“Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss”

THESIS

Submitted to the
Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya
In partial fulfillment of the requirements for the degree of
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In

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(GENETICS AND PLANT BREEDING)

By

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CERTIFICATE – I

This is to certify that the thesis entitled **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** submitted in partial fulfillment of the requirements for the Degree of **Doctor of Philosophy in Agriculture** in the Department of **Genetics and Plant Breeding** of **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya,** Gwalior is a record of the bona-fide research work carried out by **Miss Chitralekha Shyam** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of this investigation has been acknowledged by the scholar.

(Dr. M.K. Tripathi)

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CERTIFICATE - II

This is to certify that the thesis entitled **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** submitted by **Chitralekha Shyam** to the **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya**, Gwalior in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture** in the Department of **Genetics and Plant Breeding** has been approved by the External Examiner and Student's Advisory Committee after oral examination.

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Director of Instructions ....

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Date:
Place: Gwalior

Chitralekha Shyam

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LIST OF ABBREVIATIONS

Abbreviations	Descriptions
%	Percentage
^{0}C	Degree celsious
i.e.	That is
Viz.	For example
et. al.	And co – worker / and others
Anon	Anonymous
Fig	Figure
SN	Serial Number
DH	Days to heading
DF	Days to maturity
PH	Plant height
NPB	Number of primary branches
NSB	Number of secondary branches
LMR	Length of main raceme
SR	Number of silique per main raceme
SP	Number of silique per plant
SL	Silique length
SS	Number of seeds per silique
TSW	Thousand seed weight
BYPP	Biological yield per plot
SYPP	Seed yield per plot
SYPPG	Seed yield per plant gram
HI	Harvest index
GCV	Genotypic
PCV	Phenotypic coefficient variation
GA	Genetic advance
RBD	Randomized block design
CRD	Completely randomized design
S	Significance
NS	Non significance
R	Replication
SV	Source of variation
DF	Degree of freedom
EMS	Error mean sum of square
t	Treatment
SE	Standard error
CD	Critical difference
CV	Coefficient of variation
Cov	Co-variance
g	gm

Kg	Kilogram
h	hectare
h-1	Per hectare
t	tone
mt	Million tone
Min	Minimum
Max	Maximum
No.	Number
Q	Quantal
cm	Centimeter
mm	Milimeter
µl	Microlitre
ng	Nanograms
FAO	Food and Agriculture organization
WHO	World health organization
QTL	Quantitative trait loci
MAS	Marker assisted selection
Sp.	Species
DAS	Day after sowing
PC	Principal component
PF	principal factor
SM	similarity matrix
UPGMA	Unweighted pair group method
PIC	Polymorphic information content
GC	Gas chromatography
GCMS	Gas chromatography mass
NIR	Near-Infrared spectroscopy
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
HEC	Hypocotyl derivedembryogenic calli
CEC	Cotyledon derived embryogenic calli
FAE	Fatty acid elongase
bp	Base pair
PCR	Polmerase Chain Reaction
UV	Ultra voilet
SSR	Simple Sequence Repeat
AFLP	Amplified Fragment Length Polymorphism
RFLP	Ristriction Fragment Length Polymorphism
CAPS	Cleaved Polymorphic Sequence
IP	Intron Polymorphic
SNP	Single Nucleotide Polymorphic
EST	Expressed Sequence Tag
DNA	Deoxyribonucleic acid

RNA	Ribonucleic acid
CETB	Cetyltrimethyl ammonium bromide
EDTA	Ethylene diamine tetra acetic
PCI	Phenol-chloroform isoamyl alcohol
TBE	Tris Borate Edta
Etbr	Ethidium Bromide
HCL	Hydrocloric acid
DNTP	Deoxyribonucleoside triphosphate
RNase	Ribonuclease
rpm	Revolution per minute
Taq	Thermusaquaticus
TE	Tris EDTA
MS	Murashige and Skoog
2, 4-D	2, 4-Dichlorophenoxy acetic acid
Kn	Kinetin
NAA	Naphthalene acetic acid
BAP	Benzyl amino purine
IBA	Indole butyric acid
PGR	Plant growth regulator
V	Volt
@	At the rate
Hrs	Hour

CHAPTER - I

INTRODUCTION

Oilseed crops are the *second most important* determinant of agricultural economy, next only to cereals**.** Rapeseed mustard was sown in the 3000 BC in the Indus valley. The use of mustard oil is recorded in the last few centuries of the pre-Christian era. It is the third important oilseed crop in the world after soybean (Glycine max) and palm (Elaeis guineensis) oil which contributes 28.6% in the production of oilseeds. Rapeseed mustard is the second most important edible oilseed crop in India after groundnut and accounts for nearly 30% of the total oilseeds produced in the country (Damodaran et al., 2005). It is cultivated mostly under temperate climate. It is also cultivated in tropical and subtropical region. It requires cool, moist climate with about 20°C temperature and 75 cm rainfall. It can be cultivated upto a height of 800 meters above sea level. The crop can be grown well under both irrigated and rainfed conditions. It requires sandy loam to clay loam soils but thrive best on light loam soils. Do not tolerate water logging conditions or heavy soils. Soil having neutral pH is ideal for their proper growth and development in rapeseed mustard.

Classification and origin:

Rapeseed mustard group is mainly constituted by *Brassica nigra*, *Brassica oleracea*, *Brassica rapa, Brassica carinata, Brassica juncea and Brassica napus.* Out of these *Brassica. juncea* L. (Indian mustard) is the major crop of the India. Among *Brassica* species, Indian mustard (*Brassica juncea* L.) displays a great polymorphism and is a source of different types of vegetables, condiment and oilseeds. Vegetable mustard is grown predominantly in China and Japan. On Canadian prairies, however, the mustard is a major source of condiment, while in Indian subcontinent, oilseed mustard is more important than vegetable and condiment types. The term mustard is believed to be derived from the early European practice of mixing the sweet “must” of old wine with the crushed seeds to form a hot paste “hot must” or “mustum ardens” hence the modern term.

It is important oilseed crop belongs to the family cruciferae (Brassicaceae). It is mainly self-pollinating crop, however, up to 30% out-crossing does occur under natural field conditions, depending upon wind and bee activities (Rakow and Woods, 1987). *Brassica juncea* (n =18) is an amphidiploid species derived from inter specific crosses between *B. nigra* (n = 9) and *B. rapa* (n = 10). It has thirty six chromosome number and genome AABB. Middle East is considered as the primary centre of origin of Indian mustard. China, north eastern India Caucasus are considered to be the secondary centres of origin. Indian mustard (*Brassica juncea* (L.) Czern & Coss) is cultivated in Rajasthan, Haryana, Punjab, Madhya Pradesh, Uttar Pradesh, West Bengal, Gujarat, Bihar, Jharkhand, Assam, Jammu Kashmir, Uttranchal, Chhattisgarh, Orissa and Maharashtra. It is also grown under some nontraditional areas of South India including Karnataka, Tamil Nadu, and Andhra Pradesh. The oleiferous form of *Brassica juncea* (Indian mustard) is extensively grown for its oil in India, Pakistan, China, Bangladesh and Nepal. It is also cultivated in the Middle-East and East European countries. In recent years, commercial cultivation of *Brassica juncea* for oilseed production rise in UK, Canada, USA and Australia.

Area, Production and productivity:

In World level, the total area, production and productivity of rapeseed mustard were recorded 36.59 million hectare, 71.64 million tonne and 1958 kg/hectare in 2014-15. In India, it was grown on 5.79 million hectare and produced 6.31 million tonne in production and 1079 kg/hectare in productivity in 2014-15. The M.P. state level production, area and productivity were 0.713 million hectare, 0.717 million tonne and 1006 kg/hectare in 2014-15 (Anonymous, 2014).

Globally, the total area under cultivation of rapeseed mustard in 2015-16 was 35.52 million hectares, production of rapeseed mustard recorded 71.45 million tonne and productivity was recorded as 2010kg per hectare. The highest production recorded 18.38 million tonne in Canada during 2015-16 and it ranks first in the world. India ranks seventh with a record production by 6.82 million tonne. Major rapeseed mustard producing countries of the world are Canada, China, France, Germany, Poland, UK, India, Australia, Russia and Ukraine (Anonymous, 2015).

India occupies a premier position in global oilseeds scenario with 12–15% of oilseeds area. India contributes 8.3% and 19.8% of world acreage and production respectively. In India, rapeseed mustard is cultivated in an area of 5.76 million hectare with a production of 6.82 million tones with productivity of 1184kg/ha. The highest production recorded 3.26 million tonne in Rajasthan during 2015-16 and it ranks first in the India. MP ranks third with a record production by 0.7 million tonne. Major rapeseed mustard producing states of the country are Rajasthan, Haryana, Madhya Pradesh, Uttar Pradesh, West Bengal, Gujarat, Assam, Jharkhand, Bihar, Jammu and Kashmir, Chhattisgarh and others (Anonymous, 2015).

In Madhya Pradesh, the total area under cultivation of rapeseed mustard in 2015-16 was 0.62 million hectares, production recorded 0.70 million tonne and productivity was recorded as 1135 kg/ha. The highest production recorded 3.26 million tonnes in Morena during 2015-16 and it ranks first in the Madhya Pradesh. Gwalior ranks fourth with a record production by 0.7 million tonne. Major rapessed mustard producing districts of the Madhya Pradesh are Morena, Bhind, Sheopur, Gwalior, Mandsaur and other districts (Anonymous, 2015).

Globally, the total area under cultivation of rapeseed mustard in 2016-17 was 33.70 million hectares, production of rapeseed mustard recorded 69.18 million tonne and productivity was recorded as 2053kg per hectare. In India, the total area, production and productivity under cultivation of rapeseed mustard in 2016-17 were 6.65 million hectare, 7.10 million tonne and 1069 kg/hectare, respectively. In M.P., it was sown on 0.723 million hectare and produced 0.607 million tonne in production and 840 kg/hectare in productivity in 2016-2017 (Anonymous, 2016).

In World level, the total area, production and productivity of rapeseed mustard were recorded 36.68 million hectare, 72.42 million tonne and 1974 kg/hectare in 2017-18. In India, it was grown on 6.412 million hectare and produced 6.33 million tonne in production and 6979 kg/hectare in productivity in

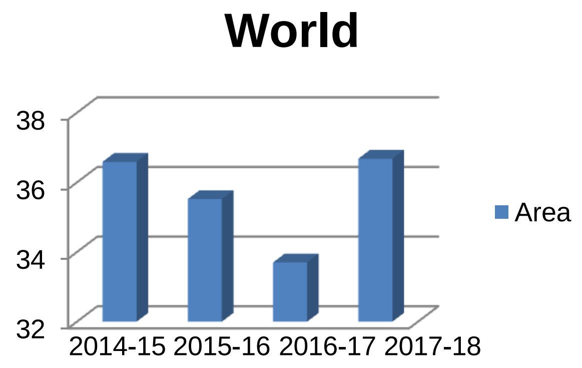


Fig 1.1: Graphical representation of area of mustard in World

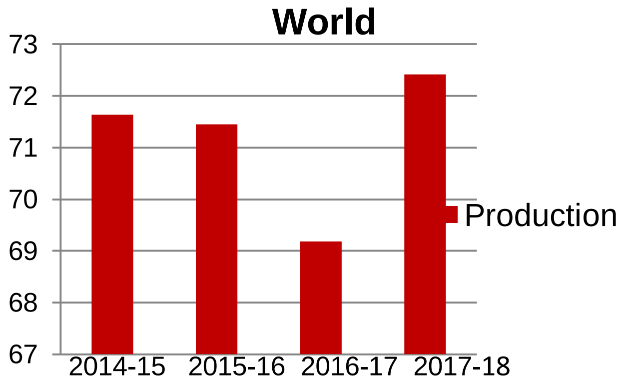


Fig 1.2: Graphical representation of production of mustard in World

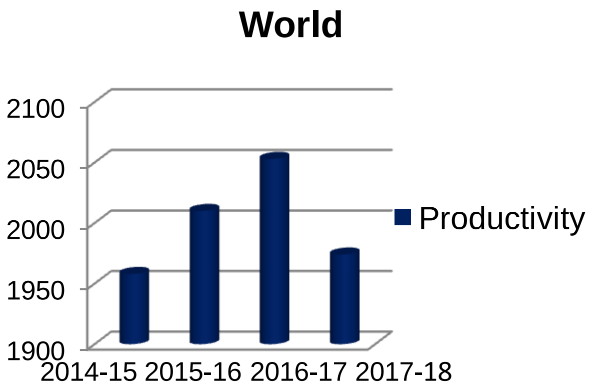


Fig 1.3: Graphical representation of productivity of mustard in World

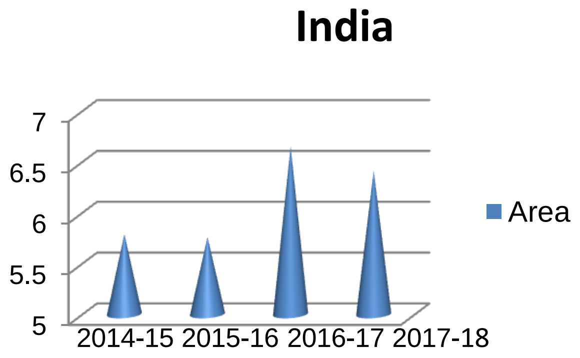


Fig 1.4: Graphical representation of area of mustard in India

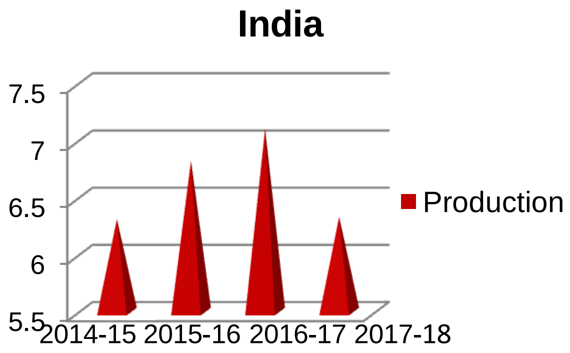


Fig 1.5: Graphical representation of production of mustard in India

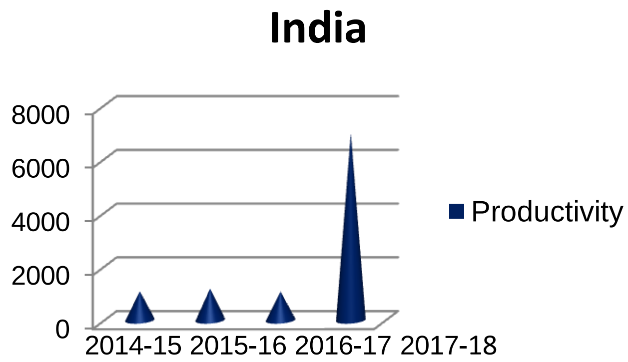


Fig 1.6: Graphical representation of productivity of mustard in India

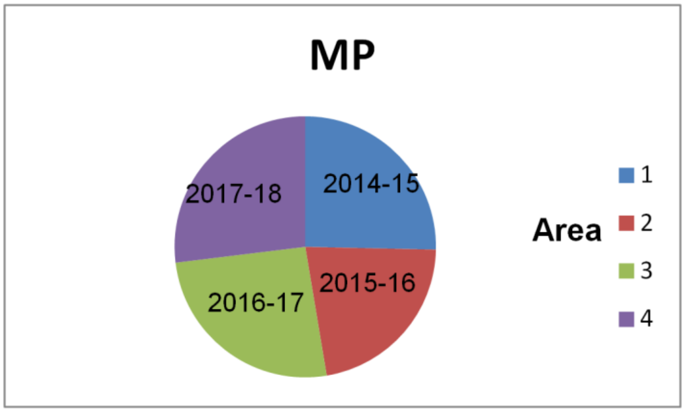


Fig 1.7: Graphical representation of area of mustard in MP

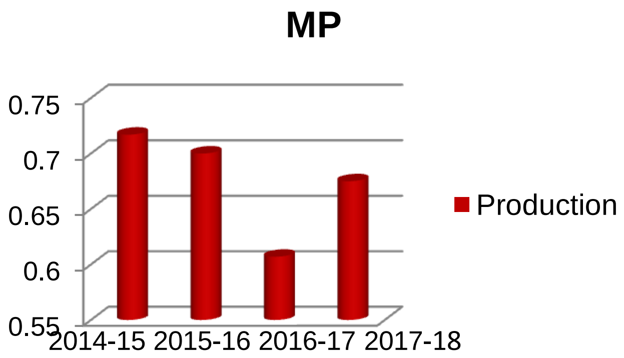


Fig 1.8: Graphical representation of production of mustard in MP

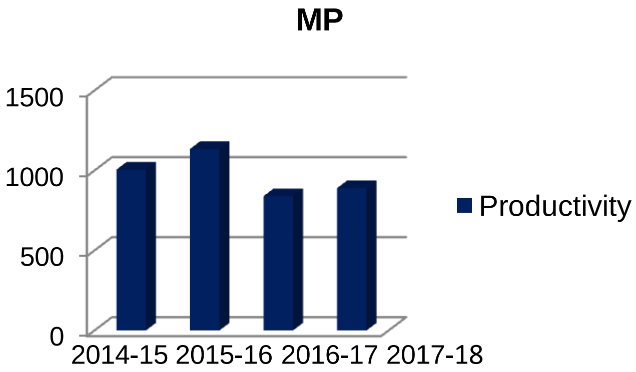


Fig 1.9: Graphical representation of productivity of mustard in MP

2017-18. The M.P. state level production, area and productivity were 0.758 million hectare, 0.675 million tonne and 890 kg/hectare in 2017-18 (Anonymous, 2017).

Genomic constitutions:

In India, there are two main cultivated species of mustard viz., Indain mustard (*Brassica juncea*) and Black mustard (*Brassica nigra)* and one mainly cultivated species of rapeseed i.e. *Brassica compestris*. Other cultivated species in mustard *Brassica junea*, *Brassica, carinata* and *Brassica nigra* and in rapeseed *Brassica compestris* and *Brassica napus*. The genus *Brassica* is one of

51 genera of Brassiceae family. The genus *Brassica* is native to Western Europe, the Mediterranean and temperate regions of Asia. Many wild species grow as weeds, especially in North America, South America, and Australia. *Sinapsis alba* is a wild species of mustard. Over 30 wild species found in *Brassica*. In *Brassica*, *Brassica* triangle is given by Nagaheru. In this triangle, *Brassica juncea (*n =18) is derived from inter specific crosses between *B. nigra* (n = 8) and *B. rapa* (n = 10). *Brassica carinata* is originated from inter specific crosses between *B. nigra* (n = 8) and *B. olerecea* (n = 9). *Brassica napus* is derived from inter specific crosses between B. olerecea (n = 9) and *B. compestris* (n = 1). *Brassica nigra* contains BB genome, *B*. *olerecea* get CC genome and *B. compestris* having AA genome. *Brassica juncea* is having AABB genome, *B. carinata* contains BBCC genome and *B. napus* is having CCAA genome. Mating system in *B. juncea*, *B. carinata* and *B. napus* are self compatible, respectively. *B. nigra*, *B. oleracea* and *B. compestris* are self incompatible, respectively.

Toxonomy:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Dilleniidae
Order: Capparales
Family: Brassicaceae
Genus: *Brassica*
Species: *juncea*

Botanical description:

Root: Tap root system, roots are branched.
Stem: Erect, branched above
Leaf: Simple, alternative
Inflorescence: Panicle
Flower: Complete, pedicillate, hermaphrodite, cruciform
Calyx: Sepal oblong
Corolla: Petal4, cruciform, yellow in colour
Androecium: Stamen6, tetradynamous

Gynocium:	Ovary cylindrical, superior
Fruit:	Yellow brown siliqua

Plant genetic resources:

The National Genebank (NBPGR) New Delhi holds 10,301 accessions comprising of 14 genera and 24 species of Brassica. India possesses rich diversity of oilseed brassicas. *Brassica rapa* var. toria, *B. rapa* var. brown sarson and *B. juncea* considered to be native of Indian gene centre (Arora, 1988) are distributed in eight agro-ecological zones of the country. Much of the diversity is concentrated in the Indo gangetic plains and sub-montane Himalayas. These are widely grown in the northern plains in the states of Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Gujarat, West Bengal, Assam, Bihar, Jharkhand, Punjab, Himachal Pradesh and Jammu and Kashmir. A large number of indigenous rapeseed-mustard collections have been made in the country by National Bureau of Plant Genetic Resources (NBPGR), Indian Agricultural Research Institute (IARI), Haryana Agricultural University and Directorate of Rapessed Mustard Research (DRMR).

In general, genetic improvement of crops can be accelerated when there is a broad genetic diversity and information on these genetic resources is available. Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of these crops. The collection of these genetic resources and the assessment of genetic diversity within and between these resources should be given priority for varietal improvement. At the same time, it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm and to increase the utilization of plant genetic resources. In a common practice, genetic improvement is easy in those species/crops, which have broad genetic diversity and the information regarding these hidden genetic resources is easily available. Thus, the morphological and biochemical profiling is a method to investigate genetic variation and to classify mustard genotypes.

Uses:

Rapessed, mustard used as an oil, it is a vegetable oil and is obtained from seeds of mustard plant. The oil is extracted from clean and sound mustard seeds, which belong to species *namely*: *compestris, juncea or napus* varieties of *Brassica*. Though this oil is nutty tasting. Edible oil is one of the most important products of various species of *Brassica*. The oil obtained is the main cooking medium in Northern India and cannot be easily replaced by any other edible oil. The seed and oil of mustard have a peculiar pungency due to presence of glucosinolate and its hydrolysis products such as allyl-isothio-cynate makes suitable for its use as condiment in the preparation of pickles and for flavoring curries and vegetables. It is also used for preparation of hair oils and medicines. It is used for making of soaps and in manufacturing of lubricants. In food, black mustard leaves (greens) are used in salads and other dishes. Also in food, black mustard seed is used as a spice and to flavor mustard condiment. Oilseed cake is used for cattle feed and organic manure. The meal cake after extraction of the oil from the seed contains 40-45 per cent protein which could be exploited as raw material for the manufacture of protein rich products intended for both animal and human consumption. Besides, green stems and leaves are a good source of fodder for cattle. The leaves of young plants are used as green vegetables as they supply enough sulphur and minerals in the diet. In the tannery, industry mustard oil is used for softening leather.

Nutrient value and health benefits:

- The mustard seed per 100 g is nutritionally rich having total carbohydrate 23.8g, total fat 39.7g, protein 20g and also present vitamins, minerals and water. Rapeseed contains total fat 100g, vitamins E, K and minerals. Mustard seeds are an excellent source of essential B-complex vitamins such as folates, niacin, thiamin, riboflavin, pyridoxine (vitaminB-6) and pantothenic acid. These vitamins are essential in the sense that body requires them from external sources to replenish. These B-complex groups of vitamins help in enzyme synthesis, nervous system function and regulating body metabolism.

- Mustard seeds contain flavonoid and carotenoid antioxidants such as carotenes, zeaxanthin, and lutein. In addition, the seeds compose a small amount of vitamin antioxidants such as vitamin A, C, and vitamin K. The seeds are an excellent source of vitamin E, gamma tocopherol; contain about 19.82 mg per 100 g (about 132% of RDA). Tocopherol is recognized for the antioxidant activity and have been observed to be useful in degenerative diseases like cancer, cataract, cardiovascular disease and aging. Tocopherols in oil act through several mechanism such as inhibiting the lipid peroxidation, chain termination, singlet oxygen quenching and radical scavenging to deactivate free radical that are produced during the oxidation of biomolecules (Qureshi et al.,1991). Vitamin E is a powerful lipid soluble antioxidant, required for maintaining the integrity of cell membrane of mucosa and skin by protecting it from harmful oxygen-free radicals. It provides various micronutrients such as beta carotene, oryzanol, tocotrienols and squalene. Beta carotene has been recognized for its ability to contribute to the synthesis of retinol (Groff et al., 1995). Oryzanol is known for its strong hypocholesterolemic properties and other beneficial effects (Rukmini and Raghuram, 1991).
- Mustards are rich source of health benefiting minerals. Calcium, manganese, copper, iron, zinc, selenium and magnesium are some of the minerals especially concentrated in these seeds. Calcium helps build bone and teeth. Manganese used by the body as a cofactor for the antioxidant enzyme *superoxide dismutase*. Copper required in the production of red blood cells. Iron is essential for the red blood cell formation and cellular metabolism. Selenium and magnesium, which gives it anti-inflammatory properties. It also helps stimulating sweat glands and helps lowering body temperature. 100 g of mustards provide 4.733 mg of niacin (vitamin B-3). Niacin is a part of nicotinamide coenzymes that help lower blood cholesterol and triglyceride levels. The rapeseed mustard seeds are high in essential oils as well as plant sterols. Some of the important sterols include such as*brassicasterol, campesterol, sitosterol, avenasterol and*

stigmasterol. Some of the glucosinolate and fatty acids in the seeds are *sinigrin, myrosin, erucic, eicosanoic, oleic, and palmitic acids.*

- Internally, mustard seeds are used for purging the body of toxins. Mustard seed is an ingredient of formulations that induce emesis, cleanse the cranial cavity and for giving decoction enema. These procedures are indicated in diseases like vomiting, insanity, flatulence, pallor, jaundice and rhinitis. In tumor of the thyroid gland as well as lymphadenitis, a paste of mustard seeds with other herbs is indicated for external application. Its seeds and oil has traditionally been used to relieve muscle pain, rheumatism and arthritic pain. In India, mustard oil is applied to the scalp and is believed to stimulate hair growth.
- Its ground seeds act as a laxative, stimulant to the gastric mucosa and increase intestinal secretion. Seed paste applied on wounds whereas paste of leaf said to heal cattle wounds (Sood *etal.,* 2010). The applications are seen indicated in diseases like leucoderma, cracked skin, fever, leprosy, wasting, epilepsy, swelling, rheumatoid arthritis, neurological disorders, gynecological disorders, breast milk disorders earache, wounds, acne vulgaris, eruptions in oral cavity and retention of placenta.

Toxic substance:

In traditional *Brassica* oilseeds, the occurrence of erucic acid is considered as anti-nutritional factor for human consumption that cause toxic affects on the heart at high enough doses. As such there is need to minimize the erucic acid content and have low erucic acid genotypes through breeding and biotechnological tools.

Major problems in Rapeseed mustard: In India, the productivity of rapeseed mustard is low due to various constraints and another quality based oil. There is an urgent need to free from these problems. Some following constraints are presented:

- ✓ **Biotic stress:**

A) **Major diseases**

Alternaria blight/dark leaf and pod spot (*Alternaria brassicae* Sacc.)
White rust/ white blister (*Albugo candida* Kuntz.)
Stem rot/stem blight (*Sclerotinia sclerotiorum* de Bary.)
Powdery mildew (*Erysipse cruciferarum/Erysiphe polygoni* DC)

B) **Major insects**

Mustard aphid (*Lipaphis erysimi*)
Saw fly (*Athalia proxima*)
Painted bug (*Bagrada cruciferarum*)

✓ **A biotic stress:**

Drought
Salinity

✓ **Quality traits**

Low erucic and glucosinolate

Breeding objectives in Rapeseed mustard: The major objectives of the *Brassica* research programme in the country are:

1. Improve oil and seed yield
2. Stabilizing yield
3. Major biotic and a biotic stresses tolerance/resistance
4. Improving oil (low erucic acid) and seed meal (low glucosinolate) quality
5. Developing suitable varieties for non-traditional areas (southern states) and rice fallows in eastern and northeastern states
6. Early maturity
7. Shattering resistance
8. Ideal plant type

Practical utility of research problems:

Genetic variability in a population can be partitioned into heritable and non heritable variation with the aid of genetic parameters such as variance, coefficient of variation, heritability and genetic advance, which serve as a basis for selection of some outstanding genotypes from existing ones.

In plant breeding, genetic diversity plays an important role because hybrids belonging to genetically diverse parents generally, display a greater heterosis than those between closely related parents. Genetic diversity may arise due to geographical separation or due to different genetic constitution of accessions. The choice of genetically diverse parents for hybridization is an important feature of any crop improvement programme for getting desirable segregants. The assessment of genetic diversity is not only important for crop improvement efforts but also for the efficient management and protection of available genetic variability. Heritability measures the degree of resemblance between the phenotypic and breeding value. It is valid strictly for the population from which they are derived. The estimates for the same character may vary considerably for different population. Differences in the estimates of heritability are due to environment variance. The environment variations depend on the condition of cultivar and management practices. Uniform conditions increases the value whereas, differences may increases the values of heritability. Genetic advance is the improvement in the mean of selection family over the base population (Lush, 1949 and Johnson *et al.*, 1955)**.** Correlation studies provide a better understanding of the association of different characters with grain yield (Dixet and Dubey, 1984) which is generally due to linkage, pleiotrophy, physiological association in developmental and biochemical pathway. Path coefficient analysis splits the genotypic correlation coefficient into measures of direct and indirect effects. It measures the direct and indirect contribution of independent variables on dependent variable. Now a day's crop improvement is based on selection and further breeding process by utilizing biotechnological approaches and molecular profiling is one of the most important tool for that.

Molecular profiling has been the preferred choice of breeding as these are more reliable & authentic and less influenced by environmental fluctuations (Vinu *et al*., 2013). The molecular marker in the other hand can be utilized for identification of marker for specific trait. Such diversity studies are helpful in categorizing the population into diverse group and that will help in development of gene pool. Molecular markers can also be used for screening plants along with phenotypic selections (Arunachalam *et al.,* 2005). These molecular markers hold on the precise information regarding genetic divergence and similarities between them. Thus identification of genomic regions associated with stress tolerance would enable researchers and breeders to develop improved cultivars with increased stress tolerance using marker assisted selection (MAS) (Ribaut *et al,.* 1997). Even, molecular markers have been used to study the evolutionary relationships in *Brassica*.

In mustard, various marker systems have been used for analyzing the genetic diversity. There is increasing number of reports where molecular markers like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNAs, (RAPDs), amplified fragment length polymorphism (AFLP) and microsatellites or simple sequence repeats (SSRs) have been used to study genome organization, varietal differences and diversity analysis in *Brassicas*. In recent years Simple Sequence Repeats (SSRs) or microsatellites have been recognized as useful molecular markers in marker assisted selection (MAS), for the analysis of genetic diversity, population analysis and other purposes in various species. So, SSR markers have become the marker of choice for linkage mapping and QTL studies of the crops due to their multi-allelic and co-dominant nature. Several hundred SSR markers have been developed from *Brassica.* Among the various types of molecular markers used, Simple Sequence Repeat (SSR) markers are the most preferred one because of their higher reproducibility, co-dominance nature and abundance, wide distribution throughout the genome, easy storability and multi-allelic variation (Powell *et al*., 1996). The strengths of SSR markers are due to its present in high numbers in eukaryotes. Also, it can be functional with low quantities of template DNA (10–50 ng/reaction) because of the PCR based technique also found effective with low quality of DNA. Microsatellites surpass other DNA based markers such as RFLPs are co-dominant but they have hybridization based problems and are less polymorphic than SSR markers, whereas RAPDs have low reproducibility therefore SSRs can analyze better genetic diversity than other molecular markers. Also, it is an improved technique which is more simple and efficient to find polymorphism. With the increasing population and improving life standards, *per capita* oil consumption has also increased therefore to meet the present oil requirements, there is an urgent need to increase the yield potential of *B. juncea* through genetic manipulations. The better utilization of a species for breeding and its adaptation to different environments depend on the level of genetic diversity it holds. As Indian mustard, contributes more than 80% to the total rapeseed-mustard production in the country and is an important component in the oilseed sector of country also it is known to be more drought tolerant and shattering resistant than *B. napus* and *B. rapa .* Vinu *et al.,* (2013) stated that for diversity studies reliability on molecular markers is worth proving and SSR markers are the stronger tools than quantitative traits in discriminating *B. juncea* genotypes.

Beside, As traditional breeding techniques failed to keep pace with demand and to provide sufficiently fast and efficient systems for crop improvement, another technology called tissue culture got developed. These techniques have certain merits over traditional methods. They produce exact copies of plants required that have desirable traits. They regenerate mature plants quickly. Multiple plants are produced in the absence of seeds or necessary pollinators to produce seeds. It conserves endangered plants. Diseases resistant plants are produced by micro propagation. Whole plants are produced regenerated from plant cells that are genetically modified. Some quality traits can improve through somaclonal variation. Hence, biotechnological tools including callus cultiure provides a good source of experimental tissue material from which whole plant can be regenerated by changing the nutrient and hormonal constituents in the culture medium and source of chromosomal variation.

In recent years, considerable research efforts have been made in tissue culture studies, transformations and molecular breeding of *Brassica****.*** Plant regeneration has been reported *via* organogenesis and somatic embryogenesis using various explants to optimize *in vitro* regeneration and development of homozygous lines through rapid and doubled haploids using microspores. Development of interspecific and intergeneric hybrids in sexually incompatible species of *Brassica* through somatic cell fusion has been made possible. Exploitation of somaclonal variation and *in vitro* selection has been attempted in recent years for improvement.

Erucic acid in the seed oil and glucosinolates in deoiled mealed are to nutrionally undesired rapeseed oil elements which affect quality of their nutrional value. The genetic manipulations of fatty acid biosynthesis pathway have been attempted. As such biotechnological, *in vitro* methods such as somaclonal variation along with conventional approaches have greater contributed towards developing desired genotype(s) for both nutritional and industrial purposes. Thus, *In vitro* cell selection is promising methods to get the low erucic acid cell line and to get low erucic acid regenerants. Few attempts have been made to standardize *in vitro* regeneration protocol for rapeseed-mustard using various explants by several workers. In view of the above facts, the study entitled **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** was carried out at Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior (M.P.).

Looking to these challenges the present study is formulated with the following objectives:

1. Assessment of various genotypes based on morpho-phsiological traits.
2. Fatty acid analysis in selected genotypes to select low erucic acid genotypes.
3. Comparative genetic diversity analysis based on morpho-physiological and biochemical traits.
4. Identification of *Brassica* genotypes for low and high erucic acid content using SSR markers.
5. To study the effectual combinations and concentrations of plant growth regulators on establishment of callus and cell suspension cultures followed by plantlet regeneration.
6. To characterize low and high erucic acid content in selected lines.
7. To select low erucic acid containing cell lines and *in vitro* regeneration.

CHAPTER - II

REVIEW OF LITERATURE

The present study entitled **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** was conductedduring years 2016-2017 and 2017-18.The literature pertains to the topic have been reviewed underthe following heads in this chapter.

2.1 Genetic diversity analysis in Genus Brassica

2.1.1 Morpho-physiological Analysis.

2.1.2 Biochemical diversity analysis based on fatty acid spectrum and erucic acid contents.

2.1.3 Molecular markers based analysis.

2.2 *In vitro* selection

2.1 Genetic diversity analysis in genus *Brassica*

2.1.1 Morpho-physiological analysis

The magnitudes of variability, heritability, genetic advance, correlation, path and genetic diversity analysis have been reported by many researchers on various genotypes for morpho-physiological traits. The same have been reviewed on the following lines.

Raliya *et al*. (2018) revealed that mean sum of squares due to genotypes (treatments) were significant for all the traits studied. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the characters evaluated. Most of the characters including yield ha^{-1} depicted high GCV, PCV and narrow sense heritability. Seed yield per hectare was found to be positively correlated with1000-seed weight, siliqua length, plant height, main shoot length and days to maturity at genotypic level. The magnitude of genotypic correlation coefficients was higher than their corresponding phenotypic coefficients. This pointed towards a strong inherent association between different traits studied and the phenotypic expression of these traits was less under the influenced by environment.

Dawar *et al*. (2018) evaluated the mean and component of variability. A positive correlation and path analysis was recorded for yield and various yield components. Seed yield per plant (g.) with plant height; number of primary branch, total no. of siliqua per plant and 1000-seed weight at genotypic level was recorded. Path coefficient analysis revealed that, the highest positive direct effect on seed yield (g) was exhibited by total no. of siliqua per plant, plant height, 1000-seed weight, Number of primary branches and number of seed per siliqua had direct positive contribution towards seed yield per plant. For mustard breeding seed per plant is variable having maximum potential of selection for improvement because this traits possessed high heritability, significant positive correlation and maximum positive direct effects with yield.

Kumar *et al*. (2018) estimated the genetic variability, heritability and genetic advance as percentage of mean for nine quantitative characters *viz.* plant height, number of primary branches, number of primary branches, number of siliquae per plant, siliqua length *(*cm), number of seeds per siliqua, number of seeds per plant, total seed yield (g) and test weight (g). Analysis of variance showed significant differences among the accession for all characters under study. Path coefficient analysiswas carried out using correlation coefficients to know the yield contributing traits having true associations with seed yield. Improvement in seed yield can be achieved by selection using the correlation and path analysis data generated in this study. Total seed yield/ plant were positively correlated with siliquae length. Number of seeds/ plant and test weight had higher phenotypic direct effects on total seed yield/ plant, revealing that indirect selection for these traits would be effective in improving seed yield. The high heritability coupled with high genetic advance for test weight would also be of great use for indirect selection for improvement in seed yield.

Devi (2018) investigated analysis of variance ratios for all the characters and were found highly significant. Coefficient of variation for GCV and PCV were found higher for biological yield per plant, grain yield per plant and silliqua on main raceme. Biological yield per plant, silliqua on main raceme, grain yield per plant and number of seed per silliqua exhibited higher heritability and higher genetic advance. Correlation study revealed that biological yield per plant and silliqua on main raceme exerted higher positive significant genotypic correlation with grain yield per plant and secondary branches per plant was found negatively correlated with grain yield per plant. Path analysis revealed that days to 50% flowering had maximum positive direct effect however the highest indirect effect of this trait was exhibited through biological yield per plant.

Gupta *et al*. (2018) assessed the correlation and path coefficients for yield and yield attributing traits under timely (TS) and late sown (LS) conditions. The genotypes were evaluated for thirteen quantitative characters *viz.,* days to 50% flowering, days to maturity, plant height (cm), number of primary branches plant^{-1}, number of secondary branches plant^{-1}, length of main raceme (cm), silique on main raceme (cm), seeds silique^{-1}, 1000-seed weight (g), biological yield plant^{-1} (g), harvest index (%), oil content (%) and seed yield plant^{-1} (g). Seed yield plant^{-1} showed highly significant and positive association with biological yield plant^{-1} followed by harvest index, silique on main raceme, length of main raceme, 1000-seed weight and secondary branches plant^{-1} both, under timely and late sown conditions. On the other hand plant height possessed highly significant and positive association with seed yield plant^{-1} only under timely sown condition. Path analysis identified biological yield plant^{-1} followed by harvest index, as major direct contributors towards seed yield plant^{-1} (both under timely and late sown conditions), while plant height emerged as most important indirect yield component under timely sown condition. Secondary branches plant^{-1} (at genotypic level) and Plant height (at phenotypic level) showed maximum indirect effect on seed yield plant^{-1}*via.,* biological yield plant^{-1} under late sown condition. The characters mentioned above should be given due consideration at the time of selection to develop stable high yielding genotypes in Indian mustard to sustain the production and productivity.

Roy *et al.* (2018) reported high heritability in conjunction with high genetic advance for seed yield per plant, number of pods per plant, leaf area index and secondary branches per plant suggesting predominant role of additive gene action for expression of these traits. The correlation study revealed that seed yield per plant was found to be positively and significantly correlated with pod length, number of seeds per pod and oil content while, negative significant association of seed yield was observed with days to first flowering andtotal biomass. Path analysis revealed that oil content, leaf area index showed the highest positive direct effect on seed yield per plant followed by number of seeds per pod and pod length which suggested that selection for oil content, leaf area index, number of seeds per pod and pod length would be quite effective in improving seed yield in late sown Indian mustard. The study also suggested importance of leaf area index, number of seeds per pod and pod length for improving seed yield in Indian mustard.

Singh *et al*. (2018) reported genetic divergence using Mahalanobis's D^2 statistics. Analysis of variance pointed towards considerable genetic variability among the 60 genotypes evaluated. On the basis of Tocher's clustering pattern, the genotypes were grouped into 9 clusters. Plant height, length of main raceme, 1000-seed weight and siliqua on main raceme were the major contributors for genetic diversity among the genotypes with 57.25%, 21.63%, 20.86% and 8.32%, respectively. The highest intra cluster distance was recorded for CVII (246.31) followed by C III (131.03) and C IX (127.63). The maximum inter cluster distance was observed between C VI and IX (2338.242) followed by C III and IX (2028.639), while the lowest in the C VIII and IX (274.3).

Amsalu *et al*. (2017) observed univariate analysis of variance and revealed the presence of considerable variability among genotypes for leaf length, length of petiole, leaf width and leaf area traits except seed yield per plant, leaf biomass per plant topped at 40th, 50th and 60th growth stage days of topping and number of intact leaves at flowering. The significant difference of results indicates that the presence of good opportunity to improve these characters using the various tested genotypes.

Saleem *et al*. (2017) reported significant diversity for different agro-morphological characters. Among all the evaluated accessions two genotypes having principal component with 26,813 and 26,817 showed great potential for seeds/silique, 1000-seed weight and seed yield/plant, respectively, these genotypes. Based on studies were recommended for future breeding programmes for achieving promising results.

Mohan *et al*. (2017) assessed 25 genotypes; these were grouped into 6 clusters based on D^2 analysis. The cluster-I with 9 strains had maximum genotypes among all the clusters followed by cluster-III, II, IV, V and VI. The inter cluster distance was recorded highest between cluster-III and cluster-IV (90.88). The minimum inter cluster distance was observed between cluster-I and IV (15.38).

Devi *et al*. (2017) showed the analysis of variance revealed significant differences among the genotypes for all characters under study. The genotypes were grouped into 7 clusters using Tocher's method, with cluster I containing maximum genotypes (18 genotypes) followed by cluster III (9 genotypes), cluster IV (8 genotypes), cluster II (6 genotypes), cluster VI (2 genotypes) while cluster V and VII with single genotype each. Root width and length ratio, siliqua per plant, main shoot length and 1000 seed weight were the major contributors for genetic diversity among the genotypes with 23%, 21.80%, 21.32% and 20.51% respectively. The cluster IV exhibited maximum intra-cluster distance followed by cluster VI (844.272) while maximum inter-cluster distance was found between cluster V and VII (7273.532). The study suggested that selection of diverse genotype containing desirable characters from the cluster and utilizing in hybridization programme will likely produce trangressive segregants and heterotic F_1s.

Rao *et al*. (2017) recorded the identification of nine principal components (PCs) which explained about 77% variability. The first principal component (PC1) explained 16.65% of the total variation. The remaining PC's explained progressively lesser and lesser of the total variation. Varimax Rotation enabled loading of similar type of variables on a common principal factor (PF) permitting to designate them as seed yield and component traits, leaf, oil and its quality factors. Based on PF scores, the genotypes *viz*., RH(OE)0801, EC597320, EC597341, EC597344, EC592579, EC592584 and JM6014(YS) have been identified superior for seed yield/plant, while the genotypes JM6009, JM6011, EC697334 and ZEM-1were found superior for oil content. Similarly, the genotypes JM6009, NUDBYJ-10, Pusa Mustard-21, RLC-2 and ZEM-2 showed superiority for erucic acid, whereas genotypes JM6004 (YS), JM6026 and EC552583 exhibited superiority for glucosinolate content. These genotypes may further be utilized in breeding programmes for evolving mustard varieties having high seed yield and oil content; and with superior oil quality. Hierarchical cluster analysis resulted into eight clusters containing one to 16 genotypes. The results of cluster and principal factor analyses confirmed each other.

Ali *et al*. (2017) recorded high level of variation for seed yield followed by maturity, glucosinolate contents, plant height and flowering time. Cluster analysis constructed by these workers categorized these 85 accessions into seven main groups, while in 2013 the same accessions were divided into six main groups. During 2012, first five principal components (PCs) accounted for 52.02% of variations among the studied accessions using morphological traits. Out of 52.02%, PC1 had 17.29%, PC2 contributed 10.13%, PC3 (9.51%), PC4 (7.98%) and the share of variability produced by PC5 was 7.11%. During 2013, the contributions of these accessions were 27.46% (PC1), 11.33% (PC2), 8.70% (PC3), 7.27% (PC4) and 6.38% (PC5) with an overall contribution of 61.14% variability.

Salam *et al*. (2017) noted presence of sufficient variability based on different biometrical analysis except for days to maturity and oil content (%). Relative magnitude of phenotypic co-efficient of variation was higher than the genotypic co-efficient of variation. The high GCV and PCV were observed by these workers for only two traits *viz*. number of branches per plant and harvest index (%). The traits plant height (cm), siliqua length (cm), number of siliquae per plant and seed yield per plant had moderate GCV and PCV. The highest heritability estimates were observed for the traits erucic acid content followed by plant height, branches per plant, seed yield per plant, siliqua length, days to 50%

flowering and harvest index (%). Genetic advance as percentage of mean was recorded higher for number of siliquae per plant, followed by seed yield per plant, days to maturity and plant height.

Kumar *et al*. (2016) reported that harvest index and total biological yield per plant exerted high significant positive correlation coefficients with seed yield at both genotypic and phenotypic level. Path analysis revealed that harvest index showed maximum positive direct effect, followed by total biological yield per plant, days to maturity, siliquae length at the time of maturity and seed yield per plant at both genotypic and phenotypic levels. According to these workers have concluded that two traits *namely* harvest index and total biological yield per plant exerted high correlation as well as direct influence on seed yield may be considered for selection and to improve the seed yield of the mustard genotypes.

Verma *et al.* (2016) performed genetic divergence analysis on the basis of. relative magnitude of D^2values; 60 genotypes of Indian mustard were grouped into five clusters and plant height, no. of siliquae on main shoot and days to maturity were found important discriminatory characters for the selection of diverse genotypes.

Worekeneh *et al*. (2016) had shown that there was significant different variation among genotypes in all traits compared in 49 Ethiopian Mustard. The significant difference pointed that geographical diversity could not necessarily be an index of variation and the factors other than geographic diversity such as genetic drift, selection pressure, closeness in pedigree and environment could be responsible for differential grouping of genotypes.

Dipti *et al*. (2016) recorded maximum value of phenotypic coefficient of variation for no. of pods on main branch (36.23) followed by oil content (31.65), siliqua density on main shoot (30.72) and thehighest genotypic coefficient of variation was observed for number of pods on main branch (33.49)followed by seed yield per plant (29.18) followed by oil content (28.89). Path coefficient analysis revealed that no. of pods on main branch had the highest direct contribution towards seed yield per plant followed by 1000-seed weight and siliqua density on main shoot (1.82) which suggested that selection for these traits would be quite effective in improving seed yield inIndian mustard. According to these investigations the mean performance ofF_1's (OR x RH 406 and OR x RH 832) was better than parental genotypes for no. of primary and secondary branches, no. of seeds per siliqua and seed yield per plant.

Neeru *et al*. (2015) measured principal factor analysis and identified 11 principal components (PCs) which explained about 75% variability. PC_1 had 13.19% of total variation in agromorphological traits, PC_2 depicted 10.07% of total morphological variability, and PC_3 accounted for 8.56%of the total variation. Varimax rotation enabled loading of similar type of variables on a common principal factor permitting to designate them as seed yield, maturity, leaf and siliqua characters and oil content factors. The genotypes JMM-937, RC-199, RH-0401(YS), Pusa Bold, Pusa Bahar and KM-888 were found to be superior on the basis of principal factor scores with regard to seed yield, its main components, and oil content, when both the principal factors were considered together. It is suggested that these genotypes may be future utilized inbreeding programmes for developing Indian mustard varieties with high seed yield and superior oil content. Hierarchical cluster analysis categorized all the 60 genotypes into 10 clusters containing one to 23 genotypes. Based on the inter-cluster distances, maximum genetic diversity was observed between clusters I and IV(221.4), followed between CII and IV clusters (200.5), C IV and C IX (191.8) and C IV and C X (181.5)indicating that genotypes from these clusters can usefully be hybridized for getting superior recombinants insegregating generations. The results of cluster and principal factor analyses confirmed each other.

Ram *et al*. (2015) while evaluating53 genotypes showed that various Indian mustard genotypesresponded differently for eight physiological traits under normal and high temperature stress conditions. Among the 53 genotypes included for the study, BPR-349-9, Urvashi, BPR-541-2, BPR-605-40, Pusa Tarak (EJ9912-13), RGN-48,BPR-549-2, DRMR-729 and DRMR-1918 were identified as heat-tolerant at seedling stage with heat susceptibility index (S) and yield stability ratio (YS), ≤ 0.5 and > 80%, respectively. The relative water content showed significant negative correlation with excised-leaf water loss (r = -0.385**) under heat stress condition. It is suggested that the higher membrane stability index and high population survival could have provided high temperature stress tolerance in genotypesDRMR-541-44 and RGN-48. Identified heat tolerant genotypes provide good resource to develop further improved genotype in future breeding programme in view of climate change.

Akabari and Niranjana (2015) studied genotypic variability for various traits. Higher values of phenotypic co-efficients of variation and genotypic co-efficients of variation were observed for number of secondary branches per plant, number of siliqua per plant and yield per plant showing higher magnitude of variability among the tested genotypes with respect to above characters. According to these workers, higher heritability estimates values were recorded for number of siliqua per plant, 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, siliqua length, protein content and plant height. These traits were less influenced by environmental factors and selection for them is fairly easy. Genetic advance as per cent of mean recorded was higher for yield per plant, number of siliqua per plant, number of secondary branches, number of seeds per siliqua, length of main branch, siliqua length, and 1000 seed weight, indicating that selection would be more useful based on these traits.

Lodhi *et al*. (2014) reported phenotypic and genotypic coefficients of variation were higher for important traits including number of secondary branches/ plant, seed yield/ plant, and 1000-seed weight. High heritability in conjunction with high genetic advance were observed for seed yield/ plant, number of secondary branches/ plant, 1000-seed weight, number of seeds/ siliqua, primary branch angle, number of primary branches/ plant, siliqua angle, siliquae on main shoot and siliqua length suggesting predominant role of additive gene action for expression of these traits. Seed yield/ plant was found to be positively and significantly correlated with number of primary branches/ plant, number of secondary branches/ plant, primary branch angle, main shoot length, siliqua length, and number of seeds/ siliqua; seed yield/ plant had negative association with oil content. Path analysis revealed that main shoot length, number of primary branches/ plant, number of seeds/ siliqua, and primary branch angle showed positive direct effect on seed yield/ plant which suggested that selection for number of primary branches/ plant, primary branch angle, main shoot length, number of seeds/siliqua would be quite effective in improving seed yield in Indian mustard.

Bind *et al*. (2014) found maximum biological yield per plant and minimum days to maturity as noticed by genotypic coefficient of variation. Heritability estimate washigh for 1000 seed weight, day to maturity, day to flowering, plant height and main shoot length. Genetic advance percent was high for biological yield per plant, 1000 seed weight, yield per plant, number of secondary branches and main shoot length. Apositive correlation with seed yield per plant both at phenotypic and genotypic levels except days to 50% flowering and days to maturity. The path coefficient analysis at genotypic level showed that biological yield per plant had the highest direct positive effect on seed yield per plant followed by harvest index, 1000seed weight, no. of seeds per siliqua and no. of primary branches. At phenotypic level, highest negative direct effect on seed yield per plant was observed for plant height.

Singh *et al*. (2014) reported variability for 18 agro-morphological traits and 6 computed variables in 62 extant varieties. The multivariate analyses based upon principal components and hierarchical cluster revealed 4 distinct groups of varieties based upon geographical region of their originating centers. Most of the variability could be accounted for leaf and stem characteristics and phenological stages. Closely resembling as well as distinct varieties were identified. Varieties from different clusters based upon genetic distance were suggested for utilization in hybridization programme.

Ray *et al*. (2014) presented test weight had the highest correlation with seed yield followed by net assimilation rate (NAR) at 40 - 75 DAS and dry matter accumulation (DMA) at 75 DAS, respectively. Out of nineteen, only two factors had given value greater than one and these components accounted for 72.42% of total variance. The loadings of each variable onto each component were analyzed from the rotated factor matrix obtained through varimax rotation. First component accounted for 51.84% of total variation and associations of traits that are highly correlated with seed yield among which NAR at 40 - 75 DAS and at 75 -110 DAS and test weight were most important. Second component with 20.58% of total variation had higher loadings of variables having comparatively lower association with seed yield and among those, relative growth rate (RGR) at 75-110 DAS, plant height at 110 DAS and number of siliquae per plant were most important.

Rathod *et al*. (2013) reported that the seed yield per plant had significant and positive correlation with harvest index, biological yield per plant, number of siliquae per plant and number of secondary branches per plant at both genotypic and phenotypic levels. According to them biological yield per plant had significant and positive association with harvest index and oil content at genotypic level. Further, path coefficient analysis of twelve yield contributing characters clearly indicated that biological yield per plant showed the highest positive direct effect on seed yield followed by harvest index, length of siliqua, days to 50 per cent flowering, oil content, number of secondary branches per plant and number of primary branches per plant, which indicated positive direct effects in descending order and other characters contributed indirectly towards seed yield in Indian mustard.

Mekonnen *et al*. (2013) evaluated association of agronomic traits of thirty-six Ethiopian mustard (*Brassica carinata*) genotypes. The correlation and path coefficient analysis were conducted for 15 and five traits respectively at phenotypic and genotypic levels. Seed yield per plot was positively correlated with oil yield per plot, biomass per plot, plant height, days to maturity, grain-filling period and secondary branches per plant and 1000-seed weight at both genotypic and phenotypic levels. However, it was negatively correlated with days to flowering, number of pod per plant, number of seeds per pod and pod length at phenotypic level whereas primary branches per plant and harvest index at genotypic level and oil content were negatively correlated with at both levels. Phenotypic and genotypic path coefficient analysis for harvest index had exerted positive direct effect on seed yield. Grain filling period and harvest index had exerted positive direct effect on oil content at genotypic level. Day to maturity, grain filling period, secondary branches per plant, harvest index and seed yield per plot had exerted negative effect on oil content at phenotypic level.

Zada *et al*. (2013) observed considerable variation in seed yield (kgha^{-1}) whereas a moderate variability was observed in plant height, main raceme length, silique/main raceme, glucosinolate contents and erucic acid. Hierarchical cluster analysis categorized the 134 accessions into seven main clusters. First three principal components (PCs) accounted for a total of 39.03% of variability among the accessions using agro-morphological traits. PC1 had 17.79% of total variation in agro-morphological traits; PC2 depicted 11.45% of total morphological variability, while PC3 accounted for 9.80% of the total variation. Based on greater yield potential, seed yield per plant, 1000-seed weight, oil contents, protein contents and oleic acid four promising genotypes were identified for breeding and variety development programmes in Indian Mustard.

Pandey *et al*. (2013) conducted D^2 analysis to measure the genetic diversity among the genotypes revealed significant differences. The 45 genotypes were grouped in 8 clusters using Tocher's method. Intra cluster distance was maximum for cluster VI followed by cluster III. The maximum inter-cluster distance was found between cluster II and III indicating high genetic divergence among genotypes of these groups. Maximum contribution towards the divergence was accountable to 1000-grain weight (46.87%) followed by seed yield/ plant (20.91%) and number of silique on main raceme (8.38%). The genotypes of cluster II and cluster VIII constitute high cluster mean for different trait along with early maturity. These genotypes may be utilized in different combinations for selection of transgressive segregants for early maturity and better performance for yield.

Shathi *et al*. (2012) grouped 25 mustard (*Brassica* sp. L) genotypes into six clusters. Cluster II had the maximum number (13) of genotypes, while Clusters IV, V, and VI had the minimum number (2) of genotypes. The highest inter-cluster distance was observed between Clusters I and IV, while the lowest inter-cluster distance was observed between Clusters V and VI. Cluster II (D =

4.91) had the highest intra-cluster distance, while Cluster II (5.607) had the lowest intra-cluster distance. The characteristics such as plant height, number of secondary branches per plant and number of siliqua per plant contributed greatest towards the divergence among evaluated mustard genotypes.

Patel and Vyas (2011) investigated heritability and genetic advance study using three high yielding varieties *viz*., GM1, GM2 and GM3 and two ‘0’ and/or ‘00’ quality genotypes NUDH YJ3 and EC 278811 over two environments created by two date of sowing. High heritability (broad sense) associated with moderate to high genetic advance was recorded for 1000-seed weight, seed yield per plant, harvest index, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic and erucic acid contents. The study suggested that these traits can be further improved through selection in segregating generations. Moderate to high heritability alongwith low genetic advance observed for days to maturity, days to flowering and oil content suggested very remote possibilities of improving these traits through straight selection; hence *inter se*-crossing of desirable recombinants keeping adequate population size could be beneficial.

Yadava *et al*. (2011) studied G x E interaction on 14 quantitative traits. The mean, range, phenotypic, genotypic and environmental variance, genotypic and phenotypic coefficient of variation, heritability in broad sense and genetic advance were calculated. Path coefficient analysis was carried out using correlation coefficients to know the yield- contributing traits having true associations with seed yield. The high heritability coupled with higher genetic advance for 1000-seed weight was found to be of significant use for indirect selection for improvement in seed yield.

Singh *et al*. (2011) recorded Cluster means for different clusters and inter cluster distances were used to judge the importance of different clusters in the improvement programme. Evaluated genotypes were grouped into thirteen clusters based on the economic traits. Cluster I was the largest, consisting of nine genotypes. Cluster X showed maximum genetic distance from cluster VII, suggesting wide diversity between them. When the quality traits were considered for clustering the genotypes grouped into six clusters, cluster VI (largest cluster) consisted of fourteen genotypes and cluster VI had the maximum inter cluster distance from cluster III which showed presence of wider diversity among the genotypes of these two clusters.

Doddabhimappa *et al*. (2009) revealed that seed yield per meter was highly and significantly correlated with seed yield per plant, number of siliquae per plant, number of primary and secondary branches per plant, biological yield per plant, 1000- seed weight, number of seeds per siliqua at both genotypic and phenotypic levels in protected and unprotected conditions. Further, path coefficient analysis of eleven yield contributing characters clearly indicated that number of siliquae per plant showed highest positive direct effect on seed yield in both protected and unprotected conditions even though number of primary branches per plant and number of secondary branches per plant, biological yield per plant, harvest index indicated positive direct effects in descending order and other characters contributed indirectly towards seed yield. The present study has clearly indicated the need for giving due weightage for number of siliquae per plant, number of secondary branches per plant, harvest index and biological yield per plant for improving seed yield in mustard.

Patil *et al.* (2006) revealed thirteen genotypes along with four check varieties viz., JL-24, GPBD-4, Dh-86 and Dh-3-3-30 were evaluated for association analysis at six different locations namely Dharwad, Sankeshwar, Nippani, Bagalkot, Raichur and Kawadimatti during kharif 2003. The correlation study revealed that pod yield per plant had significant positive association with number of pods per plant, shelling per cent and SMK per cent at minimum three locations. Path analysis also indicated that three traits viz., number of pods per plant, shelling per cent and sound mature kernel per cent had the maximum direct effect on pod yield per plant at minimum three locations

Shah *et al*. (2002) studied the correlation coefficients were worked out among various traits *viz*. days to 50% flowering, days to maturity, plant height, no. of primary and secondary branches, number and length of siliquae, test weight and seed yield per plant in F_4 progenies among different crosses during *Rabi*1997-1998. The study revealed that number of siliquae per plant had the strongest positive and significant correlation with yield at genotypic as well as phenotypic levels. The characters *viz.,* days to50% flowering, number of primary and secondary branches, plant height, number of seeds per siliquae and number of siliquae per plant had shown positive correlation mutually as well as with yield at both the levels.

2.1.2 Biochemical Diversity Analysis based on Fatty acid Spectrum and Erucic acid contents

Rai *et al*. (2018) reported significant differences (p≤0.05) among the genotypes for the oil quality, *viz*. oil content and fatty acids content. Oil content was in the range of 32.67–39.47%, 37.82–40.56% and 40.35–41.43% in *Brassica juncea*, *Brassica napus* and *Brassica rapa* seeds, respectively. The saturated fatty acid (Palmitic acid) content were in the range of 3.08–3.85, 3.70–5.15, 2.75–3.73% in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively and oleic acid content were in the range of 0.80–48.70, 16.15–37.98 and 6.21–16.15 in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively. Significant variation (p≤0.05) was also observed for linoleic acid content and linolenic acid. Linoleic acid content varied from 11.00- 45.30, 18.57-26.93 and 14.08- 18.18% in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively and linolenic acid content varied from 11.10- 26.72, 9.99- 17.23 and 9.82- 26.66% in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively. The Erucic acid, another important trait also differed significantly amongst the *Brassica* species genotypes being 0.80- 49.40, 10.04- 34.96 and 43.77- 49.99%. The minimum Erucic acid content was recorded in *Brassica juncea* genotypes PM-24 (0.80%) and significantly *at par* with PM- 21, PM-22, Pusa Karishma and Nov Gold, whereas maximum erucic acid content were recorded in Pusa Bold (49.40%), DGS-1(34.96%) and RSPT-02 (49.99%) in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively. Significant variability in fatty acids content was noted in rapeseed mustard.

Balalic *et al*. (2017) determined the variability of oil and protein content in winter rapeseed cultivars affected by seeding date. Four cultivars (Banaćanka, Slavica, Express, Valesca) were sown at six seeding dates (SD1-21 August, SD2-31 August, SD3-10 September, SD4-21 September, SD5-1 October, SD6-9 October) in four replications across two cropping seasons. The trial was arranged as a randomized complete block design. The effect of year, cultivar, and seeding date were highly significant for oil and protein content. Increased oil content in the second year (2010/2011) was related to weather conditions which were favorable for rapeseed. However, protein content was significantly higher in the first year (2009/2010). Oil content ranged between 41.19% (Valesca) and 42.69% (Express). Significantly lowest oil content across seeding dates was found in SD6 (40.67%) and highest in SD4 (41.86%) and SD1(41.61%). Valesca showed significantly highest mean protein content (21.54%). Protein content was highest in SD6 (20.18%). Oil content decreased with delayed seeding. Between oil and protein content highly significant negative correlation (r = -0.730) was stated. Seeding date showed a significant effect on oil and protein content in rapeseed. Cultivar Express and SD4 can be pointed out, based on the achieved oil content and cultivar Valesca for high protein content.

Rahman*et al*. (2017) evaluated the biochemical properties and chromatographic picturesque of various rapeseed oils namely mustard (wild and hybrid), Rai (wild and hybrid) and canola oil in both raw and fried state. The qualitative tests such as acid value, iodine value, peroxide value, saponification value, unsaponifiable matter and Rechert-Meissl value were determined to differentiate the better quality of these consumable oils. Among five types of rapeseed oils, canola oil could be the best choice for consumption due to its lower erucic acid (6%) and high polyunsaturated fatty acids content, while the erucic acid content of mustard (wild), mustard (hybrid), rai (wild) and rai (hybrid) were 51.56, 64.82, 56.31 and 67.98% respectively. Canola oil also had the lowest level of acid value, peroxide value, saponification value and Reichert Meissl value but higher level of iodine value. On the other hand, rai (hybrid) fried oil was the lowest quality of consumable oil in comparison with other forms of rapeseed oils. Raw seed oils were also better than fried oils.

