Genetic analysis of wilt resistance in intraspecific crosses of Asiatic cotton (*G. arboreum* L.)

By

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Reg. No. 09/17

A thesis submitted to

Mahatma Phule Krishi Vidyapeeth,
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In partial fulfillment of the requirements for the degree

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in

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Department of Agricultural Botany
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Rahuri, Dist. Ahmednagar

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CANDIDATE'S DECLARATION

I hereby declare that this thesis or part there of has not been submitted by me or other person to any other University or Institute for degree or diploma.

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This assistance and help received during the course of this investigation have been acknowledged.

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Place: *Rahuri*

Date: *17/05/2013* (***Rani D. Nimbalkar***)

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LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate
µg	:	Micro gram
µl	:	Microliter
bp	:	base pairs
^{0}C	:	Degree Celsius
cm	:	Centimeter (s)
DF	:	Degrees of freedom
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxy nucleoside triphosphates
e.g.	:	*Exampli gratia*
EC	:	Emulsified concentrate
EDTA	:	Ethylenediamine tetraacetic acid
et al.	:	And others (*et alli*)
etc.	:	*Et cetra* (And so on)
Fig.	:	Figure
G	:	Gram (s)
g/tex	:	Gram per tex
gca	:	General combining ability
GCV	:	Genotypic coefficient of variation
ha	:	Hectare
hr	:	Hour
i. e.	:	That is
ISSR	:	Inter simple sequence repeats
kg	:	Kilogram (s)
lit	:	Litre
m	:	Meter
M	:	Molar
mg	:	Milligram (s)
ml	:	Milliliter (s)
mm	:	Millimeter
MSS	:	Mean sum of squares
No.	:	Number
PCR	:	Polymerase chain reaction
PCV	:	Phenotypic coefficient of variation
QTL	:	Quantitative Trait Loci
RFLP	:	Restriction fragment length polymorphism
RGA	:	Resistant gene analogs
rpm	:	Revolution per minute
S	:	Standard Deviation
sca	:	Specific combining ability
SE	:	Standard error
SL	:	Soluble liquid
SSR	:	Simple sequence repeat
TE	:	Tris EDTA
TBE	:	Tris Borate EDTA
TS	:	Transverse section
U	:	Unit
UV	:	Ultraviolet
viz.,	:	*Vide licent* (Namely)
wt.	:	Weight

ABSTRACT

"Genetic analysis of wilt resistance in intraspecific crosses of Asiatic cotton (*G. arboreum* L.)"

By

Rani D. Nimbalkar

A candidate for the degree of

DOCTOR OF PHYLOSOPHY (AGRICULTURE)

in

AGRICULTURAL BOTANY

(GENETICS AND PLANT BREEDING)

Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722

2013

Research Guide: Dr. S. S. Mehetre

Major Discipline: Agricultural Botany
(Genetics and Plant Breeding)

The present investigation on "Genetic analysis of wilt resistance in intraspecific crosses of Asiatic cotton (*G. arboreum* L.)" was carried out at Cotton Improvement Project and Maharashtra State Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2009-2012. Two crosses *viz.*, AKA-7 × Dh-2 and PA-141 × Dh-2 were obtained by using AKA-7 and PA-141 as moderately resistant varieties and Dh-2 susceptible variety to *Fusarium* wilt. The F_2 populations of two crosses along with parents were screened against wilt in wilt sick soil to find out genetics related to wilt resistance in diploid cotton. Simultaneously, F_2 population of the cross AKA-7 × Dh-2 was used to identify molecular marker associated with wilt resistance. Further sequencing of the resistant band was done. Six generations of the two

crosses were evaluated for generation mean analysis to study the gene action for yield and yield contributing characters.

In the inheritance of resistance to *Fusarium* wilt the insignificance χ^2 in case-II of AKA-7 × Dh-2 and PA-141 × Dh-2, indicates that the resistance to *Fusarium* wilt is dominant and digenically governed. The data fits in 13:3 Mendelian ratio. Hence, it can be concluded that there is presence of inhibitory interaction.

In bulk segregant analysis, NLRR-inv-1/2 primer-277 bp band was found associated with *Fusarium* wilt resistance in Asiatic cotton *G. arboreum*. This primer was identified as marker for differentiating the resistance and susceptibility to *Fusarium* wilt in Asiatic cotton hence, can be utilized for marker assisted selection of resistant plants at early stages of plant growth. Although marker was based on RGA analogue primer but sequence analysis of the 300bp fragment did not reveal presence of NBS-LRR R genes. However, sequence homology analysis showed homology with *G. hirsutum* and *G. arboreum* clones having few candidate genes *viz.,* putative caffeic acid methyltransferase putative protein disulfide isomerase genes and alcohol dehydrogenase A gene, which may be likely responsible for wilt resistance.

Resistance mechanisms may have took place before or after vascular invasion by the pathogen. Physical resistance in AKA-7 was observed by little thickening of cell wall as well as reduction in the size of the cells near the epidermis. However, such structural changes were not seen in susceptible parent Dh-2. Thickening of the cell wall may be due to the lignin or phytoalaxin accumulation in the cells and further retardation of fungal growth might have been reason for resistance in AKA-7.

In the second part of the research the scaling test indicated that either A, B or C or all significantly deviated from zero for majority of characters except monopodia/plant, boll weight, ginning percentage, uniformity (%), micronaire and fibre strength in cross AKA-7 × Dh-2 while, in case of cross PA-141 × Dh-2 one of the scales was significant for majority of characters except for plant height, monopodia/plant, days to flowering, days to boll bursting, days to 50% maturity boll weight and fibre strength. Joint scaling test also resulted in highly significant chi-square value for the characters where any one scale is also found significant. Significant scaling and joint scaling tests suggested the presence of higher order interactions responsible for expression of these characters.

Analysis of gene effects revealed the predominance of dominant (h) gene effect in majority of characters studied. Additive component is higher only for plant height in AKA-7 × Dh-2. The main effect 'h' (dominant) is significant for boll per plant, boll weight, lint weight per plant, seed cotton yield per plant in both the crosses and for sympodia per plant, days to boll bursting and days to maturity in AKA-7 × Dh-2 and uniformity ratio in PA-141 × Dh-2. While, additive gene effect (d) is significant for plant height, days to boll bursting and days to maturity in AKA-7 × Dh-2 and for lint weight per plant, ginning percentage, seed cotton yield per plant in PA-141 × Dh-2 respectively. When interaction components 'i+j+l' put together, against main effects 'd+h', main effects were larger for sympodia per plant, lint weight per plant, seed cotton yield per plant in both the crosses and for days to boll burst, days to maturity in AKA-7 × Dh-2 and for uniformity in the cross PA-141 × Dh-2. The interaction component was higher for plant height, 50% flowering, 100 seed weight and span length in AKA-7 × Dh-2 and 100 seed weight, ginning percentage and micronaire value in PA-141 × Dh-2 indicating importance of gene interaction in governing these characters. The 'h' and 'l' are in opposite direction indicating duplicate type of interaction for most of all the characters except for 100 seed weight in both the crosses and ginning percentage and micronaire value in PA-141 × Dh-2. Duplicate type of gene interaction tends to reduce the heterosis effect as such is not desirable while, complimentary epistasis increases the heterosis. The additive gene effect (d) and additive × additive gene interaction (i) were found to be significant simultaneously for days to maturity in AKA-7 × Dh-2, lint weight per plant and seed cotton yield in PA-141 × Dh-2. These characters can be improved by selection. Absence of any interaction was observed in boll weight in both the crosses indicating additive dominant model is sufficient to explain the gene effects. The significant dominant component (h) in both the crosses for boll weight can be exploited by heterosis breeding. In general, mostly all characters can be improved through recurrent selection except boll weight.

1. INTRODUCTION

Cotton is the most important renewable natural textile fiber and sixth largest source of vegetable oil in the world. Cotton belongs to the genus *Gossypium* from the family malvaceae, which consists of at least 45 diploid and five allotetraploid species (Percival *et al.*, 1999, Ulloa *et al.*, 2005 and 2006). *G. hirsutum* L. (Gh, AD_1) *and G. barbadense* L. (Gb, AD_2) are modern allotetraploid cottons that together represents the most extensively cultivated species worldwide. A genome of diploid cottons produces spinnable fiber while, D genome species produce short appressed fiber.

Archaeological evidences from Harappa civilization (2300-1750 BC) clearly showed that Indian civilization had developed highly sophisticated textile craftsmanship. The world famous "Dhaka muslins" were woven from the Indian *Desi* tree species *G. arboreum* with mean fibre length of 18-24 mm, but the yarn was one of the finest ever heard of 345-356 count. For over centuries, the Indian Dhaka handloom muslins ruled the world textile trade. The British East India company was established in 1615 in India started exporting Calicoes and Dhaka muslins to Britain. After the start of textile industrialization in 18th century, spinning frames were developed to suit American cotton *G. hirsutum* of medium staple and good strength. But, the American revolutionary war during 1775-83 caused shortage of raw American cotton and British immediately turn to India as an alternative option to cultivate American cotton. In 1947, the area under *desi* cotton in India was 97% and that of American cotton was 3 %. The picture is completely reversed today and at the invent of *Bt* cotton hybrids Indian desi cotton varieties were edged out.

However, the American varieties *G hirsutum* and 'Sea Island' cotton, *G. barbadense* are more susceptible to insect and pests such as jassids, whiteflies, American bollworm and diseases like *Verticilium* wilt, para wilt and leaf curl virus. Even *Bt* cotton hybrids face all the problems except bollworm. The *Desi* cotton *G. arboreum* and *G. herbaceum* species have advantage of

resistance and tolerance to sucking pests including whiteflies, jassids and thrips. By virtue of having been cultivated for ages, the two *desi* species *G. arboreum* and *G. herbaceum*, are known to tide over biotic and abiotic stresses with the ease under the native conditions.

Cotton area (%) in India since independence

Year	American	Egyptian	Asiatic
1947	3	0.1	97
1955	32	0.2	67.8
1965	35	4	61
1970	36	4	60
1980	36.96	7.7	55.34
1990	46.6	6.5	46.9
1995	59.34	2.58	38.08
2000	73.2	1.8	25
2005	83	1.2	15.8
2010	96	0.5	3.5

Source: The Indian Express (31st Jan, 2011)

Cotton in India is grown in varied soil, climates and agricultural practices under irrigated and rainfed conditions. Approximately 65 % India's cotton is produced under rainfed conditions and 35 % on irrigated lands. The northern is almost totally irrigated, while the percentage of irrigated area is much lower in central (23 %) and southern zone (40 %) (Anonymous, 2011). Maharashtra comprises 35 % of area (4.15 million ha) under cotton, primarily under rainfed conditions, with the lowest area (3-4 %) under irrigation. *Bt* hybrids will give their performance only under irrigated conditions. Under such situations, *desi* cotton will perform better.

The quality profile of Indian cotton has also been changed. Long staple cotton which constituted 38 % prior to 2002, increased to an estimated 85 % of total cotton produced in 2010, primarily because of *Bt* cotton hybrids. However, the Confederation of Indian Textile Industries (CITI) estimates that in the 25.8 m bales utilization capacity, current requirement of Indian textile Industry is 37 % of long and extra long staple, 53 % of medium staple and

10 % of short staple (Kranthi, 2011). The demand for short staple cotton (less than 10 counts) and also coarse cotton for non woven cotton purpose such as surgical cotton, absorbent cotton and technical textiles has been increasing. With the demand for denim cotton on the rise, short staple cotton of 7-14s counts is now in higher demand especially denim export and local use. The use of cotton and synthetic blends will increase as it rose 13 % in recent years and *desi* cotton varieties and hybrids are suitable for blending. Good fibre strength and extensibility are important for blending to get good yarn properties.

The significant progress has been accomplished at national and international level in breeding for agronomic traits, fiber characteristics and pest resistance over the last 70 years. In order to maintain pace with the increased demand for the commodity, both in national and international market it is imperative to give impetus for development of new cotton varieties and hybrids with appropriate cultivation technologies in consolidated and newer levels of productivity are reached in all location specific situations.

India is the only country where all four cultivated species of cotton are grown on commercial scale and covers 11.77 million ha. India continued to maintain the largest area under cotton and second largest producer of cotton next to China with 34 % of world area and 21 % world production. The production increased from meager 2.79 million bales (170 kg lint/bale) in 1947-48 to a high of 17.6 million bales in 1996-97 and during current year it reaches up to 35.61 million bales (Anonymous, 2013) with the productivity meager 169 kg/ha to 496 kg/ha (65.40 % of world average). Despite good progress made by public sectors research and development, it is a matter of concern that the productivity started to decline from 566 kg/ha in 2008 to 496 kg/ha in 2012-13. Statewise, Maharashtra ranks 1st in area under cotton cultivation, but it ranks the lowest in per ha productivity (Anonymous, 2013). Several factors including erratic rainfall and emerging biotic and abiotic stresses declined the yield in India.

The increase in yield is delimited by number of pests and diseases in cotton. Looking only to the diseases, fungal and bacterial diseases result in

loss of about 4 % of the value of crop (Brown, 2002). The damage varies with locality and season. In certain areas the damage is so high that cotton can't be grown profitably under common methods of procedure. Among the different diseases of cotton, wilt caused *by Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) known also under the names of "black heart" and "frenching" is one of the most economically damaging disease worldwide causing yellowing, wilting, defoliating, vascular tissue damage and ultimately death of the plant. Earlier 5-60 % loss was observed in *desi*/ asiatic cotton due to *Fusarium* wilt (Dasture *et al.,* 1960).

Fusarium wilt of cotton caused by the fungus *Fusarium oxysporum* Schlechtend f. sp. *vasinfectum* (Atk.) Snyd and Hans was first identified in 1892 in USA by Atkinson. After initial identification, the disease was identified in many other countries. *Fusarium* wilt was reported in Egypt (1902) (Fahmy, 1927), India (1908) (Kulkarni,1934), Tanzania (1954) (Woods and Ebbels, 1972), California (1959) (Garber and Paxman, 1963), Sudan (1960) (Ibrahim, 1966), Israel (1970) (Dishon and Nevo, 1970), Brazil (1978) (Armstrong and Armstrong, 1978), China (1981)(Chen *et al.*,1985), Australia (1993) (Kochman, 1995) in successive years and the disease become disease of global importance in cotton growing countries at the end of next century. In addition to worldwide distribution, *fusarium* wilt occurs in all the four domesticated cottons *viz.*, *G. arboreum, G. herbaceum, G. hirsutum* and *G. barbadense*.

Till the date, eight different races and one Australian biotype were reported all over the world (Davis *et al.*, 2006). Scientists reported race 1 and 2 in United States, race 3 in Egypt, race 4 in India, race 5 in Sudan, race 6 in Brazil and race 7 and 8 in China. Armstrong and Armstrong (1958, 1960 and 1978) reported races 1 to 5; Ibrahim (1966) reported race 5 and Chen *et al.* (1985) reported races 7 and 8.

Regarding the host range, *Fusarium oxysporum* f. sp. *vasinfectum* has wider host range. It was isolated from roots of various crops and weeds. Armstrong and Armstrong (1960) reported it on tobacco and soybean, while (Grover and Singh, 1970) reported on okra. It was also reported on Chinese

lantern, cowpea, green gram, hollyhock (Armstrong and Armstrong, 1960; Smith *et al.*, 1981). Plants like sorghum, pearl millet, finger millet, sweet potato, snapdragon harbor FOV without exhibiting above ground symptoms (Armstrong and Armstrong, 1948). Several weeds in malvaceae, sterculiaceae and tiliaceae were found susceptible on artificial inoculation (Woods and Ebbels, 1972). Number of plants acts as symptomless carrier for FOV. It can be isolated even from resistant varieties of cotton without external or internal symptoms (Armstrong and Armstrong, 1948). It was also observed that incidence of FOV of cotton in a field after an absence of cotton for 12 years was still high. Looking to the wider host range, FOV of cotton will persists for longer period in absence of cotton. Thus the treat of FOV in cotton remains constant in near future.

It was first observed by Evans in 1908 (Kulkarni, 1934), at Nagpur experimental farm and then by Kulkarni from Bombay Presidency. The area under *desi*/ asiatic cotton (*G. arboreum* and *G. herbaceum*) though reduced, it is grown in some area because of many advantages such as insect resistance, drought resistance, high superior fiber quality and needs comparatively less inputs. However, the *Fusarium* wilt disease is the most limiting factor in *desi* cotton production. Out of eight different races of *F. oxysporum vasinfectum,* found all over the world, race 4 is most widely distributed in India and restricted to diploid cotton species *G. arboreum* and *G. herbaceum*. Recently this race of FOV was identified in California field infecting Pima cotton fields also infected Acala and Upland cottons (Kim *et al.,* 2005). Thus, the threat of *Fusarium* wilt race 4 is extended to *G. hirsutum*. Due to the seed and soil borne nature of the pathogen, disease control remains the foremost challenge in this area of research. The heavy use of fungicides to control this disease causes the human hazards, environmental pollution and may lead to the resistance in pathogen. To avoid these harmful effects, continuous breeding for disease resistance remains only viable environmental friendly strategy in disease management.

A technique of breeding for *Fusarium* wilt resistance in *G. arboreum* was developed which was known as "*Poona Technique*". According to this technique wilt resistance in cotton is not due to single gene but may be controlled by cumulative genes (Uppal, 1938). Efforts were made by earlier scientists/ breeders to transfer the wilt resistance from wild species to cultivated species. Crossing between promising parents combined with selection or back cross method are the most common breeding procedures.

However, genetics of resistance to *Fusarium* wilt is still hidden in case of Asiatic cotton. Though plenty of information is available in *desi* cotton germplasm, diversity, adoption, pathogen races, epidemiology and cotton biotechnology, there is little or no information available related to *Fusarium* wilt resistance using molecular markers. Molecular tagging of genes or QTL confirming FW resistance has not been previously reported in Asiatic cotton. Marker assisted selection is a reliable and faster method than classical screening (Wang *et al.,* 2009). Hence, the current study "Genetic analysis of wilt resistance in intraspecific crosses of Asiatic cotton (*G. arboreum* L.)," is undertaken with the objectives to find out inheritance pattern of *Fusarium* wilt resistance in Asiatic cotton (*G. arboreum*), to identify molecular markers related to it and also to find out gene action of various yield contributing characters.

2. REVIEW OF LITERATURE

The present research work was carried out in order to study the inheritance of *Fusarium* wilt resistance in Asiatic cotton *G. arboreum* and to identify molecular markers linked to wilt resistance in *G. arboreum* and also to find out gene action of various yield contributing characters.

The research work carried out on these aspects earlier is described below under following heads.

2.1 Inheritance of resistance to *Fusarium* wilt

2.2 *Fusarium* Wilt: Molecular Marker Study

2.3 Root anatomy

2.4 Generation mean

2.5 Qualitative characters

2.1 Inheritance of resistance to *Fusarium* wilt

Inheritance pattern of *Fusarium* wilt resistance during selection of wilt immune strains of Egyptian varieties of *G. barbadense* and the progenies of immune × susceptible crosses revealed that inheritance to resistance was of a 'cumulative' in nature (Fahmy, 1934). According to Uppal *et al.* (1940), three complementary factors which governs the resistance to *Fusarium*. Kelkar *et al.* (1947) studied the inheritance of *Fusarium* wilt resistance in Indian cotton and concluded that the resistance to *Fusarium* wilt in *G. arboreum* and *G. herbaceum* was expressed in the presence of two dominant complementary genes but the third gene present, inhibited the action of other two genes. Further, Mohamad and Darrag (1964) reported that a single dominant factor was involved in inheritance of resistance to FOV in cotton in United Arab Republic.

In Sea Island cottons (*G. barbadense*) resistance was controlled by two major genes (Smith and Dick, 1960) and also reported that where nematodes could be controlled, wilt resistance was regulated by one dominant gene. While in 1961, work of Johnes in upland cotton indicated that

resistance to the wilt was a quantitative character and at least two genes but not more than three genes pairs were involved, but additional modifying gene influence resistance.

In Acala and non-Acala Upland cottons, Kappelman (1971) reported that resistance to *Fusarium* wilt may be more complex and possibly inherited in a quantitative manner by several major genes and minor modifying genes. Further, Ebbels (1975) concluded that inheritance to *Fusarium* wilt is likely to be polygenic in *G. hirsutum,* while in *G. barbadense*, it may be controlled by relatively few genes. Hillocks (1984) also concluded that inheritance of resistance to *Fusarium* wilt was complex involving several major and minor genes. Whereas, Netzer (1982) showed that crossing between resistant *G. hirsutum* cultivars and susceptible *G. barbadense* and back crossing resistance was controlled by a dominant gene

More than 150 Pima (*G. barbadence* L.) and Acala and non Acala, Upland (*G. hirsutum* L.) entries from gene pool and a population of 32 Pima recombinant inbred lines (RILs) and six F_1 combination of hybrids developed between susceptible and resistance entries were evaluated against *Fusarium oxysporum f. sp. vasinfectum* by Ulloa *et al.* (2006) and revealed that resistance against FOV race 4 in Pima cotton was more complete than expected and may be determined by a single dominant major gene and one or more modifying minor genes (Ulloa *et al.*, 2005 and 2006). FOV resistance in Acala and non Acala Upland cottons may be more complex and may be inherited in quantitative manner by several major genes and minor modifying genes.

Inheritance of resistance to *Fusarium* wilt in other crops and root knot nematode

Zhang *et al.* (2004) using two segregating populations of cotton derived from crosses between Sure-Grow 747× Auburn 634RNR and Sure-Grow 747 × M-240RNR reported that, one or two genes are responsible for RKN resistance. Segregation of resistance phenotype in both mapping populations fit in 3:1 ratio by chi-square analysis, indicating one dominant RKN resistance gene. In

the F_2 mapping population derived from a cross between Sure-Grow 747 × M-240 RNR, segregation of the resistance phenotype also fit a 13:3 ratio, indicating possible epistatic interaction between a dominant and a recessive gene conferring RKN resistance.

Dominance of *Fusarium* wilt resistance to susceptibility and the role of two gene interactions *viz.,* inhibitory (13:3) and complementary (9:7) in pigeonpea was also confirmed by Sameer Kumar *et al.* (2009).

2.2. *Fusarium* Wilt: Molecular Marker Study

Nucleotide composition of DNA of FOV and that of cotton variety 5904-I were markedly different and the DNA of fungus belongs to the GC rich type while that of cotton to the AT rich type Ibragimov *et al.* (1977). This correlation was found between wilt resistance and the degree of homology of DNA of fungus and that of the thin fibre cotton cultivars.

In the studies of Dowd *et al.* (2004), PR-10 related genes were the most common PR genes included in hypocotyls infected with *F. oxysporum f. sp. vasinfectum.* The study revealed that function of PR-10 genes is still unknown but they evidently are involved in the response of *F. oxysporum f. sp. vasinfectum* infection because they represent the most abundant of all the genes included (20 cDNA copies). Mcfadden *et al.* (2006) utilized microarray and QPCR technology to identify *fov* genes expressed in root and hypocotyl tissues during a compatible infection of cotton. They identified 218 fungal clones representing 174 *fov* non-redundant genes as expressed in *planta*. The majority of the expressed sequences were expressed in infected roots, with only six genes detected in hypocotyl tissue. The *fov* genes identified were predominantly of unknown function or associated with fungal growth and production. 11 genes were identified which preferentially expressed in plant tissue. A putative oxido-reductase (with homology to *AtsC*) gene was found to be highly preferentially expressed in *planta.* In *Agrobacterium tumefeciencs, AtsC* is associated with virulence. Inoculation of susceptible and a partially resistant cotton cultivar with either a pathogenic or a non pathogenic isolate

of Fov revealed that the expression of the *fov AtsC* homologue was associated with pathogenicity and disease symptom formation.

An intraspecific F_2 in *G. hirsutum* L. was developed by crossing with a highly resistant cultivar Znongmiansuo 35 (ZMS35) and susceptible cultivar Junmian by Wang *et al.* (2009), to screen simple sequence repeats (SSRs) closely linked to the FW resistant gene. The results showed that FW resistance segregated in a 3:1 ratio as simple monogenic trait in $F_{2:3}$ families. Molecular mapping identified a FW resistance gene closely linked with the SSR marker $JESPR304_{280}$ in chromosome D3 (c17). A composite interval mapping method detected from QTLs for FW resistance in Chr. A7 (c7), D1 (c15), D9 (c23) and D3 respectively. Among them, one major QTL (LOD>20) was tagged near marker JESPR304 within an interval of 0.06-0.2 cM and explained over 52.5-60.9 % of the total phenotypic variance. The data confirmed the existence of a major gene in Chr.D3.

Using highly resistant and highly susceptible cultivars, Wang *et al.* (2010), F_2 populations of two intraspecific (*G. hirsutum* × *G. hirsutum*) and one interspecific (*G. hirsutum* × *G. barbadense*) crosses developed and SSR markers were used to screen genomic regions closely linked to FW resistance. The results showed that five QTLs associated with FW resistance were detected in intraspecific populations using composite interval mapping method, four loci located on Chr.2/Chr.17. Neighboring markers JESPR304 and CIR 305 explained 13.1 to 45.9 % of the phenotypic effect and found tightly linked. They suspected the possibility that the four QTLs found under different conditions were the same resistance QTL/gene and possibly presence of a major FW resistant gene in interspecific population. In the interspecific mapping population two QTLs were detected on Chr.9 and Chr. 12/26 which explained great phenotypic variance of 49.4 and 45.7 % and concluded that different resistance mechanisms were working between *G. hirsutum* and *G. barbadense* because QTLs located for FW resistance in intraspecific and interspecific populations on totally different positions.

F_2 population derived from the "Charentais-form1" × "TRG-1551" cross of melon was studied by Oumoud *et al.* (2008) for bulk segregant analysis utilizing random amplified polymorphic DNA, in order to develop molecular marker linked to the locus FOM-1. Resistance to races 0 and 2 of *F. oxysporum* is conditioned by dominant gene FOM-1. They identified three RAPD markers *viz.,* $B17_{649}$, $VO1_{578}$ and $VO6_{1092}$ are linked to FOM-1 locus out of 400 tested markers. Fragments amplified by primers $B17_{649}$ and $VO1_{578}$ were linked to coupling phase to FOM-1 at 3.5 and 4cM respectively, whereas $VO6_{1092}$ marker was linked in repulsion to same dominant allele at 15.1 cM from the FOM-1 locus. These RAPDs were cloned and sequenced in order to design primers that would amplify the target fragment. The derived sequence characterized amplified region (SCAR) markers $SB17_{645}$ and $SV01_{574}$ (645 and 574 bp), respectively were present only in resistant parents and $SV06_{1092}$ (1092bp) present only in susceptible parent.

2.2.1 R- Genes in Plants

Plants have evolved a sophisticated, multi-layered defense network to detect and respond to pathogen challenges. Inducible responses are governed by plasma membrane pattern recognition receptors (PRRs) and also cytoplasmic immune receptors encoded by resistance (R) genes. PRRs recognize relatively conserved small molecules, proteins and protein fragments, produced externally to the cell by invading pathogens, and collectively referred to as pathogen associated molecular patterns (PAMPS). By contrast, R proteins directly or indirectly perceive proteins and small molecules termed effectors that are introduced into plant cells by the pathogen. Genes encoding effectors that are recognized by R gene products, leading to effective plant resistance, are genetically defined as avirulence (avr) genes. Two modes of resistance may be distinguished: PAMP triggered immunity (PTI) that is mediated by PRRs, and effecter triggered immunity (ETI) that results from effecter recognition by R proteins and often produces a hypersensitive response, a form of localized host programmed cell death (Jones and Dang, 2006). R genes have been implicated in resistances against

diverse and taxonomically unrelated pathogens including bacteria, viruses, nematodes, insects, filamentous fungi and oomycetes. Remarkably, most resistance genes against diverse pathogens, such as viruses, bacteria and fungi, share structural similarity. In addition to being pivotal for host resistance, PRRs and R genes are thought to play a role in non-host resistance (Schulze-Lefert and Panstruga, 2011). The majority of cloned and functional R genes described within the plant kingdom contain a nucleotide binding site (NB) and leucine-rich repeat (LRR) domain, and are members of the STAND (Signal Transduction ATPase with Numerous Domains) protein family of NTPases, known as NB-LRRs (Lukasik and Takken, 2009; Van der Biezen and Jones, 1998).

2.2.2. Resistant Gene Analogues

RGA marker type based on the conserved domain of resistance genes. A number of resistance genes have been cloned from several crop species. Plant disease resistance (R) genes have been cloned and characterized from both mono- and dicotyledonous plant (Hammond - Kosack and Jones, 1997). RGA were isolated from several plant species, such as potato (Leister *et al.,* 1996), soybean (Kanazin *et al.,* 1996; Yu *et al.,* 1996), lettuce (Shen *et al.,* 1998), tomato (Ohmori *et al.,* 1998; Pan *et al.,* 2000), rice (Leister *et al.,* 1998; Mago *et al.,* 1999), barley (Leister *et al.,* 1998; Seah *et al.,* 1998), wheat (Seah *et al.,* 1998; 2000), chickpea (Huettel *et al.,* 2002), and *Medicago truncatula* (Zhu *et al.,* 2002). Genetic mapping revealed that many of the RGA either co-segregate with or are closely linked to known disease resistance loci (Kanazin *et al.,* 1996; Leister *et al.,* 1996, 1998; Zuang *et al.,* 1998; Mago *et al.,* 1999; Pan *et al.,* 2000; Shen *et al.,* 1998; Yu *et al.,* 1996). Recently Zhuang *et al.,* 2002; Rajesh *et al.,* 2002; He *et al.,* 2004; Mantovani *et al.*, 2006; Zhang *et al.,* 2007; Bart Brugmans *et al.,* 2008; Palomino *et al.,* 2008 used RGA for identification and tagging of R genes. Although not all amplified products using these RGA primers are functional disease resistance genes, they contain elements involved in signal transduction pathways in plants (Chen *et al.,* 1998). Ramalingam *et al.* (2003) showed that, in rice, RGA are associated not

only with qualitative resistance but also with quantitative response. These isolated RGA, thus, have provided useful tools to dissect, tag and isolate genes conferring both qualitative and quantitative resistance to different pathogens. Hinchliffe *et al.* (2005) also mapped nine RGA markers to homeologous chromosomes in cultivated tetraploid cotton based on linkage to existing markers that are located on these chromosomes for RKN. Further, Tan *et al.* (2003) utilized R-gene degenerate primers designed from the NBS motifs of the tobacco N protein, Arabidopsis RPS2 protein, and the flax L6 protein to amplify and clone PCR products in the 250-bp size range in cotton.

2.2.3. NBS-LRR:

NB-LRR genes comprise one of the largest gene families in plants. Based on the presence or absence of N-terminal domains, members of the NB-LRR family can be divided into two major groups. The first group contains an N terminal domain with homology to the Drosophila toll and human interleukin-1 receptor (TIR) and is referred to as TIR-NB-LRRs or TNLs. The second, non-TIR-NBLRR, group is collectively known as CNLs as some, but not all, members of this group contain a predicted coiled-coil (CC) structure in the N-terminus. This division of NB-LRR proteins is also reflected in phylogenetic analyses of the NB-ARC domains in which TNL and CNL proteins form distinct clades (Meyers *et al.*, 1999; McHale *et al.*, 2006; Meyers *et al.*, 2002). In addition, partial NB-LRRs that lack some NB-LRR specific domains and contain, for example, only TIR, TIR-NB, CC, and CC-NB domains, have been described in plant genomes (Meyers *et al.*, 2002; Guo *et al.*, 2011). NB-LRR genes are ancient in their origin and have been identified in ancestors of early land plants. NB genes with sequence homology to TNLs have been described in bryophytes (Akita and Valkonen, 2002) and TNLs and CNLs have been found in gymnosperms and eudicots (Tarr and Alexander, 2009). However, the composition of NB-LRR genes varies significantly between species (Cannon *et al.*, 2002). The unequal representation of NBLRR lineages within plant taxa has been typified by the low frequency of TNLs within the

monocotyledonous species despite the manifestation of TNLs prior to the angiosperm-gymnosperm split (Tarr and Alexander, 2009; Jiang *et al.*, 2005).

Within genomes, NB-LRR genes are organized either as isolated genes, or as linked clusters of varying size that are thought to facilitate rapid R gene evolution (Hulbert *et al.* 2001). NB-LRR gene clusters are termed homogeneous when they contain only sequences that share a recent common ancestor. In contrast, clusters that contain more distantly-related NBLRRs are referred to as heterogeneous (Friedman and Baker, 2007).

