

**GENETICAL, BIOCHEMICAL AND MOLECULAR
INVESTIGATIONS IN MUNGBEAN (*Vigna radiata* L
Wilczek) FOR SEED YIELD AND POWDERY
MILDEW RESISTANCE**

by

Amol Madhavrao Dethé

Regd. No. 0107

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1149

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722, DIST. AHMEDNAGAR,
(M.S.) INDIA**

in the partial fulfillment of requirement for the degree of
DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

CYTOGENETICS AND PLANT BREEDING

DEPARTMENT OF AGRICULTURAL BOTANY

POST GRADUATE INSTITUTE

**MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722**

~ 2005 ~

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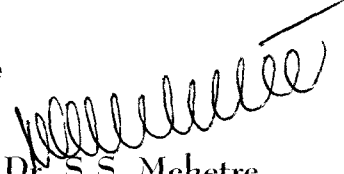
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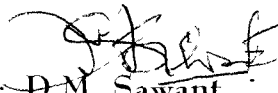
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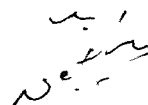
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CANDIDATE'S DECLARATION

I hereby declare that this thesis or part hereof
has not been submitted by me or any other
person to this or any other University
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or Diploma

Place : M P K V , Rahuri

Dated : 24/06/2005


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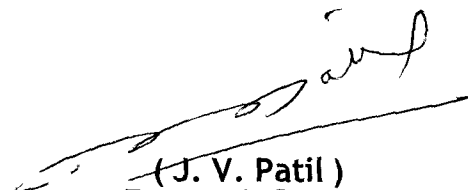
C E R T I F I C A T E

This is to certify that the thesis entitled "**Genetical, biochemical and molecular investigations in mungbean (*Vigna radiata* (L.) Wilczek) for seed yield, its components and powdery mildew resistance**", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri- 413722, Dist - Ahmednagar (Maharashtra State) in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) in CYTOGENETICS AND PLANT BREEDING**, embodies the results of a piece of *bona fide* research work carried out by **Shri. AMOL MADHAVRAO DETHE**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma

The assistance and help received during the course of this investigation is duly acknowledged.

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Place : M.P K V , Rahuri

Date : 24/06/2005


(S. H. Shinde)

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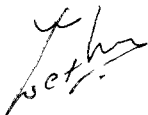
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Date: 24th June, 2005


(Amol M. Dethe)

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ABSTRACT

GENETICAL, BIOCHEMICAL AND MOLECULAR INVESTIGATIONS IN MUNGBEAN (*VIGNA RADIATA* (L.) WILCZEK) FOR SEED YIELD, ITS COMPONENTS AND POWEDERY MILDEW RESISTANCE

By

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A candidate for the degree of

Doctor of Philosophy (Agriculture)

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Research Guide	:	Dr. J. V. Patil (Professor of Agril Botany)
Department	:	Agricultural Botany
Major Field	:	Cytogenetics and Plant Breeding

The present investigations were conducted at Central Campus, MPKV, Rahuri and Agharkar Research Institute (MACS), Pune during 2000-2004 with the objective to study the heterosis and combining ability, the DNA polymorphism through molecular markers, nature and magnitude of gene action controlling yield, its components and quality traits alongwith powdery mildew resistance (*Erysiphe polygoni* D C.) through pathological and biochemical parameters in mungbean (*Vigna radiata* (L) Wilczek). Seven diverse parents were selected and intermated in half diallel fashion to evaluate the heterosis and combining ability Gene action study for yield quantitative characters was attempted on selected five crosses Whereas, three of these crosses, comprising susceptible and resistance parents to powdery mildew were chosen to know the genetics of powdery mildew resistance using two pathological and four pathogenesis-related biochemical parameters

The heterosis study revealed that the crosses, BM 4 x TARM 18, Vaibhav x TARM 18, AKM 8802 x BM 4, and PM 9341 x BM 4 displayed significant mid parent heterosis and heterobeltiosis for seed yield/ plant and some of its components, and such crosses can be exploited to isolate

transgressive segregants in subsequent generations. The parents, Vaibhav, TARM 18 and BM 4 were proved to be good general combiners with good *per se* performance for most of the traits studied, suggesting scope for their use in breeding programme. The hybrids, Vaibhav x TARM 18, PM 9341 x BM 4, BM 4 x TARM 18 and PM 9341 x BPMR 145 evinced high *sca* effects for most of the traits, from which superior transgressive segregants may be obtained.

Molecular markers namely, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) were used to screen seven parental genotypes for assessing the genetic diversity between/ among them. ISSR technique was appeared to be more efficient than the RAPD approach in detecting the polymorphism in mungbean *i.e.* 54.7% and 44.0%, respectively. Variety-specific amplifcons were generated with 21 different polymorphic primers, which revealed the genetic distance ranged between 0.116 and 0.250. Thus, the RAPD and ISSR fingerprinting has been successful in detecting the variations at intraspecific level to cluster the mungbean genotypes into different groups. DNA marker-based genetic distance (heterozygosity) was found to be significantly associated with 100 seed weight and seeds/ pod, while seed yield/ plant and its majority of components were low, but found positively associated. Such correlations based on specific positive markers, however in large, may have practical utility for predicting heterosis. This study also provides baseline information for the genetic resource collection management and identification in Indian mungbean.

In generation mean analysis for yield, its components and quality traits (protein, tryptophan and methionine), additive, dominance and non-allelic interactions were found involving in the expression of almost all these characters. However, non-additive (dominance components) gene action was predominant in inheritance of seed yield and its components; hence selection should be delayed until virtual homozygosity^{1/2} attained. Single seed descent method may be adopted to handle the segregating generations and develop pure line efficiently.

The parents Vaibhav and TARM 18 were found resistant to powdery mildew, whereas Kopargaon was the most susceptible. Pathological assessment studies projected that the speed of disease development was slower in resistant than that of susceptible parents. The host-parasite interactions quantified in terms of per cent disease index (PDI) and area under disease progress curve (AUDPC) was observed under the control of both additive and non-additive gene actions.

Pathogenesis related biochemical studies divulged that there was strong association of these biochemical characters with disease score. The levels of enzymes (chitinase, β -1, 3-glucanase and polyphenol oxidase) and potash in resistant genotypes were high as compared to their levels in susceptible genotypes. Although, there was less consistency of any type of gene action component across the crosses at pre and post infection stages, the biochemical parameters were predominated by non-additive gene action in most of the cases.

A study of gene actions for pathological and biochemical parameters suggested that early generations may be intermated in segregating population and selection may be postponed due to presence of non-additive gene action with duplicate type of epistasis in the crosses studied.

Chapter Opener Page



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INTRODUCTION

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) ($2n=2x=22$) is a leguminous species, grown as pulse crop principally for its protein-rich edible seeds, which is originated from Indian sub-continent (Vavilov, 1926, Zukoveskij, 1962; DeCandolle, 1986) and is the third important pulse after Bengalgram and Redgram. Being a short duration crop with wider adaptability, it is grown all over the country in different seasons as a sole crop and as an intercrop or mixed crop with cereals, forming important component of crop rotations.

In Maharashtra, it is the second important *kharif* crop after pigeonpea, grown on an area of 7.62 lakh ha. The production is 3.76 lakh tonnes with the productivity of 493 kg/ha (Anonymous, 2004). However, in India, it is grown on an area of 25.3 lakh ha with 346 kg/ha productivity and 9.6 lakh tonnes total production (Anonymous, 2003).

The present yield potential of improved varieties is not enough to attract the farmers/ consumers, because of relatively smaller seed size, low yield potential and susceptibility to disease. Conventional breeding method can not achieve the desirable goal without precise understanding of gene action involved for yield potential, its quality or resistance and availability of such genes in germplasm. The knowledge of combining ability provides useful tool for selection of desirable parents and cross combinations for further exploitation in breeding programmes to be employed for the desired improvement of the crop.

Mungbean, a major 'Asian pulse crop' seed are easily digestible and forms excellent source of high quality proteins in Asian diet. The protein of mungbean is less expensive than animal protein. To improve the protein level and quality of protein, the genetics of the protein or its constituents may provide useful clue for the further desired improvement.

The yield losses due to powdery mildew (*Erysiphe polygoni* D C) are reported to be 20-40% (Fernandez and Shanmugasundaram, 1987) and 100% at the seedling stage (Reddy *et al*, 1994). Under such

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conditions evolving resistant varieties seems to be the only easy approach and ecofriendly endeavor. Arrays of biochemicals are produced in the plants as a result of Host-Parasite interaction. The genetics of these pathogenesis-related biochemicals, those impart resistance to range of diseases, can be made use for planning and implementing resistance breeding programme.

The knowledge of genetic diversity and relatedness in the germplasm is a pre-requisite for crop improvement programmes. Both morphological traits and isozyme markers have been used for this purpose, but they have several disadvantages such as their limited number, environmental dependence and temporal-spatial expression. More recently, DNA markers have been reliably used in cultivar identification, diversity analysis, gene maps construction (QTLs) and tagging agronomically important genes. Another important application of DNA markers is the prediction of heterosis in hybrids. However, no comprehensive effort has been made to investigate the Indian elite mungbean using random amplified polymorphic DNAs and inter-simple sequence repeats. In a crop like mungbean, in which no other marker systems are designated and established, such techniques would be a useful method that enables rapid identification of various markers and estimate the genetic relationship and diversity of the populations.

The present investigation was, therefore, designed with the following objectives

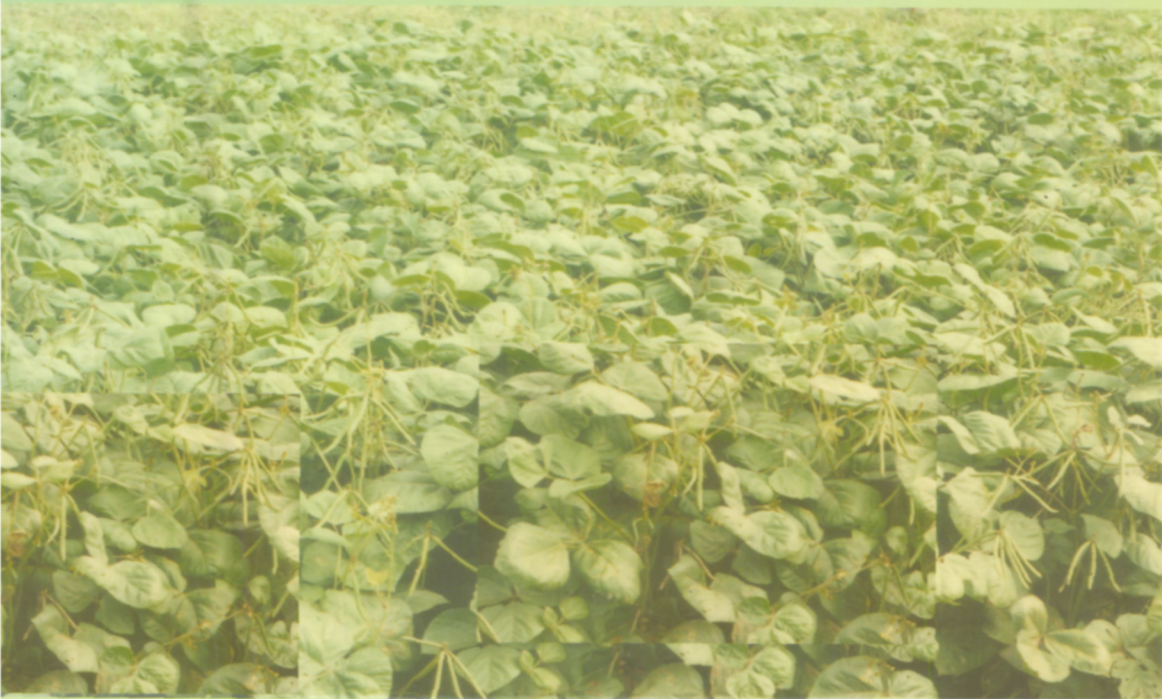
- i To estimate heterosis for grain yield and its components**
- ii To estimate the general and specific combining ability effects for seed yield and its components**
- iii To study the DNA polymorphism in parental genotypes using molecular markers**
- iv To study the genetics of seed yield, its components and nutritional quality traits through generation mean analysis**
- v To study genetics of host-parasite interaction and plant biochemical defence through generation mean analysis.**

Chapter Opener Page



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REVIEW OF LITERATURE



REVIEW OF LITERATURE

The literature pertaining to the present investigation on the following aspects has been reviewed and broadly categorized under relevant sub-titles in this chapter

2.1 Botany

2.2 Heterosis

2.3 Combining ability

2.4 DNA polymorphism by molecular markers

2.5 Gene effects from generation means

2.5.1 Gene action for seed yield, its components and quality traits

2.5.2 Gene action for pathological attributes

2.5.3 Genetics of biochemical basis of powdery mildew resistance

2.1 Botany

Plant kingdom which includes small portion of crop plants, the legumes are a large group among them, second to cereals as a source of human food. The word 'Legume' is derived from the Latin word 'lerge' which means 'to collect' indicating easy hand harvesting of the crops instead of being threshed from the plants as cereal grains. Legumes belong to family Leguminosae, the crops belonging to this family are characterized by their fruits, which are pods, and by their (usually) alternate, pinnate or trifoliate leaves (Cobley and Steele, 1976). The pulses are group of crops belonging to legume family. The word 'Pulse' is defined as the split cotyledons of dry legume seeds boiled in excess of water soften, macerated and used as soup.

Mungbean is a rapidly growing erect or suberect annual, usually 40-120 cm in height and showing considerable variation in form and adaptation. The root system may be mesophytic or xerophytic. It is frequently much branched with slight tendency to twilling its upper branches. The leaves are trifoliate with large, ovate entire or rarely lobed membranous leaflets, 5-10 cm long, with scattered hairs on both sides and light to dark green in colour.

The calyx and bracts are ovate to oblong. The yellow or greenish yellow flowers are crowded in clusters of 10 to 20 on auxiliary or terminal (mostly later) racemes. The flowers are fully self-fertile and normally self-pollinated. The pods are subcylindrical long, wide, straight or lightly curved. Normally containing 10 to 20 small seeds with globular or oblong often green but may be yellow brown or speckled black coloured.

Narsimham (1929) reported the results of preliminary studies on floral biology of mungbean. It bears typical papilionaceous flowers consisting of five petals one standard, two wings and two keels with diadelphous anther condition. Just before the opening of flowers pollen grains are seen out of their sacs on the previous night by 9 p.m. and by 11 p.m., most of the stigmas are thoroughly dusted with pollen grains. Pollinations completed in all the cases by 1.30 p.m. The minimum interval between the dehiscence of anthers and opening of flower is about 4 hours, thus causing self-fertilization. These studies were also confirmed by Bose (1932).

2.2 Heterosis

The superiority of hybrids over their parents is termed as 'heterosis', which was first recognized by Dr. J.G. Koelreuter in 1673 in artificial *Nicotiana* spp. In 1876, Darwin concluded that hybrid from unrelated plant types were highly vigorous. Subsequently, many workers reported the same in a large number of plant species. Shull (1914) coined the term 'heterosis' and defined it as a phenomenon in which crossing of two genetically dissimilar gametes (or individuals) produce increased (or decreased) vigour over their parents. Most of our present knowledge perhaps comes from the work on maize (*Zea mays*).

Bhatnagar and Singh (1964) reported heterosis for the first time in the interspecific crosses with mungbean. In pulses, Singh (1971) clarified that at present the only possibility would be to make use of heterosis for developing high yielding pure lines.

The hybrid vigour in the first generation generally reflects potentiality of its segregants in the succeeding generations. The crosses

manifesting good heterotic expression in F_1 are likely to give better segregants in later generations, when non-additive genetic effects are low (Shah and Patel, 1981) Extent of heterosis over better parent (BP), mid parent (MP) and standard variety (SV) for various characters have been studied to obtain the information of heterozygotes' improvement

2.2.1 Days to 50 % flowering

Non-significant heterosis for this character was reported by Swindell and Poehlman (1976) and Singh (1980) Ranges of mid parent heterosis and heterobeltiosis alongwith authorities are given below

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	-11.42 to 3.99	-9.99 to 10.33	Manjare (1976)
2	---	-6.35 to 6.04	Shah and Patel (1981)
3	-3.83 to 6.67	-2.56 to 17.08	Halkude (1992)
4	---	-3.19 to 22.32	Reddy <i>et al</i> (1992)
5	---	-2.56 to 16.00	Kelkar (1993)
6	-10.83 to 4.00	-5.32 to 10.19	Patil (1993)
7	-5.84 to -0.77	-5.48 to 1.56	Naidu and Satyanarayana (1993 ^a)
8	-6.27 to 1.06	-2.26 to 8.03	Naidu and Satyanarayana (1993 ^b)
9	-7.41 to 2.39	-10.71 to 0.00	Sonawane (1995)
10	-4.17 to 3.32	-2.70 to 14.4	Patil <i>et al</i> (1996 ^a)
11.	---	2.85 to 23.82	Hegde <i>et al</i> (1996 ^b)
12	-8.60 to 1.77	-4.06 to 4.91	Kute (1997)
13	-8.56 to -3.63	-6.09 to -0.52	Sawale (1999)
14	---	-10.71 to 13.00	Aher <i>et al</i> (2000 ^a)
14	---	-2.56 to 16.00	Aher and Dahat (1999)
15		-3.04 to 1.87	Aher <i>et al</i> (2000 ^b)
16	-12.50 to 34.02	-24.39 to 31.31	Reddy <i>et al</i> (2003)

2.2.2 Days to maturity

Negative heterobeltiosis for days to maturity was reported by Manjare (1976), Shah and Patel (1981), Halkude (1992), Patil (1993) and Jahagirdar (2001). Non-significant heterosis was reported by Swindell and Poehlman (1976). Aher *et al* (2000^a) observed highest negative heterobeltiosis i.e. -6.09% for this trait. Different ranges of mid parent heterosis and heterobeltiosis alongwith authorities are given below.

Sr. No	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	---	-1.84 to 22.02	Reddy <i>et al</i> (1992)
2		-3.22 to 0.92	Naidu and Satyanarayana (1993 ^a)
3	---	-2.05 to 4.45	Naidu and Satyanarayana (1993 ^b)
4	-11.70 to 1.6	-6.64 to 6.10	Sekhar <i>et al</i> (1994)
5	---	-1.00 to 15.00	Patil <i>et al</i> (1996 ^b)
6	---	0.26 to 16.66	Hegde <i>et al</i> (1996 ^b)
7	-8.95 to 3.18	-8.72 to 8.79	Kute (1997)
8	-16.00 to -1.41	-13.34 to 11.35	Reddy (1998)
9	-2.22 to -0.82	-0.18 to 3.21	Sawale (1999)
10	---	0.46 to 11.11	Aher and Dahat (1999)
11	---	-6.70 to 0.58	Aher <i>et al</i> (2000 ^b)
12	-1.64 to 22.72	-10.05 to 18.85	Reddy <i>et al</i> (2003)

2.2.3 Plant height

Positive heterobeltiosis of 5.62%, 16.3% and 10.99% was observed by Mishra *et al*. (1970), Singh and Jain (1970) and Aher *et al* (2000^a), respectively for plant height. Thimmappa (1987) and Natrajan (1992) reported significant heterosis for plant height. Various ranges of mid parent heterosis and heterobeltiosis including authorities are presented here.

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	-12.02 to 49.74	-20.17 to 45.09	Manjare (1976)
2	---	1.28 to 68.18	Shah and Patel (1981)
3	-10.95 to 13.10	-17.17 to 12.17	Halkude (1992)
4	---	-9.48 to 19.81	Reddy <i>et al.</i> (1992)
5	-7.22 to 8.27	15.28 to 6.60	Kelkar (1993)
6	---	-12.55 to 7.52	Patil (1993)
7	---	-20.52 to 12.09	Naidu and Satyanarayana (1993 ^a)
8	---	-20.07 to -2.94	Naidu and Satyanarayana (1993 ^b)
9	-17.70 to 35.20	-24.3 to 27.7	Sekhar <i>et al.</i> (1994)
10	-4.07 to 13.29	-9.90 to 10.99	Sonawane (1995)
11	---	5.78 to 19.14	Hegde <i>et al.</i> (1996 ^b)
12	-10.20 to 8.37	-12.30 to 14.90	Patil <i>et al.</i> (1996 ^a)
13	-3.94 to 13.65	-4.67 to 13.29	Kute (1997)
14	-16.95 to 35.09	-21.00 to 21.20	Reddy (1998)
15.	---	-15.28 to 6.60	Aher and Dahat (1999)
16	2.64 to 15.65	1.84 to 8.06	Sawale (1999)
17	---	-3.88 to 5.49	Aher <i>et al.</i> (2000 ^b)
18	---	-49.73 to 5.34	Joseph and Santoshkumar (2000)
19	-23.19 to 9.75	-39.90 to 5.34	Reddy <i>et al.</i> (2003)

2.2.4 Primary branches per plant

Non-significant heterobeltiosis was observed for primary branches per plant (Hegde *et al.*, 1996^a) Aher *et al.* (2000^a) estimated 16.67% heterobeltiosis for this trait. Negative heterobeltiosis as well as

standard heterosis for this trait was observed by Joseph and Santoshkumar (2000) Number of workers have reported different ranges of mid parent heterosis and heterobeltiosis as given below Bhatnagar and Singh (1964) reported heterosis to the tune of 43.9% over mid parent

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	---	-36.7 to 30.8	Singh and Jain (1970)
2	-13.19 to 51.33	-7.15 to 41.74	Kelkar (1993)
3	---	-23.57 to 44.59	Naidu and Satyanarayana (1993 ^a)
4	---	-16.59 to 94.69	Naidu and Satyanarayana (1993 ^b)
5	-7.53 to 18.52	-14.00 to 16.67	Sonawane (1995)
6	---	-8.20 to 19.60	Patil <i>et al</i> (1996 ^a)
7	---	0.00 to 45.80	Patil <i>et al</i> (1996 ^b)
8	-17.65 to 62.07	-26.47 to 62.07	Kute (1997)
9	---	-7.15 to 41.47	Aher and Dahat (1999)
10.	0.21 to 20.4	-1.60 to 8.09	Sawale (1999)
11	---	-12.73 to 2.38	Aher <i>et al</i> (2000 ^b)

2.2.5 Pod clusters per plant

For pod cluster per plant 20.6% heterobeltiosis was recorded (Singh and Jain, 1970) Singh and Singh (1974) estimated 30.6% heterobeltiosis for this trait Reddy *et al* (1982) also reported significant heterosis for this character Aher *et al* (2000^a) observed heterobeltiosis to the extent of 23.68% Ranges of mid parent heterosis and heterobeltiosis alongwith authorities are presented here

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	25 54 to 55 58	-29 03 to 55 58	Manjare and Deshmukh (1981)
2.	---	-25 02 to 42 87	Shah and Patel (1981)
3.	30 75 to 79 51	-57 34 to 37 31	Halkude (1992)
4	-24 47 to 67 54	-57 43 to 13 28	Kelkar (1993)
5	---	-43 66 to 58 15	Patil (1993)
6	---	-5 66 to 16 35	Naidu and Satyanarayana (1993 ^a)
7	---	-9 50 to 9 40	Naidu and Satyanarayana (1993 ^b)
8	-26 30 to 80 30	-34 30 to 67 00	Sekhar <i>et al</i> (1994)
9	-7 19 to 26 17	-12 66 to 23 68	Sonawane (1995)
10	---	0 00 to 36 74	Hegde <i>et al</i> (1996 ^b)
11	-8 91 to 79 03	-17 90 to 43 80	Patil <i>et al</i> (1996 ^a)
12	---	-11 76 to 29 41	Patil <i>et al</i> (1996 ^b)
13	-6 10 to 29 80	-12 50 to 19 51	Kute (1997)
14	-26 66 to 78 66	-44 82 to 45 67	Reddy (1998)
15	---	-57 43 to 13 28	Aher and Dahat (1999)
16	1 67 o 5 29	-0 70 to 4 55	Sawale (1999)
17	---	-17 72 to 25 39	Aher <i>et al</i> (2000 ^b)
18	-30 45 to 26 44	-40 64 to 18 20	Reddy <i>et al</i> (2003)

2.2.6 Pods per plant

Remarkable heterobeltiosis i.e 17 31 and 21 17% was reported for pods/ plant (Mishra *et al*, 1970 and Singh and Jain, 1970). Manjare (1976) reported heterosis in the range of -29 76 to 82 82% over mid parent Swindell and Poehlman (1976), Singh (1980), Thimmappa (1987) and Choudhary (1986) reported significant heterosis for this trait

Aher *et al* (2000^a) estimated heterobeltiosis to the extent of 49.54%

Other authors reported different ranges as given below

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	-16.75 to 71.28	-75.15 to 41.18	Manjare and Deshmukh (1981)
2	-29.18 to 109.3	-56.95 to 69.04	Halkude (1992)
3	---	-19.80 to 55.17	Reddy <i>et al</i> (1992)
4	-28.29 to 54.88	-57.05 to 24.50	Kelkar (1993)
5	---	-15.79 to 21.42	Naidu and Satyanarayana (1993 ^a)
6	---	-10.40 to 14.76	Naidu and Satyanarayana (1993 ^b)
7	-10.26 to 12.64	-15.71 to 8.95	Patil (1993)
8	-11.80 to 121.8	-33.10 to 101.4	Sekhar <i>et al</i> (1994)
9	1.75 to 56.02	-9.60 to 49.54	Sonawane (1995)
10	---	-25.50 to 69.05	Patil <i>et al</i> (1996 ^a)
11	---	-19.60 to 118.18	Patil <i>et al</i> (1996 ^b)
12	---	-11.39 to 38.64	Hegde <i>et al</i> (1996 ^b)
13	10.23 to 80.47	8.90 to 69.32	Kute (1997)
14	-5.67 to 125.7	-9.85 to 111.89	Reddy (1998)
15	---	-57.05 to 24.50	Aher and Dahat (1999)
16	5.23 to 18.64	2.56 to 15.47	Sawale (1999)
17	---	-10.19 to 27.09	Aher <i>et al</i> (2000 ^b)
18	---	-5.51 to 12.17	Joseph and Santoshkumar (2000)
19	-36.73 to 152.2	-46.69 to 152.2	Reddy <i>et al</i> (2003)

2.2.6 Seeds per pod

Significant positive heterobeltiosis was observed for seeds per pod (Mishra *et al* 1970, Singh and Jain, 1970, Manjare, 1976, Swindell and Poehlman, 1976, Shah and Patel, 1981, Halkude, 1992, Kelkar, 1993, Patil, 1993, Sonawane, 1995 and Jahagirdar, 2001) Heterobeltiosis to the extent of 155% was observed by Aher *et al* (2000^a) However, non-significant heterobeltiosis was reported by Aher and Dahat (1999) Different ranges of heterobeltiosis and mid parent heterosis alongwith workers are given below

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	---	-19 10 to 65 43	Reddy <i>et al</i> (1992)
2	---	-6 64 to 6 61	Naidu and Satyanarayana (1993 ^a)
3	---	-12 35 to 1 97	Naidu and Satyanarayana (1993 ^b)
4	-39 50 to 25 20	-39 60 to 24 00	Sekhar <i>et al</i> (1994)
5	---	0 20 to 3 20	Patil <i>et al</i> (1996 ^a)
6	---	-28 15 to 55 35	Patil <i>et al</i> (1996 ^b)
7	---	-5 32 to 7 93	Hegde <i>et al</i> (1996 ^b)
8	-3 38 to 14 82	-7 62 to 12 44	Kute (1997)
9	-5 85 to 12.24	8 57 to 7 02	Reddy (1998)
10	0 46 to 5 21	-0 92 to 2 83	Sawale (1999)
11	---	-7 23 to 9 62	Aher <i>et al</i> (2000 ^b)
12	---	0 03 to 2 74	Joseph and Santoshkumar (2000)
13	-17 81 to 30 45	-18 63 to 30 15	Reddy <i>et al</i> (2003)

2.2.7 100 seed weight

Aher *et al* (2000^a) estimated 10.0% heterobeltiosis for 100 seed weight. Various ranges of heterobeltiosis and mid parent heterosis including authorities are given hereafter.

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	---	-41.70 to 13.11	Halkude (1992)
2	---	-39.36 to 7.37	Reddy <i>et al</i> (1992)
3	---	-9.29 to 4.89	Naidu and Satyanarayana (1993 ^a)
4	---	-5.48 to 2.74	Naidu and Satyanarayana (1993 ^b)
5	-14.50 to 10.40	-20.40 to 8.30	Sekhar <i>et al</i> (1994)
6	-31.10 to 17.93	---	Sonawane (1995)
7	---	-25.00 to 13.30	Patil <i>et al</i> (1996 ^a)
8	---	-16.47 to 23.17	Patil <i>et al</i> (1996 ^b)
9	-15.50 to 23.87	-27.87 to 12.52	Kute (1997)
10	-5.00 to 15.00	-10.77 to 11.08	Reddy (1998)
11	---	-41.60 to 34.45	Aher and Dahat (1999)
12	1.49 to 9.80	1.18 to 6.01	Sawale (1999)
13	---	-10.51 to 13.41	Aher <i>et al</i> (2000 ^b)
14	---	-9.85 to 16.06	Joseph and Santoshkumar (2000)
15	-9.79 to 24.07	-14.40 to 23.29	Reddy <i>et al</i> (2003)

2.2.8 Seed yield per plant

Bhatnagar and Singh (1964) observed that interspecific crosses exhibit high degree of hybrid vigour ranging from 108 to 252%. Significant positive heterobeltiosis for seed yield/ plant was observed by Mishra *et al* (1970), Singh and Jain (1970), Singh and Singh (1974), Manjare (1976), Swindell and Poehlman (1976), Shah and Patel (1981),

Halkude (1992), Kelkar (1993), Patil (1993), Sonawane (1995) and Jahagirdar (2001)

Patil *et al* (2003) derived physiological basis of heterosis in mungbean and indicated an appreciable amount of heterosis for most of the physiological attributes alongwith the seed yield. Heterobeltiosis was observed to the extent of 55.66% (Aher *et al*, 2000^a). Swamy and Reddy (2004) observed that the parents separated by medium D^2 values generally showed high heterosis for all the characters studied. Various ranges of mid parent heterosis and heterobeltiosis alongwith workers are given below

Sr. No.	Mid Parent heterosis (%)	Heterobeltiosis (%)	Reference
1	---	-7.40 to 61.69	Natarajan (1992)
2	---	3.13 to 70.85	Reddy <i>et al</i> (1992)
3	---	-25.50 to 32.73	Naidu and Satyanarayana (1993 ^a)
4	---	-8.49 to 25.89	Naidu and Satyanarayana (1993 ^b)
5	-29.40 to 25.30	-32.80 to 50.30	Sekhar <i>et al</i> (1994)
6	---	-4.90 to 52.50	Patil <i>et al</i> (1996 ^a)
7	---	21.67 to 91.63	Patil <i>et al</i> (1996 ^b)
8	---	-10.31 to 31.97	Hegde <i>et al</i> (1996 ^a)
9	-1.96 to 88.17	-0.07 to 66.73	Kute (1997)
10	-16.27 to 94.50	18.28 to 63.80	Reddy (1998)
11	---	-42.09 to 38.42	Aher and Dahat (1999)
12	13.12 to 16.50	5.20 to 11.70	Sawale (1999)
13	---	-9.33 to 42.23	Aher <i>et al</i> (2000 ^b)
14	---	4.03 to 10.64	Joseph and Santoshkumar (2000)
15	6.54 to 70.75	-6.86 to 51.91	Patil <i>et al</i> (2003)
16	-29.42 to 110.77	-45.55 to 77.54	Reddy <i>et al</i> (2003)

2.3 Combining Ability

The concept of combining ability is useful especially in connection with 'testing' procedures, which involves the study and comparison of the performance of homozygous inbred lines in hybrid combinations. It is a technique that has been extensively used to classify the parental lines in cross combinations, which provides estimates of effects as well as variances. This concept is well established in plant breeding since it distinguishes parental lines, provides means to understand the nature and magnitude of gene action involved and also aids in devising a suitable breeding procedure.

Sprague and Tatum (1942) initially put forth the concept of combining ability. They defined general combining ability as "the average performance of a line in a series of crosses", and described it primarily to additive genetic variance or additive gene action, while specific combining ability (sca) as "those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved," which is regarded as the estimate of non-additive gene action.

Griffing (1956) gave a generalized concept for combining ability of diallel cross and outlined the procedure for determining general and specific combining ability effects and variances from diallel set of different compositions.

Allard (1960) defined general combining abilities as 'the average performance of a strain in a series of crosses' and specific combining ability as 'the deviation from the performance predicated on the basis of general combining ability'.

The account of work performed on combining ability studies in mungbean is briefly presented below

Singh and Jain (1971) studied 6 x 6 diallel in green gram to determine *sca* and *gca* effects and gene action for pod length and seed size and observed that *gca* was more important for pod length, whereas, *gca* and *sca* both were important for seed size

Singh and Singh (1972) noticed that the *gca* variances were higher than the *sca* variances, where the crosses with higher *sca* generally involved High x Low (*gca*) combinations and revealed the importance of additive as well as non-additive gene effects

Singh and Singh (1974) observed the *gca* and *sca* variances were important for yield, however *gca* variance was more important for seed size, pod number, cluster number and pods per cluster. The hybrids with high *sca* had one of the parents as high general combiner for yield and other traits

The highest *gca* estimate in parents viz, Russian-1 for seed yield per plant, B-1 for pods per plant, Madira for seeds per pod and Kopargaon-1 for test weight was reported by Tiwari and Ramanujam (1974)

Yohe and Poehlman (1975) reported that the *gca* effects for seed yield/ plant and its principal components were much larger than *sca* effects and *sca* effects being non-significant in general, suggesting the variables were predominantly controlled by loci with additive gene effects

The *gca* mean squares were significant for all the characters except for pods per plant, while *sca* mean squares were significant for plant height and branch length, as reported by Swindell and Poehlman (1976)

Luthra *et al* (1977) found that *gca* variances were significant for all the characters and were more than *sca* variance, especially for seed yield and its components and this *sca* for pod number and yield was related to *gca* of its parents as all superior hybrids comprised at least one parent with high *gca* effects

Nijhawan and Chandra (1977) revealed that analysis of components of genetic variances with a high non-additive component for grain yield. More or less similar results were obtained through analysis of variance for the combining ability. Further, they pointed out that the selection in subsequent generation of the hybrids may be concentrated in the crosses having high F_1 performance and involving good combining parents

Gupta *et al* (1978) reported that good *gca* could not be taken as an indication of good performance. The female parent H-70-16 and the male parent C-65 were the best general combiners for most of the characters

Considering the overall performance, Rao and Naggur (1979) suggested the parents Jawahar-45 and R-288-8 shall be utilized in further breeding programme to enhance the yield, where the high *sca* effects were observed in the crosses 12-67 x J-781 and Jawahar-45 x R-288-8

Both *gca* and *sca* variances were highly significant for all the traits. The variances due to *sca* were higher for seed yield, pods per plant and seeds per pod, which indicated the importance of these components for seed yield (Luthra *et al*, 1979)

Ahuja (1980) stated that highly significant differences among progenies for all the traits except for number of branches per plant. The magnitude of *gca* variances was higher than *sca* variance for all the traits except for pods per plant

Deshmukh and Manjare (1980) reported that the variances due to *gca* and *sca* were highly significant for days to flowering, days to maturity, plant height, number of pod clusters/ plant, pods/ cluster, number of pods/ plant, seed yield/ plant and 100 seed weight

In diallel selective mating, Malhotra *et al* (1980) opined that parents on the basis of *gca* may result in breaking up some undesirable linkages and release greater genetic variability. They reported the significant mean squares due to *gca* and *sca* for number of pod clusters per plant and pods per plant

Baseeruddin and Naggur (1981) found non-additive gene action for the expression of grain yield and it was suggested that the parent Jawahar-45 may be used in hybridization programme for increasing the yield in green gram

Lal *et al* (1982), revealed high *gca* effects in K-851 for grain yield and its components, PS-16 for earliness and H-70-16 for bold seeds. In cross ML-1 x K-851 high *sca* effects were observed for 100 seed weight and seed yield. Higher *gca* variances than *sca* variances were observed by Reddy *et al* (1982) for almost all the traits except for plant height indicating the predominance of additive gene action

Out of 45 hybrids from 10 x 10 half diallel, four hybrids were further studied in the F_2 and F_3 by Reddy *et al* (1983). Two of these selected hybrids showed high *sca* estimates for yield and its components and the other two had low *sca* estimates

Singh and Pathak (1983) reported that *gca* variances were significant for all the characters in F_1 and F_2 except for days to maturity and pods per plant in F_2 . The *sca* variances were significant in both the generations except for pods per plant in F_2 . The *gca* variances were higher than *sca* variances for all the traits in both the generations

Wilson *et al* (1985) reported predominant additive gene action for almost all the characters investigated

Choudhary (1986) revealed the *gca* variances were highly significant for plant height, branches per plant, pods per plant and seed yield per plant, while, *sca* variances were significant for plant height and seed yield per plant

Thimmappa *et al* (1986) found that Varsha and J-781 were the best general combiners and Varsha x Pusa Baisakhi, Varsha x J-781 and S-8 x PS-16 were the best specific combinations for seed yield and pods per plant

The *gca* variances were higher than *sca* variances for almost all characters except for number of pods per plant. The crosses having high *sca* effects involved all combinations of high, medium and low combining ability parents (Thimmappa, 1987)

Thimmappa *et al* (1987) observed that the variances due to *gca* effects were more predominant for number of branches per plant and 100 grain weight, while *sca* variances were more predominant for plant height, days to flower, pod clusters per plant, pods per cluster, pods per plant, seeds per pod and seed yield

Patel *et al* (1988) revealed that *gca* and *sca* variances were significant for all characters studied except for branches per plant and seeds per pod, respectively

Natrajan *et al* (1989) observed best *sca* effects for four characters out of six in the cross ML-65 x ADT-2

In the study of combining ability by Naidu *et al* (1992), additive gene action was found for days to maturity, plant height, pod clusters per plant, pods per plant, pod length, seeds per pod, 100 seed weight, seed protein and seed yield, while non-additive gene

action observed for days to 50% flowering, branches per plant and shoot dry weight at harvest

Reddy *et al* (1992) on comparing the relative magnitudes of the *gca* and *sca* variances revealed the predominance of additive gene effect in the expression of days to flower, days to maturity, plant height at maturity, seeds per plant and 100 seed weight and non-additive gene effects for seed yield per plant. They also noticed that the high degree of diversity among the parents might be the important factor responsible for greater magnitude of non-additive genetic variance.

Saxena and Sharma (1992) revealed that both *gca* and *sca* were significant for yield per plant, branches per plant, pods per plant, pods per cluster, pod clusters per plant and seeds per pod indicating importance of additive as well as non-additive variances, former being predominant.

From Line x Tester (4 x 5) mating design, Naidu and Satyanarayana (1993^a) reported that additive gene action was found to be predominant for days to 50% flowering, days to maturity, pods per plant and seed protein, whereas non-additive gene action for plant height, seed weight and seed yield. Both additive and non-additive gene actions were found to be important for branches per plant, pod clusters per plant, pod length and seeds per pod.

Higher *gca* to *sca* ratios indicated the predominance of additive gene actions in the expression of characters studied (Saxena and Sharma, 1993).

Tiwari *et al* (1993) stated that variances due to general and specific combining ability were significant for all characters and additive gene effects were important for branches per plant and test weight, while non-additive gene effects were important for days to maturity,

plant height, pods per plant, seed yield per plant and seed protein were predominant

Holkar (1994) noticed that mean squares due to general and specific combining ability were significant for plant height, branches per plant, days to flowering, days to maturity, 100 seed weight, pods per plant, seeds per pod, biological yield per plant and harvest index. Further, he realized that non-additive gene action played an important role in expression of majority of characters, although the additive genetic variance was substantial.

Rosaiah *et al.* (1994) reported that the 100 seed weight and seed yield were controlled by additive gene action in their studies.

Combining ability study for 10 quantitative traits revealed the significant *gca* and *sca* variances for all the characters except *gca* for plant height and *sca* for grains per pod. The *gca* : *sca* variances ratio signified the predominant role of additive variance for days to 50% flowering, days to maturity, pod length, number of grains per pod and test weight (Mansuria and Joshi, 1994).

Sonawane (1995) observed that the variances due to *gca* and *sca* were significant for all the characters except *sca* variances for plant spread and number of primary branches. Ratio of *gca* to *sca* was higher indicating the predominance of additive gene action for all the characters studied except the grain yield per plant.

Halkude *et al.* (1996) in their 8 x 8 half diallel study revealed that the variances due to *gca* and *sca* were highly significant for days to 50% flowering, days to maturity, plant height, pod clusters per plant, pods per plant, pods per cluster, number of grains per pod, 100 seed weight and grain yield per plant, while *sca* variance was non-significant for seeds per pod.

Dasgupta *et al* (1998) observed that mean squares due to *gca* and *sca* were highly significant for all the characters. However, additive gene effects were predominant for pods per cluster, harvest index and seed yield per plant. On the contrary, both additive and non-additive components of variances were equally important for plant height, days to 50% flowering, pods per plant and protein percentage.

Kute *et al* (1999) concluded that variances due to general and specific combining ability were significant for days for 50% flowering, days to maturity, plant height, pod clusters per plant, pods per cluster, pods per plant, seeds per pod, 100 seed weight and seed yield per plant, suggesting both additive and non-additive gene effects were involved in the inheritance of these characters.

The *gca* variances were highly significant for plant height, leaves per plant, pod clusters per plant, pod weight per plant, pod length and seed yield per plant. However, *sca* variances were significant for branches per plant and pods per cluster including above all characters (Jain *et al*, 2000).

Aher *et al* (2001) found significant *gca* and *sca* effects for all the attributes studied except *sca* effects for primary branches per plant.

Jahagirdar (2001) reported that the estimates of *gca* was more than the *sca* variance for days to 50% flowering and days to maturity, 100 seed weight and seed yield per plant, suggesting the predominance of additive gene action for these traits.

Combining ability study by Khattak *et al* (2001) on the components of synchronization in maturity and determinate growth habit in mungbean revealed the predominant significant additive gene action only in DDd2 (degree of determination from first pod stage to 90% pod maturity).

Singh and Dikshit (2003), in their Line x Tester analysis, found that the *sca* estimates were higher than *gca* variances for all the traits, indicating the importance of non-additive gene effects in the

expression of the traits and the cross JPM 99-124 x IPM 99-125 was the best combination not only for its high and desirable *sca* effect for yield per plant, but also for other agronomic traits including harvest index and pods per plant

2.4 DNA Polymorphism by Molecular Markers

The progress made in DNA marker technology has been tremendous and exciting. DNA markers have provided valuable tools in various analyses ranging from phylogenetic analysis to the positional cloning of genes. By virtue of their features like ubiquitous nature, detection at any developmental stages and independent of environmental effects and management, these molecular markers have a direct applicability to plant breeding programmes. DNA marker technology has found application in fingerprinting genotypes, in determining seed purity, in systemic sampling of germplasm and in phylogenetic analysis. This review emphasizes the potential of this technology for the genetic improvement of plants, particularly the *Vigna* species.

