CHARACTERIZATION OF *Moricandia* BASED RESTORER LINES IN INDIAN MUSTARD [*Brassica juncea* (L.) Czern and Coss]

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ABSTRACT

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ABSTRACT

Genotypic variability, molecular diversity and fertility restorability were studied in a set of twenty-one genotypes. That includes 15 restorers for *Moricandia* based CGMS system with 3 A lines 'CMS' with their respective 3 B lines 'Maintainer' of mustard [*Brassica juncea* (L.) Czern and Coss] which were grown in RBD design with three replications. Along with this experiment, 45 hybrids synthesized using those 3 A and 15 R lines were also grown in single replication for fertility restorability study at the Agronomy Instructional Farm, SDAU, Sardarkrushinagar during *Rabi* 2021-22. Molecular analysis work was also carried out on those 21 parental lines at biotechnology laboratory of the Department of Genetics and Plant Breeding, CPCA, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

All fifteen genotypes showed sufficient amount of variability based on eighteen qualitative descriptors. Among the eighteen visually assessed DUS descriptors, six were monomorphic, seven were dimorphic and five were polymorphic in fifteen Indian mustard genotypes. Analysis of variance for all eleven quantitative characters with all 15 genotypes showed significance differences among the characters and indicating enough amount of variability present in the material. Genotypic variance was higher than their respective environmental variance for most of the quantitative characters (except seeds per siliqua and oil content) which indicated less influence of environment on these characters. GCV and PCV was moderate for number of branches per plant, number of siliqua per plant, 1000 seed weight and seed yield per plant which indicates that noticeable amount of genetic variability presents for these characters in the present set of materials and selection of these traits will be fruitful. High heritability was found for days to maturity (80.42%), number of branches per plant (83.69%), number of siliqua per plant (77.82%) and 1000 seed weight (97.95%). The phenotypic selection would be effective for number of branches per plant, number of siliqua per plant and 1000-seed weight, as they expressed the high genetic advance coupled with high heritability.

Seed yield per plant exhibited highly significant and positive correlations with number of branches per plant (r_g= 0.966), number of siliqua per plant (r_g= 0.989), number of siliqua on main shoot (r_g= 0.978), number of seeds per siliqua (r_g= 0.923), siliqua length (r_g= 0.563) and 1000 seed weight (r_g= 0.739) at genotypic level, while highly significant and negative correlation with days to maturity (r_g= -0.619) at genotypic level. Path coefficient analysis revealed that highest positive direct effect on seed yield was noticed by number of branches per plant (1.218) followed by number of siliqua per plant (0.789), seeds per siliqua (0.774), days to maturity (0.531) and 1000 seed weight (0.184). Negative direct effect on seed yield was found with oil content (-0.530) followed by number of siliqua length (-0.457), days to flowering (-0.219) and number of siliqua on main shoot (-0.217). Overall path analysis revealed that for improving seed yield in mustard, weightage in selection should be given to days to maturity, number of branches per plant, number of siliqua per plant, seeds per siliqua and 1000 seed weight.

Molecular diversity of 21 mustard genotypes was analyzed using simple sequence repeat (SSR) analysis. DNA extraction was carried out by CTAB method. The quality and quantity of genomic DNA was good. Total 30 SSR primers were screened across all the genotypes of mustard. The polymorphism was found from six SSRs primers (BRMS-01, BRMS-08, SSR Na10-D09, SR-7223, SR-94102 and BG-48). The Polymorphic Information Content (PIC) value and total number of alleles per locus with an average of 0.50 and 2 allele per locus, respectively. Similarity indices derived from Jaccard’s similarity co-efficient were estimated on the basis of 30 SSR primers ranged from 0.22 to 0.94. Between GM*Mo*2128R and GM*Mo*2124A showed minimum similarity (0.22). A dendrogram based on the UPGMA analysis, showed 2 main clusters and 4 sub-clusters. It is also noted that some pairs of genotypes had similar genetics *i.e.,* all ‘A’ and ‘B’ lines are included in A1 sub-cluster and all ‘R’ lines are in A2, B1 and B2 sub-clusters.

The CMS line crossed with restorer line exhibited pollen fertility in most of crosses. The crosses GM*Mo*2124A × GM*Mo*2113R showed lower pollen fertility restorability (62.50%) followed by GM*Mo*2125A × GM*Mo*2115R (66.67%). The crosses GM*Mo*2118A × GM*Mo*2125R and GM*Mo*2125A × GM*Mo*2124R (98%) showed higher pollen fertility showing good restorability of both the restorer lines. At field level, siliquae set upon selfing resulted GM*Mo*2124A × GM*Mo*2113R showed lower restorability (62.40%) followed by GM*Mo*2125A × GM*Mo*2115R (66.40%). The crosses GM*Mo*2118A × GM*Mo*2125R and GM*Mo*2125A × GM*Mo*2124R (97.60%) showed higher fertility. On the basis of both the test, GM*Mo*2113R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2132R and GM*Mo*2133R are found having good restoration ability in their hybrids.

Combining all the aspects studied, it could be concluded that the number of branches per plant, number of siliqua per plant, seeds per siliqua and 1000 seed weight had a positive correlation coefficient with yield. Using present set of SSR markers, all 15 ‘R’ lines can be differentiated from ‘A’ and ‘B’ lines. Less differences between A and B lines indicated more molecular markers need to be studied. Also, ample fertility restorability is available in this material that can be used for authentication of stability in fertility restoration capacity in *Moricandia* based CGMS system of Indian mustard.

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CERTIFICATE – I

Date: 19/9/2022

This is to certify that the thesis entitled **"CHARACTERIZATION OF *Moricandia* BASED RESTORER LINES IN INDIAN MUSTARD [*Brassica juncea* (L.) Czern and Coss]"** submitted for the degree of **MASTER OF SCIENCE** in the subject of **GENETICS AND PLANT BREEDING** is a record of bonafide research work carried out by **KOLI MUKESHBHAI CHHAGANJI** under my guidance and supervision and that no part of this thesis has been submitted for any other degree, diploma, associateship, fellowship or other similar titles. The assistance and help received during the course of investigation have been fully acknowledged.

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(KOLI MUKESH C.)

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	:	Degree Celsius
A_{260}	:	Absorbance at 260 nanometers
A_{280}	:	Absorbance at 280 nanometers
AFLP	:	Amplified Fragment Length Polymorphism
ANOVA	:	Analysis of variance
bp	:	Base pairs
C.D.	:	Critical difference
C.V.	:	Coefficient of variation
C: I	:	Chloroform: Isoamyl alcohol
cm	:	Centimeter
Cov.	:	Covariance
CPCA	:	Chimanbhai Patel College of Agriculture
CTAB	:	Cetyl Trimethyl Ammonium Bromide
d.f.	:	Degree of freedom
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxyribonucleotide triphosphates
DUS	:	Distinctiveness, Uniformity and Stability
e.g.	:	Exempli gratia
EDTA	:	Ethylene diamine tetra acetic acid
EST	:	Expressed Sequence Tag
et al.	:	*Et alii* (and others)
etc.	:	Et cetera
F:	:	Forward primer
F_1	:	First filial generation
Fig.	:	Figure
G	:	Gram
GA	:	Genetic advance
GAM	:	Genetic advance as per centage of mean
GCV	:	Genotypic coefficient of variation
H^2b	:	Heritability broad sense
Ha	:	Hectare
Hr	:	Hour
i.e.	:	Id est (That is)
ISSR	:	Inter-Simple Sequence Repeat
Kg	:	Kilogram
M	:	Molar
M.S.P.	:	Mean square of products
MAS	:	Marker assisted selection
Max.	:	Maximum
M_e	:	Error mean sum of square
M_g	:	Genotypic mean sum of square
mg	:	Milli gram

Min. : Minimum
ml : Milliliter
mM : Milli molar
M_r : Replication mean sum of square
MS : Mean square
NaCl : Sodium chloride
ng : Nanogram
NIR : Near-infrared
No. : Number
P: C: I : Phenol: Chloroform: Isoamyl alcohol
PCR : Polymerase Chain Reaction
PCV : Phenotypic coefficient of variation
pg : picogram
pH : Potential of hydrogen
PIC : Polymorphic information content
pmol : Pico mole
PVP : Polyvinylpyrrolidone
r : Replication
R: : Reverse primer
RAPD : Random Amplified Polymorphic DNA
RBD : Randomized Block Design
RFLP : Restriction Fragment Length Polymorphism
r_g : Genotypic correlation coefficient
RNase : Ribonuclease
r_p : Phenotypic correlation coefficient
rpm : Revolution per minute
S.E. : Standard error
S.Ed. : Standard error of difference
S.Em. : Standard error of mean
SDAU : Sardarkrushinagar Dantiwada Agricultural University
SNPs : Single nucleotide polymorphisms
Sr. : Serial
SSE : Error sum of square
SSG : Genotypic sum of square
SSR : Replication sum of square
SSRs : Simple Sequence Repeats
SST : Total sum of square
TBE : Tris-Borate-EDTA-buffer
TE : Tris- EDTA
Tm : Melting temperature
UPGMA : Unweighted Pair Group Method with Arithmetic Mean
viz. : Videlicet (Namely)
vs. : Versus
w/v : Weight/ Volume

µl : Micro litre
σ^2_e : Variances due to error
σ^2_g : Variances due to genotype
σ^2_p : Variances due to phenotype
σ^2_r : Variances due to replication
Σp : Phenotypic standard deviation
= : is equal to
° : Degree
. : Full stop
' : Minute
× : Multiply
- : Minus
( ) : Bracket
√ : Square root
[ ] : Square bracket
, : Comma
% : Per cent
$>$: Greater than
$<$: Less than
/ : Per
σ : Sigma
; : Semi colon
: : Colon
μ : General mean

INTRODUCTION

I. INTRODUCTION

Today, agriculture has considered a commercial profession and oilseed crops are cultivated by farmers owing to good remunerative prices. Oilseeds occupy prime importance in India as well as in the world, which is evident from the impact created by the yellow revolution. The demand for oilseeds and their products is ever-increasing and has become an essential part of our daily diet in view of changing lifestyles and increasing population.

Indian mustard [*Brassica juncea* (L.) Czern and Coss.], commonly known as rai or raya, is one of the major oilseed crops cultivated in the Indian sub-continent. Mustard is a *rabi* season crop of temperate regions, which requires relatively cool and dry weather for satisfactory growth. The crop requires about 18^0C to 25^0C temperature, low humidity and practically no rains at the time of flowering. It is resistant to frost at all the growth stages. It is grown on a wide range of soil types from fairly heavy clay to light sandy soil but loamy soil is most suitable for its cultivation. The normal sowing time of mustard crop is 8th to 31st of October.

Indian mustard belongs to the family *Brassicaceae* (*Cruciferae*) and the genus *Brassica*. The family *Brassicaceae*, containing about 350 genera and 3500 species, is one of the ten most economically important plant families with a wide range of agronomic traits. The name crucifer comes from the shape of flowers, with four diagonally opposite petals in the form of a cross. Mustard includes six cultivated species, among these, *B. nigra* (n=8), *B. oleracea* (n=9), *B. campestris* (n=10) are diploids and the rest of the three, namely *B. carinata* (n=17), *B. napus* (n=19) and *B. juncea* (n=18) are amphidiploids (Nagahuru, 1935).

Indian mustard or brown mustard is a natural amphidiploid (2n=36) derived from an interspecific cross of *Brassica campestris* (2n=20) and *Brassica nigra* (2n=16). It originated in Asia with its major centre of diversity in China. It was introduced in India from China and from where it spread to Afghanistan and other countries (Vaughan, 1977).

Mustard is largely a self-pollinated crop (85-90%). However, owing to insects, especially honeybees, the extent of cross-pollination varies from 4.0 to 16.6 per cent (Rambhajan *et al*., 1991). Mustard seed is largely crushed for edible oil, which is perhaps the cheapest source of oil in our daily diet. Mustard seeds contain about

38-46 per cent oil, which is golden yellow in colour, considered among the healthiest and most nutritional cooking mediums. It is also utilized as a condiment, for medicinal uses and has industrial applications. Besides, its oil also serves as an important raw material for industrial products like soap, paints and lubricants etc. The mustard cake is also nutritious and contains about 12 per cent oil and 38-42 per cent protein but due to its high glucosinolate content, it is not suitable for animal feed (Nagraj, 1995).

India occupies the first position in area and second position in the production of mustard after China in the world and contributes 28.3 and 19.8 per cent as its share in acreage and production of rapeseed-mustard, respectively in the world. Mustard is one of the most important oilseed crops in India, grown next to groundnut. It is cultivated in an area of 68.50 lakh hectares in India with an annual production of 91.20 lakh tonnes and productivity of 1331 kg/ha. It is cultivated particularly in Rajasthan, Uttar Pradesh, Punjab, Haryana, Gujarat, Madhya Pradesh, Bihar and Jharkhand. In Gujarat, Banaskantha, Sabarkantha, Mehsana, Patan, Gandhinagar, Arvalli and Kutch are the major mustard-producing districts. An estimated area in Gujarat is 1.72 lakh hectares with a production 3.33 lakh tonnes and a productivity of 1932 kg/ha (Anonymous, 2019-20).

The nutritional and functional properties of oils are determined by their fatty acid composition and the distribution pattern of fatty acids within the triacylglycerol molecule. A good percentage of essential fatty acids (Linoleic acid; C18:2 and Linolenic acid; C18:3) in rapeseed-mustard oil makes it desirable from a nutritional point of view, but the amount of erucic acid; C22:1 (40-57%) lowers its utility as edible oil (Agnihotri *et al.,* 2007). Consumption of oil with >20% erucic acid in the diet causes myocardial fibrosis and lipidosis in monkeys (Ackman *et al.,* 1977). However, rapeseed-mustard meal contains a high amount of anti-nutritional compounds called glucosinolate (Wanasundara, 2011). Cleavage products from hydrolysis of glucosinolate reduce the feed palatability by affecting the iodine uptake by thyroid glands, especially in non-ruminants including pigs and poultry (Bell, 1984). Therefore, the amelioration of nutritional qualities by developing new varieties having alternative oil and meal characteristics has been an important objective in the quality breeding of rapeseed mustard.

The success of any breeding programme depends upon the genetic variability engraved in the breeding material. The assessment of parameters including phenotypic and genotypic coefficients of variation, heritability in a broad sense and genetic advance as per cent of mean is a pre-requisite for making an effective selection. Yield is a complex trait, polygenic in inheritance and more prone to environmental fluctuations than ancillary traits such as a number of branches per plant, seeds per siliqua, main shoot length and 1000 seed weight.

At present large number of mustard cultivars are available for commercial cultivation. A set of descriptors/characters are generally used for characterization, differentiation and protection of plant varieties. The descriptor refers to the form, structure or behaviour of accession in a germplasm collection (Begum and Kumar, 2011). Descriptors should be based on the relative characteristics of the plant by which it can be defined as a variety. These descriptors should satisfy three technical requisites of Distinctness, Uniformity and Stability (DUS). Distinctness means the variety should be clearly distinct from existing varieties. Uniformity indicates the similarity of characteristics and features among the plants of the selected variety. Stability means that the variety should produce stable results year after year. DUS testing of cultivars is required for granting Plant Breeders’ Rights (PBR) and it is conducted according to the DUS guidelines. In the DUS testing protocol, morphological features of the plant play a greater role as these are recognized as one of the most promising features which can differentiate the plant varieties.

The cytoplasmic genetic male sterility and fertility restoration (CGMS-FR) system is an efficient and heritable pollination control method in hybrid seed production. Cytoplasmic male sterility leads to the production of non-functional pollen grains, resulting from an incompatible nuclear-cytoplasmic (mitochondrial) gene interaction. This maternally inherited male sterility can be restored in the F_1 hybrids by an appropriate fertility restorer gene (Eckardt, 2006). These fertility restorer genes may be available in nature or may be introgressed from the wild species from which the CMS was developed. Cytoplasmic genetic male sterility (CGMS) system comprises male sterile (A) line, maintainer (B) line and restorer (R) line and have been successfully utilized in many crops such as maize (Rogers and Edwardson, 1952), pearl millet (Rai, 1995), sorghum, (Reddy *et al.*, 2007), rice (Shinjyo, 1975) *etc*. to produce commercial hybrids.

Molecular techniques have also critical roles in studies of phylogeny and species evolution. Molecular techniques have been applied to increase the understanding of the distribution as well as the extent of genetic variation within and between species. Several types of molecular markers such as Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR) (Tripathi *et al.*, 2012), Restriction Fragment Length Polymorphism (RFLP) (Diers and Osborn,1994), Amplified Fragment Length Polymorphism (AFLP) (Tripathi *et al.*, 2011), Simple Sequence Repeat (SSR) and Sequence-Tagged Microsatellite Sites (STMS) (Wang *et al.*, 2009) have been used in plant breeding for a wide range of applications. The main advantages of using molecular markers are that they measure the genetic diversity at DNA level, can account for the effects of selection are environment independent and are available in an enormous number. The molecular marker technique allows cultivar identification in the early stage of plant development (Williams *et al.*, 1990).

Among the various DNA-based markers, SSR is a class of genetic markers that have proven to be abundant and well distributed throughout the genome of plants (Wu and Tanksley, 1993; Chin *et. al.*, 1996 and Wu *et. al.*, 2006). SSR markers have the ability to discriminate genotypes into homozygotes and heterozygotes due to their co-dominant nature, high level of polymorphism as compared to any other molecular marker assay is achieved (Powell *et. al.*, 1996).

The line x tester mating design has been widely used in crop plants for testing the performance of genotypes in hybrid combinations and also for characterizing the magnitude and nature of gene action (Kempthorne, 1957). With these perspectives, the present investigation was planned using *Moricandia* based CMS as line x *Moricandia* based 'R' as tester mating design in Indian mustard with the following objectives.

Objectives

1) DUS characterization of restorer lines
2) To study the genetic variability among restorer lines
3) To analyze molecular diversity using microsatellite markers
4) To apprize fertility restorability of restorer lines

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The literature available pertaining to the present investigation on mustard has been reviewed and presented under the following subhead:

2.1. MORPHOLOGICAL VARIATION

2.2.GENETIC VARIABILITY

2.3. MOLECULAR DIVERSITY

2.4. FERTILITY RESTORABILITY

2.1. MORPHOLOGICAL VARIATION

The morphological characterization is the first step toward diversity analysis and conservation of plant genetic resources. Varieties are identified through distinctiveness, uniformity and stability (DUS) testing protocol. In the DUS testing protocol, morphological features of plants play a greater role. DUS characterization is recognized as one of the most promising features which can differentiate plant varieties. The promising way of determining whether descriptors could differentiate varieties or not can be best observed by studying the characteristics of field layouts at least for two years by keeping all the agronomical practices identical. Morphological characteristics of the DUS protocol prove to be very useful and convenient for varietal identification which in turn helps in the maintenance of genetic purity of varieties.

Singh *et al.* (2012) studied to identify and classify eight varieties of cabbage by DUS characterization. Among 26 morphological characteristics studied, 20 were visually assessed and six were measured. No intra-varietal variation was observed for any visual characteristics and expression characters in different varieties remained the same for two consecutive years confirming the uniformity and stability of the variety for visual characteristics.

Yadav *et al.* (2013) grouped 78 genotypes of Indian mustard for several morphological descriptors. The number of lobes varies from low to medium. Stem colour varies from light green to dark green. Wide diversity has been observed for leaf length (40.2 to 63cm) and leaf breadth (18 to 27cm). No variability was observed for leaf division, petiole enlargement, petiole section, petiole colour, flower colour and silique surface outline. In the majority of cases, it was observed that leaf angle was a prostrate type, leaf blade shape was spathulate, leaf division dentate lyrate type, leaf apex and leaf blistering was intermediate, leaf tip and lamina attitude was straight and sparse hairs were present. Petiole length, width and thickness were intermediate types. Similar, findings were also observed for petal length, breadth, silique angle, length andwidth and also for pedicel and beak length. The seed colour of all the genotypes was black colour except RH 401Y which was yellow in colour. The maximum genotypes had medium size seed (3-5g/1000 seed weight) except RH 270(bold seed>5g/1000 seed weight).

Singh *et al*. (2013[a]) classified 15 varieties of cauliflower by DUS characterization. They identified that most of the varieties exhibited absent anthocyanin colouration while the snowball group expressed seedling anthocyanin colouration. Two varieties namely Pusa Sharad and Pusa Snow Ball-1 expressed medium and 13 varieties showed long stalk length, while none of the varieties depicted short outer stem (stalk) length. As per leaf attitude, three varieties expressed erect growth habit, while two varieties showed horizontal and the rest 10 varieties showed semi-erect type growth habit. Four varieties had short leaf length, ten varieties showed medium leaf length and other varieties showed long leaf length. Considerable variation for leaf shape, variety like Pusa Meghna was of narrow elliptic leaf shape while eight varieties were of elliptic type and rest seven varieties had broad elliptic type leaf shape. On the basis of leaf lobe, most of the varieties expressed leaf lobe except Pusa Paushja, Pusa Sharad, Pusa Himjyoti, Pusa Snow Ball-1 and Pusa Shakti. On the basis of leaf colour, most of the varieties were found to have light and dark green leaves except Pusa Paushja which showed a bluish leaf colour. On the basis leaf, waxiness was absent in Pusa Meghna while five varieties exhibited medium waxiness. Five varieties expressed light, whereas Pusa Paushja and PSBK-25 showed strong leaf waxiness. The trait leaf torsion was found absent in five varieties, while five varieties expressed weak and rest varieties showed medium torsion of the leaf tip. None of the varieties showed strong torsion of the leaf tip.On the basis of leaf profile of upper side of blade character, five varieties were of flat type leaf, while six varieties showed convex and rest varieties showed concave type leaf. Among thevarieties, weak leaf puckering was absent in four varieties, whereas seven varieties expressed medium and three varieties showed strong leaf puckering characters. Seven varieties as weak leaf crimping near main vine, five as medium and three as strong, whereas none of the varieties had no leaf crimping near main vine. Three varieties with early curd initiation, while nine varieties were in the medium group and four varieties exhibited late curd initiation.

Neeru *et al*. (2017) evaluated sixty genotypes and germplasm lines and characterized for 14 quantitative and 8 qualitative traits in Indian mustard. Observations were recorded on the basis of scores given in the DUS descriptor. The majority of accessions were very late in maturity and medium in flowering. No variability was observed for leaf length and leaf width. On the basis of branches, most of the genotypes were classified under the intermediate category. Long main shoot length (31), medium number of siliquae on main shoot (46), medium density on main shoot (52), short siliqua length (51), very tall plant height (38), few numbers of seeds per siliqua (33), medium 1000-seed weight (38), medium seed yield per plant (32) and low oil content (40) were observed in most of the genotypes. For qualitative traits, most of the genotype showed dark green leaf colour, sparse hairs, dentation of leaf margin lyrate type, open leaf growth habit, yellow petal colour, semi-appressed siliqua angle with main shoot and intermediate siliqua surface texture.

Tiwari *et al*. (2017^{b}) classified 18 varieties of mustard in the field by observing six morphological characteristics of the DUS protocol. Further, they classified varieties based on three qualitative characters *viz*., leaf hairiness, time of 50 per cent flowering and maturity period and three quantitative characters *viz*., number of siliqua on the main shoot, seeds per siliqua and weight of 1000 seeds. The varieties PCR-7, Pusa Jaikishan, RH-781 and CS-52 were identified separately on the basis of hairiness, number of siliqua on main shoot and number of seeds per siliqua respectively. All the remaining varieties were classified into groups based on time of 50 per cent flowering, maturity period and weight of 1000 seeds.

Hinge and Malode (2018) evaluated morphological variation in 10 accessions of *Brassica napus* using 23 morphological traits. Out of the 23 morphological descriptors used, 10 were significantly different and proved useful in examining diversity and differentiating among accessions studied. No considerable variability was observed for leaf hairiness, leaf colour, leaf lobes, number of leaf lobes, leaf margin, leaf length, leaf width, flower colour, length of petal, width of petal, siliqua angle with main shoot, siliqua texture and number of seeds. Wide diversity has been observed for the time of flowering, siliqua length, maturity period, seed colour and seed weight. It was observed that maximum genotypes have thousand seed weight of medium size (5.0-6.0) except genotype 10367 had bold seed size (>6g). Time of flowering was late (>50 days) for all the genotypes except early flowering (<40 days) Accession no. 2. Also, it was found that the siliqua length of genotype 10362 was comparatively smaller (<4.5 cm) than other studied genotypes.

Singh *et al.* (2018) evaluated 31 genotypes of mustard using eight morphological traits. They revealed that eight varieties had small seed weight, nine varieties had medium seed weight and eight varieties had bold seed weight. Three varieties had small siliqua length, 14 varieties had medium siliqua length and eight varieties had long siliqua length. Four varieties showed early maturity, 17 varieties showed medium maturity and seven varieties showed late maturity. Two varieties showed short plant height, 17 varieties showed medium plant height and ten varieties showed tall plant height. Nine varieties showed small length of main raceme, 14 varieties showed medium length of main raceme and three varieties showed tall length of main raceme. One variety showed low seeds per siliqua, 19 varieties showed medium seeds per siliqua and six varieties showed many seeds per siliqua. Six varieties showed small beak length and 24 varieties showed medium beak length. 14 varieties showed medium days to flower initiation and 16 varieties showed late days to flower initiation.

Ashraf *et al.* (2020) evaluated 28 genotypes of turnip(*Brassica rapa* var. *rapifera* L.) with ten traits. The genotypes used in the study, upon categorization showed variation with respect to vegetative and root characters. A wide range of variation was found among the genotypes for various morphological traits. Leaf margins, all the twenty-eight genotypes showed dentate leaf margins and the colour of the upper portion of root skin showed Creamish(6), white(2), light greenish(2), pink(4), and purple(14). The colour of the lower portion of root skin showed white(15), pinkish(4), and purple(9). All the twenty-eight genotypes under study showed white flesh colour. Root shape showed flattish round root shape(19) and round root shape(9). The core colour was white in all the genotypes. Also, pithiness and root branching were absent in all the twenty-eight genotypes studied and pungency showed in seven genotypes.

Bano *et al.* (2021) evaluated 54 genotypes of *Brassica rapa* with nine traits. All the plants were short in leaf length. Leaf colour was mostly light green followed by dark green and medium green. The plant exhibited mostly narrow (44%) leaf width followed by medium (23%). The plants exhibited mostly entire (65%) leaf dentation of margin followed by dentate (17.5%) and serrate (17.5%). Present leaf hairiness was found in maximum genotypes with frequency (88%) followed by absent (12%). Leaf lobes were mostly present with frequency (70%) followed by absent (30%), and most plants exhibited a medium number of leaf lobes with frequency (70%) followed by low (18%) and long (12%). The plant mostly exhibited light yellow (79%) flower petal colour followed by yellow (21%). The seed colour was mostly brown with frequency (53%) followed by dark brown (30%) and reddish brown (12%).

Hyder *et al*. (2021) evaluated 50 genotypes of gobhi sarson (*Brassica napas* L.) with 24 traits. Highest variation present in genotypes for traits like the width of petals, seed colour, leaf dentation of margin, plant height, siliqua number on main raceme length and 1000 seed weight. Low variation is present in genotypes for traits namely leaf hairiness, leaf colour, leaf lobes, siliqua texture, main raceme length, siliqua length and oil content percentage. All the germplasm lines exhibited 100 per cent short leaf length, narrow leaf width, short petal length, yellow flower petal colour, days to 50 per cent flowering, number of seeds per siliqua and days to 80 per cent maturity and marked no differences among genotypes for these traits.

2.2. GENETIC VARIABILITY

Variation in a character is a must for improvement in that character. Heritable variations in plants are essential to the plant breeder without them plant improvement may not fruitful. The breeder’s particular task is to identify those heritable variations which will be useful for the improvement of crop plants and to concentrate genes for those characteristics into a variety.

Some of the brief reviews of the literature on genetic variability are given as under:

Allard (1960) suggested that genotypic variance must be measured with references to some particular group of environments. He also reported that genotypic variance estimated from a single test in one environment contains interaction variance in addition to the genotypic variance.

Kumar *et al*. (2000) assessed genetic variability in selected mutant lines of *Brassica juncea* L. The high genotypic and phenotypic coefficient of variation was recorded for number of primary branches per plant, number of secondary branches per plant, number of siliquae per plant and seed yield per plant.

Gupta (2005) evaluated fourteen genotypes including the European cultivar of Indian mustard. Genotypes Topa, Excel, Karat, Legend, Nikolas, Gulliver and Hermes revealed a great amount of variability for seed yield per plant, number of primary and secondary branches per plant, number of seeds per siliqua. All these traits were highly influenced by the environment as revealed by a phenotypic and genotypic coefficient of variation.

Patel *et al.* (2006) studied forty genotypes of Indian mustard for thirteen different characters and they observed a considerable amount of variability for all the characters under study. The close correspondence between GCV and PCV was observed for test weight, seed yield per plant, number of siliquae per plant and days to 50 per cent flowering.

Muhammad *et al.* (2007) studied variability, heritability and genetic advance in eight lines along with two varieties of *Brassica juncea* (L.) and they revealed that number of siliquae per plant had a higher genotypic coefficient of variability, broad sense heritability and genetic advance.

Chauhan *et al.* (2008) screened forty genotypes of Indian mustard to study the extent of genetic variability for fatty acid profile, oil, protein and glucosinolate content. They revealed that environmental effects were significant for erucic, oleic acid, glucosinolate and protein content. The genotypic coefficients of variation were high for oleic, erucic and linolenic acid. The heritability in broad sense was relatively high for oleic and erucic acid. The high heritability was associated with high genetic advance only for oleic acid suggesting the role of additive gene action in the inheritance of this character.

Yadav *et al.* (2011) carried out an experiment with thirty released varieties of Indian mustard to determine variability for seed yield and its component characters. High PCV and GCV were observed for point to the first branch, seed yield per plant, point to first siliqua, number of secondary branches per plant and 1000 seed weight. They reported 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.

Kumar (2013) studied twenty-four parents and eight F_1 crosses of Indian mustard to assess the nature of variability. They found the GCV and PCV were high for oleic, palmitic plus stearic, erucic and linolenic acid. The high heritability was associated with high genetic advance only for oleic acid suggesting the role of additive gene action in the inheritance of this character.

Kumar *et al*. (2013) examined sixty germplasm of Indian mustard for determining genetic variability and its parameters. They found that greater contribution of additive genetic component was reflected by main shoot length, number of siliquae on main branch, siliqua length, palmitic acid, oleic acid and linolenic acid with a pronounced range of variation, high heritability coupled with a high genetic advance under selection.

Bind *et al*. (2014) evaluated thirty genotypes of Indian mustard to study variability for seed yield and its related traits. They revealed that genetic variability was found maximum for biological yield per plant and minimum for days to maturity as reflected by a genotypic coefficient of variation. Heritability estimate in broad sense was high for 1000 seed weight, days to maturity, days to flowering, plant height and main shoot length. Genetic advance as per cent of mean was high for biological yield per plant, 1000 seed weight, seed yield per plant, number of secondary branches per plant and main shoot length.

Lodhi *et al*. (2014) studied ninety diverse genotypes of Indian mustard for fifteen quantitative traits. Both phenotypic and genotypic coefficients of variation were higher for important traits including number of secondary branches per plant, seed yield per plant and 1000 seed weight. High heritability in conjunction with high genetic advance was observed for seed yield per plant, number of secondary branches per plant, 1000 seed weight, number of seeds per siliqua, primary branch angle, number of primary branches per plant, siliqua angle, siliquae on main shoot and siliqua length suggesting a predominant role of additive gene action for the expression of these traits.

Akbari and Niranjana (2015) examined thirty-six Indian mustard genotypes to estimate variability, heritability and genetic advance for seed yield and its components. Higher values of phenotypic and genotypic coefficient of variation were observed for number of secondary branches per plant, number of siliquae per plant and seed yield per plant indicating the existence of higher magnitude of variability among the test genotypes for effective selection in respect of the above characters. Higher heritability estimates values were recorded for number of siliquae per plant, seed yield per plant, number of seeds per siliqua, length of main branch, days to 50 per cent flowering, 1000 seed weight, number of secondary branches per plant, siliqua length, protein content and plant height. High heritability values coupled with high genetic advance were observed in case of number of siliquae per plant, plant height, length of main branch and seed yield per plant, indicating that selection for these traits would be effective.

Sharafi *et al.* (2015) evaluated twenty accessions of six *Brassica* species to analyze variability parameters. They found the higher GCV and PCV for oil content, stearic acid, oleic acid, linolenic acid and erucic acid. Oil content, stearic acid, oleic acid, linolenic acid, linoleic acid and erucic acid showed high heritability.

Bibi *et al.* (2016) studied ten genotypes of Indian mustard to evaluate the genetic variability. They found that the high heritability coupled with high genetic advance for plant height, silique length and seed yield which gave evidence that these traits were under the control of additive genes which will be more useful in predicting the gain under selection.

Gupta and Dwivedi (2016) studied genetic variability of twelve quantitative characters among twenty-six genotypes of Indian mustard. They observed higher broad sense heritability for days to 50 per cent flowering, number of primary branches per plant, number of secondary branches per plant, plant height, 1000 seed weight and biological yield per plant. The expected genetic advance was recorded higher for biological yield per plant and the number of secondary branches per plant.

Trivedi *et al.* (2016) studied thirty-five genotypes of Indian mustard and revealed significant variability among the genotypes for all the traits under study. The estimates of the genotypic and phenotypic coefficient of variation were high for biological yield per plant, seed yield per plant, harvest index and 1000 seed weight and moderate for number of primary branches per plant, number of secondary branches per plant, number of siliquae on main branch, number of siliquae per plant and length of main branch. High heritability coupled with high genetic advance was observed for number of primary branches per plant, number of secondary branches per plant, number of siliquae on main branch, number of silique per plant, length of main branch, biological yield per plant, seed yield per plant, harvest index and 1000 seed weight.

Rathod *et al.* (2017) screened fifty genotypes of Indian mustard to study the extent of genetic variability for seed yield and its attributes. They observed that high heritability along with high GCV and high genetic gain was observed for seed yield per plant, days to 50 per cent flowering, number of primary branches per plant, number of secondary branches per plant, number of siliquae per plant and 1000 seed weight.

Tiwari *et al.* (2017^{a}) examined eighty-three genotypes of Indian mustard for determining genetic variability and its parameters. They found higher heritability coupled with higher genetic advance for number of primary branches per plant, number of secondary branches per plant, number of siliquae per plant, number of seeds per siliqua, harvest index and 1000 seed weight.

Malik *et al.* (2018) evaluated thirty-five genotypes of Indian mustard to study genetic variability for eight different traits. They reported high heritability and genetic advance for days to 50 per cent flowering, plant height and biological yield per plant. Although, number of secondary branches per plant, seed yield per plant and harvest index had moderately high heritability with moderate genetic advance indicating additive gene effects and selection pressure could be applied on them for yield improvement.

Maurya *et al.* (2018) examined fifty germplasm of Indian mustard for determining genetic variability and its parameters. They found higher estimates of PCV and GCV in the case of 1000 seed weight, number of secondary branches per plant, biological yield and seed yield per plant. High heritability coupled with high genetic advance as per cent of mean as well as high GCV were observed for yield components such as 1000 seed weight, biological yield per plant, number of secondary branches per plant, seed yield per plant,

Singh *et al.* (2018) studied sixty genotypes of Indian mustard and revealed that the magnitudes of PCV and GCV were high for seed yield per plant, 1000 seed weight, number of secondary branches per plant and length of main branch. High heritability estimates were observed for days to 50 per cent flowering, days to maturity, plant height, oil content, 1000 seed weight and seed yield per plant. The expected genetic advance as per cent of mean was high for seed yield per plant, 1000 seed weight, secondary branches per plant, plant height and length of main branch. The high heritability coupled with high genetic advance was observed for 1000 seed weight, plant height, seed yield per plant and length of main branch.