Hossain *et al*. (2015) made comparative evaluation of its physicochemical properties, seed weight, moisture, ash, carbohydrates, protein, fat, total energy and minerals. Among these varieties, the highest grain weight was obtained from BARI Sarisha-13(4.38g) and lowest grain weight obtained from BARI Sarisha-9(3.06g). In case of proximate analysis, the highest protein content and the highest carbohydrate were recorded from BARI Sarisha-15 (28%) and BARI Sarisha-13 (17.02%) respectively. The oil content of different varieties of mustard and rapeseed varied from 38.75% to 42.25%. BARI Sarisha-14(554.3 kcalg^{-1}) contained the highest amount of total energy. The highest amount of calcium content (2.7%) and the highest amount of Magnesium content (0.739%) were attained from BARI Sarisha- 9. Substantial genetic variability exists for chemical composition and nutritional traits which could be utilized to suggest the future strategy for the nutritionist, health advisors and feed manufacturers.

Sarwar *et al*. (2014) compared fatty acid profile of the commercial mustard oil (Industrially manufactured) and *ghani* (traditional method of oil processing) mustard oil. The purified fatty acid methyl esters of both the oils, analyzed by Gas-Liquid Chromatographic (GLC) analysis. Variation in percentage of erucic acid in the commercial mustard oil as compared to *ghani* showed the percentage of erucic acid (22:1) in the commercial and *ghani* mustard oils were recorded was 41.80% and 51.98% respectively.

Kumar (2013) found significant differences for all the quality characters investigated. The environmental effects significantly influenced erucic and oleic acid content. The genotype × environment interactions were found non-significant for all the characters. The coefficients of variation at phenotypic level varied from 4.6% for oil content to 50.9% for oleic acid. The genotypic coefficients of variability were higher for oleic, palmitic + stearic, erucic and linolenic acid, erucic acid and palmitic acid + stearic acid had the least genotypic variation (GCV: 16.3 to 16.9%). The heritability in broad-sense was relatively high for oleic (61.5%) and erucic acid (56.3%). 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. Erucic acid negatively and significantly correlated with the rest of the fatty acids except linolenic acid and significant correlation with oleic (r = -0.536) and eicosenoic acid (r = -0.260).

Although, oil content had very low direct effect (-0.011) on erucic acid but its positive association was the result of its strong positive indirect effect through oleic acid (0.435), which was partially neutralized by negative indirect effects (-0.112) through linolenic acid.

Toosi *et al.* (2013) analyzed the oil, protein and glucosinolate contents in the seeds of *Brasica juncea* var. Ensabi were determined as 34.6%, 32.1% and 34.8 µmol/g respectively. Fatty acid analysis of the Ensabi oil revealed the presence of significant amount of unsaturated fatty acids, namely oleic acid (18.2%) and linoleic acid (16.9%) while lower quantity of saturated fatty acids: stearic acid (1.4%) and palmitic acid (2.8%). It also contained a very high amount of erucic acid (42.0%) whereas other polyunsaturated fatty acids, such as linolenic acid (5.5%), eicosenoic acid (8.8%) showed moderate presence. This composition was similar to the one reported for rapeseed (high erucic) oil and could not meet the specifications for human nutrition due to the very high content of long chain unsaturated fatty acid, erucic acid against its absence as recommended by FAO and WHO.

Khan *et al*. (2013) revealed mustard oil has low saturated fat as compared to other cooking oils. It is basically consist of oleic acid, erucic acid and linoleic acid. Besides It is also loaded with essential vitamins. The samples analyzed for fatty acids, free fatty acids, and peroxide value, iodine number, saponification number were determined by these workers according to the standard procedures.

Kumar *et al*. (2010) conformed using partial least square regression, calibration for non-destructive estimation of erucic acid and glucosinolate contents in seeds of rapeseed-mustard by Fourier transform near infrared reflectance spectroscopy (FT-NIRS) was developed. The calibration developed showed a very close relationship between the reference method for erucic acid (gas chromatography) and glucosinolate content (palladium complex formation) and NIR spectral data from7502.1 to 5444.6 cm. The coefficients of determinations were 97.16% and 98.34% for erucic acid and glucosinolate contents, respectively. Mortuza *et al*. (2006) were characterized rapeseed/mustard cultivars (*Brassica campestris, B. juncea* and *B. napus*) for their fatty acid composition. Erucic acid was one of the main fatty acids, in proportions ranging from 21.59 to 51.57% followed by oleic acid ranging from 7.03 to 25.21%. The other major monounsaturated fatty acid had oleic acid, which accounted for 4.12–12.06%.The major polyunsaturated fatty acids were linoleic and linolenic acid, ranging from 11.79 to 16.89% and6.29 to 11.15%, respectively. Among the saturated fatty acids, palmitic acid accounted for 1.20–3.36%.Erucic acid content was negatively correlated with all other major fatty acids. The cultivars Binasarisha-3,Binasarisha-4, Binasarisha-5, MM 22-12-98, MM 2-16-98, MM 36-6-98, MM 49-3-98, MM 34-7-98 andBarisarisha-8 of *B. napus* had significantly ($P < 0.001$) low levels of erucic acid and high levels of oleic, linoleic and linolenic acids among all the cultivars.

Khan *et al*. (2008) reported highly significant genetic variation among *Brassica* populations for oil content, glucosinolate, oleic acid, linolenic acid and erucic acid contents. Non-significant ($P>0.05$) variation was recorded for protein content. According to these workers, genetic variances for most of the traits were generally 3 to 15 times greater than the environmental variance indicating significant genetic control over expression of quality traits. Recorded data showed heritability estimates were high(>0.70) for glucosinolate, linolenic acid, oleic acid and erucic acid contents, while it had low heritability (< 0.50) for protein content.

Chauhan *et al*. (2008) made comprehensive evaluation of exotic rapeseed mustard genetic resources for quality characteristics and Identified and characterized drought tolerant genotypes of Indian mustard. Further quality characteristics and their interrelationship have been reported.

Kaushik and Agnihotri (2000) studied the Indian rapeseed-mustard germplasm lines and some newly developed low-erucic-acid strains. Based onthe GLC analysis pointed out that the rapeseed-mustard varieties being commonly grown in India are characterized by high erucic acid content (30±51%) in the oil with low levels of oleic acid (13±23%). However, from the recently developed low-erucic-acid strains, several lines have been identified showing comparatively high oleic acid (60±70%), moderate to high linoleic acid(13±40%) and low linolenic acid (<10%) contents.

2.1.3 Molecular Markers based Diversity Analysis

Bharti *et al*. (2018) found based on using 4 SSR markers in 71 genotypes were clustered into three groups. All the genotypes were clustered in three groups by using DARwin software. Cluster I had seven genotypes *viz.,* RSPR-01, PM-21, PM-22, RB-50, Urvashi, Nov. Gold and Pusa Bold followed by cluster II (five genotypes *viz.,* RSPR-03, Varuna, PusaKarishma, RL-1359 and Kranti and cluster III (two genotypes *viz.,* PM-24 and NRCDR-2) for molecular characterization of erucic acid and glucosinolate.

Salam *et al*. (2018) carried out genetic diversity analysis at molecular level in 24 genotypes of toria, two major cluster with 53% genetic similarity in which two genotypes named GPT-13 and GPT-59 comes under one cluster and remaining 22 falls under second cluster. Genotypes GPT-1 and GPT-43 and genotype GPT-125 and GPT-126 exhibited 87% genetic similarity with each other. These results indicated the existence of sufficient variability among genotypes and there are very much chance of improvement either through selection or through hybridization by getting heterosis.

Patel *et al*. (2018) reported 358 SSR markers, 42 were found polymorphic and 154 were monomorphic. A total of 225 alleles, ranging from 2 to 4 were amplified. The PIC value ranged from 0.427-0.730. Jaccard’s similarity coefficients was generated between these F_2 populations. F_2 population was grouped in 2 major clusters and 37 sub-clusters at a similarity coefficient of 0.58. The allelic information indicated that the genotypes used in the present study have distinct genetic patterns. It was concluded that SSR markers are more reliable and will be helpful in selecting diverse genotypes which can be used to identify QTL related to salinity.

Thakur *et al*. (2018) assayed 124 *Brassica*-derived SSR loci, 100% cross-transferability for *B. juncea* and three subspecies of *B. rapa*, while lowest cross-transferability (91.93%) was obtained for *Eruca sativa*. The average % age ofcross-transferability across all the seven species was 98.15%. The number of alleles detected at each locus ranged from one to six with an average of 3.41 alleles per primer pair. Neighbor-Joining-based dendrogram divided all the 40 accessions into two main groups composed of *B. juncea*/*B. nigra*/*B. rapa* and *B. carinata*/*B. napus*/*B.oleracea*. C-genome of oilseed *Brassica species* remained relatively more conserved than A and B genome. A genome present in *B. juncea* and *B. napus* seems distinct from each other and hence provides great opportunity for generating diversity through synthesizing amphidiploids from different sources of A genome. *B. juncea* had least intra-specific distance indicating narrow genetic base. *B. rapa* appears to be more primitive species from which other two diploid species might have evolved.

Singh *et al*. (2017) detected polymorphism using 453 SSRs and 139 (30.7%) with 308 alleles. Polymorphic information content ranged from 0.101 to 0.668, with the average value of 0.474, revealing that much variation was present among these genotypes. The cluster analysis gave three major groups where white rust resistant genotypes were grouped in one major cluster, double low quality genotypes in second cluster while the recipients were grouped in the third cluster indicating that grouping of genotypes based on SSRs corresponded well to their known pedigree data. These observations suggested that SSRs are efficient for evaluating genetic variation and relationships among different varieties of mustard.

Saini *et al*. (2016) studied the single nucleotide polymorphism (SNP) andfatty acid elongase 1(*FAE1.1* and *FAE1.2*) gene was exploited to expedite the breeding programme. The paralogs of *FAE1* gene were sequenced from low erucic acid genotype Pusa Mustard30 and SNPs were identified through homologous alignment with sequence downloaded from NCBI Gene Bank. Two SNPs in *FAE1.1* at position 591 and 1265 and one in *FAE1.2* at 237 were found polymorphic among low and high erucic acid genotypes. These SNPs either create or change the recognition site of restriction enzymes. Transition of a single nucleotide at position 591 and 1265 in *FAE1.1*, and at position 237 in *FAE1.2*, leads to a change in the recognition site of *Hpy99I*, *BglII* and *MnlI* restriction enzymes, respectively. Two CAPS markers for *FAE1.1* and one for *FAE1.2* were developed to differentiate low and high erucic acid genotypes. The efficiency of these CAPS markers was found 100 per cent when validated in *Brassica juncea*, and *B. nigra* genotypes and used in back-cross breeding. These CAPS markers provided to facilitate marker-assisted selection for improvement of oil quality in *Brassica juncea*.

Sudan *et al.* (2016) found molecular diversity analysis of Indian and Exotic genotypes of Brassica juncea genotypes was carried out using simple sequence 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 polymorphic information content (PIC), respectively. The genotypes were grouped into three distinct clusters based on unweighted neighbour joining and population structure analyses.

Fayyaz *et al*. (2014) represented using 12 SSR primer combinations reported a total of 33 alleles, of that 32 were polymorphic loci, whereas only one was monomorphic locus. Primers BRMS-19 and BRMS-40 were highly polymorphic producing 4 bands each. Primer Ra2-D04 was less polymorphic and it produced only one band. The proportion of polymorphic loci was 95.83% which pointed towards high genetic diversity among the progenies. Considerable genetic diversity was recorded among the F_2 segregating progenies and their parents using SSR markers.

Gupta *et al*. (2014) assessed genetic variation among 23 genotypes of *Brassica juncea* L. cultivated in North India. Four different genotypes of other *Brassica* species were also included during study. The genetic diversity analysis among the total 27 genotypes showed the polymorphism percentage of more than 91% and86.66% using the fifteen reproducible RAPDs and three EST SSRs respectively. Moreover the PIC values observed corresponds to 0.303 with 4.44 marker index and 6.89 resolving power for RAPDs, however, SSRs showed the PIC value of 0.281,with 0.94 marker index and 0.269 resolving power. The parameters calculated to estimate the discriminatory power presented a significant correlation. Their high values depict the potential of primers for distinguishing the genotypes. The cluster analysis based on UPGMA separated the genotypes in two major groups. All the 23 genotypes of *Brassica juncea* are grouped in one major cluster and 4 genotypes of other *Brassica* species are grouped in different cluster.

Priyamedha *et al*. (2014) evaluated of double low quality lines (seed oil with < 2% erucic acid and glucosinolate content < 30 µmoles/gram defatted seed meal) in Indian mustard. Diversity analysis using RAPD markers produced a total of 104 loci with an average of 8.6 bands per primer. The range of amplified bands was between 300bp to 3kb. The largest fragment of 3kb was amplified with three primers, A-5, A-19 and B-05. On an average 84% similarity matrix (SM) was observed between all thirty genotypes. The most diverse genotype was RBJ-97001 with average genetic diversity of 29% while RBJ-02017 showed the highest average genetic SM (87%) to all other genotypes. The genotypes RBJ-07017 and RL-18 were observed most similar to each other with genetic SM of 95%, whereas, Raya 49/2 and RBJ-97001 were found the most diverse lines with genetic SM of 71%. Unweighed pair group method of arithmetic means cluster analysis indicated that 30 genotypes were capable of being classified into two major groups, A and B. Both groups were further divided into two sub-groups (Tahira *et al*., 2013).

Vinu *et al*. (2013) analyzed 44 Indian mustard (*Brassica juncea*) genotypes including varieties/purelines from different agro-climaticzones of India and few exotic genotypes (Australia, Poland and China). For this, they used A and B genome specific SSR markers and phenotypic data on 12 yield and yield contributing traits. Out of the 143 primers tested,134 reported polymorphism and a total of 355 alleles were amplified. Dendrograms based on Jaccard's similarity coefficients and Manhattan dissimilarity coefficients were generated based on an average linkage algorithm (UPGMA) using marker data and phenotypic data. Genotypes were grouped into four clusters based on genetic distances. Both the clustering patterns based on Jaccard's similarity and Manhattan dissimilarity coefficients, independently, discriminated the genotypes effectively as per their pedigree and origin. PCoA revealed that, the grouping of genotypes based on

SSR marker data is more convincing than phenotypic data, however, the correlation between phenotypic and genetic distance matrices was observed to be very low (r=0.11). Hence, for diversity studies reliability on molecular markers is worth proving and SSR markers found to be stronger tools than quantitative traits in discriminating *B. juncea* genotypes.

Chandra *et al*. (2013) studied 37 diverse Indian mustard genotypes including recombinant lines, indigenous lines, exotic lines with *Alternaria* blight tolerant (PAB 9511) and susceptible check (Varuna) based on genetic analysis using Ten A and C genome specific SSR markers showing 97.56% polymorphism were grouped into 5 clusters. Presence of unique band helped in the identification of specific genotype.PAB-09-1, PAB-05-16,PAB09-11, PAB-09-9, EC399312 and EC399301shared common cluster with PAB 9511 and were *at par* in their disease score index to PAB 9511 as well. The study showed these primers can be used as maker for *Alternaria* blight screening.

Yousuf *et al*. (2013) attempted four random primers to examine genetic diversity analysis of five mustard varieties. A total of 20 bands were scored corresponding to an average of five bands per primer. Level of genetic polyphorphism was in the range of 0 to 66.66%. UPGMA revealed the maximum similarity of variety Kanti with Varuna (similarity indices 0.9677) while distantly related varieties were Basanti and Rohini. The randomly amplified polymorphic DNA (RAPD) cluster pattern showed two major clusters namely cluster-I and cluster -II. Cluster -I includes Kanti and Varuna and cluster -II includes Rohini and Urvashi. The variety Basanti occupies a distinct place as revealed in the dendrogram.

Yadav and Rana (2012) reported 156 amplified bands with an average of 7.8 bands per primer out of which 115 were polymorphic. Size of amplified fragments ranged from 100 bp to 1500 bp and number varied from 4 to 14. ISSR data generated to compute genetic similarities based on Jaccrad's similarity coefficient, it ranged from 0.50 to 1.0. UPGMA (Unweighted pair-group method with arithmetic average) dendrogram based on similarity coefficient, all the Indian mustard genotypes were grouped into three major clusters Two dimensional and three dimensional analyses supported the cluster analysis observations. Genotypes, Kranti and Rohini were found to be least diverse. BEC 144 found to be most diverse genotype with aseparate cluster. Phenotypic variation was also analyzed and revealed some genetic relationship among genotypes which were evaluated for seed yield and other phenotypic traits.

Nirala *et al*. (2011) developed high density molecular map based on 707 expressed sequence tag-derived simple sequence repeat markers (EST-SSRs) and used for development of a high-density integrated map using four individual mapping populations of *B. rapa*. This map contains a total of 1426 markers, consisting of 306 EST-SSRs, 153 intron polymorphic markers, 395 bacterial artificial chromosome-derived SSRs (BAC-SSRs) and 572 public SSRs and other markers covering a total distance of 1245.9 cM of the *B. rapa* genome. Allelic diversity analysis in 24 *B. rapa* germplasm lines using 234 mapped EST-SSR markers showed amplification of 2 alleles by majority of EST-SSRs, although amplification of alleles ranging from 2 to 8 was found. Transferability analysis of 167 EST-SSRs in 35species belonging to cultivated and wild *Brassica* relatives showed 42.51% (*Sysimprium leteum*) to 100%(*B. carinata, B. juncea*, and *B. napus*) amplification. According to them EST-SSRs and high-density linkage map based on highly transferable genic markers would facilitate the molecular mapping of quantitative trait loci and the positional cloning of specific genes, in addition to marker-assisted selection and comparative genomic studies of *B. rapa* with other related species.

Tian *et al.* (2011) studied the β-ketoacyl CoA synthase encoded by fatty acid elongase 1 gene (BnFAE1.1) is a rate-limiting enzyme regulating biosynthesis of erucic acid in rapeseeds (Brassica napus). To develop low level of erucic acid in rapeseeds by intron-spliced hairpin RNA, an inverted repeat unit of a partial BnFAE1.1 gene interrupted by a spliceable intron was cloned into pCAMBIA3301, and a seed-specific (Napin) promoter was used to control the transcription of the transgene. Transgenic plants (4) harboring a single copy of transgene were generated. Expression of endogenous BnFAE1.1 gene in developing T3 seeds was significantly reduced. In mature T3 seeds, erucic acid was decreased by 60.8 to 99.1% compared with wild type seeds, and accounted for 0.36 to 15.56% of total fatty acids. The level of eicosenoic acid was also greatly decreased. Furthermore, it resulted in a significant increase in the level of oleic acid, but total fatty acid content in T3 seeds was the same with that in wild type seeds. In conclusion, the expression of endogenous BnFAE1.1 was efficiently silenced by the designed RNAi silencer, causing a significant down-regulation in the level of erucic acid.

Abbas *et al*. (2008) conducted diversity analysis using Insulin Growth like Factor (IGF) primer sets and estimated genetic relationship among 5 F_2 segregating population of *Brassica* along with 9 parental lines. Twenty-nine alleles were amplified using IGF primer sets. Mean genetic distance estimates ranged between 0.25-1.00 (G.D = 0%-100 %), respectively. Size of scorable fragments ranged from approximately 250 to >2000 bp. A high level of genetic dissimilarity (GD= up to 100%) was recorded among all the genotypes. Based on cluster analysis diverse genotypes were identified which could provide resource for breeding programmes aimed at creating genetic variability in local germplasm lines.

Saha *et al*. (2008) explored the genetic diversity and relationship among nine *Brassica* varieties off our different species using Random Amplified Polymorphic DNA (RAPD) markers. In total, 59 reproducible DNA bands were generated by four arbitrarily selected primers, of which 58 bands were proved to be polymorphic. These bands have had range from 212 to 2272 bp in size. The highest proportion of polymorphic loci (37.29 %) was found in BARI sarisha-12 (*B. rapa*) and the lowest was 8.47 % for both in BINA sarisha-4 (*B. napus*) and Rai-5 (*B.juncea*). The highest intra-varietal similarity index was found in Alboglabra while the lowest value was found inBARI sarisha-12 which suggested that the former one is the least diversified variety and the later is the most diversified variety among the studied varieties. UPGMA cluster analysis indicated that nine accessions were capable of being classified into two major groups. One group consists of BARI sarisha-12, Agrani and Sampad of *B. rapa*(2n =20, AA); Daulot and Rai-5 of *B. juncea* (2n =36, AABB) and Alboglabra (*B. oleracea,* 2n

=18 CC) whereDaulot and Rai-5 showed the lowest genetic distance of 0.049. Another group contains BINA sarisha-4, BINAsarisha-5 and BARI sarisha-13 all of which represent *B. napus* where BINA sarisha-5 and BARI sarisha-13 showed genetic distance of 0.071. The highest level of genetic distance was found between the varieties of BINA sarisha-4and Rai-5 followed by the varietal pairs BINA sarisha-4 and Daulot.

2.2 *In vitro* selection

Biswas *et al*. (2017) compared callus induction competence of *Brassica species*. Regeneration *via* direct organogenesis using cotyledon and stem explants of *Brassica napus*, *Brassica campestris* and *Brassica juncea* have been reported using various media composition and PGRs. Highest frequency of callus formation was recorded in MS medium containing 2.0 mgl^{-1} BAP, 0.5mgl^{-1}NAA and 2.0 mgl^{-1} $AgNO_3$ with both stem and cotyledon explants. Stem explants was found to be most responsive for callus induction as compared to cotyledon. Competence of genotypes used, BINA Sarisha-4 showed better callus induction, shoot regeneration on MS medium supplemented with combination of plant growth regulators supplemented with silver nitrate. The highest percentage of root induction (66.67 and 58.33%) in plantlets was recorded on ½ MS medium supplemented with 2.0mgl-1 IBA and 0.5 mgl-1 of NAA. The highestsurvival rate was found after acclimatization of plants derived from stem (77.78%) and cotyledon (64.28%) explants of BINA Sarisha-4 in pot and 64.33 and 55.55%, respectively in field.

Nasrin *et al*. (2017) revealed *In vitro* callogenesis and plant regeneration system has been established for the oilseed *Brassica* (*Brassica napus, Brassica juncea*), Various explants (hypocotyls and cotyledonary leaves with petioles of the *in vitro* grown seedlings) were used to develop callus using different combinations of hormones (2,4-D, BAP, IAA, IBA) and additives ($AgNO_3$, Casein Hydrolysate, Proline). More than 95 % callus formed was observed in medium supplemented with 0.5 mg l^{-1} 2, 4-D + 1.0 mg l^{-1} BAP. To induce regeneration of plants from the calli, MS medium supplemented with various concentrations of different auxins and cytokinins were used. The medium supplemented with 2.0

mg l^{-1} BAP+ 3.0 mg l^{-1} AgNO3 + 0.1 mg l^{-1} NAA was the best medium for regeneration for all the varieties. Efficient rooting was found in the medium with half-strength of MS plus 1.0 mg l^{-1} of IBA. Among the varieties, BARI-16 and BARI-8 showed better responses in almost all the media tested.

Lone *et al*. (2017) established regeneration procedure for mustard (*Brassica juncea* var RSPR 01, RSPR 03) utilized hypocotyls as an explants. MS medium amended with 0.5mgl^{-1} 2,4-D in combination with BAP (1, 2, 3, 4 and 5 mgl^{-1}).Regeneration frequency of 100 per cent with variety RSPR 03 was observed in the media supplemented with 0.5mgl^{-1} 2,4-D in combination with 5.0 mgl^{-1} BAP whereas RSPR 01 showed regeneration frequency of 100 per cent when 0.5mgl^{-1} 2,4-D in combination with 4.0 mgl^{-1} BAP.

Dhania *et al*. (2016) reported regeneration in *Brassica* is highly genotype dependent. *In vitro,* cotyledon was found more responsive to compared hypocotyl regeneration using BAP. There generation efficiency was also influenced by age and size of explants. This report provides new insights into the development of efficient regeneration system in *B. juncea* with high frequency multiple shoot regeneration. Significant difference in the callus induction and regeneration frequency (RF) was recorded for GSL-1 and DGS-1 two genotypes of *Brassica napus* by Lone *et al*. (2016). MS medium supplemented with 5.0 mgl^{-1} BAP and 0.5 mgl^{-1} 2, 4-D hovered shoot regeneration frequency in GSL-1.

Ahmad *et al*. (2016) examined the effect of different plant growth regulators and carbon sources on callus induction and regeneration using seven different callus induction media compositions and five different shoot induction media compositions along with controls. Among the different callus induction media compositions supplemented, media amended with 3% sucrose or glucose as a carbon source, along with 4.0 mgl^{-1} NAA, 0.4 mgl^{-1} BAP and 0.4 mgl^{-1} Kinetin resulted in maximum callus induction frequency (80% and 69%). Media supplemented with 3% sucrose or glucose, supplemented with 0.3 mgl^{-1}NAA and 3,0 mgl^{-1} BAP favored shoot induction. Among the ten genotypes tested, the advanced line NIFA Nr.2 was found to be most responsive for callus induction. Maximum shooting (12.96%) was observed for NIFA Nr.1. Three percent sucrose and glucose, the same line showed maximum shooting *i.e.* 18% and 10%, respectively. The shoots obtained were then transferred to half strength MS media with or without plant growth regulators (control media) for root development.

Zisan *et al*. (2015) reported indirect somatic embryogenesis. The immature torpedo shaped embryos (451-700 μm) and mature walking-stick type (>700 μm) embryos of five *B. rapa* varieties *namely*, Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6, BARI Sarisha-15 were cultured onto basal MS media supplemented with 1.0 mgl^{-1} BAP, 0.5 mgl^{-1} NAA and 1,0 mgl^{-1} 2,4-D. The immature embryo culture followed indirect somatic embryogenesis process and the mature embryos followed direct organogenesis. Callus induction was observed after using immature torpedo embryos. Mature embryos regenerated plantlets *via* direct organogenesis within 8-11 days. Differential response was quite clearly observed among the varieties Agrani, BARI Sarisha-6 and BINA Sarisha-6 for callus induction from immature embryos. The highest percentage of plantlet was regenerated from mature embryos in Safal and BARI Sarisha-15. The higher concentration of BAP 10 to 20 ppm on MS media + 5 ppm IBAdecreased the size of the plantlets in all the varieties except the Agrani.

Kumar and Srivastava (2015) estimated reproducible and highly efficient procedure for organogenesis using hypocotyl, cotyledon, leaf and petiole explants of Broccoli (*Brassica oleracea* L. var. italica cv. Solan green head). Various explants cultured on shoot induction medium containing different concentrations and combinations of BAP and NAA resulting efficient shoot regeneration when hypocotyl (83.33 %), cotyledon (90.11 %), leaf (62.96 %) and petiole (91.10 %) explants were cultured on MS medium supplemented with 3.5 mgl^{-1} BAP in combination with 0.019 mgl-1 NAA, 2.5 mgl^{-1} BAP+0.5 mgl^{-1} NAA, 4.0 mgl^{-1} BAP+0.5 mgl^{-1} NAA and 4.5 mgl^{-1} BAP+0.019 mgl^{-1} NAA respectively. Petiole explants found to be most responsive as compared to other cultured explants. MS medium supplemented with 0.10 mgl^{-1} NAAhovered root regeneration (100 %). The optimized efficient regeneration procedure was effectively used for genetic transformation in Broccoli.

Gerszberg *et al*. (2015) published comparative *in vitro* response of hypocotyl and cotyledon explants from 10-d-oldseedlings using different callus induction media based on MS basal medium supplemented with 1% sucrose and different concentrations and combinations of plant growth regulators. Hypocotyl explants were found to be more responsive for callus induction and subsequent organogenesis as compared to cotyledon explants in all the cultivars tested. In terms of regeneration, the cv. 'Amager' genotype was significantly more responsive than the other cultivars tested and produced the highest number of shoots/buds per explant. Among the five types of media tested, MS+8.88 µM BAP in combination with 0.53 µM NAA was found most effective for shoot regeneration. Rooting was achieved within 10–15 d on all the rooting media, but MS medium containing5.37 µM NAA produced the maximum number of strong and healthy roots. Plantlets (95%) were subsequently established in the greenhouse, and no phenotypic variation was observed among regenerated plants.

Dubey *et al*. (2014) reported different concentrations and type of plant growth regulators for callus induction and shoots proliferation among three genotypes of *Brassica* namely MM (*Brassica napus*), GM(*Brassica juncea*) and YM (*Sinapis hirta*) observed that hypocotyl portion was found to be the best for callus induction in MS+0.5mgl^{-1} 2, 4-D in combination with 0.5mgl^{-1} NAA (94%).Maximum shoot proliferation percentage was recorded with MM on MS medium fortified with 0.5mglBAP in combination with 0.5mgl^{-1} Kinetin (100%) as well as with GM on MS+1.0mgl^{-1} BAP (100%) butIt was noted that genotype MM showed shoot proliferation relatively at higherrates. Differential response quite clears competence of various genotypes of *Brassica* under *in vitro* conditions.

Trivedi and Dubey (2014) documented effect of various phytohormones *viz*., NAA and BAP for callus induction and plant regeneration. Highest callus induction frequency (91.6-100%) was observed using hypocotyls explants on MS media supplemented with BAP at 0.5-1.0 mgl^{-1} in combination with 0.5-1.0 mgl^{-1} NAA. The same combinations resulted shoot differentiation, ranging from 7 to 20 shootlets / explant. The combinations of MS medium with 2.0-2.5 mgl^{-1} BP and

0.5 mgl^{-1}NAA favored callus induction but shoot induction was found to be very less and was recorded 8.3-33.3% showing shoot formation as less as (1-2 shootlets/explants).

Alam *et al*. (2014) developed procedure for an efficient *in vitro* regeneration system for BARI sarisha-13 (*Brassica napus*L.) where hypocotyls and cotyledonary petioles obtained from *in vitro* grown seedlings were used as explants for indirect regeneration. Single and combinatorial effects of plant growth regulators (PGRs) and additives were observed. Varying concentrations of plant growth regulators was found to change efficiency of callus, shoot and root formation. Increasing 2,4-Dfrom 0.5 mgl^{-1} to above and BAP concentration from 2.0 mg^{-1} to 3.0 mg^{-1} and NAA from 0.1 mg^{-1} to 0.5 mg^{-1} decreased callus and shoot formation, respectively. Roots were obtained when NAA was used. Effects of kinetin, $AgNO_3$, casein hydrolysate (CH), proline, IBA, *etc* was also examined during different stages of regeneration.

Thakur *et al*., 2013 conformed genetic stability of *in vitro* regenerated plants was evaluated using random amplified polymorphic DNA markers. On the basis of DNA pattern, genetic uniformity of all the regenerants.

Akmal *et al*. (2011) studied high frequency somatic embryogenesis has been established in mustard crop (*Brassica juncea* L. cv. Pusa Jai kisan), in which embryogenic calli were induced from hypocotyls and cotyledons of *in vitro* germinated seedlings. The hypocotyl derived embryogenic calli (HEC) were transparent and whitish, while cotyledon derived embryogenic calli (CEC) were creamy yellow incolour. Highest embryogenic callusing frequency (98%) was obtained in cotyledons on 2.0 mgl^{-1}2, 4-D added MS medium. Hypocotylsand cotyledons derived calli were differentiated into somatic embryos at higher frequency (90-100%) on 2.0mgl^{-1} 2ip or 2.0 mgl^{-1} BAP amended medium. Embryo maturation occurred on the same embryo development medium and germination was best achieved on2.6 mgl^{-1} ABA amended medium. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and histological studies revealed that the embryos had bipolar structure and developed mainly from the epidermis of explants. Furthermore, the embryonic tissues have stored bodies and numerous cell organelles.

Alam *et al*. (2009) compared BARI Shariaha-7, Tori-7, Agrani, Daulat and Safal to observe there *in vitro* regeneration competence with different concentrations and combinations of plant growth regulators. The range of callus induction frequency was recorded in range of 12.50-87.50 %. The maximum callus induction frequency (75.00%) was recorded on MS +4.0 mgl^{-1} 2, 4-D in combination with 1.0 mgl^{-1} BAP. Among the genotypes, BARI Sharisha-7 showed the highest percentage of callus induction (60.42%). Among the treatments, shoot regeneration (75.00%) was hovered by MS + 4.0 mg.l^{-1} BAP + 1.0 mgl-1 NAA. BARI Sharisha-7 also showed the highest rate of plant regeneration (66.67%). Half strength MS medium supplemented with 1.0 mgl^{-1} IBA and 0.5 mgl^{-1} NAA hovered root induction. Plantlets with well developed roots were transferred successfully to plastic pots and subsequently to the field. Among the four genotypes, BARI Sharisha-7 and Tori-7 were successfully transferred in the pots as well as in the field but Safal was very poor in survivability both in the pots and in the field.

Bhuiyan *et al*. (2009) reported efficient plant regeneration in higher frequencies through investigating various factors such as plant growth regulator combinations, explant types and ages and addition of $AgNO_3$. MS medium supplemented with 0.1 mgl^{-1} NAA and 1.0 mgl^{-1} BA showed the maximum shoot regeneration frequency (56.67%) among the different combinations of NAA and BA. Explant type, explant age and addition of $AgNO_3$ also significantly affected shoot regeneration. Among four type of explants (cotyledon, hypocotyl, root and leaf explants), cotyledon explants was proved most responsive for shoot regeneration frequency. Hypocotyls explants resulted the highest number of shoots per explant, whereas root explants did not produce any shoot. Shoot regeneration frequency was improved significantly by adding $AgNO_3$ to the medium. Considerable variation was recorded in shoot regeneration potential of cotyledonay explants among *B. juncea* L. genotypes. The shoot regeneration frequency ranged from 47.78% for cv. Shambol to 91.11% for cv. Rai-5. In terms of the number of shoots produced per explant, *B. juncea* L. cv. Daulot showed the maximum efficiency. MS medium supplemented with 0.1 mgl^{-1} NAA showed the highest frequency of rooting. The regenerated plantlets were transferred to pot soil and grown to maturity in the greenhouse. All plants were fertile and morphologically comparable with the source plants.

Munir *et al*. (2008) documented the results indicating that among the 5 media combinations tested, MS media supplemented with 0.5mgl^{-1}IAA, 1.0 mgl^{-1} BAP, 0.5mgl^{-1} NAA and 1.0 mgl^{-1} Kinetin was the most responsive media combinations. With increasing concentration of IAA resulted decrease in callus induction. The cultivar Oscar was found to be most responsive for callus induction followed by cultivar H-19. The cultivar Rainbow was less amenable to calli formation. Among various combinations of media tested showed significantly different regeneration response except RM1 and RM6 media combinations that showed 1.0 mgl^{-1}zeatin and 0.1 mg^{-1}IAA was observed. Maximum genotypes to be best for giving higher regeneration percentage. The results effect was clearly noticed and cultivar Oscar showed regeneration relatively at higher rates. Whereas Rainbowshowed minimum regeneration potential among tested four cultivars.

Khan *et al*. (2010) used competence of six genotypes of oilseed *Brassica viz*. Tori-7, Sampad, Kallyania, BARI Sarisha-7, BARI Sarisha-8, and MM 20-3 using MS medium with different concentrations of BAP, NAA, and $AgNO_3$. For callus induction and subsequent plant regeneration potential. The highest percentage of callus induction (91.43%) was observed in Tori-7 in the media supplemented with 2 mgl^{-1} BAP, 0.1 mgl^{-1}NAA and 2.0 mgl^{-1} $AgNO_3$. Calli were maintained in order to get sufficient number of regenerants. With the increasing concentration of BAP, the highest percentage (57.14) of regenerants were found in Tori-7 followed by Sampad (33.13%) and BARI Sarisha-8 (31.42%) in MS media supplemented with 2.5 mgl^{-1} BAP, 0.1 mgl^{-1} NAA and 2.0 mgl^{-1} $AgNO_3$. Root formation was good from the regenerants transferred to half MS medium supplemented with 0.5 mgl^{-1} NAA in genotype Tori-7. Regenerated plantlets of four genotypes *viz*: Tori-7, BARI Sarisha-8, Kallyania and BARI Sarisha-7were successfully established in the field.

Roy and Saha (2006) reported reciprocal crosses between two cultivars; cv. RJ15 and cv. RLM198 of Indian mustard (*Brassica juncea*). Anther derived lines designated as A_1 plants, were raised through anther culture fromthese F_1 hybrid plants. Forty-five percent germination was obtained from distinctly shriveled and small A_1 seeds andgrown along with the F_2 plants in the same agro-climatic conditions. Subsequently the lines were compared for inheritance pattern between the lines. A normal frequency distribution curve for siliqua per plant was obtained in all the lines reflecting a similar pattern of recombination. Few seeds from the plants of each lines exhibiting high number of siliqua per plant, were subjected to analysis of erucic acid. Three plants were characterized with lower erucic acid as compared to parent cultivars of A_2 generation. The study pointed out that contrasting characters could be obtained from A_2 plants where the traits are oligo or monogenic through anther culture.

Ratan *et al*. (2001) reported influence of different PGRs using different explants *viz;* hypocotyl and cotyledon cultured on MS medium, cotyledon explants were found to be most responsive. The frequency of callus was higher on medium supplemented with 0.2 mgl^{-1} IAA in combination with 2.0 mgl^{-1} K1N.Difference for days to callus induction, fresh and dry weight of callus was recorded among various genotypes. The earliest callus induction was observed on genotype T59 after 45days. All the genotypes regenerated shoots on sub culturing. Genotypic difference was quite noticeable for fresh and dry weights of callus.Callus growth was better in genotype RH 781. None of the provided conditions favored shoot differential in genotypes included in the study.

CHAPTER - III

MATERIALS AND METHODS

The present study entitled **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** was conducted under field conditions during *Rabi* 2016-17 and 2017-18.The techniques/methods followed and materials used during the course of the present investigations are presented in this chapter.

3.1Experimental Site

Present investigation was carried out at the experimental field of department of Genetics & Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India.

3.2Geographical Situation

Madhya Pradesh is located in the central part of the country between $21^0$6' N and 26°30'N latitudes and 74°9' E and 82°48' E longitudes. In the state of Madhya Pradesh, Gwalior is located at 26.22° North Latitude and 78.18° East Longitude. The average elevation of the land of Gwalior is about 197 meters above the sea level.

3.3 Weather and Climate

Gwalior region spread over an area of 5214.00 sq km, in the Chambal river valley, the city of Gwalior is landlocked on all sides. The Gwalior climate can be termed as extremes, both in summer and winter. The summers are usually very hot and the winters very cold in Gwalior, the rains are in, however, restricted only to the monsoon months. The average annual rainfall of this region is 900 mm, most of which is concentrated in the mansoon month (from late June to early October).During the summer months, the climate of Gwalior is dominated by the scorching heat and the humidity level is also on the rise. From the month of April to the month of June. Gwalior feels summer months with temperatures soaring to a high of 45°C - 47°C. The climate of Gwalior is very humid, especially at this time of the year. The mean summer temperature in Gwalior is, however, 33°C. In the winter season, the climate of Gwalior steeps down to a chilling temperature of as low as 1 - 2°C. The mean minimum temperature in the winter months is 8.0°C in Gwalior. The relative humidity generally exceeds 82% and the wind velocity is higher during pre-monsoon period as compared to post-monsoon period. The experimental site comes under the eightth agro-climatic zone of the country, *i.e.* Central Plateau and Hills and is termed as semi– arid to dry sub-humid climate. The soil is sandy loam, low in available nitrogen, medium in phosphorus and high in potash with pH of 8.5. The average rainfall of this region recorded 4.62 mm and 8.2 mm in 2016-17 and 2017-18 respectively. The average maximum and minimum temperature at Gwalior ranged between 27.27^{o}C and 10.10^{o}C, 26.97^{0}C and 10.11^{0}C respectively during crop growth period. Meteorological data recorded during November 2016 to March 17 and November 17 to March 18 was recorded and tabulated in Table 3.1 and 3.2.

3.4 Experimental Materials

Experimental material consists of one ninety-six Indian mustard genotypes obtained from the Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior M.P. (AICRP on Rapeseed and Mustard) and IARI, New Delhi. These were collected from different parts of India and abroad (Table 3.3 and 3.4).

3.5. Experimental Detail

The field experiment was laid out in Randomized Block Design (RBD) with two replications. Each genotype was grown in a plot of one row of 2 meter length with a spacing of 30 cm apart between rows and 15 cm plant to plant. The crop was provided with protective irrigations, recommended package of practices were followed throughout the growing season.

Plate 3.1: Experimental Field View in 2016-

Table 3.1: Meteorological data recorded at Gwalior during the crop season Rabi 2016 – 17

eek No	**Year and month**	**Date**	**Temperature (^{0}C)**		**Humidity (%)**		**Rainfall (mm)**	**Evaporation (mm)**
			ax.	**Min.**	**Morning**	**Evening**		
7	November,16	19-25	9.9	9.5	84.7	27.7	000.0	2.6
8	November,16	26-2	8.8	11.0	79.8	35.4	000.0	3.6
9	December,16	3-9	6.0	8.3	98.3	50.0	000.0	1.0
0	December,16	10-16	7.2	8.9	90.8	37.8	000.0	1.8
1	December,16	17-23	5.7	6.7	86.5	38.4	000.0	2.3
2	December,16	24-31	5.7	7.44	88.7	43.3	000.0	1.8
	January, 17	1-7	3.18	9.1	95.71	58.4	000.0	1.1
	January, 17	8-14	9.84	5.7	93.14	45.85	000.0	1.6
	January, 17	15-21	1.5	6.3	94	48.71	000.0	2.2
	January, 17	22-28	6.8	10.35	94.14	50	4	1.8
	January, 17	29-4	4.04	9.22	94.28	57.71	000.0	1.82
	February, 17	5-11	4.2	9.0	88.7	51.4	000.0	1.9
	February, 17	12-18	5.3	9.3	90	42.5	000.0	3.4
	February, 17	19-25	9.5	11.9	69.7	33.5	000.0	4.4
	February, 17	26-4	0.6	12.1	82.8	37.5	000.0	4.5
0	March, 17	5-11	8.3	11.7	83.2	44	0.62	3.6
1	March, 17	12-18	9.0	10.3	83.7	34.7	000.0	4.2
2	March, 17	19-25	4.0	16.3	73.5	30.4	000.0	6.0
3	March, 17	26-2	8.7	18.9	59.5	24.4	000.0	9.0
ean			**7.27**	**10.10**	**85.85**	**41.69**	**4.62**	**3.08**

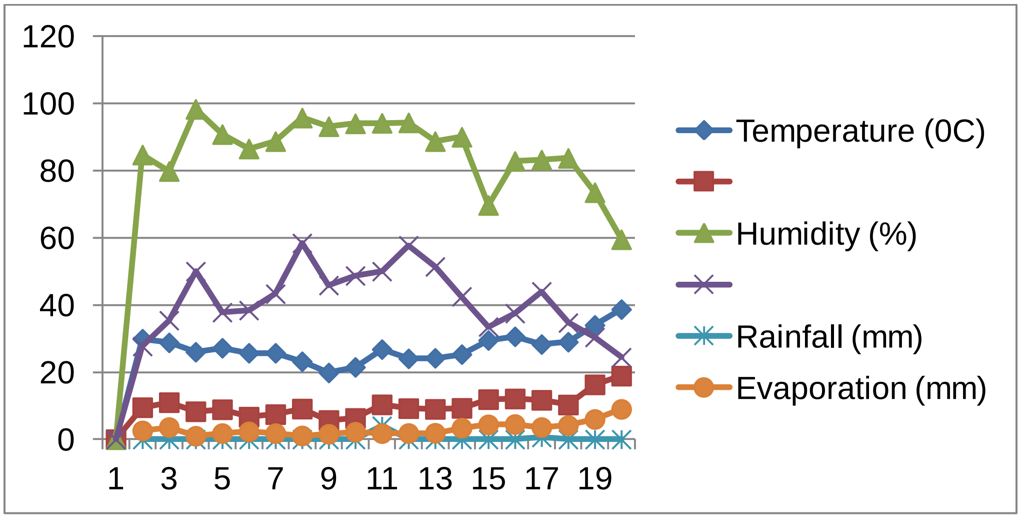


Fig 3.1: Graphical representation of meteorological data at Gwalior during the crop season Rabi 2016 – 17

Table 3.2: Meteorological data recorded at Gwalior during the crop season Rabi 2017 – 18

eek o.	Month and year period	Date	Temperature (^{0}C)		Humidity (%)		Rainfall (mm)	Evaporation (mm)
			ax.	in.	Morning	Evening		
8	Nov.-Dec17	26-2	9.1	.9	90.9	35.0	0	2.7
9	Dec, 17	3-9	4.9	0.1	87.1	47.3	6	1.8
0	Dec, 17	10-16	3.7	0.8	92.3	56.1	1	1.5
1	Dec, 17	17-23	4.0	.1	78.7	45.1	0	2.5
2	Dec, 17	24-31	5.1	.6	96.3	49.0	0	1.8
	January, 18	1-7	0.8	.5	83.2	61.5	000.0	1.3
	January, 18	8-14	3.0	.2	93.0	45.1	000.0	2.1
	January, 18	15-21	8.4	.7	93.3	40.7	000.0	2.4
	January, 18	22-28	4.4	.9	74.2	57.7	000.0	1.9
	January, 18	29-4	6.7	.3	87.7	49.4	000.0	3.1
	February, 18	5-11	4.0	.2	89.0	52.8	000.0	2.4
	Febru	1			9	6	0	2

	ary, 18	2-18	4.5	.7	0.2	7.5	00.0	.4
	Febru ary, 18	1 9-25	 .7	 1.8	8 7.5	4 1.2	0 00.0	3 .5
	Febru ary, 18	2 6-4	 2.3	 4.4	8 1.5	3 7.8	0 00.0	5 .4
 0	March , 18	5 -11	 1.3	 1.9	7 4.4	4 2.7	0 00.0	5 .2
 1	March , 18	1 2-18	 4.7	 3.9	5 4.2	3 8.8	0 00.0	5 .0
 2	March , 18	1 9-25	 4.6	 5.4	7 1.5	3 .5	0 00.0	6 .2
 3	March , 18	2 6-1	 8.0	 6.1	5 9.5	2 3.4	0 00.0	7 .8
 4	April, 18	2 -8	 9.3	 0.7	6 4.3	3 2.0	1 .2	6 .6
 ean			 **6.97**	 **0.11**	**8** **1.51**	**4** **3.51**	**0** **.43**	**3** **.5**

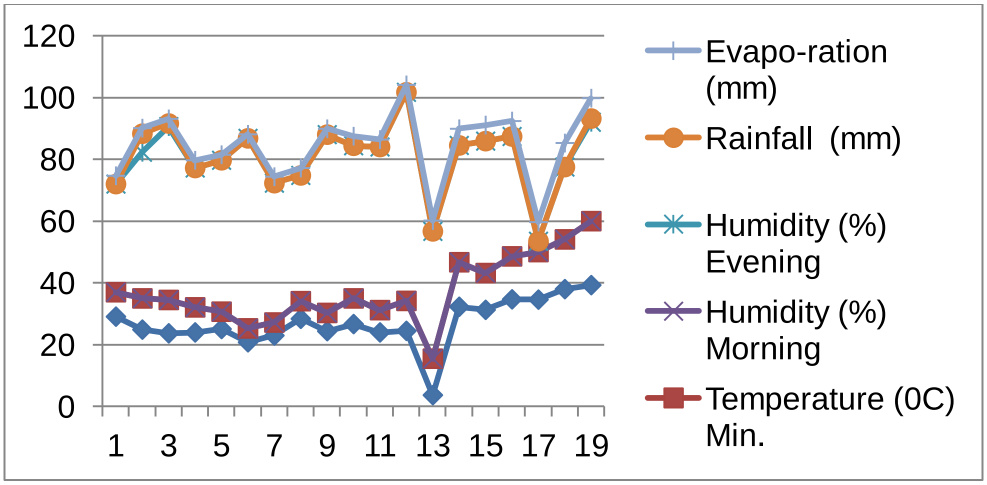


Fig 3.2: Graphical representation of meteorological data at Gwalior during the crop season Rabi 2017 – 18

Plate 3.2: Experimental Field View in 2017-

Table 3.3: List of Indian mustard genotypes along with source used in present experiment

S. No.	Genotypes	Source
1	MRNJ-1	ZARS Morena(M.P.)
2	MRNJ-2	ZARS Morena(M.P.)
3	MRNJ-3	ZARS Morena(M.P.)
4	MRNJ-4	ZARS Morena(M.P.)
5	MRNJ-5	ZARS Morena(M.P.)
6	MRNJ-6	ZARS Morena(M.P.)
7	MRNJ-7	ZARS Morena(M.P.)
8	MRNJ-8	ZARS Morena(M.P.)
9	MRNJ-9	ZARS Morena(M.P.)
10	MRNJ-10	ZARS Morena(M.P.)
11	MRNJ-11	ZARS Morena(M.P.)
12	MRNJ-12	ZARS Morena(M.P.)
13	MRNJ-13	ZARS Morena(M.P.)
14	MRNJ-14	ZARS Morena(M.P.)
15	MRNJ-15	ZARS Morena(M.P.)
16	MRNJ-16	ZARS Morena(M.P.)
17	MRNJ-17	ZARS Morena(M.P.)
18	MRNJ-18	ZARS Morena(M.P.)
19	MRNJ-19	ZARS Morena(M.P.)
20	MRNJ-20	ZARS Morena(M.P.)
21	MRNJ-21	ZARS Morena(M.P.)
22	MRNJ-22	ZARS Morena(M.P.)
23	MRNJ-23	ZARS Morena(M.P.)
24	MRNJ-24	ZARS Morena(M.P.)
25	MRNJ-25	ZARS Morena(M.P.)
26	MRNJ-26	ZARS Morena(M.P.)
27	MRNJ-27	ZARS Morena(M.P.)
28	MRNJ-28	ZARS Morena(M.P.)
29	MRNJ-29	ZARS Morena(M.P.)
30	MRNJ-30	ZARS Morena(M.P.)
31	MRNJ-31	ZARS Morena(M.P.)
32	MRNJ-33	ZARS Morena(M.P.)
33	MRNJ-34	ZARS Morena(M.P.)
34	MRNJ-35	ZARS Morena(M.P.)
35	MRNJ-36	ZARS Morena(M.P.)
36	MRNJ-37	ZARS Morena(M.P.)
37	MRNJ-38	ZARS Morena(M.P.)
38	MRNJ-39	ZARS Morena(M.P.)
39	MRNJ-40	ZARS Morena(M.P.)
40	MRNJ-41	ZARS Morena(M.P.)

S. No.	Genotypes	Source
41	MRNJ-42	ZARS Morena(M.P.)
42	MRNJ-43	ZARS Morena(M.P.)
43	MRNJ-44	ZARS Morena(M.P.)
44	MRNJ-47	ZARS Morena(M.P.)
45	MRNJ-48	ZARS Morena(M.P.)
46	MRNJ-47	ZARS Morena(M.P.)
47	MRNJ-48	ZARS Morena(M.P.)
48	MRNJ-49	ZARS Morena(M.P.)
49	MRNJ-50	ZARS Morena(M.P.)
50	MRNJ-51	ZARS Morena(M.P.)
51	MRNJ-52	ZARS Morena(M.P.)
52	MRNJ-53	ZARS Morena(M.P.)
53	MRNJ-54	ZARS Morena(M.P.)
54	MRNJ-55	ZARS Morena(M.P.)
55	MRNJ-56	ZARS Morena(M.P.)
56	MRNJ-57	ZARS Morena(M.P.)
57	MRNJ-58	ZARS Morena(M.P.)
58	MRNJ-59	ZARS Morena(M.P.)
59	MRNJ-60	ZARS Morena(M.P.)
60	MRNJ-61	ZARS Morena(M.P.)
61	MRNJ-62	ZARS Morena(M.P.)
62	MRNJ-63	ZARS Morena(M.P.)
63	MRNJ-64	ZARS Morena(M.P.)
64	MRNJ-65	ZARS Morena(M.P.)
65	MRNJ-66	ZARS Morena(M.P.)
66	MRNJ-67	ZARS Morena(M.P.)
67	MRNJ-68	ZARS Morena(M.P.)
68	MRNJ-69	ZARS Morena(M.P.)
69	MRNJ-70	ZARS Morena(M.P.)
70	MRNJ-71	ZARS Morena(M.P.)
71	MRNJ-72	ZARS Morena(M.P.)
72	MRNJ-73	ZARS Morena(M.P.)
73	MRNJ-74	ZARS Morena(M.P.)
74	MRNJ-75	ZARS Morena(M.P.)
75	MRNJ-76	ZARS Morena(M.P.)
76	MRNJ-77	ZARS Morena(M.P.)
77	MRNJ-78	ZARS Morena(M.P.)
78	MRNJ-79	ZARS Morena(M.P.)
79	MRNJ-80	ZARS Morena(M.P.)
80	MRNJ-81	ZARS Morena(M.P.)
81	MRNJ-82	ZARS Morena(M.P.)
82	MR NJ-83	ZARS Morena(M.P.)
83	MRNJ-84	ZARS Morena(M.P.)

S. No.	Genotypes	Source
84	MRNJ-85	ZARS Morena(M.P.)
85	MRNJ-86	ZARS Morena(M.P.)
86	MRNJ-87	ZARS Morena(M.P.)
87	MRNJ-88	ZARS Morena(M.P.)
88	MRNJ-89	ZARS Morena(M.P.)
89	MRNJ-90	ZARS Morena(M.P.)
90	MRNJ-91	ZARS Morena(M.P.)
91	MRNJ-92	ZARS Morena(M.P.)
92	MRNJ-93	ZARS Morena(M.P.)
93	MRNJ-94	ZARS Morena(M.P.)
94	MRNJ-95	ZARS Morena(M.P.)
95	MRNJ-96	ZARS Morena(M.P.)
96	MRNJ-97	ZARS Morena(M.P.)
97	MRNJ-98	ZARS Morena(M.P.)
98	MRNJ-99	ZARS Morena(M.P.)
99	MRNJ-100	ZARS Morena(M.P.)
100	MRNJ-101	ZARS Morena(M.P.)
101	MRNJ-102	ZARS Morena(M.P.)
102	MRNJ-103	ZARS Morena(M.P.)
103	MRNJ-104	ZARS Morena(M.P.)
104	MRNJ-105	ZARS Morena(M.P.)
105	MRNJ-106	ZARS Morena(M.P.)
106	MRNJ-107	ZARS Morena(M.P.)
107	MRNJ-108	ZARS Morena(M.P.)
108	MRNJ-109	ZARS Morena(M.P.)
109	MRNJ-110	ZARS Morena(M.P.)
110	MRNJ-111	ZARS Morena(M.P.)
111	MRNJ-112	ZARS Morena(M.P.)
112	MRNJ-113	ZARS Morena(M.P.)
113	MRNJ-114	ZARS Morena(M.P.)

S. No.	Genotypes	Source
114	MRNJ-115	ZARS Morena(M.P.)
115	MRNJ-116	ZARS Morena(M.P.)
116	MRNJ-117	ZARS Morena(M.P.)
117	MRNJ-118	ZARS Morena(M.P.)
118	MRNJ-119	ZARS Morena(M.P.)
119	MRNJ-120	ZARS Morena(M.P.)
120	MRNJ-121	ZARS Morena(M.P.)
121	MRNJ-122	ZARS Morena(M.P.)
122	MRNJ-123	ZARS Morena(M.P.)
123	MRNJ-124	ZARS Morena(M.P.)
124	MRNJ-125	ZARS Morena(M.P.)
125	MRNJ-126	ZARS Morena(M.P.)
126	MRNJ-127	ZARS Morena(M.P.)
127	MRNJ-128	ZARS Morena(M.P.)
128	MRNJ-129	ZARS Morena(M.P.)
129	MRNJ-130	ZARS Morena(M.P.)
130	MRNJ-131	ZARS Morena(M.P.)
131	MRNJ-132	ZARS Morena(M.P.)
132	MRNJ-133	ZARS Morena(M.P.)
133	MRNJ-134	ZARS Morena(M.P.)
134	MRNJ-135	ZARS Morena(M.P.)
135	MRNJ-136	ZARS Morena(M.P.)

S. No.	Genotypes	Source
136	MRNJ-137	ZARS Morena(M.P.)
137	MRNJ-138	ZARS Morena(M.P.)
138	MRNJ-139	ZARS Morena(M.P.)
139	MRNJ-140	ZARS Morena(M.P.)
140	MRNJ-141	ZARS Morena(M.P.)
141	MRNJ-142	ZARS Morena(M.P.)
142	MRNJ-143	ZARS Morena(M.P.)
143	MRNJ-144	ZARS Morena(M.P.)
144	MRNJ-145	ZARS Morena(M.P.)
145	IDM-2	ZARS Morena(M.P.)
146	IDM-8	ZARS Morena(M.P.)
147	IDM-10	ZARS Morena(M.P.)
148	IDM-11	ZARS Morena(M.P.)
149	IDM-12	ZARS Morena(M.P.)
150	IDM-15	ZARS Morena(M.P.)
151	IDM-16	ZARS Morena(M.P.)
152	IDM-25	ZARS Morena(M.P.)
153	IDM-31	ZARS Morena(M.P.)
154	IDM-41	ZARS Morena(M.P.)
155	IDM-42	ZARS Morena(M.P.)
156	IDM-53	ZARS Morena(M.P.)
157	IDM-58	ZARS Morena(M.P.)

S. No.	Genotypes	Source
158	IDM-64	ZARS Morena(M.P.)
159	IDM-66	ZARS Morena(M.P.)
160	IDM-67	ZARS Morena(M.P.)
161	IDM-69	ZARS Morena(M.P.)
162	ISC-3	Rasi seed company, Gururam (Haryana)
163	ISC-12	Rasi seed company, Gururam (Haryana)
164	ISC-17	Rasi seed company, Gurugram (Haryana)
165	ISC-18	Rasi seed company, Gurugram (Haryana)
166	ISC-20	Rasi seed company, Gurugram (Haryana)
167	ISC-23	Rasi seed company, Gurugram (Haryana)
168	MC25	Rasi seed company, Gurugram (Haryana)
169	L-4	Canada
170	L-6	Canada
171	Rohini	CSAUAT,Kanpur (U.P.)
172	Maya	CSAUAT, Kanpur (U.P.)
173	Kranti	CSAUAT, Kanpur
174	RVM-1	RMVRS UAT, Gwalior (M.P.)
175	RVM-2	RMVRS UAT, Gwalior (M.P.)
176	JM-1	RMVRS UAT, Gwalior (M.P.)
177	JM-2	RMVRS UAT, Gwalior (M.P.)
178	JM-3	RMVRS UAT, Gwalior (M.P.)
179	GM-2	SDAU, Banaskantha (Gujarat)

S. No.	**Genotypes**	**Source**
179	GM-2	SDAU, Banaskantha (Gujarat)
180	NRCDR-2	DRMR, Bharatpur (Rajasthan)
181	NRCHB101	DRMR, Bharatpur (Rajasthan)
182	DRMRIJ-31	DRMR, Bharatpur
183	DRMR150-35	DRMR, Bharatpur
184	RB50	CCS, Hisar (Haryana)
185	RH749	CCS, Hisar (Haryana)
186	CS54	CSSRI, Karnal (Haryana)
187	JD6	IARI, New Delhi
188	PM21	IARI, New Delhi
189	PM22	IARI, New Delhi
190	PM24	IARI, New Delhi
191	PM25	IARI, New Delhi
192	PM26	IARI, New Delhi
193	PM28	IARI, New Delhi
194	PM29	IARI, New Delhi
195	PM30	IARI, New Delhi
196	LES39	IARI, New Delhi

Table 3.4: Pedigree of Indian mustard varieties

S.N.		Pedigree
1	Rohini	Selection from natural population of varuna
	Maya	Varuna X KRV11
	Kranti	Selection from germplasm collected from Kanpur Dehat
	RVM-1	Selection of germplasm from Bastar, Chhattisgarh
	RVM-2	Selection from Chambal growing region (MRNJ-65)
	JM-1	Cross of L6 X Pusa bold
	JM-2	Varuna X L4
	JM-3	Varuna X YRT3
	GM-2	Selection from material collected from Vendancha, Gujarat
	NRCDR-2	MDOC43 X NBPGR36
	NRCHB101	BL4 X Pusa bold
	DRMRIJ-31	HB-9908 X HB-9916
	DRMR-150-35	RH-819 X Pusa bold
	RB50	Laxmi X RH9617
	RH-749	RH-781 X RH-9617
	CS54	B380 X NDR8603
	JD6	Pusa bold X Glossy
	PM21	Pusa bold X ZEM-2
	PM22	Pusabarani X ZEM-2
	PM24	(Pusa bold X LEB-15) X LES-29
	PM25	SEJ 8 X PusaJagannath
	PM26	VEJ Open X PusaAgrani
	PM28	SEJ 8 X PusaJagannath
	PM29	(ZEM-2 X pusabarani) X EC-287711
	PM30	Bio902 X ZEM1
	LES39	Pusabarani X ZEM-1

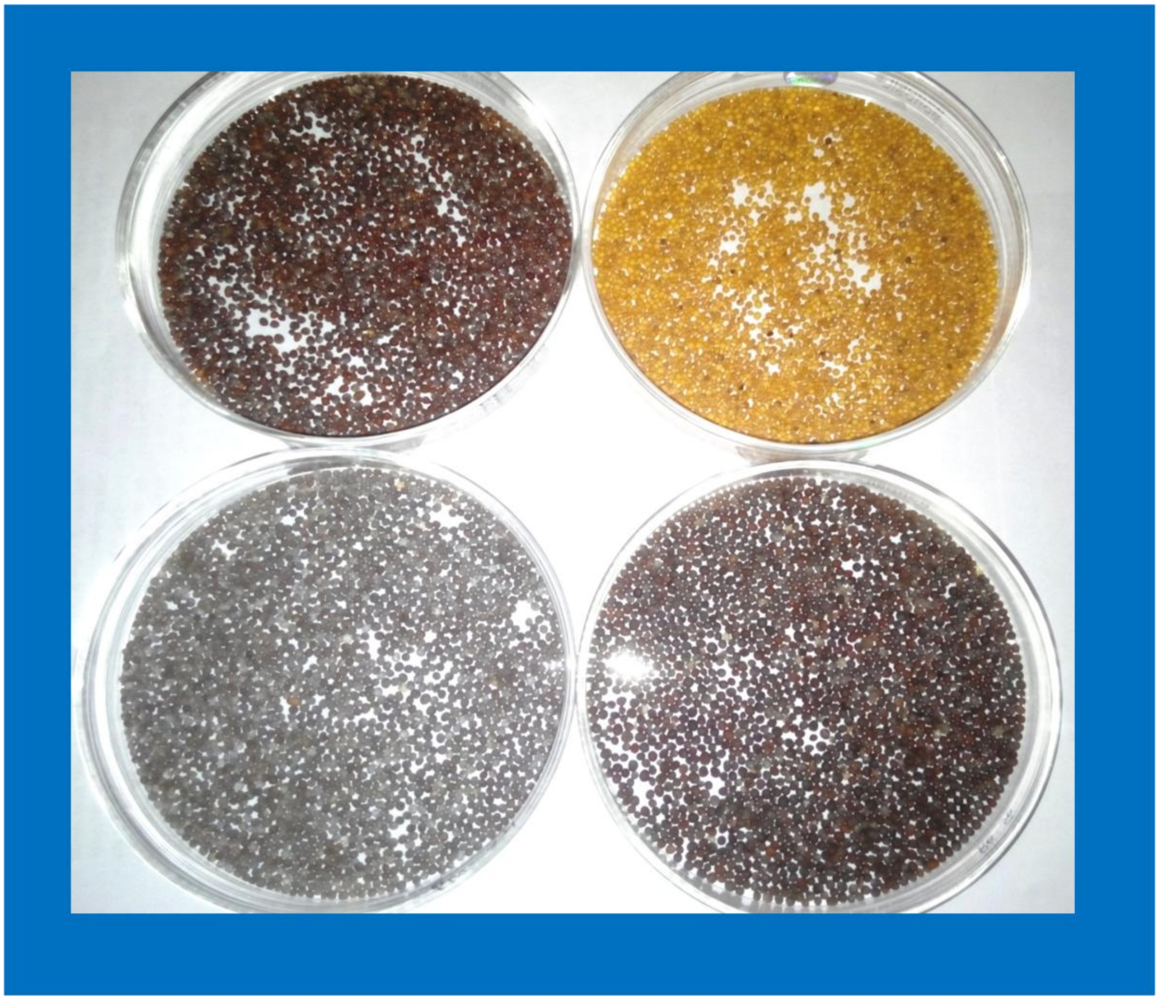


Plate 3.3: Seed colour variations in Indian mustard genotypes

3.6 Methods adopted to take observations:

a. Five plants from each plot in each replication were randomly selected for recording observations on various characters.
b. One eighty-eight genotypes with higher seed yield were used for fatty acid extraction and then characterized for low and high erucic acid content.
c. Forty-eight selected genotypes were used for molecular diversity analysis.
d. Seven genotypes were used for raising callus and cell suspension cultures with different plant growth regulators in varying concentrations and combinations.

