CONVENTIONAL AND MOLECULAR APPROACHES IN BREEDING FOR HIGH YIELD AND DISEASE RESISTANCE IN URDBEAN (*Vigna mungo* (L.) Hepper)

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1. INTRODUCTION

Grain legumes occupy unique position in Indian agriculture. Besides forming a sustainable component of Indian agriculture, they are a major source of vegetable protein to the large masses of the country that are basically vegetarian in their food habit. Combination of cereal and legume in the Indian diet is an excellent form of balanced diet. Blackgram is one of the most important grain legumes with easily digestible protein and low flatulence content. Its ability to use atmospheric nitrogen through biological nitrogen fixation is economically sounder and environmentally acceptable. According to Vavilov (1926) blackgram has originated from Indian subcontinent. Though it is grown in different countries of South and South East Asia, India is the most important producer of blackgram. The present productivity levels of blackgram in India are very low. Efforts to genetically improve the crop are still at low ebb. Further, it has been the least studied crop among the pulses and no international system under the CGIAR has this as a mandate crop (Ghafoor *et al.*, 2000).

Among the various pulses, blackgram (*Vigna mungo* (L.) Hepper) belonging to the tribe Phaseolea of family Leguminasae, is of immense importance. It is highly prized pulse, rich in phosphoric acid. In India, it is mainly grown in the states of Madhya Pradesh, Maharashtra, Uttar Pradesh, Rajasthan, Karnataka and Bihar. In Karnataka, it is grown in an area of 145.6 thousand ha with a production of 55.0 thousand tones (Anonymous 2009). However, the yield potential of the present day cultivars is very low and is plagued with a number of diseases and pests which reduce the yields considerably. Improvement of yield forms the prime objective of breeding in any crop species and blackgram is no exception to that.

Among the different constraints that are responsible for low productivity in blackgram, susceptibility to number of biotic and abiotic stresses is the major one. Blackgram suffers from several serious diseases, of which damping off, yellow mosaic virus, powdery mildew and Cercospora leaf spot are important in Karnataka.

Among the biotic stresses, powdery mildew is an important disease on blackgram with an estimated yield loss of 20-40%. Singh *et al.*, (1980). It is caused by members of the family Erysiphaceae. The pathogen after infection drains out the host nutrients causing increased respiration and transpiration with decreased photosynthesis, plant growth and yield. The disease is more severe during cool and dry months. The causal organism is an obligate parasite and artificial inoculation is possible through infected plants. But environmental factors can influence the occurrence of disease severity and hence the progress in conventional breeding is hampered. Hence, indirect selection by means of molecular markers linked to resistant genes would be very useful in increasing the accuracy and efficiency of selection for resistance against this disease.

Like powdery mildew, Yellow Mosaic Virus, a Gemini virus belonging to Begomavirus group can cause tremendous loss up to 100 per cent under severe conditions. The virus named as mungbean yellow mosaic virus (MYMV) is known to infect a few major Leguminaceae species such as blackgram, mungbean, french bean, pigeonpea and soybean causing an annual yield loss of 300 M $ (Verma *et al*,1992). MYMV was first reported in India in Delhi on *Vigna radiata* by Nariani (1960). The virus in India is not sap transmissible but easily transmitted by whitefly vector *Bemisia tabaci*. The virus shares a very narrow host range within legumes (*Cajanus cajan*, *Vigna mungo, Vigna radiata* and *Glycine max*) causing biologically indistinguishable symptoms such as faint yellow specks/ spot on young leaves and regular blotching on tip of leaves with slight leaf malformation. In severe case, there is complete yellowing of leaves and infected plants are stunted, mature late, produce very few flowers and pods. The pods are curved and reduced in size and percentage of shriveled seeds is increased. Under artificial conditions, 100 per cent yield loss is observed.

Looking to the importance of the crop in Indian agriculture and its nutritional value, it is required that research efforts be directed for improving productivity and also genetic enhancement of host plant resistance to important diseases. As revealed by literature, the work on these aspects is very meager. The conventional breeding programmes have not been very successful in breeding for resistance particularly due to inefficient selection approaches because of the complexity involved in screening of the segregating populations. The present work therefore assumes importance in this context. It is expected to throw more

light on nature of inheritance of resistance to these diseases in blackgram. The above problem of conventional breeding for crop improvement can be solved by employing new biotechnological tools such as, use of DNA markers for mapping and tagging of the marker with desirable traits. Constructing a molecular linkage map is now routine to trace the valuable alleles in segregating populations. Once the framework maps are generated, a large number of markers derived from various techniques (RFLP, AFLP, SSR *etc*) are used to saturate the maps. Hence, DNA marker based genetic linkage would enable breeders to effectively pyramid genes for resistance to biotic and abiotic stresses in an agronomically enhanced breeding populations in a much shorter time than would be possible by conventional breeding techniques.

The knowledge of variability, direction and magnitude of association between various traits is essential for a plant breeder to boost the yield level.

Since several economic characters including yield in blackgram are polygenically controlled, it is necessary to partition the observed overall phenotypic variability into heritable (genetic) and non-heritable (environment) components with the help of genetic coefficient of variation and heritability. Estimation of expected genetic advance may help in knowing the possible gain that can be made by selection. Further, it may not be advisable to base selection for increasing the yielding ability of a genotype on yield alone. It is necessary to give importance to the yield contributing characters which are relatively more heritable.

Knowledge of association between yield and yield contributing characters would be of immense aid in plant breeding programme where simultaneous improvement in two or more characters is desired to be achieved. Here again, the knowledge of simple correlations would be helpful.

The present investigation was undertaken at College of Agriculture, University of Agricultural Sciences, Dharwad. With the objective to help in enriching the genetic map of the species and also identify markers for important diseases of blackgram. The mapping populations would also serve as good resources for mapping studies in blackgram.

In the light of the above facts, present study on **"Conventional and molecular approaches in breeding for high yield and disease resistance in Urdbean (*Vigna mungo* (L.) Hepper)"** was undertaken with the following objectives.

1. To study the inheritance of host plant resistance to powdery mildew and MYMV resistance in Urdbean.

2. Identification of parental polymorphism for SSR markers.

3. Identification of molecular markers linked to powdery mildew and MYMV resistance.

4. To estimate genetic variability for yield and other yield related traits in F_2 and F_3 populations derived from the crosses LBG-17 X TAU-1 and BDU-4 X TAU-1.

5. To study association pattern between yield and its component traits.

6. To study the pattern of transgressive segregants and identification of superior families in F_3 for seed yield and disease resistance.

2. REVIEW OF LITERATURE

Blackgram (*Vigna mungo* (L.) Hepper) is a leguminous crop belonging to the family Leguminaceae and is one of the important *kharif* pulse crops grown in India. According to Vavilov (1926), India and Central Asia are the centers of origin of blackgram. The *Vigna mungo* var. *sublobata* appears to be the most probable progenitor of *Vigna mungo* and occurs wild in India and Indonesia.

Hybridization is one of the important sources of variability in blackgram. It may help to have better reshuffling of genes, which provide an additional source of variability. However, the reports to substantiate this aspect are limited in blackgram to draw any meaningful conclusions. Therefore, the available information on this aspect is supported with similar studies in other crops.

A review of scientific literature which deals with reports on genetic variability studies, estimates of heritability and genetic advance as well as nature and extent of association between different traits, inheritance for powdery mildew and mungbean yellow mosaic virus and molecular marker studies have been reviewed in this chapter under the following headings.

2.1 Inheritance of powdery mildew and mungbean yellow mosaic virus (MYMV)

2.2 Molecular marker studies

2.3 Marker trait association

2.4 Genetic variability, heritability and genetic advance

2.5 Correlation studies

2.1 Inheritance of powdery mildew and mung bean yellow mosaic virus (MYMV)

Powdery mildew

Powdery mildew caused by the fungus *Erysiphe polygoni* D.C. is a common foliar disease of mungbean, particularly in the cool-dry season. The disease epidemic covers the upper side of leaves with white floury patches. Parts of the leaves later change into brown. Yield losses due to the disease were reported to be 20-40% at the reproductive stages (Fernandez and Shanmugasundaram, 1988), but the damage can be more serious when the epidemic starts at the seedling stages (Reddy *et al*. 1994). Since all recommended mungbean varieties are susceptible to the disease, plant breeders are interested in developing resistant varieties through both conventional and/or marker-assisted breeding. Genetics and variability of resistance to powdery mildew in mungbean has been reviewed by several researchers.

Kaughal and Singh (1989) reported single recessive gene for resistance. Where as, two dominant genes (Pm1 and Pm2) have been reported to confer resistance to powdery mildew, when both *Pm-1* and *Pm-2* were present, an R0 reaction was observed. The resistant reaction was R1 when only *Pm-1* was present and R2 in the presence of *Pm-2*. In the absence of both *Pm-1* and *Pm-2*, susceptible reactions 3, 4 and 5 were observed (Reddy *et al.,* 1994).

Gene effects were estimated for powdery mildew disease reaction in mungbean to know gene action involved for powdery mildew resistance by Gawande and Patil (2003). Six generations (P_1, P_2, F_1, F_2, B_1 and B_2), of three crosses, PIP 3-85-2×TARM 18, AKM 8802×TARM 18 and PM 9339×TARM 18 were grown for recording disease incidence. Both additive and dominance gene actions were found to be important in inheritance of powdery mildew resistance (all the three characters) including non-allelic interactions. Duplicate type of epistasis was detected for disease incidence and complementary for PDI and AUDPC in most of the crosses. The selected plants from early segregating generations may be intermated to accumulate resistance genes and to fix resistance at desired level.

Chaitieng *et al.* (2002) reported the inheritance of powdery mildew resistance in four crosses between two resistant and two susceptible lines of mungbean in 2000 and 2001. Six generations, comprising P_1, P_2, F_1, F_2, BC_1 (F_1xP_1) and BC_2 (F_1xP_2), of each cross were evaluated in a randomized complete block design with three replications under field conditions and subjected to generation mean analysis. Significant additive and dominant gene effects of similar magnitude were observed indicating that these gene effects are responsible for the inheritance of powdery mildew resistance. Epistatsis was not found in any of the crosses. Frequency distributions for powdery mildew reaction in the F_2 and BC_1 were used to analyze segregation ratios. It can be concluded that the resistance to powdery mildew in all four crosses was controlled by a single dominant gene.

Six populations, *viz.,* P_1, P_2, F_1, F_2, B1-1, and B1-2, from the three crosses were planted in a randomized block design with three replications. The disease reaction, area under disease progress curve (AUDPC), and biochemical parameters *viz.*, content of reducing sugar, non-reducing sugar, total sugar, and potash content, before and after infection, were recorded on five plants from non-segregating populations viz., parents and F_1's; ten plants from backcrosses, and twenty plants from F_2 populations. The study revealed that dominance gene action was found predominant in the inheritance of these parameters, with duplicate type of epistasis in majority of cases by Gawande and Patil (2004).

Sorojjapinun *et al.* (2005) revealed that additive gene action was found to play a major role in controlling powdery mildew resistance in mungbean. Thus, a breeding method employed for self-pollinated species, such as the pedigree, bulk, or single seed descent selection should be effective in selecting for powdery mildew resistant genotypes in the progenies derived from this cross.

Khajudparn *et al.* (2007) studied the inheritance pattern of powdery mildew in three crosses. In F_2 populations, the resistance segregated in a ratio of 15R:1S for all the crosses. These results indicate that two dominant gene confers resistance to powdery mildew in each resistant line and these resistant genes are non allelic.

The inheritance studies showed that complete resistance (RO) was controlled by two dominant genes, *Pm1*, *Pm2*. The breakdown of complete resistance (RO) into moderate resistance (R2) by race-2 (Akola) has been reported. It is assumed that the change in resistance reaction is due to a mutation in the pathogen. The investigation which was carried out with a view to screen germplasm, cultivars and mutants for identification of complete resistance (RO) sources against race-2 and to study their inheritance, identified 'Mulmarada', a local mungbean cultivar from Maharashtra state of India as a complete resistance (RO) source for race-2. The inheritance of Mulmarada's resistance (RO) was studied. The F_1 and the segregation in F_2 and F_3 showed that the complete resistance (RO) in 'Mulmarada' is controlled by a single dominant gene, which is different from the earlier identified *Pm1* and *Pm2* resistance genes. Mulmarada's resistance gene is designated as *Pm3* for PM resistance (Reddy, 2009).

Nisar and Ghafoor (2009) determined the inheritance of powdery mildew in pea caused by the *Eryisiphe pisi* in F_2 generation. Result suggested that observed value count of susceptible was 33/46 and resistant 13/46. Chi squire for the expected value 3:1 ratio was calculated (0.2) and was fit for goodness by Chi squire (p>0.65), indicating the monogenic recessive inheritance for powdery mildew disease.

Waraluk *et al.* (2009) reported genetics of the resistance to powdery mildew disease in mungbean using a recombinant inbred line (RIL) population derived from a cross between the susceptible parent "KPS1" and the resistant parent "VC6468-11-1A". Five hundred and ninety-two RILs were developed by random descending from 200 F_2 plants. The population was evaluated against the fungus in field and greenhouse conditions. The results suggested that the resistance is quantitatively inherited with high heritability and predominantly additive gene action.

Mungbean yellow mosaic virus

Mungbean yellow mosaic virus, a whitefly-transmitted geminivirus, causes one of the most serious diseases of mungbean in all of South Asia. Disease incidenceas high as 100% in farmers' fields is common in the Indian subcontinent, often resulting in considerable yield losses (Varma *et al.* 1992). Soybean, blackgram, mothbean, cowpea and a few other

leguminous species have also been reported hosts of this virus. For the past several decades research has been conducted on biological characterization of the virus, virus/vector relationship, epidemiology and disease control by chemical means and resistance breeding (Varma *et al*.1992).

Despite nearly 25 years of resistance breeding efforts and the release of tolerant lines, the disease still poses a major problem to economic production of this crop in the Indian subcontinent. This has been attributed to various factors, including increase in disease and whitefly populations, and unstable levels of resistance. Thakur, *et al.* (1977) opined that resistance was controlled by a single recessive gene for YMV reaction in green gram.

The inheritance pattern for resistance against MYMV has been worked out in mungbean and blackgram, Singh (1980) reported that susceptibility was dominant over resistance with two recessive genes required for resistance and similar reports were are also observed in greengram cowpea, soybean and pea. Solanki (1981) reported recessive gene for resistance to MYMV in blackgram. The recessive and two complimentary genes controlling resistance of YMV was reported by Shukla and Pandya (1985). Verma and Singh (2000) studied the allelic relationship of resistance genes for MYMV in blackgram *(V. mungo* (L.) Hepper). The resistant donors to MYMV- 'Pant U84' and 'UPU 2', and their F_1, F_2 and F_3 generations were inoculated artificially using an insect vector, whitefly *(Bemisia tabaci* Germ.). They concluded that two recessive genes previously reported for resistance were found to be the same in both donors Verma and Singh (1989) wherein they have reported that susceptibility was dominant over resistance with two recessive genes required for resistance and similar reports were are also observed in greengram cowpea, soybean and pea.

Govindaraj and Subramanian (1992) reported oligogenic recessive resistance to MYMV in blackgram. Basak *et al.* (2004) conducted experiment to know the inheritance pattern of MYMV in blackgram. F_1, F_2 and F_3 generations were phenotyped for MYMV-reaction by forced inoculation using viruliferous white flies. A monogenic recessive control of YMV tolerance was revealed from the F_2 segregation ratio of 3:1 susceptible: tolerant, which was confirmed by the segregation ratio of the F_3 families.

Gupta *et al.* (2005) examined the inheritance of resistance to Mungbean Yellow Mosaic Virus (MYMV) in F_1, F_2, and F_3 populations of intervarietal crosses of blackgram, disease severity on F_2 plants segregated 3:1 (resistant: susceptible; R:S) as expected for a single dominant resistant gene in all resistant x susceptible crosses. The results of F_3 analysis confirmed the presence of a dominant gene for resistance to MYMV.

Singh and Singh (2006) reported the inheritance of resistance to MYMV in cross involving three resistant and four susceptible genotypes of mungbean. Susceptible to MYMV was dominant over resistance in F_1 generation of all the crosses. Observation on disease incidence of F_2 and F_3 generation indicated that two recessive gene imparted resistance against MYMV in each cross.

The SML-668 x MLYMVPR mutant and MLYMVPR mutant x Kopergaon crosses were screened for YMV reactions in F_1, F_2 and F_3. The F_1 was susceptible and F_2 segregated in a 3:1 ratio for susceptible and mutant characters and 1:2:1 genetic ratio in the F_3 generation confirmed the F_2 segregation in green gram (Reddy, 2009)

The magnitude of dominance was higher than additive component. In case of days to maturity, branches per plant, seed yield per plant, 100 seed weight, protein content and YMV incidence at least one gene or gene group exhibited dominance. This cross can be used for direct selection in segregating generation for isolation of superior homozygous lines (Patel, 2009). Genetic control of MYMV- resistance evaluated and confirmed a monogenic recessive nature by Kundagrami *et al.* (2009).

2.2 Molecular marker studies

Peakall *et al.,* (1998) investigated the transferability of 31 soybean (*Glycine max*) simple sequence repeat (SSR) loci to wild progenitor and to other legume genera. Up to 65% of the soybean primer pairs amplified SSRs within *Glycine,* but frequently, the SSRs were short and interrupted compared with those of soybeans.

Nevertheless, 85% of the loci were polymorphic within *G. clandestina.* Cross-species amplification outside of the genus was much lower (3%–13%), with polymorphism restricted to one primer pair, AG81. AG81 amplified loci in *Glycine, Kennedia,* and *Vigna* (Phaseoleae), *Vicia* (Vicieae), *Trifolium* (Trifolieae), and *Lupinus* (Genisteae) within the Papilionoideae, and in *Albizia* within the Mimosoideae.

The F_2 map constructed by Lambrides *et al.,* 2000 from 67 individuals consisted of 110 markers (52 RFLP and 56 RAPD) that grouped into 12 linkage groups. The linked markers spanned a total map distance of 758.3 cM. A recombinant inbred (RI) population derived from the 67 F_2 individuals was used for the generation of an additional linkage map. The RI map, composed entirely of RAPD markers, consisted of 115 markers in 12 linkage groups. The linked markers spanned a total map distance of 691.7 cM. Using a framework set of RFLP markers, the F_2 map was compared with another F_2 mungbean map constructed in Minnesota. In general, the order of these markers was consistent between maps.

Forty-six microsatellite DNA markers were used to evaluate genetic similarities among 90 cowpea breeding lines developed at IITA by Cheng-Dao *et al* (2001). Twenty-seven primer pairs could amplify polymorphic single-locus microsatellites from all of these materials. Two to seven alleles per primer were detected with a polymorphic information content varying from 0.02 to 0.73. By means of only five polymorphic microsatellite primers, 88 of the 90 cowpea lines could be distinguished. A dendrogram based on the microsatellite polymorphisms generally agreed with the pedigree of the cowpea lines.

The polymorphism of the microsatellites was evaluated in a panel of 21 *P. vulgaris* genotypes made up of cultivated and wild beans from the Mesoamerican and Andean pools, and nine genotypes from four *Phaseolus* species. The number of alleles per microsatellite locus ranged from 1 to14, with an average of 6 alleles per primer pair. These results indicate that microsatellites can be valuable genetic markers for assessing genetic diversity in the *P. vulgaris*. The high levels of polymorphism of these new bean micro- satellites and their wide cross-species transportability make these new markers useful for mapping and molecular characterization of *Phaseolus* species by Gaita *et al.* (2002).

A genetic linkage map of mungbean (*Vigna radiata*, 2n = 2*x* = 22) consisting of 255 RFLP loci was developed using a recombinant inbred population of 80 individuals by Humphry *et al.* (2002). The population was derived from an intersubspecific cross between the cultivated mungbean variety 'Berken' and a wild mungbean genotype 'ACC 41' (*V. radiata* subsp. *sublobata*). The total length of the map, which comprised 13 linkage groups, spanned 737.9 cM with an average distance between markers of 3.0 cM and a maximum distance between linked markers of 15.4 cM. The mungbean map was compared to a previously published map of lablab using a common set of 65 RFLP probes. In contrast to some other comparative mapping studies among members of the Fabaceae, where a high level of chromosomal rearrangement has been observed, marker order between mungbean and lablab was found to be highly conserved. However, the two genomes have apparently accumulated a large number of duplications/deletions after they diverged.

Random amplified polymorphism DNA (RAPD) and inter simple sequence repeat (ISSR) marker were used to study the DNA polymorphism in 12 gamma ray induced morphological mutants of blackgram by Souframanien and Gopalkrishna (2004). Of the 35 random and eight ISSR primers used, 15 random and five ISSR primers detected polymorphism among the mutants. Total number of polymorphic bands varied from 1 to 3 for RAPD and from 1 to 6 for ISSR. Percent polymorphism ranged from 12.5 to 50.0 for RAPD and 12.5 to 44.4 for ISSR. DNA polymorphism data revealed by RAPD and ISSR could facilitate selection of mutants to be involved in cross breeding and genome mapping. Diversity averaged 0.71. Differentiation of all 67 genotypes each from others has been successful by using of even only 6 of SSR with gene diversity from 0.66 to 0.89. Clustering of genotypes partially reflects origin and pedigree of analysed soybean accessions.

The value of these single copy marker was evident in their ability to link with two exiting RFLP–based with a base map developed for the Mesoamerican X Andean populations, DOR364 X G19833.