2.2.4 Role of enzymes and other chemicals in resistance mechanism

Dowd *et al.* (2004), while studying gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum* f. sp. v*asinfectum* concluded that, some of the complex plant defense responses that occur in cotton in response to *F. oxysporum* f. sp. *vasinfectum* infection. Cotton alcohol dehydrogenase (anaerobic stress) was induced in both tissues but another highly redundant Zn-class alcohol dehydrogenase was repressed, possibly indicating different roles for these dehydrogenases. ROS scavengers (peroxidase and oxidoreductase) were induced in hypocotyls only and were repressed in roots. The induction of antioxidant enzymes at the later stages of infection likely is a response to ROS generated as a secondary response to injury and the oxidative stress effects of infection.

The role of enzymes in cell wall strengthening phenylalanine ammonia lyase, cinnamyl alcohol dehydrogenase, peroxidase and polyphenol oxidase was reported by Ana *et al.* (2000) in *Fusarium* wilt of banana. They reported that, these enzymes were upregulated. Accumulation of lignin and lignin like material in infected plant tissues are widely reported for defense mechanism. Lignin is highly resistance to attack by micro-organisms and lignified cell walls are an effective barrier to pathogen entrance and spread. Lignification as a mechanism of disease resistance was studied by many scientists (Vance *et al.,* 1980, Davin and Lewis, 1992 and Dowd *et al.*, 2004).

Caffeic acid is an key intermediate in biosynthesis of lignin. In human being caffeic acid is an antioxidant in-vitro and also in-vivo (Olthof *et al.*, 2001). Cinnamic acid derivatives including caffeic acid and rosnarinic acid are secondary plant metabolites that have been intensely studied for their antimicrobial and antioxidant activities (Raven *et al.*, 1989, Shetty, 1997; Nascimento *et al.*, 2000, Debersac *et al.*, 2001, Bais *et al.*, 2002).

Caffioylshikimic acid (CSA), a major phenolic compound of date palm roots, represents one of the resistance factors of the host to *F. oxysporum* f. sp. *albedinis*. Caffeic acid showed a larger inhibition of the activity of various CWDE that was more than that of CSA and its inhibiting effect appeared to be more important during their production (Modafar *et al.*, 2000).

During allelopathy in plants Miller *et al.* (1991) observed that, caffeic acid concentration is increased in response to *Fusarium oxysporum* infection.

Protein disulfide isomerase is an enzyme in the endoplasmic reticulum that catalyses the formation and breakage of disulfide bonds between system residues within proteins and therefore the protein acts to protein folding. Ray *et al.* (2003) reported rapid induction of protein disulfide isomerase and defense related genes in wheat in response to blotch caused by fungal pathogen *Mycosphaerella graminicola* (Stolf *et al.*, 2011).

2.3 Root anatomy

Resistance mechanisms may took place before or after vascular invasion by the pathogen which leads to formation of lignitubers to contain invading hyphae and occlusion of xylem vessels by gel or tyloses and thus, prevents microconidial transport in the xylem (Smith *et al.*, 1981). Unlike indole acetic acid and gibberellic acid, chlorocholin chlorides reduce the size of the cells and make them denser, thus, increasing resistance by forming a biological barrier (Kertykova *et al.*, 1985).

The restriction of pathogen growth in vascular tissue is involved in the resistance of cotton to several wilt diseases (Wilhem *et al.,* 1974, Harrison and Beckman, 1982). Shi *et al.* (1993) more specifically found the restriction of

fungal spread in the vascular tissue is a characteristic of cotton plants to *Fusarium oxysporum* f. sp. *vasinfectum*. Increase in cytoplasmic content and concomitant decrease in the size of central vacuole is linked to the direct secretion of osmophilic materials into the vessels that coated the fungus. Nature of response dependent on the location of the cells relative to infected vessels. The evidences showed cytoplasmic reorganization and increase metabolic activity in contact cells. Shi *et al.* documented varied responses to *Fusarium* infection (Shi *et al.,* 1991[a], Shi *et al.,* 1991[b], Shi *et al.,* 1992). Shi *et al*. (1991[b]) observed cellular product in contact cells in resistant and susceptible varieties of US. Globular structures accumulated in vessels superficially resembling tyloses (Shi *et al.*, 1992). It is probable that, these globular structures were in fact terpenoid phytoalexin accumulations being secreted into the vessel lumen. The changes in the structure of contact cells and the associated accumulation of material in vessels were observed in resistant as well as susceptible varieties however, the responses were greater and faster in less susceptible variety (Shi *et al.*, 1992 and Christina, 2007).

The suberized exodermis and endodermis of roots, and the cutinized epidermis of subsoil stems and leaves (scales) constitute structural barriers to penetration. Preformed fungitoxic compounds or their precursors in the outer scales of bulbs also protect against penetration. Cultivar resistance is mostly due to active retardation or localization of the fungus after penetration. Physical resistance in wilt diseases, is expressed by local occlusion responses (tyloses, gums) which block or retard the vertical spread of the fungus, and by cell wall-strengthening responses. Chemical resistance is achieved by among others production of phytoalexins, formed locally at the site of the defense response. In retarding fungal growth, phytoalexins prolong the period that the host is able to form structural barriers to colonization (Baayen, 1992).

2.4 Generation mean

Mirani (1968) while studying combining ability in interspecific crosses (*G. hirsutum* × *G. barbadense*) observed that additive and dominance effects were operating in the inheritance of fibre length. Significant effects

of gca were found for fibre length and tensile strength. For fibre strength additive and dominance effect were operating and dominance effects was operating for micronaire.

Pathak and Singh (1970) investigated the inheritance of yield and yield components parents, F_1, F_2 and back cross populations of crosses between the cluster boll bearing strain PRS-72 and five strains with normal fruiting branches of *G. hirsutum* L. Dominance variance and dominance gene effects were higher than additive variance and additive genes were controlling yield and bolls per plant. Non-additive effects were more important for other yield components. There were epistatic effects for almost all the parameters in all the hybrids.

The estimates of the genetic variances and gene effects of eight different crosses involving diverse parents studied by Singh *et al.* (1972) showed prevalence of all three types of gene actions *viz.,* additive, dominance and epistasis for all characters in all the crosses. Amongst the epistatic effects dominance × dominance (d × d) interaction was prominent followed by additive × additive effects. In many cases, 'd × d' interaction were negative and thus had depressing effect on character expression. Ginning outturn had considerably higher values of dominance variance as compared to itssimpler component characters *viz.,* seed index and lint index. It was found that the ginning and fibre characters have three types of gene actions i.e. additive, dominance and epistasis. The reciprocal recurrent selection breeding procedures seems to be the best available method as it utilizes all three kinds of gene effects simultaneously.

Abo-El-Zahab and Methwaly (1979) analyzed the parental, F_1 and F_2 generations and reported that additive × additive effects influenced lint index and lint per cent. Non additive effects influenced seed cotton yield and bolls per plant, while both additive and non-additive effects governed five other traits. They further reported that F_1 hybrids showed heterosis for seed cotton yield, bolls per plant and boll weight. Reciprocal effects in both F_1 and F_2 were observed for all the traits except seed index.

Virupakshappa *et al.* (1978) reported that additive gene effect, dominance and epistasis influenced the yield of seed cotton, bolls per plant and boll weight, respectively. These results were estimated from six crosses involving four varieties in F_1, F_2 and BC_2. Krishnamurthy and Henry (1979) in combining ability analysis of *G. hirsutum* observed that dominance effect was predominant for boll weight accompanied by additive × dominance (j) type of non allelic interaction. The presence of additive type of gene action for yield and yield components was reported by Bhatade *et al.* (1980) and they suggested reciprocal recurrent selection for exploitation of available genetic variability.

Deshmukh *et al.* (1980) examined yield and six yield components in a diallel cross with reciprocals involving six varieties of *G. arboreum* L. and observed significant GCA and SCA variances for seed cotton yield, weight of seeds per boll, halo length and seed index indicating additive, dominance and epistatic gene actions. The significant GCA variances observed for G.O.T., sympodia per plant and lint index indicated predominance of additive gene action.

Heterosis for yield, plant height, bolls per plant and halo length was reported by Kalse and Vithal (1980) which revealed (a) dominance ranging from partial to over-dominance depending on the trait, (b) high genotypic variation and genetic advance, (c) additive and dominance variance of almost equal magnitude for yield, (d) importance of dominance variance for plant height, bolls per plant and halo length and (e) the predominance of additive × additive interaction among epistatic effects.

Singh *et al.* (1980) studied the nature and magnitude of genetic components of variation in 7 × 7 diallel cross experiment for seed cotton yield, boll number and boll weight in seven varieties of *G. hirsutum*. The major portion of genetic variability was due to dominance component for all these characters. The recessive genes also contributed to high seed cotton yield. Graphical analysis indicated the presence of epistatic gene action for all these characters. Exploitation of dominance component for heterosis

through hand pollination or through male sterility was suggested. Similarly, preponderance of non-additive genetic effect revealed by Bhandari *et al.* (1981) for yield and its components and fibre quality traits in 6 × 6 diallel analysis of *G. hirsutum*. While, the additive genetic effect was significant except for seed index and number of monopodial branches.

Using six generations of inter-varietal crosses Gill and Kalsay (1981) partitioned the components of generation means for seed cotton yield, bolls per plant, boll weight, G.O.T and halo length. Epistasis was involved in the inheritance of all the traits in all crosses except for boll weight in one cross. Additive genetic effects were important for most characters and dominance effects for some.

Mittal *et al.* (1981) estimated the gene effects for lint index, seed index and seeds per boll in two crosses of *G. arboreum viz.,* G-27 × NR-5 and G-27 × LD-124 by the generation mean analysis. They found additive as well as dominance gene effects were significant for lint index and seed index in G-27 × NR-5; but dominance genes effects were more prominent. Only dominance effects contributed to the inheritance of lint index in G-27 × LD-124 cross. Additive × additive interaction component identified for seed index and seed number per boll was positive and could be exploited. But negative 'i' estimates obtained for other character would inhibit the expression of the traits. Additive × dominance gene effects were significant for lint index in both crosses and for seed index in G-27 × LD-124. Duplicate type of epistasis for lint index and seed index in G-27 × NR-5 cross may hinder the progress through selection. This study revealed the importance of epistasis in genetic control of these characters.

Singh and Singh (1981) analyzed data for gene action, heritability and genetic advance on 13 *G. hirsutum* L. lines of foreign and Indian origin and three testers. Non-additive genetic variance predominated for mean fibre weight in both generations. Heritability in the narrow sense was high, ranging from 42.45 to 96.16 percent in the F_1 and F_2. Estimated genetic advance was

high for monopodia per plant and low for boll weight, halo length, seed and lint indices and GOT.

Bains *et al.* (1982) studied the genetics of seed cotton yield per plant, boll weight, boll number and seed index of an inter-varietal cross (CL 20 × H-14) of cotton. The additive and dominance effects were highly significant for all characters except yield per plant for which only additive effects were significant. The epistatic effect additive × additive was important for boll weight, boll number and seed index. The role of additive × dominance was limited only for boll weight and boll number whereas dominance × dominance component influenced the boll number and seed index. Thus, in the material under study the additive component appeared to be predominant for all characters but at the same time the opposite signs of 'h' and 'i' components indicated the presence of duplicate epistasis which may hinder the progress through the selection to which additive component is amenable.

Triple test cross analysis of inter varietal cross of upland cotton at two locations i.e. Ludhiana and Faridkot were carried out by Dhillon and Singh *et al.*(1982). Additive × additive type of epistasis was significant for ginning outturn, lint index and halo length at Ludhiana and for ginning outturn, seed index and halo length at Faridkot, while its interaction with environment was significant for seed index only. Epistasis 'j' and 'l' were significant for all characters except number of bolls at Ludhiana and for ginning outturn, seed index, lint index and halo length at Faridkot. Barring halo length this cross showed significant interaction with environment for all the characters. Both additive and dominance component of variation showed significant interaction with the environment.

Percival (1982) in F_2 half diallel cross of selected breeding stocks ranging in stature from semi-dwarf to normal investigated gene action and interaction for 16 agronomic, yield components and fibre quality characters. Significant additive and dominance variation was observed for most characters and nearly all the traits showed over-dominance.

While analyzing parentals, BC_1, BC_2 and F_2 generations of the crosses in Gossypium arboreum, Prakash (1982) revealed the importance of both additive and non-additive components of genetic variance for yield and yield related traits. Rao (1982) in diallel analysis of *G. hirsutum* observed the preponderance additive effect for boll weight, dominance effect for plant height and monopodia per plant.

Vande and Thombre (1982) carried out 7 × 7 diallel analysis of seven geographically diverse genotypes of *G. barbadense* to know the nature and magnitude of genetic component of variation for lint index, seed index, ginning percentage and halo length. Except ginning out turn, all the characters influenced by dominance or over-dominance gene action while ginning outturn was under additive gene action.

Abo-El-Zahab (1983) studied heterosis and combining ability for earliness, lint yield per plant, boll weight, lint per cent, lint index, seed index in a diallel cross of ten cultivars and reported that additive effects were pre dominant for lint yield, lint percentage, lint index, seed index and non-additive effects pre-dominant for boll weight and both types of gene action were important for earliness.

Hossain (1983) studied heritability for oil content, fibre qualities and yield in *G. hirsutum* L. from two pure line varieties and their reciprocals, F_1, F_2 and back cross hybrids. He observed that, high oil content was a continuously varying character, controlled by few major gene pairs, which differentiated the parents into relatively high and low oil types.

Ma *et al.* (1983) studied eleven characters in the P_1, P_2, F_1, F_2, BC_1 and BC_2 of the parameters showed heterosis in the filial and backcross generations. All the traits except seeds per boll, showed additive gene effects. Dominance effects were noted for seed index, seeds per boll and fibre fineness.

Nandarajan and Elongovan (1983) in line × tester studies of interspecific hybrids of *G. hirsutum* × *G. barbadense* estimated sizable

magnitude of variance due to gca indicating the importance of additive gene action in the inheritance of lint yield, ginning percentage and halo length.

Patil and Chopde (1983) in 7 × 7 diallel crosses of *G. hirsutum* × *G. barbadense* L. reported the high proportion of additive genetic component for yield per plant, days to 50 % flowering, days to boll bursting, boll weight, lint index was recorded while, both additive and non-additive genetic variance was significant for plant height, ginning percentage and mean halo length.

Silva and Alves (1983) in the triallel analysis of *G. hirsutum* observed that sympodium per plant was under additive control; while for bolls per plant dominance gene effect was predominant.

Simongulyan and Akhedov (1983) studied fibre yield and quality traits in crosses involving varieties differing in fibre quality containing seven *hirsutum* varieties and other nine *hirsutum* and sub- species *maxicanum* and *purpura-scens* of *G. tricuspidatum* L.). The genetic control of the same polygenic character differed in different varieties and such traits as fibre index, length and fineness could be controlled both by dominant and recessive genes. In the variety 173, for example, dominant genes controlled thin long fibre, while in 144 it was controlled by recessive polygenes. Analysis of F_2 to F_5 generations indicated that if favourable values of the trait studied were determined by recessive genes and the most useful plants, with long fine fibre, would appear in the F_3 to F_5 or in later generation, so the selection should not begin before the F_4 generation.

Singh *et al.* (1983) contemplated gene effects for six yield related and fibre quality characters from F_1, F_2 and back cross combinations in Upland cotton. Epistasis was observed for every trait except halo length. Both additive and dominance effects were observed for every character except bolls per plant and lint index.

Wariboko (1983) reported that his studies on gene action in Upland cotton revealed the dominance type of gene action, responsible for the lowest

number of days for the first fruiting branch and least number of days from planting to opening of first flower.

Zhou *et al.* (1983) investigated gene action for fibre length, fibre strength, fibre fineness, seeds per boll and seed index. All the traits except seeds per boll showed additive effects and dominant effects were noted in case of seeds per boll, seed index and fibre fineness.

In line × tester, Chakreshkumar *et al.* (1984) found that, yield and lint index were controlled by additive genetic effect and nonadditive effect in *G. hirsutum* L. The characters i.e. boll number, boll weight and ginning percentage were influenced by additive genetic effect, whereas seed index was influenced by nonadditive genetic effect.

Duhoon *et al.* (1984) in 9 × 9 diallel of *G. hirsutum* L., reported the preponderance of dominance gene effect for seed cotton yield, boll number, boll weight, sympodial branches and ginning out turn. Additive and dominant gene effects were equally important for plant height, monopodial branches and halo length.

Deshpande and Bhale (1984) recorded predominance of dominance gene effect for ginning outturn and lint index while studying intraspecific crosses of *G. hirsutum* however, additive component was significant for seed index but it was lower in magnitude than dominant component.

Gupta and Singh (1984) made genetic analysis for some fibre and seed traits of F_1 to F_3 and back cross generations of 11 parental diallel cross of Upland cotton and additive and dominance effects were important in the control of seed and lint indices.

Khajjidoni *et al.* (1984) crossed two *G. arboreum* lines in a line × tester analysis and ten yield related traits were investigated GCA variance was higher than SCA variance for bolls per plant, boll weight, seeds per boll and halo length indicating the importance of additive gene effects.

Khan and Tariq (1984) studied the inheritance of plant height, yield and yield components from the data in P_1, P_2, F_1 and F_2 generations in

hirsutum crosses of Mutant H1-30 with three other cultivars *viz.*, ST-731 N, HG-61 N and AUH-38 N. They reported positive heterosis for all characters and this was attributed to over-dominance, partial dominance and additive gene effects. Broad sense heritability was moderate to high for all the traits. Expected genetic advance was high for all the traits except boll weight.

Mirza and Khan (1984) studied the genetics of varietal differences for plant height, yield and its components in *G. hirsutum* L. The results indicated additive effects for most traits. The best combiners were B-557 for yield and bolls per plant, AC-134 for plant height ad 407-26 for boll weight and seed and lint indices.

Vyahalkar *et al.* (1984) reported inheritance of fur fibre related traits in *G. arboretum* L. from F_1 and F_2 hybrids of 10 × 10 diallel cross excluding reciprocals. Additive components of genetic variance were important for halo length and non-additive components for fibre strength and fibre maturity coefficient. They suggested that the F_2 material was best for selection for the improvement of fibre length.

Waldia *et al.* (1984) indicated from the data on yield and six yield related characters from a cross between ten female lines and three male testers, that gene action was predominantly non- additive for plant height, boll weight and seed cotton yield and additive for seeds per locule. For other traits studied, both components were important.

Amalraj and Gawande (1985) in the genetic analysis of yield and ginning outturn in inter-varietal hybrids of *G. hirsutum*, reported that boll weight, 100 seed weight, seed cotton yield and ginning percentage were under the control of additive effect while for bolls per plant dominance gene effect was significant.

Dhorajia *et al.* (1985) studied six generations of an inter-varietal cross 744A and G. Cot. 15 and reported that additive gene effect was significant for all the characters except seed cotton yield and ginning out turn. Dominance gene effect was also significant for all the characters except seed index and

number of seeds/ boll. Non additive gene effect was found significant in some traits.

In diallel analysis of *G. hirsutum,* plant height was under the control of nonadditive gene effect (Lather *et al.,* 1985).

Jagtap and Kolhe (1986) in 5 × 5 diallel of *G. hirsutum*, reported the presence of both additive and dominance effects in inheritance of characters *viz.,* days to first flowering, boll number, boll weight, seed cotton yield, ginning percentage and halo length. Dominance gene effect was more predominant than additive gene effect for boll number, boll weight and seed cotton yield.

Kenchana Goudar *et al.* (1986) studied the inheritance of earliness, yield and its components in 9 × 9 diallel analysis of nine diverse parents of *G. hirsutum*. Moderate to high genetic diversity was observed among parents for all characters. Except fruit points per plant and total flower produced per plant, all characters were under control of additive and dominance gene effect, while only non-allelic interactions were found operative for these two characters.

Kassam *et al.* (1986) made genetic analysis for yield and yield components in cotton and reported significant heterosis for seed cotton yield, boll weight, locules per boll, bolls per plant, lint percentage and seed index. Additive, dominance and epistatic gene effects were involved in their inheritance.

Rahman (1986) concluded genetic analysis for yield, yield components and oil % in 6 × 6 *hirsutum* diallel cross. It was revealed from Wr/Vr graphs that bolls per plant and staple length were controlled by additive and non-additive genes with the presence of non- allelic interation in both F_1 and F_2 generations, while lint index was controlled by additive type of gene action with partial dominance with some non-allelic interaction in both the generations.

Jagtap and Kolhe (1987) studied ten parents of *G. hirsutum* in partial diallel fashion to find out the components of genetic variation for days to flowering, boll number, boll weight, seed cotton yield, ginning percentage, lint index seed index, and halo length. Both additive and dominance gene action were significant for these characters. However, the significant role of dominance was observed for days to flowering, boll number, boll weight, seed cotton yield, ginning percentage, lint index and seed index. Whereas, additive gene action was important for halo length. The study also illustrated partial dominance for halo length and over dominance for all remaining 7 characters.

Dhanda *et al.* (1987) carried out biparental mating between the 120 progenies of cross H-777 × H-807, which revealed the presence of additive as well as non-additive gene effect for yield, boll weight, number of seeds per boll, halo length, fibre fineness and fibre maturity coefficient. The non-additive gene effect was significant for plant height and seed index.

Jain *et al.* (1987) in biparental mating using North Carolina Design-I for cross G-27 x H-476 observed preponderance of non-additive genetic variance for yield, number of bolls and additive gene genetic variance for seeds per locule, first fruiting node number and plant height. Whereas, weight per boll, number of locules and seed index were governed by both additive and non-additive gene action. Seed index exhibited partial dominance while weight per boll, number of locules showed over dominance.

In genetic analysis study of 6 × 6 diallel cross of *G. hirsutum* Khan (1988) reported that traits like bolls per plant and staple length were controlled by additive type of gene action with partial dominance without evidence of any non-allelic interaction, the inheritance of boll weight, seeds per boll, GOT and seed and lint indices manifested over-dominance type of gene action with no evidence of non-allelic interaction. The seed cotton yield was governed by over-dominance type of gene action complicated by non-allelic interaction.

Rahman *et al.* (1988) studied the inheritance of yield and yield components in different crosses of *G. hirsutum* L. Bolls per plant were additive and non-additive in their gene action in both F_1 and F_2 generations with presence of non- allelic interaction. Lint index was controlled by additive type of gene action with partial dominance in two generations, whereas seed cotton yield, lint % and seed index were controlled by over-dominance complicated by non- allelic interaction in both the generations.

Tomer *et al.* (1988) in line × tester analysis of *G. hirsutum* found that additive gene effect was involved in the inheritance of all quality characters *viz.,* halo length, lint index, seed index, ginning out turn and fibre fineness.

Amalraj (1989) in line × tester analysis of *G. hirsutum* × *G. barbadense*, observed the preponderance of additive genetic variance for seed cotton yield, boll number, seeds per boll, halo length and ginning percentage.

Mahmood *et al.* (1989) conducted genetic studies in 4 × 4 *hirsutum* diallel cross for gene action and inheritance of plant height, bolls per plant, boll weight and seed cotton yield. The results indicated that differences among the parents and their F_1 hybrids were highly significant. Vr/Wr graphs indicated that additive type of gene action was operative for bolls per plant and boll weight while, plant height and seed cotton yield were administered by over-dominance.

Pavasia *et al.* (1990) in 8 × 8 diallel analysis of American cotton observed that both general and specific combining ability variances were significant for all yield contributing characters suggesting the importance of both additive and non-additive gene action.

Srivastava and Kalsay (1990) in generation mean analysis of upland cotton (*G. hirsutum*) revealed the prominence of additive effect of 100 seed weight and ginning percentage. For 100 seed weight along with additive effect additive × dominance type of epistatic interaction was also significant, so was for bolls per plant.

Nandarajan and Rangaswamy (1990) in line × tester analysis of *G. hirsutum* revealed that, lint index, seed index, 2.5 % span length, uniformly ratio and fibre fineness were governed by additive gene effect indicating early fixation of superior genotypes of these traits for lint yield and maturity coefficient. Both additive and non additive gene actions were important indicating fixation of superior genotype in the later segregating generations.

Khan *et al.* (1991) reported, from 4 × 4 diallel cross of Upland cotton, that GCA effects were highly significant for plant height, boll number, boll weight, seed cotton yield, seed index and staple length. The SCA effects were highly significant for boll weight, seed cotton yield, seed index and staple length. Additive type of gene action was predominant for seed cotton yield, bolls per plant, plant height and lint index.

Verma *et al.* (1991) in line × tester of *G. hirsutum* revealed preponderance of non-additive gene effect for boll number per plant, boll weight and seed cotton yield. However, plant height, halo length, ginning out turn were controlled by additive gene action.

Sanyasi (1991) in the genetic analysis of yield and bolls per plant observed the predominance of additive × dominance type of interaction in the control of these traits.

Nandarajan and Rangaswamy (1991) assessed the gene action underlying the inheritance of fibre traits *viz.,* 2.5 % span length, fibre uniformity, fineness and maturity coefficient in fibre crosses obtained and reported additive, dominance and digenic non allelic interaction effects. However, non-additive gene action dominated the additive gene action. Hence, one or two cycles of recurrent selection followed by pedigree method of handling the segregates could be successfully adopted for improvement of the traits studied.

In line × tester analysis of interspecific hybrids (*G. hirsutum* × *G. barbadense*) Katagiri *et al.* (1992) observed that magnitude of sca variance was nearly four times higher than gca variance indicating predominance of

non-additive gene effects than additive for seed cotton yield and two crosses DRC-80 × CB-289E, DRC-68 × BCS9-95.

Khan *et al.* (1992) carried out genetic analysis for yield and its components in diallel cross of upland cotton. The mean squares of variances were highly significant for all the traits. Additive type of gene action was observed for bolls per plant while over-dominance for boll weight and seed cotton yield. No epistatic effects were observed.

Mane and Bhatade (1992) in 6 × 6 diallel analysis of *G. hirsutum* L. found that three traits of cotton *viz.,* seed cotton yield, ginning percentage and fibre length were controlled by both additive and non-additive gene effect with predominance of additive gene action. The parent G. Cot. 10 was the best general combiner for seed yield and Acala-44 for ginning percentage.

Murtaza *et al.* (1992a) studied the inheritance of yield and its components in *hirsutum* diallel cross. Additive effects with partial dominance predominated for all the characters. Epistatic effects were involved in the expression of these traits.

Murtaza *et al.* (1992b) reported gene action through quantitative genetic analysis of *hirsutum* diallel crosses. The metrical traits studied were lint index, staple length, micronaire and fibre maturity. Additive type of gene action with partial dominance complicated with some non-allelic interaction was interpreted to be involved in the inheritance pattern of the traits.

Patel *et al.* (1992) in diallel analysis of *G. hirsutum* reported pre-dominant additive gene effect for bolls per plant.

Khan and Khan (1993) studied gene action for some morphological traits in diallel crosses of upland cotton. Additive variation with some non allelic interaction was observed in the inheritance pattern of plant height and sympodia per plant. For bolls per plant, additive genetic effects with partial dominance were detected while over-dominance gene action was operative for monopodia per plant, seed cotton yield and boll weight.

Patel and Badaya (1993) in genetic analysis of *G. hirsutum* observed that boll weight was governed by dominance gene effect while for seed cotton yield additive gene effect was predominant. However, dominance × dominance type of epistasis was significant for both characters.

Rehman *et al.* (1993) performed *hirsutum* diallel analysis of varietal differences for seed and lint indices. It was concluded that lint index was governed by over-dominance gene action while additive gene action with partial dominance was observed for seed index.

Shah *et al.* (1993) evaluated gene action and combining ability in *hirsutum* diallel cross. The results indicated that traits like plant height, bolls per plant and staple length were controlled by additive type of gene action while sympodia and monpodia per plant, boll weight and GOT were governed by over- dominance type of gene action.

Ajaz-ul-Haq (1994) studied inheritance of economic traits in Upland cotton diallel cross. The Wr/Vr graph revealed over- dominance type of gene action for plant height, bolls per plant, boll weight, seed cotton yield and fibre fineness. Whereas, partial dominance was predominant for lint per cent, seed and lint indices and staple length. No non-allelic interaction was present except for bolls per plant and seed cotton yield.

Echekwu and Alabi (1994) studied diallel analysis for earliness in four *barbadense* and three *hirsutum* varieties crossed to obtain a diallel set excluding reciprocals. GCA was highly significant for days to flower, days to 50 % flowering and days to boll opening suggesting the importance of additive gene effects.

Kalsay *et al.* (1994) in half diallel analysis (9 × 9) revealed presence ofboth additive and non-additive gene action for yield contributing traits except ginning outturn where only non-additive gene action was observed.

Chabbra *et al.* (1994) in generation mean analysis of *G. hirsutum* observed the preponderance of additive gene effect for boll weight and seed cotton yield, while dominance effect was significant for bolls per plant. All

these traits were under the influence of additive × additive and additive × dominance type of non-allelic interaction. While for ginning outturn additive × additive type of interaction was significant. For 100 seed weight additive × additive and dominance × dominance type of epistatic interactions were significant.

Bhatade *et al.* (1994) in 6 × 6 diallel revealed that both additive and non-additive effects were important for yield and other traits studied although additive effect was predominant for plant height, ginning out turn and number of bolls per plant. Estimates of gca effects revealed NISD-2 and LRA-5166 were best general combiners for seed cotton yield, boll number, number of sympodia, seed index and earliness while, LRA-5166 for halo length.

Panchal *et al.* (1994) in generation mean analysis of interspecific (*G. hirsutum* × *G. barbadense*) hybrids observed that all the three kinds of gene effects (additive, dominance and epistatic) were involved in the inheritance of lint yield per plant, seed index and lint index while additive and epistatic gene effects were important for inheritance of ginning percentage and number of seeds per boll.

Taware and Patil (1994) in the genetic analysis of an interspecific cross between a pink boll worm resistant local variety of *G. hirsutum* and highly susceptible variety PSH-1 of *G. barbadense* for characters like bollworm damage, locules per boll, seeds per boll, seed cotton yield per plant, bolls per plant, lint index and fibre strength revealed the presence of epistatic interaction for all characters except for locules per boll. Additive gene effect was predominant in the inheritance of all characters followed by dominance gene effect. Additive, dominance and additive × additive gene effects were involved in the inheritance of pink bollworm resistance in the interspecific cross. For seed cotton yield per plant, bolls per plant and fibre strength the complementary epistasis was found; while duplicate epistasis was observed for pink bollworm damage. RRS was suggested for improvement in resistance and other agronomic characters.

Sayal *et al.* (1995) studied inheritance pattern of metrical traits in *hirsutum* diallel cross. Wr/Vr graphs indicated over-dominance type of gene action for plant height and boll weight whereas additive gene action was reported for bolls per plant and seed cotton yield.

Gururajan and Henry (1995) carried out generation mean analysis of two crosses of cotton i.e. KE × H-77 and EC × K-32 to study the gene effects and type of epistasis for nine boll characters. Additive gene effect was operative for all characters under study i. e. boll length, boll weight, boll breadth, length/breadth ratio, seeds/boll, seeds/locule, ginning per cent, lint index and seed index. Additive × additive and additive × dominance effects were significant for boll weight, seeds/ boll and seeds/ locule in cross EC × K-32 and for lint index in cross KE × H-77. Early generation selection and inter mating were suggested for improvement of component characters.

Panchal *et al.* (1995) in line × tester (3 × 6) analysis observed preponderance of additive gene effects for ginning percentage only. Whereas, for rest of yield contributing characters non-additive gene effects were important.

Tariq *et al.* (1995[a]) studied inheritance of boll weight, boll number and seed cotton yield in *G. hirsutum*. Significant additive and non additive effects were observed for all the traits except boll weight for additive effects were significant. The failure of regression analysis for all the traits suggested the presence of non-allelic interaction.

Tariq *et al.* (1995[b]) studied inheritance of lint %, seed and lint indices and staple length in 6 × 6 Upland cotton diallel cross. Reciprocal differences were observed for seed and lint indices but were significant for lint % and staple length. Failure of regression line from unity for lint % and staple length suggested involvement of dominance and epistasis for these traits. Additive with partial dominance was reflected from graphic analysis for seed and lint indices.

Iqbal and Khan (1996) studied effectiveness of additive dominance for seed and lint indices in *hirsutum* diallel cross in F_1 and F_2 generations. Both additive and non-additive genetic variances were observed for these traits. The test for diallel assumptions was fully satisfied by F_1s for lint index whereas seed index showed partial adequacy of additive-dominance model. Both graphic illustration and degree of dominance suggested over-dominance genetic control for lint index and partial dominance for seed index. Dominant genes were responsible for increase in lint index whereas both dominant and reciprocal genes were responsible for high expression of seed index.

Kenchana Goudar *et al.* (1996) reported the predominance of additive gene effect for seed cotton yield and bolls per plant in 9 × 9 diallel analysis of nine genotypes of *G. hirsutum* cotton.

Sayal and Sulemani (1996) performed genetic analysis in 8 × 8 *hirsutum* diallel cross. Wr/Vr graphs showed over-dominance type of gene action for all the quality traits, i.e., lint %, seed index, lint index, staple length, while seed cotton yield was governed by additive type of gene action.

