GENETIC VARIABILITY STUDIES IN M_5 GENERATION OF MUSTARD

THESIS

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Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola

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(GENETICS AND PLANT BREEDING)

BY

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Enrolment Number - PP/3281

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretationof the Thesis entitled **"GENETIC VARIABILITY STUDIES IN M_5 GENERATION OF MUSTARD"** or part thereof has neither been submitted for any other Degree or Diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Nagpur **(PRAKASH L HOSUR)**

Date: 24/06/2019 Enrolment No. : PP/3281

CERTIFICATE

This is to certify that thesis entitled **"GENETIC VARIABILITY STUDIES IN M_5 GENERATION OF MUSTARD"** submitted in partial fulfilment of the requirement for the degree of **"Master of science in Agriculture** (**Agricultural Botany)"** of Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **PRAKASH L HOSUR** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

Place: Nagpur
Date: 24/06/2019

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Place: Nagpur

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(D) Abbreviations

%	:	Per cent
ACN	:	Agriculture College Nagpur
AGE	:	Agarose Gel Electrophoresis
AICRP	:	All India Coordinated Research Project
BARC	:	Bhabha Atomic Research Center
C.D.	:	Critical difference
C.V	:	Coefficient of variation
Cm	:	Centimeter
d. f.	:	Degrees of freedom
D/W	:	Distilled Water
DNA	:	Deoxyribonucleic Acid
DNTPs	:	deoxyNucleotide Tri Phosphate
d. f.	:	Degrees of freedom
EDTA	:	Ethylene Diamine Tetra Acetic Acid
et al.	:	et alia (and associates)
etc	:	Et cetera
g	:	Grams
GA	:	Genetic Advance
GCV	:	Genotypic coefficient of variation
Gy	:	gamma rays
$h^2_{(BS)}$	:	Broad sense heritability
h^2	:	Heritability
i.e.	:	that is

Kg ha^{-1}	:	Kilogram per hectare
LN_2	:	Liquid Nitrogen
m ha	:	million hectare
m t	:	million tones
mm	:	millimetre
MS	:	Ethyl Methyl Sulphonate
No. / no.	:	Number
PCR	:	Polymerase Chain Reaction
PCV:		Phenotypic coefficient of variation
$Plant^{-1}$	:	per plant
$Plot^{-1}$	:	per plot
RIL	:	Recombinant Inbred Line
S.D.	:	Standard Deviation
S.E.(m)$\pm$	:	Standard error
$Siliqua^{-1}$	:	per siliqua
SSR	:	Simple Sequence Repeats
TBE	:	Trizma base, Boric Acid, EDTA
viz.,	:	Namely
$\sigma^2 g$	:	Genotypic variance
$\sigma^2 p$	:	Phenotypic variance

(E) THESIS ABSTRACT

a)	Title of the thesis	:	**"GENETIC VARIABILITY STUIDIES IN M_5 GENERATION OF MUSTARD"**
b)	Full name of student	:	**PRAKASH L HOSUR**
c)	Name and address of Major Advisor	:	**Dr. Beena Nair** Linseed Breeder, AICRP on Linseed and Mustard, College of Agriculture, Nagpur
d)	Degree to be awarded	:	M.Sc. (Agri.)
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ABSTRACT

The present study was conducted with the objectives to estimate genetic variability of the selected mutants based on morphological characters and selection of superior mutants from M_5 generation of mustard at AICRP on Linseed and Mustard farm of College of Agriculture Nagpur during *rabi* 2018 in M_5 generation. In *rabi* 2018, 26 advanced mutants along with four checks (Bio 902, Pusa bold, Kranti, Shatabdi) were evaluated in M_5 generation in three replication. Data were recorded on days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1},number of seeds siliqua^{-1}, length of siliqua, seed yield plant^{-1} and 1000 seed weight recorded. Diversity analysis of 10 high yielding advanced mutants was also done using 20 SSR primers.

Analysis of variance indicated that the mean square due to between family and within family were highly significant for all traits, revealing the presence of significant genetic variability between the families.

Intra class correlation (t) lead the conclusion that each family distinctly differentiated from each other and differences between individuals within a family is large for all the characters. Therefore it was suggested to assign more weightage to σ^2f than σ^2w for selection in this generation.

The genetic parameter analysis revealed the importance of number of siliqua plant^{-1} and seed yield plant^{-1} for selection of better individual mutant from the progenies, based on genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance. In M_5 generation 8 high yielding plants from 26 advanced mutants were selected.

Twenty SSR primers were used to evaluate 10 mutant genotypes of mustard. The PCR amplified products of each primer were resolved on 3% agarose gel electrophoresis. Out of 20 SSR primers screened during present study, 12 primers *viz.*,Na14E08, Na10G10, OI11B05, OI12E03,

OI10F06, Ni4H05, Ni2E12, Ni2H06, Ni2Co1, Ni4G09b, Ra2DO4 and OI10F09were found monomorphic and eight primers *viz.*, Na12E01, Na10E02, OI10E05, Na12A08, Na12D04, Na12F11, Ra2E12 and Ra2A11 were found polymorphic for the set of selected genotypes. And based on banding pattern dendrogram was generated for better understanding of the diversity among the selected mutants.

Eight mutants *viz.,* ACNMM 20, ACNMM 23, ACNMM 17, ACNMM 12, ACNMM 22, ACNMM 1, ACNMM 3 and ACNMM 9 were selected on the basis of yield and some of these mutants *viz.,* ACNMM 23, ACNMM 22, ACNMM 13, ACNMM 14, ACNMM 7 were observed to be diverse from the checks as they appeared in different clades as that of checks. Some of them were found similar to the checks viz. ACNMM 9, ACNMM 4, ACNMM 17, ACNMM 19 to BIO-902 and ACNMM 15 to Kranti as they occupied the same clade as that of the check.

These superior mutants will be further evaluated in multilocation trials of superior and diverse genotypes which can be released as variety or used in breeding programme.

CHAPTER 1

INTRODUCTION

Oilseed crops form a significant part of the agricultural economy in India. In terms of acreage, production and economic value, oilseeds are second only to food grains. Indian mustard (*Brassica juncea*) is called as “rai”, “raya” or “laha” is one of the important oilseed crops belonging to family cruciferae *(Syn. Brassicaceae)* and genus *Brassica.* Indian mustard or brown mustard, *[Brassica juncea* Czern &Coss] genome content AABB is a natural amphidiploid (2n=36) of *Brassica campestris* (2n = 20) and *Brassica nigra* (2n = 16). Mustard is largely self pollinated crop but certain amount (5-18%) of cross pollination may take place (Labana and Banga,1984).

Mustard originated in China and from there it was introduced to India from where, it spread to Afghanistan and other countries. It is grown in more than 50 countries of Asia, Europe, America and Australia covering about 25 million ha area with a total production of about 40 million tonnes.

The primary centre of origin of Indian mustard is thought to be central Asia while secondary centers in central and western China. Major Indian mustard producing countries include Canada, China, Germany, France, Australia, Pakistan, Poland and India.

India is world's fourth largest edible oil economy after the US, China and Brazil. Globally, it contributes almost six per cent of global vegetable oil production; 14 per cent of vegetable oil imports and 10 per cent of edible oils. The total market size of the Indian oilseed sector is about Rs 600 billion (US$134 billion). In India, oilseeds occupy 13 per cent of the country's gross cropped area and 3% to gross national products and 10% value of agricultural products. Soybean, groundnut and rapeseed-mustard are the major oilseed crops in India contributing nearly 84% and 88% to its total acreage and production, respectively (Average of 2012-13 to 2016-17). The average contribution of rapeseed-mustard to the total oilseed production in India was 25.3%, with its average productivity 1304 (kg/ha) during 2016-17. Though, rapeseed-

mustard is placed 2nd in terms of production, after soybean, it ranks 1st in terms of oil yield among all oilseed crops. Oilseed *Brassica* grown in India are 3. *B. rapa. B. napus and B. carinata. B. juncea* predominates and accounts for about 90% area under rapeseed-mustard crops. These crops are grown in diverse agro-climatic conditions varying from north-eastern /north-western hills to down south under irrigated or rainfed, timely or late sown and sole or mixed cropping. Indian mustard accounts for about 75-80 % of the 6.07 million hectares under these crops in the country during 2016-17 crop season.

Brassica juncea is second most important edible oilseed crop in India after groundnut and accounts for about 30% of the total oilseeds produced in the country. Indian mustard is cultivated in the states of Punjab, Rajasthan, Uttar Pradesh, Assam, Gujarat, Haryana, Madhya Pradesh, and West Bengal as a *rabi* crop.

The estimated area, production and yield of rapeseed-mustard in the world was 36.68 million hectares (mha), 72.42 million tonnes (mt) and 1974 kg/ha, respectively, during 2017-18. Globally, India account for 19.8 % and 9.8% of the total acreage and production (USDA 2016-17).In India, area under mustard cultivation is 6.07 mha producing about 7.91 million tonnes of seeds with average productivity of 1183 $kgha^{-1}$ (Anonymous, 2016-17). Area under mustard cultivation in Maharashtra was 9500 hectares with production of 3300 tones and average productivity of 308 $kgha^{-1}$ (Anonymous, 2017) and 2200 ha area was under cultivationin Vidharbha region having production of 1100 tonnes and productivity of 500 kg ha^{-1} (Anonymous, 2017). During the last seven years, there has been a considerable increase in productivity from 1840 kg/ha in 2010-11 to 1974 kg/ha in 2017-18 and production has 3150 increased from 61.64 mt in 2010-11 to 72.42 mt in 2017-18 (Anonymous, 2019).

The rapeseed-mustard crop is mainly cultivated in small holder production systems and in marginal and disadvantaged regions characterized by rain-fed farming and low input intensity in cultivation. The enhancement of crop productivity and profitability is therefore critical

for achieving inclusive farm growth and to enhance the livelihood security of the farmer producers.

1.2. Importance of study

Rapeseed-mustard is the largest contributor to the domestic edible oil production in India. Though the crop comes second in terms of oilseed production behind soybean, the higher oil content in the seeds makes this crop the largest source of domestic edible oil availability. The average contribution of rapeseed-mustard to the total oilseed production from the annual oilseeds stood at 24.9 per cent during 2012-13 to 2016-17. The crop accounted for more than 25 per cent of the domestic edible oil availability from primary sources during last ten years 2006-07 to 2015-16. The need for enhancing self sufficiency in meeting the edible oil requirements of the country is being felt increasingly, especially with rise in import dependency (almost half of total consumption), drain of foreign exchange reserves and instability in international markets. The rapeseed-mustard crop is seen as a potential source for enhancing domestic edible oil availability through crop productivity enhancement mediated by appropriate technology intervention and extension strategies.

The concept of inducing mutation and utilizing them in plant breeding was first given by Hugo De Vries (1903) for generating variability and achieving the goal of generating new strains of cultivated crop plants. Mutation induction is an important complementary method of breeding crop species. The utilization of induced mutations for the improvement of crop plants has yielded several mutants which have been used directly as new cultivars. Mutation breeding is accomplished by chemical or physical treatments followed by selection for heritable changes of specific genotypes, and this method has been used successfully in the genetic improvement of crop plants. Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement.

Gamma rays are belonging to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells

and affect different morphology, anatomy, biochemistry and physiological characters in plants, mainly depending on the level of irradiation. These effects could change in plant, the cellular structure and metabolism, like dilation of thylakoid membranes, alterationin photosynthesis, modulation of the antioxidative and accumulation of phenolic compounds.

Yield is one of the most important economic character and is the product of multiplicative interaction of contributing characters. Hence, the important objective in mustard improvement is oriented to develop varieties which have high yielding potential. The other objectives are oriented to develop new varieties with wider adaptability, early maturity, disease resistance and high oil content along with high yield potential. To achieve this goal and to bring about desired improvement in mustard, technique of mutation breeding can be exploited by plant breeders. To evolve a variety having high yield and good yield contributing traits, information on nature and magnitude of variation in available materials is necessary and mutation breeding helps in creating this variability.

Handling of M_5 generation is important as it is the efficiency of the breeder to decide the criteria to select the best mutants which has almost attained homozygosity and is almost stabilized. This ultimately can give us a useful mutant. However the extent of variability among the mutants depends upon the genetic diversity among the mutants and parents.

One of the main objectives of this experiment was to identify superior mutants for forwarding to yield trial. Individual mutants were selected from M_2 generation on the basis of high seed yield plant^{-1}, earliness, aphid resistant, powdery mildew resistance. The selected mutants from M_2 generation were raised in M_3 generation to test the performance and homozygosity. Among these mutants which were true breeding were forwarded to M_4 and further generation till homozygosity is attained.

In order to evaluate the superior mutants the present study was undertaken using the most promising mutants identified in M_4 generation of Pusa bold and Bio 902 variety of mustard planted with two mutagenic

agents i.e Gamma rays alone and in combination of Gamma rays and EMS. These selected mutants were evaluated with checks, BIO 902, Pusa Bold, Kranti, Shatabdi in preliminary yield trial for two years *i.e rabi* 2017-18 & 2018-19.

1.3 Objectives of study

1. To estimate genetic variability of the selected mutants based on morphological characters.
2. To identify superior mutants.

1.4 Scope and Limitation

B. juncea predominates and accounts for about 90% area under rapeseed-mustard crops. These crops are grown in diverse agro-climatic conditions.

The state wise productivity of rapeseed-mustard has shown a progressive increase and sustained production after TMOP in 1986, which resulted in the attainment of near self-sufficiency and the beginning of Yellow Revolution in the country as well as opening of new opportunities to compete in the international market. However, sizeable area is still under inherently poor yielding species of rapeseed-mustard under marginal and sub-marginal lands. Vulnerability of the crop to major foliar diseases and insects-pests is a matter of concern. Unfavorable climatic conditions (frost & drought) cause instability in production. Heavy fluctuation in prices of produce and inputs also influence the production.

1.5 Hypothesis

This study will help in estimating genetic variability based on morphological characters and also identifying superior mutants for yield and yield attributes which can be forwarded to next generation & help in development of superior varieties.

Chapter II

REVIEW OF LITERATURE

Exploiting natural or induced genetic diversity is a substantiated strategy in the improvement of all major food crops and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability. In recent years, induced mutations have been extensively used for genetic enhancement of the annual oilseed crops. Various mutagenic agents are used to induce favourable mutations at high frequency that include ionizing radiation and chemical mutagens. The biological effect of ionizing radiation like gamma rays depends primarily on the amount of energy that will be absorbed by the biological system of which of course, the chromosomes are the most important target. It is known that irradiation of seeds increases mutation frequency and in turn promote gene recombination and widen the mutation spectrum (Emrani, 2012).

Several positive mutations have been created in agricultural crops by using gamma irradiations. Gamma rays are the most energetic form of electromagnetic radiation, possesses the energy level from 10 kilo electron volts to several hundred kV and they are considered as the most penetrating in comparison to other radiation such as alpha and beta rays. Gamma rays belong to ionizing radiation and interact on atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level.

Development of high-yielding cultivars requires a thorough knowledge of the existing genetic variation for yield and its components. The observed variability is a combined estimate of genetic and environmental causes, of which only the former is heritable. However, estimates of heritability alone do not provide a full anticipation of the outcome in plant response to selection, but should be combined with other estimates such as genetic advance, the change in mean value

between generations, phenotypic and genotypic coefficients of variation, (Wani & Khan, 2006).

Molecular markers, such as Simple Sequence Repeats (SSRs) are powerful tools which generates information contained within germplasm collections. They are becoming increasingly important in plant variety testing (Donini *et al*. 2000). SSRs, also known as microsatellites, are short stretches of DNA sequence occurring as tandem repeats of mono-, di-, tri-, tetra-, penta- and hexa-nucleotides. Previous studies of SSRs in crop species, including SSRs in *Brassicas*. Markers are available tool for characterising germplasm. This is due to their properties of genetic co-dominance, abundance, dispersal throughout the genome, multiallelic variation, high reproducibility and amenability.

The work carried out by various scientists analysing variability studies for development of advanced lines using both morphological as well as molecular techniques in the past are given below.

2.1 Variances between family.

Thagana *et al.* (2006) studied the comparison of phenotypic characters of developed mutant lines of rapeseed (*Brassica napus* L) with the original parents. Data was taken on various characters which included 1000 seed weight, harvest index, seed yield among other characters. The entries were highly significant ($P<0.01$) for plant height, seed yield and seed weight. The mutant lines were distinct from their parents for desirable phenotypic characters including earliness and height among others.