Molecular markers developed in plant genetics, since the late 1980s were generally neutral, in the sense that their polymorphism did not contribute directly to the variation in traits of interest. With the discovery of revolutionary restriction fragment length polymorphism (RFLP) by Botstein *et al* (1980) and polymerase chain reaction (PCR) by Mullis (1990), which can be used as molecular markers, the scenario changed and a variety of molecular markers such as, several types of PCR based DNA markers have been developed including random amplified polymorphic DNAs (RAPDs) [Williams *et al* 1990, Welsh and McClelland, 1990], sequence tagged sites (STS) [Olson *et al*, 1989, Talbert *et al*, 1994], Simple sequence repeats (SSRs) or microsatellites (Beckmann and Solier, 1990, Zietkiewicz *et al*, 1994 and Roder *et al*, 1995) and amplified fragment length polymorphisms (AFLPs) [Zabeau, 1993]. Some of the important applications of molecular markers, such as elite materials characterization of elite materials and marker-

assisted backcrossing of Mendelian traits or favourable QTL alleles with major effects, are at present routinely used in many programmes (Kumar, 1999 and Asin, 2002)

This review aims at providing a global overview of the state-of-art of the application of molecular markers in *Vigna* species comprising mungbean (*Vigna radiata* L) and their potential in its breeding, as there is very scanty published literature on mungbean alone

Mungbean and cowpea are currently believed to represent divergent but parallel evolutionary lineages in genus *Vigna* (Baudin and Marechal 1985, Smartt 1985 and 1990)

Young *et al* (1992) mapped the TC 1966 bruchid resistance gene using restriction fragment length polymorphism (RFLP) markers in mungbean. Based on RFLP analysis, an individual was also identified in the F₂ population that retained the bruchid resistance gene within a tightly linked double crossover

Menancio *et al* (1993) compared mungbean (*Vigna radiata* (L) Wilczek) and cowpea (*Vigna unguiculata* (L) Walp) genomes using RFLP mapping data, and found that 90% of the probed hybridized to both the species DNA, indicating a high degree of similarity in the nucleotide sequences among these species. A higher level of polymorphism was detected in the mungbean population (75.7%) than in the cowpea population (41.2%). Further they reported that RFLP loci accounted for about 89% of the currently mapped markers with a few DNAs and RAPD markers added. The comparative mapping demonstrated that the entire linkage groups were not conserved, but several large linkage blocks were maintained in both genomes. The same maps consisting of all available markers including 20 cDNA markers in mungbean and 5 cDNA and 5 RAPD markers in cowpea have been published earlier (Fatokun *et al* 1993)

A direct application of genetic linkage maps has been in tagging genes of economic importance with molecular markers, particularly the powdery mildew resistance, where single genes conditioning resistance have been reported by various eminent workers in different crops, as mentioned below

Crop	Causal organism of powdery mildew	Resistance gene(s)	References
Wheat	<i>Erysiphe graminis f sp tritici</i>	<i>Pm1, Pm2, Pm3, Pm4</i>	Hartl <i>et al</i> , 1993, Ma <i>et al</i> , 1994
Barley	<i>Erysiphe graminis</i>	<i>ml-0, ml-a, MI(La)</i>	Schuller <i>et al</i> , 1992, Giese <i>et al</i> , 1993
Tomato	<i>Leveillula taurica (Lev)</i> Arnaud	<i>Lv</i>	Chunwongse <i>et al</i> ,1994
	<i>Oidium lycopersicon</i>	<i>OI-1</i>	Van der Beek <i>et al</i> ,1994

Young *et al* (1993) used RFLPs to map genes in mungbean that confer partial resistance to the powdery mildew fungus. The results indicated that putative partial resistance loci for powdery mildew in mungbean can be identified with DNA markers, even in a population of modest size analyzed at a single location in single year.

Ratnaparkhe *et al* (1995) detected DNA polymorphisms to distinguish the pigeonpea cultivars as well as its wild relatives using RAPD markers, thereby indicating the immense potential of RAPD in the genetic fingerprinting of pigeonpea.

Santalla *et al* (1998) revealed genetic diversity in mungbean germplasm using RAPD markers.

The "VC" lines, developed at AVRDC, tended to be much closer than the materials obtained from South Asia, when they were

analyzed by RAPD markers to determine the degree of genetic diversity among these varieties. Based on the 100 RAPD bands, the tested materials could be separated into four main clusters revealing the baseline information about the relationship between these promising materials (Anonymous, 1999)

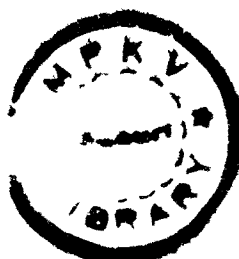
Ajibade *et al* (2000) reported the usefulness of inter-simple sequence repeats analysis to establish genetic relationships in the genus *Vigna*

Lambrigdes *et al* (2000) reported two genetic linkage maps of mungbean derived from the cross Berken x ACC 41 using 52 RFLPs and 56 RAPDs that grouped 67 F₂ individuals into 12 linkage groups. The regions of segregation distortion identified in the Australian maps did not coincide with regions of the Minnesota genome map

Chida *et al* (2000) carried out RAPD analysis to tag the *cry* locus in cowpea (*Vigna unguiculata*) containing *cry* gene which confers resistance against cucumber mosaic virus infection, which resulted in many polymorphisms in amplified DNA patterns. As a result, they obtained three RAPD markers, having the *cry* locus with the help of linkage analysis using parental and/or F₂ cowpea DNAs

Lakhanpaul *et al* (2000) used the RAPD markers to fingerprint the mungbean cultivars and analyzed the variation among them

A much low level of genetic variation was observed in cultivated and weedy adzuki beans (*Vigna angularis*) compared to wild adzuki bean, when 42 accessions were assessed with RAPD features. In addition, high genetic diversity in wild adzuki bean in subtropical highlands of Asia was regarded as an important genetic resource in its improvement (Mimura *et al*, 2000)



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Singh *et al* (2000) reported a set of certain polymorphic markers by using anchored simple sequence repeat primers in mungbean

Ubi *et al* , (2000) constructed a genetic linkage map through QTL analysis using recombinant inbred population derived from an inter-subspecific cross of cowpea (*Vigna unguiculata*), which comprised 80 mapped loci (77 RAPD and 3 morphological loci) assembled into 12 linkage groups

Cidrack *et al* (2001) carried out an analysis of the genetic variability of eight cowpea genotypes by mean of chromosome number determination and amplification of RAPD molecular markers to reveal that though, the somatic chromosome number of $2n=22$ was found in all genotypes, the RAPD markers were showed to be polymorphic with total of 68 bands being amplified by the nine decamer primers used

Ranade and Gopalakrishna (2001) characterized twelve black gram (*Vigna mungo*) varieties using RAPD technique Of the 40 random primers, only 2 primers were able to amplify 5 to 6 polymorphic bands and 6 primers amplified 3 polymorphic bands to distinguish all the blackgram cultivars

To construct a genetic linkage map for cowpea with PCR-based molecular markers, 520 random RAPD primers were screened for parental polymorphism, of which 90 RAPD markers (from 60 primers) were segregated in 75 F_2 mapping population derived from the cross of local cultivars GSC01 and GSC02, where linkage map spanned 474.1 cM across 11 linkage groups (Shim *et al* , 2001)

Xavier *et al* (2001) optimized and revealed the RAPD markers, a fast, simple and useful tool to identify divergent cowpea groups and established fingerprint profiles for the genotypes to assess phylogenetic relationships among them

Chaiteng *et al* (2002) employed both restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) analysis to map a new source of resistance to powdery mildew in mungbean, where a major resistance QTL was found on AFLP linkage group that accounted for 64.9% of the variation in resistance to powdery mildew. One of the probes developed in this study showed the potential to assist in breeding for powdery mildew resistance in mungbean.

An improved genetic linkage map was constructed for cowpea (*Vigna unguiculata*) based on the segregation of various molecular markers combining AFLP, RFLP, RAPD, biochemical markers and biological resistance traits in a population of 94 recombinant inbred lines (RILs) derived from the cross between the breeding lines, IT84S-2049 and 524B. These markers displayed usefulness for the development in cowpea breeding as well as for subsequent map-based cloning of various resistance genes (Quedraogo *et al*, 2002).

Weder *et al* (2002) investigated 62 legume seed samples representing 25 species to identify them by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). They identified 19 legume species including green gram (*Vigna radiata*) using informative primers and suggested that the technique may also be suitable to identify the remaining species studied.

Fall *et al* (2003) employed genetic characterization of cultivated Senegalese varieties of cowpea (*Vigna unguiculata*) based on DNA polymorphism by RAPD technology to recognize the national germplasm in order to eliminate the putative duplicates, and to identify elite varieties.

RAPD analysis by Massaswe *et al*, (2003) in *Vigna subterranea* classified the 12 landraces in two clusters and revealed high levels of polymorphism as well as genetic relationship within landraces. Moitra *et al* (2003) indicated that RAPD markers constitute a powerful tool for the analysis of genetic variability among different accessions of moth bean (*Vigna aconitifolia*) species.

Genetic characterization of Malawian cowpea landraces was studied to determine the pattern and extend of RAPD marker variation within and among cowpea population from different agro-ecological zones and to estimate the degree of genetic relationships among different landraces used by local farmers. The genetic distance values among accessions varied between 0.09 and 0.57 (Nkongolo, 2003)

Humphry *et al* (2003) reported a QTL map to identify a major locus conferring resistance to the powdery mildew in mungbean with RFLP clones tested against two parents, *cv* ATF 3640 (highly susceptible) and *cv* Berken (highly resistant)

Zong *et al* , (2003) compared the genetic distances within and between clusters in *Vigna angularis* revealed by AFLP analysis and found a weak genetic diversity in main cultivated accessions and the wild, weedy types from Japan, while a broad genetic diversity was shown in the wild accessions found elsewhere and in the cultivars from Himalayan region

Pandey *et al* (2004) observed DNA polymorphism in radiation induced mutants of cowpea (*Vigna unguiculata*) by DNA fingerprinting based on RAPD, where mutant specific polymorphic markers either alone or in combination were detected and stated that this DNA fingerprinting of the mutants will facilitate their identification, registration and determination of seed purity

➤ **Molecular markers in relation to heterosis**

Neutral markers and functional polymorphism offer many promising applications in plant breeding. Molecular markers can be used to better document the organization of genetic diversity between possible parental materials of new breeding programmes, to accelerate the alleles, to contribute to hybrid performance prediction, and to establish the distinctiveness of new cultivars prior to their registration (Charcosset and Gallais, 2002)

While studying the genetic basis of heterosis in rice Zhang *et al* (1994) in a diallel analysis of heterosis using two classes of molecular markers as, RFLPs and microsatellites found that the heterozygosity was significantly correlated with several attributes of performance and heterosis, and stated that the large correlations may have practical utility for predicting heterosis

Barbosa *et al* (1996) found the correlation between RFLP-based estimates of genetic distance and coefficient of parentage (CoP) was non-significant, whereas coefficient of parentage was significantly correlated with heterosis for all traits in one year only

Xiao *et al* (1996) evaluated ten elite inbred lines used in hybrid rice breeding in China and analyzed with 100 arbitrary decamer oligonucleotide primers and 22 microsatellites (SSRs) primer sets *via* polymerase chain reaction and indicated that genetic distance measures based on RAPDs and SSRs may be useful for predicating yield potential and heterosis of intra-specific hybrids

Sant *et al* (1999) investigated, if DNA markers are useful in predicting F_1 performance and heterosis in chickpea diallel set They reported that although, molecular marker-based (RAPDs, microsatellites) genetic distance did not linearly correlate to heterosis, two heterotic groups could be identified on the basis of the general marker heterozygosity

Naghia *et al*, (2002) derived molecular genetic distances among 19 hybrid rice parental lines using RAPD markers The cluster analysis based on these markers revealed close genetic relationship among rice genotypes currently used in the hybrid rice breeding programmes

2.5 Gene Effects from Generation Means

The pioneer workers were Johnson (1909), Nilson-Ehle (1909) and East (1916) to study quantitative genetics. Continuous variation was first partitioned by Fisher (1918) into additive, dominance and epistasis. Fisher (1932) suggested the method for separation of fixable and non-fixable components of variation in segregating population by the use of second and third degree statistics.

Further, Mather (1949) used additive-dominance model to describe the effect of the gene, assuming absence of interactions between non-allelic genes. Comstock and Robinson (1948), Mather (1949) and Hayman (1958) proposed the genetic models to estimate the gene effects. However, mostly the importance was given to additive and dominance gene effects.

Anderson and Kempthorne (1954) and Hayman (1957) showed the presence of epistatic gene effects in sufficient magnitude for the quantitative characters.

Cavalli (1952) proposed Joint scaling test, which combines, very effectively, several scaling tests into a unique and more general approach. Secondly, this test provides best possible estimates of all the parameters needed to describe differences among means of families, in addition to the test of adequacy of additive dominance model.

Six parameter model is the simplest model that permits estimation of all six parameters viz, mean (m), additive (d), dominance (h), additive x additive (i), additive x dominance (j) and dominance x dominance (l). The broad area of generation mean analysis is summarized also by Mather and Jinks (1971).

Thus, the real potential of individual cross can be known by the generation mean method, which estimates the types of gene action for each cross separately.

2.5.1 Gene action for seed yield, its components and quality traits

A brief review has been taken here regarding the role of various gene actions in the inheritance of yield, its components and some seed quality traits. Although, there are range of methodologies i.e. either diallel, Line x Tester or North Carolina Designs to detect gene actions, this review enlightens the gene actions mainly through generation mean analysis models as it is more concerned to the present investigation.

1. Days to 50% flowering

Godhani *et al*, (1978), Luthra *et al*, (1979), Singh (1980), Patil *et al*, (1987), Patel *et al*, (1989), Kute (1997), Gawande (2001), Khattak *et al*, (2001) and Manivannan (2002) reported that the additive component played an important role in the inheritance of days to 50% flowering, while Deshmukh and Manjare (1980), Ahuja (1980), Mansuria (1991), Hegde *et al* (1994) and Sawale (1999) recorded non-additive gene action.

As per Murthy *et al*, (1976) dominance x dominance gene interaction played an important role, while additive and dominance gene actions were noticed as important in the studies by Chhabra and Singh (1988).

Kelkar *et al* (1993) reported dominance, additive x additive and dominance x dominance gene actions. Whereas, Singh and Singh (1996) revealed involvement of additive, dominance and other digenic interactions in controlling this trait.

Both additive and non-additive gene actions were found equally important for this character (Wilson *et al*, 1985 and Dasgupta *et al*, 1998).

2. Days to maturity

The additive gene action was observed to be more important in the studies of Gupta *et al* (1978), Patil *et al* (1987), Patel *et al* (1989) and Kute (1997), while Deshmukh and Manjare (1980), Ahuja (1980), Mansuria (1981), Hegde *et al* (1994) recorded importance of non-additive gene actions in control of this character

Wilson *et al* (1985) and Gawande (2001) found than both additive and non-additive gene actions of equal importance, while according to Chhabra and Singh (1988) and Sawale (1999), the dominance gene effects play an important role, while, Kelkar (1993) indicated dominance gene effects of importance in the expression of this character

2. Plant height

Additive gene action was observed to be more important in the studies by Gupta *et al* (1978), Deshmukh and Manjare (1980), Patil *et al* (1987), Singh (1980), Gawande (2001) and Khattak *et al* (2002), whereas Ahuja (1980), Reddy *et al* (1982), Patel *et al* (1988), Natrajan *et al* (1989) and Ram (1997) revealed importance of non-additive gene actions

Wilson *et al* (1985) reported an equal importance of both the additive and non-additive gene actions for this character

Godhani *et al* (1978) and Singh and Singh (1996) indicated involvement of additive as well as dominance and epistatic gene effects in the governing of plant height while, Murthy *et al* (1976) found dominance x dominance type of gene action

Yadav and Rao (1986), Kute (1997) and Sawale (1999) reported dominance gene effects, however, Kelkar (1993) noticed dominance and dominance x dominance gene action for the plant height in their studies

3. Primary branches per plant

Saxena and Sharma (1992) and Ram (1997) observed both additive and non-additive gene action of equal importance in the control of this character. Godhani *et al* (1978) and Khattak *et al* (2002) revealed additive gene action for this character, while non-additive gene actions were reported in the study of Singh (1980), Mansuria (1991) and Manivannan (2002).

Hegde *et al* (1999), Singh and Singh (1996), Kute (1997), Sawale (1999) and Gawande (2001) noticed dominance gene action to play important role, whereas, Kelkar (1993) reported importance of dominance as well as dominance x dominance interaction effects for this character.

4. Pod clusters per plant

Predominance of additive gene effect was reported by Deshmukh and Manjare (1980), Reddy *et al* (1982) and Khattak *et al* (2002) for the character pod clusters per plant. However, dominance gene action was indicated to be more important by Yadav and Rao (1986), Hegde *et al* (1994), Ram (1997), Kute (1997) and Sawale (1999) in the expression of this trait.

Non-additive gene interactions for this character were observed by Singh and Singh (1972), Natrajan *et al* (1989), Mansuria (1991), Gawande (2001) and Manivannan (2002), Wilson *et al* (1985), Thimmappa (1987), Saxena and Sharma (1992) and Khattak (2001) reported equal importance of both additive and non-additive gene interactions for this character. Whereas, Kelkar (1993) noticed dominance, additive x additive and dominance x dominance gene action for the same.

5. Pods per plant

Patil *et al* (1987) and Khattak *et al* (2002) observed this character to be under the control of additive gene action. While, Singh and Singh (1972), Godhani *et al* (1978), Luthra *et al* (1979),

Deshmukh and Manjare (1980), Singh (1980), Natrajan *et al* (1989), Mansuria (1991), Singh and Singh (1996), Ram (1997) and Manivannan (2002) reported importance of non-additive gene action for this character

Predominance of dominance gene effects for pods per plant was reported by Hegde *et al* (1994), Sawale (1999) and Gawande (2001) However,) the involvement of both additive and non-additive variances was reported for this trait (Wilson *et al* , 1985, Singh, 1987, Saxena and Sharma, 1992 and Dasgupta *et al* , 1998)

Murthy *et al* (1976), Kelkar (1993) and Kute (1997) observed that dominance x dominance type of gene interaction was predominated in expression of pods per plant

6. Seeds per pod

Luthra *et al* (1979), Singh (1980), Patil *et al* (1987), Natrajan *et al* (1989), Mansuria (1991), Singh and Singh (1996) and Ram (1997) reported that additive gene action was important to control this character While, Deshmukh and Manjare (1980), Gawande (2001) and Manivannan (2002) observed this trait to be under the control of non-additive gene interaction

Importance of dominance gene effects were reported by Hegde *et al* (1994) and Sawale (1999) through generation mean analysis for seeds per pod

Wilson *et al* (1985), Saxena and Sharma (1997) reported equal importance of additive and non-additive gene actions, however, Malhotra (1983), Chhabra and Singh (1988) observed importance of additive as well as dominance gene actions in governing this character

Godhani *et al* (1978) indicated the additive and dominance gene effects with additive x additive type of epistasis to be responsible for expression of this trait Apart from this, other workers observed dominance x dominance gene interaction for seeds per pod expression (Murthy *et al* , 1976, Kelkar, 1993 and Kute, 1997)

7. 100 seed weight

Additive gene action revealed as a major component controlling the test weight in the studies of Singh and Singh (1972), Gupta *et al* (1978), Ahuja (1980), Deshmukh and Manjare (1980), Thimmappa (1987), Kelkar (1993), Rosaiah *et al.* (1994), Mansuria (1991), Kute (1997) and Sawale (1999)

Luthra *et al* (1979), Patil *et al* (1987), Pate *et al* (1988), Rosaiah *et al* (1995) and Gawande (2001) reported importance of non-additive gene actions were found to be important in several studies (Wilson *et al.*, 1985; Singh, 1987, Hegde *et al* , 1994 and Srinives *et al* , 1991)

Similarly, Chhabra and Singh (1988) found it under the control of both additive and dominance gene action, whereas, dominance x dominance kind of interaction was observed by Singh and Singh (1996) in controlling the 100 seed weight.

8. Seed yield per plant

Reddy *et al* (1982), Rosaiah *et al* (1995) and Dasgupta *et al* (1998) and Khattak *et al* (2002) reported the seed yield per plant in mungbean was mainly controlled by additive gene action. On the contrary to this, several workers have noticed importance of non-additive gene action (Singh and Singh, 1972, Nijhawan and Chandra, 1977, Luthra *et al.*, 1979, Rao and Naggur, 1979, Deshmukh and Manjare, 1980, Singh, 1980 and Patel *et al* , 1988)

Involvement of both additive and non-additive gene actions in controlling this character was indicated by Wilson *et al* (1985), Singh (1987), Saxena and Sharma (1992), Srinives *et al* (1991) and Kelkar (1993).

Murthy *et al.*, (1976) revealed dominance x dominance gene action and Thimmappa (1987) observed duplicate type of epistasis, while

Kute (1997) reported additive x additive gene interaction to play an important role for this trait

In the studies of Godhani *et al* (1978), Yadav and Rao (1986), Hegde *et al* (1994), Sing and Singh (1996), Ram (1997), Sawale (1999) and Gawande (2001), dominant gene action was found to be predominated in the expression of seed yield per plant

9. Protein content

Selim *et al* (1974) reported that additive gene action was important in inheritance of protein content in broad bean Tejindar and Singh (1974) reported that both the additive and non-additive components of variances in the expression of the observed protein content in green gram The non-additive component of variance was constituted by both the dominance and epistatic variances

Malhotra *et al* (1979) reported that high protein content was controlled by dominant genes and non-additive variance was involved in its expression in mungbean Wilson *et al* (1985) observed the non-additive gene action to be predominated in the expression of protein content.

Das and Kumar (1993) in a diallel analysis of pea found both additive as well as non-additive gene actions. Sharma *et al* (1994) observed similar gene actions in pigeonpea While, Sawale (1999) revealed this trait under the control of dominance gene effect with duplicate epistasis in mungbean

However, in case of methionine, in the combining ability study by Tiwari and Ramanujam (1974) revealed the role of dominance in governing this amino acid in mungbean

2.5.2 Gene actions for pathological characters

Mungbean is affected by about 60 fungal, 3 bacterial and 5 viral diseases (Mukherji and Bhasin, 1986) The fungal diseases include powdery mildew, leaf spot, root, rot, wilt, anthracnose, etc Among these, powdery mildew is the most devastating and of commonly occurring disease

Powdery mildew caused by *Erysiphe polygoni* D C reported for the first time by DeCandolle (1886) Distribution of powdery mildew in India was studied by Butler and Bisby (1931)

Powdery mildew (*Erysiphe polygoni* D C) of mungbean appears in epidemic form every year in Maharashtra State leading to heavy economic losses This disease causes 40% yield losses (Legaspi *et al* , 1978), which may be higher up to 100%, if the disease occurs at the seedling stage (Reddy *et al* , 1994)

Inheritance of powdery mildew resistance and gene action were studied by Reddy *et al* (1994) and Hegde *et al* (1996^b), respectively However, very limited reports are available on nature of gene actions for powdery mildew resistance in mungbean

According to Yohe and Poehlman (1975) powdery mildew resistance in mungbean is due to additive gene effects Studies carried out at AVRDC in 1978, revealed that the powdery mildew resistance in green gram is due to single dominant gene However, Reddy *et al* (1994) noticed two different dominant genes, *Pm1* and *Pm2* for the adult plant resistant in mungbean

From the studies of restriction fragment length polymorphism (RFLP), Young *et al* (1993) reported that a total of three genomic regions were found to have an effect on powdery mildew response in mungbean

In an intervarietal cross in mungbean, Hegde *et al* (1996^b) reported that additive and additive based gene interactions played a major role in the inheritance of powdery mildew resistance

The segregation in F₂ generation revealed that resistance to powdery mildew in mungbean is under the control of three duplicate recessive genes, which has confirmed in F₃ generation (Kute, 1997) Tyagi (1990) reported that additive and dominance components including epistasis were significant for powdery mildew resistance in pea in most of the crosses Tyagi and Srivastava (2000) noticed importance of additive and non-additive genetic variance in the expression of resistance to powdery mildew in pea

Quantitative assessments are necessary to judge the relative importance of disease The basic requirements of any disease assessment are that it should provide a practical degree of accuracy and the results should be comparable from one worker to another, from one location to another, and from one season to the next

The simplest method is to count the number and hence obtain a percentage of diseased plants This is particularly useful where the entire plant is killed However, not all diseases are so simple to assess More frequently plants are affected in different degrees, especially in foliar disease such as powdery mildew leaf blight, etc

There are different methods for assessing the disease quantitatively Few of them have been used to score the powdery mildew disease as below

1. Per cent disease index (PDI)

One of the most useful methods devised by McKinney (1923), which is still widely used in some form or other With the use of PDI, Hedge *et al* (1996^b) and Kute (1997) studied gene action and

inheritance of resistance to powdery mildew in mungbean, respectively, whereas Tyagi (1999) studied generation mean analysis for resistance to powdery mildew in field pea using PDI

2. Area under disease progress curve (AUDPC)

The apparent rate of disease development (r) is a measure of the speed at which an epidemic develops. Despite the presence of virulent pathogen and favourable environment, differences were observed in the rate of disease development on various genotypes culminating in low terminal disease severity. The slowing down of rate of infection has been attributed to the level of host resistance. Van der Plank (1963) considered this rate reducing resistance as horizontal resistance.

One of the means of quantifying this type of host resistance is to measure the area under disease progress curve (AUDPC). Slow growth and poor terminal severity will result in lesser AUDPC values. Wilcoxson *et al* (1975) quantified the area under disease progress curve as A-value. Such quantification of disease demonstrate the importance of AUDPC value as a reliable parameter to estimate and rank the performance of various host genotypes depending on their ability to retard the rate of disease development.

AUDPC values are being employed more and more in identification of partial resistance. Some of the workers of the workers used AUDPC value to study the level of resistance in different genotype viz, Yohe and Bowman (1980) in rice, Southern and Wilcoxson (1983) in wheat, Norgaard Knudsen *et al* (1986) in barley, Wilson and in cowpea, Anilkumar *et al* (1992) in field peas, Anilkumar *et al* (1994) in field peas, Cheiran *et al* (1996) in mungbean. However, the work in mungbean on gene action for resistance to powdery mildew using disease incidence and AUDPC value for scoring disease is lacking in the literature.

2.4.1 Biochemical-genetic basis of powdery mildew resistance

In plant-pathogen interactions, it is often the case that different host cultivars exhibit specific responses to different physiological races of the pathogen. From analysis of the cultivar race-specific interactions, it has become apparent that one gene in the host for resistance is complemented by a single gene in the pathogen for avirulence. This '*gene for gene hypothesis*' holds true irrespective of the number of loci in the host that confer resistance.

In many cases, an incompatible interaction is characterized by the hypersensitive response (HR) involving rapid death of the cell(s) in the immediate vicinity of the pathogen and the triggering of a wide range of "inducible defense genes". This response is now recognized to be wide spread, occurring as incompatibility between host plants and pathogens, whether the second organism is fungal, bacterial, viral or a nematode.

One of the most studying phenomena during the expression of localized resistance is the accumulation of antimicrobial compounds known as phytoalexins. The induction of enzymes involved in the synthesis of these antibiotics, and the genes encoding these enzymes, has provided a useful system for defining the sequence of events that lead from the initial recognition event to transcription of the inducible genes (Dixon, 1990). Among these, the following are under intense investigation:

- 1 Enzymes involved in the synthesis of phytoalexins, the secondary metabolites that are toxic to pathogen
- 2 Enzymes and proteins leading to the function of physical barriers to fungal or bacterial invasion through modification of plant cell wall
- 3 Inhibitors of serine endopeptidases which can inhibit major digestive enzymes of attacking organisms

4 Lytic enzymes of chitinases and β -1, 3-glucanases capable of degrading fungal cell wall

Considerable information has arisen from the activators of these defense response genes *i.e.* elicitors of stress condition or pathogens. The disease resistance of a plant is its ability to prevent, restrict or retard the disease development. Plant disease resistance apparently depends upon the interaction of more than one resistant mechanism to inhibit the invasion of the pathogen. The plants may possess pre-existing chemical inhibitors or post-infectious phytoalexins or may show HR at the site of infections, to isolate the pathogen and prevent from spreading further (reviewed Subramaniam, 1969, Ellingboe, 1981, Ayers *et al.*, 1985, Cresay, 1985, Sen, 1988, Vidyasekaran, 1988 and Bowles, 1990)

Hence, such enzymes and compounds like sugars, total phenols and potash concentrations in the plant, which contributes in disease resistance should be assayed for the gene action in the inheritance of resistance for their specific enzyme activity of different stages. Only available reference is the work done by Gawande *et al.* (2002), which performed quantitative genetic analysis for total phenols, peroxidase, polyphenol oxidase, sugars and potash content, where they revealed the enzyme activities related to degree of resistance to powdery mildew and their expression was influenced by different generations of mungbean.

As hardly any work has been done to trace out the gene action of the enzyme activities or disease-related biochemicals involved in powdery mildew fungus - mungbean interaction, a brief review of role of enzymes and potash under investigation in imparting resistance was taken and is presented hereunder.

Chitinase (EC 3.2.1.1.4) and β -1, 3-glucanase (EC 3.2.1.39)

Higher plants do not contain chitin, a β -1,4 polymer of *N*-acetyl glucosamine, or β -1, 3-glucans, or any other known endogenous substrate for chitinase and β -1, 3-glucanases. However, chitin and β -1, 3-glucans are major components in the cell wall of many fungi and are target substrates for plant chitinases and β -1, 3-glucanases. These enzymes inhibit fungal growth by hyphal tip lysis, and have synergistic effect in inhibiting fungal growth. Thus, it has been proposed that induction of chitinase and β -1, 3-glucanase is a part of the biochemical defense of plants against pathogenic fungi.

Synthesis of chitinase and β -1, 3-glucanase in response to fungal pathogens infection has been reported by various workers in many plants (Mridula, *et al* , 1992, Ward *et al* , 1991, Sharma *et al* , 1982, Subhash Chander, 1990, Zhang 2003, Tyagi, 2001, Rakshit, 2000 and Giri, 1998)

Clarke and Stone (1962) listed the recorded occurrence of β -1, 3-glucanase in 17 mono and dicotyledons and measured the activity of this enzyme in more than 20 species including two woody plants. The role of the enzyme in higher plants seems obscure in most cases, but specific β -1, 3-glucanase in grape vine was thought to be related to the seasonal removal of sieve tube callose.

The level and onset of β -1, 3-glucanase expression is often positively correlated to the level of pathogen resistance. Muskmelon and tomatoes infected with *Fusarium oxysporum* indicated a higher and more rapid increase in activity of β -1, 3-glucanase in resistant than in susceptible varieties (Netzer *et al* , 1979 and Ferraris *et al* , 1987). Tomato plants resistant to the fungal pathogen *Cladosporium fulvum* produced β -1, 3-glucanase and chitinase earlier than susceptible varieties (Jootfen *et al* , 1989). Muskmelon seedlings susceptible to the fungus

Collectorchum lagenarium did not produce more β -1,3-glucanase (Rabenantoandro *et al* , 1976)

Wyatt *et al* , (1991) reported that the increase in β -1, 3-glucanase, chitinase and peroxidase activities in tobacco correlates with the development of age related resistance to blue mold caused by *Peronospora tabacina* *Nicotiana glutinosa* x *Nicotiana debneyi* hybrid showed greater viral, bacterial and fungal resistance than the parents and this hybrid had higher β -1, 3-glucanase, chitinase, peroxidase and polyphenol oxidase (Ahl-Goy *et al* , 1993) Evidence has also been obtained that supports a role of β -1, 3-glucanase and chitinase in defense against plant pathogenic fungi in vivo Transgenic plants continuously expressing PR protein genes including basic β -1, 3-glucanase and chitinase a number of fungal pathogen (Alexander *et al* , 1993 and Jongedijk *et al* , 1995)

Joshi (1999) through generation mean analysis observed that epistatic interactions prevailed in the inheritance of the chitinase and β -1, 3-glucanase enzymes, in general in pearl millet against downy mildew

Polyphenol oxidase (PPO) activity (EC 1.10.3.1)

The association of phenolic acids with resistance to pests and pathogens in crop plants has been studied in considerable detail (Cole, 1984) The enzymes of the phenylpropanoid pathway associated with aromatic biosynthesis influence the flux of phenolic acids within the metabolic pool. The toxicity of pathogen has been shown to depend largely on the oxidation of phenolic compounds to quinones by polyphenol oxidase These acts as powerful enzyme and metabolic inhibitor because of their reactions with sulfhydryl and amino groups

(Rubin and Artisikhovskaya, 1963, Cruickshank and Perrin, 1964, Uritani *et al* , 1967, Webb, 1966 and Williams and Kuc, 1969)

The higher levels of polyphenol oxidase in resistant genotypes were observed by Goodman *et al* , (1967) Sharma and Sharma (1977) revealed that on inoculation with leaf rust in wheat, resistant lines showed higher polyphenol oxidase activity in comparison to the susceptible lines at both seedling as well as post seedling stages and also in uninoculated and inoculated samples

Additive gene action was found to be more important in the expression of polyphenol oxidase activity, which was related to the degree of resistance to downy mildew in pearl millet (Thukral, 1983) Sharma (1982) reported that both additive (d) and dominance (h) components were found to contribute to the inheritance of polyphenol oxidase activity in pea with duplicate type of epistasis

The enzymatic association (including PPO) with resistance to rust and powdery mildew in peas was described by Subhash Chander and Chandravandana (1988) Kalia and Sharma (1988) noticed higher levels of polyphenol oxidase activity in resistant cultivars of pea Subhash Chander (1990) reported the activity of this enzyme as a property of resistance in all the improved resistant lines of pea Gupta *et al* (1992) found that polyphenol oxidase activity was relatively more in leaf spot tolerant cultivars than susceptible ones in peanut Similar findings also have been obtained in several other host-pathogen interactions (Arora and Wagle, 1985, Luthra *et al* , 1988 and Guleria *et al* , 1998)

Gawande *et al* (2002) opined that the PPO activity before and after powdery mildew infection in mungbean was controlled by both additive and non-additive gene actions

Potash content

In the study of Singh and Saksena (1983), resistant cultivars of pea contained higher amount of potassium than susceptible ones indicating dominant role of potassium in host resistance to powdery mildew, the higher quantities of potassium may be contributing factor towards resistance in pea against powdery mildew

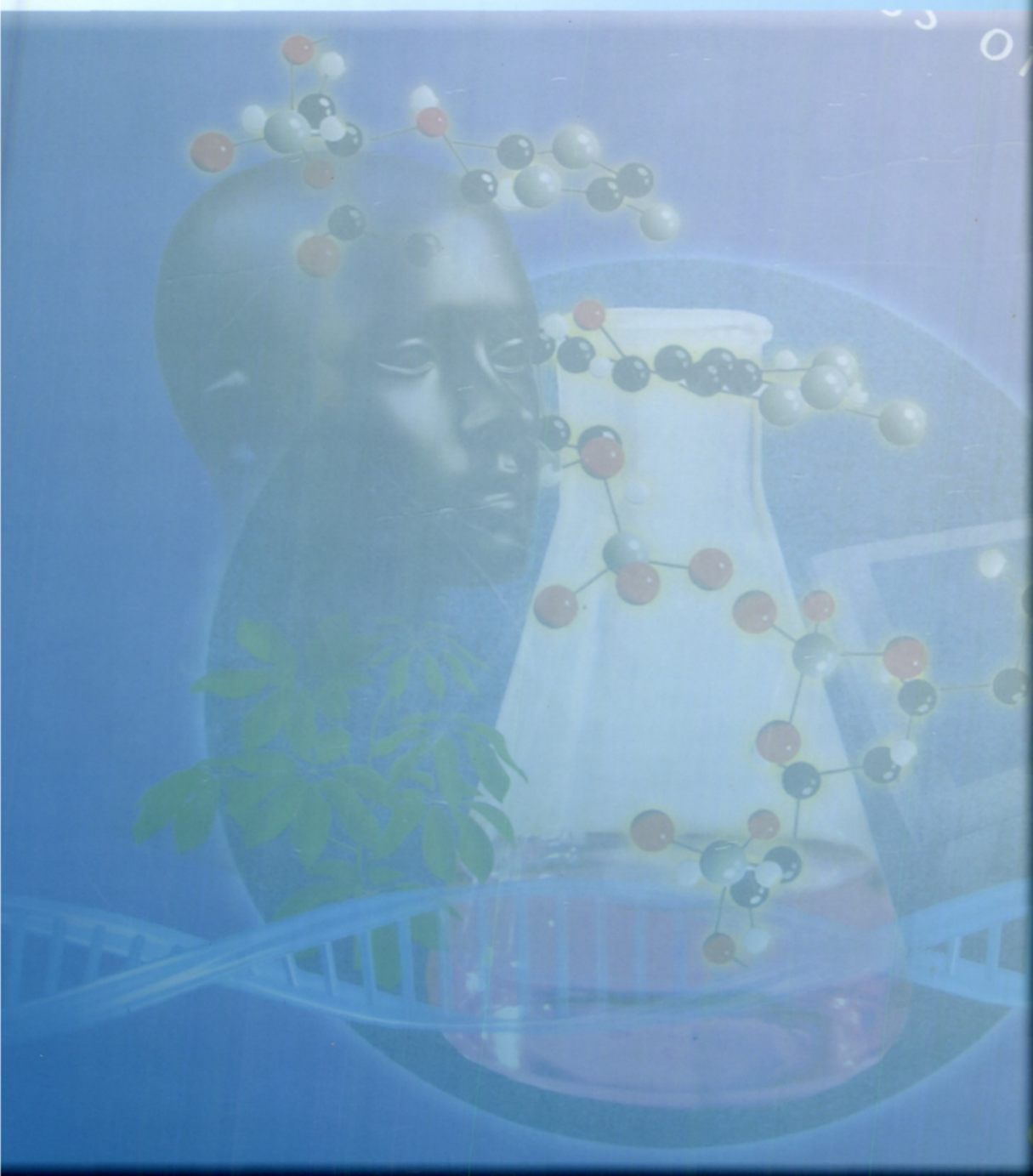
Singh (1996) stated that potash increases resistance in plant against diseases like leaf blight and stalk rot in corn, wilt and damping off in cotton, leaf spot and dollar spot in grasses, mold and mildew in soybean, black spot and stem end rot in potatoes and wild fire in tobacco

Rathi *et al* (1998) observed that resistant varieties contained higher concentration of potassium than susceptible varieties and after infection concentration decreased in all the varieties but much more decreased in susceptible than those of resistant. They concluded that balanced level of potash induces thicker cell wall, accumulation of amino acid (arginine) and production of new tissues

Potash enhances plant growth, ensuring a healthy plant with more resistance to attack / e entry following infection by viruses, bacteria, and fungi, as optimum stems and stalks, no sugar accumulation in the leaves and no accumulation of excess or unused nitrogen (Anonymous, 2000)

Gawande *et al* (2002) noticed that the dominance gene action was predominant for controlling the potash content with duplicate type of epistasis after and before the powdery mildew infection, where the potash content was positively associated with resistance

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MATERIAL AND METHODS

MATERIAL AND METHODS

The present investigations aimed at studying the heterosis and combining ability, the DNA polymorphism, nature of gene action controlling yield, its components and quality traits along with powdery mildew resistance through pathological and biochemical parameters in mungbean (*Vigna radiata* (L) Wilczek) were conducted during 2000-2004 at Post Graduate Instructional Farm, Department of Agricultural Botany, Biotechnology Centre, Biochemistry Laboratory, MPKV, Rahuri and Genetics Group Laboratory, Agharkar Research Institute (MACS), Pune. The details of material used, methods and statistical procedures followed have been given in this chapter.

3.1 MATERIAL

The parental material for the present investigation was selected from the germplasm maintained at Pulses Improvement Project, MPKV, Rahuri. Salient features of the selected parents are given in Table 3.1.

Table 3.1 Salient features of parent material used for the present investigation

Sr. No.	Parents	Evolved at	Salient features
1	Kopargaon	Nagpur (M.S)	Bold seeded, early, wider adaptability
2	AKM 8802	Akola (M S)	High yield, susceptible to powdery mildew
3	PM 9341	Rahuri (M S)	Bold seeded, high yield, susceptible to powdery mildew
4	BM 4	Badanapur (M S)	Early, high yield, susceptible to powdery mildew
5	BPMR 145	Badanapur (M S)	Bold seeded, resistant to powdery mildew
6.	Vaibhav	Rahuri (M S)	Bold seeded, high yield, resistant to powdery mildew
7	TARM 18	Akola and BARC (M S)	Early, high yield, resistant to powdery mildew

3.2 METHODS

3.2.1 Hybridization

The crosses were effected in 7 x 7 diallel fashion excluding reciprocals during summer and *kharif*, 2002 by hand emasculation and pollination. Emasculations were done during evening from 5 00 to 6 30 p.m. and emasculated buds were marked with different coloured threads for easy identification. Other flowers were removed and only emasculated buds were kept. Pollination was done next morning between 7 00 a.m. and 9 30 a.m. Five crosses viz, Kopargaon x AKM 8802, Kopargaon x TARM 18, BM 4 x BPMR 145, BPMR 145 x Vaibhav and Vaibhav x TARM 18 were selected for generation mean analysis for yield, its components and nutritional quality traits. To study the genetics of resistance to powdery mildew, three crosses viz, Kopargaon x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18 were selected (Table 3.2).

To study the generation mean analysis of yield, its components with quality traits and the genetics of resistance to powdery mildew, part of F_1 seeds harvested from above crosses were sown during Summer, 2003 and selfing of F_1 s and backcrosses were done to get F_2 , B_1 and B_2 generations.

3.2.2 Conduct of experiments

3.2.2.1 Experiment I : Heterosis and combining ability analysis

The experiment with twenty eight treatments (7 parents + 21 F_1 s) was laid out in Randomized Block Design (RBD) with three replications during *kharif* 2002 at Post Graduate Instructional Farm, M.P.K.V., Rahuri (Fig. 3.1a). All the 28 treatments were grown in single progeny row of 3 meter length with spacing of 30 cm between rows and 15cm between plants. The experimental plots were surrounded by non-experimental border rows of variety Phule M-2. Recommended package of practices was carried out to raise healthy and disease-free crop.

Table 3.2 Material for different investigations in mungbean

Heterosis and Combining ability analysis	DNA polymorphism by molecular markers	Genetics of yield, its components and quality traits	Genetics of powdery mildew resistance
Kopargaon	Kopargaon	Kopargaon x AKM 8802	Kopargaon x TARM18
AKM 8802	AKM 8802	Kopargaon x TARM 18	BPMR145 x Vaibhav
PM 9341	PM 9341	BM 4 x BPMR 145	Vaibhav x TARM 18
BM 4	BM 4	BPMR 145 x Vaibhav	P ₁
BPMR 145	BPMR 145	Vaibhav x TARM 18	P ₂
Vaibhav	Vaibhav	P ₁	F ₁
TARM 18	TARM 18	P ₂	F ₂
Kopargaon x AKM 8802		F ₁	B ₁
Kopargaon x PM 9341		F ₂	B ₂
Kopargaon x BM 4		B ₁	
Kopargaon x BPMR 145		B ₂	
Kopargaon x Vaibhav			
Kopargaon x TARM 18			
AKM 8802 x PM 9341			
AKM 8802 x BM 4			
AKM 8802 x BPMR 145			
AKM 8802 x Vaibhav			
AKM 8802 x TARM 18			
PM 9341 x BM 4			
PM 9341 x BPMR 145			
PM 9341 x Vaibhav			
PM 9341 x TARM 18			
BM 4 x BPMR 145			
BM 4 x Vaibhav			
BM 4 x TARM 18			
BPMR 145 x Vaibhav			
BPMR 145 x TARM 18			
Vaibhav x TARM 18			

3.2.2.2 Experiment II: DNA polymorphism by molecular markers

The work on molecular marker analysis was done at Genetics Group Laboratory, Agharkar Research Institute (MACS), Pune

I. Plant material and DNA isolation

Genomic DNA was extracted from the leaves of 15-day-old seedlings of seven parental genotypes grown in glasshouse, by modified CTAB (Hexacetyltrimethyl ammonium bromide) protocol of Rogers and Bendich (1988)

Young leaf tissue (10 g) was ground to fine powder in liquid nitrogen using a mortar and pestle. To this, 2x CTAB buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1% PVP and 1% β -mercaptoethanol) was added and the contents were mixed to form an emulsion, which was incubated at 60°C for 20 min. After incubation, the emulsion was allowed to cool down to room temperature, following which it was equally distributed in SS-34 centrifuge tubes. In each tube, an equal volume of Chloroform:IAA (24:1) mixture was added. The tubes were capped and gently swirled to mix the contents. The tubes were centrifuged at 10,000 rpm for 10 min at room temperature in a Sorvall RC-5B centrifuge (Du Pont, U.S.A.)

The aqueous layer formed after centrifugation was recovered and distributed in fresh SS-34 tubes. To this, an equal volume of CTAB precipitation buffer (1% CTAB, 500 mM Tris-HCl pH 8.0, 10 mM EDTA) was added, the contents were mixed and kept at room temperature for 15 min. Subsequently, the pellet of the precipitated DNA obtained by centrifuging the tubes at 10,000 rpm for 10 min at 15°C, was dried and dissolved in high salt TE buffer (1 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA). The dissolved DNA was reprecipitated by adding two volumes of chilled ethanol and the precipitated DNA was either spooled out or pelleted by centrifugation at 10,000 rpm for 10 min at 4°C.

The DNA pellet was washed with 70% ethanol, dried and dissolved in an appropriate volume of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0). To remove RNA from the samples, 100 µg/ml RNase A (DNase free) was added and incubated at 37°C for 1 h. Subsequently, an equal volume of chloroform:IAA (24:1) mixture was added, the contents were mixed and centrifuged at 10,000 rpm for 10 min at 15°C. Following centrifugation, the aqueous layer containing DNA was recovered and stored at -20°C until further use. The DNA concentration was estimated by agarose gel (0.8%) electrophoresis by comparing with known concentration of λ DNA.