To gain a better understanding of wild and weedy azuki population structures in relation to the cultigens Wang *et al.* (2004)developed simple sequence repeat (SSR) markers based on a new methodology for plant material. In the azuki bean genome, the number of

(AG)n and (AC)n motif loci per haploid genome has been estimated to be 3,500 and 2,100, respectively, indicating that (AG)n motifs are a rich source of markers. They constructed a (AG)n-SSR enriched library in azuki bean in order to obtain a comprehensive range of SSR markers efficiently. The method applied in this study resulted in a 116-fold enrichment over the non-enriched genomic library, with a high percentage (98%) of successful single-locus amplification by the primer pairs designed. Consequently, this method can be applied to construct SSR-enriched libraries suitable for large-scale sequencing

Shivaprakash *et al.* (2004) revealed the genetic diversity of blackgram landraces by amplified fragment length polymorphism markers. They have used seven primer combinations, the percent polymorphism across the samples varied from 74.5 to 93 per cent.

A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers was done by Souframanien and Gopalakrishna (2004) and revealed that ISSR markers were more efficient than the RAPD assay, as they detected 57.4% polymorphic DNA markers in *Vigna mungo* as compared to 42.7% for RAPD markers.

To make progress in genome analysis of azuki bean (*Vigna angularis*) a genetic linkage map was constructed by Han *et al.* (2005) from a backcross population of (*V. nepalensis* x *V. angularis)* x *V.*angularis consisting of 187 individuals. A total of 486 markers 205 simple sequence repeats (SSRs), 187 amplified fragment length polymorphisms (AFLPs) and 94 restriction fragment length polymorphisms (RFLPs) were mapped onto 11 linkage groups corresponding to the haploid chromosome number of azuki bean.

Chaitieng *et al.* 2006 reported the first genetic linkage map of black gram [*Vigna mungo* (L.) Hepper]. They constructed using a BC_1F_1 population consisting of 180 individuals. The BC_1F_1 population was analyzed in 61 SSR primer pairs, 56 RFLP probes, 27 AFLP loci and one morphological marker. About 148 marker loci could be assigned to the 11 linkage groups, which correspond to the haploid chromosome number of blackgram. The linkage groups cover a total of 783 cM of the blackgram genome.

A large representative collection of mungbean (*Vigna radiata* (L.) Wilczek) consisting of 415 cultivated, 189 wild and 11 intermediate accessions was analysed by using 19 SSR primers. These SSR primers showed polymorphism in wild and cultivated mungbean and were selected from those available for the related species azuki bean [*V. angularis* (Willd.) Ohwi & Ohwi]. One or more SSR primer for each linkage group (on the basis of the azuki linkage map) was analysed. In total, 309 alleles were detected and of these, about twice as many were detected in wild (257 alleles) as in cultivated accessions (138 alleles) by Chontira *et al.* 2007.

Dikshit *et al.* (2007) have done the genetic differentiation of *vigna* species using wild and cultivated species using random amplified polymormpic DNA, universal rice primer and simple sequence repeats. Comparison of three marker systems showed that SSR marker was more efficient in detecting genetic variability among all the *Vigna* species.

A total of 157 DNA polymorphisms in the collection were produced from ten primer sets when using *V. radiata* var. *sublobata* as the reference. The majority of polymorphisms detected were found in putative introns. The banding patterns varied from simple to complex as the number of DNA polymorphisms between two pooled samples increased. Numerous SNPs and INDELS ranging from 4–24 and 1–6, respectively, were detected in all fragments when pooling *V. radiata* var. *sublobata* with *V. radiata* var. *radiata*. On the other hand, when accessions of *V. radiata* var. *radiate* were mixed together and digested with CEL I relatively few SNPs and no INDELS were detected by Barkley *et al.* (2008).

A second genetic linkage map of black gram, *Vigna mungo* (L.) Hepper, was constructed by Gupta *et al.* 2008 with 428 molecular markers using an F_9 recombinant inbred population of 104 individuals. The population was derived from an inter-subspecific cross between a black gram cultivar, TU94-2, and a wild genotype, *V. mungo* var. *silvestris*. The linkage analysis at a LOD score of 5.0 distributed all 428 markers (254 AFLP, 47 SSR, 86 RAPD, and 41 ISSR) into 11 linkage groups. The map spanned a total distance of 865.1 cM with an average marker density of 2 cM. Comparison of the map with other published azuki bean and black gram maps showed high colinearity of markers, with some inversions.

Kundagrami *et al.* (2009) done a molecular analysis and revealed that defect in the NB-ARC domain of putative disease resistance R gene in the susceptible T_9, while NB-ARC domain of all the MYMV tolerant mutant lines have common functional.

Genetic diversity and relatedness were measured in 17 mungbean (*Vigna radiata* (L.) Wilczek) and 5 blackgram (*Vigna mungo* (L.) Hepper) genotypes by means of inter-simple sequence repeat (ISSR) analysis and morphological characters. Unweighted pair-group method arithmetic average (UPGMA) analysis of 19 morpho-agronomic characters showed clear separation of the genotypes into 3 major groups; cluster I containing 15 Thai cultivated mungbean varieties and breeding lines, cluster III containing 4 Thai cultivated blackgram varieties, and cluster II containing a mungbean wild relative (subspecies *sublobata*), a blackgram wild relative (subspecies *silvestris*) together with an Indian mungbean landrace by Piyada *et al.* (2010).

Ashwini *et al.* (2010) have taken each of MYMV resistant and susceptible genotypes in Mungbean and Urbean respectively for the diversity analysis using molecular markers. Twenty four RGA primers from cowpea were used to screen the twenty four genotypes. Dendrogram generated clearly indicated two big clusters at 15% similarity. All mungbean genotypes made one cluster (cluster A) except PS16, which was included in other cluster made by Urdbean genotypes (cluster B). Cluster A contained eleven genotypes while cluster B contained thirteen genotypes. Cluster A and B were further classified into two sub clusters namely A1 and A2, B1 and B2 respectively. A1 consisted of seven genotypes of which five were resistant (PANT MUNG 1, PANT MUNG 5, HUM 12, PUSA 9531, HUM 1) and two were susceptible (TARM 2, KOPERGAON 3), while A2 comprised of remaining four genotypes in which three were susceptible (TAP 7, SML 134 and SML 668), and one (AKM 8803) was resistant.

2.3 Marker trait association

Complexity in accurate screening procedure to select resistant types hindered genetic improvement through conventional approaches. The advent of molecular marker technology has provided us with avenues that help us in the efficient screening of resistant types even in the absence of disease. A DNA specific probe has been produced against the geminivirus which has caused yellow mosaic of mungbean in Thailand (Chiemsombat, 1992).

Christian *et al.* (1992) developed genomic maps for cowpea *(Vigna unguiculata* 2N=22) and mungbean *(Vigna radiata* 2N=22) based on restriction fragment length polymorphism (RFLP) markers. Using these maps, we have located major quantitative trait loci (QTLs) for seed weight in both species. Two unlinked genomic regions in cowpea contained QTLs accounting for 52 .7%o f the variation for seed weight. In mungbean there were four unlinked genomic regions accounting for 49.7%o f the variation for seed weight.

RFLP mapping of a major bruchid resistance gene in mungbean (*Vigna radiata,* L. Wilczek) was conducted by Young *et al.* (1993). They analyzed the underlying genetics, accelerate breeding, and provided a basis for map-based cloning of this gene, they have mapped the TC1966 bruchid resistance gene using restriction fragment length polymorphism (RFLP) markers. Fifty-eight F 2 progeny from a cross between TC1966 and a susceptible mungbean cultivar were analyzed with 153 RFLP markers. Resistance mapped to a single locus on linkage group VIII, approximately 3.6 cM from the nearest RFLP marker.

Mapping oligogenic resistance to powdery mildew in mungbean with RFLPs was done by Young *et al.* (1993). They have used restriction fragment length polymorphisms (RFLPs) to map genes in mungbean (*Vigna radiata)* that confer partial resistance to the powdery mildew fungus, *Erysiphe polygoni.* genotypes for 145 RFLP loci spanning 1570 centimorgans of the mungbean genome were assayed in a population of 58 F_2 plants. This population was derived from a cross between a moderately powdery mildew resistant ("VC3980A") and a susceptible ("TC1966") mungbean parent. F_3 lines derived from the F_2 plants were assayed in the field for powdery mildew response and the results were compared to the RFLP genotype data, thereby identifying loci associated with powdery mildew response. A total of three genomic regions were found to have an effect on powdery mildew response, together explaining 58 per cent of the total variation.

One QTL for texture layer on linkage group 8-9 have been identified in mungbean *(Vigna radiata,* L. Wilczek) of the cross Berken x ACC41 using RFLP and RAPD marker by Lambrides (1996) .

Pigmentation of the texture layer and green testa color have been identified on linkage group 2 in mungbean *(Vigna radiata,* L. Wilczek) of the cross Berken x ACC41 using RFLP and RAPD marker by Lambrides *et al.* (2000)

Chaitieng *et al.* 2002 used both restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) to map a new source of resistance to powdery mildew in mungbean. The RFLP loci detected by two of the cloned AFLP bands were associated with resistance and constitute a new linkage group. A major resistance quantitative trait locus was found on this linkage group that accounted for 64.9% of the variation in resistance to powdery mildew. One of the probes developed in this study has the potential to assist in breeding for powdery mildew resistance in mungbean.

A major locus conferring resistance to the causal organism of powdery mildew, *Erysiphe polygoni* D.C., in mungbean (*Vigna radiata* L. Wilczek) was identified by Humphry *et al.* 2003 using QTL analysis with a population of 147 recombinant inbred individuals. To generate a linkage map, 322 RFLP clones were tested against the two parents and 51 of these were selected to screen the mapping population. The 51 probes generated 52 mapped loci, which were used to construct a linkage map spanning 350 cM of the mungbean genome over 10 linkage groups. Using these markers, a single locus was identified that explained up to a maximum of 86% of the total variation in the resistance response to the pathogen.

Basak *et al.* (2004) developed the Yellow Mosaic Virus (YMV) resistance linked DNA marker in *Vigna mungo* from populations segregating for YMV-reaction. YMV-tolerant lines, generated from a single YMV-tolerant plant identified in the field within a large population of the susceptible cultivar T-9, were crossed with T-9, and F_1, F_2 and F_3 progenies raised. Of 24 pairs of resistance gene analog (RGA) primers screened, only one pair, RGA 1F-CG/RGA 1R, was found to be polymorphic among the parents. Selected F_2 individuals and F_3 families were genotyped with the polymorphic RGA primer pair and the polymorphism was found to be linked with YMV-reaction. To the best my knowledge, this is the first report of YMV-resistance linked DNA marker development in any crop species using segregating populations. This YMV-resistance linked marker is of potential commercial importance in resistance breeding of plants.

Miyagi *et al.* (2004) reported the construction of the first mungbean (*Vigna radiata* L. Wilczek) BAC libraries. These BAC clones were obtained from two ligations and represent an estimated 3.5 genome equivalents. This correlated well with the screening of nine random single-copy restriction fragment length polymorphism probes, which detected on average three BACs each. These mungbean clones were successfully used in the development of two PCR-based markers linked closely with a major locus conditioning bruchid (*Callosobruchus chinesis*) resistance. These markers will be invaluable in facilitating the introgression of bruchid resistance into breeding programmes as well as the further characterisation of the resistance locus.

Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata)* assessed by QTL analysis by Humphry *et al.* (2005) QTL analyses revealed four loci for hard-seededness and 11 loci for seed weight. Two of the hard-seededness loci co-localized with seed weight QTL. When seed weight was used as a covariate in the analysis of hard-seededness from the field data, two of the four hard-seeded QTL remained significant with the effect at one of these remaining unchanged.

Ghafoor *et al.* (2005) used SDS-PAGE markers for determining quantitative traits loci (QTL) in blackgram [*Vigna mungo* (L.) Hepper] germplasm, screening analysis for markers to quantitative traits revealed its significance in determining QTL in blackgram through SDS-PAGE markers.

Selvi *et al.* (2006) undertaken a study to identify RAPD marker associated with Mungbean Yellow Mosaic Virus (MYMV) resistance in mungbean *(Vigna radiata,* L. Wilczek) in the cross ML 267 X CO4. Bulked segregant analysis was employed to identify RAPD marker linked to MYMV resistance gene of ML 267. A total of 149 random decamers were surveyed, approximately 94% of these produced DNA amplification in both the parents and

bulks. 41 primers produced specific bands for resistant parent which were absent in susceptible parent. Out of 41 primers 3 primers produced specific fragments in resistant parent and resistant bulk, which were absent in the susceptible parent and bulk. Amplification of individual DNA samples out of the bulk with putative marker OPS 7_{900} only reveled polymorphism in all 8 resistant and 6 susceptible plant, indicating this marker was associated with MYMV resistance in MI 267.

QTLs were detected for seed, pod, stem and leaf-related traits using SSR marker. Most traits were controlled by between two and nine QTLs but several traits, such as pod dehiscence, were controlled by single genes. QTLs for domestication-related traits were restricted to particular regions of the azuki bean genome, especially linkage groups 1, 2, 4, 7 and 9. Linkage groups 1 and 2 had QTLs for a group of traits including pod size, germination, seed size and lower stem length. QTLs on linkage groups 7 and 9 were associated with upper stem length, maximum leaf size and pod and seed size. Pleiotropy or close linkage of genes for domestication-related traits is suggested in these regions. While some QTLs are common to azuki bean and other warm-season legumes, many are recorded for the first time in azuki bean by Isemura *et al.* (2007).

Two hundred recombinant inbred lines at the F12 generation have been developed for molecular mapping of bruchid resistance (Br) gene in TC1966 by Chen *et al.* 2007. Through bulked segregant analysis (BSA), ten randomly amplified polymorphic DNA (RAPD) markers associated with the bruchid resistance gene were successfully identified. A total of four closely linked RAPDs were cloned and transformed into sequence characterized amplified region (SCAR) and cleaved amplified polymorphism (CAP) markers. Seven CAPs developed from the identified RAPD markers showed tighter linkage with the Br gene than the original RAPD. Through transformation of RAPDs into CAPs, codominant markers for bruchid resistance were successfully obtained. Homozygous genotypes of these PCR-based markers were estimated to contribute 85% of the variance for seed damage when the insect assay was performed under favorable growth conditions for bruchid.

Quantitative trait loci (QTLs) analysis for resistance to *C. chinensis* (L.) and *C. maculatus* (F.) was conducted by Prakit Somta *et al.* 2008 using F_2 (*V. nepalensis* & *V. angularis*) and BC_1F_1 [(*V. nepalensis* & *V. angularis*) & *V. angularis*] populations derived from crosses between the bruchid resistant species *V. nepalensis* and bruchid susceptible species *V. angularis*. Based on the results from both populations seven QTLs were detected for bruchid resistance; five QTLs for resistance to *C. chinensis* and two QTLs for resistance to *C. maculatus*. The different locations found for some resistant QTL to the two bruchid species suggests different resistance mechanisms. Based on linked markers the QTL on these two linkage groups appear to be the same as previously reported in other Asian *Vigna*. However, several other QTLs were newly detected including one on LG4 that appears unrelated to seed size.

2.4 Genetic variability, heritability and genetic advance

Earlier efforts by Johannsen (1909), Nilson Ehle (1909) and East (1916) has led to the partitioning of total variability into genetic and environmental components. The classical experiment of Johannsen (1909) demonstrated that both heritable and non-heritable agencies contributed to the somatic variations in segregating populations and that variation in pure line was entirely due to environment. Nilson Ehle (1909) and East (1916) further confirmed the work of Johannsen (1909) and demonstrated how such results were obtained. Based on the study on non-segregating populations, Charles and Smith (1939), Powers (1942) and Powers *et al.* (1950) separated genetic variance from total variance by the use of estimates of environmental variance.

Estimating heritability along with genetic gain is usually more useful in predicting the resultant effect from selecting the best individual (Johnson *et al.,* 1955). The expression of a character is the result of genetic constitution of a strain and the influence of the environment on it; hence, some strains can perform well under specific environmental conditions while others may not. The environmental conditions have a significant effect on the expression of yield and other quantitative traits.

The evaluation of genotypes under different environmental conditions provide information on the relative magnitude of phenotypic and genotypic variability and the extent of genetic advance that can be made by studying the material under more than one environment has been emphasized by Comstock and Robinson (1952), Johnson *et al.* (1955). Neij and Syakudd (1957) and Athwal and Singh (1966).

The most important objective in any crop improvement programme is to increase the seed yield. Yield is the end product of action and interaction of the vital activities of the plants throughout the life cycle. Therefore, for the improvement of crop yields, an understanding of the heritable portion of the total variation for the desirable characters becomes essential. This has induced the biometricians to study variability and heritability of yield and other yield contributing components (Robinson *et al.,* 1951; Grafius, 1959 and Dewey and Lu, 1959). The major yield components in blackgram are pod number, seeds per pod and test weight. This in turn is influenced by number of other traits like plant height, number of branches, clusters *etc.* Any change in yield has to be brought from a change in one or more of these components (Grafius, 1964).

The quantitative characters are governed by large number of genes and are more influenced by the environment. The phenotype observed is not transmitted entirely to next generation. Therefore, it is necessary to know the proportion of observed variability that is heritable (Heritability). Estimating heritability along with genetic gain is usually more useful in predicting the resultant effect from selecting the best individual (Johnson *et al.,* 1955).

The review pertaining to the genetic variability, heritability and genetic advance in blackgram and greengram is reviewed for the yield and yield related traits.

Giriraj (1973) evaluated 55 varieties of mungbean and reported a wide range of genetic variability, heritability and genetic advance for plant height, total number of pods per plant, pod length, seeds per pod, 100 seed weight and seed yield per plant. Medhi *et al.* (1980) studied genetic variability and heritability for seed yield in 12 genotypes of greengram. Substantial amount of variability was observed for all the characters. In general, the difference between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were narrow for most of the characters indicating that the observed variability due to genetic factors.

Paramshivan and Rajashekharan (1980) estimated the genotypic and phenotypic contributions for the yield contributing characters in 19 varieties of greengram. A wide range was noticed for all the 11 characters studied.

Ramakrishna and Jairaj (1981) noticed high GCV and heritability associated with high genetic gain (84%) for plant height in blackgram. They also reported low genetic advance for 100 seed weight and fairly high heritability for seed yield. Shah and Patel (1981) noticed higher GCV, heritability and genetic advance for plant height, moderate heritability and genetic advance for number of clusters per plant and pods per plant while, low heritability was reported for seed yield in blackgram.

Patel and Shah (1982) in case of noticed high GCV, heritability coupled with high genetic advance for plant height. Whereas, high heritability estimates with low genetic advance was observed for number of pods per cluster, seeds per pod and 100 seed weight.

Mishra (1983) while working on variability, heritability and genetic advance in 18 varieties of blackgram having diverse origin observed that heritability estimates were high for 100 seed weight and plant height and moderate for pods per plant. Plant height, pods per plant and clusters per plant had high predicted genetic advance accompanied by high variability and moderate heritability.

In a study on heritability estimates for seven quantitative traits in greengram Thimmppa (1983) reported high heritability estimates for primary branches per plant, pods per plant, pod length and 100 seed weight, medium for plant height, clusters per plant and seed yield and low for seeds per pod.

Lieu *et al.* (1984b) observed a wide range of genetic variability for plant height, pods per plant and seed yield. While, evaluating variability for 12 characters in mungbean.

From a study on 28 F_3 progenies in blackgram Kumar and Reddy (1986) reported additive gene action for plant height, primary branches, clusters per plant and pods per plant. Pods per plant, pod length, seeds per pod, 100 seed weight and seed yield per plant recorded low to moderate heritability.

In a study by Rathnaswamy *et al.* (1986) on 24 characters limiting productivity in greengram, it was observed that variability was large for most of the characters. In a study by Lee and Lee (1987) in 30 combinations of F_2 populations of mungbean, broad sense heritability was observed to be high for plant height, number of pods per plant, 100 seed weight and seed yield. Heritability was medium for number of seeds per pod.

Ramanna and Singh (1987) revealed pronounced effect of environment for most of the characters in different genotypes of greengram. The seed yield per plant followed by pods per plant and clusters per plant showed the highest genotypic and phenotypic coefficient of variation in two seasons. In a study of 48 crosses of F_1 and F_2 Singh *et al.* (1987) reported high heritability for plant height in F_1 and F_2 and number of seeds per pod in F_2. Estimates were higher in F_2 for all traits than F_1. Estimates of genetic advance were similar to heritability in both the generations of blackgram.

Sharma and Rao (1988) reported variation for yield and yield components by analysis from F_1s, F_2s and parents of six intervarietal crosses in case of blackgram. High heritability was obtained for pod length and 100 seed weight. High heritability coupled with high genetic advance was noticed for pod length and seed yield per plant.

Iihamuddin *et al.* (1989) revealed significant differences among cultivars for all the eight quantitative characters studied in greengram. High genotypic and phenotypic variations were recorded for plant height and thousand seed weight. Further GCV and PCV were highest for yield per plant.

Reddy and Singh (1990) studied three crosses T44 x ML-5, ML-5 x LM293 and T44 x PLN15 in mungbean and reported that averages and variances were high for seed yield per plant, seeds per pod and pods per plant in F_2 generation of cross T44 x PLN15 and for pods per plant in T55 x ML-5.

Dahiya and Singh (1991) studied variability involving 61 high yielding progenies of greengram involving five selection methods. Progenies developed through selective intermating showed maximum variability and minimum for pedigree selection. Sirohi *et al.* (1994) carried out studies on genetic variability, heritability and genetic advance in 56 blackgram genotypes. The estimates of heritability and genetic advance were high for 100 seed weight, seed yield per plant and plant height.