Ali *et al.* (2010) carried out genetic analysis of some morphological traits of *Brassica napus* (Canola). The data of all the characters were recorded and subjected to analysis of variance techniques to see whether the genotypic effects among the hybrids and their parents were significant. The combined ANOVA indicated that all of the characters had significant mean sum of squares.

Siddiqui *et al.* (2009) demonstrated genetic variability induced by physical mutagen (gamma rays 750 Gy & 1000 Gy) and chemical mutagen Ethylmethane sulphonate (EMS 0.75% & 1.00% solution) alone and in combinations {(750Gy+0.75% EMS), (750 Gy+1.00% EMS), (1000 Gy+0.75% EMS) and (1000 Gy+1.00% EMS)}. However, all the mutagens showed enhanced effect for siliqua plant^{-1}and deteriorating effect for grains siliqua^{-1}. Combinations of physical and chemical mutagen have shown a considerable increase in variance for all of the traits under study enhancing the effect on primary branches. The induced variation can be exploited in the evolution of new varieties of rapeseed with improved agronomic traits.

Yadava *et al*. (2011) evaluated genetic variability and trait association in Indian mustard. Analysis of variance on 14 quantitative traits was carried out and pooled analysis over the environments was conducted. Analysis of variance revealed significant differences for individual as well as pooled data amongst genotypes for all the fourteen characters studied. Variance due to genotype was highly significant for all the fourteen characters indicating the presence of sufficient variability in the genotypes selected for this study. High magnitude of variability has been reported for days to 50% flowering, days to maturity, plant height, total siliquae plant^{-1} and seed yield (Kumar and Misra, 2007). The reason for high magnitude of variability in the present study may be due the fact that the genotypes selected were developed in different breeding programmes representing different agro-climatic conditions of the country.

Rahimi *et al.* (2011) studied the effect of gamma irradiation on qualitative and quantitative characteristics of canola (*Brassica napus* L.) to study the effect of ^{60}Cogamma irradiation on agronomic characteristics where six levels of gamma irradiation including zero (control), 100, 200, 300, 400 and 500 Gy were considered. Data was collected on plant height, specific seed weight and seed yield. Study demonstrate that 100 Gy irradiation was the best rate for increasing all traits. Higher doses of gamma irradiation decreased all traits in this study.

Singh *et al.* (2012) estimated high heritability coupled with high genetic advance % of mean for number of siliqua on main raceme, 1000 seed weight, seed yield plant^{-1} and plant height. Analysis of variance between families revealed that highly significant differences for most of the characters except days to 50% flowering, days to maturity, primary branches plant^{-1}, seeds siliqua^{-1}, and 1000 seed weight. This indicated that the families under study had wide genetic base for most of the characters and analysis of variance within families. Study shows that highly significant differences for most of the quantitative characters in all crosses except for days to maturity, primary branches plant^{-1}, number of siliquae on main raceme, seeds siliqua^{-1} in cross I and days to 50% flowering, days to maturity and primary branches plant^{-1} in cross ll, and for days to 50 % flowering, days to maturity in cross-lll and days to 50% flowering, days to maturity, seeds per siliqua in cross-IV. Significant differences between progenies indicated that differences between progenies exist for most of the traits under study.

Emrani *et al.* (2012) evaluated induced genetic variability in agronomic traits by gamma irradiation in canola (*Brassica napus* L.). The seeds were treated at 0, 800, 1000 and 1200 Gy of gamma radiation from ^{60}Co source. Days to flowering, days to maturity, plant height (cm), number of fruits plant^{-1} , number of seeds fruit^{-1}, 1000 seed weight (g) and seed yield plant^{-1} (g) were recorded using five randomly selected plants from each line in every treatment. Analysis of variance revealed that gamma doses significantly affected the variations of all traits in both generations. Two canola cultivars differed significantly for all traits with the exception of number of seeds per fruit in M_2 and number of fruits per plant in M_3 generations. The cultivar × dose interaction was also highly significant for all the traits at both generations. Therefore, these cultivars were differentially affected by the irradiation doses in terms of induction of genetic variations.

Ali *et al.* (2013) evaluated and selected rapeseed (*Brassica napus* l.) mutant lines for yield performance. The rapeseed (*Brassica napus* L.) mutant lines of M_2 generation were developed for improvement in yield at

Nuclear Institute for Food and Agriculture (NIFA), Peshawar. The data on days to 50% flowering, seed yield (kg ha^{-1}), 1000 seed weight (g) and oil yield (kg ha^{-1}) were recorded and subjected to statistical analysis to workout variance and contrast analyses of test and control treatments, and critical differences for performing treatment comparisons in augmented design. Four quantitative traits showed significant differences among all the test treatments in terms of all traits measured except 1000 seed weight while block mean square was non-significant for day to 50% flowering only.

Bind *et al*. (2013) evaluated genetic variability and character association in Indian mustard. Data were recorded on fourteen different quantitative characters. Analysis of variance revealed significant differences among the genotypes for all the characters, indicating presence of wide spectrum of variability. Significant differences were observed for all the traits among the genotypes. Genetic variability was found maximum for biological yield per plant and minimum for days to maturity as reflected by genotypic coefficient of variation.

Ahmad *et al*. (2013) estimated genetic variability of some quantitative traits in advance mutant lines of winter rapeseed (*Brassica Napus* L.). 35 advance Brassica mutant lines were evaluated for genetic variability between days to 50% flowering, plant height (cm), 1000 seed weight (g), seed yield (kg ha^{-1}) and oil yield (kg ha^{-1}). Analysis of variance was done which revealed significant differences for number of days to flowering among 35 tested genotypes against the check.

Singh *et al.* (2013) determined interrelationships among morphological and seedling characters in F_5 progenies of Indian mustard. Ten plants were randomly taken from each plot to record plant height, primary branches $plant^{-1}$, main shoot length, fruiting zone length, siliqua $plant^{-1}$, siliqua on main shoot, siliqua length, seeds $siliqua^{-1}$, seed yield $plant^{-1}$ and 1000 seed weight. The study revealed adequate variability for 18 morphological and seedling traits. The analysis of variance revealed significant differences among progenies for all the characters, except

germination percentage. Seed yield per plant, followed by siliquae per plant, and fresh seedling weight, showed higher estimates of genotypic as well as phenotypic coefficients of variation as compared with other characters suggesting that selection may be exercised directly for these traits.

Nasim *et al.* (2013) studied genetic variability for morphophysiological traits in *Brassica napus*. Data were recorded for days to flowering (initiation, half and completion), plant height, primary branches plant^{-1}, pods main raceme^{-1}, main raceme length, pod length, pod width, seeds pod^{-1} and 1000 seed weight. Highly significant differences were found for 1000 seed weight, plant height, seed pod^{-1}, pod length, pod width, days to flowering initiation, days to half flowering, days to flowering completion whereas differences were non-significant for primary branches per plant^{-1}, pods main raceme^{-1} and main raceme length.

Lohia *et al.* (2013) studied genetic variability, heritability and character association among the various traits of Indian mustard. Analysis of variance revealed significant differences among the genotypes for all the characters indicating presence of wide spectrum of variability. The magnitude of variability in some genotypes (F113 and F33) was found higher for all the characters in comparison to parental generation except days to flowering in F_2 generation. It indicated that genetic variability is found significant and in desirable direction in both the generations.

Hassan *et al.* (2014) investigated the study of effectiveness of different doses of gamma radiation (0, 5, 10 and 15 Kr) to induce new genetic variability in some agronomic traits of canola to improve the yielding ability via., selection of useful mutants under saline conditions. Results indicated significant mean square due to radiation doses, cultivars and their interaction for most of the studied traits, indicating the differential response of cultivars to radiation treatments. Higher variation was observed in the treated populations compaired to control for all

studied traits, except for plant height. High positive correlation was obtained between seed yield plant^{-1} and each of No. of branches plant^{-1} and no. of pods plant^{-1}. Some promising mutants were isolated in the M_2 generation for high yielding ability and early flowering mutants.

Rameeh (2015) studied heritability, genetic variability and correlation analysis of some important agronomic traits in rapeseed advanced lines. Twenty one rapeseed genotypes were evaluated based on randomized complete block design with three replications. Significant genotypes effects were exhibited for phenological traits, plant height, yield components except pod length and seed yield, indicating significant genetic differences among the genotypes. The high value of genetic variations of the genotypes were detected for pods per main axis, pods per plant.

Khan *et al*. (2016) studied genetic variability and heritability in F_4 populations of *Brassica napus* L. The experiment was conducted to estimate genetic variability. Significant differences were observed among genotypes, parents, F_4 populations and parents versus F_4 populations for days to 50% flowering, days to maturity, plant height, primary branches plant^{-1} and main raceme length.

Verma *et al.* (2016) evaluated eighty advanced progenies along with four check varieties of Indian mustard (*Brassica juncea* L. Czern and Coss) in an augmented block design with five blocks. Analysis of variance indicated significant variability among progenies for most of the characters studied, except primary branches plant^{-1}.

Kumar *et al*. (2018) evaluated the genetic variability, heritability and genetic advance as percentage of mean for nine quantitative characters *viz.* plant height, number of primary branches, number of siliquae plant^{-1}, siliqua length *(*cm), number seeds siliqua^{-1}, number of seeds plant^{-1}, total seed yield (g) and test weight (g) in exotic lines of Indian mustard. Analysis of variance showed significant differences among the accession for all characters under study. Number of seeds plant^{-1} and test weight had higher phenotypic direct effects on total seed

yield plant^{-1}, revealing that indirect selection for these traits would be effective in improving seed yield. Primary branches plant^{-1} exhibited positive and significant correlation with plant height ,number of secondary branches plant^{-1}, number of siliqua plant^{-1} ,number of seed siliqua^{-1} ,total number of seed, total seed yield plant^{-1} and negatively significant with test weight at both genotypic and phenotypic level, respectively.

Pawar *et al.* (2018) evaluated induced genetic variability, heritability and genetic advance in Indian mustard. Analysis of variance indicated the presence of significant genetic variability between the families for all seven characters which allowed the estimation of genetic parameters. The results obtained significant variability between the families.

They all showed that the analysis of variance recorded significant difference for all the traits under evaluation. Significant differences were observed between the progenies for all the seven characters studied.

2.2 Genetic parameter estimates

Javed *et al.* (2000) studied homogeneous seeds of oriental mustard cv. Agati Sarhein (*Brassica juncea* Coss.) when treated with different doses of gamma rays (750 to 1250 Gy) to induce genetic variability. Seventy five useful mutants selected from M_2generation were tested in progeny rows to confirm the stability of genetically altered economic traits in M_3 generation. Thirteen mutants with promising performance for yield and yield components were evaluated in preliminary yield trial. Five mutants produced significantly ($P \leq 0.05$) higher yield than parent.

Akbar *et al.* (2003) carried out variability studies in summer mustard. Eighteen lines of *Brassica juncea L.* were evaluated for plant height, number of branches plant^{-1}, number of siliqua plant^{-1}, 1000 seed weight and seed yield plant^{-1} through PCV, GCV, heritability and G.A..Number of siliqua plant^{-1} was found to be a strong parameter followed by number of branches and plant height for seed yield

improvement. Siliqua plant^{-1} had highest GCV, heritability, G.A, highly significant positive correlation and maximum direct contribution for seed yield followed by number of branches plant^{-1} and plant height. Perusal of the data revealed highest genotypic coefficient of variability for seed yield plant^{-1}, number of siliqua plant^{-1}, number of branches plant^{-1}, plant height and least G.C.V. for 1000 seed weight. Heritability was maximum for number of siliqua plant^{-1} and plant height followed by number of branches plant^{-1}, seed yield plant^{-1} and least for 1000 seed weight. Genetic coefficients of variability together with heritability are considered the good estimates of the amount of advance to be expected from selection on phenotypic performance.

Khatri *et al*. (2005) evaluated high yielding mutants of *Brassica juncea* CV. S-9 developed through Gamma rays and EMS. Homogeneous seeds of *Brassica juncea* L. cv. S-9 were treated with different doses of gamma rays (750 and 1000 Gy) and EMS (0.75% and 1.0%) to induce genetic variability for the selection of genotypes with improved quantitative and quality traits. After passing through different stages of selection, 17 promising mutants were selected for further studies. Seventeen mutants and their parents were evaluated for yield and yield components in the preliminary yield trials for two consecutive years. Three mutants were significantly superior to all other entries in grain yield and these were also found early in maturity, short statured and having high seed index.

Ali *et al.* (2010) carried out genetic analysis of some morphological traits of *Brassica napus* (Canola). A 4x4 diallel cross experiment on *Brassica napus L*. (Canola) was conducted to estimate the genetic control of some important agronomic and quality parameters. The estimation of narrow sense heritability was higher for the traits days to flowering (78.32%) seed yield plant^{-1} (76.60%), number of seeds siliqua^{-1} (75.80%), number of siliqua plant^{-1} (75.10%) for F_1 but broad sense heritability for days to flowering (83.53%), seed yield plant^{-1} (79.50%), and for plant height was 71.80%. The higher value of heritability indicated that the selection on the basis of following traits in phenotypes can be

effective and be helpful in selection of high yielding genotypes. From the estimation of genetic components of variation, it is clear that additive properties were more important in effecting the variation for all the traits.

Yadava *et al*. (2011) evaluated genetic variability and trait association in Indian mustard. They considered thirty released varieties of Indian mustard for evaluating the analysis of variance on 14 quantitative traits. Phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the observed characters. High PCV and GCV were observed for point to first branch, seed yield plant^{-1}, point to first siliqua, number of secondary branches plant^{-1} and 1000 seed weight. These characters have been reported as main yield contributing traits. 1000 seed weight and total siliqua plant^{-1} also had higher phenotypic and genotypic direct effects on seed yield plant^{-1}, revealing that indirect selection for these traits would be effective in improving seed yield. Thousand seed weight and point to first branch exhibited high heritability. Genetic advance as per cent of mean was higher for 1000 seed weight, point to first branch and point to first siliqua indicating that selection for these traits would be effective for the improvement. The high heritability coupled with high genetic advance for 1000 seed weight would also be of great use for indirect selection for improvement in seed yield.

Tahira *et al*. (2011) estimated heritability, association and selection criteria for yield components in mustard. Ten genotypes of *Brassica juncea* were sown in a Randomized Complete Block Design with four replications. At maturity randomly selected plants were tagged to record data for plant height (cm), branches plant^{-1}, seeds siliqua^{-1}, 1000 seed weight (g), oil contents (%) and yield plant^{-1} (g). Heritability in broad sense calculated for various traits showed that siliqua length, plant height and seed yield have high values. Silique length and plant height were the variables with maximum potential for selection for seed yield improvement because these traits possessed high broad sense heritability, significant positive correlation and maximum positive direct effects with yield.

Singh *et al.* (2011) carried out genetic parameters and character association studies among the various traits of Indian mustard [*Brassica juncea* (L) Czern & Coss]. High GCV coupled with high heritability was observed for seed yield and days to maturity. High magnitude of heritability and moderate genetic advance for days to 50 % flowering indicated that improvement in this trait could be done through selection. Seed yield had significant positive association with days to 50 % flowering, days to maturity, plant height, length of main shoot, number of siliquae on main shoot and 1000 seed weight, both at genotypic and phenotypic levels.

Malek *et al*. (2012) selected promising rapeseed mutants through multi location trials. Significant variations were observed for most of the characters among the three promising M_5 rapeseed mutants. The mutants also had higher number of primary branches and siliquae plant^{-1}, 1000 seed weight and required shorter maturity period than the control variety under both management practices. Selection for the desired agronomic traits was carried out in M_2, M_3 and M_4 through trials. From the mutant population, three M_5 mutants were finally selected on the basis of their promising performance for seed yield and yield contributing characters with shorter maturity period.

Kumar *et al.* (2012) studied the extent of variability for yield and yield components in the 24 gamma ray induced mutants (M_7 generation) of two genotypes of *Brassica napus* L. A highly desirable shift in mean values in the mutants as compared to national check, GSL-1 was observed for almost all the characters. The maximum value of PCV was obtained for number of grains siliqua^{-1} followed by grain yield plant^{-1} and 1000 grain weight. The characters, such as, days to 50 per cent flowering, days to maturity, length of siliqua, plant height, number of primary branches, grain yield plant^{-1} and 1000 grain weight showed high heritability (more than 80%). Among component traits, plant height, length of siliqua, no. of grains siliqua^{-1}, no. of siliquae plant^{-1} and 1000 grain weight showed strong positive correlation with seed yield plant^{-1}.