Ahmed *et al.* (1997[a]) studied inheritance of lint yield and quality related traits in 4 × 4 Upland cotton diallel cross. Over-dominance type of gene action was observed for lint %, staple length and fibre fineness, while in case of lint index, additive type of gene action with partial dominance was noticed. No epistasis were observed in the inheritance of these traits.

Ahmed *et al.* (1997[b]) performed diallel analysis for seed cotton yield in *hirsutum* cotton. Cultivars were genetically analyzed for gene action controlling phenotypic expression of seed cotton yield and its related traits. Additive type of gene action with partial dominance was observed for bolls per plant, boll weight, seed cotton yield and seed index. Epistatic effects were also reported in the expression of all the traits except boll weight.

Amudha and Ravindran (1997) in combining ability analysis of coloured cotton reported the significance of additive effect for plant height, boll weight, seed cotton yield and ginning percent whereas bolls per plant

was under control of dominance gene effect. Additive × dominance (j) type of epistatic interaction was foundplaying important role for sympodia per plant.

Hussain *et al.* (1998[a]) studied genetic mechanism for seed and lint indices in 8 × 8 Upland cotton diallel in F_1 and F_2 generations. Additive dominance model was adequate for almost all the traits in both generations except F_2 lint index where it was inadequate. Failure of regression line in F_2 lint index suggested involvement of dominance and epistasis for this trait. Additive with partial dominance was reflected from graphic analysis for seed and lint indices.

Hussain *et al.* (1998[b]) analyzed genetic mechanism for control and expression of some quantitative traits in 8 × 8 diallel cross of *G. hirsutum* L. in F_1 and F_2 generations. Hayman-Jinks additive dominance model proved to be adequate in both generations for all the traits except staple length in F_2.Wr/Vr graphs manifested additive with partial dominance type of gene action for all the traits in both the generations.

Kalwar *et al.* (1998[a]) studied diallel analysis for plant height, sympodia, bolls per plant and seed cotton yield in 4 Upland cotton cultivars and their 12 F_1 hybrids. Plant height and sympodia per plant were governed by over-dominance, bolls per plant by partial dominance and seed cotton yield by additive type of gene action.

Kalwar *et al.* (1998[b]) studied genetic model for some economic traits in four parent *hirsutum* diallel cross and investigated gene action for seed cotton yield per plant, GOT and staple length. The results indicated predominance of additive gene action for seed cotton yield per plant, over-dominance for GOT and partial dominance for staple length.

Pavasia *et al.* (1998) in 8 × 8 diallel of *G. hirsutum* reported the preponderance of additive gene effect for monopodial, sympodial branches and plant height. The variances due to GCA were higher in magnitude than sca for all characters. The parent H-777 was found to be a good general combiner for all three characters.

Yingxin and Xiangming (1998) studied the inheritance of 12 economic characters in Upland cotton. Results indicated that bolls per plant, seed cotton yield and lint % were controlled by additive and non-additive type of gene actions. Boll size, fibre length, fibre strength and fineness were administered by non-additive type of gene action.

Pavasia *et al.* (1999) reported the importance of additive gene effect for ginning percentage, seed index, lint index, 2.5 % span length and fibre fineness in 8 × 8 diallel cross among *G. hirsutum* L. genotypes.

Ahmed and Mehra (2000) studied the genetic architecture of an inter-*hirsutum* cross 43-3-6 × Pusa 19-27 through generation mean analysis which revealed the presence of dominance and epistatic interaction in genetic control of boll number, boll weight, sympodia per plant, monopodia per plant, plant height and biological yield, while only additive gene action was significant for first fruiting node number. Epistasis was absent for harvest index but both additive and dominance effects were significant.

Azhar and Ahmed (2000) investigated inheritance pattern of cotton seed oil in F_1 and F_2 of *hirsutum* diallel. Values of genetic components of variation showed that non-additive genes controlled oil content in F_1 and additive ones in F_2 with varying degree of dominance in both the generations. Depending upon the additive gene effects, estimates of narrow sense heritability were moderate in F_1 and high in F_2. Additive gene gene effects and high heritability of oil content in F_2 suggest recurrent selection for effective progress in oil content.

Kumaresan *et al.* (2000) performed genetic analysis for bolls per plant, seed cotton yield and days to first flower in Upland cotton. High values of broad sense heritability estimates were also noticed for these traits.

Pawar (2000) carried out generation mean analysis in four crosses. Both additive and dominance gene actions were found significant for days to flowering, plant height, number of sympodia per plant, number of bolls per plant, average boll weight, ginning percentage, lint index, bundle

strength and micronaire value. For seed index and uniformity ratio the dominance gene action was significant and important in all the crosses. Duplicate epistasis observed for days to flowering, plant height, number of sympodia number of bolls per plant, average boll weight, ginning percentage, bundle strength and 2.5 % span length which indicated that delayed selection would be more effective.

Phogat and Singh (2000) evaluated cross combination of American glandless × HG 625 through generation mean analysis for gossypol and fibre properties. They found that seed cotton yield per plant, lint (%), 2.5% span length, uniformity ratio, micronaire values, maturity coefficient and fibre strength were governed by all the three type of gene effects *viz.,* additive (d), dominance (h) and epistasis (i, j and l).

Subhan *et al.* (2000) studied gene action controlling boll weight, seed and lint indices and staple length in Upland cotton. Boll weight and staple length reflected additive gene action while in seed and lint indices, over dominance was observed. Non- allelic interaction was observed in staple length showing absence of epistasis providing promising aspects for isolating superior genotypes. Wr/Vr graphs for boll weight and lint index revealed that regression line showed significant deviation from unit slope and indicated epistasis.

Subhan *et al.* (2001) observed differences among hybrids and their parental lines for bolls per plant, seeds per boll and GOT. Wr/Vr graphs indicated over-dominance type of gene action for seed cotton yield per plant. Non-allelic interaction for bolls per plant, seed cotton yield and seeds per boll showing also absence of epistasis. Significant deviation from unit slop manifested presence of epistasis in lint per cent, implying effectiveness of selection in this trait.

Khan *et al.* (2002) studied gene action for quantitative traits in *G. hirsutum*. Results demonstrated that GOT % and seed index were controlled by over-dominance whereas seed cotton yield per plant was governed by additive gene action with partial dominance.

Patil and Meshram (2002) used six diverse strains of *G. hirsutum* cotton in triallel analysis to study the genetics of boll numbers per plant. This trait was appeared to be controlled predominantly by epistatic interactions i.e. additive × dominance and dominance × dominance. Additive component was present but at lower magnitude. The additive component might have been resulted due to additive effect of good general line effects exhibited by parents. Epistatic components i.e. additive and dominance may be partly due to two line specific effect of single cross. While, dominance × dominance component resulted due to interactions of lines involved in the superior three-way crosses. Therefore, the breeding strategy for improvement of this trait should be to exploit the additive component by initiating selection programme in crosses using parental lines.

Ramalingam and Sivasamy (2002) observed preponderance of additive × dominance epistatic effect (highest magnitude) for days to 50 per cent flowering suggesting delayed selection and intermating the segregants followed by recurrent selection for improving this trait.

Reddy *et al.* (2002) in studies with generation mean analysis using six parameter model for four crosses in upland cotton (*G. hirsutum*) reported all three types of gene action found significant in cross H-8 × H-16 and H-11 × H-12 for bolls per plant. For boll weight genetic variance was entirely due to additive component in H-11 × H-12. Additive, dominance and epistatic components were significant in H-25 × H-11 for this traits. All three gene action were significant in H-11 × H-12 and H-11 × H-25 for seed cotton yield.

Singh and Yadavendra (2002) carried out the generation mean analysis of four cotton crosses *viz.,* Cross-I (G. cot-10 × BC –2000 –1), Cross II (G. cot-16 × BB-2-4-3), Cross III (LRA 5166 × G-84-1/247) and Cross-IV (4716 SR × Sils-9-22) for Bartlett's index, days to 50 % flowering and lint yield using 6 parameter model of Hayman (1958). Highly significant values for mean were observed for three characters under study in all four crosses. In case of Cross-II and IV both additive and dominance gene

effects and interactions were significant whereas for Cross I and III with exception of dominance × dominance interaction both gene effects (d and h) and gene interaction (i and j) were significant for Bartlett's index. For days to 50 % flowering in cross-I all gene effects were non-significant, whereas in Cross-II except dominance × dominance interaction, all other gene effects were highly significant. Cross-IV showed highly significant additive effect and additive × dominance gene interaction. For lint yield highly significant additive, dominance and epistatic gene effects were recorded in all four crosses except non significant dominance × dominance gene interaction in cross-IV.

Thangaraj *et al.* (2002) carried out generation mean analysis in *G. hirsutum,* to assess the gene action underlying the inheritance of fibre quality attributes *viz.,* 2.5 % span length, fibre strength, uniformity ratio, short fibre per cent, micronaire and fibre elongation per cent. They observed inadequacy of simple additive-dominance model for all these traits. Additive, dominance and non-allelic interaction gene effects were responsible for genetic determination of fibre properties. The non-fixable gene effects i.e. i, j and l were predominant over fixable effect 'd' and 'h' suggesting that complicated breeding procedures would be required for improving the traits.

Laxman and Pradeep (2003) made attempt review the gene action of yield and yield contributing characters in cotton. The review is summarized in Table 2.1 excluding the references reviewed in this chapter. They made 60 three way cross hybrids derived from crossing six diverse varieties of *G. hirsutum* cotton in triallel fashion to find out the genetics of seed cotton yield. Preponderance of dominance × dominance followed by additive × additive and additive components was observed for seed cotton yield. Duplicate gene action was observed predominantly in the control of seed cotton yield. Thus, intermating in early segregating generation followed by selection has been suggested for exploitation of seed cotton yield.

Table 2.1 Review of gene action by Laxman and Pradeep (2003)

Character	Type of gene action	Authors
Plant height	Non-additive	Rajesh *et al.* (1979)
Number of sympodia	Non-additive	Kadapa *et al.* (1989); Kowsalya (1994); Ramalingam (1996)
	Additive and non-additive	Mohuiddin (1996)
Days to 50 % flowering	Non-additive	Baker and Verhalen (1975); Holla (1986)
	Additive and Non-additive	Mohuiddin (1996)
Number of bolls per plant	Non-additive	Desai *et al.* (1988); Kalsay *et al.* (1981); Singh *et al.* (1982); Dagaonkar and Malkhandale (1993); Ramalingam (1996)
	Additive and Non-additive	Desai *et al.* (1988); Waldia *et al.* (1984); Dagaonkar and Malkhandale (1993); Mohuiddin (1996); Saxena *et al.* (1998)
	Dominance × dominance	Khan (1994); Laxman (2001)
	Dominance	Singh and Singh (1985); Murthy *et al.* (1994); Khan (1994); Rathore *et al.* (1999)
Boll weight	Additive	Deswal and Lather (1983); Singh and Singh (1985); Murthy *et al.* (1994); Krishna Rao (1998); Ajuja and Tuteja (1999)
	Non-additive	Desai *et al.* (1988); Dagaonkar and Malkhandale (1993), Waldia *et al.* (1980)
	Additive and Non-additive	Gururajarao *et al.* (1977); Tyagi (1978); Desai *et al.* (1988); Bhatade *et al.* (1992); Dagaonkar and Malkhandale (1993); Saxena *et al.* (1998)
	Dominance	Singh and Singh (1985); Laxman (2001)
Seed cotton yield/plant	Additive	Singh and Singh (1985); Green and Culp (1990); Randhawa *et al.* (1991); Sankarapadian *et al.* (1998); Gururajan and Basu (1992)

	Non-additive	Desai *et al.* (1988); Dagaonkar and Malkhandale (1993); Mohuiddin (1996); Krishna Rao (1998); Mandloi *et al.* (1998); Ajuja and Tuteja (1999)
	Additive and Non-additive	Desai *et al.* (1980); Randhava *et al.* (1991); Bhatade *et al.* (1992); Dagaonkar and Maldhandale (1993); Gupta (1993); Saxena *et al.* (1998); Rathore *et al.*(1999); Singh and Singh (2001); Rao and Reddy (2002)
	Dominance	Deshpande *et al.* (1984); Singh and Singh (1985); Murthy *et al.* (1994)
Ginning percentage	Additive	Singh and Singh (1985); Green and Culp (1990); Krishana Rao (1998); Mandloi *et al.* (1998); Sankara-pandian *et al.* (1998)
	Epistasis	Randhawa *et al.* (1991)
	Dominance × dominance	Ramalingam (1996)

Mehetre *et al.* (2003[a]) analyzed the nature and magnitude of gene action for six fibre characters in five intraspecific crosses of *Gossypium hirsutum* using six generation mean performance. The magnitude of dominance effect was higher for all characters except micronaire and uniformity ratio. Epistatic components i.e. additive × additive (i) and dominance × dominance (l) were predominant indicating duplicate type of epistatic gene action in the expression of characters like 2.5 % span length, micronaire value, bundle strength, fibre quality index, uniformity ratio and extensibility(%). Significant epistatic gene effect coupled with duplicate epistasis indicated that selection through transgressive segregants could yield superior lines in subsequent generations.

Mehetre *et al.* (2003[b]) studied the nature and magnitude of gene effects governing the inheritance of quantitative traits of inter specific cross of *G. hirsutum* × *G. barbadense* (i.e. RHC-001 × RHCb-001) by using generation mean analysis. The magnitude of dominance effect was predominant for all characters except days to first boll bursting, bundle

strength and uniformity ratio indicating the possibility of heterosis breeding for improvement of these traits. The epistatic effect i.e. dominance × dominance (l) was predominant for plant height, bolls/plant, sympodia, bundle strength and uniformity ratio while both additive × additive and dominance × dominance were important for days to 50 % flowering, days to maturity, boll weight, span length, micronaire, bundle strength fibre quality index, uniformity ratio and extensibility percentage. Most of character like days to 50 % flowering, days to first boll busting, days to maturity, plant height (cm), monopodia per plant, boll weight (g), lint index, and fibre characters *viz.,* 2.5 % span length (mm), micronaire, bundle strength, fibre quality index (3.2 mm), uniformity ratio and extensibility etc., showed significant epistatic gene effect coupled with duplicate epistasis except bolls per plant, sympodia per plant, seed index, ginning percent and seed cotton yield.

Mehetre *et al.* (2003[c]) carried out generation mean analysis of an intra-hirsutum cross (AK-32A × DHY-286–1R) which revealed preponderance of dominance and epistatic interaction in the genetic control of seed cotton yield, yield components and quality characters. They reported the involvement of duplicate type gene action in the expression of days to 50 % flowering, days to first boll busting, bolls per plant, monopodia per plant, plant height (cm), boll weight (g), seed index, lint index, 2.5 % span length, micronaire value, bundle strength, fibre quality index, ginning per cent and extensibility (%) whereas involvement of complementary type of gene action for days to maturity, seed cotton yield and sympodia per plant.

Muthuswamy *et al.* (2003) in line × tester (12 × 5) analysis of *G. hirsutum*, revealed predominance of non-additive gene action for all fibre quality characters except 2.5 percent span length.

Ramlingam (2003) in triallel analysis observed preponderance of epistasis for ginning outturn. The magnitude of dominance × dominance type of epistasis was maximum as compared to additive gene effect.

Reddy and Satyanarayanan (2003) in 10 × 10 diallel revealed that non-additive gene effect was important for seed index, maturity coefficient and bundle strength. Whereas, both additive and non- additive gene effects were equally important for lint index, ginning percentage, micronaire value and seed cotton yield. For 2.5 % span length only additive gene effect was important.

Saravanan and Gopalan (2003) in 8 × 8 diallel of intraspecific (*G. hirsutum* × *G. hirsutum*) and interspecific crosses (*G. hirsutum* × *G. barbadense*) of cotton reported that all the six characters i.e. days to first flowering, plant height, number of bolls per plant, boll weight, number of seeds per boll and seed cotton yield per plant were controlled by both additive and non-additive gene effect with predominance of additive gene action.

Singh and Chahal (2003) carried out triple test cross analysis to estimate additive dominance and epistatic components of genetic variation for seed cotton yield and its component using 34 progenies of *G. hirsutum* produced by crossing 17 genotypes with two testers. Epistasis observed for all characters except number of monopodia and boll setting percentage. Both additive and dominance components were significant for all characters studied Partial degree of dominance was observed for plant height, number of fruiting branches, boll weight and harvest index, which indicated the predominance of additive genetic component for these characters. The dominance component was more prominent for number of monopodia, number of sympodia, number of bolls, boll setting percentage, average inter-nodal length, seed cotton yield and days to maturity where degree of dominance was in over dominance range.

Verma *et al.* (2004) in line × tester analysis revealed importance of non-additive gene action for seed cotton yield and its component characters. The testers RS-2013 and LH-1556 and lines CISV-31, CISV-48, CISV-12, CISV-6 and CIT-7-2 were found to be good combiners for seed cotton yield on the basis of sca effects.

In estimation of different gene actions Singh and Chahal (2005), revealed presence of epistasis for ginning outturn, lint index, 2.5 % span length and fibre strength and additive as well as dominance component for all fibre characters along with fibre fineness. The study also revealed additive component was higher than dominant component.

Reddy and Nandarajan (2006) in 8 × 8 diallel analysis of *G. hirsutum* observed the additive gene effect governing characters *viz.,* 50 % flowering, number of bolls per plant, ginning outturn, lint index and oil content, while non-additive gene effect was found in case of plant height, number of sympodia per plant and boll weight.

In an inheritance study, Murtaza *et al.* (2006) revealed additive as well as dominant effect for number of bolls per plant, seed cotton yield and plant height in diallel cross and also suggested that these additive as well as non-additive components can be utilized by adopting bi-parental mating in early generations among the selected lines in future breeding programme for improvement of seed cotton yield.

Further, in a diallel cross study of *G. arboretum* Pradeep *et al.* (2007) found additive as well as non additive gene effects for all characters except boll weight which was governed by additive gene effect and revealed that relative magnitude of dominant component was higher in magnitude in number of monopodia, number sympodia, number of bolls per plant, lint index, seed index and seed cotton yield and additive component for halo length and ginning outturn.

Gene effects in *G. arboreum* for number of bolls per plant, boll weight seed cotton yield, ginning outturn, seed index, lint index and halo length were studied by Pradeep and Sumalini (2008). They revealed additive gene effects for ginning outturn and lint index and dominant gene effects for boll weight in different crosses. Further the study also indicated additive × additive gene interaction for boll weight while, additive × additive and dominance × dominance effects for halo length and ginning outturn with duplicate type interactions and suggested biparental mating in

early segregating generation followed by recurrent selection for further improvement.

According to Akhtar *et al.* (2008) additive gene action was important for all fibre characters and also reported adequacy of additive dominance model in inheritance of staple length, fibre strength and fibre fineness.

Further, Abbas *et al.* (2008) also reported additive gene action with partial dominance for monopodial branches, sympodial branches, boll weight, seed cotton yield, lint %, fibre length, fibre strength and fibre fineness. Minhas *et al.* (2008) also reported similar results for fibre length, fibre strength and fibre fineness and over-dominance for uniformity ratio.

Ali *et al.* (2010) revealed adequacy of additive dominance model data for boll weight, seed cotton yield, lint percentage, fibre fineness and fibre strength.

Haleem *et al.* (2010) estimated gene effects using six generations in different crosses among seven Egyptian cotton varieties. They revealed that the additive-dominance model was adequate to demonstrate the genetic variation and it important in the inheritance of most studied traits. Non-allelic gene interaction was operating in the control of genetic variation in most studied traits. The epiststic effects, additive × additive (i) and dominance × dominance (h) were highly significant in most cases. The signs of (h) and (L) were opposite in all studied traits for most crosses. Also, the inheritance of all studied traits was controlled by additive and non-additive genetic effects, but dominance gene effects play the major role in controlling the genetic variation of the most studied traits. Significant negative heterosis relative to mid-parents was found for both characters, first fruiting branch per plant and days to 50 % maturity in all crosses while, significant negative heterosis above the better parent was found for first fruiting in both crosses no. 1 and 4, days to 50 % maturity in cross no. 4. Inbreeding depression estimates were found to negative and highly significant for days to 50 % flowering, first fruiting per plant and days to 50 % maturity.

Gawande (2011) reported additive, non-additive and higher order interactions for seed cotton yield, lint yield, seed weight per plant, bolls per plant, boll weight, number of monopodia, number sympodia, 100 seed weight and short fibre per cent while preponderance of dominant gene effect and dominant × dominant interaction for plant height in Phule-388 and 2.5% span length, fibre strength, ginning outturn and oil content in NHH-44 implying non-fixable gene effects can be improved through recurrent selection and also reported sole control of dominance on fibre elongation and seeds per boll in NHH-44 rewarding heterosis breeding for improvement.

2.5 Qualitative characters

Fyson (1908) concluded dominance of narrow lobe in the crosses *neglectum* (narrow) × *herbaceum* (broad). In the F_2 of two crosses (*arboreum* variety Nanking × *arboreum*) between narrow and broad lobe types Leake (1911^a and 1911^b) revealed 1:2:1 ratio.

Kottur (1923) explained modified trihybrid ratio 39:9:16 for inheritance of petal colour in the crosses full yellow of *herbaceum* and white of *arboreum*. Hutchinson (1931) established multiple allelomorph series for petal colour. Silow (1941) and Bholanath (1942) demonstrated complimentary factors for petal colour in Chinese cotton.

An extensive genetic survey of new world cottons by Stephens (1945) has shown that leaf shape is controlled by a single multiple allelomorph series having a minimum of four members, L_S, L_O, L_E and l.

Basu and Bhat (1984) studied inheritance of seven qualitative characters in cotton (*Gossypium hirsutum* L.) and concluded that colour corolla were controlled by two and one pair of genes respectively. Red pigmentation is incompletely dominant in case of corolla colour. Further, they revealed incomplete dominance for leaf shape and duplicate recessive genes governed nectariless character.

Endrizzi *et al.* (1984) concluded that leaf shape and hairiness in cotton are monogenically controlled. The gene for profuse hairiness (Pilose) and narrow okra leaf is controlled by H_2 and L_0, respectively

Mane *et al.* (1987) studied the inheritance of five qualitative characters in *G. arboreum.* They confirmed that anthocynin pigmentation governed by R_1, R_2 and R_3 (54:10) genes, petal spot R_1S (15:1) genes, petal colour (1:2:1) Ya gene, leaf nectory by monogenic (Ne) gene and bract shape by (15:1) R_1 Fg genes.

The expressivity of the two genes (H_2 and L_0) for pilose hairing and okra leaf type were studied by Rahman and Khan (1998) in F_1 and F_2 generations in different genetic backgrounds. The F_2 generation segregated into four classes of hairiness and four classes of leaf shape and fitted into the theoretical 1:2:1 ratio of partial dominance. The phenotypic expression in heterozygous condition was affected by the genetic background, i.e., modifying gene effects.

Nawab *et al.* (2011) observed incomplete dominance showing ratio 1:2:1 for leaf shape in F_2 population. F_2 populations of the three crosses segregated in 1 okra leaf (L_0L_0) : 2 an intermediate class of sub-okra (L_0l_0) : 1 normal leaf (l_0l_0).

3. MATERIAL AND METHODS

The present investigation entitled, "Genetic analysis of wilt resistance in intra specific crosses of Asiatic cotton (*G. arboreum* L.)" was conducted at Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri and State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri during 2009 to 2012.

3.1 Material

The studies involved P_1, P_2, F_1, F_2, BC_1 and BC_2 generations of intra specific crosses between resistant and susceptible varieties of *G. arboreum* L. cotton. Following genotypes were used as parents for hybridization programme of present investigation.

	Genotypes	**Special features**
1.	AKA -7	Moderately Resistant to *Fusarium* wilt
2.	PA-141	Tolerant to *Fusarium* wilt
3.	Dh-2	Susceptible to *Fusarium* wilt

The above material was procured from Agricultural Research Station, Jalgaon.

3.2 Methods

3.2.1 Testing of parental material

Soil was made sick as per the method given in the Pune technique (Uppal, 1938) with *Fusarium oxysporum* f. sp. *vasinfectum* Atk. The fungus was isolated from the roots of wilt infected cotton plants. A pilot test was undertaken to observe the virulence of *Fusarium* inoculum added in the soil. Seed of susceptible cultivar was sown in wilt sick soil in pots under glass house condition. The spore load of the *Fusarium* in soil in pots was observed when the plants showed symptoms of wilt. The parental material was tested in this sick soil under green house condition.

3.2.2 Development of breeding material

The resistant seedlings of the parents AKA-7 and PA-141 were transplanted in wilt sick soil and the susceptible parent Dh-2 was transplanted in normal soil in the field. The crosses were effected during *kharif* 2010 to develop F_1s. Hybridization was undertaken as below.

AKA -7(MR) × Dh-2(S) and

PA-141(Tolerant) × Dh-2(S)

Selfing of resistant plants of parents was done simultaneously.

The F_1s and their parents were sown in crossing block for development of backcrosses and obtaining F_2 seeds during summer 2011. The F_1s was back crossed to both the parents of respective crosses to obtain BC_1 and BC_2 seeds. F_1s were selfed simultaneously to obtain F_2 seeds. Part of F_1 seeds was kept for evaluation study. Hand emasculation method was used for development of F_1s and back crosses. Hand emasculation of mother plants was done at evening hours and these emasculated buds were pollinated with pollens of male parent in the next day morning. Pollination was carried out during morning hours because the stigma remains more receptive during this time which results into good seed set. The pollinated buds were covered with straw tube in order to avoid their contact with stray pollens in the vicinity. The crossed buds were then labeled with price lables for easy identification. To obtain the F_2 seeds, tips of the unopened buds of F_1 plants were tied with white thread to avoid opening of bud and to ensure self pollination.

3.2.3 Evaluation of breeding material

3.2.3.1 Screening of F_2 generation under green house for inheritance of resistance to *Fusarium* wilt and molecular analysis

F_2 generation was sown in wilt sick soil filled in potrays along with parents in green house conditions on 8th October, 2011, at Cotton Improvement Project, M.P.K.V., Rahuri. The disease reaction was observed for the seedling resistance. The F_2 population of the cross AKA-7 × Dh-2 was also used for finding molecular marker associated with wilt resistance in Asiatic cotton.

3.2.3.2 Evaluation of breeding material for study of inheritance of various parameters of yield

Randomized Block Design with three replications was adopted for sowing of breeding material. Each replication contains 12 treatments. A single row of non segregating generations, 3 rows of each back crosses and 8 rows of F_2 were grown in each replication. Sowing was done in the rows of 4.50 meter length, with plant to plant and row × row spacing of 22.5 cm × 45 cm, accommodating twenty plants per row. Randomized Block Design with three replications was adopted for sowing of these materials. Each replication consisted of two tiers, each tier encompassing the 17 rows, randomly allocated to P_1, P_2, F_1, F_2, B_1 and B_2 generations of either cross. Thus each replication included 34 rows in total.

3.2.3.3 Cultural Practices

Fertilizer doses and plant protection measures were followed as per the recommended schedule. The fertilizer dose of 80:40:40 kg NPK/ha was given in two splits. The 40 kg N, 40 kg P and 40 kg K was provided at sowing time. While 40kg nitrogen given 30 days after sowing The NPK were provided in the form of urea, single super phosphate and murate of potash, respectively. For control of sucking pest spraying was done with emidaclopride 200 SL @ 3ml/10 lit., whereas bollworm complex was kept under control with the spray of endosulfan 35 EC @17 ml/10lit. The operations like weeding and hoeing were done regularly as per need and stage of crop growth. Only a healthy plant per hill was maintained for recording the observations.

Table 3.1 Parental details of different generations developed.

Generation	Cross-1 (AKA-7 × Dh-2)	Cross-2 (PA-141 × Dh-2)
P_1	AKA-7	PA-141
P_2	Dh-2	Dh-2
F_1	(AKA-7 × Dh-2)	(PA-141 × Dh-2)
F_2	Selfed F_1	Selfed F_1
B_1	(AKA-7 × Dh-2) × AKA-7	(PA-141 × Dh-2) × PA-141
B_2	(AKA-7 × Dh-2) × Dh-2	(PA-141 × Dh-2) × Dh-2

3.3. Observations recorded

Following observations were recorded on single plant basis for different morphological characters and fibre quality parameters.

3.3.1 Screening of breeding material against *Fusarium* wilt under green house

100 seeds of P_1, P_2 and 200 seeds of F_2 were sown in potrays in wilt sick soil. The number of plants wilted per day were recorded after germination.

3.3.2 Quantitative characters

Randomly selected competent five plants in P_1, P_2 and F_1 generations, 10 plants in B_1 and B_2 generations and 20 plants in F_2 generation were selected for recording observations in each cross in each replication. Data on following characters were recorded on single plant basis for different quantitative characters.

3.3.2.1 Plant height (cm)

The plant height was measured from ground level to the tip of the last fully opened leaf of main stem at maturity in centimeter.

3.3.2.2 Number of monopodia per plant

Number of vegetative branches on which secondary fruiting branches grown were counted on each plant.

3.3.2.3 Number of sympodia per plant

Numbers of fruiting branches on each plant were counted.

3.3.2.4 Days to 50 % flowering

Days required to 50 per cent plant to flower with at least one flower open were counted.

3.3.2.5 Days to first boll bursting

Days required to first boll bursting were counted.

3.3.2.6 Days to maturity

Number of days after sowing upto last picking was counted.

3.3.2.7 Number of bolls per plant

It is the sum total of matured bolls actually picked in four pickings from the single plant.

3.3.2.8 Average boll weight (g)

The weight of seed cotton of five fully opened bolls of single plant at first picking was taken in grams and the average was calculated.

3.3.2.9 100 seed weight (g)

This is the weight in grams of hundred matured healthy seeds.

3.3.2.10 Lint weight per plant (g)

After ginning of the total matured bolls of single plant, the lint obtained was weighed and lint weight per plant was calculated.

3.3.2.11 Ginning percentage (%)

This is the proportion of lint to seed cotton expressed in the percentage. For which the ginning of the five bolls of single plant was done separately and ginning percentage was calculated by using following formula.

$$\text{Ginning percentage} = \frac{\text{Lint weight}}{\text{Weight of seed cotton}} \times 100$$

3.3.2.12 Seed cotton yield per plant (g)

It is the total seed cotton picked per plant in four pickings recorded in grams.

3.3.3 Fibre quality characters

The selected plants from P_1, P_2, F_1, F_2, B_1 and B_2 generation were subjected for detailed investigation on fibre properties based on the availability of lint samples.

Following characters were analyzed at Central Institute for Research on Cotton Technology (CIRCOT), Matunga, Mumbai.

3.3.3.1 2.5 % span length (mm)

It is defined as the distance spanned by specified percentage of fibre in specimen being tested expressed in millimeters. 2.5 % span length is determined by photoelectric instrument called "Fibrograph".

3.3.3.2 Fibre strength (g/tex)

Fibre strength was measured by "Stelometer". It is the force required to break the fibre of unit linear density.

3.3.3.3 Micronaire (μg/inch)

It is average weight per unit length of fibre. It is used to determine the fibre fineness and expressed in microgram per inch.

3.3.3.4 Uniformity ratio (%)

It is the ratio of 50 per cent span length to 2.5 per cent span length. It indicates the uniformity of fibre length.

3.3.4 Qualitative characters

Following observations were recorded on single plant basis for different qualitative characters on F_2 population.

3.3.4.1 Leaf shape

Leaf shape was observed visually of 5 plants in non segregating populations and in F_2 population. It was recorded as palmate (normal), semi digitate (semi okra), digitate (okra) and lanceolate (super okra).

3.3.4.2 Leaf nectory

Leaf nectories were visually observed on 5 plants in non segregating populations and all plants in F_2 population at peak flowering stage and recorded as present or absent.

3.3.4.3 Flower colour

Flower colour was visually observed on 5 plants in non segregating populations and all plants in F_2 population at peak flowering stage and recorded as white/cream/yellow/pink/red/bicolour.

3.3.4.4 Petal spot

Petal spot was visually observed on 5 plants in non segregating populations and all plants in F_2 population at peak flowering stage and recorded as present or absent.

3.3.4.5 Anther colour

Anther colour was visually observed on 5 plants in non segregating populations and all plants in F_2 population at peak flowering stage and recorded as white/cream/yellow/purple.

3.3.4.6 Stigma exertion

Stigma exertion was visually observed on 5 plants in non segregating populations and all plants in F_2 population at peak flowering stage and recorded as embedded or exerted.

3.4 Molecular analysis

The methodology followed for the different DNA marker analysis is described below.

3.4.1 Chemicals used for Isolation of genomic DNA

The genomic DNA from cotton leaves of selected plants which were screened against *fusarium* wilt from P_1, P_2, F_2 generations was isolated as per miniprep method given by Li *et al.* (2001).