II. PCR amplification

A. RAPD analysis

Random decamer oligonucleotide primers from Operon Technologies, Alameda, U.S.A., were used in this study. Primers, which produced clear and reproducible amplification patterns upon repetitive trials, were used in further investigations. PCR amplifications were performed in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.1 mM dNTPs, 0.4 mM spermidine, 5 picomoles of primer, 0.8 unit of *Taq* DNA polymerase (Perkin Elmer, U.S.A.) and 20 ng of genomic DNA per 25 µl reaction. The PCR conditions include, initial denaturation of 4 min at 94°C, followed by 5 cycles of 30 s at 92°C, 2 min at 35°C, 1.5 min at 72°C, 35 cycles of denaturation at 92°C for 5 s, annealing at 40°C for 20 s, extension at 72°C for 1.5 min with final extension at 72°C for 5 min.

B. ISSR analysis

A set of 100 ISSR primers obtained from Biotechnology Laboratory, University of British Columbia, Canada (UBC set/ISSR no 801 to 900), were used in PCR analysis. Amplifications were carried out in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.1 mM dNTPs, 2% formamide, 0.4 mM spermidine, 0.2 µM primer, 0.8 unit of *Taq* DNA polymerase (Perkin Elmer, U.S.A.) and 15 ng of genomic DNA per 25 µl

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reaction The PCR conditions included initial denaturation for 5 min at 94°C, followed by 45 cycles of 30 s at 94°C, 45 s at 50°C, 2 min at 72°C and final 5 min extension at 72°C

Polymerase chain reactions of RAPD and ISSR were performed in Perkin Elmer-480® and PTC-200® (M J Research Co , USA) thermal cycler, respectively The amplified products were electrophoresed on 1.5% agarose gel in 0.5X TAE (0.02M Tris-acetate, 0.005M EDTA pH 8.0) buffer for further detection with ethidium bromide staining

3.2.2.3 Experiment III : Generation mean analysis for yield, its components and quality traits

The experiment was conducted in Randomized Block Design (RBD) with four replications, the 26 treatments consisting parents F₁s, F₂s, B₁s and B₂s of five selected crosses viz , Kopargaon x AKM 8802, Kopargaon x TARM 18, BM 4 x BPMR 145, BPMR 145 x Vaibhav and Vaibhav x TARM 18 were grown during *Kharif*, 2003 at Post Graduate Instructional Farm, MPKV, Rahuri Each of the parent, F₁s, B₁s and B₂s were represented by single row and F₂s by four rows of three meter length with spacing 30 x 15 cm in each replication All the cultural practices were followed to ensure satisfactory crop growth

3.2.2.4 Experiment IV: Genetics of host-parasite interaction and biochemicals related to powdery mildew resistance

Four parents, 3F₁s, 3F₂s, 3B₁s and 3B₂s of three crosses viz , Kopargaon x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18 were grown in Randomized Block Design on 13th July, 2003 with four replications at Post Graduate Instructional Farm, MPKV, Rahuri Each parent, F₁, B₁ and B₂ were represented by three meter single row, whereas, F₂s were represented by four rows of three meter length Seeds were sown at spacing 45 cm between the rows and 15 cm between plants

The experiment was surrounded by pre-planted rows of susceptible variety Phule M 2 (Fig 3.1d), which were sown on



(a) Heterosis and Combining Ability Trial



(b) PCR Thermocycler machine



(c) Powdery Mildew Susceptible Powdery Mildew Resistant



**(d) Experiment surrounded by pre-planted susceptible rows of PM-2
[Figure 3.1]**

27th June, 2003 Similarly, each plot was surrounded by border rows of susceptible variety Conidia of *Erysiphe polygoni* from infected leaves were dusted on the test lines of experimental plants immediately when powdery mildew incidence was noticed on PM-2, so as to ensure enough disease pressure and uniform spread of the disease Artificial epiphytotics of powdery mildew were created by spraying the conidial suspension on the plants to ensure sufficient source of inoculum All the recommended package of practices was followed to have a satisfactory crop growth

3.2.3 Recording of experimental data

3.2.3.1 Experiment I and III

Observations were recorded in all the replications on randomly selected competitive plants Five plants in parents and F₁s, 10 plants in B₁s and B₂s, while 20 plants in F₂s were selected in each replication and observations were recorded on the following traits

1. Days to 50 % flowering

The number of days from sowing date to flowering was calculated on the date, when 50 % of the plants in a plot flowered

2. Days to maturity

Number of days required to mature the plants in a plot was recorded from the data of sowing, when maximum pods of plants were matured

3. Plant height (cm)

Plant height at maturity was recorded from ground level to the growing tip of the main shoot

4. Primary branches per plant

The number of primary branches arising from main shoot and terminally ending in pod clusters was recorded at maturity.

5. Pod clusters per plant

Number of pod clusters was counted when plants attained maturity

6. Pods per plant

Total number of pods during every picking was recorded and added together to have the total number of pods per plant

7. Seeds per pod

Randomly selected five pods from each of the observational plants were used to calculate the average number of seeds per pod in each treatment

8. 100 seed weight (g)

From the mixed seed lot of observational plants, 100 seeds were randomly taken and weighted in grams

9. Seed yield per plant (g)

Each observational plant was harvested and seed yield was recorded in grams and the average was worked out

In **Experiment III**, in addition to above nine quantitative characters following quality traits were recorded

- 1. Protein content**
- 2. Methionine**
- 3. Tryptophan**

The biochemical analysis of nutritional quality traits of seed was carried out at Department of Agricultural Chemistry and Soil Science and Plant Physiology Laboratory, Department of Agricultural Botany, MPKV, Rahuri

Results of the individual quality traits in the five crosses viz , Kopargaon x AKM 8802, Kopargaon x TARM 18, BM 4 x BPMR 145, BPMR 145 x Vaibhav and Vaibhav x TARM 18 were subjected for statistical analysis to estimate gene effects using Cavalli's (1952) Joint scaling test and Hayman's (1958) six parameter model

Method of analysis

1. Protein content (%)

The protein content of the seed was determined as per the micro-Kjeldahl method (McKenzie and Wallace, 1954) The protein percentage was calculated by multiplying the nitrogen content by a factor of 6.25

2. Tryptophan (g/16 g N)

Tryptophan present in the seed sample was determined by the calorimetric method as described by Mertz (1975)

To 0.1 g air-dried, powdered and defatted seed sample, 5 ml of fresh papain solution (technical grade) was added and incubated at 65°C overnight. After cooling, it was centrifuged and supernatant was collected. To 1 ml supernatant 4 ml of Reagent-C (i.e. solution of glacial acetic acid containing FeCl₃ · 6H₂O and 2% acetic anhydride-Reagent-A mixed with H₂SO₄ Reagent-B about one hour before use) was mixed and incubated at 65°C for 15 min. After cooling, the absorbance of orange-red colour at 545 nm against a processed blank was measured. The standard curve was calibrated using the tryptophan in the range of 0 to 50 µg.

3. Methionine (g/16 g N)

This amino acid content of seed was estimated by a method described by Horn *et al* (1946). Finely ground defatted sample (0.59) was taken. To this, 6 ml of 2 N HCL was added and autoclaved at 151 lb pressure for 1 hr. Filtrate (pH 6.5) was made up to 50 ml with water after

cooling Under alkaline condition, the liberated methionine with 3 ml of 10% NaOH, 0.15 ml of sodium nitroprusside and 1 ml of glycine solution gives yellow color, which turns red on acidification i.e. by adding 2 ml of orthophosphoric acid. The absorbance was measured at 520 nm after 10 min against the processed blank.

Standard curve was prepared by using 0, 1, 2, 3, 4 and 5 ml of standard methionine solution (0.1 g of L-Methionine in 4 ml of 20% HCL, diluted with 100 ml water i.e. 1 mg/ml).

3.2.3.2 Experiment II

Scoring and molecular markers data analysis:

DNA amplifications with RAPD and ISSR primers were repeated at least three times to ensure reproducibility. The bands were considered reproducible only when they were observed in three separate amplifications using different DNA isolations. Bands seen against heavy background smear were not scored.

For each genotype, presence of a band (1/+) or its absence (0/-) was scored for concerned primer. Pairwise comparisons of degree of band sharing were made and similarity index (SI) values were calculated by Nei's (Nei and Li, 1979) method as,

$$SI = \frac{2 N_{ab}}{N_a + N_b}$$

Where,

N_a = Total number of bands present in lane 'a'

N_b = Total number of bands present in lane 'b'

N_{ab} = Number of bands common to lanes 'a' and 'b'

A dendrogram was constructed using NTSYS-PC version 2.0 software (Rohlf, 1993) based on the degree of band sharing.

3.2.3.3 Experiment IV

A. Pathological observations related to powdery mildew resistance

The disease incidence was first ensured on the pre-planted spreader rows of susceptible variety (Phule M 2) [Reddy *et al* , 1987] The disease reaction observations were recorded on the following characters on random 5 plants from parents and F₁s, 10 plants from backcrosses and 20 plants from F₂ generations using all the leaves of selected plants of all the three crosses *viz* , Kopargaon x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18

1. Per cent disease index (PDI)

PDI was recorded by grading the leaves as per the scale (Anonymous, 1995) shown in Fig 3 2, wherein all the leaves of selected plants were graded on 1 - 5 scale (Table 3 3) and calculated using the formula given by Wheeler (1969)

Table 3.3 Disease rating scale

0 %	Grade 0
0 1 to 10 0%	Grade 1
10 1 to 25 0%	Grade 2
25 1 to 50 0%	Grade 3
50 1 to 75 0%	Grade 4
75 1 to 100 0%	Grade 5

$$\text{PDI} = \frac{\text{Sum of individual rating}}{\text{No of leaves assessed} \times \text{Maximum disease grade}} \times 100$$

Means, variances and genes effects were calculated after transforming the PDI values by *Arc sine* transformations



0 % - GRADE 0



0.1 to 10.0 % - GRADE 1



10.1 to 25.0 % - GRADE 2



25.1 to 50.0 % - GRADE 3



50.1 to 75.0 % - GRADE 4



75.1 to 100 % - GRADE 5

Fig. 3.2 Standard powdery mildew diseased leaves of mungbean

2. Area under disease progress curve (AUDPC)

The mildew severity was scored at 4 days interval from first appearance of disease i.e. from 12th August to 5th September. Mildew intensity was recorded as per cent disease index per plant. AUDPC values were calculated based on the following formula

$$\text{AUDPC (A-value)} = \sum_{i=1}^k \frac{1}{2} (S_i + S_{i-1}) \times d$$

Where,

- S_i = Disease intensity at i^{th} day of evaluation
- k = Number of successive evaluation
- d = Interval between i and $i-1$ evaluation of disease

B. Biochemical observations related to powdery mildew resistance

The disease related biochemical attributes such as enzyme activities and potash content have been analysed at two stages i.e. pre and post stages of disease infection in all the six basic generations (P_1 , P_2 , F_1 , F_2 , B_1 and B_2) in the crosses namely, Kopargaon x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18. The following four biochemical parameters were studied,

1. Chitinase activity
2. β -1, 3-glucanase activity
3. Polyphenol oxidase activity
4. Potash content

Results of the individual biochemical characters in above said crosses were subjected for statistical analysis to estimate gene effects using Cavalli's (1952) Joint scaling test and Hayman's (1958) six parameter model

Preparation of material

For pre-infection estimations *i.e.* at healthy plant conditions, the sampling was done at same hours in a day in all the experimental treatments well before the first incidence of any disease or stress on the test lines and spreader variety, as well *i.e.* between 25th to 27th July, 2003, while in the similar manner and keeping same individuals for a particular biochemical study after development of disease the sampling was carried out *i.e.* between 16th to 18th Aug , 2003 to rule-out any possibility of aging effect on developmental phenomenon The leaves from bottom, middle and top of plants were harvested, cleaned and chilled at 4⁰C and immediately analysed for polyphenol oxidase activity For chitinase and β -1, 3-glucanase enzyme assay, the acetone powder of leaves was used Another set of leaf samples was dried at 60⁰C and then ground and analysed for potash content

Method of analysis

1. Chitinase activity

The chitinase activity was assayed as per the procedure given by Reissig *et al.* (1955) with suitable modifications as outlined below

Leaves (500 mg) were extracted in 0.1 M sodium acetate buffer (pH 5.0) in a pre-chilled pestle and mortar The homogenate was filtered and centrifuged for 15 min at 12,000 rpm at 4⁰C

The reaction mixture of 0.5 ml contained 10 μ l of extract, 10 mg colloidal chitin and 20 mM sodium acetate buffer (pH 5.0) The reaction, allowed to occur for one hour at 37⁰C with 20 units of sweet almond β -glucosidase (Sigma Co , USA) Monomeric units, *N*-acetyl glucosamine were estimated using sodium tetraborate buffer (pH 9.2) and *p*-dimethyl amino benzaldehyde (DMAB) reagent Absorbance was read

at 585nm Commercially available *N*-acetyl glucosamine was used to prepare calibration curve

One unit was defined as the amount of enzyme liberating one μmol of *N*-acetyl glucosamine per min under the given assay conditions Colloidal chitin was prepared by the method of Pegg (1988)

2. β -1, 3-glucanase activity

The activity of β -1, 3-glucanase was assayed as per the method given by Keen and Yoshikawa (1983) as follows

Leaf samples of 500 g each from test lines were extracted in 0.2 M sodium acetate buffer (pH 5.2) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 15 min in a refrigerated centrifuge at 4°C and resultant supernatant liquid was retained for subsequent assay of activity.

The reaction mixture contained 2 mg laminarin (Sigma Co, USA), 20 μl of extract and 50 mM sodium acetate buffer (pH 5.2) was incubated for 20 min at 37°C in a water bath. Reducing sugar thus produced was determined by using alkaline copper reagent and arsenomolybdate reagent action by Somogyi-Nelson method (Somogyi, 1952). Absorbance was read at 540 nm. A calibration curve was prepared by using D-Glucose as a standard.

One unit (U) of β -1, 3-glucanase was defined as the amount of enzyme that produced one mg glucose per hour under the given assay conditions.

3. Polyphenol oxidase (PPO) activity

The polyphenol oxidase activity was assayed as per the procedure given by Kumar and Khan (1982).

Reaction mixture for PPO contained 2.5 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml of 0.01 M pyrogallol and 0.5 ml enzyme.

extract. The absorbance was measured at 420 nm on Spectronic-20 for 2 min and enzyme activity was expressed as $\Delta A/hr^{-1} mg^{-1}$ soluble proteins in the leaves

The soluble protein content in the extract was determined by the method described by Lowry *et al* (1951)

3. Potash content

The potash content in the leaf sample was estimated by flame photometer method as described by Chapman and Pratt (1961).

The 0.2 g finely ground samples were digested with 10 ml H_2SO_4 and 10 ml H_2O_2 in micro-Kjeldahl flasks at $250^{\circ}C$ temperature. After digestion, contents were cooled and volume made to 100 ml with distilled water. Exactly 2 ml of digested extract was diluted to 50 ml with distilled water and readings were taken on flame photometer (Sanyo Co.)

A standard solution of KCl ($10 \mu g K/ml$) in a series (0 to 5.0 ml) was pipetted in the 50 ml volumetric flasks and volume was made with distilled water. The readings were recorded on flame photometer and standard graph was prepared by plotting flame photometer readings against concentration of potassium.

The potash content in the sample was determined using standard graph and expressed as $mg g^{-1}$ dry weight.

3.2.4 Statistical analysis

3.2.4.1 Analysis of variance (ANOVA)

The data collected from all the three experiments for all the characters were subjected to statistical analysis. The 'Null hypothesis' that there were no genotypic differences in the population under study, was tested. Each character was analysed separately and the ANOVA was constructed by procedure of Panse and Sukhatme (1995) as presented in Table 3.4.

Table 3.4 Analysis of variance

Source of variation	Degrees of freedom	Mean sum of squares	F ratio
Replications	(r-1)	Mr	Mr/E
Treatments	(t-1)	Mt	Mt/E
Error	(r-1) (t-1)	E	

Where,

r = Number of replications

t = Number of treatments

The characters, which had significant treatment differences, were only subjected for further statistical analysis

3.2.4.2 Heterosis

The values of F_1 s were averaged over the replications and used in estimating heterosis percentage over mid parent (MP) i.e. mid parent or relative heterosis (H_1) and over superior parent (SP) i.e. heterobeltiosis (H_2). The parent in respective cross, which exhibited higher values for the character studied, was regarded as superior parents except for the characters earliness and maturity. For these, the parent with lower value regarded as superior, since such values are desirable one. The mid parent heterosis and heterobeltiosis were calculated and tested as specified by Turner (1953) and Hays *et al* (1955)

1. Mid parent heterosis (%)

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

Where,

F_1 = Mean of the F_1 cross

MP = Mean of the parents

2. Heterobeltiosis (%)

$$\text{Heterobeltiosis (\%)} = \frac{F_1 - SP}{SP} \times 100$$

Where,

F_1 = Mean of the F_1 cross

SP = Mean of the superior parent of that particular F_1 cross

Test of significance

Mid-parent heterosis and heterobeltiosis were tested by calculating standard error using the following formula

$$S E = [2 ME/r]^{1/2}$$

Where,

ME = Error mean sum of square

r = Number of replications

The critical differences were calculated by multiplying S E value with table 't' at 5% or 1% at error degrees of freedom

3.2.4.3 Analysis of variance for combining ability

The analysis was done according to Griffing (1956) Model I (fixed effect model), Method II (parents and F_1 s excluding reciprocals)

The mathematical model for combining ability analysis was assumed to be:

$$X_{ij} = \mu + g_i + g_j + S_{ij} + 1/bc + \sum_k e_{ijk}$$

$$i \text{ and } j = 1, 2, \dots, p$$

$$k = 1, 2, \dots, b$$

Where,

$$p = \text{Number of parents}$$

$$b = \text{Number of replications}$$

$$c = \text{Number of observations for each of the plots}$$

$$\mu = \text{Population mean}$$

$$g_i = \text{gca effect of } i^{\text{th}} \text{ parent}$$

$$g_j = \text{gca effect of } j^{\text{th}} \text{ parent}$$

$$s_{ij} = \text{sca effects of the cross between } i^{\text{th}} \text{ and } j^{\text{th}} \text{ parent}$$

$$e_{ijk} = \text{Environmental effect pertaining to the } ijk^{\text{th}} \text{ observation on } ij^{\text{th}} \text{ individual in } k^{\text{th}} \text{ block with } i^{\text{th}} \text{ as female parent and } j^{\text{th}} \text{ as male parent}$$

Assumption for model I

$$1 \quad \sum_{i=1}^p g_i = 0$$

$$2 \quad \sum_{j=1}^p S_{ij} = 0$$

Table 3.5 Analysis of variance

Source	d f	Sum of squares
Replication	(r-1)	$\frac{\sum Y^2 k}{n(n+2)/2} - \frac{Y^2}{n(n+1) r/2}$
Treatment	(t-1)	$\frac{(\sum Y_{ij})^2}{r} - \frac{Y^2}{n(n+1) r/2}$
Parents	(n-1)	$\sum_{i=j} Y^2_{ij} - \frac{[(\sum_{i=j} Y_{ij})]^2}{nr}$
Crosses	(c-1)	$\frac{\sum_{i=j} Y^2_{ij}}{r} - \frac{[(\sum_{i=j} Y_{ij})]^2}{n(n+1) r/2}$
Parents Vs Crosses	(t-n-c+1)	Treat S S – Parent S S – Crosses S S
Error	(t-1)(r-1)	Total S S – Treat S S – Replication S S
Total	(tr-1)	$\sum Y^2_{ijk} - \frac{Y^2}{n(n+1) r/2}$

Where,

r = Number of replications

t = Number of treatments

n = Number of parents

c = Number of crosses

The mean sum of squares was tested against the error variance by 'F' test.

Table 3.6 Analysis of variance for combining ability

Source	d f	S S	M S S	Expected mean S S
<i>gca</i>	(n-1)	S _g	M _g	$\sigma^2e + \frac{(n+2)}{(n-1)} \sum_i g_i^2$
<i>sca</i>	$\frac{n(n-1)}{2}$	S _s	M _s	$\sigma^2e + \frac{2}{n(n+1)} \sum_i \sum_j S_{ij}^2$
Error	M	Se	Me	σ^2e

Where,

$$S_g = \frac{1}{(n+2)} \left[\sum_i (X_{i1} + X_{i2})^2 - 4/n X^2 \right]$$

$$S_s = \sum_{i \leq j} \sum X_{ij}^2 - \frac{1}{(n+2)} \sum_i (X_{i1} + X_{i2})^2 + \frac{2}{(n+1)(n+2)} X^2$$

S_g = Sum of square due to *gca*

S_s = Sum of square due to *sca*

n = Number of parents

X_{ij} = Value of cross between ith and jth parent

X_i = Total of ith array in diallel table

X_i = Total of ith column in diallel table

X = Grand total of n² values of diallel

M_g and M_s were calculated by dividing the respective sum of squares with corresponding degrees of freedom while error mean square (M'e) was calculated by dividing error mean square by number of replications / e

$$M'e = \frac{Me}{r} = \sigma^2e$$

The following F ratios were used for testing the *gca* and *sca* effects

i To test the difference between *gca* effects

$$F [(n-1), M] = \frac{Mg}{M'e}$$

ii To test the difference between *sca* effects

$$F [n(n-1)/2, M] = \frac{Ms}{M'e}$$

Computation of *gca* and *sca* effects

The effects were estimated as follows

$$\mu = \frac{2}{n(n+1)} X$$

gca estimates of parent I

$$g = \frac{1}{(n+2)} [(X_{I1} + X_{I2}) - \frac{2}{n} X]$$

sca estimates of cross X_{ij}

$$S_{ij} = X_{ij} - \frac{1}{(n+2)} [(X_{I1} + X_{I2} + X_{J1} + X_{J2}) + \frac{2}{(n+1)(n+2)} X]$$

Standard error (S E) to test the significance of *gca* and *sca* estimates and the difference between each of the two estimates were computed using following formulae

$$S E \text{ for } gca \text{ effects } (g_i) = \sqrt{\frac{(n-1)}{n(n+2)} M'e}$$

$$S E \text{ for } sca \text{ effects } (S_{ij}) = \sqrt{\frac{n^2 + n + 2}{(n+1)(n+2)} M'e} \quad (i \neq j)$$

$$(S E) (g_i - g_j) = \sqrt{\frac{2M'e}{(n+2)}} \quad (i \neq j)$$

(S E) difference between two sca effects in different arrays is given by,

$$(S E) (S_{ij} - S_{ik}) = \sqrt{\frac{2(n+1)}{(n+2)} M'e} \quad (i \neq j, k, j \neq i)$$

Critical differences were estimated as given above

3.2.4.3 Generation mean analysis

For computation of gene effects for grain yield, its components, pathological and biochemical characters with six basic generations, Cavalli's (1952) three parameter model and Hayman's (1958) six parameter models were used

Sampling variance of generation means

The generation means were subjected to sampling variation, which can be estimated by normal statistical procedures. Replication wise variance among the individuals within each generation was estimated and then pooled over replications. The estimates of variance of a generation mean (\bar{X}) were obtained by dividing the variance within generation by the total number of individuals in that generation.

Joint Scaling Test

Further analysis of data was performed according to the method of "Joint Scaling Test" given by Cavalli (1952). It consists of estimating the parameters from the means of all the available generations followed by comparison of observed generation means with expected values derived from the estimates of the parameters of a model. The parameters are estimated by weighed least squares, taking the weights as reciprocals of the squared standard errors of each mean. The comparison between observed and expected mean was then done by assuming the sum of squares, minimized in the fitting process, to be distributed as chi-square with degrees of freedom equal to the difference of the number of parameters of a model and number of generation means.

available Number of generations available in the present study (i.e. six generations) were sufficient to test the adequacy of additive-dominance i.e. three parameter model with three degrees of freedom for Chi-square test

Sequential steps involved in the procedure of joint scaling test used have been given below

Step I: Computation of mean weights in different generations and tabulations of these values with the coefficient of parameters

Table 3.7 Generation means, weights and coefficients in three parameter model

Generations	Mean	Weights	Coefficient of parameters		
			m	d	h
P ₁	P ₂	1/V(P ₁)	1	1	0
P ₂	P ₂	1/V(P ₂)	1	-1	0
F ₁	F ₁	1/V(F ₁)	1	0	1
F ₂	F ₂	1/V(F ₂)	1	0	½
B ₁	B ₁	1/V(B ₁)	1	½	½
B ₂	B ₂	1/V(B ₁)	1	-½	½

In the table, weights are defined as reciprocal of the squared standard errors, denotes as 1/V(X) Coefficient of these parameters has been given by Mather and Jinks (1971)

Step II : Setting up the equation for estimating m, d and h

The equations were obtained by equating the observed generations means, to their expectation in terms of the parameters to be estimated For three parameter model, three equations are required to estimate m, d and h

Equations with respect of m, since there are six generations and total of six equations were set up Say for P₁ the coefficient of m is 1 so the desired equation is –

$$P_1 = 1^* \times 1^* \times \frac{1}{V(P_1)/m} + 1^* \times (-1)^{**} \times \frac{1}{V(P_1)d} \times 1^* \times 0^{**} \times \frac{1}{V(P_1)h}$$

Where,

*, **, *** = Coefficient of m, d and h, respectively

Similarly, the equations were obtained for all other generations with respect to m These equations were therefore summed to yield equation 1 with respect to m Second and third equations with respect to d and h were obtained in similar fashion Let the three equations be

$$T_{11} m + T_{12} d + T_{13} h = Tm CW_1 \quad (1)$$

$$T_{21} m + T_{22} d + T_{23} h = Tm CW_2 \quad (2)$$

$$T_{31} m + T_{32} d + T_{33} h = Tm CW_3 \quad (3)$$

Step III : Obtaining the estimates of parameters by way of matrix inversion

From the above three equations, the estimates of the parameter were obtained in the followed way

$$\begin{array}{c} \left| \begin{array}{ccc} T_{11} & T_{12} & T_{13} \\ T_{21} & T_{22} & T_{23} \\ T_{31} & T_{32} & T_{33} \end{array} \right| \\ A \end{array} \begin{array}{c} \left| \begin{array}{c} m \\ d \\ h \end{array} \right| \\ B \end{array} = \begin{array}{c} \left| \begin{array}{c} TmCW_1 \\ TmCW_2 \\ TmCW_3 \end{array} \right| \\ C \end{array}$$

Where,

A = is the information matrix

B = is the column vector of the estimates of parameters

C = is the column vector of scores

The solution then takes the general form,

$$B = A^{-1}C$$

Where,

A^{-1} = is the inverse of information matrix accordingly

$$\begin{matrix} m \\ d \\ h \end{matrix} = \begin{vmatrix} t_{11} & t_{12} & t_{13} \\ t_{21} & t_{22} & t_{23} \\ t_{31} & t_{32} & t_{33} \end{vmatrix} \begin{vmatrix} TmCW_1 \\ TmCW_2 \\ TmCW_3 \end{vmatrix} = \begin{vmatrix} K \\ L \\ M \end{vmatrix}$$

The standard errors and respective t' values of each of these estimates were obtained as under root of diagonal element of the inverse matrix as given below

$$SE (m) = \sqrt{t_{11}}$$

$$SE (d) = \sqrt{t_{22}}$$

$$SE (h) = \sqrt{t_{33}}$$

$$Tm = \frac{m}{SE (m)} = K / \sqrt{t_{11}}$$

$$Td = \frac{d}{SE (d)} = L / \sqrt{t_{22}}$$

$$Th = \frac{h}{SE (h)} = M / \sqrt{t_{33}}$$

Now, the three parameters with their standard errors are

$$m = K \pm t \sqrt{t_{11}}$$

$$d = L \pm t \sqrt{t_{22}}$$

$$h = M \pm t \sqrt{t_{33}}$$

The calculated 't' values were tested against 1.96 and 2.57, which are table values of 't' at 5 and 1 per cent level of significance, respectively

Step IV: Testing adequacy of model

The following two steps were involved

- 1 Expected means of all the generations were computed using the estimates of parameters
- 2 From observed and expected values of each generation of Chi-square (χ^2) was obtained as follows

$$\chi^2 = (\text{Observed mean} - \text{Expected mean})^2 \times \text{Weight}$$

When the pooled χ^2 value was within the acceptable probability limit (0.05), the model was taken as adequate and inferences were drawn from the estimates of parameters

Six parameter model (Hayman, 1958)

Components of generation means were analysed for three crosses using six basic generations viz, P₁, P₂, F₁, F₂, B₁ and B₂. Cavalli's (1952) joint scaling test was applied to test the adequacy of additive-dominance model. Whenever, the model was found inadequate, Hayman (1958) six parameter model was followed to estimate the different gene effects.

Various notations used for the various gene effects by Hayman (1958) were

Gene effects	Notations of Hayman (1958)
Mean	m
Additive	d
Dominance	h
Additive x Additive	i
Additive x Dominance	j
Dominance x Dominance	l

The estimates of m, d, h, i, j and l were calculated by using the means of six populations

$$m = \bar{F}_2$$

$$d = \bar{B}_1 - \bar{B}_2$$

$$h = -\frac{1}{2} \bar{P}_1 - \frac{1}{2} \bar{P}_2 - \bar{F}_1 - 4 \bar{F}_2 + 2 \bar{B}_1 + 2 \bar{B}_2$$

$$i = 2 \bar{B}_1 + 2 \bar{B}_2 - 4 \bar{F}_2$$

$$j = \bar{B}_1 + \frac{1}{2} \bar{P}_1 - \bar{B}_2 + \frac{1}{2} \bar{P}_2$$

$$l = \bar{P}_1 + \bar{P}_2 + 2 \bar{F}_1 + 4 \bar{F}_2 - 4 \bar{B}_1 + 4 \bar{B}_2$$

The variances of these gene effects were obtained as

$$V_m = V\bar{F}_2$$

$$V_d = V\bar{B}_1 + V\bar{B}_2$$

$$V_h = \frac{1}{4} V\bar{P}_1 + \frac{1}{4} V\bar{P}_2 + V\bar{F}_1 + 16 V\bar{F}_2 + 4 V\bar{B}_1 + 4 V\bar{B}_2$$

$$V_i = 16 V\bar{F}_2 + 4 V\bar{B}_1 + 4 V\bar{B}_2$$

$$V_j = \frac{1}{4} V\bar{P}_1 + \frac{1}{4} V\bar{P}_2 + V\bar{B}_1 + V\bar{B}_2$$

$$V_l = V\bar{P}_1 + V\bar{P}_2 + 4 V\bar{F}_1 + 16 V\bar{F}_2 + 16 V\bar{B}_1 + 16 V\bar{B}_2$$

Estimation of standard error

$$S E (m) = \sqrt{V_m}$$

$$S E (d) = \sqrt{V_d}$$

$$S E (h) = \sqrt{V_h}$$

$$S E (i) = \sqrt{V_i}$$

$$S E (j) = \sqrt{V_j}$$

$$S E (l) = \sqrt{V_l}$$

Estimates of 't' values for gene action

It is the ratio of the estimate of different genetic effects to their standard errors. It is used to test significance of the values of different genetic effects and calculated as

$$t (m) = m/S E (m)$$

$$t (d) = d/S E (d)$$

$$t (h) = h/S E (h)$$

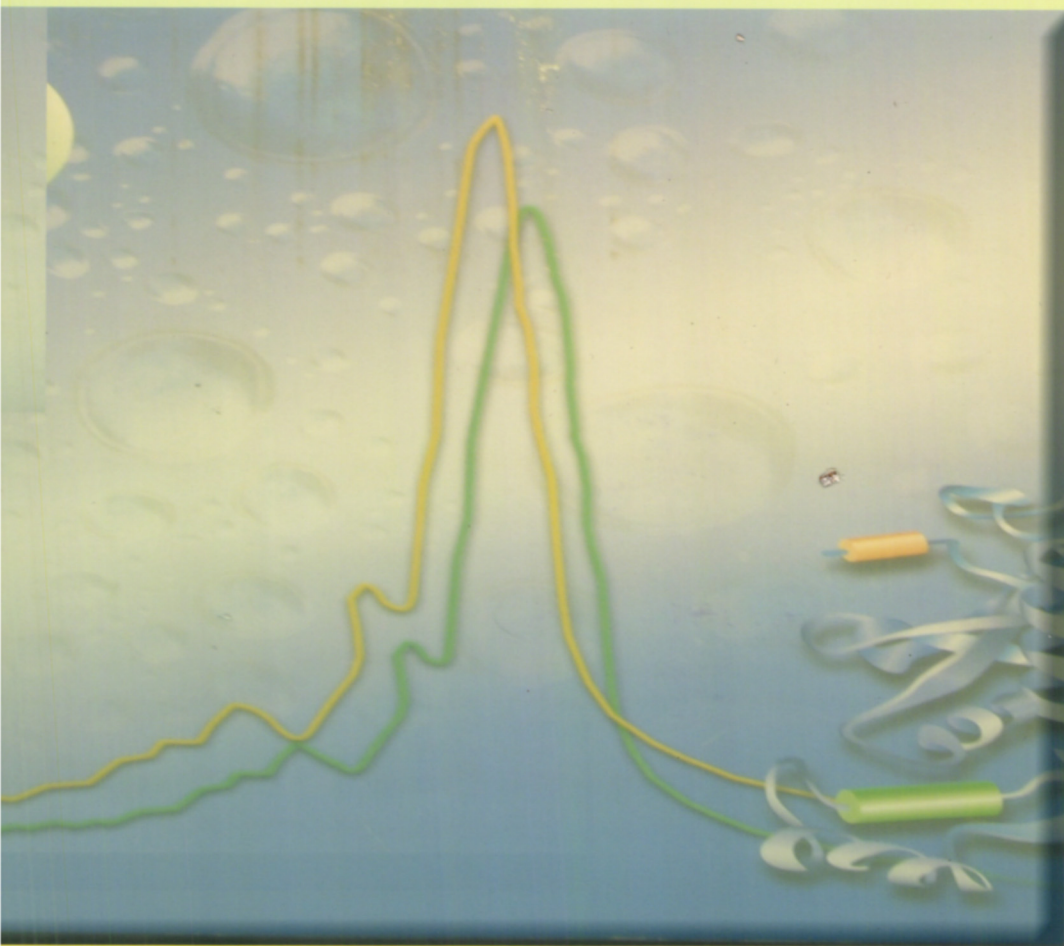
$$t (i) = i/S E (i)$$

$$t (j) = j/S E (j)$$

$$t (l) = l/S E (l)$$

The calculated values of 't' were to be compared with 1.96 and 2.57 which were the tabulated values of 't' at 5 per cent and 1 per cent of significance, respectively.

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4

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The present investigation was undertaken with views to study heterosis and combining ability through diallel analysis, the DNA polymorphism revealed by molecular analysis and to know the gene actions through generation mean analysis for seed yield, its components and quality traits in mungbean (*Vigna radiata* (L.) Wilczek). Some pathological as well as biochemical parameters related to powdery mildew (*Erysiphe polygoni* D C) resistance were also studied to know their gene action through generation mean analysis.

The results obtained in the present investigation have been grouped and presented under following subheadings

- 4.1 Heterosis and combining ability**
- 4.2 DNA polymorphism study using molecular markers**
- 4.3 Generation mean analysis for seed yield, its components and nutritional quality traits**
- 4.4 Genetics of host-parasite interaction and biochemicals related to powdery mildew resistance**

4.1 Heterosis and Combining Ability

4.1.1 Mean performance of parents and their crosses

The variation among the genotypes was highly significant for all the characters studied (Table 4.1) which indicated the presence of substantial genetic variability among the mean values of parents and their hybrids for all the traits under study.

Table 4.1 Analysis of variance

Source of variance	d f	Mean squares				
		Days to 50% flowering	Days to maturity	Plant height	Primary branches/ plant	Pod clusters/ plant
Replication	2	0 440	1 083	11 760	0 126	0 005
Genotypes	27	6 964**	13 885**	39 728**	0 148**	0 953**
Error	54	1 255	1 022	19 304	0 051	0 079

Source of variance	d f	Mean squares			
		Pods/ plant	Seeds/ pod	100 seed weight	Seed yield/ plant
Replication	2	0 893	0 015	0 227	0 171
Genotypes	27	13 656**	0 717**	0 600**	2 018**
Error	54	2 776	0 480	0 073	0 182

*, ** significant at 5% and 1% level, respectively

1. Days to 50 % flowering

The mean values of parents and crosses ranged between 35 00 to 39 33 and 34.33 to 40.33 days, respectively (Table 4 2)

Among the parents, TARM 18 and Kopargaon were the earliest to flower with mean value 35 00 days, requiring significantly less (35 33) number of days than others. The cross combination, Kopargaon x AKM 8802 recorded significantly less number of days to 50% flowering than the rest of the crosses.

2. Days to maturity

Parents and crosses had range from 61 00 to 67 33 and 60 67 to 68 33 days, respectively. Among the parents, TARM 18 (61 00) was the earliest parent requiring significantly less number of days to maturity, followed by Kopargaon (63 00).

The cross AKM 8802 x TARM 18 (60 67) required significantly less number of days to maturity than the rest of the combinations.

Table 4.2 Mean performance of parents and crosses in mungbean

Sr. No	Genotypes	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches /plant	Pod clusters / plant
Parents						
1	Kopargaon	35 00	63 00	54 11	2 20	9 07
2	AKM 8802	38 00	63 33	49 59	2 07	7 67
3	PM 9341	39 33	67 00	56 37	2 27	8 53
4	BM 4	38 00	65 00	42 80	2 00	8 00
5	BPMR 145	38 33	67 33	54 54	2 40	7 80
6	Vaibhav	38 00	67 00	60 01	2 60	9 46
7	TARM 18	35 00	61 00	53 71	2 80	8 40
Crosses						
8	Kopargaon x AKM 8802	35 33	64 00	51 14	2 00	8 47
9	Kopargaon x PM 9341	38 67	66 33	55 96	2 40	8 86
10	Kopargaon x BM 4	37 00	64 00	52 24	2 36	8 87
11	Kopargaon x BPMR 145	37 33	66 33	55 15	2 56	9 47
12	Kopargaon x Vaibhav	38 33	65 67	54 32	2 20	9 13
13	Kopargaon x TARM 18	34 33	61 00	49 67	2 67	8 93
14	AKM 8802 x PM 9341	37 33	66 66	49 23	2 40	8 13
15	AKM 8802 x BM 4	36 67	64 66	49 38	2 06	7 93
16	AKM 8802 x BPMR 145	37 67	67 67	9 38	2 40	8 00
17	AKM 8802 x Vaibhav	36 67	66 00	56 06	2 60	8 93
18	AKM 8802 x TARM 18	36 67	60 67	51 22	2 53	8 47
19	PM 9341 x BM 4	39 00	66 67	54 80	2 40	8 33
20	PM 9341 x BPMR 145	39 33	67 00	53 82	2 37	8 50
21	PM 9341 x Vaibhav	40 33	66 67	54 63	2 53	9 40
22	PM 9341 x TARM 18	38 33	63 67	58 82	2 67	8 53
23	BM 4 x BPMR 145	38 67	67 33	53 87	2 60	7 80
24	BM 4 x Vaibhav	39 00	67 33	57 18	2 53	9 67
25	BM 4 x TARM 18	35 33	63 33	55 41	2 67	8 33
26	BPMR 145 x Vaibhav	39 33	68 33	59 79	2 67	8 27
27	BPMR 145 x TARM 18	37 00	63 33	55 89	2 40	8 73
28	Vaibhav x TARM 18	37 66	64 00	64 56	2 67	9 33
	SE (m) \pm	0 6468	0 5835	2 5366	0 1301	0 1620
	CD at 5%	1 834	1 655	7 192	0 368	0 460

Cntd .

Table 4.2 (Contd...)

Sr. No	Genotypes	Pods/ plant	Seeds/ pod	100 seed weight (g)	Seed yield/ plant (g)
Parents					
1	Kopargaon	21 07	10 20	4 26	9 00
2	AKM 8802	21 33	10 35	4 13	7 78
3	PM 9341	19 33	09 50	4 61	8 61
4	BM 4	22 80	10 47	3 15	8 18
5	BPMR 145	19 31	11 46	4 47	8 48
6	Vaibhav	20 41	10 97	4 41	9 21
7	TARM 18	21 74	10 67	3 26	8 44
Crosses					
8	Kopargaon x AKM 8802	23 11	11 00	4 27	9 53
9	Kopargaon x PM 9341	18 80	10 42	4 04	8 07
10	Kopargaon x BM 4	24 04	11 21	4 77	8 90
11	Kopargaon x BPMR 145	22 80	10 57	4 34	8 26
12	Kopargaon x Vaibhav	22 48	11 00	3 51	8 98
13	Kopargaon x TARM 18	25 70	11 20	3 99	9 77
14	AKM 8802 x PM 9341	22 10	10 13	4 09	7 22
15	AKM 8802 x BM 4	25 87	11 42	4 44	9 68
16	AKM 8802 x BPMR 145	22 80	10 57	4 37	9 15
17	AKM 8802 x Vaibhav	25 28	11 55	4 03	9 93
18	AKM 8802 x TARM 18	21 26	10 37	3 27	7 99
19	PM 9341 x BM 4	23 60	11 18	4 63	10 14
20	PM 9341 x BPMR 145	18 60	10 93	4 48	9 42
21	PM 9341 x Vaibhav	19 93	10 84	4 81	8 61
22	PM 9341 x TARM 18	22 30	11 77	4 05	8 12
23	BM 4 x BPMR 145	24 27	11 10	4 07	8 48
24	BM 4 x Vaibhav	20 93	11 07	3 51	9 77
25	BM 4 x TARM 18	26 47	11 35	3 94	10 37
26	BPMR 145 x Vaibhav	20 46	11 10	4 15	8 69
27	BPMR 145 x TARM 18	21 36	11 00	3 72	8 28
28	Vaibhav x TARM 18	22 10	11 27	4 26	10 84
	SE (m) ±	0 9620	0 4397	0 1561	0 2465
	CD at 5%	2 728	1 247	0 442	0 698

3. Plant height (cm)

Mean performance for parents and crosses ranged from 42.80 to 60.01 cm and 49.23 to 64.56 cm, respectively. The parent Vaibhav (60.01 cm) was the tallest, while cross Vaibhav x TARM 18 (64.56 cm) was found significantly tall among rest of the crosses except BPMR 145 x Vaibhav (59.79 cm) and PM 9341 x TARM 18 (58.82 cm).

4. Primary branches per plant

The mean values for parents and crosses varied between limits of 2.00 to 2.80 and 2.00 to 2.67, respectively. Among the parents, TARM 18 (2.80) was significantly superior followed by Vaibhav (2.60) and BPMR 145 (2.40).

The cross combinations viz, Kopargaon x TARM 18, PM 9341 x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18 which were *at par* exhibited significant number of branches/ plant (2.67).

5. Pod clusters per plant

Pod clusters ranged between 7.67 to 9.46 and 7.80 and 9.67 for parents and crosses, respectively. The parent, Vaibhav (9.46) recorded higher number of pod clusters followed by Kopargaon (9.07) and PM 9341 (8.53).

Among the crosses, BM 4 x Vaibhav (9.67) had significantly more clusters per plant followed by Kopargaon x BPMR 145 (9.47), PM 9341 x Vaibhav (9.33) and Vaibhav x TARM 18 (9.33) than other hybrids.

6. Pods per plant

The pods per plant were ranged between 19.31 to 22.80 and 18.60 to 26.47 in parents and crosses, respectively. The parent BM 4 displayed highest pods per plant (22.80) followed by TARM 18 (21.74) and AKM 8802 (21.33).