The isolation of certain promising mutants signified the role of mutation breeding in enhancing the genetic variability in *Brassica napus* L.

Singh *et al.* (2012) estimated high heritability coupled with high genetic advance % of mean was observed for number of siliqua on main raceme, 1000 seed weight, seed yield plant^{-1} and plant height. The magnitude of high heritability and genetic advance % of mean indicated that improvement in this trait could be done through selection. High heritability coupled with high genetic advance was observed for plant height, number of siliquae on main raceme, 1000 seed weight and seed yield plant^{-1}in most crosses. So the selection was advocated for these traits indicated the presence of additive gene effects. Hence, their improvement can be done through mass selection and these results were found confirmed that the high heritability coupled with high genetic advance in % of mean for secondary branches plant $^{-1}$,seed yield plant^{-1} and number of siliqua plant^{-1} and for 1000-seed weight.

Emrani *et al.* (2012) evaluated induced genetic variability in agronomic traits by gamma irradiation in canola (*Brassica napus* L.). The seeds were treated at 0, 800, 1000 and 1200 Gy of gamma radiation from ^{60}Co source. Days to flowering , days to maturity, plant height (cm), number of fruits per plant , number of seeds per fruit , 1000 seed weight (g) and seed yield per plant (g) were recorded using five randomly selected plants from each line in every treatment. The data were averaged on the 30 M_3 lines belonging to each treatment. Several parameters were calculated to evaluate the effects of the mutagenic treatments on inducing variation on phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2) and expected genetic advance as percentage to mean (GAM). The present study reported a highly significant genotype × dose interaction for the agronomic traits in M_2 and M_3 which suggests that response to the gamma irradiation doses being genotype-dependent in terms of induction of genetic variations in canola. The results also showed the treated populations was significantly affected for all of the traits by the irradiations and the highest GCV, PCV and GAM belong to seed yield

plant^{-1}. The highest variations induced with treatment of 1000 Gy of gamma rays in most of the traits in 'RGS003' cultivar, while 800 Gy gamma rays induced similar conditions in Sarigol cultivar.

Singh *et al.* (2013) determined interrelationships among morphological and seedling characters in F_5 progenies of Indian mustard. The material for the investigation consisted of 180 advanced F_5 progenies of Indian mustard. These progenies were derived from five different crosses involving exotic (Australian and Chinese germplasm) and Indian genotypes. Ten plants were randomly taken from each plot to record plant height, primary branches plant^{-1}, main shoot length, fruiting zone length, siliquae plant^{-1}, siliquae on main shoot, siliqua length, seeds siliqua^{-1}, seed yield plant^{-1} and 1000 seed weight. The study revealed adequate variability for 18 morphological and seedling traits. Seed yield plant^{-1} followed by siliqua plant^{-1}, and fresh seedling weight, showed higher estimates of genotypic as well as phenotypic coefficients of variation as compared with other characters suggesting that selection may be exercised directly for these traits. The estimates of heritability in the present investigation were of higher magnitude (>75%) for plant height while genetic advance was highest for seed yield plant^{-1} and siliquaplant^{-1}.

Nasim *et al.* (2013) studied genetic variability for morphophysiological traits in *Brassica napus*. Ten *B.napus* genotypes were grown during 2009-10 at The University of Agriculture Peshawar, Pakistan to determine genetic variability, heritability, genetic advance and correlation between various traits. The experiment was laid out in a randomized complete block (RBD) design with three replications. Data were recorded for days to flowering (initiation, half and completion), plant height, primary branches plant^{-1}, pods main raceme^{-1}, main raceme length, pod length, pod width, seeds pod^{-1} and 1000 seed weight. Highly significant differences were found with respect to genetic variability for 1000 seed weight, plant height, seed pod^{-1}, pod length, pod width, days to flowering initiation, days to half flowering, days to flowering completion where as differences were non-significant for primary branches plant^{-1},

pods main raceme^{-1} and main raceme length. High heritability and high genetic advance were observed for flowering initiation, 50% flowering and flowering completion, plant height, seeds pod^{-1} and 1000 seed weight.

Ahmad *et al*. (2013) estimated genetic variability of some quantitative traits in advance mutant lines of winter rapeseed (*Brassica Napus* L.). Thirty five advance *Brassica* mutant lines and one check were evaluated for genetic variability between days to 50% flowering, plant height (cm), 1000 seed weight (g), seed yield (kg ha^{-1}) and oil yield (kg ha^{-1}). The mutant lines and the check were evaluated in four replications according to the design described in RBD. High genetic variability were recorded for oil yield, seed yield and number of days to flowering which demonstrated the effect of environment for the inheritance of these characters. Heritability and genetic advance were recorded high for oil yield and seed yield showing the existence of additive gene action for the expression of these traits.

Bind *et al*. (2013) evaluated genetic variability and character association in Indian mustard. Data were recorded on fourteen different quantitative characters. Estimates of genotypic and phenotypic coefficient of variation also showed significant differences for the respective traits. Maximum and minimum differences between GCV and PCV were observed for days to maturity and number of primary branches indicating the influence of environment for these traits, respectively. GCV along with heritability estimate gave the precise picture of genetic gain to be exploited through selection. High values of GCV coupled with heritability were observed for length of main shoot, seed yield and day to maturity suggesting that additive gene action might play major role in the expression of these characters and selection would be helpful in further improvement of these characters. High value of heritability and moderate genetic advance for days to 50% flowering indicated that improvement in this trait could be done through selection to some extent.

Lohia *et al.* (2013) studied genetic variability, heritability and character association among the various traits of Indian mustard. High heritability estimates revealed that these attributes exhibited more contribution of additive genetic variance and marginal role of non-additive in their inheritance. Seed yield had significant positive association with number of primary branches, number of secondary branches, number of slliqua plant^{-1}, number of seeds sillqua^{-1} and 1000 seed weight in both at genotypic and phenotypic levels. It was found that the genetic variability is significant and desirable direction in both the generations F_1 s and F_2s. High heritability estimates were recorded for days to flowering, days to maturity, plant height, 1000-seed weight, and seed yield per plant in both F_1 and F_2 generations. In this study, the high genetic gain in percent coupled with high heritability was observed for days to maturity indicating that the manifestation of the traits were primarily governed by additive effect.

Tahira *et al.* (2014) carried out seed yield improvement in Indian mustard using genetic parameters. Twenty advanced lines along with check varieties of mustard were evaluated utilizing heritability, correlation and path coefficient analysis for seed yield and yield contributing traits. Genotypic and phenotypic coefficients of variability revealed considerable genetic variability in the population. Both genotypic and phenotypic coefficients of variability were higher for seed yield followed by days to flower and plant height. Seed yield and days to flowering had high heritability accompanied by high genetic advance indicating that the heritability is due to additive gene effects and selection was effective. Plant height and branches plant^{-1} had high broad sense heritability along with low genetic advance showing that the non additive gene effects were involved in the genetic control of these traits. The traits, days to maturity, seeds pod length^{-1} and oil % showed low heritability as well as low genetics advance. These results indicated that these traits were highly influenced by the environment and selection of genotypes through these traits would be ineffective.

Ullah *et al.*(2015) studied genetic variability for morphological and quality traits in six advanced lines of *Brassica*. Data collected on plant height, pods main raceme^{-1}, pod length, seed yield plant^{-1} and 1000 seed weight. Genotypic and phenotypic variances, genotypic and phenotypic coefficients of variance (PCV) and heritability (broad sense) were computed. Most of the advanced lines varied significantly for all the studied parameters. High broad sense heritability estimates were recorded for plant height, pods main raceme^{-1}, pod length, seed yield plant^{-1}, Seed yield plant^{-1} had positive significant correlation with plant height, pod length, 1000 seeds weight.

Rameeh (2015) studied heritability genetic variability and correlation analysis of some important agronomic traits in rapeseed advanced lines. Twenty one rapeseed genotypes were evaluated based on randomized complete block design with three replications. High broad sense heritability was determined for phenological traits, plant height and seed yield demonstrating selection gain for improving these traits will be high. Genetic coefficient of variation, which is indicating the genetic diversity of the genotypes, varied from 1.93 to 18.95 for days to maturity and seed yield, respectively. Broad sense heritability estimates varied from 0.18 to 0.98 for pod length and days to end of flowering, respectively. The high values of genetic variations of the genotypes were detected for pods main axis^{-1} and pods plant^{-1}.

Khan *et al*. (2016) studied genetic variability and heritability in F_4 populations of *Brassica napus* L. The experiment was conducted to estimate genetic variability, heritability and genetic advance for important attributes in *Brassica napus* using 10 parental lines and their 21 F_4 populations. Significant differences were observed among genotypes, parents, F_4 populations and parents versus F_4 populations for days to 50% flowering, days to maturity, plant height, primary branches plant^{-1} and main raceme length. Plant height and main raceme length displayed moderate to high broad sense heritability and maximum genetic advance for most of the F_4 populations. Current results suggested the effectiveness of selection in early generations for the improvement of

these traits. Data were recorded on 10 randomly selected plants from each genotype at proper time for days to flowering, days to maturity, number of primary branches on main stem, plant height and main raceme length. They also reported high heritability coupled with high genetic advance in *Brassica napus.*

Verma *et al.* (2016) evaluated eighty advanced progenies along with four check varieties of Indian mustard (*Brassica juncea* L. Czern and Coss) in an augmented block design with five blocks. Estimate of high GCV and PCV (>30%) were observed for seed yield plant^{-1}. The estimates of moderate heritability were observed (>50%) for all the characters studied. The genetic advance expressed as percentage of mean was high (>30%) for secondary branches plant^{-1} (66.2%), siliqua plant^{-1} (48.8%), and seed yield plant^{-1} (57.2%).

Rai *et al*. (2017) worked on evaluation of recombinant inbred lines for morphological and biochemical parameters in Indian mustard (*Brassica juncea* L.). A set of 225 recombinant inbred lines (RlLs) were developed through a cross between high yielding commercially released Indian mustard cultivars. They were evaluated in F_6 and F_7 generation during the *rabi* season of 20l2-2013 and data were recorded for various morphological and biochemical parameters such as plant height, main shoot length, siliqua length, seeds siliqua^{-1}, biological yield plant^{-1}, 1000 seed weight, seed yield plant^{-1} and oil content. They observed genetic variation among RILs for all the traits based on analysis of variance along with high genetic advance and high heritability for seed yield plant^{-1}, biological yield^{-1}, 1000 seed weight. Among these, some RILs were identified as highly promising RILs on the basis of seed yield plant^{-1} which can be further used for multilocation testing for varietal release or used as parents in mustard breeding programs.

Kumar *et al*. (2018) evaluated the genetic variability, heritability and genetic advance as percentage of mean for nine quantitative characters *viz.* plant height, number of primary branches, number of siliqua plant^{-1}, siliqua length *(*cm), number seeds siliqua^{-1}, number of

seeds plant^{-1}, total seed yield (g) and test weight (g) in exotic lines of Indian mustard. The high heritability coupled with high genetic advance for test weight used for indirect selection for improvement in seed yield and branches plant^{-1}, at genotypic and phenotypic level, respectively. The highest heritability was recorded on test weight (98%) with genetic advance and expected genetic advance over percentage of mean of (1.9 and 50.0%), followed by siliqua length (52%) with genetic advance and an expected genetic advance over percentage of mean followed by number of secondary branches plant^{-1}, number of primary branches plant^{-1}, number of seed siliqua^{-1},plant height, number of siliqua plant^{-1} and total seed yield respectively.

Pawar *et al.* (2018) evaluated induced genetic variability, heritability and genetic advance in Indian mustard. 122 mutants along with four checks (Shatabdi, Kranti, Pusa bold and Bio902) were evaluated in three replications. Data were recorded on germination percentage, days to flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1} and seed yield plant^{-1}. The genetic parameters for seven characters were considered. It was found that seed yield plant^{-1} along with number of siliqua plant^{-1} exhibited high genotypic coefficient of variation, low heritability along with moderate genetic advance.

2.3 Molecular characterization

Baviskar *et al*. (2011) studied evaluation of mustard genotypes on the basis of biochemical and molecular traits. The binary data obtained from 18 random primers for thirteen genotypes were analysed and the similarity index between the genotypes was derived. Based on the similarity index, a cluster dendrogram was constructed. The thirteen genotypes were grouped into two major clusters. 28 reproducible bands were used for cluster analysis and with the help of these bands genetic diversity was estimated. Out of 28, four major bands were observed. Dendrogram was constructed and the accessions were divided into two main groups comprising 11 clusters. The result obtained from these

cluster showed minimum genetic diversity in these accession on SDS-PAGE level. Dendrogram obtained from the data of molecular data analysis revealed that the thirteen genotypes were distributed in two major and one minor cluster.

Kumar *et al*. (2011) worked on morphological and molecular characterization of *Brassica rapa*ssp yellow sarson mutants. Thirteen out of 55 gamma rays induced promising mutants of *Brassica rapa*ssp yellow sarson along with their two parents were used to characterize the genetic variability through morphological as well as ISSR markers analysis. Mutants showed wide range of variability for seed yield, yield attributes, oil content (%) and alternaria blight disease reaction. Five mutants within selected showed significantly high seed yield and high oil content as well as improved level of tolerance to alternaria blight as compared to parents. Investigation using ISSR analysis showed high degree of variation yielding 78 bands scored for the presence or absence of bands among genotypes. The mutants were put into three major clusters: cluster 11 was represented by three sub-clusters. The present investigation clearly indicated that morphological traits alone could not be considered as the true reflection of their genotypic characteristics and hence the need of molecular analysis using ISSR markers is proved.

Singh *et al*. (2013) assessed genetic diversity in Indian mustard [*Brassica juncea* (L.) Czern & coss] as revealed by agronomic traits and RAPD markers. An attempt has been made to assess the genetic diversity among 50 genotypes of Indian mustard using agronomic traits and random amplified polymorphic DNA (RAPD) markers. Variability was observed for agronomic traits viz plant height (cm), main shoot length (cm), days to flower initiation, number of siliqua on main raceme, number of seeds siliqua^{-1}, seed yield plot^{-1} (g), biological yield plot^{-1} (g). harvest index (%), days to maturity and 1000 seed weight (g). Cluster analysis based on agronomic trait revealed a high degree of diversity among the accessions. Both agronomic traits and molecular markers were effective in discriminating genotypes into different clusters, however, clustering pattern varied for both agronomic traits and molecular markers. Different

clustering pattern based on agronomic data and RAPD markers indicated lack of association between genetic and phenotypic diversity implying that polymorphism due to RAPD is not linked to phenotypic variation.

Vinu *et al.* (2013) studied the assessment of genetic diversity in *Brassica juncea* (*Brassicaceae*) genotypes using phenotypic differences and SSR markers. They evaluated the genetic diversity among 44 Indian mustard (*Brassica juncea*) genotypes including varieties/purelines from different agro-climatic zones of India and few exotic genotypes (Australia, Poland and China) using A and B genome specific SSR markers and phenotypic data on 12 yield and yield contributing traits. Out of the 143 primers tested,134 reported polymorphism and a total of 355 alleles were amplified. Dendrograms based on Jaccard's similarity coefficients and Manhattan dissimilarity coefficients were generated based on an average linkage algorithm (UPGMA) using marker data and phenotypic data. Genotypes were grouped into four clusters based on genetic distances. PCoA revealed that, the grouping of genotypes based on SSR marker data is more convincing than phenotypic data, however, the correlation between phenotypic and genetic distance matrices was observed to be very low (r=0.11).