Gupta *et al.* (2019) carried out an investigation to estimate genetic variability among thirty-five genotypes of oilseed *Brassica*, including twenty F_2/F_3

populations derived through interspecific hybridization and fifteen parents. The estimates of genotypic coefficient of variation and phenotypic coefficient of variation were comparatively higher for plant height, number of primary and secondary branches per plant, number of siliquae per plant, siliqua length, number of seeds per siliqua, test weight, seed yield per plant and oil yield per plant. High estimates of heritability were recorded for days to 50 per cent flowering, days to maturity, main shoot length, number of siliquae on main shoot, number of seeds per siliqua, plant height, number of primary and secondary branches per plant, number of siliquae per plant, siliqua length, seeds per siliqua, test weight, seed yield per plant and oil yield per plant. The estimate of genetic advance (GA) was also recorded high for number of siliquae per plant and plant height.

Iqbal *et al.* (2019) assessed genetic variability in selected twenty-eight genotypes of Indian mustard. They revealed that estimates of GCV were observed to be higher in oleic acid, linolenic acid, ecosenoic and erucic acid, while moderate GCV was recorded for palmitic plus stearic acid and glucosinolate content.

Pal *et al.* (2019) performed a variability study for thirteen characters in seven genotypes of Indian mustard for seed yield and its contributing traits. They showed that the PCV was greater than GCV for all the traits *viz.*, days to 50 per cent flowering, days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, number of siliquae per plant, biological yield per plant, siliqua length, number of seeds per siliqua, 1000 seed weight, seedyield per plant, harvest index and oil content. High heritability along with high genetic advance as per cent of mean was recorded for seed yield per plant, number of seeds per siliqua, siliqua length and number of secondary branches per plant.

Patel *et al.* (2019) studied sixty diverse genotypes of Indian mustard in order to determine the genetic variability for seed yield and its attributes. They revealed that the phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation for days to flowering, days to maturity, plant height, length of main branch, total number of branches per plant, number of siliquae on main branch, number of siliquae per plant, length of siliqua, seeds per siliqua, 1000 seed weight, oil content and seed yield per plant. A high heritability value was recorded for 1000 seed weight, oil content, days to flowering and seed yield per plant. The highest value of genetic advance as per cent mean was observed for seed yield per plant.

Pandey *et al.* (2020) evaluated forty accessions of Indian mustard germplasm for seed yield as well as quality traits and revealed significant differences among the accessions for all the traits under study. They recorded the highest estimate of phenotypic and genotypic coefficient of variation for number of secondary branches per plant and harvest index. Higher estimates of heritability coupled with higher genetic advance were observed for number of secondary branches per plant and harvest index.

Patel *et al.* (2021) evaluated five genotypes for seed yield and quality traits and revealed that the low difference between genotypic and phenotypic variances revealed that the contribution of genotypic variance to total variance was more for all the traits except days to maturity and plant height. The high values of genotypic and phenotypic coefficient of variation for the number of branches per plant, seed yield per plant, myristic acid, palmitic acid and stearic acid which indicated the potential variability available for these traits. The high heritability estimates obtained in conjunction with high genetic advance were observed for the number of branches per plant, number of siliquae per plant, seeds per siliqua, length of siliqua, 1000 seed weight, seed yield per plant, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid and glucosinolate suggesting the predominant role of additive gene action for their expression.

Kaur *et al.* (2022) evaluated five diverse parents crossed in half diallel mating design and made 10 F_1 hybrids and observed high PCV and GCV by siliquae/plant. High heritability along with high genetic advance (GA) was observed for siliquae/plant, biological yield/plant and test weight (TW). At genotypic levels, it was revealed high positive path coefficient in harvest index (HI) followed by biological yield/plant and a highly negative path coefficient exhibited in TW followed by siliquae/plant.

2.3. MOLECULAR DIVERSITY

Molecular markers are being successfully used to decode diversity among populations. Amongst the various types of molecular markersused, Simple Sequence Repeat (SSR) markers are the most preferred because of their higher reproducibility, co-dominance nature, abundance,wide dispersal throughout the genome, and multi-allelic variation (Powell *et al*., 1996). Simple sequence repeats markers have beenused for genetic diversity analysis in a number of crops.

Recently the development of molecular markers made it possible to be used in a wide range of applications including confirming the identity of hybrids in breeding programmes, determining phylogenetic relationships in related species and facilitating introgression of desirable traits from wild relatives to cultivated crop species. Breeders can use DNA maps to carry out the marker-assisted selection(Chin *et al.*,1996).

The polymerase chain reaction (PCR) has become a popular technique for molecular genome mapping. The technique ensures the amplification of specific DNA sequences by the use of primers and the enzyme *Taq* DNA polymerase.

Abbas *et al.* (2009) utilized RAPD and SSR markers for molecular analysis of genetic diversity in *Brassica* species. They observed the mean genetic distance ranged from 26-89 per cent and 5-61 per cent for RAPD and SSR, respectively. The size of scorable fragments ranged from approximately 250 to >2000 bp. A high level of genetic dissimilarity (GD= up to 100%) was estimated among the 14 genotypes.

Qu *et al.* (2012) studied genetic diversity among *B. napus* inbred lines by SSR analysis. Sixty-two primers were used which produced 249 alleles in the 90 lines and the allele number for the SSR loci ranged from 2 to 7 with a mean value of 4.0. Cluster analysis was also carried out using the UPGMA method which divided yellow seeded cote lines from black seeded cote lines into different groups.

Turi *et al.* (2012) characterized 120 different accessions of *Brassica* species with the help of SSR markers. They used 39 SSR primers which produced 162 scorable bands out of which 105 were polymorphic. The average rate of polymorphic loci was 46%, which indicates high genetic diversity among the accessions.

Singh *et al.* (2013) studied genetic diversity by using 143 SSR primers tested, 134 reported polymorphism and a total of 355 alleles were amplified. 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).

Pratap *et al.* (2014) studied genetic diversity by using SSR markers, in 20 Indian mustard genotypes for *Alternaria* blight tolerance. Genotypes DRMR 2805 and DRMR 2806 found tolerant against *Alternaria* blight in the field conditions. Genetic diversity evaluated among 20 mustard genotypes using 25 *Brassica species* derived-SSR markers, resulted into polymorphic amplicons. The tolerant genotypes DRMR 2805 and DRMR 2806 identified in this study can be used for the introgression of *Alternaria* blight tolerance into the elite mustard.

Sudan *et al. (*2016) studied molecular diversity analysis of Indian and exotic genotypes of *Brassica juncea* using simplesequence repeat (SSR) markers. Sixteen out of 32 SSR markers were found to be polymorphic and amplified 54 alleles in 23 genotypes with an average of 2.37 and 0.31 for alleles per locus and the polymorphic information content (PIC),respectively.

Patel *et al.* (2018) studied genetic diversity by using a total of 200 SSR markers from different *Brassica* species. Out of 200 SSR markers analyzed for polymorphism in two parental mustard genotypes, 51 showed polymorphic. The PIC values for various primers ranged from 0.340-0.505 with an average of 0.406. Similarity coefficient data based on the proportion of shared alleles using 51 SSR markers were used to calculate the coefficient values among the 157 F_2 plants of RB 50 × Kranti and parental *B. juncea* genotypes and subjected to UPGMA tree cluster analysis. All the 157 F_2 plants clustered in two major groups at a similarity coefficient of 0.53. Two parental varieties RB 50 and Kranti had low similarity coefficients.

Thakur *et al.* (2018) tested a set of 124 simple sequence repeat (SSR) markers previously developed for various *Brassica sp.* including *B. nigra, B. oleracea, B. napus* and *B. rapa* for cross-transferability in twelve popular varietiesof *B. juncea.* Only 81 SSRs produced clear and distinguishable amplification products. Amplified allele sizes ranged from 40 to 410 bp and the number of alleles per locus ranged from 1 to 6, with an average of 2.17 alleles/locus. Out of these 81 SSR primer pairs, the highest frequency (97.5 %) of cross-transferability was recorded for the Indian mustard variety Kranti. A high percentage of cross-transferability of these SSR markers to *B. juncea* cultivars suggested the possibility of using these markers for various applications in the genetics and genomics of *B. juncea.*

Baghel *et al.* (2020) studied genetic diversity by using a total of 20 SSR markers of 48 mustard genotypes illustrated good technical resolution and out of a total of eight, seven furnished adequate variations among diverse genotypes. In total 50 per cent polymorphism was detected. The major group restrained 48 genotypes that were divided into three major groups, the first main group ‘I’ contained 17 genotypes, the second chief group ‘II’ hold 24 genotypes and the third core group ‘III’ included 7 genotypes. Polymorphic information content (PIC) was estimated for each of the 7 SSR markers. The highest PIC value (0.6851) was documented for the molecular marker OI10-CO 5. It had 8 alleles among the 48 genotypes, whereas the lowest PIC (0.4038) was examined for the molecular marker SR-7223.

Kumar *et al.* (2020) studied genetic diversity by using a total of 58 SSR primers among 45 ‘A’ and ‘R’ lines (5 A lines and 40 restorers) of Indian mustard. Out of 58 primers, 33 primers were found to be polymorphic. A total of 95 alleles were detected across all 45 genotypes. The PCR amplified products ranged from 70 bp to 350 bp in size. The number of alleles per locus varied from 2 to 5 with an average of 2.89. The PIC value varied from 0.206 to 0.749 with an average of 0.519. The highest PIC value was obtained for BG 132 (0.749) followed by BG 114 (0.744). The UPGMA cluster analysis suggested the demarcation of 45 genotypes into six distinct groups in the dendrogram wherein three groups had single genotype (restorer line) each.

Rajpoot *et al.* (2020) studied a total of 50 SSR markers for the characterization of lines, out of which 7 SSR markers were highly polymorphic between all the germplasms of mustard. A UPGMA phonogram was constructed for all 48 germplasms and the similarity coefficient ranged from 0.00 to 0.91. A number of alleles ranged from 3 to 4, genetic diversity ranged from 71 per cent to 65 per cent with an average value of 67 per cent, heterozygosity ranged from 20 to 10 per cent with an average of 12 per cent and PIC value for markers ranged from 0.65 to 0.59 with mean PIC value 0.61. All seven SSR primers showed a PIC value above 0.5 (50%) indicating high genetic diversity in the studied plant material.

Singh *et al.* (2020) studied genetic diversity by using a total of 70 SSR markers of 95 mustard genotypes. Of 70 SSR markers, 44 were found to be polymorphic which amplified 157 alleles in 95 different genotypes with the mean value of 3.57 alleles per locus and 0.48 of average polymorphic information content (PIC). The expected heterozygosity (H_e) and observed heterozygosity (H_o) values were 0.54 and 0.81, respectively. Jaccard's dissimilarity coefficient ranged from 0.13 to 0.77. Based on the dissimilarity coefficient, M 13 and RC 47 were the most diverse genotypes (0.77).

Thakur *et al.* (2021) studied genetic diversity in 16 genotypes of Ethiopian mustard in which 125 *Brassica rapa* L. specific and 70 *Brassica nigra* L. specific genomic SSRs had been evaluated. A cross-transferability rate of 82.4 per cent (103 SSRs) and 84.3 per cent (59 SSRs) had been obtained for *B. rapa* and *B. nigra* derived SSRs, respectively, out of which 66 (64.1%) of *B. rapa* and 37 (62.7%) of *B. nigra* SSRs resulted into polymorphic amplicons. Comparison of polymorphism parameters including PIC value, gene diversity and heterozygosity values revealed that *B. nigra* SSRs were more efficient in terms of transferability rate and polymorphic potential than *B. rapa* SSRs.

Verma *et al.* (2021) studied genetic diversity among 38 germplasm lines and 5 cultivated varieties of Indian mustard using 11 microsatellite markers. A total of 65 alleles were identified. The genotypes and alleles per locus varied from 3-12. Maximum numbers of alleles were amplified with SR_922, while a minimum of 3 with CN-52 and SORF-73 markers. The mean value of polymorphic markers was 5.99.

2.4. FERTILITY RESTORABILITY

The presence of exploitable hybrid vigour, availability of cytoplasmic genetic male sterility and pollen fertility and sound seed production techniques are the prerequisites for the success of any hybrid breeding programme. In the exploitation of heterosis from potential crosses, the level of pollen fertility would likely be the key to added yield advantages. Pollen fertility/sterility has been observed in F_1. The hybrids have been tested for their pollen fertility status at the initial flowering stage of a randomly selected plant on each hybrid. To identify the sterility/fertility of pollen grains, a 2% aceto-carmine solution can be used (Alexander, 1969). Therefore, a precise understanding of the pollen fertility of different restorers is necessary for improving the efficiency and quality of restorers used in hybrid mustard breeding.

The salient studies conducted on pollen fertility in mustard are reviewed here as below:

Kirti *et al.* (1997) reported the crosses made on the *B. juncea* CMS line with the restorers R72 and R74. Fertility restored plants were selfed and also crossed as females with maintainer line *B. juncea cv.*‘Pusa Bold’. Crosses of the fertility restored plants with the maintainer line segregated in a 1:1 ratio (fertile: sterile). Selfing of restored plants in segregation of fertile and sterile plants in 3:1. This ratio fits well for monogenic segregation indicating that the fertility restoration is due to a single dominant gene.

Prakash *et al*. (1998) studied a *Moricandia arvensis* based cytoplasmic male sterility and fertility restoration system in *Brassica juncea*. Genetic information for fertility restoration was introgressed following the development of a *M. arvensis* monosomic addition line on CMS *B. juncea*. The putative restorer plant also exhibited severe chlorosis similar to CMS plants but possessed 89 per cent and 73 per cent pollen and seed fertility, respectively. This subsequently increased to 96 per cent and 87 per cent in the selfed progeny. The progeny of the cross of CMS line with the restorer line MJR 15, segregated into 1 fertile: 1 sterile. The CMS (*Moricandia*) *B. juncea*, the restorer (MJR 15) and fertility-restored F_1 plants possess similar cytoplasmic organellar genomes.

Prakash *et al.* (2001) studied the expression of male sterility in alloplasmic *B. juncea* with *Erucastrum canariense* cytoplasm and developed a fertility restoration system. The fertility restoration gene was introgressed to CMS *B. juncea* from the cytoplasmic donor *E. canariense* through pairing between chromosomes belonging to *B. juncea* with those of the *E. canariense* genome. The restorer plants had normal flowers, with well-developed anthers containing fertile pollen. Pollen and seed fertility averaged 90 per cent and 82 per cent, respectively. F_1 hybrids between CMS and the restorer were fully pollen fertile and show a normal seed set. Preliminary results indicated that restoration was achieved by a single dominant gene. The constitution of the organelle genomes of the CMS, restorer and fertility restored plants was identical as revealed by southern analysis using mitochondrial and chloroplast probes *atp* A and *psb* D, respectively.

Banga *et al.* (2003) studied alloplasmic male sterile *B. juncea* with *Enarthocarpus lyratus* cytoplasm and the introgression of a gene for fertility restoration from cytoplasm donor species. The gene for male fertility restoration (*Rf*) was introgressed from donor species through homologous pairing between A and E chromosomes in monosomic addition plants (2n = 18II+1E). The per cent of pollen fertility of restored F_1 (*lyr* CMS putative restorer) plants ranged from 60 to 80 per cent. However, this was sufficient to ensure complete seed set upon by bag selfing.

Pathania *et al*. (2003) characterized cytoplasmic male sterility (CMS) and identified the fertility restorer gene for CMS (*Diplotaxis catholica*) *Brassica juncea* derived through sexual hybridization. The fertility restorer gene was identified by crossing the CMS line with progeny plants derived from somatic hybrids of *B.*

juncea and *D. cathoilca*. Genetic segregation data indicated that a single, dominant, nuclear gene governs fertility restoration. Restored plants showed high female fertility and lacked gynoecium abnormalities. In fertility-restored plants, petal development was found to be variable, some flowers had the normal number of four petals, while others had zero to three petals. Interestingly, the trilocular character of the ovary was found to be concerned with CMS and became bilocular upon male fertility restoration. Thus, this trait appears to be affected by the interaction of nuclear and mitochondrial (*mt*) genomes.

Bhat *et al.* (2006) developed the CMS line of *B. juncea* through somatic hybridization with *Moricandia arvensis* and introgressed the fertility restorer gene into *B. juncea*. This fertility was unique that it was capable of restoring male fertility to two other alloplasmic CMS systems of *B. juncea*. A BC_1F_1 population segregating for male fertility/sterility was used for further molecular tagging of this restorer gene using the AFLP technique for cloning of *Rf* locus using the bulk segregant analysis method.

Sodhi *et al.* (2006) reported that CMS lines when crossed with lines other than the respective maintainer line were restored for fertility, implying that any variety could act as a restorer for ‘126-1’ cytoplasm in *B. juncea*. These unique features in the maintenance and restoration of CMS lines coupled with near normal floral morphology of the CMS lines allowed the use of ‘126-1’ cytoplasm for hybrid seed production. The uniqueness of ‘126-1’ has been further established by southern hybridization with mitochondrial DNA probes and by a histological study of the development of male sterile anthers.

Bhat *et al.* (2007) reported the development of an improved cytoplasmic male sterile (CMS) system of *Brassica juncea* carrying cytoplasm of the wild *Diplotaxis berthautii* and identified a restorer. Fertility restorers of *M. arvensis* and *D. catholica*-based alloplasmic CMS systems of *B. juncea* were found capable of restoring male fertility to this new CMS line. The fertility restoration was monogenic and gametophytic. Northern analysis of the CMS, fertility restored and euplasmie lines using eight mitochondrial gene probes revealed altered *atp*A expression species associated with male sterility.

Bhat *et al*. (2008) reported a unique introgression from *Moricandia arvensis* confers male fertility upon two different cytoplasmic male sterile lines of *B. juncea. B. juncea* line carrying an introgression from *M. arvensis* restored male fertility to two CMS system, either *M. arvensis* or *Diplotaxis catholica* cytoplasm.Genetics of fertility restoration was studied in the F_1, F_2, F_3 and backcross generations ofthe cross between CMS and fertility restorer lines. No male sterile plants were found in F_1-F_3 generations of the cross between CMS (*M. arvensis*) *B. juncea* and the restorer. However, 1: 1 segregation for male sterility and fertility restoration was observed when the F_1 was pollinated with non-restorer pollen from a euplasmic line. These results clearly show that restoration is monogenic and gametophytic. In CMS lines carrying *D. catholica* cytoplasm, the restorer conferred male fertility to the F_1 and showed 3: 1 and 1: 1 segregation for male fertility and sterility in F_2 and BC_1 generations, respectively indicating a monogenic, sporophytic mode of fertility restoration. The results were also supported by pollen stainability in the F_1 which was about 65 per cent in *M. arvensis* based CMS and more than 90 per cent in *D. catholica* based CMS.

Chamola *et al.* (2013) studied the effect of alien cytoplasm and fertility restorer genes on agronomical and physiological traits of *B. juncea*. The restorer gene corrected the floral defects in CMS *trachystoma* and restored yield to the level of the euplasmic line, while it did so only partly in CMS *catholica*. Restorer genes had no adverse effect on traits in any of the systems. The *Rf* gene of *Moricandia* CMS system exerted a positive effect on leaf chlorophyll content.

Gupta *et al.* (2013) developed SCAR markers for fertility restoration gene that is useful for hybrid development in mustard. Validation of primer (SCAR-3) associated with fertility restoration gene was tested in 50 restorers inbred (R-lines) lines and their F_1 derived from *Moricandia* CMS. The results showed very high correspondence with flower, pollen fertility/sterility and seed set with SCAR marker indicating the potentiality of case-specific SCAR markers for testing.

Ashutosh *et al.* (2014) studied restoration ability in a cytoplasmic male sterile system in Indian mustard by crossing two CMS lines of *Moricandia viz.*, CMSNDRE 4 and CMS *Mori* with 30 genotypes to identify restorers. Effective restorers for two CMS lines could not be identified through *Moricandia* CMS system and common partial restorers were found in the parents, NPJ 147, NPJ 112, PRE-2007-06, NPJ 135, Divya 33, DRMREJ 2010-02, Divya 22, RH 919, RRN 693 and Kranti for both CMS lines. Effects of CMS lines on seed yield and its components traits indicated that seed yield in F_1 was higher than the parents.

Sirvi (2016) studied fifty-four genotypes consisting of five *Mori* based CMS lines and eight *Mori* based restorer lines crossed in line x tester mating design. She observed visual differences in all the F_1 crosses/hybrids. The F_1 generation of all the crosses showed fertility restoration with pollen fertility except for F_1 crosses with all the five CMS lines with *Mori* ‘R’ GM 3 as a restorer line (0%) which ranged from 65.15 per cent (*Mori* ‘A’ SKM 109 x *Mori* ‘R’ Pusa Bahar) to 88.51 per cent (*Mori* ‘A’ SKM 219 x *Mori* ‘R’ SKM 301) over standard check Kranti (91.22%). Similar results were also obtained from the study of a number of siliqua set and per cent siliqua set in field conditions.

Vinu *et al.* (2017) studied the genetics of the fertility restorer gene which can restore fertility in three different male sterile systems (*Mori*, *Eru* and *Ber*) in *B. juncea* using nine different BC_1F_1 populations. Monogenic and gametophytic mode of inheritance was observed for all the populations except for the back cross population derived from Pusa Agrani (*Ber*). The per cent pollen fertility of back cross populations ranged from 30.12 per cent to 68.42 per cent. The highest pollen fertility per cent 68.42 was observed in the back cross-generation of NPJ 112 with *Moricandia* cytoplasm. In all the back cross populations few progenies exhibited more than 50 per cent pollen fertility and it was highest (20 progenies out of 40 fertile progenies) with the back cross population from SEJ 8 (*Mori*) x Mori *Rf*. This back cross population had the highest mean pollen fertility per cent with 51.56 per cent. It was observed that a few minor genes influence the pollen fertility in all the back cross populations.

Patel (2019) studied 47 genotypes comprising 12 parents, 5 CMS lines and 7 restorer lines crossed in line x tester mating design. He observed visual differences in all the F_1 crosses and standard check for pollen fertility. The crosses made by CMS lines and male fertile lines SKM 303, Pusa Agrani, PCR 7 and *Mori* ‘R’ 1-18 exhibited pollen fertility ranging from 68.26 per cent to 85.17 per cent, whereas standard check GDM 4 showed 91.36 per cent pollen fertility, while the crosses between CMS lines and male fertile lines *i.e.*, Vardan, Rohini and SKM 319 exhibited 0 per cent pollen fertility. These results were further confirmed by the ability to produce seed set upon self-fertilization. The crosses made by CMS lines and male fertile lines SKM 303, Pusa Agrani, PCR 7 and *Mori* ‘R’ 1-18 exhibited per cent siliquae set by self-pollination which ranging from 66.99 per cent to 83.82 per cent, whereas standard check GDM 4 has 89.76 per cent siliquae set, while the crosses carried out by crossing CMS lines with male fertile lines namely Vardan, Rohini and SKM 319 recorded 0 per cent siliquae set.

Vyas (2022) studied 55 genotypes comprising 13 parents, 5 CMS lines and 8 restorer lines crossed in line x tester mating design. She observed visual differences in all the F_1 crossesa nd standard check for pollen fertility. The male sterile lines exhibited (100%) pollen sterility and the pollen fertility in fertility restorer lines varied from 88.03 per cent (RSK 93R) to 98.67 per cent (RSK 96R). The F_1 generation of almost all the crosses exhibited fertility restoration with pollen fertility ranging from 51.21 (GM3 A × SKM9033 R) to 92.74 per cent (SKM 125A × GM1 R) except three F_1 hybrids exhibited male sterility, when line SKM 139 A used as female with RSK 96 R, SKM 9033 R and RSK 93R as the male parent. These results were further confirmed by the ability to produce seed set upon self-fertilization.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigation on **"Characterization of *Moricandia* based restorer lines in Indian mustard [*Brassica juncea (*L.) Czern and Coss]"** was conducted during *Rabi* 2021-22 in field at Agronomy Instructional Farm, whereas laboratory work was carried out in Biotechnology Laboratory, Dept. of Genetics and Plant Breeding, CPCA, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

3.1 Geographical and edaphic details

Sardarkrushinagar is situated at 24^0-19' North latitude and 72^0-19' East longitude with an altitude of 154.52 meter above the mean sea level. The soil of the experimental field was sandy loam with pH 7.5. The weather during the growing season was normal and favorable for crop growth (Appendix I).

3.2 Experiment materials

The experimental material including three '*Mori*' based 'A' lines and fifteen '*Mori*' based 'R' testers for the present investigation was obtained from Center for Oilseeds Research, SDAU, Sardarkrushinagar. The details of genotypes used are presented in Table 3.1.

3.3 Crossing programme

The crossing programme was carried out during *Rabi* 2020-21 at Center for Oilseeds Research, S. D. Agricultural University, Sardarkrushinagar. Three lines were crossed with fifteen testers in line × tester mating design to obtained 45 F_1 hybrids of Indian mustard. At the same time, female sterile lines (A lines) were crossed with its maintainer lines (B line) to get seeds of female parents for evaluation in the next season and the testers or restorer lines (male parents) and maintainer 'B' lines were selfed to get pure seeds.

3.3.1 Pollination

The flower opened in the morning (between 8.00 to 11.00 a.m.) on 'A' lines under net house was pollinated by collecting the pollen grains from protected flowers of male parents. The pollen grains were applied on a receptive stigma by touching anthers on stigma and these flowers were covered with white butter paper bags and labeled. The selfed and crossed siliquae were harvested separately at the time of maturity. The capsules were dried under the sunlight and seeds were collected separately.

Table 3.1: List of genotypes

Sr. No.	**Genotypes (‘A’ LINE)**	**Converted from**	**Sr. No.**	**Genotypes (‘R’ LINE)**	**Converted from**
1.	GM*Mo*2118A	SKM 303	1.	GM*Mo*2101R	RSK 88
2.	GM*Mo*2124A	RSK 90	2.	GM*Mo*2105R	GM 3
3.	GM*Mo*2125A	RSK 91	3.	GM*Mo*2113R	RSK 88
			4.	GM*Mo*2115R	RSK 96
Sr. No.	**Genotypes (‘B’ LINE)**	**Converted from**	5.	GM*Mo*2116R	RSK 95
1.	GM*Mo*2118B	SKM 303	6.	GM*Mo*2119R	GM 2
2.	GM*Mo*2124B	RSK 90	7.	GM*Mo*2120R	RSK 89
3.	GM*Mo*2125B	RSK 91	8.	GM*Mo*2122R	GM 1
			9.	GM*Mo*2124R	RSK 93
			10.	GMMo2125R	RSK 94
			11.	GM*Mo*2127R	RSK 92
			12.	GM*Mo*2128R	SKM 201P1
			13.	GM*Mo*2130R	SKM 219P1W
			14.	GM*Mo*2132R	SKM 125P2
			15.	GM*Mo*2133R	SKM 139P1

*Source: Center for Oilseeds Research, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar – 385 506

**GM*Mo*~Gujarat Mustard *Moricandia*

3.4 Morphological characterization

3.4.1 Experimental design

A set of 66 genotypes comprising 21 parents (3 ‘A’ lines, 3 ‘B’ lines and 15 ‘R’ lines) which were on 27th October in Randomized Block Design (RBD) with three replications for variability and DUS study. Along with this experiment, 45 F_1 hybrids were also grown in single replication for fertility restorability study during *Rabi* 2021-22. Each entry sown in 4 m length row with 45 cm × 10 cm spacing. The recommended agronomical practices and plant protection measures were adopted as per requirement.

3.4.2 Sampling Procedure

The observations were recorded from 60 plants or parts of plants, which divided among 3 replications (20 plants per replication) as per the guidelines of PPV and FR Authority.

3.4.3 Characteristics used for observations

Observations were recorded at different growth stages as per DUS test guidelines of PPV and FR Authority for 18 morphological descriptors for mustard

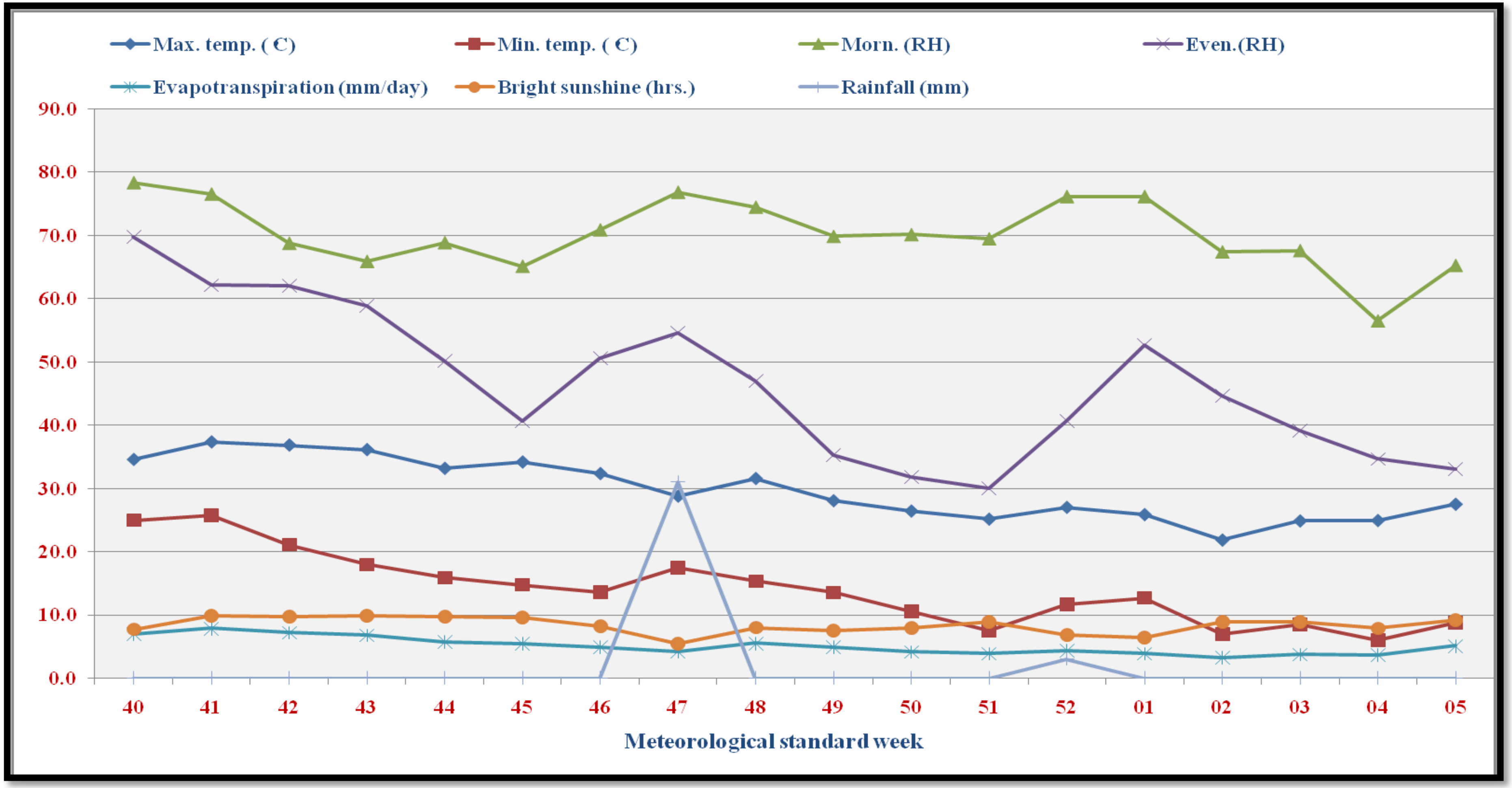


Fig. 1: Graphical representation of weekly meteorological data recorded during the crop season

A

B

PLATE-I (A): General view of experimental site,

(B): Experimental view at the time of maturity

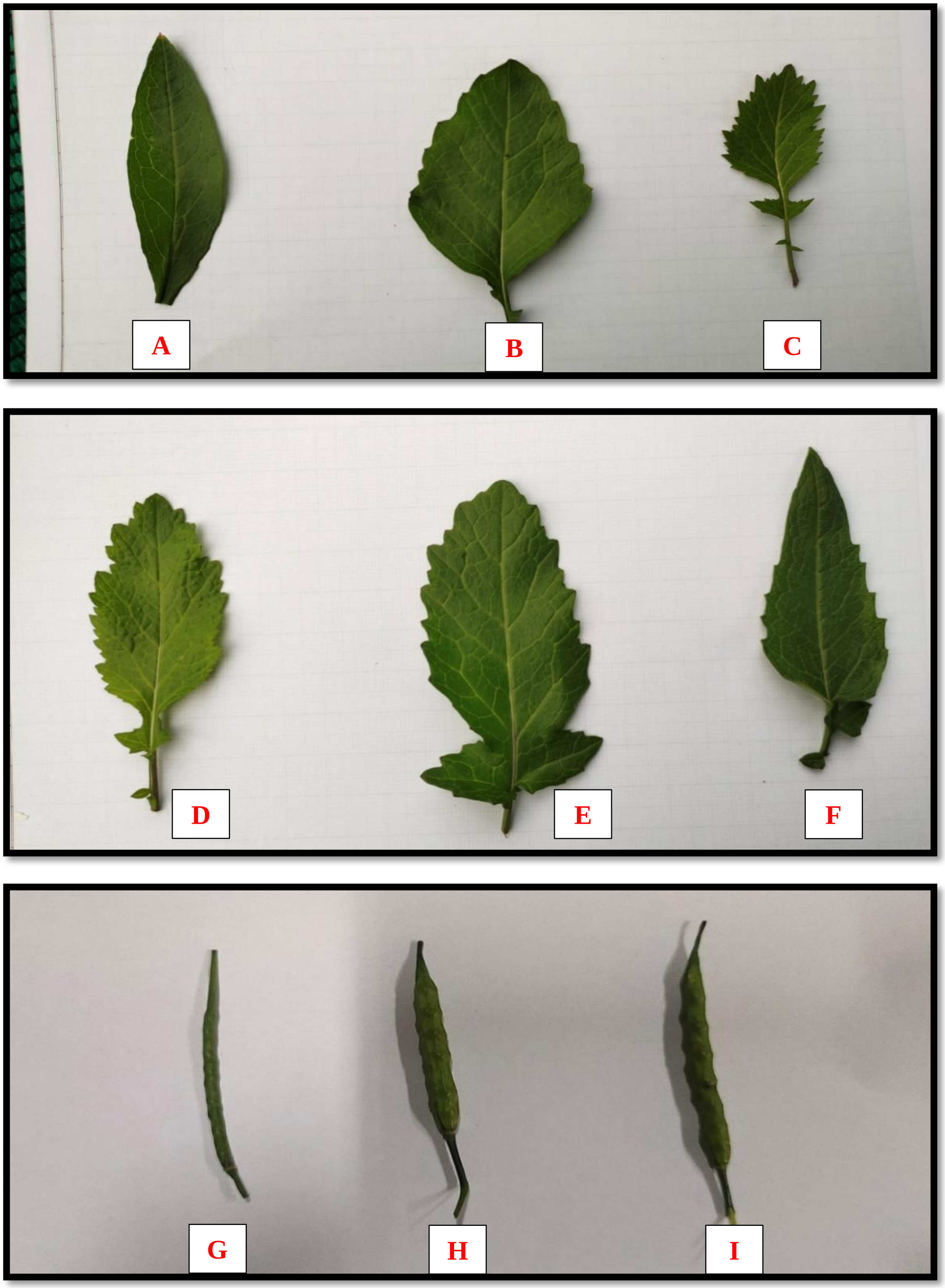


Plate II: Leaf Dentation of Margin [A: Entire, B: Dentate, C: Serrate], Leaf Colour [D: Light Green, E: Medium Green, F: Dark Green], Siliqua Texture [G: Smooth, H: Undulated, I: Constricted]

as mentioned in Table 3.2. Four types assessments were followed:

VS: Visual assessment by observations of individual plants or parts of plants.