3.7 Observations

3.7.1 Quantitative characters

3.7.1.1 Days to 50 per cent flowering: The numbers of days were recorded from date of sowing to days when 50 percent plants of the population were in flowering.

3.7.1.2 Days to maturity: The numbers of days from the date of sowing to the physiological maturity were recorded for each entry.

3.7.1.3 Plant height (cm): Height of the plant measured in centimeters at maturity from ground level to the tip of the plant.

3.7.1.4 Number of primary branches per plant: Number of branches arising from main stem was recorded at the time of harvest and their average was worked out.

3.7.1.5 Number of secondary branches per plant: Number of branches arising from primary branches was recorded at the time of harvest and their average was calculated.

3.7.1.6 Length of main raceme (cm): At the time of the harvest, length of the main raceme was measured with the help of the meter scale.

3.7.1.7Number of silique per main raceme: At the time of harvest, total numbers of silique per main raceme produced on individual plant were recorded.

3.7.1.8 Number of silique per plant: At the time of harvest, total numbers of silique produced on individual plant were recorded.

3.7.1.9Silique length (cm): Five mature silique were randomly selected from each plant. Their length was measured with meter scale and average silique length was worked out.

3.7.1.10Number of seeds per silique: Five mature and effective silique were randomly selected from each plant. These were threshed manually and average number of seeds per silique was worked out.

3.7.1.111000-seed weight (g): Weight of one thousand dry and well filled seeds was recorded for this observation.

3.7.1.12Biological yield per plot (g): Five randomly selected plants were dried and weighed together to get biological yield per plot.

3.7.1.13Seed yield per plot (g): Five randomly selected plants were dried and weighed together to get seed yield per plot.

3.7.1.14 Seed yield per plant (g): Five random plants were harvested together; threshed and average seed yield per plant was recorded in each replication.

3.7.1.15Harvest Index (%): Harvest Index was calculated as the ratio of seed yield to the total biological yield and expressed in per cent.

HI(%) = Seed yield per plant (g) ÷Biological yield per plant (g) x100

3.7.2 Qualitative characters

Following parameters were recorded for qualitative characters:

3.7.2.1. Palmitic acid %
3.7.2 .2.Oleic acid %
3.7.2.3. Linoleic acid %
3.7.2.4. Linolenic acid %
3.7.2.5 Erucic acid %
3.7.2.6 Seed oil %

3.8 Statistical analysis

Data were analyzed in RBD design in individual year and pooled over 2 years.

Table 3.5: Analysis of variance of pooled analysis (2year) in RBD

source	**d.f.**	**Expectation MS**
Environment	e-1	$\sigma^2{}_e + GR\sigma^2{}_e$
Replication	e(r-1)	$\sigma^2{}_e + GE\sigma^2{}_r$
Genotype	g-1	$\sigma^2{}_e + RE\sigma^2{}_g$
GXE interaction	(g-1) (e-1)	$\sigma^2{}_e + \sigma^2{}_{g\,x\,e}$
Pooled error	e(r-1) (g-1)	$\sigma^2{}_e$

3.8.1 Standard error: S.E. = $\sqrt{2EMS}/r$

Where,

S.E. = Standard error

EMS = Error mean sum of square

r = Replication

3.8.2 Coefficient of variance

CV (%) = $\frac{\sqrt{EMS}}{X} * 100$

Where,

CV = Coefficient of variance

EMS = Error mean sum of square used as standard deviation from ANOVA table.

X = Mean

3.8.3 Genotypic and phenotypic coefficients of variation:

(a) Genotypic coefficient of variation

The genotypic coefficient of variation, which measures the magnitude of genetic variation present in a particular character, was estimated as per the formula suggested by Burton (1952);

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

Where,

$\sigma^2 g$ = Genotypic variance

$\bar{X}$ = Mean

(b) Phenotypic coefficient of variation

The phenotypic coefficient of variation, which measures the magnitude of phenotypic variation present in a particular character, was estimated as per the formula suggested by Burton (1952);

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

Where,

σ^2p = Phenotypic variance

$\overline{X}$ = Mean

The PCV and GCV are classified as follows as suggested by Sivasubramanian and Madhavamenon (1973).

Low: Less than 10%

Moderate: 10-20%

High: More than 20%

3.8.4Heritability in broad sense

It is the ratio of genotypic variance to the phenotypic variance, was calculated according to formula suggested by Lush, 1940;

$$h^2 = \frac{\sigma^2{}_g}{\sigma^2{}_p} \times 100$$

Where,

h^2 = Heritability

σ^2g = Genotypic variance

σ^2p = Phenotypic variance

Heritability values are categorized by Johanson*et al.* (1955).

Low: Less than 30%

Moderate: 30-60%

High: More than 60%

3.8.5 Genetic advance

The expected genetic advance under selection was estimated by following the method suggested by Johnson *et al.* (1955);

$$GA = k \, x\sigma_p x \, h^2$$

Where,

GA = Genetic advance

k = Selection differential (value of k at 5% selection intensity = 2.06)

σ_p= Phenotypic standard deviation.

h^2 = Heritability

3.8.6 Genetic advance expressed as percentage of mean

The genetic advance expressed as percentage of mean was computed as under;

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

Where,

GA = Genetic advance under selection

$\overline{\text{X}}$= General mean

The range of genetic advance as percent of mean is classified as suggested by Johanson *et al.* (1955).

Low: Less than 10%

Moderate: 10-20%

High: More than 20%

3.8.7 Correlation coefficient analysis

The correlation coefficients were worked out to determine the degree of association of a character with yield and also among the yield components. Genotypic and phenotypic correlations were calculated by using the formula given by Weber and Moorthy, 1952; Miller (1958);

$$r_p = \frac{Covp(XY)}{\sqrt{\sigma^2 p(X).\sigma^2 p(Y)}}$$

$$r_g = \frac{Covg(XY)}{\sqrt{\sigma^2 g(X).\sigma^2 g(Y)}}$$

Where,

rp = phenotypic correlation

rg = genotypic correlation

Covp(XY) = phenotypic covariance between X and Y

Covg(XY) = genotypic covariance between X and Y

σ^2p(X) and σ^2p (Y) = phenotypic variance of X and Y

σ^2g(X) and σ^2g (Y) = genotypic variance of X and Y

Estimates of correlation coefficients were compared against r-values given in Fisher and Yates (1963) table at (n-2) df at the probability levels of 0.05 and 0.01 to test their significance.

3.8.8Path coefficient analysis: Path coefficient is a standardized partial regression coefficient, which measures the direct influence of one variable upon another and allows partition of correlation coefficient into components of direct and indirect effects. The proportion of direct and indirect contributions of various characteristics to the total correlation coefficients with grain yield per plant, was estimated through path coefficient analysis as suggested by Wright (1921, 1935) and elaborated by Dewey and Lu (1959). To estimates various direct and indirect effect, the following set of simultaneous equations were formed and solved.

$$r_{1y} = P_{1y} + r_{12}P_{2y} + r_{13}P_{3y} + \cdots + r_{1i}P_{iy}$$

$$r_{2y} = r_{2y}P_{1y} + P_{2y} + r_{23}P_{3y} + \cdots + r_{2i}P_{iy}$$

$$r_{iy} = r_{i1}P_{1y} + r_{12}P_{2y} + r_{13}P_{3y} + \cdots + P_{iy}$$

$Where,$

$r_{1y}\ to\ r_{iy} = Cofficient\ of\ correlation\ between\ causal\ factor\ 1\ to\ i\ and\ dependent\ character\ y$

$r_{12}\ to\ r_{i-1,i} = Coefficient\ of\ correlation\ among\ causal\ factors\ themselves, and$

$P_{1y}\ to\ P_{iy} = Direct\ effect\ of\ characters\ 1\ to\ i\ on\ character\ y$

Residual effect, which measures the contribution of the characters not considered in the causal scheme, was obtained as:

$$Residual\ effect(P_{RY}) = \sqrt{1 + R^2}$$

$Where,$

$$R^2 = \sum_{iy} P^2{}_{iy} + 2\sum_{\substack{i\neq j\\ i>j}} P_{iy}P_{jy}r_{ij}$$

Above all the experimental data for various quantitative and qualitative parameters were statistically analyzed by the OPSTAT software at the Computer Centre, Department ofStatistics, CCS HAU, Hisar (Haryana).

3.8.9 Genetic divergence analysis

Principal component analysis or canonical (vector) analysis is a sort of multivariate analysis where canonical vectors or roots representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively, are derived (Rao, 1952). It reflects the importance of the largest contributor to the total variation at each axis of differentiation. Principal component analysis was done on PAST v3.14 software based on correlation matrix (normalized variance-covariance) because the variables are measured in different units; this implies normalizing all variables using division by their standard deviations.

Euclidean distance cluster analysis was also used for diversity analysis and formed clusters. Euclidean distance cluster analysis was done XLSTAT 2014.5.03 software based.

3.9 Fatty acid analysis:

Fatty acid analyses of 188 genotypes (Germplasm lines and varieties) were carried out at Quality Lab, Division of Genetics, Indian Agricultural Research Institute, New Delhiand further fatty acid profiling of 2 soma clones were carried at Central Instrumental Laboratory, Department of Botany, Banaras Hindu University, Varanasi, UttarPradesh, India.

Procedure for preparation of Methyl esters:

Five grams of each *Brassica* seed sample was taken in a pestle and mortar and ground well into a fine powder. The powder was transferred to a completely dry test tube (50 ml capacity, 25x150mm). Five ml methanol wasadded to each tube followed by two drops of conc. H_2SO_4. These tubes containing oil:methanol:Acid mixtures were incubated in a water bath, at 65^oC for an hour. The tubes were cooled to room temperature and 2.0 ml of hexane was added in each tube. The tubes were shaken well using a vortex mixer and allowed to stand till the hexane layer separated out which contains methyl esters. The hexane layer (1.0-1.5ml) was removed carefully using micropipette and transferred into a 2.0 ml screw capped vial. Small amount of anhydrous sodium sulphate was added to each vial to remove moisture, if any. The vials were stored in a refrigerator in vaccum desiccators if samples were not analyzed on the same day. One ml of the hexane layer containing methyl esters was injected into pre-conditioned gas chromatograph.

The individual fatty acids were identified by their relative retention times and comparing with known standards. Percent fatty acid composition was determined by measuring area under each peak. Fatty acids were analyzed using Gas liquid chromatography (Perkin Elmer Claurus 500) fitted with megabore column (30 meter long and 0.53mm) packed with OV-101, polymer of methyl silicone, using Flame ionizationdetector (FID). The conditions maintained were Column temperature: 150^oC-270^0C, Injector Temperature: 250^oC and Detector temperature: 250^oC. GLC was programmed for the temperature at the rate of 10^OC per minute increase and finally it was maintained at 270^oC. Each sample took 15 minutes (approx) for its analysis.

3.10 Seed oil percentage analysis

The oil extraction was carried out using Soxhlet method at Department of Soil Science& Agricultural Chemistry,College of Agriculture, RVSKVV, Gwalior (M.P.).

Oil Content (%):

The oil was extracted from samples of seeds obtained randomly from each replication of each treatment by using Soxhlet method.

Principle:

Oil from a known quantity of seed is extracted from petroleum ether. It is then distilled off completely, dried and the oil is weighed and % oil was calculated.

Material:

Seed sample, Petroleum ether, Whatman No. 2 filter paper and soxhlet apparatus.

Procedure:

Oil content was estimated by Soxhlet method as described by Sadasivam and Manickam (1966). In this method the seed samples were dried in the oven at 70^0 C for removal of moisture. After removal of moisture, the seed samples were finely ground with the help of pestle and mortar. 10g of dried sample was taken in a thimble which is to be placed in extraction chamber of soxhlet apparatus. The sample was extract with solvent petroleum ether for a period of 6 hours without interruption at 65-70°C temperature to generate solvent vapors, which passed through the distillation column. Cold water was circulated around the distillation column for proper condensation of solvent vapors. The condensed solvent trickled down drop by drop on the sample packet in the extraction chamber. The condensed solvent extracted the oil from the sample. Once the siphon tube got filled, the oil and solvent mixture was siphoned and collected in the bottom flask. On heating, the solvent vapors alone arise up and condensed in the condensing unit. The condensed solvent dropped back to the extraction chamber for further extraction of oil from the sample and the cycle was repeated. After specified time of extraction, the sample packet was removed. The solvent (Petroleum ether) was evaporated on a water bath to remove the odor. Then the flask was placed along with content into oven at $80\text{-}100^0$ C for 30 min. It has been removed after 30 min and allowed to cool in desicator and weighed. The percentage of oil extracted was calculated as follows.

$$\text{Oil \%} = \text{W2} = \frac{W3 - W2}{W1} x\ 100$$

Where:

W1 = Weight of sample

W2 = Weight of flask

W3 = Weight of flask + oil

3.11 Molecular studies:

Based on morpho-physiological and biochemical data of 196 Indian mustard genotypes, molecular characterization of selected 48 genotypes was carried out at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology &Biotechnology, College of Agriculture, Gwalior (M.P.). The molecular characterization was performed as follows:

3.11.1 Molecular markers:

Highly polymorphic random SSRs, gene based SSRs and gene based CAPS markers were used during present investigation.

3.11.2 Genomic DNA Extraction

For DNA isolation, fresh leaf tissue was collected from 3-4 week old plants grown in field. DNA was isolated using modified **CTAB method** (Murray & Thompson, 1980). The following protocol was used to isolate genomic DNA of Indian mustard:

Plate 3.4: Molecular Laboratory Works

Fresh young leaves were taken in sterilized mortar and pestle and pre-warmed (65^{0}C for 30 mins) DNA extraction buffer (2% w/v CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-Cl, milli-Q water and pH8.0) and 0.2% of β-mercaptoethanol was added and emulsion was transferred to 2.0 ml eppendorf tubes.

↓

Kept at 65^{0}C in water bath for 45-50 mins by repeated mixing by gentle swirling in every 10-15 mins. The tubes were allowed to cool to room temperature.

↓

Then 800µl of PCI (Phenol: Chloroform: Isoamyl 25:24:1) was added followed by a gentle swirling for 3-4 min.Then centrifugation was done at 10000 rpm for 10 min at 25˚C.

↓

The aqueous separate phase was then transferred to the fresh clean 2.0ml eppendorf tube. Three-five µl of RNase was added and the contents were mixed and incubated at 37˚Cfor 30-45 mins.

.

↓

RNase may be added after DNA quantification also and the measurement of the quantity of RNase was dependent on the appearance of RNA on the gel during quantification.

↓

Eighty µl of 1/10 volume of Sodium acetate and 2 volume of absolute ethanol was added to the aqueous solution.It was quickly mixedby gentle inversion for five to six times and incubated at -20˚C for 30-40 min or at 4˚C for overnight.

↓

Pellet the DNA by centrifugation at 10,000 rpm for 10 min and the supernatant was discarded.

↓

DNA pellet was washed with 200 µl of 70% ethanol by centrifugation at 10,000 rpm for 5 min then the supernatant wasdiscardedand air dried till the smell of ethanol gone out.

↓

The DNA pellet was dissolved in 100 microlitre TE (100 mM TrisCl and 0.5mM EDTA, pH 8.0) buffer or Nuclease Free Water according to DNA pellet size and stored at 4˚C for complete dissolution.

3.11.3Purification of DNA

Major contaminants of crude DNA preparation are RNA, protein and polysaccharides. Inclusion of CTAB in DNA extraction buffer helps in elimination of polysaccharides to a large extent. The RNA was removed by treating the sample with DNase free RNase (10.0 $mgml^{-1}$) denatured at 37^0C. Proteins including RNase can be removed by treatment with phenol: chloroform: isoamylalcohol (25:24:1).

3.11.4Quantification and Dilution of DNA

For quantification, 1µl of DNA samples, along with known quantity of λ uncut DNA (100ng or 200 ng) was loaded on 0.8% agarose gel. The electrophoresis was carried out at 80 volts for 30 min. DNA was stained with ethidium bromide and observed under UV. The quantity of DNA was determined by comparing the florescence of sample with that of standards after quantification.

To analyze actual quantity of DNA present in sample, DNA samples isolated from each line were quantified on Nano Drop Spectroscopy. Quantification of DNA is done to know the quantity of DNA present in 1 µl of extracted crude sample.After that, DNA was diluted with TE buffer or Nuclease free water such that the final concentration of DNA was approximately25 ng/µl for SSR marker. Quantity of DNA should not be less as primers wouldn’t be able to find there complementary sequences.

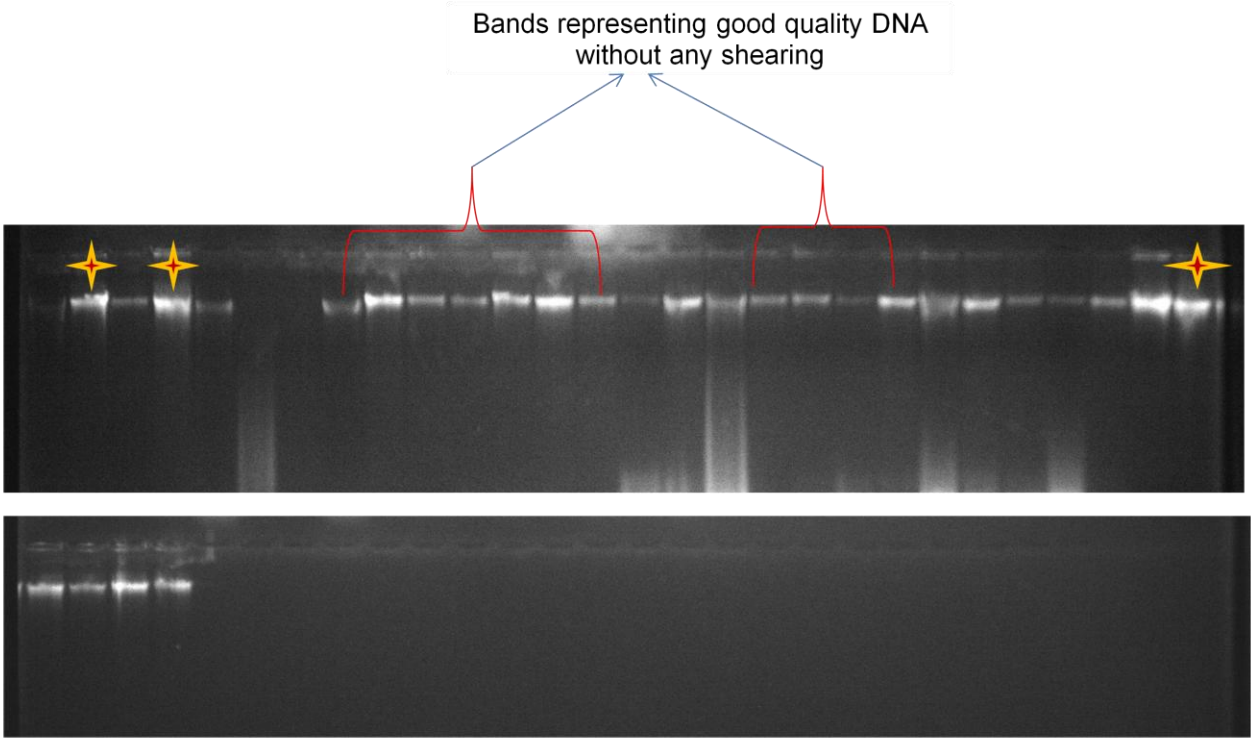
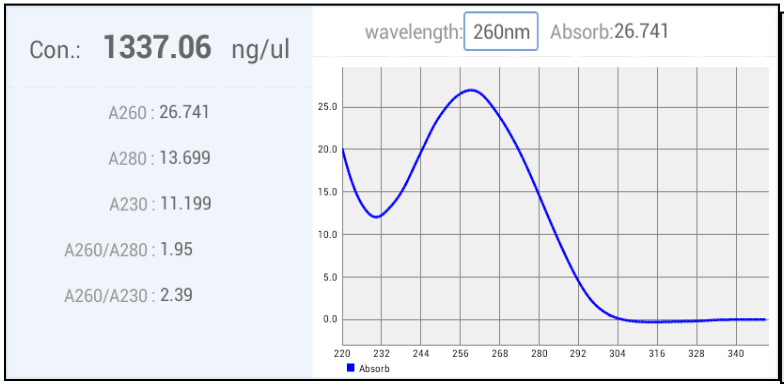


Plate 3.5: Image representing DNA bands of Indian mustard genotypes using 0.8% agarose gel electrophoresis, ✧ - represents band with slight shearing in DNA

3.11.5 PCR amplification of DNA

PCR analysis was done using SSR markers and CPAS markers to identify polymorphism between 48 lines.



Con.: **1337.06** ng/ul

A260 : 26.741

A280 : 13.699

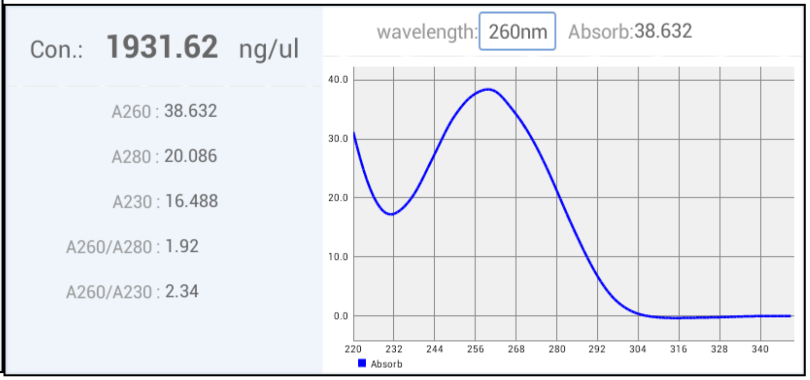
A230 : 11.199

A260/A280 : 1.95

A260/A230 : 2.39

wavelength: 260nm Absorb:26.741

PM21



Con.: **1931.62** ng/ul

A260 : 38.632

A280 : 20.086

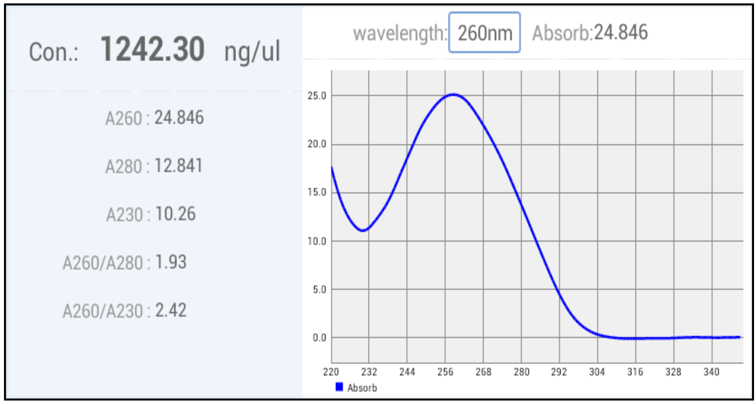
A230 : 16.488

A260/A280 : 1.92

A260/A230 : 2.34

wavelength: 260nm Absorb:38.632

PM22



Con.: **1242.30** ng/ul

A260 : 24.846

A280 : 12.841

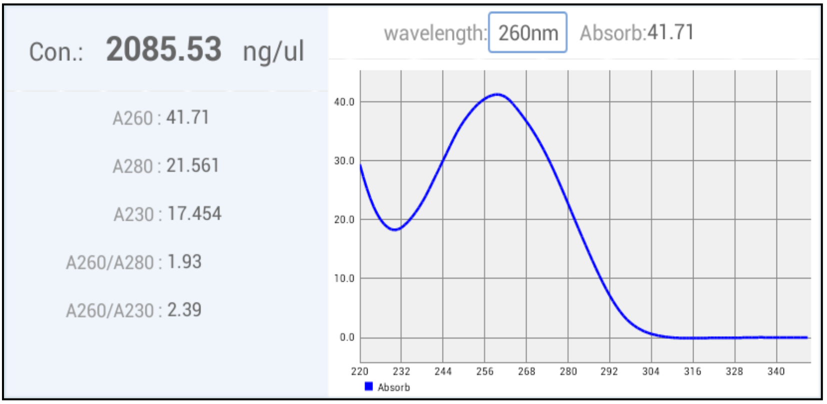
A230 : 10.26

A260/A280 : 1.93

A260/A230 : 2.42

wavelength: 260nm Absorb:24.846

PM24



Con.: **2085.53** ng/ul

A260 : 41.71

A280 : 21.561

A230 : 17.454

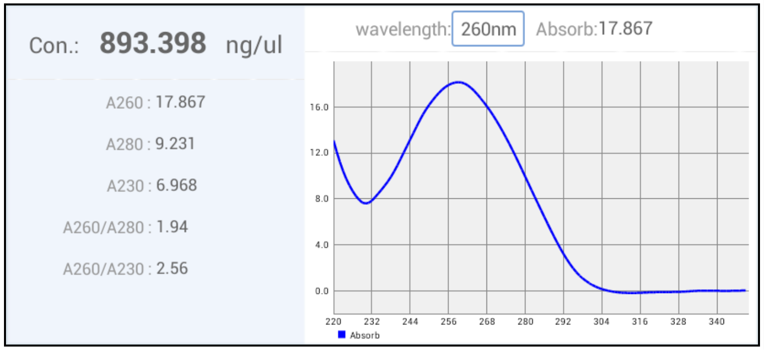
A260/A280 : 1.93

A260/A230 : 2.39

wavelength: 260nm Absorb:41.71

PM29

Fig 3.3: DNA Quantification of some Indian mustard genotypes *viz.*, PM21, PM22, PM24 and PM29


Con.: **893.398** ng/ul

wavelength: 260nm Absorb:17.867

A260 : 17.867

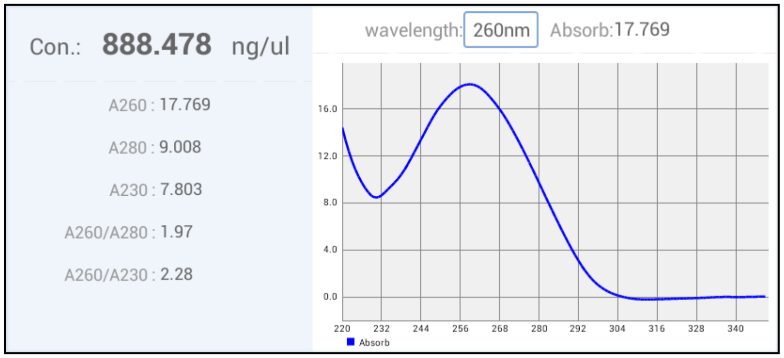
A280 : 9.231

A230 : 6.968

A260/A280 : 1.94

A260/A230 : 2.56

PM30


Con.: **888.478** ng/ul

wavelength: 260nm Absorb:17.769

A260 : 17.769

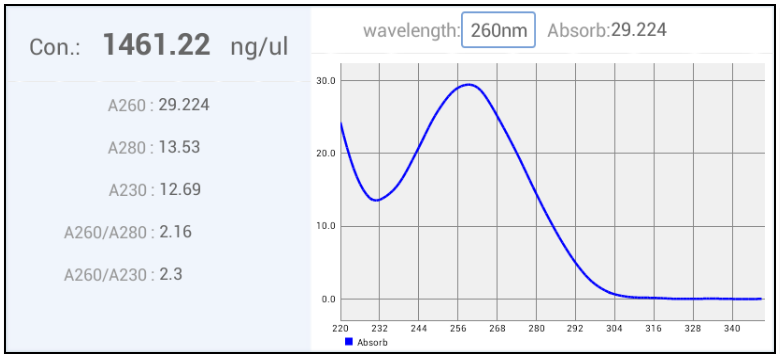
A280 : 9.008

A230 : 7.803

A260/A280 : 1.97

A260/A230 : 2.28

LES


Con.: **1461.22** ng/ul

wavelength: 260nm Absorb:29.224

A260 : 29.224

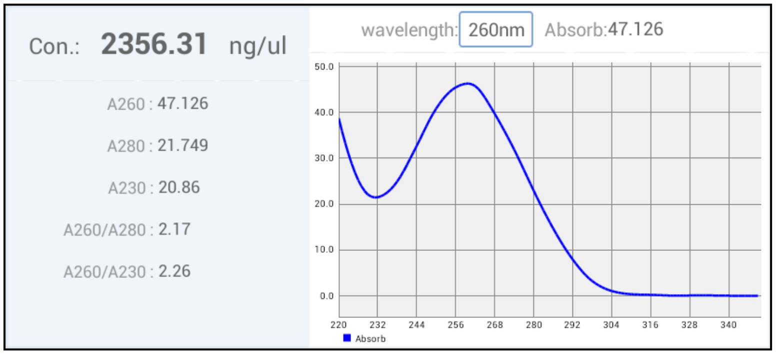
A280 : 13.53

A230 : 12.69

A260/A280 : 2.16

A260/A230 : 2.3

L4


Con.: **2356.31** ng/ul

wavelength: 260nm Absorb:47.126

A260 : 47.126

A280 : 21.749

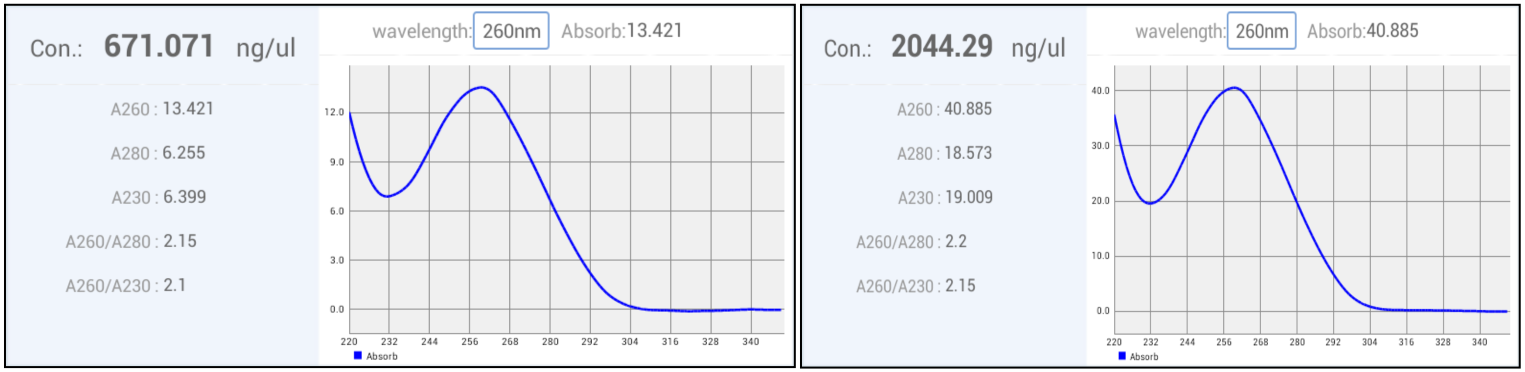
A230 : 20.86

A260/A280 : 2.17

A260/A230 : 2.26

L6

Fig 3.4: DNA Quantification of some Indian mustard genotypes *viz.,* PM30, LES39, L4 and L6



Con.: 671.071 ng/ul

A260 : 13.421

A280 : 6.255

A230 : 6.399

A260/A280 : 2.15

A260/A230 : 2.1

wavelength: 260nm Absorb:13.421

GM2

Con.: 2044.29 ng/ul

A260 : 40.885

A280 : 18.573

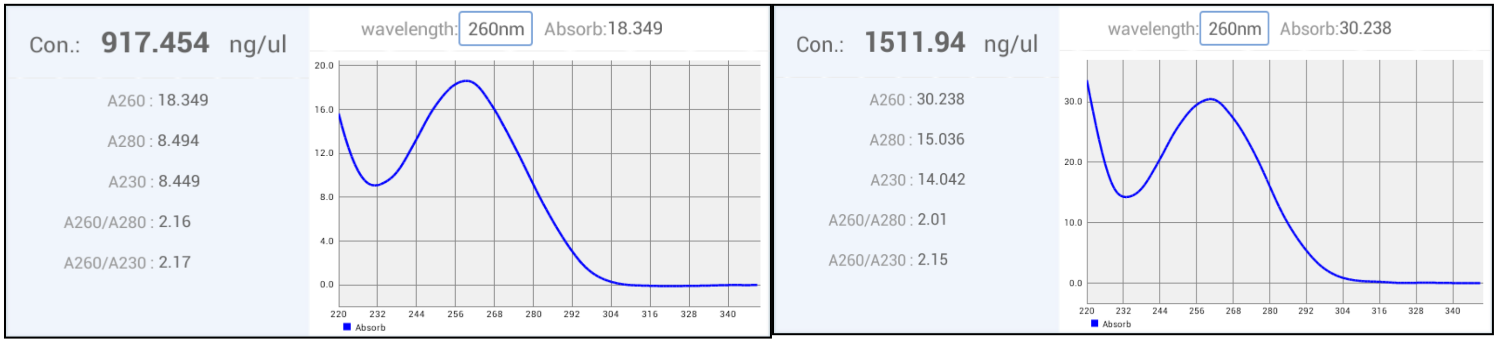
A230 : 19.009

A260/A280 : 2.2

A260/A230 : 2.15

wavelength: 260nm Absorb:40.885

CS54



Con.: **917.454** ng/ul

A260 : 18.349

A280 : 8.494

A230 : 8.449

A260/A280 : 2.16

A260/A230 : 2.17

wavelength: 260nm Absorb:18.349

Con.: **1511.94** ng/ul

A260 : 30.238

A280 : 15.036

A230 : 14.042

A260/A280 : 2.01

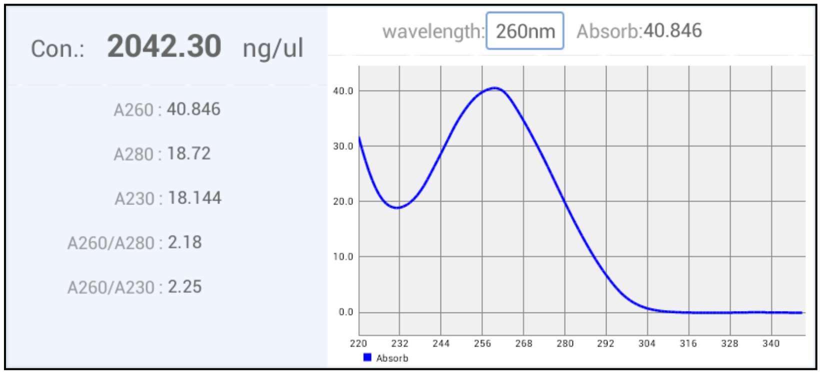
A260/A230 : 2.15

wavelength: 260nm Absorb:30.238

R

RH74

Fig 3.5: DNA Quantification of some Indian mustard genotypes *viz.,* GM2, CS54, RB50 and RH749



Con.: **2042.30** ng/ul

wavelength: 260nm Absorb:40.846

A260 : 40.846

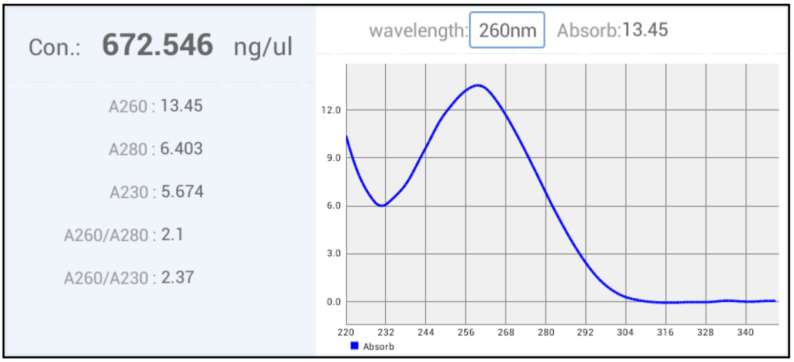
A280 : 18.72

A230 : 18.144

A260/A280 : 2.18

A260/A230 : 2.25

Maya



Con.: **672.546** ng/ul

wavelength: 260nm Absorb:13.45

A260 : 13.45

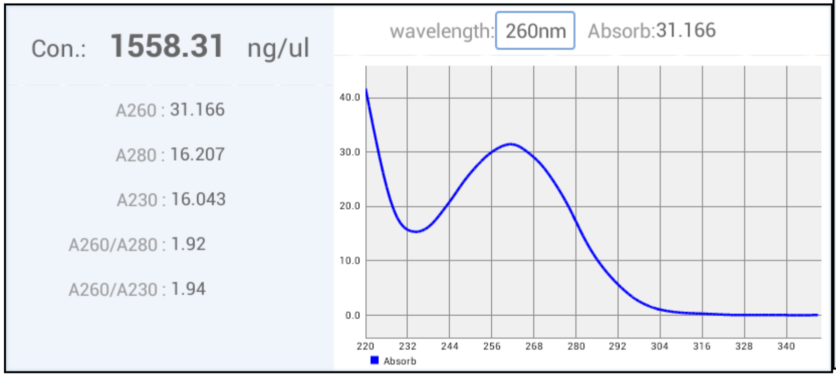
A280 : 6.403

A230 : 5.674

A260/A280 : 2.1

A260/A230 : 2.37

NRCDR2



Con.: **1558.31** ng/ul

wavelength: 260nm Absorb:31.166

A260 : 31.166

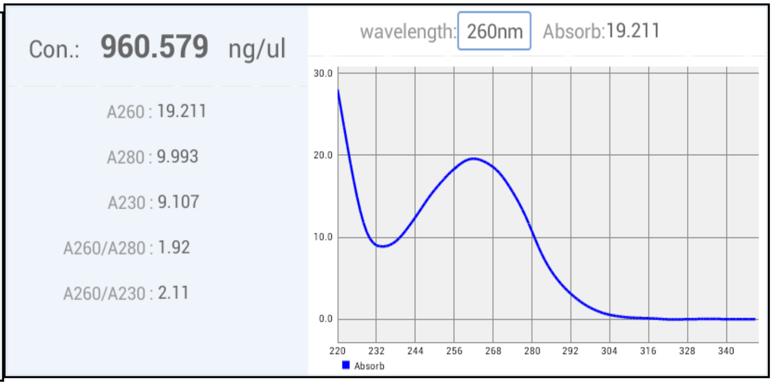
A280 : 16.207

A230 : 16.043

A260/A280 : 1.92

A260/A230 : 1.94

DRMRIJ31



Con.: **960.579** ng/ul

wavelength: 260nm Absorb:19.211

A260 : 19.211

A280 : 9.993

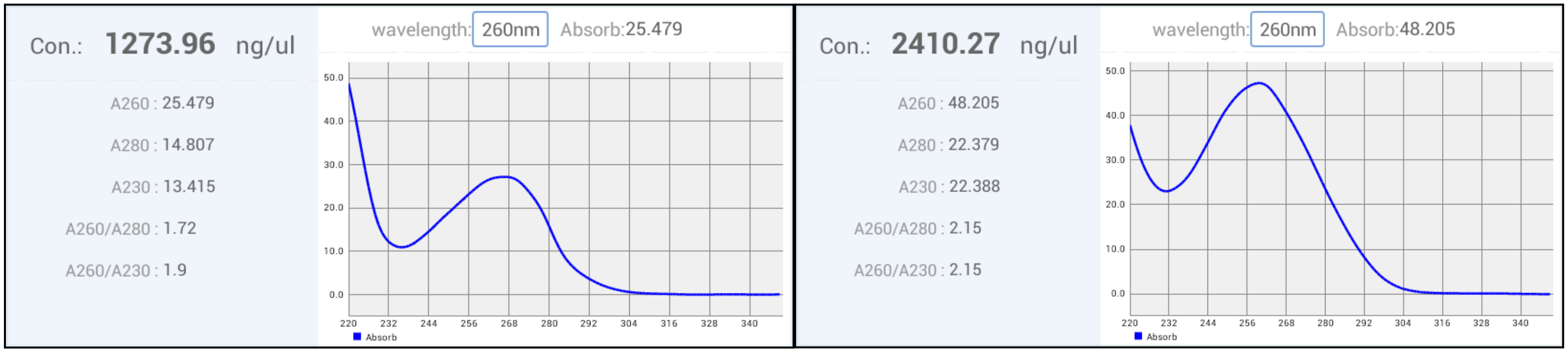
A230 : 9.107

A260/A280 : 1.92

A260/A230 : 2.11

DRMR150-

Fig 3.6: DNA Quantification of some Indian mustard genotypes *viz.,* Maya, NRCDR2, DRMRIJ31 and DRMR150-35



Con.: **1273.96** ng/ul

wavelength: 260nm Absorb:25.479

A260 : 25.479

A280 : 14.807

A230 : 13.415

A260/A280 : 1.72

A260/A230 : 1.9

Con.: **2410.27** ng/ul

wavelength: 260nm Absorb:48.205

A260 : 48.205

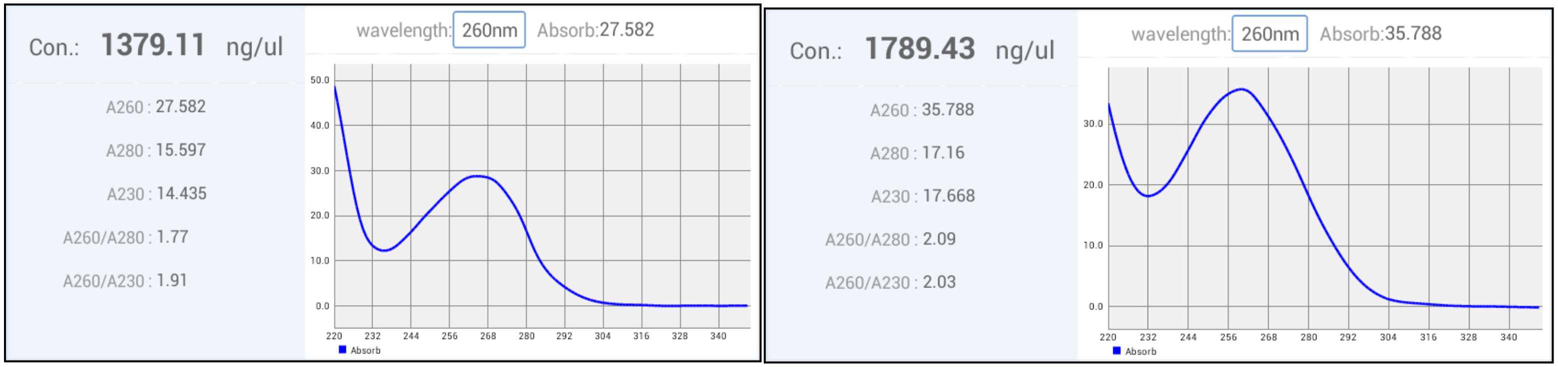
A280 : 22.379

A230 : 22.388

A260/A280 : 2.15

A260/A230 : 2.15

JM2 RVM1



Con.: **1379.11** ng/ul

wavelength: 260nm Absorb:27.582

A260 : 27.582

A280 : 15.597

A230 : 14.435

A260/A280 : 1.77

A260/A230 : 1.91

Con.: **1789.43** ng/ul

wavelength: 260nm Absorb:35.788

A260 : 35.788

A280 : 17.16

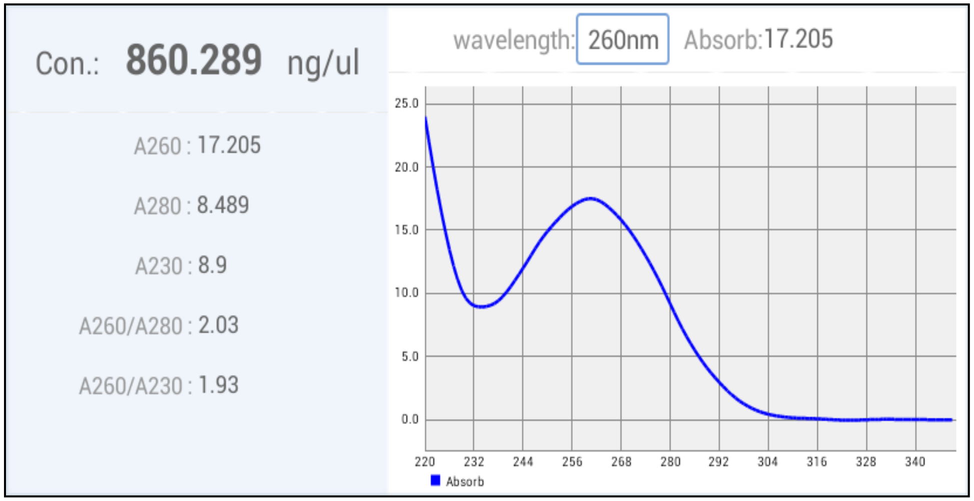
A230 : 17.668

A260/A280 : 2.09

A260/A230 : 2.03

RVM2 Rohini

Fig 3.7: DNA Quantification of some Indian mustard genotypes *viz.,* JM2, RVM1, RVM2 and Rohini



Con.: **860.289** ng/ul

A260 : 17.205

A280 : 8.489

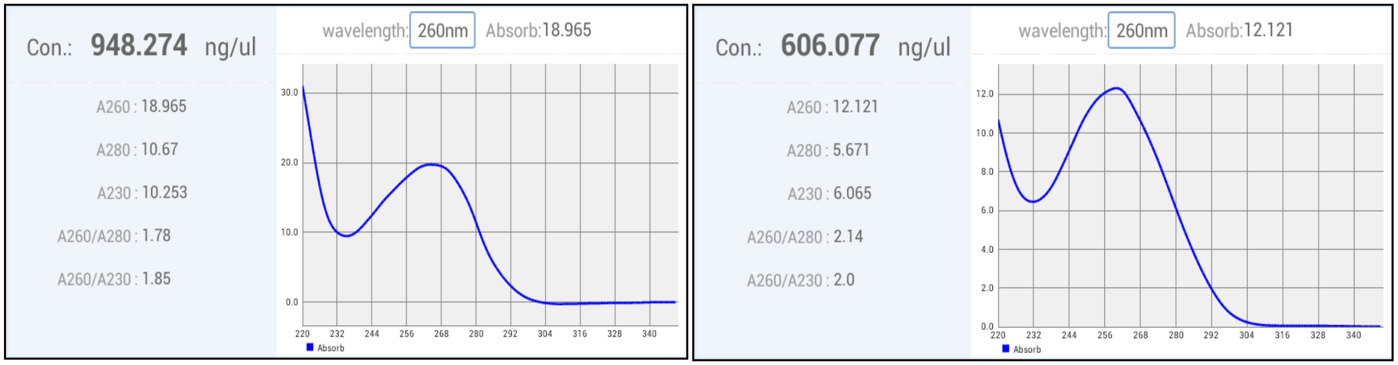
A230 : 8.9

A260/A280 : 2.03

A260/A230 : 1.93

wavelength: 260nm Absorb:17.205

JD6


Con.: **948.274** ng/ul

wavelength: 260nm Absorb:18.965

A260 : 18.965

A280 : 10.67

A230 : 10.253

A260/A280 : 1.78

A260/A230 : 1.85

MRN

Con.: **606.077** ng/ul

wavelength: 260nm Absorb:12.121

A260 : 12.121

A280 : 5.671

A230 : 6.065

A260/A280 : 2.14

A260/A230 : 2.0

ISC3

Fig 3.8: DNA Quantification of some Indian mustard genotypes *viz.,* JD6, MRNJ19 and

Protocol for PCR Analysis was as Follows:

Taken a PCR plate and labeled it for each genomic DNA.

↓

Added 1 µl of template DNA into the wells of PCR plate

↓

Prepared the following PCR reaction cocktail in the cocktail tube for required number of reactions

↓

Added 9 µl of the PCR reaction mixture into each well, which was already loaded with 1µl of template DNA making the final volume to 10 µl

↓

Covered the PCR plate and centrifuged it at about 500 rpm for 1 minute to bring the contents to the bottom of the wells.

↓

Performed the PCR reaction in Programmable Thermo Cycler

↓

The amplification was carried out for 35 cycles of Verti96 well of BIO-RAD T100 Thermal Cycler. Temperature profiles followed for PCR are being presented in Table 3.7.

3.11.6 Visualization of amplified products in agarose gel electrophoresis

3.0 per cent agarose gels were used for separation and visualization of PCR amplified products of SSR and CAPS markers. Gels were casted in electrophoresis unit.

3.11.7. Assembling and pouring the gel

The ends of the 400 ml gel tray securely sealed with strips of colored lab tape. The tape was firmly pressed to the edges of the gel tray to form a fluid-tight seal. For making 3 %. agarose gel 12.0 g agarose is added in to 300 ml of TBE having 1X concentration in a measuring bottle then subjected to 15 minutes heating in microwave oven. After that the melted gel was added with 15 μl ethidium bromide subsequently mixing and pouring of gel in to the 400 ml gel tray Now four combs each having 28 wells was placed on the gel tray having notches for appropriate fixation of the combs. After that gel was allowed to cool for 30 minutes. Now cooled gel poured in 400 ml gel tray and having four combs was placed in gel tank filled with 1X TBE buffer. Combs were removed gently.

3.11.8. Gel Electrophoresis

Two μl loading dye (10x) was added to PCR products. Finally, 10 μl of each sample was loaded into the wells for facilitating the sizing of the various alleles. MAGB and 100 bp DNA Ladder (APSLABS) was loaded in the first well (6 μl). Gel was run at 120 volts till the dye reached bottom of the gel for about 90 minutes.

3.11.9. Visualization of bands

After completion of gel electrophoresis, gels were subjected to documentation with UV image analyzer and subsequently gel picture having DNA as a band was saved to the desktop connected with UV image analyzer (UVP Gel Doc-It Imager, Analytikjena).

3.11.10. Detection of polymorphism using simple sequence repeat (SSR) primers and Cleaved amplified polymorphism

Total 24 random SSR primers were used for detection of polymorphism out of which 23 SSR primers showed polymorphism and 1 primer showed monomorphism. Another 2 gene specific SSR primers and CAPS were also used.

Reaction	**Stock concentration**	**Volume**
Nuclease free water		6.75
PCR buffer with $MgCl_2$	10X	1
DNTPs mix	1mM	0.15
Primer (Forward +Reverse)	5pmol	0.5+0.5
Taq DNA polymerase	3U/ml	0.1
DNA template	50ng/ml	2.0
Total		**10**

Table 3.6: PCR mix for one reaction

Table 3.7: Temperature profile used for PCR amplification

Steps	**Temperature**	**Duration**	**Cycle**	**Activity**
1	95^{0}C	5 min	1	Denaturation
2	95^{0}C	30 sec		Denaturation
3	56^{0}C	30sec	35	Anealing
4	72^{0}C	2 min		Extension
5	72^{0}C	7 min	1	Final Extension
6.	4^{0}C	∞	1	Storage

3.11.11Scoring of data

The banding pattern of population developed by each set of primer was scored separately. For estimating the size of DNA of each sample the band position was compared with a base pair of standard marker presence of band in a particular base pair position was scored as “A, B, C,D, E” and absence of band that particular base pair position was scored as blank.

3.11.12. Development of genotypic data using SSR markers and CAPS markers

Genotypic data using primers was developed.

3.11.13. Reagents and solutions

3.11.13.1Reagents and solutions for DEB

a.10% CTAB:

100 g of CTAB powder was dissolved in sterile Milli-Q water and the volume was adjusted to 1litre.

b. Tris-Cl (1M):

121.1 g of Tris-Cl salts was dissolved in 800 ml of sterile Mill-Q water. pH was adjusted to 8.0 with concentrated 1N HCl. The final volume was adjusted to 1 litre with Milli-Q water and sterilized by autoclaving.

c. EDTA (0.5M; pH-8):

186.12 g of EDTA was dissolved in 800 ml of milli-Q water. The pH was adjusted to 8 using NaOH pellets. Final volume was adjusted to 1litre with milli-Q water and sterilized by autoclaving.

d. NaCl (4M):

233.8 g of NaCl was dissolved in 800 ml of Milli-Q water. The final volume was adjusted to 1litre with Milli-Q water and sterilized by autoclaving.

e.DNA extraction buffer (100ml):

Tris-Cl (1M, pH-8) 5 ml

EDTA (0.5M, pH-8) 2 ml

NaCl (4M) 17.5 ml

10% CTAB 10ml

β - mercaptoethanol 0.2ml

Milli – Q water 15.3ml

f. TE buffer:

1M Tris-Cl (pH-8) 1 ml

0.5M EDTA (pH-8) 0. 2 ml

The final volume was adjusted to 100ml with 98.8ml Milli-Q water.

g.70% Ethanol:

70ml of absolute ethanol was added in 30ml of distilled water.

3.11.13.2. Reagents for PCR

a. Primers:

Highly variable microsatellite markers procured from ILS/SIGMA-SviBiosolutions Private limited. Primer (Forward and Reverse) are diluted according to their ηmol concentration, for stock preparation SIGMA water is added 10 times more to its ηmol concentration and then 10µl of each primer (F& R) was taken in Eppendorf tube and added 80 µl of SIGMA water to makes 100 µl.

b. dNTPs: (dATP/dCTP/dGTP/dTTP)

10 mM stock of dNTP (Thermo scientific) was used. Stock preparation- taken 10µl of each dNTPs (*i.e*. dATP/dCTP/dGTP/dTTPs) in 1.5 ml of Eppendorf tube, mixed well by vortexing, final volume is 40µl having 100 mMdNTPs stock concentration. For dilution 10 µldNTPs of stock solution was taken in 1.5 ml Eppendorf tube and 990 µl SIGMA water was addedto the tube, so the total volume became 1000 µl. This makes 1mM dNTPs was ready to be used for PCR.

c. PCR buffer (10X) included 20mM $MgCl_2$:

Thermo scientific's PCR buffer (10X) included 20mM $MgCl_2$was used.

d. *Taq* DNA polymerase:

5U/µl of 500U*Taq*(Thermo Scientific) was used for PCR.

e. Nuclear free water:

Himedia's nuclear free water was used.

3.11.13.3Reagents and solutions for electrophoresis

a. 10X TBE buffer:

Tris base 108 g
Boric Acid 55g
EDTA (0.5M) 40 ml
Distilled water 800 ml
Final volume was adjusted to 1 liter with distilled water.

b. Tank buffer (1X TBE):

10 ml of 10X TBE was taken and 90 ml of distilled water was added.

c. 6X loading dye:

Bromophenol Blue 6X loading dye (Thermo Scientific) was used.

d. 100bp DNA Ladder:

APSLAB's 100bp DNA ladder was used.

3.11.14 Molecular Analysis

The SSR and CAPS profiles were scored based on the size of fragments amplified across selected mustard genotypes. The major allelic frequency, polymorphism information content and genetic distance based clustering was performed with Un weighted Pair Group Method for Arithmetic Average (UPGMA) tree using Power Marker v 3.25 and the dendrogram was constructed using MEGA 4.0 software. The population structure was inferred using Structure Harvester. The relation between genetic similarity identified by SSR markers and taxonomic distance measured by mean genetic distance was analyzed using Jaccard's Similarity Index and average taxonomic distance calculated by NTSYS-pc v 2.1.

Table 3.8: List of random SSR markers

.N.	Marker name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
	BRMS-093	TCCAAGTAGACCGAATCAAGAGAGT	ATAAATCGAACCTGAAACCATGTCT
	BRMS- 098	TGCTTGAGACGCTGCCACTTTGTTC	CATTCCTCCCCACCACCTTCACATC
	BRMS- 240	CAAGAGTATTTGTGTGGGTTGACTC	AAATAACGAACGGAGAGAGAGAGAG
	BRMS- 324	AACTTAACCGAAACCGAGSTAGGTG	AATCTCGAAATTCATCGACTTCCTC
	CB -10065	CGGCAATAATGGACCACT	CGGCTTTCACGCAGACTTCG
	SORF -73	CGTGGGCCAAGCTTAGATTA	CGTTCAAGAAGACACAGATCAAA
	SR-7223	AGGACCCGACTTTCCTTGTT	ACCAAACTCGGCGTACAAAT
	SR-9222	CACCGAACAAAACTGAGGGT	CGTTTCACTGCGTTCTACCA
	SR 94102	ATCCCCAAACTACCCTCACC	AGGATGAGCAAAGGAAAGCA
0	SR-9447	AAATTCGAAAATGCAAACGG	CCAATCTTGGAACAATAGAAGATG
1	OI10-CO 5	GGCTACAAAATGTTTGATAAGCTCT	ACCTGAAAGAGAGGCTACACAT
2	SSR Na10-B08	AGAGAAAAACACTTCCCGCC	GTGAGCTTTGCGAAACACG
3	SSR Na10-B10	GTCGGGTTTGAGTGAGTTGG	CATCGCAGATCCTTCTCTCC
4	SSR Na10-B11	TTTAACAACAACCGTCACGC	CTCCTCCTCCATCAATCTGC
5	SSR Na10-C01	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC
6	SSR Na10-C03	TTGGGTGTCTTTGTTACCCC	ACCGAGAAGACTGATACGGG
7	SSR Na10-C06	TGGATGAAAGCATCAACGAG	ATCAATCAACACAAGCTGCG
8	SSR Na10-C08	GTTTGGTTCAGAGGCAGAGG	CTATCGCTGCAGAAGAAGGG
9	SSR Na10-D03	ATGATTTGCCTTGAAATGCC	GATGAAACAATAACCTGAGACACAC
0	SSR Na10-D07	CTACTTTGATGGACACTTGCC	TCTGAAGTTGATTAGTCGGTCC
1	SSR Na10-D08	TCCATTCATTAAAATCGGCG	TTCTGATCCCTTTCTCTCCC
2	SSR Na10-D09	AAGAACGTCAAGATCCTCTGC	ACCACCACGGTAGTAGAGCG
3	SSR Na10-D11	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG
4	SSR CN-52	5′-CCGGCTTGGTTCGATACTT-3′	5′-TTGCGAATCTTTAAGGGACG-3′

Table 3.9: List of gene specific SSR markers

.N.	Marker name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
	FAE1	ATGACGTCCGTTAACGTA	AAGACTTGTCGTCAGCTCCA
	Sal-SRK-I	GATTATCTCGTGTCTGAATG	GGTAATGTCGAATCTCTCCT

Table 3.10: List of gene based CAPS markers

.N.	ene	Primer name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Restriction enzyme	Amplicon size (bp)
	AE 1.1	CAPS1265	ACGTTAGGTCCGTTGATTCTTC	GGGTATCTGTCGATGCAATGT	BG1II	427
	AE1.2	CAPS237	TAACCATCGCTCCACTCTTTG	TCAAGAAGTCAAGCCACGAC	MnII	219

3.12 *In vitro* studies

(A) For callus culture and cell suspension culture:

Based on morpho-physiological and biochemical data of 196 Indian mustard genotypes, callus culture of selected 7 genotypes were established in Plant Tissue Culture Lab at Plant Molecular Biology & Biotechnology Department, College of Agriculture, RVSKVV,Gwalior (M.P.).Six genotypes (CS-54, GM-2, RB50, NRCDR-2, Rohini and PM30) were selected based on their growth, performances of yield and low and high erucic acid content among one eighty-eight Indian mustard genotypes. These six genotypes were used for establishment and initiation of callus and cell suspension cultures utilizingvarious plant growth regulators in different concentrations individually as well as in combinations.

Method

The general methodology for media involved preparation of stock solution (10x to 100x) using high purity chemicals obtained from Hi-media and Sigma, USA. The formulation of Murashige & Skoog (1962) basal media was followed throughout all the experiments. The media wassupplemented with different plant growth regulators individually and in combinations for various stages. The MS dehydrated media (Hi-Media) was used for making different stock solution of 1 litre. The dehydrated MS media (Hi-Media) devoid of sucrose, PVP, agar and PGRs was used. Sucrose and PGRs were added as per requirement during different culture phases for callus induction and cell suspension cultures followed by plantlet regeneration.

3.12.1. Preparation of stock solutions:

The formulation of Murashige and Skoog (1962) basal medium was followed throughout all the experiments, as it is most widely used enriched media in contemporary for tissue culture studies. A general composition of basal MS medium is given in the Table 3.8.

Table 3.11: Chemical Composition of Murashige and Skoog (1962) Media

S.No.	Constituents	Amount (mg/l)
I Inorganic compound		
(a)	**Macro nutrients**	
	NH_4NO_3	1650
	KNO_3	1900
	$MgSO_4.7H_2O$	370
	$CaCl_2.2H_2O$	440
	KH_2PO_4	170
(b) Micro nutrients		
	H_3BO_3	6.2
	$MnSO_4.4H_2O$	22.3
	ZNSO4.7H_2O	8.6
	KI	0.83
	$NaMoO_4.2H_2O$	0.25
	$CuSO_4.5H_2O$	0.025
	$CoCl_2.6H_2O$	0.025
(c) Iron source		
	Na_2EDTA	37.25
	$FeSO_4$7H2O	27.85
II Organic compound		
(a)	Vitamins	
	Nicotinic acid	0.5
	Thiamine HCl	0.1
	PyridoxinHCl	0.5

	Myoinositol	100
(b) Amino acid		
	Glycine	2.0
(C) Carbon source		
	Sucrose	30000
III Gelling agent		
	Agar	8000
IV Other		
	pH 5.8	

3.12.2 Stock solution of vitamins

To make 50 ml of vitamins stock solution, nicotinic acid 25 mg dissolved in small amount of sterile distilled water and the final volumes was made to 50 ml and allowed to cool and stored in refrigerator at 0^0C. Similarly the other two vitamins pyridoxine HCl (25.0 mg) and thiamine HCl (5.0mg) were prepared and stored.

3.12.3 Stock solution of plant growth regulators

Plant growth regulators were not soluble in water so they were firstly dissolved in NaOH or alcohol then water was added to get final volume. Fifty mg of 2, 4-D was dissolved in 1.0 ml 1N NaOH and final volume was made up to 50 ml with double distilled water. Fifty mg of NAA was dissolved in 1.0 ml 1N NaOH and final volume was made up to 50 ml double distilled water. Fifty mg of auxin Indole 3-butyric acid (IBA) was initially dissolved in the 1.0 ml 1N NaOH and then volume was made up to the final volume of 50 ml by adding double distilled H_2O. Fifty mg of cytokinin 6- benzyl amino purine (BAP) were first dissolved in 1.0 ml 1N NaOH and final volume was made up to 50 ml with double distilled water.