Following chemicals were used for isolation and purification of genomic DNA.

DNA extraction buffer (EB): (Fulton *et al.*, 1995)

100 µl M Tris-base

5 µl M EDTA

Sodium bisulfite, 0.4 g/100 ml buffer (Before using)

0.35 M sorbitol

Lysis buffer (LB): (Fulton *et al.*, 1995; Murray and Thompson, 1980).

0.2 M Tris-base (pH 8.0),

50 µl M EDTA (pH 8.0)

2 M NaCl

55µl M CTAB

Five percent Sarkosyl (N-Lauroylsarcosine, sodium salt).

TE buffer

Tris (10 mM)

EDTA (0.1 mM)

Other chemicals

3 M Na-COO-CH3 (Sodium acetate)

100 % Isopropanol

70 % Ethanol

100 % Ethanol

Chloroform : Isoamyl alcohol (24:1)

Phenol: Chloroform (1:1)

RNAase (10 mg/ml)

Liquid nitrogen

3.4.2 Protocol for DNA extraction

1. Leaf tissue preparation

1. Harvest about 0.05-0.1 g young leaves (2.0-2.5 cm in diameter).
2. Fold leaves and place in a 1.5 ml microcentrifuge tube.

3. Keep tissue samples on ice.
4. Freeze tissue with liquefied N_2 and grind to a fine powder in a 1.5 ml micro-centrifuge tube with a pellet pestle.

2. DNA extraction

1. Add 600 µl extraction buffer (EB) and vortex 40-60 s until thoroughly mixed.
2. Centrifuge 6500-7000 *g*-force for 15 min at room temperature.
3. Discard supernatant.
4. Add 250 µl EB to pellet and vortex 40-60 s.
5. Add 5 µl RNase (pancreatic RNAse A) 10 mg/ml (optional).
6. Add 600 µl lysis buffer (LB) and 60 µl L 5 % Sarkosyl. Invert tube 20-40 times until thoroughly mixed.
7. Incubate at 65°C for 15 min.
8. Add 500 µl of chloroform:isoamyl alcohol (CIA, 24:1, v/v) and vortex samples 40-60 s to mix contents.
9. Centrifuge 6500-7000 *g*-force for 5-10 min at room temperature.
10. Pipette aqueous supernatant into a new clean 1.5 ml microcentrifuge tube.
11. Repeat CIA extraction.

3. DNA precipitation

1. Add 1 volume of cold isopropanol (-20^{0}C).
2. Gently mix by tube inversion until DNA precipitates.
3. Centrifuge 6500-7000 *g*-force for 10-15 min.
4. Discard the supernatant.
5. Wash DNA pellet with 70 % ethanol.
6. Dry the pellet 30 min and dissolve DNA into 30-60 µl L H_2O or TE buffer.

3.4.3 DNA purification

1. Equal volume of phenol : chloroform (1:1) added after DNA pellet dissolved in TE.
2. Mixture was spinned 11000 rpm for 10 minutes at 4^{0}C.
3. Supernant was taken out to which 0.1 volume of 3 M sodium

acetate + 0.6 volume of 100 % ethanol was added.

4. For precipitation DNA was kept in deep freeze for 1-2 hours.
5. After deep freezing solution was centrifuged 12000 rpm for 10 minutes at 4^0C.
6. DNA pallet was saved by decanting the supernant.
7. The wash of 70% ethanol (200 µl) was given to purify DNA at 3000 rpm for 3 minutes.
8. DNA was air dried until ethanol smell vanishes off after which DNA was dissolved in TE buffer (10mM Tris, 1mM EDTA).

3.4.4 Determination of quality of genomic DNA

Confirmation of DNA in the sample was carried out on 0.8 % agarose gel containing ethidium bromide @ 0.5 µl /10 ml. 5 µl of genomic DNA of each sample along with 2 µl standard lambda DNA (50 ng/ µl) mixed with 6X tracking dye ($1/6^{th}$ volume) was loaded in agarose well and subjected to electrophoresis at 80 V. After completion of $2/3^{rd}$ run, gel was observed under UV rays in gel documentation system. Thus DNA yield and quality was confirmed by comparison with standard lamda DNA.

3.4.5 Determination of quantity of genomic DNA

DNA quantification was carried out on spectrophotometer instrument called 'Nanodrop'. At first 1µl distilled water was feed to Nanodrop as initialization of the instrument followed by 1µl TE as blank respectively. Then 1µl diluted DNA was measured at 260nm as well as 280nm wavelength. The absorbance of 260/280 ratio was recorded. A pure sample of DNA shows the ratio of 260/280 as 1.8. The ratio less than 1.8 indicate the contamination in the preparation either with proteins or phenol. While, the value higher than 1.8 indicates the presence of RNA in the preparation.

3.4.6 Polymerase chain reaction

3.4.6.1 Chemicals used for PCR:

Various components required for carrying out the PCR reaction are listed as below-

Template DNA

The purified genomic DNA extracts of the parents and the F_2 progenies were used as template DNA.

Primers

PCR amplification was performed with Inter simple Sequence Repeats (ISSR), Microsatellites (SSR) and Resistant Gene Analogues (RGA) primers that are available in State Level Biotechnology Laboratory, MPKV, Rahuri. These custom synthesized primers were procured from M/S Bangalore Genei Pvt. Ltd. A total of 45 ISSR, SSR and RGA primers were used for amplification. The SSR primers (Table 3.6) were of different series like BNL (Brookhaven National Laboratory, NY, USA), CIR (Centre International en Recherche Agronomique Pour le Development, Cedex, France), JESPR (after the names of principle investigators from Texas A & M University, College Station, TX, USA). The commercial design random ISSR primers were procured from M/S Bangalore Genei Pvt. Ltd (Table 3.3 and 3.4). The details of RGA primers presented in Table 3.8. The working concentrations of random primers (5 pM per µl) and specific (2 pM per µl) primers were prepared using the autoclaved sterile distilled water.

Taq DNA polymerase

Taq DNA polymerase (3 units per µl) was obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

dNTP mix

10mM dNTP mix was obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

Taq Buffer A:

10X Taq buffer A (Tris pH 9.0, KCL, 15mM $MgCl_2$, gelatin) was obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

Water:

Double distilled sterilized RNAse free water was used to make the final volume 20ml.

Table 3.2 PCR reaction mixture used for DNA amplification using ISSR Primers.

Sr. No.	Components	Stock concentration	Volume used per tube (20µl)
1	Template DNA	30 ng/µl	1.00
2	Primer	1 µM	1.00
3	Taq DNA buffer A	10 X	2.00
4	Taq DNA polymerase	3 U/µl	0.30
5	dNTPs mix	2.5 mM each	2.00
8	Sterile distilled water	-	13.7
Total			20.00

DNA amplification:

ISSR analysis of genomic DNA was carried out by PCR reaction in a palm cycler using 17 ISSR primers.

Table 3.3 ISSR primers and used anneal temp

Sr. No.	Primers	Used anneal Temp (°C)
1.	ISSR-8336	46
2.	ISSR-834	52
3.	ISSR-841	48
4.	ISSR-827	46
5.	ISSR-857	52
6.	ISSR-8036	46
7.	IS-8	47
8.	IS-12	55
9	IS-13	45
10.	ISSR-8081	46
11.	ISSR-803	48.3
12.	ISSR-804	50

Table 3.4 Base pair sequences of Nagpur ISSR primers used

Sr No.	Primer	Primer Sequence (5'-3')	Annealing Temp °C	Used Annealing Temp °C
1	IS-2	AGCAGCAGCAGCGG	55.4	55
2	ISN-801	AGCAGCAGCAGCAT	51.1	51
3	IS-6	CACACACACACAAC	43.2	43
4	IS-11	GTGTGTGTGTGTTA	40.9	50
5	ISN-814	CAGGAGAGAGAGAGA	44.2	44

Table 3.5 PCR reaction mixture used for DNA amplification using SSR Primers.

Sr. No.	Components	Stock concentration	Volume used per tube(µl)
1	Template DNA	30 ng/µl	1.00
2	Primer (Forward)	1 µM	1.00
3	Primer (Reverse)	1 µM	1.00
4	Taq DNA buffer A	10 X	2.00
5	Taq DNA polymerase	3 U/µl	0.30
6	dNTPs mix	2.5 mM each	2.00
7	Sterile distilled water	-	12.70
Total			**20.00**

DNA amplification:

SSR analysis of genomic DNA was carried out by PCR reaction in a palm cycler using 12 SSR primers.

Table 3.6 Base pair sequences of SSR primers used

Sr. No.	Primer		Sequence of Primers (5' to 3')	Chromosome No.	T_{ann} (^{0} C)	Used T_{ann} (^{0} C)
1.	BNL 1047	Forward Primer	GCTTGTCATCTCCATTGCTG	qMVChr22	58	53.8
		Reverse primer	TAGCCCGGTTCATGTTCTTC		58	
2.	BNL 1672	Forward Primer	TGGATTTGTCCCTCTGTGTG	qLYChr23, qFL3Chr09	58	53.8
		Reverse primer	AACCAACTTTTCCAACACCG		56	
3.	BNL 3435	Forward Primer	CGTGGATTTAAGCACCGATT	qLYChr26	56	53.8
		Reverse primer	TAAGAAATGGTGTTGCAATTACC		58	
4.	BNL 3580	Forward Primer	CTTGTTTACATTCCCTTCTTTATACC	qFL4Chr01	62	53.8
		Reverse primer	CAAAGGCGAACTCTTCCAAA		56	
5.	BNL 3627	Forward Primer	TATGGGCCTGTCCACCTAAG	qMV7, qMVA02, qFS2A02b	60	53.8
		Reverse primer	CAAAGCAACATGCACACACA		56	
6.	JESPR 230	Forward Primer	GGGACTAAAGAAGTAATTATGCC	qFSChr09	59	53.8
		Reverse primer	GAAACCCTTGGCCATGAG		56	
7.	BNL 1059	Forward Primer	CCTTCTCTGACACTCTGCCC	qMVChr14a+14b, qFL1Chr14	63	60
		Reverse primer	TGTATTCTCTTCTTTTCCTTATACTTTT		60	
8.	BNL 3147	Forward Primer	ATGGCTCTCTCTGAGCGTGT	qFL3Chr09	60	58.1
		Reverse primer	CGGTTCAGAGGCTTTGTTGT		58	

Table 3.6 Contd..

9.	JESPR 134	Forward Primer	GTCAGAGTCTTCGGGTTGTC	qLYChr05a	60	58.0
		Reverse primer	GTAACAGCAGAGAAGTCGGTG		61	
10.	BNL 3510	Forward Primer	GCACCAGTGCTCAGACACACA	qLYChr26	63	56.3
		Reverse primer	ATNTGAGTTGAAATCTGCCGTAA		58	
11.	BNL 3867	Forward Primer	TAATTGAGTTGTTTTCTTACTTGCC	qLYChr26	59	56.3
		Reverse primer	TGCCAATTTAGCAATCACCA		54	
12.	JESPR 151	Forward Primer	CTGGACTAAAAACCTTAACTGG	qFSChr23	58	56.3
		Reverse primer	CTCGATTCTAACTCAATCACG		57	
13.	CIR 089	Forward Primer	CTCCATTCCTCGTTTG	C1-LG/Chr, qFL4Chr01	48	49.0
		Reverse primer	AGATTTCGTTTCCCATT		45	
14.	CIR 413	Forward Primer	TTAAAGCTCACACACACA	qMVD03, D03-LG/Chr	49	49.0
		Reverse primer	CAACAGTAACGAAGAACAAT		52	
15.	CIR 244	Forward Primer	TGGAAGGTGATGTTCTAA	A02, qFS1A02b	49	56.3
		Reverse primer	GATCAAAGAGCAAACTAATC		52	
16.	CIR 354	Forward Primer	CACAATCCTCAGCCA	A02 03,qFLA02, new qFSA02A	46	56.3
		Reverse primer	AGAGAAGGAAAGAGGAAA		49	
17.	BNL 3090	Forward Primer	GAAATCATTGGAAGAACATATACTACA	qFL4Chr01	61	58.1
		Reverse primer	TTGCTCCGTATTTTCCAGCT		56	
18.	JESPR 289	Forward Primer	CATTGCATTTTGCCCC	qFL4Chr01	48	
		Reverse primer	AATCTAGCGCACAAGGGC		56	

Table 3.7 PCR reaction mixture used for DNA amplification using RGA Primers.

Sr. No.	Components	Stock concentration	Volume used per tube(20µl)
1	Template DNA	30 ng/µl	1.00
2	Primer (Forward)	1 µ M	1.00
3	Primer (Reversed)	1 µM	1.00
4	Taq DNA buffer A	10 X	2.00
5	Taq DNA polymerase	3 U/µl	0.30
6	dNTPs mix	2.5 mM each	2.00
7	Sterile distilled water	-	12.7
Total			**20.00**

DNA amplification:

RGA analysis of genomic DNA was carried out by PCR reaction in a palm cycler using 10 RGA primers.

3.4.6.2 PCR amplification programme

The PCR amplification for different molecular primer analysis as performed according to Williams *et al.* (1990) with certain modifications. The PCR amplification programme is described below:

Table 3.9 PCR amplification programme

Sr. no.	Steps	Temperature (°C)	Duration (min.)	Cycles
1	Denaturation	95	5	1
2	Denaturation	94	1	40
3	Annealing	As per primer	1	
4	Extension	72	1	
5	Final extension	72	10	1
6	Hold	04	Until removed	

Table 3.8 Primer sequences used for the amplification cotton resistant gene analogs (RGA) Liu Er-ming *et al.* (2005)

Sr. No.	Primer ID	Motif	Motif sequence	Primer sequence (5' to 3')	Used T_{ann} (^{0}C)
RGA1	NLRR-inv1	The LRR region of the tobacco *N* gene		TGCTACGTTCTCCGGG	40
	NLRR-inv2			TCAGGCCGTGAAAAATAT	
RGA2	NLRR-fwd	The LRR region of the tobacco *N* gene		TAGGGCCTCTTGCATCGT	51.1
	NLRR-rev			TATAAAAAGTGCCGGACT	
RGA3	XLRR-for	The LRR region of *Xa21* in rice		TCCGTTGGACAGGAAGGAG	40
	XLRR-rev			TCCCATAGACCGGACTGT T	
RGA4	XLRR-inv1	The LRR region of *Xa21* in rice		TTGTCAGGCCAGATACCC	51.1
	XLRR-inv2			GAGGAAGGACAGGTTGCC	
RAG5	Pto-kin1	The kinase domain of gene *Pto* in tomato		GCATTGGAACAAGGTGAA	40.5
	Pto-kin2			AGGGGGACCACCACGTAG	
RGA6	Pto-kin3	The kinase domain of gene *Pto* in tomato		TAGTTCGGACGTTTACAT	40.5
	Pto-kin4			AGTGTCTTGTAGGGTATC	
RGA7	PLTR-fwd	P-loop GLPE	GMGGVG KTT	GGNATGGGNGTNGGNAAR ACNACN	42.7
	PLTR-rev		GLPLALK VLG	NCANCARAANGGNTGNGG NGGGAANGG	
RGA8	PNTR-fwd	P-loop R-NSB-D	GGVGKTT	GGNGGNGTNGGNAANACN AC	42.7
	PNTR-rev		CFLYCAL FP	CGRAANARNSHRCARTANV NRAARC	
RGA9	PCRE-fwd	Kinase-2 EGF	LILDDVW	TGATACTGGATGATGTCTG G	51.1
	PCRE-rev		EGFIRNT	GTGCTTCTTATGAACCCTT C	
RGA10	PCf-fwd	LRR LRR	SNKLHGPI	WSNAAYAARYTNCAYGGNC CNAT	42.7
	PCf-rev		GEIPQQLA	GCNARYTGTCKNGGNATYT CNCC	

Degeneracy code: N-A,G,C or T, R-A or G, H-A, C or T, S=C or G, V= A,C or G, W= A or T, Y= C or T, K= G or T

3.4.6.3 Gel electrophoresis

Separation of amplified DNA product was done by agarose gel electrophoresis on 1.2 % agarose gel for ISSR primers and 2 % agarose gel for SSR and RGA primers. The gel was stained with ethidium bromide to detect the amplified bands and visualized under UV trans-illuminator. The gel was then photographed on the gel documentation unit with Alpha Erase FC Programme.

❖ **Requirements for gel electrophoresis**

i) Electrophoretic unit
ii) Gel documentation unit
iii) UV transilluminator
iv) Ethidium bromide(10 mg/ml)
v) Loading dye1 percent (w/v) bromophenol blue + 20 percent (w/v) Ficoll + 10 mM EDTA
vi) Agarose
vii) 10 X TBE (1 M Tris base, 830 mM boric acid,10mM EDTA, pH 8.0)
viii) 1X TBE (The 10ml of 10 X buffer was diluted to 100ml distilled water.
ix) PCR product
x) 100bp step up ladder

3.4.7 Bulked Segregant Analysis

Bulked segregant analysis (Michelmore *et al.*, 1991) involves screening for differences between two pooled DNA samples derived from a segregating population that originated from a single cross. Each pool, or bulk, contains individuals selected to have identical genotypes for a particular genomic region ("target locus or region") but random genotypes at loci unlinked to the selected region. Therefore, the two resultant bulked DNA samples differ genetically only in the selected region and are seemingly heterozygous and monomorphic for all other regions. In this study, the F_2 population of the cross AKA-7 × Dh-2 was used for Bulk segregant analysis.

1. F_2 population was screened against *Fusarium* wilt in wilt sick soil.
2. After germination, daily the disease reaction was scored visually.

3. Leaf samples were taken from individual plant.
4. The plants showing highly resistant reaction were identified to make one group.
5. The plants showing highly susceptible reaction were identified to make another group.
6. The DNA was extracted from these individual leaf sample as per method described in this chapter 3.4.1.
7. The quality and quantity of these DNA samples was checked by using gel electrophoresis method on 0.8 % agarose gel as well as on nanodrop machine.
8. The individual DNA samples from highly resistant group were mixed together in such a way that equal quantity of DNA from each sample would come in the DNA mixture. This formed the resistant bulk.
9. Similarly, the individual DNA samples from highly susceptible group were mixed together in such a way that equal quantity of DNA from each sample would come in the DNA mixture. This formed the susceptible bulk.
10. Resistant and susceptible bulks were diluted /vacuum concentrated to a volume so that the bulks contained equimolar concentration of 30ng/µl DNA.
11. These bulks were used for polymorphism study of molecular markers.
12. The different amplified markers were scored as present (+) and absent (-) for each sample.

3.4.8 Sequencing and homology search

Custom sequences of PCR purified product of 300bp (appro.) fragment eluted in PCR were obtained from m/s Banglore Genei Ltd. and analyzed. Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequences at the 3' and 5' ends using the sequence analysis tools (ChromasLite2.01 software).

3.5 Root anatomy

Both the Susceptible- Dh-2 and Resistant- AKA-7 varieties were sown in previously prepared wilt sick soil. Fine transverse sections of roots of 8-10 days old seedlings were taken with the help of simple razor blade and stained with differential stain. Observations were recorded when the susceptible cotton strain Dh-2 showed first wilting symptom. Different parts of root viz; conduction region, root hair region and elongation region were observed under microscope.

3.6 Statistical analysis

The individual plant data was used for statistical and molecular analysis. These procedures have been outlined under different headings.

3.6.1 Genetic study of disease resistance

Pearson (1900) χ^2 test was used to describe the magnitude of the discrepancy between theory and observation. It is calculated with following formula.

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

Where, O refers to observed frequencies and e refers to the expected frequencies. Significance of χ^2 was tested at (n- 1) degrees of freedom.

3.6.2 Generation mean analysis

The data collected for various characters over single plant from different generations was used for statistical analysis.

3.6.2.1 Scaling tests

Adequacy of additive and dominance effect was detected by scaling tests *viz.*, A, B, and C to reveal the presence or absence of intergenic interaction by using formulae given by Mather (1949).

A = 2 B_1 – P_1 – F_1

B = 2 B_2 – P_2 – F_1

C = 4 F_2 –2 F_1 – P_1 – P_2

Where,

P_1 = Mean of P_1

P_2 = Mean of P_2

F_1 = Mean of F_1

F_2 = Mean of F_2

B_1 = Mean of B_1

B_2 = Mean of B_2

Variances of these tests were calculated to test the significance these test as

$VA = 4\ V(B_1) + V(P_1) + V(F_1)$

$VB = 4\ V(B_2) + V(P_2) + V(F_1)$

$VC = 16\ V(F_2) + 4\ V(F_1) + V(P_1) + V(P_2)$

Where,

VA = Variance of A; VB = Variance of B;

VC = Variance of C; VP_1 = Variance of P_1;

VP_2 = Variance of P_2; VF_1 = Variance of F_1;

VF_2 =Variance of F_2; VB_1 = Variance of B_1

VB_2 = Variance of B_2.

Square root of these variances provided respective standard errors which were used to test their significance. The calculated values of 't' compared with 1.96 table value of 't' at 5 per cent level of significance. If the calculated value of these scales is higher than 1.96; then it is considered significant and *vice versa*. The type of epistasis is revealed by the significance of specific scale as given below,

1) The significance of A and B scales indicates the presence of all the three type of non-allelic gene interactions *viz.,* additive × additive (i), additive × dominance (j) and dominance × dominance (l)
2) The significance of 'C' scale suggests dominance × dominance (l) type of non-allelic gene interaction.

3) The significance of 'D' scale reveals additive × additive type of gene interaction

4) Significance of both 'C' and 'D' scales indicates additive × additive (i) and dominance × dominance type of gene interactions

Further, joint scaling test suggested by Cavalli (1952) was also employed for the detection of interallelic interactions.

3.6.2.2 Estimation of gene effects

To provide information on the nature of gene action governing the traits under study, all six generation means were calculated following the method by Hayman (1958). The notations for various gene effects used in this study were those adopted by Hayman (1958) such as:

Gene effect	**Notations**
i) Mean	m
ii) Additive gene effect	d
iii) Dominance gene effect	h
iv) Additive × Additive interaction effect	i
v) Additive × Dominance interaction effect	j
vi) Dominance × Dominance interaction effect	l

The estimates of m, d, h, i, j and l were worked out by using the following equations of Hayman (1958).

$$m = F_2$$

$$d = B_1 - B_2$$

$$h = F_1 - 4F_2 - \tfrac{1}{2}P_1 - \tfrac{1}{2}P_2 + 2B_1 + 2B_2$$

$$i = 2B_1 + 2B_2 - 4F_2$$

$$j = B_1 - \tfrac{1}{2}P_1 - B_2 + \tfrac{1}{2}P_2$$

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

The variances of these estimates of gene effects were obtained as;

$Vm = V(F_2)$

$Vd = V(B_1) + V(B_2)$

$Vh = V(F_1) + 16\,V(F_2) + \frac{1}{4}\,V(P_1) + \frac{1}{4}\,V(P_2) + 4\,V(B_1) + 4\,V(B_2)$

$Vi = 4\,V(B_1) + 4(B_2) + 16\,V(F_2)$

$Vj = V(B_1) + \frac{1}{4}\,V(P_1) + V(B_2) + \frac{1}{4}\,V(P_2)$

$Vl = V(P_1) + V(P_2) + 4\,V(F_1) + 16\,V(F_2) + 16\,V(B_1) + 16\,V(B_2)$

The square roots of these variances provided standard error of respective parameter for testing the significance. The significance of the parameters estimated by comparing values of calculated 't' for each parameter with that value given in 't' table. The 't' values for m, d, h, i, j and l were calculated by dividing estimated value of each parameters by their respective standard error. Significance of the gene effects were tested by comparing the calculated 't' values with table 't' i.e. 1.96 and 2.58 at 5 % and 1 % level of significance respectively.

2. Standard deviation of sample

$$S = \frac{\Sigma (X_i - X)^2}{n-1}$$

3. Standard error of mean

$$SE(X) = \frac{S}{n}$$

4. Variance (S^2)

$$S^2 = \frac{\Sigma (Xi - X)^2}{n - 1}$$

Where,

X_i = Variate value of i^{th} sample

X = arithmetic mean

S = Standard deviation for the sample

n = Number of samples

4. EXPERIMENTAL RESULTS

The present investigation entitled "Genetic analysis of wilt resistance in intraspecific crosses in Asiatic cotton (*G. arboreum* L.)" was carried out to study the inheritance of wilt {*Fusarium* (*F. oxysporum*)} resistance in Asiatic cotton *G. arboreum*, to identify molecular markers linked to wilt resistance in *G. arboreum* and to find out gene action of various yield contributing characters. The results obtained in relation to above aspects are presented here under following heads *viz.,*

1. Inheritance of resistance to *Fusarium* wilt
2. Molecular analysis
3. Root anatomy
4. Generation mean
    A. Mean performance
    B. Analysis of variance
    C. Scaling tests
    D. Gene effects

4.1 Inheritance of resistance to *Fusarium* wilt

The inheritance of resistance to *Fusarium* wilt was studied by screening the F_2 generation along with parents of both the crosses in wilt sick soil in potrays (Plate 1 and Plate 2). A control of susceptible parent Dh-2 was grown in non inoculated and inoculated soil in potray. Observations were recorded number of days to wilt and number of plants wilted per day.

Cross I: AKA-7 × Dh-2

The results of wilting of parental plants per day and average score calculated is given in Table 4.1.

Case 1

Average score calculated from the observed frequency of number of plants wilted per day in susceptible parent showed that plants wilted before 5.93 days after germination were highly susceptible to wilt. Similarly, average

score calculated in resistant parent AKA-7 from observed frequency showed that plants wilted after 8.63 days after germination were considered as resistant to wilt i.e., plants wilted before 8.63 days were considered as susceptible to wilt. Using the score of resistant parent, the F_2 population screened against *Fusarium* wilt was grouped into two classes *viz.*, susceptible and resistant. Amongst the 196 F_2, 159 plants were found susceptible while, 37 were resistant (Table 4.2).

Screening of F_2 population of cross AKA-7 × Dh-2 against *Fusarium* wilt revealed that the number of susceptible plants were higher than number of resistant plants. Hence, the χ^2 value was calculated considering susceptible being monogenic dominant over the resistance (Table 4.3). The calculated χ^2 value was less than table χ^2 at 1 d.f. and 1 % probability level hence, χ^2 value was found non-significant which indicated that there is no difference between observed and expected frequencies and hence the F_2 population was segregated in the ratio of 3 susceptible: 1 resistant.

Table: 4.1 Scoring of parental material of the cross AKA-7 × Dh-2

Days to wilt after germination	**No of plants wilted per day after germination**							
	AKA-7				**Dh-2**			
	R-I	**R-II**	**Total**	**Score**	**R-I**	**R-II**	**Total**	**Score**
1	0	0	0	0	1	0	0	1
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	2	2	8
5	2	2	4	20	15	18	33	165
6	3	3	6	36	6	7	13	78
7	3	5	8	56	2	5	11	77
8	4	6	10	80	5	6	7	56
9	7	9	16	144	2	2	4	36
10	3	3	6	60	0	0	0	0
11	1	2	3	33	0	0	0	0
12	2	2	4	48	0	0	0	0
13	0	1	1	13	0	0	0	0
14	0	2	2	28	0	0	0	0
Total	**25**	**35**	**60**	**518**	**31**	**40**	**71**	**425**
Average Score				**8.63**				**5.98**

Table 4.2 Screening of F_2 population of cross AKA-7 × Dh-2 against *Fusarium* wilt

Days to wilt after germination	No of plants wilted per day after germination in F_2 population of the cross AKA-7 × Dh-2
	Total
1	0
2	0
3	1
4	13
5	35
6	46
7	35
8	29
9	12
10	6
11	8
12	7
13	4
Total	**196**

Table: 4.3 Segregation of Susceptible and Resistant plants in F_2 population AKA-7 × Dh-2

F_2 segregation	Classes		Total
	Susceptible	**Resistant**	
Observed (O)	159	37	196
Expected (E)	147	49	196
D= O-E	12	12	--
χ^2**= D²/E**	0.979	2.94	3.91(NS)

Case II

Screening of parental material of AKA-7 × Dh-2 indicated that all the plants of susceptible parent Dh-2 wilted up to 9th day after germination (Table 4.1).This margin of 9th day was used for classification of F_2 population. Plants which wilted up to 9th day after germination were considered as susceptible and remaining plants which wilted after 9 days of germination were considered as resistant to wilt. Thus, out of 196 F_2 plants, 171 plants were found susceptible and 25 plants were found resistant (Table 4.2). It was

observed that the number of susceptible plants were much higher than the number of resistant plants. The χ^2 was calculated considering that the resistance was dominant and digenically governed (Table 4.4). The calculated χ^2 value was less than table χ^2 at 1 d.f. and 1 % probability level hence, χ^2 value was found non-significant which indicated that there is no difference between observed and expected frequencies and hence the F_2 population was segregated in the ratio of 13 susceptible: 3 resistant.

Table: 4.4 Segregation of Susceptible and Resistant plant plants in F_2 population of AKA-7 × Dh-2

F_2 segregation	**Classes**		**Total**
	Susceptible	**Resistant**	
Observed (O)	171	25	196
Expected (E)	159.25	36.25	196
D= O-E	11.75	11.75	--
χ^2= D²/E	0.87	3.76	4.62(NS)

Cross II: PA-141 × Dh2

The results of wilting of parental plants per day and average score calculated are given in Table 4.5.

Case 1

Average score calculated from the observed frequency of number of plants wilted per day in susceptible parent showed that, plants wilted before 5.98 days after germination were highly susceptible to wilt. Similarly, average score calculated in resistant parent PA-141 from observed frequency showed that, plants wilted after 8.98 days after germination were considered as resistant to wilt i.e. plants wilted before 8.98 days were considered as susceptible to wilt. Using the score of resistant parent, the F_2 population screened against *Fusarium* wilt was grouped into two classes *viz.*, susceptible and resistant. Out of 155 plants, 102 plants were found susceptible, whereas, 53 plants were resistant (Table 4.6).

Screening of parental material of the PA-141 × Dh-2 indicated that the number of susceptible plants were higher than number of resistant plants (Table 4.6). The χ^2 calculated on the basis of F_2 population considering the susceptible being monogenic dominant to resistance (Table 4.7). The calculated χ^2 value was higher than table χ^2 at 1 d.f. and 1 % probability level hence, χ^2 value was found significant which indicated that there is difference between observed and expected frequencies and hence the F_2 population was not segregated in the ratio of 3 susceptible: 1 resistant. There might be different mode of inheritance.

Table: 4.5 Scoring of parental material of the cross PA-141 × Dh-2

Days to wilt after germination	**No of plants wilted per day after germination**							
	PA-141				**Dh-2**			
	R-I	**R-II**	**Total**	**Score**	**R-I**	**R-II**	**Total**	**Score**
1	0	0	0	0	1	0	0	1
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	2	2	8
5	1	1	2	10	15	18	33	165
6	1	1	2	12	6	7	13	78
7	3	3	6	42	2	5	11	77
8	6	5	11	88	5	6	7	56
9	8	9	17	153	2	2	4	36
10	4	5	9	90	0	0	0	0
11	1	3	4	44	0	0	0	0
12	1	1	2	24	0	0	0	0
13	1	1	2	26	0	0	0	0
14	0	1	1	14	0	0	0	0
Total	26	30	56	503	31	40	71	425
Average Score				**8.98**				**5.98**

Table 4.6 Screening of F_2 population of the cross PA-141 × Dh-2 against *Fusarium* wilt

Days to wilt after germination	No of plants wilted per day after germination in F_2 population of the cross PA-141 × Dh-2
	Total
1	0
2	0
3	2
4	5
5	10
6	30
7	25
8	30
9	25
10	22
11	6
12	0
13	0
Total	**155**

Table 4.7 Segregation of Susceptible and Resistant plants in F_2 population PA-141 × Dh-2

F_2 segregation	Classes		Total
	Susceptible	Resistant	
Observed (O)	102	53	155
Expected (E)	116.25	38.75	155
D= O-E	-14.25	14.25	0
χ^2 **= D²/E**	1.75	5.24	6.99**

Case II

Screening of parental material of the PA-141 × Dh-2 (Table 4.6) revealed that all the plants of susceptible parent Dh-2 wilted upto 9th day after germination. This margin of 9th day was used for classification of F_2 population. Plants wilted up to 9 days after germination was considered as susceptible and remaining plants which wilted after 9 days of germination were considered as resistant to wilt. Thus 127 F_2 plants were found susceptible and 28 F_2 plants were found resistant (Table 4.6). It was observed that the number of susceptible plants were much higher than number of resistant plants. The χ^2 was calculated considering the resistance was dominant digenically governed (Table 4.8). The calculated χ^2 value was less than table χ^2 at 1 d.f. and 1 % and 5 % probability level i. e. χ^2 value found insignificant which indicated that there was no difference between observed and expected frequencies. Hence the F_2 population segregated in the ratio of 13 susceptible: 3 resistant.

