	10504.28	5.08	1.68
GCA	9.00	239314.64	26590.52	12.85	2.02
SCA	45.00	2641920.93	58709.35	28.38	1.55
Error	70.00	144811.49	2068.74		

APPENDIX – VI

Analysis of variance for combining ability (plant height (two months))

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.06	0.0275	1.02	3.13
Crosses	23	5.90	0.257	9.53	1.68
GCA	9	19393.58	2154.84	80056.81	2.02
SCA	45	66464.57	1476.99	54873.21	1.55
Error	70	1.88	0.03		

APPENDIX – VII

Analysis of variance for combining ability (plant height (six months))

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	5.05	2.5241	8.64	3.13
Crosses	23	76.13	3.310	11.32	1.68
GCA	9	4391.44	487.94	1669.41	2.02
SCA	45	67962.60	1510.28	5167.19	1.55
Error	70	20.46	0.29		

APPENDIX – VIII

Analysis of variance for combining ability (diameter (two months))

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.08	0.0389	3.52	3.13
Crosses	23	6.71	0.292	26.36	1.68
GCA	9	103.47	11.50	1039.49	2.02
SCA	45	1206.02	26.80	2423.22	1.55
Error	70	0.77	0.01		

APPENDIX – IX

Analysis of variance for combining ability (diameter (six months))

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.94	0.4696	17.55	3.13
Crosses	23	21.43	0.932	34.81	1.68
GCA	9	153.51	17.06	637.39	2.02
SCA	45	1510.36	33.56	1254.26	1.55
Error	70	1.87	0.03		

APPENDIX – X

Analysis of variance for combining ability (No. of leaves (two months))

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.14	0.0686	1.16	3.13
Crosses	23	8.96	0.389	6.56	1.68
GCA	9	717.68	79.74	1343.59	2.02
SCA	45	8092.87	179.84	3030.18	1.55
Error	70	4.15	0.06		

APPENDIX – XI

Analysis of variance for combining ability (No. of leaves (six months))

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.81	0.4071	3.03	3.13
Crosses	23	11.06	0.481	3.58	1.68
GCA	9	1200.94	133.44	994.31	2.02
SCA	45	18742.05	416.49	3103.47	1.55
Error	70	9.39	0.13		

APPENDIX – XII**Analysis of variance for combining ability (internode length (two months))**

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.01	0.0028	1.39	3.13
Crosses	23	1.19	0.052	25.52	1.68
GCA	9	26.09	2.90	1435.30	2.02
SCA	45	321.30	7.14	3535.03	1.55
Error	70	0.14	0.002		

APPENDIX – XIII**Analysis of variance for combining ability (internode length (six months))**

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.02	0.0085	0.90	3.13
Crosses	23	1.75	0.076	8.05	1.68
GCA	9	57.38	6.38	675.44	2.02
SCA	45	682.36	15.16	1606.47	1.55
Error	70	0.66	0.01		

APPENDIX – XIV**Analysis of variance for combining ability (leaf area)**

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	192.01	96.01	2.36	3.13
Crosses	23	20.06	0.87	0.02	1.68
GCA	9	2098.19	233.13	5.74	2.02
SCA	45	180563.72	4012.53	98.72	1.55
Error	70	2845.31	40.65		

APPENDIX – XV**Analysis of variance for combining ability (ether extract %)**

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.93	0.47	5.29	3.13
Crosses	23	0.05	0.002	0.03	1.68
GCA	9	58.20	6.47	73.45	2.02
SCA	45	5238.66	116.41	1322.34	1.55
Error	70	6.16	0.09		

APPENDIX – XVI
Analysis of variance for combining ability (crude protein %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	11.34	5.67	3.25	3.13
Crosses	23	0.001	0.00	0.00	1.68
GCA	9	744.61	82.73	47.39	2.02
SCA	45	68073.41	1512.74	866.42	1.55
Error	70	122.22	1.75		