Fayyaz *et al.* (2014) worked on genetic diversity analysis of *Brassica napus* / *Brassica campestris* progenies using microsatellite markers. A set of 90 genotypes (2 parental lines and their 88 F_2 progenies) was characterized separately using 24 microsatellite or SSR markers to cover the diversity. The 12 SSR primer combinations generated a total of 33 alleles, of that 32 were polymorphic loci, whereas only one was monomorphic locus. The proportion of polymorphic loci was 95.83% which indicates high genetic diversity among the progenies. The average number of polymorphic alleles per locus was 2.66. The PIC values ranged from0.395 for primer Ra2-E03 to 0.726 for primer BRMS-019 with an average genetic diversity (PIC value) of 0.584 per locus**.** Seven primers showed PIC values above 0.5 (50%) indicating high genetic diversity in the studied plant materials. Pair-wise similarity indices among 90 genotypes ranged from 0.3 to 0.95. Dendrogram obtained

through UPGMA clustering of F_2progenies depicted eight main groups using similarity coefficient of 0.70.

Pratap *et al*. (2014) studied on development and evaluation of alternaria blight tolerant lines in Indian mustard (*Brassica juncea*). Their study was aimed to evaluate and develop alternaria blight tolerant lines in Indian mustard [*Brassica juncea* (L) Czern & Coss]. A total of 14 alternaria blight tolerant lines were selected from 214 lines in F_8 generation. Amongst the selected lines, DRMR-2803, DRMR-2805 and DRMR-2806 showed the least per cent mean disease severity values of 11.4, 10.4 and 9.4, respectively. However, only DRMR-2805 exhibited seed yield plant^{-1} higher than the check variety Varuna. They evaluated the selected lines for performance, for three consecutive years (2010-13), which varied considerably for most yield parameters. The most pronounced range was obtained for plant height, siliquae on main shoot, main shoot length, and seed yield plant^{-1}, number of primary branches plant^{-1}, siliqua length, number of seeds siliqua^{-1}, 1000 seed weight. Oil content (%) exhibited narrow range of variation. RAPD analysis of selected alternaria blight tolerant lines, along with tolerant, and susceptible checks, clearly distinguished the individual lines to their available pedigree data. Highly promising tolerant lines identified in the present study may be used as potential donors for transferring altemaria blight tolerance in high yielding mustard varieties. The alternaria blight tolerant breeding line showing seed yield plant^{-1} almost at par with check variety will be released as a variety after multi location testing.

Singh *et al*. (2016) studied morphological and molecular diversity among full-sib progenies of Indian mustard (*Brassica juncea* L.). Twenty-one full-sib progenies derived through 3 cycles of selection were evaluated for agro-morphological traits in complete randomized block design. Out of the 150 genic-SSR markers used in this study, 65 markers (43.3%) were found to be polymorphic and amplified products of varying sizes in range of 100-400 bp while 85 SSRs (56.6%) were monomorphic.

Raza *et al.* (2018) worked on genetic diversity analysis of *Brassica* species using PCR-based SSR markers. Ten SSR markers produced overall 21 alleles with an average of 2.1 alleles per primer. Out of 21, 18 alleles showed polymorphism (85.71%) and 3 alleles showed monomorphism (14.28%). Polymorphic information content (PIC) varied from 0.37 to 0.71, with an average of 0.66 per primer. A dendrogram classified the genotypes into two main clusters. Cluster-A is further divided into cluster-C, which consists of *B. carinata* and *B. oleracea*. *B. napus* and *B. juncea* each form an independent cluster. Cluster-B consists of *B. nigra* and *B. campestris,* meaning that these two species are closely related to each other. The results indicated that these species can be isolated from each other at the molecular level by using molecular markers.

2.4 Criteria of selection

Javed *et al.* (2000) treated homogeneous seeds of Oriental mustard cv. Agati Sarhein (*Brassica juncea* Coss.) with different doses of gamma rays to induce genetic variability for the selection of genotypes with improved agronomic traits. Seventy five useful mutants selected from M_2 generation were tested in progeny rows to confirm the stability of genetically altered economic traits in M_3 generation. Thirteen mutants with promising performance for yield and yield components were evaluated in preliminary yield trial. Five mutants produced significantly higher yield than the parent.

Khatri *et al.* (2005) treated homogeneous seeds of *Brassica juncea* L. cv. S-9 with different doses of gamma rays and EMS to induce genetic variability for the selection of genotypes with improved quantitative and quality traits. After passing through different stages of selection, 17 promising mutants were selected for further studies. Seventeen mutants and its parents were evaluated for yield and yield components in the preliminary yield trials for two consecutive years. Three mutants S 97-75/36, S 97-1.0E/20 and S 97-1.0E/21 were significantly ($p \leq 0.05$) superior to all other entries in grain yield and these

were also found early in maturity, short statured and having high seed index.

Siddiqui *et al.* (2009) demonstrated genetic variability induced by physical mutagen and chemical mutagen alone and in combinations. However, all the mutagens showed enhanced effect for siliqua plant^{-1} and deteriorating effect for grains siliqua^{-1}. Combinations of physical and chemical mutagen have shown a considerable increase in variance for all of the traits under study enhancing the effect on primary branches. The induced variation can be exploited in the evolution of new varieties of rapeseed with improved agronomic traits.

Tahira *et al.*(2011) determined the best selection criteria for yield improvement in mustard (*Brassica juncea*). Ten genotypes of *Brassica juncea* were sown in a Randomized Complete Block Design with four replications. At phenotypic level, seed yield plant^{-1} had significant positive correlation with oil percentage while highly significant positive correlation with plant height and siliqua length. The genetic correlation was positive and highly significant with plant height, branches plant^{-1}, silique length, weight of 1000 seed and oil percentage. A positive and highly significant genetic relationship was found between plant height and branches plant^{-1}, siliqua length and seeds siliqua^{-1}, oil percentage and 1000 seed weight.

Vim *et al.* (2011) developed new varieties of rapeseed (*Brassica napus* L.), by treating the seeds of three varieties ‘Naehan’, ‘Tammi’, and ‘flaila’ with proton ion beams and gamma rays (0-2000 Gy), and then the characteristics of the mutants induced were examined upto M_5 generation to select the lines with fixed useful traits. In M_5 generation, they had selected several lines that were highly fixed for some useful traits such as plant height, maturity and flower size; one line with both earlier maturity and shorter stem than wild type, one line with only earlier maturity, two lines with shorter stem, one line with large flower. Among them, NP600-1-1-198-2 was found superior for its distinction from the original variety, uniformity and stability.

Malek *et al.* (2012) irradiated seeds of the well adapted and popular mustard variety BARI sarisha-11 with gamma ray using ^{60}Co gamma cells. Irradiated seeds were grown as M_1 during 2004-05. Selection was made from M_2 generation during 2005-06. Desirable mutants were confirmed in M_4 generation during 2007-08 and ten true breeding mutants having higher seed yield plant^{-1}with desirable morphological characters and yield attributes were selected for evaluation in yield trial.

Ahmed *et al.* (2013) undertook studies to evaluate rapeseed mutants for yield and yield components at an early stage of entry selection in non-replicated trial. Sixty five mutant entries were selected. The data on days to 50% flowering, seed yield (kg ha^{-1}), 1000 seed weight (g) and oil yield (kg ha^{-1}) were recorded and analyzed to workout variance and contrast analyses. Significant differences were revealed for all the traits among the mutants and with the control except 1000 seed weight. Three mutant lines were significantly superior both in seed yield and oil yield. Ten mutant lines took significantly less number of days to 50% flowering. The evaluation and selection of superior genotypes in early generation with better yield potential were done.

Hassan and Haleem (2014) carried out research work to select early flowering mutants among 17 canola (*Brassica napus* L.) lines. Promising mutants were evaluated and selected under saline new reclaimed lands conditions. They were isolated from M_2 generation after seed treatment of canola variety i.e. Serw 4 by 15 Kr of gamma rays. In the M_3 generation selection was practiced based on early flowering compared to the mother parent. Ten mutants were selected and evaluated in two preliminary trials with their mother parent and check variety i.e. Serw 6. All mutants were found to be significant earlier than the parent and the check variety in two trials. From the combined data, the mutants, M11-2-4 and M11-2-1 were found to be the best in earliness with good performance in most other traits. These two earlier mutants may be put into more future advanced evaluation trials in different locations before registration.

Malek *et al*. (2016) evaluated seven mutants along with the mother variety in randomized complete block design with four replicates at four rapeseed growing areas of Bangladesh during 2013-2014 to observe their performances regarding seed yield and yield attributes, and to select promising mutants having higher seed yield with short maturity period. Analysis of variance showed highly significant variations among the mutants and the check for most of the characters studied in individual location and combined over locations. Combined means over locations showed that the six mutants matured earlier except the mutant RM-03-07 and most of the mutants produced higher number of branches plant^{-1}compared to the mother variety. Results over different locations also showed that the three mutants RM-01-07, RM-10-07 and RM-04-07 produced significantly higher seed yield (1912, 1846 and 1862 kg/ha, respectively) which was 15.1, 12.1 and 11.1% higher than the mother variety, Binasarisha-4 with seed yield of 1661 kg ha^{-1}. These three mutants had also the higher number of siliqua than the mother variety. This suggests that gamma rays irradiation can be fruitfully applied to develop mutants with higher seed yield and other improved agronomic traits in Oleiferous *Brassica*.

Dutta *et al*. (2017) carried out an experiment on the performance of various mustard mutants developed at Bhaba Atomic Research Centre, Mumbai and tested at Bidhan Chandra Krishi Viswavidyalaya, West Bengal during the winter season of 2013-14, 2014-15 and 2015-16 at 13 locations across four agro climatic zones. Among the test entries, two yellow coated mustard TM-204 and TM-143 were included in the trial. The experiment was laid out in Randomized Complete Block Design having four replications. Statistical analysis of the data showed significant differences for all the parameters except days to 50 per cent flowering, days to maturity and 1000 seed weight. Based on three years trial TM 204 showed 10-15 per cent yield advantage and TM 143 showed 7-12 per cent yield advantage over two check varieties Kranti (National Check) and Pusa Bold (Zonal Check).

Chapter III

MATERIAL AND METHODS

The present research work entitled **"**Genetic variability studies in M_5 generation of mustard" was conducted during *rabi* 2017-18, *rabi* 2018-19 at AICRP on linseed and mustard farm, College of Agriculture. Nagpur and the laboratory facilities were utilized from BARC. Trombay, Mumbai. The material used and methodology followed in this research work were described below**.**

3.1 Materials required.

Dry healthy seed of *Brassica juncea,* Pusa bold and BIO 902 treated with gamma rays and EMS. The gamma rays treatment of 900, 1000, 1100, 1200, 1300Gy (^{60}Co) was done at BARC Trombay, Mumbai. Each of these treatments was treated with 0.5% aqueous solution of EMS.

The harvested seed of M_1 generation were used to raise M_2 generation in 2015-2016. During *rabi* 2016-17, 61 mutants were identified from Pusa bold and 61 mutants were identified from BIO 902 during M_3 generation. These identified mutants along with 4 checks (Pusa bold, BIO 902, Kranti, Shatabdi) were planted in *rabi* 2017-2018 for assessment. Among them were identified 26 high yielding mutants from M_4 generation selected to raise in 2018-19 in randomized block design with three replication for assessment.

3.2 Methods adopted

During *rabi* 2018-19, 26 genotypes were raised along with four check varieties in randomised block design with three replications for evaluation. Experimental details were as given below in table 1.

Table 1. Experiment details

Design of experiment	Randomized block design
No. of replications	3
Number of treatments	26 identified mutants of M_4 + 4 checks
Plot size	3 X 1.5 m^2
Spacing	0.45 x 0.10 m^2
No. of rows $plot^{-1}$	3
No. of plants row^{-1}	30
Plants under observation	5
Date of sowing	13/11/2018
Date of harvesting	As per the maturity.

The M_5 generation was observed for different parameters. Observations were recorded for different characters on randomly selected 5 plants for each mutant line. And all the recommended package of practices and plant protection measures were taken as per the schedule to raise a healthy crop.

Table 2. Pedigree of advanced mutant lines.

Sr. No.	Name of mutant	Pedigree of mutant	Character of mutant
1.	ACNMM1	1000GY M-2-7-5-1	Bold seed
2.	ACNMM2	1000GY M-2-7-5-2	Bold seed
3.	ACNMM3	1100GY M-3-104-23-3	High yield
4.	ACNMM4	1100GY M-3-104-23-4	High yield
5.	ACNMM5	1200GY M-4-12-41-1	Bold seed
6.	ACNMM6	1300GY M-5-17-11-1	High yield
7.	ACNMM7	1300GY M-5-17-3-5	Long siliqua

8.	ACNMM8	1300GY M-5-18-31-1	High yield
9.	ACNMM9	1300GY M-5-18-31-4	High yield
10.	ACNMM10	1300GY M-5-107-28-5	High yield
11.	ACNMM11	1300GY M-5-107-34-3	High yield
12.	ACNMM12	(900GY+EMS)M-6-109-12-8	Appressed
13.	ACNMM13	(1200GY+EMS)M-9-35-60-1	More branches
14.	ACNMM14	(1200GY+EMS)M-9-38-38-4	Bold seed
15.	ACNMM15	(1300GY+EMS)M-10-44-34-5	Bold and early
16.	ACNMM16	900GY M-11-47-14-5	Early
17.	ACNMM17	900GY M-11-51-36-1	High yield
18.	ACNMM18	900GY M-11-51-36-2	High yield
19.	ACNMM19	900GY M-11-51-36-6	High yield
20.	ACNMM20	1100GY M-12-60-23-12	High yield
21.	ACNMM21	1300GY M-15-70-47-15	Appressed
22.	ACNMM22	1300GY M-15-68-51-5	Bold seed
23.	ACNMM23	1300GY M-15-68-51-7	Bold seed
24.	ACNMM24	1300GY M-15-68-51-12	Bold seed
25.	ACNMM25	(900GY+EMS)M-16-74-34-11	Appressed
26.	ACNMM26	(1300GY+EMS)M-19-126-60-14	Bold seed
27.	BIO-902	Check	
28.	Pusa Bold	Check	
29.	Kranti	Check	
30.	Shatabdi	Check	

3.3 Recording of experimental data

Five plants from each treatment were randomly selected from each replication for recording the observations on the following characters.

1) Days to 50 % flowering (on plot basis)

The date on which 50% of plants flowered was recorded on plot basis in each treatment and the days to 50% flowering was calculated from the date of sowing.

2) Days to maturity (on plot basis)

Days to maturity was calculated by recording the days taken by the plot to reach physiological maturity from the date of sowing.

3) Plant height (cm)

Plant height was recorded in centimeters at maturity from ground level to the highest point of main inflorescence of five randomly selected plants from each genotype.

4) Number of siliqua plant^{-1}

The number of siliqua of five randomly selected plants were counted prior to harvest and mean number of siliqua plant^{-1} was recorded**.**

5) No of seeds siliqua^{-1}

The number of seeds siliqua^{-1} was recorded by counting individual seeds from few randomly selected siliqua of the five randomly selected plants and mean number of seeds siliqua^{-1} was calculated.

6) 1000 seed weight (g)

1000 seed were counted from five randomly selected plants and weighed in gram separately on precision electronic balance and mean 1000 seed weight plant^{-1} was recorded.

7) Seed yield plant^{-1} (g)

The seed yield obtained from five randomly selected plants were weighed in gram separately on precision electronic balance and mean seed yield plant^{-1} was calculated.

8) Primary branches plant^{-1}

Number of branches originating from the main stem which gave raise to other siliqua bearing branches were counted from five randomly selected plants at maturity.

9)Siliqua length (cm)

The siliqua length was recorded in centi meter by measuring 25 individual siliqua of the five randomly selected plants and mean siliqua length was calculated.

3.4 Statistical analysis

Following statistical parameters was used to estimate the genetic variability, heritability and variation cause during M_5 generation.

1) Analysis of variance to estimate between families and within families variances.
2) Intra class correlation (t).
3) Estimation of genetic parameters.
    a) Genotypic variance
    b) Phenotypic variance
    c) Heritability (broad sense)
    d) Genotypic coefficient of variation (%)
    e) Phenotypic coefficient of variation (%)
    f) Genetic advance (G.A)
    g) Standard error (S.E)
    h) Co-efficient of variation (C.V) (%)
    i) Critical difference (C.D).