Table 4.8 Segregation of Susceptible and Resistant plants in F_2 population PA-141 × Dh-2

F_2 segregation	**Classes**		**Total**
	Susceptible	**Resistant**	
Observed (O)	127	28	155
Expected (E)	125.94	29.06	155.00
D= O-E	1.06	-1.06	0.00
χ^2**= D²/E**	0.01	0.04	0.05(NS)

4.2 Molecular analysis

Bulk Segregant Analysis (Plate 3, Plate 4 and Plate 5) was done using three different types of markers *viz.,* ISSR (17), SSR (18) and RGA (10) primers. Total of 45 primers were used out of which 43 primers showed amplification. 20 primers yielded polymorphic pattern, while 23 showed monomorphic amplification pattern and remaining two primer *viz.*, CIR-413(SSR 18) and NLRR-fwd-rev (RGA 2) failed to amplify. Results of polymorphism study of these primers are presented in Table 4.9, Table 4.10 (a,b,c) and Table 4.11(a,b,c).

4.2.1 ISSR Primers

In all, 78 bands were produced on amplification by using 17 ISSR primers with 14.10 % aggregate polymorphism (Table 4.10a and Table 4.11a). Out of 78 bands observed, 59 bands were monomorphic in all 4 DNA samples. Out of rest 19 bands, 4 bands were observed in 3 samples (ISSR-841-1640, ISSR-8081- 258, IS-2-589, ISN-801-684 primers), 7 bands were observed in 2 samples (ISSR-841-1911bp, ISSR-8036-1006bp, ISSR-8036-280bp, ISSR-8036-152bp, IS-8-4445bp, IS-2-589bp and ISN-801-344bp) and 8 bands were unique (IS-8-756bp, IS-2-732bp, IS-2-665bp, IS-2-485bp, IS-2-409bp, IS-2-337bp, IS-2-306bp, ISN-801-1494bp), respectively.

In IS-2, all eight bands amplified were polymorphic, with three bands were observed only in resistant parent (RP) and one band was observed only in susceptible parent (SP) while, three bands were observed only in bulk samples. Other than this primer, ISN-801 also showed polymorphism with 3 out of 6 bands amplified being polymorphic of which one band amplified only in susceptible parent (SP). Other than these primers IS-8036, IS-8, ISSR - 8081, IS-841 and ISN-801 also showed polymorphism while, remaining primers showed monomorphism. In IS-8-756 bp band was observed only in resistant bulk.

4.2.2 SSR Primers

Eighteen different SSR primers were used for bulk segregant analysis of wilt resistance, out of which 17 primers got amplified. In all 35.42 % aggregate polymorphism was observed in SSR primers (Table 4.10b and Table

4.11b). In eight primers polymorphic banding pattern was observed; while rest nine primers were uninformative as they yielded monomorphic amplification pattern.

On SSR amplification with these 17 primers, a total of 48 bands were produced out of which 23 bands were monomorphic. No amplification was observed in susceptible parent (SP) with the primer BNL-3867. Out of 5 bands amplified by BNL-3867, 4 were monomorphic in rest 3 samples. BNL-3627-465bp and JESPR-151- 499bp bands got amplified only in resistant parent (RP) while, BNL-1059-339bp band amplified only in susceptible parent (SP).

However, five bands observed only in bulked DNA samples of which two bands (BNL-1059-150bp and JESPR-151-672bp) got amplified only in resistant bulk (RB) while three (BNL-3627-457bp, BNL-3867-204bp and CIR-413-480bp) bands got amplified only in susceptible bulk. SSR being co-dominant marker, plants should have bands inherited from either of the parent therefore; this band may be due to non specific amplification.

4.2.3 RGA markers

Ten RGA primers were used for molecular study in current research, out of which nine RGA primers showed amplification. In all 24.32 % aggregate polymorphism was observed in RGA primers (Table 4.10c and Table 4.11c). Total of 37 bands amplified, 27 bands were monomorphic. Interestingly, NLRR-inv1-2 primer amplified band at 277bp (approx.300bp) present only in resistant parent (RP) as well as resistant bulk (RB). In order to further verify its suitability for marker resistance it was further checked for individual F_2 population. The NLRR-inv1-2-277bp band showed amplification in resistant parent and resistant F_2 population, while it was absent in susceptible parent and susceptible F_2 population (Plate 6). This band is likely a candidate for molecular tagging of wilt resistance in *desi* cotton. It is required that this NLRR-inv1-2-277bp band should be converted into locus specific SCAR marker. The XLRR-inv1-2-1410bp band got amplified only in resistant parent. Four bands amplified in both the parents in NLRR-fwd-rev, PCRE- fwd-rev and PCF- fwd-rev, but not amplified in bulk DNA samples.

Table 4.9 Discrimination of wilt resistance by using different primers

Sr. No.	Particulars	Primer	Primer No	Total	
1	Polymorphic	ISSR	ISSR-841, ISSR-8036, IS-8, ISSR-8081, IS-2, ISN-801	6	20
		SSR	BNL-3435, BNL-3627, JESPR-230, BNL-1059, BNL-3147, JESPR-134, BNL-3867, JESPR-151, CIR-413	9	
		RGA	NLRR-inv1/2, XLRR-fwd/rev, XLRR-inv1/2, PCRE-fwd/rev, PCF-fwd/rev	5	
2	Monomorphic	ISSR	ISSR-8336, ISSR-834, ISSR-827, ISSR-857, IS-12, IS-13, ISSR-803, ISSR-804, IS-6,IS-11, ISN-814	11	23
		SSR	BNL-1047, BNL-1672, BNL-3580, BNL-3510, CIR-089, CIR-244, CIR-354, BNL-3090	8	
		RGA	Pto-kin1/2, Pto-kin3/4, PLTR-fwd/rev, PLTR-fwd/rev	4	
3	Unamplified	SSR	JESPR-289	1	2
		RGA	NLRR-fwd/rev	1	

Table 4.10a Summarized results of random primers used for ISSR analysis

Sr. No.	Particular	Total
1	Total number of random primers used	17
2	Total number of polymorphic primers	6
3	Total number of monomorphic primers	11
4	Total number of unamplified primers	0
5	Total number of bands generated	78
6	Total number of polymorphic bands	11
7	Total number of monomorphic bands	59
8	Total number of unique bands	08
9	Aggregate polymorphism (%)	14.10 %

Table 4.10b Summarized results of SSR primers used for molecular analysis

Sr. No.	Particular	Total
1	Total number of random primers used	18
2	Total number of polymorphic primers	9
3	Total number of monomorphic primers	8
4	Total number of unamplified primers	1
5	Total number of bands generated	48
6	Total number of polymorphic bands	17
7	Total number of monomorphic bands	23
8	Total number of unique bands	08
9	Aggregate polymorphism (%)	35.42 %

Table 4.10c Summarized results of RGA primers used for molecular analysis

Sr. No.	Particular	Total
1	Total number of random primers used	10
2	Total number of polymorphic primers	5
3	Total number of monomorphic primers	4
4	Total number of unamplified primers	1
5	Total number of bands generated	37
6	Total number of polymorphic bands	9
7	Total number of monomorphic bands	27
8	Total number of unique bands	01
9	Aggregate polymorphism (%)	24.32 %

Table 4.11a. Results of molecular analysis carried out for 4 DNA samples *viz.*, resistant parent (RP), resistant bulk (RB), susceptible parent (SP) and susceptible bulk (SB) using ISSR markers.

ISSR markers									
Total bands	Sr No	Marker	Band No.	Mol. Wt	Samples				No of samples
					RP	RB	SP	SB	
1	1	ISSR-8336	1	1084	1	1	1	1	4
2			2	544	1	1	1	1	4
3			3	336	1	1	1	1	4
4			4	293	1	1	1	1	4
5			5	255	1	1	1	1	4
6			6	189	1	1	1	1	4
7	2	ISSR -834	1	1389	1	1	1	1	4
8			2	869	1	1	1	1	4
9			3	407	1	1	1	1	4
10			4	348	1	1	1	1	4
11	3	ISSR-841	1	1911	0	1	0	1	2
12			2	1640	1	1	0	1	3
13			3	628	1	1	1	1	4
14			4	563	1	1	1	1	4
15			5	484	1	1	1	1	4
16			6	406	1	1	1	1	4
17	4	ISSR -827	1	909	1	1	1	1	4
18			2	297	1	1	1	1	4
19	5	ISSR-857	1	2283	1	1	1	1	4
20			2	825	1	1	1	1	4
21			3	730	1	1	1	1	4
22			4	607	1	1	1	1	4
23			5	447	1	1	1	1	4
24			6	329	1	1	1	1	4
25	6	ISSR -8036	1	1006	0	1	0	1	2
26			2	280	0	1	0	1	2
27			3	190	1	1	1	1	4
28			4	152	0	1	0	1	2
29	7	IS-8	1	4445	1	0	1	0	2
30			2	2032	1	1	1	1	4
31			3	929	1	1	1	1	4
32			4	756	0	1	0	0	1
33	8	IS-12	1	956	1	1	1	1	4
34			2	810	1	1	1	1	4
35	9	IS-13	1	549	1	1	1	1	4

Table 4.11a. Continued....

Total bands	Sr No	Marker	Band No.	Mol. Wt	Samples				No of samples
					RP	RB	SP	SB	
36	10	ISSR -8081	1	615	1	1	1	1	4
37			2	460	1	1	1	1	4
38			3	323	1	1	1	1	4
39			4	258	0	1	1	1	3
40			5	184	1	1	1	1	4
41	11	ISSR- 803	1	1332	1	1	1	1	4
42			2	809	1	1	1	1	4
43			3	645	1	1	1	1	4
44			4	558	1	1	1	1	4
45			5	425	1	1	1	1	4
46			6	323	1	1	1	1	4
47			7	250	1	1	1	1	4
48			8	219	1	1	1	1	4
49	12	ISSR -804	1	981	1	1	1	1	4
50			2	783	1	1	1	1	4
51			3	483	1	1	1	1	4
52			4	350	1	1	1	1	4
53	13	IS-2	1	911	1	1	0	1	3
54			2	732	0	0	1	0	1
55			3	665	1	0	0	0	1
56			4	589	0	1	0	1	2
57			5	485	1	0	0	0	1
58			6	409	0	0	0	1	1
59			7	337	1	0	0	0	1
60			8	306	0	0	0	1	1
61	14	ISN-801	1	1494	0	0	1	0	1
62			2	891	1	1	1	1	4
63			3	684	0	1	1	1	3
64			4	459	1	1	1	1	4
65			5	344	0	1	0	1	2
66			6	258	1	1	1	1	4
67	15	IS-6	1	1378	1	1	1	1	4
68			2	1058	1	1	1	1	4
69			3	849	1	1	1	1	4
70			4	546	1	1	1	1	4
71	16	IS-11	1	1410	1	1	1	1	4
72			2	1035	1	1	1	1	4
73			3	760	1	1	1	1	4
74			4	500	1	1	1	1	4
75			5	282	1	1	1	1	4
76	17	ISN-814	1	834	1	1	1	1	4
77			2	626	1	1	1	1	4
78			3	329	1	1	1	1	4

Table 4.11b Results of molecular analysis carried out for 4 DNA samples *viz.*, resistant parent (RP), resistant bulk (RB), susceptible parent (SP) and susceptible bulk (SB) using SSR markers.

SSR Markers									
Total bands	Sr No	Marker	Band No.	Mol Wt	Samples				No of samples
					RP	RB	SP	SB	
1	1	BNL-1047	1	479	1	1	1	1	4
2			2	381	1	1	1	1	4
3			3	255	1	1	1	1	4
4			4	162	1	1	1	1	4
5			5	122	1	1	1	1	4
6	2	BNL-1672	1	282	1	1	1	1	4
7			2	108	1	1	1	1	4
8	3	BNL-3435	1	158	0	1	1	1	3
9	4	BNL-3580	1	179	1	1	1	1	4
10	5	BNL-3627	1	1172	1	1	1	1	4
11			2	974	1	1	1	1	4
12			3	810	1	1	1	1	4
13			4	465	1	0	0	0	1
14			5	457	0	0	0	1	1
15			6	297	1	1	1	1	4
16			7	234	1	1	1	1	4
17	6	JESPR-230	1	974	1	0	1	0	2
18			2	211	1	1	1	1	4
19	7	BNL-1059	1	339	na	0	1	na	1
20			2	150	na	1	0	na	1
21	8	BNL-3147	1	131	0	1	1	0	2
22	9	JESPR-134	1	837	1	0	1	1	3
23			2	339	1	1	1	1	4
24			3	245	1	1	1	1	4
25			4	146	1	1	1	1	4
26	10	BNL-3510	1	232	1	1	0	1	3

Table 4.11b. Continued....

Total bands	Sr No	Marker	Band No.	Mol Wt	Samples				No of samples
					RP	RB	SP	SB	
27	11	BNL-3867	1	937	1	1	0	1	3
28			2	411	1	1	0	1	3
29			3	280	1	1	0	1	3
30			4	204	0	0	0	1	3
31			5	171	1	1	0	1	3
32	12	JESPR-151	1	733	1	0	1	1	3
33			2	672	0	1	0	0	1
34			3	605	0	1	1	1	3
35			4	499	1	0	0	0	1
36			5	419	0	1	1	0	2
37			6	390	1	1	0	1	3
38			7	280	1	1	1	0	3
39			8	223	1	1	0	1	3
40	13	CIR-089	1	167	1	1	1	1	4
41	14	CIR-413	1	1009	na	1	1	1	3
42			2	480	na	0	0	1	1
43			3	213	na	1	1	1	3
44	15	CIR-244	1	710	1	1	1	1	4
45			2	167	1	1	1	1	4
46	16	CIR-354	1	473	1	1	1	1	4
47			2	116	1	1	1	1	4
48	17	BNL-3090	1	196	1	1	1	1	4
49	18	JESPR-289	NA						

Table 4.11c Results of molecular analysis carried out for 4 DNA samples *viz.*, resistant parent (RP), resistant bulk (RB), susceptible parent (SP) and susceptible bulk (SB) using RGA markers.

RGA Markers									
Total bands	Sr No	Marker	Band No.	Mol Wt	Samples				No of samples
					RP	RB	SP	SB	
1	1	NLRR-inv1/2	1	824	1	1	1	1	4
2			2	504	1	1	1	1	4
3			3	446	1	1	1	1	4
4			4	277	1	1	0	0	2
5	2	NLRR-fwd/rev	NA						0
6	3	XLRR-for/rev	1	1486	1	0	1	0	2
7			2	1315	1	1	1	1	4
8			3	977	1	1	1	1	4
9			4	673	1	0	1	0	2
10			5	558	1	1	1	1	4
11			6	468	1	1	1	1	4
12			7	342	1	1	1	1	4
13			8	209	1	1	1	1	4
14	4	XLRR-inv1/2	1	1410	1	0	0	0	1
15			2	834	1	1	1	1	4
16			3	676	1	1	0	1	3
17			4	568	1	1	0	1	3
18			5	414	1	1	1	1	4
19			6	319	1	1	1	1	4
20			7	292	1	1	0	1	3
21			8	241	1	1	0	1	3
22	5	Pto-kin1/2	1	2220	1	1	1	1	4
23			2	1251	1	1	1	1	4
24			3	1134	1	1	1	1	4
25			4	872	1	1	1	1	4
26			5	442	1	1	1	1	4
27	6	Pto-kin3/4	1	1548	1	1	1	1	4
28			2	1474	1	1	1	1	4
29			3	1293	1	1	1	1	4
30			4	1172	1	1	1	1	4
31			5	682	1	1	1	1	4
32			6	589	1	1	1	1	4
33	7	PLTR-fwd/rev	1	503	1	1	1	1	4
34	8	PNTR-fwd/rev	1	477	1	1	1	1	4
35	9	PCRE-fwd/rev	1		1	1	1	1	4
36			2		1	0	1	0	2
37	10	PCf-fwd/rev	1	1262	1	0	1	0	2
38			2	796	1	1	1	1	4

4.2.4 Sequencing and homology search

Custom sequences of PCR purified product of 300bp (appro.) fragment eluted in PCR were obtained from m/s Banglore Genei Ltd. and analyzed. Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequences at the 3' and 5' ends using the sequence analysis tools (ChromasLite2.01 software) (Plate 7).

4.2.4.1 Consensus gene sequences of wilt resistance gene analogs and highly similar sequences obtained by MEGABLAST search from NCBI database

Consensus gene sequences of wilt resistance gene analogs and highly similar homologous sequences obtained by MEGABLAST search from NCBI database is given in Table 4.12

The 300 bp fragment which was observed to be co-segregating with *Fusarium* wilt resistance in *G. arboreum* was sequenced using the NBS NLRR-inv1 and NLRR-inv2 primers (Plate 8). On sequence homology analysis using online BLAST-N software with highly similar sequences gave 9 hits. All these highly similar nine hits were from *Gossypium* genus. Maximum identity was shown by AC243195.1 with 93 % coverage; followed by AC243103.1 with 91% identity and 44 % coverage. Further, AC243148.1, EF457753.1 and AC243162.1 showed 85 %, 88 % and 87 % identity with 50 %, 47 % and 48 % coverage respectively.

4.2.4.2 Consensus gene sequences of wilt resistance gene analogs and somewhat similar homologous sequences matching to flowering plants obtained by BLASTn search from NCBI database

Somewhat similar BLAST analysis with flowering plants gave 21 hits Table 4.13. It was observed that this sequence showed 94 % coverage (91 % identity) with *G. hirsutum* (AC243135.1) clone MXO19A11-jhl (Yu *et al.*, 2010) and *G. arboreum* clone (AC243103.1) GM258G19-jhj (Yu *et al.*, 2010), 95 % coverage (90 % identity) with *G. hirsutum* clone MX086N22-jhl, 47 % coverage and 88 and 84 % identity with *G. hirsutum* (EF457753.1) and *G.*

arboreum clone GM 276 M14-jhh (AC243105.1)respectively, 49 % coverage and 87 % match with *G. hirsutum* clone MX169F24-jhp (AC243162.11), 48 % coverage and 81 and 77 % match with *G. hirsutum* clone MX146F18-joh (AC243160.1) and *G. arboreum* clone GAH174K06-jlo (AC243080.1), respectively. *G. hirsutum* clone MX146F17-jlq (AC243159.1) showed 46 % coverage and 69 % match. Out of these maximum coverage of 94 % and maximum identity of 91 % was shown by *G. hirsutum* MX019A11-jhj (AC243135.1) and G. arboreum clone GM258G19-jhf (AC243103.1).

4.2.4.3 Consensus gene sequences of wilt resistance gene analogs and somewhat similar homologous sequences matching to all organisms obtained by BLASTn search from NCBI network server.

BLAST analysis with somewhat similar sequences led to 118 hits among all organism out of which first 17 hits were of *Gossypium* genome. It was observed that wilt sequence showed 94 % coverage (91 % identity) with *G. hirsutum* (AC243135.1) and *G. arboreum* clone (AC243103.1) while, it was followed by *G. hirsutum* clone MX086N22-jhl with 95 % coverage (90 % identity). Among the 17 *Gossypium* sequences, 8 sequences were from *G. hirsutum* with coverage ranging from 95 to 48 % and maximum identity ranging 94 to 72 % while, five were from *G. arboreum* with coverage ranging from 94 to 41 % and maximum identity ranging 91 to 71 %. Three sequences were from *G. raimondii* with coverage ranging from 93 to 43 % and maximum identity ranging 79 to 71 %, and one from *Gossypioides kirkii* (AC 243087.1) with coverage 44 % and maximum identity 72 %. Other than *Gossypium* genus, three *Carica papaya* BAC clones viz., AC238619.1, AC239138.1 and AC238629.1 have shown 67, 66 and 66 per cent identity with 32, 40 and 40 per cent converge to the wilt sequence. Other organisms though have shown 100 % identity the coverage observed was less than 11 %.

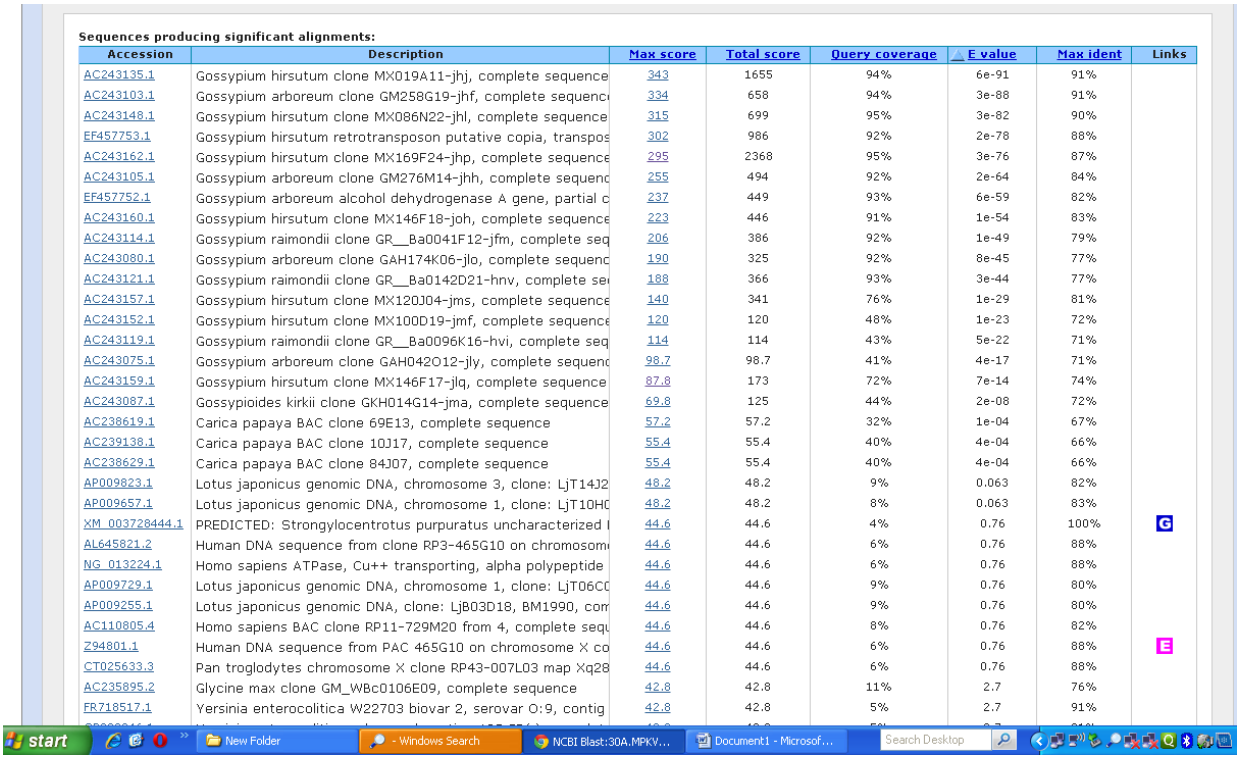
Table 4.12 Consensus gene sequences of wilt resistance gene analogs and highly similar sequences obtained by BLASTn search at NCBI network server

Accession	Homologous gene bank accessions	Coverage	% identity
AC243135.1	*G. hirsutum* clone MX019A11-jhj, complete sequence	93 %	91 %
AC243103.1	*G. arboreum* clone GM258G19-jhf, complete sequence	44 %	91 %
AC243148.1	*G. hirsutum* clone MX086N22-jhl, complete sequence	50 %	85 %
EF457753.1	*G. hirsutum* retrotransposon putative copia, transposon	47 %	88 %
AC243162.1	*G. hirsutum* clone MX169F24-jhp, complete sequence	48 %	87 %
AC243105.1	*G. arboreum* clone GM 276 M14-jhh, complete sequence	47 %	84 %
AC243160.1	*G. hirsutum* clone MX146F18-joh, complete sequence	48 %	81 %
AC243080.1	*G. arboreum* clone GAH174K06-jlo, complete sequence	48 %	77 %
AC243159.1	*G. hirsutum* clone MX146F17-jlq, complete sequence	46 %	69 %

Table 4.13 Consensus gene sequences of wilt resistance gene analogs and somewhat similar homologous sequences matching to flowering plants obtained by BLASTn search at NCBI network server.

Accession	Homologous gene bank accessions	Coverage	% identity
AC243135.1	*G. hirsutum* clone MX019A11-jhj, complete sequence	94 %	91 %
AC243103.1	*G. arboreum* clone GM258G19-jhf, complete sequence	94 %	91 %
AC243114.1	*G. hirsutum* clone MX086N22-jhl, complete sequence	95 %	90 %
EF457753.1	*G. hirsutum* retrotransposon putative copia, transposon	47 %	88 %
AC243162.11	*G. hirsutum* clone MX169F24-jhp, complete sequence	49 %	87 %
AC243105.1	G. arboreum clone GM 276 M14-jhh, complete sequence	47 %	84 %
AC243160.1	*G. hirsutum* clone MX146F18-joh, complete sequence	48 %	81 %
AC243080.1	*G. arboreum* clone GAH174K06-jlo, complete sequence	48 %	77 %
AC243159.1	*G. hirsutum* clone MX146F17-jlq, complete sequence	46 %	69 %
AC243157.1	*G. hirsutum* clone MX120J04-jms, complete sequence	15 %	78 %

Table 4.14 Consensus gene sequences of wilt resistance gene analogs and somewhat similar homologous sequences matching to all organisms obtained by BLASTn search at NCBI network server.



Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AC243135.1	Gossypium hirsutum clone MX019A11-jhj, complete sequence	343	1655	94%	6e-91	91%	
AC243103.1	Gossypium arboreum clone GM258G19-jhf, complete sequenc	334	658	94%	3e-88	91%	
AC243148.1	Gossypium hirsutum clone MX086N22-jhl, complete sequence	315	699	95%	3e-82	90%	
EF457753.1	Gossypium hirsutum retrotransposon putative copia, transpos	302	986	92%	2e-78	88%	
AC243162.1	Gossypium hirsutum clone MX169F24-jhp, complete sequence	295	2368	95%	3e-76	87%	
AC243105.1	Gossypium arboreum clone GM276M14-jhh, complete sequenc	255	494	92%	2e-64	84%	
EF457752.1	Gossypium arboreum alcohol dehydrogenase A gene, partial c	237	449	93%	6e-59	82%	
AC243160.1	Gossypium hirsutum clone MX146F18-joh, complete sequence	223	446	91%	1e-54	83%	
AC243114.1	Gossypium raimondii clone GR__Ba0041F12-jfm, complete seq	206	386	92%	1e-49	79%	
AC243080.1	Gossypium arboreum clone GAH174K06-jlo, complete sequenc	190	325	92%	8e-45	77%	
AC243121.1	Gossypium raimondii clone GR__Ba0142D21-hnv, complete se	188	366	93%	3e-44	77%	
AC243157.1	Gossypium hirsutum clone MX120J04-jms, complete sequence	140	341	76%	1e-29	81%	
AC243152.1	Gossypium hirsutum clone MX100D19-jmf, complete sequence	120	120	48%	1e-23	72%	
AC243119.1	Gossypium raimondii clone GR__Ba0096K16-hvi, complete seq	114	114	43%	5e-22	71%	
AC243075.1	Gossypium arboreum clone GAH042O12-jly, complete sequenc	98.7	98.7	41%	4e-17	71%	
AC243159.1	Gossypium hirsutum clone MX146F17-jlq, complete sequence	87.8	173	72%	7e-14	74%	
AC243087.1	Gossypioides kirkii clone GKH014G14-jma, complete sequence	69.8	125	44%	2e-08	72%	
AC238619.1	Carica papaya BAC clone 69E13, complete sequence	57.2	57.2	32%	1e-04	67%	
AC239138.1	Carica papaya BAC clone 10J17, complete sequence	55.4	55.4	40%	4e-04	66%	
AC238629.1	Carica papaya BAC clone 84J07, complete sequence	55.4	55.4	40%	4e-04	66%	
AP009823.1	Lotus japonicus genomic DNA, chromosome 3, clone: LjT14J2	48.2	48.2	9%	0.063	82%	
AP009657.1	Lotus japonicus genomic DNA, chromosome 1, clone: LjT10H0	48.2	48.2	8%	0.063	83%	
XM_003728444.1	PREDICTED: Strongylocentrotus purpuratus uncharacterized l	44.6	44.6	4%	0.76	100%	G
AL645821.2	Human DNA sequence from clone RP3-465G10 on chromosom	44.6	44.6	6%	0.76	88%	
NG_013224.1	Homo sapiens ATPase, Cu++ transporting, alpha polypeptide	44.6	44.6	6%	0.76	88%	
AP009729.1	Lotus japonicus genomic DNA, chromosome 1, clone: LjT06C0	44.6	44.6	9%	0.76	80%	
AP009255.1	Lotus japonicus genomic DNA, clone: LjB03D18, BM1990, com	44.6	44.6	9%	0.76	80%	
AC110805.4	Homo sapiens BAC clone RP11-729M20 from 4, complete sequ	44.6	44.6	8%	0.76	82%	
Z94801.1	Human DNA sequence from PAC 465G10 on chromosome X co	44.6	44.6	6%	0.76	88%	E
CT025633.3	Pan troglodytes chromosome X clone RP43-007L03 map Xq28	44.6	44.6	6%	0.76	88%	
AC235895.2	Glycine max clone GM_WBc0106E09, complete sequence	42.8	42.8	11%	2.7	76%	
FR718517.1	Yersinia enterocolitica W22703 biovar 2, serovar O:9, contig	42.8	42.8	5%	2.7	91%	

4.3 Root Anatomy

Infection was found in the susceptible as well as resistant cotton varieties. Infection in resistant variety AKA-7 was started 3 days later as compared to susceptible variety Dh-2. Little thickening of cell wall as well as reduction in the cell size near the epidermis was observed in the moderately resistant parent AKA-7. However, such structural changes were not seen in susceptible parent, Dh-2. It was also observed that the entry of pathogen which started from the root hair region and then upwards. The mycelium entered the root hair, then it grew in the epidermal layer and then it passed the cortex layer and entered in the xylem vessels crossing the endodermis. It developed in xylem vessels and blocked them completely, which became a barrier for upward conduction of water through the vascular system. Ultimately it led to the death of plants (Plate 9).

4.4 Generation mean

The results of generation mean analysis of various yield and yield contributing characters of both the crosses are presented under the heads;

1. Mean performance
2. Analysis of variance
3. Scaling test
4. Gene effects

4.4.1 Mean performance over generations

Mean performances observed for sixteen characters of six generations of two crosses *viz.,* AKA-7 × Dh-2 and PA-141 × Dh-2 are represented in Table 4.15 and Table 4.16. The mean performance of the basic generations *viz.,* P_1, P_2, F_1, F_2, B_1 and B_2 of the crosses showed substantial variability in the material for most of the characters (Plate 10 and 11).

4.4.1.1 Cross I (AKA-7 × Dh-2)

The mean performances for sixteen characters in cross AKA-7 × Dh-2 are presented in Table 4.15.

Highest plant height was recorded by B_2 generation (181.50cm); followed by F_2 (178.00 cm) and P_2 (175.87cm). Monopodia per plant recorded the highest 1.20 in B_2 generation followed by F_2 (1.17) and B_1 (1.10). The B_2 generation recorded the highest (12.33) sympodia per plant, followed by B_1 generation (11.97) and F_1 (11.93).

The 50 % flowering recorded highest in P_2 (86.47) and lowest in P_1 (78.93); while all the remaining generations had values intermediate between P_1 and P_2 for days 50 % flowering. Days to 50 % flowering recorded second lowest in F_1 generation. Similarly, days to first boll bursting and days to maturity recorded the same trend. Highest days to first boll bursting was recorded in P_2 (121.73) and lowest days to first boll bursting was recorded in P_1 (110.80). Highest days to maturity was recorded in P_1 (151.47) and lowest days to maturity was recorded in P_2 (140.67), while all the remaining generations had values intermediate between P_1 and P_2 for both characters.

Number of bolls per plant and boll weight are the most important characters directly contributing towards seed cotton yield. Number of bolls per plant was found to be the highest in B_1 generation (17.33); followed by F_1 (17.07) and B_2 (16.33) generations, respectively. The hybrid generation was found superior to both the parents. The hybrid produced highest boll weight (2.60 g/boll) as compared to the parents P_1 (2.30 g/boll) and P_2 (2.26 g/boll). The highest boll weight of F_1 generation was followed by B_1 (2.54 g/boll) and B_2 (2.51 g/boll).

Hybrid generation expressed increased trend for 100 seed weight with highest magnitude (6.57g) as compared to both the parents. It was followed by P_2 (6.56g) and B_2 (6.09g) generations, respectively.

The hybrid generation exhibited highest (37.22 %) ginning percentage. It was followed by B_2 (37.16 %) and F_2 (36.98 %) generations, respectively.