Five cross combinations viz, BM 4 x TARM18 (26 47), AKM 8802 x BM 4 (25 87), Kopargaon x TARM 18 (25 70), AKM 8802 x Vaibhav (25 28), BM 4 x BPMR 145 (24 27) displayed significantly more number of pods per plant

7. Seeds per pod

The mean performance of this trait varied between limits of 9 50 to 11 46 and 10 13 to 11 77 for parents and crosses, respectively. The parent, BPMR 145 (19 31) followed by Vaibhav (10 97) recorded highest seeds per pod

Eighteen hybrids were found with higher significant means than the rest of the crosses, in which PM 9341 x TARM 18 (11 77) followed by AKM 8802 x Vaibhav (11 55) recorded higher number of seeds/ plant

8. 100 seed weight (g)

Among the parents highest 100 seed weight was displayed by PM 9341 (4 61 g) followed by BPMR 145 (4 47 g) and Vaibhav (4 41 g). The mean values for this character ranged from 3 15 to 4 61 g and 3 27 to 4 81 g for parent and hybrids, respectively

The cross combination, PM 9341 x Vaibhav (4 81 g) exhibited significantly higher test weight than the rest of crosses followed by Kopargaon x BM 4 (4 77 g) and PM 9341 x BM 4 (4 63 g) among the six hybrids with highly significant means

9. Seed yield per plant (g)

The mean values for this trait among parents and crosses varied between 7 78 to 9 21 g and 7 22 to 10 48 g, respectively. The parents, Vaibhav produced highest (9 21 g) seed yield per plant followed by Kopargaon (9 00 g) and PM 9341 (8 61 g)

The crosses, Vaibhav x TARM 18 (10 84 g) evinced significantly higher mean value for seed yield per plant followed by BM 4 x TARM 18 (10 37 g) and PM 9341 x BM 4 (10 14 g)

4.1.2 Heterosis

The magnitudes of mid parent heterosis (H_1) and heterobeltiosis (H_2) have been presented in Table 4.3 and the range for them has been depicted in Fig. 4.1 and 4.2

1. Days to 50 % flowering

The negative heterosis i.e. earliness is desirable, hence cross combinations having negative heterosis for days to 50 % flowering are of immense value in breeding programme

The mid parent heterosis and heterobeltiosis ranged from 3.51 to 5.02% and -3.51 to 10.48%, respectively. None of the crosses showed significantly negative heterosis over MP as well as BP for this character. Whereas, two crosses viz., Kopargaon x Vaibhav and PM 9341 x Vaibhav showed significant positive mid parent heterosis and eight crosses exhibited significant heterobeltiosis

Highest negative mid parent heterosis as well as heterobeltiosis of -3.51% was recorded by two crosses viz., AKM 8802 x BM 4 and AKM 8802 x Vaibhav.

2. Days to maturity

The heterosis over mid and better parents ranged from -4.46 to 2.05% and -0.55 to 5.29%, respectively. Only hybrid, AKM 8802 x TARM 18 (-4.46%) exhibited significant desirable heterosis over mid parent. While, none of the crosses showed significantly negative heterobeltiosis as well as positive relative heterosis for this trait. Ten crosses showed significant positive heterobeltiosis

The hybrid AKM 8802 x TARM 18 (-4.46%) followed by Kopargaon x TARM 18 (-1.16%) evinced highest negative heterosis over mid parent. The highest estimates of heterobeltiosis in the favourable direction were exhibited by the cross AKM 8802 x TARM 18 (-0.55%) followed by PM 9341 x Vaibhav (-0.50%)

Table 4.3 Percentage of mid parent heterosis (H_1) and heterobeltiosis (H_2) in mungbean

Crosses		Days to 50% flowering	Days to maturity	Plant height	Primary branches / plant	Pod clusters/ plant	Pods/ plant	Seeds/ pod	100 seed weight	Seed yield/ plant
pargaon x M 8802	H ₁	-3.20	-1.03	-1.27	-1.64	1.6	9.21	8.11	5.04	13.6**
	H ₂	0.95	1.59	-5.30	-3.23	-5.93*	8.36	6.28	3.39	5.89
pargaon x A 9341	H ₁	4.04	2.05	1.41	12.50	1.14	-6.78	6.84	-6.16	-8.4*
	H ₂	10.48**	5.29**	-0.72	5.88	-1.48	-10.48	4.17	-12.36*	-10.4**
pargaon x A 4	H ₁	1.37	0.00	7.93	18.33*	4.31	9.79	9.54	33.55**	3.61
	H ₂	5.71*	1.59	-3.26	18.33	-1.48	5.45	7.10	19.42**	-1.07
pargaon x PMR 145	H ₁	1.82	1.79	1.62	16.67*	12.7**	13.1*	-1.55	2.56	5.89
	H ₂	6.67*	5.29**	1.11	6.94	5.19*	8.57	-7.85	-2.83	2.85
pargaon x Aibhav	H ₁	5.02*	1.03	-4.71	-4.35	-1.08	8.60	4.93	-16.7**	-1.34
	H ₂	9.52**	4.23**	9.49	-15.38*	-3.52	7.08	0.3	-20.6**	-2.46
pargaon x ARM 18	H ₁	-1.90	-1.61	-7.77	11.11	2.68	20.2**	8.39	10.05	12.0**
	H ₂	-1.90	0.00	-8.02	-4.76	-0.74	18.2**	5.0	-0.08	8.52*
M 8802 x A 9341	H ₁	-3.45	0.25	7.44	12.5	-1.61	9.59	3.93	-4.99	-13.1**
	H ₂	-1.75	1.01	-12.67	5.88	-4.69	5.25	1.33	-11.28*	-16.2**
M 8802 x A 4	H ₁	-3.51	-1.27	10.75	3.33	-0.83	18.1**	11.56*	24.23**	19.6**
	H ₂	-3.51	-0.51	2.77	3.33	-0.83	13.45*	9.08*	11.08*	18.3**
M 8802 x PMR 145	H ₁	-1.31	1.50	-5.52	9.09	1.27	13.12*	-1.65	3.19	11.0**
	H ₂	-0.88	2.53	-9.45	0.00	0.00	8.57	-7.94	-2.24	7.86
M 8802 x Aibhav	H ₁	-3.51	-0.75	1.91	13.04	2.29	22.1**	6.39	-4.32	3.78
	H ₂	-3.51	0.00	-6.59	0.00	-5.63*	20.4**	1.7	-8.83	-3.04
M 8802 x ARM 18	H ₁	0.46	-4.46**	-1.22	5.56	3.25	-0.49	0.32	-10.05	-2.84
	H ₂	4.76	-0.55	-4.63	-9.52	0.79	-2.19	-2.81	-18.3**	-5.41
A 9341 x A 4	H ₁	1.30	1.01	10.93	20.0*	-1.96	12.92*	9.28	13.65**	16.8**
	H ₂	2.63	2.56*	-2.15	20.0*	-7.41**	3.51	6.85	-7.33	11.5**
A 9341 x PMR 145	H ₁	1.72	-0.25	-2.61	7.58	1.19	-2.9	1.86	-5.39	7.95*
	H ₂	2.61	0.00	-3.88	-1.39	-5.56*	-3.68	-4.65	-10.40*	4.85
A 9341 x Aibhav	H ₁	4.76*	-0.50	-5.88	10.14	1.81	1.15	3.43	2.23	-5.47
	H ₂	6.14*	-0.50	-9.03	-2.56	-0.70	-2.35	-1.12	-3.73	-6.55
A 9341 x ARM 18	H ₁	3.60	-0.52	7.23	11.11	-1.92	9.47	13.87*	-1.98	-6.88
	H ₂	9.52**	4.37**	5.04	-4.76	-5.19*	2.56	10.31*	-19.0**	-9.74*
A 4 x PMR 145	H ₁	1.31	1.76	10.45	18.18*	-1.27	14.76*	3.42	9.09	2.93
	H ₂	1.75	3.59**	-1.23	8.33	-2.50	5.55	-3.2	-8.87	0.00
A 4 x Aibhav	H ₁	2.63	2.02	11.02	10.14	10.7**	-3.56	5.56	-5.26	13.5**
	H ₂	2.63	3.59**	-4.72	-2.56	2.11	-8.99	0.91	-20.5**	6.04
A 4 x ARM 18	H ₁	-3.20	0.53	14.60*	11.11	1.63	18.3**	9.9	25.92**	26.1**
	H ₂	0.95	3.83**	3.18	-4.76	-0.79	15.1*	6.47	20.8**	22.8**
PMR 145 x Aibhav	H ₁	3.51	1.99	3.97*	15.94*	-5.34*	3.86	1.06	-1.23	0.99
	H ₂	3.51	1.99	-0.37	2.56	-12.7**	0.26	0.91	-5.89	-5.65
PMR 145 x ARM 18	H ₁	1.37	-1.04	2.83	0.00	6.50**	4.88	1.54	2.52	0.69
	H ₂	5.71*	3.83**	1.62	-14.29*	3.97	-1.73	0.00	-6.92	-1.97
Aibhav x ARM 18	H ₁	3.20	0.00	-4.02	-8.05	7.28**	5.89	4.00	17.3**	24.3**
	H ₂	7.62**	4.92**	-9.06	-11.11	3.70	1.64	2.42	6.50	20.4**
E D ±	H ₁	0.792	0.715	3.107	0.159	0.198	1.178	0.538	0.191	0.302
	H ₂	0.915	0.825	3.587	0.183	0.229	1.360	0.621	0.220	0.348

*, ** Significant at 5% and 1% level, respectively

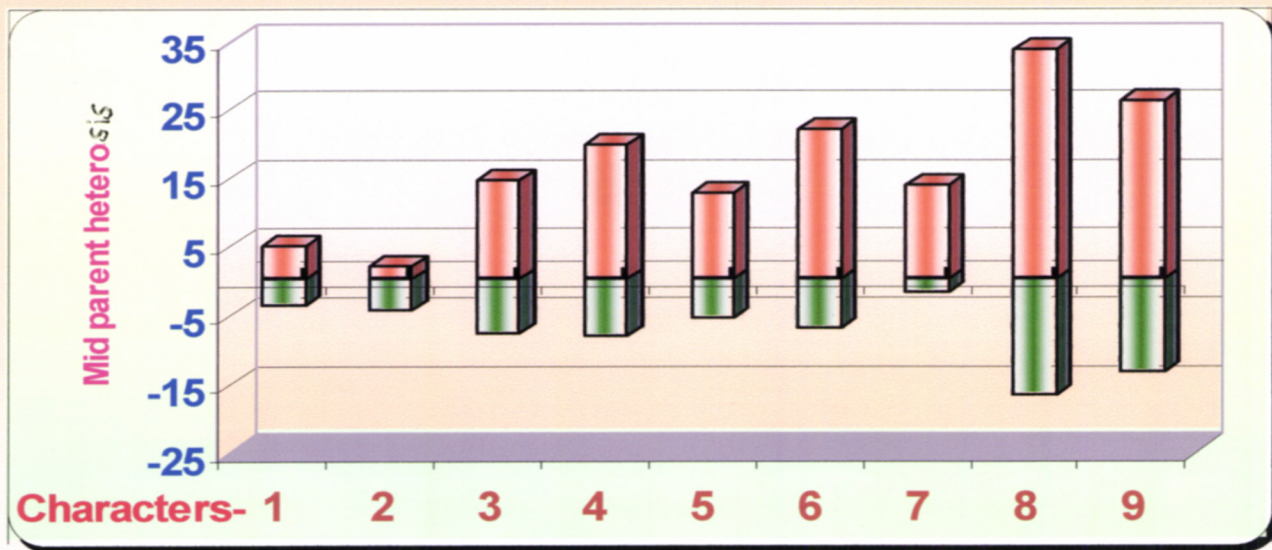


Fig. 4.1 Ranges of mid parent heterosis for different characters

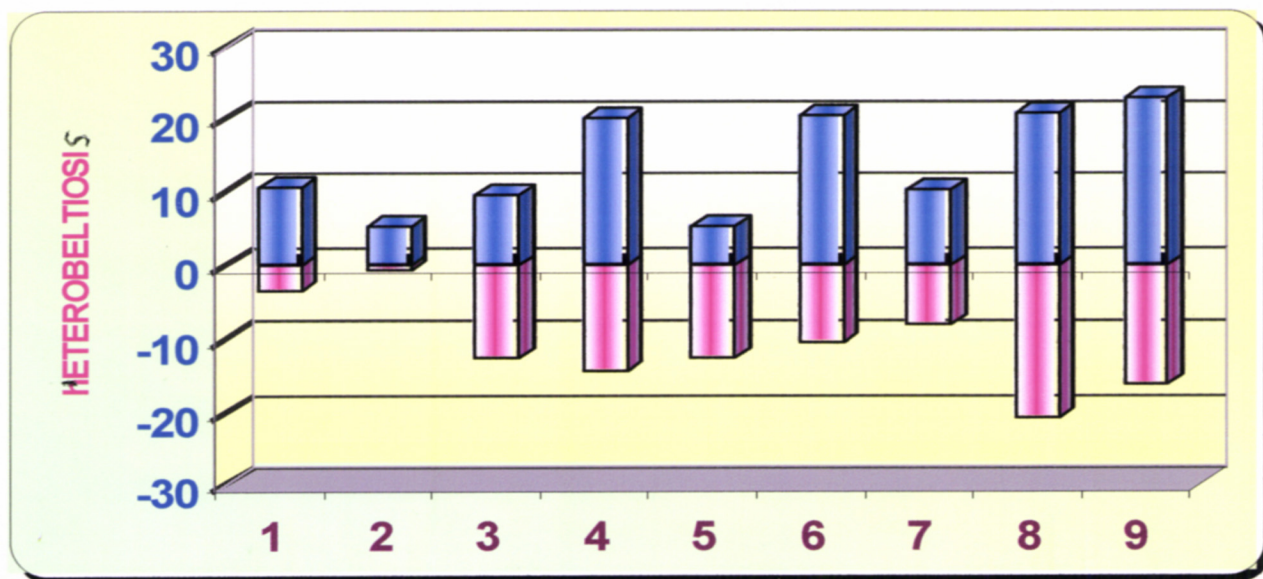


Fig. 4.2 : Ranges of heterobeliosis for different characters

Characters

- | | |
|--------------------------|-----------------------------|
| 1. Days to 50% flowering | 2. Days to maturity |
| 3. Plant height | 4. Primary branches / plant |
| 5. Clusters / plant | 6. Pods / plant |
| 7. Seeds / plant | 8. 100 seed weight |
| 9. Seed yield / plant | |

3. Plant height

The extent of heterosis over mid parent and better parent for this character ranged from -7.77 to 14.60% and -12.67 to 9.49%, respectively. Out of 21 crosses two showed significant positive relative heterosis, however, none of the crosses recorded significant negative heterosis over mid parent as well as significant positive heterosis over mid and better parent. The highest significant positive relative heterosis was exhibited by the cross BM 4 x TARM 18 (14.60%) followed by BM 4 x Vaibhav (11.02%), while the highest positive heterobeltiosis was displayed by the hybrid Kopargaon x Vaibhav (9.49%).

4. Primary branches per plant

The variation for mid parent heterosis and heterobeltiosis for this character was ranged from -8.05 to 20.0% and -14.29 to 20.0%, respectively. Among 21 crosses, only the cross PM 9341 x BM 4 (20.0%) evinced highest significant heterotic effect over mid parent and better parent. Five crosses showed significant positive mid parent heterosis.

5. Pod clusters per plant

Highest percentage of heterosis over mid parent was displayed by the cross Kopargaon x BPMR 145 (12.7%) followed by BM 4 x Vaibhav (10.7%). Out of four highly significant heterotic combinations, only one cross was found with significant negative relative heterosis. The range of variation for heterosis over mid parent and better parent was observed from -5.34 to 12.7% and -12.7 to 5.19%, respectively. The only cross combination Kopargaon x BPMR 145 (5.19%) manifested highest significant positive heterobeltiosis, followed by BPMR 145 x TARM 18 (3.97%) and Vaibhav x TARM 18 (3.70%), but they were non-significant whereas none of the crosses exhibited negative significant heterobeltiosis.

6. Pods per plant

The mid parent heterosis and heterobeltiosis were ranged from -6.78 to 22.1% and -10.48 to 20.4%, respectively. Eight and four crosses exhibited significant positive relative heterosis and heterobeltiosis, respectively. The cross AKM 8802 x Vaibhav (22.1%) followed by Kopargaon x TARM 18 (20.2%) and BM 4 x TARM 18 (18.3%) was found with highest highly significant mid parent heterosis, whereas the same hybrid followed by Kopargaon x TARM 18 (18.2%) and BM 4 x TARM 18 (15.1%) recorded highly significant heterobeltiosis of 20.4% for this trait.

7. Seeds per pod

The estimate of mid parent heterosis and heterobeltiosis varied from -1.65 to 13.87% and -7.94 to 10.31%, respectively.

Among, the 21 crosses, PM 9341 x TARM 18 ($H_1 = 13.87\%$ and $H_2 = 10.31\%$) manifested highest significant mid parent heterosis as well as heterobeltiosis in desirable direction followed by AKM 8802 x BM 4 ($H_1=10.31\%$ and $H_2=9.08\%$) for number of seeds/ pod.

8. 100 seed weight

The heterosis over mid parent varied from -16.7 to 33.55% with the heterobeltiosis ranging from -20.6 to 20.8%. Five and three cross combinations showed significant positive heterosis over mid parent and better parent, respectively.

Highest significant positive relative heterosis was recorded in the hybrid Kopargaon x BM 4 (33.55%) followed by BM 4 x TARM 18 (25.92%) and AKM 8802 x PM 9341 (24.23%). Whereas BM 4 x TARM 18 (20.8%) registered highest significant positive heterobeltiosis followed by Kopargaon x BM 4 (19.42%) and AKM 8802 x BM 4 (11.08%).

9. Seed yield per plant

The range of relative heterosis and heterobeltiosis was observed between -13.1 to 26.1% and -16.2 to 22.8%, respectively. Nine and five cross combinations recorded significant positive heterosis over mid parent and better parent, respectively.

The cross combination BM 4 x TARM 18 ($H_1=26.1\%$ and $H_2=22.8\%$) followed by Vaibhav x TARM 18 ($H_1=24.4\%$ and $H_2=20.4\%$) and AKM 8802 x BM 4 ($H_1=79.6\%$ and $H_2=18.3\%$) registered highest percentage of highly significant positive mid parent heterosis as well as heterobeltiosis for seed yield per plant, among 21 crosses.

4.1.3 Combining Ability Analysis

4.1.3.1 Analysis of variance

The analysis of variance presented in Table 4.4 divulged that, the mean sum of squares due to treatments and crosses were highly significant for all the characters except seeds per pod. The parents showed highly significant differences for most of the characters except seeds per pod.

The analysis of variance for combining ability (general and specific combining ability) revealed that the variances due to parents / i.e. general combining ability effects were significant for all the traits except primary branches per plant and seeds per pod. The variances due to specific combining ability effects were also highly significant for almost all the characters under study (Table 4.5).

Table 4.4 Analysis of variance

Source of variance	d f	Mean squares				
		Days to 50% flowering	Days to maturity	Plant height	Primary branches/ plant	Pod clusters/ plant
Replication	2	0 440	1 083	11 760	0 126	0 005
Genotypes	27	6 964**	13 885**	39 728**	0 148**	0 953**
Parents	6	8 603**	17 413**	90 135**	0 249**	1 328**
Crosses	20	6 776**	13 521**	25 796*	0 112*	0 839**
Parents Vs Crosses	1	0 893	0 016	15 931*	0 260*	1 003**
Error	54	1 255	1 022	19 304	0 051	0 079

Source of variance	d f	Mean squares			
		Pods/ plant	Seeds/ pod	100 seed weight	Seed yield/ plant
Replication	2	0 893	0 015	0.227	0 171
Genotypes	27	13 656**	0 717	0 600**	2 018**
Parents	6	4 872*	1 146	1 048**	0 693**
Crosses	20	14 620**	0 454	0 489**	2 303**
Parents Vs Crosses	1	47 086**	3 421*	0 126*	4 267**
Error	54	2 776	0 580	0 073	0 182

*, ** significant at 5% and 1% level, respectively

Table 4.5 Analysis of variance for combining ability

Source of variance	d f	Mean squares				
		Days to 50% flowering	Days to maturity	Plant height	Primary branches/ plant	Pod clusters/ plant
SCA	6	8 061**	18 414**	32 489**	0 134**	1 020**
GCA	21	0 682*	0 690*	7 744*	0 025	0.117**
Error	54	0 410	0 341	6 035	0 017	0 026

Source of variance	d f	Mean squares			
		Pods/ plant	Seeds/ pod	100 seed weight	Seed yield/ plant
SCA	6	9 674**	0 337*	0 351**	0 641**
GCA	21	3 089**	0 211*	0 157**	0 682**
Error	54	0 925	0 193	0 024	0 061

*, ** significant at 5% and 1% level, respectively

4.1.3.2 General combining ability effects

The estimates of general combining ability effects for individual parents involved in the present investigation are tabulated in Table 4.6

Looking to the general combining ability effects of various parents, it was observed that Vaibhav (P_8) was one of the best general combiners as it has shown high *gca* in desirable direction for five characters out of nine studies. Of these characters, the performance of Vaibhav was significant for days to 50% flowering (-0.762), plant height (0.928), primary branches/ plant (0.107), pod clusters/ plant (0.533) including seed yield/ plant (0.317). The parent TARM 18 (P_7) was the other best parent which showed desirable high *gca* effects for days to 50% flowering (-1.238), days to maturity (-2.677), primary branches/ plant (0.196) and pods/ plant (0.606). Kopargaon (P_1) produced significant *gca* effects in desirable direction for three characters *viz*, days to 50% flowering (-1.053), days to maturity (-0.974) and pod clusters/ plant (0.333). The parent BM 4 (P_4) expressed highest positive highly significant *gca* effects for the two traits *viz*, pods/ plant (1.506) and seed yield/ plant (0.248).

The parents, which produced significant *gca* effects in desirable direction for the respective characters were Kopargaon (P_1) for days to flowering, days to maturity, primary branches per plant and pod cluster per plant, AKM 8802 (P_2) for days to 50% flowering, plant height and pods per plant, PM 9341 (P_3) and BPMR 145 (P_5) for only one trait *i.e.* 100 seed weight with highest *gca* effect of 0.272 in P_3 , BM 4 (P_4) for pods per plant and seed yield per plant and parent Vaibhav (P_6) and TARM 18 (P_7) for five and four characters, respectively.

Table 4.6 Estimates of general combining ability effects of parents

Sr No	Genotypes	Days to 50% flowering	Days to maturity	Plant height	Primary branches/ plant	Pod clusters/ plant
1	Kopargaon	- 1 053**	- 0 974*	-0 387	-0 093*	0 333**
2	AKM 8802	- 0 460*	0 026	- 2 509**	-0 145**	-0 400**
3	PM 9341	1 243	0 989*	1 086	0 015	-0 004
4	BM 4	0 132	0 138	- 2 188**	- 0 089*	-0 215**
5	BPMR 145	0 614*	1 397*	0 758	0 040	-0 278**
6	Vaibhav	-0 762*	1 101*	2 928**	0 107**	0 533**
7	TARM 18	-1 238**	- 2 677*	0 313	0 196**	0 030
	SE (m) \pm	0 199	0 180	0 782	0 040	0 050

Sr No	Genotypes	Pods/ plant	Seeds/ pod	100 seed weight (g)	Seed yield/ plant (g)
1	Kopargaon	0 205	-0 126	0 063	0 126
2	AKM 8802	0 652*	-0 178	-0 017	-0 369**
3	PM 9341	-1 470**	-0 295*	0 272**	-0 296**
4	BM 4	1 506**	0 149	-0 132**	0 248**
5	BPMR 145	-0 922**	0 140	0 134**	-0 124
6	Vaibhav	-0 578	0 159	0 026	0 317**
7	TARM 18	0 606*	0 151	-0 346**	0 098
	SE (m) \pm	0 297	0 136	0 048	0 076

*, ** significant at 5% and 1% level, respectively

4.1.3.3 Specific combining ability effects

The estimation of specific combining ability effects for 21 crosses in respect of the characters under study is presented in Table 4.7

1. Days to 50 per cent flowering

The extent of *sca* effects in desirable direction (*i.e.* negative) was found up to -1.194. Among 21, only two crosses *viz.*, AKM 8802 x Vaibhav (-1.194) and TARM 18 (-1.130) manifested significant negative *sca* effects for days to 50 % flowering.

2. Days to maturity

The cross combination AKM 8802 x TARM 18 (-1.94) exhibited highly significant negative *sca* effect followed by AKM 8802 x BM 4 (-0.789), however, excluding these two hybrids, there were seven crosses with negative *sca* effects. Three crosses had undesirable significant *sca* effects, including AKM 8802 x BPMR 145 (0.991) with highest positive *sca* effect.

3. Plant height

Among 21 crosses, only the cross PM 9341 x TARM 18 (4.64) evinced highest positive significant *sca* effect. However, 12 crosses recorded *sca* effect in desirable direction. None of the hybrids recorded negative significant *sca* effect for this trait.

4. Primary branches per plant

The combination BM 4 x BPMR 145 (0.219) followed by AKM 8802 x Vaibhav (0.208) showed highest significant positive *sca* effect for number of primary branches per plant. Whereas, BPMR 145 x TARM 18 (-0.27) and Kopergaon x Vaibhav recorded significant negative *sca* effects.

Table 4.7 Estimates of specific combining ability effects of crosses in mungbean

Crosses	Days to 50% flowering	Days to maturity	Plant height	Primary branches/plant	Pod clusters / plant	Pods/ plant	Seeds/ pod	100 seed weight	Seed yield/ plant
302 x 302	-0.713	-0.315	0.263	-0.192	-0.075	0.105	0.438	0.12	0.85**
302 x 41	0.917	1.056*	1.492	0.079	-0.071	-2.09*	-0.029	-0.41**	-0.69**
302 x 145	0.361	-0.426	1.043	0.119	0.140	0.178	0.321	0.74**	-0.394
302 x 18	0.213	0.648	1.007	0.190	0.81**	1.363	-0.313	0.04	0.332
302 x IV	1.065	0.278	-1.994	-0.244*	-0.34**	0.705	0.101	-0.69**	-0.383
302 x 18	-0.935	-0.611	-4.028	0.134	-0.038	2.73**	0.309	0.17	0.62**
41 x 41	-1.009	0.389	-3.123	0.131	-0.071	0.764	-0.260	-0.28*	-1.04**
41 x 145	-0.565	-0.789*	2.311	-0.129	-0.060	1.55**	0.680*	0.48**	0.89**
41 x 145	0.046	0.991*	-2.635	0.075	0.069	0.916*	-0.271	0.14	0.721
41 x IV	-1.194*	-0.389	1.864	0.208*	0.192	3.06**	0.306	-0.09	0.059
41 x 18	0.806	-1.94**	-0.357	0.053	0.229	-2.15**	-0.473	-0.48**	-0.66**
41 x 145	0.065	0.278	2.123	0.075	-0.056	1.410	0.463	0.38*	1.16**
41 x 145	-0.083	-0.648	-1.790	-0.088	0.173	-1.161	0.222	-0.04	0.93**
41 x IV	0.769	-0.685	-3.194	0.012	0.262*	-0.173	0.113	0.41**	-0.337
41 x 18	0.769	0.093	4.64*	0.056	-0.101	1.010	1.044*	0.014	-0.60**
145 x 145	0.361	0.537	1.528	0.219*	-0.32*	1.539*	-0.055	-0.04	-0.563
145 x IV	0.546	0.833*	2.671	0.086	0.74**	-2.15**	-0.107	-0.49**	0.279
145 x 18	-1.130*	0.611	3.516	0.131	-0.090	2.20**	0.191	0.31*	1.10**
145 x IV	0.398	0.574	2.331	0.090	-0.60**	-0.187	-0.066	-0.11	-0.43*
145 x 18	0.065	-0.648	1.050	-0.27**	0.37**	-0.471	-0.157	-0.18	-0.62*
IV x 18	0.583	0.315	-2.447	-0.066	0.162	-0.083	0.090	0.47**	1.50**
±	0.580	0.504	2.277	0.112	0.145	0.863	0.395	0.140	0.221

*, ** significant at 5% and 1% level, respectively

5. Pod clusters per plant

Among four hybrids with significant desirable *sca* effects, the cross Kopargaon x BPMR 145 (0 803) was found highest pod clusters followed by BM 4 x Vaibhav (0 74), BPMR 145 x TARM 18 (0 373) and PM 9341 x Vaibhav (0 262)

6. Pods per plant

The best significant positive *sca* effect was depicted by the cross AKM 8802 x Vaibhav (3 06) followed by Kopargaon x TARM 18 (2 73), BM 4 x TARM 18 (2 20) and AKM 8802 x BM 4 (1 55) However, Kopargaon x PM 9341 (-2 09), AKM 8802 x TARM 18 (-2 15) and BM 4 x Vaibhav (-2 18) recorded significant negative *sca* effects

7. Seeds per pod

Only two crosses, PM 9341 x Vaibhav (1 04) and AKM 8802 x BM 4 (0 68) registered significant positive *sca* effects The combinations PM 9341 x BM 4 (0 463), Kopargaon x AKM 8802 (0 438) and Kopargaon x BM 4 (0 321) were the others showing non-significant, but magnitudinally high *sca* estimates, for seeds per pod Nine hybrids exhibited negative *sca* effects, but they were non-significant

8. 100 seed weight

The highest and highly significant estimate of *sca* was recorded by the hybrid Kopargaon x BM 4 (0 74), followed by AKM 8802 x BM 4 (0 48), Vaibhav x TARM 18 (0 47) and PM 9341 x Vaibhav (0 41) The *sca* effects for test weight were significant positive in six crosses, while four crosses exhibited significant negative *sca* effects

9. Seed yield per plant

The extent of *sca* effects in desirable direction for seed yield was found up to 1 50 The best specific combination was Vaibhav x TARM 18 (1 50) followed by PM 9341 x BM 4 (1 16), BM 4 x TARM 18 (1 10) and PM 9341 x BPMR 145 (0 93) Among 21 hybrids, seven and six crosses displayed positive and negative significant *sca* estimates, respectively

4.2 DNA Polymorphism Study by Molecular Markers

In recent years, molecular markers have been developed based on more detailed knowledge of genome structure. Considerable emphasis has been laid on the use of molecular markers in practical breeding and genotype identification. Molecular markers are molecules that could be used to trace a desired gene(s) in examined genotypes. In fact, a piece of DNA or a protein can be used as a marker. Earlier approaches that made selection of specific traits easier were based on the evaluation of morphological traits, isozymes, storage proteins like glutenins, gliadins, hordeins etc. However, DNA markers seem to be the best candidates for efficient diagnostic evaluation of the genotypes in the population.

This investigation attempts to study an account of different molecules currently available for genome mapping. Mungbean, one of the most important pulse crops in the Asia, is grown extensively throughout the India. However, its productivity is not high enough to fulfill the requirements of an increasing population. One major reason for the low productivity of cultivated mungbean, *Vigna radiata*, is its narrow genetic base and its sexual incompatibility with other *Vigna* wild types in natural interspecific crosses. Thus the knowledge of interspecific genetic diversity and relatedness in the germplasm is essential for the crop improvement. This study was undertaken to determine the degree of genetic diversity among the Indian elite mungbean cultivars with the help of two different DNA marker systems namely, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR). Further, the relationship of DNA marker-based genetic distance (genetic diversity) with heterosis was examined and discussed to assess, whether such PCR-based markers were useful for evaluating germplasm and predicating hybrid performance/ heterosis in mungbean.

In the study presented here, RAPD and ISSR markers were employed to study DNA polymorphism among the seven parental mungbean genotypes.

4.2.1 Optimization of amplification conditions

Assays to optimize the template concentration were conducted over the range of 0.5 to 200 ng. A constant banding pattern was obtained at a template concentration between 5 and 30 ng. Ultimately, a template DNA concentration of 12.5 ng/ 25 µl reaction was selected for the PCR amplification. Figure 4 a and 4 b depicts a representative picture of good polymorphic PCR amplifications using different primers of RAPD and ISSR. The co-migrating bands provided an internal control by which to monitor reproducibility of the amplification patterns. Further more, a low magnesium concentration (1.5 mM) in the PCR reaction was found to be optimal for the purpose of producing clear and reproducible DNA fingerprints.

To determine the degree of heterogeneity within the population, we extracted DNA from different individuals of the same accessions and used this for PCR amplification. In general, the RAPD and ISSR patterns were found to be almost identical in all cases. Successive analyses were performed routinely on DNA extracted from leaves pooled from at least ten individuals of the same accessions.

4.2.2 Polymorphism by randomly amplified DNA (RAPD) markers

In an attempt to examine the potential of RAPD markers for their ability to identify mungbean cultivars, a set of 210 random primers from Operon Technologies (Alameda, USA) of series A to H, K to P, T, U, V and X was used for initial screening to amplify DNA from these seven mungbean accessions. In total, fifty-six 10-mer primers were found to amplify all the seven varieties, out of which only five primers viz , OPC 19, OPH 13, DPH 18, OPV 19 and OPM 06 were revealed to be polymorphic and reproducible, while remaining (52) were monomorphic and/ or very less consistent. Detailed results for each polymorphic primer are given in the Table 4.8

Table 4.8 Random primers producing polymorphic markers among the mungbean cultivars

Operon Random Primer	10-mer Sequence (5' to 3')	Average No. of bands/cultivar	No. of Polymorphic bands (P)	Total No. of bands (T)	% Polymorphism (P/T x 100)
OPC 19	GTTGCCAGCC	5 14	20	39	51 28
OPH 13	GACGCCACAC	5 43	16	40	40 00
OPH 18	GAATCGGCCA	4 28	20	33	60 61
OPM 06	CTGGGCAACT	7 85	22	58	37 93
OPV 19	GGGTGTGCAG	7 57	21	55	38 18

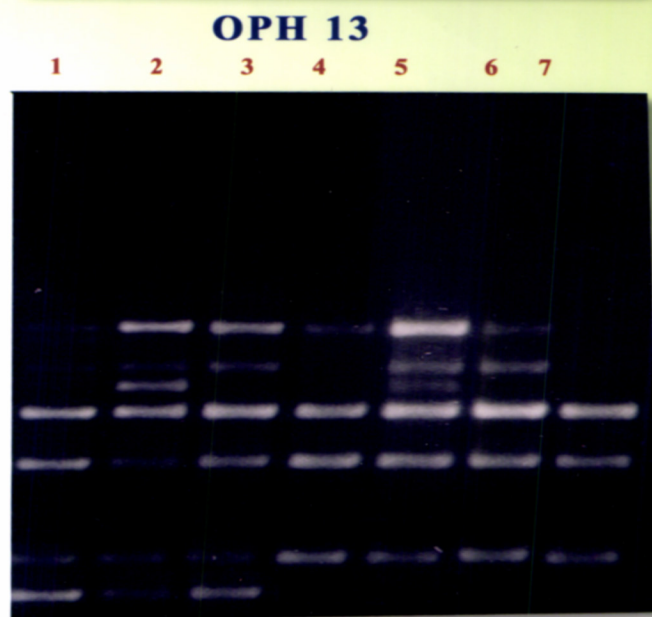
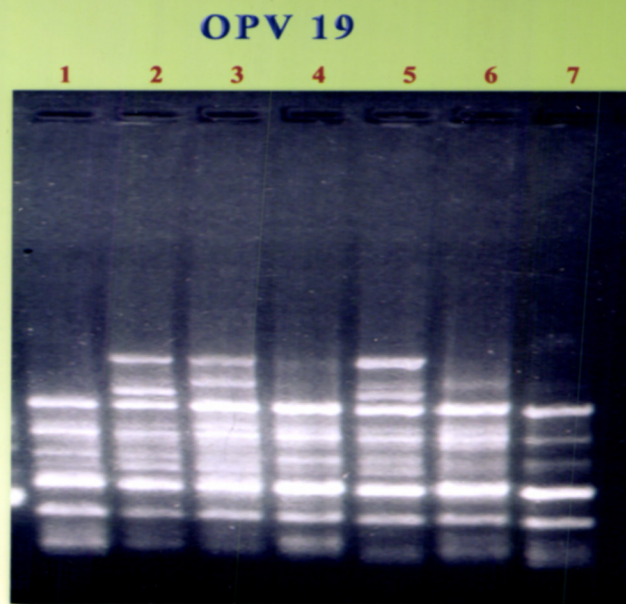
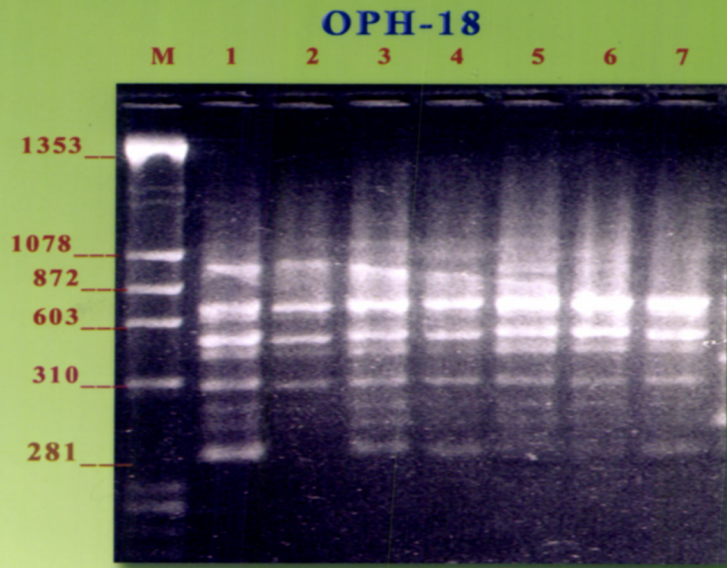
Table 4.9 Random primers producing polymorphic markers among the mungbean cultivars

ISSR-UBC Primer	Sequence (5' to 3')	Average No of bands/cultivar	No. of Polymorphic bands (P)	Total No. of bands (T)	% Polymorphism (P/T x 100)
808	AGA GAG AGA GAG AGA GC	7 42	18	52	34 62
823	TCT CTC TCT CTC TCT CC	6 85	41	48	85 42
834	AGA GAG AGA GAG AGA GYT	7 71	20	53	37 73
835	AGA GAG AGA GAG AGA GYC	4 85	40	50	80 00
840	GAG AGA GAG AGA GAG AYT	7 71	33	54	61 11
841	GAG AGA GAG AGA GAG AYC	4 29	9	30	30 00
842	GAG AGA GAG AGA GAG AYG	5 71	12	40	30 00
846	CAC ACA CAC ACA CAC ART	5 57	21	39	53 84
847	CAC ACA CAC ACA CAC	4 29	13	30	43 33
857	ACA CAC ACA CAC ACA CYG	8 29	26	58	44 82
873	GAC AGA CAG ACA GAC A	10 14	35	71	49 30
880	GGA GAG GAG AGG AGA	7 29	26	51	70 59
888	VHV GTG TGT GTG TGT GT	9 43	18	65	27 27
890	BDB CAC ACA CAC ACA CA	7 29	17	50	34 00
899	CAT GGT GTT GGT CAT TGT TCCA	8 86	32	62	50 00
900	ACT TCC CCA CAG GTT AAC ACA	8 71	34	61	55 73

Single letter abbreviations for mixed base populations

H= (A,C,T i.e not G), V= (A,C,G i.e not T), B= (C,G,T i.e not A), D= (A,G,T i.e not C),
Y= (C,T), N= (A,G,C,T), R= (A,G)

Fig.4.3 RAPD fingerprints of mungbean genotypes with different primers



M~ Ø X-174/ Hae III digest marker

1 - Kopargaon 2 - AKM 8802 3 - PM 9341 4 - BM 4
5 - BPMR 145 6 - Vaibhav 7 - TARM 18

Different primers varied in their ability to detect polymorphism. The average number of bands per primer per accession ranged between 2 and 9 with an average number of 5.88 bands/genotype. Primer OPH 18 revealed the highest polymorphism of 60.61% followed by OPV 19 (51.28%), whereas OPM 06 exhibited the lowest polymorphism (37.93%) [Table 4.8]. The polymorphism obtained in seven mungbean cultivars with RAPD primers showed a distinct variation, where variety-specific amplifications were observed (Fig 4.3). The number of bands for each primer which produced a polymorphic band pattern varied from 3 (OPM 06) to 8 (OPH 18). The lengths of the amplification products varied from 0.40 kb to 2.0 kb.

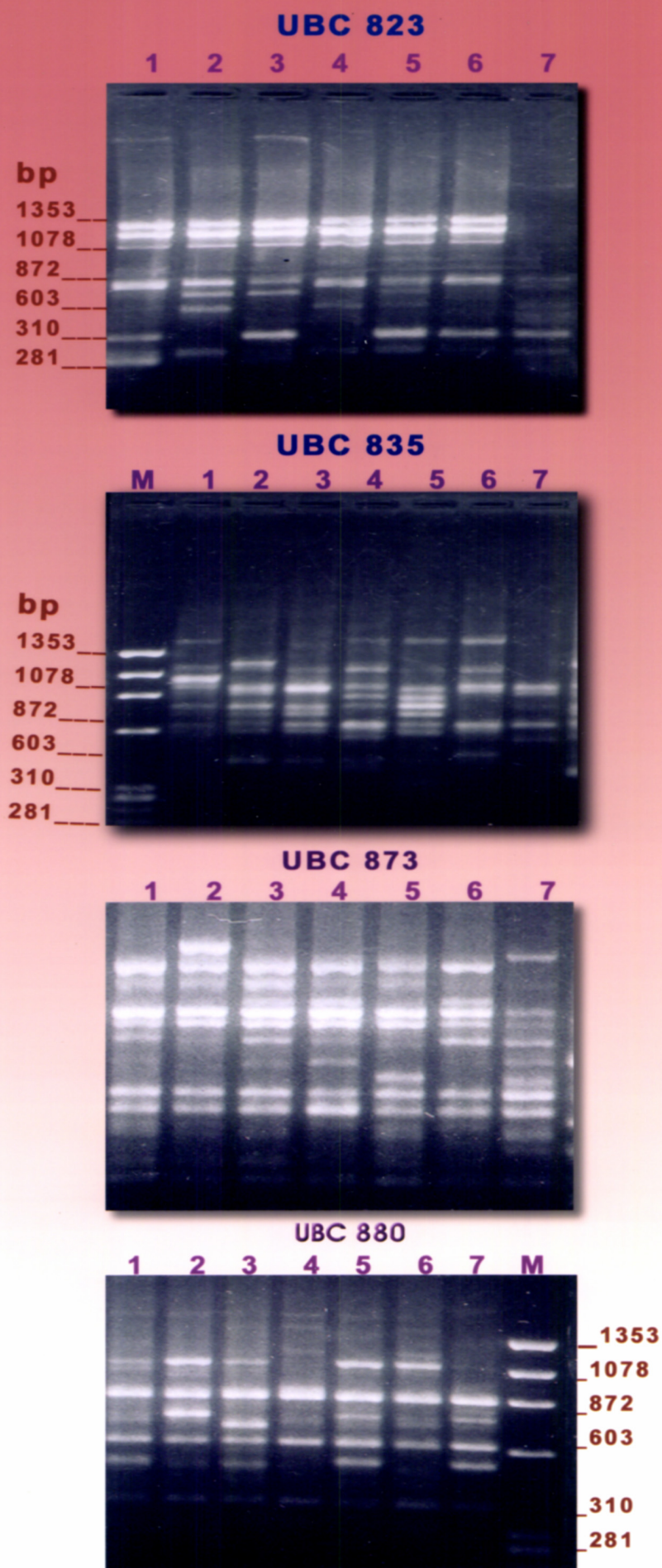
Based on the RAPD patterns, a pairwise comparison was made between all the genotypes for each primer. A total of 225 amplification products were scored, out of which 99 bands were found polymorphic in nature, i.e. 44.0% polymorphism was generated *via* RAPD analysis.

4.2.3 Polymorphism of inter-simple sequence repeats (ISSR) DNA markers

In another approach, the inter-simple sequence repeat (ISSR)-PCR based marker system was applied for analyzing DNA polymorphism in these mungbean varieties (parental genotypes). Hundred primers (UBC Set # 9 i.e. 801 to 900) from Biotechnology Laboratory, University of British Columbia (Vancouver, Canada) were used for initial screening to obtain stable, clear and polymorphic DNA fingerprints. Sixty-two ISSR primers were found to amplify all mungbean DNAs. Finally, 16 primers yielded reproducible and polymorphic banding patterns namely, UBC-ISSR 808, 823, 834, 835, 840, 841, 842, 846, 847, 857, 873, 880, 888, 890, 899 and 900 (Table 4.9).

Each primer produced amplification products between 3 and 11 depending upon the various cultivars' DNA, with an average number of bands of 7.15 per primer per accession. The lengths of the amplification products varied from 200 bp to 1.3 kb. Total 724 amplicons were produced, out of which 396 products were polymorphic to reveal 54.7% polymorphism *via* ISSR-PCR analysis in seven mungbean genotypes.