VG: Visual assessment by single observation of a group plants or parts of plants.

MS: Measurement of a number of individual plants or parts of plants.

MG: Measurement by single observation of a group plants or parts of plants.

Table 3.2: Study of qualitative descriptors for fifteen Indian mustard genotypes

Sr. No.	Qualitative Descriptors	States	Example varieties	Stage of Observation	Type of assessment
1.	Leaf: Hairiness	Absent	Basanti, RH 781	50-60	VS
		Sparse	Varuna, Pusa Bold		
		Dense	CS 52, Geeta		
2.	Leaf: Colour	Light green	NDRE 4	50-60	VG
		Medium green	Varuna, BIO 902		
		Dark green	GM 1		
3.	Leaf: Lobes	Absent	-	50-60	VS
		Present	Varuna		
4.	Leaf: Number of lobes	Low (1-2)	-	50-60	MS
		Medium (3-5)	Kranti		
		High (>5)	CS 52, RH 819		
5.	Leaf: Dentation of margin	Entire	-	50-60	VS
		Dentate	Varuna, BIO 902		
		Serrate	NDRE 4		
6.	Leaf: Length (cm)	Short (≤ 25)	NDRE 4	50-60	MS
		Medium (26- ≤30)	Varuna		
		Long (>30)	RH 781, PCR 7		
7.	Leaf: Width (cm)	Narrow (<10)	NDRE 4	50-60	MS
		Medium (10-12)	Varuna, GM 1		
		Broad (>12)	RH 781, PCR 7		
8.	Flower: Colour of petals	White	-	60-62	VG
		Light yellow	Pusa Mahak		
		Yellow	BIO902		
9.	Flower: Length of petals	Short (<1.2)	NDRE 4	60-62	MS
		Medium (1.2-1.5)	Pusa Bold, Rohini		
		Long (>1.5)	-		

10.	Flower: Width of petals	Narrow (<0.6)	-	60-62	MS
		Medium (0.6-0.7)	Basanti, BIO902		
		Broad (>0.7)	RL 1359		
11.	Siliqua: Length	Short (<4.5)	Kranti	85	MS
		Medium (4.5-5.0)	RH 30		
		Broad (>5.5)	Pusa bold		
12.	Siliqua: Length of beak	Short (<0.8)	Geeta	85	MS
		Medium (0.8-≤1.2)	PBR 97, PCR 7		
		Long (>1.2)	Pusa Bahar		
13.	Siliqua: Angle with main shoot	Appressed	S. Asech	85	VG
		Semi appressed	Rohini, Geeta		
		Open	Varuna		
14.	Siliqua: Texture	Smooth	-	85	VS
		Undulated	Varuna		
		Constricted	Basanti		
15.	Siliqua: Number of siliqua on main shoot	Very few (≤ 40)	NDRE 4	85	MS
		Few (41- ≤50)	Varuna		
		Medium (51-≤60)	Rohini		
		Many (>60)	Geeta		
16.	Seed: Colour	Yellow	Basanti	100	VG
		Reddish brown	CS 52		
		Brown	RCC 4		
		Dark brown	BIO 902		
		Black	-		
17.	Seed: Size	Small (<5.0g)	Kranti	100	MG
		Medium (4.0-6.0)	Rohini		
		Bold (>6.0)	Pusa Bold		
18.	Seed: Oil (%)	Low (<38)	-	100	MG
		Medium (38-<42)	CS 52, Varuna		
		High (42-46)	Rohini		
		Very High (>46)	-		

3.5 Characters studied

The observations were recorded on five randomly selected plants from each genotype in each replication. Final mean values were used for statistical analysis.

3.5.1 Days to flowering

The number of days from the date of sowing to the date of emergence of flowers (anthesis) on 50 per cent plants in a plot.

I. Early (≤40 days)
II. Medium (41- ≤50 days)
III. Late (>50 days)

3.5.2 Days to maturity

The number of days was recorded from the date of sowing to drying of siliquae on main branch in 80 per cent plant.

I. Early (≤110 days)
II. Medium (111- ≤130 days)
III. Late (131- ≤150 days)
IV. Very late (> 150 days)

3.5.3 Plant height (cm)

The height of the selected plant was measured in centimetre from ground level to the tip of the main shoot at the time of maturity.

I. Short (≤130)
II. Medium (131 - ≤150)
III. Tall (151-≤170)
IV. Very tall (>170)

3.5.4 Number of branches per plant

The total number of branches bearing siliquae of selected plants was counted at the time of maturity.

3.5.5 Number of siliqua per plant

At the time of maturity, seeds bearing siliquae on each selected plant was counted.

I. Very few (≤40)
II. Few (41-≤50)
III. Medium (51- ≤60)
IV. Many (>60)

3.5.6 Seeds per siliqua

Five siliquae from each selected plant were collected and number of seeds per siliqua was counted and average value computed.

I. Very few (≤12)
II. Few (13 -≤16)
III. Medium (17- ≤20)
IV. Many (>20)

3.5.7 Length of siliqua (cm)

Length of siliqua was measured for the seed-bearing portion of the siliqua of five selected plants in centimetre and average was computed.

I. Short (<4.5)
II. Medium (4.5-5.5)
III. Long (>5.5)

3.5.8 Seed yield per plant (g)

Selected five plants were harvested, threshed, cleaned and weighed in gram and average on per plant was computed.

3.5.9 1000 seed weight (g)

Thousand seeds were counted from the bulk seed yield of selected plant from each replication and to be weighed in gram.

I. Small (<5 g)
II. Medium (5 – 6 g)
III. Bold (>6 g)

3.5.10 Oil content (%)

A random sample (Approximately 60.0 g) of bulk seeds of each genotype from replicated samples were taken and oil content was determined in percentage through Nuclear Magnetic Resonance (NMR) Technique (Tiwari *et al.,* 1974).

I. Low (<38)
II. Medium (38 - <42)
III. High (42- 46)

3.5.11 Pollen fertility

Percent pollen fertility was calculated by counting fertile and sterile pollens under light microscope using 2 per cent aceto-carmine test (Alexander, 1969).

- **Field study**

Selfing was done using butter paper bag on each tagged plant. Number of plants setting pods in selfed bags was considered fertile and computed as percent on plant basis for each genotype.

3.6 Statistical analysis

Pollen fertility/sterility was observed in 3 CMS lines, 15 restorer lines and F_1 population. All population have been tested for their pollen fertility status (Alexander, 1969) at the initial flowering stage of randomly selected plant. To identify sterility/fertility of pollen grains in laboratory, 2 per cent aceto-carmine solution has been used. Ten well developed flower buds were collected randomly from each plant from different branches of the plant at the time of anthesis (9- 10 a.m.). From each bud, the anthers were collected on a microscope's slide and crushed with a drop of two per cent aceto-carmine stain and examined under a light microscope (40X). Two such microscopic fields were examined for each plant. The round and well stained pollen grains were counted as fertile, while shriveled hyaline pollen grains were scored as sterile. The total for all the microscopic fields were worked out and the fertile pollens were expressed as percentage of total pollen grains observed in individual plants. Based on the number of stained and unstained pollen grains, the fertility status was computed as follows:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of round and stained pollen (fertile pollen)}}{\text{Total number of pollen grains examined}} \times 100$$

3.6.1 Analysis of variance (ANOVA)

The analysis of variance for randomized block design was done for each character as per Panse and Sukhatme (1978). The statistical model used for analysis of variance was based on the following linear model.

$$Y_{ij} = \mu + R_i + G_j + E_{ij}$$

Where,

Y_{ij}= An observation of j^{th} genotype in i^{th} replication,

μ= General mean,

R_i=An effect of i^{th} replication,

G_j= An effect of j^{th} genotype,

E_{ij}= Uncontrolled variation associated with j^{th} genotype in i^{th} replication,

I = Number of replications (1, 2… i) and

j = Number of genotypes (1, 2… j).

The format of analysis of variance is given as under:

Table 3.3: Analysis of variance for experimental design

Source of variation	**Degree of freedom**	**Sum of square**	**Mean square**	**Expected mean sum of square (EMS)**
Replications	(r-1)	SSR	MS_R	$\sigma_e^2 + g\sigma_r^2$
Genotypes	(g-1)	SSG	MS_G	$\sigma_e^2 + r\sigma_g^2$
Error	(r-1) (g-1)	SSE	MS_e	σ_e^2
Total	(rg-1)	SST	-	-

Where,

r = Number of replications,

g = Number of genotypes,

MS_R= Mean sum of square due to replications,

MS_G= Mean sum of square due to genotypes and

MS_e= Mean sum of square due to error.

Significance of mean sum of square due to replications (MS_R) and genotypes (MS_G) were tested against error mean sum of square (MS_e).

The Standard error of mean (S.Em.) was calculated using following formula

$$S.\ Em = \sqrt{MS_e}\ /r$$

The critical difference (C.D.) to compare the mean of any two genotypes was calculated using following formula

$$C.\ D. = S.\ Em \times \sqrt{2} \times t$$

Where,

't' = Table value of 't' at 5% level of significance at error degree of freedom.

The coefficient of variation (CV) was calculated by using the following formula.

$$C.V.\ \% = \frac{\sqrt{MS_e}}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = General mean of a character.

3.6.2 Estimation of variance components

Total variation was partitioned into phenotypic (σ_p^2), genotypic (σ_g^2) and environmental variance (σ_e^2) based on expectation of mean square for respective source of variation described in ANOVA (Table3.3).

3.6.2.1 Estimate of phenotypic, genotypic and environmental variances

Phenotypic, genotypic and environmental variances were calculated as per methods suggested by Johnson *et al.* (1955).

[a] Phenotypic variance (σ_p^2)

It is the sum of the variances contributed by genetic causes and environmental factors. It was calculated as under:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

Where,

σ_p^2= Phenotypic variance,

σ_g^2= Genotypic variance and

σ_e^2= Environment variance.

[b] Genotypic variance (σ_g^2)

It is the variance contributed by genetic causes or the occurrence of difference among individuals due to differences in their genetic make-up.

It was calculated as under:

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

Where,

σ_g^2 = Genotypic variance,

MS_g = Genotypic mean sum of square of the character,

MS = Error mean sum of square of the character, and

r = Number of replications.

[c] Environmental variance (σ_e^2)

The mean sum square of error is converted as the environmental variance.

$$\sigma_e^2 = MS_e$$

Where,

σ_e^2 = Environmental variance, and

MS_e = Error mean sum of square

3.6.2.2 Phenotypic and genotypic coefficient of variation

The formula suggested by Burton (1952) was employed to calculate phenotypic and genotypic coefficient of variation.

[a] Genotypic coefficient of variation (GCV)

$$\text{GCV (\%)} = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

[b] Phenotypic coefficient of variation (PCV)

$$\text{PCV (\%)} = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100$$

Where,

σ_p^2, σ_g^2 = Phenotypic and genotypic variance, respectively, and

$\bar{X}$ = Mean value of character.

Genotypic coefficient of variation and phenotypic coefficient of variation were classified as suggested by Sivasubramanian and Madhavamenon (1973) as follows:

< 10 % = Low

10 - 20 % =Moderate

> 20 % = High

3.6.2.3 Heritability (Broad sense)

It is the proportion of phenotypic variability that is due to genetic reasons. It was computed in per cent using the formula given by Allard (1960).

$$h^2_{(b)}(\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

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

σ^2_g = Genotypic variance, and

$\sigma^2 p$ = Phenotypic variance.

Heritability percentage was categorized as demonstrated by Robinson *et al.* (1949).

< 30 % = Low

30 - 60 % = Moderate

> 60 % = High

3.6.2.4 Genetic advance (GA) as percentage of mean

The expected genetic advance (GA) was calculated for each character by adopting the procedure as suggested by Allard (1960).

$$\text{GA} = \text{K} \times \frac{\sigma_g^2}{\sigma_p^2} \times \sigma_p$$

Where,

K = Standardized selection differential, (K = 5 % selection intensity coefficient).

σ_p = Phenotypic variance

$\frac{\sigma_g^2}{\sigma_p^2}$ = Heritability in broad sense, and

The genetic advance expressed as per cent of mean was estimated as under.

$$\text{GA (\% of mean)} = \frac{\text{GA}}{\overline{\text{X}}} \times 100$$

Where,

GA = Expected genetic advance, and

$\overline{\text{X}}$ = Mean value of the character

The genetic advance as per cent of mean was categorized as demonstrated by Johnson *et al*. (1955).

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

3.6.3 Correlation coefficient

The genotypic, phenotypic and environmental correlation coefficient for all the traits was worked out with grain yield and among themselves. The data were subjected to co-variance analysis from which various components of mean sum of product were estimated.

Table 3.4: Analysis of co-variance

Source	d.f.	Mean square of products (M.S.P.)	Expected MSP
Replications (r)	(r-1)	-	-
Genotypes (g)	(g-1)	MSP_1	Co $\sigma^2_{e1.2}$ + r Co $\sigma^2_{g1.2}$
Error	(r-1) (g-1)	MSP_2	Co $\sigma^2_{e1.2}$

Where,

r = Number of replications,

MSP_1 = Mean sum of products due to genotypes between character first and character second, and

MSP_2 = Mean sum of products due to error between character first and character second.

The phenotypic, genotypic and error variances and co-variances were used for calculating the phenotypic, genotypic and environmental correlation coefficients respectively (Al. Jibouri *et al.*, 1958).

(a) **Phenotypic correlation coefficient ($r_{p1.2}$):**$= \frac{\mathrm{CoV}_{p1.2}}{\sqrt{\sigma^2_{p1}\times\sigma^2_{p2}}}$

(b) **Genotypic correlation coefficient ($r_{g1.2}$):**$= \frac{\mathrm{CoV}_{g1.2}}{\sqrt{\sigma^2_{g1}\times\sigma^2_{g2}}}$

(c) **Environmental correlation coefficient ($r_{e1.2}$):**$= \frac{\mathrm{CoV}_{e1.2}}{\sqrt{\sigma^2_{e1}\times\sigma^2_{e2}}}$

Where,

$\mathrm{Cov}_{p1.2}$ = Phenotypic covariance for a pair of characters first and second,

$\mathrm{Cov}_{g1.2}$ = Genotypic covariance for a pair of characters first and second,

$\mathrm{Cov}_{e1.2}$ = Environmental covariance for a pair of characters first and second,

σ^2_{g1}, σ^2_{p1} = Genotypic and phenotypic variance for character first,

σ^2_{g2}, σ^2_{p2} = Genotypic and phenotypic variance for character second, and

σ^2_{e1}, σ^2_{e2} = Error variance for characters first and second.

Test of significance:

The significance of correlation coefficient was tested using the following equation.

$$t = \frac{r}{\sqrt{(1-r^2)}} \times \sqrt{n-2}$$

Where,

r = Correlation coefficient, and

n = Number of pairs of observation.

3.6.4 Path coefficient analysis

The cause and effect interrelationship between two variables cannot be estimated from simple correlation coefficient analysis. Therefore, the data were subjected to a standard regression analysis known as path analysis to unravel whether the association of different traits with yield is due to their direct effects or it is a consequence of their indirect effect *via* some other traits. The path coefficient analysis was carried-out according to the method suggested by Wright (1921) and used by Dewey and Lu (1959).

The path coefficients were obtained by solving simultaneous equations which represent the basic relationship between correlation and path coefficient.

$$r_{ny} = P_{ny} + r_{n2}P_{2y} + r_{n3}P_{3y} + \ldots\ldots + r_{nx}P_{xy}$$

Where,

r_{ny} = Correlation coefficient between one casual factor and dependent character *i.e.*,yield,

P_{ny} = Path coefficient between the character and yield.

$r_{n2}, r_{n3} \ldots . r_{nx}$ = Represents the correlation coefficient between that character and each other yield component in turn

$$\begin{pmatrix} r1y \\ r2y \\ r3y \\ . \\ . \\ . \\ rny \end{pmatrix} = \begin{pmatrix} 1 & r12 & r13 \ldots\ldots\ldots r1n \\ r21 & 1 & r23 \ldots\ldots\ldots r2n \\ r31 & r32 & 1 \ldots\ldots\ldots r3n \\ . & . & . \\ . & . & . \\ . & . & . \\ rn1 & rn2 & rn3 \ldots\ldots\ldots 1 \end{pmatrix} \begin{pmatrix} P1y \\ P2y \\ P3y \\ . \\ . \\ . \\ Pny \end{pmatrix}$$

[A] [B] [C]

Where,

[A] = Correlation matrix between yield and biometrical characters,

[B] = Correlation matrix between different biometrical characters

and

[C] = Path coefficient matrix.

The value of [C] matrix can be obtained as under,

$$[A] = [B] \cdot [C]$$

Thus,

$$[C] = [A]\,[B]^{-1}$$

The path value means the direct effects were calculated as follows,

$$P_{iy} = \Sigma\, c_{i1} r_{iy}$$

The indirect effects of a particular character through other characters were obtained by multiplication of direct path and particular correlation coefficient between those two characters, respectively.

$$\text{Indirect effect} = r_{ij} \text{ x } P_{ij}$$

Where,

i and j = 1 to n

The residual effect is a composite variable that includes all other unaccounted factors affecting seed yield in this study and is assumed to be independent of the remaining variables. It was calculated as under:

$$\text{Residual effect (X)} = (1 - R^2)^{0.5}$$

Where,

$$R^2 = P_{1y}r_{1y} + P_{2y}r_{2y} + \ldots\ldots\ldots + P_{ny}r_{ny}$$

3.7 Assessment of molecular diversity by using microsatellite markers

3.7.1 Genomic DNA Extraction

Genomic DNA of 21 genotypes was extracted from fresh tender leaves using CTAB (Cetyltrimethyl Ammonium Bromide) extraction method described by Doyle and Doyle, 1987 with required modifications.

3.7.1.1 Glasswares, polywares and equipments

All the glassware used in the experiment were obtained from Borosil and J-sil, while the plastic wares from Axygene, Imperical and Tarsons. The equipments and instrument which used are given in Table 3.5.

Table 3.5: Glasswares, polywares and equipments

Sr. No.	Equipments and Instruments	Company/ Manufacture
1.	Weighing balance	Precisa, Switzerland.
2.	Double distillation unit	Borosil, India.
3.	pH meter	GeNei, India.
4.	Autoclave	Equitron Medica Pvt. Ltd., India.
5.	Hot water bath	Macro Scientific Works Pvt. Ltd., India.
6.	Table top cooling centrifuge	Remi Lab World, India.
7.	Hybridization oven	Labnet International, U.S.A.
8.	-20 °C Freezer	Eppendorf, North America.
9.	Microwave oven	LG, South Korea.
10.	Gel electrophoresis unit	Scie-Plas Ltd., U.K.
11.	Gel documentation machine	Alpha Innotech Corporation, U.S.A.
12.	Nano-Drop spectrophotometer	BioSpectrometer, Eppendrof, Germany.
13.	Mini centrifuge	GeNei, India.
14.	Vortexer	GeNei, India.
15.	Mini PCR plate spinner	Genaxy Scientific Pvt. Ltd., India.
16.	Thermal cycler	Eppendorf, Germany.

3.7.2 Protocol for genomic DNA extraction

DNA extraction:

- The fresh leaves (~1.5 g) were taken from 10-12 days old seedling, washed with distilled water twice and dried well.
- Leaf samples were ground to fine powder in liquid nitrogen using sterile pestle and mortar.
- The powder was transferred to 2 ml micro-centrifuge tubes followed by addition of 1 ml 2.5% CTAB extraction buffer and 10μl β-mercaptoethanol to the tubes.
- The contents were thoroughly stirred and incubated at 65°C in a water bath for 60 minutes along with continuous mixing after every 15 minutes.

3.7.3 Reagents and chemicals

Table 3.6: Composition of extraction buffer (CTAB buffer)

Sr. No.	Reagents	Concentration	
1.	**70 % ethanol**	70 ml absolute ethanol in 30 ml distilled water	
2.	**DNA extraction buffer (CTAB buffer)**	Tris-HCl (100 mM) pH 8.0	10 ml from 1 M Tris-HCl stock solution for 100 ml
		EDTA (25 mM) pH 8.0	5 ml from 0.5 M EDTA stock solution for 100 ml
		CTAB (2.5% w/v)	2.5 g for 100 ml
		NaCl 1.5 M	8.76 g for 100 ml
		PVP (1% w/v) (Polyvinylpyrrolidone)	1 g for 100 ml
		Double distilled water	Volume makes up to 100 ml
3.	**Phenol: Chloroform: Isoamyl Alcohol (25:24:1)**	25 ml phenol: 24 ml chloroform: 1 ml isoamyl alcohol for 50 ml	
4.	**Chloroform: Isoamyl Alcohol (24: 1)**	24 ml chloroform: 1 ml isoamyl alcohol for 25ml	
5.	**Isopropanol**	100% and used chilled	
6.	**RNase**	30 mg RNase was dissolved in 1 ml of buffer at the time of use	
7.	**TE buffer (pH 8.0)**	Tris-HCl (10 mM) pH 8.0	1 ml from 1 M Tris-HCl stock solution for 100 ml
		EDTA (1 mM) pH 8.0	200 μl from 0.5 M EDTA stock solution for 100 ml
		Nuclease-free water	As requirement

Purification:

- Then the contents were kept at room temperature to cool down for 10-15 min.
- Tubes were centrifuged at 12,000 rpm for 10 min at 27-30°C.
- The supernatant was transferred to a fresh micro centrifuge tube and the debris remained were discarded.
- Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to each tube and mixed by gently inverting followed by centrifugation of tubes at 12,000 rpm for 10 minutes at 27-30°C.
- The supernatant was treated with RNase by adding 10 μl DNase free RNase in each tube and incubated at 37°C for 30 min.
- After incubation, an equal volume of chloroform: isoamyl alcohol (24:1) was filled in the tubes and mixed gently by inverting followed by centrifugation at 12,000 rpm for 10 minutes at 27-30°C.
- An aqueous phase was transferred to the fresh micro centrifuge tube followed by C: I treatment as mentioned above.

Precipitation:

- An upper aqueous phase was transferred to a fresh tube containing 150 μl of 3 M sodium acetate and equal amount of 100 % chilled isopropanol and was mixed gently by inverting.
- Thick, bright pellets of DNA were observed.
- Tubes were kept for 2 hrs. to up to overnight at -20°C for better precipitation of DNA.
- Contents in the tubes were centrifuged at 10,000 rpm for 15 minutes at 4°C.
- The supernatant was discarded while pellets were air dried at room temperature and washed with 200 μ1 of 70 % ethanol by centrifuging tubes at 7,000 rpm at 4°C for 5 minutes.
- Ethanol wash was repeated and supernatant was discarded from tubes.
- Pellets were air dried until the smell of ethanol got removed.
- Further, pellets were dissolved in 200 μl of Tris-EDTA (1X TE) buffer at room temperature and were stored at -20°C until the use.

3.7.4 Purity check by agarose gel electrophoresis

The purity of genomic DNA samples was checked by agarose gel electrophoresis (Scie-Plas Ltd., UK). Gel casting plate was washed with distilled water and dried.

Agarose gel was prepared by dissolving 0.8 g of agarose in 100 ml 0.5 X TBE buffer and heated in a microwave oven until it forms a transparent solution. Ethidium bromide (10 mg/ml) 5µl/100 ml in TBE buffer was added to it after cooling it approximately 40ºC. Gel solution was then poured into the gel casting plate inserted with appropriate comb. After solidification of gel, the plate was placed in electrophoresis chamber and sub-merged in electrophoresis tank filled with 0.5 X TBE buffer before those combs were removed gently. 4 µl genomic DNA samples were mixed with 2 µl of 6x gel loading dye and were loaded in the wells. The standard DNA marker (50 or 100 bp) was also included along with the samples. Electrophoresis was carried out at constant voltage (80 V, one hour). Visualization of genomic band was carried out using gel documentation system (Alpha Innotech Corporation, USA) under the presence of UV light.

3.7.5 Quantification of DNA samples

Spectrophotometry was performed to determine DNA concentration by using Nanodrop 2000 (Biospectrometer, Eppendrof, Germany) at absorbance ratio 260/280 nm. For quantification, 2 µl of DNA was loaded into the well of spectrophotometer and the concentrations at A_{260}/A_{280} were measured. Pure DNA has an A_{260}/A_{280} ratio 1.8 to 2.0 in TE buffer. Strong absorbance at 280 nm, resulting in a low A_{260}/A_{280} ratio, indicates the presence of contaminants such as proteins in the sample. Such samples were discarded and not taken for further analysis. Based on the quantification data, a portion of DNA samples were diluted with appropriate quantity of nuclease free water to yield a working concentration of 20ng/µl and stored at −20°C for further molecular analysis.

3.7.7 PCR amplification of SSR markers

PCR amplification of diluted DNA samples (50 ng/µl) was performed in thermal cycler using various SSR primers. The SSR primers used to study the polymorphism present in the various *Brassica juncea* L. genotypes are mentioned in **Table 3.7**. PCR (Eppendorf, Germany) reactions for SSR markers were carried out in a reaction volume of 15 µl. The PCR mixture contained 1.5 µl 10X PCR buffer, 0.3 µl 10mM dNTPs, 0.1 µl 3U *Taq* DNA polymerase, 1.5 µl 5.0 pmoles/µl primer pair, 1.0 µl 20.0 ng/µl template DNA and 10.6 µl nuclease free water. The amplification was programmed for initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94 °C denaturation for 50 seconds, annealing of primer at annealing temperature for

45 seconds and extension at 72 °C for 1 minute. The cycling programme was terminated by a final extension step at 72 °C for 7 minutes.

The amplification products along with standard 100bp DNA marker were separated by electrophoresis on 3 % agarose gels with 5X TBE buffer, stained with ethidium bromide (EtBr) (10 μl/100 ml TBE) and photographed under UV light using FluorChem FC 2 gel documentation system (Alpha Innotech Corporation, USA).

3.7.8 Data analysis

The SSR reproducible bands of DNA fragments were scored as present (1) or absent (0) and were compared with each other. The data were entered into simple text file. Assembly of all these profiles formed a data matrix. The data matrix was read by NTSYS-pc version 2.20 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software) developed by Rohalf (1998) and analysed by SIMQUAL (Similarity for quantitative data) program with Jaccard's similarity coefficient (Jaccard, 1908).

The resulted similarity matrix was entered into SAHN (Sequential agglomerative, hierarchical, and nested clustering method) clustering program, a plant matrix was produced and dendrogram constructed using UPGMA (Unweighted pair-group method with arithmetic averages). The assumptions underlying the use of UPGMA clustering is equal rate of evolution among all dendrogram branches. Dendrogram of publication quality were produced from the output plant file of SAHN by PLANT (plant display) program in graphics mode.

3.7.9 Polymorphism information content (PIC)

Polymorphic information content (PIC) is a measure of locus polymorphism counted for markers used in linkage analysis.

PIC was counted for bi or multi-allelic locus following formulas:

$$\text{PIC} = 1 - \Sigma x_i^2$$

Where,

X_i is the relative frequency of the i^{th} allele of the SSR loci

PIC for co dominant marker, determine the number of alleles in the examined locus and then number or frequencies of alleles in the study group (sum of alleles frequencies must equal 1). For co dominant marker range of PIC value is between 0.0-1.0 (Miks and Binkowski, 2018).

Table 3.7: List of SSR primers used in the present study

Sr. No.	Primer Name	Sequence (5’ to 3’)	Base	Tm (°C)	References
1	BR_A01	F : CCGTTTTTATGTCACAAATCT	21	52.01	Sharma *et al.*, 2018
		R : AAACAAAACGAACTTTGTCAG	21	52.01	Sharma *et al.*, 2018
2	BR_A03	F : ATCGTCTCTTTCGTCTTGTCT	21	55.92	Sharma *et al.*, 2018
		R : CGTAAAACTGAAACCATTCAC	21	53.97	Sharma *et al.*, 2018
3	BR_A04	F :ATGGAATCTGCTCATCTCAC	20	55.25	Sharma *et al.*, 2018
		R :TAAGCTGCAATGATCAAAGAT	21	52.01	Sharma *et al.*, 2018
4	BR_A04	F :TTTGAACGATACACAACAACA	21	52.01	Sharma *et al.*, 2018
		R :GTTGGTCCACGAGTAAAAGAT	21	55.92	Sharma *et al.*, 2018
5	BR_A05	F :ATAAAGATTTGATGGGAGGAG	21	53.97	Prajapati *et al.*, 2014
		R :GGTGGAGGAGGATAGTTGTAG	21	59.82	Prajapati *et al.*, 2014
6	BRMS-01	F :GGTGGCTCTAATTCCTCTGA	20	57.30	Prajapati *et al.*, 2014
		R :ATCTTTCTCTCACCAACCCC	20	57.30	Prajapati *et al.*, 2014
7	BRMS-05	F :ACCTCCTGCAGATTCGTGTC	20	59.35	Prajapati *et al.*, 2014
		R :GCTGACCTTTCTTACCGCTC	20	59.35	Prajapati *et al.*, 2014
8	BRMS-07	F :AAATTGTTTCTCTTCCCCAT	20	51.15	Prajapati *et al.*, 2014
		R :GTGTTAGGGAGCTGGAGAAT	20	57.30	Prajapati *et al.*, 2014
9	BRMS-08	F :AGGACACCAGGCACCATATA	20	57.30	Prajapati *et al.*, 2014
		R :CATTGTTGTCTTGGGAGAGC	20	57.30	Prajapati *et al.*, 2014

Table 3.7 Continue

10	BRMS-14	F :CCGTAAGGAATATTGAGGCA	20	55.25	Prajapati *et al.*, 2014
		R :TTCCCAATTCTCAAACGGTA	20	53.20	Prajapati *et al.*, 2014
11	BRMS-27	F :GTGCTTGATGAGTTTCACATTG	22	56.53	Prajapati *et al.*, 2014
		R :GCAGGCGTTGCCTTTATGTA	20	57.30	Prajapati *et al.*, 2014
12	BRMS-30	F :TCAGCCTACCAACGAGTCATAA	22	58.39	Prajapati *et al.*, 2014
		R :AAGGTCTCATACGATGGGAGTG	22	60.25	Prajapati *et al.*, 2014
13	BRMS-31	F:TGCCACCAATGACAATGACACTAC	25	61.34	Prajapati *et al.*, 2014
		R:GATGCACTGGGACCACTTACATTTT	25	61.34	Prajapati *et al.*, 2014
14	BRMS-37	F :CTGCTCGCATTTTTTATCATAC	22	54.66	Prajapati *et al.*, 2014
		R :TACGCTTGGGAGAGAAAACTAT	22	56.53	Prajapati *et al.*, 2014
15	BRMS- 240	F:CAAGAGTATTTGTGTGGGTTGACTC	25	61.34	Rajpoot *et al.*, 2020
		R:AAATAACGAACGGAGAGAGAGAGAG	25	61.34	Rajpoot *et al.*, 2020
16	BRMS- 324	F :AACTTAACCGAAACCGAGSTAGGTG	25	62.98	Rajpoot *et al.*, 2020
		R :AATCTCGAAATTCATCGACTTCCTC	25	59.70	Rajpoot *et al.*, 2020
17	SSR Na10-D09	F :AAGAACGTCAAGATCCTCTGC	21	57.87	Rajpoot *et al.*, 2020
		R :ACCACCACGGTAGTAGAGCG	20	61.40	Rajpoot *et al.*, 2020

Table 3.7 Continue

18	SSR Na10-D11	F :GAGACATAGATGAGTGAATCTGGC	24	61.01	Rajpoot *et al.*, 2020
		R :CATTAGTTGTGGACGGTCGG	20	59.35	Rajpoot *et al.*, 2020
19	SR- 7223	F :AGGACCCGACTTTCCTTGTT	20	57.30	Rajpoot *et al.*, 2020
		R :ACCAAACTCGGCGTACAAAT	20	55.25	Rajpoot *et al.*, 2020
20	SR- 9222	F :CACCGAACAAAACTGAGGGT	20	57.30	Rajpoot *et al.*, 2020
		R :CGTTTCACTGCGTTCTACCA	20	57.30	Rajpoot *et al.*, 2020
21	SR – 94102	F :ATCCCCAAACTACCCTCACC	20	59.35	Rajpoot *et al.*, 2020
		R :AGGATGAGCAAAGGAAAGCA	20	55.25	Rajpoot *et al.*, 2020
22	BG41	F :TCCTCCGACAACAACAACTCAA	22	58.39	Kumar *et al.*, 2020
		R :ATCTAACCCGTCTGCGAATCTG	22	60.25	Kumar *et al.*, 2020
23	BG48	F :CACGAAAGCTGTAGAGGCATGA	22	60.25	Kumar *et al.*, 2020
		R :TCTTTTCCTGTCCATGAGATTCAA	24	57.59	Kumar *et al.*, 2020
24	BG100	F :TGGTAAGTGTACTTCCATATAATGTTT	27	57.37	Kumar *et al.*, 2020
		R :TGTGATGGATTGAATCGAGGTC	22	58.39	Kumar *et al.*, 2020
25	BG114	F :GCAAAATCCATTGGTAATCAGGA	23	57.08	Kumar *et al.*, 2020
		R :TGGGCAAGTCACACTCACTCA	21	59.82	Kumar *et al.*, 2020

Table 3.7 Continue

26	BG132	F :AATGCCCATCCCTCCTTGAT	20	57.30	Kumar *et al.*, 2020
		R :TTTGAACACTCATTGTGGTGATGA	24	57.59	Kumar *et al.*, 2020
27	BRMS-017	F :GGAAAGGGAAGCTTCATATC	20	55.25	Pratap *et al.*, 2014
		R :CTGGAAAGCATACACTTTGG	20	55.25	Pratap *et al.*, 2014
28	BRMS-033	F :GCGGAAACGAACACTCCTCCCATGT	25	66.26	Pratap *et al.*, 2014
		R :CCTCCTTGTGCTTTCCCTGGAGACG	25	67.90	Pratap *et al.*, 2014
29	BRMS-011	F :GAACGCGCAACAACAAATAGTG	22	58.39	Pratap *et al.*, 2014
		R :CGCGTCACAATCGTAGAGAATC	22	60.25	Pratap *et al.*, 2014
30	BrgMS339	F :CTACCTGAAGATGACCCAGACG	22	62.12	Pratap *et al.*, 2014
		R :GCATACAAACCTCGTCCTAAGC	22	60.25	Pratap *et al.*, 2014

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

The experimental results obtained from the present study on “Characterization of *Moricandia* based restorer lines in Indian mustard [*Brassica juncea* (L.) Czern and Coss]” are presented and discussed under following sub-headings:

4.1 Variation through qualitative descriptors

4.2 Variation through quantitative characters

4.3 Correlation coefficient analysis

4.4 Path coefficient analysis

4.5 Molecular diversity

4.6 Fertility restorability

4.1 Variation through qualitative descriptors

To distinguish mustard varieties, observation was recorded in fifteen restorers on 18 morphological characteristics, as per the DUS test guidelines of PPV and FR authority. Mustard germplasm has been reported with vast variability including leaf hairiness, leaf colour, leaf length and width, leaf lobes, flower colour, length and width of petals, siliqua length, siliqua angle with main shoot, siliqua texture, seeds per siliqua and seed colour.

All fifteen genotypes (restorers) showed similar characteristics in terms of leaf lobes (present), leaf width (medium), flower colour of petals (yellow), flower of width (medium), siliqua length (short), seed oil (low percentage). While, rest of the qualitative descriptors showed sufficient variation among genotypes. Study of qualitative descriptors for fifteen genotypes showed differences of all eighteen qualitative descriptors among fifteen Indian mustard genotypes studied given in Table 4.1. and Plate III to Plate VI. Results revealed sufficient amount of variability based on qualitative descriptors.