The highest (14.52g) lint weight per plant recorded in F_1 generation; followed by B_1 (13.79 g) and B_2 (13.72 g) generations, respectively. The highest seed cotton yield (39.03 g) per plant was recorded in hybrid. It was followed by B_1 (37.88 g) and B_2 (36.93 g). Four generations *viz.,* F_1, F_2, B_1 and B_2 showed increased trend in seed cotton yield as compared to both the parents for both the characters.

The highest 2.5 % span length (25.97 mm) was recorded in P_2 generation; followed by F_1 (25.90 mm) and F_2 (25.80 mm). F_1 generation expressed intermediate value as compared to both the parents.

Both the parental generations expressed the highest uniformity ratio (49.67 %) among all the six generations. It was followed by B_1 (49.58 %), F_2 (49.43 %) and F_1 (49.33 %) generations respectively.

The P_1 generation showed highest (4.60 µg/inch) micronaire value. It was followed by B_1 (4.39 µg/inch), F_2 (4.35 µg/inch) and F_1 (4.33 µg/inch) generations. F_1 generation expressed intermediate value as compared to both parents.

The P_1 generation displayed the highest fibre strength (21.33 g/tex); followed by F_1 (20.87 g/tex), F_2 (20.58 g/tex) and B_1 (20.55 g/tex)

generations, respectively. F_1 generation expressed intermediate value as compared to both the parents.

4.4.2 Cross II (PA-141 × Dh-2)

The mean performances for sixteen characters in cross PA-141 × Dh-2 are presented in Table 4.16.

Both the parents differed with very less margin for plant height. P_1 recorded 175.33 cm height and P_2 recorded 181.33 cm height. Little increasing trend recorded in B_2, F_2, F_1 and B_1 generations in increasing order over the parents. B_1 generation recorded the highest plant height (185.17 cm).

Monopodia per plant recorded the same value (1.07) in P_1, P_2, F_1 and B_2 generations. The highest monopodia per plant recorded in F_2 generation (1.08). The highest magnitude (11.53) for number of sympodia per plant was recorded in F_1 generation; followed by B_1 generation (10.70) and P_1 generation (10.53).

The lowest number of days to 50 per cent flowering (84.27) recorded in P_1 generation; followed by F_1 generation (84.93). Very little variation was observed for days to first boll bursting in six different generations. B_2 generation recorded the lowest days to first boll burst (120.07); followed by P_2 (120.20) and F_1 (120.80) generations, respectively. Similarly, very low variation was observed in means of different generation for days to maturity. The lowest number of days to maturity (150.57) was recorded in B_2 generation; followed by F_1 (150.80) and P_2 (151.07) generations, respectively.

The hybrid generation recorded the highest number of bolls per plant (16.87); followed by B_1 (15.90) and B_2 (14.20). It expressed superiority over both the parents. The highest boll weight (2.70 g) was observed in F_1 generation. It was followed by B_1 (2.54 g), F_2 (2.54 g), B_2 (2.52 g) generations, respectively. All these generations showed superiority over both the parents.

The hybrid generation expressed the increased 100 seed weight having the highest magnitude (7.02 g) over P_1 (6.71 g) and P_2 (6.62 g).

The highest ginning percentage (37.54 %) recorded in F_1 generation; followed by B_1 (36.63 %) and B_2 (36.26 %) generations, respectively.

Lint weight per plant was the highest (15.19 g) in F_1 generation; followed by B_1 (12.93 g), P_1 (11.7 g) and B_2 (10.68 g). The hybrid generation expressed highest value (40.47 g) for seed cotton yield per plant followed by B_1 (35.22 g) and P_1 (32.71 g) generations, respectively. The lowest mean value for seed cotton yield was recorded in F_2 generation.

The F_1 generation showed the highest (27.27 mm) 2.5 % span length. It was followed by F_2 (26.48 mm), B_2 (26.12 mm) and both the parents P_2 (25.93mm) and P_1 (25.73mm).

The highest uniformity ratio 50.00 % was observed in P_1 generation. It was followed by P_2 (49.67 %), F_2 (49.60 %) and B_2 (48.75 %) generations, respectively.

The highest (4.47 µg/inch) micronaire value was reported in P_1 generation. It was followed by P_2 (4.37 µg/inch), F_1 (4.27 µg/inch) and B_1 (4.24µg/inch) generations.

The fibre strength was the highest in P_1 generation (22.03 g/tex), followed by F_2 (21.42 g/tex), B_1 (21.09 g/tex), B_2 (20.69 g/tex) and F_1 (20.23 g/tex) generations respectively. The F_1 generation expressed intermediate value as compared to both parents.

4.5 Analysis of Variance

Analysis of variance for various characters of six generations of both the crosses *viz.,* AKA-7 × Dh-2 and PA-141 × Dh-2 is given in Table 4.17.

Out of sixteen characters studied, the mean sum of squares were significant in AKA-7 × Dh-2 in all the characters due to generations, except monopodia per plant, ginning percentage and fibre characters whereas, in case of PA-141 × Dh-2, mean sum of squares due to generations were highly significant for sympodia per plant, number of bolls per plant, boll weight, 100 seed weight, lint weight per plant, ginning percentage, seed cotton yield per plant, 2.5 % span length and uniformity ratio.

Table 4.15 Mean performance for various characters in six generations of AKA-7 × Dh-2 in cotton

Character	Generations					
	P_1	P_2	F_1	F_2	B_1	B_2
Plant height (cm)						
Mean	116.33	175.87	165.07	178.00	167.87	181.50
S E±	1.37	0.99	1.26	3.46	4.34	4.35
Monopodia per plant						
Mean	1.07	1.07	1.07	1.17	1.10	1.20
S E±	0.06	0.06	0.06	0.05	0.06	0.07
Sympodia per plant						
Mean	6.93	10.53	11.93	10.87	11.97	12.33
S E±	0.23	0.32	0.21	0.49	0.55	0.58
Days to 50% flowering						
Mean	78.93	86.47	82.33	83.75	84.23	85.37
S E±	0.43	0.40	0.35	0.53	0.62	0.64
Days to first boll bursting						
Mean	110.80	121.73	119.67	116.95	117.17	119.67
S E±	0.38	0.37	0.45	0.61	0.75	0.48
Days to maturity						
Mean	140.67	151.47	149.40	146.78	146.47	149.60
S E±	0.35	0.22	0.39	0.58	0.76	0.52
Bolls per plant						
Mean	8.87	13.93	17.07	15.88	17.33	16.73
S E±	0.22	0.32	0.33	0.79	0.78	0.91
Boll weight (g)						
Mean	2.30	2.26	2.60	2.50	2.54	2.51
S E±	0.01	0.03	0.01	0.05	0.06	0.05
100 seed weight (g)						
Mean	5.60	6.56	6.57	6.04	5.83	6.09
S E±	0.03	0.05	0.02	0.10	0.13	0.12

Table 4.15 contd.....

Character	Generations					
	P_1	P_2	F_1	F_2	B_1	B_2
Lint weight per plant (g)						
Mean	7.02	10.31	14.52	13.07	13.79	13.72
S E±	0.24	0.46	0.30	0.72	0.63	0.79
Ginning (%)						
Mean	35.79	35.90	37.22	36.84	36.96	37.16
S E±	0.44	0.52	0.51	0.39	0.49	0.47
Seed cotton yield per plant (g)						
Mean	19.63	28.72	39.03	35.35	37.88	36.93
S E±	0.67	1.20	0.61	1.87	1.78	1.99
2.5 % Span Length (mm)						
Mean	25.63	25.97	25.90	25.80	25.34	25.45
S E±	0.19	0.27	0.42	0.11	0.16	0.18
Uniformity ratio (%)						
Mean	49.67	49.67	49.33	49.43	49.58	49.00
S E±	0.33	0.33	0.67	0.14	0.23	0.17
Micronaire (µg/inch)						
Mean	4.60	4.27	4.33	4.35	4.39	4.21
S E±	0.27	0.03	0.13	0.05	0.07	0.12
Fibre strength (g/tex)						
Mean	21.33	20.30	20.87	20.58	20.55	20.24
S E±	0.42	0.23	0.58	0.19	0.21	0.20

Table 4.16 Mean performance for various characters in six generations of PA-141 × Dh-2 in cotton

Character	Generations					
	P_1	P_2	F_1	F_2	B_1	B_2
Plant height (cm)						
Mean	175.33	181.33	183.33	183.20	185.17	182.67
S E±	2.08	1.58	1.20	3.65	3.50	3.97
Monopodia per plant						
Mean	1.07	1.07	1.07	1.08	1.03	1.07
S E±	0.07	0.07	0.07	0.04	0.03	0.05
Sympodia per plant						
Mean	10.53	10.07	11.53	7.13	10.70	10.20
S E±	0.36	0.56	0.51	0.33	0.40	0.42
Days to 50% flowering						
Mean	84.27	85.67	84.93	86.05	85.73	86.30
S E±	0.34	0.21	0.18	0.56	0.75	0.70
Days to first boll bursting						
Mean	121.80	120.20	120.80	121.50	121.53	120.07
S E±	0.53	0.86	0.63	0.60	0.84	0.80
Days to maturity						
Mean	151.80	151.07	150.80	152.25	152.17	150.57
S E±	0.53	0.66	00.63	0.75	1.05	0.85
Bolls per plant						
Mean	14.13	13.80	16.87	10.28	15.90	14.20
S E±	0.34	0.38	0.32	0.53	0.74	0.63
Boll weight (g)						
Mean	2.38	2.25	2.70	2.54	2.54	2.52
S E±	0.03	0.02	0.02	0.06	0.06	0.08
100 seed weight (g)						
Mean	6.71	6.62	7.02	6.28	6.36	6.32
S E±	0.04	0.04	0.04	0.09	0.12	0.10

Table 4.16. Contd.....

Character	Generations					
	P_1	P_2	F_1	F_2	B_1	B_2
Lint yield per plant (g)						
Mean	11.76	10.63	15.19	8.02	12.93	10.68
S E±	0.21	0.27	0.26	0.51	0.72	0.67
Ginning (%)						
Mean	36.02	36.13	37.54	35.47	36.63	36.26
S E±	0.21	0.41	0.36	0.53	0.43	0.52
Seed cotton yield per plant (g)						
Mean	32.71	29.69	40.47	22.74	35.22	30.16
S E±	0.65	1.09	0.52	1.41	1.81	1.70
2.5 % Span Length (mm)						
Mean	25.73	25.93	27.27	26.48	25.90	26.12
S E±	0.23	0.12	0.32	0.19	0.13	0.15
Uniformity ratio (%)						
Mean	50.00	49.67	48.00	49.60	48.71	48.50
S E±	0.58	0.33	0.58	0.19	0.26	0.16
Mironaire (µg/inch)						
Mean	4.47	4.37	4.27	4.05	4.24	4.09
S E±	0.12	0.12	0.07	0.06	0.09	0.10
Fibre strength (g/tex)						
Mean	22.03	19.83	20.23	21.42	21.09	20.69
S E±	0.65	0.44	0.12	0.32	0.31	0.27

Table 4.17 Analysis of variance for various characters of six generations

Characters	Mean sum of squares for various characters					
	AKA-7 × Dh-2			PA-141 × Dh-2		
Source of variation	**Replication**	**Generations**	**Error**	**Replication**	**Generations**	**Error**
D.F.	2	5	10	2	5	10
Plant height (cm)	91.39	1758.72**	39.93	27.48	35.43	17.46
Monopodia/ plant	0.04	0.01	0.02	0.02	0.00	0.01
Sympodia/ plant	2.26	12.01**	1.15	0.09	6.83*	1.30
Days to 50 % flowering (Days)	5.30*	29.28**	1.22	0.96	1.72	1.63
Days to first boll bursting (Days)	7.67	43.47**	3.67	4.50	1.63	2.44
Days to maturity (Days)	6.08	42.56**	2.52	5.44	1.58	3.60
No. of bolls/ plant	3.78	31.35**	1.43	0.04	15.30**	1.29
Boll weight (g)	0.00	0.05**	0.01	0.03	0.07*	0.01
100 Seed weight (g)	0.25	0.46**	0.06	0.01	0.25**	0.03
Lint yield (g/plant)	0.36	24.81**	0.81	1.22	17.56**	0.65
Ginning (%)	0.58	1.25	1.27	0.75	2.05*	0.42
Seed cotton yield/plant (g)	0.47	166.86**	6.72	4.39	106.19**	5.31
2.5 % Span Length(mm)	0.43	0.18	0.11	0.09	1.02**	0.08
Uniformity ratio (%)	0.81	0.20	0.40	0.68	1.42*	0.34
Micronaire (µg/inch)	0.04	0.05	0.06	0.01	0.12	0.06
Fibre strength (g/tex)	0.63	0.49	0.31	0.66	1.21	0.46

** Significant at 1 % level of significance.
* Significant at 5 % level of significance.

4.6 Scaling Test

Simple scaling test i.e. A, B, C of Mather (1949) and joint scaling test of Cavalli (1952) were used to detect presence of epistasis by using the data of various generations in both the crosses. The estimates of both scaling tests for sixteen characters of crosses AKA-7 × Dh-2 and PA-141 × Dh-2 are presented in Table 4.18.

The scaling test (Table 4.18) indicated that either A, B or C significantly deviated from zero for all the characters except monopodia/plant, boll weight, ginning percentage, uniformity (%), micronaire and fibre strength in cross AKA-7 × Dh-2 while, in case of cross PA-141 × Dh-2 the one of the scales was significant for all the characters except plant height, monopodia/plant, days to flowering, days to boll bursting, days to 50 % maturity boll weight and fibre strength. Joint scaling test also resulted into high significant chi-square value for the characters where any one scale is also found significant. Significant scaling and joint scaling tests revealed the presence of epistasis in characters while, non significance of scaling test as well as joint scaling tests indicated absence of epistasis and only additive- dominance model was adequate for the inheritance of those characters. Considering the tests, main and interaction effects were compared as per six/three parameter model and presented in Table 4.19.

4.7 Gene effects

The data collected for different characters in six generations of two crosses was analyzed to workout gene effects by following six parameters model given by Hayman (1958) as well as three parameter model (Jinks and Jones, 1958). The results obtained are given Table 4.19 and described as follows:

4.7.1 Plant height (cm)

The magnitude of additive (d) gene effect was significant at 5 % level of significance with negative sign and the magnitude of dominance (h)

effect was insignificant in AKA-7 × Dh-2. Amongst inter-allelic interactions, additive × dominance (j) effect was significantly positive at 1 % level of significance; while, dominance × dominance (l) interaction was negatively significant at 5 % level of significance in AKA-7 × Dh-2.

4.7.2 Sympodia per plant

The dominance gene effect (h) was positively significant at 1 % level of significance having higher magnitude than additive effect (d) in both the crosses.

The estimates of additive × additive (i) interaction was significant with positive sign and that of dominant × dominant (l) was highly significant with negative sign in AKA-7 × Dh-2, while in PA-141 × Dh-2, estimates of additive × additive (i) interaction and dominant × dominant (l) interactions were highly significant with positive and negative signs.

4.7.3 Days to 50 % flowering

In AKA-7 × Dh-2, estimates of additive as well as dominant gene effects were insignificant however, dominant gene effect (h) found higher in magnitude than additive gene effect (d).

All the digenic inter-allelic interactions *viz.*, additive × additive (i), additive × dominant (j) and dominant × dominant (l) were highly significant in AKA-7 × Dh-2.

4.7.4 Days to first boll bursting

Additive gene effect (d) was negatively significant and dominant gene effect (h) was positively significant in AKA-7 × Dh-2. The dominant gene effect (h) was higher in magnitude than additive gene effect (d) with positive magnitude.

In AKA-7 × Dh-2, additive × additive (i) interaction found significant at 5 % level of significance and additive × dominant (j) inter-allelic interaction found significant at 1 % level of significance.

Table 4.18 Scaling tests for various characters for six generations

Character	AKA-7 × Dh-2				PA-141 × Dh-2			
	A	B	C	X^2	A	B	C	X^2
Plant height (cm)	54.33**	22.07*	89.67**	79.14**	11.467	0.467	9.067	10.98 (NS)
Monopodia/ plant	0.07	0.27	0.40	3.86 (NS)	-0.07	0.07	0.07	1.11 (NS)
Sympodia/ plant	5.07**	2.20	2.13	22.79**	-0.67	-1.20	-15.13**	86.02**
Days to 50 % flowering	7.20**	1.93	4.93*	31.22**	2.27	2.00	4.4	7.24 (NS)
Days to first boll burst	3.86**	-2.07	-4.07	13.42**	0.47	-0.87	2.40	1.22 (NS)
Days to maturity(Days)	4.87**	-1.67	-3.80	15.12**	1.73	-0.73	4.53	4.52 (NS)
No. of Bolls/plant	8.73*	2.47	6.33*	33.07**	0.80	-2.27	-20.53**	85.47**
Boll weight (g)	0.18	0.16	0.24	7.29 (NS)	0.01	0.10	0.12	0.70 (NS)
100 seed weight (g)	-0.51	-0.96**	-1.14**	25.99**	-1.02**	-1.00**	-2.26**	74.71**
Lint weight/ plant (g)	6.04**	2.61	5.91*	25.31**	-1.10	-4.46**	-20.68**	100.45**
Ginning (%)	0.92	1.31	1.22	1.72 (NS)	-0.30	-3.15**	-5.32*	10.74*
Seed cotton yield/plant (g)	17.09**	6.09	14.97*	26.11**	-2.74	-9.84**	-52.39**	83.60**
2.5 % Span Length (mm)	-0.85	-0.97	-0.21	8.57*	-1.20*	-0.96*	-0.27	13.13*
Uniformity ratio (%)	0.17	-1.00	-0.28	7.48 (NS)	-0.57	-0.17	2.73	13.99*
Micronaire (µg/inch)	-0.16	-0.19	-0.14	1.13 (NS)	-0.26	-0.45	-1.18**	14.98*
Fibre strength (g/tex)	-1.09	-0.68	-1.05	2.67 (NS)	-0.08	1.31	3.36	7.51 (NS)

** Significant at 1 % level of significance.
*Significant at 5 % level of significance.

4.7.5 Days to maturity

The estimates of additive (d) and dominant (h) gene effects were significant at 5 and 1 % levels of significance in AKA-7 × Dh-2. Dominant component (h) had much higher and positive magnitude than additive component (d).

Amongst the inter-allelic interactions, all three types of interaction *viz.,* additive × additive (i), additive × dominant (j) and dominant × dominant (l) were significant in AKA-7 × Dh-2.

4.7.6 Number of bolls per plant

The dominant gene effect (h) was found positively significant at 1 % level of significance in AKA-7 × Dh-2 and PA-141 × Dh-2. It was much higher in magnitude than the additive gene effect (d).

Amongst the inter-allelic interactions, additive × dominant (j) and dominant × dominant (l) epistasis were significant in AKA-7 × Dh-2 at 5 and 1% level of significance. In PA-141 × Dh-2, additive × additive (i) and dominant × dominant (l) type of epistasis found highly significant. In AKA-7 × Dh-2, dominant × dominant (l) interaction was higher in magnitude as compared to additive × additive interaction (i) with negative sign. In PA-141 × Dh-2, estimates of dominant × dominant (l) interaction was little lower in magnitude as compared to additive × additive interaction (i) with opposite sign.

4.7.7 Boll weight (g)

As scaling tests and χ^2 tests found insignificant gene effect were calculated as per three parameter model. As per three parameter model dominant (h) gene effect was found significant in both the crosses *viz.*, AKA-7 × Dh-2 and PA-141 × Dh-2.

4.7.8 100 seed weight (g)

Both additive (d) as well as dominant gene effect (h) found insignificant in AKA-7 × Dh-2 and PA-141 × Dh-2.

The estimates of dominant × dominant (l) type of interaction found significant in both the crosses at 5 % level of significance.

Table 4.19 Gene effects for various characters in the crosses

Characters	Cross No.	Gene Effects						Type of Epistasis
		M	d	h	i	j	l	
Plant height (cm)	C1	178.00**	-13.63*	5.70	-13.27	16.13**	-63.13*	Duplicate
Sympodia/ plant	C1	10.87**	-0.37	8.33**	5.13*	1.43	-12.40**	Duplicate
	C2	7.13**	0.50	14.50	13.27**	0.27	-11.40**	Duplicate
Days to 50% flowering	C1	83.75**	-1.13	3.83	4.2	2.6**	-13.33**	Duplicate
Days to first boll burst	C1	116.95**	-2.50**	9.26**	5.87*	2.97**	-7.67	Duplicate
Days to maturity	C1	146.78**	2.13*	10.33**	7.00*	3.27**	-10.20*	Duplicate
Bolls/plant	C1	15.82**	0.60	10.53**	4.87	3.13*	-16.07**	Duplicate
	C2	10.28**	1.70	21.97**	19.07**	1.53	-17.60**	Duplicate
Boll weight (g)	C1	2.29**	0.02	0.32*	--	--	--	--
	C2	2.32**	0.07	0.39*	--	--	--	--
100 seed weight (g)	C1	6.04**	-0.26	0.16	-0.33	0.22	1.79*	Complimentary
	C2	6.28**	0.04	0.60	0.25	-0.01	1.77*	Complimentary
Lint weight/ plant (g)	C1	13.07**	0.07	8.60**	2.74	1.71	-11.38*	Duplicate
	C2	8.02**	2.25*	19.12**	15.12*	1.68	-9.56**	Duplicate
Ginning (%)	C2	35.47**	1.37*	3.33	1.87	1.43*	1.57	Complimentary
Seed cotton yield/plant (g)	C1	35.35**	0.95	23.07*	8.21	5.50*	-31.40*	Duplicate
	C2	22.74**	5.06*	49.08**	39.81**	3.55	-27.23**	Duplicate
2.5 % Span Length(mm)	C1	25.80**	-0.11	-1.51	-1.61*	0.06	3.44*	Duplicate
	C2	26.48**	-0.22	-0.46	-1.89*	-0.12	4.05**	Duplicate
Uniformity ratio (%)	C2	49.60**	-0.04	-5.31**	-3.47**	-0.20	4.21*	Duplicate
Micronaire (µg/inch)	C2	4.05**	0.15	0.32	0.47	0.10	0.23	Complimentary

C1= AKA-7 × Dh-2 and C2= PA-141 × Dh-2

** Significant at 1 % level of significance.
* Significant at 5 % level of significance.

4.7.9 Lint weight per plant

The dominant gene effect (h) was found positively significant at 1 % level of significance in AKA-7 × Dh-2. The magnitude of dominant gene effects was much higher than additive gene effect in AKA-7 × Dh-2. In PA-141 × Dh-2, both additive (d) as well as dominant (h) effects were found positively significant at 5 and 1 % level of significance. Similar to AKA-7 × Dh-2, estimates of dominant gene effects was much higher than additive gene effect in PA-141 × Dh-2.

Among the inter-allelic interactions, dominant × dominant (l) type of epistasis found positively significant in AKA-7 × Dh-2 at 5 % level of significance, while in PA-141 × Dh-2, additive × additive (i) and dominant × dominant (l) epistasis were found significant at 5 and 1 % level of significance.

4.7.10 Ginning Percentage (%)

In case of PA-141 × Dh-2, the additive gene effect (d) was positively significant at 5 % level of significance.

In PA-141 × Dh-2, additive × dominant (j) interaction found significant at 1 % level of significance. The magnitude of 'l' was higher than 'i' and 'j' components.

4.7.11 Seed cotton yield per plant (g)

In AKA-7 × Dh-2, the dominant component (h) was found positively significant having higher magnitude as compared to additive component. In PA-141 × Dh-2, additive (d) and dominant (h) gene effects found significant at 5 and 1% level of significance, respectively. In both the crosses, the magnitude of dominant component (h) was higher than additive component (d).

Estimates of inter-genic interaction additive × dominant (j) and dominant × dominant (l) interactions were found positively significant in AKA-7 × Dh-2 at 5 % level of significance. In PA-141 × Dh-2, additive × additive (i) and dominant × dominant (l) interactions were found highly significant.

4.7.12 2.5 % Span length (mm)

In AKA-7 × Dh-2, the dominance gene effect (h) was magnitudinally higher than additive gene effect (d), but both components were insignificant with negative sign. Among inter-genic interactions additive × additive (i) and dominant × dominant (l) components were significant at 5 % level of significance. The 'l' component had highest magnitude among epistatic interactions. Similar results were observed in PA-141 × Dh-2.

4.7.13 Uniformity ratio (%)

In PA-141 × Dh-2, dominant gene effect (h) was found significant at 1% level of significance. It showed much higher value than additive gene component (d). Both the components expressed negative sign.

Among the inter allelic interactions, in PA-141 × Dh-2, additive × additive (i) and dominant × dominant (l) interactions were found significant at 1 and 5 % level of significance, respectively. The estimates of both the interactions were higher in magnitude having additive × additive interaction (i) negative sign and dominant × dominant interaction (l) positive sign.

4.7.14 Micronaire (μg/inch)

In PA-141 × Dh-2, both additive and dominant components of gene action were found insignificant with same sign. The dominant component (h) had double magnitude (approx.) than additive component (d) in PA-141 × Dh-2. None of the interaction found significant.

4.8 Inheritance of qualitative characters

Variations were observed in leaf shape of AKA-7 × Dh-2 and flower colour in both the crosses (Plate 12 and 13). No differences were observed in parents of both crosses in petal spot, anther colour, anther exertion and nectary. Petal spot was present, anther colour was yellow, anther was exerted and nectarines were present in all generations in both the crosses, while no differences were observed leaf shape for cross PA-141 × Dh-2.

Leaf shape: It was observed that the 272 plants, F_2 population of the cross AKA -7 × Dh-2 segregated in the proportion 216 super okra and 56 okra. There were no significant differences between the observed and expected values when the expected population considered to be segregated in the ratio 3:1. The calculated χ^2 found less than table χ^2.

Flower colour: It was observed that the 259 plants, F_2 population of the cross AKA -7 × Dh-2 segregated in the proportion 179 cream and 80 white. There were no significant differences between the observed and expected values when the expected population considered to be segregated in the ratio 3:1. The calculated χ^2 found less than table χ^2 at 1 % significance level.

In the cross PA-141 × Dh-2, the 203 plants, F_2 population segregated in the proportion 1 dark yellow, 68 yellow, 75 light yellow, 59 cream and 1 white. There were no significant differences between the observed and expected values when the expected population considered to be segregated in the ratio 1:21:20:21:1. The calculated χ^2 found less than table χ^2 at 1 % significance level.

Table 4.20 Inheritance of qualitative characters.

Inheritance of qualitative characters in AKA-7 × Dh-2

Leaf Shape				
Generation		**Super okra**	**Okra**	**Total**
P_1			All	
P_2		All		
F_1		All		
F_2	O	216	56	272
	E	204	68	272
χ^2		**0.71**	**2.12**	**2.82**
Flower Colour				
Generation		**Cream**	**White**	**Total**
P_1			All	
P_2		All		
F_1		All		
F_2	O	179	80	259
	E	194.25	64.75	259
χ^2		**1.20**	**3.59**	**4.79**

Inheritance of qualitative characters in PA-141 × Dh-2

Flower Colour							
Generation		**Dark yellow**	**Yellow**	**Light Yellow**	**Cream**	**White**	**Total**
P_1			All				
P_2					All		
F_1				All			
F_2	O	1	68	75	59	1	203
	E	3	67	63	67	3	203
χ^2		**1.49**	**0.03**	**2**	**1**	**1**	**5.98**

5. DISCUSSION

Cotton breeding programs focus on improved resistance to a variety of pathogens, integrating pathogen resistance phenotypes into high-yielding, high-fiber quality cultivars. Conventional cotton breeding selections rely mainly on bioassays such as severity of disease symptoms and on the ability of the breeder to identify desirable traits for generation advancement. These techniques can be extremely subjective and in the case of bioassays, can lead to false selections due to environmental factors such as non-uniform distribution of pathogens in the field. Marker assisted selection is a reliable and faster method than classical screening. This method also has advantage of screening at early crop growth stages. Current study was undertaken to identify molecular marker associated with *Fusarium* wilt resistance along with classical screening in diploid cotton. Further, sequencing of molecular band associated with *Fusarium* wilt resistance was carried out and the results obtained are discussed here. The results obtained in relation to generation mean analysis of two crosses were discussed in the second part.

5.1 Inheritance of resistance to *Fusarium* wilt

Screening of F_2 derived from two crosses i.e., AKA-7 × Dh-2 and PA-141 × Dh-2 was undertaken in the current study. The average score in terms of days to wilt in the three parents i.e., AKA-7, PA-141 and Dh-2 was 8.63, 8.98 and 5.93. In the first cross (AKA-7 × Dh-2), both single gene dominance (3:1) and two gene epistatic (13:3) interaction were observed when resistance classification was based on either 8.63 or 9 days of germination, respectively, with susceptibility dominant over tolerance in case-I while, resistance was dominant over susceptibility with presence of epistasis in case-II. However, in the second cross (PA-141 × Dh-2) only the epistatic interaction was observed as the F_2 segregated in the ratio of 13 susceptible : 3 resistant. Segregation of the F_2 population of both the crosses indicated that resistance was dominant over the susceptibility and governed by two genes. Segregation of the F_2 populations in 13:3 ratio indicated that the first

dominant gene governed the resistance to *Fusarium* wilt and second dominant gene inhibited the action of first gene. Uppal *et al.* (1940) reported that three complementary factors governed the resistance to *Fusarium.* Further, Kelkar *et al.* (1947) concluded that the resistance to *Fusarium* wilt in *G. arboreum* and *G. herbaceum* was expressed in the presence of two dominant complementary genes but the third gene present, inhibited the action of other two genes.

Zhang *et al.* (2004) using two segregating populations derived from crosses between Sure-Grow 747 × Auburn 634 RNR and Sure-Grow 747 × M-240 RNR reported that one or two genes are responsible for RKN resistance. Segregation of the resistance phenotype in both mapping populations showed 3:1 ratio by chi-square analysis, indicating one dominant RKN resistance gene. In the F_2 mapping population derived from a cross between Sure-Grow 747 × M-240 RNR, segregation of the resistance phenotype also fit a 13:3 ratio, indicating possible epistatic interaction between a dominant and a recessive gene conferring RKN resistance.

Dominance of *Fusarium* wilt resistance to susceptibility and the role of two gene interactions *viz.,* inhibitory (13:3) and complementary (9:7) in pigeonpea was also confirmed by Sameer Kumar *et al.* (2009).

5.2 Molecular analysis

Bulk segregant analysis was done using three different types of markers *viz.,* ISSR (17), SSR (18) and RGA (10) primers. Amongst these 45 primers, 43 primers showed amplification, out of which 20 primers yielded polymorphic pattern, while 23 showed monomorphic amplification. Among phenotypic markers, only one set of RGA primer showed differentiation between resistance and succeptibility hoping most reliable in MAS for identification of FW resistance in positive direction.

5.2.1 Significance of RGA marker analysis in combination with BSA

In the spectrum of applied research, crop scientists have benefited greatly from identification and mapping of R genes that are linked to pathogen and pest resistance phenotypes. Identification of new sequence-specific R-gene markers that are associated with resistance phenotypes facilitates marker-assisted selection (MAS) and allows for introgression of resistance genes into susceptible elite cultivars. In this instance, through the use of DNA markers, the loci containing the resistance genes of choice can be tracked through generations of selection. Clearly, identification of R-gene-linked DNA markers and/or genes that confer natural resistance to pests would be greatly beneficial to the cotton farmer in terms of overhead expenditures.

In the current research, among all the primers only one Resistant Gene Analogue primer set *viz.,* NLRR-inv1 and NLRR-inv2 showed amplification of 277bp band in resistant parent and resistant F_2 population, while it was absent in susceptible parent and susceptible F_2 population. This was the only primer differentiating the resistant and susceptible in segregating population. Further, on verification in F_2 resistant plant progenies it also showed the same band at 277bp (approx.) confirming its suitability for resistance.

The RGA primers used in the present study were based on the conserved sequences present in resistant genes from various plant species. When these conserved sequence based primers are used in polymerase chain reaction, if they amplify the R-gene region, they are called Resistant Gene Analogues (RGA) (Timmerman *et al.,* 2000; Garcia-Mas *et al.,* 2001). By utilizing the conserved motif of resistance genes, a PCR-based approach to identify new resistance genes and to develop markers tightly linked to resistance genes were used in various crop species (Kanazin *et al.*, 1996; Leister *et al.*, 1996; Yu *et al.*, 1996).

On individual F_2 progeny analysis, NLRR-inv1-2-277bp band amplified only in the plants which survived for more than nine days in FOV wilt sick soil while none of the plant that died prior to it amplified the same. This band

is likely candidate for molecular tagging of wilt resistance in *desi* cotton. It is required that this NLRR-inv1-2-277bp band should be converted into locus specific SCAR marker. The same RGA primers linked with blast resistance were first reported by Zuang *et al.* (1998).