Table3.12: List of Plant Growth Regulators

S.No.	Hormones	Required amount for stock solution (mg)	Amount of solvent required to dissolve	Amount of water added (ml)
1	2,4-D	10	1 ml IN NaOH	100ml
2	NAA	10	1 ml IN NaOH	100ml
3	IBA	10	1 ml IN NaOH	100ml
4	BAP	10	1 ml IN NaOH	100ml
5	TDZ	10	1 ml IN NaOH	100ml

Plate 3.6: ***In Vitro*** **Laboratory Works View**

3.12.4 Preparation of culture media

800ml of distill water was taken

↓

4.41g MS powder was added

↓

30.0gm sucrose was added

↓

PGRs were added

↓

Volume was made up to 1 liter

↓

pH was adjusted to 5.8 to 6.5
(1N NaOH – High)
(HCl – Low)

↓

Magnetic stirrer put in this solution

↓

Subjected for melting onHotplate for 20 min

↓

7.5 gm agar was added to make semisolid

↓

Poured in sterilized culture bottles/petriplates and subjected to autoclaving

3.12.5 Sterilization of culture media

Prepared culture mediawas sterilized in autoclave at 121^{0}C and 15 psi for 20 min. The heat sensitive PGRs were filter sterilized and added aseptically to the autoclaved media.

3.12.6Surface sterilization and inoculation of seed

Establishment of contamination free cultures is the first step in the development of the regeneration protocol for any plant species. In the present study, healthy and medium sized seeds have been taken for germination. The seeds were given treatment with Tween-20 for 10 min and then washed with double distilled water. The seeds were then treated with Bavastin (Carbendazim at 0.25%, w/v) for 2 min followed by a washing with distilled water. The seeds were further treated with70% ethanol for 2 min followed by washing with distilled water followed by surface sterilization by immersing the seed in 0.1% mercuricchloride ($HgCl_2$) for 1-2 min and finally with sterilized double distilled water. About 7-8seeds were transferred aseptically with the help of forceps to each culture bottle (7-8 cm diameter) containing 50 ml of MS medium under aseptic condition and incubated at 25± 2^0C under photoperiod of 16 hr light and 8hr dark.

Table 3.13: List of surface sterilizing agents

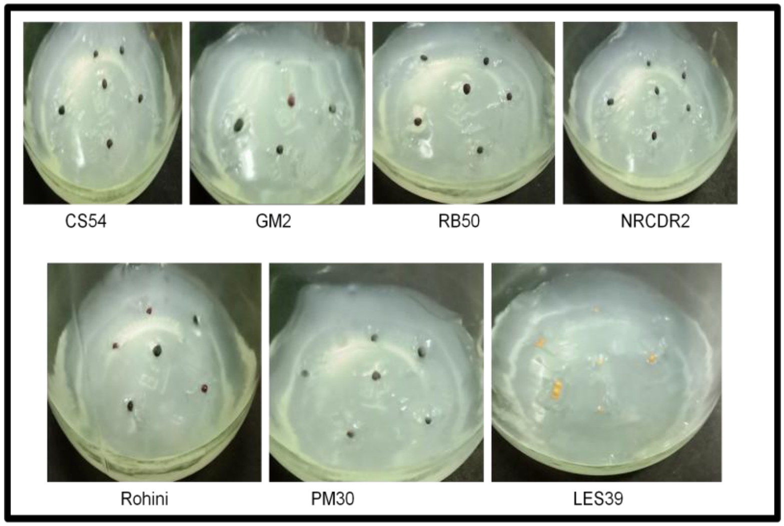
S.No.	**Surface sterilizing agents**	**Percent of steriliants**	**Time**
1.	Twin 20	5%	10min
2.	Bavastin	0.25%	2min
3.	Ethanol	70%	2min
4.	$HgCl_2$	0.1%	1-2min

3.12.7 Preparation of explants

Three explants *viz*: hypocotyls, cotyledon and seed were used for culture establishment. About 0.8-1.0 cm long pieces of hypocotyls of 10 to 15days old Indian mustard seedlings were cut with the help of sterile blades. Hypocotyls below the first true leaf from 3-4 weeks old *in vitro* seedlings were taken. 0.1 cm cotyledon explants from *in vitro* seeding and 1 to 2 mm seed were also taken. All the aseptic operations were carried out inside the Laminar Air Flow Cabinet.

3.12.7 Transfer of explants

All the explants were placed on a sterilized filter papers taken in a Petri dish and were cut into small pieces (0.5 to 1.0cm) using sterilized surgical blades. Explants cut to proper size were then transferred with the help of a pair of sterilized forceps on to culture flasks containing desired amount of sterilized media.



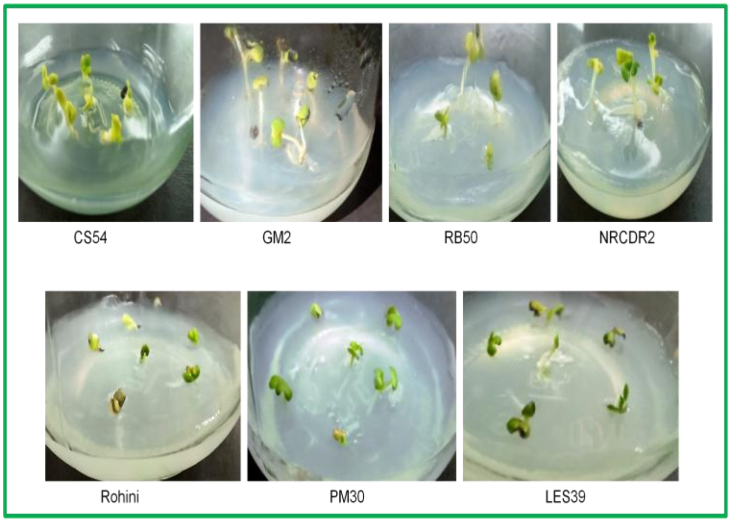
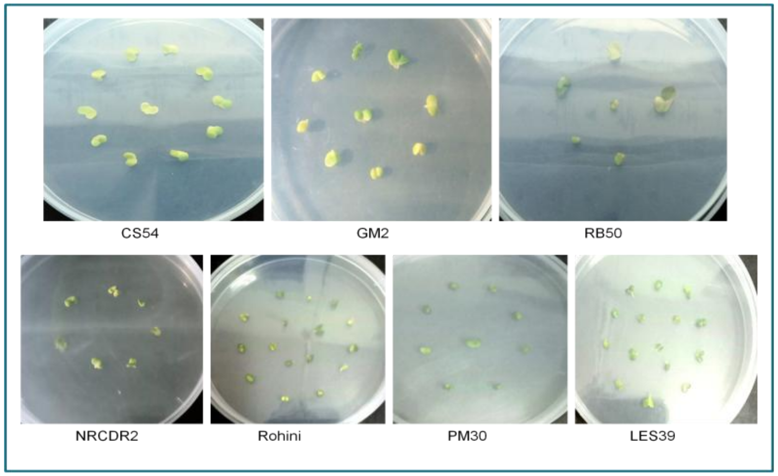


Plate 3.7: Seed explants of various Indian mustard genotypes

Plate 3.8: Seed germination of different Indian mustard genotypes



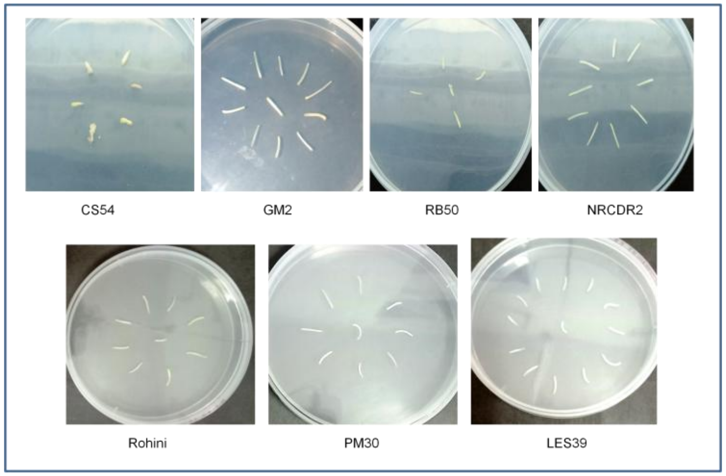


Fig 3.9: Cotyledon explants of various Indian mustard genotypes

Fig 3.10: Hypocotyl explants of different Indian mustard genotypes

Table 3.14 Preliminary experiments with different types and concentrations of plant growth regulators (auxin and cytokinin) used for optimization of *in vitro* response

S. No.	**Treatment code**	**Plant growth regulators (in mgl^{-1})**				
		2,4- D	NAA	BA	BAP	TDZ
1.	MSD	0.5	-		-	-
2	MSD	1.0	-	-	-	-
3	MS2D	2.0	-		-	-
4	MS3D	3.0	-		-	-
5	MS4D	4.0	-		-	-
6	MS.5B	-	-		0.5	-
7	MSB	-	-		1.0	-
8	MS5B.5D	0.5	-		5.0	-
9	MSB.5N	-	0.5		1.0	-
10	MSNT	-	1.0		-	1.0
11	MS.5IBA	-	-	.5	-	-
12	MSIBA	-	-	.0	-	-

3.12.8 Incubation and maintenance of culture

All cultures were incubated in racks inside a culture room with controlled conditions of light, temperature and humidity. The cultures were exposed to photoperiodic cycle of 16 hours cool white fluorescent light and 8 h darkness at 25 ± 2ºC and 70RH maintained inside the culture room. It was observed daily. After 7-10 days, callus formation occurred from explants and these were further maintained by sub-culturing to fresh medium.

Table3.15: List of culture media used for callusinduction

S. No.	Treatment code	Plant Growth regulators (in mgl^{-1})
1	MS2D	2.0, 2,4-D
2	MS3D	3.0, 2,4-D
3	MS4D	4.0, 2,4-D
4	MS.5B	0.5, BAP

Table 3.16: Different treatments for friable callus induction, cell suspension culture and shoot formation

. No.	Treatment code	friable callus induction	cell suspension culture	shoot formation
	MS2D	Yes	Yes	-
	MS3D	Yes	-	-
	MS2D	-	Yes	-
	MS3D	Yes	Yes	-
	MS5B.5D	-	-	Yes
	MSB.5N	-	-	Yes
	MSNT	-	-	Yes

3.12.9 Induction for organogenetic calli

Well developed callus were cut into small pieces and transferred to the maintenance media. After 15-20 days, shoot formation occurred from callus and these shoots werecut and further transferred to shootmediafor multiple shoot proliferation.

3.12.10 Induction of shootlets

Morphogeniccalli was transferred to shooting media for proper growth and development.

Table3.17: Different treatments for Morphogenic calli and shoot initiation

S. No.	Treatment code	Plant Growth regulators (in mgl^{-1})
1	MS.5B	0.5BAP
2	MS5B.5D	5.0BAP+0.52,4-D
3	MSB.5N	1.0BAP+0.5NAA
4	MSNT	1.0NAA+1.0TDZ

3.12.11 Induction ofroots

For rootinduction, elongated and well developed shoots of 3–5 cm length were placed on rooting media. After 10 days root formation occurred. Fully developed plantlet with root was transferred to polyhouse for hardening.

Table3.18:Culture media combination for root induction

S. No.	Treatment code	Plant Growth regulators (in mgl^{-1})
1	MS.5I	MS media+0.5IBA

3.12.12Establishment of cell suspension cultures

Well developed friable callus (0.5 to 2.0 g fresh mass) was transferred in liquid medium for raisingfriable cell suspension cultures. Fresh weight was taken. Relative growth rate was calculated on the basis of increment in fresh weight after culturing of embryogenic calli on different liquid medium after 2-7 weeks of initial culture.

3.12.13Regeneration of plant from embryoid/cell clumps

Cell clumps/ embryoids were transferred in regeneration media.

3.12.14Observations were recorded as follows:

(a) Callus culture

For seedlings, cotyledons and hypocotyls explants cultures, observations were recorded at 3 different stages:

Stage-I: after 2-3 weeks of initial culturing,

Stage-II: after 4-5 weeks of sub culturing of calli on regeneration media and

Stage-III: when the complete plantlets were obtained. All the observations were based on initial culture media, irrespective of regeneration medium or rooting medium. During the present study, following observations were recorded:

1. **Number of callus forming explants / 100 explants plated**: Cultured explants on different culture media recorded for callus formation after 3-4 weeks (**Stage -I** observation).
2. **Number of morphogeniccalli / 100 explants plated**: Calli showing morphogenic ability considered as Morphogenic calli after 3-4 weeks (**Stage-I** observation).
3. **Number of plants / shoots regenerated / 100 explants**: Total no. of plantlets (shoots) regenerated (directly or indirectly after transferring into regeneration medium) recorded as regenerated plantlets after 3-4 weeks of reculturing of calli on regeneration medium (**Stage-II** observation).
4. **Number of complete plantlets/100 explants:** Complete plantlets (shoots with roots) recorded as complete plantlets after 3-4 weeks of reculturing of calli on rooting medium (**Stage-III** observation).

(b) Cell suspension culture

For embryogenic cell suspension culture, observations were recorded at 3 stages;

Stage-I: after 2-7 weeks of initial culturing.

Stage-II: after 3-4 weeks of reculturing of cell clumps/embryoids on regeneration media and

Stage-III: when the complete plantlets will be obtained. All the observations based on initial culture media, irrespective of regeneration medium or rooting medium.

1.Relative growth rate: Relative growth rate calculated on the basis of increment in fresh weight after culturing of embryogenic calli on different liquid medium for raising embryogenic cell suspension culture after 2-7 weeks of initial culture (**Stage-I** observation).

2. Number of plantlets/clumps/embryoids: Frequency of plantlets regeneration calculated as percentage of cell clumps/ embryoids with plantlets from total cell clumps/ embryoids subcultured after 3-4 weeks on regeneration media (**Stage-II** observation).

3. Number of complete plantlets/100 embryoids: Complete plantlets (shoot + roots) regenerated recorded as complete plantlets after transferring into rooting media. (**Stage-III** observation).

In vitro shooting

For *in vitro* shooting, observations were recorded for three parameters. All the observations were based on shooting media, irrespective of initial or regeneration medium. During the present experimentation following observations were recorded:

1. No. of shoots / responding explants: Total numbers (s) of shoots initiated per explants cultured on different culture media recorded for shoot (s) per responding explant after 3-4 weeks.

2. No. of shoots/ 100 cultured: Total number (s) of shoot (s) proliferation on shooting media considered as shoot proliferating efficiency after 3-4 weeksof culture.

3. Mean shoot length: Mean length of shoots wererecorded after 3-4 weeks.

In vitro rooting

For *in vitro* rooting, observations were recorded for three parameters. All the observations were based on rooting media, irrespective of initial or regeneration medium. During the present experimentation following observations were recorded:

1. No. of roots / responding shootlets: Total numbers (s) of roots initiated per shootlet transferred on different rooting media recorded for nos. of root (s) per responding shootlet after 3-4 weeks.

2. No. of root proliferating shootlets / 100 cultured shootlets: Total number (s) of root proliferating shootlets cultured on different rooting media considered as root proliferating efficiency after 3-4 weeks.

3. Mean root length: Mean length of roots recorded after 3-4 weeks.

(c) Hardening

After rooting, plantlets with sufficient roots were removed from the culture medium carefully, washed thoroughly till complete agar media was removed from the plantlets surface. The rooted plantlets were transferred to the nursery pot containing vermiculate, FYM and sand (1:1:1) for 30 days with maintaining light, humidity and temperature under green house conditions. After 30 days well developed regenerants were transferred in Net House for 45 days before transfer to field conditions.

(d) Transfer of regenerants to field: After 45 days, well developed regenerants were transferred under field conditions.

(e) *In vivo* testing of regenerated plants for erucic acid content

The plantlets regenerated from *in vitro* cultures subjected to fatty acids analysis to characterize low and high erucic acid containing regenerants.

(f) *In vitro* selection, maintenance and regeneration of somaclones from callus lines

Callus cultures of two lines (CS54 and PM30) maintained by continuous sub-culturing were cut into small pieces (≈60 mg). Five pieces of callus were picked randomly and cultured in petridish on selective media (MS+0.5mg l^{-1}BAP) for organogenesis and regeneration and on non-selective media (MS+0.5mgl^{-1} 2, 4-D + 5.0mgl^{-1}BAP, MS+1.0 mgl^{-1} BAP + 0.5mgl^{-1}NAA) for maintenance of organogenetic callus lines.

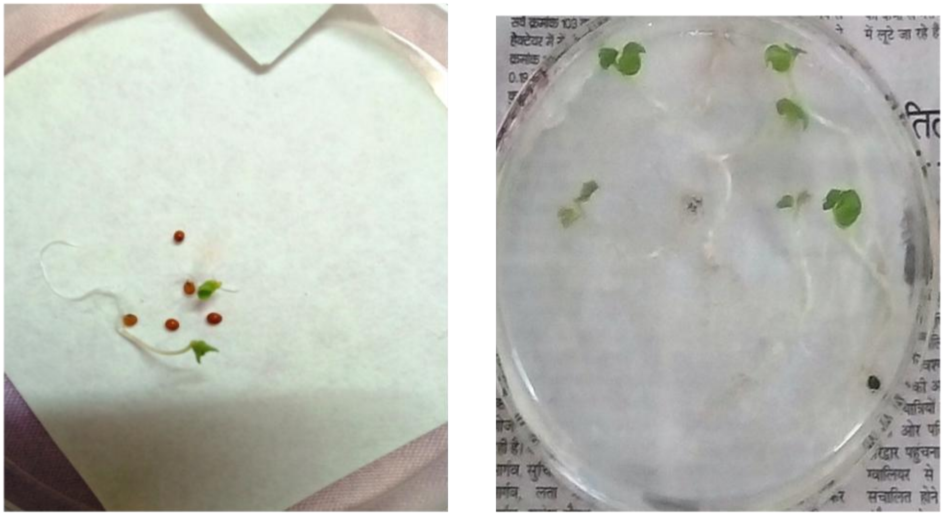
After one month of incubation on selection medium callus pieces or parts showing green shoot primordia were separated and transferred to shoot regeneration medium (MS+0.5mgl^{-1}BAP). Well developed regenerated shoots were separated after 45 days and transferred again on fresh regeneration media in glass jars to obtain multiple shoots. Shootlets were allowed to grow in culture room conditions (Temperature25±2˚C, Humidity 75% and Light intensity (1600Lux) for16 hour light and 8 dark. Well developed regenerated shootlets of suitable length (<7.5cm) and normal shape were transferred to rooting medium (MS+0.5mgl^{-1}IBA) until the sufficient roots were developed.

Regenerated shoots with well developed roots designated as somaclones 1(CS54) and somaclone 2 (PM30) were removed from the jars and washed thoroughly in running tap water to wash out all the agar media. Rooted plants were handled gently and transferred to pots in potting mixture Vermiculite: FYM: Sand (1:1:1). Pots were placed in Green house controlled conditions (Temperature 28^{0}C, Humidity 70% and Photoperiod regimes 16 hour light and 8 hour dark) for 30 days. After 30 days these were transferred to net house conditions for 45 days before transfer to field conditions during January 2019 at Research Farm of college of Agriculture, RVSKVV, Gwalior (M.P.) till and grown to maturity and seed setting. Seeds were collected for fatty acid profiling and subjected to fatty acid analysis.

Seed of CS 54 soma clone

Seed of PM 30 soma clone

Plate 3.11: Seeds of soma clones of Indian mustard genotypes



Seed germination of CS 54 soma clone

Seed germination of PM 30 soma clone

Plate 3.12: Seed germination of soma clones of Indian mustard genotypes

3.12.15Statistical analysis

The data generated from the various *in vitro* experiments were subjected to statistical analysis in Completely Randomized Design (CRD).

Table 3.19 Analysis of variance in CRD

Source of variation	**Degree of freedom**	**Sum of square**	**Mean sum of square**
Genotype/treatment	(t-1)	TrSS	TrMS
Error	t(r-1)	ErSS	EMS
Total	rt-1		

3.12.15.1 Standard error

It is the measure of the mean differences between sample estimates of mean (X) and the population parameters (μ) *i.e*. it is the measures of uncontrolled variation present in a sample;

S.E. = $\sqrt{2EMS}/r$

Where,

S.E. = Standard error

EMS = Error mean sum of square

r = Replication

3.12.15.2 Coefficient of variance

A measure of variation which is dependent of the unit of measurement provided by the standard deviation expressed as percentage of mean. This is known as CV (%);

CV (%) = $\frac{\sqrt{EMS}}{X} * 100$

Where,

CV = Coefficient of variance

EMS = Error mean sum of square

X = Mean

3.13 Microscopy and photography

Stereo zoom microscope was used for photography of callus and somatic embryos of different developmental stages. Photography was taken by Nikon camera with16 megapixel.

CHAPTER – IV

RESULTS

The results of the present experimentation on **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** conducted during session 2016 to 2019 are presented in this chapter. The experimental findings with regards to genetic parameters of variation, biochemical and molecular characterizations and In *vitro selection* studied are being summarized and discussed under the following sub heads:

4.1 Assessment of genotypes based on morpho- physiological traits

4.1.1 Analysis of variance

Pooled analysis of variance over 2 years (Table 4.1) revealed that highly significant variability were present among different genotypes for all the characters under study *viz*., days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, length of main raceme, number of silique per main raceme, number of silique per plant, silique length, number of seeds per silique, 1000 seed weight, biological yield per plot, seed yield per plot and plant and harvest index.

4.1.2 Mean performance of different characters

Mean performance and range of different characters recorded from the population of Indian mustard are presented in Table 4.2 and Appendices A. Performance of individual genotypes for quantitative characters is described as under:

4.1.2.1 Days to 50 % flowering

Days to 50% flowering ranged from 53.70 to 105.53 days, with a mean performance of 72.42 days. Genotype MRNJ-102 required early days to complete 50% flowering (53.70) intimately followed by PM28 (54.03), PM25 (55.04), MRNJ33 (55.22), MRNJ-12 (55.70) MRNJ-22 (55.78), MRNJ-25 (56.18) MRNJ-78 (56.70) and CS-54 (56.52) whereas, late days to 50 % flowering was recorded for genotype IDM 10 (105.53 days).

4.1.2.2 Days to maturity

Days to maturity ranged from 222.75 to 295.7 days, with a mean performance of 269.31 days. Genotype JD-6 required early days to maturity (222.75) closely followed by MRNJ-25 (242), MRNJ-33 (245.6), MRNJ-24 (252.3), MRNJ-102 (252.7), MRNJ-31(254.8), MRNJ-133 (256.45), PM-26 (256.65) and pm-25 (258.35) whereas, the maximum days to maturity was recorded for genotype MRNJ-56 (295.7days).

4.1.2.3 Plant height (cm)

Plant height ranged from 224cm to 343.95 cm, with a mean performance of 296.90 cm. Genotype MRNJ-127 (343.95cm) had maximum plant height followed by MRNJ-130 (340.95cm), MRNJ-128 (335.70cm), MRNJ-62 (333cm), MRNJ-126 (330.6cm), MRNJ-129 (331.40cm) MRNJ-56 (327.85cm), MRNJ-131 (322.05cm) and NRCDR-2 (317.65cm) whereas, the lowest plant height was documented for genotype PM-25 (224.0).

4.1.2.4 Number(s) of primary branches per plant

Number of primary branches per plant was documented in range of 7.8 to13.0, with a mean performance of 10.55. Genotype Karishma (13.0) produced highest number of primary branches followed by PM-30 (12.80), PM-29 (12.35), PM-24 (12.35), MRNJ-18 (12.2), MRNJ-94 (12.1), MRNJ-91 (12.05), MRNJ-137 (12.05) and DRMR-150-35 (12) whereas, the lowest value of plant height was recorded in genotype MRNJ-73 (7.8).

4.1.2.5 Number(s) of secondary branches per plant

Number of secondary branches per plant ranged from 11.45 to 20.65, with mean performance of 15.21. Maximum number of secondary branches per plant was recorded for genotype IDM-42 (20.65) narrowly followed by MRNJ-4 (20.55), MRNJ-104 (19.45), IDM-25 (19.15), IDM-53 (19.1), MRNJ-105 (18.95), MRNJ-92 (18.95), MRNJ-25 (18.75), MRNJ-12 (18.65) and Maya (18.65) whereas, minimum value of number of secondary branches per plant was recorded for genotype RB-50 (11.45).

4.1.2.6 Length of main raceme (cm)

Length of main raceme was evidenced in range of 86.5cm to 185.4cm with mean performance of 131.32cm. Genotype MRNJ-138 (185.4cm) had highest length of main raceme intimately followed by a group of three genotypes, *viz*: MRNJ-90(180.45cm), MRNJ-125 (179.35cm) and IDM-12 (179.1) with *at par* performance. A group of 6 genotypes was next to them including DRMR-150-35 (171.2), MRNJ-119 (171.25), MRNJ-135 (171.20), MRNJ-45 (169.4), MRNJ-22 (169.5) and Karishma (167.45) whereas, the lowest value of length of main raceme was recorded for genotype MRNJ-138 (185.4).

4.1.2.7 Numbers of silique/main raceme

Silique/main raceme was ranged from 58.23 to 125.03, with mean performance of 89.98. Maximum silique/main raceme was recorded for genotype MRNJ-35 (125.03) intimately followed by PM-24 (122.39), MRNJ-71 (121.65), MRNJ-57 (121.56), MRNJ-92 (119.94), MRNJ- 80 (119.91), MRNJ-38 (118.53), MRNJ-79 (116.06) MRNJ-90 (116) and MRNJ-87 (115.45). whereas, minimum value of silique/main raceme was recorded for genotype MRNJ-134 (58.23).

4.1.2.8 Numbers of silique per plant

Silique per plant ranged from 215.15 to 586.8, with mean performance of 366.31. Maximum silique per plant was witnessed for genotype MRNJ-73 (586.8) followed by genotypes: MRNJ-143 (508.8), MRNJ-66 (493.55), MC-25 (489.15), IDM-66 (485.7), IDM-53 (486.4), L-4 (479.3), MRNJ-3 (472.4), MRNJ-105 (469.8) and Maya (468.15). The lowest value of silique per plant was recorded for genotype MRNJ-44 (215.15).

4.1.2.9 Silique length (cm)

Silique length in cm was ranged between 6.92 to 12.77, with mean performance of 9.66.Genotype MRNJ-48 (12.77) demonstrated highest silique length intimately followed by MRNJ-64 (12.42), PM-29 (11.94), RB-50 (11.90), MRNJ-75 (11.70), Karishma (11.56), CS-54 (11.47), PM-30 (11.34), MRNJ-40 (11.8) and MRNJ-1 (11.6), however, minimum value of silique length was recorded for genotype MRNJ-135 (6.92).

4.1.2.10 Numbers of Seeds per silique

Seeds per silique ranged from 17.75 to 40.07, with mean performance of 26.54. Maximum seeds per silique was recorded for genotype MRNJ-15 (40.07) intimately followed by MRNJ-16 (35.6), MRNJ-99 (34.87), MRNJ-34 (33.65), MRNJ-4 (33.10), MRNJ-82 (33.02), MRNJ-1 (33), MRNJ-115 (32.65), MRNJ-114 (32.12) and MRNJ-105 (32.7) whereas; the lowest value of seeds per silique was recorded for genotype MRNJ-131 (17.75).

4.1.2.11 1000 seed weight (g)

1000 seed weight in gram was ranged from 7.05 to 16.14, with mean worth of 10.69. Genotype L-6 (16.14) showed the highest 1000 seed weight intimately followed by genotypes : PM-30 (15.85), PM-21 (15.71), MRNJ-143 (15.35), ISC-18 (15.1), RVM-2 (14.88), IDM-15 (14.86), MRNJ-36 (14.57) and IDM-69 (14.52), whereas; the lowest value of 1000 seed weight was recorded for genotype MRNJ-6 (7.05).

4.1.2.12 Biological yield per plot (g)

Biological yield per plot was documented in range of 1607.95 to 3639.10, with mean performance of 2484.88. Genotype MRNJ-26 (3639.1) yielded highest biological material per plot followed by genotypes: MRNJ-87 (3337.45), MRNJ-75 (3221.7), MRNJ-69 (3219.1), MRNJ-34 (3153.3), MRNJ-74 (3081.9), MRNJ-27 (2967.75) and MRNJ-73 (2961.25). The lowest count was addressed for genotype JD-6 (1607.95).

4.1.2.13 Seed yield per plot (g)

Seed yield per plot was ranged between 255.04g to 898.75g, with mean performance of 343.06g. Maximum seed yield per plot was evidenced for genotype MRNJ-60 (898.75g) followed by MRNJ-16 (591.61), MRNJ-7 (576.52), MRNJ-25 (545.64), MRNJ-36 (543.05), MRNJ-40 (496.97), MRNJ-2 (470.75), PM-29 (470.26), MRNJ-15 (450.77) and MRNJ-143 (439.40). Significant minimum counts was recorded for genotype IDM-15 (255.04).

4.1.2.14 Seed yield per plant (g)

Seed yield per plant was ranged between 11.17g to 40.54g, with mean performance of 21.95g. Genotype MRNJ-118 (40.54) has maximum seed yield per plant intimately followed by genotypes : MRNJ-37 (40.34g), JM-3 (38.82g), MRNJ-133 (37.26g), RVM-2 (35.42g), JM-1 (34.97g), MRNJ-108 (34.81g), MRNJ-103 (34.44g), MRNJ-121 (34.7g) and JM-2 (34.02g), however, the lowest value of seed yield per plant was recorded for genotype MRNJ-11 (11.17g).

Table 4.1: Pooled analysis of variance for 15 quantitative characters over 2 years in Indian mustard

Continued…

Source	**D.F.**	**Days to 50% flowering**	**Days to maturity**	**Plant height (cm)**	**No. of primary branches**	**No. of secondary branches**	**Length of main raceme (cm)**	**No. of silique per main raceme**	**No. of silique per plant**
Environment	1	24.19**	1055.70**	42.25**	125.83**	1008.64**	74.33**	26345.12**	3721.68**
Genotype	195	175.45**	157.70**	397.03**	1.47**	7.38**	662.50**	444.97**	13033.82**
GXE	195	5.4	42.4	43.5	1.4	6.8	21.4	104.7	500.4
Pooled error	390	2.9	7.5	14.9	0.4	1.3	24.9	25.7	336.3
Total	**391**	**90.3**	**102.5**	**219.8**	**1.8**	**9.7**	**341.3**	**341.5**	**6759.3**

Source	**D.F.**	**Silique length (cm)**	**No.of seeds per silique**	**1000 seed weight (g)**	**Biological yield per plot (g)**	**Seed yield per plot (g)**	**Seed yield per plant (g)**	**Harvest index (%)**
Environment	1	5.95**	43.59**	7.43**	1797.0	0.003	4.6	1.9
Genotype	195	2.69**	20.08**	7.72**	201373.23**	8500.18**	72.33**	82.36**
GXE	195	1.7	3.4	1.4	4860.9	39.5	3.4	1.2
Pooled error	390	0.6	2.7	0.9	20136.8	107.1	2.7	4.5
Total	**391**	**2.2**	**11.8**	**4.5**	**102857.9**	**4258.9**	**37.8**	**41.7**

4.1.2.15 Harvest Index (%)

Harvest Index ranged from 16.77% to 65.61%, with mean performance of 28.28%. Genotype MRNJ-60 (65.61%) had maximum harvest Index followed by MRNJ-7 (50.72%), GM-2 (50.41%), MRNJ-25 (47.60%), MRNJ-16 (45.58%), JD-6 (45.47%), PM-29 (43.43%), IDM-41 (40.59%), MRNJ-40 (42.40%) and MRNJ-36 (40.02%).While significant minimum value was recorded for genotype MRNJ-26 (16.77).

4.1.3 Genotypic and phenotypic coefficient of variations

Coefficient of variation was calculated at respective phenotypic and genotypic levels. In general, phenotypic coefficient of variation was higher in magnitude than that of genotypic for all the characters (Table 4.2).

4.1.3.1 Genotypic coefficient of variation (GCV)

Genotypic coefficients of variation for different characters were presented in Table 4.2. The existence of higher magnitude of genetic variability was evident through high values of genotypic coefficient of variation for majority of characters. The highest genotypic coefficient of variation was noted for seed yield per plant in gram (26.72) followed by harvest index and number of silique per plant. The seed yield per plot (18.95g), 1000 seed weight (16.67g), number of silique per main raceme (14.36), length of main raceme (13.63), days to 50% flowering (12.72 days), biological yield per plot (12.61g) and number of seeds per silique (10.89g) had moderate values while silique length (7.32cm), plant height (4.47cm), number of secondary branches (3.59), days to maturity (2.81days) and number of primary branches (1.58) exhibited very low genotypic coefficient of variation.

4.1.3.2 Phenotypic coefficient of variation (PCV)

Phenotypic coefficients of variation for all the characters are presented in Table 4.2 . The highest phenotypic coefficient of variation was observed for seed yield per plant in gram (28.03), harvest index (22.84) and number of silique per plant (22.45), 1000 seed weight (19.91), seed yield per plot (19.04), number of silique per main raceme (18.22), number of secondary branches (17.49), silique length (15.32), length of main raceme (14.08), days to 50% flowering (13.12), biological yield per plot (12.92), number of seeds per silique (12.89) and number of primary branches (11.41) had moderate values. Plant height (4.99) and days to maturity (3.71) exhibited very low PCV values.

Table 4.2: Pooled estimates of grand mean, range, GCV, PCV, heritability and genetic advance for 15 quantitative characters over 2 years in Indian mustard

S.N0.	Characters	Mean	Range		GCV (%)	PCV (%)	h^2	GA	GA (%)
			Min	max					
1	**Days to 50% flowering**	72.42	53.70	105.53	12.72	13.12	94.05	9.20	25.42
2	**Days to maturity**	269.31	222.75	295.70	2.81	3.714	57.59	5.93	4.40
3	**Plant height (cm)**	296.90	224.00	343.95	4.47	4.99	80.26	12.27	8.26
4	**No. of primary branches**	10.55	7.80	13.00	1.58	11.41	1.92	0.02	0.45
5	**No. of secondary branches**	15.22	11.45	20.65	3.59	17.49	4.21	0.11	1.52
6	**Length of main raceme (cm)**	131.33	86.50	185.40	13.63	14.08	93.74	17.85	27.19
7	**No. of silique per main raceme**	89.98	58.23	125.03	14.36	18.22	62.08	10.49	23.31
8	**No. of silique per plant**	366.31	215.15	586.80	21.61	22.45	92.60	78.46	42.84
9	**silique length (cm)**	9.67	6.92	12.77	7.32	15.32	22.81	0.34	7.20
10	**No. of seeds per silique**	26.54	17.75	40.07	10.89	12.89	71.38	2.51	18.96
11	**1000 seed weight (g)**	10.70	7.05	16.14	16.67	19.91	70.05	1.53	28.74
12	**Biological yield per plot (g)**	2484.88	1607.95	3639.10	12.61	12.92	95.28	315.16	25.36
13	**Seed yield per plot (g)**	343.06	255.04	898.75	18.95	19.04	99.07	66.68	38.87
14	**Seed yield per plant (g)**	21.95	11.17	40.54	26.72	28.03	90.93	5.76	52.50
15	**Harvest index (%)**	28.28	16.77	65.61	22.52	22.84	97.20	6.47	45.75

4.1.4 Heritability (broad sense)

The broad sense heritability estimates for different characters are given in Table 4.2. High heritability was recorded for seed yield per plot (99.07%), harvest index (97.20%), biological yield per plot (95.28%), days to 50% flowering (94.05%), length of main raceme (93.74%), number of silique per plant (92.60%), seed yield per plant in gram (90.93%), plant height (80.26%), number of seeds per silique (71.38%), 1000 seed weight (70.05%) and number of silique per main raceme (62.08%). Moderate heritability estimates were documented for days to maturity (57.59%). However, silique length (22.81%), number of secondary branches (4.21%) and number of primary branches (1.92%) exhibited low estimates of heritability.

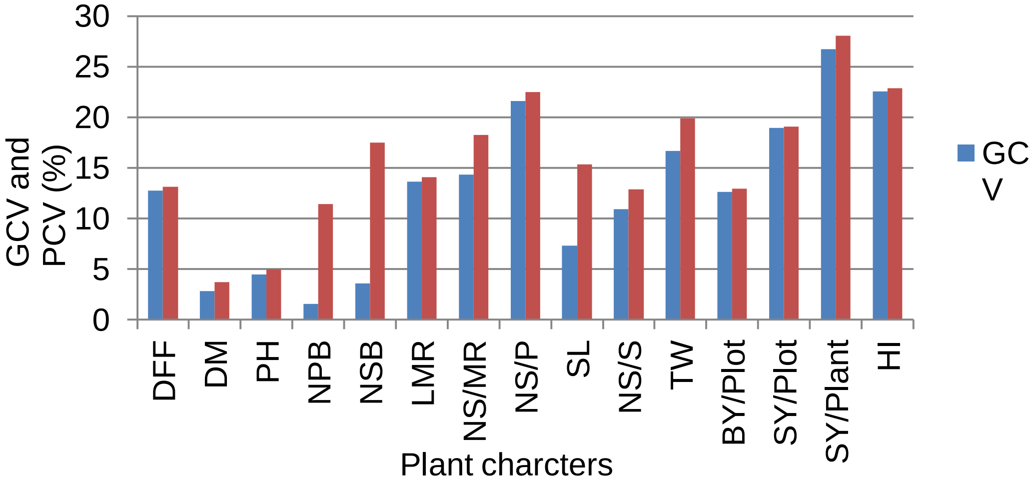


Fig 4.1: Genotypic (GCV) and phenotypic co-efficient of variation (PCV) for different quantitative traits over pooled environment (Rabi 2016-17& Rabi 2017-2018) in Indian mustard

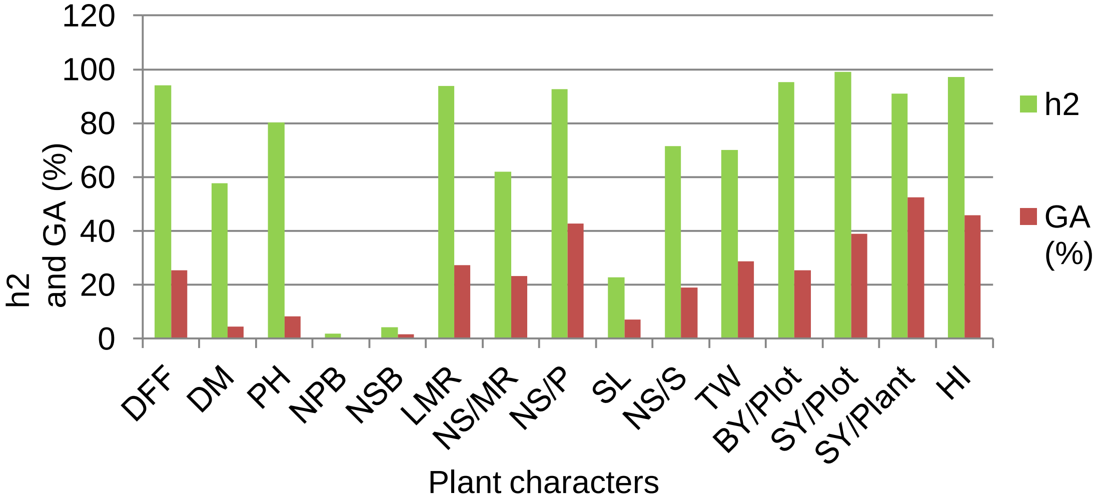


Fig 4.2: Heritability (h^2) and genetic advance as percent of mean (GAM) for quantitative different traits over pooled environment (Rabi 2016-17 & Rabi 2017-2018) in Indian mustard

4.1.5 Genetic advance as percentage of mean

The estimation of genetic advance expressed as percentage of mean (Table 4.2) were found high for seed yield per plant (52.50), harvest index (45.75), number of silique per plant (42.84), seed yield per plot (38.87), 1000 seed weight (28.74), length of main raceme (27.19), days to 50% flowering (25.42), biological yield per plot (25.36) and number of silique per main raceme (23.31). The values were moderate for number of seeds per silique (18.96). It was low for plant height (8.26), silique length (7.20), days to maturity (4.40), number of secondary branches (1.52) and number of primary branches (0.45).

4.1.6 Correlation coefficients

Phenotypic and genotypic correlation coefficient between seed yield per plant and its contributing characters were presented in Table 4.3 and 4.4.

4.1.6.1 Genotypic correlation coefficients

Days to 50% flowering exhibited significant positive correlation with days to maturity (0.750), plant height (0.236) and 1000 seed weight (0.210), whereas, significant negative correlation was documented with biological yield per plot (-0.111), length of main raceme (-0.114), number of seeds per silique (-0.162) and silique length (-0.186).

Days to maturity showed significant positive correlation with number of primary branches (1.084) and number of silique per main raceme (0.190). It also was exhibited significant negative correlation with silique length (-0.151), length of main raceme (-0.152), seed yield per plant (-0.161), number of silique per plant (-0.172) and number of secondary branches (-0.554).

Plant height had significant positive correlation with 1000 seed weight (0.346), length of main raceme (0.114) and number of secondary branches (0.113), whereas, showed significant negative correlation with biological yield per plot (-0.104), seed yield per plot (-0.109), number of seeds per silique (-0.132), silique length (-0.154), number of silique per main raceme (-0.207) and number of primary branches (-0.623).

Number of primary branches showed highly significant positive correlation with number of silique per main raceme (1.249), number of secondary branches (0.979), seed yield per plot (0.446), harvest index (0.404) and silique length (0.315), whereas, showed significant negative correlation with seeds per silique (-0.179), length of main raceme (-0.311), number of 1000 seed weight (-0.478) and seed yield per plant (-0.686).

Number of secondary branches had significant positive correlation with number of silique per plant (0.522), seed yield per plant (0.497) and number of seeds per silique (0.138). It showed significant negative correlation with length of main raceme (-0.278), silique length (-0.813), 1000 seed weight (-0.843) and number of silique per main raceme (-0.959).Length of main raceme showed positive and negative correlations however, all were found non-significant.

Number of silique per main raceme exhibited significant positive correlation with silique length (0.253), seed yield per plot (0.201) and biological yield per plot (0.135 whereas, significant negative correlation was documented with seed yield per plant (-0.106), number of seeds per silique (-0.141), number of silique per plant (-0.175) and 1000 seed weight (-0.203).

Number of silique per plant showed significant positive correlation with seed yield per plant (0.436). However, It showed significant negative correlation with number of seeds per silique (-0.102).

Silique length showed significant positive correlation with number of seeds per silique (0.217) and 1000 seed weight (0.189) whereas, significant negative correlation was addressed with seed yield per plant (-0.152).

Number of seeds per silique showed positive and negative correlations, however all were found non-significant.1000 seed weight had significant negative correlation with seed yield per plot (-0.118) and biological yield per plot (-0.279). Biological yield per plot showed significant negative correlation with harvest index (-0.562). Seed yield per plot recorded significant negative correlation with harvest index (0.787).

Table 4.3: Genotypic correlation coefficient for 15 quantitative traits over pooled years in Indian mustard

	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches	No. of secondary branches	Length of main raceme (cm)	No. of silique per main raceme	No. of silique per plant	silique length (cm)	No. of seeds per silique	1000 seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Seed yield per plant (g)	Harvest index (%)
Days to 50% flowering	1														
Days to maturity	0.750**														
Plant height (cm)	0.236**	-0.033^{NS}													
No. of primary branches	-0.074^{NS}	1.084**	-0.623**	1											
No. of secondary branches	0.013^{NS}	-0.554**	0.113*	0.979**	1										
Length of main raceme (cm)	-0.114*	-0.152**	0.114*	-0.311**	-0.278**										
No. of silique per main raceme	-0.097^{NS}	0.190**	-0.207**	1.249**	-0.959**	0.075^{NS}									
No. of silique per plant	-0.050^{NS}	-0.172**	-0.099^{NS}	-0.067^{NS}	0.522**	0.019^{NS}	-0.175**								
silique length (cm)	-0.186**	-0.151**	-0.154**	0.315**	-0.813**	-0.029^{NS}	0.253**	-0.047^{NS}							
No. of seeds per silique	-0.162**	-0.018^{NS}	-0.132**	-0.179**	0.138**	0.044^{NS}	-0.141**	-0.102*	.217**	1					
1000 seed weight (g)	0.210**	-0.009^{NS}	0.346**	-0.478**	-0.843**	-0.019^{NS}	-0.203**	0.094^{NS}	0.189**	-0.082^{NS}	1				
Biological yield per plot (g)	-0.111*	-0.024^{NS}	-0.104*	-0.052^{NS}	0.007^{NS}	0.085^{NS}	0.135**	-0.085^{NS}	0.055^{NS}	-0.014^{NS}	-0.279**				
Seed yield per plot (g)	0.000^{NS}	0.085^{NS}	-0.109*	0.446**	-0.011^{NS}	0.034^{NS}	0.201**	-0.004^{NS}	0.041^{NS}	0.025^{NS}	-0.118*	.038^{NS}			
Seed yield per plant (g)	0.027^{NS}	-0.161**	0.094^{NS}	-0.686**	0.497**	0.048^{NS}	-0.106*	0.436**	-0.152**	-0.031^{NS}	-0.001^{NS}	-0.028^{NS}	0.038^{NS}		
Harvest index (%)	0.049^{NS}	0.030^{NS}	-0.020^{NS}	0.404**	-0.047^{NS}	-0.029^{NS}	0.078^{NS}	0.057^{NS}	0.056^{NS}	0.030^{NS}	0.083^{NS}	-0.562**	0.787**	0.057^{NS}	11

4.1.6.2 Phenotypic correlation coefficients

Days to 50% flowering had significant positive correlation with days to maturity (0.551), plant height (0.217) and 1000 seed weight (0.176). It showed significant negative correlation with biological yield per plot (-0.105), number of seeds per silique (-0.125) and length of main raceme (-0.130).Days to maturity exhibited significant negative correlation with seed yield per plant (-0.128), length of main raceme (-0.131) and number of silique per plant (-0.143).Plant height had significant positive correlation with 1000 seed weight (0.254), whereas, significant negative correlation was documented with no. of silique per main raceme (-0.132).

Number(s) of primary branches had significant positive correlation with number of silique per main raceme (0.188).Number of secondary branches recorded significant positive correlation with number of silique per plant (0.136) and seed yield per plant (0.105). However, it showed significant negative correlation with 1000 seed weight (-0.117).

Number(s) of silique per main raceme had significant positive correlation with seed yield per plot (0.167), biological yield per plot (0.130) and silique length (0.105), Whereas, significant negative correlation was found with number of silique per plant (-0.115). Number(s) of silique per plant exhibited significant positive correlation with seed yield per plant in gram (0.413) and silique length had significant positive correlation with number of seeds per silique (0.105).

1000 seed weight recorded significant negative correlation with biological yield per plot (-0.211).Biological yield per plot had significant negative correlation with harvest index (-0.568) and seed yield per plot recorded significant positive correlation with harvest index (0.775).

4.1.6.3 Simple correlation coefficient among morphological traits using SPSS V19 software

Significance of correlation of different quantitative characters was analyzed using SPSS V19 software (Table 4.5). Mean value of the data of morpho-physiological traits was documented for correlation coefficient at 0.01 and 0.05 probability significant levels. Days to 50% flowering was found highly significant and positively correlated with days to maturity (r=0.631), plant height (r=0.226), 1000 seed weight (r=0.191) at 1% level of significance. It showed significant negative correlation with number of seeds per silique (r=-0.142) at 5 % level of significance. Days to maturity showed significant negative correlation with number of silique per plant (r=-0.154) and seed yield per plant (r=-0.141) at 5% significance level. Plant height exhibited highly significant positive correlation with 1000 seed weight ($r=0.294^{**}$) at 1% significant level and negative correlation with number (s) of silique per main raceme (r=-0.163) at 5% significance level. Number of primary branches had highly significant and positive correlation with number(s) of silique per main raceme (r=0.252) at 1% significance level. Number(s) of secondary branches showed significant positive correlation with number(s) of silique per plant (r=0.169), seed yield per plant (r=0.144) and negative correlation with number(s) of silique per main raceme (r=-0.147), 1000 seed weight (0-.197) at 5% and 1 % levels of significance, respectively. Number(s) of silique per main raceme showed significant positive correlation with silique length (r=0.142*) and seed yield per plot (r=0.181*) at 5 % level of significance. Number(s) of silique per plant had significant positive correlation with seed yield per plant (r=0.424) at 1 % level of significance. 1000 seed weight showed highly significant negative correlation with biological yield per plot BYD (r=0-.241) at 1% probability level. Biological yield per plot exhibited highly significant negative correlation with harvest index (r=-0.565) at 1 % level of significance. Seed yield per plot showed highly significant positive correlation with harvest index (r=0.781) at 1 % probability level.

	ays to 50% flowering	ays to maturity	lant height (cm)	o. of primary branches	o. of secondary branches	ength of main raceme (cm)	o. of silique per main raceme	o. of silique per plant	ilique length (cm)	o. of seeds per silique	1 000 seed eight (g)	iological yield per plot (g)	eed yield per plot (g)	eed yield per plant (g)	arvest index (%)
Days to 50% flowering															
Days to maturity	.551**														
Plant height (cm)	.217**	0.027^{NS}													
No. of primary branches	.006^{NS}	.032^{NS}	0.080^{NS}												
No. of secondary branches	.016^{NS}	0.032^{NS}	0.018^{NS}	0.012^{NS}											
Length of main raceme (cm)	0.130**	0.131**	.090^{NS}	0.040^{NS}	0.067^{NS}										
No. of silique per main raceme	0.070^{NS}	.073^{NS}	0.132**	.188**	0.036^{NS}	.056^{NS}									
No. of silique per plant	0.044^{NS}	0.143**	0.090^{NS}	0.019^{NS}	.136**	.015^{NS}	0.115*								
silique length (cm)	0.068^{NS}	0.014^{NS}	0.039^{NS}	.087^{NS}	0.043^{NS}	0.004^{NS}	.105*	0.002^{NS}							
No. of seeds per silique	0.125*	.005^{NS}	0.075^{NS}	.001^{NS}	.011^{NS}	.030^{NS}	0.074^{NS}	0.055^{NS}	.105*						
1000 seed weight (g)	.176**	0.029^{NS}	.254**	0.000^{NS}	0.117*	0.013^{NS}	0.079^{NS}	.093^{NS}	.078^{NS}	0.070^{NS}					
Biological yield per plot (g)	0.105*	0.024^{NS}	0.094^{NS}	.020^{NS}	.012^{NS}	.082^{NS}	.130*	0.077^{NS}	.036^{NS}	0.019^{NS}	- 0.211**				
Seed yield per plot (g)	0.000^{NS}	.064^{NS}	0.098^{NS}	.055^{NS}	.015^{NS}	.032^{NS}	.167**	0.004^{NS}	.015^{NS}	.024^{NS}	- 0.097^{NS}	.035^{NS}			
Seed yield per plant (g)	.029^{NS}	0.128*	.066^{NS}	0.088^{NS}	.105*	.049^{NS}	0.064^{NS}	.413**	0.047^{NS}	0.036^{NS}	0 .016^{NS}	0.020^{NS}	.036^{NS}		
Harvest index (%)	.047^{NS}	.028^{NS}	0.016^{NS}	.047^{NS}	0.012^{NS}	0.030^{NS}	.058^{NS}	.053^{NS}	.002^{NS}	.027^{NS}	0 .063^{NS}	0.568**	.775**	.049^{NS}	

Table 4.4: Phenotypic correlation coefficient for 15 quantitative traits over pooled years in Indian mustard

Table 4.5: Correlations coefficient for 15 quantitative traits over pooled years using SPSS V19 software in Indian mustard

	F	M	H	PB	SB	MR	R	P	L	S	SW	YD	GYD	S GYDP	I
D F		631**	226**	.003	013	.122	.081	.047	.100	.142*	191**	.108	000	. 028	047
D M	631**		.029	115	.092	.139	117	.154*	.050	.004	.021	.024	073	- .141*	029
P H	226**	.029		.117	002	101	.163*	.094	.071	.099	294**	.099	.103	. 079	.018
N PB	.003	115	.117		016	.059	252**	.020	096	.015	.042	009	082	- .128	072
N SB	013	.092	002	016		.086	.147*	169*	.109	026	.197**	009	009	. 144*	.015
L MR	.122	.139	101	.059	.086		064	017	.011	036	.016	083	033	. 049	.029
S R	.081	117	.163*	252**	.147*	064		.140	142*	.101	.128	131	181*	- .082	067
S P	.047	.154*	.094	.020	169*	017	.140		.015	.076	093	.081	.004	. 424**	055
S L	.100	.050	.071	096	.109	.011	142*	.015		133	106	040	022	- .076	018
S S	.142*	.004	.099	.015	026	036	.101	.076	133		.075	.017	025	- .034	028
T SW	191**	.021	294**	.042	.197**	.016	.128	093	106	.075		.241**	.106	. 008	072
B YD	.108	.024	.099	009	009	083	131	.081	040	.017	.241**		036	- .024	.565**
S GYD	000	073	.103	082	009	033	181*	.004	022	025	.106	036		. 037	781**
S GYDP	028	.141*	079	.128	144*	049	.082	424**	.076	.034	008	.024	037	1	053
H I	047	029	.018	072	.015	.029	067	055	018	028	072	.565**	781**	. 053	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

DF= Days to 50 % flowering, **DM**= days to maturity, **PH**= Plant height, **NPB**= Number of primary branches, **NSB**= Number of secondary branches, **LMR**= Length of main raceme, **SR**= Number of silique per main raceme, **SP**= Number of silique per plant, **SL**= Silique length, **SS**= Number of seeds per silique, **TWS**= 1000 seed weight, **BYD**= Biological yield per plot, **SGYD**=Seed yield per plot, **SGYDP**= Seed yield per plant, **HI**=Harvest index.

4.1.7. Path coefficient analysis

Path coefficient analysis was carried out at genotypic and phenotypic levels are presented in Table 4.6 and 4.7.

4.1.7.1 Genotypic path coefficient analysis

Path coefficient analysis was carried out at genotypic level, considering seed yield per plant (g) as dependent variable. The direct and indirect effects have been presented.

Direct Effects

Genotypic path coefficient analysis revealed that the maximum positive direct effect of number(s) of silique per plant (0.719) on seed yield per plant (g) followed by harvest index (0.270), plant height (0.207), days to 50% flowering (0.712), number(s) of seeds per silique (0.110), biological yield per plot (0.053) and number(s) of silique per main raceme (0.046), whereas highest negative direct effect on seed yield per plant was exhibited by 1000 seed weight (-0.674) followed by number(s) of secondary branches (-0.452), number(s) of primary branches (-0.342), silique length (-0.265), length of main raceme (-0.245), days to maturity (-0.130) and seed yield per plot (-0.065).

Indirect Effects

The indirect effect had been explained for only those characters which had shown significant correlation values with seed yield per plant (g).

Days to maturity

Days to maturity showed maximum positive indirect effect *via* number(s) of secondary branches (0.251) followed by days to 50% flowering (0.129), silique length (0.040), length of main raceme (0.037), number(s) of silique per main raceme (0.009), harvest index (0.008) and 1000 seed weight (0.006), whereas negative indirect effect *via* number(s) of primary branches (-0.371) followed by number(s) of silique per plant (-0.124), plant height (-0.007), seed yield per plot (-0.006), number(s) of seeds per silique (-0.002) and biological yield per plot (-0.001).

Number(s) of primary branches

Number of primary branches exhibited positive indirect effect on seed yield per plant through 1000 seed weight (0.322), harvest index (0.109), length of main raceme (0.076) and number of silique per main raceme (0.058) but negative indirect effect via number of secondary branches (-0.443), days to maturity (-0.141), plant height (-0.129), silique length (-0.083), number of silique per plant (-0.048), seed yield per plot (-0.029), number of seeds per silique (-0.020), days to 50% flowering (-0.013) and biological yield per plot (-0.003).

Number(s) of secondary branches

Number(s) of secondary branches showed maximum positive indirect effect *via* 1000 seed weight (0.568), number(s) of silique per plant (0.375), silique length (0.216), days to maturity (0.072), length of main raceme (0.068), plant height (0.023), number(s) of seeds per silique (0.015), days to 50% flowering (0.002), seed yield per plot (0.001) and biological yield per plot (0.0004), whereas negative indirect effect *via* number(s) of primary branches (-0.335), number of silique per main raceme (-0.044) and harvest index (-0.013).

Number(s) of silique per main raceme

Number(s) of silique per main raceme showed positive indirect effect through number(s) of secondary branches (0.434) followed by 1000 seed weight (0.137), harvest index (0.021) and biological yield per plot (0.007), whereas negative indirect effect through number(s) of primary branches (-0.427), number(s) of silique per plant (-0.126), silique length (-0.067), plant height (-0.043), days to maturity (-0.025), length of main raceme (-0.018), days to 50% flowering (-0.017), number(s) of seeds per silique (-0.016) and seed yield per plot (-0.013).

Number(s) of silique per plant

Number(s) of silique per plant exhibited positive indirect effect *via* number(s) of primary branches (0.023), days to maturity (0.022), harvest index (0.015), silique length (0.012) and seed yield per plot (0.0003) but negative indirect effect *via* number(s) of secondary branches (-0.236), 1000 seed weight (-0.063), plant height (-0.020), number(s) of seeds per silique (-0.011), days to 50% flowering (-0.009) and number(s) of silique per main raceme (-0.008).

Silique length

Silique length showed positive indirect effect through number(s) of secondary branches (0.368), number(s) of seeds per silique (0.024), days to maturity (0.020), harvest index (0.015), number(s) of silique per main raceme (0.012), length of main raceme (0.007) and biological yield per plot (0.003). It showed negative indirect effect *via* 1000 seed weight (-0.127), number(s) of primary branches (-0.108), number(s) of silique per plant (-0.034), days to 50% flowering (-0.0320), plant height (-0.0319) and seed yield per plot (-0.003).

4.1.7.2 Phenotypic path coefficient analysis

Path coefficient analysis was carried out at phenotypic level, considering seed yield per plant (g) as dependent variable. The direct and indirect effects have been summarized as under:

Direct Effects

Phenotypic path coefficient analysis exerted that the maximum positive direct effect of seed yield per plant (g) was positively and directly depended up on number(s) of silique per plant (0.4076), days to 50% flowering (0.1124), harvest index (0.0888), plant height (0.0875), biological yield per plot (0.0668), number(s) of secondary branches (0.0404), length of main raceme (0.0294), number(s) of silique per main raceme (0.0135) and number(s) of seeds per silique (0.0061), whereas highest negative direct effect on seed yield per plant was interconnected with days to maturity (0.1247), number(s) of primary branches (-0.0723), 1000 seed weight (-0.0527), silique length (-0.0288) and seed yield per plot (-0.0218).

Indirect Effects

The indirect effect had been explained for only those characters which had shown significant correlation values with seed yield per plant (g).

Days to maturity

Days to maturity showed maximum positive indirect effect *via* days to 50% flowering (0.0620), harvest index (0.0025), 1000 seed weight (0.0015), number(s) of silique per main raceme (0.0010), silique length (0.0004) and number(s) of seeds per silique (0.00003), whereas negative indirect effect through number of silique per plant (-0.0582), length of main raceme (-0.0039), plant height (-0.0024), number of primary branches (-0.0023), biological yield per plot (-0.0016), seed yield per plot (-0.0014) and number of secondary branches (-0.0013).

Number(s) of secondary branches

Number of secondary branches exhibited maximum positive indirect effect through number of silique per plant (0.0555), 1000 seed weight (0.0062), days to maturity (0.0040), days to 50% flowering (0.0018), silique length (0.0012), number of primary branches (0.0009), biological yield per plot (0.0008) and number of seeds per silique (0.0001) but negative indirect effect *via* length of main raceme (-0.0020), plant height (-0.0016), harvest index (-0.0011), number of silique per main raceme (-0.0005) and seed yield per plot (-0.0003).

Number(s) of silique per plant

Number(s) of silique per plant exhibited maximum positive indirect effect through days to maturity (0.0178), number(s) of secondary branches (0.0055), harvest index (0.0047), number(s) of primary branches (0.0013), length of main raceme (0.0004), seed yield per plot (0.00009) and silique length (0.00005), While, negative indirect effect through plant height (-0.0079), biological yield per plot (-0.0052), days to 50% flowering (-0.00493), 1000 seed weight (-0.00490), number(s) of silique per main raceme (-0.0016) and number(s) of seeds per silique (-0.0003).