Borah and Hazarika (1995) studied genetic variability in exotic collections of greengram and noticed high estimates of genotypic variance for plant height and number of pods per plant. High estimates of heritability along with high genetic advance and GCV were observed for plant height, number of pods per plant, seed yield per plant, number of clusters per plant and number of primary branches per plant.

Tiwari *et al.* (1996) evaluated six parents and their 15 F_2s in green gram and reported high variability in F_2s for clusters per plant, pod length and 100 seed weight and high heritability for 100 seed weight and cluster per plant. High heritability was associated with low genetic advance in greengram.

Byregowda *et al.* (1997b) evaluated 18 blackgram genotypes of diverse origin for PCV, GCV, heritability and genetic advance. Sufficient variability was recorded in the material for grain yield per plant, pods per plant, branches per plant and plant height. High heritability values associated with high genetic advance were obtained for grain yield per plant and pods per plant. High heritability in conjunction with medium genetic advance was obtained for 100 seed weight and branches per plant.

Vikas *et al.* (1998) evaluated 18 parents and their 45 F_1s for 12 seed yield related traits in greengram and reported high components of genetic variation, heritability and genetic advance for plant height, number of clusters per plant, days to 50 per cent flowering, number of pods per plant and seed yield.

Sharma (1999) evaluated nine parents and their 15 F_2 progenies and reported high heritability for all the traits studied. High heritability and genetic advance were observed for pods per plant, seeds per plant, 100 seed weight and seed yield in greengram.

Appreciable amount of heritability and genetic advance for seed yield and its associates was reported from a study on 22 mungbean genotypes by Khairnar *et al.* (2003) indicating presence of gene action. Reddy *et al.* (2004) who evaluated 69 elite and exotic mungbean for variability reported formation of 11 clusters and branches per plant was found to have maximum variation followed by branches per plant, pods/plant, number of clusters per plant, and grain weight per plant.

Ghafoor and Ahmad (2005) evaluated agronomic traits of 111 genotypes of black gram *Vigna mungo* (L.) Hepper, mainly from Pakistan, to determine the extent of genetic diversity. High genetic variance was observed for days to flowering, days to maturity, number of branches per plant, number of pods per plant, biomass per plant, grain yield per plant and harvest index, whereas low genetic variance was observed for pod length, seeds per pod and 100 seed weight in both years studied.

Seven blackgram genotypes were tested for 10 physical characters which determine seed quality. There were significant variations between genotypes for 100 seed weight and volume before and after soaking, hydration capacity, swelling capacity, hydration index and swelling index. Variation of soaked seed density was low Ghosh and Panda (2006).

Pervin *et al.* (2007) observed a wide range of variability in blackgram for five quantitative traits. Heritability in the broad sense with genetic advance expressed as percentage of mean was comparatively low. Study was conducted involving 40 genotypes of blackgram for genetic variability of seed yield and its component traits. The estimates of PCV values were higher than GCV. High estimates of GCV were observed for crude fiber content and hundred seed weight. Moderate GCV estimates were observed for grain yield per plant. Very high heritability was seen for hundred seed weight. High genetic advance as per cent of mean was observed for hundred seed weight and days to 50% per cent flowering indicating under the control of additive gene effects, may serve as better source for breeding programme to develop high yielding varieties by Konda *et al.* (2009).

Singh *et al.* (2009) Genetic variability, heritability and genetic advance were estimated for various quantitative characters in forty genotypes of mungbean The study exhibited high estimates of heritability for number of pods per plant, 100 seed weight and biological yield. The estimates of PCV were higher than those of GCV for all the characters, which indicate greater G x E interaction. The number of pods per plant, 100 seed weight, seed yield and biological yield exhibited high coefficients of variation and genetic gain in most of the environments and hence these are the most important characters for improving the genotypes.

Arulbalachandran *et al.* (2010) observed high genetic variability, heritability and genetic advance for all quantitative traits in blackgram mutants. Genotypic and phenotypic variance, coefficient of variance, heritability, genetic advance, was evaluated for yield and its contributing characters in 26 mungbean genotypes. High heritability (broad) along with high genetic advance in percent of mean was observed for plant height, number of pods per plant, number of seeds per pod, 1000-grain weight and grain yield per plant. (Rahim *et al.,* 2010).

2.5 Inter-character correlations

Correlation study indicates the degree of interdependence of plant characters which forms an important tool in selection of pertinent genotype. Most of the plant breeding programmes are aimed at augmentation of yield, which is an intricate character dependent on many other component characters which are further related among themselves. Thus, rendering the correlation study incompetent.

Luthra and singh (1978) estimated genotypic and phenotypic correlations in F_3 populations of blackgram and reported that seed yield was strongly and positively correlated with branch number, cluster number and pod length in blackgram.

Singh and Sharma (1981) observed positive and significant correlation between plant height and pods per plant in greengram.

Mishra (1983) observed that the pods per plant, cluster per plant and days to maturity had high positive correlation with seed yield both at genotypic level and phenotypic level from the study on eighteen varieties of blackgram.

Srinivasan *et al.* (1985) and Choi *et al.* (1986) observed positive correlation of seed yield with number of seeds per pod and 100- seed weight in greengram.

Wanjari (1986) studied character association in greengram. He observed that at phenotypic level grain yield was positively associated with pods per plant and pod length, while at genotypic level grain yield was positively associated with pods per plant, pod length and cluster per plant.

Damodaran *et al.* (1989) studied the interrelationship between yield and yield components in 28 genotypes of blackgram and reported that pods per plant had high positive genotypic (0.7597) and phenotypic (0.7235) direct effect on seed yield, while branches per plant had low direct effect on seed yield. It was concluded that, pods per plant followed by clusters per plant and 100-seed weight are major yield components in blackgram.

Boomikumaran and Rathinam (1991) studied the correlation of yield component characters with yield and among themselves and their influence on grain yield of greengram which was determined by study of genotypic, phenotypic and environmental correlations in 49 diverse genotypes of greengram and it was observed that characters except pod length and 100- seed weight were significantly correlated with grain yield and among themselves.

Verma (1992) concluded that seed yield had positive and significant correlation with 100-seed weight, number of days to maturity and number of primary branch and plant height both at genotypic and phenotypic levels in blackgram.

Naidu and Rosaiah (1993) evaluated nine parents and 20 F_1 s and F_2s in greengram and reported that plant height, clusters per plant , pods per plant, seeds per pod and 100- seed weight showed positive correlation with seed yield in all the populations.

Reddy *et al.* (1994) reported that in mungbean pods per plant and seeds per pod had strong positive correlation with seed yield as well as among themselves. The same yield components were reported to have positive direct effect on seed yield.

Rao (1995) studied phenotypic and genotypic coefficients of variations, estimates of heritability and expected genetic advance. Simple coefficient of correlations was calculated for seed yield per plant and eight related traits in 19 *Vigna mungo* accessions from different regions of India. The data suggested that selection of individual plants for high, number of seed per pod and number of primary branches would be effective in the improvement of seed yield.

Singh *et al.* (1995) revealed positive correlations of plant height, pods per plant and clusters per plant and seeds per pod with seed yield from data derived from 400 randomly chosen F_2 plants from each of the four crosses in greengram.

From the studies of Suryawanshi and Rao (1995) in 46 genotypes of blackgram it was observed that pods per plant, pod length, seed per pod had positive correlation with seed yield.

Hegde *et al.* (1996) reported that grain yield was positively and significantly correlated with clusters per plant, pods per plant, pod length and seeds per pod from the data derived in 11 yield related traits in F_2 progenies from crosses of ML-329 X Chinamung.

Mahto and Mahto (1997) while working on 11 cultivars of blackgram found that seed yield was highly and positively correlated with 100-seed weight, days to 50 pr cent flowering, plant height, number of primary branches per plant, number of seeds per pod and days to maturity.

Islam *et al.* (1999) evaluated 53 genotypes and observed positive correlation of seed yield with plant height, number of primary branches per plant, pod length, pod length, number of seed per pod and 100 seed weight in greengram.

Ghafoor *et al.* (2000) observed a high correlation of grain yield with branches, pods per plant, seeds per pod, seed weight and biological yield and indicated the importance of these characters in determining yield potential for blackgram.

The correlation studies among 10 traits of 25 genetically diverse black gram cultivars revealed that in the kharif season in Annamalai. Seed yield was positively and significantly correlated with all the characters studied. The 100-seed weight showed the highest positive direct effect on seed yield, followed by the number of pods per plant (Kumar *et al.,* 2003).

For the most important yield characters like pods per plant and seed yield per plants phenotypic coefficient of variation and genotypic coefficient of variation were maximum at 20 kR gamma ray treatment and 40 mM EMS treatment by Deepalakshmi and Kumar (2004).

Srividhya *et al.* (2005) did correlation analysis in the 15 F_2 crosses derived from 6 x 6 diallel cross in urdbean and revealed that seed yield/plant was positively and significantly associated with pods/plant, c1usters/ plant, seeds/pod, 100-seed weight and biomass and concluded that the selection criteria based on pods/plant, clusters/plant, seeds/ pod, 100-seed weight, days to maturity and plant height Will give fruitful results for yield Improvement in urdbean.

In Annamalainagar, during rabi 2001, genetic variability study was carried out with black gram in the segregating populations of three crosses, involving 4 parents. Observations were recorded for plant height, number of branches, cluster and pods per plant, pods per cluster, pod length, seeds per pod, 100-seed weight and seed yield. The cross LBG 645/LBG 20 recorded high estimates of phenotypic coefficient of variation and genotypic coefficient of variation for plant height, number of branches per plant, number of seeds per pod and seed yield by Veeramani *et al.* (2005).

An experiment comprising sixteen genotypes of blackgram was laid out by Aher *et al.* (2006) and reported the data regarding correlation coefficient between morpho-physiological and yield and yield attributes which indicated that grain yield was positively and significantly correlated to factors like NAR, total dry matter, clusters per plant, pods per plant, grain per pods and test weight. Similarly, total dry weight was positively correlated with clusters per plant, number of pods per plant and test seed weight.

The study of interrelationships among yield components in early generations (F_2 to F_4) of blackgram revealed that the seed yield is an inherent function primarily of pod number and harvest index. A stable trend of positive correlation was also observed between plant height and pod number, pod length, seeds per pod, 100-seed weight, harvest index and grain yield per plant. More over, pod length and seeds per pod showed a positive relationship in almost all the segregating generations of the crosses. A change in degree and magnitude of correlation coefficients in different crosses and in different segregating generations was also observed in the present study which may be attributed to the differences in gene association of the parental lines and the differential segregation and recombination occurring in the early segregating generations by Bhagowati and Hazarika (2006).

180 germplasm lines of mungbean comprising both indigenous and exotic collections along with checks were evaluated by Mallikarjuna *et al.* (2006) in augmented block design for yield and its components during *rabi* 2002-03. The traits plant height and number of clusters per plant recorded highly significant and positive association with grain yield, while number of seeds per pod showed negative association with seed yield.

The study conducted by Konda *et al.* (2008) revealed that the branches per plant, clusters per plant, pods per plant, seeds per pod, pod length and hundred seed weight exhibited significant and positive correlation with yield per plant.

Makeen *et al.* (2009) revealed that the phenotypic correlation estimates showed that pods/plant and clusters/plant had highly significant positive correlation with seed yield/plant. In addition the trait like 100-seed weight and plant height showed moderate positive association with seed yield, suggesting that these traits may be given second priority in the selection programme in blackgram.

3. MATERIAL AND METHODS

The present investigation was primarily undertaken to know the genetics and identify molecular markers linked to disease resistance along with variability estimation in segregating population for productivity traits. The details of experiment conducted is presented below.

Experimental Site

The field experiment was conducted in the G-block at Main Agricultural Research Station, Dharwad. Geographically, Dharwad is situated at 15^0 31' North latitude and 75^0 07' East longitude at an altitude of 678 m above mean sea level with an average rainfall about 800mm. The soil type of the experimental block was vertisol with a pH in the range of 7 to 7.5. The molecular experiment was conducted in Molecular Marker Laboratory of Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Dharwad.

The material used and methodology followed are presented under the following headings.

3.1 Inheritance and molecular markers linked to powdery mildew disease

3.2 Inheritance and molecular markers linked to MYMV

3.3 Variability parameters

3.4 Inter-character correlations

3.5 Transgressive segregants, high yielding and disease resistant lines

3.6 Statistical analysis

3.1 Inheritance and molecular markers linked to powdery mildew disease

3.1.1 Experimental material

The experimental material for studying powdery mildew disease was developed using two contrasting parental lines, LBG 17 and TAU-1, for disease reaction. TAU-1 is the agronomically superior line but highly susceptible to powdery mildew disease whereas LBG 17 is resistant to the disease. The salient features of the genotypes used in the study are given in Table 1.

3.1.2 Generation of experimental material:

Experimental material was generated as shown below.

Summer 2008:   (LBG17 X TAU-1)

$\downarrow$

F_1

$\downarrow$

F_2

Kharif 2008: F_2

$\downarrow$ By SSD

F_3

Kharif 2009: F_3

$\downarrow$

F_4
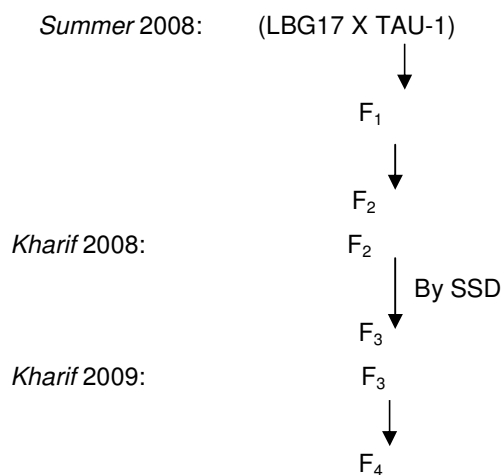
Table 1. Salient features of parents used in the present study

Characters	Parents		
	TAU-1	LBG-17	BDU-4
Origin/Source	BARC, Trombay, Maharashtra	Regional Research Station, Lam Farm Guntur, Andrapradesh	ARS, Bidar
Plant type	Semi erect	Erect	Erect
Maturity (days)	78.0	85.0-90.0	75.0-80.0
Number of Pods per plant (#)	38.5	24.0	29.6
Number of seeds per pod (#)	6.5	5.5	6.1
Pod length (cm)	4.8	4.3	4.6
100 seed weight (g)	4.9	4.2	4.6
Seed yield/plant (g)	12.0	9.9	10.5
Disease reaction for Powdery mildew (score)	Highly Susceptible	Resistant	Susceptible
Disease reaction for MYMV (score)	Susceptible	Moderately Susceptible	Resistant

3.1.3 Experimental details

3.1.3.1 Study on inheritance of powdery mildew disease resistance

LBG-17 was crossed to TAU-1 to produce F_1 seeds. It was advanced to F_2 generation during *summer* 2008. During first week of August (*kharif* 2008) the F_2 of the cross was planted along with parents and 300 F_2 plants to ensure required congenial condition for the development of powdery mildew diseases. The experiment was conducted without replications as it was segregating material. The sowing was done with a spacing of 30 cm between rows and 10 cm between plants with row length of 5 meters. Each F_2 was raised with minimum of 300 plant population. All standard agronomic practices were followed to raise a good crop. At the time of harvest, observations were recorded on all the plants including their parents and F_1s for quantitative characters.

Table 2. Powdery mildew disease reaction scale

Scale	Score
0	R (Resistant)
1	MR (Moderately Resistant)
2	T (Tolerant)
3	MT (Moderately Tolerant)
4	MS (Moderately Susceptible)
5	S (Susceptible)
6	HS (Highly Susceptible)

Powdery mildew assays

Screening of both the parents, their F_1 and 300 F_2 plants was carried out on 45 days old crop by following different inoculation techniques *viz.*,

a) Dusting conidia with camel hair brush: Leaves of the plant were first moistened by sprinkling water and then powdery mildew conidia were dusted slowly on them by using camel hair brush.

b) Spraying the spores suspension: Powdery mildew conidial suspension was used for inoculation by spraying with a hand sprayer.

Plants were scored following the procedure given by Humphry *et al* (2003) for powdery mildew response by the percentage of leaf covered by the disease. An average was taken for each set of three leaves per plant. Plants were scored as 0 (no visible infection), 1 (up to approximately 20% leaf coverage), 2 (approximately 21–40% leaf coverage), 3 (approximately 41-60% leaf coverage), 4 (approximately 61-80% leaf coverage), 5 (81-100% of leaf covered), and 6 (whole leaf covered and plant drying). (Table 2)

3.1.3.2 Molecular marker studies

3.1.3.2.1 DNA isolation of parents and F_2s

Total cellular DNA (Deoxy ribonucleic acid) of each 300 F_2 plants along with parents LBG-17 and TAU-1 was extracted by CTAB method.

3.1.3.2.2 Quantification of DNA- Agarose gel electrophoresis

2 µl of each sample was loaded on to 0.8% agarose gel and was electrophoressed at 100 volts. After electrophoresis gel was stained with ethidium bromide solution (10µg/ml) for 15 min and viewed under trans-illuminator. Quantification was done based on the intensity of the bands.

3.1.3.2.3 Normalization of DNA concentration for PCR

Normalization of DNA was done to bring all DNA concentrations to a relatively equal level (20ng/µl) by appropriate dilutions. Dilutions were done with TE buffer.

3.1.3.2.4 Polymerase chain reaction (PCR):

Amplification was carried out on Master Cycler Gradient Eppendorf PCR.

3.1.3.2.5 Agarose gel electrophoresis:

The PCR products were run on 3% gels to verify amplification. The gel was electrophoressed in 1x TAE at 100V for 80-120 min. The gel was then stained in ethidium bromide solution (1µg /ml) for 15 min. and de-stained with water and observed on a UV Tran illuminator. Agarose gels don't give as high resolution as PAGE, the monomorphic markers and the markers which showed less base pair size on agarose gel were arrayed on PAGE.

3.1.3.2.6 Polyacrylamide gel electrophoresis (PAGE)

SSR analysis was carried out following the protocol described by Paunaud et al. (1996) with some modifications.

Casting of the gel included the following procedure

The two plates (a notched plate and outer plate) (BIORAD) were cleaned thoroughly with warm water and detergent. They were then cleaned with Sterile Distilled water and wiped with tissue paper. Finally, they were wiped with ethanol. The ethanol removes any detergent that might be left on the plates after washing.

Preparation of notched (large) plate

About 300µl of repel silane dissolved in 700ml of aceto-ethanol solution was applied and spread uniformly with the tissue paper using wearing gloves. The plate was allowed to dry for 5 min.

Preparation of outer plate

Bind silane solution was prepared by mixing 5µl of bind silane in 1ml of aceto-ethanol solution and dispensed on the plate and spread uniformly with tissue paper. The plate was allowed to dry for 5 min.

Preparation of Gel Sandwitch

The spacers (0.4mm) were placed along the vertical edges of the notched plate. The outer plate was placed on the notched plate with coated side facing the spacers taking care to avoid contact between the two-coated surfaces. The straight side of the shark-tooth comb (0.4mm) was introduced in such a way that no air bubble is trapped and the jagged edge of the comb is even with the edge of the notched plate. About 60ml of acryl amide gel solution (5%) was prepared by adding 600 µl of 10% ammonium per sulfate (APS) and 60µl of TEMED.(N,N,N',N'-Tetra methyl ethylene di amine) in a 100 ml beaker.

The gel solution was introduced steadily from the bottom, till it reaches the top. The gel was allowed to polymerize for 20 min at room temperature.

Setting up gel assembly in electrophoresis apparatus

The outer surface of the glass plates were cleaned with a wet paper towel. The assembly was placed vertically in the electrophoresis tank.

The 1X TBE buffer was then added from the top of the apparatus, till it reached the tip of the plates. The comb was removed carefully. Using the syringe, air bubbles and non-polymerized acryl amide was removed from the top of the gel. The gel was pre-run for 45 to 60 min. at 100W to achieve a gel temperature of 50ºC.

Loading and running samples in PAGE apparatus

After the PCR run, 0.5µl of 3X of loading dye was added and the samples were denatured at 95 ºC for 4 min. in a thermocycler.

The samples were chilled immediately by placing them on ice (denatured samples can be stored at –20 ºC for a week).

After the pre-run, the well area was rinsed with a high volume pipette, to remove the excess urea and un-polymerized solution by taking care not to disturb the flat well surface. The shark toothcomb with the jagged surface was carefully inserted into the well area till the point the tips of the teeth just touch the surface of the gel.

About 3µl of denatured samples was loaded per lane. Loading is completed with in 20 min. to prevent the gel temperature falling below 40 ºC.

Gel run

The gel was run at 70W, 2000V maximum and 50mA with a temperature at 50 ºC. When the xylene-cyanol dye front had run about 2/3 rd of the gel length, the gel run was stopped.

Fixing and silver staining PAGE gels

The following reagents were prepared for silver staining of the polyacrylamide gel.

Fixer solution

10% acetic acid: Glacial acetic acid solution of 2 liters was made by dissolving 200 ml of acetic acid in 1800 ml of double distilled water.

Silver staining solution:

1.4 g of Silver nitrate was dissolved in 1400 ml of double distilled water, to which 2 ml of formaldehyde (37%) is added. The solution was mixed well and stored at room temperature in ambered colored container.

Developer solution:

45 g of Sodium carbonate was dissolved in 1500 ml of double distilled water. 300 µl of Sodium thiosulphate (10mg/ml) and 2.0 ml of Formaldehyde (37%) were added to the Sodium carbonate solution just before transferring the gel into solution.