Fig.4.4 ISSR fingerprints of mungbean cultivars with different primers



M~ Ø X-174/ Hae III digest marker

1 - Kopargaon 2 - AKM 8802 3 - PM 9341 4 - BM 4
5 - BPMR 145 6 - Vaibhav 7 - TARM 18

Table 4.10 Genetic distances between mungbean cultivars based on similarity index values from RAPD analysis

Cultivars	Kopargaon	AKM 8802	PM 9341	BM 4	BPMR 145	Vaibhav
Kopargaon	0 000					
AKM 8802	0 084	0 000				
PM 9341	0 083	0 084	0 000			
BM 4	0 264	0 369	0 263	0 000		
BPMR 145	0 200	0 200	0 300	0 467	0 000	
Vaibhav	0 200	0 200	0 200	0 334	0 250	0 000
TARM 18	0 143	0 238	0 238	0 249	0 235	0 177

Table 4.11 Genetic distances between mungbean cultivars based on similarity index values from ISSR analysis

Cultivars	Kopargaon	AKM 8802	PM 9341	BM 4	BPMR 145	Vaibhav
Kopargaon	0 000					
AKM 8802	0 189	0 000				
PM 9341	0 138	0 157	0 000			
BM 4	0 191	0 200	0 187	0 000		
BPMR 145	0 146	0 118	0 133	0 223	0 000	
Vaibhav	0 142	0 200	0 108	0 154	0 175	0 000
TARM 18	0 223	0 243	0 239	0 234	0 206	0 256

Table 4.12 Genetic distances between mungbean cultivars based on similarity index values from RAPD and ISSR analysis

Cultivars	Kopargaon	AKM 8802	PM 9341	BM 4	BPMR 145	Vaibhav
Kopargaon	0 000					
AKM 8802	0 178	0 000				
PM 9341	0 132	0 149	0 000			
BM 4	0 197	0 214	0 193	0 000		
BPMR 145	0 150	0 125	0 148	0 238	0 000	
Vaibhav	0 147	0 200	0 116	0 166	0 181	0 000
TARM 18	0 215	0 242	0 238	0 235	0 213	0 250

In the present study, amplifications by different primers were informative to distinguish between the cultivars under study and produced cultivar-specific patterns (Fig 4.4). The ISSR primer UBC 823 yielded highest polymorphism (85.42%) followed by UBC 835 (80.0%), UBC 880 (70.59%), UBC 840 (61.11%) and UBC 900 (55.73%), while primer UBC 888 detected lowest (25.76%) polymorphism among the 16 polymorphic ISSR primers.

4.2.4 Genetic distances among/ between cultivars (Parents)

Nei's estimate of similarity, based on the probability that an amplified fragment from one plant (genotype) will also be found in another, was used to generate a similarity matrix (Nei and Li, 1979). Based on RAPD analysis, the distances among the seven cultivars (parents) ranged from 0.083 (Kopargaon — PM 9341) with an average of 0.227 across all these intra-specific genotypes (Table 4.10). It was minimum (0.108) between PM 9341 and Vaibhav and maximum (0.256) between the cultivars, Vaibhav and TARM 18 from the similarity index of ISSR analysis with a mean genetic distance of 0.184 (Table 4.11).

When these estimates pooled together to reveal overall Nei's genetic distance based on 495 non-redundant polymorphic marker variants/ bands (99 RAPD variants, 396 ISSR variants) with average of 6.51 amplifications per primer per cultivar for the overall molecular marker analysis with 52.16% polymorphism (Table 4.12). These results indicated that the ultimate genetic distance was maximum, i.e. 0.250 between varieties BPMP 145 and TARM 18, while it was lowest between PM 9341 and Vaibhav (0.116). Dendrogram was constructed on the basis of these variants (polymorphic bands scored) from the entire data set of RAPDs and ISSRs.

4.2.5 Variety-specific markers

Figure 4 3 and 4 4 depicts a representative picture of the electrophoretic pattern of PCR-amplified DNA fragments of mungbean cultivars using different RAPD and ISSR primers, respectively. In the present study, no single primer was able to distinguish between all the seven cultivars. However, "genotype-specific" informative amplification patterns were observed.

Table 4 13 lists certain variety-specific bands obtained from molecular marker analysis, wherein the marker is designated as the primer code (RAPD) or number (ISSR), followed by molecular weight in base pairs (bp) of the amplified band which were unique to different individuals. Other fragments were present in more than two cultivars and were still polymorphic. The reproducibility of these results was evaluated by replicating the marker analysis in all the accessions with these primers.

Table 4.13 Variety-specific DNA markers with different primers in mungbean

Sr. No.	Variety	Specific bands
1	Kopargaon	OPH 18 ₃₁₀ , OPH 13 ₁₀₇₈ , OPC 19 ₆₀₃ , UBC 823 ₆₀₃ , UBC 857 ₈₇₂ , UBC 881 ₃₁₀
2	AKM 8802	OPH 13 ₈₇₂ , OPV 19 ₁₀₇₈ , UBC 823 ₈₇₂ , UBC 835 ₁₀₇₈ , UBC 873 ₁₃₅₃ , UBC 887 ₃₁₀ , UBC 881 ₈₇₂
3	PM 9341	OPV 19 ₁₀₇₈ , UBC 880 ₆₀₃ , UBC 823 ₁₀₇₈
4	BM 4	UBC 83 ₈₇₂ , UBC 835 ₆₀₃ , UBC 900 ₈₇₂
5	BPMR 145	OPH 13 ₈₇₂ , OPV 19 ₁₀₇₈ , UBC 881 ₁₀₇₈ , UBC 835 ₆₀₃
6	Vaibhav	OPM 06 ₆₀₃ , OPC 19 ₁₀₇₈ , UBC 900 ₂₈₁
7	TARM 18	UBC 835 ₃₁₀ , UBC 808 ₃₁₀ , UBC 823 ₈₇₂

4.3 Generation Mean Analysis for Yield, its Components and Quality Traits

Five crosses viz, Kopargaon x AKM 8802, Kopargaon x TARM 18, BM 4 x BPMR 145, BPMR 145 x Vaibhav and Vaibhav x TARM 18 were investigated to study the gene actions for yield, its contributing characters and quality attributes through generation mean analysis. The crosses were selected considering the involvement of the six parents in five crosses to represent maximum diversity. Hence, this experiment involves 26 treatments involving six parents, five of F_1 s, F_2 s, B_1 s and B_2 s each during *kharif* 2003. Analysis of variance for nine quantitative and three quality characters revealed substantial variability among the treatments for all the characters studied due to significant differences among the genotypes.

4.3.1 Mean performance of parents F_1 s, F_2 s, B_1 s and B_2 s

The mean performance for different characters over six generations of the five crosses is given in Table 4.14.

The F_1 means in almost all the crosses indicated the dominance of earliness. The means among the parents ranged from 33.67 (TARM 18) to 38.33 (Vaibhav) for days to 50% flowering. None of the F_1 s or back crosses or F_2 s found earlier to flower than the parent TARM 18 except the hybrid (F_1) Kopargaon x TARM 18, which required only 33 days for 50 per cent flowering. While the backcross B_2 [(Vaibhav x TARM 18) x TARM 18] (63.0) found earliest to mature.

The parent Vaibhav (57.7 cm) was found tallest than rest of the parents, whereas the backcross (B_2) [(BPMR 145 x Vaibhav) x Vaibhav] recorded maximum plant height (57.9 cm). For primary branches per plant Vaibhav (2.60) exhibited highest mean value, while the cross Vaibhav x TARM 18 (9.13) were registered highest pod clusters/ plant among all the parents and different generations, respectively. The parent Kopargaon (22.62) exhibited highest pods per plant, while the F_1 , Kopargaon x AKM 8802 (24.07) recorded highest mean for pods/ plant.

Table 4.14 Mean performances of Parents, F₁s, F₂s and Backcrosses for yield, its components and quality traits

Cross		Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches/plant	Pod clusters/plant	Pods/plant
Cross I Kopargaon X AKM 8802	P ₁	34 00 ± 0 40	64 67 ± 0 23	55 76 ± 1 16	2 40 ± 0 08	8 73 ± 0 12	22 62 ± 0 69
	P ₂	37 67 ± 0 84	68 00 ± 0 40	48 43 ± 0 74	2 07 ± 0 09	7 73 ± 0 04	19 77 ± 0 72
	F ₁	34 67 ± 0 47	66 00 ± 0 40	57 56 ± 0 38	2 27 ± 0 09	8 33 ± 0 17	22 30 ± 0 49
	F ₂	37 00 ± 0 40	68 30 ± 0 23	48 96 ± 0 55	1 87 ± 0 04	7 73 ± 0 12	18 90 ± 0 43
	B ₁	34 33 ± 0 62	64 67 ± 0 47	53 80 ± 0 93	2 27 ± 0 09	8 40 ± 0 08	22 73 ± 0 77
	B ₂	35 67 ± 0 84	67 67 ± 0 62	48 26 ± 0 53	2 07 ± 0 12	7 60 ± 0 08	20 70 ± 0 40
Cross II Kopargaon X TARM 18	P ₁	34 00 ± 0 40	65 00 ± 0 40	55 76 ± 1 16	2 40 ± 0 08	8 73 ± 0 48	22 62 ± 0 69
	P ₂	33 67 ± 0 23	64 00 ± 0 40	47 54 ± 0 91	2 53 ± 0 09	8 73 ± 0 09	21 13 ± 0 82
	F ₁	33 00 ± 0 00	63 67 ± 0 47	51 13 ± 0 80	2 53 ± 0 12	8 73 ± 0 12	24 07 ± 1 13
	F ₂	36 00 ± 0 40	67 00 ± 0 40	48 66 ± 0 61	1 80 ± 0 08	8 33 ± 0 04	17 97 ± 0 90
	B ₁	36 00 ± 0 40	65 00 ± 0 40	54 10 ± 0 57	2 13 ± 0 12	8 53 ± 0 12	21 17 ± 0 84
	B ₂	33 67 ± 0 47	64 00 ± 0 40	49 63 ± 0 39	2 67 ± 0 04	8 87 ± 0 12	22 53 ± 0 56
Cross III BM 4 X BPMR 145	P ₁	34 67 ± 0 62	65 67 ± 0 23	49 53 ± 0 73	2 17 ± 0 19	7 93 ± 0 17	19 57 ± 0 96
	P ₂	36 67 ± 0 62	67 67 ± 0 62	55 76 ± 1 01	1 87 ± 0 12	8 53 ± 0 09	19 60 ± 0 69
	F ₁	35 67 ± 0 62	66 00 ± 0 40	56 23 ± 0 97	2 20 ± 0 08	8 20 ± 0 21	20 33 ± 0 79
	F ₂	37 67 ± 0 62	67 33 ± 0 84	52 13 ± 0 81	1 80 ± 0 08	7 87 ± 0 12	16 57 ± 0 81
	B ₁	35 00 ± 0 40	64 00 ± 0 81	52 03 ± 1 13	2 13 ± 0 04	8 47 ± 0 12	22 67 ± 0 37
	B ₂	37 00 ± 0 81	68 00 ± 0 70	55 90 ± 1 11	2 40 ± 0 08	8 47 ± 0 09	19 03 ± 0 45
Cross IV BPMR 145 X Vaibhav	P ₁	37 00 ± 0 81	67 67 ± 0 62	56 06 ± 0 81	1 87 ± 0 12	8 53 ± 0 09	19 60 ± 0 69
	P ₂	38 33 ± 0 23	68 33 ± 0 47	57 70 ± 0 55	2 60 ± 0 08	8 87 ± 0 09	21 10 ± 1 17
	F ₁	37 33 ± 0 47	67 67 ± 0 23	57 00 ± 0 68	2 53 ± 0 04	8 93 ± 0 09	22 70 ± 0 72
	F ₂	38 66 ± 0 62	68 67 ± 0 23	53 26 ± 1 03	2 00 ± 0 08	8 67 ± 0 12	20 53 ± 0 60
	B ₁	36 67 ± 0 47	67 33 ± 0 62	52 46 ± 1 32	2 40 ± 0 08	8 73 ± 0 04	19 50 ± 0 90
	B ₂	38 33 ± 0 25	68 33 ± 0 23	57 90 ± 0 72	2 60 ± 0 14	8 73 ± 0 17	22 0 ± 0 86
Cross V Vaibhav X TARM 18	P ₁	38 33 ± 0 25	68 33 ± 0 47	57 70 ± 0 55	2 60 ± 0 08	8 87 ± 0 09	21 10 ± 1 17
	P ₂	33 67 ± 0 23	64 00 ± 0 40	47 54 ± 0 91	2 51 ± 0 10	8 73 ± 0 09	21 33 ± 0 69
	F ₁	36 00 ± 0 40	63 33 ± 0 62	51 56 ± 0 81	2 76 ± 0 18	9 13 ± 0 12	23 33 ± 0 66
	F ₂	36 67 ± 0 62	67 33 ± 0 62	49 66 ± 1 11	2 27 ± 0 12	8 80 ± 0 08	21 50 ± 1 16
	B ₁	37 67 ± 0 47	66 67 ± 0 47	56 00 ± 0 69	2 47 ± 0 09	9 00 ± 0 14	20 60 ± 0 89
	B ₂	35 00 ± 0 40	63 00 ± 0 40	51 00 ± 0 52	2 13 ± 0 09	9 07 ± 0 12	23 87 ± 0 73

Table 4.14 (Contd...) Mean performances of Parents, F₁s, F₂s and Backcrosses...

Cross		Seeds/ pod	100 seed weight (g)	Seed yield/ plant (g)	Protein content (%)	Tryptophan (g/16gN)	Methionine (g/16gN)
Cross I Kopargaon X AKM 8802	P ₁	10 67 ±0 12	4 67 ±0 09	9 03 ±0 05	23 57 ±0 35	0 88 ±0 002	0 66 ±0 006
	P ₂	9 47 ±0 24	4 10 ±0 06	8 08 ±0 06	22 37 ±0 66	0 79 ±0 002	0 59 ±0 006
	F ₁	10 67 ±0 17	4 69 ±0 13	9 86 ±0 21	24 30 ±0 53	0 88 ±0 002	0 68 ±0 010
	F ₂	9 13 ±0 17	4 01 ±0 01	9 04 ±0 07	21 00 ±0 60	0 78 ±0 006	0 55 ±0 010
	B ₁	10 73 ±0 17	4 80 ±0 08	10 44 ±0 21	23 47 ±0 20	0 90 ±0 004	0 70 ±0 010
	B ₂	10 20 ±0 28	4 28 ±0 14	9 35 ±0 27	23 30 ±0 38	0 79 ±0 002	0 60 ±0 010
Cross II Kopargaon X TARM 18	P ₁	10 67 ±0 12	4 67 ±0 09	9 03 ±0 05	23 57 ±0 47	0 88 ±0 002	0 66 ±0 006
	P ₂	10 60 ±0 21	3 50 ±0 12	7 72 ±0 29	22 03 ±0 23	0 81 ±0 004	0 51 ±0 010
	F ₁	10 47 ±0 28	4 09 ±0 04	10 78 ±0 32	23 63 ±0 52	0 88 ±0 002	0 64 ±0 010
	F ₂	10 27 ±0 24	3 77 ±0 09	9 68 ±0 19	22 37 ±0 33	0 77 ±0 01	0 48 ±0 004
	B ₁	10 47 ±0 18	4 21 ±0 11	10 82 ±0 18	23 40 ±0 32	0 90 ±0 004	0 69 ±0 008
	B ₂	11 00 ±0 16	3 39 ±0 09	10 09 ±0 36	23 00 ±0 35	0 89 ±0 004	0 45 ±0 010
Cross III BM 4 X BPMR 145	P ₁	10 47 ±0 24	3 22 ±0 05	9 74 ±0 10	21 50 ±0 42	0 78 ±0 008	0 50 ±0 008
	P ₂	10 40 ±0 21	4 26 ±0 06	9 04 ±0 36	23 53 ±0 17	0 89 ±0 004	0 50 ±0 006
	F ₁	10 40 ±0 24	4 37 ±0 11	10 27 ±0 35	25 07 ±0 12	0 90 ±0 002	0 53 ±0 006
	F ₂	9 67 ±0 12	3 80 ±0 22	8 16 ±0 10	22 80 ±0 35	0 86 ±0 004	0 41 ±0 002
	B ₁	10 00 ±0 14	3 87 ±0 06	10 99 ±0 31	20 50 ±0 22	0 76 ±0 008	0 51 ±0 007
	B ₂	11 00 ±0 08	4 15 ±0 30	9 61 ±0 31	24 73 ±0 34	0 90 ±0 01	0 51 ±0 008
Cross IV BPMR 145 X Vaibhav	P ₁	10 40 ±0 21	4 26 ±0 06	9 04 ±0 36	23 53 ±0 17	0 89 ±0 004	0 50 ±0 006
	P ₂	11 07 ±0 12	4 70 ±0 06	10 94 ±0 37	23 73 ±0 46	0 88 ±0 01	0 50 ±0 008
	F ₁	11 07 ±0 12	4 54 ±0 10	11 56 ±0 21	25 57 ±0 23	0 89 ±0 008	0 55 ±0 001
	F ₂	10 00 ±0 08	4 23 ±0 05	9 81 ±0 29	22 57 ±0 32	0 82 ±0 006	0 50 ±0 002
	B ₁	11 13 ±0 12	4 21 ±0 08	9 99 ±0 07	24 87 ±0 55	0 89 ±0 004	0 96 ±0 002
	B ₂	10 93 ±0 17	4 87 ±0 06	11 74 ±0 18	22 77 ±0 59	0 89 ±0 002	0 57 ±0 010
Cross V Vaibhav X TARM 18	P ₁	11 07 ±0 12	4 70 ±0 06	10 94 ±0 37	23 73 ±0 46	0 88 ±0 01	0 50 ±0 008
	P ₂	10 07 ±0 17	3 50 ±0 12	7 72 ±0 29	22 10 ±0 64	0 81 ±0 004	0 50 ±0 008
	F ₁	10 53 ±0 12	3 63 ±0 20	11 87 ±0 36	22 63 ±0 66	0 92 ±0 008	0 59 ±0 006
	F ₂	10 13 ±0 12	3 68 ±0 21	10 53 ±0 44	20 17 ±0 55	0 82 ±0 009	0 49 ±0 004
	B ₁	10 93 ±0 12	4 87 ±0 01	11 12 ±0 47	22 60 ±0 28	0 89 ±0 002	0 52 ±0 007
	B ₂	9 87 ±0 04	4 16 ±0 08	11 93 ±0 30	22 47 ±0 23	0 90 ±0 006	0 49 ±0 004

7-5974

For seeds per pod, parent Vaibhav (11.07) and the generation B₁ i.e. [(BPMR 145 x Vaibhav) x BPMR 145] (11.13) showed maximum mean values. The test weight was highest in the parent Vaibhav (4.70 g), however it was maximum in the two backcrosses viz., B₂ [(BPMR 145 x Vaibhav) x Vaibhav] and B₁ [(Vaibhav x TARM 18) x Vaibhav] (4.87 g). In case of seed yield per plant, among parents Vaibhav (10.94g) yielded highest, whereas the heterotic combination Vaibhav x TARM 18 (11.87 g) evinced highest seed yield than any of the generations and parents, as well.

Protein content, one of the seed quality attributes was highest in Vaibhav (23.73%) and F₁ (BPMR 145 x Vaibhav) with 25.57% among the parents and generations, respectively. The parent BPMR 145 (0.89) and F₁, Vaibhav x TARM 18 (0.92) registered highest means for tryptophan content (g/16gN). For methionine content (g/16gN), Kopargaon (0.66) displayed highest mean value among the parents, while the backcross B₁ [(Kopargaon x TARM 18) x Kopargaon] (0.69) had maximum mean overall the generations.

4.3.2 Gene effects for seed yield, its components and quality traits

Data was analyzed by joint scaling test of Cavalli (1952) to detect the adequacy of additive-dominance model. When Chi-square test was non-significant i.e. epistasis was absent, m, d and h were estimated as per Cavalli (1952). However, in presence of epistasis i.e. significant estimates of Chi-square values, the six parameter model of Hayman (1958) was applied to estimate the components m, d, h, i, j and l (Table 4.16 and 4.17), which indicates inadequacy of three parameter model.

The scaling tests for nine quantitative characters and three quality traits (Table 4.15) emphasized that the simple additive-dominance model was inadequate to explain total genetic variability in these crosses for different traits except for days to 50% flowering, pod clusters/ plant, pods/ plant in crosses BPMR 145 x Vaibhav and Vaibhav x TARM 18,

Table 4.15 Scaling tests of generation means of mungbean crosses for yield, its components and quality traits

Cross	Scales			
	A	B	C	D
1. Days to 50% flowering				
C I	-0 002	-1 00	7 00**	4 00**
C II	5 00**	0 67	10 33**	2 33*
C III	-0 34	1 67	8 30**	3 33*
C IV	-1 00	1 00	4 67	2 34
C V	1 00	0 33	2 67	0 67
2. Days to maturity				
C I	-1 33	1 34	8 66**	4 33**
C II	1 33	0 33	11 67**	5 00**
C III	-3 67*	2 33	3 99	2 66
C IV	-0 67	0 66	3 34**	1 67*
C V	1 67	-1 33	10 33**	5 00**
3. Plant height (cm)				
C I	-5 74**	-9 46**	-23 5**	-4 13**
C II	1 30	0 59	-10 90**	-6 40**
C III	-1 70	-0 20	-9 23*	-3 67
C IV	-8.13**	1 10	-14 70**	-3 83
C V	2 73	2 89	-9 71*	-7 66**
4. Primary branches/ plant				
C I	-0 13	-0 20	-1 53**	-0 60**
C II	-0 67*	0 27	-2 80**	-1 20**
C III	-0 10	0 73**	-1 23**	-0 93**
C IV	0 40	0 07	-1 53**	-1 00**
C V	-0 42	-1 00**	-1 55*	-0 07
5. Pod clusters / plant				
C I	-0.26	-0 86**	-2 20**	-0 53
C II	-0 40	0 27	-1 60**	-0 73**
C III	0 80**	0 20	-1 39**	-1 20**
C IV	0 008	-0 33	0 59	0 13
C V	0 00	0 27	-0 67	-0 47
6. Pods / plant				
C I	0 54	-0 68	-11 39**	-5 63**
C II	-4 35*	-0 13	-20 02**	-7 76**
C III	5 43**	-1 87	-13 56**	-8 56**
C IV	-3 30	0 26	-3 97	-0 47
C V	-3 23	3 07	-3 10	-1 47

*, ** significant at 5% and 1% level, respectively

Table 4.15 (Contd...)

Cross	Scales			
	A	B	C	D
7. Seeds/ pod				
C I	0.13	0.26	-4.94**	-2.68**
C II	-0.20	0.93	-1.13	-0.93
C III	-0.87	1.20**	-2.99**	-1.66**
C IV	0.80*	-0.27	-3.60**	-2.06**
C V	0.26	-8.65**	-1.67*	-0.53
8. 100 seed weight (g)				
C I	0.26	-0.21	-2.08**	-1.06**
C II	-0.34	-0.81**	-1.26**	-0.05*
C III	0.15	-0.32	-1.03	-0.40
C IV	-0.38	0.50**	-1.10**	-0.61**
C V	1.40**	1.19**	-0.76	-1.68**
9. Grain yield/ plant (g)				
C I	1.99**	0.76	-0.68	-1.72**
C II	1.83**	1.68*	0.40	-1.55**
C III	1.97**	-0.08	-6.65**	-4.27**
C IV	-0.61	0.97	-3.87**	-2.15**
C V	-0.57	4.27**	-0.28	-1.99
Quality characters				
10. Protein content				
C I	-0.93	-0.07	-10.53**	-4.76**
C II	-0.40	0.33	-3.39	-1.66*
C III	-5.57**	0.86	-3.97**	0.37
C IV	0.63	-3.76**	-8.13**	-2.50*
C V	-1.16	0.20	-10.43**	-4.73**
11. Tryptophan				
C I	0.03**	-0.09**	-0.33**	-0.13**
C II	0.03**	0.08**	-0.36**	-0.24**
C III	-0.15	-0.01	-0.07	0.05
C IV	-0.008	0.02	-0.26**	-0.14**
C V	-0.01	0.07**	-0.22**	-0.14**
12. Methionine				
C I	0.06*	-0.07*	-0.42**	-0.21**
C II	0.07**	-0.25**	-0.55**	-0.18**
C III	-0.01	-0.01	-0.42**	-0.19**
C IV	2.95	0.09*	-0.10**	-1.57
C V	-0.55**	-0.11**	-0.20**	-0.01

*, ** significant at 5% and 1% level, respectively

seeds/ pod in the cross Kopargaon x TARM 18 and 100 seed weight in cross BM 4 x BPMR 145. The significant estimates of scaling tests warranted for the use of six parameter model to estimate gene effects

1. Days to 50 % flowering

Estimates of additive (d) gene action were found to be significant in all the crosses except Kopargaon x AKM 8802 and BPMR 145 x Vaibhav, while dominant (h) gene action was significant in all the crosses except Vaibhav x TARM 18. According to Hayman's (1958) model, all the first three crosses exhibited significant negative additive x additive (i) interactions. The additive x dominance (j) and dominance x dominance (l) non-allelic interactions were found significant in crosses Kopargaon x TARM 18 and Kopargaon x AKM 8802, respectively. The estimates of dominance (h) gene action and dominance x dominance (l) interactions with opposite signs were observed in the cross Kopargaon x AKM 8802 (Table 4.16)

2. Days to maturity

The fitting of the six parameter digenic interaction model gave highly significant estimates of additive (d) effect in the crosses Kopargaon x AKM 8802, BM 4 x BPMR 145 and Vaibhav x TARM 18, while dominance (h) effect was found to be negative and significant in all the crosses except BM 4 x BPMR 145.

The crosses Kopargaon x AKM 8802 and Kopargaon x TARM 18 exhibited significant additive x additive (i) component and dominance x dominance (l) non-allelic effect with opposite signs, while these effects with additive x dominance (j) component found to be positive significant in Vaibhav x TARM 18.

3. Plant height

The six-parameter digenic interaction model was found to be adequate in all the five crosses for plant height (Table 4 16) Both the major gene effects (d and h) except dominance (h) effect in BPMR 145 x Vaibhav were significant

The crosses Kopargaon x TARM18 and Vaibhav x TARM18 revealed highly significant additive x additive (i) and dominance x dominance (l) non-allelic interactions with opposite signs Only cross BM 4 x BPMR 145 had similar signs of (h) and (l) effects

4. Primary branches per plant

According to Hayman's (1958) model, six parameter model, both the genetic effects (d and h) as well as non-allelic interactions (i, j and l) were highly significant in the crosses, Kopargaon x TARM 18 and BM 4 x BPMR 145 and with opposite signs of (h) and (l) effects, which was also evident in BPMR 145 x Vaibhav

5. Pod clusters per plant

The fitting of the additive-dominance model indicated the absence of non-allelic interactions in the crosses BPMR 145 x Vaibhav and Vaibhav x TARM 18, while the perfect fit six-parameter model applicable to the first three crosses, with significant dominance (h) component (Table 4 16)

Additive x additive (i) type of gene interaction was found to be highly significant in the crosses, Kopargaon x TARM 18 and BM 4 x BPMR 145, where dominance x dominance (l) component was negatively significant in the latter one The cross Kopargaon x AKM 8802 exhibited significant estimates of additive (d), dominance (h) and additive x dominance (j) components

Character	Cross	χ^2	Component						Type of epistasis	
			m	d	h	i	j	l		
1.	Days to 50% flowering	C I	16 70**	-37 0	-1 33	-9 16**	-8 00**	0 5	9 01*	Duplicate
		C II	10 13**	36 0	2 33**	-5 50**	-4 67*	2 17**	-1 00	--
		C III	9 38*	37 67	-2 0*	-6 68*	-6 67*	-1 0	5 34	--
		C IV	6 26	42 34	-0 67	-9 67*	-	-	-	--
		C V	1 98	37 35	2 33**	-1 34	-	-	-	--
2.	Days to maturity	C I	54 04**	68 33	-3 00**	-8 99**	-8 67**	-1 34	8 67*	Duplicate
		C II	37 54**	67 00	1 00	-10 83**	-10 00*	0 50	8 33*	Duplicate
		C III	11 02*	67 33	-4 00**	-5 99	-5 33	-3 0**	6 67	--
		C IV	10 03**	68 67	-1 0	-3 67*	3 34*	-0 67	3 34	Duplicate
		C V	4 65*	67 33	3 67**	-12 82**	10 0*	1 50*	9 65*	Duplicate
3.	Plant height (cm)	C I	100 13**	48 96	5 53**	13 73**	8 26**	1 87	6 93	--
		C II	20 71**	48 67	4 47**	12 27**	12 80**	0 35	-14 7**	Duplicate
		C III	5 79*	52 13	-3 87*	-10 91*	7 34	-0 75	-5 43*	Complementary
		C IV	18 88**	53 27	-5 43**	7 78	7 67	-4 61**	-0 63	--
		C V	13 03**	49 67	5 00**	14 27**	15 33**	-0 08	-20 95**	Duplicate
4.	Primary branches/plant	C I	31 33**	1 87	0 20	1 23**	1 20**	0 03	-0 86	--
		C II	75 98**	1 80	-0 54**	2 47**	2 40**	-0 47**	-2 00**	Duplicate
		C III	32 30**	1 80	-0 27**	2 05**	1 87**	-0 42**	-2 50**	Duplicate
		C IV	26 75**	2 00	-0 20	2 30**	2 00**	0 16	-2 47**	Duplicate
		C V	14 87**	2 27	0 34*	0 34	0 13	0 29*	1 29	--
5.	Pod clusters/plant	C I	20 58**	7 73	0 80**	1 17*	1 07	0 30*	0 06	--
		C II	20 80**	8 33	-0 33	1 47**	1 47**	-0 33	1 34	--
		C III	18 06**	7 86	0 00	2 37**	2 40**	0 33	-3 40**	Duplicate
		C IV	5 73	8 44	-0 17*	0 42	-	-	-	--
		C V	5 28	7 87	0 07	2 47*	-	-	-	--

*, ** significant at 5% and 1% level, respectively

C I - Kopargaon x AKM 8802

C II - Kopargaon x TARM 18

C III - BM 4 x BPMR 145

C IV - BPMR 145 x Vaibhav

C V - Vaibhav x TARM 18

Table 4.16 (Contd...) Estimates of gene effects for yield and its components in mungbean

Character	Cross	χ^2	Component						Type of epistasis	
			m	d	h	i	j	l		
6.	Pods/ plant	C I	33.36**	18.90	2.03	12.37**	11.26**	0.60	-11.14**	Duplicate
		C II	26.24**	17.96	-1.36	17.72**	15.553**	-2.11	-11.04	Duplicate
		C III	46.24**	16.57	3.64**	17.88**	17.13**	3.65**	-20.70**	Duplicate
		C IV	6.54	19.41	-0.75	1.18	-	-	-	--
		C V	8.86	18.28	-0.11	7.82	-	-	-	--
7.	Seeds/ pod	C I	43.42**	9.13	0.53	5.93**	5.33**	-0.07	-5.73**	Duplicate
		C II	8.36	8.76	0.04	4.30	-	-	-	--
		C III	62.98**	9.66	-1.0**	3.3**	3.33**	-1.04**	-3.66**	Duplicate
		C IV	100.1**	10.0	0.20	4.46**	4.13**	0.53*	-4.66**	Duplicate
		C V	19.76**	10.13	1.07**	1.35	1.07	0.56**	-0.47	--
8.	100 seed weight (g)	C I	115.5**	4.00	0.52**	2.41**	2.13**	0.23	-2.17**	Duplicate
		C II	20.81**	3.77	0.82**	0.10	0.10	0.23	1.05	--
		C III	2.56	2.88	-0.52**	2.18	-	-	-	--
		C IV	37.0**	4.23	-0.66**	1.28**	1.23**	-0.44**	-1.35*	Duplicate
		C V	58.00**	3.67	0.70**	2.88**	3.35**	0.10	-5.95**	Duplicate
9.	Seed yield/ plant (g)	C I	28.97**	9.04	1.09**	4.75**	3.44**	0.61	-6.20**	Duplicate
		C II	17.21**	9.68	0.72	5.51**	3.10**	0.07	-6.62**	Duplicate
		C III	111.9**	8.16	1.38**	9.43**	8.55**	1.03	-10.43**	Duplicate
		C IV	15.64**	9.81	-1.75**	5.80**	4.23**	-0.8*	-4.6**	Duplicate
		C V	36.68**	10.53	-0.80	6.62**	3.98*	-2.42	-7.68**	Duplicate

*, ** significant at 5% and 1% level, respectively

C I - Kopargaon x AKM 8802

C II - Kopargaon x TARM 18

C III - BM 4 x BPMR 145

C IV - BPMR 145 x Vaibhav

C V - Vaibhav x TARM 18

6. Pods per plant

The six-parameter digenic interaction model was found adequate for this trait in first three crosses, in which the highly significant dominance (h) gene effect coupled with additive x additive (i) interaction were occurred along with negative and significant dominance x dominance (l) type of non-allelic interaction except in Kopargaon x TARM 18

The cross BM 4 x BPMR 145 only revealed highly significant estimates of additive (d) gene effect and additive x dominance (j) type of non-allelic gene interaction

7. Seeds per pod

All the crosses except Kopargaon x TARM 18 showed adequacy for Hayman's model (1958) [Table 4 16] Highly significant estimates of dominance (h) and non-allelic (i and l) components of genetic variation, where in the dominance (h) gene effects and dominance x dominance (l) gene interaction with opposite signs were indicated by the crosses Kopargaon x AKM 8802, BM 4 x BPMR 145 and BPMR 145 x Vaibhav

The crosses BM 4 x BPMR 145, BPMR 145 x Vaibhav and Vaibhav x TARM 18 gave significant estimates of additive x dominance (j) type of interaction, whereas significant additive (d) major gene effect was exhibited by the crosses BM 4 x BPMR 145 and Vaibhav x TARM 18

8. 100 seed weight

All the crosses except Kopargaon x TARM 18 were explained according to Hayman's six-parameter model, for this character. The additive (d) gene effect was found to be highly significant in all the crosses, however was negative in BM 4 x BPMR 145 and BPMR 145 x Vaibhav, while the dominance (h) gene action coupled with negative significant dominance x dominance (l) interaction in the crosses, Kopargaon x AKM 8802, BPMR 145 x Vaibhav and Vaibhav x TARM 18. The cross BPMR 145 x Vaibhav only showed negative and significant estimate of additive x dominance (j) kind of non-allelic interaction.

9. Seed yield per plant

The six parameter digenic interaction model fitted for all the crosses revealed highly significant estimates of dominance (h) gene effects coupled with highly significant dominance x dominance (l) type of gene interactions with negative sign. All the crosses exhibited highly significant additive x additive (i) component except Vaibhav x TARM 18, where as only BPMR 145 x Vaibhav gave significant negative estimate of additive x dominance (j) interaction. The major gene effect additive (d) was found to be highly significant in the crosses Kopargaon x AKM 8802, BM 4 x BPMR 145 and BPMR 145 x Vaibhav (Table 4 16)

10. Protein

According to Hayman's (1958) model, dominance (h) gene effects coupled with negative dominance x dominance (l) non-allelic interactions were found to be highly significant in the crosses, Kopargaon x AKM 8802 and Kopargaon x TARM 18. The major gene effect additive (d) along with additive x dominance (j) type of interaction were found

highly significant in BM 4 x BPMR 145 and BPMR 145 x Vaibhav. All the crosses except BM 4 x BPMR 145 showed significant estimates of additive x additive (i) interaction (Table 4 17)

11. Tryptophan

The fitting of six-parameter model gave significant estimates of additive (d) gene effect in the crosses, Kopargaon x AKM 8802 (C I) and BM 4 x BPMR 145 (C III) with negative sign in latter one. Highly significant estimates of dominance (h) gene action with non-allelic interactions (i and l) was evident in all the crosses except BM 4 x BPMR 145 (C III). While, additive x dominance (j) type of component was found highly significant in all the crosses except cross V, Vaibhav x TARM 18.

12. Methionine

Both the major gene effects (d and h) and non-allelic interactions (i, j and l) were found highly significant in the crosses, Kopargaon x AKM 8802 and Kopargaon x TARM 18 with opposite signs of (h) and (l) therein. In the cross BM 4 x BPMR 145 dominance (h) effect and non-allelic (i and l) interactions were highly significant, whereas the cross Vaibhav x TARM 18 indicated highly significant additive (d) gene effect and non-allelic (j and l) components (Table 4 17).

Thus, almost all the crosses for different quantitative yield characters and quality traits as above exhibited duplicate type of epistasis, except the cross BM 4 x BPMR 145 for plant height and BPMR 145 x Vaibhav for tryptophan, where the complementary type of epistasis was revealed.

Table 4.17 Estimates of gene effects for nutritional quality traits in mungbean

Character	Cross	χ^2	Component						Type of epistasis
			m	d	h	i	j	l	
10. Protein	C I	21 36**	21 0	0 17	10 87**	9 53**	-0 43	-8 55**	Duplicate
	C II	6 79**	22 36	0 40	4 16*	3 33*	-0 37	-3 27	
	C III	79 6**	22 8	-4 23**	1 81	-0 73	-3 22**	5 43*	
	C IV	38 63**	22 56	2 10**	6 93**	5 00*	2 20*	-1 87	
	C V	21 37**	20 17	0 13	9 18**	9 46**	-0 68	-8 50**	Duplicate
11. Tryptophan	C I	364 7**	0 78	0 11**	0 32**	0 27**	0 06**	-0 21**	Duplicate
	C II	128 1**	0 77	0 01	0 52**	0 78**	-0 03**	-0 60*	Duplicate
	C III	110 8**	0 86	-0 15**	-0 05	-0 13	-0 09**	0 35**	Duplicate
	C IV	113 5*	0 82	-0 01	0 27**	0 27**	-0 02*	0 30**	Complementary
	C V	63 35**	0 82	-0 01	0 35**	0 28**	-0 05	-0 34**	Duplicate
12. Methionine	C I	64 92**	0 55	0 10**	0 46**	0 41**	0 07**	-0 40**	Duplicate
	C II	380 00**	0 48	0 23**	0 43**	0 36**	0 16**	-0 18**	Duplicate
	C III	630 1**	0 41	-0 002	0 42**	0 40**	0 39**	-0 001	
	C IV	27 20**	0 50	1 43	3 19	3 14	1 43	-6 18	
	C V	92 8**	0 50	0 03**	0 12	0 03	0 03**	0 14**	

*, ** significant at 5% and 1% level, respectively

C I - Kopargaon x AKM 8802

C II - Kopargaon x TARM 18

C III - BM 4 x BPMR 145

C IV - BPMR 145 x Vaibhav

C V - Vaibhav x TARM 18

4.2 Genetics of Host-Parasite Interaction and Biochemical Defense to Powdery Mildew

The host-parasite interaction and biochemical study on pathogenesis related enzyme activity and potash content in six generations of three different crosses viz , Kopargaon x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18 using powdery mildew (*Erysiphe polygoni*) resistant (TARM 18, BPMR 145 and Vaibhav) and susceptible (Kopargaon) parents was carried out to know the role of different pathological and biochemical traits associated with resistance and gene action involved in their inheritance

The analysis of variance for two pathological parameters and four biochemical attributes (at two stages i.e pre and post infection) revealed that there was substantial variation among various generations for all the traits under investigation

4.4.1 Mean performance of parents F₁s, F₂s and back crosses

The mean performance for different characters i.e two pathological and four biochemicals at two stages (pre and post infection) for 16 treatments over six generations for the three crosses have been presented (Tables 4 18 to 4 20)

The parent Kopargaon was found to be the most susceptible among all the genotypes having highest mean values for the pathological parameters (Table 4 18) viz , per cent disease index (PDI=67.27) and area under disease progress curve (AUDPC=1013.83) While, the parents Vaibhav and TARM 18 found resistant with lowest PDI of 12.46 and AUDPC of 240.20, respectively, followed by BPMR 145 (PDI=19.48, AUDPC= 296.70)

The F₁ mean of almost all the three crosses remained intermediate between their respective parents or approached the upper/lower limit of their parents for these pathological parameters

Table 4.18 Mean performances of Parents, F₁s F₂s and Backcrosses for pathological parameters

Cross		Percent disease index	A U D P C
Cross A Kopargaon X TARM 18	P ₁	67.27 (55.30)	1013.83 (31.38)
	P ₂	23.52 (23.81)	240.20 (15.39)
	F ₁	39.96 (39.08)	319.06 (17.69)
	F ₂	51.74 (46.19)	720.96 (24.55)
	B ₁	67.57 (55.55)	896.90 (29.72)
	B ₂	30.32 (33.26)	314.66 (17.66)
Cross B BPMR 145 X Vaibhav	P ₁	19.48 (26.13)	296.70 (16.89)
	P ₂	12.46 (20.60)	246.16 (15.59)
	F ₁	22.99 (28.54)	443.86 (20.69)
	F ₂	36.96 (37.31)	500.33 (22.30)
	B ₁	15.02 (22.68)	189.50 (13.74)
	B ₂	16.37 (23.81)	195.10 (13.94)
Cross C Vaibhav X TARM 18	P ₁	12.46 (20.60)	246.16 (15.59)
	P ₂	23.52 (23.81)	240.20 (15.39)
	F ₁	19.82 (26.41)	240.80 (15.50)
	F ₂	27.94 (31.78)	296.03 (17.17)
	B ₁	9.72 (17.86)	163.66 (13.98)
	B ₂	20.63 (26.80)	223.40 (14.90)

† : Pre Infection, II : Post Infection,

A U D P C : Area Under Disease Progress Curve

Values in parentheses are transformed

Table 4.19 Mean performances of Parents, F₁s, F₂s and Backcrosses

Cross		<i>Disease-related Biochemical Characters</i>			
		Chitinase activity (U mg ⁻¹ leaf powder)		β-1,3-glucanase activity (U mg ⁻¹ leaf powder)	
		I	II	I	II
Cross A Kopargaon X TARM 18	P ₁	1 83 ±0 05	2 01 ±0 03	1 02 ±0 03	2 00 ±0 05
	P ₂	2 56 ±0 10	3 20 ±0 02	1 92 ±0 09	3 07 ±0 05
	F ₁	2 39 ±0.19	3 18 ±0 02	1 64 ±0 04	2 50 ±0 20
	F ₂	1 51 ±0.18	2 43 ±0 14	1 68 ±0 15	2 40 ±0 14
	B ₁	1 96 ±0 03	2 66 ±0 22	1 71 ±0 16	3 02 ±0 23
	B ₂	2 76 ±0.04	3 22 ±0 05	1 96 ±0 11	2 99 ±0 05
Cross B BPMR 145 X Vaibhav	P ₁	2 33 ±0 05	3 04 ±3 25	1 26 ±0 01	2 30 ±0 11
	P ₂	2 33 ±0 03	3 36 ±0 10	2 31 ±0 19	3 08 ±0 03
	F ₁	2 40 ±0 01	3 36 ±0 10	1 80 ±0 12	3 22 ±0 09
	F ₂	2 21 ±0 05	3 08 ±0 04	1 84 ±0 04	2 73 ±0 08
	B ₁	2 46 ±0 02	3 43 ±0 06	2 07 ±0 07	3 15 ±0 03
	B ₂	2 39 ±0 04	3 47 ±0 04	1 37 ±0 10	2 23 ±0 11
Cross C Vaibhav X TARM 18	P ₁	2 33 ±0 03	3 36 ±0 10	2 31 ±0 19	3 08 ±0 03
	P ₂	2 56 ±0 10	3 20 ±0 02	1 92 ±0 09	3 07 ±0 05
	F ₁	2 38 ±0 01	3 24 ±0 06	1 97±0 03	3 25 ±0 03
	F ₂	2 27 ±0 03	3 07 ±0 03	2 02 ±0 02	3 29 ±0 06
	B ₁	2 32 ±0 02	3 15 ±0 07	2 07 ±0 03	3 13 ±0 06
	B ₂	2 46 ±0 06	3 17 ±0 09	2 12 ±0 05	3 27 ±0 03

I : Pre Infection, II : Post Infection

Table 4.20 Mean Performances of Parents, F₁s, F₂s and Backcrosses

Cross		<i>Disease-related Biochemical Characters</i>			
		Polyphenol oxidase activity ($\Delta A/hr^{-1}mg^{-1}protein$)		Potash content ($mg\ g^{-1}dry\ weight$)	
		I	II	I	II
Cross A Kopargaon X TARM 18	P ₁	2.49 ± 0.04	3.26 ± 0.09	43.27 ± 0.26	32.50 ± 0.60
	P ₂	2.61 ± 0.03	5.12 ± 0.05	47.25 ± 0.43	38.33 ± 0.18
	F ₁	2.64 ± 0.07	4.47 ± 0.11	47.10 ± 0.15	37.50 ± 0.52
	F ₂	2.81 ± 0.09	4.91 ± 0.03	41.42 ± 0.37	36.20 ± 0.33
	B ₁	2.16 ± 0.07	4.11 ± 0.05	42.50 ± 0.57	31.58 ± 0.47
	B ₂	3.07 ± 0.07	4.29 ± 0.03	49.27 ± 0.48	38.33 ± 0.38
Cross B BPMR 145 X Vaibhav	P ₁	2.91 ± 0.05	4.29 ± 0.02	39.00 ± 0.17	29.42 ± 0.51
	P ₂	3.00 ± 0.05	4.66 ± 0.17	44.80 ± 1.32	38.42 ± 0.76
	F ₁	3.02 ± 0.05	4.65 ± 0.14	44.80 ± 0.45	34.40 ± 0.51
	F ₂	2.78 ± 0.01	3.66 ± 0.11	38.42 ± 0.37	29.39 ± 0.48
	B ₁	3.20 ± 0.07	4.94 ± 0.05	37.50 ± 0.38	31.67 ± 0.35
	B ₂	3.15 ± 0.08	4.59 ± 0.13	47.40 ± 0.38	36.50 ± 0.15
Cross C Vaibhav X TARM 18	P ₁	3.00 ± 0.05	4.66 ± 0.17	44.80 ± 1.32	38.42 ± 0.76
	P ₂	2.61 ± 0.03	5.12 ± 0.05	47.25 ± 0.43	38.33 ± 0.18
	F ₁	2.86 ± 0.13	4.55 ± 0.15	44.25 ± 0.37	34.80 ± 0.82
	F ₂	2.51 ± 0.04	4.22 ± 0.05	40.00 ± 0.84	31.68 ± 0.87
	B ₁	3.21 ± 0.07	4.75 ± 0.19	48.00 ± 0.55	40.25 ± 0.78
	B ₂	3.42 ± 0.05	5.30 ± 0.06	45.50 ± 0.57	35.00 ± 0.80

I : Pre Infection, II : Post Infection

Table 4.21 Scaling tests of generation means for crosses of mungbean for powdery mildew resistance

Cross	Scales							
	A		B		C		D	
Pathological Parameters								
1. Per cent disease index								
CA	16.72		3.63		27.50		3.57	
CB	-9.31**		-1.52		45.41**		28.12**	
CC	-11.28		3.35		29.87**		18.9**	
2. Area under disease progress curve								
CA	10.37		2.24		16.06		1.72	
CB	-10.11**		-8.41**		15.35*		17.0**	
CC	-3.12		-1.08		6.70*		5.45**	
Disease-related biochemical characters								
1. Chitinase activity								
	Before infection				After infection			
	A	B	C	D	A	B	C	D
CA	-0.3	0.57*	-3.14**	-1.71**	0.13	0.06	-1.86**	-1.03**
CB	0.18*	0.06	-0.63**	-0.44**	0.47**	0.33*	-0.68*	-0.75**
CC	-0.07	-0.02	-0.57**	-0.23**	-0.19	-0.09	-0.63**	-0.17
2. β-1, 3-glucanase activity								
CA	0.75*	0.36	0.51	-0.30	1.54**	0.41	-0.44	-1.20**
CB	1.08**	-1.36**	0.19	0.24	0.78**	-1.84**	-0.89*	0.09
CC	0.19*	0.34*	0.24	-0.15	-0.06	0.22**	0.50	0.17
3. Polyphenol oxidase activity								
CA	-0.81**	0.90**	0.85*	0.38	0.48**	-1.04**	2.32**	1.44**
CB	0.47**	0.27	-0.84**	-8.0**	0.94**	-0.13**	-3.60**	-2.20**
CC	0.56**	1.37**	-1.30**	-1.62**	0.68	0.92**	-1.64**	-1.62**
4. Potash content								
CA	-5.57**	4.26**	-16.9**	-7.80**	-6.5**	0.94	-0.73	2.40**
CB	-9.33**	3.43**	-19.3**	-6.70**	-0.36	0.33	-18.2**	-9.06**
CC	7.23**	-0.60	-20.4**	-13.53**	6.97**	-2.99	19.9**	-11.9**

*, ** significant at 5% and 1% level, respectively

C A- Kopargaon x TARM 18, C B- BPMR 145 x Vaibhav, C C- Vaibhav x TARM 18

Among the plant biochemicals related to powdery mildew namely, chitinase activity was found to be highest in B₂ [(Kopargaon x TARM 18) x TARM 18] before infection i.e. 2.76. Whereas, it was lowest of 1.51 in F₂ (Kopargaon x TARM 18) x TARM 18] at the same stage. After infection chitinase activity was highest (3.47) in B₂ [(BPMR 145 x Vaibhav) x Vaibhav], while lowest activity of 2.01 was recorded in parent Kopargaon.