1) Analysis of variance to estimate between families and within families variances

The data was analyzed statistically for testing the significance of between families and within families' variances for all the characters as per the method given by Sharma (2006),

Sources of Variation	Degrees of Freedom	Sum of squares		F Cal
		Observed	Expected	
Between families $\sigma^2 f$	(f-1)	FMS	TMS	$\frac{FMS}{WMS}$
Within families $\sigma^2 w$	f(k-1)	WMS	$\sigma^2 w$	
Total	(kf-1)	TMS		

Where,

F = No. of progenies

k = No. of plants observed in each progeny

Following the expectations in above table, different estimates of variances components were calculated as follows:

$\sigma^2 f$ (variance between families) $= \frac{FMS - WMS}{k}$

$\sigma^2 w$ (variance within families) = WMS

$\sigma^2 p$ (Total variance) $= \sigma^2 f + \sigma^2 w$

2) Intra class correlation (t)

In the segregating generation there exists a clear cut relationship between family mean and individual values in that family for a character .Such an association is called intra class correlation and symbolized as 't' to distinguish it from inter class correlation 'r' .Its calculation is also different from that of 'r'. . While 'r' is the ratio of covariance and root of product of two variances, 't' is the ratio of two variances i.e. between

family variances and total phenotypic variances. The $\sigma^2 p$ is the function of $\sigma^2 f+\sigma^2 w$.

$$t = \sigma^2 f / \sigma^2 p$$

3) Estimation of genetic parameters

The data were subjected to the estimation of the following genetic parameters

a) Genotypic variance $\sigma^2 f = \frac{FMS-WMS}{k}$

Where,

FMS = between family mean squares.

WMS= within family mean squares.

b) Phenotypic variance $\sigma^2 p = \sigma^2 f + \sigma^2 w$

c) Heritability in broad sense

Heritability in broad sense which is the heritable variation were estimated as the ratio of genotypic variance to the phenotypic variance and were expressed in percentage (Hanson *et al.* 1956)

Heritability in % (h^2) = $\boldsymbol{\sigma}^2 g / \boldsymbol{\sigma}^2 p \times 100$

Where,

$\boldsymbol{\sigma}^2 g$ = Genotypic variance

$\boldsymbol{\sigma}^2 p$ =Phenotypic variance

The estimates of heritability were categorized into low, moderate, high according to Robinson *et al.* (1949) as

< 30% = Low

30-60 % = Moderate

> 60% = High

d) The genotypic and phenotypic coefficient of variation were computed according to Burton (1953)

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sigma_g}{\overline{X}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sigma_p}{\overline{X}} \times 100$$

Where,

σ_g = Genotypic standard deviation.

σ_p = Phenotypic standard deviation.

$\overline{X}$ = General mean of the character.

GCV and PCV were categorized according to Sivasubramanian and Menon (1973) as follows,

< 10 %	=	Low
10-20 %	=	Moderate
> 20 %	=	High

e) Genetic advances (G.A)

The extent of genetic advance expected by selecting ten percent of the superior progeny was calculated by using formula given by Robinson *et al.* (1949).

$$G.A = k.\sigma_p.h^2$$

Where,

K - Coefficient of selection at 10 per cent selection intensity

σ_p - Phenotypic standard deviation

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

The genetic advance as percentage of mean was categorized as low, moderate and high as per the criteria of Johnson *et al.* (1955) and the same is as given below,

< 10 % = Low

10-20 % = Moderate

> 20 % = High

Genetic advance as per cent of mean (GAM)

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

Where,

GA = Genetic advance

$\overline{X}$ = General mean of character

f) S.E = $\sqrt{\frac{WMS}{r}}$

g) C.V = $\frac{S.D.}{\overline{x}} x\ 100$

h) C.D (5%) = S.E ($\overline{m}$) x $\sqrt{2}$ x t value at error d.f.

3.5 SSR marker for polymorphism in mustard

The present study related to SSR marker polymorphism was conducted at Nuclear Agricultural Biotechnology Division at BARC, Mumbai. The details of material used and methods adopted during course of investigation were described as under.

3.5.1 Experimental Material:

Ten high yielding mutant lines from Bio902 in M_4 generation was selected along with variety Bio902 and Kranti were selected for molecular analysis. The features of lines used for the study was given below in table 3.

Table 3: Pedigree of mutants selected for molecular analysis with their characters.

Sr.No.	Mutant name	Mutant Pedigree	Mutant Character
1.	ACNMM4	1100GY M-3-104-23-4	High Yield
2.	ACNMM7	1300GY M-5-17-3-5	Long Siliqua
3.	ACNMM9	1300GY M-5-18-31-4	High Yield
4.	ACNMM13	(1200GY+EMS)M-9-35-60-1	More Branches
5.	ACNMM14	(1200GY+EMS)M-9-38-38-4	Bold Seed
6.	ACNMM15	(1300GY+EMS)M-10-44-34-5	Bold Early
7.	ACNMM17	900GY M-11-51-36-1	High Yield
8.	ACNMM19	900GY M-11-51-36-6	High Yield
9.	ACNMM22	1300GY M-15-68-51-5	Bold Seed
10.	ACNMM23	1300GY M-15-68-51-7	Bold Seed
11.	BIO-902	Control	
12.	Kranti	Control	

DNA extraction

Genomic DNA was isolated using CTAB method. One gram of plant sample was hand grinded to fine powder in liquid nitrogen using sterilized mortar and pestle. The ground leaf tissue powder was mixed thoroughly with pre-warmed CTAB extraction buffer at 65°C. Tissues were mixed thoroughly by gentle inversions of the tubes and incubated in water bath at 65°C for 1h. Chloroform-iso amyl alcohol (24:1) was added to each sample and inverted twice to mix followed by centrifugation. Seven volume of chilled iso-propanol was added to each sample and inverted once to mix. The mixture was kept at -20°C for 20 minutes. DNA was precipitated by centrifugation at 10,000 rpm for 5 minutes. Supernatant was discarded and pellets were air dried. The dried pellet was dissolved in 500 µl of MQ water. Five µLRNaseA (10

mg/mL) was added to each sample and incubated at 37°C for 30 min to remove the RNA contamination from the samples.

Purification of DNA

DNA samples, which did not form a clear solution, were purified by treating with phenol. Samples were re-extracted with equal volumes of phenol:chloroform:isoamyl alcohol (25:24:1) followed by precipitation of DNA and was washed twice with 70 % ethanol and air dried. DNA pellet were dissolved in 50 µl of TE buffer and stored at 4°C till further use.

DNA quantification

DNA concentration of each sample was quantified by using a Spectrophotometer at UV absorption of 260 nm, assuming 1 OD at 260 nm was equal to 50 µg of DNA. The concentration of DNA was estimated from the following formula:

Concentration of DNA (µg/ml) = A260 x 50 x dilution factor.

Quality of DNA

DNA samples were analyzed using 0.8% TAE- agarose gel to check its integrity. Gels were stained with ethidium bromide and visualized under UV transilluminator and then photographed with Bio Rad gel documentation system. A DNA smear indicates poor quality where as single intact band of high molecular weight shows DNA as pure. Samples of poor quality were re-extracted. The ratio of OD at 260 and 280 was calculated to check the purity of DNA sample.

Buffer compositions used for DNA extraction in the present study were;

100 mMTris HCL pH 8 – 10 ml

1.4 M NaCl – 28ml

20 mM EDTA – 4ml

2 % CTAB- 10ml

PCR amplification

The PCR reaction was performed in thermo cycler. The reaction components and reaction cycle was mentioned below. The SSR primers (forward and reverse) used in this study was mentioned in table 4.

PCR reaction components used in the present study;

Nanopure H_2O -10µl

PCR Buffer (10X)- 2 µl

MgCL2 (25mM) -2.5 µl

dNTPs (mix) (10 mM)- 1 µl

Primer forward (5pmol) -1 µl

Primer reverse (5pmol)- 1 µl

Taq polymerase (1 unit/µl)-0.5 µl

DNA Template (30ng/µg)- 2.0 µl

Total reaction volume**:** 20 µl

PCR Program :

Initial denaturation 95° C for 5 min

Denaturation 95° C for 30 sec

Annealing 55° C for 30 sec

Initial elongation 72° C for 1 min

Elongation 72° C for 10 min

Hold at 4° C for infinite

Number of cycles: 35

Table 4: List of 20 SSR markers used in the present study.

S.No	Primer name	Forward primer	Reverse primer
1	Na12E01	ATTCCATGACTCCATTGTC	AAATCCCTTGTCTCTGTCG
2	Na10E02	TCGCGCATGTAATCAAAATC	TGTGACGCATCCGATCATAC
3	Na14E08	TTACTATCCCCTCTCCGCAC	GCGGATTATGATGACGCAG
4	Na10G10	TGGAAACATTGGTGTTAAGGC	CATAGATTCCATCTCAAATCCG
5	OI10E05	GCCAGAAACAGGAGAAATGG	GAAGCCGAAGAAAATAAGCG
6	OI11B05	TCGCGACGTTGTTTTGTTC	ACCATCTTCCTCGACCCTG
7	OI12E03	CTTGAAGAGCTTCCGACACC	GACGGCTAACAGTGGTGGAC
8	OI10F06	CATTGGTTTAGTCATTTCGTCG	AATTCAAAAACTGCCGAACG
9	Ni4H05	GAAAACACACCACCAAACCC	CCATAGAGTTCTTGTTTCTCTCTC
10	Ni2E12	TTATCTGCTTGTCTTGGGGC	AAGGAAATCGTCTCACTTGG
11	Ni2H06	CATCAGATCCGACGAAATCC	TCCTTTGGACTGTGAAAAACG
12	Ni2C01	GAGTATGAGAGATGGGAATCCG	GACTGAGCAGCTTGGAGACC
13	Ni4G09b	AAAAACTGGACCCAATTCC	GGTTAGGTCATAAACCCAAAGC
14	Na12A08	AACACTTGCAACTTCATTTTCC	CATTGGTTGGTGAATTGACAG
15	Na12D04	ACGGAGTGATGATGGGTCTC	CCTCAATGAAACTGAAATATGTGTG
16	Na12F11	CCTCACATCGTCTTCTTCATCC	TCACATCAGTCCATGGTTCC
17	Ra2DO4	TGGATTCTCTTTACACACGCC	CAAACCAAAATGTGTGAAGCC
18	Ra2E12	TGTCAGTGTGTCCACTTCGC	AAGAGAAACCCAATAAAGTAGAACC
19	Ra2A11	GACCTATTTTAATATGCTGTTTTACG	ACCTCACCGGAGAGAAATCC

20	OI10F09	AGAGAGCGAGATTG ATTGGC	AAACGACCACGAGTGA TTCC

Visualization of amplified products in agarose gel.

In order to visualize the PCR product, agarose gel electrophoresis was carried out. For making 3% gel, 3 g of agarose was disssolved in 100 ml of 1 X TBE buffer [10X TBE buffer (108 g Trisbase, 55 g Boric acid, 7.5 g EDTA, pH 8.0). After adding 2 µl of Ethidium bromide (10 mg/ml) dye, the solution was poured onto the gel casting tray fitted with comb. After solidification, the gel was transferred in the tank containing 1X TBE. The samples were mixed with appropriate amount of 6X gel loading dye (0.25 % Bromo phenol blue and 0.25 % Xylene Cyanol) and loaded onto the wells along with 100 bp ladder .Electrophoresis was carried out at a constant voltage of 40 V till the dye moved to the other end of the gel. The gel was observed under UV light transilluminator and documented.

Data analysis and detection of genetic diversity for SSR markers;

All SSR fragments were scored manually and converted into binary data, i.e., 1 for presence of band and 0 for absence of band. Polymorphism information content (PIC) was calculated using formula given by Roldán-Ruiz et al. (2000).

$$PICi = 2fi\ (1 - fi)$$

Where, PICi was the polymorphic information content of marker i, *fi* was the frequency of the marker bands present and (1−*fi*) was frequency of marker bands absent.

The marker index can be calculated by the multiplication of the PIC value of each primer combination with the EMR (effective multiplex ratio) value as given by Varshney et al. (2007).

$$MI = PIC \times EMR$$

Where, EMR was the effective multiplex ratio, defined as the product of the total number of loci fragments per primer (n) and the fraction of polymorphic loci/fragments (β) i.e.:

$$\mathbf{EMR = n.\ \beta}$$

Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient with the help of DARwin (Perrier and Jacquemoud-Collet 2006). The robustness of each dendrogram was evaluated by bootstrap analysis.

3.6 Place / duration / season of experiment.

The research was conducted at AICRP on linseed and mustard farm, College of Agriculture. Nagpur during *rabi* 2017-18 and *rab*i 2018-19.

Chapter IV

RESULTS AND DISCUSSION

The present investigation entitled “Genetic variability studies in M_5 generation of mustard” was carried out to estimate the genetic diversity among 26 mutant lines. The result obtained from this work are presented and discussed in this chapter under the following sub heads:-

4.1 Analysis of variance.

4.2 Mean performance of selected mutants.

4.3 Estimate of genetic parameters

4.3.a Coefficient of variation

4.3.b Mean and range

4.3.c Genotypic and phenotypic co-efficient of variation

4.3.dHeritability and genetic advance.

4.4 SSR marker studies

4.4.1 DNA quantification

4.4.2 Primer selected for SSR marker study.

4.4.3 Polymorphic information content

4.4.4 SSR banding pattern

4.4.5 Assessment of genetic diversity on the basis of SSR markers.

4.5. Selection of superior mutants.

4.1 Analysis of variance for experimental design

The observations were recorded on 26 mutants along with four checks for nine characters. Analysis of variance observed significance for between family and within family genetic variation for all the

Table 5. Analysis of variance for different character in M_5 generation.

Mean sum of square										
Source of variation	**df**	**Days to 50%flowering**	**Days to maturity**	**Plant height (cm)**	**Number of Primary branches plant^{-1}**	**Number of siliqua plant^{-1}**	**Length of siliqua (cm)**	**Number of seeds siliqua^{-1}**	**Seed yield plant^{-1}(g)**	**1000 seed weight (gm)**
Between families	29	12.03**	13.36**	2097.83**	4.77**	13223.59**	0.71**	2.78**	90.96**	0.98**
Within families	420	5.30	5.10	475.85	2.0	4248.30	0.25	1.52	10.38	0.29
Intra class correlation(t)		0.110	0.298	0.351	0.185	0.084	0.123	0.110	0.052	0.548

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

characters studied. The results of this analysis were presented in (Table 5). The data on the analysis of variance resulted in highly significant mean squares due to between family for nine characters studied i.e. plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1}, seed yield plant^{-1} 1000 seed weight, siliqua length and seed siliqua^{-1}, days to maturity and days to 50% flowering. Qurban Ali *et al.* (2010) carried out genetic analysis of some morphological traits of *Brassica napus* (Canola). They indicated the presence of significant genetic variability between the families for the characters which allowed the estimation of genetic parameters. In accordance to this results significant variability between the families were also reported by Pawar *et al.* (2018) in mustard.

The intra class correlation (t) estimated in M_5 generation ranged from 0.548 for 1000 seed weight to 0.052 for number of seeds siliqua^{-1}. Highest intra class correlation value was observed for 1000 seed weight (0.548) followed by days to maturity (0.351),seed yield plant^{-1} (0.341), days to 50% flowering (0.298), plant height (0.185), number of siliqua plant^{-1} (0.123), length of siliqua (0.110),number of primary branches plant^{-1} (0.084) and number of seeds siliqua^{-1}(0.052). This revealed that 55%, 35%, 34%, 30%, 19%, 12%, 11%, 8%, 5% of variation for 1000 seed weight, days to maturity, seed yield plant^{-1}, days to 50% flowering, plant height , number of siliqua plant^{-1}, length of siliqua, number of primary branches plant^{-1} and number of seeds siliqua^{-1} respectively were due to differences within families and 45%, 65%, 66%, 70%, 81%, 88%, 89%, 92%, 95% were due to between families. Bind *et al*. (2013) observed maximum genetic variability for biological yield per plant.

This indicated that each family distinctly differentiated from each other and differences between individuals within a family is large for all the characters. Therefore it was suggested to assign more weightage to σ^2f than σ^2w for selection in this generation.

4.2 Mean performance of the selected mutants

The mean performances for nine characters studied during 2018-19 are presented in table 6.

A. Days to 50% flowering.

The data on mean value for days to 50% flowering ranged from 56.30 days to 48 days. Among the selected mutants ACNMM 19 (48.00 days) followed by ACNMM 16 (48.33 days) and ACNMM 14 (48.33 days) were the earliest to attain 50 % flowering. Among the selected mutants ACNMM 3 (53 days) followed by ACNMM 4 (54.33 days) and ACNMM 2 (56.33 days) attained late 50% flowering.