APPENDIX – XVII
Analysis of variance for combining ability (crude fiber %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	3.28	1.64	2.37	3.13
Crosses	23	0.12	0.005	0.01	1.68
GCA	9	770.85	85.65	124.09	2.02
SCA	45	69321.80	1540.48	2231.87	1.55
Error	70	48.32	0.69		

APPENDIX – XVIII
Analysis of variance for combining ability (total ash %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.88	0.44	0.76	3.13
Crosses	23	64.43	2.801	4.82	1.68
GCA	9	195.16	21.68	37.34	2.02
SCA	45	21813.55	484.75	834.70	1.55
Error	70	40.65	0.58		

APPENDIX – XIX
Analysis of variance for combining ability (nitrogen free extract %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Replication	2	0.01	0.0041	0.76	3.13
Crosses	23	535.33	23.275	4365.07	1.68
GCA	9	4103.27	455.92	85503.59	2.02
SCA	45	456397.90	10142.18	1902074.17	1.55
Error	70	0.37	0.01		

APPENDIX – XX

Variance and covariance of parents for germination and survival per cent

Parents	Germination %			Survival %		
	Vr	Wr	Wr+Vr	Vr	Wr	Wr+Vr
SO-1	146.065	-0.047	146.018	173.13	-21.79	151.33
SO-2	18.935	3.278	22.213	171.96	-17.56	154.40
SO-4	24.905	-0.454	24.450	155.39	-10.07	145.32
SO-8	58.141	-4.973	53.168	136.91	9.60	146.51
CH-2	46.462	8.604	55.066	166.53	74.01	240.54
SI-15	192.215	-2.135	190.080	188.05	-5.96	182.09
SO-3	74.480	15.158	89.638	101.20	-36.63	64.58
SI-6	118.797	-7.420	111.377	65.01	-6.87	58.14
BI-4	109.091	6.915	116.006	144.41	-23.36	121.05
HA-4	135.946	-4.010	131.936	52.53	2.24	54.77

APPENDIX – XXI

Variance and covariance of parents for height

Parents	Two months			Six months		
	Vr	Wr	Wr+Vr	Vr	Wr	Wr+Vr
SO-1	0.031	0.022	0.052	0.128	0.018	0.146
SO-2	0.021	-0.008	0.013	0.205	-0.021	0.184
SO-4	0.086	-0.024	0.062	1.056	-0.033	1.023
SO-8	0.173	-0.009	0.164	2.750	-0.001	2.749
CH-2	0.043	-0.006	0.037	0.886	-0.029	0.857
SI-15	0.069	-0.005	0.064	1.010	0.018	1.028
SO-3	0.570	-0.021	0.549	8.149	-0.266	7.883
SI-6	0.666	-0.001	0.664	10.549	-0.546	10.003
BI-4	0.756	-0.032	0.724	11.161	-0.583	10.578
HA-4	0.655	0.098	0.753	9.224	0.394	9.619

APPENDIX – XXII

Variance and covariance of parents for diameter

Parents	Two months			Six months		
	Vr	Wr	Wr+Vr	Vr	Wr	Wr+Vr
SO-1	0.030	0.025	0.055	0.030	0.025	0.055
SO-2	0.023	-0.003	0.020	0.023	-0.003	0.020
SO-4	0.126	-0.026	0.101	0.126	-0.026	0.101
SO-8	0.181	-0.020	0.161	0.181	-0.020	0.161
CH-2	0.074	-0.004	0.070	0.074	-0.004	0.070
SI-15	0.120	-0.002	0.118	0.120	-0.002	0.118
SO-3	0.076	-0.004	0.072	0.076	-0.004	0.072
SI-6	0.129	-0.003	0.126	0.129	-0.003	0.126
BI-4	0.196	-0.008	0.188	0.196	-0.008	0.188
HA-4	0.156	0.015	0.171	0.156	0.015	0.171