The results are in conformity with the results of Yadav *et al.* (2013), Neeru *et al.* (2017), Hinge and Malode (2018) and Bano *et al.* (2021) for leaf hairiness, leaf colour, leaf lobes, flower colour of petals, seed colour, siliqua texture.

Table 4.1: Study of qualitative descriptors for fifteen Indian mustard genotypes (restorers)

Sr. No.	Qualitative Descriptors	States	Example varieties	Stage of Observation	Type of assessment
1.	Leaf: Hairiness	Absent	GM*Mo*2130R	50-60	VS
		Sparse	GM*Mo*2105R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2120R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2132R, GM*Mo*2133R		
		Dense	GM*Mo*2101R, GM*Mo*2119R, GM*Mo*2127R, GM*Mo*2128R		
2.	Leaf: Colour	Light green	GM*Mo*2113R, GM*Mo*2120R, GM*Mo*2125R, GM*Mo*2132R	50-60	VG
		Medium green	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2130R, GM*Mo*2133R		
		Dark green	GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2127R, GM*Mo*2128R		
3.	Leaf: Lobes	Absent	-	50-60	VS
		Present	All fifteen restorers		
4.	Leaf: Number of lobes	Low	GM*Mo*2105R	50-60	MS
		Medium	GM*Mo*2101R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R, GM*Mo*2132R, GM*Mo*2133R		
		High	-		
5.	Leaf: Dentation of margin	Entire	-	50-60	VS
		Dentate	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2120R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R		
		Serrate	GM*Mo*2113R, GM*Mo*2119R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2132R, GM*Mo*2133R		

Plate III: Siliqua of various genotypes [A: GM*Mo*2101R, B: GM*Mo*2105R, C: GM*Mo*2113R, D: GM*Mo*2115R, E: GM*Mo*2116R, F: GM*Mo*2119R, G: GM*Mo*2120R, H: GM*Mo*2122R]

Plate IV: Siliqua of various genotypes [A: GM*Mo*2124R, B: GM*Mo*2125R, C: GM*Mo*2127R, D: GM*Mo*2128R, E: GM*Mo*2130R, F: GM*Mo*2132R, G: GM*Mo*2133R]

Plate V: Seeds of various genotypes [A: GM*Mo*2101R, B: GM*Mo*2105R, C: GM*Mo*2113R, D: GM*Mo*2115R, E: GM*Mo*2116R, F: GM*Mo*2119R, G: GM*Mo*2120R, H: GM*Mo*2122R]

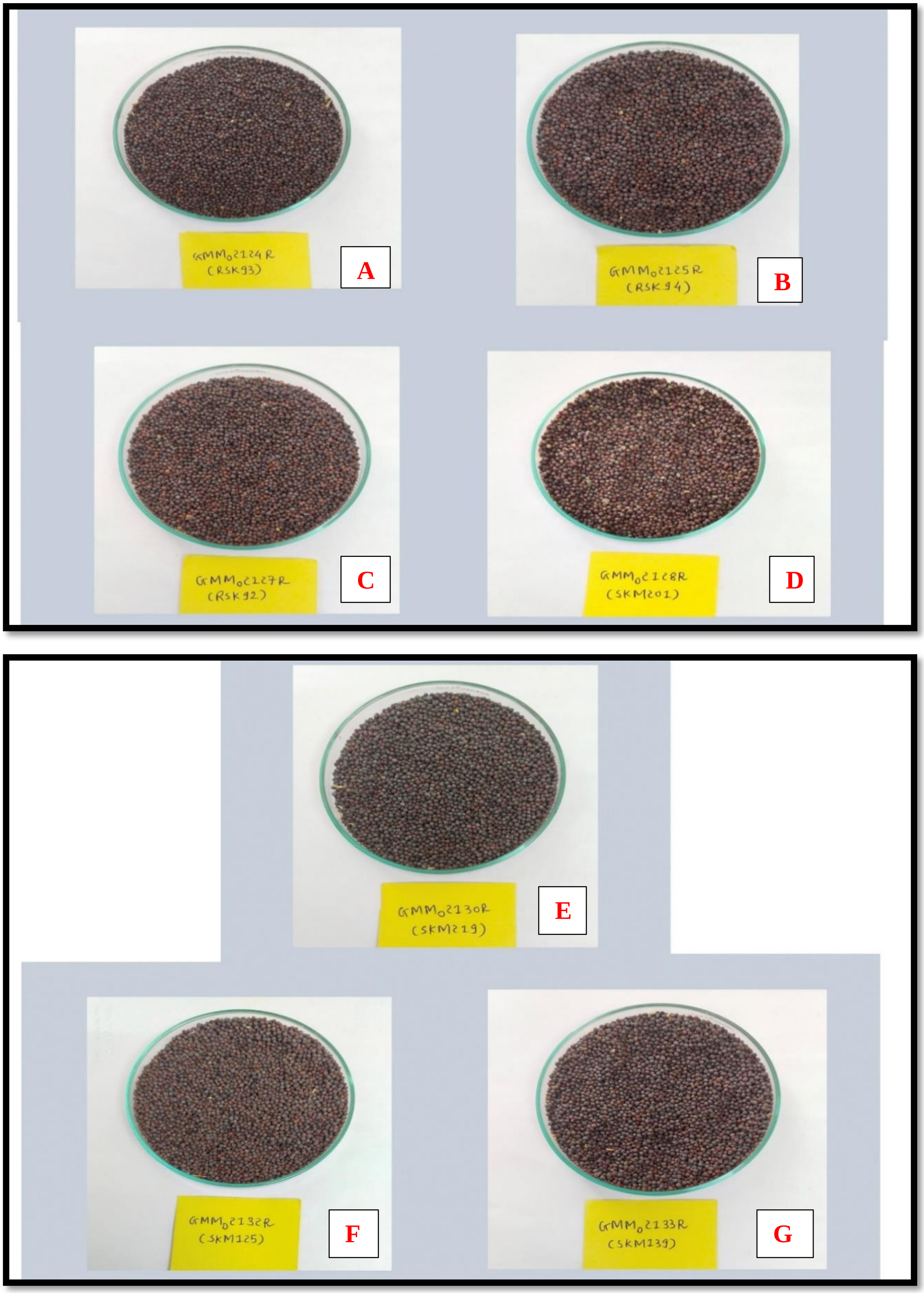


Plate VI: Siliqua of various genotypes [A: GM*Mo*2124R, B: GM*Mo*2125R, C: GM*Mo*2127R, D: GM*Mo*2128R, E: GM*Mo*2130R, F: GM*Mo*2132R, G: GM*Mo*2133R]

Table 4.1 Continue

Sr. No.	**Qualitative Descriptors**	**States**	**Example varieties**	**Stage of Observation**	**Type of assessment**
6.	Leaf: Length (cm)	Short	GM*Mo*2101R, GM*Mo*2125R, GM*Mo*2128R, GM*Mo*2130R	50-60	MS
		Medium	GM*Mo*2113R, GM*Mo*2119R, GM*Mo*2122R, GM*Mo*2124R		
		Long	GM*Mo*2105R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2120R, GM*Mo*2127R, GM*Mo*2132R, GM*Mo*2133R		
7.	Leaf: Width (cm)	Narrow	-	50-60	MS
		Medium	All fifteen restorers		
		Broad	-		
8.	Leaf: Number of lobes	White	-	60-62	VG
		Light yellow	-		
		Yellow	All fifteen restorers		
		Orange	-		
9.	Flower: Length of petals	Short	GM*Mo*2105R, GM*Mo*2120R	60-62	MS
		Medium	GM*Mo*2101R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R, GM*Mo*2132R, GM*Mo*2133R		
		Long	-		

Table 4.1 Continue

Sr. No.	Qualitative Descriptors	States	Example varieties	Stage of Observation	Type of assessment
10.	Flower: Width of petals	Narrow	-	60-62	MS
		Medium	All fifteen restorers		
		Broad	-		
11.	Siliqua: Length	Short	All fifteen restorers	85	MS
		Medium	-		
		Broad	-		
12.	Siliqua: Length of beak	Short	GM*Mo*2101R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2132R	85	MS
		Medium	GM*Mo*2105R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R, GM*Mo*2133R		
		Long	-		
13.	Siliqua: Angle with main shoot	Appressed	GM*Mo*2115R, GM*Mo*2130R, GM*Mo*2132R	85	VG
		Semi appressed	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2120R, GM*Mo*2127R, GM*Mo*2128R		
		Open	GM*Mo*2113R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2133R		
14.	Siliqua: Texture	Smooth	-	85	VS
		Undulated	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2133R		
		Constricted	GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2120R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R, GM*Mo*2132R		

Table 4.1 Continue

Sr. No.	**Qualitative Descriptors**	**States**	**Example varieties**	**Stage of Observation**	**Type of assessment**
15.	Siliqua: Number of siliqua on main shoot	Very few	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2122R, GM*Mo*2130R	85	MS
		Few	GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2132R, GM*Mo*2133R		
		Medium	-		
		Many	-		
16.	Seed: Colour	Yellow	-	100	VG
		Reddish brown	GM*Mo*2122R		
		Brown	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2132R		
		Dark brown	-		
		Black	GM*Mo*2116R, GM*Mo*2130R, GM*Mo*2133R		
17.	Seed: Size	Small	GM*Mo*2122R	100	MG
		Medium	GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R, GM*Mo*2132R, GM*Mo*2133R		
		Bold	-		
18.	Seed: Oil (%)	Low	All fifteen restorers	100	MG
		Medium	-		
		High	-		
		Very High	-		

4.2 Variation through quantitative characteristics

4.2.1 Analysis of variance

The analysis of variance indicating the mean sum of squares for eleven quantitative characters for experimental design was carried out to ascertain the genuine differences among genotypes (restorers). The data presented in Table 4.2.

Table 4.2: Analysis of variance for different quantitative characters in Indian mustard

Sr. No.	Characters	Mean Sum of Square		
		Replications	Genotypes	Error
Degree Of Freedom		**2**	**14**	**28**
1	Days to flowering	2.69	8.69 **	4.10
2	Days to maturity	1.86	122.49 **	9.20
3	Plant height(cm)	354.24	951.17 **	191.76
4	Number of branches per plant	0.94	18.50 **	1.13
5	Number of siliqua per plant	147.50	1922 **	166.73
6	Number of siliqua on main shoot	12.31	33.96 **	9.12
7	Number of seed per siliqua	0.42	3.05 **	0.65
8	Length of siliqua	0.001	0.25 **	0.05
9	Seed test weight	0.01	2.03 **	0.01
10	Oil content (%)	1.58	1.35 **	0.86
11	Yield per plant	15.10	40.27 **	9.36

*, ** Significant at 0.05 and 0.01 per cent level of significance, respectively.

The analysis of variance (Table 4.2**)** revealed highly significant differences among the genotypes (restorers) for all quantitative characters, which indicated the presence of high amount of genetic variability for all the characters studied and there is an ample scope to identify desirable genotypes to improve various traits. Similar results were reported by Muhammad *et al.* (2007), Yadav *et al.* (2011), Bind *et al.* (2014), Lodhi *et al.* (2014), Akbari and Niranjana (2015), Bibi *et al.* (2016), Gupta and Dwivedi (2016), Trivedi *et al.* (2016), Rathod *et al.* (2017), Tiwari *et al.* (2017^a), Maurya *et al.* (2018), Gupta *et al.* (2019), Patel *et al.* (2021) and Kaur *et al.* (2022).

4.2.2 Variability

The phenotypic and genotypic variances for each quantitative trait were obtained from analysis of variance. The other parameters *viz.,* genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (H^2b) and genetic advance as per cent of mean (GA as per cent of mean) were computed from variance components and mean values and these data are presented in Table 4.3. The mean values of various genotypes for eleven quantitative traits studied are presented in Appendix II.

4.2.2.1 Days to flowering

4.2.2.1.1 Mean performance

The character showed less variability among genotypes and a low range of variability (38.00 to 43.33 days) with a general mean of 41.07 days was recorded. The genotype GM*Mo*2130R was the earliest for days to flowering (38.00 days) followed by GM*Mo*2113R (38.57 days) and GM*Mo*2101R (38.67 days). The genotype GM*Mo*2116R (43.33 days) significantly took maximum days for flowering.

4.2.2.1.2 Variance components

The results revealed that genotypic variance (1.53) contributed a less portion to phenotypic variance (5.63) in expression of the character, thus indicating high influence of the environment on the expression of this character. Low genotypic variance and phenotypic variance were also observed by Akbari and Niranjana (2015), Patel *et al.* (2019) and Saiyad *et al.* (2020) in Indian mustard.

4.2.2.1.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (3.01 %) were low and PCV (5.78 %) were low indicating the presence of less variability in the population for days to flowering. Singh *et al.* (2018), Chaurasiya *et al.* (2019), Kumar *et al.* (2019), Tripathi *et al.* (2019), Pandey *et al.* (2020) and Saiyad *et al.* (2020) reported low GCV and PCV for this trait.

4.2.2.1.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was comparatively low (3.23 %) coupled with low heritability (27.14 %), indicates non-additive gene action and selection for such trait may not be rewarding. Sikarwar *et al.* (2017) observed low genetic advance coupled with low heritability for this trait.

4.2.2.2 Days to maturity

4.2.2.2.1 Mean performance

The character showed less variability among genotypes and low range of variability (103.33 to 124.73 days) with a general mean of 118.13 days was recorded. The genotype GM*Mo*2130R was the earliest in maturity (103.33 days) followed by GM*Mo*2122R (111.33 days) and GM*Mo*2105R (112.00 days). The late maturing genotypes were GM*Mo*2125R (124.73 days) followed by GM*Mo*2116R (124.13 days).

4.2.2.2.2 Variance components

The results revealed that genotypic variance (37.76) contributed a more portion to phenotypic variance (46.96) in expression of the character, this indicating a less influence of the environment on the expression of this character. Saiyad *et al.* (2020) also reported the same result for days to maturity.

4.2.2.2.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (5.20 %) and PCV (5.80 %) were low indicating the presence of less variability in the population for days to maturity. Similar results were also observed by Malik *et al.* (2018), Raliya *et al.* (2018), Chaurasiya *et al.* (2019), Kumar *et al.* (2019), Patel *et al.* (2019), Tripathi *et al.* (2019), Pandey *et al.* (2020) and Saiyad *et al.* (2020).

4.2.2.2.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was low (9.61 %) coupled with high heritability (80.42 %), indicates non-additive gene action and selection for such trait may not be rewarding. These results are in agreement with the findings of Chaurasiya *et al.* (2019), Kumar *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.3 Plant height (cm)

4.2.2.3.1 Mean performance

The wide range of variability was recorded for plant height from 132.33 to 196.67 cm with a general mean of 174.40 cm. The genotypes GM*Mo*2116R (196.67 cm) was registered with the highest plant height over the mean followed by GM*Mo*2125R (193.67 cm) and GM*Mo*2115R (188.33 cm). The genotype GM*Mo*2130R (132.33 cm) was registered the lowest plant height over the mean.

4.2.2.3.2 Variance components

The results revealed that genotypic variance (253.14) contributed a major portion to phenotypic variance (444.89) in expression of the character, indicating less influence of the environment on the expression of this character.

4.2.2.3.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (9.12 %) were low and PCV (12.09 %) were moderate indicating the presence of poor to medium variability in the population for plant height. Low GCV and moderate PCV in plant height were also found by Raliya *et al.* (2018), Singh *et al.* (2018), Kumar *et al.* (2019) and Pandey *et al.* (2020).

Table 4.3: Range, mean, genotypic, phenotypic and environmental variances, GCV, PCV, H^2 (broad sense), GA and GA as per cent of mean for different quantitative characters in mustard

Characters	Mean	Range	(σ^2_g)	(σ^2_p)	(σ^2_e)	GCV (%)	PCV (%)	H^2b (%)	GA	GA as % of Mean
Days to flowering	41.07	38.00- 43.33	1.53	5.63	4.10	3.01	5.78	27.14	1.33	3.23
Days to maturity	118.13	103.33-124.73	37.76	46.96	9.20	5.20	5.80	80.42	11.35	9.61
Plant height (cm)	174.40	132.33-196.67	253.14	444.89	191.76	9.12	12.09	56.90	24.72	14.18
Number of branches per plant	14.08	9.60-17.13	5.79	6.92	1.13	17.09	18.69	83.69	4.54	31.21
Number of siliqua per plant	156.16	113.00-192.40	585.09	751.82	166.73	15.49	17.56	77.82	43.96	28.15
Number of siliqua on main shoot	34.92	27.40-38.33	8.28	17.40	9.12	8.24	11.94	47.59	4.10	11.71
Number of seeds per siliqua	13.72	11.53-15.33	0.80	1.45	1.83	6.52	8.79	55.00	1.37	9.96
Length of siliqua(cm)	4.02	3.42-4.45	0.07	0.12	0.06	6.40	8.54	56.10	0.40	9.87
1000 seed weight (g)	4.80	3.59-5.86	0.67	0.69	0.01	17.08	17.26	97.95	1.67	34.82
Oil content (%)	36.83	35.54-37.83	0.16	1.02	0.86	1.10	2.74	16.05	33.38	90.64
Seed yield per plant (g)	22.30	15.60-27.00	10.30	19.66	9.36	14.40	19.89	52.41	4.79	21.47

4.2.2.3.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was moderate (14.18 %) coupled with moderate heritability (56.90 %). Suggested prediction of genetic response of selection is not helpful in improvement of this trait. Moderate heritability coupled with moderate genetic advance as per cent of mean was also reported by Saiyad *et al.* (2020).

4.2.2.4 Number of branches per plant

4.2.2.4.1 Mean performance

The wide range of variability was recorded for number of branches per plant from 9.60 to 17.13 with a general mean of 14.08. The genotypes GM*Mo*2116R (17.13) was registered the highest number of branches per plant over the mean followed by GM*Mo*2105R (17.00) and GM*Mo*2115R (17.00). The genotypes GM*Mo*2133R (9.60) registered the lowest number of branches per plant over the mean followed by GM*Mo*2128R (10.60).

4.2.2.4.2 Variance components

The results revealed that genotypic variance (5.79) contributed a major portion to phenotypic variance (6.92) in expression of the character, indicating less influence of the environment on the expression of this character. The results showed a low difference between genotypic variance and phenotypic variance are in accordance with the findings of Akbari and Niranjana (2015), Patel *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.4.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (17.09 %) and PCV (18.69 %) were moderate indicating the presence of moderate variability in the population for number of branches per plant. These results are supported by Gupta *et al.* (2019) and Pandey *et al.* (2020).

4.2.2.4.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was high (31.21 %) coupled with high heritability (83.69 %), which indicates that most likely the heritability is influenced by less environmental effects and additive gene effects, thus selection for this trait is effective. The results are in accordance with the findings of Gupta *et al.* (2019) and Pandey *et al.* (2020).

4.2.2.5 Number of siliqua per plant

4.2.2.5.1 Mean performance

The wide range of variability was recorded for number of siliqua per plant from 113 to 192.40 with a general mean of 156.16. The genotypes GM*Mo*2105R (192.40) was registered with the highest number of siliqua per plant over the mean followed by GM*Mo*2116R (190.23) and GM*Mo*2119R (182.25).

4.2.2.5.2 Variance components

The results revealed that genotypic variance (585.09) contributed a more portion to phenotypic variance (751.82) in expression of the character, indicating less influence of the environment on expression of this character. Akbari and Niranjana (2015) and Patel *et al.* (2019) also reported a low difference between genotypic and phenotypic variance for this trait.

4.2.2.5.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (15.49 %) and PCV (17.56 %) were moderate indicating the presence of moderate variability in the population for number of siliqua per main shoot. The moderate variability observed in this investigation is in accordance with the results reported by Rathod *et al.* (2017).

4.2.2.5.4 Genetic advance (% of mean) and heritability

The per cent genetic advance was moderate (28.15 %) coupled with high heritability (77.82 %). Suggested prediction of genetic response of selection is helpful in improvement of this trait. This result is supported by Bind *et al.* (2014).

4.2.2.6 Number of siliqua on main shoot

4.2.2.6.1 Mean performance

The wide range of variability was recorded for number of siliqua on main shoot from 27.40 to 38.33 with a general mean of 34.92. The genotypes GM*Mo*2130R (38.33) was registered with the highest number of siliqua on main shoot over the mean followed by GM*Mo*2115R (38.33) and GM*Mo*2125R (37.93).

4.2.2.6.2 Variance components

The results revealed that genotypic variance (8.28) contributed a less portion to phenotypic variance (17.40) in expression of the character, indicating more influence of the environment on expression of this character. Akbari and Niranjana (2015) and Patel *et al.* (2019) also reported a more influence of environment variance than genotypic variance for this trait.

4.2.2.6.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (8.24 %) were low and PCV (11.94 %) were moderate indicating the presence of moderate variability in the population for number of siliqua on main shoot. The moderate variability observed in this investigation is in accordance with the results reported by Rathod *et al.* (2017).

4.2.2.6.4 Genetic advance (% of mean) and heritability

The per cent genetic advance was moderate (11.71 %) coupled with moderate heritability (47.59 %). Suggested prediction of genetic response of selection is not helpful in improvement of this trait. This result is supported by Bind *et al.* (2014).

4.2.2.7 Number of seeds per siliqua

4.2.2.7.1 Mean performance

The low range of variability was observed in this trait, which varied from 11.53 to 15.33 seeds. The mean value was 13.72. Among all genotypes, the highest seeds per siliqua was recorded in genotypes GM*Mo*2130R (15.33) followed by GM*Mo*2105R (15.13). The genotype GM*Mo*2124R (11.53) was registered with the lowest seeds per siliqua over the mean.

4.2.2.7.2 Variance components

The results revealed that genotypic variance (0.80) contributed a lower portion of phenotypic variance (1.45) in expression of the character which indicates that more influence of the environment. Similar results were found by Akbari and Niranjana (2015) and Saiyad *et al.* (2020).

4.2.2.7.3 Genotypic and phenotypic coefficients of variation

The values of GCV (6.52 %) and PCV (8.79 %) were low suggesting less variability available for this trait and a small difference between them indicated the environment had little effect on the expression of this trait. Tripathi *et al.* (2019) and Pandey *et al.* (2020) reported low GCV and PCV values for this trait.

4.2.2.7.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was low (9.96 %) which accompanied by moderate heritability (55.00 %). A similar result for this trait was obtained by Chaurasiya *et al.* (2019).

4.2.2.8 Length of siliqua (cm)

4.2.2.8.1 Mean performance

The character showed less variability among genotypes and a low range of variability (3.42 to 4.45 cm) with a general mean of 4.02 cm. The genotype

GM*Mo*2130R had the longest length of siliqua (4.45 cm) followed by GM*Mo*2116R (4.37 cm). The genotype GM*Mo*2124R (3.42 cm) had significantly minimum length of siliqua.

4.2.2.8.2 Variance components

The results revealed that genotypic variance (0.07) contributed a major portion to phenotypic variance (0.12) in expression of the character, this indicating less influence of the environment on the expression of this character. Similar results were found by Akbari and Niranjana (2015) and Saiyad *et al.* (2020).

4.2.2.8.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (6.40 %) and PCV (8.54 %) were low indicating presence of poor variability in the population for length of siliqua.

4.2.2.8.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was comparatively low (9.87 %) coupled with moderate heritability (56.10 %). Such result was found by Shekhawat *et al.* (2014).

4.2.2.9 1000 seed weight (g)

4.2.2.9.1 Mean performance

1000 seed weight is an important quality parameter to determine the boldness of seed and price of seed at the time of sale, so higher mean performance is desirable. The character showed less variability among genotypes and a low range of variability (3.59 to 5.86 g) with a general mean of 4.80 g. A higher mean value was determined for genotype GM*Mo*2130R (5.86 g) followed by GM*Mo*2105R (5.83 g). The genotypes GM*Mo*2133R (3.59 g) was determined with the lowest mean for 1000 seed weight.

4.2.2.9.2 Variance components

The results revealed that genotypic variance (0.67) contributed a major portion to phenotypic variance (0.69) in expression of the character, thus indicating very less influence of the environment on the expression of this character. The present findings are in accordance with Akbari and Niranjana (2015), Patel *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.9.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (17.08 %) and PCV (17.26 %) were moderate indicating the presence of moderate variability in the population for 1000 seed weight. A similar result for this trait was obtained by Rathod *et al.* (2017), Singh *et al.* (2018), Chaurasiya *et al.* (2019), Kumar *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.9.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was high (34.82 %) accompanied with high heritability (97.95 %) revealed that the character is governed by additive gene action and high heritability is being exhibited due to low environmental effects, so selection for such trait may be rewarding. The results are akin with those of Rathod *et al.* (2017), Malik *et al.* (2018), Singh *et al.* (2018), Raliya *et al.* (2018), Yadav and Pandey (2018), Chaurasiya *et al.* (2019), Gupta *et al.* (2019), Tripathi *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.10 Oil content (%)

4.2.2.10.1 Mean performance

The wide range of variability was recorded for oil content from 35.54 to 37.83 per cent with a general mean of 36.83 per cent. The genotype GM*Mo*2105R (37.83 %) had registered the highest oil content over the mean followed by GM*Mo*2113R (37.57 %). The genotype GM*Mo*2119R (35.54 %) was registered with the lowest oil content over the mean.

4.2.2.10.2 Variance components

The magnitude of genotypic variance (0.16) and phenotypic variance (1.02) exhibited more differences between them which suggested a more influence of the environment on this trait. A similar finding was reported by Patel *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.10.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (1.10 %) and PCV (2.74 %) were low indicating presence of poor variability in the population for oil content. The present findings are in accordance with Rathod *et al.* (2017), Malik *et al.* (2018), Singh *et al.* (2018), Raliya *et al.* (2018), Chaurasiya *et al.* (2019), Gupta *et al.* (2019), Patel *et al.* (2019), Tripathi *et al.* (2019) and Saiyad *et al.* (2020).

4.2.2.10.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was high (90.64 %) coupled with low heritability (16.05 %), revealing the additive gene action and low environmental influence suggested prediction of genetic response of selection is more helpful in improvement of this trait. This result supported by Chaurasiya *et al.* (2019).

4.2.2.11 Seed yield per plant (g)

4.2.2.11.1 Mean performance

The high range of variability was observed in this trait and it was varied from 15.60 to 27.00 g. The mean seed yield per plant was 22.30 g. Among all the genotypes, the highest seed yield per plant was recorded in genotype GM*Mo*2105R (27.00 g) followed by GM*Mo*2116R (27.00 g). The lowest seed yield per plant was recorded by GM*Mo*2127R (15.60 g).

4.2.2.11.2 Variance components

The results revealed that genotypic variance (10.30) contributed a major portion to phenotypic variance (19.66) in expression of the character, this indicating less influence of the environment on the expression of this character. Akbari and Niranjana (2015), Lyngdoh *et al.* (2017), Patel *et al.* (2019) and Saiyad *et al.* (2020) also reported a low difference between genotypic and phenotypic variance for this trait.

4.2.2.11.3 Genotypic and phenotypic coefficients of variation

The estimates of GCV (14.40 %) and PCV (19.89 %) were moderate indicating the presence of moderate variability in the population for seed yield per plant. Rout *et al.* (2018) reported a moderate GCV and PCV for this trait.

4.2.2.11.4 Genetic advance (% of mean) and heritability

The genetic advance as per cent of mean was high (21.47%) coupled with moderate heritability (52.41%) which indicated the possibility of improving and fixing this character through selection. Bind *et al.* (2014) reported moderate heritability coupled with high genetic advance as per cent of mean for this trait.

The overall discussion on variability parameters proved that the traits *viz.*, number of branches per plant, number of siliqua per plant, number of siliqua on main shoot, 1000 seed weight and seed yield per plant possessed considerable to high genetic variability due to more differences in the genetic makeup of genotypes and was controlled by additive gene effects. Selection practiced on these traits for these genotypes would lead to an improvement.

4.3 Correlation coefficient analysis

In plant breeding programmes, breeder handles several traits often together because seed yield is a polygenic and complex character and it depends on number of yield attributing traits. So, for genetic improvement in seed yield and for quicker assessment of high yielding genotypes in selection programme, it is required to study relationship among these quantitative component traits and their association with seed yield.

The phenotypic correlation is the association between characters which is observed directly. Only phenotypic correlation coefficient is not enough to evaluate the association because phenotypic correlation coefficient is the result of environment and genotypic interaction. The genotypic correlation predicts the correlated response and evaluates the relative influence of one quantitative character on another, which helps in the construction of selection indices.

Hence, the correlation coefficients at genotypic and phenotypic levels were estimated between seed yield and its component traits and among the component traits which is presented in Table 4.4.

4.3.1 Days to flowering

Days to flowering was highly significant and positively correlated with days to maturity (r_g= 0.751, r_p= 0.350) and plant height (r_g= 0.718, r_p= 0.345) at genotypic and phenotypic level. It was highly significant and negatively correlated with test weight at both level (r_g= -0.656, r_p= -0.344). While, the traits number of branches per plant, number of siliqua on main shoot, seeds per siliqua, length of siliqua and seed yield per plant showed non-significant association with days to flowering. Highly significant and positive association of days to flowering with plant height and days to maturity was also reported by Prasad and Patil (2018), Pal *et al.* (2019), Pandey *et al.* (2020) and Saiyad *et al.* (2020), while the highly significant and negative association with 1000 seed weight and also reported by Prasad and Patil (2018).

4.3.2 Days to maturity

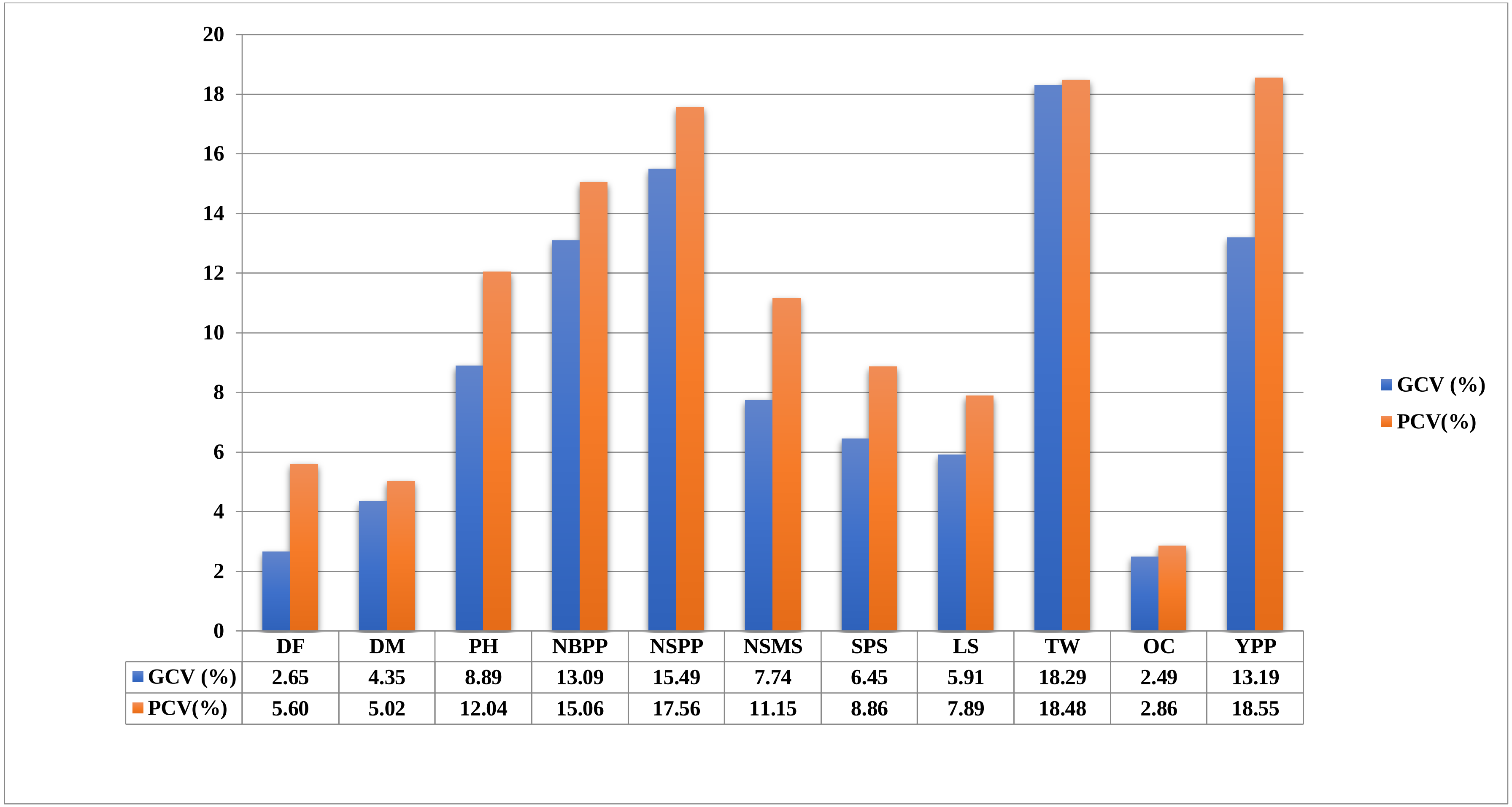
Days to maturity was highly significant and negatively correlated with seed yield per plant (r_g=-0.619, r_p= -0.397) at both the levels. It was significant and negatively correlated with seeds per siliqua (r_g= -0.695, r_p= -0.453) at both levels. It had non-significant and negative correlation with number of branches per plant (r_g= -0.359) and number of siliqua on main shoot (r_p= -0.483) at genotypic and phenotypic level

Table 4.4: Genotypic and phenotypic correlation among eleven quantitative characters in Indian mustard

Characters	r	DM	PH	NBPP	NSPP	NSMS	SPS	SL	TW	OC	SYPP
DF	r_p	0. 350*	0.345*	0.028	0.083	-0.110	0.053	-0.242	-0.344*	-0.258	0.151
	r_g	0.751**	0.718**	-0.100	0.121	-0.370	0.007	-0.192	-0.656**	0.329	-0.102
DM	r_p		0.642**	-0.287	-0.442**	-0.190	-0.453*	-0.269	-0.620**	0.205	-0.397**
	r_g		-0.906**	-0.359	-0.483	-0.483	-0.695**	-0.411	-0.708**	0.234	-0.619**
PH	r_p			-0.031	-0.137	-0.0.61	-0.333*	-0.262	-0.173	0.055	-0.235
	r_g			-0.143	-0.210	-0.389	-0.600*	-0.429	-0.213	0.109	-0.184
NBPP	r_p				0.722**	0.594**	0.396**	0.243	0.496**	0.136	0.646**
	r_g				0.909**	0.901**	0.723**	0.486	0.572*	0.639*	0.966**
NSPP	r_p					0.522**	0.515**	0.343*	0.558**	0.145	0.823**
	r_g					0.840**	0.783**	0.399	0.663*	0.376	0.989**
NSMS	r_p						0.016	0.325*	0.051	-0.249	0.405**
	r_g						0.756**	0.390	0.510	0.469	0.978**
SPS	r_p							0.348*	0.294*	0.077	0.424**
	r_g							0.546*	0.366	0.088	0.923**
SL	r_p								0.147	-0.124	0.322**
	r_g								0.178	-0.396	0.563**
TW	r_p									-0.053	0.536**
	r_g									0.024	0.739**
OC	r_p										-0.053
	r_g										0.446

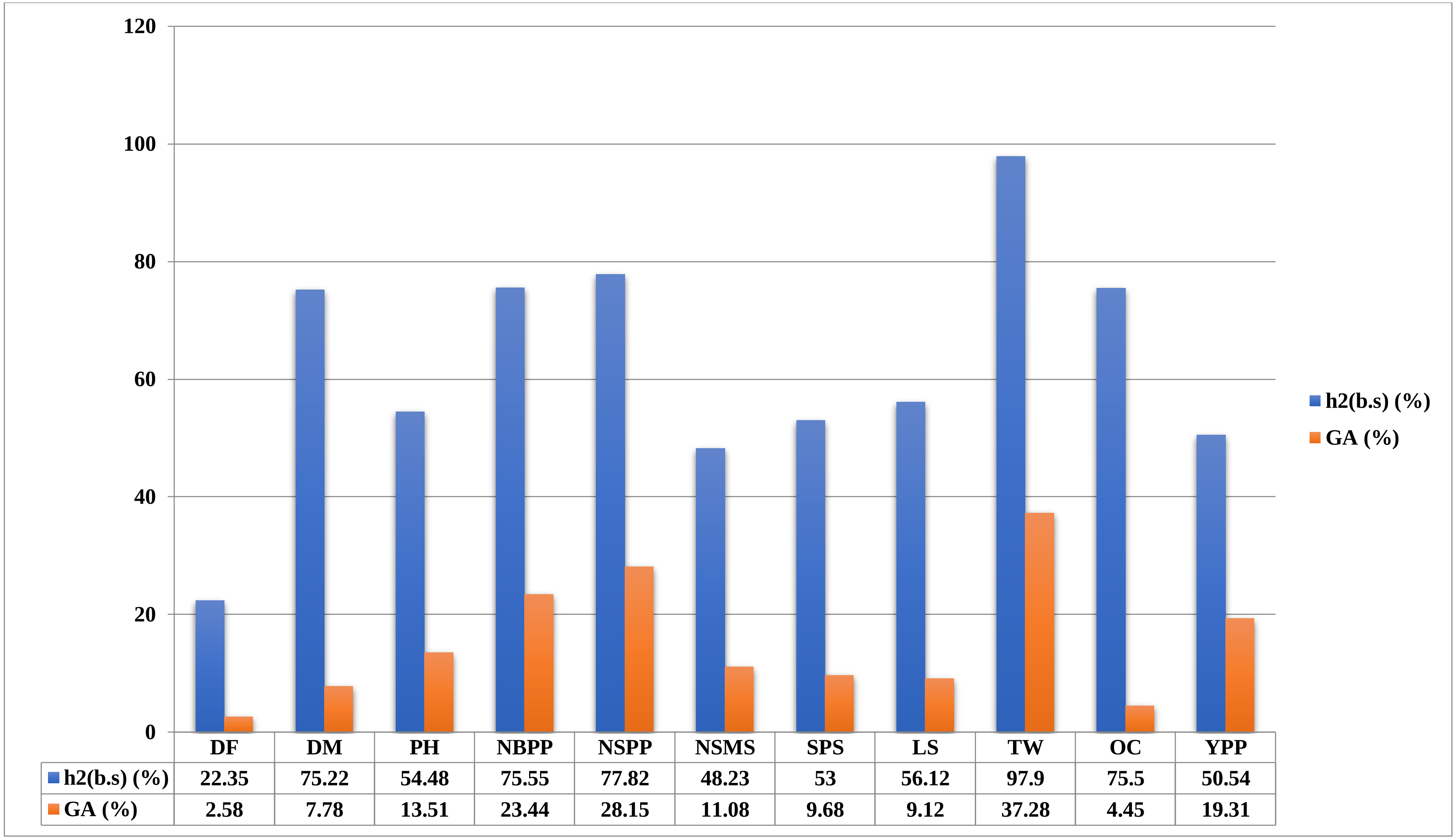
Significant at 5 and 1 per cent level of significance, respectively.