B. Days to maturity

The data on mean value for days to maturity ranged from 99.5 days to 106.00 days. Among selected mutants ACNMM 22 (99.5days) followed by ACNMM 16 (100 days) and ACNMM 17 (100.50 days) matured earliest. Among the selected mutants ACNMM 4, ACNMM 7, ACNMM 9, ACNMM 11, ACNMM 19, ACNMM 21 (106.00 days) followed by ACNMM10, ACNMM 25 (105.5 days) and ACNMM5, ACNMM 6, ACNMM 12, ACNMM 18, ACNMM 20, ACNMM 23 (105 days) matured late.

C. Plant height at maturity

The data on mean value for plant height at maturity ranged from 210.33 cm to 157.46 cm. Among the selected mutants ACNMM 18 (157.46 cm) was shortest followed by ACNMM 22 (161.60cm) and ACNMM 14 (162.46cm). Among the selected mutants ACNMM 15 (210.33 cm) was the tallest followed by ACNMM 10 (198.73cm) and ACNMM 8 (193.73cm).

D. Number of branches plant^{-1}

The data on mean value for number of branches plant^{-1} ranged from 5 to 7.06. Among the selected mutants ACNMM 1 (7.06) followed

by ACNMM 9 (6.46) and ACNMM 6 (6.46) recorded maximum number of branches plant^{-1}, while least number of branches plant^{-1} among selected mutants were exhibited by ACNMM 22 (5) followed by ACNMM 18, ACNMM 19 (5.06) and ACNMM 3, ACNMM 5, ACNMM 10 with (2.00).

E. Number of siliqua plant^{-1}

The data on mean of number of siliqua plant^{-1} ranged from 155.86 to 246.40. The number of siliqua plant^{-1} was maximum in selected mutants ACNMM 11 and ACNMM 19 (246.40) followed by ACNMM 18 (246.26), ACNMM 1 (230.33), while it was minimum in ACNMM 19 (155.86) followed by ACNMM 8 (157.2) and ACNMM 21 (159.26).

F. Siliqua length (cm)

The data on mean values of length of siliqua ranged from 4.14 cm to 4.84 cm. The length of siliqua was maximum in selected mutants ACNMM 15 (4.84 cm) followed by ACNMM 8 (4.73 cm) and ACNMM 22 (4.68 cm), while it was minimum in selected mutants ACNMM 25 (4.14cm) followed by ACNMM 23 (4.16cm) and among ACNMM 19 (4.18cm).

G. Number of seeds siliqua^{-1}

The data on mean value for number of seeds siliqua^{-1} ranged from 8.53to 9.93. The number of seeds siliqua^{-1} was maximum in selected mutants ACNMM 3 (9.93) followed by ACNMM 11 (9.86) and ACNMM 5 (9.80) was maximum, while it was minimum in selected mutants ACNMM 10 (8.53) followed by ACNMM 16, ACNMM18, ACNMM 19 (8.66) and ACNMM 20, ACNMM 25 (8.733).

H. Seed yield plant^{-1} (g)

The data on seed yield plant^{-1} ranged from 3.22 g to 12.99 g. The seed yield plant^{-1} was maximum in selected mutants ACNMM 12 (12.99 g) followed by ACNMM 9 (11.15g) and ACNMM 11, ACNMM 19 (10.98

Table 6. Mean performance of selected 26 mutants along with checks for various characters.

Sr. No.	Progeny	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of Primary branches plant^{-1}	Number of siliqua plant^{-1}	Length of siliqua (cm)	Number of seeds siliqua^{-1}	Seed yield plant^{-1}(g)	1000 seed weight (gm)
1.	ACNMM1	49.0	102	176.20	7.07	230.33	4.58	9.20	8.88	3.1
2.	ACNMM2	56.3	102	174.87	5.67	246.00	4.56	9.60	7.58	5.75
3.	ACNMM3	53.0	101	175.47	5.27	181.53	4.59	9.93	9.49	5.45
4.	ACNMM4	54.3	106	188.20	6.20	179.27	4.41	8.93	6.69	5.4
5.	ACNMM5	52.7	105	177.80	5.27	214.00	4.43	9.80	7.79	5.25
6.	ACNMM6	51.3	105	189.33	6.33	186.60	4.30	9.73	6.59	5.85
7.	ACNMM7	52.0	106	172.13	6.27	219.93	4.64	9.20	8.63	5.55
8.	ACNMM8	50.7	104	193.73	6.00	157.20	4.73	9.60	5.65	5.1

9.	ACNMM9	49.3	106	190.13	6.47	155.87	4.53	9.00	11.15	5.35
10.	ACNMM10	51.0	105.5	198.73	5.27	193.00	4.37	8.53	3.23	5
11.	ACNMM11	49.3	106	189.87	5.67	246.40	4.21	9.87	10.99	4.15
12.	ACNMM12	48.7	105	186.67	5.93	184.80	4.37	9.27	12.99	4.35
13.	ACNMM13	48.7	106	167.13	5.53	179.13	4.88	8.80	6.96	5.45
14.	ACNMM14	48.3	102.5	162.47	5.73	181.93	4.33	8.80	6.70	6.2
15.	ACNMM15	48.0	101	210.33	5.67	199.67	4.85	8.93	9.87	6.15
16.	ACNMM16	49.3	100	175.00	5.53	193.00	4.65	8.67	3.23	5.3
17.	ACNMM17	48.7	100.5	166.40	6.27	222.27	4.46	9.60	10.73	4.85
18.	ACNMM18	49.3	105	157.47	5.07	246.27	4.33	8.67	8.12	5.4
19.	ACNMM19	48.0	106	170.07	5.07	246.40	4.19	8.67	10.99	4.3
20.	ACNMM20	48.3	105	184.00	6.20	160.87	4.43	8.73	9.06	5.75
21.	ACNMM21	48.3	106	185.40	6.33	159.27	4.59	8.93	4.56	4.4

22.	ACNMM22	50.7	99.5	161.60	5.00	190.20	4.69	9.47	10.60	4.85
23.	ACNMM23	49.3	105	187.33	5.60	198.27	4.16	9.13	9.43	4.2
24.	ACNMM24	51.0	104.5	169.93	5.47	198.47	4.59	9.07	9.84	5.5
25.	ACNMM25	49.0	105.5	193.07	6.20	165.80	4.15	8.73	8.74	5.65
26.	ACNMM26	48.7	104	183.27	6.00	199.73	4.49	9.80	8.61	6
27.	BIO-902	48.7	105	184.67	4.80	166.27	4.21	9.67	8.01	5.65
28.	Pusa Bold	48.3	106.5	177.33	4.60	151.27	4.48	9.53	8.56	5.8
29.	Kranti	49.3	106.5	175.67	6.20	167.27	4.90	8.87	8.46	5.9
30.	Shatabdi	50.0	103	176.53	6.13	158.13	4.84	8.93	10.90	5.4
CV(%)		4.61	2.17	12.12	24.58	33.83	11.10	13.44	38.21	10.24
S.E(m) ±		1.63	1.60	15.42	1.0	46.09	0.35	0.87	2.28	0.38
CD (5%)		4.60	4.52	42.87	2.78	128.10	0.98	2.43	6.33	1.09
Mean		49.99	104.17	180.03	5.76	192.64	4.50	5.76	8.43	5.24

g), while it was minimum in selected mutants ACNMM 10, ACNMM 16 (3.22 g) followed by ACNMM 21 (4.56g) and ACNMM 8 (5.65g).

I. 1000 seed weight

The data on 1000 seed weight ranged from 3.10 g to 6.20 g. The 1000 seed weight was maximum in ACNMM 14 (6.20g) followed by selected mutants ACNMM 14 (6.15 g) and ACNMM 26 (6g), while it was minimum in selected mutants ACNMM 1 (3.10 g) followed by ACNMM 11 (4.15 g) and ACNMM 23 (4.2 g).

In the present study, ACNMM 12 was found to be having highest mean yield of 12.99 g, along with number of siliqua (184.8). ACNMM 11 was found having high mean yield (10.98) along with highest number of siliqua (246.4) and high number of seeds siliqua^{-1} (9.86). ACNMM 14 was found having highest 1000 seed weight (6.2 g).

4.3 Estimates of genetic parameters

Mean, range, genotypic variance, phenotypic variance, heritability (broad sense), genotypic coefficient of variation(%), phenotypic coefficient of variation(%), genetic advance (G.A), standard error (S.E) and co-efficient of variation (C.V) (%) were calculated for nine characters in M_5 generation and are presented in (Table 7).

4.3.a. Coefficient of variation

Significant differences were observed between the genotypes for all nine characters studied. The coefficient of variation (CV) ranged from 2.17% to 38.21% for various characters (Table 7). The low coefficient of variation (≤ 20%) was observed for the characters seed siliqua^{-1} (13.44%), plant height (12.12%), siliqua length (11.10%), 1000 seed weight (10.24%), days to 50% flowering (4.61%), days to maturity (2.17%), and which showed the best genetic potential and its genetic influence. High coefficient of variation (> 20%) was observed for seed yield plant^{-1} (38.21%), number of siliqua plant^{-1} (33.83%), number of primary branches plant^{-1} (24.58%) which indicated more influence of

environmental fluctuation. Similar results were observed by Siddiqui *et al.* (2009) where the maximum coefficient of variation was observed for grain yield per plant yield (37.87%) followed by siliqua per plant (29.99%)

4.3.b Range and mean

The grand mean recorded for various characters of 26 genotypes including 4 checks were found to be 49.99 days for days to 50% flowering, 104.17 days for maturity, 180.03 cm for plant height, 5.76 for number of primary branches plant^{-1}, 192.64 for number siliqua plant^{-1}, 8.43 for seed yield plant^{-1} and 5.24 for 1000 seed weight, 4.50 for length of siliqua, 9.19 for number of seeds siliqua^{-1} (Table 7). Wide range of variation was exhibited for number of siliqua plant^{-1} (444), plant height (140), seed yield plant^{-1} (18.8), days to maturity (14) and days to 50% flowering (13) whereas number of primary branches plant^{-1} (8), 1000 seed weight (3.4), number of seeds siliqua^{-1} (3), length of siliqua^{-1} (2.43) exhibited low range of variation. Wide range of variation for yield and yield components were reported by Ahmed *et al.* (2013) in mustard.

4.3.c Genotypic and phenotypic co-efficient of variation

The values of genotypic and phenotypic coefficients of variation are presented in Table 7. In general, as expected, phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV). The magnitude of both GCV and PCV varied with the traits.

High genotypic coefficient of variation was observed for seed yield plant^{-1} (27.48%). Number of siliqua plant^{-1} (15.79%) and 1000 seed weight (11.27%) possessed moderate genotypic coefficient of variation while number of primary branches plant^{-1} (7.45%), plant height (5.78%), length of siliqua (3.89%) and number of seeds siliqua^{-1}(3.15%),days to 50% flowering (3) and days to maturity (1.59) exhibited low genotypic coefficient of variation. In accordance with these results high and low genotypic coefficient of variation for seed yield and days to

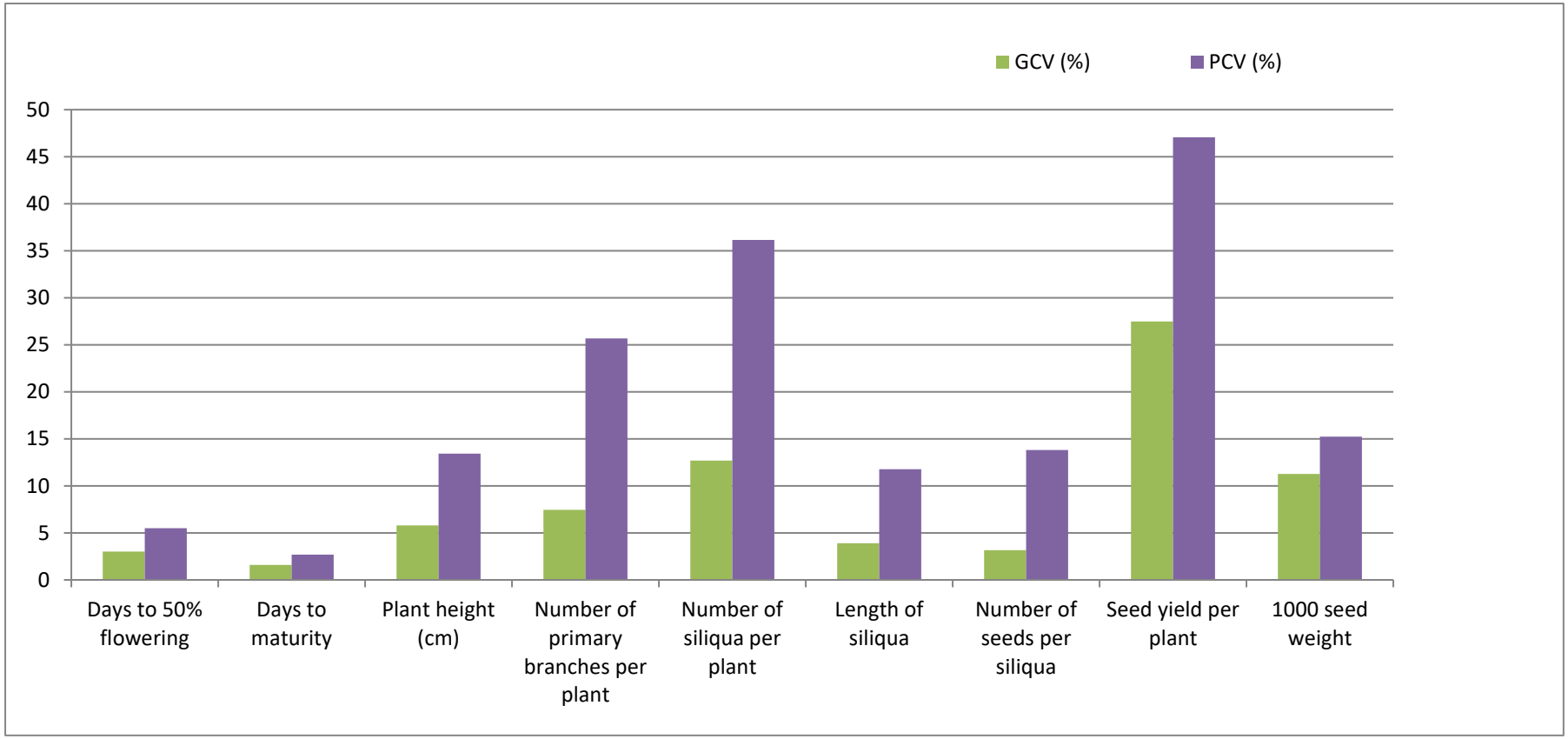


Fig-1: GCV and PCV percent estimated in M_5 generation

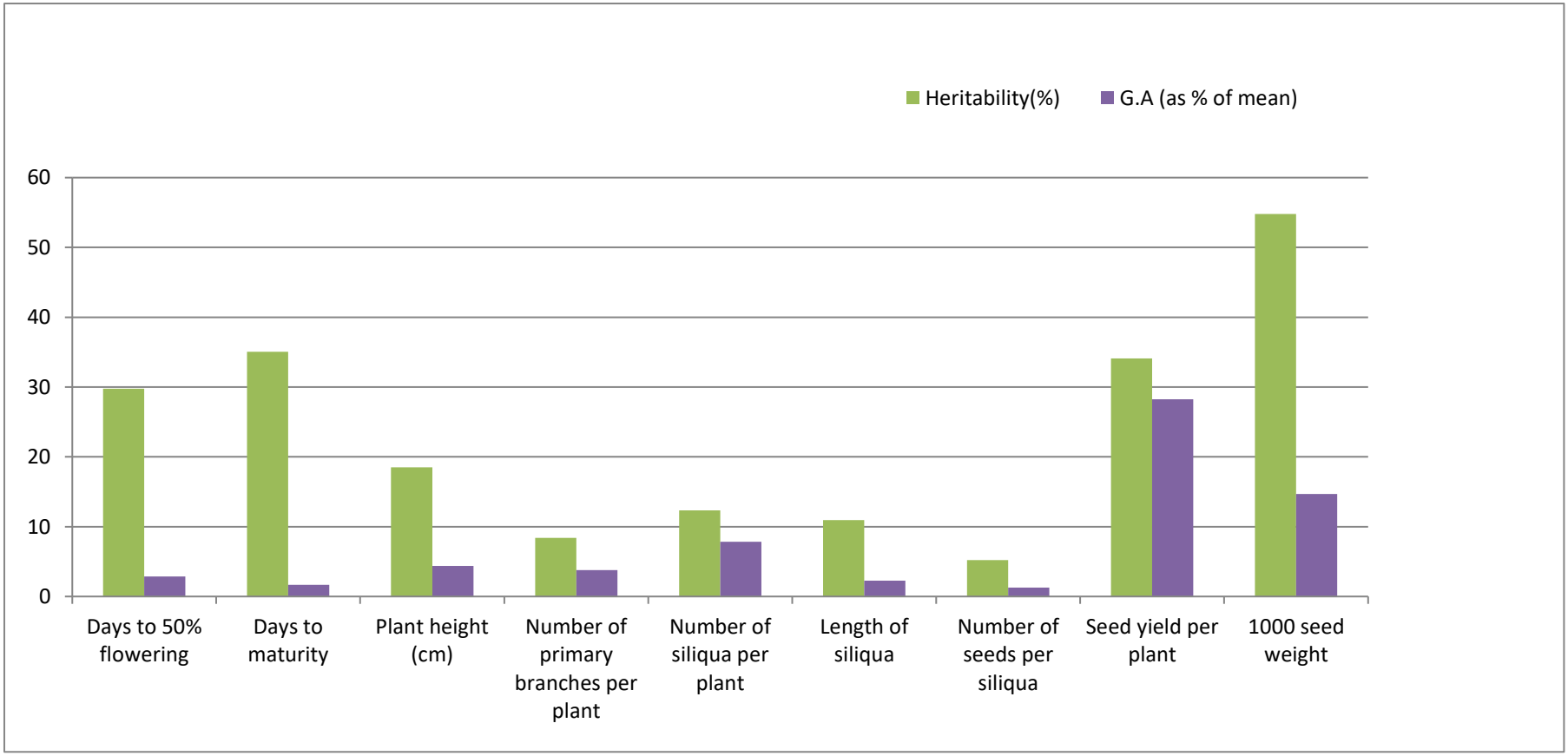


Fig.2: Heritability and GA as percentage of mean in M_5 generation

Table 7. Genetic parameters estimates for different character in M_5 generation.