After electrophoresis, the buffer from the upper and lower buffer chambers was drained and the sandwitch from the apparatus was removed. The gel was placed in a plastic tray and the following steps were followed in a sequence.

Step 1 : Fixing - Acetic acid (10%) for 20 min.

Step 2 : Washing - Distilled water for 2 min. (two times)

Step 3 : Staining - Silver Nitrate (1%) for 18 min.

Step 4 : Washing – Distilled water for 5-8 sec.

Step 5 : Developing – Developer solution (3M $NaCO_3$+2ml formaldehyde + 300µl 10% sodium thiosulphate) for 3-5 min

Step 6 : Stop – Acetic acid (10%) 10 min.

Step 7 : Washing – Double distilled water for 10 min.

All the steps were done with constant shaking. The volumes of solutions used in each were typically 2 liters per gel.

Gel documentation

After the PAGE gels were dried completely, it was photographed using digital camera and the gel images were preserved for scoring and future reference.

3.1.3.2.7 Scoring the bands

For SSR markers scores were given as below,

Parental type 1 (P1),

Parental type 3 (P2) and

Heterozygote 2, respectively

3.1.3.2.8 Parental polymorphic survey

DNA of the LBG-17and TAU-1 was extracted and analyzed for polymorphism using SSR primers. A total of 469 SSR primers *viz*., soybean SSR primer pair, Common bean SSR primer pair, redgram and Azuki bean SSR primer were used to survey the parental polymorphism. The primers were chosen based on their distribution in the genome and earlier reports. Polymorphic primers were noted. A primer was considered polymorphic, if it amplified different bands in the parents.

3.1.3.2.9 Bulk segregant analysis of cross LBG17 X TAU-1 for powdery mildew

Bulk Segregant Analysis was carried out with polymorphic SSR obtained from screening of the two parental genotypes. The F_2 individuals of the cross LBG17 X TAU-1 were used for Bulk Segregant Analysis (BSA). Resistant and susceptible DNA bulks were prepared from F_2 individuals by pooling aliquots containing equal amount of DNA (20ng/micro liter) from each of the eight resistant and eight susceptible F_2 individuals based on their reaction to Powdery mildew disease (Michelmore *et al.*, 1991).

3.2 Inheritance and molecular markers linked to MYMV

3.2.1 Experimental material

The experimental material for studying MYMV disease was developed using two contrasting parental lines, BDU-4 and TAU-1, for disease reaction. TAU-1 is the agronomically superior line but very much susceptible MYMV disease whereas BDU-4 is resistant to the disease. The salient features of the parents used are given in   Table 1.

3.2.2 Generation of experimental material

Experimental material was generated as shown below.

Summer 2008:   (BDU-4 X TAU-1)

F_1

F_2

Summer 2009 F_2

By SSD

F_3

Summer 2010 F_3

F_4
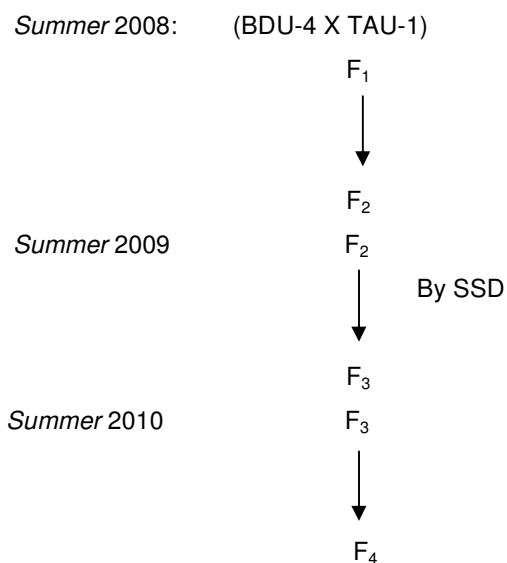
3.2.3 Experimental details

3.2.3.1 Study on inheritance of MYMV disease resistance

Single crosses (F_1s) were advanced to F_2 generation during *summer* 2008 further the population was advanced to F_3 generation in *summer* 2009. The experiment was conducted without replications as it was segregating material. The sowing was done with a spacing of 30 cm between rows and 10 cm between plants with row length of 5 meters. Each F_2 was raised with minimum of 300 plant population. All standard agronomic practices were followed to raise a good crop. At the time of harvest, observations were recorded on all the plants including their parents and F_1s for quantitative characters. The mapping population was subjected to disease screening at 50 days after sowing.

MYMV assay

For artificial inoculation, white flies were collected from the infected plants and confined in a susceptible plant in the cages showing typical YMV symptoms for 24 hrs using a small, transparent glass trapper with a cap and pipe and multiplied there its self. The same whiteflies were collected in the trap and released on the healthy plants and the viruliferous insects were allowed to feed on the leaf for 24 h. After acquisition feeding, the flies were used for 3–5 transfers for inoculation feeding, thereby allowing the viruliferous flies to transmit YMV into the plant (Plate 1 and 2). Plants were screened for yellow mosaic reaction at the pod formation stage using the scale given by (Selvi *et al.*, 2006). (Table 3).

Table 3. MYMV disease reaction scale

Scale	Score
1	R (Mottling of leaves covering 0.1-5.0% of the leaf area)
3	MR (Mottling of leaves covering 5.1-10.0% of the leaf area)
5	MS (Mottling and yellow discoloration of 10.1-25% of the leaf area)
7	S (Mottling and yellow discoloration of leaves on 25.1-50% of the leaf area)
9	HS (Severe yellow mottling on more than 51% and up to 60% of the leaf area)

3.2.3.2 Molecular marker studies

3.2.3.2.1 DNA isolation of parents and F_2s

Total cellular DNA (Deoxy ribonucleic acid) of 300 F_2 plants along with parents BDU-4 and TAU-1 was extracted by CTAB method.

3.2.3.2.2 Same as 3.1.3.2.2

3.2.3.2.3 Same as 3.1.3.2.3

3.2.3.2.4 Same as 3.1.3.2.4

3.2.3.2.5 Same as 3.1.3.2.5

3.2.3.2.6 Same as 3.1.3.2.6

3.2.3.2.7 Same as 3.1.3.2.7

3.1.3.2.8 Parental polymorphic survey

DNA of the BDU-4 and TAU-1 was extracted and analyzed for polymorphism using SSR primers. A total of 469 SSR primers *viz*., soybean SSR primer pair, Common bean SSR primer pair, redgram and Azuki bean SSR primer were used to survey the parental polymorphism. The primers were chosen based on their distribution in the genome and earlier reports. Polymorphic primers were noted. A primer was considered polymorphic, if it amplified different bands in the parents.

3.1.3.2.9 Bulk segregant analysis of cross BDU-4 X TAU-1 for powdery mildew

Bulk Segregant Analysis was carried out with polymorphic SSR obtained from screening of the two parental genotypes. The F_2 individuals of the cross BDU-4 X TAU-1 were used for Bulk Segregant Analysis (BSA). Resistant and susceptible DNA bulks were prepared from F_2 individuals by pooling aliquots containing equal amount of DNA (20ng/micro liter) from each of the eight resistant and eight susceptible F_2 individuals based on their reaction to Powdery mildew disease (Michelmore *et al.*, 1991).

Plate 1. Collected insects in the aspirator bottles

Plate 2. Maintenance of white flies in the culture cages

3.3 Variability parameters

To know the extent of variability present in the population, mean, range, variance, PCV, GCV, heritability and genetic advance were calculated in F_2 and F_3 generations of both the crosses *viz.*, LBG 17 X TAU-1 and BDU-4 X TAU-1. The following observations were recorded on all the plants in F_2 populations and 20 plants in each of the parents and F_1s. The characters studied and techniques adopted to record the observations are given below.

i) Number of pods per plant: The total numbers of pods per plant were counted at the time of harvest.

ii) Pod length: Pod length in centimeters (cm) was obtained by averaging the length of ten randomly selected pods at the time of harvest.

iii) Number of seeds per pod: Number of seeds per pod was obtained as a mean number of seeds of ten randomly selected pods at the time of harvest.

iv) 100-seed weight: weight of 100 seeds in grams (g) was recorded.

v) Seed yield per plant: Weight of seeds in grams (g) from each plant was recorded at the time of harvest.

3.4 Inter- character correlations

In all the generations, the simple correlation coefficients were calculated to determine the degree of association of different characters with seed yield and also among yield components in each of the populations separately. Correlation coefficients were compared against Table 'r' values at (n-2) df at the probability levels of 0.05 and 0.01 to test their significance.

3.5 Transgressive segregants, high yielding and disease resistant lines

From the segregating populations, the number of plants which performed better than mean of check plus one standard deviation for seed yield and yield related traits were selected as transgressive segregants. High yielding (more than check TAU-1) and disease resistant F_3 families were identified.

3.6 Statistical analysis

Statistical analysis of data was done on a personal computer using software packages like, MS-EXCEL, SAS and GMENDEL for different purposes. All the data were analyzed without any transformation.

3.6.1 Chi- square test

The goodness of test to Mendelian segregation of banding pattern in the segregating populations was tested by Chi- square test.

$$X^2 = \frac{\Sigma(oi-ei)^2}{ei}$$

oi = observed frequency of bands

ei = expected frequency of bands

The significance of Chi- square value was tested against Table value with   (n-1) degrees of freedom, wherein is the total number of segregating classes (Stansifield, 1986).

3.6.2 Marker phenotypic association analysis

Single marker analysis to detect main effect of genes was performed by the method of Liu *et al.* (1998). Significant association of tested marker with a gene for powdery mildew disease was detected by primary ANOVA.

Contribution of a marker –linked gene to a total variation in powdery mildew disease in F_2 lines was estimated by linear regression. All statistical procedures were performed with Statistical Analysis for Social science (SAS) (1995).

3.6.3 Marker segregation and linkage analysis for powdery mildew and MYMV diseases

The putative linked SSR markers from BSA were used for linkage analysis of 300 F_2 individuals of the crosses LBG17 X TAU-1 and BDU-4 X TAU-1, segregating for powdery mildew and MYMV, respectively. Chi- square test was performed to examine the goodness of fit between the expected Mendelian ratios for the segregation data of the two linked SSR markers. Linkage between the two markers was identified using GMENDEL software (Holloway and Knapp 1993).

3.6.4 Analysis of variance

The mean and variances were analyzed based on the formula given by Singh and Chaudhary (1977).

3.6.4.1 Mean

$$\overline{y} = \frac{1}{n} \sum_{i=1}^{n} (\Sigma yi)$$

3.6.4.2 Variance

$$\text{Variance} = \frac{1}{n-1} \left\{ \sum_{i=1}^{n} (Yi-\overline{Y})^2 \right\}$$

Where, Yi = Individual value

$\overline{Y}$ = Population mean

$$\text{Standard deviation (SD)} = \sqrt{\text{variance}} = \frac{\Sigma d^2}{N}$$

Where,

d = Deviation of individual value from mean and

N = Number of observations

3.6.5 Estimation of genetic parameters

Genotypic and phenotypic variances and coefficients of variance were computed based on mean and variance calculated by using the data of unreplicated treatments.

3.6.5.1 Phenotypic variance

The individual observations made for each trait on F_2 population is used for calculating the phenotypic variance.

Phenotypic variance $((\sigma^2 p) = \text{Var } F_2$.

Where, Var F_2 = variance of F_2 population

3.6.5.2 Environmental variance

The average variance of parents and their corresponding F_1 is used as environmental variance for single crosses.

$$\text{Environmental variance} = (\sigma^2 e) \quad \frac{\text{Var } P_1 + \text{Var } P_2 + \text{Var } F_1}{3}$$

Where, Var P_1 = Variance of P_1 parent

   Var P_2 = Variance of P_2 parent and

   Var F_1 = variance of corresponding F_1 cross

3.6.5.3 Genotypic variance

   Genotypic variance $(\sigma^2 g) = \sigma^2 p - \sigma^2 e$

Where,

   $\sigma^2 p$ = Phenotypic variance

   $\sigma^2 e$ = Environmental variance

3.6.5.4 Genotypic and phenotypic coefficient of variation

   The genotypic and phenotypic coefficient of variation was computed according to Burton and Devane (1953).

   Genotypic coefficient of variance (GCV)

$$\text{GCV} = \frac{\sqrt{\sigma^2 g}}{\overline{X}} \times 100$$

Phenotypic coefficient of variance (PCV)

$$\text{PCV} = \frac{\sqrt{\sigma^2 p}}{\overline{X}} \times 100$$

Where,

   $\sigma^2 g$ = Genotypic variance

   $\sigma^2 p$ = Phenotypic variance and $\overline{X}$ = General mean of the character

3.6.5.5 Heritability

   Heritability in broad sense was estimated as the ratio of genotypic to phenotypic variance and expressed in percentage (Hanson *et al.,* 1956).

$$\text{Heritability } [h^2(bs)] \, (\%) = \frac{\sigma^2 p}{\sigma^2 g} \times 100$$

Where,
   $\sigma^2 g$ = Genotypic variance
   $\sigma^2 p$ = Phenotypic variance

3.6.5.6 Genetic advance (GA)

   This was worked out as per the formula proposed by Johnson *et al.* (1955).

   $GA = k. \, \sigma_p. \, H.$

Where,

   k   = Intensity of selection

   σ_p = Phenotypic standard deviation

   H   = Heritability in broad sense

   The value of 'k' was taken as 2.06 assuming 5 per cent selection intensity.

3.6.5.7 Genetic advance expressed as percentage over mean (GAM).

$$GAM = \frac{GA}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = General mean of the character

3.6.6 Inter-character correlation analysis

Simple correlations were computed by using the formula given by Weber and Moorthy (1952) as given below.

$$rP_{xy} = \frac{Covxy}{\sqrt{Vx. Vy}}$$

Where, Cov xy = Covariance between the characters x and y

Vx = Variance of the character x

Vy = variance of the character y

4. EXPERIMENTAL RESULTS

The F_2 populations of the cross *viz.*, LBG-17 X TAU-1 and BDU-4 X TAU-1 were evaluated for yield and yield related traits and disease reaction. The data obtained was subjected to statistical analysis to find out the inheritance pattern of powdery mildew and MYMV disease resistance. Molecular study was carried out with SSR markers to find out the parental polymorphism for MYMV and powdery mildew disease and to identify the marker linked to gene resistant for powdery mildew.

Success of any plant breeding programme depends on the extent of variability present in a crop. The presence of genetic variability for economic traits is a key factor for improving the local adopted varieties with regard to specific traits. Incidentally the parental lines used for developing mapping populations were also divergent for many of the traits related to productivity. Therefore an effort was made to estimate variability and other related parameters for yield and yield components and also association study have done.

The results obtained from the investigation are presented under the following headings

4.1 Screening and inheritance studies for powdery mildew and MYMV disease resistance

4.2 Molecular marker studies

4.3 Analysis of mean, range and variance

4.4 Variability parameters

4.5 Inter-character correlations

4.6 Transgressive segregants

4.7 Identification of high yielding and disease resistant lines

4.1 Screening and inheritance studies for powdery mildew and MYMV disease resistance

4.1.1 Powdery mildew

4.1.1.1 Screening of parents

Both the parents *viz.,* LBG-17 (P_1) and TAU-1 (P_2) were subjected to artificial screening under epiphytotic condition. Screening was done using the 0 to 6 scale as shown in Table 2 which is given by Humphry *et al.* (2003). The mean score for powdery mildew reaction of P_1 was found to be 0.2 (resistant), and that of P_2 was found to be 5.8 (susceptible) which are shown in Table 4 (Plate 3).

4.1.1.2 Screening of the F_2 population of the cross LBG17 x TAU-1

Three hundred F_2 segregants of the cross LBG17 X TAU-1 were screened artificially using same procedure as that for the parental screening in *kharif* 2008. The population was grouped as per the scale given by Humphry *et al.* (2003) and presented in Table 2. Per cent incidence of powdery mildew was recorded by taking average for each set of three leaves per plant. Among 300 segregants evaluated, 161 F_2 segregants showed no visible infection and were scored as '0' hence grouped under resistant category. While 59 plants had showed approximately 20% leaf coverage by mycelial growth and hence it was grouped under moderately resistant category with grade '1'. 57 F_2 plants showed approximately 21-40% leaf coverage and therefore they were grouped as tolerant with grade '2'. Only 7 plants had approximately 41-60% leaf coverage by mycelial growth and were grouped as moderately tolerant with grade '3'. Six plants showed approximately 61-80% leaf coverage and were grouped as moderately susceptible with grade '4'. Three plants showed 81-100% of leaf coverage with mycelia growth and graded as '5', and finally 7 plants having whole leaf covered with disease and showing drying symptoms were graded as '6'. These two last groups were characterized as susceptible and highly susceptible respectively as shown in Table 5.

Table 4. Mean disease score of parental lines of the cross LBG-17 X TAU-1 for powdery mildew

Disease	Parents	Score
Powdery mildew*	LBG-17	0.2
	TAU-1	5.8

* 0-6 Scale

Table 5. Frequency of F_2 segregants of the cross LBG-17 X TAU-1 showing different grades of resistance/susceptibility to powdery mildew

Resistance/Susceptibility grade	Reaction	Frequency of F_2 segregants
0	Resistant	161
1	Moderately resistant	59
2	Tolerant	57
3	Moderately tolerant	7
4	Moderately susceptible	6
5	Susceptible	3
6	Highly susceptible	7
Total		300

Plate 3. Screening parents for powdery mildew disease in artificial condition

4.1.1.3 Screening of the F_3 population of the cross LBG17 X TAU-1

The 300 F_3 families of the cross LBG17 X TAU-1 were screened artificially using same procedure as that for the parental screening in *kharif* 2009. Each F_3 family was classified as r_0, r_1, r_2 and s types. In the F_3 generation, progenies of all susceptible F_2 plants were true breeding for the susceptible reaction while those showing r_0, r_1 and r_2 reactions were either true breeding resistant or else segregated as expected.

4.1.1.4 Inheritance of powdery mildew resistance

Using highly resistant LBG-17 as a donor and TAU-1 highly susceptible but agronomically superior cross was effected to produce mapping population. The F_2 population of the cross comprising 300 individual segregants was subjected to screening for recording observations on reaction to powdery mildew diseases during *kharif* 2008 following the standard 0 to 6 scale. The parent and F_1 were also subjected to find out their diseases reaction. The reaction grades 0, 1 and 2 representing resistant, moderately resistant and tolerant reaction were grouped together to consider it as resistant group. The reaction grades 3, 4, 5 and 6 representing moderately tolerant, moderately susceptible, susceptible and highly susceptible were grouped together to classify them as susceptible. Further the resistant, moderately resistant and susceptible reaction represented by 0, 1 and 2 grades were referred to as r_0, r_1 and r_2 respectively while 3, 4, 5, and 6 were grouped as susceptible and referred to as s.   as indicated in materials and method chapter grades 0 to 6 indicate extent of infection ranging from no visible infection (0 grade) to whole leaf showing infection along with drying of plant (grade 6) . Chi square test indicated that resistance to powdery mildew is governed by two dominant genes which as symbolized as PM_1 and PM_2. The analysis also indicated that two genes are independent but interact to govern inheritance of resistance to powdery mildew disease. The presence of two dominant genes PM_1 and PM_2 results in to r_0 reaction (no allele interaction and 0 grade), while resistant reaction is r_1 (per cent leaf infection is 1 to 20%) in presence of PM_1 and resistant reaction is r_2 (percent leaf infection is 21 to 40%) in presence of other dominant gene PM_2. The absence of both the dominant genes PM_1 and PM_2 leads to susceptible reaction `s` including remaining four grades 3, 4, 5 and6.

All the F_1 plants showed an r_0 reaction while the F_2 segregated into r_0, r_1, r_2 and s types according to a dihybrid ratio of 9:3:3:1 (Table 6 and Plate 4) in the cross. This indicated the presence of two dominant resistance genes which have been designated as PM_1 and PM_2. In the F_3 generation, progenies of all susceptible F_2 plants were true breeding for the susceptible reaction while those showing r_0, r_1 and r_2 reactions were either true breeding resistant or else segregated as expected (Table 7 and Plate 5). The pooled segregation showed a good fit to the expected ratios of 1:2:2:4:1:2:1:2:1. Segregation within the families was as expected for digenic ratios.

4.1.2 MYMV

4.1.2.1 Screening of parents

The parental genotypes BDU-4 and TAU-1 was screened during summer 2008 under artificial condition with abundant whitefly population. 1-9 scale given by Selvi *et al* (2006) was used to classify the plants as resistant and susceptible. The parent BDU-4 recorded a mean score of 1.2 and was characterized as resistant. The other parent TAU-1 recorded a mean score of 7.6 and was categorized as susceptible as shown in Table 8 (Plate 6).

4.1.2.2 Screening of the F_2 population of the cross BDU-4 X TAU-1

F_2 population was screened under epiphytotic condition in summer 2008 under cages using the procedure same as that for parental screening. 231 plants were found to be resistant with no diseases symptoms. And 69 plants showed susceptible reaction as shown in Table 9 (Plate 7).

4.1.2.3 Screening of the F_3 population of the cross BDU-4 X TAU-1

The F_3 families of the cross BDU-4 X TAU-1 were screened following the same procedure as that used for parents. Each F_3 family was classified as resistant (homozygous), susceptible (homozygous), or segregating (heterozygous) lines. 92 families were found to be resistant, 139 families showed segregation for MYMV resistance and susceptibility and 67 lines were found to be homozygous susceptible (Plate 8).