Days to flowering, **DM** = Days to maturity**, PH**= Plant height (cm), **NBPP**= Number of branches per plant, **NSPP**= Number of siliqua per plant, **NSMS**= Number of siliqua shoot, **SL**= Siliqua length (cm), **NSPS**= Number of seed per siliqua, **TW**= Test weight (g), **OC**= Oil content, **SYPP**= Seed yield per plant (g)



	DF	DM	PH	NBPP	NSPP	NSMS	SPS	LS	TW	OC	YPP
🟦 GCV (%)	2.65	4.35	8.89	13.09	15.49	7.74	6.45	5.91	18.29	2.49	13.19
🟧 PCV(%)	5.60	5.02	12.04	15.06	17.56	11.15	8.86	7.89	18.48	2.86	18.55

Fig. 2: Graphical representation of genotypic and phenotypic coefficient of variation for 11 characters in Indian mustard



	DF	DM	PH	NBPP	NSPP	NSMS	SPS	LS	TW	OC	YPP
🟦 h2(b.s) (%)	22.35	75.22	54.48	75.55	77.82	48.23	53	56.12	97.9	75.5	50.54
🟧 GA (%)	2.58	7.78	13.51	23.44	28.15	11.08	9.68	9.12	37.28	4.45	19.31

Fig. 3: Graphical representation of heritability $_{(b.s)}$ and genetic advance expressed as percent of mean for 11 characters in Indian mustard

respectively. Patel *et al.* (2021) reported a highly negative and significant correlation between 1000 seed weight and seed yield per plant and highly positive and significant correlation between plant height.

4.3.3 Plant height

Plant height was significantly and negatively associated with seeds per siliqua (r_g= -0.600, r_p= -0.333) at both levels. It had non-significant and negative association with number of branches per plant (r_g= -0.143, r_p= -0.031), number of siliqua on main shoot (r_g= -0.389, r_p= -0.061), siliqua length (r_g= -0.429, r_p= -0.262), 1000 seed weight (r_g= -0.213, r_p= -0.173), and seed yield per plant (r_g= -0.184, r_p= -0.235) at both levels. Pal *et al.* (2019) reported a negative and significant correlation between seeds per siliqua.

4.3.4 Number of branches per plant

Number of branches per plant was highly significant and positively associated with number of siliqua per plant (r_g= 0.909, r_p= 0.722), number of siliquae on main shoot (r_g= 0.901, r_p= 0.594), number of seeds per siliqua (r_g= 0.723, r_p= 0.396), 1000 seed weight (r_g= 0.572, r_p= 0.496) and seed yield per plant (r_g= 0.966, r_p= 0.646) at both the levels. It was significant and positively associated with oil content (r_g = 0.639) at genotypic level. A significant and positive correlation of number of branches per plant with number of siliqua on main shoot and seed yield per plant were also obtained by Patel *et al.* (2019) at the genotypic level only and Saiyad *et al.* (2020) reported at both genotypic and phenotypic levels.

4.3.5 Number of siliqua per plant

Number of siliqua per plant exhibited a highly significant and positive correlation with seed yield per plant (r_g= 0.989, r_p= 0.823). It was highly significant and positively associated with number of siliqua on main shoot (r_g= 0.840, r_p= 0.522), seeds per siliqua (r_g= 0.783, r_p= 0.515), 1000 seed weight (r_g= 0.663, r_p= 0.558) at both levels. It was significant and positively associated with siliqua length (r_p = 0.343) at phenotypic level. It had non-significant and positive correlation with oil content (r_g= 0.376, r_p= 0.145) at both the levels. A highly significant and positive correlation of number of siliqua per plant with seed yield per plant was observed by Bind *et al.* (2014).

4.3.6 Number of siliqua on main shoot

Number of siliqua on main shoot exhibited a highly significant and positive correlation with seed yield per plant (r_g= 0.978, r_p= 0.405). It was highly significant and positively associated with siliqua length (r_p = 0.756) at phenotypic level. It had non-significant and positive correlation with 1000 seed weight (r_g= 0.510, r_p= 0.051) at both the levels. A highly significant and positive correlation of number of siliqua on main shoot with seed yield per plant was observed by Bind *et al.* (2014).

4.3.7 Number of seeds per siliqua

Number of seeds per siliqua had highly significant and positive correlation with seed yield per plant (r_g= 0.923, r_p= 0.424) at both the levels. It had significant and positive correlation with siliqua length (r_g= 0.546, r_p= 0.348) at both levels and significant and positively correlated with oil content (r_p= 0.294) at phenotypic level. Patel *et al.* (2019) observed a significant and positive correlation between seeds per siliqua and length of siliqua. Kumar *et al.* (2018) and Chaurasiya *et al.* (2019) also reported a significant and positive correlation of seeds per siliqua with length of siliqua and seed yield per plant at genotypic and phenotypic levels, respectively. Prasad and Patil (2018) and Saiyad *et al.* (2020) reported a significant and positive correlation of seeds per siliqua with length of siliqua and seed yield per plant at both the levels as observed in the present study.

4.3.8 Length of siliqua

Length of siliqua had significant and positive association with seed yield (r_g= 0.563, r_p= 0.322) at both the levels. It was non-significantly and positively associated with 1000 seed weight (r_g= 0.178, r_p= 0.147) at both the levels. Singh *et al.* (2016) reported a significant and positive correlation of length of siliqua with seed yield per plant.

4.3.9 1000 seed weight

1000 seed weight was significant and positively correlated with seed yield per plant (r_g= 0.739, r_p= 0.536) at both levels. Saqib (2011) reported a significant and positive correlation of 1000 seed weight with seed yield per plant.

4.3.10 Oil content (%)

Oil content was non-significant and negatively (r_p= -0.053) correlated with seed yield per plant at phenotypic level while non-significant and positive (r_g= 0.446) correlated with seed yield per plant at genotypic level. Sharafi *et al.* (2015) and Saiyad *et al.* (2020) observed similar results as observed in present investigation.

4.3.11 Seed yield per plant

Seed yield per plant was highly significantly and positively correlated with number of branches per plant (r_g= 0.966, r_p= 0.646), number of siliqua on main shoot

(r_g= 0.978, r_p= 0.405), number of siliqua per plant (r_g= 0.989, r_p= 0.823), number of seeds per siliqua (r_g= 0.923, r_p= 0.424), length of siliqua (r_g= 0.563, r_p= 0.322) and 1000 seed weight (r_g=0.739, r_p= 0.536) at both the levels. It had also a highly significant and negative association with days to maturity (r_g= -0.619, r_p= -0.397) at both levels. It exhibited a non-significant and negative association with plant height (r_g= -0.184, r_p= -0.235) at both the levels. It had non-significant and negative as well as a positive correlation with days to flowering (r_g= 0.102, r_p= 0.151) at genotypic and phenotypic levels, respectively. It had non-significant and negative correlation with plant height (r_g= -0.184, r_p= -0.235) at both levels. A significant and positive association of seed yield per plant with number of branches per plant, number of siliqua on main shoot and number of seeds per siliqua was observed by Saiyad *et al.* (2020). Doddabhimppa *et al.* (2009), Akbari and Niranjana (2015) and Pandey *et al.* (2020) reported a significant and positive association of seed yield per plant with number of siliqua per plant. Doddabhimppa *et al.* (2009), Lodhi *et al.* (2014), Tiwari *et al.* (2017) and Prasad and Patil (2018) observed positive association of seed yield per plant with seeds per siliqua. A significant and negative correlation of seed yield per plant with days to flowering was found by Prasad and Patil (2018) and Saiyad *et al.* (2020).

The degree of correlation observes among attributes depends on the relation between them and on segregating genes which contribute to the variation. Some of the genes may increase both the traits causing the positive correlation, while other decrease one and increase other causing negative correlation. Negative correlations arise because of competition among traits for common precursors which are having restricted supply.

The genetic correlation is mainly due to pleiotropy. Also, linkage is a cause of transient correlation in a population derived from crosses between diverse strains. In majority of the cases, the value of genotypic correlations was higher than phenotypic correlations which indicated strong inherent association between various traits at genotypic level. However, in some cases phenotypic correlations were slightly higher than their genotypic correlations due to the influence of non-genetic causes (environmental factors) on the genotypic correlation.

High genetic correlation indicates that the two characters could be substantially same and will produce little difference regards to environment in which selection is carried out (Falconer, 1989). On the basis of present investigation of interrelationship, it is presumed that, for improving grain yield in mustard for undertaken experimental material an ideal plant type would be early maturing, greater number of siliqua per plant, greater number of siliqua on main shoot, more number of branches per plant with a medium to long siliqua, more number of seeds per siliqua and high 1000-seed weight. Hence, these characters should be used as selection criteria for improving seed yield.

4.4 Path coefficient analysis

In the selection programme, correlation study alone can serve the purpose, when smaller numbers of variables are considered. For solving this complexity path analysis (Wright, 1921 and Dewey and Lu, 1959) is used to divide the correlation into indirect and direct effects, so that in selection programme, relative merits of each character are established and their number is reduced.

Seed yield per plant is the result of indirect and direct effects of various yield contributing traits. Path coefficient analysis was carried out by considering seed yield per plant as a dependent character (effect) while ten characters as independent characters (causes). Genotypic correlation coefficient of different traits with seed yield was partitioned into direct and indirect effects, to know the contribution of different traits toward the seed yield. The estimation of indirect and direct effects of several quantitative traits on seed yield given in Table 4.5.

Direct effect of component traits on seed yield provides an idea about reliability on indirect selection to be made through that character for yield increment. In present study, highest positive direct effect on seed yield was noticed by number of branches per plant followed seed per siliqua, days to maturity and 1000 seed weight.

Negative direct effect on seed yield was found with days to flowering followed by plant height, number of siliqua per plant, siliqua length and oil content. This indicated that, if these characters are selected directly then, it will result in poor selection so indirect causal factors must be considered especially the characters contributing positively.

4.4.1 Days to flowering *vs.* seed yield per plant

A non-significant and negative association was found between days to flowering and seed yield per plant (r_g= -0.102). The direct effect of days to flowering was negative and moderate (-0.219). The indirect effect of this trait on seed yield per plant was positive and high for days to maturity (0.399). While positive and negligible indirect effect *via* number of siliqua per plant (0.095), number of siliqua on main shoot (0.080), seeds per siliqua (0.005) and siliqua length (0.088). Indirect effects *via* number of branches per plant (-0.122), 1000 seed weight (-0.121), oil content (-0.174) and plant height (-0.037) was negative and low. Pal *et al.* (2019) recorded negative and negligible direct effects of this trait on seed yield per plant.

4.4.2 Days to maturity *vs.* Seed yield per plant

The days to maturity had a highly significant and negative correlation with seed yield per plant (r_g= -0.619), however its direct effect was positive and high (0.531). The indirect effects of days to maturity through number of siliqua on main shoot (0.105) and siliqua length (0.196) was positive in direction and low in magnitude, while negative and high indirect effects through number of siliqua per plant (-0.384) number of branches per plant (-0.438) and seeds per siliqua (-0.538). The indirect effect was negative and low in magnitude for days to flowering (-0.165), 1000 seed weight (-0.131) and oil content (-0.124). Singh *et al.* (2003) also found positive and high direct effects of days to maturity on seed yield per plant.

4.4.3 Plant height *vs.* Seed yield per plant

The non-significant and negative association was observed between plant height and seed yield per plant (r_g= -0.184). The direct effect of plant height was negative and negligible (-0.052). The indirect effect of this trait on seed yield per plant was positive and high *via* days to maturity (0.481) and positive and low for siliqua length (0.196). While its indirect effects *via* number of siliqua on main shoot (0.084) was positive and negligible in magnitude while for negative and negligible for 1000 seed weight (-0.039) and oil content (-0.058). The indirect effects were negative and low in magnitude for days to flowering (-0.157), number of siliqua per plant (-0.165) and number of branches per plant (-0.174). Rout *et al.* (2018) found a negative and negligible direct effect of plant height on seed yield per plant as observed in the present study.

4.4.4 Number of branches per plant *vs.* Seed yield per plant

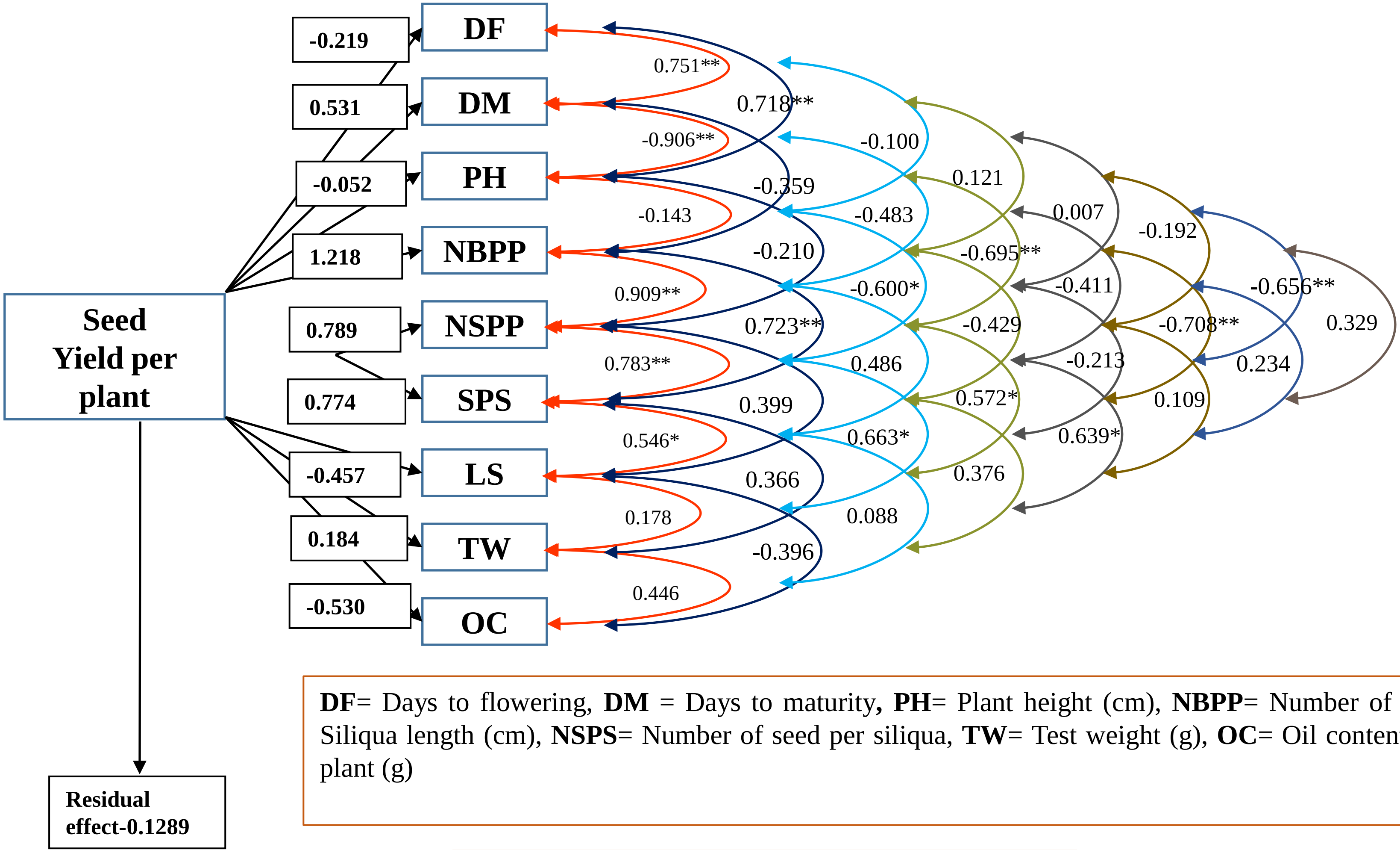
The number of branches per plant had a highly significant and positive correlation with seed yield per plant (r_g= 0.966), however its direct effect was positive and very high (1.218). The indirect effect of this trait on seed yield per plant was positive and high in magnitude for number of seed per plant (0.718), seeds per siliqua (0.560) and positive with low indirect effect for 1000 seed weight, while days to flowering (0.022) and plant height (0.007) were positive and negligible in magnitude. The negative and high indirect effect for oil content (-0.339) while negative and moderate

Table 4.5: Direct (bold) and indirect effects of different quantitative characters on seed yield in Indian mustard (Residual effect = -0.1289)

Characters	DF	DM	PH	NBPP	NSPP	NSMS	SPS	LS	TW	OC	r_g with Y
DF	**-0.219**	0.399	-0.037	-0.122	0.095	0.080	0.005	0.088	-0.121	-0.174	-0.102
DM	-0.165	**0.531**	-0.047	-0.438	-0.384	0.105	-0.538	0.188	-0.131	-0.124	-0.619**
PH	-0.157	0.481	**-0.052**	-0.174	-0.165	0.084	-0.465	0.196	-0.039	-0.058	-0.184
NBPP	0.022	-0.191	0.007	**1.218**	0.718	-0.196	0.560	-0.222	0.106	-0.339	0.966**
NSPP	0.010	-0.212	0.077	0.525	**0.789**	0.123	-0.016	-0.107	0.119	-0.183	0.989**
NSMS	0.081	-0.256	0.020	1.098	0.662	**-0.217**	0.585	-0.178	0.094	-0.249	0.978**
SPS	-0.002	-0.369	0.031	0.880	0.617	-0.164	**0.774**	-0.250	0.068	-0.047	0.923**
LS	0.042	-0.218	0.022	0.592	0.315	-0.085	0.423	**-0.457**	0.033	0.210	0.563**
TW	0.144	-0.376	0.011	0.697	0.499	-0.111	0.284	-0.081	**0.184**	-0.013	0.739**
OC	-0.072	0.124	-0.006	0.778	0.297	-0.102	0.068	0.181	0.004	**-0.530**	0.446

*, ** Significant at 5 and 1 per cent level of significance, respectively.

DF= Days to flowering, **DM** = Days to maturity**, PH**= Plant height (cm), **NBPP**= Number of branches per plant, **NSPP**= Number of siliqua per plant, **NSMS**= Number of siliqua main shoot, **SL**= Siliqua length (cm), **SPS**= Number of seed per siliqua, **TW**= Test weight (g), **OC**= Oil content, **r_g with Y**= Genetic correlation with seed yield per plant (g)



DF= Days to flowering, **DM** = Days to maturity**,** **PH**= Plant height (cm), **NBPP**= Number of branches per plant, **SL**= Siliqua length (cm), **NSPS**= Number of seed per siliqua, **TW**= Test weight (g), **OC**= Oil content, **SYPP**= Seed yield per plant (g)

Fig. 4: Genotypic path diagram in Indian mustard

indirect effect for siliqua length (-0.222). The negative and low indirect effects for days to maturity (-0.191) and number of siliqua on main shoot (-0.196) on seed yield per plant were observed. Sudan *et al.* (2004), Singh *et al.* (2003), Kardum and Singh (2005), Verma *et al.* (2008), Joshi *et al.* (2009) and Singh and Singh (2010) also found positive and high direct effects of number of branches per plant on seed yield per plant.

4.4.5 Number of siliqua per plant *vs.* Seed yield per plant

The highly significant and positive association was found between number of siliqua per plant and seed yield per plant (r_g= 0.989). The direct effect of number of siliqua per plant was positive and high (0.789). The positive and high indirect effect was observed *via* number of branches per plant (0.525). While it's indirect effects for number of siliqua on main shoot (0.123) and 1000 seed weight (0.119) was positive and low in magnitude. The indirect effect of this trait on seed yield per plant was positive and negligible *via* days to flowering (0.010) and plant height (0.077). While its indirect effects for days to maturity (-0.212) and oil content (-0.183) was negative and moderate in magnitude. Kardum and Singh (2005) and Shekhar *et al.* (2012) also found positive and high direct effects of number of siliqua per plant on seed yield per plant.

4.4.6 Number of siliqua on main shoot *vs.* Seed yield per plant

The highly significant and positive association was found between number of siliqua on main shoot and seed yield per plant (r_g= 0.978). The direct effect of number of siliqua on main shoot was negative and moderate (-0.217). The positive and high indirect effects were observed for number of branches per plant (1.098), number of siliqua per plant (0.662) and seeds per siliqua (0.585). The indirect effect of this trait on seed yield per plant was positive and negligible *via* days to flowering (0.081), plant height (0.020) and 1000 seed weight (0.094). While its indirect effects for days to maturity (-0.256) and oil content (-0.249) was negative and moderate in magnitude. Kardum and Singh (2005) and Shekhar *et al.* (2012) also found positive and high direct effects of number of siliqua on main shoot on seed yield per plant.

4.4.7 Seeds per siliqua *vs.* Seed yield per plant

The number of seeds per siliqua had a highly significant and positive correlation with seed yield per plant (r_g= 0.923), however its direct effect was positive and high (0.774). The indirect effects of number of seeds per siliqua through number of branches per plant (0.880) and number of siliqua per plant (0.617) were positive and high. The indirect effects of plant height (0.031) and 1000 seed weight (0.068) recorded positive and negligible. Days to maturity (-0.369) was negative in direction and high also siliqua length (-0.250) recorded negative and moderate in magnitude. Number of siliqua on main shoot (-0.164) recorded negative and low in magnitude, while negative and negligible indirect effects was observed through days to flowering (-0.002) and oil content (-0.047) on seed yield per plant were observed. Sudan *et al.* (2004), Singh and Singh (2004), Acharya (2006), Singh and Singh (2010) and Pandey *et al.* (2020) also found positive and high direct effects of seed per siliqua on seed yield per plant.

4.4.8 Length of siliqua *vs.* Seed yield per plant

The significant and positive association was observed between length of siliqua and seed yield per plant (r_g= 0.563). The direct effect of length of siliqua was negative and high (-0.457). The indirect effect of this trait on seed yield per plant was positive and high for number of branches per plant (0.592), seeds per siliqua (0.423) and number of siliqua per plant (0.315). The indirect effect through oil content (0.210) was positive and moderate, whereas days to flowering (0.042), plant height (0.022) and 1000 seed weight (0.033) was positive and negligible. Negative and moderate indirect effect recorded in days to maturity (-0.218) and number of siliqua on main shoot (-0.085) was negative and negligible in magnitude. Pal *at el.* (2019) also observed negative and high direct effect of length of siliqua on seed yield per plant.

4.4.9 1000 seed weight *vs.* Seed yield per plant

The 1000 seed weight had a highly significant and positive correlation with seed yield per plant (r_g= 0.739), however its direct effect was positive and low (0.184). The indirect effects of 1000 seed weight through number of branches per plant (0.697) and number of siliqua per plant (0.499) were positive and high in magnitude, seeds per siliqua (0.284) was positive and moderate in magnitude, days to flowering (0.144) was positive and low in magnitude and plant height (0.011) was positive and negligible. While negative and high indirect effect was recorded in days to maturity (-0.376), negative and low indirect effect was observed through number of siliqua on main shoot (-0.111), whereas siliqua length (-0.081) and oil content (-0.013) recorded negative and negligible in magnitude indirect effect on seed yield per plant. Bind *et al.* (2014), Ompal *et al.* (2018) and Pal *et al.* (2019) reported similar positive and low direct effect of 1000 seed weight on seed yield per plant.

4.4.10 Oil content *vs.* seed yield per plant

The genotypic correlation between oil content and seed yield per plant was positive and non-significant (rg = 0.446). Its direct effect on seed yield per plant was negative and high (-0.530). The positive indirect effect of this trait on seed yield per plant *via* number of branches per plant (0.778) and number of siliqua per plant (0.297) were high, while days to maturity (0.124) and siliqua length (0.181) were moderate and *via* number of seed per siliqua (0.068) and 1000 seed weight (0.004) were negligible. The negative indirect effect of this trait on seed yield per plant *via* days to flowering (-0.072) and plant height (-0.006) were negligible, while this effects through number of siliqua on main shoot (-0.102). Gupta *et al.* (2018) reported that oil content had negative direct effect on yield per plant.

Direct effect of any component characters on seed yield per plant gave an idea about reliability of indirect selection to be made through that character to bring the improvement in yield. If both the correlation coefficient and the direct effect were high and positive then correlation explained its true relationship and a selection for that character would be effective. If the correlation coefficient was positive but the direct effect was negative or negligible, in such relations the indirect causal factors are to be considered simultaneously for selection. When correlation coefficient was negative but the direct effect was positive and high in such cases direct selection for such traits would be practiced to reduce the undesirable direct effect.

An important consideration to formulate the path diagram is that all the important causal factors affecting the seed yield should be included. Being a complex trait, seed yield per plant gets affected by so many factors. So, it was not be feasible to include all the traits. The residual effect decides how best the causal factors accounts to the variability in seed yield.

While combined result of path analysis and correlation coefficient revealed that characters with positive correlation coefficient and positive direct effect should be selected, so that correlation explains its true relationship and a selection for these characters (number of branches per plant, number of siliqua per plant, number of seeds per siliqua, 1000 seed weight) will become effective. While days to maturity showed negative correlation coefficient, but the direct effect is positive so direct selection should be practiced for this trait to reduce the undesirable indirect effect.

4.5 Molecular diversity

In this experiment, twenty-one Indian mustard genotypes were subjected to amplification by SSR (Simple sequence repeat) marker to study molecular diversity.

4.5.1 Isolation of genomic DNA

The genomic DNA from twenty-one Indian mustard genotypes were isolated by using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method suggested by Doyle and Doyle, (1987) with modifications.

4.5.2 Checking the purity of extracted genomic DNA by agarose gel electrophoresis

Purity off extracted genomic DNA was evaluated by agarose gel electrophoresis using 0.8 per cent agarose gel as explained by Primrose and Twyman (2013). Result showed intact band of genomic DNA indicating good quality of DNA (Plate).

4.5.3 Quantification of genomic DNA

The pre-requisite step before amplification in PCR is to know the quality and quantity of extracted genomic DNA, which was done by the Nanodrop 2000. The quality of genomic DNA was represented as O.D. (A_{260}/A_{280}) while quantity was represented as concentration in ng/µl in Table 4.6.

All the samples showed A_{260}/A_{280} ratio between 1.62 to 1.85 and average ratio was 1.76. The concentrations of genomic DNA of all samples were ranged from 58.5 to 1803.6 ng/µl with average of 658.3 ng/µl.

4.5.4 Amplification by SSR primers

Total 30 SSR primers were used for screening of twenty-one Indian mustard genotypes for assessment of molecular diversity. Out of 30 SSR primers, six primers pairs were found to be polymorphic; 14 primers found monomorphic while rest of primers not amplified properly among the Indian mustard genotypes. The result obtained using six polymorphic primers out of total 30 have been presented in Table 4.7. The SSR amplification profiles obtained in present study with primers are shown in plates.

4.5.4.1 Pooled analysis of SSR amplification

Six polymorphic primers amplified a total 12 reproducible DNA fragments with an average 2 bands per primer. The results were in accordance with Prajapati *et al.* (2014), Kumar *et al.* (2020) and Rajpoot *et al.* (2020). The PIC value of SSR primers were 0.50 (BR_A01, BRMS-08, SSR Na10-D09, SR- 7223, SR – 94102 BG48). The results are in accordance with Pratap *et al.* (2014) got PIC 0.50.

This PIC range was lower than Kumar *et al.* (2020) with an average 0.51. The size of PCR amplified DNA fragment ranged from 94 bp to 720 bp. The results were in accordance with Kumar *et al.* (2020).

Table 4.6: Quantification of genomic DNA extracted from Indian mustard genotypes

Sr. No.	Genotypes	DNA concentration (ng/µl)	Optical Density (OD) A_{260}/A_{280}
1.	GM*Mo*2118A	836.9	1.79
2	GM*Mo*2124A	1181.2	1.67
3	GM*Mo*2125A	63.8	1.68
4	GM*Mo*2118B	672.3	1.70
5	GM*Mo*2124B	83.6	1.80
6	GM*Mo*2125B	158.8	1.85
7	GM*Mo*2101R	144.8	1.85
8	GM*Mo*2105R	285.6	1.70
9	GM*Mo*2113R	86.5	1.77
10	GM*Mo*2115R	840.6	1.83
11	GM*Mo*2116R	683.4	1.81
12	GM*Mo*2119R	886.9	1.76
13	GM*Mo*2120R	58.9	1.62
14	GM*Mo*2122R	58.5	1.82
15	GM*Mo*2124R	1001.4	1.84
16	GM*Mo*2125R	838.2	1.76
17	GM*Mo*2127R	1803.6	1.81
18	GM*Mo*2128R	686.1	1.76
19	GM*Mo*2130R	513.2	1.63
20	GM*Mo*2132R	1546.3	1.76
21	GM*Mo*2133R	1393.3	1.76

Table 4.7: Particulars of SSR primers analysis in Indian mustard genotypes

Sr. No.	Primer Name	No of amplified bands	Total no. of alleles	Product size (bp)	PIC value	Polymorphism
1	BRMS-01	50	2	94-210	0.50	Yes
2	BRMS-08	46	2	125-720	0.50	Yes
3	SSR Na10-D09	64	2	150-385	0.50	Yes
4	SR- 7223	21	2	424-471	0.50	Yes
5	SR – 94102	21	2	305-400	0.50	Yes
6	BG48	31	2	209-455	0.50	Yes
Total:		233	12	-	-	-
Average:		38.83	2	-	0.50	-

4.5.5 Similarity Index and Clustering pattern

Based on SSR data, cluster analysis was performed using genetic similarity value (Table 4.8 and 4.9) and dendrogram (Fig. 5) was generated showing genetic relationships among different Indian mustard genotypes. Similarity index values among Indian mustard genotypes ranged from 0.22 (between GM*Mo*2128R and GM*Mo*2124A) to 0.94 (between GM*Mo*2133R and GM*Mo*2132R), which indicates considerable amount of variation among all the genotypes. The maximum similarity index value of 0.94 was observed between GM*Mo*2133R andGM*Mo*2132R followed by 0.90 between GM*Mo*2125A and GM*Mo*2124A, GM*Mo*2115R and GM*Mo*2101R, GM*Mo*2113R and GM*Mo*2105R. Similarity indices 1.00 indicated most similar genotypes while, GM*Mo*2128R found most diverse genotypes. The results were confirmed by Kumar *et al.* (2020).

The dendrogram was prepared based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) by using Jaccard's similarity coefficient with the help of NTSYS-pc version 2.20 software.

Result of dendrogram indicated formation of two clusters *i.e.,* Clusters A and B. Cluster A and B having 65 per cent similarity. Cluster A was further divided into A1 and A2 sub-clusters. Fifteen genotypes were grouped in one major cluster A. Sub-cluster A1 included GM*Mo*2118A, GM*Mo*2124A, GM*Mo*2125A, GM*Mo*2118B, GM*Mo*2124B and GM*Mo*2125B. Sub-cluster A2 comprises of genotypes GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2122R and GM*Mo*2124R. Cluster B also subdivided into B1 and B2 sub-clusters. Six genotypes were grouped in one major cluster B. Sub-cluster B1 included GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2130R, GM*Mo*2132R and GM*Mo*2133R. Sub-cluster B2 comprises of only one genotype GM*Mo*2128R. The results were confirmed by Kumar *et al.* (2020).