The β -1, 3-glucanase activity was highest in parent Vaibhav (2.31), whereas lowest activity of 1.02 in Kopargaon was recorded before infection. Highest (3.29) activity of β -1, 3-glucanase was noticed in F₂ of cross Vaibhav x TARM 18, while it was found lowest (2.00) in Kopargaon. The level of polyphenol oxidase enzyme was found highest i.e. 3.42 and 5.30 at both the stages of infection, respectively in B₂ [(Vaibhav x TARM 18) x TARM 18], whereas it was lowest in B₁ [(Kopargaon x TARM 18) x Kopargaon] (2.16) and parent Kopargaon (3.26) before and after infection, respectively (Table 4.19).

The potash content in the leaves was highest in B₂ [(Kopargaon x TARM 18) x TARM 18] and lowest in B₁ [(BPMR 145 x Vaibhav) x BPMR 145] before infection, however, levels were decreased in all the genotypes after infection, where it was highest in B₁ [(BPMR 145 x Vaibhav) x BPMR 145] and lowest in F₂ of BPMR 145 x Vaibhav (Table 4.20).

The overall biochemical analysis revealed that the levels of enzymes activities were remarkably elevated to higher magnitude in resistant parents than susceptible one, while there was reduction in potash content after disease infection.

4.4.2 Gene effects for pathological and biochemical characters (pre and post infection)

To detect the presence of epistasis, the joint scaling test of Cavalli (1952) was applied. The mean (m), additive (d) and dominance (h) were measured as per Cavalli (1952), when epistasis was absent. The estimates of genetic parameters based on six parameter model as

suggested by Hayman (1958) were estimated, when epistasis was present *i.e.* significant estimates of Chi-square values occurred (Tables 4 22 to 4 23)

The scaling tests for two pathological and four biochemical characters suggested an inadequacy of additive-dominance model for these crosses for all the characters related to powdery mildew resistance, except only the cross, Kopargaon x TARM 18 for per cent disease index at both the stages (pre and post infection) [Table 4 21] Thus, the significance of scaling tests warranted for the use of six parameter model to cast-out the gene effects

4.3.2.1 Gene effects for pathological characters

1. Per cent disease index (PDI)

The three parameter additive-dominance model indicated the absence of non-allelic interactions in cross Kopargaon x TARM 18 with highly significant additive (d) gene effect (Table 4 22)

According to Hayman (1958) the major gene effect dominance (h) and all the non-allelic components of interaction (i, j and l) were found to be significant in the crosses BPMR 145 x Vaibhav and Vaibhav x TARM 18, where the dominance (h) effect and dominance x dominance (l) components had opposite sign. The third cross showed highly significant negative additive (d) gene effect

2. Area under disease progress curve (AUDPC)

The additive (d) component of genetic variation was found to be significant only in cross Kopargaon x TARM 18 in which no epistatic interaction was detected. However, six parameter model revealed the negative dominance (h) effect with negative additive x additive (i) and dominance x dominance (l) non-allelic interactions as highly significant in the crosses, BPMR 145 x Vaibhav and Vaibhav x TARM 18. The additive x dominance (j) component was highly significant in the second cross only (Table 4 22)

Table 4.22 Estimates of gene effects for pathological parameters in Mungbean

Character	Cross	χ^2	Component						Type of epistasis	
			m	d	h	i	j	l		
1.	Per cent disease index	C A	4.71	47.7	15.74**	5.58	-	-	-	-
		C B	22.66**	37.31	-1.13	-51.06**	56.25**	-3.9*	67.1**	Duplicate
		C C	45.05**	31.78	-8.94**	-33.60**	-37.8**	-7.32*	45.7**	Duplicate
2.	AUDPC	C A	4.91	26.83	7.99**	0.03	-	-	-	-
		C B	66.9**	22.31	-0.20	-29.42**	-33.87**	-0.85**	52.4**	Duplicate
		C C	10.23**	17.17	-0.92	-10.9**	-10.90**	-1.02	15.1**	Duplicate

*, ** significant at 5% and 1% level, respectively

C A- Kopargaon x TARM 18

C B- BPMR 145 x Vaibhav

C C- Vaibhav x TARM 18

4.3.2.2 Gene effects for pathogenesis related biochemical characters (pre and post infection)

The estimates of genetic parameters were worked out for both the stages *i.e.* before and after the disease infection. The simple additive dominance model (Cavalli, 1952) was found to be inadequate for all the biochemical attributes in all three crosses studied. Hayman's six parameter model revealed that gene actions controlling these attributes were changed in some of the crosses after infection (Table 4.23)

1. Chitinase activity

At both the stages *i.e.* before and after infection, the estimates of dominance (h) effect and all non-allelic interactions (i and l) were found highly significant in the crosses, Kopargaon x TARM 18 and BPMR 145 x Vaibhav, where the additive (d) gene effect and additive x dominance (j) component were also significant in the first cross. In both of these crosses, the dominance (h) and dominance x dominance (l) component showed opposite signs (Table 4.23)

In case of third cross, Vaibhav x TARM 18 the principal gene effect (d and h) were found significant with highly significant additive x additive (i) interaction before infection

2. β -1, 3-glucanase activity

Before infection the highly significant estimates of additive (d) effect and additive x dominance (j) non-allelic interaction in cross BPMR 145 x Vaibhav, while in cross Vaibhav x TARM 18 the dominance x dominance (l) interaction was found to be highly significant and negative

Table 4.23 Estimates of gene effects for pathogenesis-related biochemical characters in mungbean

Character	Cross	χ^2	Component						Type of epistasis	
			m	d	h	i	j	l		
1.	Chitinase activity (Pre infection)	C A	51.7**	1.50	-0.80**	3.62**	3.42**	-0.43**	-3.70**	Duplicate
		C B	18.32**	2.21	0.06	0.95**	0.87**	0.06	-1.18**	Duplicate
		C C	13.34**	2.27	-0.14*	0.41*	0.47**	-0.02	-0.037	Duplicate
	(Post infection)	C A	11.94**	2.42	0.56*	2.63**	2.05*	0.04**	-2.25**	Duplicate
		C B	51.02**	3.08	-0.04	1.70**	1.48**	0.07	-2.28**	Duplicate
		C C	9.50*	3.07	-0.02	0.36	0.34	-0.5	-0.05	--
2.	β -1, 3-glucanase activity (Pre infection)	C A	7.12*	1.68	-0.26	0.77	0.60	0.19	-1.72	--
		C B	68.65**	1.84	0.70**	-0.46	-0.47	1.22**	0.75	--
		C C	8.75*	2.02	-0.05	0.30	0.29	0.07	-0.82**	--
	(Post infection)	C A	13.6**	2.40	0.03	2.36**	2.39**	0.56	-4.35**	Duplicate
		C B	115.7**	2.74	0.92**	0.85*	-0.18	1.31**	1.25*	Complementary
		C C	9.76*	3.29	-0.13*	-0.16	-0.34	-0.14	0.18	--

*, ** significant at 5% and 1% level, respectively

Cntd...

C A- Kopergaon x TARM 18

C B- BPMR 145 x Vaibhav

C C- Vaibhav x TARM 18

Table 4.23 (Contd...) Estimates of gene effects for pathogenesis-related biochemical characters in mungbean

Character	Cross	χ^2	Component						Type of epistasis	
			m	d	h	I	j	l		
3.	Polyphenol oxidase activity (Pre infection)	C A	71.99**	2.81	-0.92**	-0.66	-0.76	0.85**	0.67	--
		C B	99.3**	2.78	0.05	1.66**	1.58**	0.10	-2.33**	Duplicate
		C C	268.1**	2.51	-0.21*	3.28**	3.23**	-0.4**	-5.16**	Duplicate
	(Post infection)	C A	488.8**	4.91	-0.17**	-2.60**	-2.87**	0.76**	3.43**	Duplicate
		C B	126.7**	3.66	0.35*	4.58**	4.41**	0.54**	-5.22**	Duplicate
		C C	98.41**	4.21	-0.54**	3.09**	3.24**	-0.12	-4.84**	Duplicate
4.	Potash content (Pre infection)	C A	160.3**	41.93	-6.87**	17.49**	15.60**	-4.92**	-14.3**	Duplicate
		C B	255.7**	38.96	-10.1	15.55**	13.4**	-6.38**	-7.5**	Duplicate
		C C	68.51**	40.0	2.80**	25.18**	27.07**	3.91**	-33.7**	Duplicate
	(Post infection)	C A	34.28**	36.20	-6.54**	-2.70	-4.80**	-3.71**	10.33**	Duplicate
		C B	81.4**	29.66	-5.06**	18.51**	18.13**	-0.35	-18.1**	Duplicate
		C C	56.9**	31.67	5.26**	20.25**	233.9**	4.98**	-27.8**	Duplicate

*, ** significant at 5% and 1% level, respectively

C A- Kopargao x TARM 18

C B- BPMR 145 x Vaibhav

C C- Vaibhav x TARM 18

After infection, the highly significant dominance (h) gene action coupled with negative dominance x dominance (l) component was found in the cross Kopargaon x TARM 18. While, cross BPMR 145 x Vaibhav indicated significant additive (d) effect with positive dominance x dominance (l) non-allelic component.

3. Polyphenol oxidase activity

As per the Hayman's model (1958), in first cross the additive (d) effect and additive x dominance (j) was found highly significant at both the stages, while after infection the other major gene effect (h) and non-allelic interactions (i and l) were also found highly significant (Table 4.23).

In the crosses, BPMR 145 x Vaibhav all the components of genetic variation were significant except additive (d) effect and additive x dominance (j) interaction at both the stages. Whereas, significant estimates of all genetic parameters were noticed at both stages in Vaibhav x TARM 18, except additive x dominance (j) component at former stage.

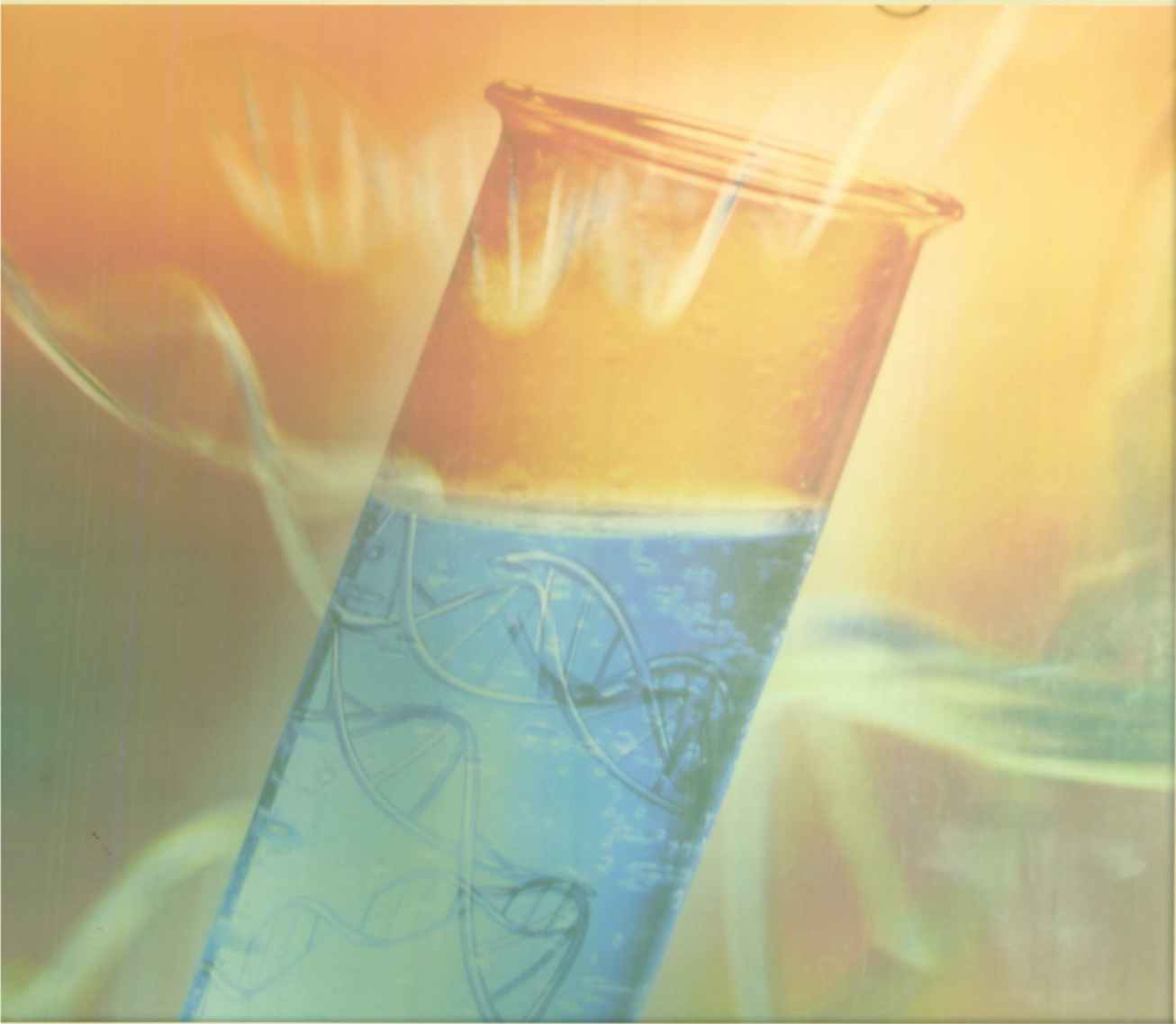
4. Potash content

At both the stages of infection, all three crosses indicated highly significant estimates of major gene effects (d and h) as well as non-allelic (i, j and l) interactions, except additive (d) effect before infection and additive x dominance (j) component after infection in the second cross (Table 4.23).

The estimates of dominance (h) gene action and dominance x dominance (l) gene interaction displayed opposite signs in all three crosses investigated at both the stages.

Thus, overall gene effects of pathological and biochemical attributes evinced the involvement of duplicate type of epistasis to govern these characters in powdery mildew resistance in almost all the cases except the complementary gene action for β -1, 3-glucanase activity after infection in cross B (BPMR 145 x Vaibhav).

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DISCUSSION



DISCUSSION

The execution of a plant breeding programme is largely based on the genetics of quantitative characters associated with yield, quality traits, pest-disease resistance or any economic trait concerned to the breeder. Such quantitative characters require sound understanding of their genetic architecture to make breeding methodology a success. Since, the quantitative traits are controlled by polygenic system, it becomes rather difficult to adjudge which particular combination of parents would yield better results and likely to produce desirable segregants permitting scope for rigorous selection.

Programs based on sound biometrical background provide a precise explanation of the genetic phenomenon, providing a greater confidence to the plant breeder in genetic interpretation of the material under investigation.

High yielding varietal development coupled with biotic and abiotic stresses resistance/ tolerance is relatively more difficult in self-pollinated crops than cross-pollinated ones, because of existence of limited natural genetic variability and difficulties in exploitation of hybrid vigour due to their mode of pollination. Keeping this in view, efforts were made in present investigation to study the extent of heterosis, to identify the best combiner and high yielding genotypes, to study DNA polymorphism *via* molecular markers and also to study gene action underlying yield, its components and some quality traits alongwith powdery mildew resistance related attributes. The results of present investigation are discussed below under suitable headings.

5.1 Heterosis and Combining Ability

Presence of adequate variability among the genotypes was revealed through highly significant differences among themselves for all the traits studied. The parents and hybrids also manifested highly significant differences for most of the traits except for seeds per pod. The parents *vs* crosses also showed significant differences for all the characters except days to 50% flowering and days to maturity implied the presence of heterosis in the cross combinations.

Many crosses exceeded their performance beyond the lower and/or upper/higher limit of parents for various characters in desirable direction. Based on the mean performance, the best crosses identified for each character were Kopargaon x AKM 8802 (days to 50% flowering), AKM 8802 x TARM 18 (days to maturity), Vaibhav x TARM 18 (plant height, primary branches/ plant and seed yield/ plant), BM 4 x Vaibhav (pod cluster/ plant), BM 4 x TARM 18 (pods/ plant), PM 9341 x TARM 18 (seeds/ pod) and PM 9341 x Vaibhav (100 seed weight)

5.1.1 Heterosis

Heterosis is the measure of deviations of progeny means from parental means. The manifestation of hybrid vigour was calculated in terms of mid parent heterosis (MP) and heterobeltiosis (BP). Kadambavanasundaram (1980) proposed that the heterotic expression over standard or best variety should alone be given due importance for commercial exploitation of hybrid vigour. Hence, the hybrids with significantly higher values of heterobeltiosis have been well taken into account in the present investigation (Table 5.1)

For earliness, a negative heterosis for days to 50% flowering and days to maturity is desirable. However, none of the cross combinations exhibited significant negative mid parent heterosis and heterobeltiosis for days to 50% flowering. Non-significant heterosis of days to 50% flowering was reported by Swindell and Poehlman (1976), Singh (1980) and Gawande (2001) [Table 5.1 and 5.2]

Only one cross AKM 8802 x TARM 18 showed significant negative heterosis over mid parent, whereas, none of the crosses exhibited significant negative heterobeltiosis for days to maturity. The parents Kopargaon and TARM 18 were early maturing, but could not produce F_1 with negative significant heterobeltiosis. This suggests that while selecting parents and crosses for early maturity due considerations should be given to mean performance of parents and F_1 s rather than to magnitude of heterosis. These findings are in agreement with Naidu and Satyanarayana (1993^b), Patil *et al.*, (1996^a), Sawale (1999) and Gawande (2001)

Table 5.1 Crosses exhibiting heterobeltiosis in desirable direction for different traits

Character	Heterobeltiosis (%)	Mean Performance	Cross
Days to 50% flowering	-3.51	36.67	AKM 8802 x BM 4
	-3.51	36.67	AKM 8802 x Vaibhav
Days to maturity	-0.55	60.47	AKM 8802 x TARM 18
	-0.51	64.47	AKM 8802 x BM 4
	-0.50	66.67	PM 9341 x Vaibhav
Plant height (cm)	9.49*	54.32	Kopargaon x Vaibhav
	5.04	58.82	PM 9341 x TARM 18
	3.18	54.41	BM 4 x TARM 18
Primary branches/plant	20.0*	2.40	PM 9341 x BM 4
	18.33	2.37	Kopargaon x BM 4
Pod clusters/plant	5.19*	9.47	Kopargaon x BPMR 145
	3.97	8.73	BPMR 145 x TARM 18
	3.70	9.33	Vaibhav x TARM 18
Pods/plant	20.40**	25.29	AKM 8802 x Vaibhav
	18.20**	25.70	Kopargaon x TARM 18
	15.10*	26.47	BM 4 x TARM 18
	13.45*	25.87	AKM 8802 x BM 4
	8.57	22.80	Kopargaon x BPMR 145
Seeds/pod	10.31	11.77	PM 9341 x TARM 18
	9.08	11.42	AKM 8802 x BM 4
	7.10	11.21	Kopargaon x BM 4
100 seed weight (g)	20.8**	3.94	BM 4 x TARM 18
	19.42**	4.77	Kopargaon x BM 4
	11.08*	4.44	AKM 8802 x BM 4
Seed yield/plant (g)	22.8**	10.37	BM 4 x TARM 18
	20.4**	10.84	Vaibhav x TARM 18
	18.3**	9.68	AKM 8802 x BM 4
	11.5**	10.04	PM 9341 x BM 4

*, ** Significant at 5% and 1% level, respectively

Only one cross AKM 8802 x TARM 18 showed significant negative heterosis over mid parent, whereas, none of the crosses exhibited significant negative heterobeltiosis for days to maturity. The parents Kopargaon and TARM 18 were early maturing, but could not produce F_1 with negative significant heterobeltiosis. This suggests that while selecting parents and crosses for early maturity, due considerations should be given to mean performance of parents and F_1 s rather than to the magnitude of heterosis. These findings are in agreement with Naidu and Satyanarayana (1993^b), Patil *et al* (1996^a), Sawale (1999) and Gawande (2001).

Out of 21 hybrids, the BM 4 x TARM 18 evinced the highest mid parent heterosis (14.60%) for plant height followed by BM 4 x Vaibhav, while the highest positive heterobeltiosis was displayed by the hybrid, Kopargaon x Vaibhav (9.49%), where none of the crosses was found significant for heterobeltiosis. The significant positive relative heterosis and heterobeltiosis for plant height was reported by Thimappa (1987), Kute (1997), Joseph and Santoshkumar (2000), Sekhar *et al* (1994), Hegde *et al* (1996^b), Reddy (1998), Aher and Dahat (1999), Aher *et al* (2000^a), Gawande (2001) and Reddy *et al* (2003).

The only hybrid, PM 9341 x BM 4 manifested highest significant heterosis over mid parent as well as better parent. The present findings are in line with Sonawane (1995), Kute (1997), Sawale (1999), Aher *et al* (2000^{a&b}), Joseph and Santoshkumar (2001), Gawande (2001) and Reddy *et al* (2003).

Highest significant mid parent heterosis and non-significant heterobeltiosis was evinced by the cross Kopargaon x BPMR 145 for pod clusters/plant which is in accordance with earlier reports by Kelkar, (1993), Patil (1993), Sekhar *et al* (1994), Kute (1997), Aher and Dahat (1999), Aher *et al* (2000^b), Gawande (2001) and Reddy *et al* (2003).

Out of 21 crosses, eight and four crosses exhibited significant positive heterosis over mid parent and better parent, respectively. The hybrid, AKM 8802 x Vaibhav followed by Kopargaon x TARM 18 displayed highest mid parent heterosis and heterobeltiosis. The crosses involving parent AKM 8802, as one of the parents exhibited significant heterosis over mid parent and better parent, indicating its importance in transferring this character in F_1 as has been reported earlier by Sonawane (1995), Kute (1997), Aher and Dahat (1999), Sawale (1999), Aher *et al* (2000^a), Gawande (2001) and Reddy (2003).

In the present investigation, the mid parent heterosis and heterobeltiosis were amounted upto 13.87% and 10.31%, respectively for seeds/ pod, an important yield component in mungbean. Two cross combinations *viz*, PM 9341 x TARM 18 and AKM 8802 x BM 4 evinced significant mid parent and better parent heterosis. Kute (1997), Sawale (1999), Aher *et al* (2000^{a&b}), Joseph and Santoshkumar (2000) and Gawande (2001) also arrived at similar conclusions.

Five and three crosses manifested significant positive heterosis over mid parent and better parent, respectively, where highest relative heterosis was observed in Kopargaon x BM 4 (33.55%) followed by BM 4 x TARM 18 (25.92%), whereas hybrid BM 4 x TARM 18 (20.8%) showed highest significant heterobeltiosis followed by Kopargaon x BM 4 (19.42%). The crosses consisting BM 4 as one of the parents mostly exhibited significant heterosis, indicating its significant contribution in producing better F_1 with high test weight. Aher *et al* (2000^b), Joseph and Santoshkumar (2000) and Reddy *et al* (2003) observed heterobeltiosis for this character, while Kute (1997), Reddy (1998), Aher and Dahat (1999), Sawale (1999), Aher *et al* (2000^a) and Gawande (2001) noticed positive heterobeltiosis for 100 seed weight.

Seed yield, the complex character, decides the economic worth of the hybrids. The high expression of heterosis for seed yield was evident in the present investigation. Similar results were reported earlier by Patil *et al* (1992), Paroda and Singh (1998), Kute (1997), Sawale (1992), Aher and Dahat (1999), Aher *et al* (2000^{a&b}), Joseph and Santoshkumar (2000), Gawande (2001) and Reddy *et al* (2003). Among 21 hybrids five crosses evinced significant and positive heterosis over mid parent and better parent, respectively. The hybrid BM 4 x TARM 18 followed by Vaibhav x TARM 18 and AKM 8802 x BM 4 displayed highest significant mid parent heterosis and heterobeltiosis for seed yield/ plant.

Heterosis over mid parent and better parent indicated that, in general, magnitude of positive heterosis was higher than negative heterosis. This might be attributed to both epistasis and over dominance. Based on the *per se* performance and heterosis among the crosses, it could be concluded that hybrids *viz*, Kopargaon x TARM 18, AKM 8802 x BM 4, PM 9341 x BM 4, BM 4 x TARM 18 and Vaibhav x TARM 18 were found better for most of traits. It clearly suggests that while selecting best cross combinations this evidence needs to be given due consideration.

In the present study, significant positive heterosis for seed yield was associated with heterosis for earliness, plant height, pods/ plant, clusters/ plant, and 100 seed weight in most of the heterotic combinations. Manjre and Deshmukh (1981), Choudhary (1986), Gawande (2001) and Reddy *et al* (2003) reported similar results. This would clearly indicate that heterosis for yield was through heterosis for individual yield components or additive or synergistic effects of the components characters or alternatively due to the multiplicative effect of partial dominance of component characters. The different magnitude for heterosis for various characters in F_1 over the parental means in the present study indicated over all dominance or positively acting genes and increased diversity between the parental genotypes in the expression of heterosis. A few modifier genes with negative effect might also be involved in the expression of heterosis.

5.1.2 Combining Ability

The knowledge of combining ability is a prerequisite to isolate the best specific combination and to study the combining ability of the parents with diverse genetic background. The *gca* effect is controlled by fixable additive genes (Simmonds, 1979) and high *gca* would produce transgressive segregates in F_2 or later generations (Singh and Singh, 1985). Hence, the process of development of high yielding varieties may be addressed in a better way by understanding the nature of gene action and also to investigate the nicking ability of the parents.

Mean squares due to *gca* and *sca* were significant for almost all the characters. However, the relative importance of *gca* and *sca* when compared, revealed the preponderance of additive gene effects for days to 50% flowering, days to maturity, plant height and pods/ plant. On the contrary both *gca* and *sca* mean squares were equally important for primary branches/ plant, pod clusters/ plant, 100 seed weight and seed yield/ plant. This indicated the importance of both additive and non-additive effects in the genetic control of seed yield/ plant.

The results of *gca* effects (Table 5.2) indicated significant and highly significant variation for all the traits studied. High *gca* coupled with high *per se* performance is the indication of an outstanding best parent with reservoir of superior genes. Therefore, *gca* effects along with mean performance may be taken into account for parental selection.

The parents, Kopargaon and TARM 18 were found to be best general combiners for days to 50% flowering and days to maturity. For test weight the parents PM 9341 and BPMR 145 were appeared to be good general combiners.

The parents, BM 4 and AKM 8802 exhibited maximum *gca* effects for the trait pods/ plant. The parents, Vaibhav and Kopargaon registered high *gca* estimates for pod clusters/ plant, while for primary branches/ plant TARM 18 and Vaibhav were found to be best general combiners. However, among the seven parents, none of the parents had significant desirable *gca* effect for seeds/ pod.

The parent Vaibhav (0 317) followed by BM 4 (0 248) expressed highest *gca* effect for the ultimate trait seed yield/ plant, which was also best general combiner for tallness among seven parents. Thus, this indicated that Vaibhav may be selected as the best general combiner as this parent exhibited significantly positive and high *gca* effects for five characters including seed yield per plant. Interestingly, *per se* performance of Vaibhav was also high. The parent TARM 18 manifested high *gca* effects for four characters *viz*, days to 50% flowering, days to maturity, primary branches per plant and pods per plant. The parents Kopargaon and BM 4 displayed good *gca* effects for three and two characters, respectively. The above four parents have good *per se* performance for most of the traits suggesting their potential in further breeding programme to isolate desirable transgressions for yield and its related traits. Similar findings have been reported by various workers (Luthra *et al*, 1977, Godhani *et al*, 1978, Deshmukh and Manjre, 1980, Malhotra *et al*, 1980, Thimmappa *et al*, 1987, Saxena and Sharma, 1992, Mansuria and Joshi, 1994, Kute, 1997, Dasgupta *et al*, 1998, Jain *et al*, 2000, Gawande, 2001 and Singh and Dikshit, 2003).

Table 5.2 Parents showing high *gca* in desirable direction for different traits

Sr. No	Genotype	No. of traits	<i>gca</i> effects	Character
1	Kopargaon	3	-1 05**	Days to 50% flowering
			-0 974*	Days to maturity
			0 333**	Pod clusters/ plant
2	AKM 8802	1	0 652*	Pods/ plant
3	PM 9341	1	0 272**	100 seed weight
4	BM 4	2	1 506**	Pods/plant
			0 248**	Seed yield/ plant
5	BPMR 145	1	0 614*	Days to 50% flowering
6	Vaibhav	5	0 762*	Days to 50% flowering
			0 928**	Plant height
			0 107*	Primary branches/ plant
			0 533**	Pod clusters/ plant
			0 317**	Seed yield/plant
7	TARM 18	4	-1 24**	Days to 50% flowering
			-2 68*	Days to maturity
			0 20**	Primary branches/ plant
			0 61*	Pods/ plant

*, ** Significant at 5% and 1% level, respectively

Table 5.3 Some important crosses showing high sca for different traits

Sr. No.	Genotype	No. of traits	sca effects	Character
1	Kopargaon x TARM 18 H x H	2	2 73**	Pods/ plant
			0 62**	Seed yield/plant
2	AKM 8802 x BM 4 L x H	5	-0 759*	Days to maturity
			1 55**	Pods/plant
			0 680*	Seeds/ pod
			0 48**	100 seed weight
			0 89**	Seed yield/ plant
3	AKM 8802 x Vaibhav L x H	3	-1 194*	Days to maturity
			0 208*	Primary branches/ plant
			3 06**	Pods/ plant
4	PM 9341 x BM 4 L x H	2	0 38*	100 seed weight
			1 16**	Seed yield/ plant
5	PM 9341 x TARM 18 L x H	2	4 64*	Plant height
			1 044*	Seeds/ pod
6	BM 4 x TARM 18 L x H	4	-1 130*	Days to 50% flowering
			2 20**	Pods/ plant
			0 31	100 seed weight
			1 10**	Seed yield/ plant
7	Vaibhav x TARM 18 H x H	2	0 47**	100 seed weight
			1 53**	Seed yield/ plant

*, ** Significant at 5% and 1% level, respectively

high x high type combinations not necessarily result in to high sca effects This might be due to internal cancellation of gene effects in such parents as suggested by Jones (1958) However, in the crosses, AKM 8802 x BM 4, AKM 8802 x Vaibhav, PM 9341 x TARM 18 and BM 4 x TARM 18, one parent was good general combiner and other was poor/ average general combiner and other was poor/ average general combiner for most of the traits. This might be due to presence of genetic diversity in the form of heterozygous loci for these characters Similar situations were also observed by Singh and Singh (1974), Luthra *et al* (1979), Thimmappa (1987), Kute (1997), Dasgupta *et al* (1998) and Gawande (2001)

Considering the *per se* performance, general combining ability effects of parents, specific combining ability effects of hybrids and heterotic performance for yield and its principal components in the F_1 hybrids the combinations namely, AKM 8802 x BM 4, BM 4 x TARM 18 and Vaibhav x TARM 18 were appeared to be the most promising Hence the desired size of F_2 population of these crosses can be grown to isolate high yielding lines of mungbean The high degree of diversity among the parents might be degree of diversity among the parents might be the important factor responsible for greater magnitude of non-additive genetic variance These crosses would provide transgressive segregants and may be utilized in further breeding programme either in biparental mating or in diallel selective mating (Jenson, 1970) for multiple parent's input into the central gene pool for isolating high yielding lines from advanced generations To release greater amount of genetic variability, Malhotra *et al* (1980) evinced that diallel selective mating among the parents on the basis of *gca* may result in breaking up some undesirable linkages

5.2 DNA Polymorphism Study by Molecular Markers

Mungbean (*Vigna radiata* (L.) Wilczek) is a tropical Legume species that provides important and inexpensive sources of dietary protein to the people of Asia and other parts of the world. Despite the major position of mungbean as an important legume crop, very little information about its DNA markers based genetics is available. DNA polymorphisms have been extensively employed as means of assessing genetic diversity, which is a prerequisite for any crop improvement programme. Traditionally phenological and morphological characters have been used for the identification of cultivars and their relatives, however, these characters may not be significantly distinct and usually require growing the plants to full maturity prior to identification (Charcosset and Moreau, 2004). Biochemical markers such as electrophoresis of seed proteins/ enzymes detects very little polymorphism. Hence, discrimination of elite mungbean varieties was attempted using PCR-based DNA markers.

The polymerase chain reaction (PCR) provides a simple, faster, safer and less expensive means for genome analysis compared with RFLPs. A single, short oligonucleotide primer can amplify specific sequences for genomic DNA through PCR. Randomly amplified polymorphic DNA sequences (RAPDs) obtained by the use of random decamer primers in PCR have been extensively used as molecular markers for tagging genes, saturating and constructing existing molecular maps, etc (Kumar, 1999). Inter-simple sequence repeats polymorphism has emerged as a relatively new, reliable and speedy marker system for germplasm evaluation. These primers are 20 to 25 bases in length and contain a microsatellite motif and a one or two base 3' anchor. Thereby they amplify the region lying between two microsatellite containing sites. ISSRs have been used in various plants for assessment of variability in the population. As the ISSR primers were used under stringent amplification conditions, but are comparable to RAPD primers in cost and operation of the assay. In our first effort of molecular characterization of

genetic diversity in a set of elite mungbean cultivars used in Indian breeding programmes (particularly in Maharashtra, Madhya Pradesh and Gujarat States) RAPD as well as ISSR markers were used and hierarchical distribution of genetic diversity was observed among them, the results of which have been discussed in this section. Further, these two marker systems are compared for their suitability in the evaluation of mungbean genotypes.

5.2.1 Polymorphism using RAPD and ISSR markers

Seven varieties, as parents used in the diallel mating set were selected on the basis of their phenotypic characters and used for analyzing DNA polymorphism by advocating two marker systems viz., RAPD and ISSR. Both RAPD and ISSR fingerprinting has been successful in detecting variation in these cultivars in the present investigation.

Out of 210 decamer Operon random primers and 100 UBC-ISSR primers, five RAPD and sixteen ISSR primers amplified polymorphic patterns that revealed 44.0% and 54.7% polymorphism, respectively. Different primers varied in their ability to detect polymorphism. A total of 468 variants (bands) were produced by these 21 primers, which were polymorphic in nature across seven cultivars with an average of 6.51 bands/ primer (Table 4.8 and 4.9). The level of polymorphism detection was high in ISSR primers compared to random primers and also it was more reproducible with ISSR.

Although 100 ISSR primers were used for initial screening consisted of di-, tri-, and tetra-nucleotide repeat motifs, all the 16 polymorphic primers contained dinucleotide motifs with a single or two base 3' anchor. Eight of these primers contained (AG)_n repeat motifs, in general.

Thus, when these two marker systems were compared for their efficiency as tools for diversity analysis in mungbean genotypes, the informative potential of ISSR primers was found higher than the RAPD primers. Though the RAPD also detected polymorphism among these

cultivars, it required large number of primers for initial screening to obtain the primers with polymorphic amplifications. In earlier works on tetraploid wheat (Pujar, 2001), chickpea (Sant *et al.*, 1999) and blackgram (Souframanien *et al.*, 2002), it was shown that ISSRs could reveal a high degree of polymorphism. Our data revealed relatively low polymorphism in mungbean with RAPD markers and hence these markers may not be that much reliable as ISSR in accessing the genetic diversity in these Indian mungbean cultivars. Extensive DNA polymorphism, however, has been reported using RAPD markers in several other crop plants (Hilu and Stalker, 1995, Ratnaparkhe *et al.*, 1995, Morell *et al.*, 1994, Ranade and Sane, 1996 and Kumar, 1999). Similar kind of results with ISSR markers were reported by Singh *et al.* (2000) in mungbean and Ajibade *et al.* (2000) in *Vigna* genus.

Different primers varied in their ability to detect diversity across seven mungbean genotypes under study. A precise comparison of efficiency of these two methods of assessing diversity is thus not possible, at least within the range of material of the present investigation in mungbean. But the variety-specific (highly polymorphic) markers revealed from both these marker-systems would constitute a 'core set' of useful primers those can be further used as an initial set for genome analysis, such as construction of genetic linkage map or tagging the gene of interest or development of polymorphic probes in mungbean.

5.2.2 Genetic distance among cultivars and their clustering

In order to quantify level of polymorphism detected, Nei's genetic distances were computed for all 21 combinations of the seven parental genotypes considering the RAPD and ISSR approaches individually as well as together (Table 4.12). Based on the RAPD markers alone, the genetic distance ranged from 0.083 to 0.467, while that calculated on the basis of ISSR markers ranged from 0.108 to 0.256. However, genetic distance based on both molecular markers together varied from 0.116 to 0.250, which was used to construct the dendrogram (Fig 5.1).

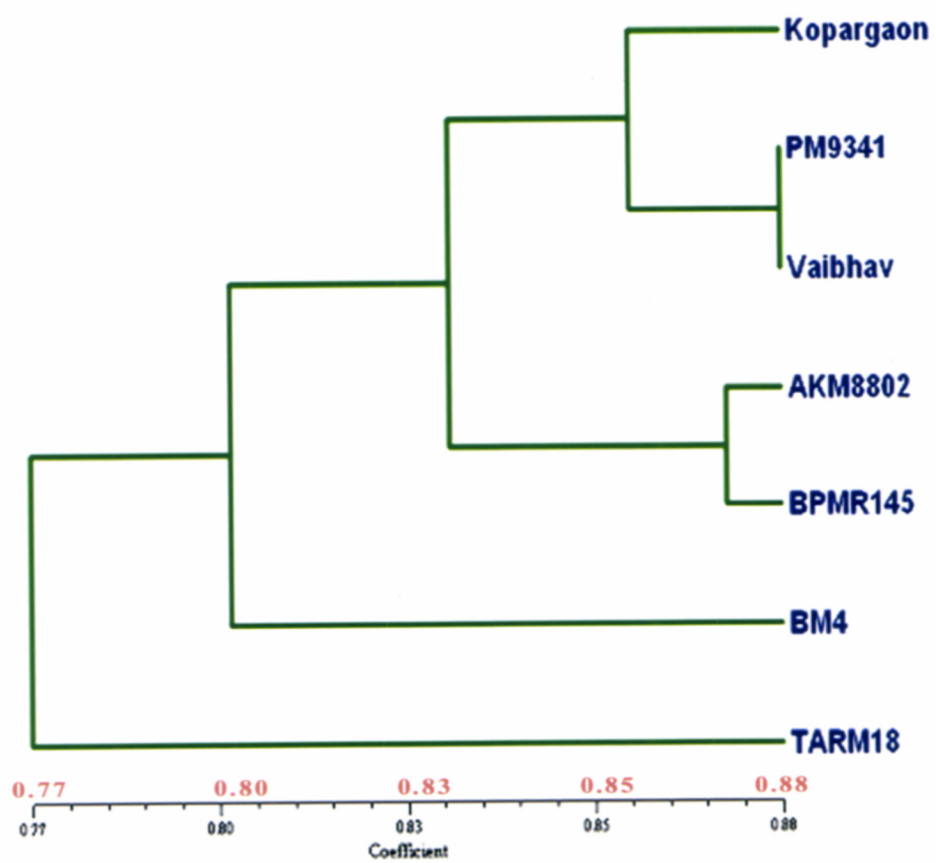


Fig. 5.1 Combined dendrogram through RAPD and ISSR analysis of parental mungbean genotypes

From the dendrogram, it can be seen that seven parental mungbean cultivars cluster into a major group containing five cultivars (Kopargaon, PM 9341, Vaibhav, AKM 8802 and BPMR 145), while remaining two cultivars, BM 4 and TARM 18 grouped-out from the others. The major group consists of two subgroups, Kopargaon, PM 9341 and Vaibhav forming one subgroup and AKM 8802 and BPMR 145 forming the other. PM 9341 and Vaibhav (PM 9339) from the first subgroup share a common parentage (KDM-1 and TARM 18), both bold seeded, are genetically closest at distance of 0.116, however, the Kopargaon is a local selection and therefore the clustering based on its pedigree can not be commented upon. TARM 18, emerged as a most out grouped individual in cluster analysis, is a mutant derivative (pedigree- PDM 54 x TARM 2) with small seeds and resistant to powdery mildew which is extensively used as a one of the donors in resistance breeding in Indian subcontinent. The BM 4 (J 781 x Mugi), which is also grouped-out from the main cluster, is of early and small seeded in nature. On the other hand, in another subgroup AKM 8802 (susceptible to powdery mildew) and BPMR 145 (resistant to powdery mildew and bold seeded) clustered with a low genetic distance of 0.181 only. This might be due to limited polymorphism revealed through the present set of polymorphic markers, which might explored less unlike regions between these two genomes.