4.1.2.4 Inheritance of resistance to mungbean yellow mosaic virus

Using BDU-4 a highly resistant parent as a pollen parent, hybridization was carried out with TAU-1 a susceptible cultivar having good agronomic background as female parent. The parents, F_1 and F_2 generations of this cross were sown at University of Agricultural Sciences, Dharwad in summer season of 2009.

The inheritance study based on F_2 data indicated the ratio of 231 resistant plants and 69 susceptible plants shows a good fit to expected 3:1(Resistant: Susceptible) ratio suggesting typical single dominant gene governing resistant reaction against MYMV resistance (Table 10 & Plate 9).

In summer 2009, YMV-reaction of F_3 plants and families from the same cross were assessed by forced inoculation in field condition. The F_3 population of the cross involving resistant and susceptible parents showed 1 resistant (92): 2 segregating (139): 1 susceptible (67) segregation ratio confirming role of single dominant gene controlling resistance to MYMV (Table 11 & Plate 10).

4.2 Molecular marker studies

4.2.1 Powdery mildew

4.2.1.1 Screening of parental genotypes using SSR markers for powdery mildew

Four hundred and sixty-eight legume specific SSR primers from soybean, common bean, redgram and azuki bean were used for screening parental genotypes *viz.*, LBG-17 and TAU-1. A total of 32 (Table 12 and Plate 11) primers were polymorphic between the parents of the cross LBG-17 X TAU-1 indicating low polymorphism in the parental genotypes used for the study using SSR marker.

4.2.1.2 Bulk Segregant Analysis (BSA) in F_2 population of the cross LBG17 X TAU-1 using SSR markers for powdery mildew resistance.

Table 6. Inheritance of host plant resistance to powdery mildew disease in F_2 population of cross LBG-17 X TAU-1 in blackgram

Resistant/Susceptible Reaction	Frequency
Resistant 'r_0' plants	161
Resistant 'r_1' plants	59
Resistant 'r_2' plants	57
Susceptible 's' plants	23
Total	300
χ^2 value* (9:3:3:1)	1.88

* Value for significance of p= 0.05 is 7.815.

SSR primers polymorphic between the parents of the tagging population of the cross LBG-17 X TAU-1 were subjected to bulk segregant analysis to identify putatively linked markers for powdery mildew disease resistance. Out of thirty two SSR markers that were polymorphic between the parents of the cross LBG-17 X TAU-1, five SSR_S were found to be polymorphic between resistant and susceptible F_2 bulks of cross LBG-17 X TAU-1 (Plate 12) for powdery mildew disease. For reconfirmation of these five markers again these markers were analysed on individual ten extreme resistant and susceptible plants. Of the five markers two SSR primers were found to be polymorphic indicating that these two markers are putatively linked to powdery mildew resistant gene (Plate13).

4.2.1.3 SSR marker and powdery mildew association analysis.

The two polymorphic markers identified based on analysis of bulked extremes were used to screen the 300 F_2 individuals to establish the association between the respective marker and phenotype. One way ANOVA was carried out using marker genotype as groups. The ANOVA on B-210 marker and B-230 marker genotypes as groups for powdery mildew resistance established significant association between marker (B-210 and B-230) and phenotypic trait (powdery mildew) (Table 13). The single marker ANOVA revealed that B-210 marker linked to the powdery mildew and B-230 linked to powdery mildew accounted for 33.5 and 36.04% of the total variation for powdery mildew resistance in F_2 population respectively. These results indicated possible detection of two genes for powdery mildew resistance.

4.2.1.4 Segregation behavior of SSR markers in F_2 population of the cross LBG17 X TAU-1 for resistance to powdery mildew resistance.

The two putatively linked SSR markers obtained from Bulk Segregant Analysis were subjected to individual SSR analysis of 300 F_2 individuals to analyse the segregation pattern of the markers. Chi-square test was performed to examine the goodness of fit between the observed and excepted SSR marker bands. Both SSR markers *viz.*, B-210 and B-230 exhibited Mendelian segregation ratio of 1:2:1 (Plate 14) which is typical ratio of Co-dominant marker (Table 14 and Plate 14). In our study powdery mildew disease is governed by two gene but marker data showed segregation ratio of 1:2:1 which is typical ratio for one gene. This implies that both the marker are linked to the single gene among the two reported genes and both the marker they are located on the same linkage group with the genetic distance of 28.8 cM.

LBG-17(R)   X   **TAU-1 (Susceptible)**

F₁   +

Segregation for susceptibility and resistance in F2 population
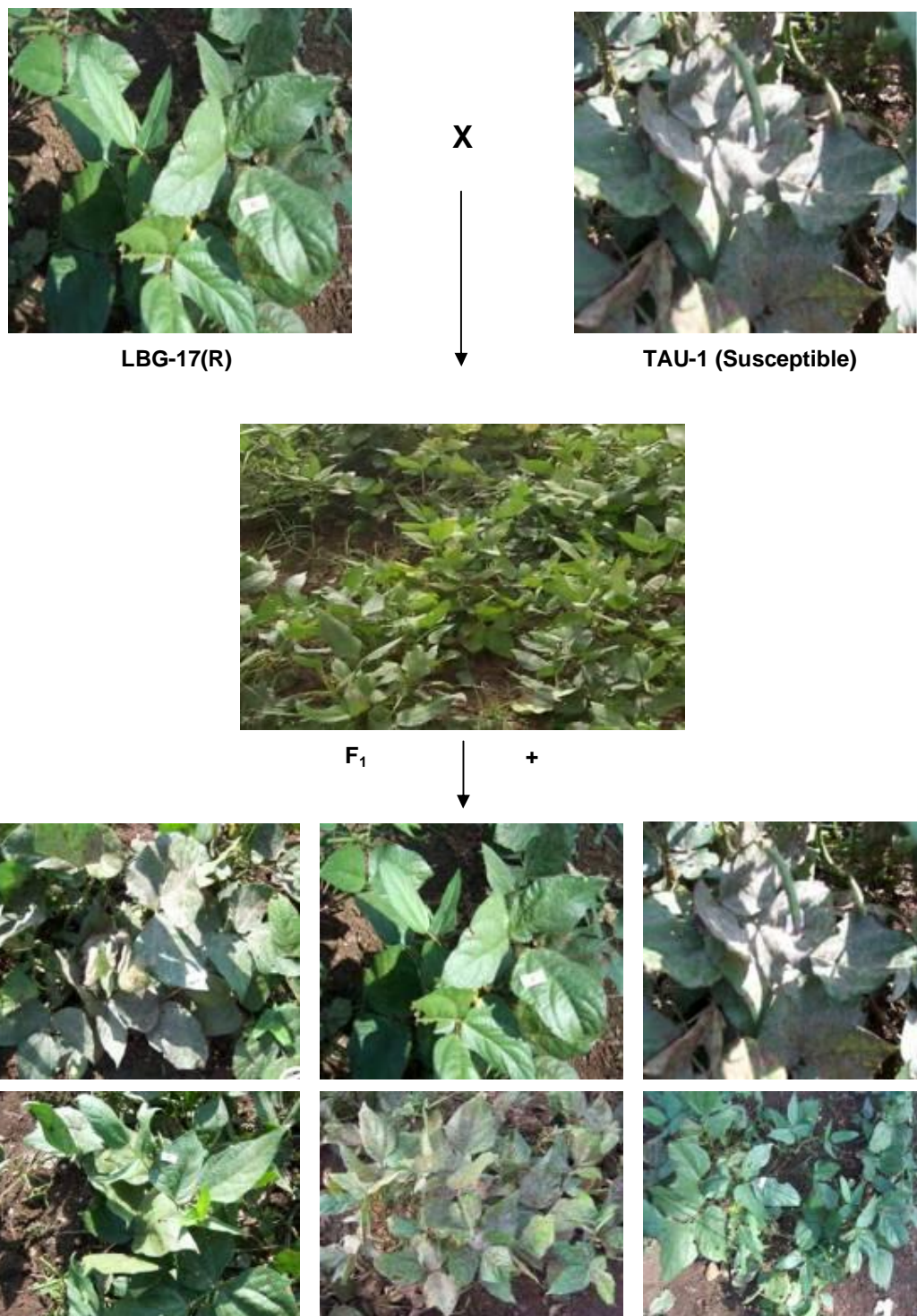
Plate 4. Screening of segregating material for powdery mildew disease reaction

Table 7. Confirmation of inheritance pattern for resistance to powdery mildew disease based on F_3 population of the cross LBG-17 X TAU-1 in blackgram

Resistant/Susceptible Reaction	Segregation Pattern	Frequency
r_0	r_0 (homozygous)	26
	r_0, r_1 (segregation)	43
	r_0, r_2 (segregation)	29
	r_0, r_1, r_2, s (segregation)	61
r_1	r_1 (homozygous)	24
	r_1, s (segregation)	30
r_2	r_2 (homozygous)	23
	r_2, s (segregation)	42
S	s (homozygous)	16
Total		294
χ^2 value* (1:2:2:4:1:2:1:2:1)		13.17

* Value for significance of p= 0.05 is 15.5.

| Heterozygons line | Susceptible line | Resistant line | Susceptible |

Plate 5. Segregation for susceptibility and resistance in F$_3$ population for powdery mildew disease

Table 8. Mean disease score of parental lines of the cross BDU-4 X TAU-1 for MYMV in blackgram

Disease	Parents	Score
MYMV**	BDU-4	1.2
	TAU-1	7.6

**0-9 Scale

Plate 6. Screening parents for mungbean yellow mosaic virus (MYMV) disease in artificial condition

Table 9. Frequency of F$_2$ segregants of the cross BDU-4 X TAU-1 of blackgram showing different grades of resistance/susceptibility to MYMV

Resistance/ Susceptibility grade	Reaction	Frequency of F$_2$ segregants
1	Resistant	85
3	Moderately resistant	146
5	Moderately susceptible	27
7	Susceptible	23
9	Highly susceptible	19
Total		300

Plate 7. Cages in the field for screening F$_2$ population for MYMV disease reaction

BDU-4(R)

TAU-1(S)

F1   **+**

Segregation for susceptibility and resistance in F$_2$ population

Plate 8. Screening of segregating material for MYMV disease reaction

Table 10. Inheritance of host plant resistance to MYMV disease in F_2 population of cross BDU-4 X TAU-1 in blackgram

Disease Reaction	Frequency
Resistant plants	231
Susceptible plants	69
Total	300
χ^2 value* (3:1)	0.64

* Value for significance of p= 0.05 is 3.84.

Plate 9. Cages in the field for screening F3 population for MYMV disease reaction

Table 11. Confirmation of inheritance pattern for resistance to MYMV disease based on F$_3$ population of the cross BDU-4 X TAU-1 in blackgram

Disease Reaction	Frequency
Homozygous resistant lines	92
Segregating lines	139
Homozygous susceptible lines	67
Total	298
χ^2 value* (1:2:1)	5.54

* Value for significance of p= 0.05 is 5.99.

Plate 10. Segregation for susceptibility and resistance in F$_3$ population for MYMV disease

Table 12. SSR primers used for molecular analysis of powdery mildew disease in blackgram

Particulars	
Total number of primers screened	469
Total number of primers amplified	263
Polymorphic primers between LBG-17 and TAU-1	32
Primers linked with disease powdery mildew	2
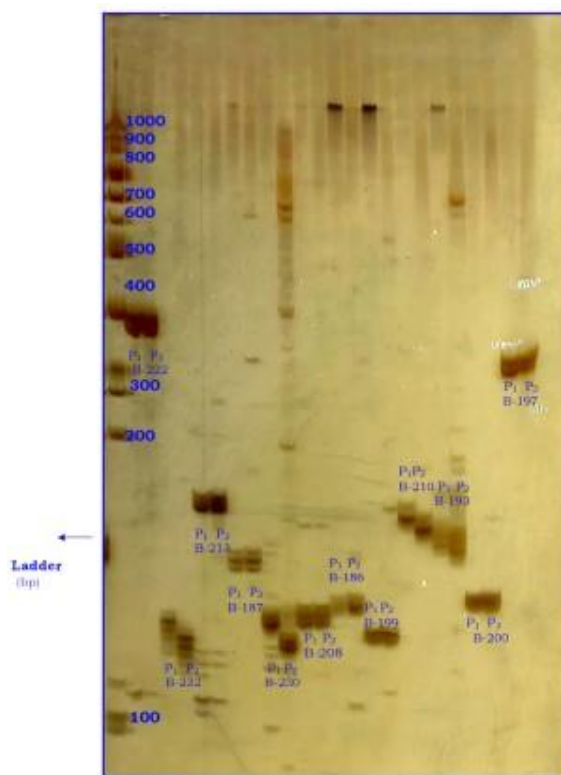
P1=TAu-1   P2=LBG-17

Plate 11. SSR markers showing polymorphism between parents- LBG 17 and TAU-1
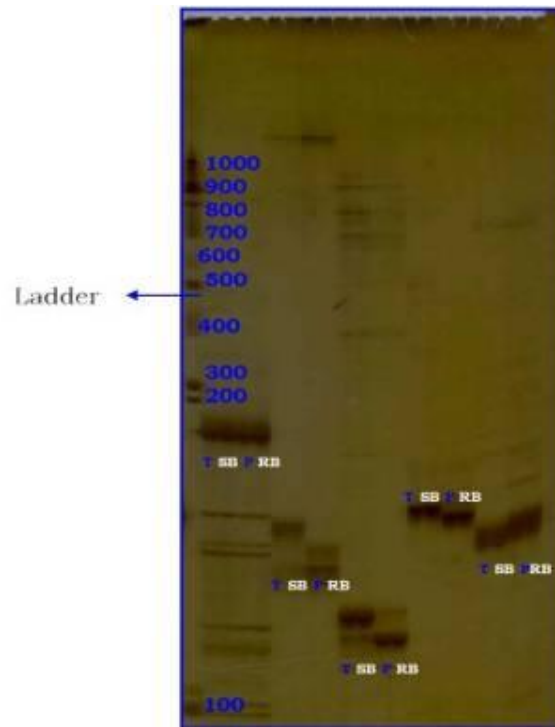
Plate 12. Bulk Segregant Analysis showing polymorphism between resistant and susceptible bulks
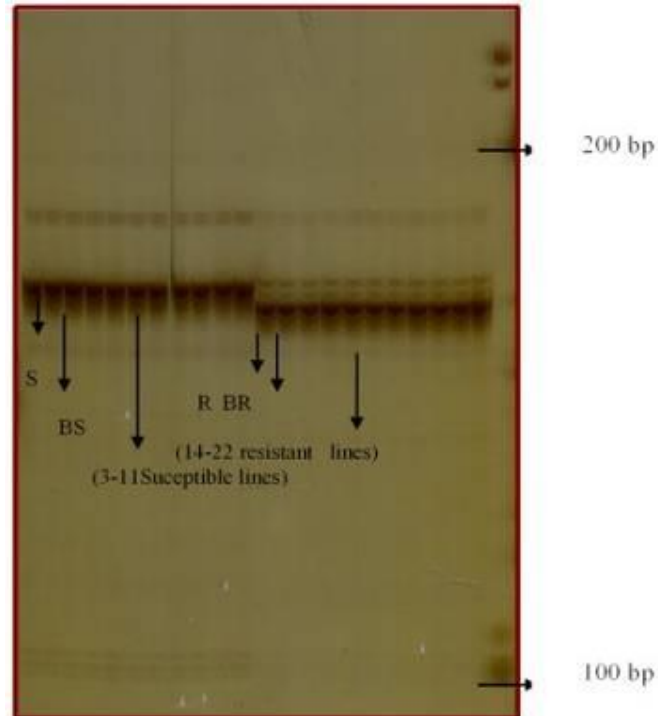
Plate 13. Bulk Segregant Analysis with primer B-210 on extreme resistant and susceptible plants

Table 13. Analysis of variance of the powdery mildew disease in F_2 population

Marker	Source	df	SS	MSS	F	F crit	P	Variance explained (%)
B-210								
	Model	2	58177.99	29088.99	75.01	3.03	4.25E-27	33.56
	Error	297	115164.70	387.75				
	Total	299	173342.70					
B-230								
Model	Model	2	62480.64	31240.32	83.69	3.03	4.25E-27	36.04
Error	Error	297	110862.00	373.27				
Total	Total	299	173342.70					

4.2.2 MYMV

4.2.2.1 Screening of parental genotypes using SSR markers for MYMV disease

Four hundred and sixty-eight legume specific SSR primers from soybean, common bean, redgram and azuki bean were used for screening parental genotypes *viz.*, BDU-4 and TAU-1. 24 SSR markers were found to be polymorphic between the parents indicating low polymorphism in the parental genotypes used for the study using SSR markers (Table 15 & Plate15).

4.2.2.2 Bulk Segregant Analysis (BSA) in F_2 population of the cross BDU-4 x TAU-1 using SSR markers for MYMV disease resistance.

SSR primers polymorphic between the parents of the tagging population of the cross BDU-4 X TAU-1 were subjected to bulk segregant analysis to identify putatively linked marker for MYMV disease resistance. None of the SSR markers showed polymorphism between resistant and susceptible bulks.

4.3 Analysis of mean, range and variance

Mean and range were computed for five quantitative traits *viz.,* number of pods per plant, number of seeds per pod, pod length (cm), 100- seed weight (g) and seed yield per plant (g) in F_2, F_3 populations of the crosses LBG17 X TAU-1 and BDU-4 X TAU-1. The results are presented in Table 16 and 17, respectively.

4.3.1 LBG17 X TAU-1

4.3.1.1 F_2 population

The F_2 population of this cross exhibited high variance for number of pods per plant (19.63) and seed yield per plant (16.43), whereas less variance was observed for the remaining traits. The lowest variance was observed for pod length (0.179). Number of pods per plant ranged from 8 to 43 with a mean of 24.56; number of seeds per pod ranged from 5.6 to 10.5 with a mean of 8.17; pod length ranged from 3.96 to 7.68 cm with a mean of 6.13 cm; 100- seed weight ranged from 2.6 to 6.9 g with a mean of 4.85g and seed yield per plant ranged from 3.87 to 26 g per plant with a mean of 9.6g.

4.3.1.2 F_3 population

The F_3 population of this cross exhibited high variance for number of pods per plant (18.71) and seed yield per plant (15.1), whereas less variation was observed for the remaining traits. The lowest variance was observed for the trait pod length (0.22). Number of pods per plant ranged from 7.9 to 42.10 with a mean of 24.16; number of seeds per pod ranged from 5.6 to 10.3 with a mean of 7.80; pod length ranged from 3.8 to 7.6 cm with a mean of 5.94 cm; 100- seed weight ranged from 2.5 to 6.8 g with a mean of 4.72g and seed yield per plant ranged from 3.6 to 25.3 g per plant with a mean of 9.39g.

4.3.2 BDU-4 X TAU-1

4.3.2.1 F_2 population

The F_2 population of this cross exhibited high variance for number of pods per plant (40.82) and seed yield per plant (16.4). Less variance was observed for the remaining traits. The lowest variation was observed for the trait pod length (0.18). Number of pods per plant ranged from 8 to 39 with a mean of 30.10; number of seeds per pod ranged from 3.3 to 7 with a mean of 6.32; pod length ranged from 3.3 to 6.4 cm with a mean of 5.2 cm; 100- seed weight ranged from 2.3 to 6.7 g with a mean of 6.05g and seed yield per plant ranged from 5.5 to 21.90 g per plant with a mean of 13.17 g.

4.3.2.2 F_3 population

The F_3 population of this cross exhibited high variance for number of pods per plant (42.9) and seed yield per plant (15.27). Less variance was observed for the remaining traits and the lowest variation was observed for the trait pod length (0.23).