Table 4.6: Genotypic path-coefficient direct and indirect effects of various characters on seed yield per plant over pooled years in Indian mustard

	ays to 50% flowering	ays to maturity	lant height (cm)	o. of primary branches	o. of secondary branches	ength of main raceme (cm)	o. of silique per main raceme	o. of silique per plant	ilique length (cm)	o. of seeds per silique	000 seed weight (g)	iological yield per plot (g)	eed yield per plot (g)	arvest index	enotypic correlation with seed yield per plant (g)
Days to 50% flowering	**0.172**	-0.098	0.049	0.025	-0.006	0.028	-0.004	-0.036	0.049	-0.018	-0.142	-0.006	0.000	0.013	0.027^{NS}
Days to maturity	0.129	**-0.130**	-0.007	-0.371	0.251	0.037	0.009	-0.124	0.040	-0.002	0.006	-0.001	-0.006	0.008	0.161^{**}
Plant height (cm)	.041	.004	**.207**	.213	0.051	0.028	0.010	0.071	.041	0.014	0.233	0.006	.007	0.005	$.094^{NS}$
No. of primary branches	0.013	0.141	0.129	**0.342**	0.443	.076	.058	0.048	0.083	0.020	.322	0.003	0.029	0.109	0.686^{**}
No. of secondary branches	.002	.072	.023	0.335	**0.452**	.068	0.044	.375	.216	.015	.568	.0004	.001	0.013	0.497^{**}
Length of main raceme (cm)	0.020	.020	.024	.106	.126	**0.245**	.003	.014	.008	.005	.013	.005	0.002	0.008	$.048^{NS}$
No. of silique per main raceme	0.017	0.025	0.043	0.427	.434	0.018	**.046**	0.126	0.067	0.016	.137	.007	0.013	.021	0.106^{*}
No. of silique per plant	0.009	.022	0.020	.023	0.236	0.005	0.008	**.719**	.012	0.011	0.063	0.005	.0003	.015	$.436^{**}$
silique length (cm)	0.0320	.020	0.0319	0.108	.368	.007	.012	0.034	**0.265**	.024	0.127	.003	0.003	.015	0.152^{**}
No. of seeds per silique	0.028	.002	0.027	.061	0.062	0.011	0.007	0.073	0.058	**.110**	.056	0.001	0.002	.008	0.031^{NS}
1000 seed weight (g)	.036	.001	.072	.163	.381	.005	0.009	.068	0.050	0.009	**0.674**	0.015	.008	.023	0.001^{NS}
Biological yield per plot (g)	0.019	.003	0.022	.018	0.003	0.021	.006	0.061	0.015	0.002	.188	**.053**	0.002	0.152	0.028^{NS}
Seed yield per plot (g)	.000	0.011	0.023	0.153	.005	0.008	.009	0.003	0.011	.003	.080	.002	**0.065**	.213	$.038^{NS}$
Harvest index	.008	0.004	0.004	0.138	.021	.007	.004	.041	0.015	.003	0.056	0.030	0.051	**.270**	$.057^{NS}$

Residual effect = 0.599

Table 4.7: Phenotypic path-coefficient direct and indirect effects of various characters on seed yield per plant over pooled years in Indian mustard

	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches	No. of secondary branches	Length of main raceme (cm)	No. of silique per main raceme	No. of silique per plant	silique length (cm)	No. of seeds per silique	1000 seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Harvest index	*genotypic correlation with seed yield per plant (g)*
Days to 50% flowering	0.1124	-0.0687	0.0190	-0.0005	0.0006	-0.0038	-0.0009	-0.0179	0.0020	-0.0008	-0.0093	-0.0070	0.00001	0.0041	0.029^{NS}
Days to maturity	0.0620	-0.1247	-0.0024	-0.0023	-0.0013	-0.0039	0.0010	-0.0582	0.0004	0.00003	0.0015	-0.0016	-0.0014	0.0025	-0.128
Plant height (cm)	0.0244	0.0034	0.0875	0.0058	-0.0007	0.0027	-0.0018	-0.0367	0.0011	-0.0005	-0.0134	-0.0063	0.0021	-0.0015	0.066^{NS}
No. of primary branches	0.0007	-0.0040	-0.0070	-0.0723	-0.0005	-0.0012	0.0025	-0.0076	-0.0025	0.00001	0.00001	0.0013	-0.0012	0.0042	-0.088^{NS}
No. of secondary branches	0.0018	0.0040	-0.0016	0.0009	0.0404	-0.0020	-0.0005	0.0555	0.0012	0.0001	0.0062	0.0008	-0.0003	-0.0011	0.105
Length of main raceme (cm)	-0.0147	0.0163	0.0079	0.0029	-0.0027	0.0294	0.0008	0.0062	0.0001	0.0002	0.0007	0.0055	-0.0007	-0.0026	0.049^{NS}
No. of silique per main raceme	-0.0078	-0.0091	-0.0116	-0.0136	-0.0015	0.0016	0.0135	-0.0468	-0.0030	-0.0005	0.0041	0.0087	-0.0036	0.0052	-0.064^{NS}
No. of silique per plant	-0.0049	0.0178	-0.0079	0.0013	0.0055	0.0004	-0.0016	0.4076	0.0001	-0.0003	-0.0049	-0.0052	0.0001	0.0047	0.413
silique length (cm)	-0.0077	0.0017	-0.0035	-0.0063	-0.0017	-0.0001	0.0014	-0.0007	-0.0288	0.0007	-0.0041	0.0024	-0.0003	0.0002	-0.047^{NS}
No. of seeds per silique	-0.0141	-0.0006	-0.0066	-0.0001	0.0004	0.0009	-0.0010	-0.0223	-0.0030	0.0061	0.0037	-0.0013	-0.0005	0.0024	-0.036^{NS}
1000 seed weight (g)	0.0198	0.0036	0.0223	0.0000	-0.0047	-0.0004	-0.0011	0.0379	-0.0022	-0.0004	-0.0527	-0.0141	0.0021	0.0056	0.016^{NS}
Biological yield per plot (g)	-0.0118	0.0029	-0.0082	-0.0014	0.0005	0.0024	0.0018	-0.0314	-0.0010	-0.0001	0.0111	0.0668	-0.0008	-0.0504	-0.020^{NS}
Seed yield per plot (g)	-0.00004	-0.0080	-0.0086	-0.0040	0.0006	0.0010	0.0023	-0.0016	-0.0004	0.0002	0.0051	0.0024	-0.0218	0.0688	0.036^{NS}
Harvest index	0.0052	-0.0035	-0.0014	-0.0034	-0.0005	-0.0009	0.0008	0.0216	-0.0001	0.0002	-0.0033	-0.0379	-0.0169	0.0888	0.049^{NS}

Residual=0.793

4.2 Fatty acid analysis of selected genotypes to select low erucic acid containing genotype (s)

4.2.1 Analysis of variance

Analysis of variance carried out for all the 6 parameters has been presented in Table 4.8. Analysis of variance indicated that significant amount of variability were present among different genotypes for all the parameters *viz.*, palmitic acid, oleic acid, linoleic acid, linolenic acid, erucic acid and oil percentage.

Table 4.8: Analysis of variance for biochemical parameters in Indian mustard

Source of Variation	F	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Oil (%)
Replication		0.57*	2.39**	8.94**	0.03	15.14**	0.96
Treatment	87	5.14**	38.36**	30.20**	4.18**	146.65**	12.5**
Error	87	0.11	0.18	0.68	0.29	0.36	4.06

4.2.2 Mean Performance of different characters

Mean performance and range of different biochemical parameters were recorded are presented in Table 4.9 and Appendices B.

4.2.2.1 Palmitic acid (%)

Palmitic acid (%) ranged from 3.94% to13.27%, with a mean performance of 6.821%. Genotype IDM-67 synthesized maximum palmitic acid content (13.27%), intimately followed by genotypes ISC-12 (12.17%), PM28 (12.05%), ISC-17 (10.97%), L-4 (10.65%), MRNJ-122 (10.32%), PM21 (10.04%), MRNJ-135 (9.66%), MRNJ-126 (9.65%) and MRNJ-142 (9.39%) whereas, minimum was recorded for genotype MRNJ-21 (3.94%).

4.2.2.2 Oleic acid (%)

The content of oleic acid (%) was varied between 6.06% to 37.1% with a mean value of 15.36%. Genotype ISC-18 synthesized maximum oleic acid (37.1%) closely followed by genotypes MRNJ-30 (32.21%), MRNJ-25 (26.82%), PM30 (25.6%), LES-39 (23.15%), PM24 (22.75%), MRNJ-81 (22.46%), MRNJ-92 (22.45%), MRNJ-9 (22.31%) and MRNJ-131 (22.31%) whereas, minimum was recorded for genotype RVM-1 (6.06%).

4.2.2.3 Linoleic acid (%)

Linoleic acid (%) ranged between 14.9% to 37.33%, with a mean count of 24.01%. Genotype ISC-12 exhibited highest linoleic acid (37.33%) intimately followed by genotypes PM22 (36.2%), LES-39 (35.85%), IDM-67 (35.62%), MRNJ-126 (32.79%), ISC-18 (32.79%), MRNJ-122 (32.63%), PM29 (32.55%), ISC-17 (31.70%) and L-4 (31.22%) however, the lowest was recorded for genotype Maya (14.97%).

4.2.2.4 Linolenic acid (%)

Linolenic acid (%) ranged between 8.29% to 16.75% with a mean performance of 11.34. Maximum linolenic acid was noted in genotype LES-39 (16.75) followed by PM28 (15.3), PM-25 (15.25), MRNJ-128 (14.32), MRNJ-108 (14.31), MRNJ-12 (14.27), MRNJ-63 (14.08), L-4 (14.02), NRCHB101 (14.01) and PM24 (14.00) whereas, lowest was exhibited in genotype MRNJ-42 (8.29).

4.2.2.5 Erucic acid (%)

Erucic acid (%) ranged from 0.92% to 51.44% with a mean value of 30.99%. Among different genotypes, Maya (51.44%) synthesized maximum erucic acid intimately followed by JM-1 (46.24%), RVM-2 (45.06%), IDM-41 (43.75%), IDM-25 (42.55%), RH74.9 (42.3%), MRNJ-54 (42.39%), RVM-1 (41.7%), MRNJ-125 (41.66%) and CS-54 (41.36%). However, genotype PM29 was revealed minimum erucic acid content.

4.2.2.6 Oil percentage

Oil percentage varied from 20.5% to 40.5% with a mean performance of 34.68%. Genotypes NRCDR-2, JM-1, Rohini, CS-54, RB-50 and PM28 performed highest oil percentages with *at par* performance followed by GM-2, RH 74.9 and JD6 (39.5), MRNJ-40, MRNJ-45, MRNJ-46, MRNJ-114 NRCHB101, JM-3, Maya, PM25 and PM26 (38.5%), whereas genotype MRNJ98 (20.5%) was showed in minimum oil percentage.

4.2.3 Genotypic and phenotypic coefficient of variation

Coefficient of variation was calculated at respective phenotypic and genotypic levels. Phenotypic coefficient of variation was higher in magnitude than that of genotypic for all the characters (Table 4.8).

Table 4.9: Genetic parameters of variations for biochemical parameters in Indian mustard

Parameters	Mean	Range		PCV (%)	GCV (%)	h^2	GA	GA (%)
		in.	ax.					
Palmitic acid (%)	6.82	3.94	13.27	23.74	23.25	95.95	3.20	46.92
Oleic acid (%)	15.37	6.07	37.10	28.56	28.43	99.05	8.96	58.28
Linoleic acid (%)	24.01	14.97	37.34	16.36	16.00	95.63	7.74	32.24
Linolenic acid (%)	11.35	8.29	16.75	13.18	12.29	86.94	2.68	23.60
Erucic acid (%)	30.99	0.93	51.44	27.66	27.60	99.51	17.58	56.71
Oil (%)	4.76	29.50	41.00	8.32	5.95	51.18	3.04	8.77

4.2.3.1 Genotypic coefficient of variation (GCV)

Genotypic coefficients of variation for different traits are presented in Table 4.9. The existence of higher magnitude of genetic variability was evident through higher values of genotypic coefficient of variation for majority of traits. The highest genotypic coefficient of variation was observed for oleic acid (28.43%) followed by erucic acid (27.60%), palmitic acid (23.25%). Linoleic acid (16.00%) and linolenic acid (12.29%) had moderate values. While, oil percentage (5.95%) exhibited very low GCV.

4.2.3.2 Phenotypic coefficient of variation (PCV)

Phenotypic coefficients of variation for all the parameters are given in Table 4.9. The highest phenotypic coefficient of variation was recorded for oleic acid (28.56%) followed by erucic acid (27.66%) and palmitic acid (23.74%). Linoleic acid (16.36%) and linolenic acid (13.18%) was showed moderate PCV values. However, oil percentage (8.32%) observed very low value of PCV.

4.2.4 Heritability (broad sense)

The broad sense heritability estimates for different factors are given in Table 4.9. Erucic acid (99.51) had possessed high heritability followed by oleic acid (99.05), palmitic acid (95.95), linoleic acid (95.63) and linolenic acid (86.94). While moderate heritability was estimated for oil percentage (51.18).

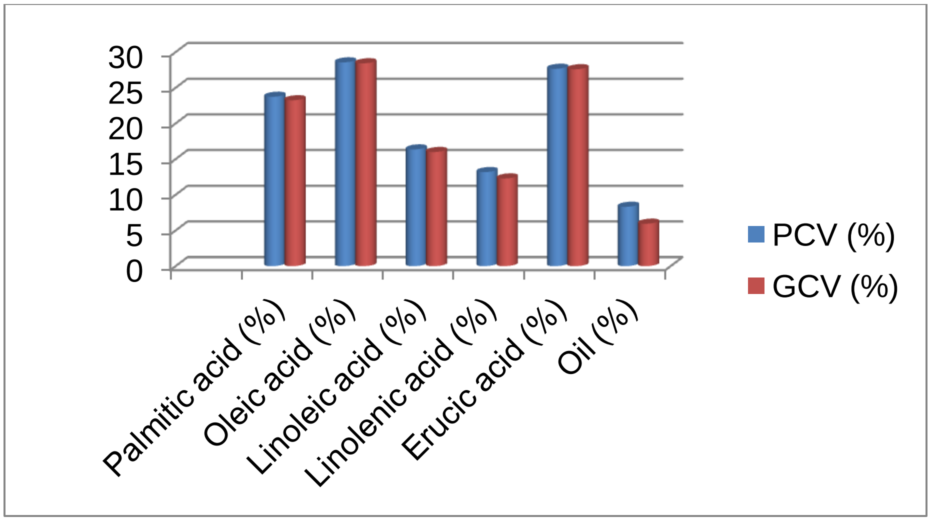


Fig 4.3: Genotypic (GCV) and phenotypic co-efficient of variation (PCV) for biochemical traits in Indian mustard

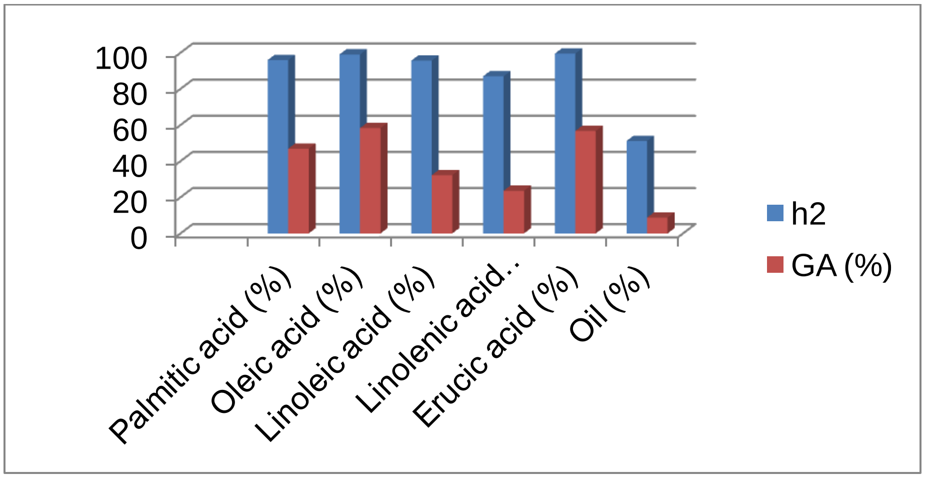


Fig 4.4: Heritability and Genetic Advance as % of mean for biochemical traits in Indian mustard

4.2.5 Genetic advance as percentage of mean

The estimation of genetic advance expressed as percentage of mean were found higher for oleic acid (58.28) followed by erucic acid (56.71), palmitic acid (46.92)), linoleic acid (32.24) and linolenic acid (23.60).The value was found low for oil percentage (8.77).

4.2.6 Correlation coefficients

Phenotypic and genotypic correlation coefficient between erucic acid and its contributing traits were presented in Table 4.10 and 4.11.

4.2.6.1 Genotypic correlation coefficients

Palmitic acid (%) exhibited significant positive correlation with linoeic acid (0.798) and linolenic acid (0.248). Whereas, significant negative correlation with erucic acid (-0.402) and oleic acid (-0.173). Oleic acid (%) showed significant negative correlation with linolenic acid (-0.117) and erucic acid (-0.325). Linoeic acid (%) perused significant positive correlation with linolenic acid (0.381). Whereas, significant negative correlation with erucic acid (-0.685) and oil percentage (-0.106).Linolenic acid (%) showed significant negative correlation with erucic acid (-0.390).

Table 4.10: Genotypic correlation coefficient for biochemical parameters in Indian mustard

Parameter	**Palmitic acid (%)**	**Oleic acid (%)**	**Linoeic acid (%)**	**Linolenic acid (%)**	**Erucic acid (%)**	**il (%)**
Palmitic acid (%)	1					
Oleic acid (%)	-0.173**	1				
Linoeic acid (%)	0.798**	0.053	1			
Linolenic acid (%)	0.248**	-0.117*	0.381**	1		
Erucic acid (%)	-0.402**	-0.325**	-0.685**	-0.390**	1	
Oil (%)	-0.094	-0.011	-0.106*	0.073	-0.037	

4.2.6.2 Phenotypic correlation coefficients

Palmitic acid (%) demonstrated significant positive correlation with linoeic acid (0.768) and linolenic acid (0.224). Whereas, significant negative correlation with oleic acid (-0.170) and erucic acid (-0.394).Oleic acid (%) showed significant negative correlation with linolenic acid (-0.110) and erucic acid (-0.322). Linoeic acid (%) possessed significant positive correlation with linolenic acid (0.354). However, significant negative correlation with erucic acid (-0.669) and Linolenic acid (%) showed significant negative correlation with erucic acid (-0.365).

Table 4.11: Phenotypic correlation coefficient for biochemical parameters in Indian mustard

Parameters	Palmitic acid (%)	Oleic acid (%)	Linoeic acid (%)	Linolenic acid (%)	Erucic acid (%)	il (%)
Palmitic acid (%)	1					
Oleic acid (%)	-0.170**	1				
Linoeic acid (%)	0.768**	0.05	1			
Linolenic acid (%)	0.224**	-0.110*	0.354**	1		
Erucic acid (%)	-0.394**	-0.322**	-0.669**	-0.365**	1	
Oil (%)	-0.063	-0.008	-0.064	0.03	-0.027	

4.2.6.3 Simple correlation coefficient among biochemical traits using SPSS V19 software

Significance among biochemical parameters was analyzed using SPSS V19 software (Table 4.12). Mean value for all biochemical parameters was considered for significance at 0.01 and 0.05 probability levels. Palmitic acid was found highly significant and positively correlated with linoleic acid (r=0.783) and linolenic acid (r=0.235) at 1% level of significance. Palmitic acid exhibited significantly and negatively correlated with oleic acid (r=-0.171) at 5 % and erucic acid (r=-0.398) at 1% significance level. Oleic acid showed highly significant and negative correlation with erucic acid (r=-0.323) at 1 % level of significance. Linoleic acid had highly significant positive correlation with linolenic acid (r=0.367) and negative correlation with erucic acid (r=-0.677) at 1 % level of significance. Linolenic acid exhibited highly significant negative correlation with erucic acid (r=-0.377) at 1 % level of significance.

Table 4.12: Correlations coefficient for biochemical parameters using SPSS V19 software in Indian mustard

	PA	OA	LA	LNA	OP	EA
PA	1	-.171*	.783**	.235**	-.030	-.398**
OA	-.171*	1	.051	-.113	-.058	-.323**
LA	.783**	.051	1	.367**	-.049	-.677**
LNA	.235**	-.113	.367**	1	.053	-.377**
OP	-.030	-.058	-.049	.053	1	-.039
EA	-.398**	-.323**	-.677**	-.377**	-.039	1

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed)

PA= Palmitic acid, **OA**= Oleic acid, **LA**= Linoeic acid, **LNA**= Linolenic acid, **OP**= Oil percentage, **EA**= Erucic acid.

4.2.7 Path coefficient analysis

Path coefficient analysis was carried out at genotypic and phenotypic level in Table 4.13 and 4.14.

Table 4.13: Genotypic path-coefficient direct and indirect effects of various biochemical parameters on erucic acid in Indian mustard

Parameters	**Palmitic acid (%)**	**Oleic acid (%)**	**Linoeic acid (%)**	**Linolenic acid (%)**	**Oil (%)**	**Genotypic correlation with erucic acid (%)**
Palmitic acid (%)	**0.213**	0.046	-0.628	-0.042	0.009	-0.402**
Oleic acid (%)	-0.037	**-0.267**	-0.041	0.020	0.001	-0.325**
Linoeic acid (%)	0.170	-0.014	**-0.787**	-0.064	0.010	-0.685**
Linolenic acid (%)	0.053	0.031	-0.300	**-0.168**	-0.007	-0.390**
Oil (%)	-0.020	0.003	0.084	-0.012	**-0.092**	-0.037

4.2.7.1 Genotypic path coefficient analysis

Path coefficient analysis was carried out at genotypic level considering erucic acid (%) as dependent variable. The direct and indirect effects have been presented in Table 4.13.

Direct Effects

Genotypic path coefficient analysis revealed that the maximum positive direct effect of palmitic acid (0.213) was on erucic acid, whereas highest negative direct effect on erucic acid was imposed by linoeic acid (-0.787) followed by oleic acid (-0.267), linolenic acid (-0.168) and oil percentage(-0.092).

Indirect Effects

The indirect effect had been explained for only those characters which has showed significant correlation values with erucic acid (%).

Palmitic acid (%)

Palmitic acid showed maximum positive indirect effect *via* oleic acid (0.046) and oil percentage (0.009), whereas negative indirect effect *via* linoeic acid (-0.628) and linolenic acid (-0.042).

Oleic acid (%)

Oleic acid showed maximum positive indirect effect *via*. linolenic acid (0.020) and oil percentage (0.001) whereas negative indirect effect *via*. palmitic acid (-0.037) and linoeic acid (-0.041).

Linoeic acid (%)

Linoeic acid exhibited maximum positive indirect effect *via.* palmitic acid (0.170) and oil (0.010) whereas negative indirect effect *via* oleic acid (-0.014) and linolenic acid (-0.064).

Linolenic acid (%)

Linolenic acid demonstrated maximum positive indirect effect *via* palmitic acid (0.053) and oleic acid (0.031) whereas negative indirect effect *via*. linoeic acid (-0.300) and linolenic acid (-0.168).

Table 4.14: Phenotypic path-coefficient direct and indirect effects of various biochemical parameters on erucic acid in Indian mustard

4.2.7.2 Phenotypic path coefficient analysis

Parameters	**Palmitic acid (%)**	**Oleic acid (%)**	**Linoeic acid (%)**	**Linolenic acid (%)**	**Oil (%)**	**Phenotypic correlation with erucic acid (%)**
Palmitic acid (%)	**0.124**	0.049	-0.530	-0.040	0.004	-0.394**
Oleic acid (%)	-0.021	**-0.286**	-0.035	0.020	0.0005	-0.322**
Linoeic acid (%)	0.095	-0.014	**-0.691**	-0.063	0.004	-0.669**
Linolenic acid (%)	0.028	0.031	-0.244	**-0.178**	-0.002	-0.365**
Oil (%)	-0.008	0.002	0.044	-0.005	**-0.061**	-0.027

Path coefficient analysis was carried out at phenotypic level considering erucic acid (%) as dependent variable. The direct and indirect effects have been presented in Table 4.14.

Direct Effects

Phenotypic path coefficient analysis demonstrated that the maximum positive direct effect of palmitic acid (0.124) was exhibited on erucic acid, whereas highest negative direct effect on erucic acid was imposed by linoeic acid (-0.691) followed by oleic acid (-0.286), linolenic acid (-0.178) and oil percentage(-0.061).

Indirect Effects

The indirect effect had been explained for only those characters which had shown significant correlation values with erucic acid (%).

Palmitic acid (%)

Palmitic acid demonstrated positive indirect effect *via* oleic acid (0.049) and oil percentage (0.004), whereas negative indirect effect *via* linoeic acid (-0.530) and linolenic acid (-0.040).

Oleic acid (%)

Oleic acid showed maximum positive indirect effect *via*. linolenic acid (0.020) and oil percentage (0.0005), whereas negative indirect effect *via*. palmitic acid (-0.021) and linoeic acid (-0.035).

Linoeic acid (%)

Linoeic acid exhibited maximum positive indirect effect *via*. palmitic acid (0.095) and oil percentage (0.004), whereas negative indirect effect *via*. linolenic acid (-0.063) and oleic acid (-0.014).

Linolenic acid (%)

Linolenic acid showed maximum positive indirect effect *via.* oleic acid (0.031) and palmitic acid (0.028), while negative indirect effect by way of linoeic acid (-0.244) and oil (-0.002).

4.3 Comparative genetic diversity analysis based on morpho-physiological and biochemical traits

Genetic divergence analysis was performed on the basis of principal components and Euclidean distance cluster analysis.

4.3.1 Genetic diversity analysis based on morpho-physiological traits

4.3.1.1 Principal component analysis

Fifteen principal components of the data over pooled years were given in Table 4.15 and 4.16. First principal component had 13.55 of the total variances for the different morphological traits.

PC1

First principle component showed primarily the variations in days to 50% flowering (0.27), days to maturity (0.275), plant height (0.024), number of primary branches (0.139), number of silique per main raceme (0.113), silique length (0.014),1000 seed weight (0.132), seed yield per plot (0.491) and harvest index (0.629).

PC2

Second principle component contributed a total of 12.56%, having days to 50% flowering (0.318), days to maturity (0.023), plant height (0.406), number of secondary branches (0.095), number of silique per plant (0.231), 1000 seed weight (0.371) and seed yield per plant (0.265).

Table 4.15: Total variance explained by different principal components over pooled years in Indian mustard

PC	Eigen value	% variance
1	2.033	13.554
2	1.885	12.565
3	1.829	12.193
4	1.422	9.483
5	1.198	7.983
6	1.157	7.715
7	1.016	6.774
8	0.912	6.077
9	0.851	5.670
10	0.699	4.658
11	0.690	4.598
12	0.564	3.761
13	0.455	3.035
14	0.273	1.821
15	0.017	0.114

PC3

The principal component three accounted for 12.19 % of divergence and among those values recorded for number of secondary branches (0.218), length of main raceme (0.131),number of silique per plant (0.398), number of seeds per silique (0.095), seed yield per plot (0.232), seed yield per plant (0.364) and harvest index (0.283).

PC4

The total contribution of fourth components were 9.48 %, having days to 50% flowering (0.270), days to maturity (0.308), number(s) of primary branches (0.084), number(s) of secondary branches (0.464), number(s) of silique per plant (0.230), biological yield per plot (0.211), seed yield per plot (0.089) and seed yield per plant (0.256).

PC5

The component fifth explained 7.98 % of the total variation and was contributed by days to 50% flowering (0.080), plant height (0.153), number(s) of primary branches (0.153), length of main raceme (0.356), number(s) of silique per main raceme (0.438), number(s) of silique per plant (0.256), silique length (0.032), 1000 seed weight (0.158), biological yield per plot (0.262), seed yield per plot (0.092) and seed yield per plant (0.318).

PC6

The sixth component estimated 7.71 % variance imposed by days to maturity (0.025), number(s) of primary branches (0.402), number(s) of secondary branches (0.014), number(s) of silique per main raceme (0.071), number(s) of silique per plant (0.380), silique length (0.470), number of seeds per silique (0.015), 1000 seed weight (0.266) and seed yield per plant (0.083).

PC7

The seventh principal component accounted for 6.77 % of diversity and among those values noted for days to 50% flowering (0.245), days to maturity (0.299), length of main raceme (0.298), number(s) of silique per plant (0.149), silique length (0.397), number of seeds per silique (0.588), 1000 seed weight (0.013), biological yield per plot (0.255), seed yield per plot (0.128) and seed yield per plant (0.311).

PC8

The eighth component possessed 6.07 % variance by days to 50% flowering (0.039), plant height (0.487), number(s) of primary branches (0.551), number(s) of secondary branches (0.565), length of main raceme (0.257), number(s) of silique per main raceme (0.029), silique length (0.127), number(s) of seeds per silique (0.170), 1000 seed weight (0.018) and biological yield per plot (0.007).

PC9

The 9thprincipal component accounted for 5.67 % of divergence and among those values noted for days to 50% flowering (0.008), plant height (0.274), number(s) of secondary branches (0.208), silique length (0.423), 1000 seed weight (0.066), biological yield per plot (0.393), seed yield per plot (0.312), seed yield per plant (0.010) and harvest index (0.035).

PC10

The total contribution of 10th principal component was 4.65 %, created by plant height (0.051), number(s) of primary branches (0.405), number (s) of silique per plant (0.113), number(s) of seeds per silique (0.204), 1000 seed weight (0.297), biological yield per plot (0.448) and seed yield per plot (0.287).

PC11

Principal component 11 revealed that it showed 4.59 variance with plant height (0.317), number of primary branches (0.030), number of silique per main raceme (0.432), number of seeds per silique (0.319) and seed yield per plant (0.507).

PC12

The 12th component recorded 3.76 % variance by number of secondary branches (0.369), number of silique per main raceme (0.484), number of silique per plant (0.102), number of seeds per silique (0.228), 1000 seed weight (0.568), biological yield per plot (0.101) and seed yield per plot (0.042).

PC13

The total contribution of 13th principal component was 3.03%, having days to maturity (0.097), plant height (0.454), number(s) of silique per main raceme (0.173), number(s) of silique per plant (0.610), number(s) of seeds per silique (0.123), seed yield per plot (0.005) and harvest index (0.006).

PC14

Principal component 14th contributed a total of 1.82 % variation, caused by days to maturity (0.658), plant height (0.114), number (s) of secondary branches (0.107), length of main raceme (0.018), silique length (0.017), 1000 seed weight (0.148), seed yield per plot (0.027) and seed yield per plant (0.119).

PC15

This component showed 0.11 % variance created by days to maturity (0.045), number(s) of secondary branches (0.017), length of main raceme (0.010), number(s) of silique per main raceme (0.001), 1000 seed weight (0.0002), biological yield per plot (0.422) and harvest index (0.700).

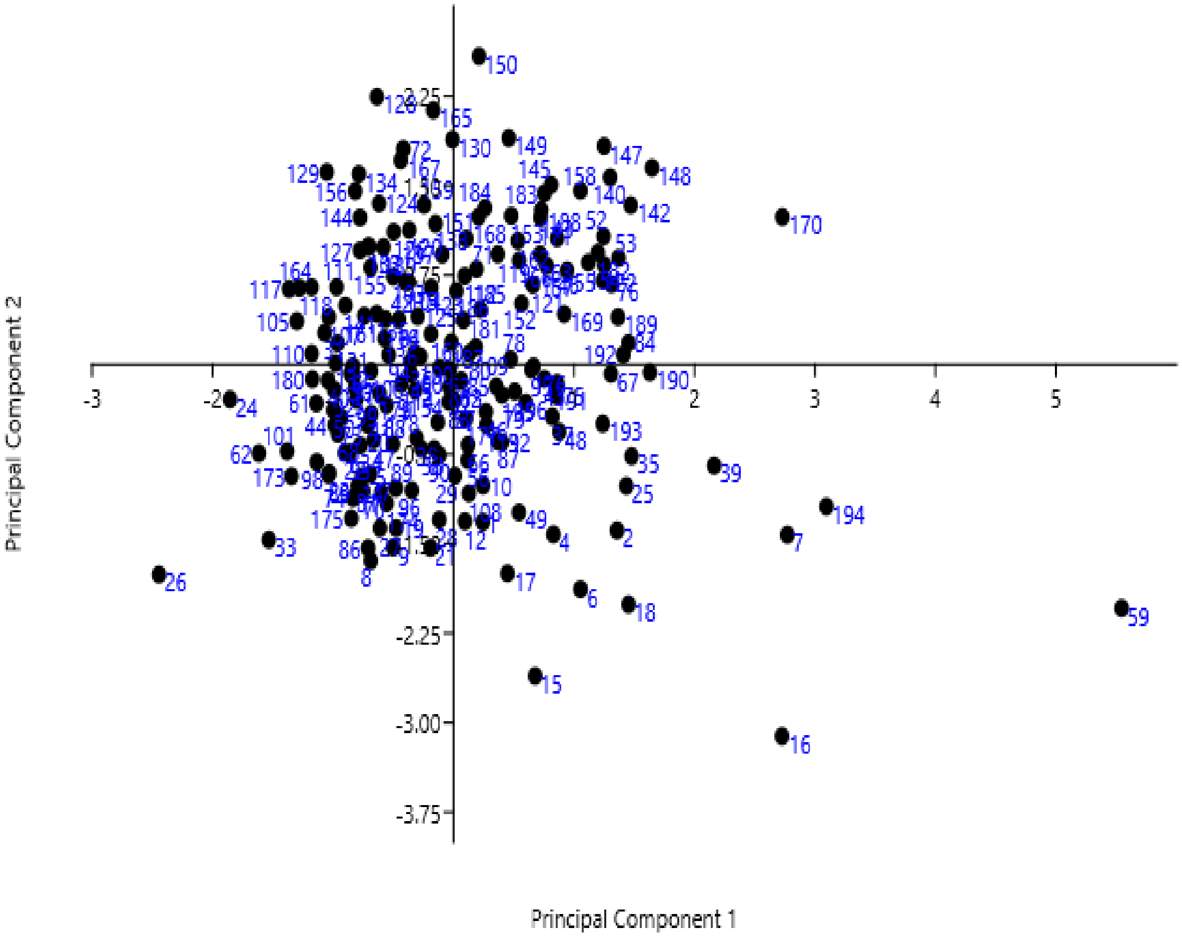


Fig 4.5: Scatter plot for the genetic relationship among 196 *Brassica juncea L.* as revealed by first and second principal components

4.3.1.2 Euclidean distance cluster analysis

The choice of genetically diverse parents for hybridization technique is an important feature of any crop improvement programme for getting desirable segregants. The multivariate analysis based on Euclidean distance cluster analysis is used for divergence analysis. These genotypes from diverse clusters can be utilized in breeding programme depending upon the breeding objectives.

4.3.1.2.1 Clustering pattern in genotypes

A set of 196 genotypes of Indian mustard were subjected to cluster analysis for 15 different parameters. Based on these values, 16 clusters were formed (Table 4.17). Results of cluster analysis revealed that the cluster 5 was the largest which consist of 40 genotypes followed by cluster 1 (37 genotypes), cluster 7 (34 genotypes), cluster 3 (31 genotypes), cluster 6 (18 genotypes), cluster 4 (17 genotypes), cluster 2 and 10 (each having 4 genotypes), cluster 16 (3 genotypes), cluster 15 (2 genotypes) and cluster 8, 9, 11, 12, 13 and 14 (1 genotype each).

4.3.1.2.2 Intra and inter cluster distance

The average intra and inter cluster distances among the 16 clusters are presented in Table 4.18. The maximum inter- cluster distance was observed between cluster 9 and cluster 16 (998.7) followed by between cluster 9 and cluster 15 (856.4), cluster 10 and cluster 16 (795.4), cluster 4 and cluster 9 (792.7), cluster 13 and cluster 16 (720.9), cluster 1 and cluster 9 (715.5), cluster 14 and cluster 16 (669.5), cluster 2 and cluster 9 (656.9), cluster 10 and cluster 15 (653.3) and cluster 12 and cluster 16 (635.1). The minimum inter-cluster distance (82.4) was recorded between clusters 1 and 4. However, the intra-cluster distances was noted zero.

4.3.1.2.3 Cluster means for various characters

The cluster means from various characters are presented in Table 4.19. Results of the analysis revealed that the cluster 8 had the highest mean value for days to maturity (143.88), number(s) of primary branches (5.85) and number(s) of seeds per silique (17.80). Similarly; genotypes included in cluster 9 recorded the highest mean value for silique length (5.65) and biological yield per plot (1819.55). The cluster 11 revealed maximum values for length of main raceme (83.65), silique per main raceme (53.51) and 1000 seed weight

Table 4.16: Principal components for morpho-physiological characters over pooled years in Indian mustard

Parameters	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10	C 11	C 12	C 13	C 14	C 15
Days to 50% flowering	.271	.318	0.432	.270	.080	0.001	.245	.039	.008	0.068	0.107	0.017	0.088	0.688	0.016
Days to maturity	.275	.023	0.502	.308	0.009	.025	.299	0.087	0.159	0.077	0.060	0.005	.097	.658	.045
Plant height (cm)	.024	.406	0.118	0.239	.153	0.278	0.014	.487	.274	.051	.317	0.169	.454	.114	0.003
No. of primary branches	.139	0.279	0.108	.084	.153	.402	0.207	.551	0.315	.405	.030	0.273	0.118	0.026	0.012
No. of secondary branches	0.067	.095	.218	.464	0.234	.014	0.092	.565	.208	0.303	0.194	.369	0.147	.107	.017
Length of main raceme (cm)	0.099	0.046	.131	0.236	.356	0.445	.298	.257	0.482	0.197	0.383	0.014	0.131	.018	.010
No. of silique per main raceme	.113	0.427	0.115	0.007	.438	.071	0.054	.029	0.035	0.354	.432	.484	.173	0.117	.001
No. of silique per plant	0.017	.231	.398	.230	.256	.380	.149	0.120	0.179	.113	0.244	.102	.610	0.040	0.003
Silique length (cm)	.014	0.202	0.001	0.337	.032	.470	.397	.127	.423	0.376	0.216	0.287	0.018	.017	0.014
No. of seeds per silique	0.030	0.137	.095	0.129	0.547	.015	.588	.170	0.235	.204	.319	.228	.123	0.123	0.0002
1000 seed weight (g)	.132	.371	0.088	0.436	.158	.266	.013	.018	.066	.297	0.122	.568	0.316	.148	.00020
Biological yield per plot (g)	0.385	0.268	0.128	.211	.262	0.164	.255	.007	.393	.448	0.121	.101	0.007	0.023	.422
Seed yield per plot (g)	.491	0.258	.232	.089	.092	0.277	.128	0.014	.312	.287	0.131	.042	.005	.027	0.574
Seed yield per plant (g)	0.016	.265	.364	.256	.318	.083	.311	0.061	.010	0.025	.507	0.212	0.454	.119	0.003
Harvest index	.629	0.050	.283	0.080	0.074	0.107	0.058	0.012	.035	0.020	0.025	0.049	.006	0.025	.700

Table 4.17: Grouping of 196 Indian mustard genotypes in various clusters

Cluster number	No. of genotypes	Name of genotypes
1	37	MRNJ-1, MRNJ-6, MRNJ-10, MRNJ-18, MRNJ-33, MRNJ-39, MRNJ-44, MRNJ-49, MRNJ-53, MRNJ-54, MRNJ-72, MRNJ-76, MRNJ-77, MRNJ-79, MRNJ-85, MRNJ-90, MRNJ-92, MRNJ-98, MRNJ-101, MRNJ-105, MRNJ-120, MRNJ-123, MRNJ-125, MRNJ-129, MRNJ-135, MRNJ-138, IDM-8, IDM-25, IDM-42, Rohini, DRMRIJ-31, Kranti, PM-25, MC-25, ISC-12, PM-22 and Karishma
2	4	MRNJ-2, MRNJ-7, MRNJ-25 and MRNJ-40
3	31	MRNJ-3, MRNJ-9, MRNJ-14, MRNJ-17, MRNJ-20, MRNJ-29, MRNJ-31, MRNJ-30, MRNJ-35, MRNJ-37, MRNJ-38, MRNJ-41, MRNJ-47, MRNJ-58, MRNJ-64, MRNJ-66, MRNJ-80, MRNJ-94, MRNJ-103, MRNJ-106, MRNJ-107, MRNJ-108, MRNJ-110, MRNJ-113, MRNJ-119, MRNJ-140, MRNJ-142, IDM-53, JM-2, ISC-3 and ISC-17
4	17	MRNJ-4, MRNJ-131, MRNJ-143, MRNJ-141, IDM-12, MRNJ-144, IDM-10, IDM-31, IDM-41, IDM-58, IDM-64, Maya, JM-1, L-4, ISC-18, PM-24 and PM-29
5	40	MRNJ-5, MRNJ-13, MRNJ-15, MRNJ-19, MRNJ-22, MRNJ-23, MRNJ-24, MRNJ-27, MRNJ-42, MRNJ-48, MRNJ-45, MRNJ-56, MRNJ-57, MRNJ-62, MRNJ-67, MRNJ-68, MRNJ-70, MRNJ-71, MRNJ-74, MRNJ-82, MRNJ-83, MRNJ-84, MRNJ-89, MRNJ-95, MRNJ-99, MRNJ-100, MRNJ-111, MRNJ-112, MRNJ-117, MRNJ-118, MRNJ-130, MRNJ-133, MRNJ-136, MRNJ-145, IDM-69, CS-54, PM-26, DRMR-150-35, RB-50 and RH-74.9
6	18	MRNJ-8, MRNJ-28, MRNJ-46, MRNJ-51, MRNJ-55, MRNJ-61, MRNJ-65, MRNJ-78, MRNJ-91, MRNJ-93, MRNJ-96, MRNJ-97, MRNJ-115, MRNJ-126, MRNJ-127, MRNJ-132, IDM-67 and PM-28
7	34	MRNJ-11, MRNJ-12, MRNJ-21, MRNJ-43, MRNJ-50, MRNJ-52, MRNJ-59, MRNJ-81, MRNJ-86, MRNJ-88, MRNJ-102, MRNJ-104, MRNJ-109, MRNJ-114, MRNJ-116, MRNJ-121, MRNJ-122, MRNJ-124, MRNJ-128, MRNJ-134, MRNJ-137, MRNJ-139, IDM-11, IDM-15, IDM-16, IDM-66, RVM-1, RVM-2, JM-3, NRCDR-2, NRCHB-101, L-6, PM-21 and PM-30
8	1	MRNJ-16
9	1	MRNJ-26
10	4	MRNJ-34, MRNJ-69, MRNJ-75 and MRNJ-87
11	1	MRNJ-36
12	1	MRNJ-60
13	1	MRNJ-63
14	1	MRNJ-73
15	2	IDM-2 and ISC-20
16	3	GM-2, JD-6 and ISC-23

Table 4.18: Inter and intra cluster distance of 196 genotypes in Indian mustard

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1																
2	18.8	.0														
3	02.8	64.7	.0													
4	2.4	68.2	76.9	.0												
5	10.4	60.1	14.4	87.8	.0											
6	89.7	66.2	3.9	71.2	37.7	.0										
7	8.1	09.2	22.9	69.3	24.7	02.8	.0									
8	37.8	45.2	49.7	04.8	76.0	33.2	76.2	.0								
9	15.5	56.9	16.4	92.7	05.4	35.0	29.1	40.8	.0							
10	12.4	52.7	14.4	89.5	02.1	32.8	26.3	39.9	05.0	.0						
11	83.7	93.3	24.9	49.0	26.4	66.4	15.7	14.4	75.9	77.0	.0					
12	05.9	97.7	10.3	61.2	86.8	02.7	53.5	80.6	25.5	57.1	09.9	.0				
13	40.6	93.1	53.5	20.8	41.5	54.7	53.0	83.6	85.6	8.9	41.9	36.1	.0			
14	91.8	27.4	95.2	57.9	35.3	55.4	17.1	58.3	66.2	87.4	57.7	21.7	95.0	.0		
15	46.5	37.2	48.1	6.1	52.4	22.8	28.2	59.6	56.4	53.3	22.6	10.9	77.0	37.3	.0	
16	83.7	64.9	85.7	11.8	93.7	67.1	69.7	95.9	98.7	95.4	55.0	35.1	20.9	69.5	46.0	.0

Table 4.19: Mean performance of individual clusters for 15 quantitative characters in Indian mustard

Class	50 flower	maturity	plant height	p branch	s branch	l m rac	s / m rac	s / plant	s length	s / silique	1000 s wei	bio /plot	seed/plot	s yield/p	hi
1	36.68	134.85	146.79	5.23	7.45	67.22	45.16	187.20	4.79	13.20	5.39	1104.68	166.60	10.97	15.22
2	34.29	131.25	143.97	5.33	7.79	68.26	50.18	205.82	5.23	12.62	4.48	1173.21	261.24	12.44	22.45
3	35.79	134.69	147.46	5.37	7.81	65.63	46.29	212.42	4.74	12.79	5.00	1305.83	164.54	11.98	12.64
4	37.88	135.57	150.23	5.35	7.78	64.39	41.80	218.64	4.73	13.37	6.07	1029.39	176.08	11.84	17.30
5	35.70	134.89	147.90	5.29	7.41	66.97	45.65	178.41	4.86	13.35	5.18	1414.94	170.10	11.01	12.10
6	35.35	133.85	150.61	5.25	7.38	62.63	45.12	131.21	4.73	13.08	5.22	1285.78	165.54	8.71	12.94
7	36.71	135.02	150.03	5.20	7.82	64.43	42.96	169.87	4.91	13.65	5.53	1190.69	160.64	10.69	13.67
8	32.56	143.88	136.80	5.85	6.93	64.55	51.01	143.38	5.30	17.80	4.19	1298.58	295.81	8.84	22.79
9	32.85	130.43	139.55	5.00	8.35	67.78	47.74	162.25	5.65	13.96	4.54	1819.55	153.48	8.42	8.39
10	35.32	133.09	146.94	5.53	7.84	66.68	48.96	166.04	5.28	13.58	5.11	1616.44	179.17	10.21	11.11
11	33.60	129.95	147.63	5.30	7.73	83.65	53.51	229.93	5.20	12.95	7.29	1364.03	271.53	10.62	20.01
12	40.06	137.30	156.48	5.43	8.80	65.95	53.28	124.10	4.25	11.97	4.42	1388.13	449.38	11.34	32.81
13	35.02	132.55	151.10	5.00	7.30	83.15	46.24	114.90	5.17	14.40	5.44	1538.65	150.65	8.32	9.63
14	40.37	136.25	147.43	3.90	8.75	54.60	42.55	293.40	3.67	13.88	5.83	1480.63	193.35	15.96	13.17
15	37.92	135.96	151.04	5.08	8.08	56.89	43.13	149.59	5.14	13.41	6.52	963.64	167.07	12.36	17.27
116	34.87	129.24	155.78	5.45	6.67	61.20	44.86	180.65	4.99	12.80	6.35	821.42	168.77	11.07	21.83

(7.29). The cluster 12 had high mean values for plant height (156.48), number(s) of secondary branches (8.80), seed yield per plot (449.38) and harvest index (32.81) whereas, the cluster 14 exhibited highest mean value for days to 50 % flowering (40.37), silique per plant (293.40) and seed yield per plant (15.96).

4.3.2 Genetic diversity analysis based on biochemical traits

4.3.2.1Principal component analysis

Data of six principal components is given in Table 4.20 and 4.21. In the first principal component had 41.19 % of the total variance for the different biochemical traits.

PC1: First principal component showed 41.19 % variations including palmitic acid (0.505), oleic acid (0.046), linoeic acid (0.594) and linolenic acid (0.355).

PC2: Second principal component estimated 20.38 % variations with palmitic acid (0.274), linoeic acid (0.011), linolenic acid (0.201), oil percentage (0.170) and erucic acid (0.345).

PC3: The principal component three accounted for 17.13 % diversity and among those values recorded for oleic acid (0.104), linolenic acid (0.256) and oil percentage (0.915).

PC4: The total contribution of 4th principal components was 13.41 %, including palmitic acid (0.405), oleic acid (0.035), linoeic acid (0.196), oil percentage (0.355) and erucic acid (0.060).

PC5: Principal component 5th contributed a total of 5.57 % variation, with palmitic acid (0.401), oleic acid (0.499), linoeic acid (0.031), linolenic acid (0.314) oil percentage(0.080) and erucic acid (0.696).

PC6: The sixth component explained 2.29 % of the total variation and was contributed by linoeic acid (0.771), oil (0.040) and erucic acid (0.322).

Table 4.20: Total variance explained by different principal components in Indian mustard

PC	Eigen value	% variance
1	2.471	41.192
2	1.222	20.381
3	1.028	17.138
4	0.805	13.419
5	0.334	5.576
6	0.137	2.293

Table 4.21: Principal components for biochemical parameters in Indian mustard

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Palmitic acid (%)	0.506	0.275	-0.217	0.405	0.401	-0.545
Oleic acid (%)	0.047	-0.857	0.104	0.035	0.499	-0.037
Linoeic acid (%)	0.595	0.012	-0.111	0.196	0.031	0.771
Linolenic acid (%)	0.356	0.202	0.256	-0.816	0.314	-0.046
Oil (%)	-0.006	0.170	0.915	0.355	0.080	0.040
Erucic acid (%)	-0.511	0.346	-0.166	0.060	0.696	0.322

4.3.2.2 Euclidean distance cluster analysis

Genetic divergence among 188 Indian mustard genotypes along with checks for qualitative assessment was studied using Euclidean distance cluster analysis. The clustering pattern of all the genotypes, intra and inter cluster distance and cluster means was described.

4.3.2.2.1 Clustering pattern in genotypes

The 188 genotypes were grouped into 18 different clusters. The highest number of genotypes appeared in cluster 2, which contains 82 genotypes followed by cluster 1 (36 genotypes), cluster 4 (18 genotypes), cluster 5 (17 genotypes), cluster 3 (12 genotypes), cluster 8, 10, 14 and 17 (3 genotypes each), cluster 9 and 11 (2 genotypes each) and cluster 6, 7, 12, 13, 15, 16 and 18 (1 genotype each).

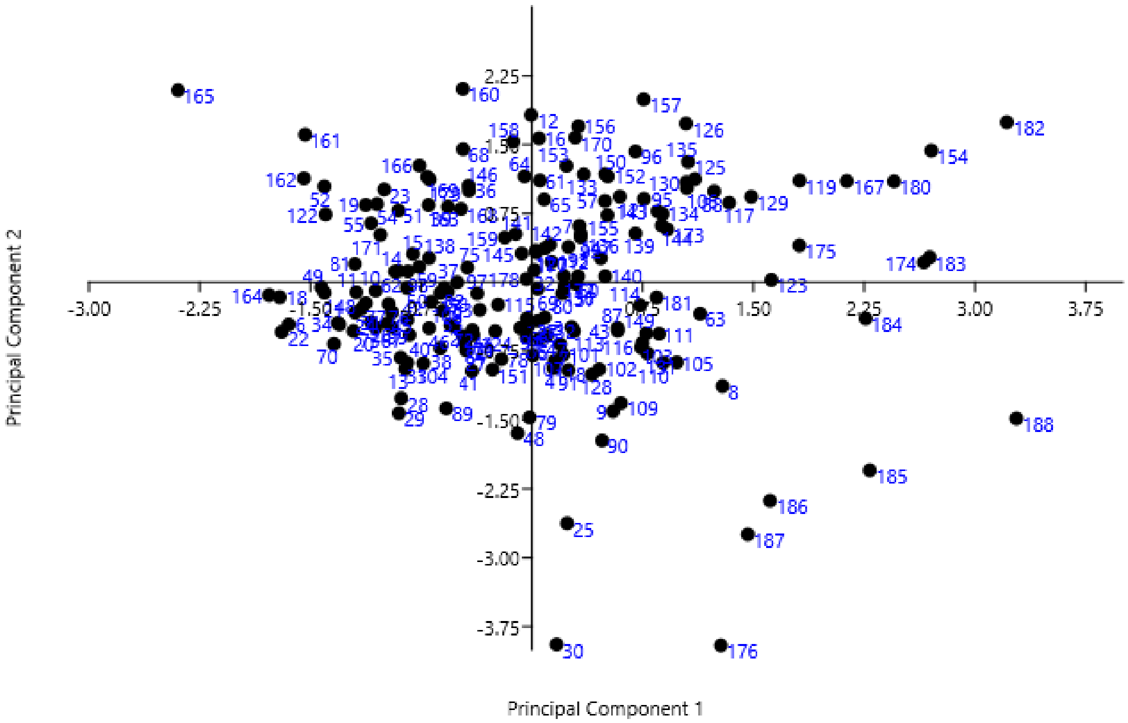


Fig 4.6: Scatter plot of the genetic relationship among 188 *Brassica juncea L.* genotypes as revealed by first and second principal components

4.3.2.2.2 Intra and inter cluster distance

The highest inter cluster distance was recorded between cluster 18 and cluster 12 (57.46) followed by between cluster 17 and cluster 12 (55.45), cluster 16 and cluster 12 (55.04), cluster 14 and cluster 12 (53.02), cluster 15 and cluster 12 (51.12), cluster 18 and cluster 11 (49.72), cluster 16 and cluster 11 (47.99), cluster 17 and cluster 11 (47.93), cluster 14 and cluster 11 (46.25) and cluster 12 and cluster 7 (45.06). However, the minimum inter-cluster distance (6.95) was recorded between clusters 3 and 11. The intra cluster distances was observed zero in all clusters.

4.3.2.2.3 Cluster means for various characters

The cluster means of six different biochemical parameters is given in Table 4.24. The cluster mean for palmitic acid (%) was highest in cluster 8 (11.70) and the lowest in cluster 7 (4.16). Cluster 13 (37.10) had highest cluster mean value for oleic acid (%). whereas the lowest mean value for oleic acid (%) was exhibited by the cluster 12 (6.08). The highest and lowest cluster mean value for linoleic acid (%) was observed for cluster 16 (36.20) and cluster 12 (14.97) respectively, while the highest and lowest cluster mean were recorded for linolenic acid (%) by cluster 18 (16.75) and cluster 15 (9.80) respectively. Cluster 10 had the highest cluster mean (39.83) for oil percentage (%) and the lowest cluster mean (33.17) for this character was recorded in cluster 8. The cluster mean for erucic acid (%) was highest for cluster 12 (51.44) and lowest for cluster 17 (1.06).

Table 4.22: Grouping of 188 Indian mustard genotypes in various clusters

Cluster number	**No. of genotypes**	**Name of genotypes**
1	36	MRNJ1, MRNJ7, MRNJ-59, MRNJ-67, MRNJ-87, MRNJ-90, MRNJ-94, MRNJ-95, MRNJ-97, MRNJ-98, MRNJ-108, MRNJ-120, MRNJ-122, MRNJ-123, MRNJ-124, MRNJ-128, MRNJ-129, MRNJ-135, MRNJ-136, MRNJ-139, MRNJ-140, MRNJ-142, IDM-8, IDM-10, IDM-11, IDM-12, IDM-31, IDM-53, IDM-64, IDM-66, IDM-69, L-4, MC-25, ISC-3, ISC-17 and ISC-20
2	82	MRNJ2, MRNJ3, MRNJ4, MRNJ5, MRNJ10, MRNJ11, MRNJ13, MRNJ14, MRNJ15, MRNJ17, MRNJ20, MRNJ21, MRNJ-22, MRNJ-24, MRNJ-26, MRNJ-27, MRNJ-28, MRNJ-29, MRNJ-33, MRNJ-34, MRNJ-35, MRNJ-36, MRNJ-37, MRNJ-39, MRNJ-40, MRNJ-42, MRNJ-43, MRNJ-44, MRNJ-46, MRNJ-47, MRNJ-48, MRNJ-49, MRNJ-52, MRNJ-55, MRNJ-58, MRNJ-60, MRNJ-62, MRNJ-64, MRNJ-68, MRNJ-69, MRNJ-71, MRNJ-72, MRNJ-73, MRNJ-74, MRNJ-75, MRNJ-76, MRNJ-77, MRNJ-78, MRNJ-79, MRNJ-80, MRNJ-82, MRNJ-84, MRNJ-85, MRNJ-86, MRNJ-88, MRNJ-91, MRNJ-96, MRNJ-99, MRNJ-100, MRNJ-101, MRNJ-102, MRNJ-103, MRNJ-104, MRNJ-106, MRNJ-109, MRNJ-110, MRNJ-115, MRNJ-116, MRNJ-117, MRNJ-118, MRNJ-121, MRNJ-127, MRNJ-130, MRNJ-

		138, MRNJ-143, MRNJ-144, IDM-2, IDM-15, IDM-16, IDM-58, DRMR-150-35 and ISC-23
3	12	MRNJ6, MRNJ18, MRNJ-51, MRNJ-57, MRNJ-83, IDM-41, DRMRIJ-31, JM-3, Rohini, GM-2, CS-54 and RH-749
4	18	MRNJ8, MRNJ9, MRNJ-45, MRNJ-50, MRNJ-65, MRNJ-81, MRNJ-89, MRNJ-92, MRNJ-93, MRNJ-105, MRNJ-107, MRNJ-111, MRNJ-113, MRNJ-114, MRNJ-119, MRNJ-131, MRNJ-137 and IDM-42
5	17	MRNJ12, MRNJ16, MRNJ19, MRNJ-23, MRNJ-38, MRNJ-41, MRNJ-53, MRNJ-54, MRNJ-56, MRNJ-61, MRNJ-63, MRNJ-66, MRNJ-70, MRNJ-125, IDM-25, RVM-1 and L-6
6	1	MRNJ-25
7	1	MRNJ-30
8	3	MRNJ-126, IDM-67 and ISC-12
9	2	MRNJ-145 and JD-6
10	3	NRCDR-2, NRCHB-101 and RB-50
11	2	RVM-2 and JM-1
12	1	Maya
13	1	ISC-18
14	3	PM-25, PM-28 and PM-21
15	1	PM-26
16	1	PM-22
17	3	PM-24, PM-29 and PM-30
18	1	Karishma

Table 4.24: Mean performance of individual clusters for 6 biochemical parameters in Indian mustard

Class	Palmitic acid (%)	Oleic acid (%)	Linoeic acid (%)	Linolenic acid (%)	Oil (%)	Erucic acid (%)
1	8.46	12.26	27.23	11.70	33.69	30.84
2	6.06	16.89	22.20	11.07	34.49	32.81
3	5.59	14.32	19.77	10.66	37.75	40.27
4	7.59	20.25	26.08	11.52	34.56	25.10
5	6.26	9.74	22.04	11.29	33.71	37.92
6	5.26	26.82	25.13	11.39	34.50	21.53
7	4.16	32.22	23.11	12.03	33.50	16.05
8	11.70	13.39	35.25	10.58	33.17	20.82
9	5.86	10.78	19.59	10.60	38.00	28.78
10	7.61	10.57	26.37	13.31	39.83	36.23
11	5.05	10.27	18.79	10.86	39.00	45.65
12	4.49	6.08	14.97	10.06	38.50	51.44
13	7.37	37.10	32.79	11.45	33.50	27.05
14	10.21	10.02	29.27	14.55	38.33	1.06
15	6.30	10.10	25.20	9.80	38.50	1.55
16	6.85	11.75	36.20	11.10	35.50	1.13
17	6.20	23.32	29.95	12.28	35.83	1.06
18	7.35	23.15	35.85	16.75	37.50	1.24

Table 4.23: Inter and intra-cluster distance of 188 genotypes in Indian mustard

Clu-ster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.00	7.58	13.21	9.97	9.41	17.72	25.55	13.34	9.56	8.58	18.31	25.58	25.76	30.47	29.97	31.14	32.04	33.32
2		0.00	8.90	9.39	8.84	15.33	22.84	18.98	8.54	10.25	15.55	23.15	23.59	33.90	32.40	35.04	33.35	35.57
3			0.00	17.88	6.99	23.39	30.66	26.02	12.03	9.45	6.95	14.76	29.75	41.02	39.34	42.60	41.58	43.59
4				0.00	17.13	7.90	15.72	13.01	12.69	15.76	24.53	32.33	18.28	26.89	26.05	27.40	24.63	26.64
5					0.00	23.91	31.46	22.60	10.48	8.11	10.02	16.55	31.37	38.19	36.85	39.52	40.14	41.97
6						0.00	8.11	18.09	18.81	22.79	30.27	38.03	14.15	27.71	26.43	27.74	21.40	24.13
7							0.00	24.16	25.67	30.66	37.53	45.06	15.79	28.67	27.17	28.72	18.99	22.64
8								0.00	19.31	19.82	31.24	38.51	25.03	21.96	23.27	20.51	23.61	23.56
9									0.00	10.74	16.95	23.65	29.89	29.95	27.83	32.38	32.26	34.88
10										0.00	12.64	20.09	29.55	35.44	34.93	36.80	37.83	38.60
11											0.00	8.17	36.03	46.25	44.60	47.99	47.93	49.72
12												0.00	43.71	53.02	51.12	55.04	55.45	57.46
13													0.00	38.24	38.28	36.47	29.68	30.24
14														00	7.40	9.07	14.31	15.15
15															0.00	11.61	14.52	18.28
16																0.00	13.22	12.89
17																	0.00	7.68
18																		0.00

4.4 To characterize low and high erucic acid content in selected germplasm lines

The erucic acid content in the oil of all *B. juncea* genotypes was given in Table 4.25, 4.26 and 4.27. Nine genotypes *viz*., PM-25 (1.00), PM-26 (1.55), PM-28 (1.14), PM-21 (1.05), PM-22 (1.13), PM-24 (1.18), PM-29 (0.93), PM-30 (1.08) and Karishma (1.24) had erucic acid content less than 2 per cent.

Fifty seven genotypes *namely*., MRNJ-4 (29.03), MRNJ-5 (29.24), MRNJ-8 (21.30), MRNJ-9 (25.15) MRNJ-25 (21.53), MRNJ-27 (29.69), MRNJ-28 (29.92), MRNJ-30 (16.05), MRNJ-45 (25.92), MRNJ-50 (25.53), MRNJ-58 (29.59), MRNJ-62 (28.55), MRNJ-65 (24.00), MRNJ-68 (29.45), MRNJ-73 (29.17), MRNJ-81 (26.47), MRNJ-82 (28.73), MRNJ-89 (26.30), MRNJ-90 (27.08), MRNJ-91 (29.40), MRNJ-92 (23.78), MRNJ-93 (27.06), MRNJ-97 (29.11), MRNJ-103 (28.04), MRNJ-104 (28.09), MRNJ-105 (26.26), MRNJ-107 (23.63), MRNJ-108 (27.20), MRNJ-109 (28.27), MRNJ-111 (23.88), MRNJ-113 (23.36), MRNJ-114 (24.86), MRNJ-116 (27.77), MRNJ-117 (27.25), MRNJ-119 (25.16), MRNJ-120 (26.91), MRNJ-121 (29.05), MRNJ-122 (27.07), MRNJ-126 (20.67), MRNJ-127 (29.79), MRNJ-128 (26.64), MRNJ-129 (29.72), MRNJ-131 (26.99), MRNJ-135 (24.89), MRNJ-137 (26.65), MRNJ-138 (29.67), MRNJ-140 (28.87), MRNJ-143 (29.40), MRNJ-145 (25.91), IDM-8 (29.98), IDM-42 (25.62), IDM-67 (22.16), L-4 (24.22), MC-25 (29.93), ISC-12 (19.64), ISC-17 (24.79) and ISC-18 (27.05) were showed moderate erucic acid (%) having 2 to 30 %.

However one twenty-two genotypes contains high erucic acid including MRNJ-1 (36.02), MRNJ-2 (35.16), MRNJ-3 (30.15), MRNJ-6 (40.00), MRNJ-7 (33.95), MRNJ-10 (37.27), MRNJ-11 (37.57), MRNJ-12 (35.33), MRNJ-13 (33.68), MRNJ-14 (37.14), MRNJ-15 (39.30), MRNJ-16 (33.99), MRNJ-17 (34.02), MRNJ-18 (40.11), MRNJ-19 (39.30), MRNJ-20 (36.15), MRNJ-21 (35.78), MRNJ-22 (37.94), MRNJ-23 (40.44), MRNJ-24 (37.25) MRNJ-26 (35.38), MRNJ-29 (32.65), MRNJ-33 (36.15), MRNJ-34 (30.35), MRNJ-35 (34.83), MRNJ-36 (37.44), MRNJ-37 (32.72), MRNJ-38 (36.78), MRNJ-39 (33.54), MRNJ-40 (31.29), MRNJ-41 (37.16), MRNJ-42 (35.15), MRNJ-43 (30.93), MRNJ-44 (35.12), MRNJ-46 (30.27), MRNJ-47 (35.79), MRNJ-48 (33.82), MRNJ-49 (30.07), MRNJ-51 (39.60), MRNJ-52 (35.30), MRNJ-53 37.33), MRNJ-54 (42.39), MRNJ-55 (33.43), MRNJ-56 (38.35), MRNJ-57 (38.02), MRNJ-59 (31.08), MRNJ-60 (33.61), MRNJ-61 (35.89), MRNJ-63 (33.67), MRNJ-64 (36.05), MRNJ-66 (34.12), MRNJ-67 (32.17), MRNJ-69 (34.16), MRNJ-70 (37.06), MRNJ-71 (31.08), MRNJ-72 (39.10), MRNJ-74 (32.31), MRNJ-75 (35.15), MRNJ-76 (31.04), MRNJ-77 (33.29), MRNJ-78 (36.33), MRNJ-79 (38.23), MRNJ-80 (30.32), MRNJ-83 (38.27), MRNJ-84 (35.44), MRNJ-85 (32.26), MRNJ-86 (31.92), MRNJ-87 (30.88), MRNJ-88

(30.93), MRNJ-94 (31.31), MRNJ-95 (31.29), MRNJ-96 (31.08), MRNJ-98 (31.21), MRNJ-99 (32.77), MRNJ-100 (36.98), MRNJ-101 (35.33), MRNJ-102 (33.01), MRNJ-106 (32.98), MRNJ-110 (34.44), MRNJ-115 (32.75), MRNJ-118 (32.81), MRNJ-123 (34.03), MRNJ-124 (30.73), MRNJ-125 (41.66), MRNJ-130 (32.11), MRNJ-136 (30.66), MRNJ-139 (33.87), MRNJ-142 (30.64), MRNJ-144 (30.30), IDM-2 (30.16), IDM-10 (37.20), IDM-11 (34.74), IDM-12 (32.82), IDM-15 (30.37), IDM-16 (36.16), IDM-25 (42.55), IDM-31 (34.73), IDM-41 (43.76), IDM-53 (34.97), IDM-58 (33.81), IDM-64 (33.83), IDM-66 (34.69), IDM-69 (30.16), NRCDR-2 (36.72), NRCHB-101 (36.18), DRMRIJ-31 (39.89), DRMR-150-35 (34.97), RVM-1 (41.70), RVM-2 (45.07), JM-1 (46.24), JM-3 (41.10), Rohini (37.82), Maya (51.44), GM-2 (40.97), L-6 (36.93), CS-54 (41.36), RB-50 (35.80), RH-74.9 (42.30), ISC-3 (30.21), ISC-20 (32.69), ISC-23 (34.03) and JD-6 (31.65) had erucic acid content greater than 30 per cent.