APPENDIX – XXIII

Variance and covariance of parents for number of leaves

Parents	Two months			Six months		
	Vr	Wr	Wr+Vr	Vr	Wr	Wr+Vr
SO-1	0.109	0.087	0.195	0.020	0.003	0.023
SO-2	0.072	0.008	0.080	0.044	-0.001	0.043
SO-4	0.126	-0.062	0.064	0.081	0.005	0.086
SO-8	0.211	-0.041	0.170	0.350	-0.013	0.337
CH-2	0.107	0.000	0.107	0.036	-0.007	0.029
SI-15	0.135	-0.037	0.098	0.037	-0.013	0.024
SO-3	0.699	-0.001	0.698	0.414	-0.071	0.342
SI-6	0.720	-0.182	0.539	0.651	-0.060	0.591
BI-4	0.843	-0.165	0.678	0.607	-0.036	0.571
HA-4	0.783	-0.010	0.773	0.498	0.066	0.564

APPENDIX – XXIV

Variance and covariance of parents for internodel length

Parents	Two months			Six months		
	Vr	Wr	Wr+Vr	Vr	Wr	Wr+Vr
SO-1	0.008	0.007	0.004	0.007	0.006	0.014
SO-2	0.006	-0.001	0.015	0.006	-0.002	0.004
SO-4	0.016	-0.005	0.011	0.012	-0.003	0.008
SO-8	0.034	-0.001	0.011	0.071	-0.001	0.070
CH-2	0.013	-0.002	0.032	0.008	-0.003	0.005
SI-15	0.020	0.012	0.019	0.011	0.001	0.012
SO-3	0.020	-0.001	0.030	0.026	-0.001	0.025
SI-6	0.030	0.000	0.033	0.065	-0.007	0.058
BI-4	0.038	-0.002	0.041	0.061	-0.004	0.057
HA-4	0.037	0.004	0.035	0.032	0.003	0.035

APPENDIX – XXV
Variance and covariance of parents for leaf area

Parents	Vr	Wr	Wr+Vr
SO-1	11.802	-0.926	10.876
SO-2	19.138	12.678	31.816
SO-4	36.312	-6.797	29.515
SO-8	125.452	10.923	136.376
CH-2	11.992	-8.044	3.948
SI-15	19.936	6.511	26.447
SO-3	15.436	3.460	18.896
SI-6	72.081	-15.537	56.544
BI-4	50.000	-8.942	41.058
HA-4	34.927	2.210	37.137

APPENDIX – XXVI
Variance and covariance of parents for ether extract (%)

Parents	Vr	Wr	Wr+Vr
SO-1	0.049	0.005	0.054
SO-2	0.098	-0.012	0.086
SO-4	0.084	0.027	0.111
SO-8	0.350	-0.121	0.229
CH-2	0.283	0.060	0.342
SI-15	0.082	-0.010	0.072
SO-3	0.282	0.076	0.357
SI-6	0.199	0.062	0.261
BI-4	0.230	0.121	0.350
HA-4	0.103	-0.009	0.094

APPENDIX – XXVII
Table. Variance and covariance of parents for crude protein (%)

Parents	Vr	Wr	Wr+Vr
SO-1	1.091	-0.004	1.087
SO-2	1.122	-0.004	1.118
SO-4	0.322	0.007	0.328
SO-8	0.516	0.022	0.538
CH-2	0.468	0.021	0.489
SI-15	0.584	-0.012	0.572
SO-3	0.588	0.182	0.770
SI-6	0.904	0.271	1.175
BI-4	0.774	0.284	1.058
HA-4	0.665	0.138	0.803

APPENDIX – XXVIII

Variance and covariance of parents for crude fiber (%)

Parents	Vr	Wr	Wr+Vr
SO-1	0.18	0.01	0.19
SO-2	0.13	0.02	0.15
SO-4	0.78	0.07	0.85
SO-8	1.64	-0.11	1.53
CH-2	0.09	-0.01	0.09
SI-15	0.70	-0.10	0.60
SO-3	0.47	0.13	0.60
SI-6	1.38	-0.22	1.16
BI-4	1.21	-0.28	0.93
HA-4	0.92	0.20	1.12

APPENDIX – XXIX

Variance and covariance of parents for total ash (%)