Table 14. Segregation behavior of SSR primers to powdery mildew disease in F_2 generation of the cross LBG-17 X TAU-1 in blackgram

SSR primers	Total number of F_2 Plants	Observed Polymorphic bands			Expected Polymorphic bands			Ratio	χ^2 (Cal)	χ^2 (Tab.)
		RR	Rr	rr	RR	Rr	rr			
B-256	300	88	131	81	75.16	149.66	75.16	1:2:1	4.97	5.99
B-260	300	58	163	79	75.16	149.66	75.16	1:2:1	5.30	5.99

200 bp

Segregation for
Susceptibility
and resisatnce
(1 to 50 plants)

P1P2 1 2 3 4 5 6 7 8 9 10 11 1213141516 171819

100 bp

Segregation for
Susceptibility
and resisatnce
(1 to 50 plants)

20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 P₁ P₂ 40 41 42 43 44 45 46 47 48 49 50
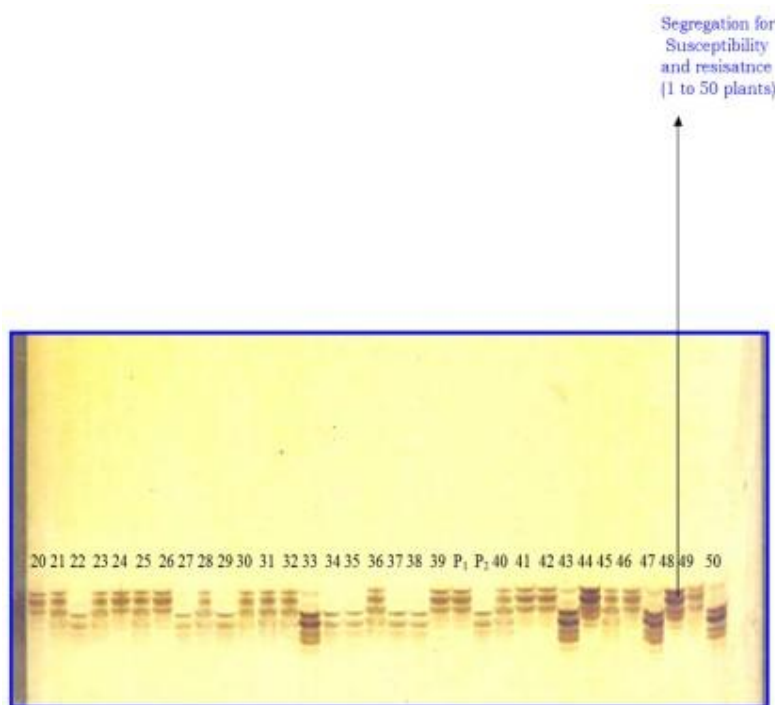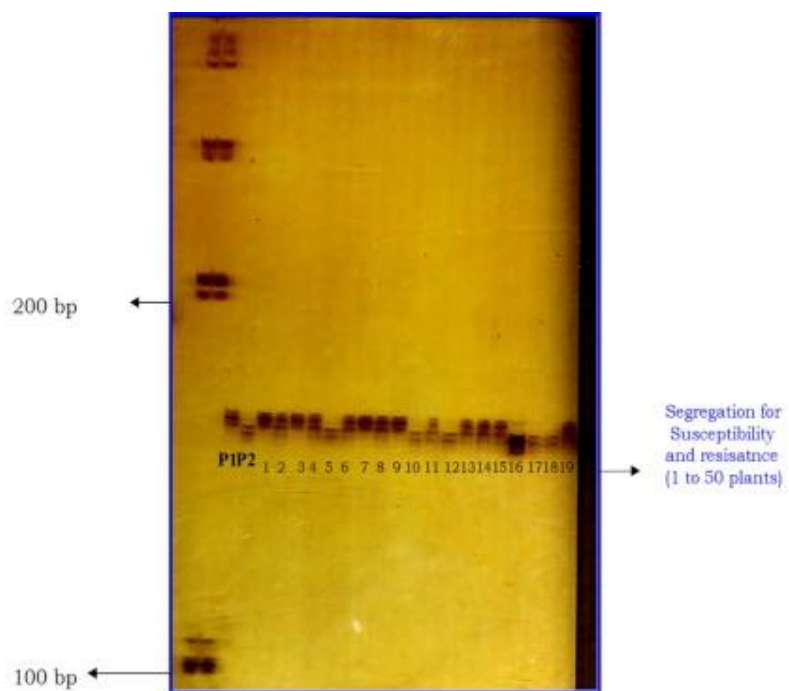
Plate 14. Segregation for the marker B-210 in the population
LBG 17 X TAU-1

P1=TAU-1 P2=LBG-17

Table 15. SSR primers used for molecular analysis of MYMV disease in blackgram

Particulars	
Total number of primers screened	469
Total number of primers amplified	263
Polymorphic primers between BDU-4 and TAU-1	24
Primers linked with disease MYMV	None
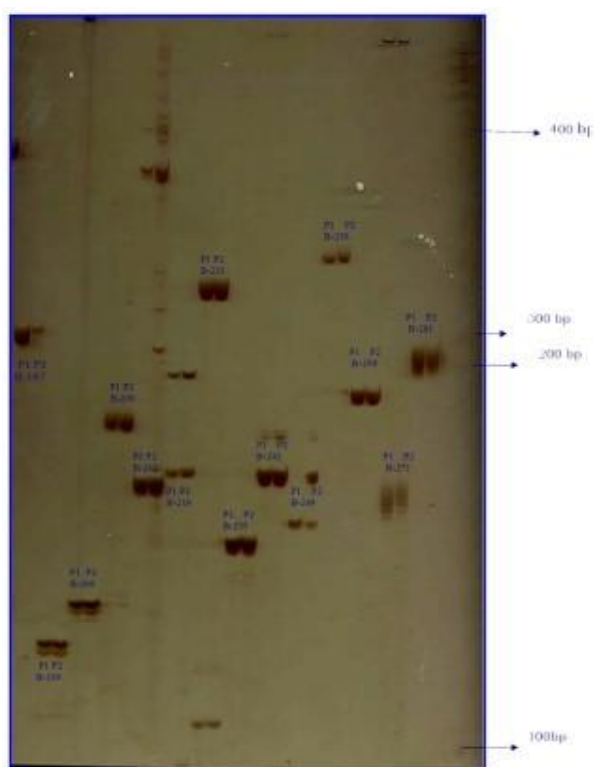
Plate 15. SSR markers showing polymorphism between parents, BDU-4 and TAU-1

Table 16. Mean, range and variance values for five traits in segregating F$_2$ population of LBG17 X TAU-1 and BDU-4 X TAU-1 in blackgram

Characters	LBG17 X TAU-1			BDU-4 X TAU-1			Parental mean		
	Mean ± SD	Range	Variance	Mean± SD	Range	Variance	TAU-1	LBG-17	BDU-4
Number of pods per plant(#)	24.56 ± 0.26	8.00-43.00	19.63	30.10 ± 0.39	8.0-39.0	40.82	38.50	32.00	29.60
Number of seeds per pod(#)	8.17 ± 0.04	5.60 -10.50	0.68	6.32 ± 0.04	3.3-7.0	0.53	6.50	6.20	6.10
Pod length(cm)	6.13 ± 0.02	3.96 -7.68	0.18	5.22 ± 0.02	3.3-6.4	0.18	4.80	4.30	4.60
100-seed weight(g)	4.85 ± 0.04	2.60-6.90	0.53	6.05 ± 0.06	2.3-6.7	1.08	4.90	4.20	4.70
Seed yield per plant(g)	9.63 ± 0.23	3.87-26.00	16.43	13.17 ± 0.23	5.5-21.9	16.40	12.30	9.85	10.50

Table 17. Mean, range and variance values for five traits in segregating F$_3$ population of LBG17 X TAU-1 and BDU-4 X TAU-1 in blackgram

Characters	LBG17 X TAU-1			BDU-4 X TAU-1			Parental mean		
	Mean ± SE	Range	Variance	Mean± SE	Range	Variance	TAU-1	LBG-17	BDU-4
Number of pods per plant(#)	24.16 ± 0.25	7.90-42.10	18.71	31.04 ± 0.37	7.50-39.40	42.90	36.50	30.50	28.98
Number of seeds per pod(#)	7.80 ± 0.48	5.60-10.30	0.68	6.60 ± 0.04	2.90-6.83	0.48	6.10	5.99	5.93
Pod length(cm)	5.94 ± 0.02	3.80-7.60	0.22	5.53 ± 0.03	3.40-6.20	0.23	4.96	4.51	4.82
100-seed weight(g)	4.72 ± 0.04	2.50-6.80	0.54	5.90 ± 0.58	2.20-6.50	1.00	4.83	4.43	4.65
Seed yield per plant(g)	9.39 ± 0.22	3.6-25.30	15.10	13.10 ± 0.22	3.40-21.50	15.27	11.99	8.96	9.62

Number of pods per plant ranged from 7.5 to 39.4 with a mean of 31.04; number of seeds per pod ranged from 2.9 to 6.83 with a mean of 6.60; pod length ranged from 3.4 to 6.20 cm with a mean of 5.53 cm; 100- seed weight ranged from 3.60 to 6.50 g with a mean of 5.90g and seed yield per plant ranged from 3.4 to 21.50 g per plant with a mean of 13.10 g.

4.4 Variability parameters

Variability parameters were computed for five quantitative traits *viz.,* number of pods per plant, number of seeds per pod, pod length, 100- seed weight and seed yield per plant in F_2, F_3 populations of the crosses LBG17 X TAU-1 and BDU-4 X TAU-1. The results are presented in Table 18 and 19, respectively.

4.4.1 LBG17 X TAU-1

4.4.1.1 F_2 population

All the traits except seed yield and number of seeds per pod *viz.,* number of pods per plant (18.04% and 14.48%), pod length (13.35% and 11.30%) and 100 seed weight (15.05% and 11.12%) recorded moderate GCV and PCV estimates. While seed yield per plant recorded high PCV and GCV values (42.08% and 28.46%), respectively, seeds per pod recorded low PCV and GCV values (10.03% and 7.85%), respectively.

Heritability in broad sense was high for number of pods per plant (64.44%), seeds per pod (61.19%), pod length (71.64%) and 100 seed weight (54.60%), while seed yield per plant (45.74%) recorded moderate estimate of broad sense heritability value.

Genetic advance was high for number of pods per plant (5.88) and seed yield per plant (3.82). Low for the trait seeds per pod (1.03), pod length (1.21cm) and 100 seed weight (0.82g).

Genetic advance as percent mean was high for number of pods per plant (23.95%), and seed yield per plant (39.65%), where as it was moderate for seeds per pod (12.65%), pod length (19.71%) and 100 seed weight (16.93%).

4.4.1.2 F_3 population

All the traits except seed yield *viz.,* number of pods per plant (17.9% and 14.43%), number of seeds per pod (10.6% and 8.54%), pod length (7.91% and 6.59%) and 100 seed weight (15.57% and 11.21%) recorded moderate GCV and PCV estimates, while seed yield per plant recorded high PCV and GCV values (41.5% and 26.28%).

Heritability in broad sense was high for number of pods per plant (64.9%), seeds per pod (64.99%), pod length (69.23%) and 100 seed weight (51.85%). While seed yield per plant (40.09%) recorded moderate estimate of broad sense heritability value.

Genetic advance was high number of pods per plant (5.79) and seed yield per plant (3.22), low for the trait seeds per pod (1.11), pod length (0.67) and 100 seed weight (0.78).

Genetic advance as percent mean was high for number of pods per plant (45.67%), 100 seed weight (41.1%) and seed yield per plant (38.87%),whereas it was moderate for seeds per pod (13.15%), pod length (17.32%).

4.4.2 BDU-4 X TAU-1

4.4.2.1 F_2 population

High PCV and GCV estimate were observed for number of pods per plant (33.18% and 28.16%), 100 seed weight (25.52% and 23.91%). Moderate PCV and GCV values were observed for number of seeds per plant (13.46% and 9.44%). On the other hand low GCV and PCV were noticed for pod length (8.01% and 7.52%).

Heritability in broad sense was high for number of pods per plant (72.02%), pod length (88.24%) and 100 seed weight (87.80%). Seeds per pod (49.25%) and seed yield per plant (40.53%) showed moderate estimate of broad sense heritability value.

Table 18. Estimates of components of variability, heritability (broad sense), expected genetic advance and genetic advance over mean for five traits in segregating F$_2$ population of LBG17 X TAU-1 and BDU-4 X TAU-1 in blackgram

Characters	LBG17 X TAU-1					BDU-4 X TAU-1				
	PCV	GCV	h^2%	GA	GAM	PCV	GCV	h^2%	GA	GAM
Number of pods per plant	18.04	14.48	64.44	5.88	23.95	33.18	28.16	72.02	10.04	49.23
Number of seeds per pod	10.03	7.85	61.19	1.03	12.65	13.46	9.44	49.25	0.74	13.65
Pod length	13.35	11.30	71.64	1.21	19.71	8.01	7.52	88.24	0.75	14.55
100-seed weight	15.05	11.12	54.60	0.82	16.93	25.52	23.91	87.80	2.11	57.70
Seed yield per plant	42.08	28.46	45.74	3.82	39.65	31.01	19.74	40.53	3.41	46.16

Table 19. Estimates of components of variability, heritability (broad sense), expected genetic advance and genetic advance over mean for five traits in segregating F$_3$ population of LBG17 X TAU-1 and BDU-4 X TAU-1 in blackgram

Characters	LBG17 X TAU-1					BDU-4 X TAU-1				
	PCV	GCV	h^2%	GA	GAM	PCV	GCV	h^2%	GA	GAM
Number of pods per plant	17.90	14.43	64.90	5.79	23.97	33.52	27.26	66.13	8.93	45.67
Number of seeds per pod	10.60	8.54	64.99	1.11	14.18	13.32	9.22	47.92	0.68	13.15
Pod length	7.91	6.59	69.23	0.67	11.29	9.67	9.02	86.96	0.86	7.32
100-seed weight	15.57	11.21	51.85	0.78	16.63	24.88	22.28	80.20	1.66	41.10
Seed yield per plant	41.50	26.28	40.09	3.22	34.28	29.83	23.73	63.26	5.09	38.87

Genetic advance was high for umber of pods per plant (10.04), 100 seed weight (2.11) and seed yield per plant (3.41) and low for the trait seeds per pod (0.74) and pod length (0.75) and 100 seed weight (2.11).

Genetic advance as per cent of mean values was high for number of pods per plant (49.23%), 100 seed weight (57.70%) and seed yield per plant (46.16%) and low for seeds per pod (13.65%) and pod length (14.55%).

4.4.2.2 F_3 population

High PCV and GCV estimate were observed for number of pods per plant (33.52% and 27.26%), 100 seed weight (24.88% and 22.28%) and seed yield per plant(29.83% and 23.73%),. Moderate PCV and GCV values were noticed for number of seeds per plant (13.32% and 9.22%). On the other hand low GCV and PCV were recorded for pod length (9.67% and 9.02%).

Heritability in broad sense was high for number of pods per plant (66.13%), pod length (86.96%), 100 seed weight (80.2%) and seed yield per plant (63.26%), while seeds per pod (47.92%) recorded moderate estimate of broad sense heritability value.

Genetic advance was high for number of pods per plant (8.93), and seed yield per plant (5.09), low for seeds per pod (0.68) and pod length (0.86) and 100 seed weight (1.66).

Genetic advance as per cent of mean values was high for number of pods per plant (45.67%), 100 seed weight (41.1%) and seed yield per plant (38.87%) and low for seeds per pod (13.15%) and pod length (17.32%).

4.5 Inter-character correlations

The phenotypic correlation among five characters was estimated to determine the nature of relationship in the two F_2 populations and the results are presented below (Table 20).

4.5.1 F_2 population of the cross LBG17 X TAU-1

Association of seed yield with its contributing characters

Seed yield per plant exhibited significant positive association with pods per plant (0.46), pod length (0.19) and 100 seed weight (0.48), and nonsignificant positive association with seeds per pod.

Association among other seed yield contributing characters

Number of pods per plant exhibited significant positive association with 100 seed weight (0.30) and seed yield per plant (0.46) and positive non significant association with seeds per pod and pod length. Number of seeds per pod exhibited significant association with pod length (0.21) and 100 seed weight (0.11). Pod length exhibited significant positive association with 100 seed weight (0.12).

4.5.2 F_2 population of the cross BDU-4 X TAU-1

Association of seed yield with its contributing characters

Seed yield per plant exhibited significant positive association with pods per plant (0.84), pod length (0.14), 100 seed weight (0.55), and seeds per pod (0.22).

Association among other seed yield contributing characters

Number of pods per plant exhibited significant positive association with seeds per pod (0.23), pod length (0.17) and 100 seed weight (0.63). Number of seed per pod exhibited significant association with pod length (0.65) and 100 seed weight (0.23). Pod length exhibited significant positive association with 100 seed weight (0.25).

Table 20. Phenotypic correlation coefficient of seed yield per plant with other characters in F_2 populations of LBG17 X TAU-1 and BDU-4 X TAU-1 in blackgram

Population	LBG17 X TAU-1				BDU-4 X TAU-1			
Characters	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4
X_2	0.10				0.23**			
X_3	0.04	0.21**			0.17**	0.65**		
X_4	0.30**	0.11*	0.12*		0.63**	0.23**	0.25**	
X_5	0.46**	0.04	0.19**	0.48**	0.84**	0.22**	0.14*	0.55**

** Significant at 1.0 per cent level of probability   * Significant at 5.0 per cent level of probability

X_1 – Number of pods per plant

X_2 – Number of seeds per pod

X_3 – Pod length

X_4 – 100 Seed weight

4.6 Transgressive segregants

4.6.1 LBG17 XTAU-1

4.6.1.1 F_2 population

The extent of transgressive segregation for yield and its component traits is shown in Table 21.

The number of transgressive segregants and per cent of transgressive segregation for seeds per pod was 51 and 17 per cent respectively. This was followed by 100 seed weight for which 49 F_2 segregants showed transgressive segregation which accounted for 16.33 per cent. Pod length and seed yield per plant were next in order with 34 segregants shows transgressive segregation for pod length which amounted to 11.33 per cent. Similarly for seed yield per plant 29 F_2 segregants transgressed the check or better parent yield which accounted to 9.67 per cent. The least number and per cent of transgressive segregation was observed for number of pods per plant.

4.6.1.1 F_3 population

The extent of transgressive segregation for yield and its component traits is shown in Table 22.

The extent to which F_3 families showed better performance over the check or better parent for individual trait followed same pattern as that of F_2 transgressive segregation except for seed yield per plant. As many as 62 families were superior to the check for seeds per pod which were the highest among all the families, this was followed by 100 seed weight for which 43 families were found to superior over better parent. Percentage wise it was 20.66 for seeds per pod and 14.33 for 100 seed weight. The per cent of F_3 families being superior performance for pods per plant and seed yield per plant was 6.33 and 5.66 respectively.

4.6.2 BDU-4 XTAU-1

4.6.2.1 F_2 population

The extent of transgressive segregation for yield and its component traits is shown in Table 23.

Fifty six F_2 segregants showed transgressive segregation for seed weight amounting to 18.66 per cent, following this was number of seeds per pod for which 49 segregants were superior to check accounting to 15.66 per cent. Next in order was seed yield per plant for which 31 plants showed transgressive segregation accounting for 10.30 per cent, for pods per plant and pod length the extent of transgressive segregation was less.

4.6.2.2 F_3 population

The extent of transgressive segregation for yield and its component traits is shown in Table 24.

Seventeen per cent of F_3 families showed superior performance than check for 100 seed weight which was followed by seed yield per plant (12.44%). Number of seeds per pod and pod length were next in order with 11.00 and 10.67 per cent families showing better performance over check respectively, for number of pods per plant it was 7.33 per cent.

4.7 Identification of high yielding and disease resistant lines

4.7.1 Identification of high yielding and powdery mildew resistant lines

An attempt was made to identify high yielding and powdery mildew resistant segregants in the F_2 population of the cross LBG-17 X TAU-1, on the basis of the two criteria, 12 progeny segregants were identified. The segregants reading more than the mean plus one standard deviation along with per cent disease score less than 20 per cent were identified for this purpose which can be seen from the Table 25.

Table 21. Frequency of transgressive segregants for seed yield and its component traits in F_2 population of the cross LBG17 X TAU-1 in black gram

Characters	Numbers	Per cent
Number of pods per plant (#)	13	4.33
Number of seeds per pod (#)	51	17.00
Pod length (cm)	34	11.33
100 seed weight (g)	49	16.33
Seed yield per plant (g)	29	9.67

Table 22. Frequency of superior families for seed yield and its component traits in F_3 population of the cross LBG17 X TAU-1 in black gram

Characters	Numbers	Per cent
Number of pods per plant (#)	19	6.33
Number of seeds per pod (#)	62	20.66
Pod length (cm)	30	10.00
100 seed weight (g)	43	14.33
Seed yield per plant (g)	17	5.66

Table 23. Frequency of transgressive segregants for seed yield and its component traits in F_2 population of the cross BDU-4 X TAU-1 in black gram

Characters	Numbers	Per cent
Number of pods per plant (#)	16	5.33
Number of seeds per pod (#)	47	15.66
Pod length (cm)	7.0	2.33
100 seed weight (g)	56	18.66
Seed yield per plant (g)	31	10.33

Table 24. Frequency of superior families for seed yield and its component traits in F_3 population of the cross BDU-4 X TAU-1 in black gram

Characters	Numbers	Per cent
Number of pods per plant (#)	22	7.33
Number of seeds per pod (#)	33	11.00
Pod length (cm)	32	10.67
100 seed weight (g)	51	17.00
Seed yield per plant (g)	37	12.44

Table 25. High yielding and powdery mildew resistant segregants in F_2 generation of LBG-17 X TAU-1 and their performance in F_3 generation in blackgram

S. No	Pedigree in F_2	F_2		F_3			
		Yield (g per plant)	Disease severity (%)	Yield (g per plant)	Range (F_3)	Disease severity (%)	Range (F_3)
1	2	20.6	0.0	17.50	12.6-20.22	8.5	0-10
2	19	22.2	0.0	21.77	18.2-22.10	0.0	0
3	58	15.6	15.6	18.60	15.3-19.25	10.5	0-20
4	85	21.5	0.0	20.92	16.32-21.20	0.0	0
5	135	19.9	0.0	14.50	12.30-16.20	22.0	0-25
6	164	19.1	0.0	18.14	15.42-20.32	22.4	0-30
7	170	14.2	0.0	13.61	10.50-15.65	20.0	10-25
8	185	21.2	16.7	13.20	10.26-15.22	21.0	10-25
9	196	21.2	0.0	20.90	18.36-22.12	5.6	0-10
10	233	13.5	0.0	22.22	19.63-23.12	0.0	0
11	254	17.6	16.5	15.33	10.56-16.58	24.3	10-25
12	292	13.2	12.6	16.88	12.35-18.63	15.3	10-20
13	TAU-1	12.3	85.5	11.99	-	86.3	-

Table 26. High yielding and MYMV resistant segregants in F$_2$ generation of BDU-4 X TAU-1 and their performance in F$_3$ generation in blackgram

S. No	Pedigree in F$_2$	F$_2$		F$_3$	Range (F$_3$)	Disease severity (%)	Range (F$_3$)
		Yield (g per plant)	Disease severity (%)	Yield (g per pl)			
1	12	16.50	9.8	15.30	10-50-16.25	5.3	5-8
2	17	17.20	5.0	16.90	12.23-17.85	2.5	1-5
3	22	19.20	1.0	17.90	16.53-8.62	26.0	1-5
4	63	13.90	9.8	12.50	10.23-15.48	10.0	10
5	129	14.20	8.0	15.60	12.56-16.83	8.5	8-10
6	133	14.60	10.0	16.70	13.45-18.26	9.6	8-10
7	165	14.00	9.8	16.90	12.59-18.21	10.0	10
8	208	13.50	10.0	12.60	10.56-14.3	8.0	5-10
9	294	15.40	1.0	16.50	13.59-18.57	1.5	1-5
10	TAU-1	11.80	48.6	11.40	-	50.0	-

The yield of these segregants range from 13.2g per plant (F_2-292) to 22.2 g per plant (F_2-19) as compared to check yield 12.3 g per plant. Further the per cent disease severity of these 12 superior F_2 segregants ranged from 0 to 16.7 per cent, as many as 8 of the 12 superior segregants showed zero per cent disease severity. The per cent disease severity was 85.5 per cent in check variety. The progeny rows derived from these promising F_2 segregants were evaluated again for yield and disease incidence in F_3 (Table 24). The yield of F_3 family ranged from 13.2 g per plant to 22.0 g per plant, the disease severity ranged from 0 to 24.3 per cent. Three of the F_3 families, F_3-19, F_3-85 and F_3-233 which had zero per cent disease severity again confirmed to show zero per cent disease severity indicating their highly resistance nature to powdery mildew. Check variety TAU-1 showed per cent disease severity of 86.3 per cent.