Table 4.25: Low erucic acid containing genotypes in Indian mustard

S.No.	Genotypes	Value (%)
1	PM-25	1.00
2	PM-26	1.55
3	PM-28	1.14
4	PM-21	1.05
5	PM-22	1.13
6	PM-24	1.18
7	PM-29	0.93
8	PM-30	1.08
9	Karishma	1.24

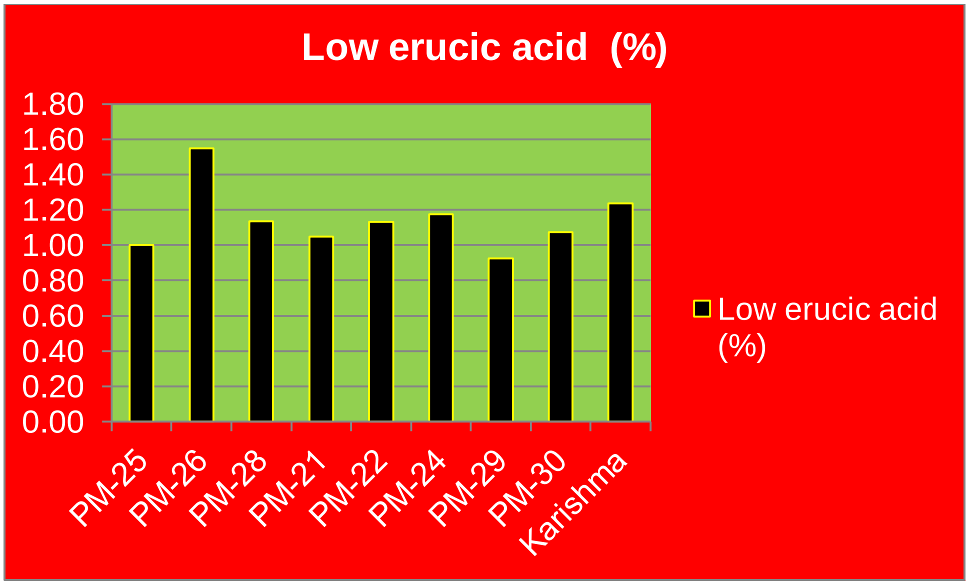


Fig 4.7: Graphical representation of low erucic acid (%) containing Indian mustard genotypes

Table 4.26: Moderate erucic acid (%) genotypes in Indian mustard

1	MRNJ-4	29.03	6	MRNJ-81	26.47	1	MRNJ-113	23.36	6	MRNJ-138	29.67
2	MRNJ-5	29.24	7	MRNJ-82	28.73	2	MRNJ-114	24.86	7	MRNJ-140	28.87
3	MRNJ-8	21.30	8	MRNJ-89	26.30	3	MRNJ-116	27.77	8	MRNJ-143	29.40
4	MRNJ-9	25.15	9	MRNJ-90	27.08	4	MRNJ-117	27.25	9	MRNJ-145	25.91
5	MRNJ-25	21.53	0	MRNJ-91	29.40	5	MRNJ-119	25.16	0	IDM-8	29.98
6	MRNJ-27	29.69	1	MRNJ-92	23.78	6	MRNJ-120	26.91	1	IDM-42	25.62
7	MRNJ-28	29.92	2	MRNJ-93	27.06	7	MRNJ-121	29.05	2	IDM-67	22.16
8	MRNJ-30	16.05	3	MRNJ-97	29.11	8	MRNJ-122	27.07	3	L-4	24.22
9	MRNJ-45	25.92	4	MRNJ-103	28.04	9	MRNJ-126	20.67	4	MC-25	29.93
10	MRNJ-50	25.53	5	MRNJ-104	28.09	0	MRNJ-127	29.79	5	ISC-12	19.64
11	MRNJ-58	29.59	6	MRNJ-105	26.26	1	MRNJ-128	26.64	6	ISC-17	24.79
12	MRNJ-62	28.55	7	MRNJ-107	23.63	2	MRNJ-129	29.72	7	ISC-18	27.05
13	MRNJ-65	24.00	8	MRNJ-108	27.20	3	MRNJ-131	26.99			
14	MRNJ-68	29.45	9	MRNJ-109	28.27	4	MRNJ-135	24.89			
15	MRNJ-73	29.17	0	MRNJ-111	23.88	5	MRNJ-137	26.65			

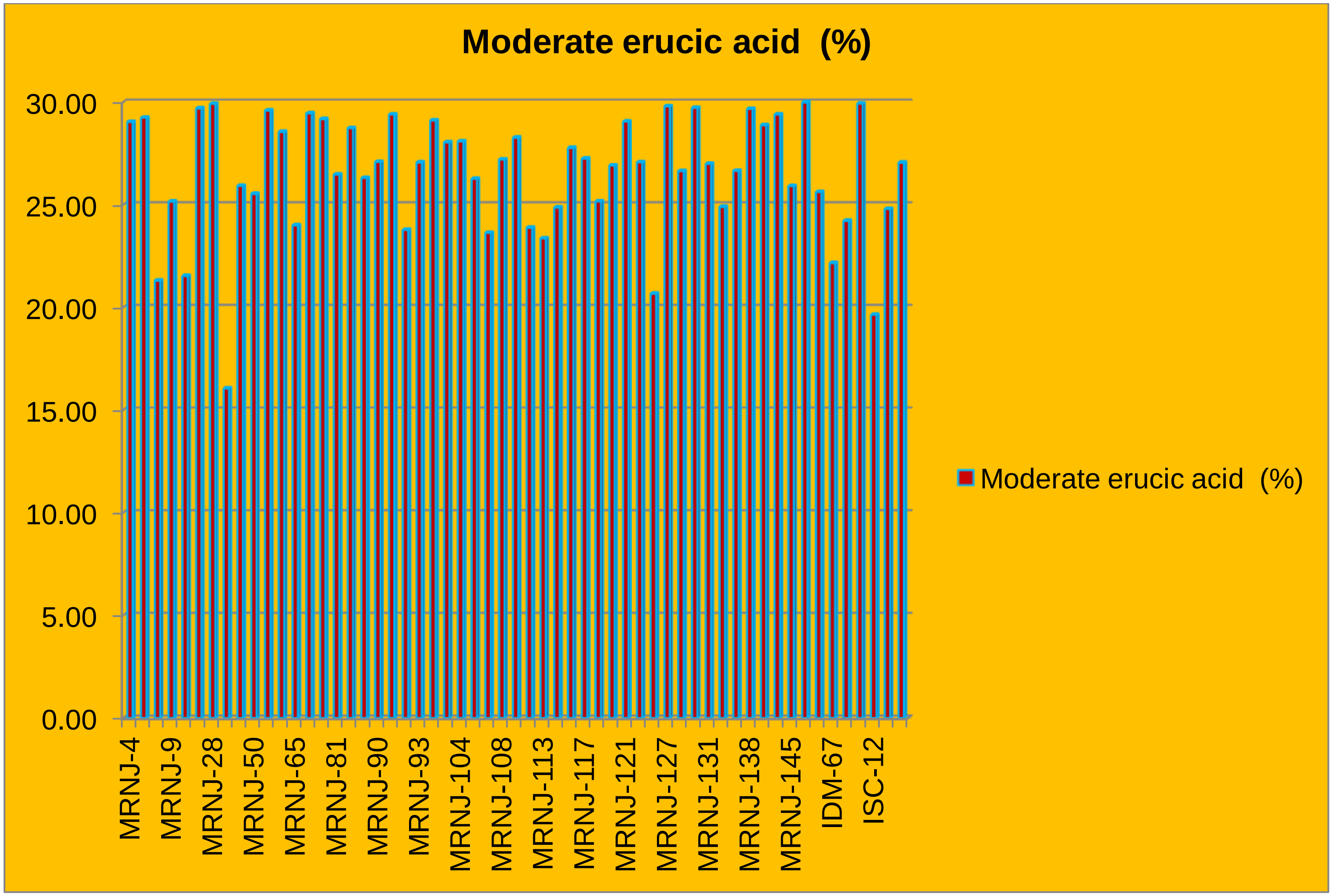


Fig 4.8: Graphical representation of moderate erucic acid (%) containing Indian mustard genotypes

1	MRNJ-1	36.02	26	MRNJ-36	37.44	51	MRNJ-66	34.12	76	MRNJ-101	35.33	101	IDM-64	33.83
2	MRNJ-2	35.16	27	MRNJ-37	32.72	52	MRNJ-67	32.17	77	MRNJ-102	33.01	102	IDM-66	34.69
3	MRNJ-3	30.15	28	MRNJ-38	36.78	53	MRNJ-69	34.16	78	MRNJ-106	32.98	103	IDM-69	30.16
4	MRNJ-6	40.00	29	MRNJ-39	33.54	54	MRNJ-70	37.06	79	MRNJ-110	34.44	104	NRCDR-2	36.72
5	MRNJ-7	33.95	30	MRNJ-40	31.29	55	MRNJ-71	31.08	80	MRNJ-115	32.75	105	NRCHB-101	36.18
6	MRNJ-10	37.27	31	MRNJ-41	37.16	56	MRNJ-72	39.10	81	MRNJ-118	32.81	106	DRMRIJ-31	39.89
7	MRNJ-11	37.57	32	MRNJ-42	35.15	57	MRNJ-74	32.31	82	MRNJ-123	34.03	107	DRMR-150-35	34.97
8	MRNJ-12	35.33	33	MRNJ-43	30.93	58	MRNJ-75	35.15	83	MRNJ-124	30.73	108	RVM-1	41.70
9	MRNJ-13	33.68	34	MRNJ-44	35.12	59	MRNJ-76	31.04	84	MRNJ-125	41.66	109	RVM-2	45.07
10	MRNJ-14	37.14	35	MRNJ-46	30.27	60	MRNJ-77	33.29	85	MRNJ-130	32.11	110	JM-1	46.24
11	MRNJ-15	38.55	36	MRNJ-47	35.79	61	MRNJ-78	36.33	86	MRNJ-136	30.66	111	JM-3	41.10
12	MRNJ-16	33.99	37	MRNJ-48	33.82	62	MRNJ-79	38.23	87	MRNJ-139	33.87	112	Rohini	37.82
13	MRNJ-17	34.02	38	MRNJ-49	30.07	63	MRNJ-80	30.32	88	MRNJ-142	30.64	113	Maya	51.44
14	MRNJ-18	40.11	39	MRNJ-51	39.60	64	MRNJ-83	38.27	89	MRNJ-144	30.30	114	GM-2	40.97
15	MRNJ-19	39.30	40	MRNJ-52	35.30	65	MRNJ-84	35.44	90	IDM-2	30.16	115	L-6	36.93
16	MRNJ-20	36.15	41	MRNJ-53	37.33	66	MRNJ-85	32.26	91	IDM-10	37.20	116	CS-54	41.36
17	MRNJ-21	35.78	42	MRNJ-54	42.39	67	MRNJ-86	31.92	92	IDM-11	34.74	117	RB-50	35.80
18	MRNJ-22	37.94	43	MRNJ-55	33.43	68	MRNJ-87	30.88	93	IDM-12	32.82	118	RH-74.9	42.30
19	MRNJ-23	40.44	44	MRNJ-56	38.35	69	MRNJ-88	30.93	94	IDM-15	30.37	119	ISC-3	30.21
20	MRNJ-24	37.25	45	MRNJ-57	38.02	70	MRNJ-94	31.31	95	IDM-16	36.16	120	ISC-20	32.69
21	MRNJ-26	35.38	46	MRNJ-59	31.08	71	MRNJ-95	31.29	96	IDM-25	42.55	121	ISC-23	34.03
22	MRNJ-29	32.65	47	MRNJ-60	33.61	72	MRNJ-96	31.08	97	IDM-31	34.73	122	JD-6	31.65
23	MRNJ-33	36.15	48	MRNJ-61	35.89	73	MRNJ-98	31.21	98	IDM-41	43.76			
24	MRNJ-34	30.35	49	MRNJ-63	33.67	74	MRNJ-99	32.77	99	IDM-53	34.97			
25	MRNJ-35	34.83	50	MRNJ-64	36.05	75	MRNJ-100	36.98	100	IDM-58	33.81			

Table 4.27: High erucic acid (%) genotypes in Indian mustard

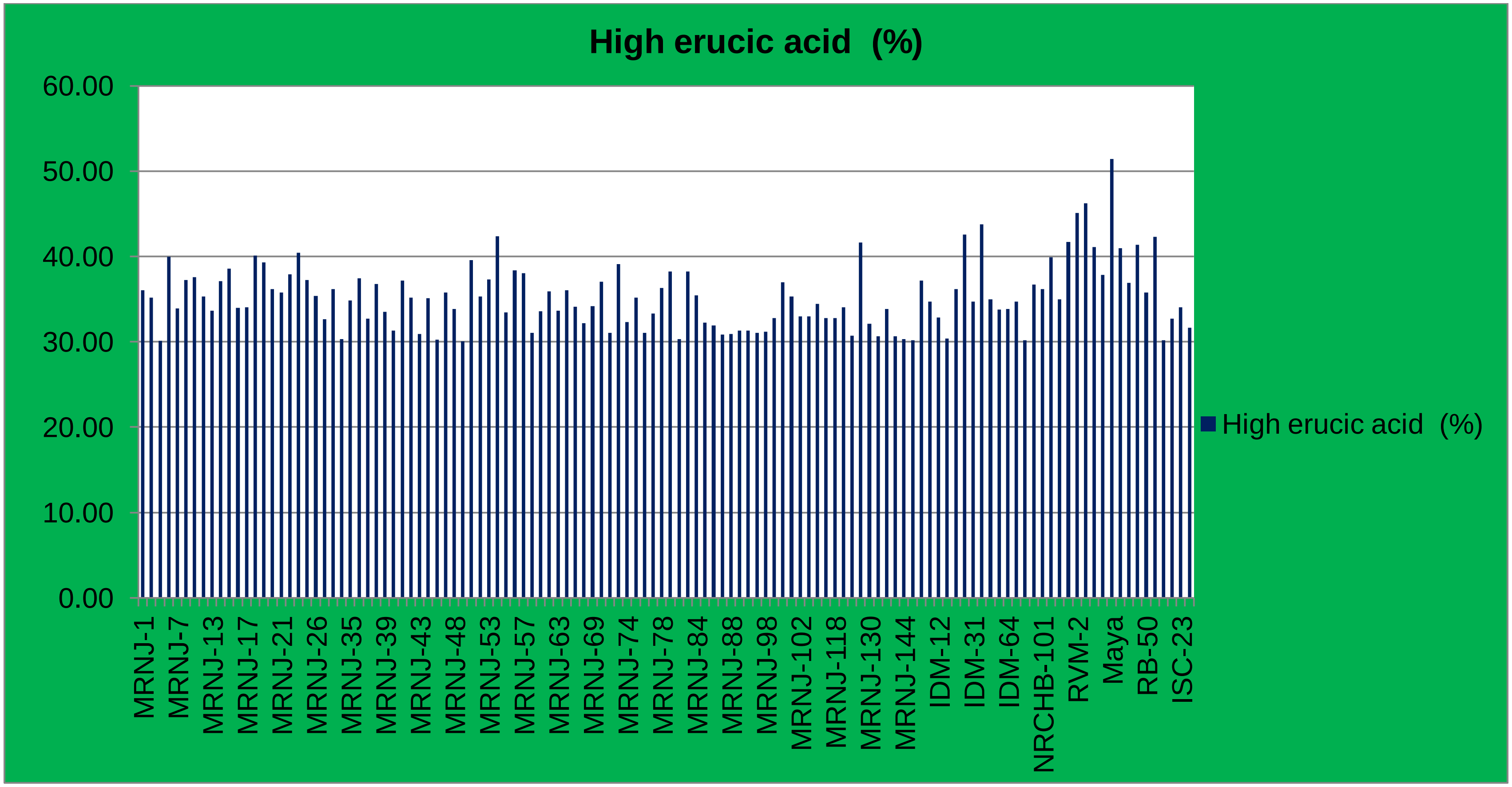


Fig 4.9: Graphical representation of high erucic acid (%) containing genotypes in Indian mustard

4.5 Identification of *Brassica* genotypes for low and high erucic acid content using SSR and CAPS molecular markers

Characterization of Indian mustard genotypes on the basis of highly polymorphic SSR, gene based SSR and CAPS molecular markers was done and results were analyzed as follow:

4.5.1 Characterization of *Brassica* genotypes using SSR markers

High quality DNA was used for amplification of highly polymorphic SSR in 48 genotypes of mustard. Working concentration of 25ng/ul DNA was used to amplify all the markers. A total of 50 SSR markers set were amplified with DNA samples of two Indian mustard genotypes (Qu *et al.,* 2012). Out of 50 SSR markers 27 were found monomorphic and 23 were polymorphic. These 23 polymorphic markers were amplified in selected 48 Indian mustard genotypes.

The representative gel showed amplified monomorphic and polymorphic banding patterns of selected primers. Total 109 alleles were amplified across 23 markers among all the 48 genotypes. Major allele frequency, allele number, gene diversity, heterozygosity and Polymorphic Information Contents were analyzed. The major allele frequency of the SSR markers varied in range of 0.31 for SSR Na10-B08 to 0.65 of SSR Na10-D07with an average of 0.44.The genotype number ranged from minimum 3 of SR-7223 and SSR Na10-B10 to maximum 8 of SSR Na10-B08 with mean value of 4.87. A total of 109 alleles were identified with an average of 4.47 alleles per locus for polymorphic SSR markers. The major allele number of the markers varied in range of 6 for BRMS-093, BRMS-098, sR-9447, SSR Na10-B08 and SSR Na10-C06 to 3 of SR-7223.Genetic diversity varied from minimum 0.55 of SSR Na10-D07 to maximum 0.77 of BRMS- 098 with mean value of 0.68. Heterozygosity ranged from 0.00 of BRMS-093, BRMS- 098, BRMS- 240, BRMS- 324, CB -10065, SORF -73, SR- 7223, SR- 9222,sR 94102, sR-9447, OI 10-CO 5,SSR Na10-B11, SSR Na10-C01,SSR Na10-C03, SSR Na10-C06, SSR Na10-C08, SSR Na10-D03, SSR Na10-D07, SSR Na10-D08, SSR Na10-D09 and SSR Na10-B11 to 0.83 of SSR Na10-B10. Mean value of heterozygosity was 0.09.Polymorphism information content (PIC) value of the markers varied from minimum 0.51 for SSR Na10-D07 to maximum 0.73 for primer BRMS- 098 with a mean value of 0.62.The primer which showed highest gene diversity (0.77) and PIC value (0.73) was observed in BRMS-098, which is representing 77% polymorphism probability of the SSR marker. The lowest gene diversity and PIC value (0.51) was observed for the primer SSR Na10-D07, indicating 51% polymorphic nature of the marker. All 23 primers representing PIC value more than 50%, representing higher capability of diversity and polymorphic nature of the primers.

Table 4.28: List of SSR markers analyzed, major allele frequency, genotype No., allele No., gene diversity, heterozygosity and PIC values used for genetic diversity in Indian mustard

Markers	MajorAlleleFrequency	Genotype No	Allele o.	Gene Diversity	Hetero-zygosity	PIC lue
BRMS-093	0.48	6.00	6.00	0.71	0.00	0.68
BRMS-098	0.33	6.00	6.00	0.77	0.00	0.73
BRMS-240	0.33	4.00	4.00	0.74	0.00	0.69
BRMS-324	0.56	4.00	4.00	0.60	0.00	0.55
CB -10065	0.46	5.00	5.00	0.69	0.00	0.65
SORF -73	0.42	5.00	5.00	0.69	0.00	0.63
SR- 7223	0.44	3.00	3.00	0.62	0.00	0.54
SR- 9222	0.52	4.00	4.00	0.63	0.00	0.57
sR 94102	0.38	5.00	5.00	0.74	0.00	0.70
sR-9447	0.52	6.00	6.00	0.64	0.00	0.59
OI 10-CO5	0.44	4.00	4.00	0.62	0.00	0.55
SSRNa10-B08	0.31	8.00	6.00	0.75	0.77	0.71
SSR Na10-B10	0.42	3.00	4.00	0.71	0.83	0.66
SSR Na10-B11	0.35	5.00	5.00	0.73	0.00	0.68
SSR Na10-C01	0.54	4.00	4.00	0.60	0.00	0.53
SSR Na10-C03	0.44	5.00	5.00	0.65	0.00	0.58
SSRNa10-C06	0.38	6.00	6.00	0.75	0.00	0.71
SSR Na10-C08	0.40	4.00	4.00	0.69	0.00	0.63
SSR Na10-D03	0.48	4.00	4.00	0.63	0.00	0.56
SSR Na10-D07	0.65	5.00	5.00	0.55	0.00	0.51
SSR Na10-D08	0.44	5.00	5.00	0.68	0.00	0.62
SSR Na10-D09	0.38	4.00	4.00	0.68	0.00	0.62
SSR Na10-D11	0.51	7.00	5.00	0.65	0.44	.60
Mean	**0.44**	**4.87**	**4.74**	**0.68**	**0.09**	**0.62**

The dendrogram was made based on scoring done for the entire polymorphic SSR markers using the software Power Marker v3.25. The genetic relationships among Indian mustard genotypes were presented. Major three clusters were obtained *i.e.,* C-I, C-II and C-III, in which cluster C-I consisted of 4 genotypes, RB-50, NRCDR-2, DRMR150-35 clustered together and JD6 in other. Cluster- II had 7 genotypes. Cluster C-II was again divided into sub clusters ‘C-II (a)' and 'C-II (b)', cluster C-II (a) divided into 2 sub clusters. Sub cluster C-II a (i) consisted of CS-54, Maya and GM-2, another sub cluster C-II a (ii) consisted of RVM-2, RVM-1, Rohini and JM-2. Sub cluster C-II (b) consisted of RH74.9 and DRMRIJ-31. Cluster C-III was having 37 genotypes and separated into sub clusters 'C-III (a)' and 'C-III (b)'.Cluster c-III (a) divided into 2 sub clusters c-III a (i) and c-III a (ii). Cluster c-III a (i) consisted of MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, PM-25, PM-22, PM-21, PM-29, PM-24, LES-39 and PM-30. Cluster-III a (ii) again divided into two sub clusters *viz.,* c-III a (ii a) and c-III a (ii b).C-III a (ii a) consisted of MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13 and MRNJ-12. C-III a (ii b) consisted of MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4 MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6. 'C-III (b)' consisted of MRNJ-26 and MRNJ-25.Most informative cluster C-III (a) contained 33 genotypes including MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6 indigenous and exotic germplasm representing good quantitative traits and PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30 were released varieties. These varieties having low erucic acid, timely sown and irrigated condition were grouped with these germplasm so, may be having that traits.

Table 4.29: Cluster groups of Indian mustard genotypes and their characteristics using highly polymorphic SSR markers

Major clusters	Sub clusters	No of germplasm and varieties	Name of germplasm and varieties	Remark
C-I,		4	RB-50, NRCDR-2, DRMR150-35 and JD6	Suitable for rainfed and salinity condition (RB50), Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew *&A. blight* (DRMR150-35),

				Drought (rainfed), suitable for early (September) and late (November) sowing under irrigated situations (JD6)
C-II	C-II (a)	7	CS-54, Maya, GM-2, RVM-2, RVM-1, Rohini and JM-2	Salinity tolerant (CS54), Suitable for irrigated condition and white rust resistance (Maya), Nontraditional areas (GM2), Early maturity and high oil content (RVM1), Suitable for rainfed and late sown conditions (RVM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Suitable for rainfed condition and white rust resistance (JM2)
	C-II (b)	2	RH749 and DRMRIJ-31	Suitable for irrigated condition (RH749), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31)
C-III	C-III (a)	33	MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-	Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for

			21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6	timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39)
	C-III (b)	2	MRNJ-26 and MRNJ-25	

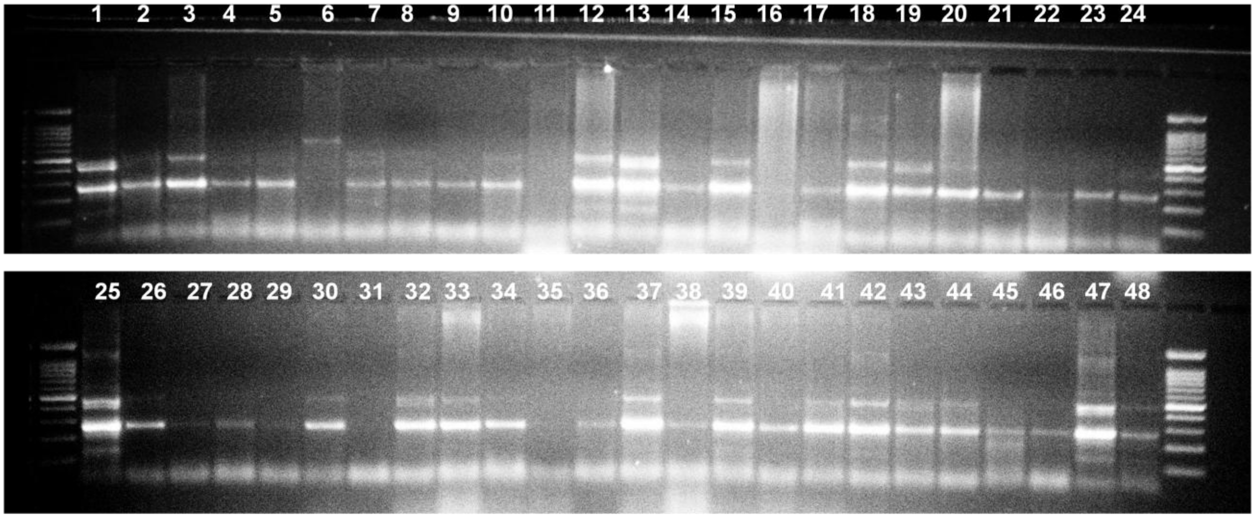


Plate 4.1: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-C08, represent 1 MRNJ-1, 2 MRNJ-2, 3 MRNJ-3, 4 MRNJ-4, 5 MRNJ-5, 6 MRNJ-6, 7 MRNJ-7, 8 MRNJ-8, 9 MRNJ-9, 10 MRNJ-10, 11 MRNJ-11, 12 MRNJ-12, 13 MRNJ-13, 14 MRNJ-14, 15 MRNJ-15, 16 MRNJ-16, 17 MRNJ-17, 18 MRNJ-18, 19 MRNJ-19, 20 MRNJ-20, 21 MRNJ-21, 22 MRNJ-22, 23 MRNJ-23, 24 MRNJ-24, 25 MRNJ-25, 26 ISC-3, 27 JM-2, 28 RVM-1, 29 RVM-2, 30 Rohini, 31 Maya, 32 NRCDR-2,

33 DRMRIJ-31, 34 DRMR-150-35, 35 GM-2, 36 CS-54, 37 RB-50, 38 RH749, 39 JD-6, 40 L-4, 41 L-6, 42 PM-25, 43 PM-21, 44 PM-22, 45 PM-24, 46 PM-29, 47 PM-30, 48 LES-39

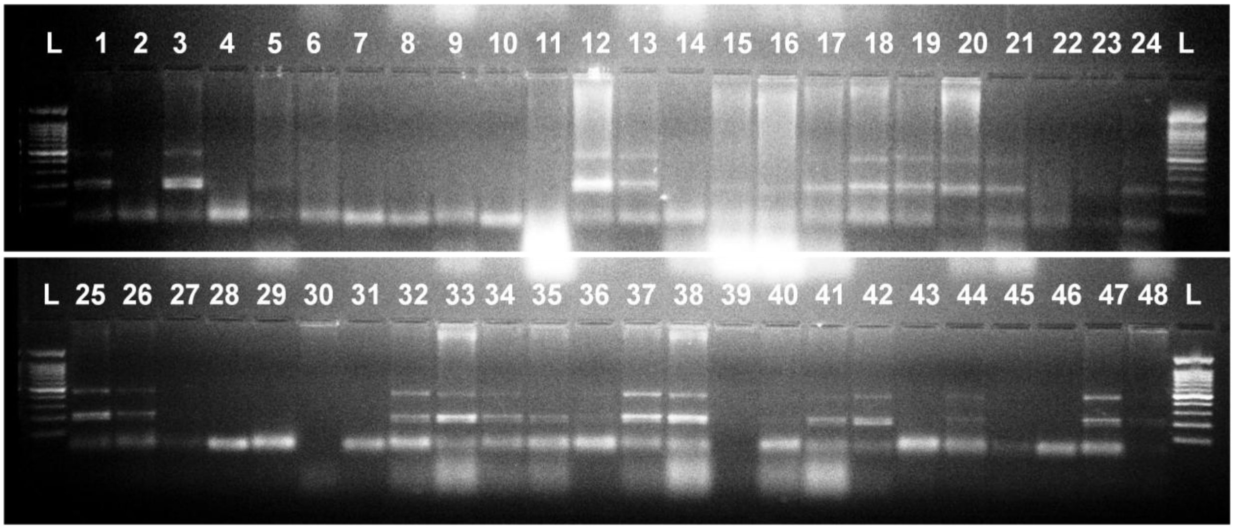


Plate 4.2: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-D03

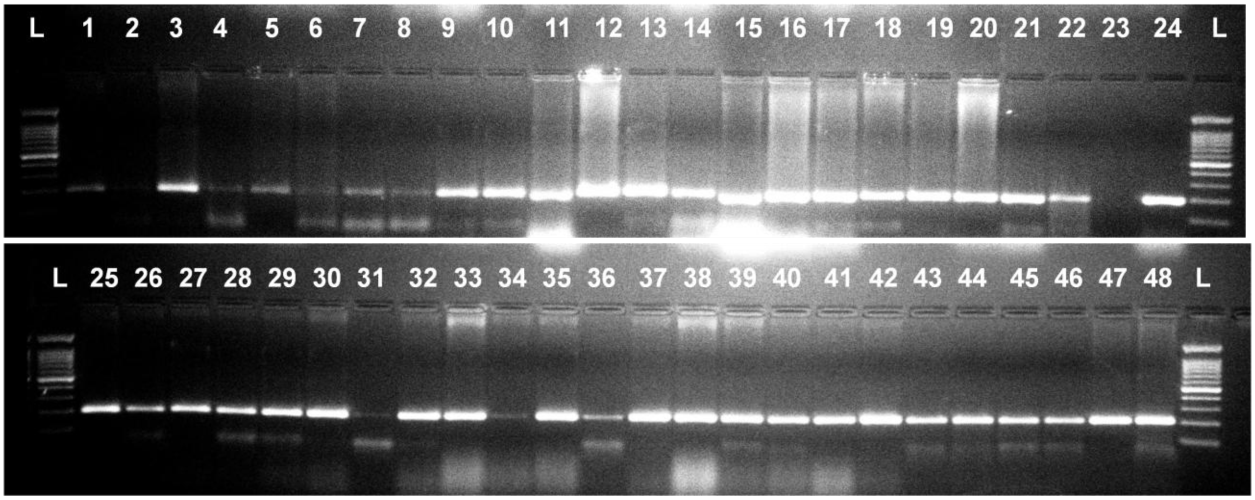
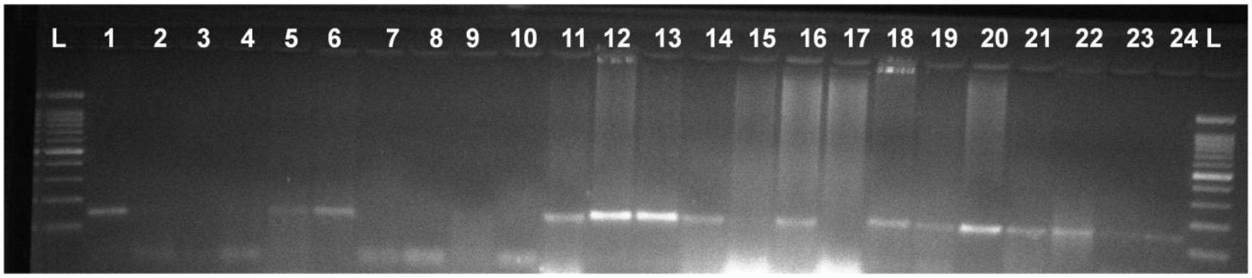


Plate 4.3: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-C06



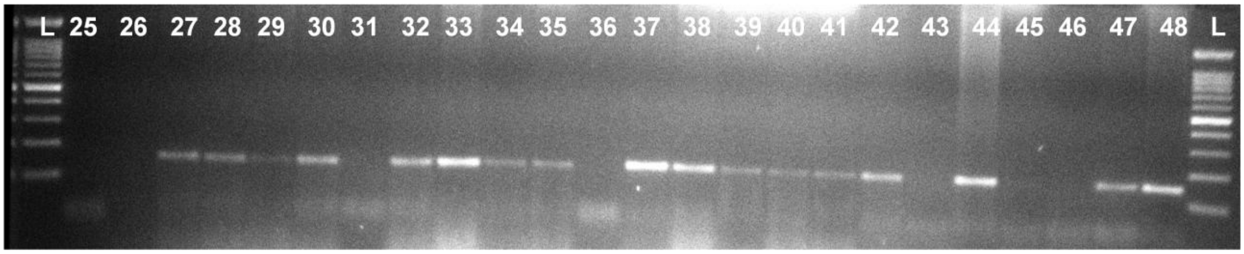


Plate 4.4: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-B11

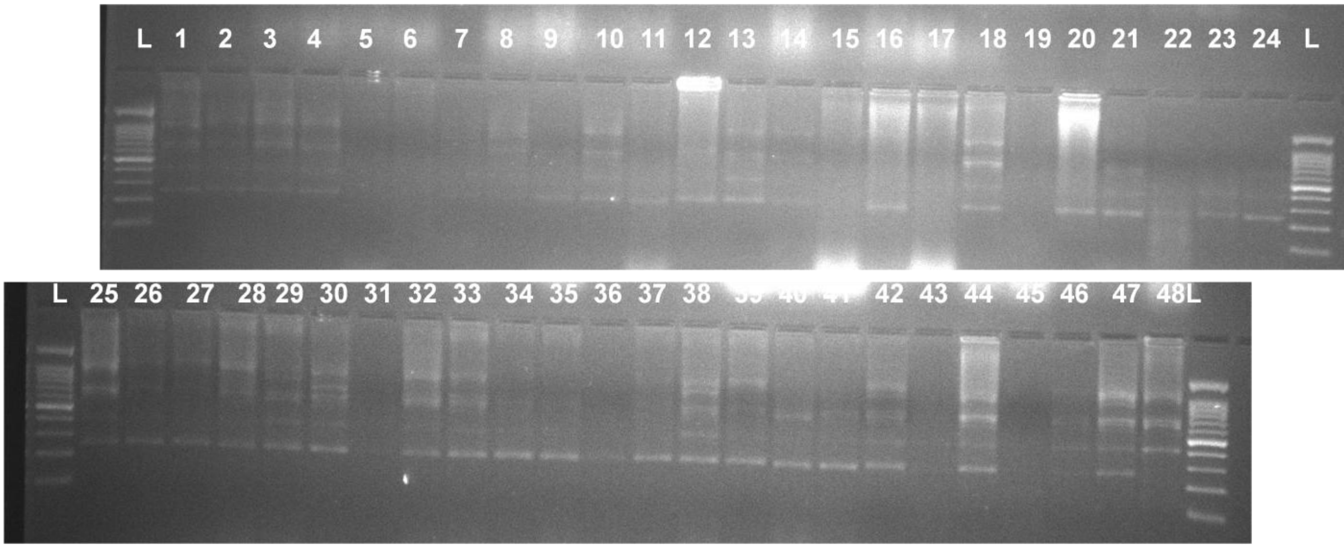


Plate 4.5: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-C01

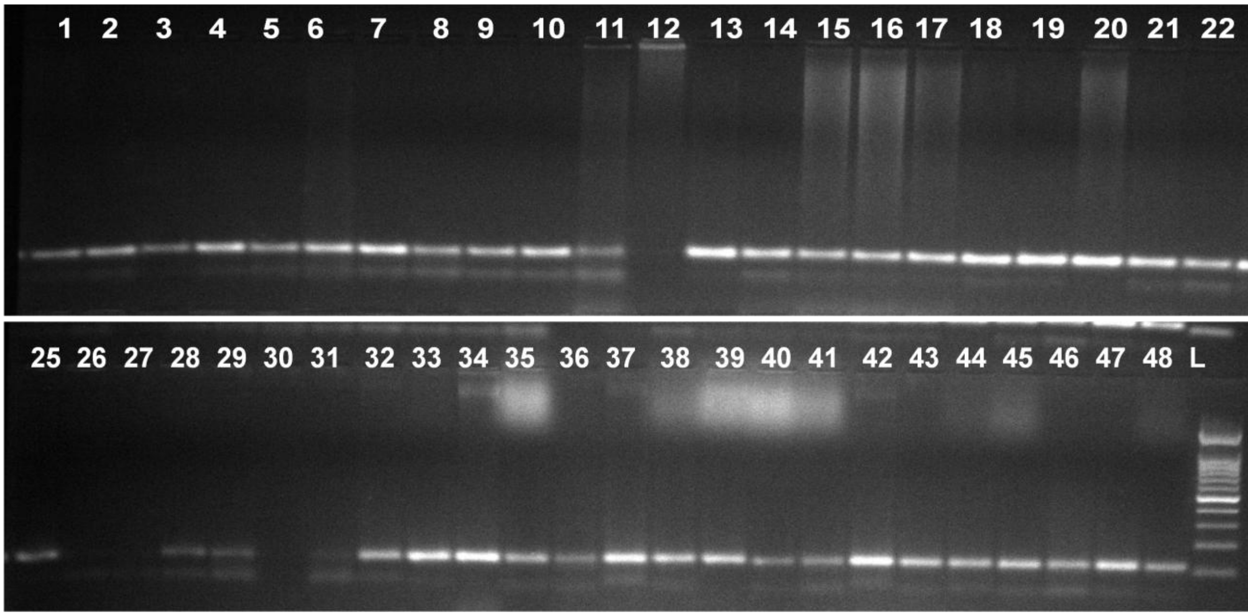


Plate 4.6: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Primer SORF -73

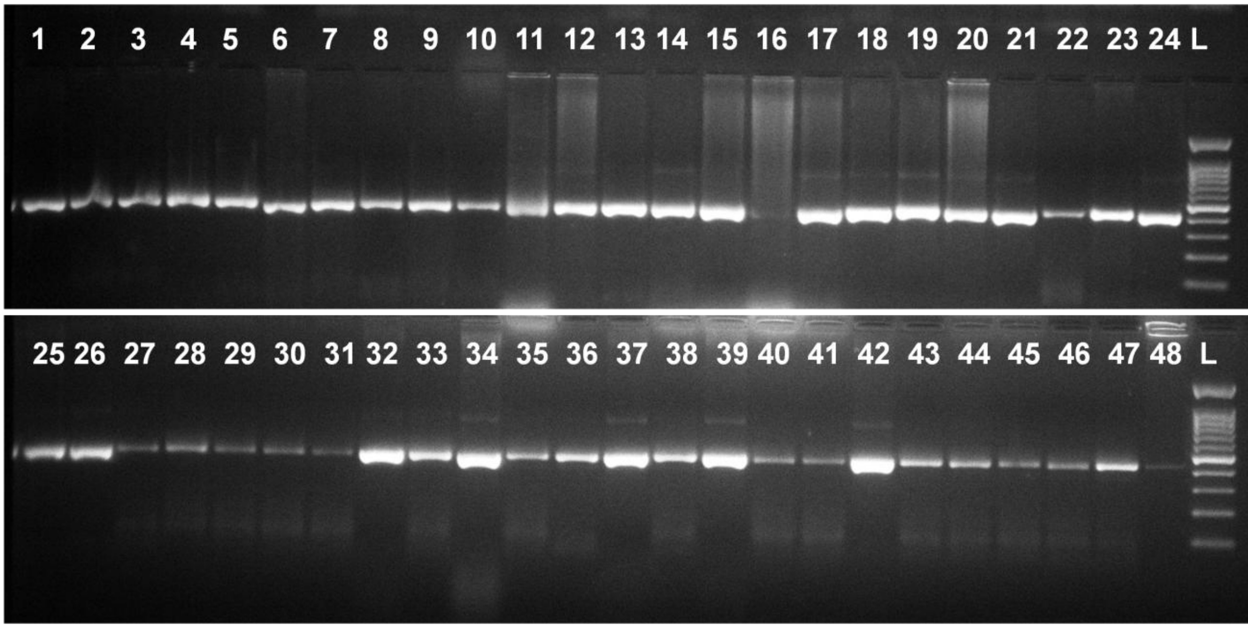


Plate 4.7: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Primer SR- 7223

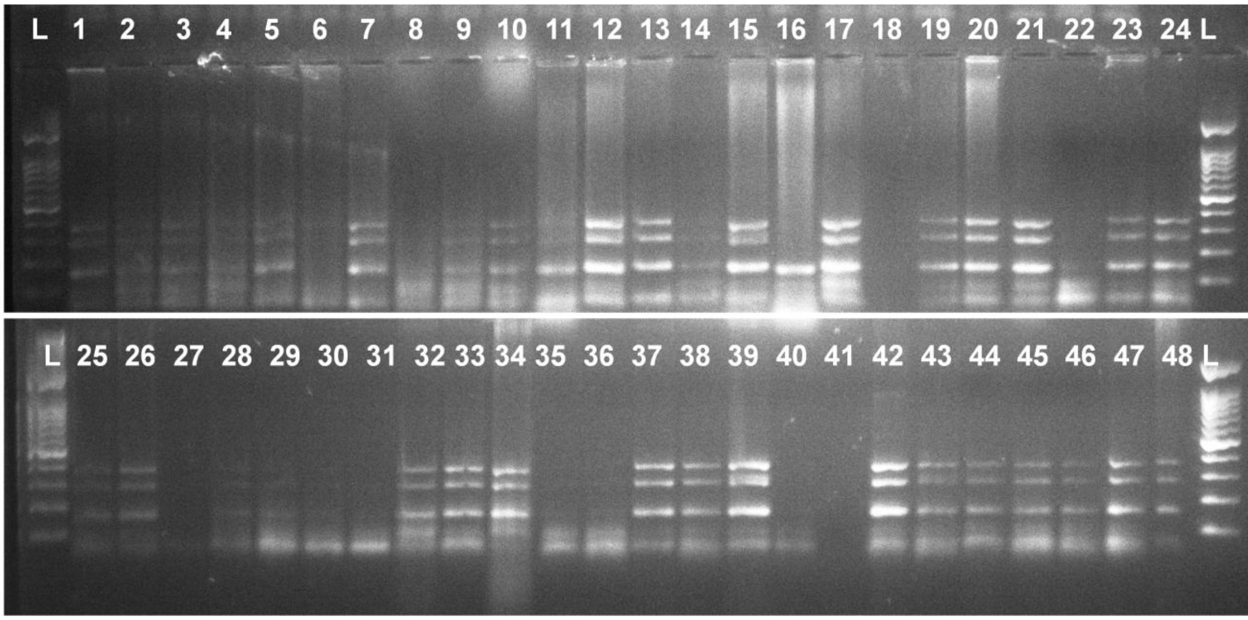


Plate 4.8: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-D09

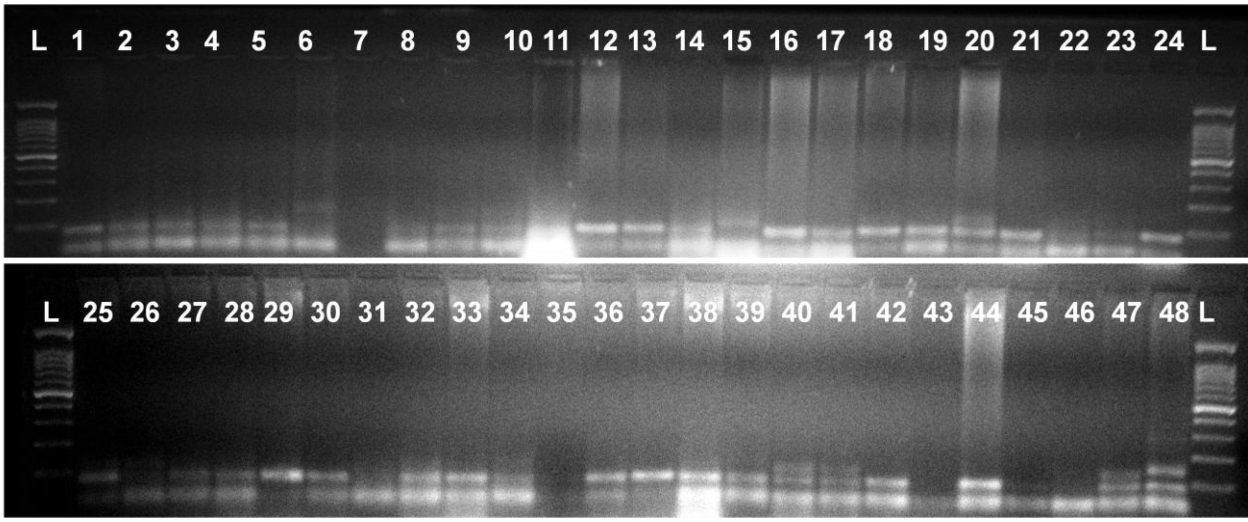


Plate 4.9: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using Primer SSR Na10-B08

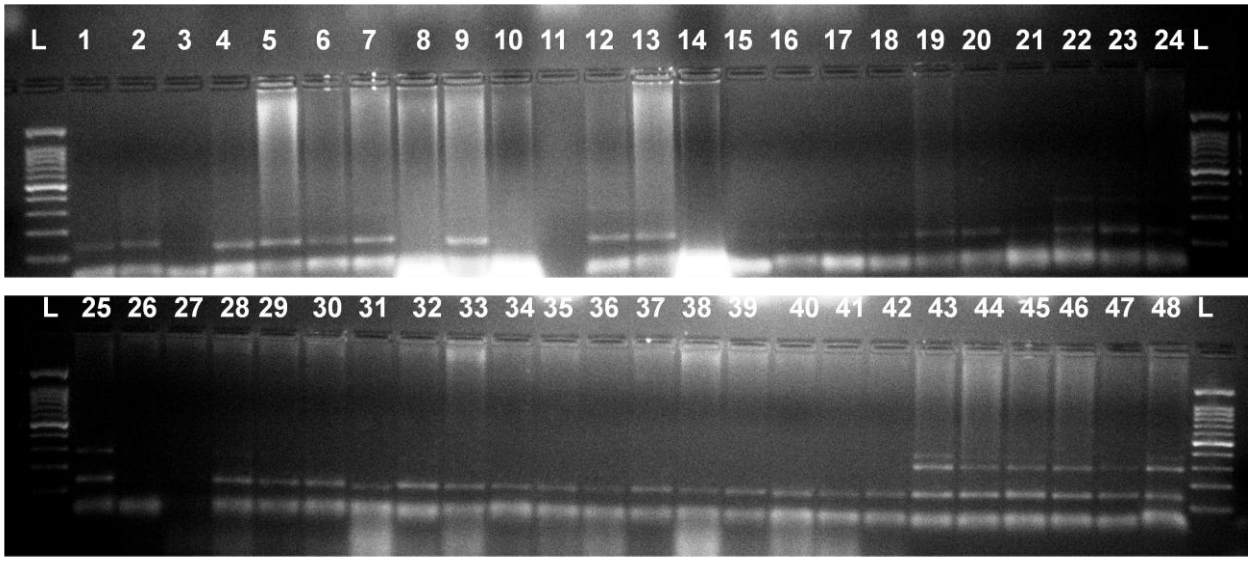
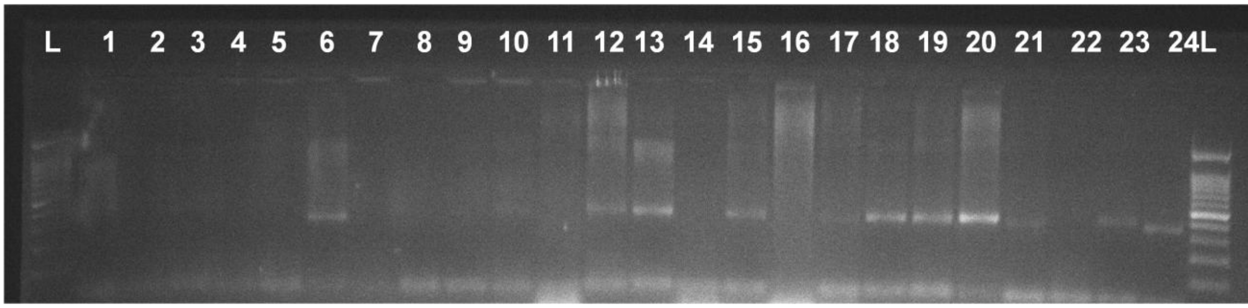


Plate 4.10: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Na10-B10



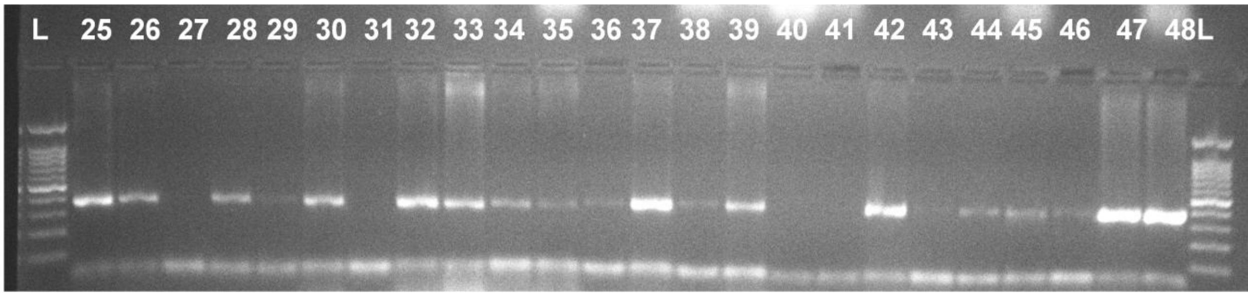


Plate 4.11: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Primer sR-9447

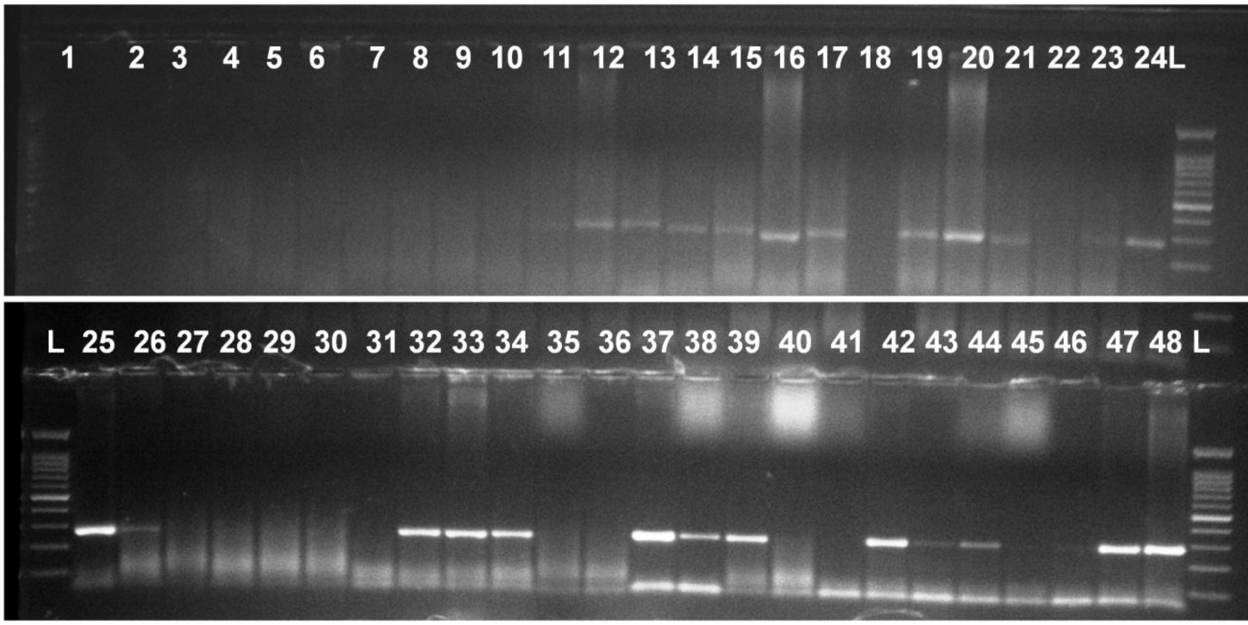


Plate 4.12: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Primer BRMS-093

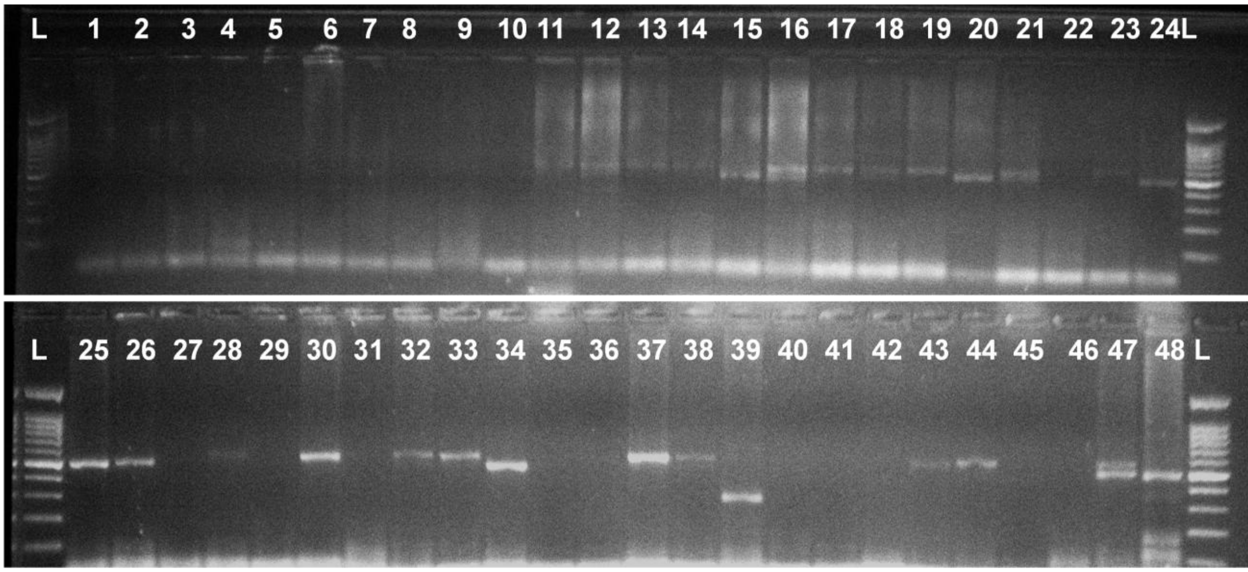


Plate 4.13: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Primer SR- 9222

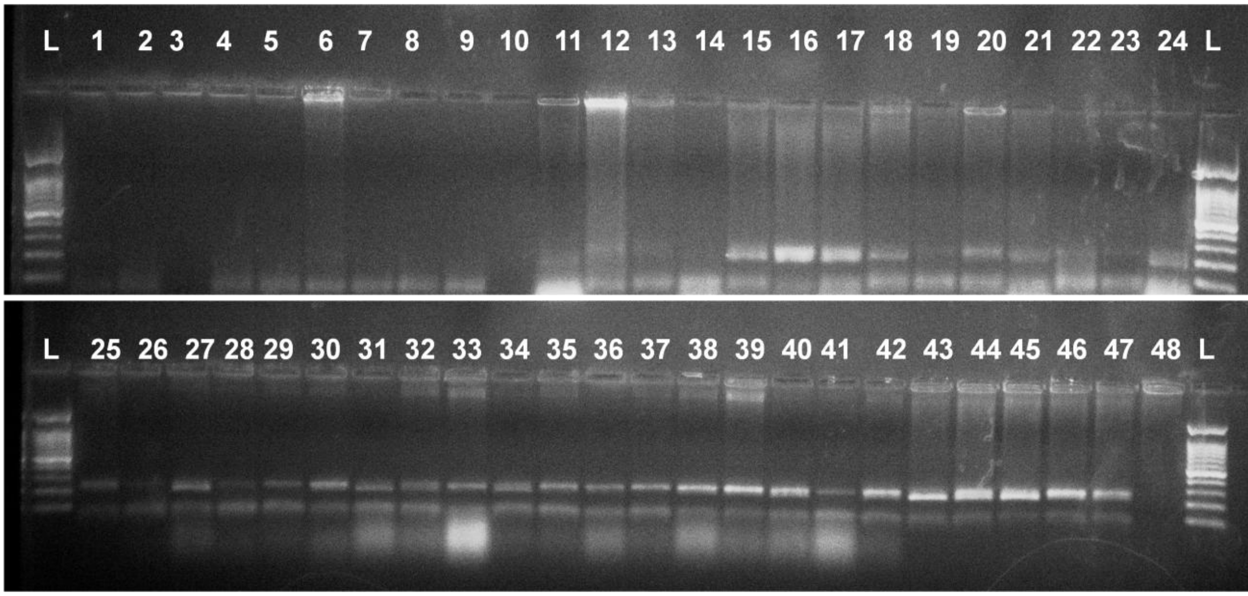


Plate 4.14: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using SSR Primer BRMS- 324

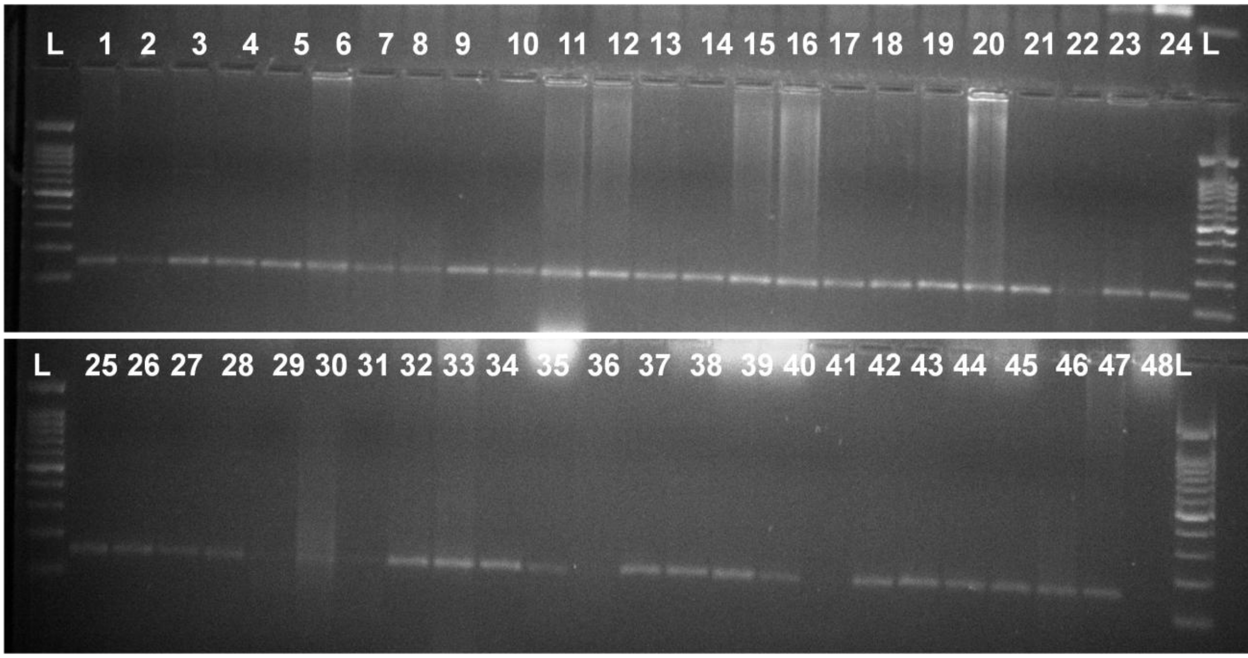


Plate 4.15: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using monomorphic SSR Primer CN -52

4.5.2 Screening of *Brassica* genotypes for erucic acid and self-incompatibility using gene based SSR markers

Statistical data of gene specific SSR markers is given in Table 4.30. Maximum major allele frequency was 0.43 of Sal-SRK-I and minimum 0.37of FAE1 with an average of 0.40. A total of 10 alleles were identified with an average of 5 alleles per locus for polymorphic SSR markers. Allele number of both markers were 5.00 and also mean was 5.00. Highest gene diversity was 0.71 of Sal-SRK-I and minimum 0.70 of FAE1 with an average of 0.70. Maximum polymorphic information content was 0.66 of Sal-SRK-I and minimum 0.64 of FAE1 with mean of 0.65. The primer which showed highest gene diversity (0.71) and PIC value (0.66) was observed in Sal-SRK-I, representing 71% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.64) was observed for the primer FAE1, indicating 64% polyporphic nature of the marker. Both primers representing PIC value more than 50%, representing highly polymorphic nature of primer, can further be used for screening purpose.

Table 4.30: List of gene based SSR markers analyzed, major allele frequency, allele No, gene diversity, and PIC values used for erucic acid and self-incompatibility in Indian mustard

Markers	Major allele frequency	Allele No.	Gene diversity	PIC value
FAE1	0.37	5.00	0.70	0.64
Sal-SRK-I	0.43	5.00	0.71	0.66
Mean	0.40	5.00	0.70	0.65

Population structure was made based on scoring done for gene specific SSR markers using the software Power Marker v3.25. The genetic relationships among Indian mustard genotypes were presented. Major three clusters were formed *i.e*., C-I, C-II and C-III. Cluster I makes group of 4 various genotypes like CS-54, GM-2, DRMR-150-35 and DRMRIJ-31. Cluster II was having 4 genotypes having MRNJ-6, MRNJ-5, RH-74.9 and RB-50. Cluster III had 40 genotypes. Cluster III can further be divided into two sub clusters namely c-III (a) and c-III (b). C-III (a) again separated into two sub clusters *viz.,* c-III a (i) and c-III a (ii). Cluster-III (b) consisted of MRNJ-8 and MRNJ-7. Cluster-III a (i) make group of NRCDR-2 and Maya genotypes. Cluster-III a (ii) again divided into two sub cluster *i.e*., c-III a (ii a) and c-III a (ii b). C-III a (ii a) consisted of MRNJ-17, MRNJ-15, MRNJ-14, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-18, MRNJ-24, MRNJ-23, MRNJ-20, PM-21, PM-25, L-6, L-4, MRNJ-19, MRNJ-1, PM-22, MRNJ-22, MRNJ-21, LES-39, PM-30, PM-29, PM-24 MRNJ-2, ISC-3, MRNJ-25, MRNJ-16, MRNJ-11, JD-6, MRNJ-9, MRNJ-4, MRNJ-3, RVM-1, JM-2, Rohini, c-III a (ii b) consisted of RVM-2. Most informative cluster C-III (a) contained 38 genotypes including NRCDR-2, Maya, MRNJ-17, MRNJ-15, MRNJ-14, MRNJ-

13, MRNJ-12, MRNJ-10, MRNJ-18, MRNJ-24, MRNJ-23, MRNJ-20, PM-21, PM-25, L-6, L-4, MRNJ-19, MRNJ-1, PM-22, MRNJ-22, MRNJ-21, LES-39, PM-30, PM-29, PM-24 MRNJ-2, ISC-3, MRNJ-25, MRNJ-16, MRNJ-11, JD-6, MRNJ-9, MRNJ-4, MRNJ-3, RVM-1, JM-2, Rohini and RVM-2 indigenous and exotic germplasm representing good quantitative traits and NRCDR-2, Maya, PM-25, PM-21, PM-29, PM-24, LES-39, PM-30, JD-6, RVM-1, JM-2, Rohini and RVM-2 were released varieties (Table 4.20).