The conducted study revealed that sub-clusters were clearly observed among Indian mustard genotypes so potential utilization of SSRs for explaining genetic diversity in Indian mustard is justified. It is also noted that some pairs of genotypes had similar genetics *i.e.,* all 'A' and 'B' lines are included in A1 sub-cluster and all 'R' lines are in A2, B1 and B2 sub-clusters. Using present set of markers, clearcut differentiation was observed between 'R' lines and 'A' lines along with 'B' lines. Less differences between 'A' and 'B' lines indicated more molecular markers need

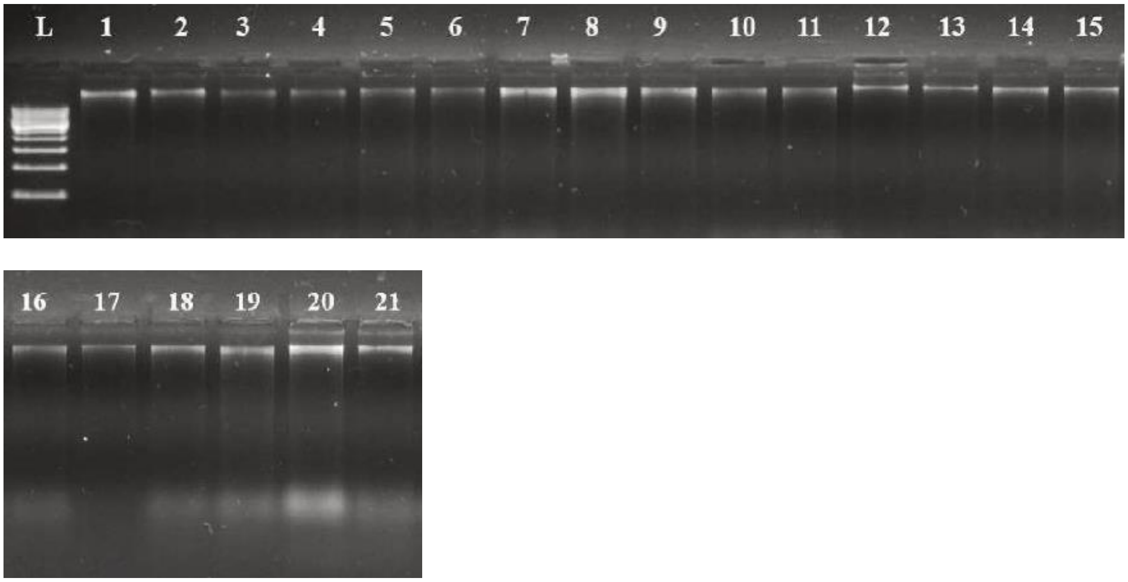


Plate VII: Separation of genomic DNA of 21 genotypes of Indian mustard on 0.8% agarose gel electrophoresis

L: DNA ladder (100 bp)

1. GM*Mo*2118A
2. GM*Mo*2124A
3. GM*Mo*2125A
4. GM*Mo*2118B
5. GM*Mo*2124B
6. GM*Mo*2125B
7. GM*Mo*2101R
8. GM*Mo*2105R
9. GM*Mo*2113R
10. GM*Mo*2115R
11. GM*Mo*2116R
12. GM*Mo*2119R
13. GM*Mo*2120R
14. GM*Mo*2122R
15. GM*Mo*2124R
16. GM*Mo*2125R
17. GM*Mo*2127R
18. GM*Mo*2128R
19. GM*Mo*2130R
20. GM*Mo*2132R
21. GM*Mo*2133R

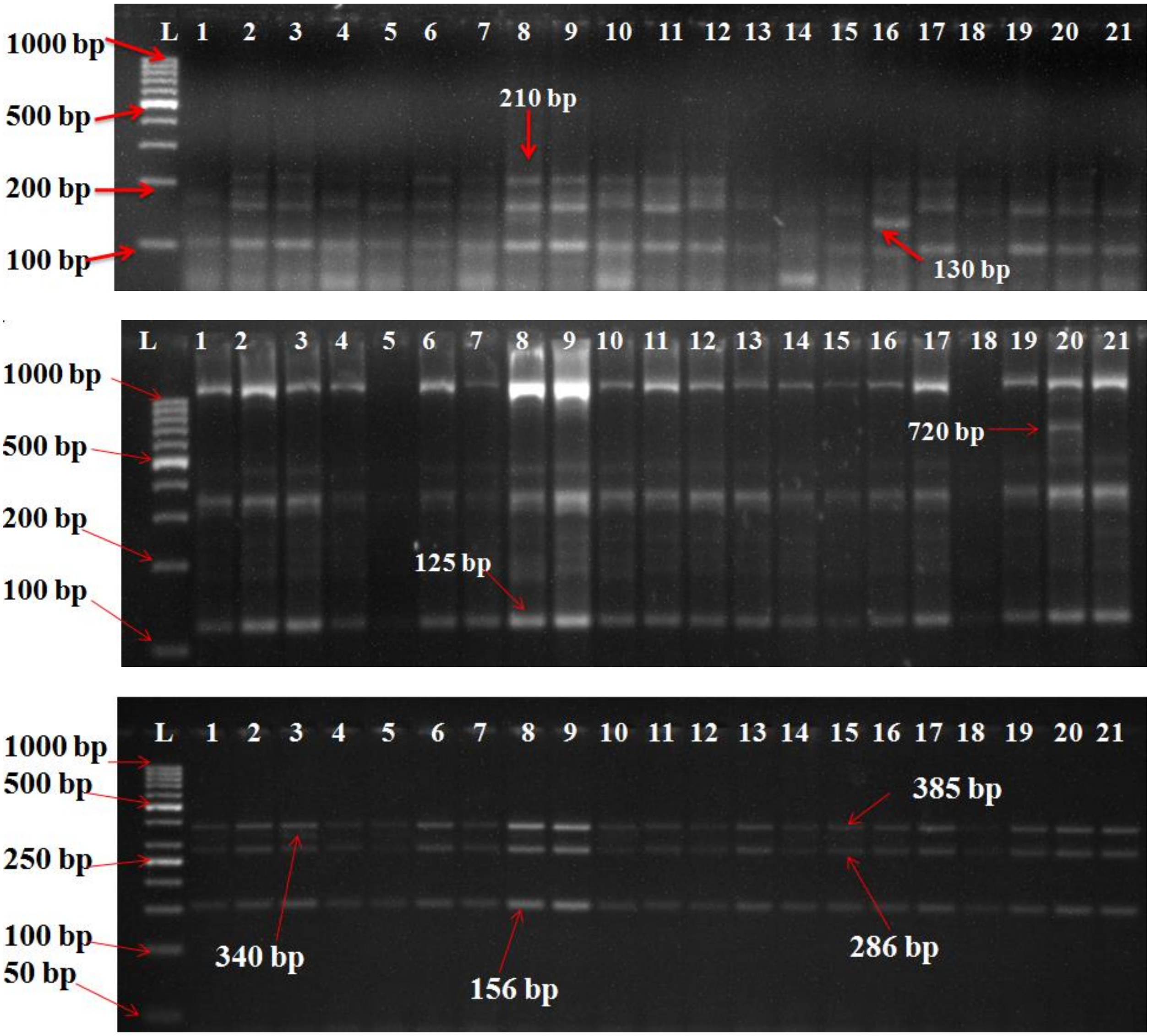


Plate VIII: SSR amplification of 21 genotypes of Indian mustard with primers BRMS- 01, BRMS- 08, SSR Na10- D09, respectively

L: DNA ladder (50 and 100 bp)

1. GM*Mo*2118A
2. GM*Mo*2124A
3. GM*Mo*2125A
4. GM*Mo*2118B
5. GM*Mo*2124B
6. GM*Mo*2125B
7. GM*Mo*2101R
8. GM*Mo*2105R
9. GM*Mo*2113R
10. GM*Mo*2115R
11. GM*Mo*2116R
12. GM*Mo*2119R
13. GM*Mo*2120R
14. GM*Mo*2122R
15. GM*Mo*2124R
16. GM*Mo*2125R
17. GM*Mo*2127R
18. GM*Mo*2128R
19. GM*Mo*2130R
20. GM*Mo*2132R
21. GM*Mo*2133R

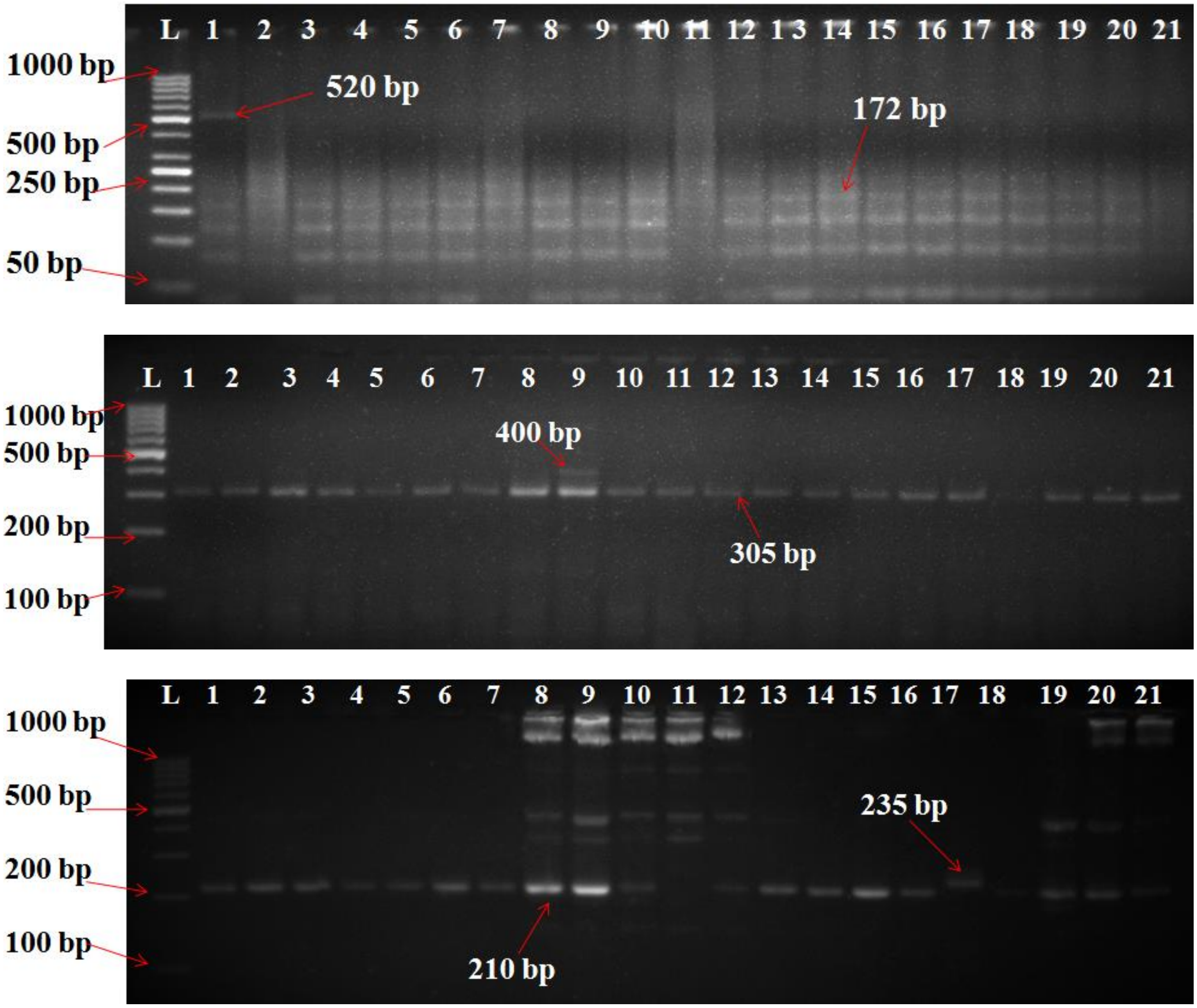


Plate IX: SSR amplification of 21 genotypes of Indian mustard with primers SR- 9222, SR- 94102, BG48, respectively

L: DNA ladder (100 bp)

1. GM*Mo*2118A
2. GM*Mo*2124A
3. GM*Mo*2125A
4. GM*Mo*2118B
5. GM*Mo*2124B
6. GM*Mo*2125B
7. GM*Mo*2101R
8. GM*Mo*2105R
9. GM*Mo*2113R
10. GM*Mo*2115R
11. GM*Mo*2116R
12. GM*Mo*2119R
13. GM*Mo*2120R
14. GM*Mo*2122R
15. GM*Mo*2124R
16. GM*Mo*2125R
17. GM*Mo*2127R
18. GM*Mo*2128R
19. GM*Mo*2130R
20. GM*Mo*2132R
21. GM*Mo*2133R

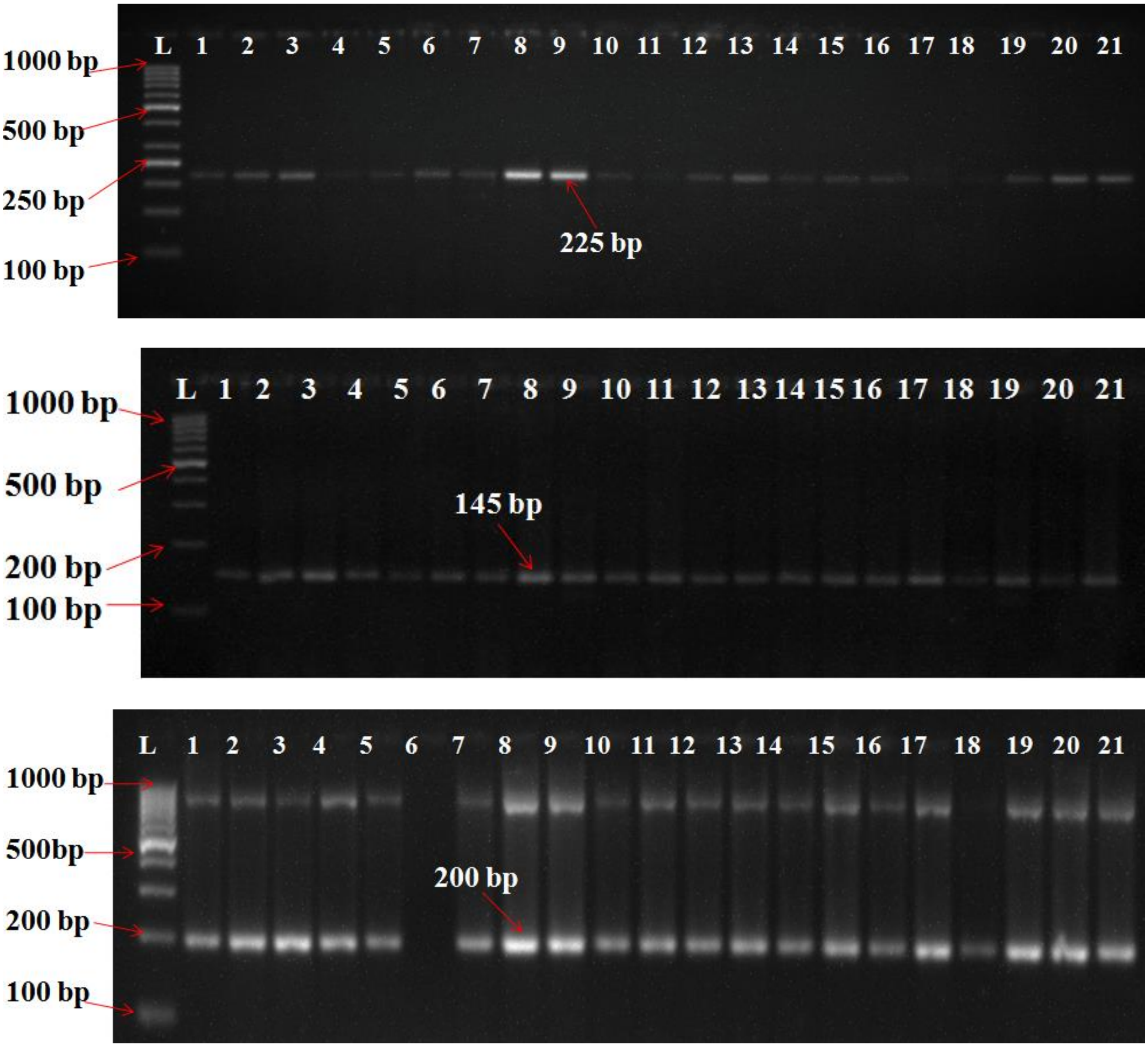


Plate X: SSR amplification of 21 genotypes of Indian mustard with primers BG- 114, BR_A03, BRMS- 01, respectively

L: DNA ladder (100 bp)

1. GM*Mo*2118A
2. GM*Mo*2124A
3. GM*Mo*2125A
4. GM*Mo*2118B
5. GM*Mo*2124B
6. GM*Mo*2125B
7. GM*Mo*2101R
8. GM*Mo*2105R
9. GM*Mo*2113R
10. GM*Mo*2115R
11. GM*Mo*2116R
12. GM*Mo*2119R
13. GM*Mo*2120R
14. GM*Mo*2122R
15. GM*Mo*2124R
16. GM*Mo*2125R
17. GM*Mo*2127R
18. GM*Mo*2128R
19. GM*Mo*2130R
20. GM*Mo*2132R
21. GM*Mo*2133R

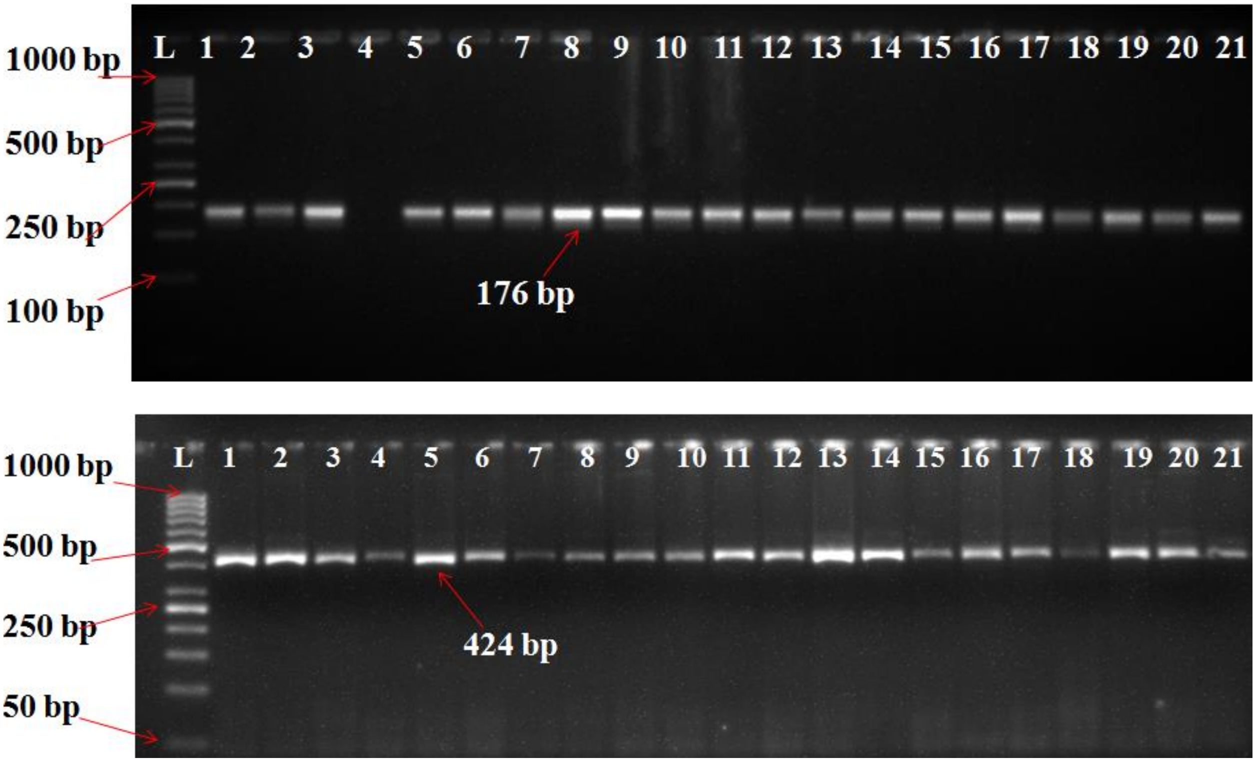


Plate XI: SSR amplification of 21 genotypes of Indian mustard with primers BRMS- 324, SR- 7223, respectively

L: DNA ladder (50 and 100 bp)

1. GM*Mo*2118A
2. GM*Mo*2124A
3. GM*Mo*2125A
4. GM*Mo*2118B
5. GM*Mo*2124B
6. GM*Mo*2125B
7. GM*Mo*2101R
8. GM*Mo*2105R
9. GM*Mo*2113R
10. GM*Mo*2115R
11. GM*Mo*2116R
12. GM*Mo*2119R
13. GM*Mo*2120R
14. GM*Mo*2122R
15. GM*Mo*2124R
16. GM*Mo*2125R
17. GM*Mo*2127R
18. GM*Mo*2128R
19. GM*Mo*2130R
20. GM*Mo*2132R
21. GM*Mo*2133R

to be studied. Therefore, for further diversity analysis, the markers used in this study will be a valuable resource together with the markers from other researchers.

Table 4.8: Distribution of genotypes based on the clusters from UPGMA dendrogram from SSR primers

Cluster	**Sub-cluster**		**Genotypes**	**No. of genotypes**
A	**A1**	**A1:1**	GM*Mo*2118A, GM*Mo*2124A, GM*Mo*2125A, GM*Mo*2124B	**4**
		A1:2	GM*Mo*2118B, GM*Mo*2125B	**2**
	A2	**A2:1**	GM*Mo*2101R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2122R, GM*Mo*2124R	**7**
		A2:2	GM*Mo*2105R, GM*Mo*2113R	**2**
B	**B1**	**B1:1**	GM*Mo*2125R, GM*Mo*2127R	**2**
		B1:2	GM*Mo*2130R, GM*Mo*2132R, GM*Mo*2133R	**3**
	B2		GM*Mo*2128R	**1**

Table 4.9: Similarity matrix for Jaccard’s coefficient for twenty-one Indian mustard genotypes based on SSR markers

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
GM*Mo*2118A	1.00																				
GM*Mo*2124A	0.84	1.00																			
GM*Mo*2125A	0.85	0.9	1.00																		
GM*Mo*2118B	0.62	0.67	0.76	1.00																	
GM*Mo*2124B	0.74	0.7	0.8	0.65	1.00																
GM*Mo*2125B	0.65	0.62	0.71	0.74	0.68	1.00															
GMMo2101R	0.59	0.64	0.73	0.75	0.62	0.7	1.00														
GM*Mo*2105R	0.54	0.58	0.67	0.68	0.57	0.64	0.81	1.00													
GM*Mo*2113R	0.48	0.52	0.6	0.61	0.5	0.57	0.73	0.9	1.00												
GM*Mo*2115R	0.68	0.73	0.82	0.76	0.64	0.71	0.9	0.82	0.74	1.00											
GM*Mo*2116R	0.62	0.59	0.68	0.62	0.5	0.65	0.75	0.68	0.61	0.85	1.00										
GM*Mo*2119R	0.65	0.7	0.64	0.57	0.45	0.6	0.7	0.64	0.57	0.8	0.83	1.00									
GM*Mo*2120R	0.59	0.64	0.65	0.59	0.48	0.55	0.71	0.81	0.73	0.81	0.75	0.79	1.00								
GM*Mo*2122R	0.5	0.54	0.63	0.57	0.46	0.52	0.68	0.77	0.7	0.77	0.71	0.67	0.85	1.00							
GM*Mo*2124R	0.52	0.5	0.58	0.52	0.42	0.62	0.64	0.73	0.65	0.73	0.75	0.7	0.8	0.76	1.00						
GM*Mo*2125R	0.35	0.33	0.41	0.4	0.31	0.48	0.5	0.65	0.58	0.52	0.52	0.48	0.57	0.68	0.71	1.00					
GM*Mo*2127R	0.4	0.44	0.52	0.52	0.42	0.55	0.64	0.65	0.58	0.65	0.67	0.55	0.57	0.54	0.64	0.71	1.00				
GM*Mo*2128R	0.29	**0.22**	0.3	0.23	0.37	0.37	0.33	0.43	0.36	0.36	0.42	0.3	0.4	0.38	0.56	0.47	0.47	1.00			
GM*Mo*2130R	0.24	0.23	0.26	0.29	0.25	0.36	0.33	0.48	0.42	0.36	0.35	0.36	0.45	0.38	0.52	0.6	0.52	0.5	1.00		
GM*Mo*2132R	0.26	0.3	0.32	0.31	0.32	0.38	0.4	0.54	0.61	0.42	0.36	0.38	0.52	0.44	0.52	0.59	0.59	0.42	0.72	1.00	
GM*Mo*2133R	0.27	0.26	0.29	0.27	0.28	0.39	0.36	0.5	0.57	0.38	0.38	0.39	0.48	0.4	0.55	0.62	0.55	0.44	0.76	**0.94**	1.00

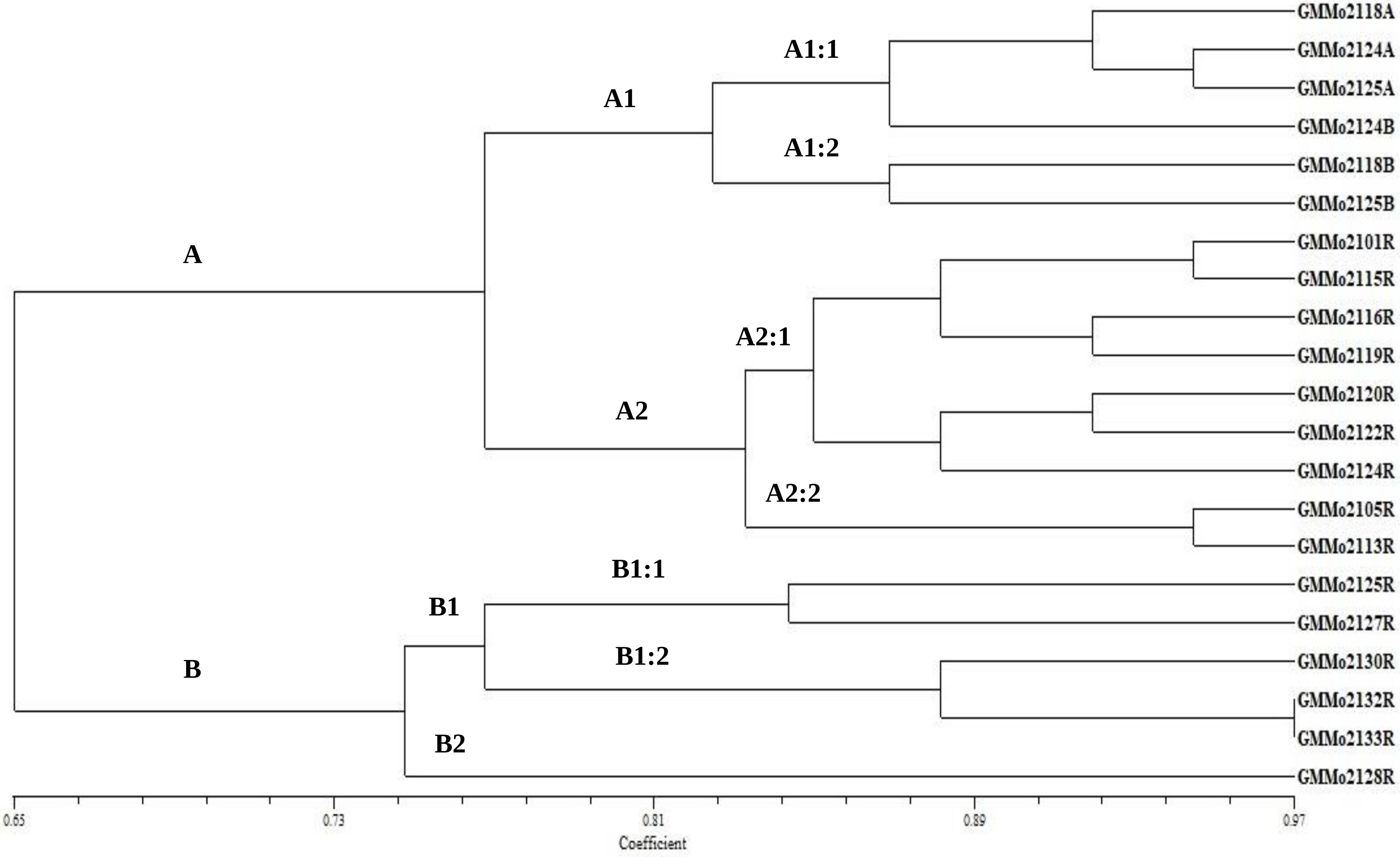


Fig. 5: UPGMA dendrogram of twenty-one genotypes of Indian mustard

4.6 Fertility Restorability

The present study was conducted using three cytoplasmic male sterile (CMS) lines 'A' with three maintainer lines 'B' and fifteen diverse male fertile lines 'R' to find out good fertility restorability to *Moricandia arvensis* cytoplasmic background at Agronomy Instructional Farm, S. D. Agricultural University, Sardarkrushinagar. Forty-five crosses (F_1's) were developed through line × tester mating design using three male sterile lines (GM*Mo*2118A, GM*Mo*2124A, GM*Mo*2125A) and fifteen pollen fertile testers (GM*Mo*2101R, GM*Mo*2105R, GM*Mo*2113R, GM*Mo*2115R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2122R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2128R, GM*Mo*2130R, GM*Mo*2132R, GM*Mo*2133R) for the morphological characterization and to confirm stability of cytoplasmic male sterile (CMS) lines.

This study was carried out by visual observation of freshly opened and fully developed flowers for the presence (male fertile) or absence (male sterile) of the pollen in F_1 hybrids. Pollen grains collected from mature flower bud of five randomly selected plants from each row of F_1 plants were dusted on glass slide, a drop of 2 per cent aceto-carmine stain was added and pollen staining ability examined under light microscope (40X) for authentication of stability in fertility restoration in their crosses (F_1's) as per method proposed by Alexander, 1969. The selfing of five buds of tagged plant of F_1 was done to examine ability or inability of the plant to produce selfed seeds by using butter paper bag for field confirmation.

Visual observation for presence or absence of the fertile pollen by dusting of the mature flower bud open during the morning and recorded the presence or absence of yellow pollen dust. The presence of yellow pollen dust was indicated as fertile pollen and absence of yellow pollen dust as sterile pollen indicating plant fertility status.

The pollen fertility was observed under light microscope and on the basis of its staining properties, pollen grains which were round, deep to light red colour consider as fertile pollen and shriveled, hyaline, transparent or light yellow colour consider as sterile pollen of different parents (Plate XII to XX).

Table 4.10: Pollen fertility status of F_1 hybrids in Indian mustard

Sr. No.	Genotypes	S	F	Total pollen observed	Plant Fertility	Pollen Fertility (%)
1	GM*Mo*2118A x GM*Mo*2101R	3	47	50	F	94.00
2	GM*Mo*2118A x GM*Mo*2105R	3	47	50	F	94.00
3	GM*Mo*2118A x GM*Mo*2113R	2	48	50	F	96.00
4	GM*Mo*2118A x GM*Mo*2115R	3	47	50	F	94.00
5	GM*Mo*2118A x GM*Mo*2116R	3	47	50	F	94.00
6	GM*Mo*2118A x GM*Mo*2119R	2	48	50	F	96.00
7	GM*Mo*2118A x GM*Mo*2120R	3	47	50	F	94.00
8	GM*Mo*2118A x GM*Mo*2122R	4	46	50	F	92.00
9	GM*Mo*2118A x GM*Mo*2124R	4	46	50	F	92.00
10	GM*Mo*2118A x GM*Mo*2125R	1	49	50	F	98.00
11	GM*Mo*2118A x GM*Mo*2127R	1	49	50	F	84.62
12	GM*Mo*2118A x GM*Mo*2128R	4	46	50	F	78.94
13	GM*Mo*2118A x GM*Mo*2130R	2	48	50	F	85.71
14	GM*Mo*2118A x GM*Mo*2132R	2	48	50	F	96.00
15	GM*Mo*2118A x GM*Mo*2133R	2	48	50	F	96.00
16	GM*Mo*2124A x GM*Mo*2101R	3	47	50	F	94.00
17	GM*Mo*2124A x GM*Mo*2105R	4	46	50	F	83.33
18	GM*Mo*2124A x GM*Mo*2113R	3	47	50	F	62.50
19	GM*Mo*2124A x GM*Mo*2115R	3	47	50	F	94.00
20	GM*Mo*2124A x GM*Mo*2116R	2	48	50	F	96.00
21	GM*Mo*2124A x GM*Mo*2119R	1	49	50	F	77.78
22	GM*Mo*2124A x GM*Mo*2120R	3	47	50	F	94.00
23	GM*Mo*2124A x GM*Mo*2122R	4	48	50	F	88.88
24	GM*Mo*2124A x GM*Mo*2124R	4	48	50	F	96.00
25	GM*Mo*2124A x GM*Mo*2125R	3	47	50	F	87.50
26	GM*Mo*2124A x GM*Mo*2127R	2	48	50	F	96.00
27	GM*Mo*2124A x GM*Mo*2128R	5	45	50	F	82.60
28	GM*Mo*2124A x GM*Mo*2130R	3	47	50	F	85.71
29	GM*Mo*2124A x GM*Mo*2132R	4	46	50	F	90.00
30	GM*Mo*2124A x GM*Mo*2133R	3	47	50	F	85.71
31	GM*Mo*2125A x GM*Mo*2101R	3	47	50	F	77.78
32	GM*Mo*2125A x GM*Mo*2105R	4	46	50	F	92.00
33	GM*Mo*2125A x GM*Mo*2113R	2	48	50	F	88.88
34	GM*Mo*2125A x GM*Mo*2115R	3	47	50	F	66.67
35	GM*Mo*2125A x GM*Mo*2116R	2	48	50	F	96.00
36	GM*Mo*2125A x GM*Mo*2119R	4	46	50	F	82.35
37	GM*Mo*2125A x GM*Mo*2120R	2	48	50	F	96.00
38	GM*Mo*2125A x GM*Mo*2122R	3	47	50	F	90.00
39	GM*Mo*2125A x GM*Mo*2124R	1	49	50	F	98.00

Table 4.10 Continue

40	GM*Mo*2125A x GM*Mo*2125R	3	47	50	F	94.00
41	GM*Mo*2125A x GM*Mo*2127R	4	46	50	F	92.00
42	GM*Mo*2125A x GM*Mo*2128R	4	46	50	F	92.00
43	GM*Mo*2125A x GM*Mo*2130R	3	47	50	F	78.57
44	GM*Mo*2125A x GM*Mo*2132R	2	48	50	F	90.00
45	GM*Mo*2125A x GM*Mo*2133R	2	48	50	F	88.24

The CMS line crossed with restorer line exhibited pollen fertility in crosses GM*Mo*2124A × GM*Mo*2113R showed lower pollen fertility restorability (62.50%) followed by GM*Mo*2125A × GM*Mo*2115R (66.67%). The crosses GM*Mo*2118A × GM*Mo*2125R and GM*Mo*2125A × GM*Mo*2124R (98%) showed higher pollen fertility restorability (Table 4.10). At field level, siliquae set upon selfing resulted GM*Mo*2124A × GM*Mo*2113R showed lower pollen fertility restorability (62.40%) followed by GM*Mo*2125A × GM*Mo*2115R (66.40%). The crosses GM*Mo*2118A × GM*Mo*2125R and GM*Mo*2125A × GM*Mo*2124R (97.60%) showed higher pollen fertility restorability (Table 4.11). Similar results were obtained by Sirvi (2016) and Vyas (2022).