4.7.2 Identification of high yielding and MYMV resistant lines

The promising segregants showing higher yield (more than mean yield of check plus one standard deviation) and resistance reaction to MYMV (less than 10 per cent of disease severity) were identified (Table 26). The yield of the nine promising segregants was ranged from 13.5 g per plant (F_2-165) to 19.2 g per plant (F_2-22) as against the mean yield of check 11.8 g per plant. Per cent disease severity of F_2-22 and F_2-294 was one and that of F_2-17 was five. These promising F_2 segregants were advanced to F_3 as progeny rows and evaluated as families for yield and per cent disease severity. The progeny of F_2 segregants showed similar yield advantage which ranged from 12.60 g per plant (F_3-165) to 17.90 g per plant (F_3-22) as compared to yield of check TAU-1 which was 8.40 g per plant. Segregants, F_2-17, F_2-22 and F_2-294, which were highly resistant to MYMV through consistency in F_3 also. The MYMV incidence of check evaluated along with F_2 population was 48.6 per cent and it was 50 per cent in F_3.

5. DISCUSSION

Although blackgram is an important legume species, research attention paid to it is negligible and the productivity of blackgram is low. There are several constraints that lead to the reduction of yield in this legume. Both biotic and abiotic stresses cause heavy losses resulting in reduced yields. Relatively the loss due to biotic stress is more than the loss caused due to abiotic stresses. Among the biotic stresses powdery mildew and MYMV are important diseases on blackgram. Powdery mildew accounts to an estimated yield loss of 20-40% (Singh *et al.*, 1980) and annual loss due to MYMV is around 300 M$ (Verma *et al.*, 1992). Therefore resistance breeding assumes greater importance in improving and enhancing yield. In Karnataka TAU-1, the most widely grown blackgram variety is under commercial cultivation. Although it is high yielding and widely adopted, it is highly susceptible to powdery mildew and MYMV. It shows powdery mildew disease incidence to the extent of 85 per cent and MYMV to the extent of 50 per cent, indicating high degree of susceptibility. It is therefore, important to incorporate resistance to both the diseases in TAU-1. Powdery mildew disease is more severe during rainy season while, MYMV during summer. Therefore powdery mildew resistant TAU-1 will become a boon to the farmers during rainy season where as MYMV resistant TAU-1 will be useful to those farmers who grow blackgram during summer season especially in paddy fallow areas.

An early study conducted in the Department of Genetics and Plant Breeding at U.A.S Dharwad. 110 collections were screened against these two diseases indicated that LBG-17 is resistant to powdery mildew while BDU-4 is found highly resistant to MYMV. These two genotypes were therefore selected as donors for the improvement of the above said diseases. Using TAU-1, as agronomic base, was crossed to LBG-17 for enhancing resistance to powdery mildew and was also crossed to BDU-4, as a donor, for improving resistance to MYMV. It is also worth mentioning the importance of identifying molecular markers linked to such serious diseases which help in improving efficiency and accuracy of selection for such diseases.

Constructing a molecular linkage map is now routine to trace the valuable alleles in segregating populations. Once the framework maps are generated, a large number of markers derived from various techniques (RFLP, AFLP, SSR *etc*) are used to saturate the maps. Hence, DNA marker based genetic linkage would enable breeders to effectively pyramid genes for resistance to biotic and abiotic stresses in an agronomically enhanced breeding populations in a much shorter time than would be possible by conventional breeding techniques. The possibility of isolating and selecting transgressive segregants for both yield and disease resistance has also been explored.

The two crosses that is LBG-17 X TAU-1 and BDU-4 X TAU-1 representing diverse combination for powdery mildew and MYMV would serve as ideal mapping populations respectively for these two diseases. Like in any other legumes the variability in blackgram is also limited, obviously in order to enhance the probability of selecting better types variation has to be generated. Among different approaches used for creating variation, hybridization is one of the important approaches to generate variation for targeted traits.

All forms of crop improvement activities through breeding contemplate an eventual boost in genetic potential for yield. Since yield is polygenically controlled and highly influenced by environment, selection based on yield alone is not effective. The breeder hence, develops into proposition of selecting for high yield indirectly through yield associated and highly heritable characters after eliminating environmental components of phenotypic variation. An attempt to improve a character by selection would be futile unless a major portion of variation is heritable. This depends entirely on the magnitude of genetic variability in the source population in respect of yield and its components. Most of the genetic variability available today in plant collection is the result of spontaneous mutation, recombination and exposure to natural selection. Over centuries, various crop plants have moulded themselves to the needs of nature through forces of evolution. Since the beginning of human civilization, man has become another force mending the trends of evolution of crop plants. The agriculturist right from prehistoric days has chosen plants and grown them according to his needs. As time passed and man's pursuit for better genotypes progressed the concept of hybridization evolved as a means to generate more variability through recombination. This variability

generated is a pre-requisite for any breeding programme aimed at improving the yield and other characters. Thus, it is imperative to have information on both genotypic and phenotypic coefficients of variation to get an idea regarding the heritability of character. The information on phenotypic coefficient of variation and heritability will be handy for prediction of the possible genetic advance by selection for the character. Besides, the knowledge of correlation and path coefficient analysis would assist setting up selection indices. The genetic parameters such as genotypic coefficient of variability and genetic advance helps to split the total variability into heritable and non-heritable components.

The population generated as mentioned in earlier paragraph would become important for improving yield besides incorporating resistance to these species. The present study aimed at developing the mapping populations, the two crosses were advanced through filial generation and during the period of study both the populations were advanced up to F_3. Therefore they provide an opportunity not only to study the inheritance pattern of two diseases but also an opportunity to assess variation for yield and its component traits to look the possibility of selecting for higher productivity as well as possessing desire level of resistance to these two diseases accordingly. Keeping all these in view, the investigation was carried out involving the genotypes TAU-1, LBG-17 and BDU-4 with which two crosses *viz.,* LBG-17 X TAU-1 and BDU-4 X TAU-1 were developed with the objectives of studying inheritance pattern of disease resistance to powdery mildew and MYMV, developing mapping populations, identifying the marker linked to resistance for two diseases and also to study variation for yield and its component traits with possibility of looking for promising lines combining resistance and productivity. The results obtained from this study are discussed in the following paragraph.

5.1 Screening and inheritance of powdery mildew and MYMV disease resistance

5.2 Molecular marker studies.

5.3 Mean, Range and Variance

5.4 Variability parameters

5.5 Inter-character correlations

5.6 Transgressive segregants, high yielding and disease resistant lines

5.1 Screening and inheritance of powdery mildew and MYMV disease resistance

5.1.1 Powdery mildew

5.1.1.1 Screening for powdery disease

It is one of the most common diseases in all blackgram growing areas. The disease is caused by *Erysiphae polygoni* D.C. It is most severe during the dry season and practically non-existent during the wet season. The temperature from 22 to 26°C and relative humidity from 80 to 88 per cent are favourable for the development of the disease (Ilag, 1978). The fungus attacks all parts of the plant except roots. The initial symptoms are faint, slightly dark areas developing over the leaf later turning into small, white powdery spots. These spots enlarge, coalesce and develop into a complete coating by a white to dirty white powder consisting of mycelium and conidia. Defoliation takes place in case of severe infections. Pods are not formed and if formed they bear subnormal seeds. Powdery mildew is an obligate parasite and therefore, disease reaction can be studied in the field or greenhouse. Inoculation can be performed using infected plants and dusting spores onto the target plants, but environmental factors can influence the severity of the infection. A previous study by Thakur and Agrawal (1995) suggested that environmental factors had a large effect on the extent and severity of powdery mildew. In field condition disease spread was slow. However, under greenhouse conditions with disease inoculation, a much heavier and faster disease attack was observed in our study. The disease symptom occurred on blackgram seedlings at 10 days after inoculation. Mycelia were formed on most seedlings some of which died within two weeks after the symptoms were detected in green house condition.

Very little information is available in the literature about host plant resistance to powdery mildew in blackgram. Genotypic differences in the severity of infection (per cent leaf area infected; the latent period of the pathogen; days for appearance of visual symptoms, namely pustules) have been observed. The above parameters are widely used as a standard practice for assessment of fungal disease reactions (Johnson, 1984). Based on the above parameters, a rating scale given by Humphry e*t al.,* (2003) was adopted for screening the material against the powdery mildew in the present study.

F_1, F_2 and F_3 material along with parents was screened under both natural and artificial condition. There was high incidence of disease observed on TAU-1 compared to the resistant parent which showed a disease incidence grade of 0.2. The F_1 also showed similar reaction indicating dominance of resistance. In F_2 and F_3 material segregation for resistance and susceptibility was observed in 9:3:3:1 and 1:2:2:4:1:2:1:2:1 respectively which is expected where two genes govern the expression of the trait.

5.1.1.2 Inheritance of powdery mildew

The causal organism for blackgram is *Erysiphae polygoni* D.C. The same organism also affects mungbean causing powdery mildew. To our knowledge there have been few reports on inheritance of host plant resistance to powdery mildew. However previous studies on mungbean using both qualitative and quantitative genetic models suggested that there were several modes of inheritance of powdery mildew resistance. Yohe and Poehlman (1975) and Waraluk *et al.* (2009) reported that the resistance was controlled by several genes with additive effect. Work at AVRDC (Anon. 1979) suggested that a monogenic dominant resistance gene controlled the trait. Gawande and Patil (2003) reported that it is governed by more than one gene with both additive and dominance gene actions. Reddy *et al.* (1994) found that powdery mildew resistance is governed by two major dominant genes. Young *et al.* (1993) identified three QTL associated with powdery mildew resistance in mungbean, while Chaitieng *et al.* (2002) and Humphry *et al.* (2003) found one QTL responsible for the resistance. These reports indicate that different resistant sources may carry different resistance genes. This could be ascribed to the material used in the study and it suggests that there could be more genes governing resistance to MYMV in mungbean as also inferred by Reddy *et al.* (1994).

In our study, results indicated that powdery mildew resistance is controlled by two dominant genes (PM-1 and PM-2). The phenotypic reaction of these lines could be distinguished by different host pathogen interactions described as r_1 and r_2 respectively as was described by Reddy *et al.* (1994) in their studies.

5.1.2 MYMV

5.1.2.1 Screening for MYMV disease

Yellow mosaic virus disease is one of the important diseases occurring on blackgram. Mungbean Yellow Mosaic Virus (MYMV), a whitefly-transmitted geminivirus, causes disease in a variety of leguminous crops, but the most seriously affected are blackgram, mungbean, and soybean in the Indian sub-continent. This virus is assigned to the genus Begomovirus within the family Geminiviridae. Host plant resistance is the most common and efficient method of MYMV control. In blackgram, two symptom types- yellow mosaic and necrotic mottle, can be distinguished (Nair and Nene, 1974). Other crops, including cowpea, develop similar symptoms as a result of infection with MYMV. The necrotic mottle is usually associated with resistance (Verma *et al.*, 1992). Plants exhibiting necrotic mottle symptoms are not as severely affected as the plants exhibiting typical yellow mosaic. This suggests that the necrotic mottle is a type of resistant reaction to the disease (Biswas and Varma, 2001). For MYMV reaction on test material, the infector or spreader row method is recommended because it is simple and efficient (Nene *et al.*, 1972). YMV infection under artificial condition was monitored in summer season. At 20 days of crop growth no infection was noticed. Then onwards YMV symptoms started appearing. After 50 days, there was spectacular increase in YMV infection. By 80 days YMV infection in the population was 70 per cent or more. Such high level of summer infection may be attributed to the prevailing environmental condition.

Such favorable environmental factors influence considerably the high build up of white fly, the vector of YMV promoting the spread of YMV. Infection build up was observed abundantly during 60- 70 days of crop growth in summer and thus differential genotypic response was judged in summer during this specific stage of crop growth.

5.1.2.2 Inheritance of mungbean yellow mosaic virus

The lines involved in the cross BDU-4 X TAU-1 were screened during summer 2008 under artificial condition with abundant whitefly population. The parent BDU-4 recorded a mean score of 1.2 and was found to be resistant and similar pattern was observed on F_1. The second parent TAU-1, recorded a mean score of 7.6 and falls under the category susceptible. In F_2 and F_3 segregation for resistance and susceptibility was observed in 3:1 and 1:2:1 respectively as expected in Mendelian ratio for single gene governing resistance for disease.

There are conflicting reports about the genetics of resistance to MYMV, claiming both resistance and susceptibility to be dominant. In blackgram, resistance was found to be monogenic dominant (Dahiya *et al.,*1977, Kaushal and Singh, 1988). The digenic recessive nature of resistance was reported by Singh (1980), Dwivedi and Singh (1985), Verma and Singh (1989), and Singh *et al.* (1998). Monogenic recessive control of MYMV resistance has also been reported (Reddy and Singh, 1990). In the present study, an attempt was made to investigate the inheritance of disease resistance to the virus in an intervarietal crosses of blackgram. The putative dominant gene is completely dominant in the F_1 and segregates 3:1 (R:S) in the F_2 as explained earlier.

The F_3 population of the crosse involving resistant and susceptible parents showed 1 resistant: 2 segregating: 1 susceptible segregation, suggesting that a single gene controls resistance. Thus the results of the present study confirm earlier reports made by Dahiya *et al*. (1977), Kaushal and Singh (1988) and Gupta *et al.* (2005). The results obtained by Singh (1980) and Reddy and Singh (1990), which suggested monogenic/digenic recessive control of resistance and Honda and Ikegami (1986) who suggested two recessive genes for resistance in a different host species, i.e. mungbean, are thus at variance with our report. This could be ascribed to the material used in the study.

5.2 Molecular marker studies

5.2.1 Powdery mildew

5.2.1.1 Parental polymorphism and Bulk Segregant Analysis

Parental polymorphism using 469 SSR primer combinations were carried out with parents LBG-17 (resistance for powdery mildew disease), BDU-4 (resistance for MYMV) and TAU-1 (susceptible for both the disease) to identify polymorphic markers. Out of 469 SSR primers used, only 32 were polymorphic between the parents LBG-17 and TAU-1 and 24 were polymorphic between the parents BDU-4 and TAU-1. Parental polymorphic survey using SSR markers indicated low polymorphism which may be due to its cross species nature as SSR markers used were from different legumes which is expected in microsatellites.

Bulk Segregant Analysis (BSA) is a rapid method for identifying markers that are linked to the genes of interest in specific regions of the genome (Michelmore *et al.* 1991). In the present study, SSR markers polymorphic between the parents of the cross LBG-17 X TAU-1 were subjected to Bulk Segregant Analysis to identify putative markers linked to gene resistant for powdery mildew. Two SSR markers polymorphic in parents of the above cross were also found to be polymorphic between resistant and susceptible bulks indicating that markers are putatively linked to resistant gene in blackgram. Earlier in mungbean also RFLP markers were reported to be linked with the resistance genes by Humphry *et al.* (2003). Chaitieng *et al.* (2002) also reported AFLP marker associated with resistant gene in mungbean. To our knowledge, this is the first report of SSR marker associated with powdery mildew disease resistance in blackgram.

5.2.1.2. SSR marker and powdery mildew association analysis.

The location of genes using QTL analysis is a labourious and time consuming process since all the polymorphic markers are supposed to be screened on the segregating population. In recent years, some markers tightly linked to genes were found by using BSA.

BSA was first reported by Michelmore *et al.* (1991) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. Using BSA combining SSR and single markers analysis, we have identified two SSR markers which are potentially linked to powdery mildew resistant genes in blackgram. By testing the 300 F_2 individuals, genetic linkage analysis indicated that two SSR markers, B-210 and B-230 were highly linked to the powdery mildew resistance gene. They accounted for 33.5 and 36.04 per cent of the total variation in powdery mildew resistance in F_2 population. Therefore, the two genetic loci, especially B-210 and B-230 suggested a promising application of molecular-assisted selection for improving powdery mildew resistance in blackgram breeding programmes.

Further in future, identification of markers linked to powdery mildew resistant gene would prove useful in initiating the cloning and characterization of powdery mildew resistant genes using one or more of the currently available methods for map based cloning. The study described above represents the first step towards reaching goals for improvement of blackgram for powdery mildew disease resistance using DNA markers.

5.2.2 MYMV

5.2.2.1 Parental polymorphism and Bulk Segregant Analysis

Similarly an exercise was made to identify SSR markers linked to MYMV disease resistance. Parental polymorphism was assayed using 469 SSR primers, out of these 263 primers were amplified however, only 24 primers were found to be polymorphic between the parental lines, BDU-4 and TAU -1. The BSA using 24 polymorphic primers on F_2 population failed to show any association of a primer with MYMV disease resistance. It only suggested that some more polymorphic markers needs to be screened to identify linked marker to disease resistance.

5.3 Mean, Range and Variance

TAU-1, one of the parents used in the present investigation as agronomic base, is high yielding and widely adapted variety. It was crossed to LBG 17 to develop mapping population and identify markers for powdery mildew resistance. Similarly, it was crossed to BDU-4 for developing mapping population and identify marker for resistance to MYMV disease. Incidentally, variability estimates were made on these two populations to assess the worth of the populations for isolating high yielding lines besides looking for disease resistance.

Hybridization is widely accepted conventional approach to develop the desired variability for productivity and its related traits. Often high yielding widely adapted agronomic base is used as one of the parents while the other parent used to supplement some of the desirable traits probably other character. In order to assess the variability important parameters like mean, range, variance, coefficient of variation, heritability and genetic advances are computed so that the analysis would indicate the worth of the population for carrying it further.

The mean performances in two different segregating populations in respect of five quantitative characters are given in Table 16. As already highlighted earlier, TAU-1, a widely adapted popular cultivar, is distinctly different from other two genotypes *viz.* LBG-17 and BDU-4, especially in respect of yield and yield related traits. TAU-1 has number of seeds per pod and high seed weight (4.9g/100 seeds compared to other two genotypes). It is also quite distinct being semi erect with high pod length and very high seed yield per plant. But with respect to disease resistance for powdery mildew and MYMV, it was susceptible, while LBG 17 was resistant to powdery mildew and BDU-4 was resistant to MYMV.

When TAU-1 was crossed with LBG 17, the F_2 population of the cross showed higher mean values than the check (TAU-1) in respect of number of seeds per pod and pod length. However, it showed relative on par values with mean of check in respect of seed weight. F_2 mean was less than the check in respect of pods per plant and seed yield. Similarly in other cross, BDU-4 X TAU-1, F_2 population showed higher mean value than the check in respect of pod length, 100 seed weight and seed yield per plant. However, it showed relative on par value with mean of check in respect of number of seeds per pod and F_2 mean value was less than the check in respect of number of pods per plant.

Shift in the mean values towards positive direction was apparent for the character seed yield per plant in the population of the cross BDU-4 X TAU-1 as compared to other cross. Hence, it may be concluded that in blackgram, the increase in mean values as a result of hybridization indicates, scope for further improvement in traits like number of pods per plant, number of seeds per pod, and pod length, test weight and other characters in subsequent generations (F_3 and F_4), there by facilitating selection of transgressive segregants in later generations. The results are in line with the findings of Parveen *et al.* (2007).

The critical parameters are range and variance which decide the higher extreme value of the cross. The report of Konda *et al.* (2009) gives higher ranges for all the five quantitative characters observed under similar study. Among the LBG17 X TAU-1 and BDU-4 X TAU-1 population, for all the characters wide range was observed in LBG17 X TAU-1 population. The range observed was wider for number of pods per plant, number of seeds per plant, pod length, test weight and seed yield per plant in F_2 population. While, F_2 recorded wide range than F_3 for all the characters, overall, there is high amount of variation among the F_2 population. This might be due to diversity among parental genotypes. Similar results were obtained by Salimath *et al.* (2007) in F_2 and F_3 population of cowpea.

A critical examination of range of values revealed some interesting and useful information on some characters. The range for number of pods per plant was wide in both the F_2 populations. The upper value of range for seed yield in F_2 segregants was nearly two times higher than mean seed yield of commercial cultivar like TAU-1 indicating the scope for selecting the high yielding segregants from these populations.