Parameters	Days to 50%flowering	Days to maturity	Plant height (cm)	Number of Primary branches plant^{-1}	Number of siliqua plant^{-1}	Length of siliqua (cm)	Number of seeds siliqua^{-1}	Seed yield plant^{-1}(g)	1000 seed weight (gm)
CV (%)	4.61	2.17	12.12	24.58	33.83	11.10	13.44	38.21	10.24
S.E(m) ±	1.63	1.60	15.42	1.0	46.09	0.35	0.87	2.28	0.38
C.D (5%)	4.60	4.52	42.87	2.78	128.10	0.98	2.43	6.33	1.09
Mean	49.99	104.17	180.03	5.76	192.64	4.50	9.19	8.43	5.24
Range	59 - 46 (13)	107 - 93 (14)	268 – 128 (140)	11 – 3 (8)	492 - 48 (444)	5.43 – 3 (2.43)	11 – 8 (3)	19.8 - 1 (18.8)	6.4 - 3 (3.4)
Genotypic variance	2.24	2.75	108.13	0.18	598.35	0.03	0.08	5.37	0.35
Phenotypic variance	7.54	7.85	583.98	2.19	4846.65	0.28	1.61	15.76	0.64
GCV (%)	3	1.59	5.78	7.45	12.70	3.89	3.15	27.48	11.27
PCV (%)	5.49	2.69	13.42	25.68	36.14	11.77	13.80	47.07	15.23
Heritability(%)	29.75	35.07	18.52	8.41	12.35	10.96	5.21	34.09	54.80
G.A	1.44	1.73	7.88	0.22	15.13	0.10	0.12	2.38	0.77
G.A (as % of mean)	2.88	1.66	4.37	3.80	7.85	3.80	1.27	28.24	14.69

maturity were also reported by Rameeh *et al.(*2015) and high genotypic variance was observed by Bind *et al.* (2013) and Singh *et al.* (2013).

High phenotypic coefficient of variation was observed for seed yield plant^{-1} (47.07%), number of siliqua plant^{-1} (36.14%) and number of primary branches plant^{-1} (25.68%). Moderate phenotypic coefficient of variation was observed for 1000 seed weight (15.23%), number of seeds siliqua^{-1} (13.80%), plant height (13.42%) and length of siliqua (11.77%), and low phenotypic coefficient of variation for days to 50% flowering (5.49%) and days to maturity (2.69%). In accordance to these results high genotypic coefficient of variation and phenotypic coefficient of variation for seed yield plant^{-1} were also reported by Emrani *et al.* (2012). 1000 seed weight and no. of siliqua plant^{-1} along with yield plant^{-1} also had higher phenotypic and genotypic direct effects on seed yield plant^{-1}, revealing that indirect selection for these traits would be effective in improving seed yield which were also reported by Yadava *et al*. (2011).

4.3.d Heritability and genetic advance.

Heritability and genetic advance are important selection parameters as they provide an idea about the effectiveness of the selection of a genotype based on phenotypic performance. Genetic advance estimates are normally more helpful in predicting the gain under selection than heritability estimates alone. A trait having high heritability and high genetic advance is considered under the control of additive genes thus highlighting the usefulness of plant selection on the basis of phenotypic performance.

Heritability percent ranged from 54.80% (1000 seed weight) to 5.21% (number of seeds siliqua^{-1}). High heritability was observed for 1000 seed weight (54.80%), moderate heritability was observed for days to maturity (35.07%) seed yield plant^{-1} (34.09%), and low heritability was observed for days to 50% flowering (29.75%), plant height (18.52%), number of siliqua plant^{-1} (12.35%), length of siliqua (10.96%), number of primary branches plant^{-1} (8.41%) and number of

seeds siliqua^{-1} (5.21%). In accordance to these results high heritability for 1000 seed weight, yield plant^{-1},days to maturity and 50% flowering were reported by Kumar *et al*. (2012) and Lohia *et al*.(2013). Akbar *et al*. (2003) and Yadava *et al*. (2011) also observed high heritability for 1000 seed weight.

Genetic advance as a percentage of mean was observed highest for seed yield plant^{-1} (28.24%), moderate for 1000 seed weight (14.69%) and low for number of siliqua plant^{-1}(7.85%), days to maturity (7.82%), plant height (4.37%), number of primary branches plant^{-1} (3.80%), days to 50% flowering (2.88%), length of siliqua (2.27%), number of seeds siliqua^{-1}(1.27%) and days to maturity (1.66%). High heritability coupled with high genetic advance for 1000 seed weight and seed yield plant^{-1}were also reported by Singh *et.al*. (2012).And Similar to these results, moderate to low genetic advance as a percentage of mean was also reported by Kumar *et al*. (2012).

When all the genetic parameters for all nine characters were considered, it was found that seed yield plant^{-1} exhibited high genotypic coefficient of variation and phenotypic coefficient of variation, moderate heritability along high genetic advance as a percentage of mean. Number of siliqua plant^{-1} exhibited moderate genotypic coefficient of variation and high phenotypic coefficient of variation, low heritability with low genetic advance as a percentage of mean. Number of primary branches plant^{-1} exhibited low genotypic coefficient of variation and high phenotypic coefficient of variation, low heritability with low genetic advance as a percentage of mean. Low genotypic and moderate phenotypic coefficient of variation along with low heritability and low genetic advance was observed for plant height, number of seeds siliqua^{-1}and length of siliqua. Low GCV, PCV with moderate heritability low genetic advance was observed for days to maturity. Moderate GCV, PCV, heritability and genetic advance was observed for 1000 seed weight where as low GCV, PCV, heritability and genetic advance was observed for days to 50% flowering. This indicated that seed yield plant^{-1}exhibited moderate heritability with moderate genetic advance and

were influenced by additive gene action in there traits in M_5 generation and helps as a criteria for selection. A character exhibiting high broad sense heritability might not necessarily give high genetic advance. Therefore, selection should not be based solely on heritability (broad sense) but due consideration should be given to genetic advance as well.

4.4 SSR marker studies

4.4.1 DNA quantification:

DNA was isolated from mutant, parent (Bio 902) and national check (Kranti) concentration of DNA was estimated using DNA Nanodrop Technology To check the purity absorbance ratio (A_{260}/A_{280}) was calculated (Table 8).

Nucleic acids have absorbance maxima at 260 nm. The ratio of this absorbance maximum to the absorbance at 280 nm has been used as a measure of purity in both DNA and RNA extractions. A 260/280 ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA.

Abnormal 260/280 ratios usually indicate that a sample is contaminated by residual phenol, guanidine, or other reagent used in the extraction protocol, in which case the ratio is normally low. Inaccurate ratios may also be encountered at very low concentrations (< 10 ng/µl) of nucleic acids.

In the present study DNA was isolated from mutant parent (Bio 902) and national check (Kranti). Concentration of DNA was estimated using DNA Nanodrop Technology. To check the purity, absorbance ratio (A_{260}/A_{280}) was calculated. DNA concentration varied from 15.82 ng/µl to 72.46 ng/µl and DNA purity ranged from 1.62 to 1.88 (Table 8). The ratio of absorbance at 260/280 was found ~1.8 through which we can conclude the extracted DNA as pure. And none of the ratios found ~2.0 indicating that there isn’t any RNA contamination and other residues.

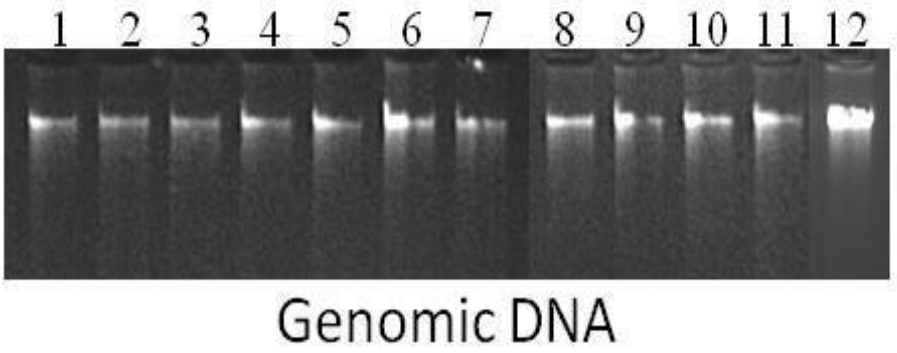


Plate 1. Agarose gel electrophoresis of 50 ng DNA of each sample.

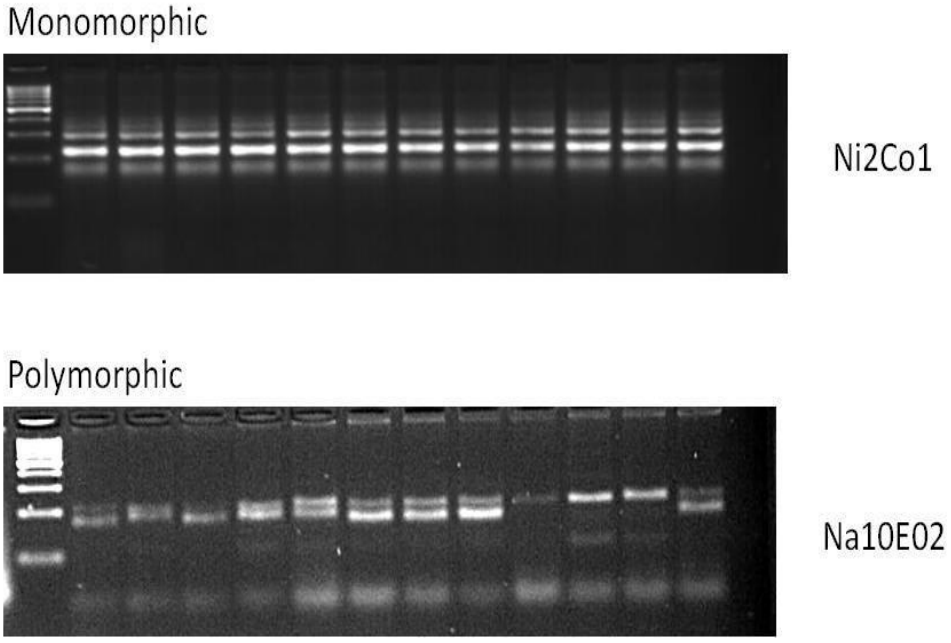


Plate 2. SSR banding profile of 10 selected mutants along with checks amplified with primers Ni2Co1and Na10E02.

Table 8: DNA quantification and purity assessment

Sr. No.	Mutant name	Absorbance at 260 nm	Ratio of absorbance at 260/280	Concentration in ng/µl
1	ACNMM4	0.680	1.68	34.01
2	ACNMM7	0.540	1.73	27.02
3	ACNMM9	0.492	1.64	24.63
4	ACNMM13	0.505	1.83	25.25
5	ACNMM14	0.625	1.75	31.25
6	ACNMM15	1.000	1.63	50.00
7	ACNMM17	0.564	1.86	28.24
8	ACNMM19	0.316	1.74	15.82
9	ACNMM22	0.390	1.62	19.53
10	ACNMM23	0.649	1.88	32.46
11	BIO-902	0.684	1.73	34.24
12	KRANTI	1.449	1.69	72.46

DNA was also visualised on 1% agarose gel to check the integrity of the DNA. The volume of the each DNA sample was calculated for 25 ng and 50 ng. Approximately 50 ng of each DNA sample was used for agarose gel electrophoresis and 25 ng of DNA was used for PCR (Plate 1). PCR Product size ranged from 100 to 350bp (Plate 2). SSR profile revealing monomorphism and Polymorphism for 10 mutant along with parent (Bio-902) and national check (Kranti). 1st lane is 100bp ladder as a molecular weight marker, Lane 2-13, genotype sequence as mentioned in Table 8, Lane14 - -Ve control (Plate 2).

4.4.2 Primer selected for SSR marker study.

Twenty SSR primers were used to evaluate 10 mutant genotypes of mustard. The PCR amplified products of each primer were resolved on 3% agarose gel electrophoresis. Out of 20 SSR primers screened during present study, 12 primers *viz.*,Na14E08, Na10G10, OI11B05, OI12E03, OI10F06, Ni4H05, Ni2E12, Ni2H06, Ni2Co1, Ni4G09b, Ra2DO4, OI10F09 were found monomorphic and eight primers *viz.*, Na12E01, Na10E02, OI10E05, Na12A08, Na12D04, Na12F11, Ra2E12, Ra2A11 were found polymorphic for the set of selected genotypes.

4.4.3 Polymorphic Information Content (PIC)

The polymorphic information content (PIC) value of 8 SSR loci were calculated across 10 mutant genotypes and are presented in table 9.Eight markers showed polymorphism in 10 mutant lines analyzed. The PIC values calculated for these 8 polymorphic primers were in the range of 0.08 (Ra2E12) to 0.48 (Na12F11 and OI10E05) with an average of 0.343 per marker (Table.7). Similar work was also conducted by Fayyaz *et.al.* (2014) where 12 SSR primer combinations generated a total of 33 alleles, of that 32 were polymorphic loci, whereas only one was monomorphic locus.

4.4.4 SSR banding pattern

The primer Na12E01 amplified 2 fragments in which both were observed to be polymorphic and none found monomorphic. The percentage of polymorphism was found to be 100%. The polymorphic information content calculated for primer Na12E01 was 0.38. EMR (effective multiplex ratio) was found to be 2 and the marker index (MI) was found to be 0.76.

The primer Na10E02 amplified 3 fragments in which all three were found to be polymorphic and none found monomorphic. The percentage of polymorphism was found to be 100%. The polymorphic information content calculated for primer Na10E02 was 0.40. EMR

(effective multiplex ratio) was found to be 3 and the marker index (MI) was found to be 1.21.

The primer OI10E05 amplified 2 fragments in which one was observed to be polymorphic and anorther found to be monomorphic. The percentage of polymorphism was 50% with polymorphic information content of 0.48. EMR (effective multiplex ratio) was found to be 1 and the marker index (MI) was found to be 0.48.