Table 4.31: Tabular representation of major and sub clusters formed from population structure of Indian mustard in gene based SSR markers

Major clusters	Sub clusters	No. of germplasm lines and varieties	Name of germplasm lines and varieties	Remark
C-I,		4	CS-54, GM-2, DRMR-150-35 and DRMRIJ-31	Salinity tolerant (CS54), Nontraditional areas (GM2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew &*A. blight* *(*DRMR-150-35), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31)
C-II		4	MRNJ-6, MRNJ-5, RH-749 and RB-50	Suitable for irrigated condition (RH749), Suitable for rainfed and salinity condition (RB50)
C-III	C-III (a)	38	NRCDR-2, Maya, MRNJ-17, MRNJ-15, MRNJ-14, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-18, MRNJ-24, MRNJ-23, MRNJ-20, PM-21,	Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Suitable for irrigated condition and white rust resistance

			PM-25, L-6, L-4, MRNJ-19, MRNJ-1, PM-22, MRNJ-22, MRNJ-21, LES-39, PM-30, PM-29, PM-24 MRNJ-2, ISC-3, MRNJ-25, MRNJ-16, MRNJ-11, JD-6, MRNJ-9, MRNJ-4, MRNJ-3, RVM-1, JM-2, Rohini and RVM-2	(Maya), Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39), Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations (JD6), Suitable for rainfed condition and white rust resistance (JM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Early maturity and high oil content (RVM1),

				Suitable for rainfed and late sown conditions (RVM2)
	C -III (b)	2	MRNJ-8 and MRNJ-7	

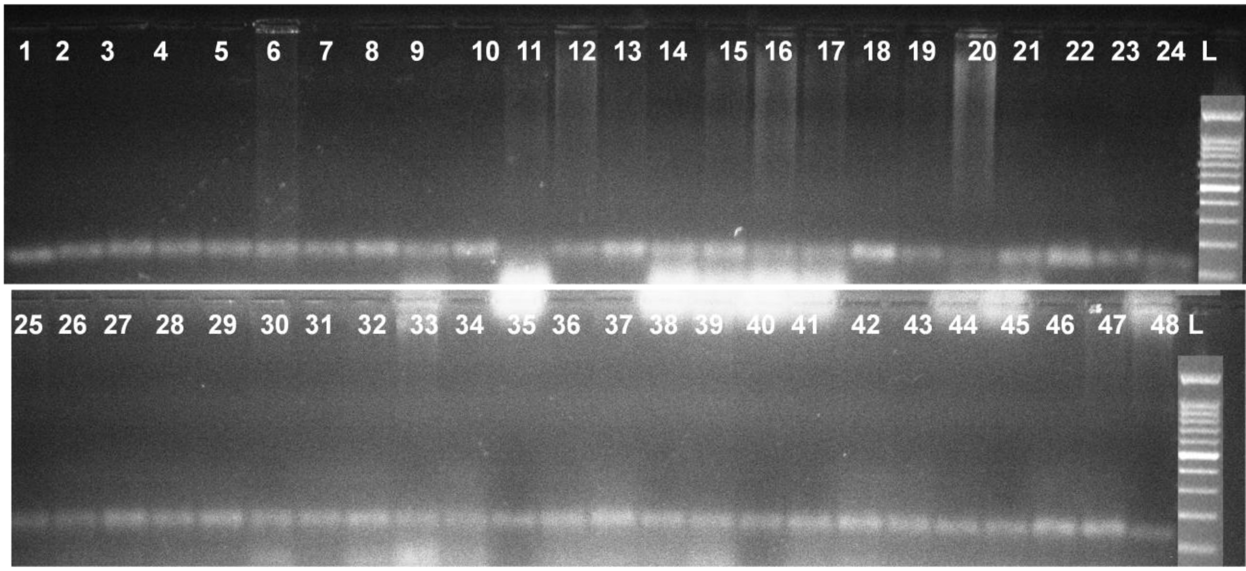


Plate 4.16: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using gene based SSR FAE1 primer

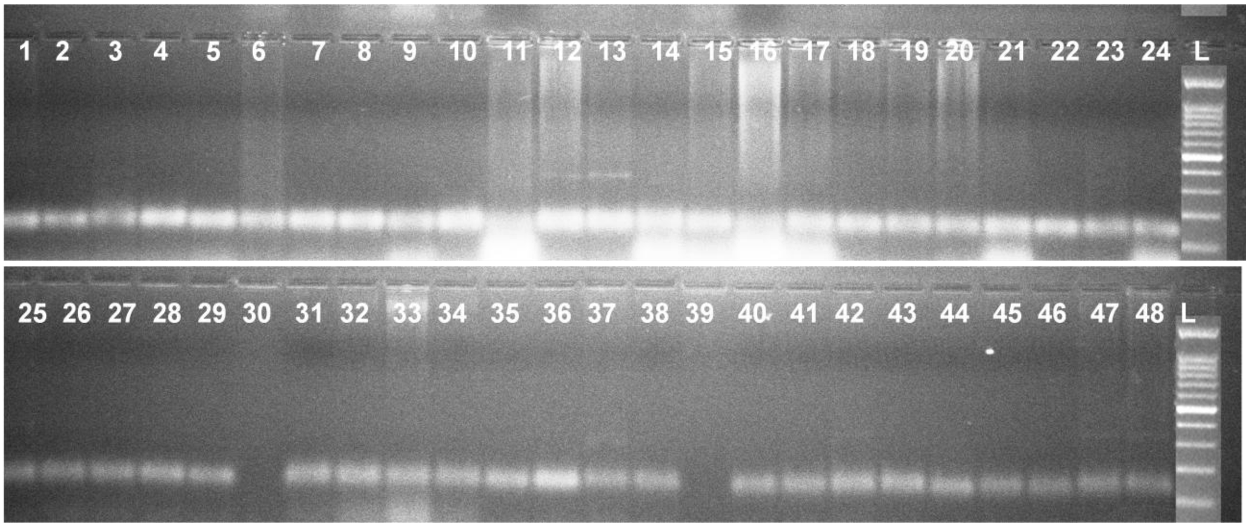


Plate 4.17: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using gene based SSR Sal-SRK-I primer

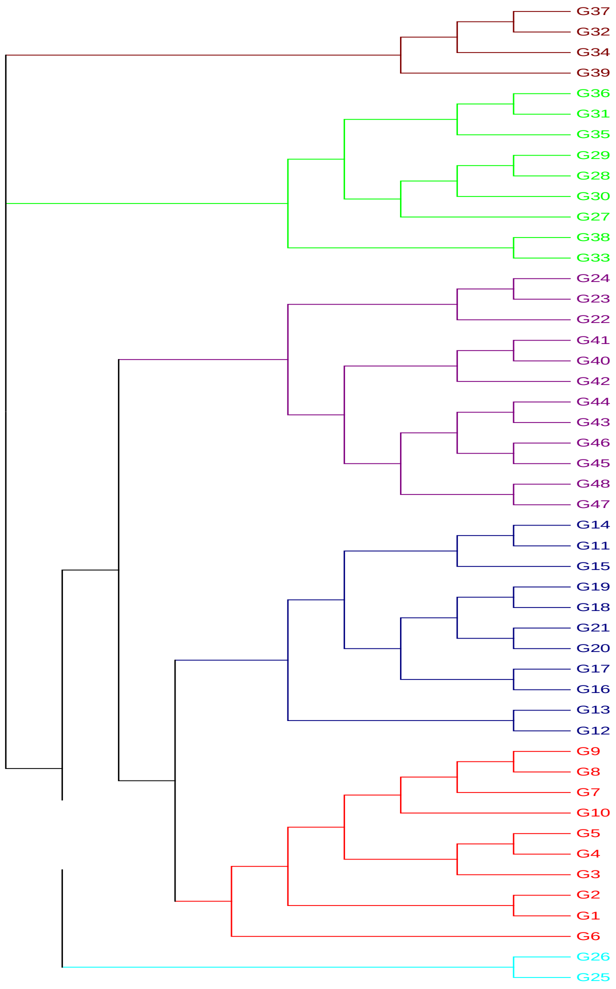


Fig 4.10: Dendogram of highly polymorphic SSR markers using power marker v3.25 software in Indian mustard

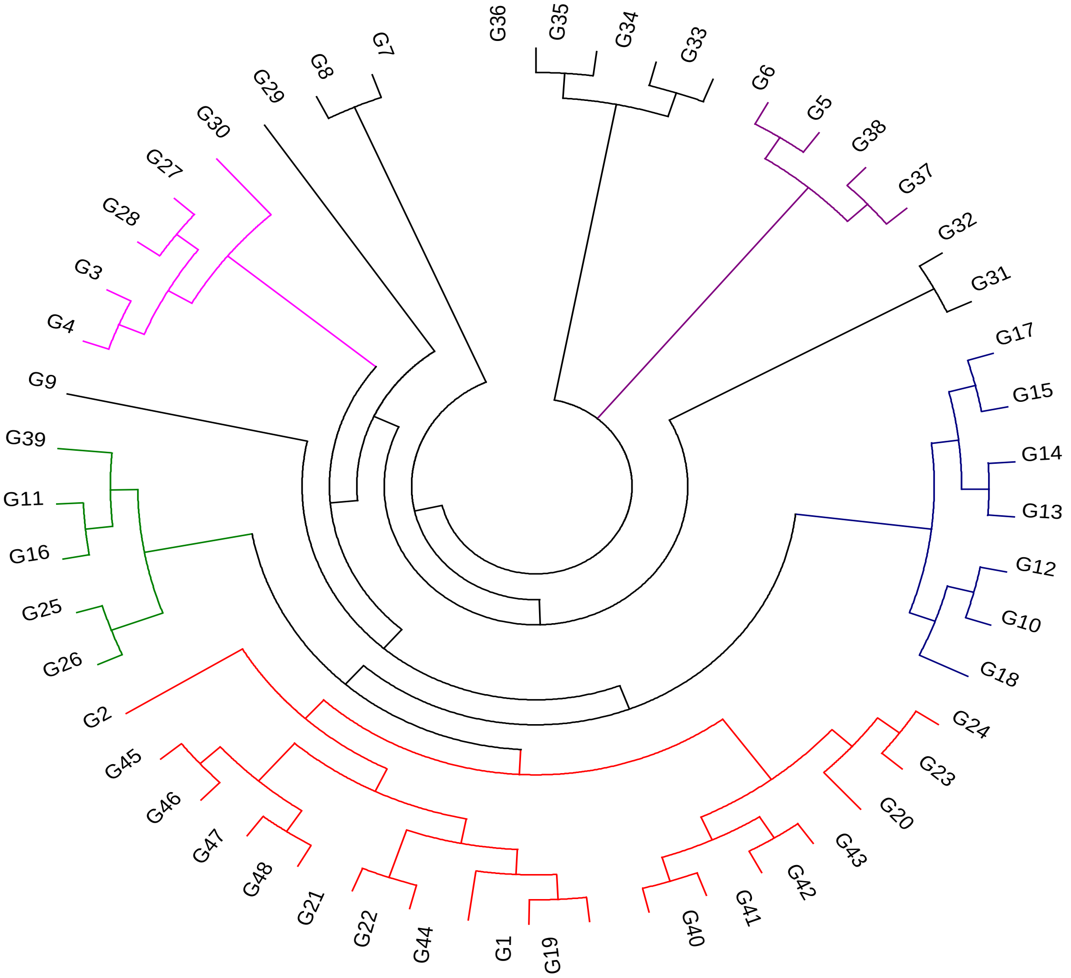


Fig 4.11: Phylogenetic tree analysis of gene based SSR marker data analysis using power marker v3.25 software in Indian mustard

4.5.3 Molecular characterization of *Brassica juncea L* genotypes through gene based CAPS markers (erucic acid)

Statistical analysis for gene based CAPS markers were done to observe major allele frequency, PIC and allele numbers. Maximum major allele frequency 0.50 was found forCAPS237 and minimum 0.31 for CAPS1265 with an average of 0.41. Highest genotype number was 5.00 of CAPS237 and lowest 4.00 of CAPS1265 with mean 4.50. A total of 9 alleles were identified with an average of 4.5 alleles per locus for polymorphic SSR markers. Maximum Allele number 5.00 was observed forCAPS237 and minimum 4.00 for CAPS1265 with mean value 4.50. Maximum gene diversity was consisted 0.74 forCAPS1265 and minimum gene diversity 0.66 for marker CAPS237 with an average of 0.70. Highest polymorphic information content 0.69 was found for markerCAPS1265 and lowest 0.61 forCAPS237 with mean PIC value 0.65. Above results were presented in Table 4.21. The primer which showed highest gene diversity (0.74) and PIC value (0.69) was CAPS1265, which is representing 74% polymorphism probability of the marker. Lowest gene diversity and PIC value (0.61) was observed for the primer CAPS237, indicating 61% polyporphic nature of the marker. Both primers showing PIC value more than 50%, so these primers having maximum polymorphic capacity and can be used for mustard molecular breeding for selected trait.

Table 4.32: Statistical values of gene based CAPS markers for major allele frequency, allele number, gene diversity, and Polymorphic Information contents in Indian mustard

Markers	**Major allele frequency**	**Allele No.**	**Gene diversity**	**PIC value**
CAPS237	0.50	5.00	0.66	0.61
CAPS1265	0.31	4.00	0.74	0.69
Mean	**0.41**	**4.50**	**0.70**	**0.65**

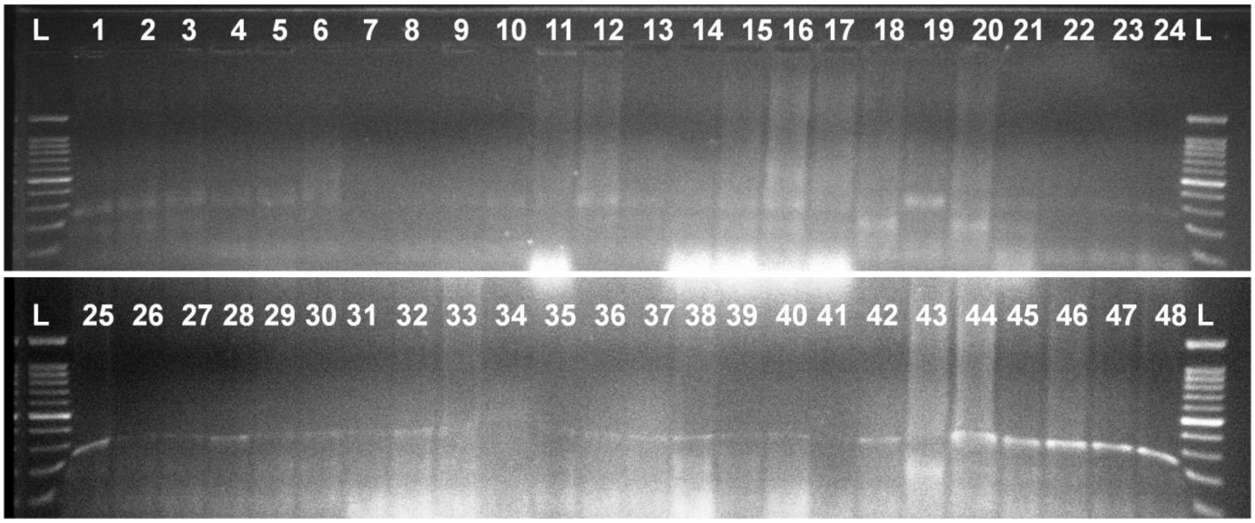


Plate 4.18: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using CAPS237

Phylogenetic analysis was done based on scoring of gene specific CAPS markers using the software Power Marker v3.25. The genetic relationships among Indian mustard genotypes were presented. Major three clusters were formed *viz.,* C-I, C-II and C-III. Cluster I were having 2 genotypes *namely*, JD6 and RB50. Cluster II was found 42 genotypes and divided into two clusters *viz*., c-II (a) and c-II (b). C-II (a) again separated into two sub-clusters, c-II a (i) and c-II a (ii). C-II a (i) again divided into two sub clusters *viz.,* c-II a (I a) and c-II a (ib). C-II a (I a) was consisted genotypes viz., RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24 and PM22. C-II a (I b) was making groups of 24 genotypes *namely*, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21 and MRNJ-24. C-II a (ii) was consisted of only one genotype RVM1-. C-II (b) contained only one genotype L-4. C-III consisted of 4 genotypes including RVM-2, Rohini, NRCDR-2 and CS-54. Most interesting group was formed in cluster II (a), including 41 indigenous and exotic genotypes RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1. RH749, Maya, PM25, PM29, PM24, PM22, JM-2, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30 and RVM-1 showed release varieties suitable for irrigated and rainfed condition, white rust resistance, high temperature tolerant, suitable for early sown, low erucic acid, timely sown, non-traditional areas, bold seeded, high oil content and high yielding variety, early maturity and tolerant to powdery mildew & *A. blight,* these germplasms falling along-with these varieties may be having these characteristics features.

Table 4.33: Tabular representation of major and sub clusters formed from population structure of Indian mustard in gene based CAPS markers

Major clusters	Sub clusters	No. of germplasm and varieties	Name of germplasm and varieties	Remarks
C-I,		2	JD6 and RB50	Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations (JD6), Suitable for rainfed and salinity condition (RB50)
C-II	C-II (a)	41	RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1	Suitable for irrigated condition (RH749), Suitable for irrigated condition and white rust resistance (Maya), Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown

				and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39), Suitable for rainfed condition and white rust resistance (JM2), Nontraditional areas (GM2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew *&A. blight* (DRMR150-35), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31), Early maturity and high oil content (RVM1)
	C -II (b)	1	L-4	
C -III		4	RVM-2, Rohini, NRCDR-2 and CS-54	Suitable for rainfed and late sown conditions (RVM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Suitable for rainfed& Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Salinity tolerant (CS54)

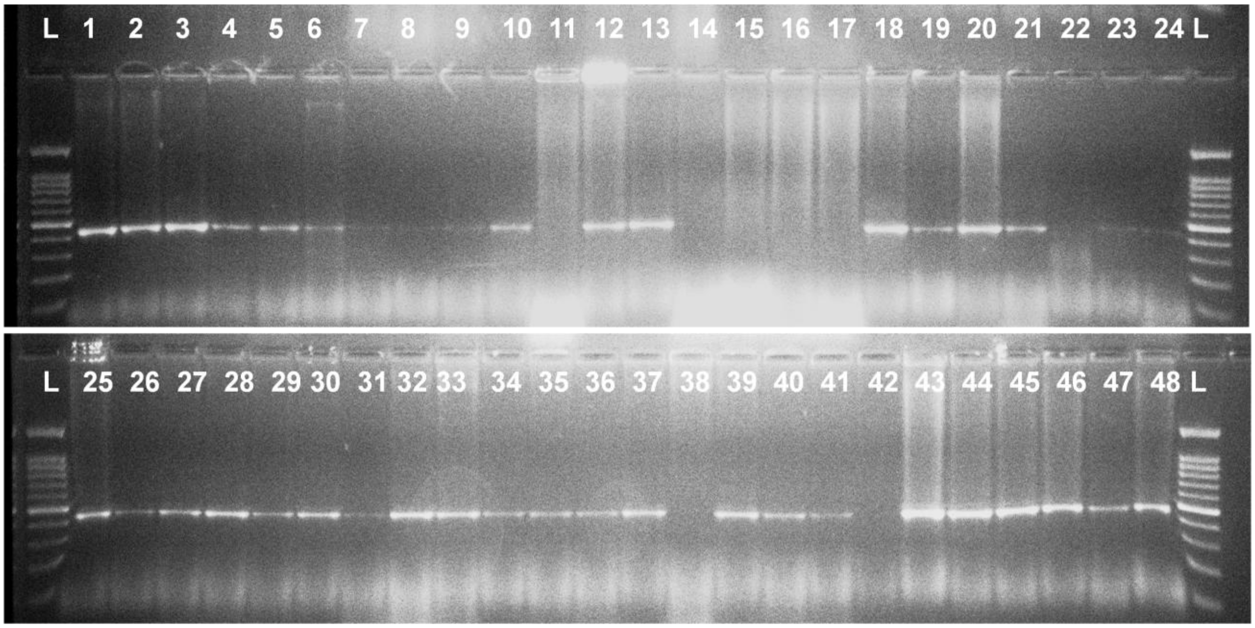


Plate 4.19: Agarose gel showing banding pattern of PCR product of 48 genotypes in Indian mustard using CAPS1265

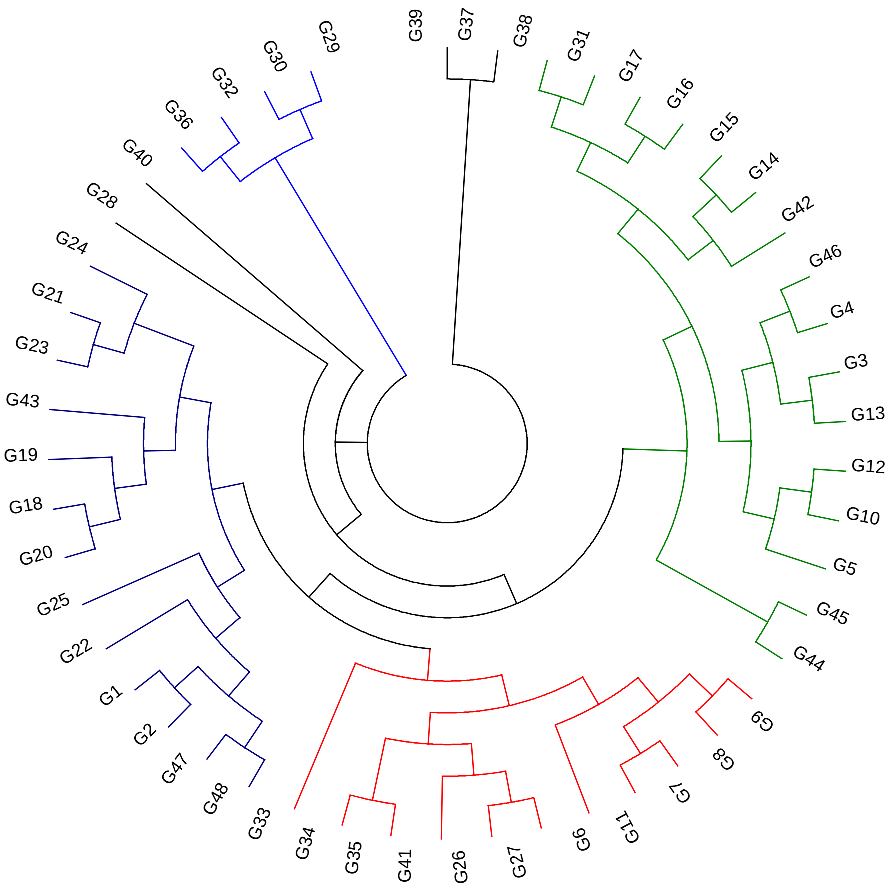


Fig 4.12: Dendrogram of gene based CAPS marker data analysis using power marker v3.25 software in Indian mustard

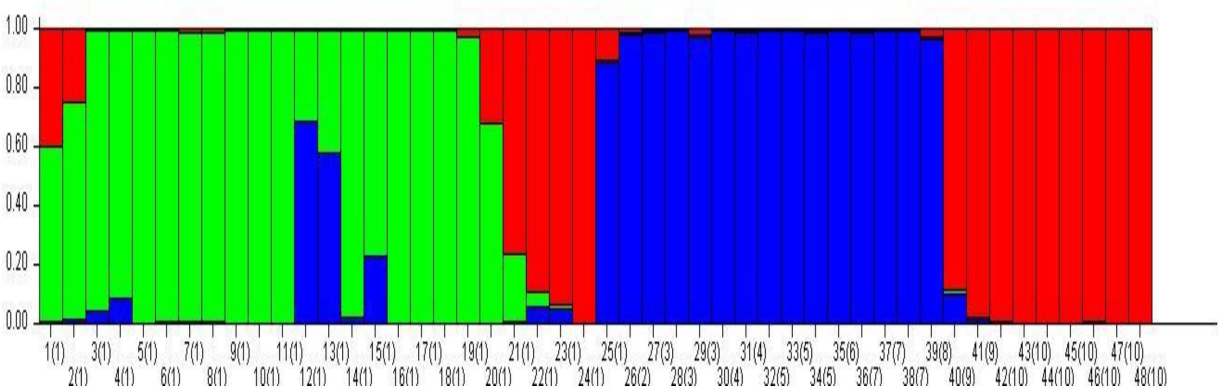


Fig 4.13 Model based population structure of Indian mustard genotypes with K = 3, using 28 Primers

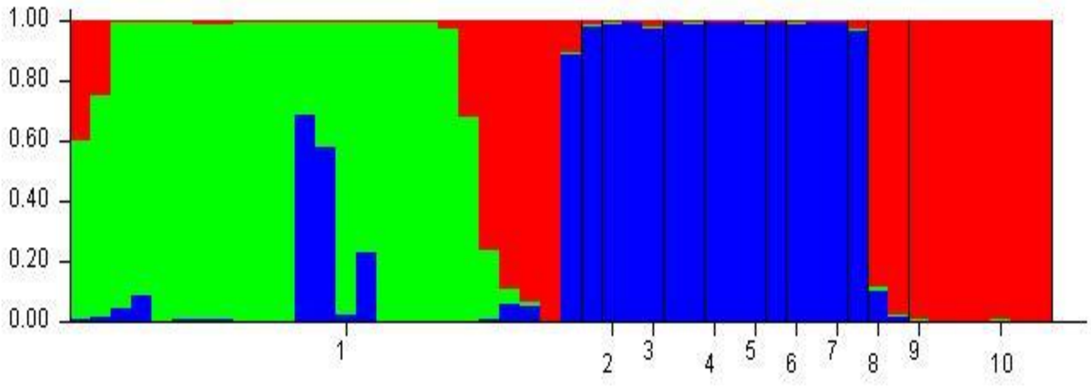


Fig 4.14: Single line analysis based population structure plot of Indian mustard genotypes with K=3, using 28 Primers

4.5.4 Population structure

The population structure of the 48 Indian mustard genotypes was estimated using STRUCTURE v2.3.3 software based on SSR and CAPS markers. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K = 3. The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values, with into three groups (fig 4.13 and 4.14) using a membership probability threshold of 0.80. First population represented by green colour contained 10 genotypes. Second population (red) represented 8 genotypes and third (blue)11genotypes. Admixture genome was represented by subpopulation of 5 genotypes green and red, 5 other genotypes represented green and blue, 3 genotypes represented all three populations *i.e*., green, red and blue and 4 genotypes red and blue subpopulations.

4.6 To study the effectual combinations and concentrations of plant growth regulators for establishment of callus and cell suspension cultures followed by plantlet regeneration and to select low erucic acid containing cell lines and *in vitro* regeneration

(A) Establishment and maintenance of callus and cell suspension cultures followed by plantlet regeneration

(1) Callus culture

(a) Selection of genotypes and explants types

Varied response of seven genotypes (CS-54, GM-2, RB50, NRCDR-2, Rohini, PM30 and LES-39) based on their plant growth; higher yield and low and high erucic acid content were utilized for initiation and establishment of *in vitro* cultures. The first need of *in vitro* studies is to select appropriate explant(s) which could be used effectively for initiation and establishment of callus and celll suspension cultures followed by plantlet regeneration, therefore for selecting suitable genotypes and explant(s), different explants type's *viz.,* cotyledon, hypocotyl and seed were collected from seven genotypes and cultured on MS medium supplemented with different combination and concentration of plant growth regulators. Cotyledon, hypocotyls and seed showed callus initiation, shoot regeneration, multiplication and rooting. Further various responsive explants were used for the initiation and establishment of callus cultures. Data recorded for number of explants producing callus from various culture media combinations, number of explants producing shoots, number of explants and shoots producing roots.

(b) To study the effectual concentration and combinations of plant growth regulators

(i) Callus initiation

(1) Hypocotyls explants

Hypocotyl explants were used for callus induction, but callus of very small size was evidenced.

(2) Mature Cotyledon explants

For initiation of callus cultures, mature cotyledons were cultured on MS media supplemented with different concentrations of 2, 4-D (1.0, 2.0, 3.0 and 4.0 mgl^{-1}). Callus initiation usually started from the cut ends of cotyledon sections. Very high response of callus induction was observed from most of the genotypes inoculated on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D. Among the seven genotypes tested for callus initiation, genotype: GM-2 (83.33 %) was found most responsive in terms of callus induction (%) with white brown and friable callus followed by genotypes RB50 (77.58 %) with white cream in colour and friable and granular in texture, CS-54 (72.72%) Cream and light in colour and compact in texture, PM-30 (71.11%) with white colour and loose texture, Rohini (69.23%)

white cream in color and friable in texture and LES-39 (65.78%) white brown in colour and loose in texture. These calluses were transferred on to shooting media. However, no response was recorded.

(3) Seed explants

For initiation of callus cultures from seed explants, mustard genotypes were cultured on MS media supplemented with different concentrations of 2, 4-D (1.0, 2.0, 3.0 and 4.0 mgl^{-1}). Higher callus induction was observed on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D. Genotype NRCDR-2 (84.6%) was found the most response for callus induction among seven *Brassica* genotypes with white cream and light compact callus followed by genotypes GM-2 (83.3%) and cream and friable in texture, CS-54 (81.8%) and dark brown and friable in texture, RB50 (81.3%) and white cream and friable in texture, Rohini (80.0%) and brown, friable and granular in texture, LES-39 (71.4%) and brown and friable texture. However genotype PM-30 (50.0%) had the lowest count with cream and light compact in texture.

For initiation of callus cultures from seed explants, different genotypes were also cultured on MS media supplemented with 0.5 mgl^{-1} BAP. Among all genotypes, genotype RB50 was found the most responsive for callus induction (88.8%) with white and light compact colour and texture followed by genotypes: PM-30 (87.5%) with cream, light and compact, Rohini (85.7%) light green in colour and compact in texture, GM-2 (83.3%) ith white and compact, NRCDR-2 (75.0%) white and compact, LES-39 (71.4%) cream light and compact. While genotype CS-54(66.6%) was found to be the lowest performer in this regard with cream in colour and compact in texture.

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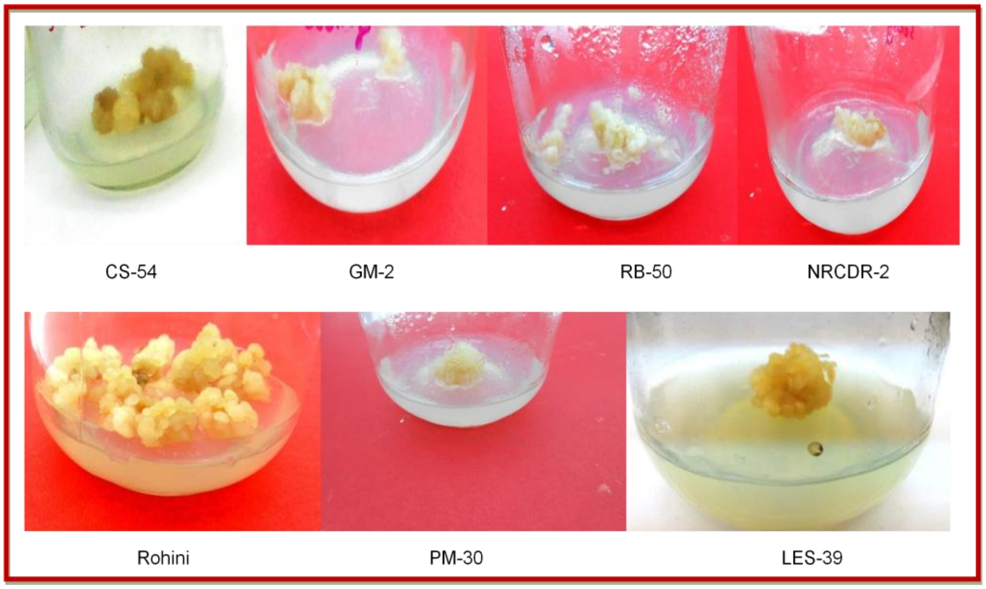
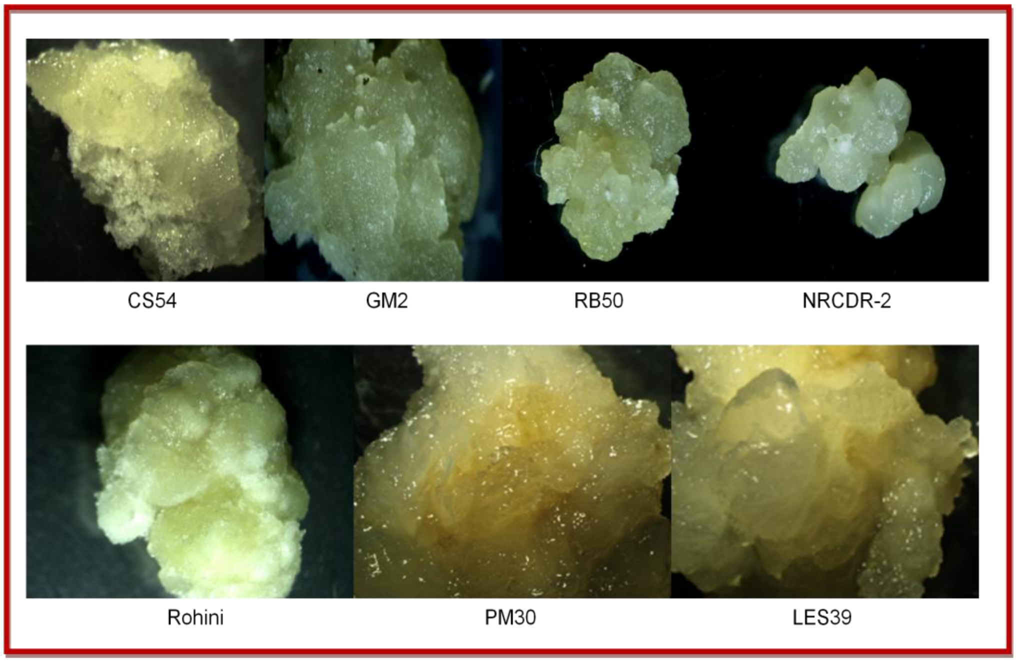
Plate 4.20 Callus induction from cultured mature cotyledons on MS3D medium of different Indian mustard genotypes



CS-54 GM-2 RB-50 NRCDR-2

Rohini PM-30 LES-39

Plate 4.21: Callus induction from cultured seeds on MS3D medium of different Indian mustard genotypes



CS54 GM2 RB50 NRCDR-2

Rohini PM30 LES39

4.22: Callus induction from cultured seeds on MS0.5B medium of different Indian mustard genotypes

Table 4.34: Effect of genotypes on Callus induction from cultured mature cotyledons on MS3D medium (MS+ 3.0mgl^{-1},2,4-D) in Indian mustard

S.No.	Genotypes	Callus induction (%)	Callus colour and texture
1.	CS-54	72.72	Cream and light compact
2.	GM-2	83.33	White brown and friable
3.	RB50	77.58	White cream, friable and granular
4.	NRCDR-2	51.11	White and friable
5.	Rohini	69.23	White cream and friable
6.	PM-30	71.11	White and loose
7.	LES-39	65.78	White brown and loose
C.D.(0.05)		**0.070**	
SE(m)		**0.020**	
SE(d)		**0.028**	
C.V.		**0.040**	

Table 4.35: Effect of genotypes on Callus induction from cultured seed on MS3D medium (MS+ 3.0mgl^{-1},2,4-D) in Indian mustard

.No.	Genotypes	Callus induction (%)	Callus color and texture
1.	CS-54	81.8	Dark brown and friable
2.	GM-2	83.3	Cream and friable
3.	RB50	81.3	White cream and friable
4.	NRCDR-2	84.6	White cream and light compact
5.	Rohini	80.0	Brown, friable and granular
6.	PM-30	50.0	Cream and light compact
7.	LES-39	71.4	Brown and friable
C.D.(0.05)		**0.255**	
SE(m)		**0.072**	
SE(d)		**0.102**	
C.V.		**0.134**	

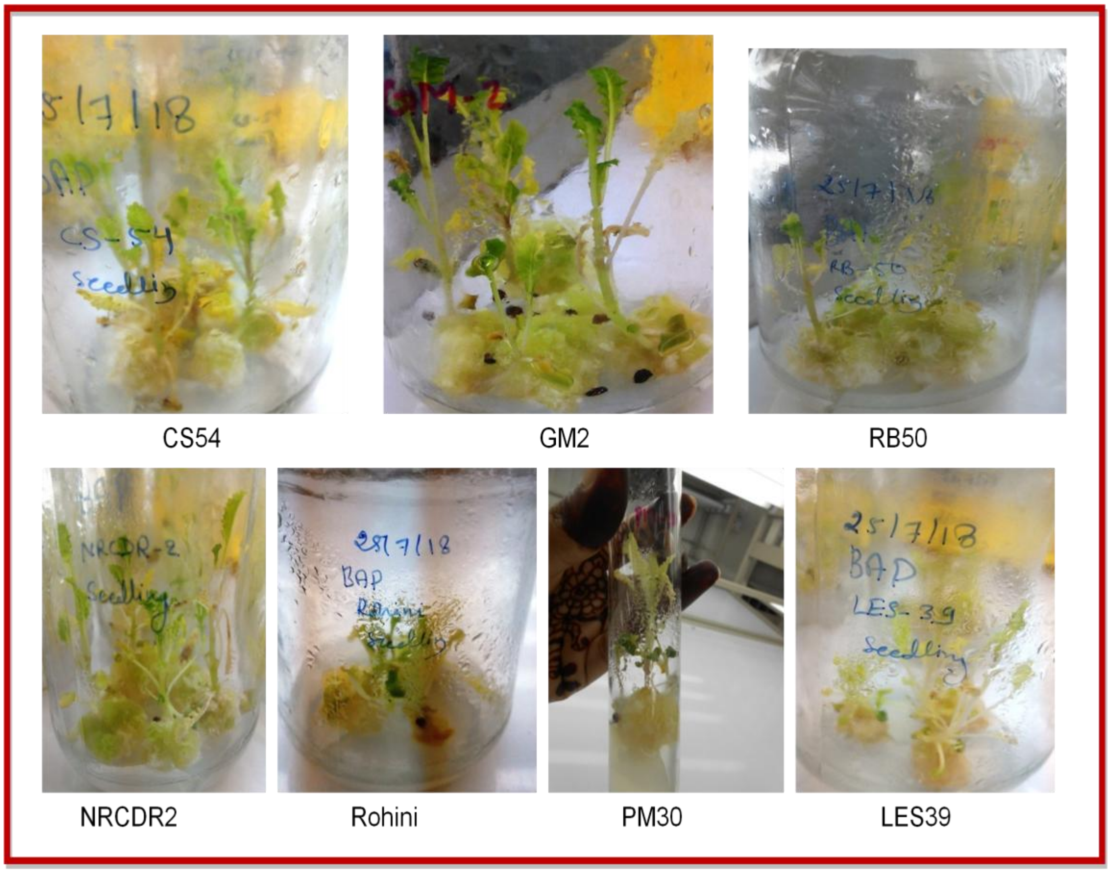
Table 4.36: Effect of genotypes on callus induction from cultured seed on MS.5B medium (MS+ 0.5mgl^{-1}BAP) in Indian mustard

S.No.	Genotypes	Callus induction (%)	Callus color and texture
1.	CS-54	66.6	Cream and compact
2.	GM-2	83.3	White and compact
3.	RB50	88.8	White and light compact
4.	NRCDR-2	75.0	White and compact
5.	Rohini	85.7	Light green and compact
6.	PM-30	87.5	Cream and light compact
7.	LES-39	71.4	Cream and light compact
C.D.(0.05)		**0.198**	
SE(m)		**0.056**	
SE(d)		**0.080**	
C.V.		**0.100**	

(ii) Shoot regeneration

Shoot regeneration were not attained from cultured hypocotyls and cotyledon explants, only seed explants were formed shootlets. Plant growth regulator BAP was found the best for induction of morphogenic callus flowed by shoots formation followed by culture medium amended with 2, 4-D in combination with BAP and BAP in combination with NAA. The best result for morphogenic callus induction was obtained on MS medium supplemented with 0.5 mgl^{-1} BAP. Genotype CS-54 (87.4%) formed morphogenic calli in highest numbers among seven genotypes followed by genotypes GM-2 (85.6%), RB50 (83.2%), Rohini (80.2%), PM-30 (75.0%) and NRCDR-2 (66.5%). While genotype LES-39 (60.0%) had the minimum count The best result for shoot induction was also obtained on MS medium fortified with 0.5 mgl^{-1} BAP. Among tested genotypes NRCDR-2 (87.7%) produced maximum shoot lets followed by genotypes RB50 (80.0%), LES-39 (76.6%), GM-2 (75.5%), Rohini (74.2%), CS-54 (74.0%) and PM-30 (70.0%).

Genotype PM-30 (9) was found the most response in terms of average no. of shoots/explants following by genotypes NRCDR-2 (8), CS-54 (8), GM-2 (6), LES-39 (6), RB-50 (5) and Rohini (5). Shoot proliferation/shootlet had maximum in genotype PM-30 (7) followed by genotypes CS-54 (6), LES-39 (5), NRCDR-2 (3), GM-2 (2), RB50 (2) and Rohini (1). Mean shoot length was recorded highest in genotype PM-30 (7.5) followed by genotypes LES-39 (7.2), CS-54 (6.5), RB50 (6.5), GM-2 (6.0), NRCDR-2 (5.5) and Rohini (3.3).



CS54 GM2 RB50

NRCDR2 Rohini PM30 LES39

Plate 4.23: Morphogenic calli and plantlet regeneratio from cultured seeds on MS0.5B medium of different Indian mustard genotypes

Table 4.37: Effect of genotypes on formation of morphogenic calli and plantlet regeneration from cultured seed on MS.5B medium in Indian mustard

S.No.	Genotypes	Callus features	Morphogenic calli (%)	Shoot regeneration (%)
1	CS-54	Cream and compact	87.4	74.0
2	GM-2	White and compact	85.6	75.5
3	RB50	White and light compact	83.2	80.0
4	NRCDR-2	White and compact	66.5	87.7
5	Rohini	Light green and compact	80.2	74.2
6	PM-30	Cream and light compact	75.0	70.0
7	LES-39	Cream and light compact	60.0	76.6
C.D.(0.05)			**0.641**	**0.212**
SE(m)			**0.182**	**0.062**
SE(d)			**0.257**	**0.088**
C.V.			**0.334**	**0.115**

Table 4.38: Effect of genotypes on shoot proliferation and mean shoot length from cultured seed on MS.5B medium in Indian mustard

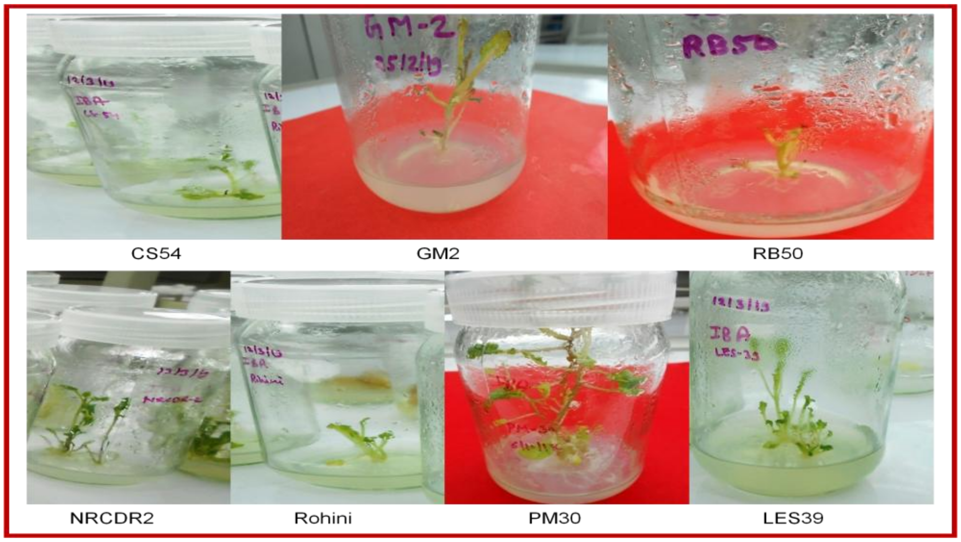
S.No.	Genotypes	Average no. of shoots/explants	Shootproliferation/shootlet	Mean shoot length
1	CS-54	8	6	6.5
2	GM-2	6	2	6.0
3	RB50	5	2	6.5
4	NRCDR-2	8	3	5.5
5	Rohini	4	1	3.3
6	PM-30	9	7	7.5
7	LES-39	6	5	7.2
C.D.(0.05)		**0.203**	**0.203**	**0.203**
SE(m)		**0.060**	**0.060**	**0.060**
SE(d)		**0.085**	**0.085**	**0.085**
C.V.		**1.262**	**2.191**	**1.409**

Observations were recorded after transferring on shooting media after 30-35 days.

(iii) *In vitro* rooting

The best result for root formation was recorded on MS medium supplemented with 0.5 mg1^{-1} IBA. Among genotypes, genotype CS-54 and GM-2 (75%) were formed maximum roots followed by gentypesRB50, NRCDR-2, Rohini, PM-30 and LES-39 (66.6 %)

Average no. of roots/explants was attained in genotypes CS-54 (7) followed by genotypes LES-39 (6), GM-2 (5), PM-30 (5), RB50 (4), NRCDR-2 (4) and Rohini (3). Root proliferation/shootlet was recorded highest in genotypes CS-54 (3) followed by GM-2 (3), LES-39 (3), RB50 (2), NRCDR-2 (2), Rohini (2) and PM-30 (2). Mean root length was noted maximum in genotypes RB50 (6.0) followd by PM30 (5.8), GM-2 (5.5), LES-39 (5.5), NRCDR-2 (4.7), CS-54 (4.5) and Rohini (3.8).



CS54 GM2 RB50

NRCDR2 Rohini PM30 LES39

Plate 4.24: Root initiation from cultured seeds on MS0.5I medium of different Indian mustard genotypes

Table 4.39: *In vitro* rooting response of Indian mustard

S.N0.	Genotypes	Root induction (%)	Average no. of roots/explant	Root proliferation/shootlet	Mean root length
1.	CS-54	75%	7	3	4.5
2.	GM-2	75%	5	3	5.5
3.	RB50	66.6 %	4	2	6.0
4.	NRCDR-2	66.6 %	4	2	4.7
5.	Rohini	66.6 %	3	2	3.8
6.	PM-30	66.6 %	5	2	5.8
7.	LES-39	66.6 %	6	3	5.5
C.D.		**0.180**	**0.203**	**0.232**	**0.280**
SE(m)		**0.053**	**0.060**	**0.068**	**0.082**
SE(d)		**0.075**	**0.085**	**0.096**	**0.116**
C.V.		**0.091**	**1.695**	**3.758**	**2.287**

Note: observations were recorded after 15 days transferring in rooting media

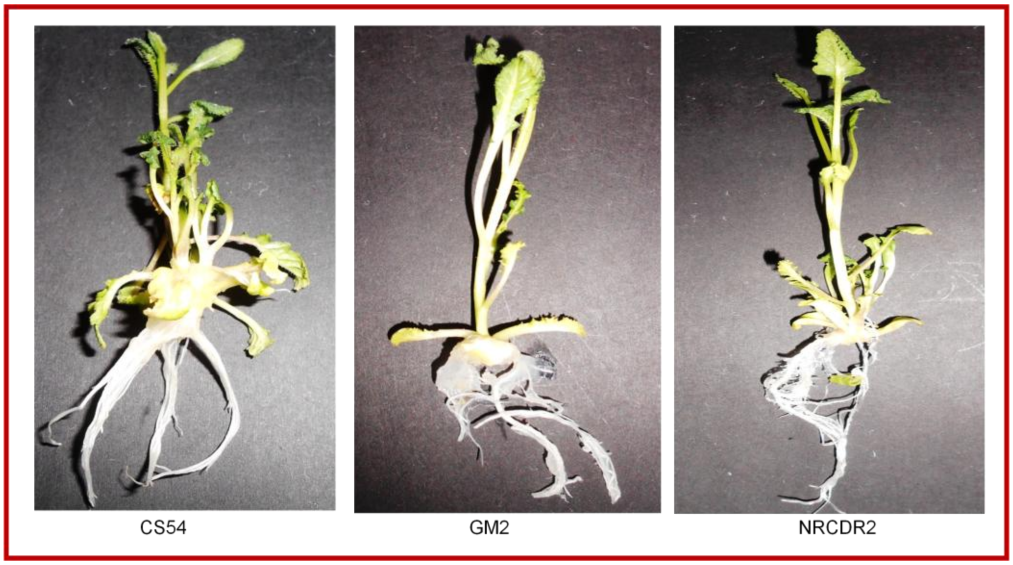
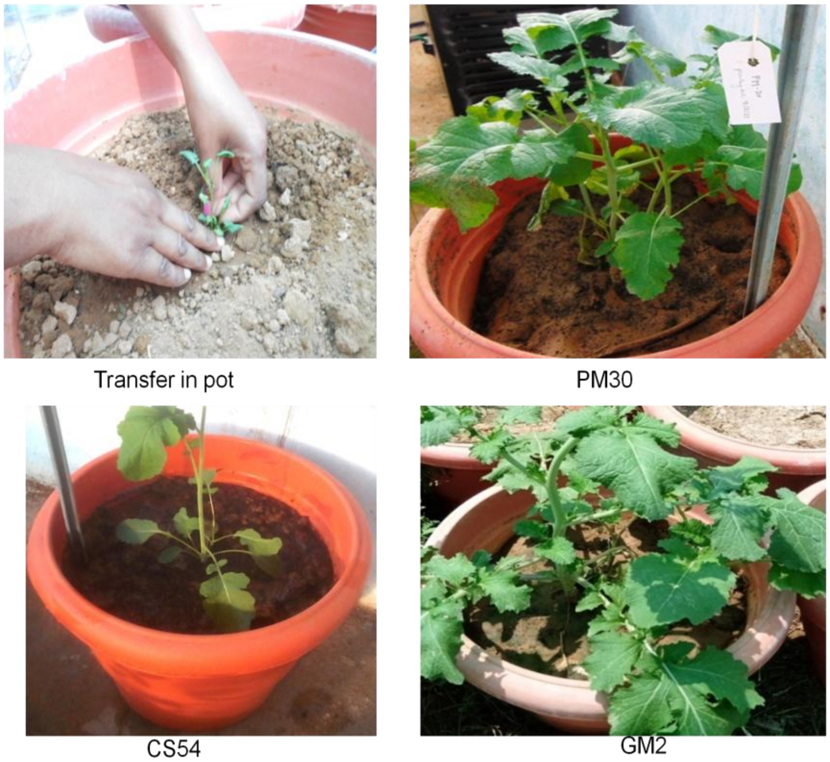


Plate 4.25: Shoot and Root views of some Indian mustard genotypes



Transfer in pot

PM30

CS54

GM2

Plate 4.26: Regenrants transferred in Green House for hardening of Indian mustard genotypes

CS54

GM2

PM30

Plate 4.27: Regenerants transferred in Net House for hardening of Indian mustard genotypes

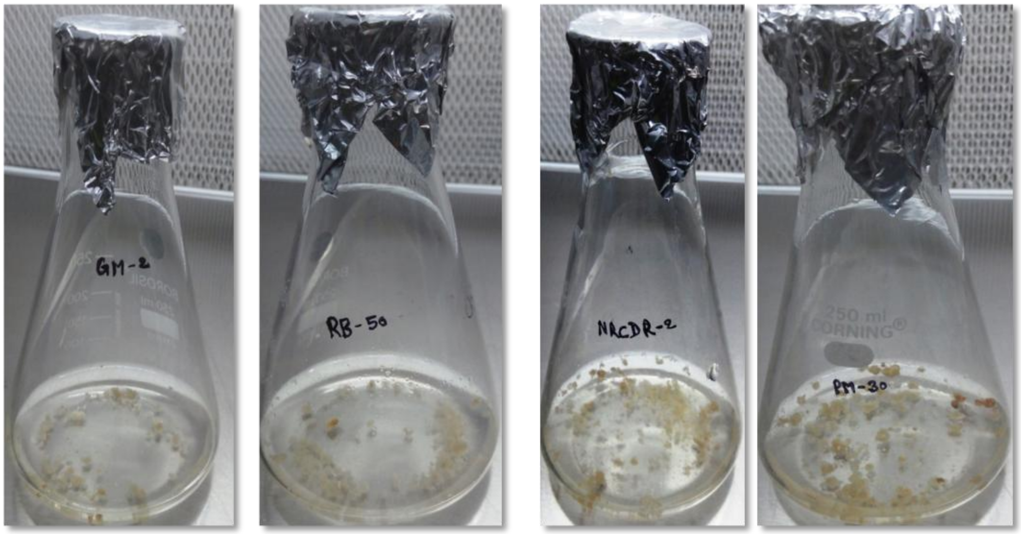

CS54 PM30

Plate 4.28: Regenerants transferred in field conditions of Indian mustard genotypes

(2) Cell suspension cultures

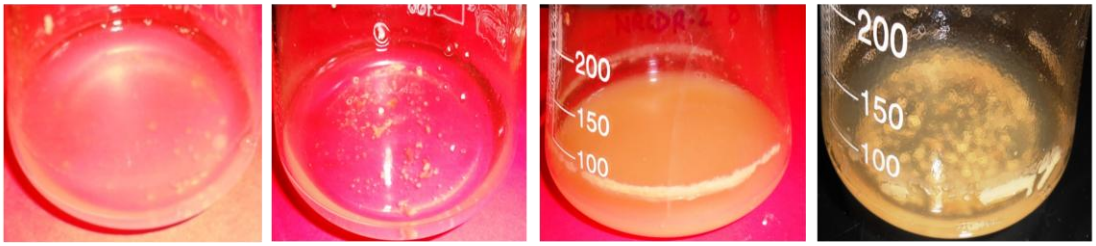
(a) Embryogenic cell suspension culture from cotyledon explants

The calli obtained from mature cotyledons were medium in size, friable in texture and white brown in colour. Friable callus was found suitable for initiating cell suspension cultures. Two gram friable callus was placed in fresh liquid media containing 50 ml of liquid medium. Suspension culture raised from embryogenic callus produced embryoids of different shapes and sizes. Genotype RB50 followed two stages of somatic embryogenesis *i.e*. globular and heart shaped. These stages were not showed by remaining genotypes. Other than in genotype CS54, cell aggregates and single cells observed. After 45 days increment in fresh weight was observed. Maximum relative growth rate was attained in genotype GM-2 (1860%), followed by genotypes RB50 (1830%), Rohini (1660%), NRCHB101 (1450%), NRCDR-2 (1250%), CS-54 (1180%) and PM-30 (975%).



GM2 RB50 NRCDR2 PM30

Plate 4.29: Initiation of cell clumps formation from cotyledonary explants on MS medium supplemented with 3 mg/l 2, 4-D in various genotypes of Indian mustard



GM2 RB50 NRCDR2 PM30

Plate 4.30: Growth stages of embryogenic cell suspension cultures after 5 weeks of initiation from cotyledonary explants on MS medium supplemented with 3 mg/l 2, 4-D in various genotypes of Indian mustard

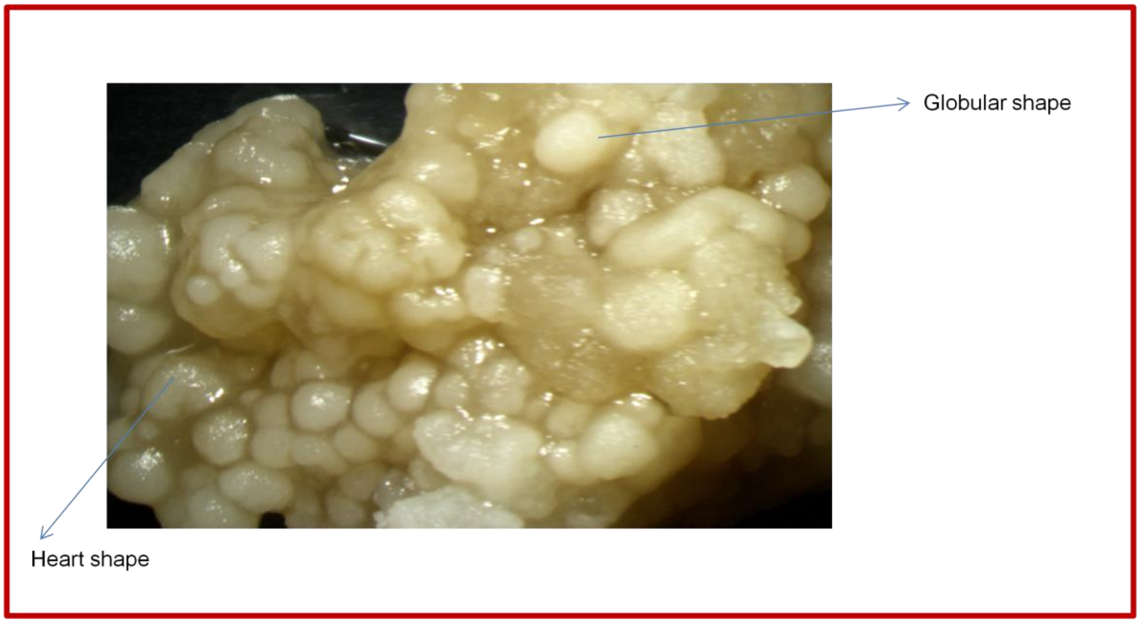


Plate 4.31: Globular and heart shape stages of embryogenesis after 35 days of cell suspension initiation on MS medium supplemented with 3 mg/l 2, 4-D using cotyledon explants in RB50 genotype of Indian mustard

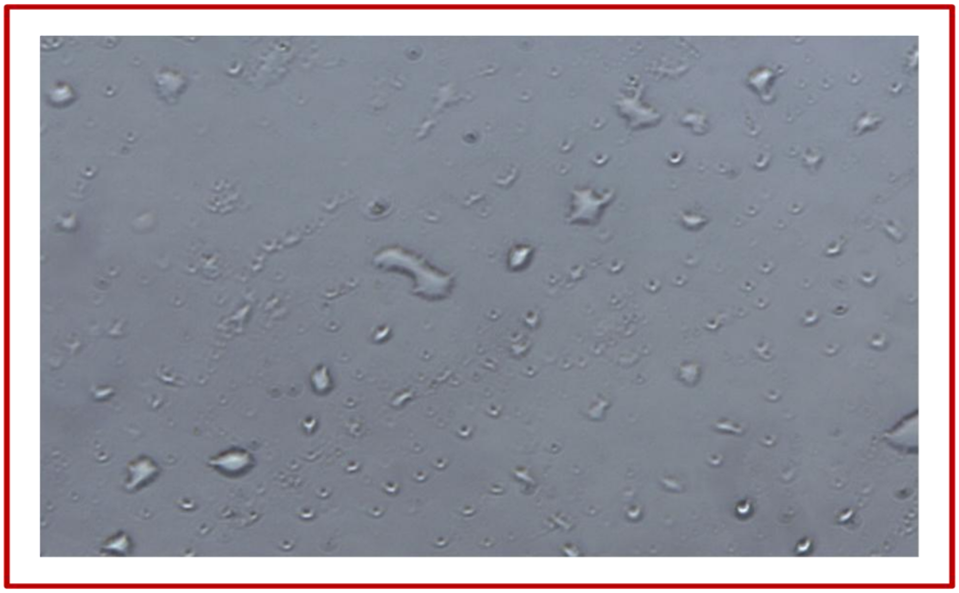


Plate 4.32: Cells aggregates and single cells after 35 days of cell suspension initiation on MS medium supplemented with 3 mg/l2, 4-D using cotyledon explants in CS-54 of Indian mustard

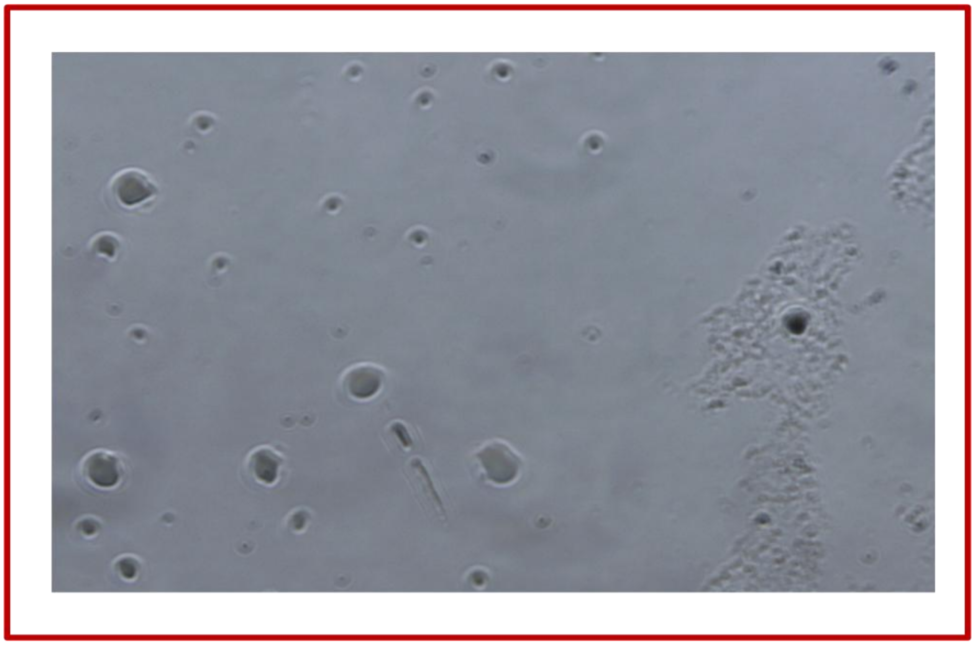


Plate 4.33: Single cells after 35 days of cell suspension initiation on MS medium supplemented with 3 mg/l2, 4-D cell suspension culture using cotyledon explants in CS-54 of Indian mustard

Table 4.40: Effect of genotypes on initiation of embryogenic cell suspension cultures from callus raised from cotyledon explants on MS3D liquid medium in Indian mustard

S.No.	Genotypes	Embryogenic cell suspension culture	
		Increment in fresh weight (g)	Relative growth rate (%)
1	CS-54	11.8	1180
2	GM-2	18.6	1860
3	RB50	18.3	1830
4	NRCDR-2	12.5	1250
5	Rohini	16.6	1660
6	NRCHB101	14.5	1450
7	PM30	9.75	975

- Evaluation was made after 45 days in culture.
- Mean of two observations in increment of fresh weight.
- Initial fresh weight was taken 2.0 g friable callus per flask containing 50ml liquid media

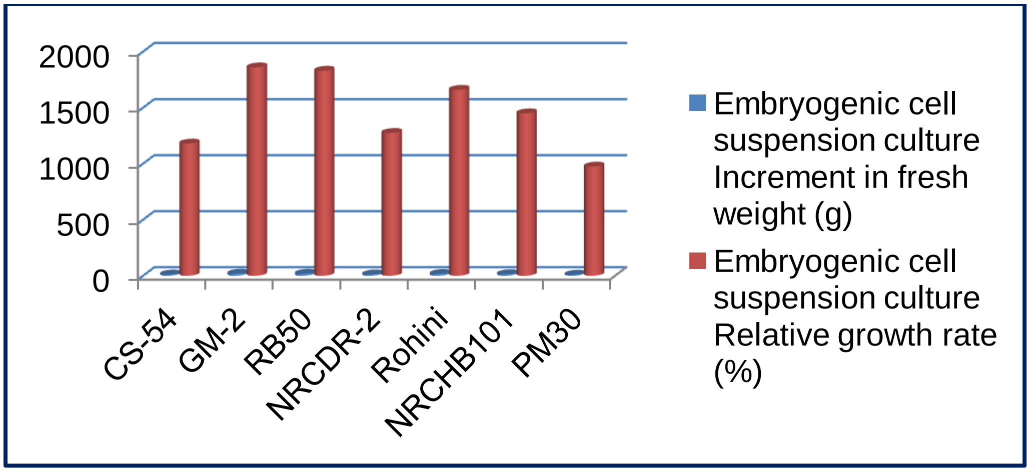


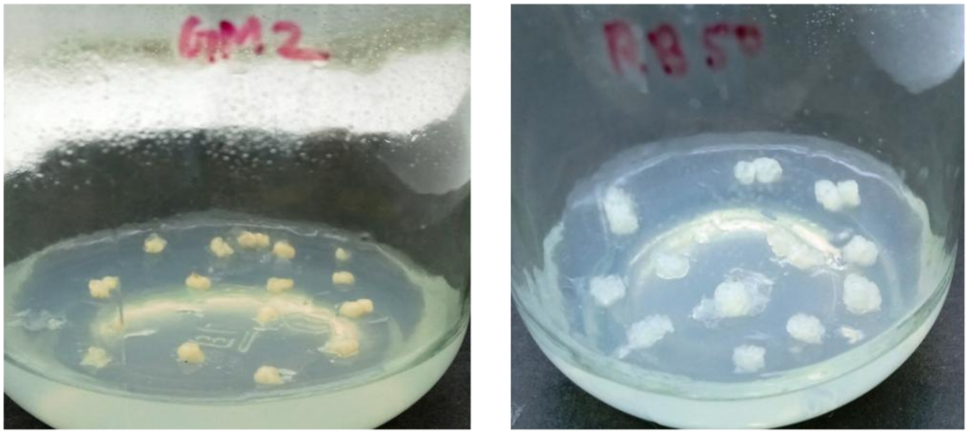
Fig 4.15: Graphical representation MS3D medium on growth of embryogenic cell suspension culture derived from mature cotyledon explants in Indian Mustard (*Brassica juncea*)

Table 4.41: Plant regeneration efficiency from cultured cell clumps and embryoids obtained from cell suspension cultures in Indian mustard

Genotypes	**Shoot regeneration (%)**	
Culture media	**MS.5D.5B**	**MS.5N.5B**
CS-54	30.0	37.5
GM-2	50.0	33.3
RB50	28.5	42.8
NRCDR-2	20.0	25.0
Rohini	16.6	44.4
PM-30	33.3	66.6
NRCHB101	25.0	14.2
C.D.	**0.193**	**0.328**
SE(m)	**0.057**	**0.096**
SE(d)	**0.080**	**0.136**
C.V.	**0.276**	**0.362**

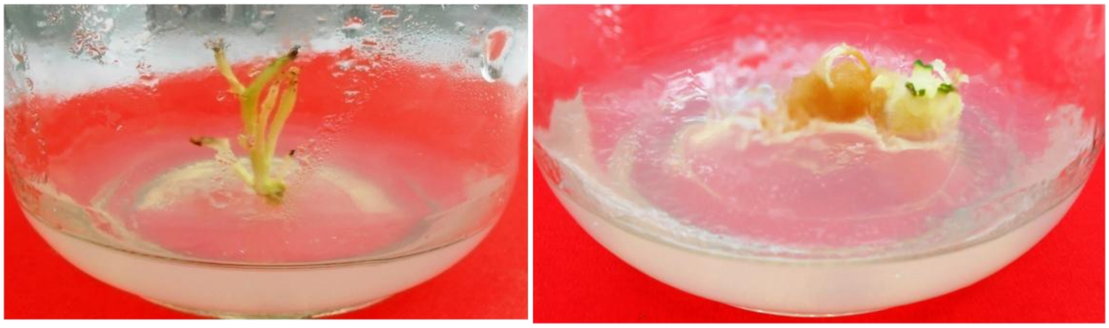
(b) Shoot regeneration from cell clumps/embryoids

After 4-6 weeks, cell clumps/ embryoids were placed on different concentrations and combinations of auxins and cytokinins for regeneration. Maximum plantlet regeneration was achieved on the regeneration media MS.5N.5B from the cell clumps/embryoids of genotype PM-30 (66.6%) followed by genotypes Rohini (44.4%), RB-50 (42.8%), CS-54 (37.5%), GM-2 (33.3%) and NRCDR-2 (25.0%). However the lowest plantlet regeneration was attained with genotype NRCHB101 (14.2%). On regeneration medium MS.5D..5B, maximum plantlet regeneration was recorded in genotype GM-2 (50.0%), followed by genotypes PM-30 (33.3%), CS-54 (30.0%), RB-50 (28.5%), NRCHB101 (25.0%) and NRCDR-2 (20.0%) while minimum count was for Rohini (16.6%).