Table 4.11: Number of siliquae set and per cent of siliquae set of F_1 hybrids in Indian mustard

Sr. No.	Genotypes	Total flower bud kept	Total siliquae set	Per cent siliquae set
1	GM*Mo*2118A x GM*Mo*2101R	125	117	93.60
2	GM*Mo*2118A x GM*Mo*2105R	125	117	93.60
3	GM*Mo*2118A x GM*Mo*2113R	125	120	96.00
4	GM*Mo*2118A x GM*Mo*2115R	125	117	93.60
5	GM*Mo*2118A x GM*Mo*2116R	125	117	93.60
6	GM*Mo*2118A x GM*Mo*2119R	125	120	96.00
7	GM*Mo*2118A x GM*Mo*2120R	125	117	93.60
8	GM*Mo*2118A x GM*Mo*2122R	125	115	92.00
9	GM*Mo*2118A x GM*Mo*2124R	125	115	92.00
10	GM*Mo*2118A x GM*Mo*2125R	125	122	97.60
11	GM*Mo*2118A x GM*Mo*2127R	125	105	84.00
12	GM*Mo*2118A x GM*Mo*2128R	125	98	78.40
13	GM*Mo*2118A x GM*Mo*2130R	125	106	84.80
14	GM*Mo*2118A x GM*Mo*2132R	125	120	96.00
15	GM*Mo*2118A x GM*Mo*2133R	125	120	96.00
16	GM*Mo*2124A x GM*Mo*2101R	125	117	93.60
17	GM*Mo*2124A x GM*Mo*2105R	125	104	83.20
18	GM*Mo*2124A x GM*Mo*2113R	125	78	62.40
19	GM*Mo*2124A x GM*Mo*2115R	125	117	93.60

Table 4.11 Continue

20	GM*Mo*2124A x GM*Mo*2116R	125	120	96.00
21	GM*Mo*2124A x GM*Mo*2119R	125	97	77.60
22	GM*Mo*2124A x GM*Mo*2120R	125	117	93.60
23	GM*Mo*2124A x GM*Mo*2122R	125	111	88.88
24	GM*Mo*2124A x GM*Mo*2124R	125	120	96.00
25	GM*Mo*2124A x GM*Mo*2125R	125	109	87.20
26	GM*Mo*2124A x GM*Mo*2127R	125	120	96.00
27	GM*Mo*2124A x GM*Mo*2128R	125	103	82.40
28	GM*Mo*2124A x GM*Mo*2130R	125	107	85.60
29	GM*Mo*2124A x GM*Mo*2132R	125	112	89.60
30	GM*Mo*2124A x GM*Mo*2133R	125	107	85.60
31	GM*Mo*2125A x GM*Mo*2101R	125	96	76.80
32	GM*Mo*2125A x GM*Mo*2105R	125	115	92.00
33	GM*Mo*2125A x GM*Mo*2113R	125	111	88.88
34	GM*Mo*2125A x GM*Mo*2115R	125	83	66.40
35	GM*Mo*2125A x GM*Mo*2116R	125	120	96.00
36	GM*Mo*2125A x GM*Mo*2119R	125	102	81.60
37	GM*Mo*2125A x GM*Mo*2120R	125	120	96.00
38	GM*Mo*2125A x GM*Mo*2122R	125	112	89.60
39	GM*Mo*2125A x GM*Mo*2124R	125	122	97.60
40	GM*Mo*2125A x GM*Mo*2125R	125	117	93.60
41	GM*Mo*2125A x GM*Mo*2127R	125	115	92.00
42	GM*Mo*2125A x GM*Mo*2128R	125	115	92.00
43	GM*Mo*2125A x GM*Mo*2130R	125	97	77.60
44	GM*Mo*2125A x GM*Mo*2132R	125	112	89.60
45	GM*Mo*2125A x GM*Mo*2133R	125	110	88.00

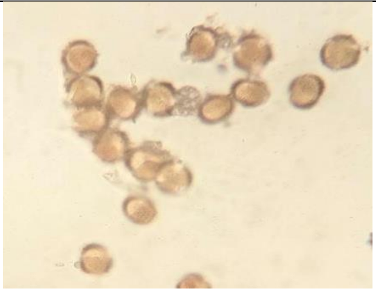
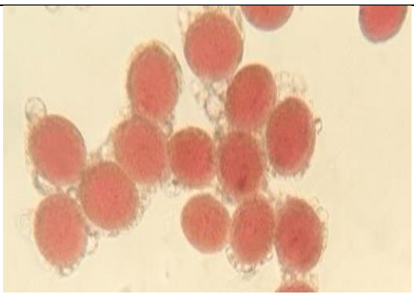
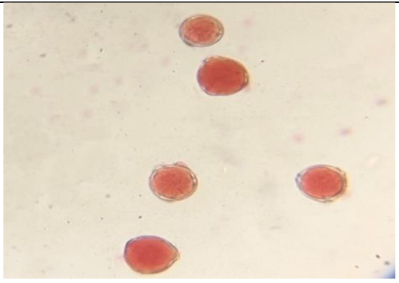
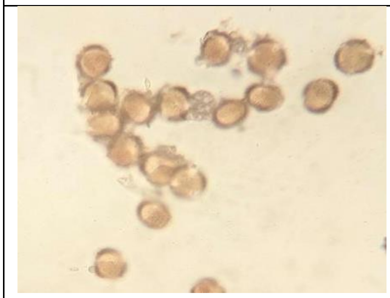
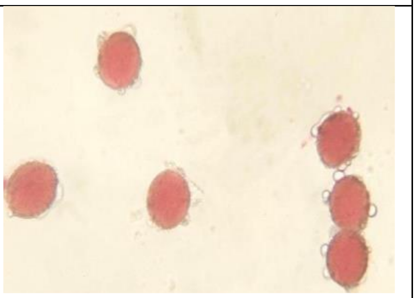
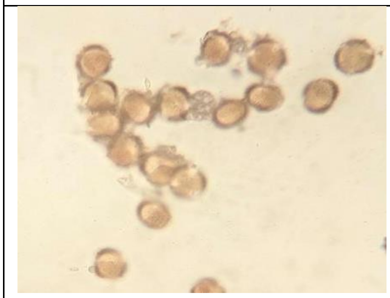
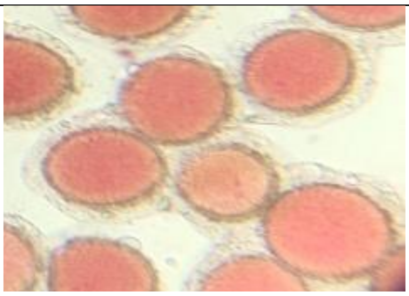
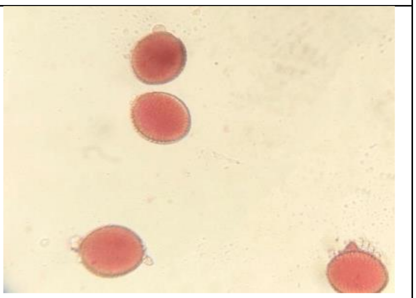
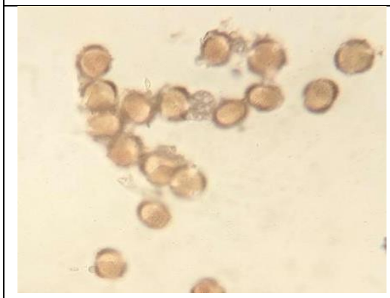
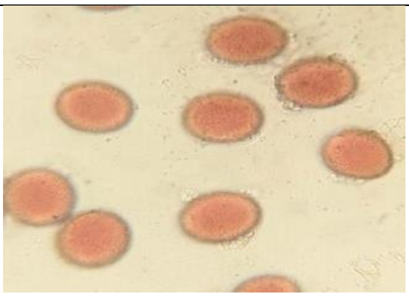
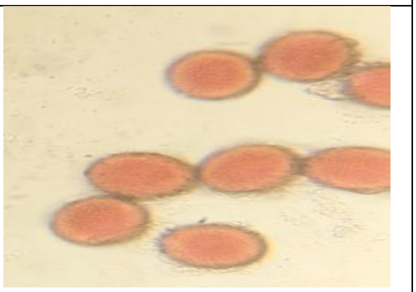
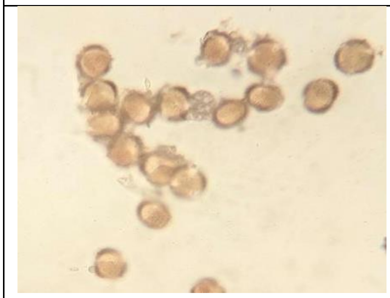
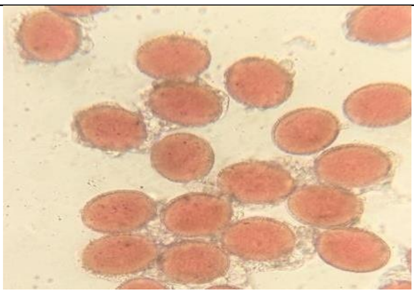
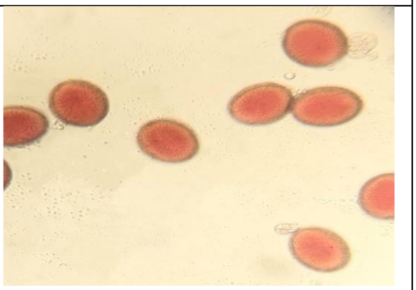
Female	**F_1**	**Male**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2101R**	**GM*Mo*2101R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2105R**	**GM*Mo*2105R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2113R**	**GM*Mo*2113R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2115R**	**GM*Mo*2115R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2116R**	**GM*Mo*2116R**

Plate XII: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

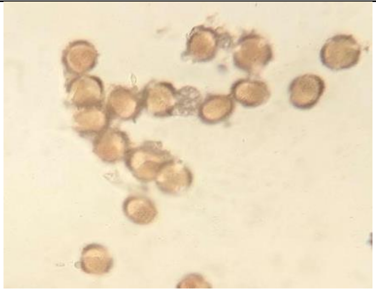
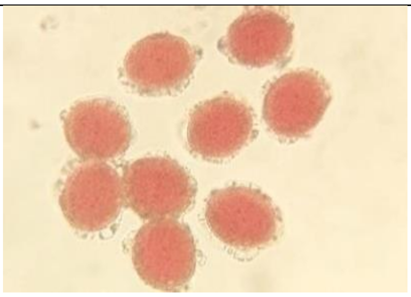
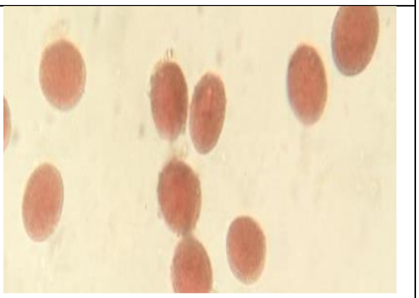
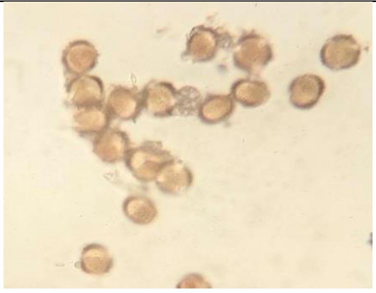
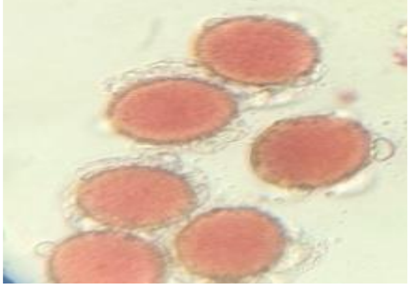
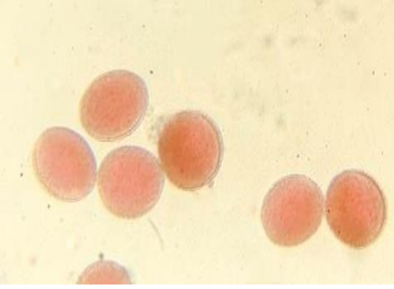
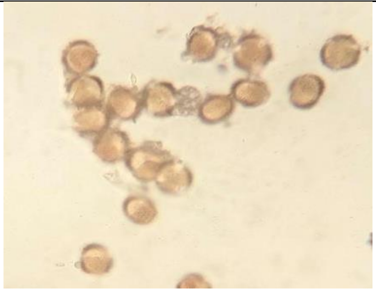
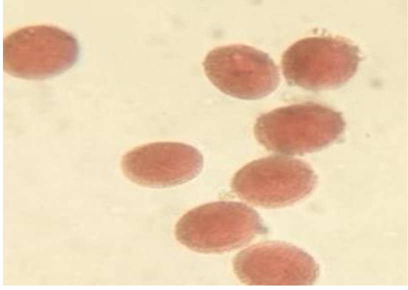
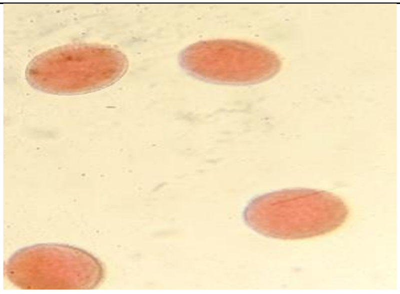
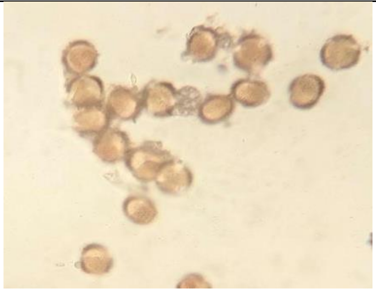
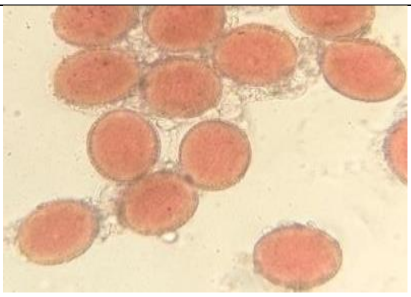
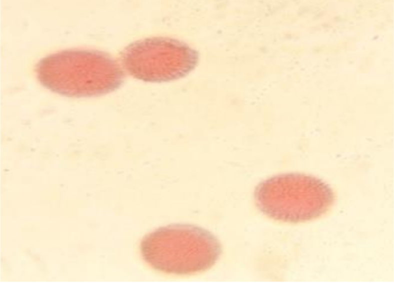
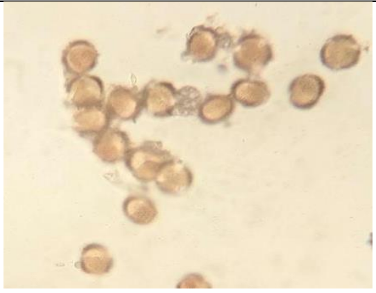
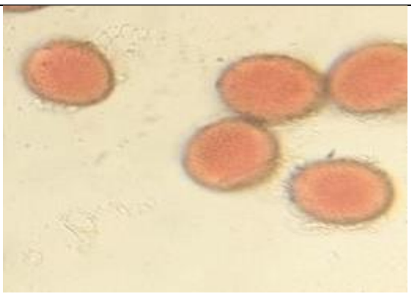
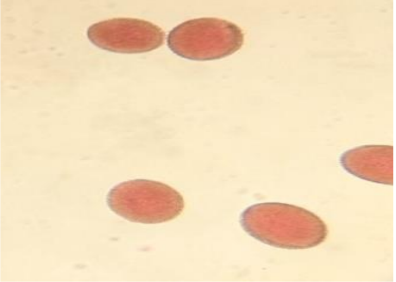
Female	F_1	Male
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2119R**	**GM*Mo*2119R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2120R**	**GM*Mo*2120R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2122R**	**GM*Mo*2122R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2124R**	**GM*Mo*2124R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2125R**	**GM*Mo*2125R**

Plate XIII: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

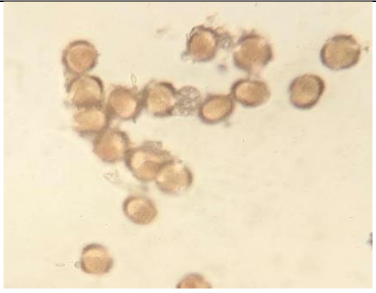
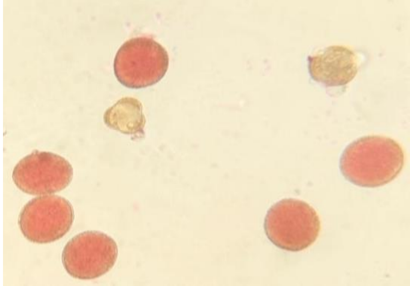
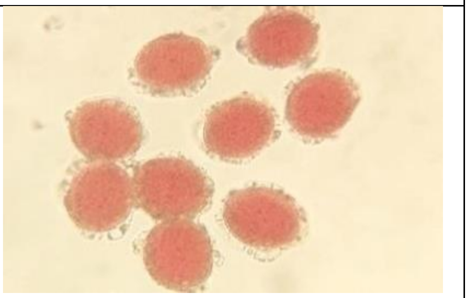
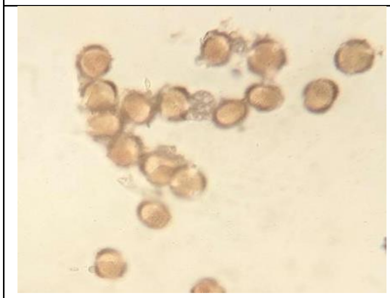
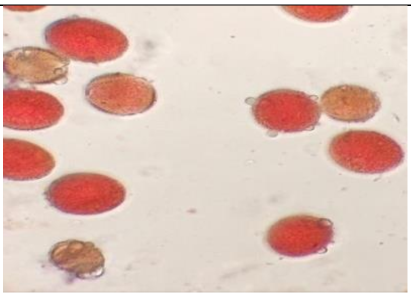
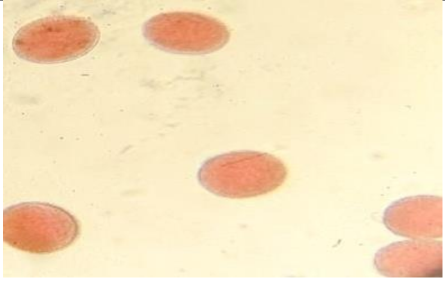
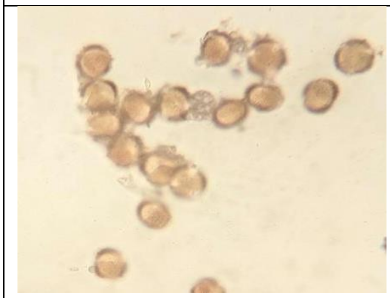
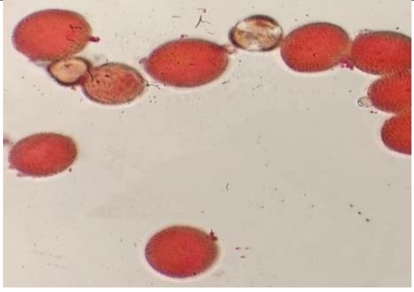
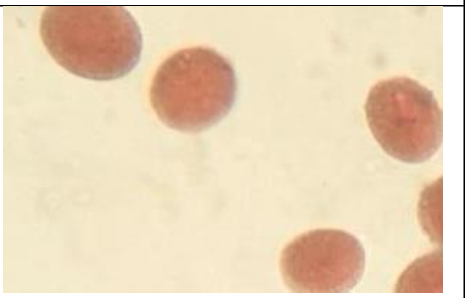
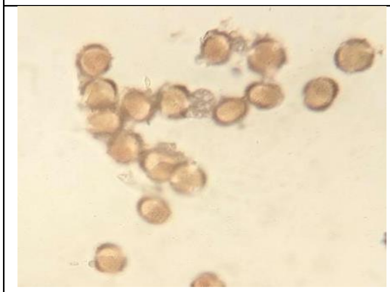
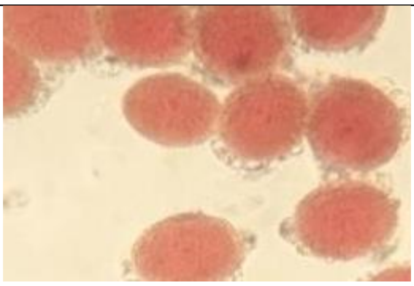
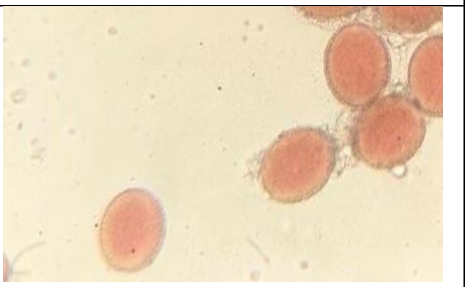
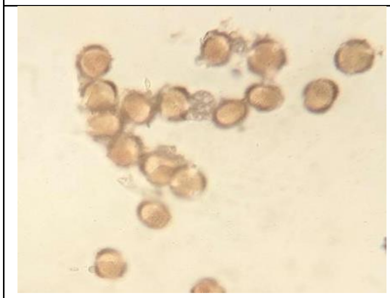
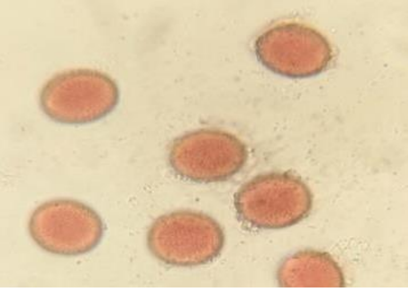
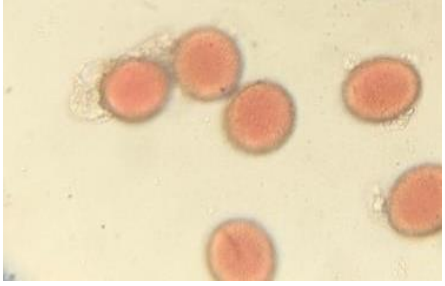
Female	**F_1**	**Male**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2127R**	**GM*Mo*2127R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2128R**	**GM*Mo*2128R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2130R**	**GM*Mo*2130R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2132R**	**GM*Mo*2132R**
		
GM*Mo*2118A	**GM*Mo*2118A × GM*Mo*2133R**	**GM*Mo*2133R**

Plate XIV: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

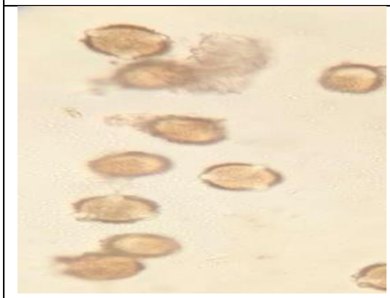
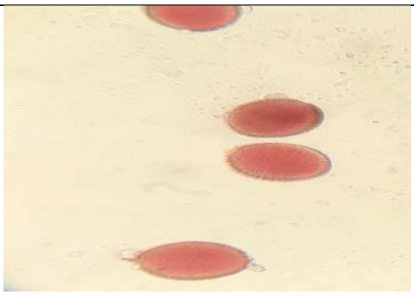
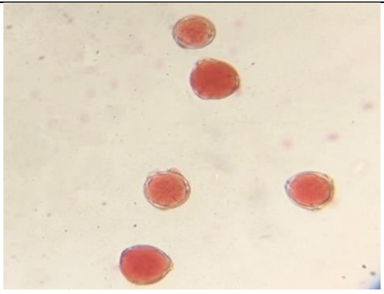
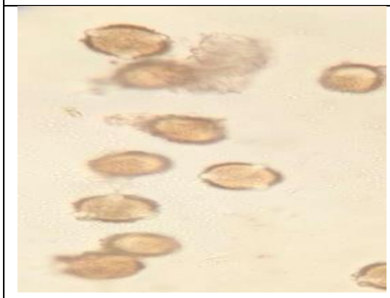
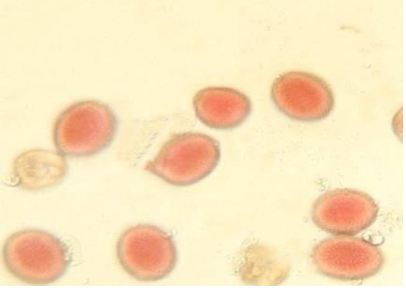
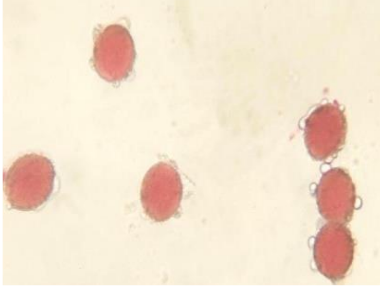
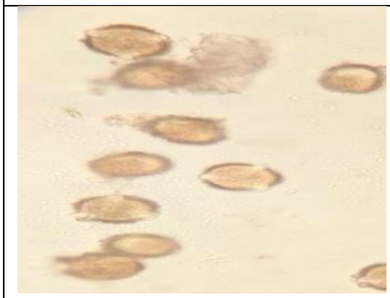
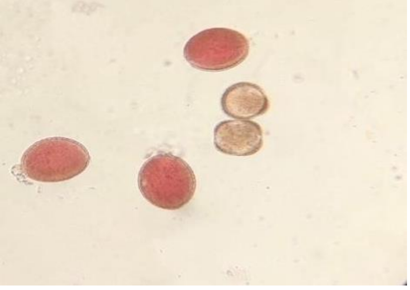
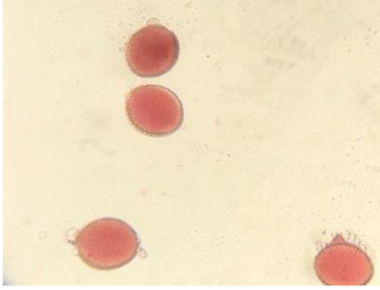
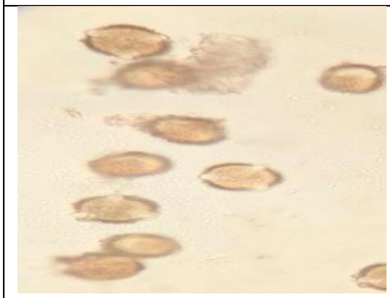
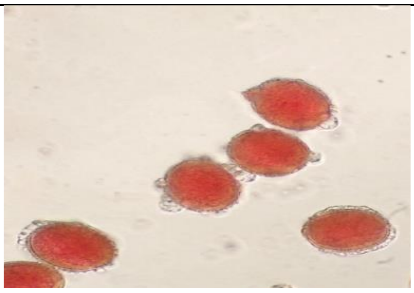
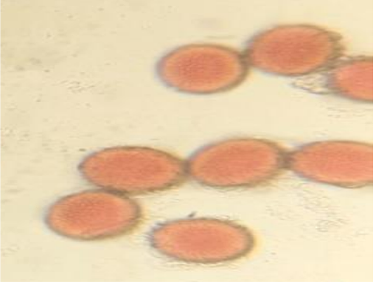
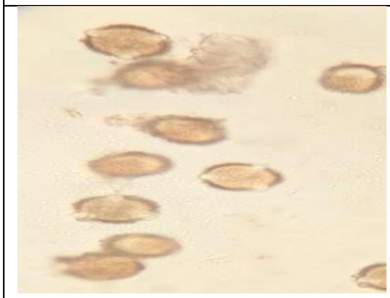
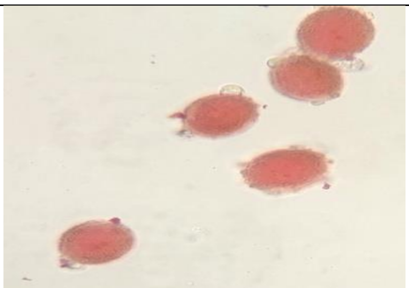
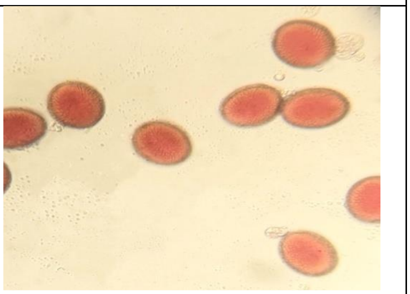
Female	**F$_1$**	**Male**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2101R**	**GM*Mo*2101R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2105R**	**GM*Mo*2105R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2113R**	**GM*Mo*2113R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2115R**	**GM*Mo*2115R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2116R**	**GM*Mo*2116R**

Plate XV: Unstained and stained pollen grains of sterile lines, restorer lines and F$_1$ crosses

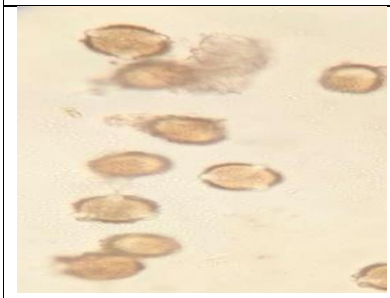
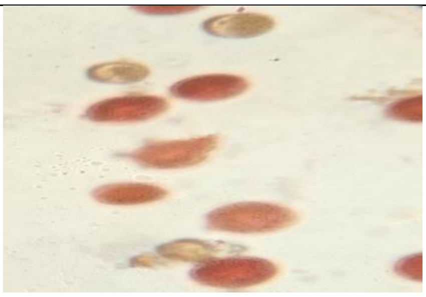
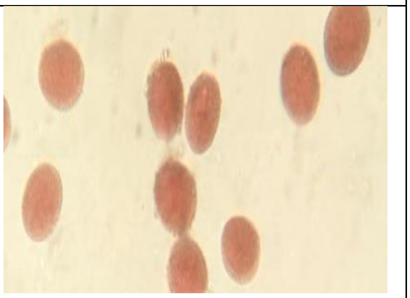
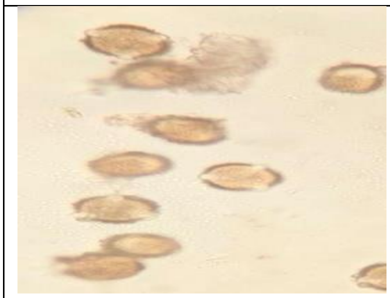
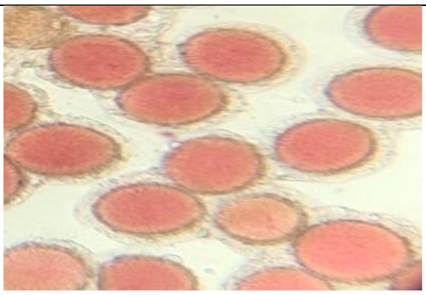
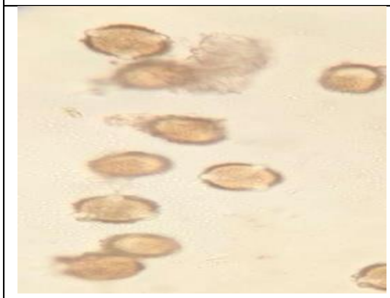
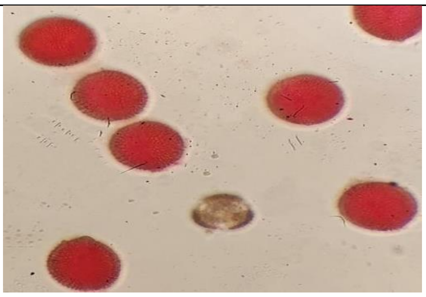
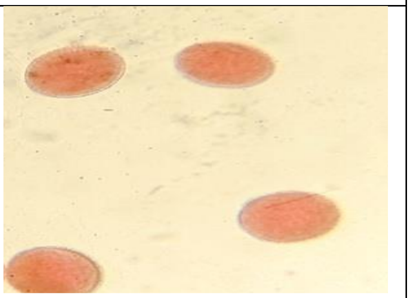
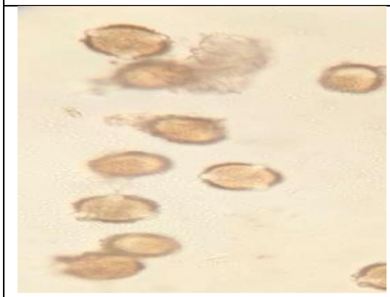
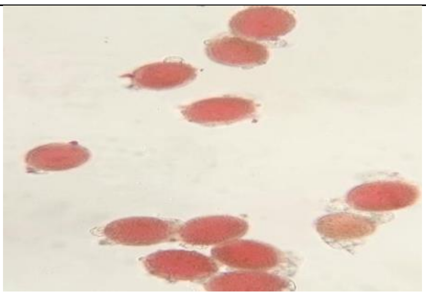
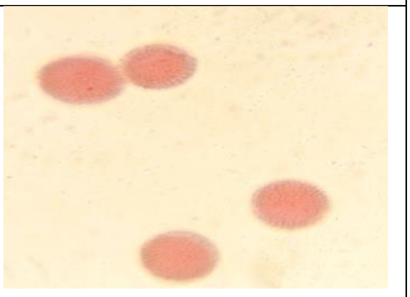
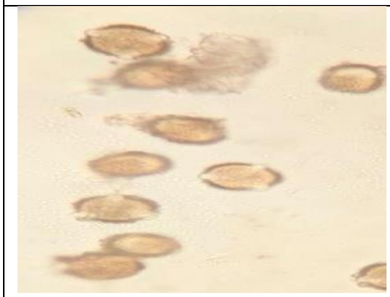
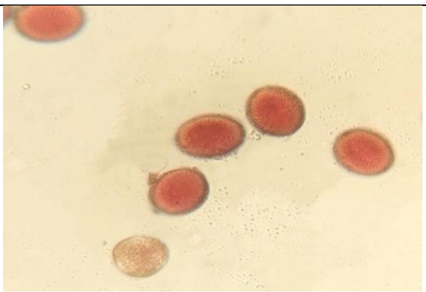
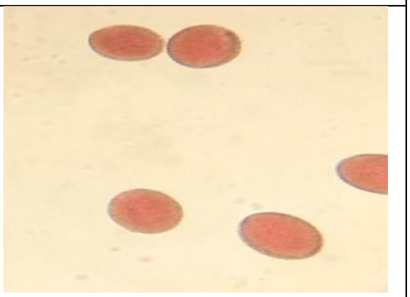
Female	**F_1**	**Male**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2119R**	**GM*Mo*2119R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2120R**	**GM*Mo*2120R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2122R**	**GM*Mo*2122R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2124R**	**GM*Mo*2124R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2125R**	**GM*Mo*2125R**