The primer Na12A08 amplified total 3 fragments in which two were observed to be polymorphic and one found monomorphic with the percentage of polymorphism of 66.67%. The polymorphic information content calculated for primer Na12A08 was 0.27. EMR (effective multiplex ratio) was found to be 2 and the marker index (MI) was found to be 0.54.

The primer Na12D04 amplified 2 fragments in which both were observed to be polymorphic and none found monomorphic. The percentage of polymorphism was found to be 100%. The polymorphic information content calculated for primer Na12D04 was 0.39. EMR (effective multiplex ratio) was found to be 2 and the marker index (MI) was found to be 0.79.

The primer Na12F11 amplified 2 fragments in which one was found to be polymorphic and the another found monomorphic. The percentage of polymorphism was found to be 50%. The polymorphic information content calculated for primer Na12F11 was 0.48. EMR (effective multiplex ratio) was found to be 1 and the marker index (MI) was found to be 0.48.

The primer Ra2E12 amplified 2 fragments in which one fragment found to be polymorphic and other monomorphic. The percentage of polymorphism was 50%. The polymorphic information content calculated for primer Ra2E12 was 0.08. EMR (effective multiplex ratio) was found to be 1 and the marker index (MI) was found to be 0.08.

Table 9. Details of informative markers based on PIC, EMR and MI.

Sr. No.	Marker	Number of Fragments				PIC	EMR	MI
		Total	Monomorphic	Polymorphic	%Polymorphism			
1	Na12E01	2	0	2	100	0.38	2	0.76
2	Na10E02	3	0	3	100	0.40	3	1.21
3	OI10E05	2	1	1	50	0.48	1	0.48
4	Na12A08	3	1	2	66.67	0.27	2	0.54
5	Na12D04	2	0	2	100	0.39	2	0.79
6	Na12F11	2	1	1	50	0.48	1	0.48
7	Ra2E12	2	1	1	50	0.08	1	0.08
8	Ra2A11	2	0	2	100	0.27	2	0.53

The primer Ra2A11 amplified 2 fragments in which both were observed to be polymorphic and none found monomorphic. The percentage of polymorphism was found to be 100%. The polymorphic information content calculated for primer Ra2A11 was 0.27. EMR (effective multiplex ratio) was found to be 2 and the marker index (MI) was found to be 0.53.

Twelve primers; Na14E08, Na10G10, OI11B05, OI12E03, OI10F06, Ni4H05, Ni2E12, Ni2H06, Ni2Co1, Ni4G09b, Ra2DO4 and OI10F09 were found monomorphic in all 10 genotypes. Out of 18 amplified bands, 4 were monomorphic and 14 were polymorphic (Table 7). The percent polymorphism varied from 66.67 to 100% with an average of 77%. The maximum numbers of polymorphic bands (3 bands) were obtained using Na10E02 primer with 100% polymorphism.

In present study, polymorphic information content (PIC) value ranged from 0.08 to 0.48. The highest PIC value was found in two primers OI10E05 and NA12F11 (0.48) followed by NA10E02 (0.40). The primer Na12A08 observed minimum polymorphism with PIC Value of 0.27. Among the primers used in the present study, Na10E02 was highly informative since it recorded high polymorphic information content (PIC), effective multiplex ratio (EMR) and marker index (MI) value of 0.40, 3 and 1.21 respectively. High PIC value indicates high degree of polymorphism among the genotypes which helps to estimate genetic distance with more and more precision. In the present study two primers *viz.*, OI10E05 and NA12F11 (0.48) showed high PIC values indicating their utility for assessment of genetic diversity. Thus it is necessary to obtain information about genetic diversity from polymorphic primers only so that genetically divergent genotypes can be effectively identified. Raza *et al*. (2018) also obtained similar PIC values ranging from 0.37-0.71. The ten advanced mutant genotypes studied were diverged into three super clusters. Both super cluster 1 and 2 were the largest including 5 genotypes each.

Thus, as similarity index goes on decreasing, the degree of divergence goes on increasing. The degrees of divergence or similarity helps in identifying genetically diverse genotypes.

4.4.5. Assessment of genetic diversity on the basis of SSR markers.

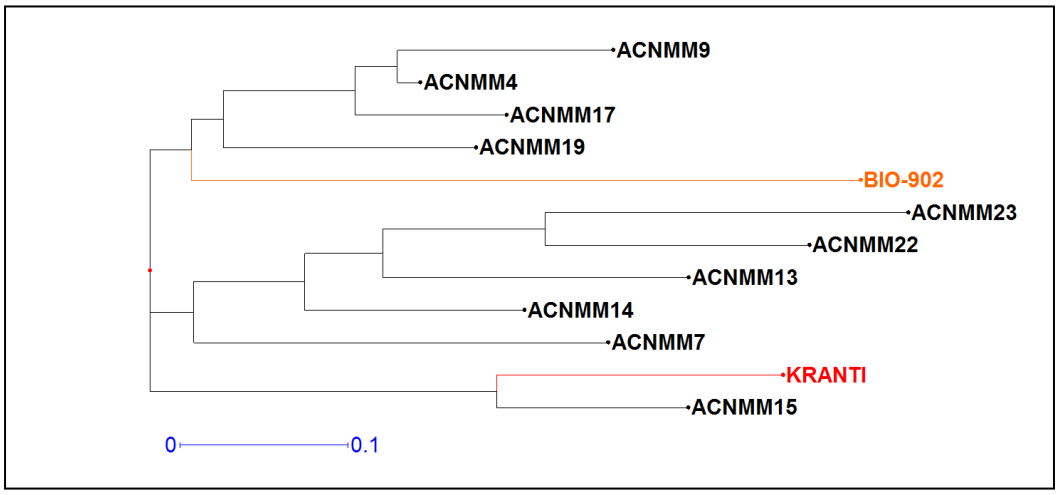
In any crop improvement programme, genetic diversity plays an important role. In fact, it is an essential prerequisite while initiating a breeding programme. For enabling better exploitation of genetic resources, it is desirable to know the genetic diversity at morphological as well as molecular levels (Kumar *et al*.2011).

Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient (fig 3).

The mutants were grouped into three major clusters at 0 similarity coefficient, cluster 1 were separated into two sub clusters having ACNMM 9, ACNMM 4, ACNMM 17, ACNMM 19 in one sub cluster and the check variety BIO-902 into separate sub cluster indicating these mutant lines were closely related to BIO-902.

Similarly, the cluster 2 was also divided into two sub clusters having ACNMM 23, ACNMM 22, ACNMM 13, ACNMM 14 in one sub cluster where as ACNMM 7 separated into individual sub cluster in cluster 2indicating these mutant lines were neither related to any of the given parents and check variety considered and were found independent. The cluster 3 was again separated into 2 sub clusters one with check variety Kranti and another with ACNMM 15 which indicates ACNMM 15 is closely related to check variety Kranti. Similar work was also conducted by Singh *et al.*(2016). Out of the 150 genic-SSR markers used in their study, 65 markers (43.3%) were found to be polymorphic and amplified products of varying sizes in range of 100-400 bp while 85 SSRs (56.6%) were monomorphic.

Fig.3 Dendrogram derived from banding pattern of SSR marker analysis of 10 mutants and 2 parents.



Results suggested that there are 3 clades. First clade consists of four mutant lines along with Bio902. Second clade consist of 5 mutant lines without any checks, whereas third clade consist of one mutant which the variety Kranti suggesting that the 5 mutants in clade 2 are diverse from the checks (Fig.3). Similar work was also conducted by Vinu *et al.* (2013) where genotypes were grouped into four clusters based on genetic distances.

4.5. Selection of superior mutants.

One of the main objectives of the present study was to identify superior mutants for forwarding to yield trial. Selection for seed yield in mustard based on single characters may not be effective. On the other hand it is very cumbersome process for a breeder to involve a large number of component characters simultaneously in the selection programme. Hence, knowledge of major yield components is necessary for involving effective selection criteria. Individual superior plants from M_5 generation over the check variety were selected considering seed yield plant^{-1}, siliqua plant^{-1}, 1000 seed weight. This revealed that the above mentioned characters were influenced by additive gene action and selection would be effective in improving these traits, hence were considered as criteria for selection in this study.

Table 10. Superior mutants selected for M_5 geneation.

Sr. no	Name of the mutant	Progeny selected	Character of progeny	Plant no. selected	Seed yield plant^{-1} (g)	No. of siliqua plant^{-1}	No. of primary branches plant^{-1}	Plant Height (cm)
1	ACNMM20	1100GY M-12-60-23-12-2	High yield	2	20.2	324	6	175
2	ACNMM23	1300GY M-15-68-51-7-3	Bold seed	3	19.8	492	4	189
3	ACNMM17	900GY M-11-51-36-1-4	High yield	4	19.6	345	7	168
4	ACNMM12	(900GY+EMS)M-6-109-12-8-10	Appressed	10	19.5	243	7	213
5	ACNMM22	1300GY M-15-68-51-5-3	Bold seed	3	19.2	259	4	175
6	ACNMM1	1000GY M-2-7-5-1-2	Bold seed	2	19.1	373	8	190
7	ACNMM3	1100GYM-3-104-23-3-2	High yield	2	19	342	4	170
8	ACNMM9	1300GY M-5-18-31-4-4	High yield	4	18	281	6	240

In the present study nine characters viz., seed yield plant^{-1}, number of siliqua plant^{-1}, number of branches plant^{-1}, 1000 seed weight, plant height, days to flowering and days to maturity, number of seed siliqua^{-1}, length of siliqua which showed varying GCV, heritability and genetic advance as percentage of mean considered for selection in M_5 generation. Similarly, Malek *et al.* (2012) selected ten true breeding mutants having higher seed yield plant^{-1} with desirable morphological characters and yield attributes and were selected for evaluation in yield trial.

Eight superior mutants were selected from 26 treatments grown in 3 replications of M_5 generation (Table 10).

The highest yielding mutant was ACNMM 20 (1100GY M-12-60-23-12-2) which was selected for the character high yield. ACNMM 20 yielded 20.2g and possessed higher number of siliqua (324) with higher number of primary branches (6). The next high yielding mutant selected from ACNMM 23 (1300GY M-15-68-51-7-3) having selected for bold seed character yielded yield of 19.8 grams, with highest number of siliqua (492) and branches (4). SSR marker studies of this mutant showed that it was found in clade no. 2 which did not include the checks BIO-902 and Kranti. Thus the mutant was found high yielding as well as diverse from the check variety. Another mutant which was high yielding was ACNMM 17 (900GY M-11-51-36-1-4) with an yield of 19.6 g, 345 number of siliqua having seven branches. Diversity analysis of ACNMM 17 mutant also revealed that it was found in clade no.1 which was in close relation to check variety BIO-902.

Another mutant ACNMM 12 ((900GY+EMS) M-6-109-12-8-10) possessed an yield of 19.5g, number of siliqua (243) and primary branches (7) which was followed by ACNMM 22 (1300GY M-15-68-51-5-3) with an yield of 19.2g, 259 no. of siliqua and four branches and was also found in clade no. 2 making it as diverse from the checks.The next highest yielding mutant ACNMM 1 (1000GY M-2-7-5-1-2) with an yield of 19.1 g, 373 number of siliqua and with highest number of

primary branches (8), which was followed by ACNMM 3 (1100GYM-3-104-23-3-2) with an yield of 19 g , 342 number of siliqua and four number of primary branches and lastly ACNMM 9 (1300GY M-5-18-31-4-4) with an yield of 18 g, having 281 number of siliqua and six number of primary branches. Diversity analysis of ACNMM 9 revealed that it has close relation with BIO-902 which found in clade no.1.

None of the selected mutants were found to be bold seeded. All the mutant selected (8) possessed yield greater than 17.8 grams which was fixed by the higher yielding check variety Kranti.

All these eight mutants which were selected from M_5 generation were mainly on the basis of higher seed yield and higher siliqua plant^{-1}. All these mutants will be forwarded for one or more generation so that homozygosity will be attained and the superior genotypes can be selected for forwarding to yield trials in further generation.

Chapter V
SUMMARY AND CONCLUSIONS

The present study entitled “Genetic variability studies in M_5 generation of mustard” was conducted to estimate between family and within family variances, to estimate genetic parameters and to identify superior mutants for forwarding to next generation on the basis of yield and yield contributing characters. For achieving these objectives, present study was carried out at AICRP on Linseed and Mustard farm farm of College of Agriculture Nagpur during *rabi* 2018 in M_5 generation. During *rabi* 2017, 26 mutants were selected from M_4 generation were grown along with four checks (Pusa Bold, Bio 902, Kranti, Shatabdi) in Randomized Block Design in three replications to get M_5 generation. Data were recorded on five randomly selected plant from each mutants and checks for days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1}, seeds siliqua^{-1}, siliqua length, seed yield plant^{-1}and 1000 seed weight for bold seeded mutant were estimated for selecting superior mutants in M_5 generation.

Analysis of variance indicated that the mean squares due to families were highly significant for all nine characters i.e. days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of siliqua plant^{-1}, seeds siliqua^{-1}, siliqua length, seed yield plant^{-1}and 1000 seed weight. This allowed further estimation of genetic parameters.

The ratio of two variances between family variances (σ^2f) and total phenotypic variances (σ^2p) estimated as intra class correlation (t) lead to conclusion that differences between individuals within family is large and each family differentiated distinctly from the other one at lower level. Hence, more weightage was given to σ^2f than σ^2w, and were suggested to be considered for selection.

In M_5 generation the high coefficient of variation was observed for seed yield plant^{-1}, number of siliqua plant^{-1} and number of primary branches plant^{-1} indicating more influence of environmental fluctuation.

The genetic parameters estimated for nine characters lead to the conclusion that high genotypic coefficient of variation, moderate heritability along with high genetic advance as a percentage of mean were found for seed yield plant^{-1}.

Selection of individual plant exhibiting significant superiority over the check for seed yield plant^{-1} was considered as a criteria of selection.

The result of between family variance, within family variance and intra class correlation (t) lead to the inference that weightage should be given to σ^2f than σ^2w during selection. Hence, instead of selecting superior progenies for forwarding to yield trial, 8 individual plants out of 26 mutants exhibiting significant superiority over check for seed yield plant^{-1} was identified for their evaluation in multilocation trials.

Two main aspects of genetic diversity, marker in formativeness (polymorphic and overall efficiency of informative fragment detection) and marker performance (overall efficacy of a primer set used in determining polymorphism level, genetic diversity, and discriminatory power) were evaluated. So in present study to investigate the genetic relationships and diversity within and among 10 high yielding mutant lines from M_5 generation along with BIO 902 and Kranti using 20 SSR primers is done. Among them twelve primers were found to be monomorphic (Na14E08, Na10G10, OI11B05, OI12E03, OI10F06, Ni4H05, Ni2E12, Ni2H06, Ni2Co1, Ni4G09b, Ra2DO4 and OI10F09) and eight primers (Na12E01, Na10E02, OI10E05, Na12A08, Na12D04, Na12F11, Ra2E12 and Ra2A11) were found polymorphic pattern which used for further analysis. Out of 18 amplified bands, 4 were monomorphic and 14 were polymorphic. The percent polymorphism varied from 66.67 to 100% with an average of 77%. The maximum numbers of polymorphic bands (3 bands) were obtained using Na10E02 primer with 100% polymorphism. PIC values ranged from 0.08 to 0.48 with an average of 0.34 per primer combination. The primer Na12A08 observed minimum polymorphism with PIC Value of 0.27. Among the primers used in the present study, Na10E02 was highly informative since it recorded high polymorphic information content (PIC), effective

multiplex ratio (EMR) and marker index (MI) value of 0.40, 3 and 1.21 respectively.

Eight mutants viz. ACNMM 20, ACNMM 23, ACNMM 17, ACNMM 12, ACNMM 22, ACNMM 1, ACNMM 3, ACNMM 9 were selected on the basis of yield and some of these mutants viz. ACNMM 23, ACNMM 22, ACNMM 13, ACNMM 14, ACNMM 7 were observed to be diverse from the checks as they appeared in different clades as that of checks. Some of them were found similar to the checks viz. ACNMM 9, ACNMM 4, ACNMM 17, ACNMM 19 to BIO-902 and ACNMM 15 to Kranti as they occupied the same clade as that of the check.

These superior mutants will be further evaluated in multilocation trials of superior and diverse genotypes can be released as variety or used in breeding programme.

Chapter VI

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