Thus, RAPD and ISSR fingerprinting has been successful in detecting variation in this intraspecific mungbean cultivars' clustering. The variations (polymorphisms) examined in the present study represent gross genetical as well as morphological or physiological alternations, which might be due to change at a single locus or at more than one loci, i.e. polymorphisms revealed by molecular markers in the present study may be due to single base change in the primer target site as well as deletion or insertion of DNA sequence (Caetano-Anolles *et al*, 1991). The narrow genetic base of the green gram accessions was revealed in the RAPD analysis study by Lakhanpaul *et al*, 2000 which also confirms present results of RAPD investigation. The study also provides baseline information for the management of genetic resource collection and diagnostic evaluation/ identification in Indian mungbean.

5.2.3 Potential of DNA markers in predicating heterosis in mungbean

The development of molecular genetic markers that detect variation at the DNA sequence level has made it possible to obtain solutions to the problems that were previously inaccessible to genetic manipulation. The prediction of hybrid performance has always been a primary objective in the entire hybrid crop breeding programmes (Hallauer and Miranda, 1988). Earlier analyses demonstrated important features regarding the correlations of molecular marker heterozygosity with heterosis and/or F_1 performance. Therefore, an attempt was made here to correlate the heterosis with molecular marker heterozygosity, in different traits including seed yield, which is the first study of its kind in elite Indian mungbean cultivars.

We observed that the hybrids were more heterotic for seed yield than for yield component traits. This is obvious because yield (Y) is generally multiplicative function of its contributing characters, such as 100 seed weight, number of seeds/ pod, number of clusters/ plant etc.

Table 5.4 Correlations between DNA marker-based genetic distance and heterosis for seed yield and its components

Sr. No	Parameters	Genetic distance	
1	Genetic distance	1 000	
2	Days to 50% flowering	MP	-0 204
		BP	-0 068
3	Days to maturity	MP	-0.437*
		BP	0 052
4	Plant height (cm)	MP	0 373
		BP	0 209
5	Primary branches/ plant	MP	-0 120
		BP	-0 168
6	Pod clusters/ plant	MP	-0 007
		BP	0 088
7	Pods/ plant	MP	0 349
		BP	0 274
8	Seeds / pod	MP	0 395
		BP	0.446*
9	100 seed weight (g)	MP	0.434*
		BP	0 245
10	Seed yield/ plant (g)	MP	0 358
		BP	0 351

*, ** significance at 5 % and 1 %, respectively

MP and BP ~ Heterosis over mid and better parent, respectively

The DNA marker-based genetic distances was found to be significantly and positively associated with mid parent heterosis for 100 seed weight and heterobeltiosis for seeds/ pod, while there was negative significant association with mid parent heterosis for days to maturity. Although, the correlations appeared to be low for heterosis in seed yield/ plant and its other components, they were positive in most of the cases including seed yield/ plant (Table 5.4). Such correlations based on positive markers (specific heterozygosity), however in large, may have practical utility in predicting the heterosis in mungbean. In order to add predictive power to present molecular markers-based genetic distance, it may be necessary to select markers that are associated with the traits to be predicted.

It was also evident that significant heterosis resulted for hybrids by crossing parents were belonging to two different groups/ subgroups and hybrids obtained after crossing parents from the same group gave poor heterosis for yield, in general. Thus, results suggest that the concept of genetic divergence for maximum expression of heterosis has certain limitation in mungbean. It was suggested earlier that hybrids showing heterosis were usually developed from parental lines diverse in relatedness, ecotype, geographic origin etc (Yuan, 1985). However, in maize heterosis manifested by hybrids developed from genetically diverse varieties was less than that between varieties, which were to be genetically less diverse (Moll *et al*, 1965). In wheat, rice, chickpea and cotton low correlation was reported between heterosis and DNA-based genetic distance (Barbosa-Neto *et al*, 1996, Xiao *et al*, 1996, Sant *et al*, 1999 and Kaur and Chahal, 2001).

A possible problem associated with the detection of a single gene effect in a diallel system is the frequent sporadic correlations among marker loci even when located on different chromosomes due to the small

number of parental lines used in the analysis. Such sporadic correlations would often cause false positives in the detections. Apparently, crosses between extremely divergent parents create a situation in which the harmonious functioning of alleles is disrupted. Consequently, the physiological functions are not efficient, resulting in low heterosis. In fact, doubts have been expressed about the usefulness of increased genome coverage for calculating marker distance and correlating it with hybrid performance/ heterosis to improve the efficiency of the prediction (Melchinger *et al* , 1990, Boppenmaier *et al* , 1993 and Sant *et al* , 1999). Alternatively the identification of marker loci and genotypes significantly associated with traits of interest was suggested.

Interestingly, if a majority of markers are located in the chromosome regions, where gene for higher yield, its components or powdery mildew resistance had been reported in previous studies based on segregation analysis, then such a remarkable agreement between the locations of gene of interest and positive markers (*i.e.* specific heterozygosity) can strongly indicate that DNA marker-based diallel analysis may be very useful for detecting the presence of gene of interest, its location as well as predicting the heterosis. Thus, correlations calculated using specific heterozygosity based on the positive markers would be of more significance than those based on general heterozygosity. But given that, a high percentage of false positives may obscure the profitability of these strategies (Asins, 2002), so as to avoid mismatch between theory and practice that instructed and assisted by recourse of quantitative genetics (Arunachalam, 2001). It is, therefore, essential that specific DNA markers be developed in this system for an efficient and reliable estimation of genetic distance for predicting heterosis in mungbean.

5.3 Generation Mean Analysis for Seed Yield, its Components and Quality Traits

Presence of genetic variability in the population and understanding the type and relative magnitude of generation involved for different characters either for agronomic traits or disease related traits, are pre-requisites for manipulating quantitatively inherited characters in a systematic breeding program. Its success depends upon both, the amount of variability present in a population with which plant breeder is dealing and so its efficient management and utilization. For this purpose, it is essential to have a thorough understanding of the nature of genetic system that accounts for the variation of different characters under study. If additive effects are greater, the chance of fixing superior genotype will be more in the subsequent generations through selection. If dominance and epistatic interactions *i.e.* non-additive gene effects are predominant, the selection will have to be postponed to later generations (Panse, 1942). However, Cockerham (1961) stated that the relative merits of the current methods of selection with regards to unknown epistatic gene actions, and it is yet critical to determine, the most efficient breeding procedures, when epistasis is operative.

Standard hybridization and selection procedures could take advantages of epistasis, if it is of the additive type (additive 'd' and additive x additive 'i'). Other types of epistasis (additive x dominance 'j', dominance x dominance 'l', etc) are not fixable by selection in self-fertilized crops and therefore, they would not be helpful to develop pure line-cultivars, but may be useful in the development of hybrids. So as to develop homozygous pure line cultivar, the masking effects of epistasis bears no consequence and hence selection is to be delayed till virtual homozygosity attained, since only additive types of non-allelic interaction are present in pure lines. Keeping these theories in view, the nature of gene action and gene effects involved in the inheritance of yield, its related components and some quality traits are discussed hereunder.

5.3.1 Mean performance of generations

The mean performance for different characters varied over six generations for the five crosses (Table 4.14). The F_1 means in most of the crosses approached to mid parental values or exceeded the high parent performance, indicating their dominance (partial/ over) in respective cross for different traits studied. Among the parents, TARM 18 was earliest in day to 50% flowering, whereas the B_2 [(Vaibhav x TARM 18) x TARM 18] was earliest to mature and had highest number of pod clusters/ plant, pods/ plant, seed yield/ plant and tryptophan content. Highest protein content (25.07%) was recorded in the hybrid BM 4 x BPMR 145. This implies that due consideration should be given to these *per se* performances of the generations alongwith the gene action inferred therein, while selecting for improvement in the respective cross(s).

5.3.2 Gene effects for seed yield, its components and quality traits

The non-significant estimates of Chi-square values for the characters under study in the respective crosses indicated the adequacy of additive-dominance model and suggested the role of either additive or dominance gene action in the expression of these characters. Significance of scaling tests in the rest of the crosses indicated inadequacy of additive-dominance model and thus suggested the presence of gene interaction in the expression of these traits.

Hayman (1958) emphasized that it is not possible to obtain epistasis free expectations of the generation means from m , d and h of six parameter model. Hence, he concluded that the approximation to epistasis free expectations, would therefore be derived from m , d and h estimated on the assumption of no epistasis i.e. m , d and h of three parameter model of Cavalli (1952). These expectations are independent of the definition of m , d and h , and 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 possible interactions between two alleles at each of two loci. These are the interactions when there is homozygosity at both loci ($i e 'i'$), that when there is homozygosity at one locus and heterozygosity at the other ($i e 'j'$) and that when there is heterozygosity at both the loci ($i e 'i'$). Thus ' i ' is the interaction between additive effects at the two loci, ' j ' the interaction between an additive effect at one and a dominance effect at the other and ' l ' is the interaction between the dominance effects at two loci (Jinks, 1983)

The six parameter model (Hayman, 1958) was applied in almost all cases except few of them for estimation of the various gene effects in view of interactions indicated by the Cavalli's (1952) joint scaling test (Table 4 15)

The signs of effects ' d ' and ' j ' depends upon the particular parent being considered as P_1 and P_2 for computational work. If value of P_1 is larger than P_2 then the sign of ' d ' and ' j ' would be positive, and *vice-versa*. 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 deduced the net direction of the dominance and a tentative classification of the type of non-allelic interaction into complementary (' h ' and ' l ' with same signs) or duplicate (' h ' and ' l ' with opposite signs).

Five crosses viz, Kopargaon x AKM 8802 (Cross I), Kopargaon x TARM 18 (Cross II), BM 4 x BPMR 145 (Cross III), BPMR 145 x Vaibhav (Cross IV) and Vaibhav x TARM 18 (Cross IV) were studied to estimate gene actions for yield and its components

1. Days to 50% flowering

Significance of gene effects (components) was not consistent over the crosses. The significance of additive (d) and dominance (h) effects in different crosses indicated their importance in expression of days to 50% flowering. The magnitude of additive component was greater in cross II, cross III and cross V, while the dominance component was higher in cross I and cross IV. Thus, predominance of additive gene action was found controlling this character for most of the crosses studied (Table 4.16). Importance of additive component was also reported by earlier workers (Singh, 1980, Patil *et al*, 1987, Patel *et al* 1989, Kute, 1997, Khattak *et al*, 2001 and Manivannan 2002).

Duplicate type of epistasis was observed only in the cross I. The significance of additive x additive (i) type of non allelic interaction for this character revealed its importance in selection program. Kelkar (1993) and Gawande (2001) also reported similar interaction for days to 50% flowering.

2. Days to maturity

Both additive and dominance gene effects were found important in the expression of this trait in the crosses, I and V, in which almost all the epistatic interaction were found to be involved. Whereas, in cross II and cross IV dominant effect (h) and in cross III additive effect (d) were found significant, suggesting their role in the expression of this. However, the predominance of non-additive (dominant component) gene action was appeared in almost all the crosses under study, which confirms the earlier findings (Deshmukh and Manjare, 1980, Ahuja, 1980, Mansuria, 1991, Hegde *et al* 1994, Kelkar, 1994 and Gawande, 2001).

The estimates of additive x additive (i) component of epistasis was most consistent in the crosses, which is fixable and could be utilized in selection program, which was also reported earlier by Kelkar (1993) and Gawande (2001). Duplicate type of epistasis was observed for this trait in all the crosses except cross III.

3. Plant height

The significance of additive and dominance components in almost all the crosses indicated their equal importance in the inheritance of plant height as well as that these genes responsible to plant height were partially dispersed. The relative magnitude of dominance component *i.e.* non-additive gene action was greater than respective additive component, in general, to suggest its important role in the expression of this character. This predominance of dominance gene action is in accordance with the earlier reports of Ahuja (1980), Reddy *et al* (1982), Yadav and Rao (1996), Patel *et al* (1988), Natarajan *et al* (1989), Kute (1997), Sawale (1999), Gawande (2001) and Khattak *et al* (2002). Duplicate type of non allelic interaction was noticed in the crosses, II and IV, while it was complementary type only in BM 4 x BPMR 145 (cross III) for plant height.

4. Primary branches/ plant

Most of the crosses except the fifth one, Vaibhav x TARM 18 were found with significant dominance major gene effect proving its importance in the expression of this trait, where as significant estimates of 'i', 'j' and 'l' in these crosses also demonstrated their role for the same. In the crosses II, III and IV the dominance component, while in cross V additive component was appeared to be predominant. However, the relative magnitude for non-additive gene action was greater across the crosses, where the duplicate epistatic interaction was prevailed, which confirms the earlier reports (Mansuria, 1991, Kelkar, 1993, Hegde *et al*, 1994, Singh and Singh, 1996, Kute, 1997, Sawale, 1999, Gawande, 2001, Khattak *et al*, 2002 and Manivannan, 2002).

5. Pod clusters/ plant

As per model of Cavalli (1952), additive (d) and dominance (h) effects were predominant in cross IV and V, respectively, while significance of gene effects varied in the model of Hayman (1958) for first three crosses, where they found equally important, in the expression of pod clusters/ plant. Only cross III, BM 4 x BMR 145 revealed duplicate epistasis for this character. Wilson *et al* (1985), Thimmappa (1987), Saxena and Sharma (1992) and Khattak (2001) reported equal importance of both additive and non-additive gene action for the same.

6. Pods/ plant

The relative magnitude of dominance component was found greater than additive in the first three crosses *via* Hayman's (1958) model. The preponderance of additive (d) and additive x additive (i) gene actions with duplicate type of epistasis revealed their potential in controlling this character, which supports the findings of Patil *et al* (1987) and Khattak *et al* (2002). The fixable, additive x additive (i) component could be exploited through selection programme.

7. Seeds/ pod

Significance of all the components varied across the crosses as per Hayman's (1958) model, where dominance component was found with relatively higher magnitude than additive in all the crosses except cross V, which had only significant component of additive (d) gene effect (Table 4.16). Thus, these significant component observed in respective crosses indicates their importance in governing the trait, seeds/ pod. However, the dominance (h) and dominance x dominance (l) interaction *i.e.* non-additive gene action was appeared to be predominant in the expression of this character as has been reported by earlier workers (Godhani, 1978, Wilson, 1985, Kelkar, 1993, Hegde *et al*, 1994, Kute, 1997, Sawale, 1999 and Gawande, 2001). Duplicate kind of epistatic interaction was prevailed in most of the crosses for seeds/pod.

8. 100 seed weight

The additive component was found to be important in cross II and III, while in cross I, IV and V, the dominance component was found important suggesting their equal importance in expression of 100 seed weight. However, in most of the crosses non-additive gene action appeared to be predominant, wherein the duplicate type of non-allelic interaction was obtained for this trait. Earlier workers Kelkar (1993), Hegde *et al* (1994), Kute (1997), Sawale (1999) and Gawande (2001) also revealed the importance of non-additive component for this trait.

9. Seed yield/ plant

Gene effects for seed yield/ plant were found most consistent among crosses, though differed for significance of additive components in cross II and V (Table 4.16). Dominance (h) component was much higher as compared to additive (d) component in all the crosses studied. While among digenic interactions dominance x dominance (l) followed by additive x additive (i) gene interactions were predominated. Duplicate type of epistasis was detected for this trait for all the crosses. This revealed the complexity of gene action underlying the ultimate trait of seed yield/ plant. Gamble (1962) has rightly pointed out that where the inheritance of quantitative traits becomes more complex, the contribution of dominance gene effect to the inheritance becomes greater.

Significance of additive x additive (i) component in all the crosses suggests its importance in the control of this trait, which can be well exploited through selection programme as it contributes for fixing genes in subsequent generations. These findings are in accordance with earlier reports of Deshmukh and Manjare (1980), Mansuria (1991), Hegde *et al* (1994), Rao and Naggur (1979), Singh and Singh (1996), Ram (1997), Kute (1997), Sawale (1999) and Gawande (2001).

10. Protein

The major gene effect, additive (d) was found significant in cross III and IV. While dominance gene effect (h) was significant in all the crosses, except cross III, indicating their importance in controlling the protein content of the mungbean seed (Table 4.17). Predominance of dominant component was observed in all the crosses under study. Thus, non-additive gene action was found to govern the seed protein in present investigation with duplicate type of epistatic interaction in two crosses (CI and CV).

For improvement in protein content, the selection method can be advocated as the significance of 'i' type of interaction appeared in the crosses. Similar conclusions were made by Malhotra (1979), Wilson *et al* (1985), Tejindar and Singh (1974) and Sawale (1959).

11. Tryptophan

The significance of additive and dominance components in all the crosses indicates their importance in expression of this amino acid content. The magnitude of dominant component was observed greater than of additive in all the crosses proved its predominance in the control of this trait, which suggested non-additive gene action in the inheritance of tryptophan in mungbean.

Duplicate epistatic interaction for all the crosses, except the fourth cross BMR 145 x Vaibhav, where complementary epistasis was observed for this character.

12. Methionine

Like in tryptophan, methionine was also found to be under control of non-additive (dominance 'h' and dominance x dominance 'l') gene action. However, other components (d, i, j and l) were also appeared to be important in the expression of this trait. The varied significant

estimates of components indicated that the genes responsible for the inheritance of methionine content in mungbean seed were partially dispersed in its genome. Methionine, the only limited 'Sulphur' containing amino acid in the seed can be elevated to its higher levels through efficient selection programme as the 'i' type of interaction prevailed across the crosses studied. However, Tiwari and Ramanujam (1974) also reported dominance gene action to control this trait in their combining ability study.

Thus, importance of additive, dominance and interaction effects with preponderance of later two as well as existence of duplicate type of epistasis in two crosses was revealed through generation mean analysis of this trait. Improvement of such characters may be expected through standard genetic procedures that first exploit additive gene effects.

On the basis of the limited material and number of generations used in this study, the additive, dominance and epistatic gene effects were found to contribute significantly for the inheritance of various characters. However, out of a total sixty cross-character combinations, none of the gene effects (except mean μ average effects) were significant for all the five crosses. This could be due to insufficient genetic diversity among the parental lines or sampling error.

The dominance effects, whether significant or not, exceeded the corresponding additive effects in almost all the crosses for all the characters, which indicated the presence of either over dominance or complete dominance. Predominance of non-additive component in the inheritance of seed yield, its components and seed protein in mungbean has been reported by several workers. Present study thus divulged that inter-allelic interactions at digenic level played a greater role in the inheritance of seed yield. Thus, breeder should follow such breeding methods which can mop-up the genes to form superior gene constellation interacting in a favourable manner.

Though, all the components of gene action were found to be operating in controlling all the characters, including yield, dominance (h) effect comprising epistatic interaction was found predominant. For almost all the traits studied, the duplicate type of epistasis was detected in majority of crosses. Therefore, improvement may be expected by exploiting the additive genetic variance first / e by mild selection intensity in the earlier and intense in the later generations. The early generation isolates may be intermated to break undesirable linkages, so as to accumulate favourable additive genes. The diallel selective mating system (Jensen, 1970), which allows the infusion of new germplasm at various stages of breeding seems to be the alternative strategy.

Reciprocal recurrent selection probably would be useful and will exploit amount of both fixable and non-fixable effects. The use of recurrent selection method as suggested by Compton (1968) seems to be the best available method, as it will utilize all the three types of gene effects and increase inter-pollinations. However, such selection programme in case of seed proteins and its quality traits for elevating their levels can only be implemented, where adequate facilities for easy and rapid laboratory analysis is available on large scale, so that material can be screened to select the potential genotypes and to plan systematic breeding scheme in line of nutritional value enhancement in mungbean.

However, in single seed descent method, a sustainable variability present in early generations can be maintained upto complete homozygosity, as variability in F_2 generations remains very high and selection in early generations may result in drift of some valuable recombinations. Hence, pure lines may developed by adopting single seed descent method which can efficiently exploit the genetic variation arising from additive and additive type epistasis.

5.4 Genetics of Host-Parasite Interaction and Biochemical Defence to Powdery Mildew

Plants and diseases have co-evolved. Said in other words, the history of plant diseases/ pests is as old as agriculture itself. Disease incidence is essentially the outcome of a compatible interaction between two unrelated, but conflicting forces, i.e. the host (plant) and the parasite (pathogen). Notwithstanding, however, a conducive environment is equally important for occurrence of disease in the host due to causal pathogen. Thus, the knowledge of the genetics of host-parasite relationship is useful mainly to understand the genetic basis of disease resistance in crop plants.

Powdery mildew (*Erysiphe polygoni* D C) is the most devastating disease of mungbean in South East Asia (Park and Yang, 1978). Its epidemic form covers all parts of the plant with white floury patches, thereby adversely affecting the photosynthetic activity of the plants which in turn reduces yield as well as its market price, thus causing enormous economic loss to the farmers. It is therefore imperative to breed a variety resistant to this disease due to non-availability of cheap, effective and eco-friendly fungicide to control this disease when occurs in epidemic form. With this view, the present investigation highlights mainly on the genetics of resistance to powdery mildew *via* pathological as well as disease-related biochemical parameters. In a breeding programme, it is necessary to screen the breeding material by creating artificial epiphytotics, hence effective screening technique is pre-requisite as a safeguard against escape. For this reason, care was taken to maintain the disease pressure by pre-sowing of border rows of susceptible variety. The mildew severity was scored at 4 days interval from the day of first appearance of disease to a period before maturity.

Three crosses *viz.*, Kopargaon x TARM 18 (cross A), BPMR 145 x Vaibhav (cross B) and Vaibhav x TARM 18 (cross C) were investigated for this study. The mean performance of different

generations involved and nature of gene action underlying the inheritance of powdery mildew resistance through pathological as well as biochemical parameters have been discussed here under

5.4.1 Pathological parameters

The degree of variation for resistance to powdery mildew was measured and quantified by evaluating the host-parasite interaction in terms of two parameters *viz*, per cent disease index (PDI) and area under disease progress curve (AUDPC). These characters were studied to know the rate of development of disease on susceptible and resistant varieties including their various generations and thereby know the gene action involved for controlling powdery mildew resistance. Low values for these characters indicated the degree of resistance of the genotype, whereas higher values indicated the degree of susceptibility.

Although the sources for powdery mildew resistance were mostly lacking in mungbean, fortunately in 1994 the variety TARM 18 was developed jointly by BARC, Trombay and Dr PDKV, Akola through mutation breeding. This variety provides protection against powdery mildew. The variety, Vaibhav (PM 9339) was evolved through hybridization between KDM-1 x TARM 18, which also enjoying resistance of TARM 18. BPMR 145 is also resistant type with the parents, J 781 and Mugi (wild type) released in 2001. However, Kopergaon, a local selection, is highly susceptible to powdery mildew.

Table 5.5 Pedigree of the parents and their reaction to powdery mildew

Parent	Pedigree	Reaction
Kopergaon	A local selection (<i>i.e.</i> Kopergaon-1)	Susceptible
TARM 18	PDM 54 x TARM 2 (Mutant)	Resistant
BPMR 145	J 781 x Mugi	Resistant
Vaibhav	KDM 1 x TARM 18	Resistant

Despite the presence of virulent pathogen and favorable environment, differences were observed in the rate of disease development. F_2 generations of all the crosses studied, exhibited varying degree of resistance, which warranted the involvement of quantitative genes controlling the powdery mildew resistance in these genotypes. The apparent rate of disease development is a measure of the speed at which an epidemic develops. The slowing down of the rate of infection has been attributed to the level of host resistance. Van der Plank (1963) considered this rate as horizontal resistance. Uniformity in the trend noticed in the material studied for disease index (%) and area under disease progress curve (A-value), suggests relevance and their realisticness in the assessment of the uniform quality of host-parasite interaction, i.e. resistance/ susceptibility. This demonstrates the reliability of these parameters in quantification of host-parasite interactions.

From the mean performances, it revealed that the parent Kopargaon was found to be the most susceptible, while the Vaibhav and TARM 18 as resistant which evident from the scores of PDI (Fig 5.2) and AUDPC (Fig 5.3). The F_1 s of cross A, Kopargaon x TARM 18 for PDI and AUDPC and cross C, Vaibhav x TARM 18 for AUDPC remained intermediate between their respective parents for these pathological characters, which indicated that the resistance in these crosses is partially dominant over susceptibility. However, in case of cross B (PDI and AUDPC) and cross C (only PDI), the F_1 mean slightly exceeded the upper limit of their parents, which might be due to mutual cancellation of few gene effects.

Among the segregating generations, the disease scores for backcrosses and F_2 generations in BPMR 145 x Vaibhav (CB) and Vaibhav x TARM 18 (CC) were much lesser than that of Kopargaon x TARM 18 (CA). All the six generations in cross B and cross C showed almost the same speed of disease development, i.e. very slow in the beginning and medium at maturity as compared to the susceptible parent Kopargaon. In resistant parent Vaibhav and TARM 18,

Fig. 5.2 Per cent Disease Index

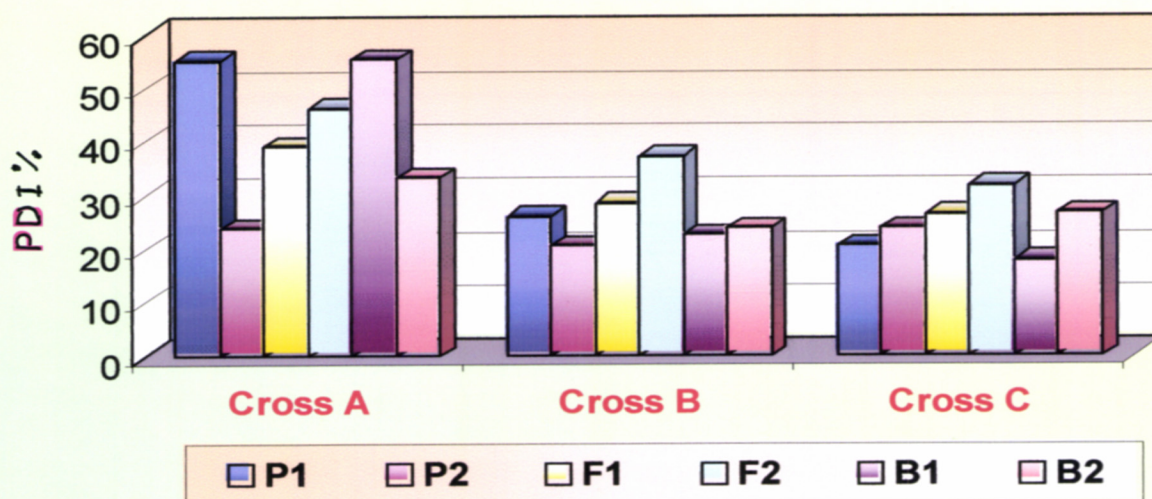
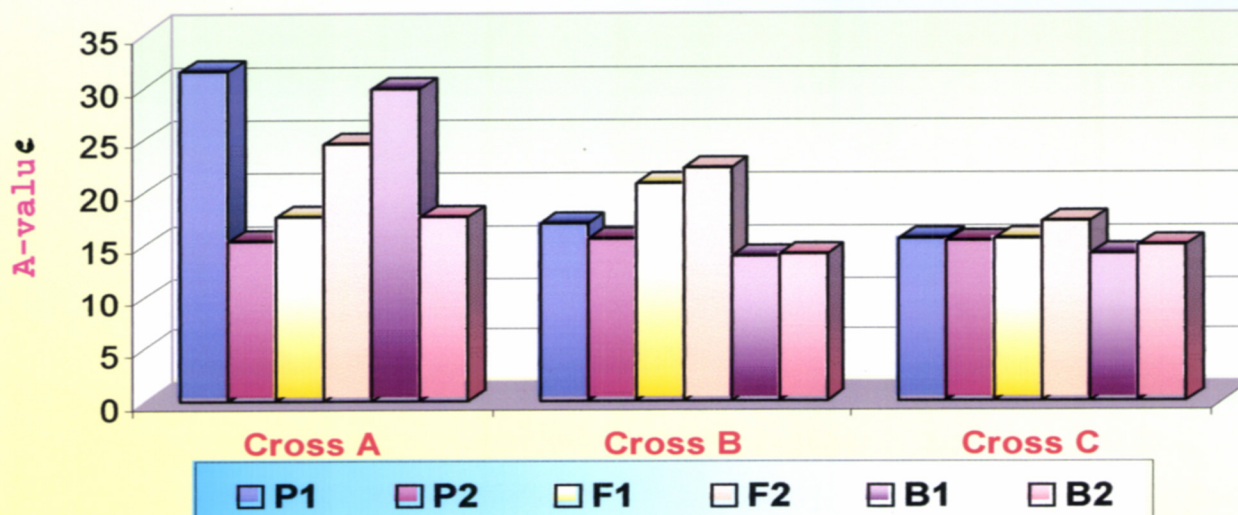


Fig. 5.3 Area Under Disease Progress Curve



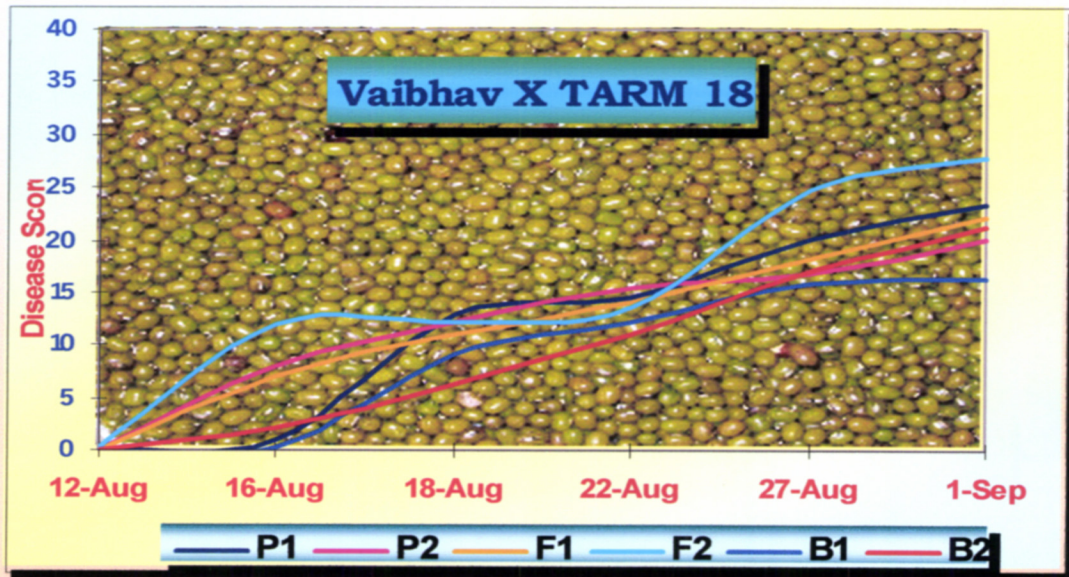
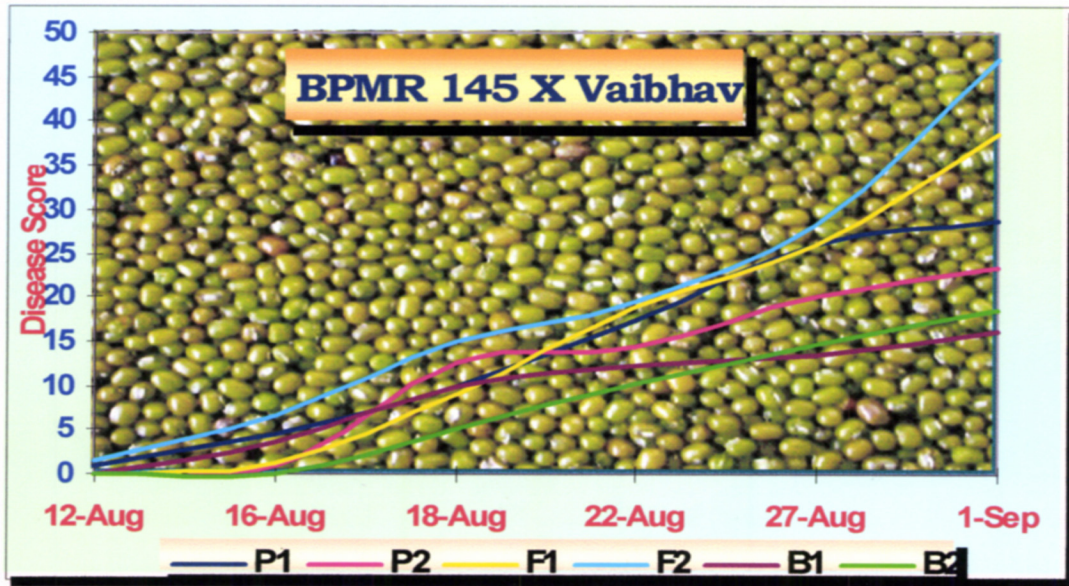
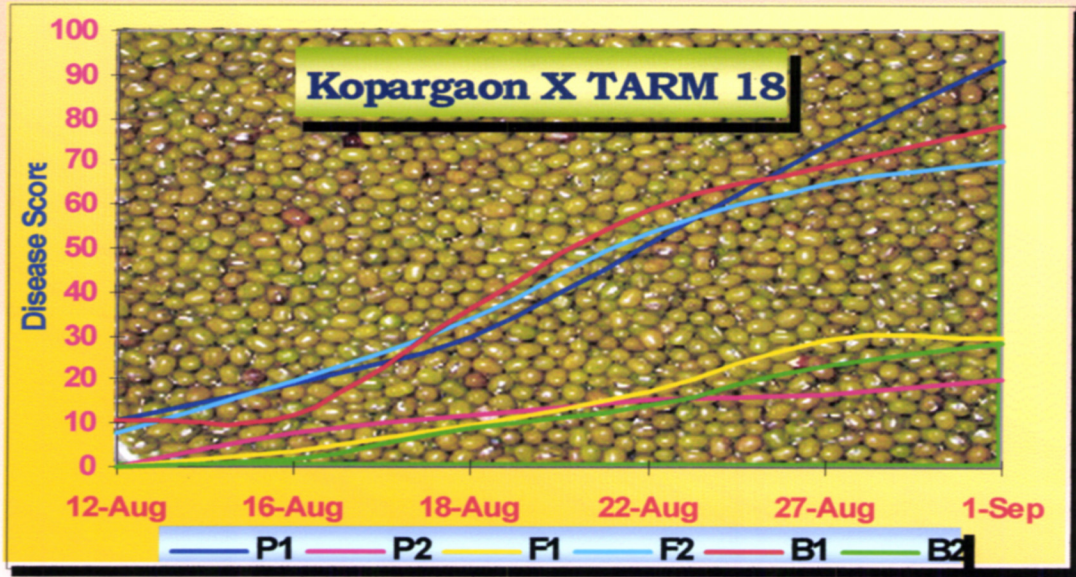


Fig. 5.4 Disease progress curves in three crosses

the rate of development of disease was much slower than the susceptible Kopargaon (Fig 5 4) Whereas, in the case of Kopargaon x TARM 18 the speed of disease development was found to be medium in the beginning and later on, it was very quick. Intermediate performance of rest of the generations (F₁, F₂, B₁ and B₂) was observed for disease development Thus, the mean performance studies revealed that AUDPC is the most reliable parameter for assessment of disease quantification in mungbean, as has been reported earlier by Kumar *et al* (1998) and Gawande and Patil (2003)

5.4.2 Gene effects for pathological characters

The complexity of powdery mildew resistance has been already reported and reviewed Hence to know the nature of gene action underlying it and formulate breeding strategies, the gene action study was undertaken and discussed here. For that instance, it was assumed that the genetics of per cent disease index and AUDPC implies the genetics of host-parasite interaction between various generations of host and *Erysiphe polygoni* D C fungus, the parasite

Except for the first cross, Kopargaon x TARM 18, the three parameter model for disease scores was found inadequate to decipher all the genetic variation Hence, epistatic six parameter model (Hayman, 1958) has to be considered as suggested from joint scaling test of Cavalli (1952) [Table 4 22].

Per cent disease index (PDI) and Area under disease progress curve (AUDPC)

Among the major gene effects, additive effect (d) in cross A, Kopargaon x TARM 18 (as per Cavalli, 1952), while dominance effect (h) in the remaining two crosses were found to be important in the expression of per cent disease index as well as AUDPC However, predominance of non-additive component (dominance 'h' and dominance x dominance 'l') much higher than its counterpart was observed in two crosses

(i.e. BPMR 145 x Vaibhav and Vaibhav x TARM 18) with duplicate type of epistasis for these traits. Thus, the presence of epistasis suggested that these traits are governed by more than one gene. These findings are in agreement with Hegde *et al.* (1996) in mungbean and Tyagi (1999) and Tyagi and Srivastava (2000) in Pea for PDI, while Gawande and Patil (2003) the only earlier report in mungbean for AUDPC, where the presence of complementary epistasis was also detected for these traits.

Thus, the presence of both additive and dominance gene actions were revealed to be operative in the inheritance of these pathological characters quantifying the mungbean (host) – powdery mildew fungus (parasite) interactions. In such a context, breeders should adopt relevant method to accumulate the resistance genes for powdery mildew resistance/ tolerance in one genotype. Though, both the gene actions were found to be involved in above three crosses for these characters, non-additive based interactions were most predominant with duplicate epistatic interactions. Hence, selection may be delayed in case of BPMR 145 x Vaibhav and Vaibhav x TARM 18, while selecting for resistant plant. However, improvement may be expected by exploiting the additive genetic variances first, and at the same time retaining the non-additive genetic variances in the population. Such system will guarantee the full utilization of both the components of gene action and will eventually lead to fixation of the resistance at the desired level. Simple selection method like single seed descent may be opted to handle the segregating generations with efficiency.

5.4.3 Pathogenesis-related biochemical characters

The obligate nature of *Erysiphe polygoni* D.C. makes the possibility of culturing it artificially a distant probability. Breeders have to depend on natural outbreaks of the pathogens. Although field/ green house/ laboratory screening methods are in use to test resistance and susceptibility of the host plant to the disease, these methods are at mercy of several environmental factors and availability of labour, besides being

time consuming and expensive. In this regard to select resistant plants and avoid the chances of escapes, it would be therefore, worthwhile to assay the resistance to powdery mildew in terms of biochemical genetic parameters, which are less influenced by the environment and strongly associated with the pathogenesis/ disease scores

Disease resistance or susceptibility of a variety is a manifestation of series of physiological and biochemical responses of the host to the pathogen (Mahadevan, 1982). Considerable information has arisen from the use of 'elicitors' that activate plant defence genes and mimic the 'gene for gene response' in being able to induce a response only in host cultivars on which that pathogen race is avirulent. Elicitor activity once defined mainly in the context of a bioassay leading to phytoalexin accumulation (Bowles, 1990). The induction of enzymes involved in the synthesis of these antibiotics and the genes encoding these enzymes, has provided a useful system for defining the sequence of events that lead from initial recognition to transcription of the inducible genes. From these studies, it has become apparent that the plant system may possess pre as well as post formed chemical inhibitors to restrict the invading pathogen and prevent the infection (Ayers *et al*., 1985, Creasy, 1985, Sen, 1988 and Vidyasekaran, 1988)

5.4.4 Enzymic associations with powdery mildew resistance

In the present investigation, chitinase (EC 3.2.1.14), β -1, 3-glucanase (EC 3.2.1.39) and polyphenol oxidase (PPO) (EC 1.10.3.1) activities were assayed at pre and post infection stages, as a property of resistance based on several studies done to prove their roles in the plant defence. Thus plant contains the range of enzymes known to perform an array of cellular mechanisms to defend themselves against invading pathogens. Such mechanism culminate in a number of physical and biochemical changes, including lignification and suberization of plant cell wall, biosynthesis and accumulation of secondary metabolites. Such as phytoalexins and *de novo* synthesis of pathogenesis-related (PR) proteins with antifungal properties.