The combination of RGA markers and BSA appeared to be a very efficient approach to identify tightly linked markers to disease resistance genes. There are many reports available which explain the use of RGA markers to tag disease resistance genes in various crops (Kanazin *et al.,* 1996; Hayes and Maroof, 2000).

5.2.2 Confirmation of dominant behavior of *Fusarium* wilt resistance gene

In the current studies using two segregating populations derived from the crosses between AKA-7 × Dh-2 and PA-141 × Dh-2 indicated that, one or two genes are responsible for wilt resistance. Segregation of the resistance phenotype in both mapping populations fit in 13:3 ratio by chi-square analysis, indicating possible epistatic interaction between a dominant and a recessive gene conferring wilt resistance. Hinchliffe *et al.* (2005) also reported same results while mapping of markers for RKN using RGAs.

However, in the F_2 mapping population derived from a cross between AKA-7 × Dh-2, segregation of the resistance phenotype also fit a 1:3 ratio by chi-square analysis, indicating wilt susceptible gene is dominant one.

It has been reported that most of the R-genes that have been characterized in the NBS-LRR class were dominantly inherited. No resistant genes which are genetically found to be recessive, have been isolated and characterized (Rajesh *et al.,* 2002). In the current study it was observed that, the resistance to *Fusarium* wilt in desi cotton is dominant and governed by two genes indicating the presence of epistatic interaction. These results are in conformity with the findings of Rajesh *et al.* (2002).

The inheritance of resistance to *Fusarium* wilt was studied by screening the F_2 generation of the cross AKA-7 × Dh-2 along with parents in

wilt sick soil. Average score calculated from the observed frequency of number of plants wilted per day in susceptible parent showed that, plants wilted before 5.93 days after germination were highly susceptible to wilt. Similarly, average score calculated in resistant parent AKA-7 showed that, plants wilted after 8.63 days after germination were considered as resistant to wilt. In F_2 population, plants started wilting 3rd days after germination. The highest wilting was observed on 6th day after germination. In the molecular analysis, the NLRR-inv1-2-277bp band was observed in plants wilted after 10th day onwards after germination in the F_2 population. This indicated that the resistant band observed in F_2 plants remained after the period when all the seedlings of susceptible varieties got wilted which is in conformity with the screening results of wilt sick soil (Case II). This confirms the dominant nature of wilt resistance.

5.2.3 Cloning and characterization of NLRR-inv1-2-277bp band

The NLRR-inv1-2 is the only primer differentiating the resistant and susceptible in segregating population. Thus, the NLRR-inv1-2-277bp band products may represent candidate disease resistance genes in plants. In some cases the RGA sequences themselves may represent candidate R-genes. Although not all amplified products using these RGA primers are functional disease resistance genes, they contain elements involved in signal transduction pathways in plants (Chen *et al.,* 1998). However, in order to generate more information regarding the nature of this RGA fragment, it will be necessary to isolate the complete gene(s).

RGA marker type based on the conserved domain of resistance genes. A number of resistance genes have been cloned from several crop species. Recently Jie-Yun Zhuang *et al.,* 2002; Rajesh *et al.,* 2002; He *et al.,* 2004; Mantovani *et al.*, 2006; Zhang *et al.,* 2007; Bart Brugmans *et al.,* 2008; Palomino *et al.,* 2008 used RGA for identification and tagging of R genes.

Using degenerated primers based on the conserved sequence of leucine rich repeats (LRR) of the *RPS2* gene of *Arabidopsis thaliana* and the *N* gene of tobacco, Leister *et al.* (1996) demonstrated that 15 of 28 loci identified by

PCR-derived probes showed sequence homology to known resistance genes and six of them were correlated by map position with known resistance genes. The copy number of NBS-LRR genes might vary widely within a species, and the loci of the genes might rapidly rearrange (Leister *et al.* 1998). Using primers derived from the nucleotide binding site of *N* and *RPS2*, Yu *et al.* (1996) mapped five of the eleven subfamilies to the vicinity of known soybean resistance genes. A large proportion of resistance gene analogs (RGAs) amplified were clustered in the chromosomal regions of respective crop species where known resistance genes had been located.

The NBS-LRR genes are abundant in plants. Whole-genome sequence analysis revealed that there are 150 to 175 NBS-LRR genes in the *Arabidopsis* genome (Dang and Jones 2001; Meyers *et al.*, 2003; Richly *et al.,* 2002), constituting about 0.6per cent of its 25,000 genes (The Arabidopsis Genome Initiative 2000) and there are approximately 600 NBS-LRR genes in the rice genome (Goff *et al.*, 2002; Meyers *et al.,* 1999), constituting about 1.5 per cent of its 40,000 genes.

The overall sequence homology of the NBS-LRR genes may vary significantly, several short motifs of their encoding proteins, such as NBS and LRR, are highly conserved. These conserved motifs have enabled rapid isolation of the NBS-LRR genes or resistance gene analogs (RGA) from different plant species by using a polymerase chain reaction (PCR)-based approach with degenerate oligonucleotide primers designed from these domains. Ramalingam *et al.* (2003) showed that, in rice, RGA are associated not only with qualitative resistance but also with quantitative response. These isolated RGA, thus, have provided useful tools to dissect, tag and isolate genes conferring both qualitative and quantitative resistance to different pathogens. Nevertheless, little is known about how the NBS-LRR gene family as an entity is organized, functions and evolves in plant genomes, especially in polyploid plant genomes.

Palomino *et al.* (2008) constructed a composite linkage map based on two interspecific recombinant inbred line populations derived from crosses

between *Cicer arietinum* (ILC72 and ICCL81001) and *Cicer reticulatum*. Seven RGA PCR-based markers (5 CAPS and 2 dCAPS) were developed and successfully genotyped in the two progenies. Six of them have been mapped in different linkage groups where major quantitative trait loci conferring resistance to *ascochyta* blight and *fusarium* wilt have been reported. Genomic locations of RGAs were compared with those of known *Cicer* R-genes and previously mapped RGAs. Association was detected between RGA05 and genes controlling resistance to *fusarium* wilt caused by races 0 and 5.

Hinchliffe *et al.* (2005) also mapped nine RGA markers to homeologous chromosomes in cultivated tetraploid cotton based on linkage to existing markers that are located on these chromosomes for RKN.

Tan *et al.* (2003) utilized R-gene degenerate primers designed from the NBS motifs of the tobacco N protein, Arabidopsis RPS2 protein, and the flax L6 protein to amplify and clone PCR products in the 250-bp size range in cotton. The 250-bp amplification products were representative of the genomic DNA sequences transcribed and translated into the region between the kinase-2 and GLPL motifs of plant R proteins. This approach enabled cloning of 33 putative cotton RGAs containing the highly conserved NBS R-protein motif.

Plant disease resistance (R) genes have been cloned and characterized from both mono- and dicotyledonous plant (Hammond - Kosack and Jones, 1997). It is amazing that almost all R genes identified so far, despite of their specificity, encode polypeptides that share similar structural motifs. These genes are categorized into the following groups based on amino acid sequence similarity; those containing both repeats (LRRs) near C-terminus and a nucleotide binding site (NBS) near N-terminus confirming race specific genes (Rajesh *et al.,* 2002).

He *et al.* (2004) utilized degenerate primers for cloning of 61 of cotton unique sequences containing high similarity to R genes of a 560-bp fragment in cotton.

5.2.4 Sequencing of NLRR-1-2-277bp

NLRR-inv amplified intergene region between two R genes NBS-NLRR domain. On sequence homology analysis using online MEGABLAST software, all the highly similar nine hits were from *Gossypium* genus, while in somewhat similar homology search gave 21 hits. Out of these maximum coverage of 94per cent and maximum identity of 91 per cent was shown by *G. hirsutum* MXO19A11-jhj (AC243135.1) and *G. arboreum* clone GM258G19-jhf (AC243103.1).

The region which showed maximum homology with AC243135.1 *G. hirsutum* and AC243103.1 *G. arboreum* in *ncbi.nlm.nib.gov* site mostly comprised of retro-transposons (Table-5.1). However, few complete cloning sequence genes (7) were also reported from same site *viz.*, alcohol dehydrogenase A gene, putative FAD-dependent oxidoreductase, putative protein disulfide isomerase gene, putative integral membrane protein gene, putative caffeic acid methyltransferase gene, and partial coding sequence of photosystem II protein gene.

Among these, three genes *viz.*, alcohol dehydrogenase A gene, putative protein disulfide isomerase genes and putative caffeic acid methyltransferase gene may be candidate genes for wilt.

The other genes *viz.*, photosystem II protein gene, putative FAD-dependent oxidoreductase, putative integral membrane protein genes are housekeeping genes required for maintenance of regular cellular/ plant activities.

Dowd *et al.* (2004), while studying gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum* f. sp. v*asinfectum* concluded that some of the complex plant defense responses that occur in cotton in response to *F. oxysporum* f. sp. *vasinfectum* infection. Cotton alcohol dehydrogenase (anaerobic stress) was induced in both tissues but another highly redundant Zn-class alcohol dehydrogenase was repressed, possibly indicating different roles for these dehydrogenases. ROS scavengers (peroxidase and oxidoreductase) were

Table 5.1 Sequences producing significant alignments.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AC243135.1	*Gossypium hirsutum* clone MX019A11-jhj, complete sequence	335	806	93 %	2e-88	91 %
AC243103.1	*Gossypium arboreum* clone GM258G19-jhf, complete sequence	311	311	44 %	4e-81	91 %
EF457753.1	*Gossypium hirsutum* retrotransposon putative copia, transposon GORGE3-like, retrotransposon putative gypsy, and transposon putative MuDR, complete sequence; alcohol dehydrogenase A gene, complete cds; transposon copia-like and myosin pseudogene, complete sequence; putative FAD-dependent oxidoreductase and putative protein disulfide isomerase genes, complete cds; retrotransposon, complete sequence; putative integral membrane protein gene, complete cds; retrotransposon putative gypsy, complete sequence; putative caffeic acid methyltransferase gene, complete cds; transposons, retrotransposons putative gypsy, and transposon, complete sequence; putative caffeic acid methyltransferase gene, complete cds; transposon, complete sequence; and photosystem II protein gene, partial cds	287	287	47 %	6e-74	88 %
AC243162.1	*Gossypium hirsutum* clone MX169F24-jhp, complete sequence	278	550	48 %	4e-71	87 %
AC243148.1	*Gossypium hirsutum* clone MX086N22-jhl, complete sequence	268	268	50 %	2e-68	85 %
AC243105.1	*Gossypium arboreum* clone GM276M14-jhh, complete sequence	233	233	47 %	8e-58	84 %

induced in hypocotyls only and were repressed in roots. The induction of antioxidant enzymes at the later stages of infection likely is a response to ROS generated as a secondary response to injury and the oxidative stress effects of infection.

The role of enzymes in cell wall strengthening phenylalanine ammonia lyase, cinnamyl alcohol dehydrogenase, peroxidase and polyphenol oxidase was reported by Ana *et al.* (2000) in *Fusarium* wilt of banana and also reported that these enzymes were upregulated. Accumulation of lignin and lignin like material in infected plant tissues are widely reported for defense mechanism. Lignin is highly resistance to attack by micro-organisms and lignified cell walls are an effective barrier to pathogen entrance and spread.

Caffioylshikimic acid (CSA), a major phenolic compound of date palm roots, represents one of the resistance factors of the host to *F. oxysporum* f. sp. *albedinis*. Caffeic acid showed a larger inhibition of the activity of various CWDE that was more than that of CSA and its inhibiting effect appeared to be more important during their production (Modafar *et al.*, 2000).

Caffeic acid is an key intermediate in biosynthesis of lignin. In human being caffeic acid is an antioxidant in-vitro and also in-vivo (Olthof *et al.*, 2001). Cinnamic acid derivatives including caffeic acid and rosnarinic acid are secondary plant metabolites that have been intensely studied for their antimicrobial and antioxidant activities (Raven *et al.*, 1989, Shetty; 1997, Nascimento *et al.*, 2000, Debersac *et al.*, 2001, Bais *et al.*, 2002).

During allelopathy in plants Miller *et al.* (1991) observed that, caffeic acid concentration is increased in response to *Fusarium oxysporum* infection.

Lignin is made up of linked phenyl propane units and is very resistant to solubilization. The caffeic acid comes as a precursor of lignin i.e. phenyl propane. Lignin deposition is known to occur in response to pathogen attack. Therefore, repression of genes for lignin biosynthesis may represent suppression of defense response by the pathogen in roots (Dowd *et al.*, 2004). and also observed that lignin content in infected and uninfected hypocotyle

tissues, there was no apparent difference in quantity of lignin but may be differences in lignin composition (syringyl: guiacyl ratio) rather than in overall lignin content. Lignan biosynthesis represents a previously unreported response to vascular wilts. Lignans are dimmers of monolignols, which are known to possess antifungal activity (Davin and Lewis, 1992). Lignin inforce the cell wall thereby reducing the spread of pathogen as well as cell wall deposition of phenolic compounds.

Lignification as a mechanism of disease resistance was studied by Vance *et al.* (1980). The biochemical pathway for lignin subunit formation consists of successive hydroxylation and 0-methylation of the aromatic ring and conversion of the side chain carboxyl to alcohol function. Out of three mono-lignols Coniferyl, Sinapyl and P-Coumaryl, alcohols forms a complex three dimentional lignin polymer by successive polymerization reaction. The coniferyl and synapyl alcohols methylated in S-adenosyl methionine dependent reaction. Methylation of hydroxylated monomeric lignin precursor represents a critical step in lignin biosynthsis. This methylation is carried out by caffeic acid-3-O-methyl transferase.

Protein disulfide isomerase is an enzyme in the endoplasmic reticulum that catalyses the formation and breakage of disulfide bonds between system residues within proteins and therefore the protein acts to protein folding. Ray *et al.* (2003) reported rapid induction of protein disulfide isomerase and defense related genes in wheat in response to blotch caused by fungal pathogen *Mycosphaerella graminicola* (Stolf *et al.*, 2011).

5.3 Root anatomy

In the current study, little thickening of cell wall as well as reduction in the size of the cells near the epidermis is observed in the moderately resistant parent AKA-7 however, such structural changes were not seen in susceptible parent Dh-2. Thickening of the cell wall may be due to the lignin accumulation in the cells and further retardation of fungal growth might have reason of resistance in AKA-7. Resistance mechanisms may took place before

or after vascular invasion by the pathogen which led to formation of lignitubers to contain invading hyphae and occlusion of xylem vessels by gel or tyloses and thus, prevents microconidial transport in the xylem (Smith *et al.*, 1981). Chlorocholin chlorides reduce the size of the cells and make them denser, thus, increasing resistance by forming a biological barrier (Kertykova *et al.*, 1985).

The restriction of pathogen growth in vascular tissue is involved in the resistance of cotton to several wilt diseases (Wilhem *et al.,* 1974, Harrison and Beckman, 1982). Shi *et al.* (1993) more specifically found that 'the restriction of fungal spread in the vascular tissue is a characteristic of cotton plants to *Fusarium oxysporum* f. sp. *vasinfectum*. Increase in cytoplasmic content and concomitant decrease in the size of central vacuole is linked to the direct secretion of osmophilic materials into the vessels that coated the fungus. Nature of response dependent on the location of the cells relative to infected vessels. The common response is contact cells showed evidences of cytoplasmic reorganization and increase metabolic activity. Shi *et al.* (Shi *et al.,* 1991[a], Shi *et al.,* 1991[b], Shi *et al.,* 1992) documented varied responses to *Fusarium* infection. Shi *et al.* (1991[b]) observed cellular product in contact cells in resistant and susceptible varieties of US. Globular structures accumulated in vessels superficially resembling tyloses (Shi *et al.*, 1992). It is probable that, these globular structures were in fact terpenoid phytoalexin accumulations being secreted into the vessel lumen. The changes in the structure of contact cells and the associated accumulation of material in vessels were observed in resistant as well as susceptible varieties however, the responses were greater and faster in less susceptible variety (Shi *et al.*, 1992 and Christina, 2007). Further, Baayen (1992) concluded that suberized exodermis and endodermis of roots constitute structural barriers to penetration. Preformed fungitoxic compounds or their precursors also protect against penetration. Cultivar resistance is mostly due, however, to active retardation or localization of the fungus after penetration. Physical resistance in wilt diseases is expressed by local occlusion responses (tyloses, gums)

which block or retard the vertical spread of the fungus, and by cell wall-strengthening responses. Chemical resistance of phytoalexins as defense response is achieved retarding fungal growth, phytoalexins prolong the period that the host is able to form structural barriers to colonization (Baayen,1992).

5.4 Generation Mean

The success of the plant breeding strategies mainly depends on the support of the genetic information on the inheritance of major quantitative traits of economic importance. It is proved by earlier research that quantitative characters of economic importance are under the control of polygenes. The estimation of different types of gene effects and determination of their contribution to heterosis is an important step in commercially exploiting hybrid vigour. The relative magnitude of additive and non-additive genetic variances will decide the breeding methodology to be adopted for exploiting the existing genotypic variation in subsequent generations. Certain assumptions are made in the model to estimate the gene actions

1. Parents are homozygous for the characters under study.
2. Absence of linkage, multiple alleles and lethal genes.
3. Absence of differential variability and differential fertility.
4. No interactions involved between non-allelic genes.

Keeping these in mind, the present study was undertaken to estimate different gene effects governing the quantitative traits in Asiatic cotton.

5.4.1 Mean performance

The mean performance of the cross I (AKA-7 × Dh-2) and cross II (PA-141 × Dh-2) (Table 4.15 and Table 4.16) are discussed here.

5.4.1.1 Cross-I: AKA-7 × Dh-2

The mean performance of the basic generations P_1, P_2, F_1, F_2, B_1 and B_2 of AKA-7 × Dh-2 showed existence of substantial variability in the material for plant height, sympodia per plant, days to 50 % flowering, days to boll bursting, days to maturity, number of bolls per plant, boll weight, 100 seed

weight, lint yield per plant and seed cotton yield per plant. Parents showed wide divergence for plant height, sympodia per plant, days to 50per cent flowering, days to boll bursting, days to maturity, number of bolls per plant, 100 seed weight, lint yield per plant and seed cotton yield per plant.

In general, F_1 performance was better than either of the parents for sympodia per plant, number of bolls per plant, boll weight, 100 seed weight, lint yield per plant, ginning percentage and seed cotton yield per plant. Superiority of F_1 performance over the parents in sympodia per plant, number of bolls per plant, 100 seed weight, lint yield per plant, ginning percentage and seed cotton yield per plant may be due to contribution of P_2 for these traits. P_1 seems to be contributing towards micronaire and fibre strength but not reflected in F_1. Intermediate values of F_1 for plant height, days to 50per cent flowering, days to boll bursting, days to maturity, 2.5 per cent span length, micronaire value and fibre strength showed presence of partial dominance.

Decline in the performance of F_2 for days to boll bursting, days to maturity, sympodia per plant, 100 seed weight, lint yield per plant, ginning percentage, seed cotton yield and 2.5 per cent span length suggested presence of dominance and epistatic interactions in expression of these traits. Similar results were obtained by Mehetre *et al.* (2003a).

Significant increase in plant height in F_2, showed presence of transgressive segregant in F_2 population indicating importance of additive gene action for plant height.

Decrease in F_1 performance was observed in uniformity ratio. This may be due to preponderance of non-allelic interaction as compared to main effects.

The B_1 generation showed better performance for plant height, days to 50per cent flowering, days to first boll bursting, days to maturity, bolls per plant, boll weight, seed cotton yield per plant, uniformity ratio, micronaire

value, fibre strength in comparison to B_2 generation while, B_2 generation was better for 100 seed weight, ginning percentage and 2.5 per cent span length.

5.4.1.2 Cross-II: PA-141 × Dh-2

The mean performance expressed presence of variability for sympodia per plant, bolls per plant, boll weight, 100 seed weight, lint weight per plant, seed cotton yield, 2.5 per cent span length, uniformity ratio and fibre strength in all six generations.

The hybrid was better in performance for plant height, sympodia per plant, bolls per plant, boll weight, 100 seed weight, lint weight per plant, ginning percentage, seed cotton yield per plant and 2.5 per cent span length over the parents. The P_1 seems to be contributed towards increase in performance of F_1 for sympodia per plant, bolls per plant, boll weight, 100 seed weight, lint weight per plant, seed cotton yield while, P_2 seems to be contributed towards plant height, ginning percentage and 2.5 per cent span length. Probably, the parents carried dominant genes for respective traits (Mehetre *et al.*, 2003[a]).

Intermediate performance of F_1 over parents for days to 50per cent flowering, days to boll bursting and fibre strength showed partial dominance for these characters.

Significant decline in F_2 mean performance for sympodia per plant, bolls per plant, boll weight, 100 seed weight, lint weight per plant, ginning percentage, seed cotton yield, 2.5 per cent span length and micronaire value indicate the presence of dominance and epistatic interactions in expression of these traits (Mehetre *et al.*, 2003[a] and 2003[b]).

In plant height, monopodia per plant and days to boll bursting, the F_2 mean performance tended towards F_1 mean which might be due to linkage or complementary factor or both. Similar results were obtained by (Mehetre *et al.*, 2003[b]). In case of days to 50 per cent flowering, days to maturity, 2.5 per cent span length, uniformity ratio and micronaire value the F_2 mean exceeded

both the parental means, indicating presence of transgressive segregant due to additive type of gene action.

The B_1 generation was better for sympodia per plant, bolls per plant, boll weight, 100 seed weight, lint weight per plant, ginning percentage, seed cotton yield, uniformity ratio, micronaire value and fibre strength while, B_2 generation was better for monopodia per plant, days to boll bursting, days to maturity and 2.5per cent span length.

5.4.2 ANOVA

The results of the analysis of variance for various traits of the crosses presented in Table 4.17 are discussed here.

The mean sum of squares were highly significant for plant height, number of sympodia per plant, days to 50per cent flowering, days to boll bursting, days to maturity, number of bolls per plant, boll weight, 100 seed weight, lint yield per plant, seed cotton yield per plant in AKA-7 × Dh-2 while, in PA-141 × Dh-2, mean sum of squares were highly significant for number of sympodia per plant, number of bolls per plant, boll weight, 100 seed weight, lint yield per plant, ginning percentage, seed cotton yield per plant, 2.5 per cent span length and uniformity ratio.

The significance of mean sum of squares for above characters indicated that all the six generations *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 of respective crosses differed significantly for these characters, i.e., there is presence of good variability among generations.

5.4.3 Generation mean analysis

Presence of genetic variability in the population and relative magnitude of gene action involved for different characters are prerequisites for manipulating quantitatively inherited characters in a systematic breeding programme. Its success depends upon both, the amount of variability present in a population with which plant breeder is dealing and so it's efficient management and utilization.

Fisher (1918) first partitioned the continuous variation into additive, dominance and epistasis. Fisher *et al.* (1932), suggested the method for separation of fixable and non-fixable components of variation in segregating population by the used of second and third degree statistics. The earlier methods of determining the genetic components of quantitative variability were based on the assumptions that non-allelic interactions among genes have a rather negligible bias on the estimates of additive and dominant components of variation. To analyze the role of epistasis, however, a genetic model developed by Hayman (1958) and Jinks and Jones (1958) which partitioned epistatic effects into different components from the means of six generations of a cross, *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 when the P_1 and P_2 being two homozygous parents. Likewise, the procedures for analysis of first degree statistics were developed by Mather (1949) and Cavalli (1952). In the present study, efforts have been made to gather information on 16 characters among two crosses involving three parents.

5.4.3.1 Scaling tests

The A, B, C scaling tests (Mather, 1949) and joint scaling test (Cavalli, 1952) were applied prior to the use of the six-parameter model (Hayman, 1958) for the estimation of various genetic components.

The scaling test (Table 4.18) indicated that, either A, B, C or all the three scales deviated from zero for all the characters in both the crosses indicating presence of epistasis due to inter-allelic interactions. Either of one, two or three scales found significant in both the crosses except monopodia per plant, boll weight, ginning percentage, uniformity ratio, fibre fineness, fibre strength in AKA-7 × Dh-2 and plant height, monopodia per plant, days to 50 per cent flowering, days to boll burst, days to maturity and boll weight in PA-141 × Dh-2.

For the above characters Joint scaling test also resulted into significant chi-squre value indicating inadequacy of three parameter model.

The scaling test and joint scaling test agreed closely with each other except 2.5 per cent span length in AKA-7 × Dh-2, uniformity ratio in PA-141 × Dh-2. In 2.5 per cent span length in AKA-7 × Dh-2 and uniformity ratio in PA-141 × Dh-2 Joint scaling test was significant while, Scaling test was insignificant. The gene effects using six parameter model revealed presence of inter-allelic interactions. Ketaka *et al.* (1976[a] and 1976[b]) and Singh and Singh (1978) concluded that joint scaling test was more accurate for indication of interacting crosses. The findings of present study agreed with these conclusions.

5.4.3.2 Gene action

The non-significant estimates for characters indicated the adequacy of the model and suggested the role of either additive and/or dominance gene effect in the expression of these characters. Significance of the scaling tests in rest of characters indicated inadequacy of additive-dominance model and suggested the presence of gene interaction in expression of these characters. Inadequacy of the six parameter model may be due to one or more of the following reasons.

1. Presence of trigenic or higher order non-allelic interaction
2. Some of the assumptions have not been satisfied. For example, g × e interactions might be involved in the inheritance of the characters being investigated.
3. Linkage between two pairs of interacting genes. For those crosses, where neither of A, B, C scaling test nor joint scaling test was significant, estimates of m, d, h were worked out using three parameter model, while six parameter model was applied to all those crosses showing significant scaling tests.

Hayman (1958) emphasized that it is not possible to obtain epistasis-free expectations of the generation means from m, d and h of the six-parameter model. Hence, he concluded that our approximation to epistasis free expectations will therefore, be derived from m, d and h estimated on the

assumption of no epistasis. These expectations are independent of the definition of m, d and h and are unique.

The presence of non-allelic interactions can be detected and their effects simultaneously estimated by fitting models which incorporate their contribution to the means, variances etc. of the generations obtainable from cross. Three components are required to define all interactions between two alleles at each of two loci. These are the interactions when there is homozygosity at both loci (i), that when there is homozygosity at one locus and heterozygosity at the other (j) and that when there is heterozygosity at both loci (l). Thus (i) is the interaction between the additive effects at two loci, (j) the interaction between an additive effect at one locus and dominance effect at the other and (l) the interaction between the dominance effects at two loci (Jinks, 1983).

The six parameter model (Hayman, 1958) was applied in all cases for estimation of the various gene effects in view of interactions indicated by both scaling tests. (Mather's 1949, A, B and C scaling tests and Cavalli's 1952 joint scaling test).

The signs of the effects 'd' and 'j' depend upon the particular parent being considered as P_1 and P_2 for computational work. If the value of P_1 is larger than P_2 then 'd' and 'j' would be positive, if the value P_2 is larger than P_1 then the sign of 'd' and 'j' will take negative sign. The interpretation of the gene effects 'd' and 'j' were required to be considered on their numerical value without regard to their sign. Only (h) and (l) reflect the net direction of the underlying gene action and from these can be deducted the net direction of the dominance and a tentative classification of the type of non-allelic interaction into complementary ([h] and [l] have same sign) or duplicate ([h] and [l] have opposite sign).

5.4.4 Gene effects of various yield contributing characters

5.4.4.1 Plant height

Gene effects governing plant height varied for two crosses. From the data presented in Table 4.19 it is seen that, additive and epistatic component

of gene effects are important for inheritance of the plant height in AKA-7 × Dh-2; while additive dominant model was adequate in PA-141 × Dh-2. In the epistatic component additive × dominant (j) and dominant × dominant (l) interactions were found significant. In general, interaction component (i+j+l) if put together is much higher than the main effects (d+h). Epistatic component playing major role for inheritance of plant height. The negative sign of the 'i' indicated that, there would be decrease in expression of the character in successive generations. The 'h' and 'l' components were having opposite signs, indicating presence of duplicate type of interaction.

The importance of additive gene action for the plant height was noticed by Kalsay *et al.* (1981), Rao (1982), Mirza and Khan (1984), Singh and Singh (1985), Jain *et al.* (1987), Amudha and Ravindran (1997), Khan *et al.* (1991), Verma *et al.* (1991), Shah *et al.* (1993), Bhatade *et al.* (1994) and Pavasia *et al.* (1998) while, additive and epistatic gene effects were reported by Khan and Khan (1993), Singh and Chahal (2003). Additive × additive type of gene action was reported by Kalsay and Vithal (1980). Dominance × dominance type of gene action was found by Ramalingam (1996), Mehetre *et al.* (2003b). Duplicate type of gene action was reported by Pawar (2000), Mehetre *et al.* (2003[b]), Mehetre *et al.*(2003c). Results of the current study are in conformity with the above scientists.

However, various types of gene actions were reported by different scientists in different breeding material. Non-additive type of gene action was reported by Rajesh *et al.* (1979), Bhatade *et al.* (1980), Kalsay and Vithal (1980), Rao (1982), Waldia *et al.* (1984), Lather (1985), Dhanda *et al.* (1987), Mahmood *et al.* (1989) and Sayal *et al.* (1995), Kalwar *et al.* (1998a), Mehetre *et al.*(2003[c]) and Reddy and Nandarajan (2006). Additive as well as non-additive type of gene effects were reported by Patil and Chopde (1983), Pawar (2000), Saravanan and Gopalan (2003), Murtaza *et al.* (2006). Further, Duhoon *et al.* (1984) and Saravanan and Gopalan (2003) reported additive and dominance effects. According to Pawar (2000) plant height is governed by additive, dominance and epistasis type of gene actions. Ahmed

and Mehra (2000) reported dominance and epistasis gene effects while, Sandhu *et al.* (1992) reported dominance and Sayal *et al.* (1995) reported over dominance gene effects while studying gene actions in cotton.

5.4.4.2 Number of monopodia per plant

Insignificant differences in generations for number of monopodia per plant in both the crosses indicating further improvement was not possible in this character.

Different workers had reported different gene effects in inheritance of number of monopodia per plant. Additive type of gene action was reported by Kalsay *et al.* (1981), Bhandari *et al.* (1981), Kenchana Goudar *et al.* (1996), and Pavasia *et al.* (1998) while, non-additive type of gene action was reported by Rao (1982), Khan and Khan (1993), Shah *et al.* (1993), Mohuiddin (1996), Mehetre *et al.* (2003c). According to Pradeep *et al.* (2007) it was governed by both additive and non-additive gene actions. Further, Gawande (2011) found additive, non-additive as well as epistasis gene actions for monopodia per plant. Dominance type of gene action was reported by Rao (1982), Singh and Chahal (2003) however, duplicate dominance epistasis was reported by Mehetre *et al.* (2003b and c). Dominance × dominance was reported by Ahmed and Mehra (2000).

5.4.4.3 Number of sympodia

Significant dominant effect in AKA-7 × Dh-2 and much higher magnitude of dominant component (h) in PA-141 × Dh-2 indicated that the trait was governed by dominance gene effect. In addition to dominance, additive × additive (i) found positively significant in both the crosses while, dominant × dominant gene effect (l) found negatively significant. The epistatic component together (i+j+l) is higher than the main effects together (d+h) indicating importance of non allelic interactions in controlling genetic variation. Though dominant component (h) playing important role, the negative and opposite sign of 'l' showed presence of duplicate gene action which hinders the expression of total heterosis for the trait. The magnitude of

'l' is higher than 'i' and 'j' in AKA-7 × Dh-2 indicating that genes responsible for the inheritance of the character were highly or partially dispersed. Singh *et al.* (1972) suggested that the improvement of such character may be expected through standard genetic procedures which first exploit additive (d) gene effects. Simultaneously, care should be taken to see that dominance variance were not dissipated rather than concentrated. Reciprocal recurrent selection seems to be effective for improving this trait. In PA-141 × Dh-2, only epistasis effect playing important role rather than main effects.

The research findings are in concurrence with the findings of Duhoon *et al.* (1984), Ahmed and Mehra (2000) and Mehetre *et al.* (2003[b]) where dominant gene effects is playing important role. Further, non additive type of gene action was reported by many scientist *viz.*, Silva and Alves (1983), Kadapa *et al.* (1989), Kowsalya (1994), Ramalingam (1996) and Reddy and Nandarajan (2006) while, over dominance was recorded by Shah *et al.* (1993) which indicated non fixable type of gene effect. The current research finding is also in conformity with Pawar (2000) who found duplicate gene action in expression of the character. Rao (1982) and Ahmed and Mehra (2000) noticed only epistatis for the inheritance of the trait. The study of Kenchana Goudar *et al.* (1996), Pavasia *et al.* (1990), Bhatade *et al.* (1994), Mohuiddin (1996) and Pradeep *et al.* (2007) explained the role of both additive and non-additive gene effects, while, Singh and Chahal (2003) and Gawande (2011) reported additive, non- additive and epistasis for the trait.