Plate XVI: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

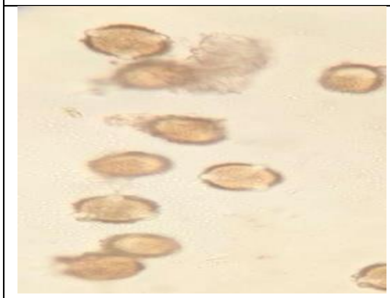
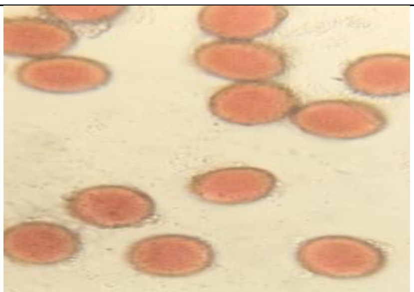
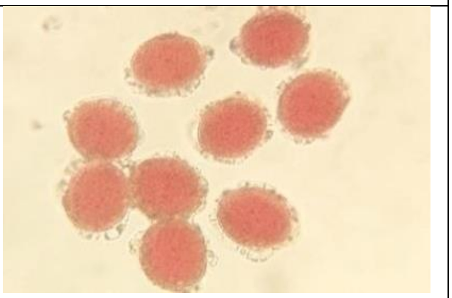
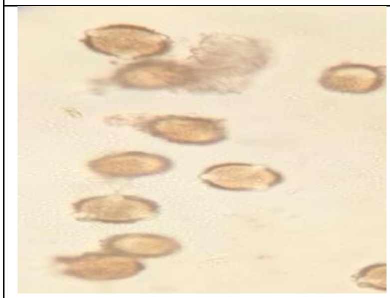
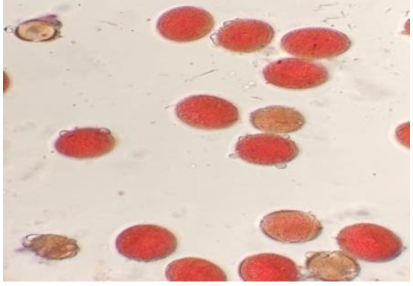
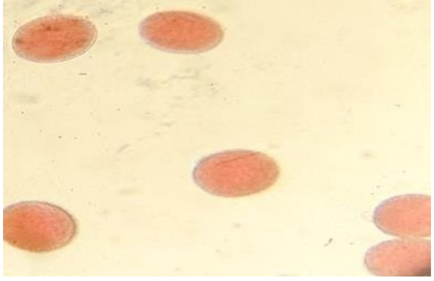
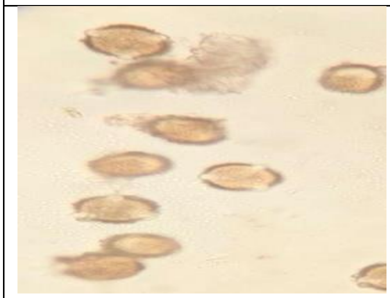
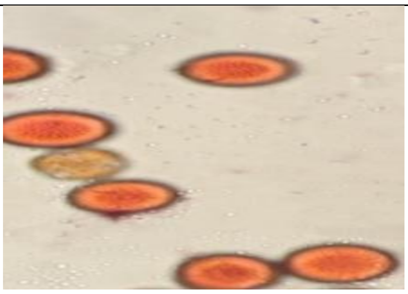
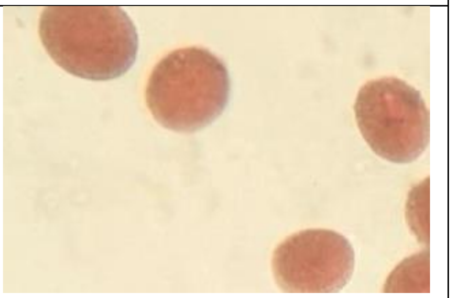
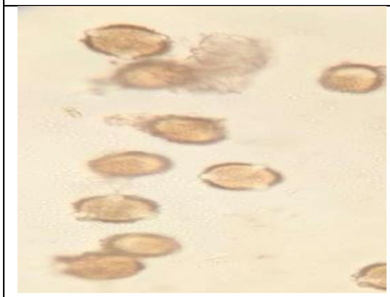
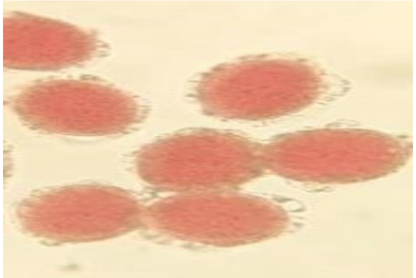
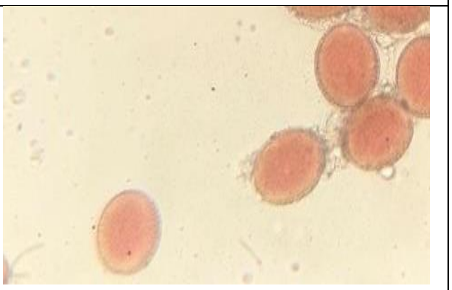
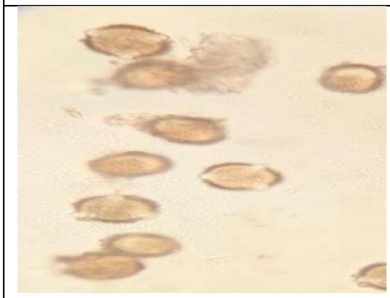
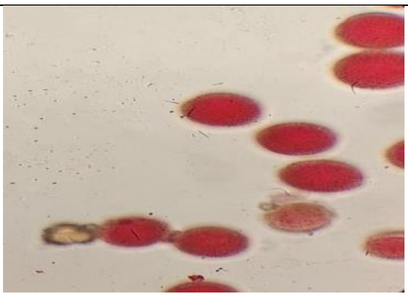
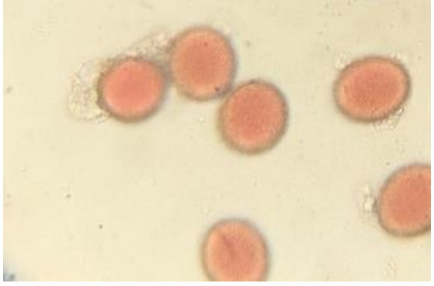
Female	**F_1**	**Male**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2127R**	**GM*Mo*2127R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2128R**	**GM*Mo*2128R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2130R**	**GM*Mo*2130R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2132R**	**GM*Mo*2132R**
		
GM*Mo*2124A	**GM*Mo*2124A × GM*Mo*2133R**	**GM*Mo*2133R**

Plate XVII: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

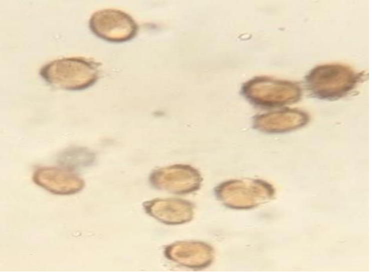
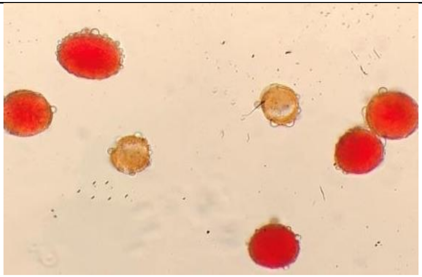
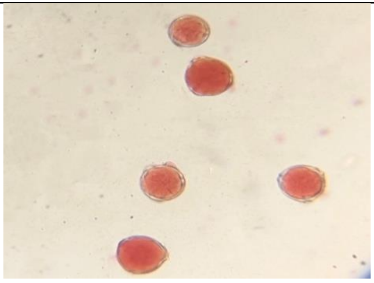
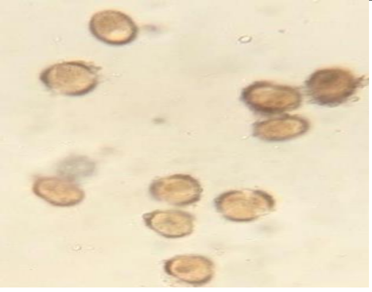
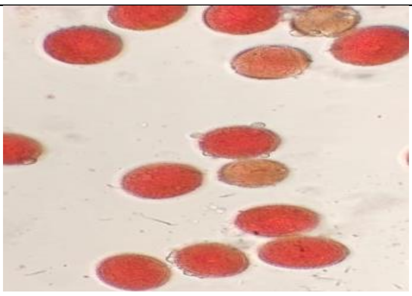
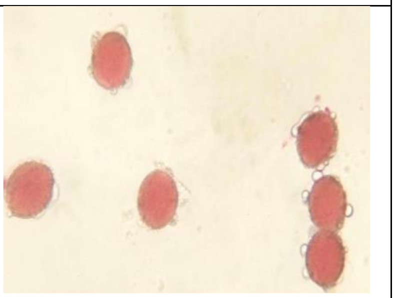
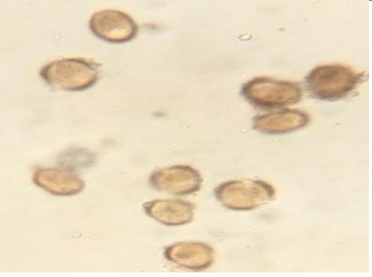
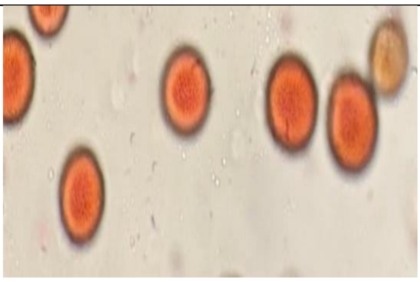
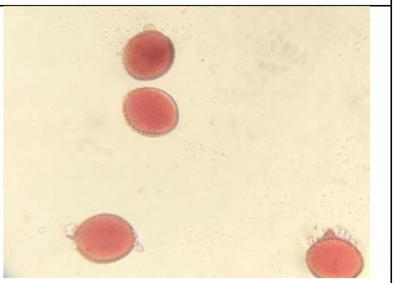
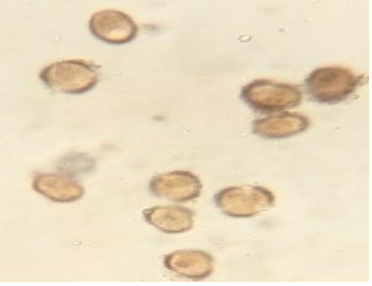
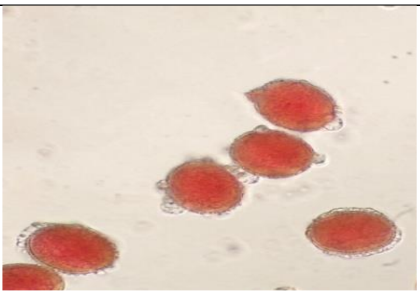
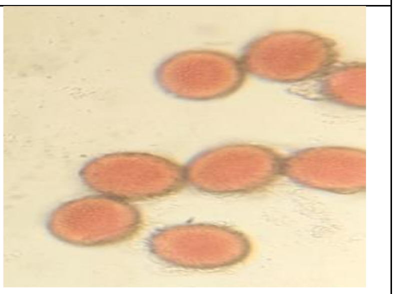
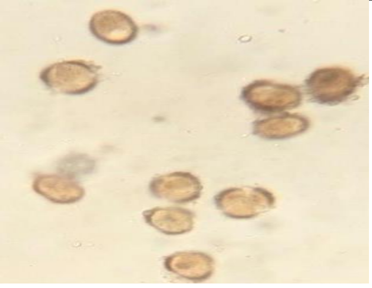
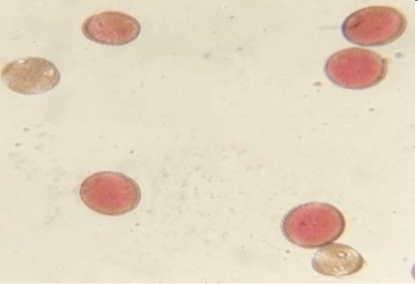
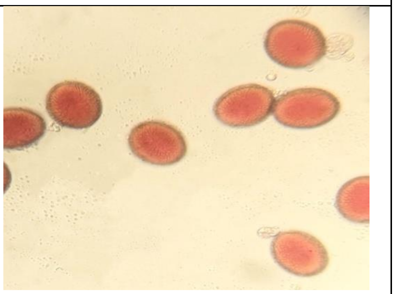
Female	F_1	Male
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2101R**	**GM*Mo*2101R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2105R**	**GM*Mo*2105R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2113R**	**GM*Mo*2113R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2115R**	**GM*Mo*2115R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2116R**	**GM*Mo*2116R**

Plate XVIII: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

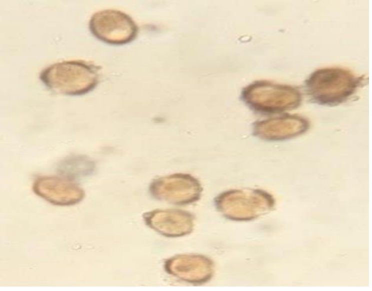
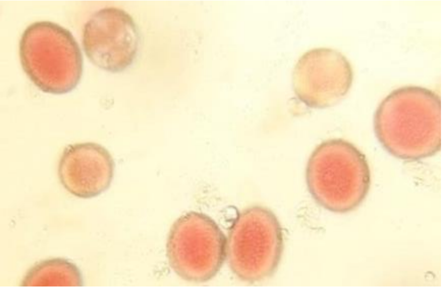
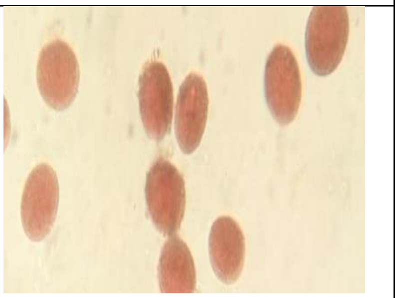
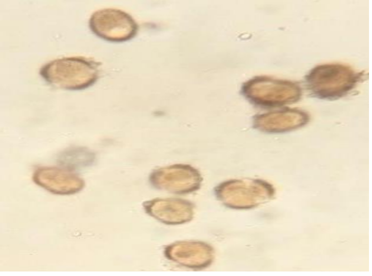
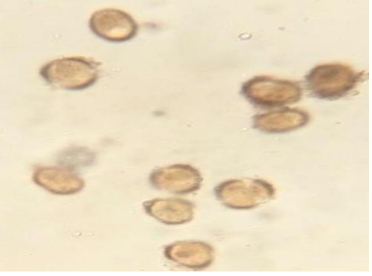
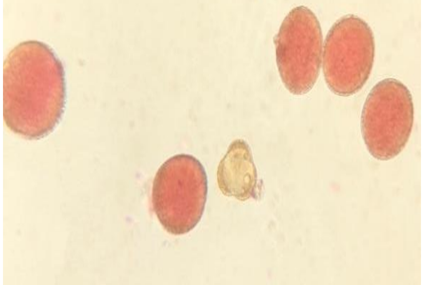
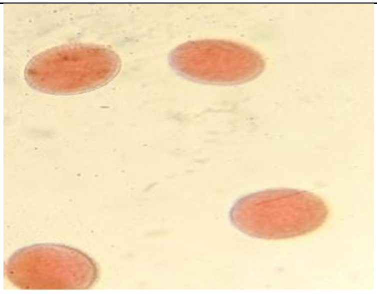
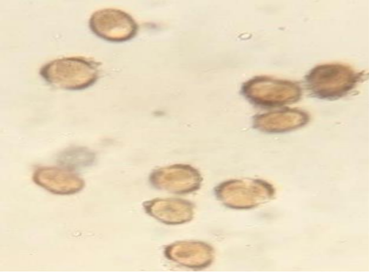
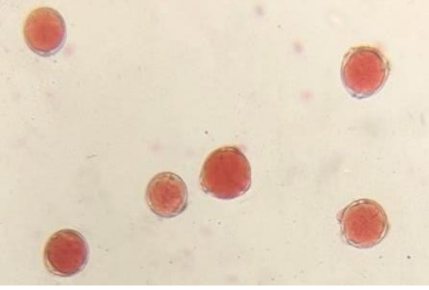
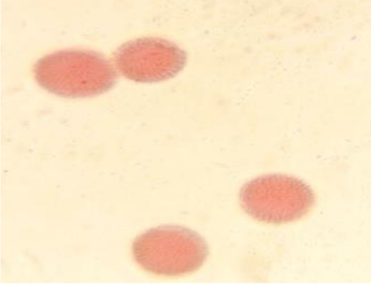
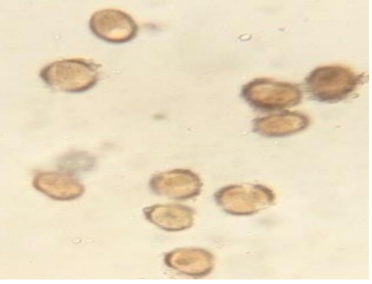
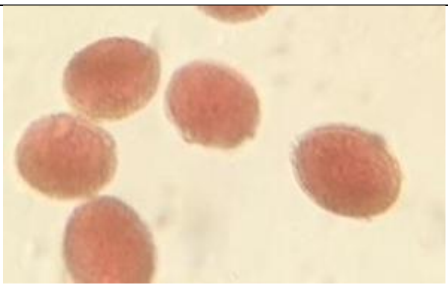
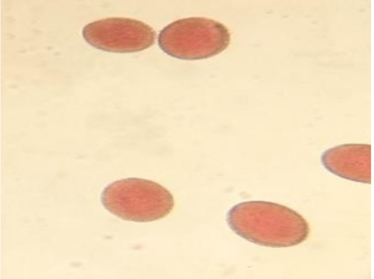
Female	**F$_1$**	**Male**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2119R**	**GM*Mo*2119R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2120R**	**GM*Mo*2120R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2122R**	**GM*Mo*2122R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2124R**	**GM*Mo*2124R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2125R**	**GM*Mo*2125R**

Plate XIX: Unstained and stained pollen grains of sterile lines, restorer lines and F$_1$ crosses

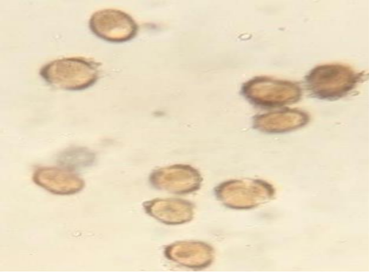
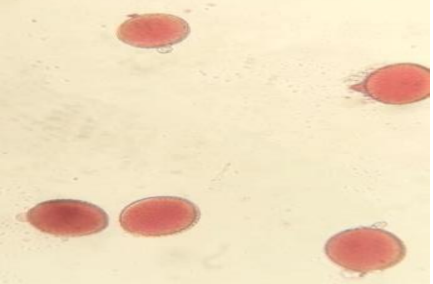
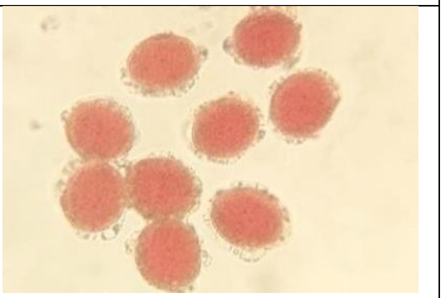
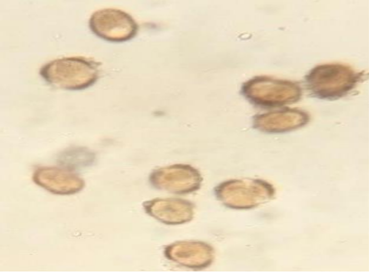
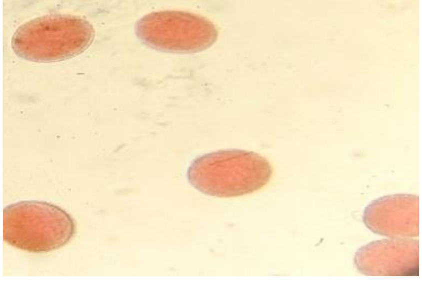
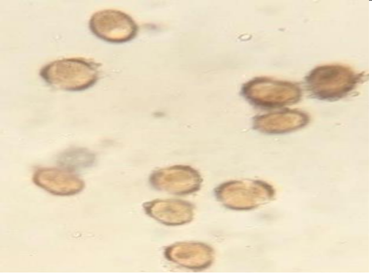
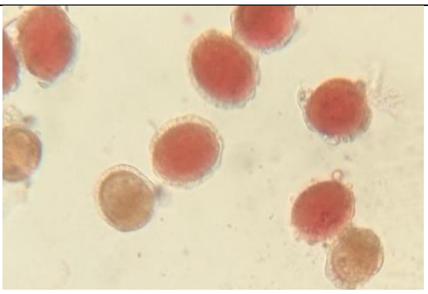
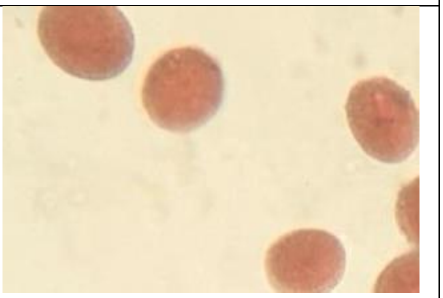
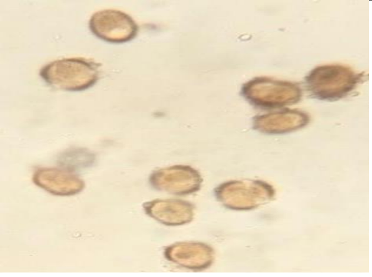
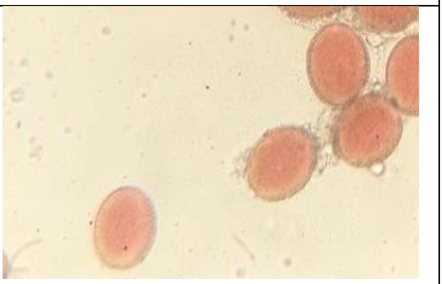
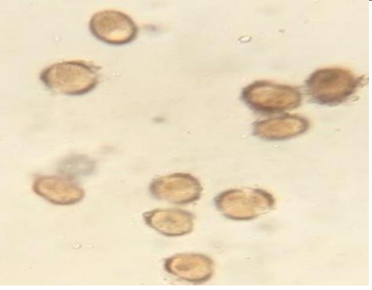
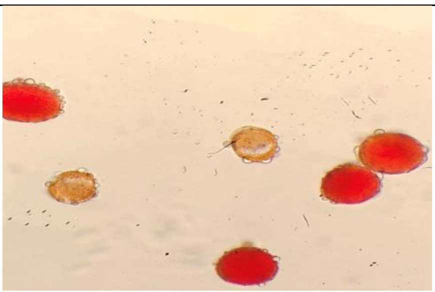
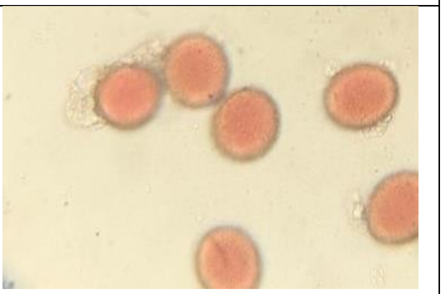
Female	**F_1**	**Male**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2127R**	**GM*Mo*2127R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2128R**	**GM*Mo*2128R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2130R**	**GM*Mo*2130R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2132R**	**GM*Mo*2132R**
		
GM*Mo*2125A	**GM*Mo*2125A × GM*Mo*2133R**	**GM*Mo*2133R**

Plate XX: Unstained and stained pollen grains of sterile lines, restorer lines and F_1 crosses

SUMMARY AND CONCLUSIONS

V. SUMMARY AND CONCLUSIONS

The present investigation entitled “Characterization of *Moricandia* based restorer lines in Indian mustard [*Brassica juncea* (L.) Czern and Coss]” was conducted during *Rabi* 2021-22 at Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar with following objectives:

1. DUS characterization of restorer lines
2. To study the genetic variability among restorer lines
3. To analyze molecular diversity using microsatellite markers
4. To apprize fertility restorability of restorer lines

The experimental material comprised of twenty-one diverse genotypes of Indian mustard collected from Center for Oilseeds Research, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, All the genotypes were sown on 27th October 2021 at Agronomy Instructional Farm, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. A set of twenty-one genotypes includes 15 restorers for *Moricandia* based CGMS system with 3 A lines 'CMS' with their respective B lines 'Maintainer' of mustard [*Brassica juncea* (L.) Czern and Coss] grown in a RBD design with three replications along with 45 hybrids in L×T mating design were also grown in single replication for fertility restorability. The experimental material was evaluated for eighteen qualitative descriptors with eleven quantitative characters *viz.*, days to flowering, days to maturity, plant height (cm), number of branches per plant, number of siliquae per plant, number of siliqua on main shoot, seeds per siliqua, length of siliqua (cm), 1000 seed weight (g), oil content (%) and seed yield per plant (g) to study the extent of DUS characterization, genetic variability, correlation coefficient, path coefficient, molecular diversity analysis and pollen fertility restorability in Indian mustard.

The important features of the results of various aspects studied are summarized under:

[1] All fifteen genotypes (restorers) showed similar characteristics in terms of leaf lobes (present), leaf width (medium), flower colour of petals (yellow), flower width of petals (medium), siliqua length (short) and seed oil (low percentage). While the rest of the twelve qualitative descriptors showed sufficient variation among genotypes. Results revealed a sufficient amount of variability based on qualitative descriptors among 15 Indian mustard genotypes.

[2] Range of *per se* performance of genotypes for days to flowering was 38.00 (GM*Mo*2130R) to 43.33 (GM*Mo*21116R); for days to maturity was 103.33 (GM*Mo*2130R) to 124.73 (GM*Mo*2133R); for plant height was 132.33 cm (GM*Mo*2130R) to 196.67 cm (GM*Mo*2116R); for number of branches per plant was 9.60 (GM*Mo*2133R) to 17.13 (GM*Mo*2116R); for number of siliqua per plant was 113 (GM*Mo*2127R) to 192.40 (GM*Mo*2105R); number of siliqua on main shoot was 27.40 (GM*Mo*2128R) to 38.33 (GM*Mo*2130R,GM*Mo*2115R); for number of seeds per siliqua was 11.53 (GM*Mo*2124R) to 15.33 (GM*Mo*2130R); for siliqua length was 3.42 cm (GM*Mo*2124R)to 4.45 cm (GM*Mo*2130R); for 1000 seed weight was 3.59 g (GM*Mo*2133R) to 5.86 g (GM*Mo*2130R); for oil content was 35.54 per cent (GM*Mo*2119R) to 37.83 per cent (GM*Mo*2105R); and for seed yield per plant was 15.60 g (GM*Mo*2127R) to 27.00 g (GM*Mo*2105R).

[3] Among the eighteen visually assessed DUS descriptors, six were monomorphic, seven were dimorphic and five were polymorphic in fifteen Indian mustard genotypes.

[4] The analysis of variance for all eleven quantitative characters with all fifteen genotypes showed significance differences among the genotypes and indicating enough amount of variability present in the material.

[5] Genotypic variance was higher than their respective environmental variance for days to flowering, days to maturity, plant height, number of branches per plant, number of siliqua per plant, number of siliqua on main shoot, siliqua length, 1000 seed weight and seed yield per plant which indicated less influence of environment on these characters except for seeds per siliqua and oil content.

[6] Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was moderate for number of branches per plant, number of siliqua per plant, 1000 seed weight and seed yield per plant which indicates that noticeable amount of genetic variability presents for these characters in the present set of materials and selection of these traits will be fruitful. Close PCV values to GCV values for most of the traits suggests that improvement by phenotypic selection is possible.

[7] High heritability was found for days to maturity (80.42%), number of branches per plant (83.69%), number of siliqua per plant (77.82%) and 1000 seed weight (97.95%) indicating less influence of environment on the expression of these characters. The direct selection for these traits would be effective for improvement in seed yield.

[8] The high heritability estimates coupled with high genetic advance were noticed for number of branches per plant, number of siliqua per plant and 1000 seed weight. These may be a sign of additive gene action and therefore; these characters are important in selection programme and direct selection of these characters would be fruitful.

[9] Seed yield per plant exhibited highly significant and positive correlations with number of branches per plant (r_g= 0.966 and r_p= 0.646), number of siliqua per plant (r_g= 0.989 and r_p= 0.823), number of siliqua on main shoot (r_g= 0.978 and r_p= 0.405), number of seeds per siliqua (r_g= 0.923 and r_p= 0.424), siliqua length (r_g= 0.563 and r_p = 0.322) and 1000 seed weight (r_g= 0.739 and r_p= 0.536) at both genotypic and phenotypic levels, while highly significant and negative correlations with days to maturity (r_g= -0.619 and r_p= -0.397) at both genotypic and phenotypic levels. Therefore, an ideal plant type would be one with early maturity, a greater number of branches per plant, a greater number of siliqua per plant, greater number of siliqua on main shoot, more number of seeds per siliqua, medium to long siliqua and bold seeded.

[10] Path coefficient analysis revealed that highest positive direct effect on seed yield was noticed by number of branches per plant (1.218) followed by number of siliqua per plant (0.789), seeds per siliqua (0.774), days to maturity (0.531) and 1000 seed weight (0.184). Negative direct effect on seed yield was found with oil content (-0.530) followed by number of siliqua length (-0.457), days to flowering (-0.219) and number of siliqua on main shoot (-0.217). Overall path analysis revealed that for improving seed yield in Indian mustard, weightage in selection should be given to days to maturity, number of branches per plant, number of siliqua per plant, seeds per siliqua and 1000 seed weight.

[11] Combined results of path analysis and correlation coefficient revealed that characters (number of branches per plant, number of siliqua per plant, seeds per siliqua, 1000 seed weight) with positive correlation coefficient and positive direct effect should be selected, so that correlation explains its true relationship and a selection for these characters will become effective. While days to maturity showed a negative correlation coefficient, but the direct effect was positive so direct selection should be practiced for this trait to reduce the undesirable indirect effects.

[12] Molecular diversity was studied with a set of 30 SSR primers. DNA extraction was carried out by the CTAB method. The quality and quantity of genomic DNA was good. Range of O. D. (A_{260} /A_{280}) was 1.62 to 1.85. The average DNA concentration was obtained as 658.3 ng/µl.

[13] Out of thirty SSR primers, six primers (BRMS-01, BRMS-08, SSR Na10-D09, SR- 7223, BG48 and BG100) were polymorphic with an average of 2 bands per primer in the cultivars studied.

[14] The amplified fragmented ranged from 94 bp to 720 bp. The PIC values of these SSR markers are 0.50.

[15] The dendrogram, based on Jaccard's similarity coefficient was able to distribute all the genotypes of Indian mustard in two clusters and four sub-clusters. It is also noted that some pairs of genotypes had similar genetics *i.e.,* all 'A' and 'B' lines are included in A1 sub-cluster and all 'R' lines are in A2, B1 and B2 sub-clusters indicating clearcut differentiation of 'A' and 'B' lines from 'R' lines.

[16] Among the Indian mustard genotypes; Between GM*Mo*2128R and GM*Mo*2124A shows minimum similarity (0.22) on the basis of SSR amplification data. These results concluded that SSR markers could be efficiently used to study divergence among Indian mustard genotypes.

[17] The presence of yellow pollen dust was indicated as fertile and the absence of yellow pollen dust indicated as sterile represented plant fertility status. The F_1 generation of all crosses exhibited fertility restoration with pollen fertility and also siliquae set by selfing.

[18] The CMS line crossed with the restorer line exhibited pollen fertility in most of crosses. The cross GM*Mo*2124A × GM*Mo*2113R showed lower pollen fertility restorability (62.50%) followed by GM*Mo*2125A × GM*Mo*2115R (66.67%). The crosses GM*Mo*2118A × GM*Mo*2125R and GM*Mo*2125A × GM*Mo*2124R (98%) showed higher pollen fertility showing good restorability of both the restorer lines. At the field level, siliquae set upon selfing resulted in GM*Mo*2124A × GM*Mo*2113R showing lower restorability (62.40%) followed by GM*Mo*2125A × GM*Mo*2115R (66.40%). The crosses GM*Mo*2118A × GM*Mo*2125R and GM*Mo*2125A × GM*Mo*2124R (97.60%) showed higher pollen fertility. On the basis of both the test, GM*Mo*2113R, GM*Mo*2116R, GM*Mo*2119R, GM*Mo*2120R, GM*Mo*2124R, GM*Mo*2125R, GM*Mo*2127R, GM*Mo*2132R and GM*Mo*2133R are found having good restoration ability in their hybrids.

Conclusion

Combining all the aspects studied, it could be concluded that the number of branches per plant, number of siliqua per plant, seeds per siliqua and 1000 seed weight had a positive correlation coefficient with yield. Using present set of SSR markers, all 15 ‘R’ lines can be differentiated from ‘A’ and ‘B’ lines. Less differences between A and B lines indicated more molecular markers need to be studied. Also, ample fertility restorability is available in this material that can be used for authentication of stability in fertility restoration capacity in *Moricandia* based CGMS system of Indian mustard.

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APPENDICES

APPENDICES

Appendix – I

Weekly meteorological data recorded during the crop season (October-2021 to February-2022) at Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

Month and year	Std. Weeks	Temperature (°C)		Relative Humidity (%)		Rain fall (mm)	Bright sunshine (hrs./ day)	Wind velocity (km/ hr)	Pan Evapo-ration (mm/ day)
		Max.	Min.	Morn.	Even.				
November 2021	40	34.7	25.0	78	70	0.0	7.7	3.9	7.0
	41	34.4	25.8	77	62	0.0	9.8	2.1	7.9
	42	36.9	21.1	69	62	0.0	9.7	2.2	7.3
	43	36.2	18.0	66	59	0.0	9.9	4.7	6.8
	44	33.3	15.9	69	50	0.0	9.7	2.3	5.8
	45	34.3	14.8	65	41	0.0	9.5	2.8	5.5
December 2021	46	32.4	13.6	71	51	0.0	8.2	2.7	4.9
	47	28.9	17.5	77	55	31.0	5.4	2.6	4.3
	48	31.6	15.4	74	47	0.0	8.0	2.6	5.5
	49	28.1	13.6	70	35	0.0	7.5	2.9	4.9
January 2022	50	26.5	10.6	70	32	0.0	8.0	2.7	4.2
	51	25.2	7.6	70	30	0.0	8.9	3.3	3.9
	52	27.1	11.7	76	41	3.0	6.8	2.8	4.4
	1	25.9	12.7	76	53	0.0	6.4	2.4	3.9
February 2022	2	21.9	7.0	67	45	0.0	8.9	4.4	3.3
	3	24.9	8.5	68	39	0.0	8.9	2.8	3.8
	4	25.0	6.0	57	35	0.0	7.9	4.1	3.7
	5	27.6	8.8	65	33	0.0	9.2	3.2	5.1
	6	27.9	9.5	64	31	0.0	9.0	2.7	5.8

Source : Agricultural Meteorology Department, C. P. College of Agriculture, Sardarkrushinagar

Appendix - II

Mean performance of fifteen genotypes for yield and yield contributing characters in Indian mustard

No	GENOTYPE	DF	DM	PH	NBPP	NSPP	NSMS	SPS	SL	TW	OC	SYPP
1	GM*Mo*2101R	38.67	113.67	173.33	15.13	170.53	37.47	13.60	3.98	5.79	37.13	24.60
2	GM*Mo*2105R	41.27	112.00	172.33	17.00	192.40	35.67	15.13	4.07	5.83	37.83	27.00
3	GM*Mo*2113R	38.57	114.33	173.43	15.23	170.43	37.57	13.70	3.88	5.69	37.57	24.70
4	GM*Mo*2115R	41.00	123.47	188.33	17.00	165.83	38.33	13.07	4.33	4.88	36.23	24.33
5	GM*Mo*2116R	43.33	124.13	196.67	17.13	190.23	36.67	14.13	4.37	4.91	37.34	27.00
6	GM*Mo*2119R	43.23	114.00	176.67	15.17	182.25	35.40	15.07	4.06	5.43	35.54	26.30
7	GM*Mo*2120R	42.47	121.80	185.67	10.93	132.33	33.00	13.47	3.67	4.16	36.96	19.17
8	GM*Mo*2122R	41.27	111.33	145.33	15.00	180.00	37.60	13.80	4.03	4.61	37.15	24.73
9	GM*Mo*2124R	40.67	122.30	186.00	11.53	125.20	29.67	11.53	3.42	5.27	36.61	17.47
10	GM*Mo*2125R	43.00	124.73	193.67	15.00	149.33	37.93	13.87	3.54	4.09	37.16	20.60
11	GM*Mo*2127R	39.47	123.73	184.00	11.93	113.00	33.33	13.00	4.14	4.10	36.99	15.60
12	GM*Mo*2128R	41.87	118.33	180.73	10.60	127.13	27.40	12.53	4.18	4.11	36.00	18.70
13	GM*Mo*2130R	38.00	103.33	132.33	15.00	160.00	38.33	15.33	4.45	5.86	35.99	24.10
14	GM*Mo*2132R	41.40	120.00	155.33	15.00	146.07	33.00	14.27	4.06	3.65	37.53	20.57
15	GM*Mo*2133R	41.80	124.73	172.00	9.60	137.60	33.00	13.40	4.03	3.59	36.41	19.67
	MEAN	41.07	118.13	174.40	14.08	156.16	34.92	13.72	4.02	4.80	36.83	22.30
	RANGE	38.00-43.33	103.33-124.73	132.33-196.67	9.60-17.13	113.00-192.40	27.40-38.33	11.53-15.33	3.42-4.45	3.59-5.86	35.54-37.83	15.60-27.00
	S.Em ±	1.17	1.75	7.99	0.61	7.45	1.74	0.47	0.13	0.07	0.35	1.77
	C.D. @ 5 %	3.39	5.07	23.16	1.78	21.60	5.05	1.35	0.38	0.20	1.00	5.12
	C.V.%	4.93	2.57	7.94	7.55	8.27	8.65	5.89	5.66	2.47	1.63	13.72

Where,

DF= Days to flowering, **DM** = Days to maturity**,** **PH**= Plant height (cm), **NBPP**= Number of branche per plant, **NSPP**= Number of siliqua per plant, **NSMS**= Number of siliqua on main shoot, **SPS**= Seeds per siliqua, **SL**= Siliqua length (cm), **TW**= Test weight (g), **OC**= Oil content (%), **SYPP**= Seed yield per plant (g)

CERTIFICATE

This is to certify that I have no objection for supplying to any scientist only two copy or any part of this thesis at a time through reprographic process, if necessary for rendering reference service in a library or documentation centre.

Place : SARDARKRUSHINAGAR
Date : 14 SEPTEMBER, 2022

(KOLI MUKESH C.)