In general, hybridization increased the range of all the polygenic traits studied. An upper limit of range was found to increase in two populations for all the characters studied except for 100 seed weight where in moderate range was found. This shows that hybridization has been useful to enhance the variability. Therefore, it would offer chances for the breeder to isolate genotypes having desirable combination of characters as transgressive segregants for group of important characters which leads to higher number of pods per plant, pod length and seed yield per plant. In the present study, higher range is in accordance with the earlier reports made by Arulbalachandran *et al.* (2010), Konda *et al.* (2009) and Parveen *et al.* (2007).

The mean values for the five traits were on par with each other in the two generations.

5.4 Variability parameters

The genetic gain through selection depends on the quantum of variability and extent to which it is heritable. Further heritability estimate in narrow sense which accounts for additive variation is more reliable than heritability estimates in broad sense. In present study heritability estimates have been made following F_2-F_3 regression method and hence are more reliable. The possibility of improving productivity and its component traits through segregation of these crosses appear to be promising. Besides desirable range of expression, that is, more positive towards desirable direction, the GCV and heritability values also resembled high. Further indicating the possibility of getting better success by selection in these two populations.

There were large differences in the variances for most of the characters under study in two different populations in two generation. Among all the segregating populations, the population BDU-4x TAU-1 exhibited higher variances for number of pods per plant, 100 seed weight than the populations LBG-17x TAU-1. For remaining traits both the populations were on par with each other in F_2 and F_3 populations. The higher variances for most of the characters indicated the presence of sufficient amount of variability which had been generated in segregating populations (Tiwari *et al.,* 1996).

5.4.1 Phenotypic and genotypic coefficient of variation

Although range can provide a preliminary idea about the variability but coefficient of variation is reliable as it is independent of unit of measurement. The extent of variability as measured by PCV and GCV also gives information regarding the relative amount of variation in different populations. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) showed wide variation for most of the characters in both the F_2

populations. Similar results were reported by Rahim *et al*, (2010), Singh *et al.* (2009) and Konda *et al.* (2009), as expected, PCV values were invariably higher than GCV for all the characters. The difference between PCV and GCV values reflect the influence of environmental effect which was high for number of pods per plant, number of seed per pod and seed yield per plant and low for remaining traits under study.

In general, F_2 population of the cross BDU-4 x TAU-1 exhibited higher PCV and GCV values for pods per plant, number of seeds per pod and 100 seed weight. The progenies of the cross LBG17 X TAU-1 exhibited the highest values for seed yield per plant indicating that there is greater scope for selection for improvement of these characters in desirable direction and also greater diversity among the progenies for these characters. Similar reports of higher coefficient of variation was also reported by Byregowda *et al.* (1997b), Borah and Hazarika (1995) and Patel and Shah (1982b).

However, in the present investigation, pod length and number of seed per pod had moderate GCV and PCV values in the two populations in two generations. These results are in accordance with results of Singh *et al* (2009) and Konda *et al.* (2009. Low to moderate GCV and PCV values for above two characters indicate the influence of the environment on these traits and also limited scope of selection for improvement of these characters.

The GCV and PCV values for number of pods per plant and seed yield were high in both the F_2 populations. Concurrent results to this effect were also reported by Konda *et al.* (2009) and Singh *et al* (2009).

The high, medium and low PCV and GCV indicate the potentiality with which the characters express. However, GCV is considered to be more useful than PCV for assessing variability since, it depends on the heritable portion of variability. The difference between GCV and PCV for pods per plant and seed yield per plant were high indicating the greater influence of environment on the expression of these characters, whereas, for remaining other traits were least influenced by environment.

The results of the above experiments showed that variability can be created by hybridization. However, the variability generated to a large extent depends on the parental genotype and the trait under study.

5.4.2 Heritability and genetic advance

It is not the magnitude of variation but the extent of heritable variation, which matters most for achieving gains in selection programme. The coefficient of variation indicates only the extent of variation for a character and does not discriminate the variability into heritable and non-heritable portion. The heritability worked out in broad sense would suggest how far the variation is heritable and selection is effective. Though the heritability estimates are the true indicators of genetic potentiality of the genotypes which can be used as a tool for selection (Islam *et al.*, 1999), changes in the values of the heritability due to fluctuations of the environmental factors detract for total dependence on such estimates. However, heritability estimates when considered in conjunction with the predicted genetic gain form a reliable tool for selection. They indicate the expected genetic advance of a character in response to the certain selection pressure imposed on them.

Most of the characters exhibited low to moderately high heritability in two segregating populations. High heritability values of more than 80 per cent have been observed for pod length and 100 seed weight in the cross BDU-4 x TAU-1 in both the generations. These results indicating high heritabilities for pod length and 100 seed weight and are in accordance with the report of Arulbalachandran *et al.* (2010), Rahim *et al.* (2010) and Konda *et al.* (2009).

Low to moderately high heritability estimates were observed for all five traits in, LBG17 X TAU-1 population in both the generation and number of pods per plant, seeds per pod and seed yield in the population of BDU-4x TAU-1 in both the generations. These results are also in line with the findings of Mishra (1983). Low to moderate broad sense heritability for seed yield and important yield component traits such as number of pods per plant and seeds per pod indicate the greater influence of environment in the expression of these traits. Thus, the improvement of yield through yield components should be based not only on simple selection but also on progeny test as blackgram is considered to be highly plastic as far as the expression of the morphological characters are concerned.

High heritability for some of the traits like 100 seed weight, pod length and pods per plant indicated that these traits are generally governed by additive gene effects.

Genetic advance can however, help to predict the extent of improvement that can be achieved for the traits. A high genetic gain along with high heritability would suggest suitable conditions for making effective selection. The genetic advance was high for the traits seed yield per plant and number of pods per plant in two populations in two generations (Rahim *et al.*, 2011), (Arulbalachandran *et al.*, 2010), (Singh *et al.,* 2009) and Konda *et al.,* 2009). The high genetic advance coupled with moderate to high heritability estimates for these traits suggested the importance of additive genetic variance for these traits.

Even though, number of pods per plant, seed yield per plant recorded moderately high heritability in two segregating populations, with comparatively high genetic advance indicate the substantial contribution of additive genetic variance in the expression of these characters. But other characters like number of seed per pod, pod length and 100 seed weight recorded moderate to high heritability in two segregating populations with low genetic advance indicating the greater role of non-additive genetic variance and epistatic and dominant environmental factors controlling the inheritance of these traits.

With regard to number of pods per plant BDU-4 X TAU-1 was found more promising, where there is more possibility of getting better gain since the mean of the F_2 population was high in BDU-4 X TAU-1 than LBG 17 X TAU-1 besides higher heritability and thus resulting in higher genetic advance over mean (Table 18). With respect to number of seeds per pod both the populations appear to be equally promising where as with respect to pod length LBG 17 X TAU-1 population was found to be better with higher GCV and genetic advance. For 100 seed weight BDU-4 X TAU-1 has advantage over LBG 17 X TAU-1 with higher GCV and heritability values resulting into considerably high expected genetic gain. With respect to seed yield per plant although both the populations have more or less similar variability estimates. Since the mean of F_2 population of BDU-4 X TAU-1 is higher, there are better chances of releasing superior lines in this population.

Comparison of genetic advance as per cent mean value in two populations in F_2 and F_3 generations revealed higher genetic advance for number of pods per plant, and seed yield per plant and low to moderate genetic advance with high heritability for seeds per pod, pod length and 100 seed weight. The heritability estimates of pod length, 100 seed weight were high with moderate genetic advance as per cent mean compared to other traits, hence, priority should be given to these traits in formulating selection strategies on the basis of these characters to realize better gains by selection.

The trend of mean and nature of variability was same in F_3 population as observed in F_2 populations. Therefore, whatever inference made for productivity and its component traits on the basis of F_2 results holds good for F_3 population also.

It is interesting to note here that mean performance in respect of yield per plant and its component traits were same in both F_2 and F_3 of the cross. It may be noted that F_2 was advanced to F_3 by selecting the productive segregants in F_2. Consequently, the fact that selection has maintained the mean performance of these traits clearly indicate that additive effects may be governing the expression of these traits as it is additive variance, which is fixable (Falconer, 1960).

Comparison of performance of F_3 with F_2 generation revealed a considerable reduction in variance in F_3 for important character like seed yield and other characters due to reduction in heterozygosity compared to F_2.

Based on the population mean and estimate of genetic gain, the possible improvements for different traits in F_4 population with 5 per cent selection intensity are given in Table 27. The results clearly indicate that both the populations have equal potential for pod length, however for number of pods per plant, 100 seed weight and seed yield per plant, BDU-4 X TAU-1 looks to be superior over LBG 17 X TAU-1. With regard to seed yield per plant BDU-4 X TAU-1 has advantage with higher expected mean of F_4 (18.19g per pl) than LBG 17 X TAU-1 (12.61g per pl). However, for selection of lines combining productivity and powdery mildew resistance the targeted population of LBG 17 X TAU-1 has to be explored and for developing lines with higher yield and MYMV resistance population of BDU-4 X TAU-1 has to be explored.

Table 27. Prediction of F_4 mean on the basis of estimated genetic gain computed from F_3 data in two populations of blackgram

Characters	LBG17 X TAU-1			BDU-4 X TAU-1		
	F_3 mean	Genetic gain	Expected mean in F_4	F_3 mean	Genetic gain	Expected mean in F_4
Number of pods per plant(#)	24.16	5.79	29.95	31.04	8.93	39.97
Number of seeds per pod(#)	7.80	1.11	8.91	6.60	0.68	7.28
Pod length(cm)	5.94	0.67	6.61	5.53	0.86	6.39
100-seed weight(g)	4.72	0.78	5.50	5.90	1.66	7.56
Seed yield per plant(g)	9.39	3.22	12.61	13.10	5.09	18.19

5.5 Inter-character correlations

Quality and quantity seldom go together and all the efforts of plant breeders are aimed at bringing these together. Hence, a knowledge of inter relationships existing among various characters is necessary when selection for simultaneous improvement of these traits is to be most effective. If two-favourable characters are associated, selection for one character will automatically be good enough for the other. Grafius (1959) reported that there may not be genes for yield as such, but operate only through its components. So correlation analysis provides the information on nature and magnitude of the association of different components characters with seed yield, which is regarded as highly complex trait in which the breeder is ultimately interested. So it is a matter of great importance to the plant breeders to find out as to which of the characters are correlated with yield and also how they are associated among themselves, if negative association between characters is due to pleiotropic effects it would be very difficult to obtain the desired combinations while if linkage is involved, special breeding programmes are needed to break these linkage blocks.

It was observed that generally seed yield is strongly and positively associated with the yield components like pod number, seeds per pod, pod length and 100 seed weight in two segregating populations except for seeds per pod in the population LBG17 X TAU-1. The strong correlation values were recorded between yield and pod number, correlation coefficient between seed yield and seeds per pod was positive but of moderate intensity.

Pod number and pod length were found to be positively associated with pods per plant in the cross LBG17 X TAU-1 where as these traits were significantly associated with pods per plant in the cross BDU-4x TAU-1. Similar results of significant association of these traits with seed yield have been reported by Makeen *et al.* (2009), Konda *et al.* (2008), Mallikarjuna Rao *et al.* (2006) and Bhagowati and Hazarika (2006).

The coefficients of correlation between 100 seed weight was significant with remaining other traits in both the populations. The trait pod length was also significantly associated with number of pods per plant in both the populations. These results are in line with the earlier reports of Bhagowati and Hazarika (2006).

The reason for the low correlation between seeds per pod and pod length may be because of the inadequate opportunities for recombination (Quinsberry *et al.*, 1975). While reviewing the studies on correlation made in several crop plants, it has been observed that strength and direction of correlation in different character combination depend on the nature of experimental material and environmental condition in which they have been studied (Falconer, 1967). However, based on the present study, it can be said that more emphasis will have to be given for all the characters under study. From the above observations the improvement in blackgram yield appears possible through selection for aforesaid characters.

5.6 Transgressive segregants, high yielding and disease resistant lines

Even though, the segregating populations are usually assessed using their means and variability, these parameters alone will not indicate the worth of different populations for effecting the selection. It was therefore, considered necessary to compare the different populations for isolation of transgressive segregants. In the present study, number of transgressive segregants was identified mainly for seed yield and its component traits in different populations on the basis of superior performance of progenies over the better parent (TAU-1) with one standard deviation value in desirable direction for each of the component traits in F_2 and F_3 generations.

The frequency of transgressive segregants in two F_2 populations is presented in Table 21 and 22. The frequency was higher for seeds per pod and seed weight in both the populations followed by seed yield per plant and there was no much variation in per cent transgressive segregation for different traits in the two populations except for pod length, which was higher in LBG 17 X TAU-1 (11.33 %) than in BDU-4 X TAU-1 (2.33 %).

The frequency transgressive segregants in two F_3 populations are presented in Table 23 and 24. As observed in case of F_2 proportion of superior families was more or less same for pods per plant, pod length and 100 seed weight in the two populations. However, for

seeds per pod, it was high in LBG 17 X TAU-1 (20.66 %) than in BDU-4 X TAU-1 (11 %). For productivity BDU-4 X TAU-1 appear to be more promising with 12.44 % of families superior to TAU-1 as against 5.66 % of the families in LBG 17 X TAU-1 population. However, for selection of powdery mildew resistance and high yielding population, LBG 17 X TAU-1 has to be explored and for MYMV resistant and high yield, BDU-4 X TAU-1 population has to be explored.

Considering this, from the cross LBG 17 X TAU-1, the 12 most promising segregants resistant to powdery mildew with high yield levels than the check (TAU-1) are listed in Table 25. As can be seen, 8 of the 12 segregants showed no disease reaction and yield per plant ranged from 13.5 g per plant to 22.2 g per plant as compared to 85 per cent disease severity and 12. 3 g per plant yield level of check. Other four segregants yielded higher than the check with disease severity less than 20 per cent.

Among the 8 segregants with zero disease reaction, three were found to be breeding true for powdery mildew disease reaction. These lines along with other lines showing disease reaction well below 20 per cent level could form a material of potentiality to derive promising cultivars for commercial cultivation.

Similarly, from the cross BDU-4 X TAU-1, promising segregants showing higher yield and resistance reaction to MYMV (less than 10 per cent of disease severity) are listed in Table 26. The yield of nine promising segregants was significantly higher than the mean yield of check (TAU-1), 11.8 g per plant. Incidentally, the segregant (F_2-22) with highest yield among the 9 selected, was found to show least disease severity (1%) and thus can be a potential line for commercial release. In addition to this, few other segregants too have higher yield levels with disease severity significantly lower than the check. Segregants, F_2-17, F_2-22 and F_2-294, which were highly resistant to MYMV through consistency in F_3 also. This indicated true breeding nature of these segregants for yield and disease reaction.

The findings revealed that the parents involved in the study differed for many genes which resulted in creating large amount of genetic variability for yield and yield components in segregating generation. This suggests the scope of this material and the parents in future breeding programme. These superior transgressive segregants obtained should be maintained and forwarded to further generation to stabilize them.

Future line of work

The results of the present investigation indicated the variability for productivity and disease related traits can be generated by hybridization involving selected parents.

1. The material generated can be forwarded by Single Seed Descent Method to develop RIL_S, which can be used for marker validation studies.

2. SSR marker linked to powdery mildew disease resistant gene can be used for Marker Assisted Breeding.

3. More SSR markers need to be screened to identify marker linked to MYMV and to saturate the blackgram linkage map.

4. The linkage between marker and the trait could be strengthened by adding more number of gene specific SSR markers and EST_S (Expressed Sequence Tag) from related *Vigna* species.

5. In the present study, hybridized populations involving three genotypes *viz.,* LBG-17, BDU-4 and TAU-1 parents resulted in increased variability, heritability and GAM values. These populations need to be handled under different selection schemes for improving productivity.

6. Transgressive segregants selected and isolated for high yield and disease resistance can be advanced to subsequent generations to develop promising cultivars.

6. SUMMARY AND CONCLUSIONS

In the present investigation, an attempt was made to know the inheritance pattern and to identify molecular markers linked to powdery mildew and Mungbean Yellow Mosaic Virus (MYMV) disease resistance through bulk segregant analysis (BSA). In order to generate desirable variability the experimental material was generated by carefully selecting the parental material aiming for improvement of yield and disease resistance of adapted cultivar. Efforts were also made to assess the variability generated by hybridization using parameters like phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability and genetic advance and further to understand the inter-relationship among the component traits of seed yield through correlation studies in blackgram in F_2 population. The field experiment was carried out at Main Agricultural Research Station, College of Agriculture, Dharwad. Observations on quantitative characters *viz.*, pods per plant, number of seeds per pod, pod length, 100 seed weight and seed yield per plant were recorded on two F_2 populations *viz.*, LBG17 X TAU-1 and BDU-4 X TAU-1. Powdery mildew and MYMV disease incidence were recorded on the population of LBG17 X TAU-1 and BDU-4 X TAU-1, respectively. The results obtained in the present investigation are summarized below.

1. Inheritance study for powdery mildew disease indicated that it is governed by two dominant genes. The F_2 segregated into a dihybrid ratio of 9:3:3:1 in the cross LBG-17 X TAU-1. This indicated the presence of two dominant genes governing resistance, which have been designated as PM_1 and PM_2. In the F_3 generation pooled segregation analysis showed a good fit to the expected ratio of 1:2:2:4:1:2:1:2:1 segregation within the families, which was as expected for digenic ratios. Chi-square test for F_2 data of the cross BDU-4 X TAU-1 suggested that MYMV resistance in blackgram is governed by a single dominant gene. The F_3 population showed 1 resistant: 2 segregating: 1 susceptible, segregation pattern suggesting that a single gene governs the resistance.

2. A total of 32 primers were polymorphic between the parents of the cross LBG-17 X TAU-1 and 24 primers were polymorphic between the parents of the cross BDU-4 X TAU-1. BSA results confirmed two SSR markers putatively linked to powdery mildew resistant gene and none with MYMV resistance.

3. Upon subjecting the 300 F_2 individuals for genotyping, genetic linkage analysis indicated two SSR markers, B-210 and B-230, closely linked to the powdery mildew resistant gene and they accounted for 33.5 and 36.04 per cent of the total variance for powdery mildew resistance.

4. The population of the cross BDU-4 X TAU-1 recorded highest variance for number of pods per plant and 100 seed weight and for the remaining traits, it was on par compared to the other population. Both the populations recorded wide range of values for all the characters under study particularly for the traits like seed yield, seeds per pod and number of pods per plant.

5. The study of PCV and GCV indicated high variability for seed yield per plant followed by number of pods per plant. Moderate to low variability was noticed for other traits. Most of the characters under study exhibited low to moderately high heritability in both the segregating populations. High heritability values of more than 81 per cent have been estimated for 100 seed weight and pod length in the cross BDU-4 X TAU-1.

6. The study of phenotypic correlation indicated significant positive association of seed yield per plant with other traits in both the populations except for seeds per pod in LBG-17 X TAU-1.

7. The frequency of transgressive segregants was higher for seeds per pod and seed weight in both the populations followed by seed yield per plant. There was not much variation in per cent transgressive segregation for different traits in the two populations except for pod length, which was higher in LBG 17 X TAU-1 (11.33 %) than in BDU-4 X TAU-1 (2.33 %).

8.   Nine superior, high yielding (more than check) and powdery mildew disease resistant (disease severity less than 20 %) lines which were identified as a potential source for developing promising cultivars resistant to powdery mildew for commercial cultivation. Similarly, for MYMV disease, eight superior lines showing higher yield levels than the check with significantly lower disease severity (less than 10 %) were identified. These can be of greater importance in MYMV disease resistance breeding programmes.

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CONVENTIONAL AND MOLECULAR APPROACHES IN BREEDING FOR HIGH YIELD AND DISEASE RESISTANCE IN URDBEAN (*Vigna mungo* (L.) HEPPER

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ABSTRACT

A study was conducted to understand the inheritance of resistance to powdery mildew and mungbean yellow mosaic virus (MYMV) in urdbean during 2008 to 2010. TAU-1, a high yielding but powdery mildew susceptible variety was crossed to LBG-17 which is resistant to powdery mildew. The F_1, F_2 and F_3 populations were evaluated along with parents for resistance to powdery mildew under artificial condition. The study indicated that two independent dominant genes together control the resistance reaction in the host plant. Further, attempts were made to identify SSR markers inked to powdery mildew disease following the bulk segregants analysis in F_2 population of this cross. Out of 469 SSR primers used for screening parental polymorphism, 32 primers could differentiate the two parents and two were found to be closely linked to powdery mildew disease resistance.

Similarly inheritance of resistance to MYMV was studied by crossing TAU-1, (susceptible to MYMV disease) with BDU-4, a resistant genotype. The evaluation of F_1, F_2 and F_3 and parental lines indicated the role of a dominant gene in governing the inheritance of resistance to MYMV. Attempts to identify the marker linked to MYMV did not give satisfactory result with 469 primers used for the study. Since TAU-1 is a high yielding cultivar, the F_2 and F_3 populations developed using this as one of the parents for inheritance study for diseases, were also evaluated for productivity and its component traits. F_2 evaluation revealed high variability and also transgressive segregation. Similarly, evaluation of F_3 families further based on selections made in F_2 confirmed the superiority of few families in both the populations. Twelve families showing resistance to powdery mildew and high yield and nine F_3 families showing resistance to MYMV and high yield were identified. It is suggested to carry forward these promising selections further to develop high yielding and disease resistant lines.