However, results of the study are in contradictory to finding of Khan and Khan (1993) where they reported additive and epistasis, Ramalingam (1996) and Amudha and Ravindran (1997) reported additive × dominant effect and Mehetre *et al.* (2003[c]) reported complementary epistatis.

5.4.4.4 Number of days to 50 per cent flowering

Higher magnitude of dominant gene effect (h) as compare to additive gene effect in AKA-7 × Dh-2 indicated gene effect is not fixable. Significant additive × dominant (j) and dominant × dominant (l) indicated preponderance

of epistatic gene effect in inheritance of days to 50 per cent flowering. Magnitude of 'l' is higher than 'i' and 'j' type of interactions indicating that genes responsible for the inheritance of this character were highly or partially dispersed (Joshi, 1995). The opposite signs of 'h' and 'l' suggested duplicate type of interaction in the trait. Though 'h' is in positive direction the duplicate action in negative direction seems to be beneficial for reduction in number of days to 50 per cent flowering. Duplicate dominance was also reported by Mehetre *et al.* (2003 b and c). According to Haleem (2010) the additive × dominant (j) parameter is significant and non significant, positive or negative indicating that dominance is towards increasing and decreasing, respectively. However, Ramalingam and Sivasamy (2002) observed preponderance of additive × dominance epistatic effect (highest magnitude) for the trait suggesting delayed selection and intermating the segregants followed by recurrent selection for improving this trait. Further 'j' type of interaction was also recorded by Singh and Yadvendra (2002) for 50 per cent flowering.

Other scientists reported various types of gene actions for days to 50per cent flowering. Additive gene action was reported by Rao (1982), Patil and Chopde (1983), Echekwu and Alabi (1994), Reddy and Nandarajan (2006), non-additive gene action was reported by Baker and Verhalen (1975), Khajjidoni *et al.* (1984), Holla (1986), Mehetre *et al.* (2003[c]) and additive and non-additive gene actions was reported by Patil and Chopade (1983) and Mohuiddin (1996).

5.4.4.5 Number of days to boll bursting

Significance of additive (d) as well as dominant (h) component and additive × additive (i) and additive × dominant (j) interactions for days to boll bursting indicated the importance of all effects for the trait in AKA-7 × Dh-2. Higher magnitude of dominant (h) effect suggested preponderance of dominant gene effect than additive (d). The 'h' and 'l' in opposite direction suggested duplicate type of gene interaction governed the trait. The negative sign of 'l' is beneficial for expression of the character. These finding are in

conformity with Mehetre *et al.* (2003b and c). Similar to days to 50per cent flowering, magnitude of 'l' was higher than 'i' and 'j' type of interactions in days to boll bursting, indicating that genes responsible for inheritance of the character were highly or partially dispersed. On the contrary, Patil and Chopade (1983), Echekwu and Alabi (1994) reported additive type of gene action for the trait.

5.4.4.6 Number of days to maturity

Significance of additive dominance gene effects as well as all three type of inter allelic interactions in AKA-7 × Dh-2, indicated the presence of all these gene actions for inheritance of the trait. Significant and highest magnitude of dominant (h) and dominance × dominance (l) suggested that days to maturity is predominantly under the control of dominant genes and dominant × dominant interaction. The 'h' and 'l' in opposite direction suggested duplicate type of gene interaction governed the trait. The estimates of 'l' is negative and that of 'h' is positive, hence it would tend to reduce the heterosis which is desirable for days to maturity and help the negative additive gene effect to fix the genes for earliness during further selection. Estimates of 'l' was higher than 'i' and 'j' type of interactions in number of days to maturity, indicating that genes responsible for inheritance of the character were highly or partially dispersed.

The results of the study are in concurrence with Singh and Chahal (2003). Mehetre *et al.* (2003b and c) noticed duplicate dominance which also supported the results obtained in the study.

5.4.4.7 Number of bolls per plant

Significant and high estimates of dominance 'h' in both the crosses indicated that the character is under the control of dominance. However, opposite signs of 'h' and 'l' suggested duplicate type of interaction is playing the role in expression of the character in both the crosses which will reduce the actual heterosis effect. The additive × dominant and dominant × dominant interaction effects in AKA-7 × Dh-2 and additive × additive and dominant ×

dominant in PA-141 × Dh-2 are significant. In general, the interaction component altogether (i+j+l) is higher than the main effects together (d+h) indicating importance of interactions in governing the variability in different generations. Significant positive value of 'i' indicated increase in expression of the character in successive generation. Higher estimates of 'l' than 'i' and 'j' type of interactions, indicated that the genes responsible for inheritance of the character were highly or partially dispersed. According to Singh *et al.* (1972), improvement in such character is accomplished by exploiting additive effect first and simultaneous dissipation of dominance variance rather than concentration.

Different scientists reported different effects for number of bolls per plant. Results of the current study are in line with Pathak and Singh (1970), Virupakshappa *et al.* (1978), Kalsay and Vithal (1980), Duhoon *et al.* (1984), Amlraj and Gawande (1985), Singh and Singh (1985), Jagtap and Kolhe (1987), Patel *et al.* (1992), Sandhu *et al.* (1992), Chhabra *et al.* (1994), Murthy *et al.* (1994), Khan (1994), Amudha and Ravindran (1997), Rathore *et al.* (1999), Ahmed and Mehra (2000) and Mehetre *et al.* (2003b and c). These scientists reported dominance component for number of bolls per plant. Duplicate Epistatis was reported by Pawar (2000) and Mehetre *et al.* (2003 b and c). Current study is in conformity with findings of these scientists. Further, Abo-El-Zahab and Methwaly (1979), Desai *et al.* (1988), Kalsay and Vithal (1980), Gill and Kalsay (1981), Singh *et al.* (1982), Silva and Alves (1983), Verma *et al.* (1991), Dagaonkar and Malkhandale (1993), Jain *et al.* (1987) and Ramalingam (1996) reported non-additive type of gene action.

However, additive and non additive gene actions were reported by Desai *et al.* (1988), Gill and Kalsay (1981), Kalsay *et al.* (1981), Patil and Chopade (1983), Waldia *et al.* (1984), Jagtap and Kolhe (1986, 1987), Pavasia *et al.* (1990), Randhawa *et al.* (1991), Bhatade *et al.* (1992), Dagaonkar and Malkhandale (1993), Gupta (1993), Bhatade *et al.* (1994), Kalsay *et al.* (1994), Kenchana Goudar *et al.* (1996), Mohuiddin (1996), Saxena *et al.* (1998), Yingxin and Xiangming (1998), Pawar (2000), Saravanan and Gopalan

(2003), Murtaza *et al.* (2006), Pradeep *et al.* (2007). Whereas, additive, dominance and epistasis gene actions were reported by Bains *et al.* (1982), Kassam *et al.* (1986), Rahman (1986), Rahman *et al.* (1988), Tariq *et al.* (1995a), Reddy *et al.*(2002), Singh and Chahal (2003) and Gawande (2011).

In the different interaction effects, additive × additive gene effect was noticed by Kalsay and Vithal (1980), Srivastava and Kalsay (1990), Chhabra *et al.* (1994), dominance × dominance gene effect by Chhabra *et al.* (1994), Khan (1994), Laxman (2001), Patil and Meshram (2002), Mehetre *et al.* (2003^{b}) while, additive × dominance gene effect by Srivastava and Kalsay (1990), Sanyasi (1991), Chhabra *et al.* (1994) and Patil and Meshram (2002).

5.4.4.8 Boll weight

As per three parameter model significant dominant (h) gene effect in both the crosses *viz.*, AKA-7 × Dh-2 and PA-141 × Dh-2 revealed importance of dominance in governing the character. Absence of interactions suggested the adequacy of the basic additive dominance model to explain the expression of the character. Absence of all the effects was also reported by Pradeep and Sulamini (2008). As per Haleem (2010), the absence of significant 'h' component would imply no dominance between the two parents and dominance effects were not important in the control of the trait in the cross.

Different scientists noticed different effects for boll weight. Additive gene action for boll weight was reported by Dhillon and Singh (1982), Gill and Kalsay (1981), Bains *et al.* (1982), Rao (1982), Deswal and Lather (1983), Patil and Chopde (1983), Chakreshkumar *et al.* (1984), Khajjidoni *et al.* (1984), Amalraj and Gawande (1985), Singh and Singh (1985), Jagtap and Kolhe (1987), Chhabra *et al.* (1994), Murthy *et al.* (1994), Gururajan and Henry (1995), Tariq *et al.* (1995a), Ahmed *et al.* (1997b), Amudha and Ravindran (1997), Krishna Rao (1998), Pavasia *et al.* (1998), Ajuja and Tuteja (1999) and Pradeep *et al.* (2007) while, non additive gene action was reported by Desai *et al.* (1988), Waldia *et al.* (1980), Gill and Kalsay (1981), Bains *et al.* (1982), Rao

(1982), Abo-El-Zahab (1983), Waldia *et al.* (1984), Verma *et al.* (1991), Katageri *et al.* (1992), Dagaonkar and Malkhandale (1993), Ramalingam (1996), Reddy and Nandarajan (2006).

Simultaneous involvement of additive and non-additive gene actions were reported by Gururajarao *et al.* (1977), Tyagi (1978), Desai *et al.* (1988), Waldia *et al.* (1984), Dhanda *et al.* (1987), Jain *et al.* (1987), Pavasia *et al.* (1990), Bhatade *et al.* (1992), Sandhu *et al.*(1992), Dagaonkar and Malkhandale (1993), Gupta (1993), Saxena *et al.* (1998), Saravanan and Gopalan (2003), Jagtap and Kolhe (1986 and 1987), Pawar (2000), while simultaneous involvement of additive, non-additive and epistasis gene actions were reported by Kassam *et al.* (1986), Reddy *et al.* (2002), Gawande (2011). Further, Krishnamurthy and Henry (1979), Duhoon *et al.* (1984), Singh and Singh (1985), Jagtap and Kolhe (1987), Patel and Badaya (1993), Laxman (2001), Mehetre *et al.* (2003[b]), Mehetre *et al.* (2003[c]), Pradeep and Sumalini (2008) recorded dominance for boll weight and Khan *et al.* (1992), Khan and Khan (1993), Shah *et al.* (1993), Sayal *et al.* (1995) reporded over dominance. Among the interactions, additive × additive was noticed by Bains *et al.* (1982), Chhabra *et al.* (1994), Gururajan and Henry (1995), Mehetre *et al.* (2003[b]), Pradeep and Sumalini (2008), additive × dominance by Krishnamurthy and Henry (1979), Bains *et al.* (1982), Srivastava and Kalsay (1990), Patel and Badaya (1993), Chhabra *et al.* (1994), Gururajan and Henry (1995) and dominance × dominance by Patel and Badaya (1993) and Mehetre *et al.* (2003[b]).

5.4.4.9 100 seed weight

Dominant × dominant (l) interaction was found significant in 100 seed weight of both the crosses suggesting importance of the interaction in creation of the variability in the generation. Dominant component (h) though insignificant it has same sign of 'l' showing presence of complimentary type of gene action. Findings of the current research are in conformity with Chabbra *et al.* (1994).

Importance of interaction effects also reported by Shrivastava and Kalsay (1990) and Gawande (2011). In the contrary, Amlraj and Gawande (1985) reported additive gene action for the trait.

5.4.4.10 Lint weight per plant

Significant and high estimates of dominant component (h) and dominant × dominant (l) interaction for lint weight per plant suggested preponderance of dominant (h) and dominant × dominant (l) effects in control of lint weight. In addition to this in PA-141 × Dh-2, additive gene effect and additive × additive interaction effect found significant and in positive direction indicated importance of additive genes which would help to fix the gene effect in early generation selection. However, 'h' and 'l' are in opposite direction indicated duplicate type of gene interaction which would reduce the dominant effects. Jagtap and Kolhe (1986) stated that, when additive effects are larger than non-additive, it is suggested that selection in early generations would be effective, while if the non-additive portion are larger than additive the improvement of the character needs intensive selection through later generations, when epistatic effects were significant for the trait, the possibility of obtaining the desirable segregants through intermating in early generations by breaking the undesirable linkage for handling the above crosses for rapid improvement. Results of the current study are in agreement with Abo-El-Zahab (1983), Nandarajan and Elongovan (1983), Panchal *et al.* (1994), Singh and Yadavendra (2002) and Gawande (2011).

5.4.4.11 Ginning percentage

Additive gene action found to be significant in PA-141 × Dh-2. However, value of dominant gene action is higher than additive gene action. In addition to additive gene action, additive × dominant (j) interaction found significant. The value of additive × dominant (j) is significantly positive, indicated dominance was towards increasing direction. Ramalingam and Sivasamy (2002) stated preponderance of additive × dominance epistatic effect (highest magnitude) for the trait is suggesting delayed selection and intermating the

segregants followed by recurrent selection for improving this trait 'j' type of integration. The dominant gene effect (h) and dominant × dominant (l) interaction having similar sign indicated complimentary gene action.

The current study is in line of Deshmukh *et al.* (1980), Vande and Thombre (1982), Nandarajan and Elongovan (1983) Chakreshkumar *et al.* (1984), Amalraj and Gawande (1985), Singh and Singh (1985), Jagtap and Kohle (1987), Tomer *et al.* (1988), Green and Culp (1990), Srivastava and Kalsay (1990), Verma *et al.* (1991), Bhatade *et al.* (1994), Gururajan and Henry (1995), Panchal *et al.* (1995), Arnudha and Ravindran (1997), Krishna Rao (1998), Mandloi *et al.* (1998); Pavasia *et at.* (1998), Sankarapandian *et al.*(1998), Pavasia *et al.* (1999), Reddy and Nandarajan (2006) and Pradeep and Sumalini (2008) who found additive gene action for ginning percentage. Further, Sandhu *et al.* (1992) observed both additive and dominance gene effects along with digenic interactions

The following scientists reported different interactions effects for ginning percentage *viz.,* Gill and Kalsay (1981), Panchal *et al.* (1994), Singh *et al.* (1972), Singh and Chahal (2005), Dhillon and Singh (1982), Randhawa *et al.* (1991), Chhabra *et al.* (1994), Pradeep and Sumalini (2008), Ramalingam (1996), Ramlingam (2003), Pawar (2000) and Mehetre *et al.* (2003c).

5.4.4.12 Seed cotton yield per plant

High and significant estimates of dominant gene effects in both the crosses indicated that dominant component is playing major role for seed cotton yield per plant in both the crosses. In addition to dominant effect, additive gene effect is also significant in PA-141 × Dh-2. In interaction component, additive × dominant (j) and dominant × dominant (l) in AKA-7 × Dh-2 and additive × additive (i) and dominant × dominant (l) interaction in PA-141 × Dh-2 found significant. The interaction factor altogether (i + j + l) is higher as compare to total main effect (d + h) suggested the importance of epistasis along with main effect in creation of variability in the trait. The

dominant (h) and dominant × dominant (l) interaction having opposite direction indicated duplicate type of gene action. Significant high dominant effect alongwith higher value of additive × additive (i) as compare to additive × dominant (j) suggested intensive selection in later generation and intermating the desirable segregant would improve the seed cotton yield in both the crosses.

Dominance gene effect was reported by Pathak and Singh (1970), Deshpande *et al.* (1984), Duhoom *et al.* (1984), Dhorajia *et al.* (1985), Singh and Singh (1985), Jagtap and Kolhe (1987), Sandhu *et al.* (1992), Murthy *et al.* (1994) and Mehetre *et al.* (2003[b]), while, additive and dominance effects were reported by Jagtap and Kolhe (1986 and 1987) and Kenchana Goudar *et al.*(1986). Further, additive and non-additive gene effects were obtained by Desai *et al.* (1988), Kalsay and Vithal (1980), Rao (1982), Chakreshkumar *et al.* (1984), Kenchana Goudar *et al.* (1986), Dhanda *et al.* (1987), Pavasia *et al.* (1990), Randhava *et al.* (1991), Bhatade *et al.* (1992), Mane and Bhatade (1992), Dagaonkar and Maldhandale (1993), Gupta (1993), Kalsay *et al.* (1994), Kenchana Goudar*et al.* (1996), Mohuiddin (1996), Saxena *et al.* (1998), Yingxin and Xiangming (1998), Rathore *et al.* (1999), Singh and Singh (2001), Rao and Reddy (2002), Reddy and Satyanarayanan (2003), Saravanan and Gopalan (2003), Murtaza *et al.* (2006) and Pradeep *et al.* (2007). All the gene effects additive, dominance and epistasis were reported by Tariq *et al.* (1995[a]), Gawande (2011), Deshmukh *et al.* (1980), Kassam *et al.* (1986), Phogat and Singh (2000), Reddy *et al.* (2002) and Singh and Chahal (2003).

The additive × additive was reported by Chhabra *et al.* (1994) and Laxman and Pradeep (2003) while, dominance × dominance by Patel and Badaya (1993), Chhabra *et al* (1994), Laxman (2001) and Laxman and Pradeep (2003). Laxman and Pradeep (2003) also reported duplicate epistasis for seed cotton yield per plant. Results of current study are in conformity with these scientists.

5.4.4.13 2.5 per cent span length

None of the main effects were found significant for 2.5 per cent span length. However, additive × additive and dominant × dominant interactions were found significant. This indicated the importance of interactions are playing major role in governing 2.5 per cent span length. Similar findings were also noticed by Mehetre *et al.* (2003a and b). The dominant (h) and dominant × dominant (l) interaction having opposite sign indicated duplicate gene action. Duplicate epistasis was reported by Pawar (2000) and Mehetre *et al.* (2003[a] and c). Negatively significant additive × additive interaction indicated that gene effects can not be fixed after selection. Other scientist reported various gene actions such as additive by Nandarajan and Rangaswamy (1990), Shah *et al.* (1993), Pavasia *et al.* (1999), Reddy and Satyanarayanan (2003), Akhtar *et al.* (2008) and Minhas *et al.* (2008); Non-additive by Yingxin and Xiangming (1998); additive and epistasis by Subhan *et al.* (2000), Additive, dominance and epistasis by Nandarajan and Rangaswamy (1991), Phogat and Singh (2000), Thangaraj *et al.* (2002) and Singh and Chahal (2005); dominance and epistasis by Tariq *et al.* (1995a) and Mehetre *et al.* (2003a,b and c).

5.4.4.14 Uniformity ratio

Significant additive gene effect in AKA-7 × Dh-2, indicated that uniformity ratio is under preponderance of additive gene effects. Nandarajan and Rangaswamy (1990) also reported additive gene effect for uniformity ratio. All the interaction effects were found insignificant suggesting adequacy of the additive dominance model in AKA-7 × Dh-2. In PA-141 × Dh-2, negatively significant dominant effect (h) indicated the importance of dominance in creation of the variability in different generations. According to Haleem *et al.* (2010), negative value of 'h' in the trait indicated the alleles responsible for fewer traits were over dominant over the alleles controlling high value. Significance of 'i' and 'l' interactions showed that the interactions played major role in governing the trait. Opposite sign of 'h' and 'l' indicated duplicate

type of gene action. Similar results were obtained by Mehetre *et al.* (2003[a] and [b]). In contrary to this Pawar (2000) reported dominance gene action, while Minhas *et al.* (2008) reported over dominance for uniformity ratio. Further, Nandarajan and Rangaswamy (1991), Phogat and Singh (2000), Thangaraj *et al.* (2002) recorded additive, dominance and epistasis type of gene actions for uniformity ratio.

5.4.4.15 Micronaire value

None of the main effects found significant in both the crosses. Also interactions found insignificant showing adequacy of additive-dominance model in both the crosses. However, additive × additive gene effect found higher in PA-141 × Dh-2 which indicated that this effect will help in fixing the additive gene effect after selection. Same sign of 'h' and 'l' indicated complementary type of gene action governing the trait.

5.4.4.16 Fibre strength

Insignificant differences in generations for fibre strength in both the crosses indicated further improvement was not possible in this character. None of the main effects as well as interaction effects were found significant for the fibre strength. This may be due to sampling error or due to simultaneous inclusion of different components during estimation or it could also be due to almost equal genetical potential of parents involved in the study. For such character, Gamble (1962) has pointed out that where the inheritance of quantitative traits becomes more complex, the contribution of dominant gene effects to their inheritance becomes greater.

5.5 Inheritance of qualitative characters

5.5.1 Leaf shape:

As the there were no significant differences observed in the observed and expected frequencies for leaf shape for cross AKA-7 × Dh-2 and the fit was good for the ratio 3super okra :1 okra, the character is monogenically governed. Super okra is monogenic dominant over the okra. Fyson (1908)

concluded dominance of narrow lobe in the crosses *neglectum* (narrow) × *herbaceum* (broad). In the F_2 of two crosses (*arboreum* variety Nanking × *arboreum*) between narrow and broad lobe types Leake (1911^a and 1911^b) revealed 1:2:1 ratio.

5.5.2 Flower colour:

As there were no significant differences between the observed and expected values for the flower colour for the cross AKA-7 × Dh-2 and the fit is good for the ratio 3 cream : 1 white, it seems that the character is mono-genically governed and cream colour is monogenic dominant over white.

In the second cross, PA-141 × Dh-2, there was no significant differences between the observed and expected values when the expected population considered to be segregated in the ratio of 1 dark yellow : 21 yellow : 20 light yellow : 21 cream : 1 white. It revealed that flower colour in the second cross was governed by three different genes.

It also revealed that the flower colour in AKA-7 × Dh-2 was governed by three genes as Dh-2 was the common parent for both the crosses. Hutchinson (1931) established multiple allelomorph series for petal colour. Silow (1941) and Bholanath (1942) demonstrated complimentary factors for petal colour in Chinese cotton. Kottur (1923) explained modified trihybrid ratio 39:9:16 for inheritance of petal colour in the crosses full yellow of *herbaceum* and white of *arboreum*. However, Mane *et al.* (1987) reported that the petal colour was governed by a single gene (1:2:1) with incomplete dominance in *G. arboreum*.

6. SUMMARY AND CONCLUSION

Asiatic cotton breeding programs focus on improved resistance to a variety of pathogens, integrating mainly *Fusarium* wilt resistance phenotypes into high-yielding, high-fiber quality cultivars. Conventional cotton breeding selections rely mainly on bioassays such as severity of disease symptoms and on the ability of the breeder to identify desirable traits for generation advancement. These techniques can be extremely subjective and in the case of bioassays, can lead to false selections due to environmental factors such as non-uniform distribution of pathogens in the field. Also, genetics of resistance to *Fusarium* wilt is still hidden in case of Asiatic cotton and hence continuous need for breeding for disease resistance remains only viable environmental friendly strategy in disease management. Simultaneously, due to the wider host range, FOV of cotton will persist for longer period even in absence of cotton. Current reference indicates that FOV race-4 also attacks *G. hirsutum.* Thus the treat of FOV in cotton remains constant in near future. Though lot of information is available related to *desi* cotton, there is little or no information available related to *Fusarium* wilt resistance using molecular markers in Asiatic (*desi*) cotton. Marker assisted selection will be very useful in screening for disease resistance as it is a reliable and faster method than classical screening for identification of the *Fusarium* wilt resistance strains in earlier stages of plant growth.

With this view, breeding material of two crosses AKA-7 × Dh-2 and PA-141 × Dh-2 was developed with AKA-7, PA-141 as moderately resistant and Dh-2 as highly susceptible parents during *kharif* 2010 and 2011 at Cotton Improvement Project, M.P.K.V., Rahuri. Parents along with the F_2 population were screened in *kharif* 2011 against *Fusarium* wilt to understand the genetics of *Fusarium* wilt. Identification of the molecular marker associated with *Fusarium* wilt resistance was carried out by using screened F_2 population of AKA-7 × Dh-2 cross along with its parent and ISSR, SSR and RGA markers

at State Biotechnology Laboratory, MPKV, Rahuri, in 2012. In addition to this, further Custom sequences of PCR purified product of 300bp (appro.) fragment eluted in PCR were obtained from m/s Banglore Genei Ltd.

In the second part of the study, the breeding material *viz*; P_1, P_2, F_1, F_2, B_1 and B_2 of these crosses was evaluated in *kharif* 2011 to find out gene effects of yield and yield contributing characters.

6.1 Inheritance of resistance to *Fusarium* wilt

The nonsignificant χ^2 in case-I of AKA-7 × Dh-2, data fitted in 3:1 mendelian ratio indicating resistance to *Fusarium* wilt was monogenically governed and susceptibility was dominant to resistance; However, the nonsignificant χ^2 in case-II of AKA-7 × Dh-2, indicated that the resistance to *Fusarium* wilt was dominant and digenecally governed. The data fitted in 13:3 Mendelian ratio. Hence, it could be concluded that there was presence of inhibitory interaction.

The significance χ^2 in case-I of PA-141 × Dh-2, data did not fit in 3:1 Mendelian ratio indicating resistance to *Fusarium* wilt was not monogenically governed. The insignificance χ^2 in case-II of PA-141 × Dh-2 indicated that the resistance to *Fusarium* wilt was dominant and digenecally governed. The data fitted in 13:3 Mendelian ratio. Hence, it could be concluded that there was presence of inhibitory interaction.

6.2 Molecular analysis

6.2.1 Bulk segregant analysis

The NLRR-inv-1/2 primer-277 bp band was found associated with *Fusarium* wilt resistance in Asiatic cotton *G. arboreum*. It was present in resistant parent (RP) as well as in resistant bulk (RB) which was absent in both susceptible parent (SP) and susceptible bulk (SB). Further, this band was also present in all individual resistant F_2 plants while, it was absent in all

F_2 susceptible plants. This primer was identified as marker for differentiating the resistance and susceptibility to *Fusarium* wilt in Asiatic cotton hence, would be utilized for marker assisted selection of resistant plants at early stages plant growth in future.

6.2.2 Sequencing

The cross AKA-7 × Dh-2, 300bp (apprpx.) band was observed co-segregating with *Fusarium* resistance in *G. arboreum*. Although marker was based on RGA analogue primer but, sequence analysis of the 300bp fragment did not reveal presence of NBS-LRR R genes. However, sequence homology analysis showed homology with *G. hirsutum* and *G. arboreum* clones having few candidate genes *viz.,* putative caffeic acid methyltransferase, putative protein disulfide isomerase genes and alcohol dehydrogenase A gene, which might be responsible for *Fusarium* wilt resistance.

6.3 Root anatomy

Resistance mechanisms may have taken place before or after vascular invasion by the pathogen. Physical resistance mechanism in AKA-7 was observed by little thickening cell wall as well as reduction in the size of the cells near the epidermis. However, such structural changes were not seen in susceptible parent Dh-2. Thickening of the cell wall may be due to the lignin or phytoalaxin accumulation in the cells and further retardation of fungal growth might be the reason for resistance in AKA-7.

6.4 Gene Action

6.4.1 Analysis of Variance

All the characters except monopodia per plant, ginning percentage and fibre characters differ significantly in AKA-7 × Dh-2 while, in PA-141 × Dh-2 sympodia per plant, number of bolls per plant, boll weight, 100 seed weight, lint weight per plant, ginning percentage, seed cotton yield per plant, 2.5% span length and uniformity ratio were found significant suggesting all the generations of the crosses studied differed significantly from each other

indicating presence of good amount of variability for these characters among generations.

6.4.2 Scaling test

The scaling test indicated that either A, B or C significantly deviated from zero for all the characters except monopodia/plant, boll weight, ginning percentage, uniformity (%), micronaire and fibre strength in cross AKA-7 × Dh-2 while, in case of cross PA-141 × Dh-2 one of the scales was significant for all the characters except plant height, monopodia/plant, days to flowering, days to boll bursting, days to 50% maturity boll weight and fibre strength. Joint scaling test also resulted in high significant chi-square value for the characters where any one scale was also found significant. All the three scaling tests were significant for days to plant height in AKA-7 × Dh-2 and 100 seed weight in PA-141 × Dh-2 suggesting presence of higher order interactions responsible for expression of these characters. Significant scaling and joint scaling tests revealed the presence of epistasis in characters while, non significance of scaling test as well as joint scaling tests indicated absence of epistasis and only additive- dominance model was adequate for the inheritance of those characters.

6.4.3 Generation mean analysis

A. Interacting crosses: (6 parameter model)

- Amongst 16 characters studied dominant component 'h' was higher than additive component 'd' for most of all characters. Additive component was higher only for plant height in AKA-7 × Dh-2.
- The main effect 'h' (dominant) was significant for sympodia per plant, days to boll bursting, days to maturity, boll per plant, boll weight, lint weight per plant, seed cotton yield per plant while, additive gene effect (d) was significant for plant height, days to boll bursting and days to maturity in AKA-7× Dh-2. In PA-141 × Dh-2, main effect 'h' was significant for bolls per

plant, boll weight, lint weight per plant, seed cotton yield per plant and uniformity ratio while, additive gene effect 'd' was significant for lint weight per plant, ginning percentage, seed cotton yield per plant.

- When interaction components 'i+j+l' were put together, against main effects 'd+h', main effects are larger for sympodia per plant, lint weight per plant, seed cotton yield per plant in both the crosses, days to boll burst, days to maturity in AKA-7 × Dh-2 and uniformity in PA-141 × Dh-2. The interaction component is higher for plant height, 50% flowering, 100 seed weight and span length in AKA-7 × Dh-2 and 100 seed weight, ginning percentage and micronaire value in PA-141 × Dh-2.
- The 'h' and 'l ' are in opposite direction indicates duplicate type of interaction for most of all the characters excepting 100 seed weight in AKA-7 × Dh-2 and PA-141 × Dh-2 and ginning percentage and micronaire value in PA-141 × Dh-2. Duplicate type of gene interaction tends to reduce the heterosis effect as such is not desirable while, complimentary epistasis increases the heterosis.
- The additive gene effect (d) and additive × additive gene interaction (i) found significant simultaneously for days to maturity in AKA-7 × Dh-2, lint weight per plant and seed cotton yield in PA-141 × Dh-2. These characters can be improved by progeny row selection.

B. Non interacting crosses: (Three parameter model)

When epitasis is non significant or absent the main effects, account for the phenotypic mean performance of the cross. When the dominant component (h) is significant the characters can be improved by heterosis breeding and when additive component (d) found significant the characters can be improved by selection. The dominant component (h) found significant for boll weight in AKA-7 × Dh-2 and PA-141 × Dh-2. The cause of heterosis may be over dominance.

The breeding programme as per the gene effects of different yield contributing characters is summarized in Table 6.1 and 6.2.

Table 6.1 Breeding strategies suggested for improvement of various characters in AKA-7 × Dh-2

Sr.No.	Characters	Gene effect	Epistatic interaction effect	Breeding strategies
1	Plant height	Additive	Add × dom (j), Dom × dom (l)	Recurrent selection
2	Sympodia/ plant	Dominance	Add × add (i), Dom × dom(l)	Recurrent selection
3	Bolls/plant	Dominance	Add × dom (j), Dom × dom(l)	Recurrent selection
4	Days to 50% flower	Dominance	Add × dom (j), dom × dom (l)	Recurrent selection
5	Days to first boll bursting	Additive and dominance	Add × add (i) and Add × dom (j)	Recurrent selection
6	Days to maturity	Additive and dominance	Add × add (i), add × dom (j) & dom × dom (l)	Recurrent selection
7	Boll weight	Dominance	--	Heterosis
8	100 seed weight	--	Dom × dom (l)	Heterosis
9	Lint weight/plant	Dominance	Dom× dom (l)	Recurrent selection
10	Seed cotton yield/plant	Dominance	Add × dom (j) & dom × dom (l)	Recurrent selection
11	2.5 % Span length	--	Add × add (i), dom × dom (l)	Recurrent selection

Table 6.2 Breeding strategies suggested for improvement of various characters in PA-141 × Dh-2

Sr.No.	Characters	Gene effect	Epistatic interaction effect	Breeding strategies
1	Sympodia/ plant	Dominance	Add × add (i), Dom × dom (l)	Recurrent selection
2	Bolls/plant	Dominance	Add × add (i), Dom × dom (l)	Recurrent selection
3	Boll weight	Dominance	--	Heterosis breeding
4	100 seed weight	--	Dom × dom (l)	Heterosis
5	Lint weight/plant	Additive and dominance	Add × add (i), dom × dom (l)	Recurrent selection
6	Ginning percentage	Additive	Add × dom (i), dom × dom (l)	Recurrent selection
7	Seed cotton yield/plant	Additive and dominance	Add × add (i), dom × dom (l)	Recurrent selection
8	2.5 % Span length	--	Add × add (i), dom × dom (j)	Recurrent selection
9	Uniformity ratio (%)	Dominance	Add × add (i), dom × dom (j)	Recurrent selection

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GENETIC ANALYSIS OF WILT RESISTANCE IN INTRASPECIFIC CROSSES OF ASIATIC COTTON (*G. arboreum* L.)

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