Parents	Vr	Wr	Wr+Vr
SO-1	0.304	0.007	0.311
SO-2	0.183	0.000	0.183
SO-4	2.515	0.078	2.593
SO-8	0.990	-0.285	0.704
CH-2	0.409	0.102	0.512
SI-15	0.421	-0.102	0.319
SO-3	0.264	0.023	0.287
SI-6	0.590	-0.135	0.455
BI-4	0.566	-0.013	0.553
HA-4	1.751	0.146	1.898

APPENDIX – XXX

Variance and covariance of parents for nitrogen free extract (%)

Parents	Vr	Wr	Wr+Vr
SO-1	2.443	0.646	3.089
SO-2	1.565	0.689	2.254
SO-4	4.930	-1.005	3.926
SO-8	9.888	-0.239	9.649
CH-2	1.380	-0.810	0.570
SI-15	7.053	-0.858	6.196
SO-3	3.365	1.106	4.471
SI-6	5.906	-0.241	5.665
BI-4	4.980	-1.265	3.715
HA-4	3.848	1.658	5.506

APPENDIX – XXXI

Analysis of variance for heterosis (plant height for two months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.06	0.03	0.67	
Tret	23	5.90	0.26	6.26	1.77
Error	46	1.88	0.04		
total	71	7.84			

APPENDIX – XXXII

Analysis of variance for heterosis (plant height for six months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	5.05	2.52	5.67	
Tret	23	76.13	3.31	7.44	1.77
Error	46	20.46	0.44		
total	71	101.64			

APPENDIX – XXXIII

Analysis of variance for heterosis (collar diameter for two months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.08	0.04	2.31	
Tret	23	6.71	0.29	17.33	1.77
Error	46	0.77	0.02		
total	71	7.56			

APPENDIX – XXXIV

Analysis of variance for heterosis (collar diameter for six months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.94	0.47	11.53	
Tret	23	21.43	0.93	22.88	1.77
Error	46	1.87	0.04		
total	71	24.24			

APPENDIX – XXXV

Analysis of variance for heterosis (number of leaves for two months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.14	0.07	0.76	
Tret	23	8.96	0.39	4.31	1.77
Error	46	4.15	0.09		
total	71	13.25			

APPENDIX – XXXVI

Analysis of variance for heterosis (number of leaves for six months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.81	0.41	1.99	
Tret	23	11.06	0.48	2.36	1.77
Error	46	9.39	0.20		
total	71	21.27			

APPENDIX – XXXVII

Analysis of variance for heterosis (internode length for two months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.01	0.003	0.92	
Tret	23	1.19	0.052	16.77	1.77
Error	46	0.14	0.003		
total	71	1.33			

APPENDIX – XXXVIII

Analysis of variance for heterosis (internode length for six months data)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.02	0.01	2.99	
Tret	23	1.75	0.08	26.85	1.77
Error	46	0.13	0.003		
total	71	1.89			

APPENDIX – XXXIX

Analysis of variance for heterosis (crude protein %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.001	0.0005	1.2708	
Tret	23	260.777	11.3381	26948.0626	1.7668
Error	46	0.019	0.0004		
total	71	260.797			

APPENDIX – XL

Analysis of variance for heterosis (ether extract %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.05	0.03	29.10	
Tret	23	21.41	0.93	987.78	1.77
Error	46	0.04	0.00		
total	71	21.50			

APPENDIX – XLI

Analysis of variance for heterosis (crude fiber %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.12	0.06	9.93	
Tret	23	75.37	3.28	543.52	1.77
Error	46	0.28	0.01		
total	71	75.77			

APPENDIX – XLII

Analysis of variance for heterosis (total ash %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	2.11	1.06	1.20	
Tret	23	64.43	2.80	3.17	1.77
Error	46	40.65	0.88		
total	71	107.19			

APPENDIX – XLIII

Analysis of variance for heterosis (nitrogen free extract %)

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	F table
Rep	2	0.18	0.09	10.89	
Tret	23	535.33	23.28	2868.48	1.77
Error	46	0.37	0.01		
total	71	535.88			

CURRICULUM VITAE

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