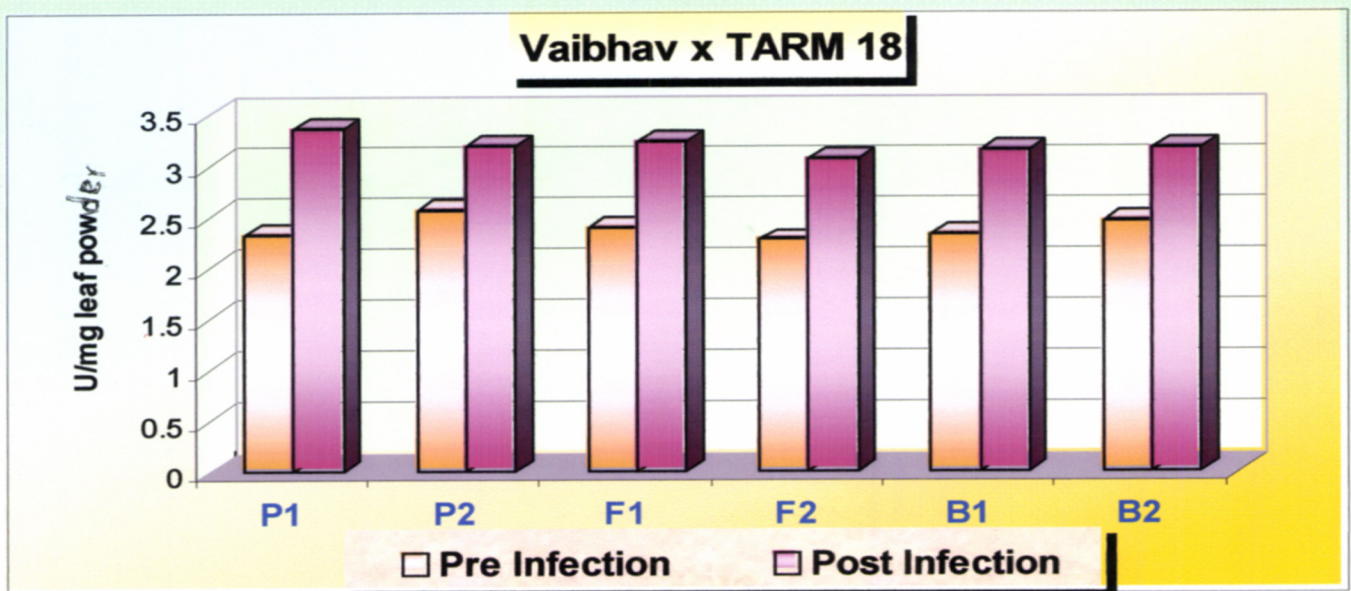
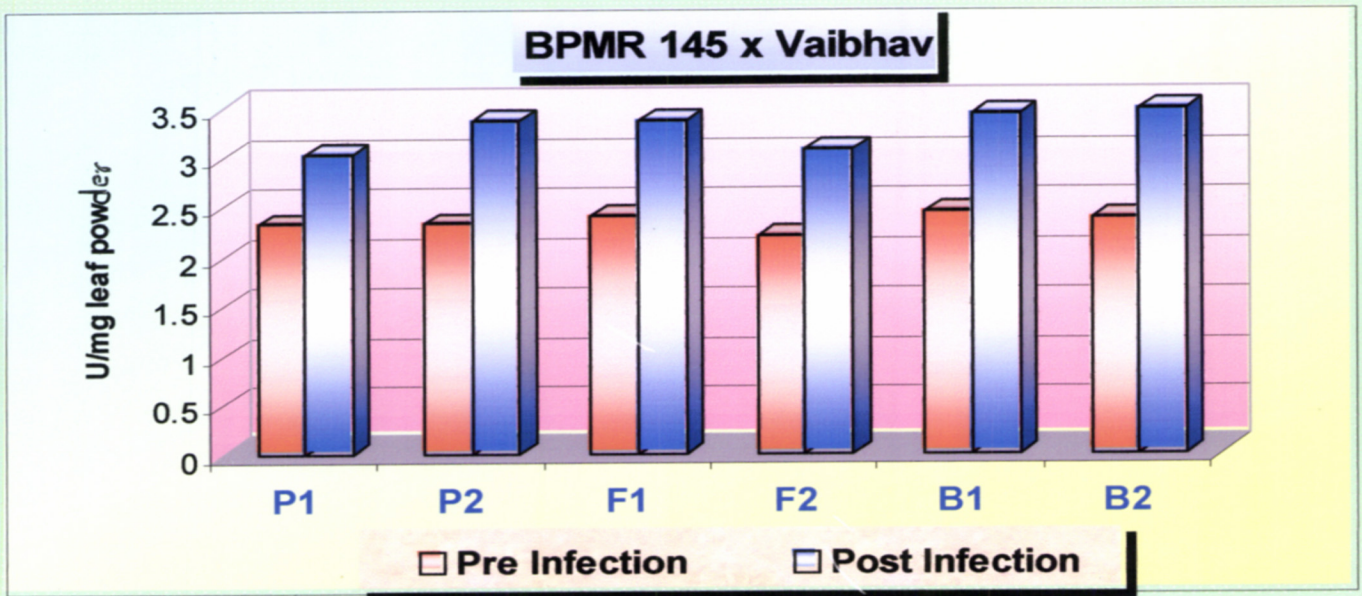
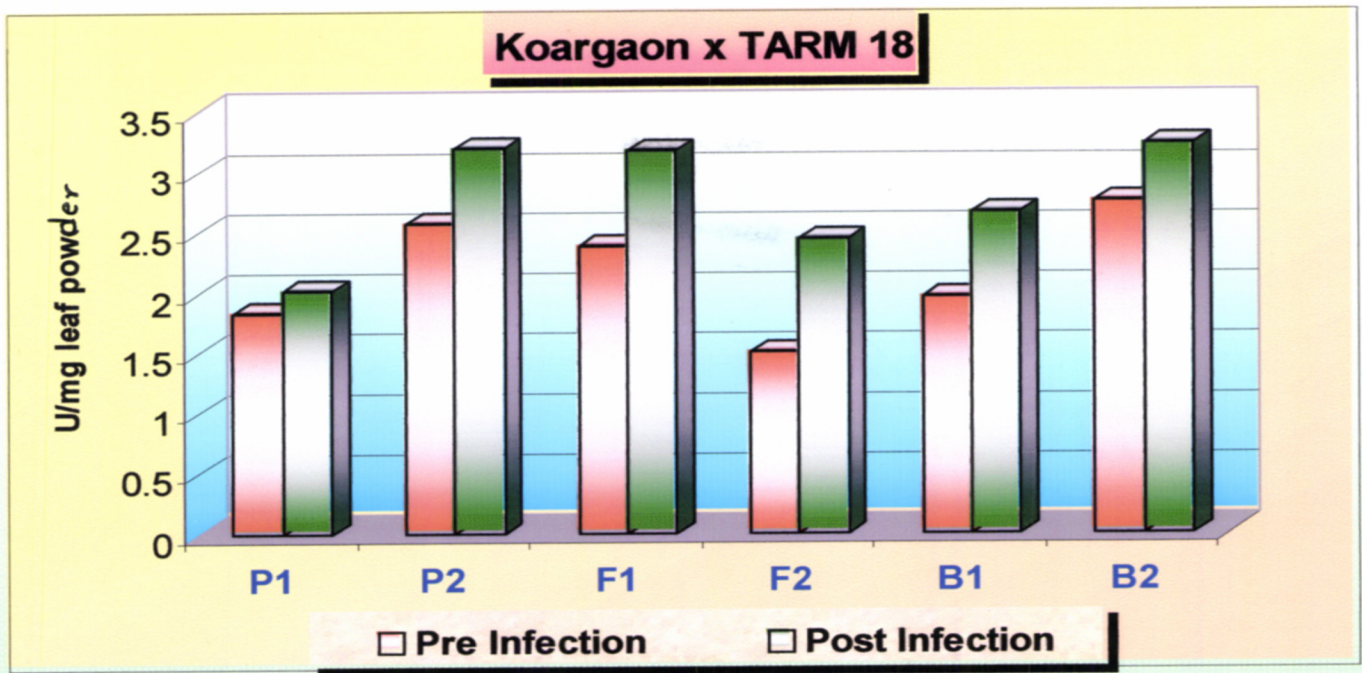
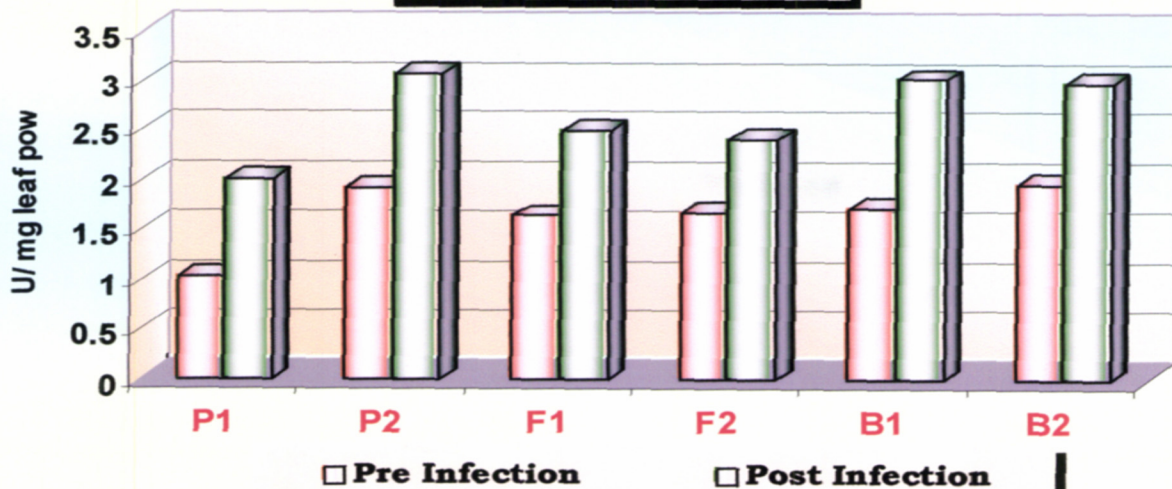
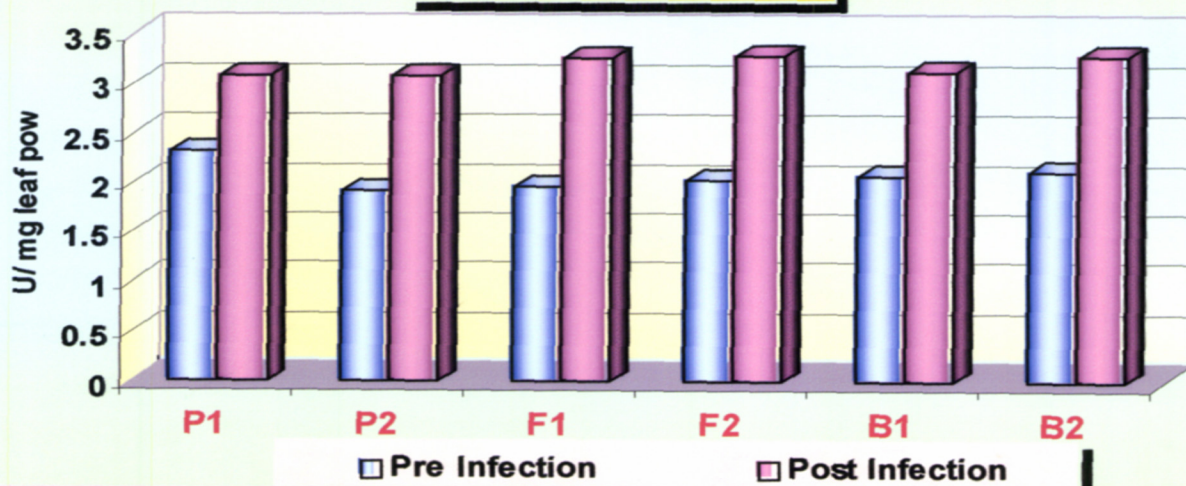


Fig. 5.5 Pre and Post infection activity of Chitinase

Kopargaon x TARM 18



BPMR 145 x Vaibhav



Vaibhav x TARM 18

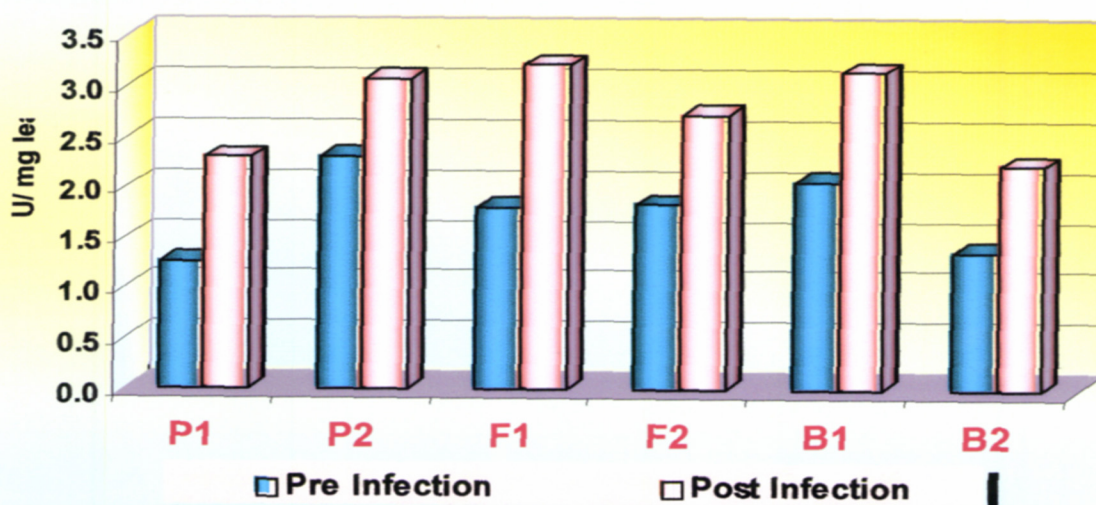


Fig. 5.6 Pre and Post infection activities of B-1, 3-glucanase

PR proteins, like chitinases (PR-3) and β -1, 3-glucanases, (PR-2) have been studied in several systems with respect to physical properties, relationship to the corresponding mRNAs and cDNAs and gene activation following pathogen infection or elicitor treatment. Role of these enzymes in host-pathogen interaction has been reviewed and discussed earlier.

Though, there was increase in chitinase activity also in susceptible genotypes after infection, however, the degree of increase in activity was not as much as recorded in resistant genotypes. Chitinase activity in F_1 s of the crosses under study was found intermediate to their respective parents, while B_2 s of cross A and B exceeded their resistant male parents, at both the stages of infection. The performance of F_2 s at both the levels was, in general to be less than their respective low mean parents (Fig 5.5). This parameter was found negatively associated with disease score at both pre and post infection, which reveals its role in powdery mildew resistance (Table 5.6 and 5.7).

There were clear differences in the β -1, 3-glucanase activity in resistant and susceptible genotypes before as well as after infection. Also, drastic changes in the degree of increase in resistance genotypes were observed as compared to susceptible genotypes after infection may be due to activation of resistant gene(s) (Fig 5.6). Most of the generations mean remained intermediate between their respective parental values except in the cross C (Vaibhav x TARM 18), where all of them (F_1 , F_2 , B_1 and B_2) found beyond the upper limit of the parent Vaibhav at both the stages of infection. Similar observations were noticed in various host-parasite interactions (Jootfen *et al*, 1989, Wyatt *et al*, 1991, Alexander *et al*, 1993 and Jongedijk *et al*, 1995). PPO activity was negatively correlated with all other parameters at both the infection stages showed its strong association with powdery mildew resistance including the AUDPC (Table 5.6 and 5.7),

Polyphenol oxidase (PPO) catalyzes the oxidation of many mono- and di-phenols as well as aromatic amines to the highly toxic quinones, which are positively and significantly associated with disease resistance (Rubin and Artisikhovskaya, 1963 and Misagi, 1982). PPO also

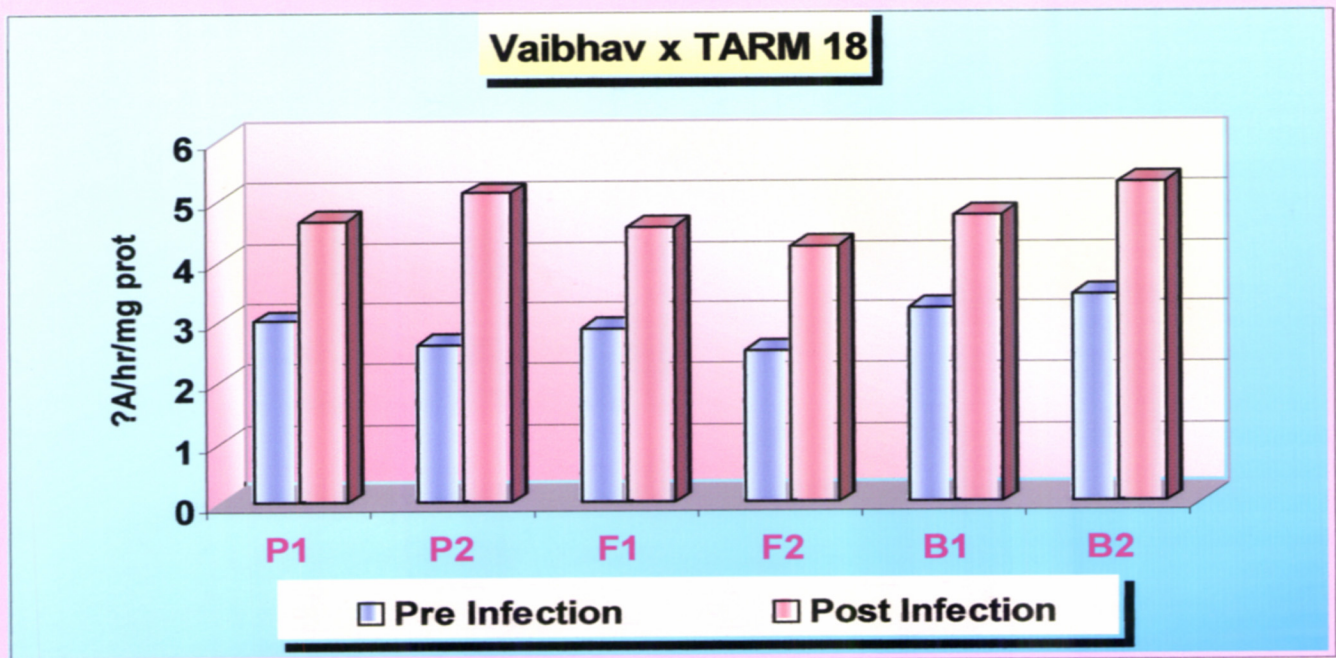
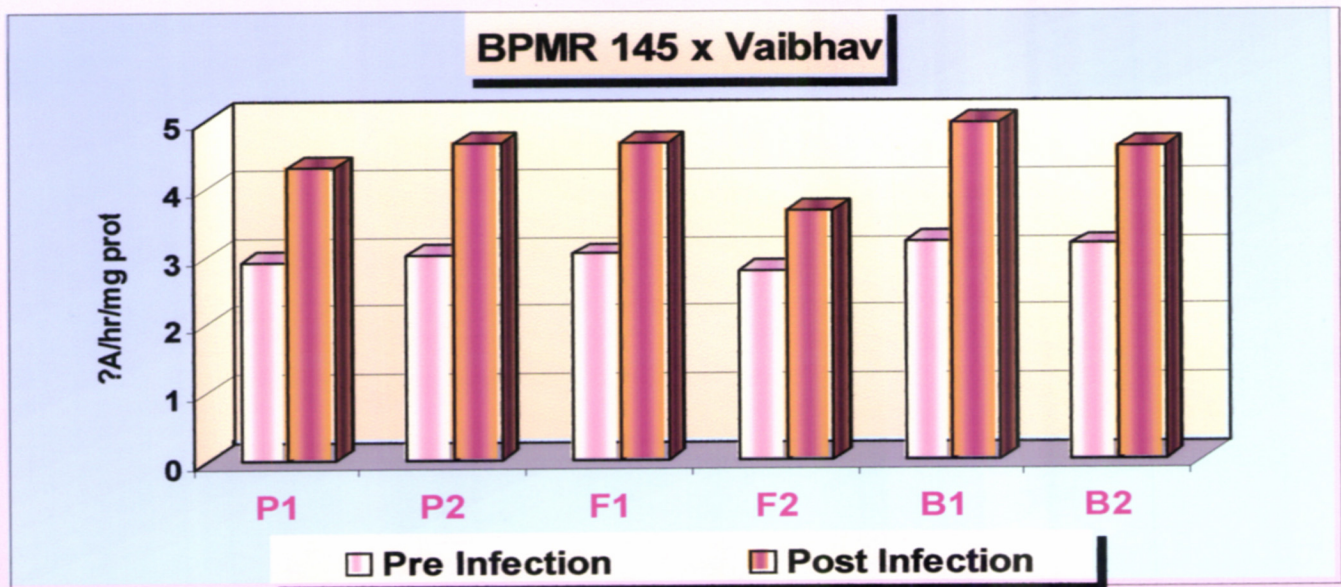
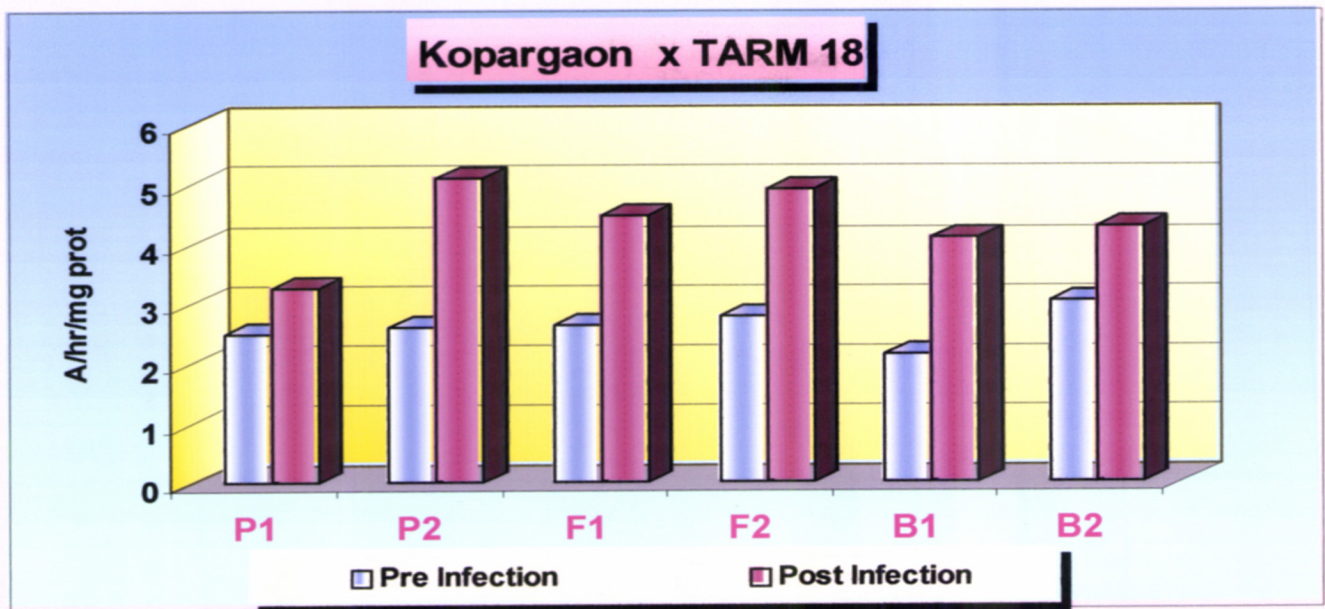
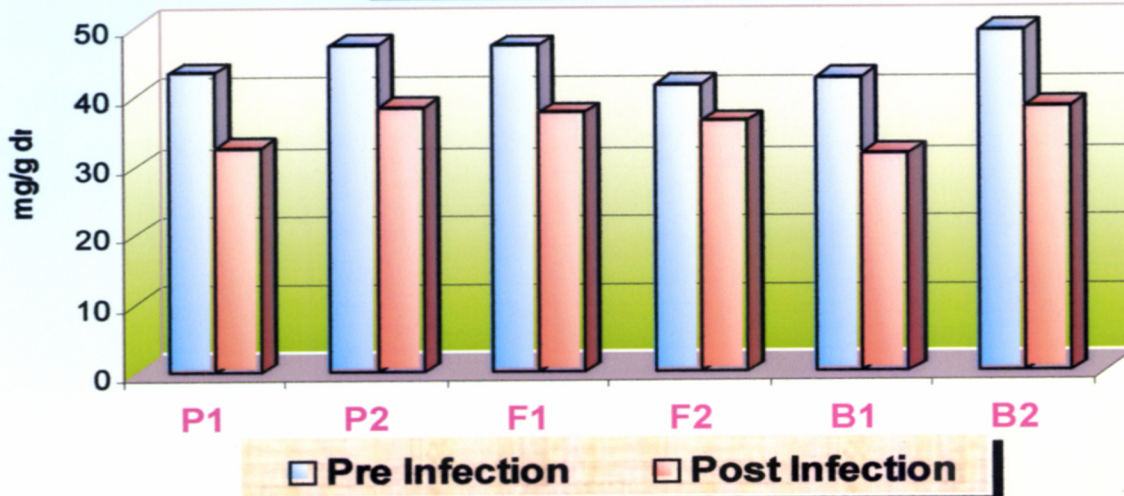
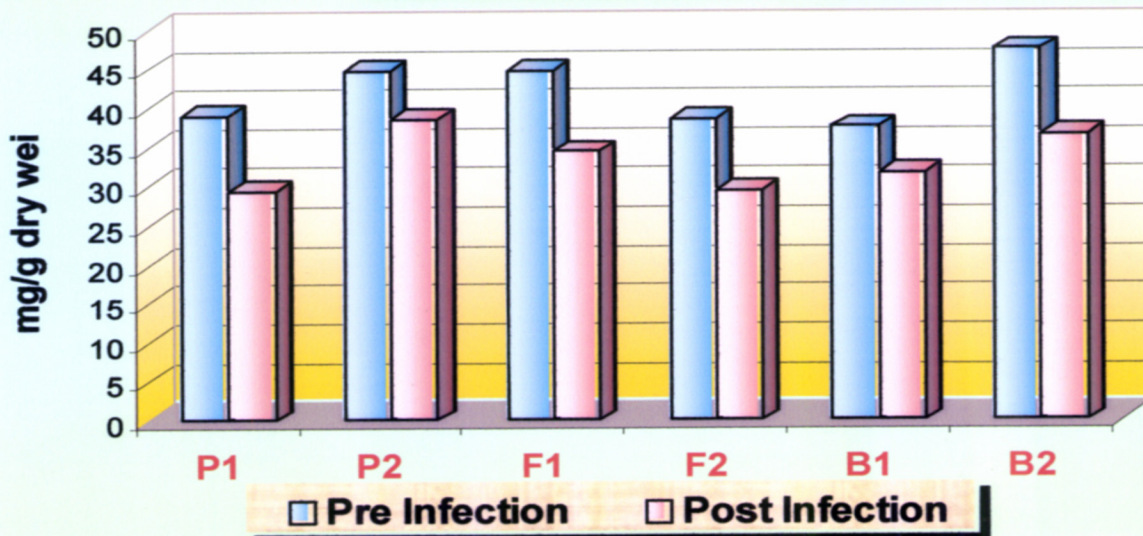


Fig. 5.7 Pre and Post infection activities of Polyphenol oxidase

Kopargaon x TARM 18



BPMR 145 x Vaibhav



Vaibhav x TARM 18

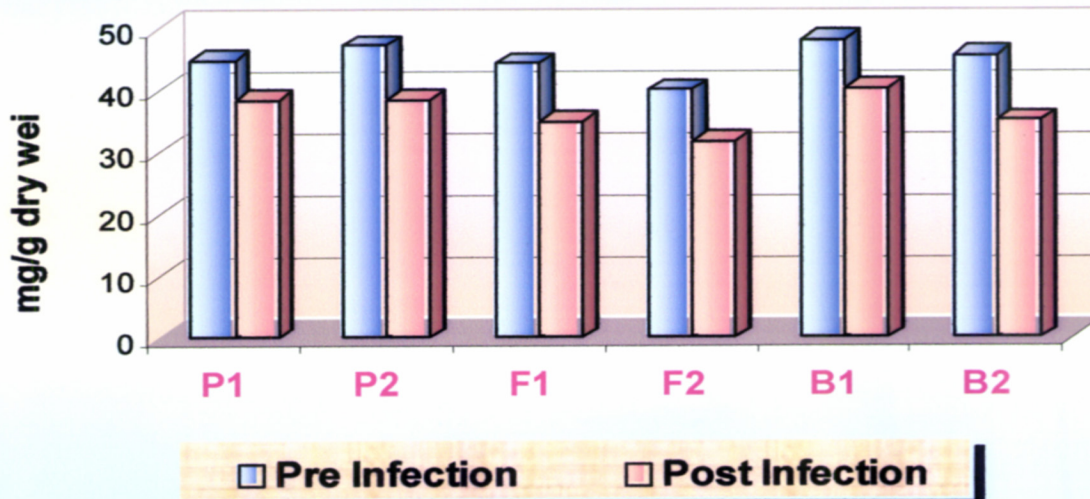


Fig. 5.8 Potash content at Pre and Post infection

gets accumulated in response to infection (Webb, 1966, Goodman, *et al*, 1967, Pollock and Drysdale, 1976) and it has role in lignification (Ride, 1975) Further, the levels of such enzymes were reported higher in resistant plant as compared to susceptible (Gowda *et al*, 1989, Kalia and Sharma, 1988, Guleria *et al*, 1998) Like that of earlier enzymes assayed, polyphenol oxidase activity was also observed to be more in the resistance lines and negatively associated with disease score at both the stages of infection, which confirms the results of Gawande *et al* (2003) Although, the F₁s activity was skewed towards their respective parents with high means, the performance of generation B₂ recorded greater than of parents in all the three crosses (Fig 5 7) As reviewed and discussed earlier, PPO activity also been found associated with disease resistance in several plant-pathogen systems

In case of the potash, the present investigation revealed that resistant genotypes contained higher levels of concentration than susceptible (Fig 5 8) However, the levels of potash decreased after infection in all the genotypes, which confirms the results of Rath *et al* (1988) This higher level of potash in resistant lines than of susceptible usually imparts resistance by cell wall thickening and non-accumulation of sugars and unused nitrogen (Anonymous, 2000) The F₁s performances were within the limits of their parents mean values for all the crosses Similar trend was not detected in B₁s and B₂s performance for the potash content The potash content was also observed negatively correlated with the disease score at pre as well as post infection (Table 5 6 and 5 7).

Thus, as far as the segregating generations are concern, the performance of all these biochemicals were not consistent that may be due to presence of large number of recombinant segregants or due to environmental effects Most of the F₁s were found between the limits of their parents in all the crosses, however, certain genotypes performed better or worse than their respective parents before and/or after infection, which may be due to heterotic effect in F₁s There is possibility of the synergism among these enzymes as evident from the positive significant correlations between them at pre and post infection

Table 5.6 Simple correlations between AUDPC and biochemical parameters (Pre infection)

Parameters	Chitinase activity	β -1, 3-glucanase activity	Polyphenol oxidase activity	Potash
AUDPC	-0.571**	-0.495**	-0.522**	-0.281*
Chitinase	--	0.278*	0.349**	0.311*
β -1, 3-glucanase activity	--	--	0.282*	0.168
Polyphenol oxidase activity	--	--	--	0.125

Table 5.7 Simple correlations between AUDPC and biochemical parameters (Post infection)

Parameters	Chitinase activity	β -1, 3-glucanase activity	Polyphenol oxidase activity	Potash
AUDPC	-0.694**	-0.433**	-0.484**	-0.332*
Chitinase	--	0.421**	0.408**	0.210
β -1, 3-glucanase activity	--	--	0.331*	0.195
Polyphenol oxidase activity	--	--	--	0.440**

*, ** Significant at 5% and 1%, respectively

5.4.5 Gene effects for biochemical characters

The scaling tests indicated the inadequacy of the additive-dominance model for the biochemical traits in all three crosses studied (Table 4 21) Hence, only the epistatic model was appropriate for these four biochemical parameters (Table 4 23) The nature and magnitude of the gene actions involved therein are discussed in this section

1. Chitinase activity

The additive major gene effect (d) was found important only in first cross, Kopargaon x TARM 18, while the dominance gene effect (h) was found operative in all the crosses at both the pre and post infection this, non allelic interactions were observed involving in all the crosses at both the stages except cross C, Vaibhav x TARM 18 after infection this may be assigned to the large sampling error for chitinase activity in this case The relative magnitude of dominance components was greater across the crosses, which noted the predominance of non-additive gene action in the control of chitinase expression Duplicate type of epistasis was revealed at pre and post infection as well However, the significant estimates of additive x additive (l) non-allelic interaction for this character was also most consistent across the crosses at both the stages of infection suggested that this fixable component could be utilized in selection programme

2. β -1, 3-glucanase activity

Only additive major gene effect (d) was noticed in BPMR 145 x Vaibhav (cross B) alongwith additive x dominance (j) type of non-allelic interaction, while dominance x dominance (l) interaction operated in Vaibhav x TARM 18 (cross C) for β -1, 3-glucanase activity

before infection, although these estimated could not decipher any clue for presence of any epistasis before infection

At post infection, the predominance of dominance components in cross A, Kopargaon x TARM 18 and the additive components in other crosses, B (BPMR 145 x Vaibhav) and C (Vaibhav x TARM 18) indicated their importance in governing this character. Thus, duplicate as well as complementary type of epistasis was appeared in the cross A and B, respectively The β -1, 3-glucanase activity, is thus controlled in a complex manner by additive, dominance and epistasis as well.

Though, the enzymes chitinase and β -1, 3-glucanase are being frequently assayed at recent in regard to plant resistance research, there is no reports regarding the persual of generation mean analysis through these enzymes in mungbean or other *Vigna* species However, chitinases and β -1, 3-glucanases are reported as integral components of the response in plants to various infections and stress conditions Plants are demonstrably been transformed with chitinase genes, which have shown improved viability towards disease resistance (as reviewed earlier) These proteins could therefore be used as marker traits for identifying mungbean lines resistant to *Erysiphe polygoni* D C pathogen Further, their use in developing transgenic mungbean plants with improved resistance can be suggested Although, much is known about the classes of molecules that induce the genes for chitinases and glucanases; little has been elucidated about the biochemical pathways by which induction is facilitated (Bowles, 1990 and Tyagi *et al.*, 2001) Because this may further strengthens the viewpoint that co-ordinated induction of chitinase and β -1, 3-glucanase activity is a probable synergistic action of both the enzymes to fight against fungal diseases, which is also evident from the positive results of the present investigation

3. Polyphenol oxidase activity

Results clearly indicated the predominance of dominance components in all the cases, with significance of non allelic interactions (i, j and l) at both the stages of infection except in cross A, where additive (d) effect with non allelic interaction 'j' was found operative for polyphenol oxidase activity before infection only. Thus, this character was found under the control of non additive (dominance and dominance x dominance) gene action.

A duplicate type of epistasis was revealed in both the stages of infection by almost all the crosses, suggesting more than one gene underlying this enzyme activity and its inheritance. Thukral (1983) and Joshi (1999) found additive gene action in pearl millet, whereas Kalia and Sharma (1988) in pea and Gawande *et al* (2003) in mungbean reported additive and dominance gene effects with duplicate as well as complementary type of epistasis.

4. Potash content

Almost all the estimates of gene action components revealed their significance across the crosses at both pre and post infection stages; except in the cross B (BPMR 145 x Vaibhav) for 'd' and 'j' components at pre and post infection, respectively. Relative magnitude of dominance components in general, exceeded the corresponding additive effects in almost all the crosses at both the stages of infection. This revealed the preponderance of non-additive gene action in the expression of potash content.

The duplicate type of epistasis was observed in all the cases, and also associated with significant additive x additive (i) component offered an opportunity of improvement by selection for this character, which confirms the earlier findings of Gawande *et al* (2003).

Overall performance of all the disease-related biochemicals indicates the inconsistency over generations, crosses and so on inoculation with pathogen. The present investigations revealed that resistant genotypes possess an inherent capacity to produce high levels of enzymes related to defence. In this context, we can say that resistance reaction at biochemical level is common in both susceptible and resistant lines except that the onset of reaction is much faster in resistant lines indicating that recognition, signal transduction and activation of pathways for resistance reaction are regulated differently.

Epistatic interactions prevailed in the inheritance of all the pathogenesis-related biochemicals, yet duplicate dominant type was recognized as unique to all. Although, these enzyme activities may not be directly useful in the breeding of resistant lines, they will certainly facilitate the development of hybrids or varieties, which will be relatively stable against environmental influences.

Thus, additive as well as non-additive gene actions were prevailed with duplicate type of epistasis in governing all the various yield as well as disease-related characters studied through generation mean analysis at least in these three common crosses. The cross 'Vaibhav x TARM 18' proved to be very promising projecting its high heterosis, best general combining ability and high sca effects for seed yield, its components along with consistent duplicate type of gene action with presence of additive type component across the yield, nutritional quality traits and pathogenesis-related parameters studied. The nature of gene action in the present investigations, suggests that early generation isolates should be intermated in segregating populations in order to accumulate favourable additive genes. This system will ensure the full utilization of both additive and non-additive gene actions and will eventually lead to the fixation of the desired character(s) at desired level for a comprehensive improvement.

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SUMMARY AND CONCLUSION



SUMMARY AND CONCLUSIONS

The present investigation entitled, “**Genetical, biochemical and molecular investigations in mungbean (*Vigna radiata* (L.) Wilczek) for seed yield, its components and powdery mildew resistance**”, was designed and executed at Department of Agril Botany, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri and Agharkar Research Institute (MACS), Pune during 2000-2004 with following objectives

- 1. To estimate heterosis for grain yield and its components**
- 2. To estimate the general and specific combining ability effects for seed yield, and its components**
- 3. To study the DNA polymorphism in parental genotypes using molecular markers**
- 4. To study the genetics of seed yield, its components and nutritional quality traits through generation mean analysis**
- 5. To study genetics of host-parasite interaction and plant biochemical defence through generation mean analysis.**

The heterosis and combining ability studies involved seven diverse parents of mungbean and their 21 F_1 s derived through diallel mating system. The DNA polymorphism study was undertaken in the same above seven parental genotypes by using PCR-based molecular marker analysis. Gene action study for yield quantitative characters was based upon five of the total 21 crosses *viz*, Kopargaon x AKM 8802, Kopargaon x TARM 18, BM 4 x BPMR 145, BPMR 145 x Vaibhav and Vaibhav x TARM 18, while three crosses namely, Kopargaon x TARM 18, BPMR 145 x Vaibhav and Vaibhav x TARM 18 comprising susceptible and resistant parents to powdery mildew were chosen to work out the genetics of powdery mildew resistance.

Observations were recorded on nine quantitative traits viz , days to 50% flowering, days to maturity, plant height, primary branches/ plant, clusters/ plant, pods/ plant, seeds/ pods, 100 seed weight, seed yield/ plant alongwith three seed quality traits viz , protein, tryptophan and methionine contents. While two pathological parameters i.e. per cent disease index and area under disease progress curve and four biochemical characters viz , chitinase activity, β -1, 3-glucanase activity, polyphenol oxidase activity and potash content were estimated at both pre and post disease infection stages.

Combining ability was worked out as per Griffing (1956) and gene effects were determined using three parameter model of Cavalli (1952) and six parameter model of Hayman (1958). In molecular markers study, a dendrogram was constructed using NTSYS-PC_2.0 software based on Nei's (Nei and Li, 1979) similarity index values. The results obtained are summarized and concluded hereunder under different subheads.

6.1 Heterosis and Combining Ability

The cross combinations, BM 4 x TARM 18, Vaibhav x TARM 18, AKM 8802 x BM 4 and PM 9341 x BM 4 exhibited significant mid parent heterosis and heterobeltiosis for seed yield/ plant and some of its components. Though this study focused the scope for exploiting heterosis, but being self-pollinated crop, it can only be made use of through isolation of transgressive segregants in subsequent generations.

None of the parents was observed to be good general combiner for all the traits studied. However, the parent Vaibhav depicted high *gca* for seed yield/ plant, days to 50% flowering, plant height, primary branches/ plant and pod clusters/ plant. Another parent, TARM 18 found to be good general combiner for days to 50% flowering, days to maturity, primary branches/ plant and pods/ plant followed by BM 4, for seed yield/ plant and pods/ plants. These parents displayed good

per se performance for most of the characters suggesting scope for their use in further breeding programme

For the ultimate trait, seed yield/ plant, the best specific combinations were Vaibhav x TARM 18, PM 9341 x BM 4 x TARM 18 and PM 9341 x BPMR 145, those can be further exploited for isolating superior genotypes

Among the 21 crosses, none of the crosses exhibited significant *sca* effect for all the characters Whereas, the hybrid AKM 8802 x BM 4 evinced significant *sca* effects for five characters *viz* , seed yield/ plant, days to maturity, pods/ plant, seeds/ pod and 100 seed weight Another cross, BM 4 x TARM 18 exhibited significant *sca* effects for seed yield/ plant, days to 50% flowering, pods/ plant and 100 seed weight, while AKM 8802 x Vaibhav displayed significant *sca* effects for days to maturity, primary branches/ plant and pods/ plant Rest of the hybrids were found non-significant or significant for one or two traits These crosses involved one good general combiner parent and other poor/good general combiner, which also revealed that high x high *gca* combination was not necessarily result into high *sca* effect This might be due to internal cancellation of the genes in these parents

6.2 DNA polymorphism study using molecular markers

Molecular markers such as random amplified polymorphic DNA (RAPDs) and inter-simple sequence repeats (ISSRs) were advocated to study genetic diversity in seven elite parental mungbean varieties In general, ISSR markers were found more efficient than the RAPD markers in detecting polymorphism in these genotypes *i.e* 44 0% and 54 7% respectively

Among the 210 RAPD and 100 ISSR primers screened, 5 and 16 respectively, were observed to generate polymorphic and reproducible amplifications Variety-specific amplifications markers were produced by these different polymorphic markers can be used to

constitute a core set of primers to evaluate genetic diversity or construct genetic linkage maps in mungbean. The dendrogram, constructed on the basis of similarity index values, grouped the mungbean genotypes into the major cluster containing five cultivators (Kopargaon, PM 9341, Vaibhav, AKM 8802 and TARM 18), while two cultivars, BM 4 and TARM 18 grouped-out from this major cluster. The genetic distance based on molecular markers varied from 0.116 (between PM 9341 and Vaibhav) to 0.250 (between Vaibhav and TARM 18).

To investigate, if DNA markers are useful in predicting heterosis in mungbean, these markers-based genetic distances were correlated with the mid parent heterosis and heterobeltiosis for seed yield and its components. Although, heterosis for only two traits viz., 100 seed weight and seeds/pod were found to be significantly correlated with marker-based genetic distance, most of them were low, but positively, associated. Also certain heterotic groups can be identified on the basis of this markers heterozygosity. It is therefore, essential that specific DNA markers (positive markers) be developed in this system for an efficient and reliable estimation of genetic distance for predicting F_1 performance/heterosis in mungbean.

Thus, the RAPD and ISSR fingerprinting has been successful in detecting polymorphism (genetic diversity) among/between mungbean cultivars to provide baseline information for the management of genetic resource collection and identification.

6.3 Generation mean analysis for yield, its components and quality traits

Analysis of variance for parents, F_1 s, F_2 s, B_1 s and B_2 s exhibited significant differences for seed yield, its components and quality traits. In most of the cases, a simple additive-dominance model (Cavalli, 1952) was not adequate and hence Hayman's (1958) six parameter model has to be considered as evidenced from joint scaling test.

Present study divulged that all additive, dominance and epistatic components were found operating in the inheritance of almost all the character studied. Predominance of non-additive (dominance and dominance x dominance) gene action was prevailed in the expression of seed yield/ plant, its components and seed quality traits (protein, methionine and tryptophan content) with duplicate type of epistasis in majority of the crosses investigated. Hence, selection should be delayed until virtual homozygosity is attained to achieve the improvement in these traits. In the improvement of these characters reciprocal recurrent selection may also prove fruitful. Single seed descent method may be adopted to develop pure lines.

6.4 Genetics of host-parasite interaction and biochemicals related to powdery mildew resistance

From the pathological parameters (PDI and AUDPC) evaluated, the Vaibhav and TARM 18 demonstrated their high resistance to powdery mildew, while Kopargaon was appeared to be highly susceptible.

Additive as well as non-additive gene actions were noticed in governing the pathological parameters, per cent disease index and area under disease progress curve. However, dominance (h) and dominance x dominance (l) interactions were found much greater than the additive components in the crosses, BPMR 145 x Vaibhav and Vaibhav x TARM 18 alongwith duplicate type of epistasis.

The pathogenesis-related biochemical investigation divulged that there was strong association with disease score at both the stages of infection except for potash content after infection.

The enzyme activities (chitinase, β -1, 3-glucanase and polyphenol oxidase) and potash content were at higher levels in resistant genotypes than that of susceptible ones at both pre and post infection.

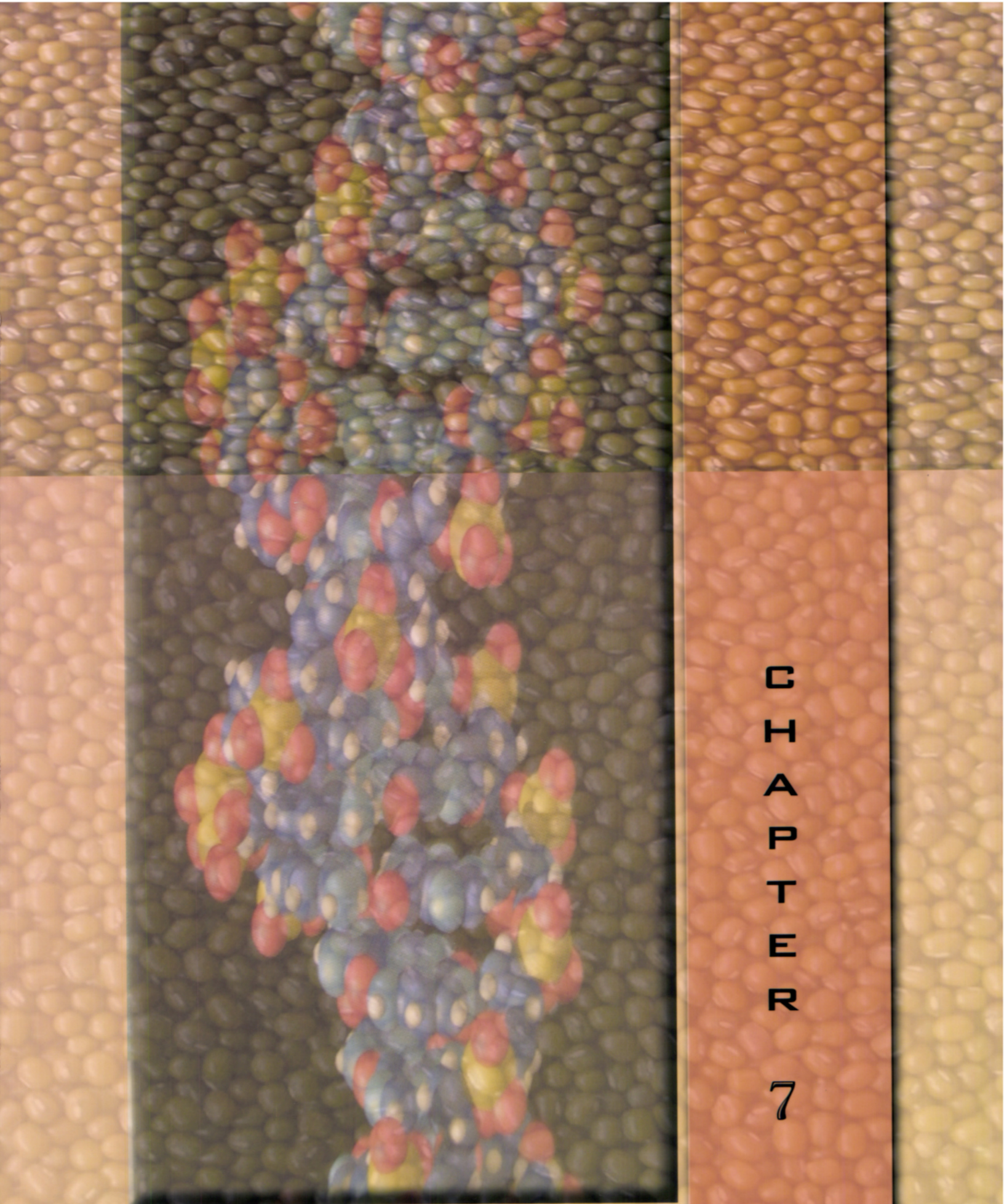
It was also evident that the enzyme levels were remarkably elevated after infection and magnitude of increase was higher in resistant genotypes than of susceptible except in case of potash content, which was found to be decreased after infection in both resistant and susceptible genotypes. However, in resistant genotypes the decrease was less than in susceptible after infection.

These biochemical characters *i.e.* enzymes and potash were prominently governed by non-additive gene action before and after infection, although both additive and non-additive gene actions appeared to be important in their expression. However, in most of the cases the non-additive (dominance and dominance x dominance) components were predominated along with duplicate type of epistasis.

Similar trend in pathological and biochemical characters indicated that the powdery mildew resistance through these traits may be improved by delaying the selection and intermating the early generations in segregating population. From the results obtained in these investigations, to isolate desirable sergeants, among five crosses, Vaibhav x TARM 18 may be expected to be the most promising.



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