GM2 RB50

Plate 4.34: Embryoids/cell clumps transferred on regeneration medium on MS medium supplemented with 0.5 mg/l2, 4-D + 5.0 mg/lBAP , 1 mg/lBAP + 0.5 mg/lNAA using cotyledon explants in genotypes of Indian mustard



GM2 RB50

Plate 4.35: Regeneration from embryogenic suspension derived cells on MS medium supplemented with 0.5 mg/l2, 4-D + 5.0 mg/lBAP , 1 mg/lBAP + 0.5 mg/lNAA using cotyledon explants in genotypes of Indian mustard

(**B**) ***In vitro*** **selection for low euricic acid**

The potential and limitations of the technique of tissue culture for production of new genotype(s) with desired characteristics for agricultural use having different status of yield parameters has been reported in considerable number of plant species including *Brassica species.* The procedures and conditions optimized during present studies for various genotypes. The optimized procedure worked well and may be utilized in future for *in vitro* selection and regeneration of somaclones in two genotypes CS-54 and PM30. Callus lines differentiated shoot organogenesis which rooted well to get soma clones. These genotypes were selected on the basis of erucic acid content. With an assumption that cultured cells represent metabolically at least in part, the cells of the whole plant, metabolic pathway for pathway to particular metabolite (*i.e.* erucic acid) production also operate in cells and cell lines selected on the basis of particular metabolite and yield may operate subsequent derived regenerates. As such the experimentation was carried out to select the cell lines and analyze them for erucic acid content and select out low and high euricic acid containing regenerants.

(a) Callus lines

Two somaclones *namely* : CS-54 and PM30 were found at maturity.

(b) Regeneration of somaclones

Well growing callus cell lines of CS-54 and PM30 were subjected to MS medium for shoot organogenesis. Well developed plantlets from these two lines (CS-54 and PM30) were transferred under green house conditions. Morphological observations were recorded and tabulated in Table 4.42 after maturity samples were collected and subjected for fatty acid analysis through Gas Chromatography Mass Spectroscopy (GCMS) and data were recorded.

(i) Morphological observations of somaclones

Two somaclones were compared on the basis of morphological parameters which included plant height, nos of primary branches, nos of secondary branches 50 % flowering, days to maturity, siliqua length, number of seed per siliqua, number of siliqua per plant, biological yield per plant, seeds per plant and seed yield per plant. Soma clone of CS-54 was showed plant height (115cm), primary branches (4), secondary branches (8), 50% flowering in 70 days, days to maturity after transferring in field condition (100 days), siliqua length (5 cm), number of seed per siliqua (8), number of siliqua per plant (130), biological yield per plant (13 g), seeds per plant (1040) and seed yield per plant (1.90g), In another somaclone obtained from PM30 line showed plant height (90cm), primary branches (2), secondary branches (2), 50% flowering in 68 days, days to maturity after transferring in field conditions (115 days), siliqua length (4.3cm), number of seeds per siliqua (7), number of siliqua per plant (140), biological yield per plant (20.86 g), seeds per plant (840) and seed yield per plant (1.6 g).

Table 4.42: Morphological observations for somaclones CS-54 and PM30 regenerated from callus cultures in Indian Mustard (*Brassica juncea*)

Characters/Somaclones	**CS54**	**PM30**
Days to 50 % flowering	70	68
Days to maturity	100	115
Plant height	115	90
Primary branches	4	2
Secondary branches	8	2
Siliqua length	5	4.3
Number of seed per siliqua	8	7
Number of siliqua per plant	130	140
Biological yield per plant (g)	13	20.86
Seeds per plant	1040	840
Seed yield per plant (g)	1.9	1.6

- Transferred under green house conditions (temperature 28^{0}C, humidity 70% and photoperiod regimes16 hour light and 8 hour dark) of both somaclones in mid December 2018 for 15 days.
- Transferred under field conditions of both somaclones in January 2019.
- Morphological observation were recorded after 60 days of CS-54.
- Morphological observation were recorded after 75 days of PM30.

(ii) Fatty acid profiling of somaclones

The results for fatty acids composition showed significant difference among 188 genotype lines of *Brassica juncea.* The most principle fatty acids detected were the unsaturated fatty acids of palmitic acid, oleic acid, linoleic acid, linolenic acid and erucic acid. These were the five major fatty acids composed of the seed oil. Erucic acid is one of the important fatty acids in *Brassica species*. This 22 carbon fatty acid is harmful to the human health. In the present study, genotype lines CS54 and PM30 showed high and lower erucic acid contents. Genotypes without this fatty acid or lower than 2 % are nutritionally suitable for human health. Attempts to select out zero erucic acid have been developed and reported in *Brassica juncea*.

In present study, fatty acid analysis of various genotypes showed varied erucic acid content from very high erucic to low content. High erucic acid containing lines are industrially useful and important raw material whereas low erucic acid containing oil is important for human health point of view. In the present study *in vitro* selection approach and regeneration of lines from callus carried out for developing low erucic acid content line(s). The regenerated variants with fatty acid composition having low erucic acid could be regenerated.

Seeds of the two somaclones (CS54 and PM30) collected after maturity were dried to 5 % moisture content and grounded samples were extracted as described in Chapter 3- Materials and Methods. The data were recorded for fatty acid profiling of somaclones (CS54 and PM30) derived *via* callus lines of genotypes are illustrated in Table 4.44, 4.45 and Fig 4.16. Fatty acid profiling and content of somaclone (CS54) showed variation for saturated and unsaturated concentration of fatty acids. Total 39 fatty acid components were detected. The % value ranged between 2.88 to 10.90. The highest content (10.90) was recorded for Ethy 9,12,15-octadecatrienoate,9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and n-Propy1 9,12,15-octadecatrienoate at RT 10.4 on given conditions. Lowest (2.88 %) was recorded for Ethyl 9, 12, 15-octadecatrienoate, 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and n-Propyl 9,12,15-octadecatrienoate at 11.4 RT.

Fatty acid profile analysis of PM 30 somaclone revealed different fatty acid contents analyzed with variations. Total 27 fatty acid compounds were detected. The highest percentage (25.36 %) was recorded for Glycidyl oleate, 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester and 9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E) eluated at 21.39 RT. The lowest percentage(1.62%) was recorded for 1-Heptacosanol, Hexacosyl pentafluoropropionate, Hexacosyl heptafluorobutyrate, 17-Pentatriacontene, Oleic acid, 3-(octadecyloxy)propyl ester and Octadecane, 3-ethyl-5-(2-ethylbutyl) expressed at 11.99 RT. On comparing erucic acid content and profiling of mother plant and somaclones regenerated from CS54 and PM30 lines. It was interesting to record that plants regenerated through *in vitro* selection. Erucic acid content recorded was 5.48 % as compared to erucic acid of mother plants of genotype line CS54 (Table 4.43).

Table 4.43: Fatty acid compositions of CS-54 of Indian mustard (*Brassica juncea*)

S.No.	Compounds	Value (%)
1	Palmitic acid (%)	5.91
2	Oleic acid (%	12.89
3	Linoleic acid (%)	2.96
4	Linolenic acid (%)	9.41
5	Erucic acid (%)	41.36

Table 4.44 Comparison of erucic acid content (%) in intact plant and after callus culture in CS-54 of Indian mustard (*Brassica juncea*)

S.N.	Variety	Erucic acid content (%)	
		Intact plant	**After callus culture (somaclone)**
1.	CS-54	41.36	5.48

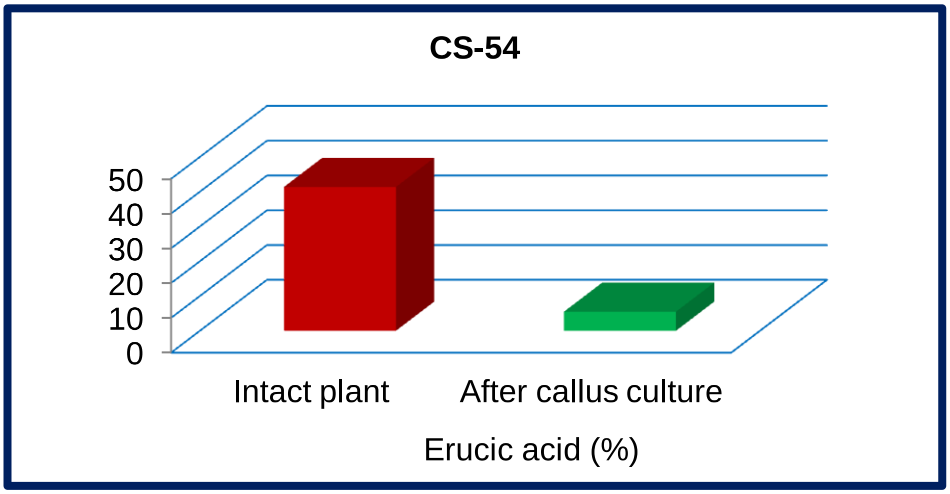


Fig 4.16: Graphical representation of comparison of erucic acid content (%) in intact plant and after callus culture of CS-54 variety in Indian Mustard (*Brassica juncea*)

Table 4.45: Fatty acid contents of somaclone obtained through selected callus line of CS-54 variety in Indian mustard (*Brassica juncea*)

S. No.	Fatty acid components	value (%)
1	1-Decanol, 2-octyl-	4.01%
2	Heptafluorobutyric acid, n-octadecyl ester	4.01%
3	1-Dodecanol, 2-hexyl	4.01%
4	Hexadecanoic acid, methyl ester	9.30%
5	Pentadecanoic acid, 13-methyl-, methyl ester	9.30%
6	Pentadecanoic acid, 14-methyl-, methyl ester	9.30%
7	2,5-Furandione, dihydro-3-isooctadecyl-	6.69%
8	L-Serinamide,1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-his	6.69%
	tidyl-N,1-L-tryptophyl-N,N,N2,O-tetramethyl-	
9	4,6-Dipropyl-nonan-5-one	6.69%
10	Ethyl iso-allocholate	6.69%
11	7,8-Epoxylanostan-11-ol, 3-acetoxy-	6.69%
12	Docosanoic acid, 1,2,3-propanetriyl ester	6.69%
13	Ethy 9,12,15-octadecatrienoate	10.90%
14	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	10.90%
15	n-Propy1 9,12,15-octadecatrienoate	10.90%
16	Ethyl 9,12,15-octadecatrienoate	2.88%
17	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	2.88%
18	n-Propyl 9,12,15-octadecatrienoate	2.88%
19	Methyl 11-docosenoate	5.48%
20	13-Docosenoic acid, methyl ester, (Z)-/Erucic acid	5.48%
21	Cis-13-Docosenoyl chloride	5.48%
22	Ethyl iso-allocholate	9.49%
23	(5á)Pregnane-3,20á-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	9.49%
24	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5	9.49%
	,6-b]oxiren-4-one,	
	8,8a-bis(acetyloxy)-2a-[(acetyloxy)methyl]-1,	
	1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-6b	
	-hydroxy-3a-methoxy-1,1,5,7-tetramethyl-,	
	[1aR-(1aà,1bá,1cà,2aà,3aà,6aà,6bà,7à,8á,8aà)]-	
25	17-(1,5-Dimethylhexyl)-2,3-dihydroxy-10,13	9.49%
	-dimethyl-1,2,3,7,8,9,10,11,12,13,14,15,16,1	
	7-tetradecahydrocyclopenta[a]phenanthren-6- one	
26	Propanoic acid,	9.49%

	2-(3-acetoxy-4,4,14-trimethylandrost-8-en-1	
	7-yl)-	
27	2,4a-Oxymethano-1,2,3,4,4a,4b,5,6,7,8,8a,9-d	9.49%
	odecahydrophenanthren-9-one,	
	8-cyanomethyl-2-methoxy-7-methoxycarbon	
	yl-1,1,7-trimethyl-	
28	Glycidyl oleate	6.05%
29	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-	6.05%
30	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	6.05%
31	9,12,15-Octadecatrienoic acid,2-phenyl-1,3-dioxan-5-yl ester	3.8%
32	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	3.68%
33	Butyl 6,9,12,15-octadecatetraenoate	3.68%
34	Glycidyl oleate	3.17%
35	2,3-Dihydroxypropyl cis-13-docosenoate	3.17%
36	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-	3.17%
37	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	4.77%
38	Trilinolein	4.77%
39	Tricyclo[20.8.0.0(7,16)]triacontane,1(22),7(16)-diepoxy-	4.77%

Table 4.46: Fatty acids characterized using GC-MS in somaclone regenerated from callus of genotype CS-54 in Indian mustard (*Brassica juncea*)

S.No.	Compound characterized	Apex RT	Start RT	End RT	Area	%Area	Height	%Height	Probability
	1-Decanol, 2-octyl-	5.24	5.16	5.35	3E+07	4.01	7E+06	7.11	3.54
	Heptafluorobutyric acid, n-octadecyl ester								3.4
	1-Dodecanol, 2-hexyl								3.27
	Hexadecanoic acid, methyl ester	7.4	7.23	7.68	6E+07	9.3	1E+07	10.72	68.41
	Pentadecanoic acid, 13-methyl-, methyl ester								12.82
	Pentadecanoic acid, 14-methyl-, methyl ester								2.77
	2,5-Furandione, dihydro-3-isooctadecyl-	9.66	9.59	10.04	4E+07	6.69	5E+06	4.55	7.43
	L-Serinamide,1-methyl-5-oxo-L-prolyl-N,1-dimethyl-L-his								5.1
	tidyl-N,1-L-tryptophyl-N,N,N2,O-tetramethyl-								
	4,6-Dipropyl-nonan-5-one								3.39
	Ethyl iso-allocholate								26.54
	7,8-Epoxylanostan-11-ol, 3-acetoxy-								17.67
	Docosanoic acid, 1,2,3-propanetriyl ester								4.81
	Ethy 9,12,15-octadecatrienoate	10.4	10.23	10.68	7E+07	10.9	1E+07	11.31	10.24
	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-								8.65
	n-Propy1 9,12,15-octadecatrienoate								6.8
	Ethyl 9,12,15-octadecatrienoate	11.4	11.27	11.65	2E+07	2.88	2E+06	1.83	10.24
	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-								8.65
	n-Propyl 9,12,15-octadecatrienoate								6.8
	Methyl 11-docosenoate	15.33	15.17	15.65	3E+07	5.48	5E+06	5.1	49.9
	13-Docosenoic acid, methyl ester, (Z)-								13.33
	Cis-13-Docosenoyl chloride								12.29

	Ethyl iso-allocholate	17.05	16.93	17.39	6E+07	9.49	8E+06	7.64	11.95
	(5á)Pregnane-3,20á-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate								7.48
	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5								5.13
	,6-b]oxiren-4-one,								
	8,8a-bis(acetyloxy)-2a-[(acetyloxy)methyl]-1,								
	1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-6b								
	-hydroxy-3a-methoxy-1,1,5,7-tetramethyl-,								
	[1aR-(1aà,1bá,1cà,2aà,3aà,6aà,6bà,7à,8á,8aà)]-								
	17-(1,5-Dimethylhexyl)-2,3-dihydroxy-10,13								21.34
	-dimethyl-1,2,3,7,8,9,10,11,12,13,14,15,16,1								
	7-tetradecahydrocyclopenta[a]phenanthren-6-								
	One								
	Propanoic acid,								14.64
	2-(3-acetoxy-4,4,14-trimethylandrost-8-en-1								
	7-yl)-								
	2,4a-Oxymethano-1,2,3,4,4a,4b,5,6,7,8,8a,9-d								14.07
	odecahydrophenanthren-9-one,								
	8-cyanomethyl-2-methoxy-7-methoxycarbon								
	yl-1,1,7-trimethyl-								
	Glycidyl oleate	21.09	20.97	21.33	4E+07	6.05	6E+06	5.6	71.7
	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-								4.87
	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester								4.68
	9,12,15-Octadecatrienoic acid,2-phenyl-1,3-dioxan-5-yl ester	23.73	23.62	23.9	2E+07	3.68	3E+06	3.09	13.64
	Methyl 2-hydroxy-octadeca-9,12,15-trienoate								3.94
	Butyl 6,9,12,15-octadecatetraenoate								3.78

0	Glycidyl oleate	7.61	7.56	7.75	2 E+07	3 .17	4 E+06	3 .91	34.58
	2,3-Dihydroxypropyl cis-13-docosenoate								6.97
	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-								3.8
1	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	2.19	2.06	2.37	3 E+07	4 .77	4 E+06	3 .92	7.41
	Trilinolein								4.78
	Tricyclo[20.8.0.0(7,16)]triacontane,1(22),7(16)-diepoxy-								4.23

Table 4.47: Fatty acid compositions of PM30 variety of Indian mustard (*Brassica juncea*)

S.No.	Compounds	Value (%)
1	Palmitic acid (%)	5.5
2	Oleic acid (%	25.6
3	Linoleic acid (%)	2.75
4	Linolenic acid (%)	15.55
5	Erucic acid (%)	1.075

Table 4.48 Comparison of erucic acid content (%) in intact plant and after callus culture in PM30 variety of Indian mustard (*Brassica juncea*)

S. No.	Variety	Erucic acid content (%)	
		Intact plant	**After callus culture (somaclone)**
1.	PM 30	1.075	Not detected

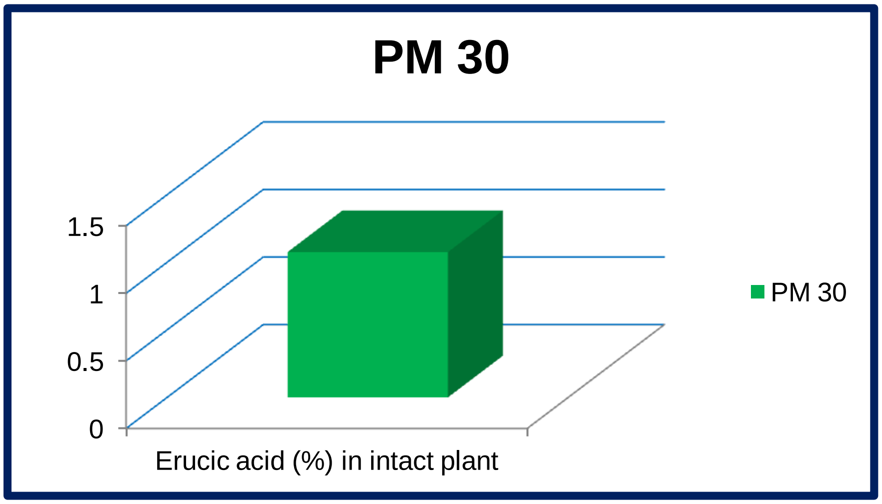


Fig 4.17: Graphical representation of erucic acid content (%) in intact plant of PM 30 variety in Indian Mustard (*Brassica juncea*)

Table 4.49: Fatty acid compositions detected from somaclone regenerated from callus of PM 30 genotype of Indian mustard (*Brassica juncea*)

S.No.	Fatty acids	Value (%)
1	1-Octadecene	2.71%
2	E-15-Heptadecenal	2.71%
3	Nonacos-1-ene	2.71%
4	Hexadecanoic acid, methyl ester	9.72%
5	Pentadecanoic acid, 13-methyl-, methyl ester	9.72%
6	Hexadecanoic acid, 2-methyl-	9.72%
7	9-Octadecenoic acid (Z)-, methyl ester	11.46%
8	cis-13-Octadecenoic acid, methyl ester	11.46%
9	trans-13-Octadecenoic acid, methyl ester	11.46%
10	1-Heptacosanol	1.62%
11	Hexacosyl pentafluoropropionate	1.62%
12	Hexacosyl heptafluorobutyrate	1.62%
13	17-Pentatriacontene	1.62%
14	Oleic acid, 3-(octadecyloxy)propyl ester	1.62%
15	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.62%
16	Glycidyl palmitate	2.11%
17	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	2.11%
18	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	2.11%
19	Glycidyl oleate	25.36%
20	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	25.36%
21	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-	25.36%
22	Phenol, 2,4-bis(1,1-dimethylethyl)-,phosphite (3:1)	4.31%
23	Silane, diethylheptyloxyoctadecyloxy-	4.31%
24	Methylenebis(2,4,6-triisopropylphenylphosphine)	4.31%
25	Phenol, 2,4-bis(1,1-dimethylethyl)-,phosphite (3:1)	4.31%
26	1-Cholestanone, O-allyloxime	4.31%
27	Cannabinol, pentafluoropropionate	4.31%

Fig 4.50: Fatty acid characterized using GC-MS of somaclone regenerated from callus of genotype PM30 in Indian mustard (*Brassica juncea*)

S.No.	Compound characterized	Apex RT	Start RT	End RT	Area	%Area	Height	%Height	Probability
1	1-Octadecene	5.24	5.19	5.32	2.14E+08	2.71	79764789	10	3.6
	E-15-Heptadecenal								3.32
	Nonacos-1-ene								3.06
2	Hexadecanoic acid, methyl ester	7.42	7.16	7.91	7.67E+08	9.72	75578774	9.47	78.51
	Pentadecanoic acid, 13-methyl-,methyl ester								9.57
	Hexadecanoic acid, 2-methyl-								2.76
3	9-Octadecenoic acid (Z)-, methyl ester	9.79	9.64	10.21	9.05E+08	11.46	87487645	10.96	15.76
	cis-13-Octadecenoic acid, methyl ester								11.75
	trans-13-Octadecenoic acid, methyl ester								10.38
4	1-Heptacosanol	11.99	11.86	12.25	1.28E+08	1.62	13276684	1.66	3.86
	Hexacosyl pentafluoropropionate								3.26
	Hexacosyl heptafluorobutyrate								3.14
	17-Pentatriacontene								21.44
	Oleic acid, 3-(octadecyloxy)propyl ester								14.27
	Octadecane, 3-ethyl-5-(2-ethylbutyl)-								11.22
5	Glycidyl palmitate	17.13	17.06	17.35	1.66E+08	2.11	27307391	3.42	86.71
	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester								2.04
	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester								1.48
6	Glycidyl oleate	21.39	21.05	22.24	2E+09	25.36	98040231	12.29	89.2
	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester								2.17
	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)-								1.62
7	Phenol, 2,4-bis(1,1-dimethylethyl)-,phosphite (3:1)	28.27	27.71	28.48	3.4E+08	4.31	28193887	3.53	90.45
	Silane, diethylheptyloxyoctadecyloxy-								2.2
	Methylenebis(2,4,6-triisopropylphenylphosphine)								1.55
	Phenol, 2,4-bis(1,1-dimethylethyl)-,phosphite (3:1)								47.29
	1-Cholestanone, O-allyloxime								8.23
	Cannabinol, pentafluoropropionate								6.14

CHAPTER – V
DISCUSSION

Successful breeding programme depends on to select out the most diverse and promising genotypes important in economically significant characters. Other than, improved breeding technique will be helpful to achieve the targets in the crop improvement. The important and new crop improvement techniques like plant tissue culture techniques, genetic engineering, somatic hybridization, marker assisted selection etc contributed significantly to develop improved cultivars, control of diseases, insects and weeds.

Indian mustard is a major oilseed crop of India. Its oil is an significant dietary component in India. It has received substantial attention from the plant breeders for developed various cultivars *viz.*, semi dwarf, disease and insect resistant, low erucic acid and glucosinolate containing genotypes, heat tolerance and salinity resistance *etc.*In India, breeding efforts have been underway to reduce glucosinolate content in the seed of rapeseed mustard varieties up to 30 micro moles/g defatted seed meal (low or 0) and erucic acid up to 2% (low or 0) as well as combining both to develop double zero or double low varieties.

To attain above said goals, the present study was carried out in *Brassica juncea* L. (Indian mustard) involving 196 diverse genotypes. Analysis of variance wasfound significant that suggested presence of considerable amount of variability in studied materials for further improvement of various traits. Acquaintance of the nature and magnitude of genetic variability are indispensable previous to starting breeding techniques. Correspondingly, heritability and genetic advance are cooperative in selecting superior individuals. Correlation studies give a clear depiction of characters alliance which is generally as a result of linkage, pleiotrophy, physiological relationship in developmental and biochemical alleyway. The characters whichare correlated are of much interest since transform in one character influenced the other one. Path coefficient analysis is basically a standardized partial regression coefficient, which splits the correlation coefficient into direct and indirect effects. In other words, it deals the direct and indirect contribution of assorted independent characters on a dependent character. In plant breeding, genetic diversity participates a vital task because hybrids between lines of diverse origin generally, display a greater heterosis than those between closely related parents. Genetic diversity arises due to geographical separation or due to genetic barriers to crossability. The choice of genetically diverse parents for hybridization is an chief feature of any crop improvement programme for getting desirable segregants. New breeding technique(s), chromosomal variation, *in vitro* selection in conjunction with somaclonal variation, secondary metabolites production through callus and cell suspension cultures including molecular breeding and genetic engineering have become important tools in the hands of plant breeders for enhancing the selection efficiency to encounter different difficulties. The results obtained from the present investigation have been discussed under the following heads.

5.1 Morpho-physiological traits

5.1.1 Analysis of variance

Pooled variances revealed that genotypes were highly significantly differing for all the characters *viz*., plant height, number(s) of primary branches, number(s) of secondary branches, days to 50% flowering, days to maturity, length of main raceme, number(s) of silique per main raceme, number(s) of silique per plant, silique length, number(s) of seeds per silique, 1000 seed weight, biological yield per plot, seed yield per plot, seed yield per plant and harvest index. The similar results were also addressed by Shekhawat *et al.* (2014), Akabari *et al.* (2015), Salam *et al.* (2017), Sikarwar *et al.* (2017) and Dawar *et al.* (2018).

5.1.2 Genotypic and phenotypic coefficients of variations

The highest genotypic coefficient of variation (GCV) was observed for seed yield per plant (g) followed by harvest index and number(s) of silique per plant. The seed yield per plot, 1000 seed weight, number(s) of silique per main raceme, length of main raceme, days to 50 % flowering, biological yield per plot and number(s) of seeds per silique had moderate values while silique length, plant height, number(s) of secondary branches, days to maturity and number(s) of primary branches exhibited very low genotypic coefficient of variation. Present results were authenticate with the findings of previous workers including Akbar *et al.* (2003) for highseed yield per plant and low in plant height, Yadav *et al.* (2011) for moderate in 1000 seed weight and low in days to maturity, plant height, siliqua length, number(s) of seeds per siliqua, Lodhi *et al.* (2014) for moderate in length of main raceme, number(s) of siliqua per main raceme, number(s) of seeds per siliqua and low in plant height, Dawar *et al.* (2018) for high in seed yield per plant and moderate in number(s) of seeds per siliqua, and Raliya *et al.* (2018) for moderate in days to 50 % flowering, number(s) of siliqua per main raceme, length of main raceme and low in number(s) of seeds per siliqua.

The highest phenotypic coefficient of variation was observed for seed yield per plant (g), harvest index and number(s) of silique per plant while 1000 seed weight, seed yield per plot, number(s) of silique per main raceme, number(s) of secondary branches, silique length, length of main raceme, days to 50% flowering, biological yield per plot, number(s) of seeds per silique and number(s) of primary branches had moderate values. Plant height and days to maturity exhibited very low PCV. These results are in confirmation with the findings of Tripathi *et al.* (2013), for moderate in number(s) of seeds per siliqua and low in plant height and days to maturity by Synerm *et al*. (2014), for moderate in days to 50 % flowering and number(s) of primary branches by Amsalu *et al*. (2017) and for highest in seed yield per plant, moderate in length of main raceme and 1000 seed weight and low in days to maturity by Singh *et al*. (2018).

5.1.3Heritability (broad sense) and genetic advance as a percentage of mean

The effectiveness of selection depends upon genetic advance of the character selected along with heritability. High heritability had possessed for seed yield per plot, harvest index, biological yield per plot, days to 50 % flowering, length of main raceme, number(s) of silique per plant, seed yield per plant, plant height, number(s) of seeds per silique, 1000 seed weight and number(s) of silique per main raceme. Moderate heritability was estimated for days to maturity. While Silique length, number(s) of secondary and primary branches exhibited low estimates of heritability. It was also reported by Lodhi *et al*. (2014) for moderate heritability in days to maturity, Akabari *et al*. (2015) for high heritability for number (s) of siliqua per plant and seed yield per plant, Salam *et al*. (2017) for high heritability for number(s) of seeds per siliqua and seed yield per plant, Sekharwar *et al*. (2017) for maximum heritability for1000 seed weight, Dawar *et al*. (2018) for highest for number(s) of siliqua per plant and number(s) of seeds per siliqua and Singh *et al*., (2018) for maximum heritability for days to 50 % flowering and number(s) of siliqua per main raceme.

Genetic advance expressed as percentage of mean found high for seed yield per plant, harvest index, number of silique per plant, seed yield per plot, 1000 seed weight, length of main raceme, days to 50% flowering, biological yield per plot and number(s) of silique per main raceme. The values were moderate for number(s) of seeds per silique. It was low for plant height, silique length, days to maturity, number (s) of secondary and primary branches. Similar findings were also reported for low GA for days to maturity by Bind *et al*. (2014), maximum for number(s) of siliqua per main raceme and low fordays to maturity and plant height by Lodhi *et al*. (2014),highest for seed yield per plant and low for days to maturity by Akabari *et al*. (2015), moderate for number(s) of seeds per siliqua by Bibi *et al*. (2016) and highest for number(s) of siliqua per plant by Dawar *et al*. (2018).

5.1.4 Genotypic and phenotypic correlation coefficients

5.1.4.1 Genotypic correlation coefficients

Days to 50% flowering exhibited significant positive correlation with days to maturity, plant height and 1000 seed weight, whereas, significant negative correlation with biological yield per plot, length of main raceme, number(s) of seeds per silique and silique length. Days to maturity showed significant positive correlation with number(s) of primary branches and silique per main raceme, while significant negative correlation with silique length, length of main raceme, seed yield per plant, number(s) of silique per plant and secondary branches. Plant height had significant positive correlation with 1000 seed weight, length of main raceme and number(s) of secondary branches, whereas, significant negative correlation with biological yield per plot, seed yield per plot, number(s) of seeds per silique, silique length, number(s) of silique per main raceme and number(s) of primary branches. Number(s) of primary branches showed highly significant positive correlation with number(s) of silique per main raceme, number(s) of secondary branches, seed yield per plot, harvest index and silique length, whereas, significant negative correlation with seeds per silique, length of main raceme, number(s) of 1000 seed weight and seed yield per plant. Number(s) of secondary branches had significant positive correlation with number(s) of silique per plant, seed yield per plant and number(s) of seeds per silique while significant negative correlation with length of main raceme, silique length, 1000 seed weight and number(s) of silique per main raceme. Length of main raceme showed positive and negative correlations which all were found non-significant. Number(s) of silique per main raceme exhibited significant positive correlation with silique length, seed yield per plot and biological yield per plot whereas, significant negative correlation with seed yield per plant, number(s) of seeds per silique, number(s) of silique per plant and 1000 seed weight. Number(s) of silique per plant showed significant positive correlation with seed yield per plant and significant negative correlation with number(s) of seeds per silique. Silique length recorded significant positive correlation with number(s) of seeds per silique and 1000 seed weight whereas, significant negative correlation with seed yield per plant. Number(s) of seeds per silique showed positive and negative correlations which all were found non-significant. One thousand seed weight had significant negative correlation with seed yield per plot and biological yield per plot, whereas biological yield per plot showed significant negative correlation with harvest index. Seed yield per plot recorded significant negative correlation with harvest index. Similar results were reported by Rathod *et al*. (2013) for days to 50 % flowering for significant positive correlation with days to maturity and plant height, days to maturity for significant positive correlation with primary branches, plant height for positive correlation with number(s) of secondary branches and significant negative correlation with number(s) of seeds per siliqua, by Amsalu *et al*. (2017) for days to 50 % flowering significant positive correlation with days to maturity and in days to maturity for significant negative correlation with seed yield per plant, by Devi *et al*. (2018) for days to 50 % flowering for significant positive correlation with days to maturity and for plant height also significant positive correlation with number(s) of secondary branches and by Raliya *et al*. (2018) for days to 50 % flowering significant positive correlation with days to maturity and 1000 seed weight.

5.1.4.2 Phenotypic correlation coefficients

Days to 50% flowering had significant positive correlation with plant height, days to maturity and 1000 seed weight. It showed significant negative correlation with biological yield per plot, number(s) of seeds per silique and length of main raceme. Days to maturity exhibited significant negative correlation with seed yield per plant, length of main raceme and number(s) of silique per plant. Plant height had significant positive correlation with 1000 seed weight, whereas, significant negative correlation with Number(s) of silique per main raceme. Number(s) of primary branches had significant positive correlation with number(s) of silique per main raceme. Number(s) of secondary branches exhibited significant positive correlation with number(s) of silique per plant and seed yield per plant, whereas significant negative correlation with 1000 seed weight. Number(s) of silique per main raceme had significant positive correlation with seed yield per plot, biological yield per plot and silique length, whereas, significant negative correlation with number(s) of silique per plant. Number(s) of silique per plant displayed significant positive correlation with seed yield per plant. Silique length had significant positive correlation with number(s) of seeds per silique. One thousand seed weight recorded significant negative correlation with biological yield per plot. Biological yield per plot had significant negative correlation with harvest index. Seed yield per plot showed significant positive correlation with harvest index. Present results were in accordance with the findings of earlier researchers. For plant height significant positive correlation with 1000 seed weight and in number(s) of secondary branches per plant also significant positive correlation with number(s) of siliqua per plant (Doddabhimappa *et al*.,2009). For days to 50 % flowering significant positive correlation with days to maturity and for plant height also significant positive correlation with 1000 seed weight (Gupta *et al*., 2018). For days to 50 % flowering: significant negative correlation with length of main raceme, number(s) of seeds per siliqua and 1000 seed weight as well as for days to maturity also displayed significant negative correlation with length of main raceme, number(s) of siliqua per plant and seed yield per plant (g) and for number(s) of siliqua per plant: significant positive correlation with seed yield per plant (g) (Kumar *et al*., 2018) and for days to 50 % flowering: significant positive correlation with days to maturity and plant height (Raliya *et al*., 2018).

5.1.5 Genotypic and phenotypic path coefficient analysis

5.1.5.1 Genotypic path coefficient analysis

The maximum positive direct effect of number(s) of silique per plant on seed yield per plant (g) followed by harvest index, plant height, days to 50% flowering, number(s) of seeds per silique, biological yield per plot and number(s) of silique per main raceme were evidenced, whereas highest negative direct effect on seed yield per plant were found on 1000 seed weight followed by number(s) of secondary branches, number(s) of primary branches, silique length, length of main raceme, days to maturity and seed yield per plot. Similar findings were also documented by Kardam *et al*. (2005) for number(s) of siliqua per plant, number(s) of siliqua per main raceme and number(s) of seeds per siliqua as they exerted positive direct effect on seed yield per plant (g), Afrin *et al*. (2011) as they reported that plant height and number(s) of siliqua per plant had positive direct effect on seed yield per plant (g) and number(s) of primary and secondary branches had negative direct effect on seed yield per plant (g)..Yadav *et al*. (2011) evidenced that seed yield per plant had positive direct effect on number(s) of siliqua per plant. Dawar *et al*. (2018)reported positive direct effect on number(s) of siliqua per plant, plant height, number(s) of seeds per siliqua on seed yield per plant (g) and negative direct effect on siliqua length and Gupta *et al*. (2018) told that harvest index, number(s) of seeds per siliqua, plant height had positive direct effect on seed yield per plant (g) and length of main raceme and 1000 seed weight had negative direct effect with seed yield per plant (g).

5.1.5.2 Phenotypic path coefficient analysis

The maximum positive direct effect of number(s) of silique per plant on seed yield per plant (g) followed by days to 50% flowering, harvest index, plant height, biological yield per plot, number(s) of secondary branches, length of main raceme, number(s) of silique per main raceme and number(s) of seeds per silique, whereas highest negative direct effect on seed yield per plant was found by days to maturity, number(s) of primary branches, 1000 seed weight, silique length and seed yield per plot. Similar findings are in conformity with the Kardam *et al*. (2005) who were reported that number(s) of siliqua per plant, number(s) of seeds per siliqua and number(s) of silqua per main raceme had positive direct effect on seed yield per plant (g). Yadav *et al*. (2011) reported that number(s) of siliqua per plant had positive direct effect on seed yield per plant (g). Dawar *et al*. (2018) demonstrated that seed yield per plant had positively directly affected by number(s) of siliqua per plant, plant height, number(s) of seeds per siliqua and had negatively directly affected by siliqua length. Gupta *et al*. (2018) reported that seed yield per plant had positive direct effect by harvest index, number(s) of secondary branches per plant, number(s) of seeds per siliqua and negative direct effect by 1000 seed weight.

5.2 Biochemical analysis

5.2.1 Analysis of variance

Analysis of variance carried out for the 6 characters. The data revealed that genotypes were highly significant differed for all the characters *viz*., palmitic acid, oleic acid,linoleic acid,linolenic acid,erucic acid and oil content. These results are in line of earlier reports of Khan *et al*. (2008) and Ali *et al*. (2013).

5.2.2Genotypic and phenotypic coefficient of variation:

The highest genotypic coefficient of variation was observed for oleic acid followed by erucic acid, palmitic acid. Linoleic acid and linolenic acid had moderate values. Oil percentage exhibited very low GCV. It was estimated moderate GCV for linolenic acid by Chauhan *et al*. (2008) and Belete*et al*. (2011), low GCV in oil content by Ali *et al*., (2013) and also moderate in linoleic acid by Kumar *etal*. (2013).

The highest phenotypic coefficient of variation was recorded for oleic acid, erucic acid and palmitic acid. Linoleic acid and linolenic acid were showed moderate values. Oil percentage demonstrated very low PCV. Previous worker Ali *et al*. (2013) has also reported low PCV value for oil content. Similarly, Kumar *et al*. (2013) has been reported higher PCV for erucic acid, moderate for linoleic acid and low for oil content.

5.2.3Heritability (broad sense) andgenetic advance as percentage of mean

High heritability had possessed for erucic acid, oleic acid, palmitic acid, linoleic acid and linolenic acid. Moderate heritability was estimated for oil percentage. Similar finding has also been addressed earlier by Khan *et al*. (2008) as they reported maximum heritability for oleic and erucic acid.

The estimation of genetic advance expressed as percentage of mean was found higher for oleic acid, erucic acid, palmitic acid, linoleic acid and linolenic acid, while low for oil percentage. Similar results were conformity for high genetic advance for oleic and linolenic acid (Chauhan *et al*., 2008), low for oil percentage(Ali *et al*., 2013), maximum for oleic and linolenic acid and low for oil percentage (Kumar *et al*., 2013).

5.2.4Genotypic and phenotypic correlation coefficients

5.2.4.1 Genotypic correlation coefficients

Palmitic acid (%) exhibited significant positive correlation with linoleic acid and linolenic acid. Whereas, significant negative correlation with erucic acid and oleic acid. Oleic acid (%) showed significant negative correlation with linolenic acid and erucic acid. Linoleic acid (%)showed significant positive correlation with linolenic acid, whereas, significant negative correlation with erucic acid and oil percentage. Linolenic acid (%) demonstrated significant negative correlation with erucic acid. These results were in an accordance with the earlier results where linoleic acid had significant positive correlation with linolenic acid and oleic acid whereas significant negative correlation could be recorded with erucic acid (Belete *et al*. 2011).Similar finding was reported by Tahira *et al*. (2015) where palmitic acid was significantly correlated with linolenic acid and oleic acid and significant negative correlation with erucic acid was reported.

5.2.4.2 Phenotypic correlation coefficients

Palmitic acid (%) showed significant positive correlation with linoleic acid and linolenic acid. Whereas, significant negative correlation with oleic acid and erucic acid. Oleic acid (%) showed significant negative correlation with linolenic acid and erucic acid. Linoleic acid (%) recorded significant positive correlation with linolenic acid, while significant negative correlation with erucic acid. Linolenic acid (%) demonstrated significant negative correlation with erucic acid. It was also recorded for palmitic and significant negative correlation with erucic acid, oleic acid and negative correlation with linoleic and erucic acid, as reported earlier by Chauhan *et al*. (2008). According to Kumar *et al*. (2013) and Tahira *et al*. (2015) oleic acid content was negatively correlated with linolenic,linoleic and erucic acid, whereas significant positive correlation was found between palmitic acid with linolenic acid and oleic acid and significant negative correlation with erucic acid by Kumar *et al*. (2013) and palmitic acid for significant positive correlation with linolenic acid and oleic acid for significant negative correlation with erucic acid by Tahira *et al*. (2015).

5.2.5Genotypic and phenotypic path coefficient analysis

5.2.5.1 Genotypic path coefficient analysis

Genotypic path coefficient analysis revealed that the maximum positive direct effect of palmitic acid was on erucic acid, while highest negative direct effect on erucic acid was found by linoleic acid followed by oleic acid,linolenic acid and oil percentage. Earlier workers also reported positive direct effect of palmitic acid and negative direct effect of oil percentage, oleic and linoleic acid on erucic acid (Tahira *et al*., 2015).

5.2.5.2 Phenotypic path coefficient analysis

Phenotypic path coefficient analysis revealed the maximum positive direct effect of palmitic acid on erucic acid, whereas highest negative direct effect on erucic acid was estimated by linoleic acid, oleic acid, linolenic acid and oil percentage. The researchers, Chauhan *et al*. (2008) and Kumar *et al*. (2013) have also found negative direct effect of oleic and linoleic acid on erucic acid.

5.3 Comparative genetic diversity analysis based on morpho-physiological and biochemical traits

Fifteen principal components were found in *Brassica juncea* over pooled years. First principal component had maximum of the total variance for the morphological traits. Similar results were conformity with the findings of Ray *et al*. (2014), Singh *et al*. (2014), Neeru *et al*. (2015), Verma *et al*. (2016) and Rao *et al*. (2017).The multivariate analysis based on Euclidean distance cluster analysis was used for diversity analysis. One-ninety-six genotypes of Indian mustard were subjected to cluster analysis for 15characters and 16 clusters were formed. Maximum inter-cluster distance was observed between cluster 9 and cluster 16.Present results are supported by earlier works of Singh *et al*. (2007), Doddabhimappa *et al*. (2010), Panday *et al*. (2013), Devi *et al*. (2017) and Mohan *et al*. (2017).

Six principal components were found. First principal component had highest total varianceforthe biochemical traits. These findings were in agreement with results reported by Chauhan *et al*. (2008) and Ali *et al*. (2015).Genetic divergence among Indian mustard genotypes for biochemical assessment was studied using Euclidean distance cluster analysis. The 188 genotypes were grouped into 18 clusters. The highest numbers of genotypes appeared in cluster 2, which contains 82 genotypes. The highest inter-cluster distance was found between cluster 18 and cluster 12.

5.4 To characterize low and high erucic acid content in selected lines

Nine genotypes of Indian mustard had low erucic acid content. Fifty-seven and one twenty-two genotypes were showed moderate and high erucic acid contents, respectively. Earlier Saini *et al*. (2016) and Rai *et al*. (2018) also reported diversity in erucic acid content among studied genotypes.

5.5 Molecular studies

Molecular breeding is one of the significant and fast approaches for crop improvement in mustard (Kapadia *et al*., 2019; Pushpa*et al*., 2016).Molecular markers are useful in characterization of genetic diversity, gene mapping, MAS including crop evolution. There are many types of molecular markers as RFLP, AFLP, RAPD, CAPS, SSR, SNP, SCAR, EST, ISSR, STS *etc*. Several types of DNA markers were used in Indian mustard for various aspects *viz*., RFLP (Pradhan *etal*., 2003), AFLP (Pradhan *et al*.,2003; Genet *et al*., 2005), RAPD (Saha *et al*.,2008; Tahira *et al*., 2013; Yousuf *et al*., 2013; Gupta *et al*., 2014 ; Iqbal *et al*., 2015), EST SSRs (Nirala *et al*., 2011; Gupta *et al*., 2014), ISSR (Yadav *et al*., 2012 ; Salam *et al*., 2018), SSR (Chandra *et al*., 2013; Verma *et al*., 2016; Bharti *et al*., 2018; Patel *et al*., 2018, Thakur *et al*., 2018), SNP (Atri*et al.,* 2019), CAPS (Saini *et al*., 2016) and IP (Singh *et al*., 2015). Erucic acid is one of the promising traits having multipurpose use and there are several reports where molecular markers have been used for screening and characterization (Bharti *et al*., 2018 and Saini *et al*., 2016). Gene based SSR markers have been used for erucic acid and glucosinolate (Farzad *et al*., 2013; Bharti *et al*., 2018). Gene based markers are easy to analyze and cost effective approach and being used widely for molecular breeding approaches. Polymorphic SSR markers have their own applications for diversity analysis and reporting new markers for specific traits. In current study both genes based as well as random polymorphic SSR markers were used for diversity analysis of mustard genotypes specifically in relation to erucic acid contents.

5.5.1 Molecular diversity analysis using SSR markers in Indian mustard

Diversity analysis of Indian mustard genotypes was done using 23 highly polymorphic SSR markers. All amplified 109 alleles were observed across 23 markers in 48 genotypes. A total of 109 alleles were identified with an average of 4.47 alleles per locus for polymorphic SSR markers. Genetic diversity varied from minimum 0.55 of SSR marker Na10-D07 to maximum 0.77 of BRMS-098 with mean value of 0.68. Polymorphism information content (PIC) value of the markers varied from minimum 0.51 for SSR Na10-D07 to maximum 0.73 with primer BRMS-098 with a mean value of 0.62.The primer which showed highest gene diversity (0.77) and PIC value (0.73) was observed in BRMS-098, which is representing 77% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.51) was observed for the primer SSR Na10-D07, indicating 51% polymorphic nature of the marker. All 23 primers representing PIC value more than 50%, so these primers can further be used for diversity analysis.

The dendrogram was made based on scoring done for the entire polymorphic SSR marker using the software Power Marker v3.25. The genetic relationships among Indian mustard genotypes were presented. Major three clusters were obtained i.e., C-I, C-II and C-III, in which cluster C-I consisted of4 genotypes. Cluster C-II had 9 genotypes. Cluster C-III was contained 35 genotypes. Most important cluster C-III contained 35 genotypes including MRNJ-24, MRNJ-23, MRNJ-22, L-6,

L-4, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6 germplasm lines representing best quantitative and qualitative traits and PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30 were notified varieties. These varieties having low erucic acid, timely sown and irrigated condition was grouped with these germplasm lines so, may be having that traits. The results were similar with the results of Yadav *et al*. (2012),studied among 30 Indian mustard genotypes using 20 ISSR primers showing 156 amplified bands with an average of 7.8 bands per primer out of which 115 were polymorphic. Size of amplified fragments ranged from 100 bp to 1500 bp and number varied from 4 to 14. Similarity matrix was utilized to construct the UPGMA dendrogram. In cluster analysis, based on similarity coefficient, Indian mustard genotypes were categorized into three major clusters. Study of Vinu *etal*. (2013) concluded that out of the 143 primers tested, 134 reported polymorphism and a total of 355 alleles were amplified. The UPGMA based dendrogram representing genetic similarity among different accessions grouped the 44 genotypes into four clusters at 40% genetic distance First cluster comprised of nine varieties of which eight were developed at IARI, New Delhi. In six of these varieties, except two early maturing varieties Pusa Agrani and Pusa Tarak, Varuna is involved as one of the parents directly orthrough the ancestry. The ninth one, Varana is a very old selection from Varanasi (Uttar Pradesh).

Fayyaz*et al*. (2014) used 90 genotypes for characterization using 24 microsatellite, but 12 SSR primers combinations generated a total of 33 alleles, of that 32 were polymorphic loci, whereas only one was monomorphic locus. The average number(s) of polymorphic alleles per locus was 2.66. The PIC values ranged from 0.395 for primer Ra2-E03 to 0.726 for primer BRMS-019 with an average genetic diversity of 0.584 per locus. Dendrogram obtained through UPGMA clustering of F_2progenies depicted eight main groups using similarity coefficient of 0.70.

Similar to our study Gupta *et al*. (2014) also used 15 reproducible RAPDs and 3 EST SSRs for genetic variation across 22 Indian mustard genotypes. The polymorphism percentage of more than 91% and 86.66% was observed respectively. Moreover the PIC values observed corresponds to 0.303, with 4.44 marker index and 6.89 resolving power for RAPDs, however, SSRs showed the PIC value of 0.281, with 0.94 marker index and 0.269 resolving power. The cluster analysis based on UPGMA separated the genotypes in two major groups. All the genotypes of *Brassica juncea* are grouped in one major cluster and genotypes of other *Brassica* species are grouped in different cluster.

Prajapat *et al*. (2014) studied genetic diversity analysis of 30 *Brassica* genotypes belonging to four cultivated species using 24 SSR markers. With a 72% polymorphism, a total of 84 alleles varied from 1 to 8 (BRMS 14) with a mean of 2.79 alleles were observed. Nine, out of 24 SSRs produced 100% polymorphism. The amplicon size ranged from 99bp (BRMS-26) to 383bp (BRMS-31). The highest allele frequency of 0.933 was for BRMS-03 andBRMS-17 whereas PIC ranged from 0.79 (BRMS-31) to 0.12 (BRMS-003). BRMS-17 gave specific bands for *Brassica carinata*. In four clusters, all 30 accessions were grouped into their respective clusters on the basis of species.

It was also reported by Patel *et al*. (2018) performed genetic diversity among F_2 plants of the cross RH 30×CS 52 in Indian mustard (*Brassica juncea*). They reported that out of 358 SSR markers, 42 were polymorphic and 154 were monomorphic. A total of 225 alleles, ranging from 2 to 4 were amplified. The PIC (Polymorphic Information Content) value ranged from 0.427-0.730. In our study, 23 primers showed polymorphism among 48 Indian mustard genotypes with PIC ranged from 0.51 to 0.73with an average of 0.62. F_2 population was grouped in 2 major clusters and 37 sub-clusters at a similarity coefficient of 0.58.

Similarly, Salam *et al*. (2018) investigated genetic diversity in 24 genotypes using 4 ISSR markers, two major clusters were formed with 53% genetic similarity. The UPGMA cluster analysis revealed two main clusters and nine sub-clusters. The dandogram indicated based on above maker study formed two major clusters *namely* ‘A’ and ‘B’. Two genotypes named GPT-13 and GPT-59 comes under one cluster ‘A’ with 53% genetic similarity while, remaining 22 genotypes falls under second cluster B with 66% genetic similarity.

Habib *et al*. (2019) presented diversity among *Brassica juncea* genotypes. Twenty *Brassica juncea* L. genotypes generated 79 polymorphic alleles from 15 SSR primer pairs with an average of 5.26 alleles per locus. The polymorphism percentage was recorded to be the maximum in the case of the primer Ni4-D04 (80%) and the minimum in the case of 3 different primers *namely:*A06-20686249, Ni3-D03 and A05-20242013 (33.3%). The polymorphism information content (PIC) values ranged from 0.41 (A01-13393871) to 0.94 (A05- 25290881), reflecting the presence of high allelic variation. The magnitude of similarity coefficient between RH749 and Kranti (0.368) was found to be the maximum amongst pair-wise combinations of genotypes. The dendrogram generated with UPGMA cluster analysis revealed six major clusters.

Khurshid *et al*. (2019) reported genetic divergence among Pakistani oilseeds *Brassica* cultivars using morphometric and microsatellite markers using 31 cultivars of four different *Brassica species i.e*. *Brassica carinata*, *B. juncea*, *B. naps*and *B. rapa* including two exotic cultivars. The PCR-based DNA polymorphism among 30 cultivars revealed significant genetic variation and depicted dissimilarity among cultivars except Dacca-raya and Sultan-raya. Cultivars were classified into 2 major groups and 4 small sub-groups according to their morphological characteristics, origin of cultivars and related species. Cultivars like Khanpur-raya, Bahawalpur-raya and Toria-sathi formed no association with other cultivars and positioned independently in the dendrogram.

5.5.2 Screening for erucic acid in Indian mustard genotypes using gene based SSR markers

Two gene based SSR markers for erucic acid were used to screening mustard germplasm lines. Maximum major allele frequency was 0.43 of Sal-SRK-I and minimum 0.37of FAE1 with an average of 0.40. A total of 10 alleles were identified with an average of 5 alleles per locus for polymorphic SSR markers. Allele number of both markers were 5.00 and also mean was 5.00. Highest gene diversity was 0.71 of Sal-SRK-I and minimum 0.70 of FAE1 with an average of 0.70. Maximum polymorphic information content was 0.66 of Sal-SRK-I and minimum 0.64 of FAE1 with mean of 0.65. The primer which showed highest gene diversity (0.71) and PIC value (0.66) was observed in Sal-SRK-I, which is representing 71% polymorphism probability of the marker. Lowest gene diversity and PIC value (0.64) was observed for the primer FAE1, indicating 64% polymorphic nature of the marker. Both primers representing PIC value more than 50%, so these primers can further be used. The genetic relationships among Indian mustard genotypes were characterized. Major three clusters were formed *i.e*., C-I, C-II and C-III. Cluster I makes group of 4 various genotypes like CS-54, GM-2, DRMR-150-35 and DRMRIJ-31. Cluster II was having 4 genotypes having MRNJ-6, MRNJ-5, RH-74.9 and RB-50. Cluster III had 40 genotypes. Abbas *et al.* (2008) exhibited genetic relationship among 5 F_2 segregating population of *Brassica* along with 9 parental lines. On an average 29 alleles were amplified using IGF primer sets (SSR). Mean genetic distance ranged from 0.25-1.00 (G.D = 0%-100 %), respectively. Size of scorable fragments ranged from 250 to>2000 bp. A high level of genetic dissimilarity (GD= up to 100%) was estimated among all genotypes. Entries were grouped in clusters using cluster analysis. Sarikamis *et al*. (2010) have got successful amplifications with genomic DNA from kale genotypes with the SSR marker Ol12FO2 and bands of expected sizes were obtained. It was determined that Ol12FO2 was polymorphic among kale genotypes. It was successful amplification of markers within different vegetable *Brassica*s. Chen *et al*. (2011) evaluated allelic diversity in the A and B genomes of oilseed *B. juncea* using SSR markers covering the A and B genomes in a large collection of landraces, cultivars and breeding lines from India, China, Europe and Australia. 500 polymorphic alleles from both A and B genomes divided 119 oilseed *B. juncea* accessions from Australia (AU), China (CN), Europe (EU) and India (IN) into 2 major groups at the genetic dissimilarity level of 0.47: Groups 1 and 2 both contained Indian and Chinese *B. juncea* accessions, while European and Australian accessions were found only in Group 2. Allocation of accessions to Groups was consistent across the A and B genomes, indicating that *B. juncea* had similar patterns of genetic dissimilarity in both A and B genomes. Chandra *et al*. (2013) estimated 37 diverse Indian mustard genotypes using 10 Aand C genome specific SSR markers for the development of molecular profile. A total of 41 bands were amplified that showed 97.56% polymorphism. The UPGMA (Unweighted Pair Group Method with Arithmetic mean) dendrogram was constructed using Jaccard'ssimilarity coefficients based on SSR marker data generated on 37 genotypes. UPGMA ordered the populations of 37 genotypes into four clusters. These results were in accordance with the result of Singh *et al*. (2017) as they used 453 SSRs and 139 (30.7%) showed polymorphism with 308 alleles. PIC ranged from 0.101 to 0.668, with the average value of 0.474, revealing that much variation was present among these genotypes. The cluster analysis gave three major groups where white rust resistant genotypes were grouped in cluster 1, double low quality genotypes in cluster 2 while the recipients were grouped in the cluster 3 indicating that grouping of genotypes based on SSRs corresponded. Similarly, Bharti *et al*. (2018) comprised 71 genotypes for molecular characterization of erucic acid and glucosinolate using 4 SSR markers. A total of 56 amplified bands were obtained, out of which 17 were polymorphic. All the genotypes were clustered in three groups. Cluster I had seven genotypes followed by cluster II five and cluster III two genotypes.

Thakur *et al*. (2018) found 124 *Brassica*-derived SSR loci assayed, 100% cross transferability was obtained for *B. juncea* and 3subspecies of *B. rapa*, while the lowest cross-transferability (91.93%) was obtained for *Eruca sativa*. The average % age ofcross-transferability across all the 7 species was 98.15%. Number(s) of alleles detected at each locus ranged from one to six with an average of 3.41 alleles per primer pair. Dendrogram divided all the40 accessions into two main groups composed of *B. juncea/B. nigra/B. rapa and B. carinata/B. napus/B. oleracea.*

Kapadia *et al*., (2019) screened molecular markers for powdery mildew resistance in Indian mustard. The molecular analysis for powdery mildew resistance in *Brassica* spp*.* was carried out with four females, three males, F_{1S} and F_{2S} generation. The SSR markers *viz,* OI10-B12 and OI10-C01 clearly distinguished between susceptible and resistant bulks of interspecific cross GM-3 x PusaSwarnim.

Monika *et al*. (2019) screened out 200 SSR markers for polymorphism in two parental *Brassica juncea* genotypes (RB 50, drought tolerant and Kranti, drought susceptible), 51 were found polymorphic. The polymorphic markers were used to screen F_2 population resulted a total of 108 alleles were identified in the RB 50 and Kranti and the parental *B. juncea* genotypes. The PIC values for various primers ranged from 0.340-0.505 with an average of 0.406. All the 157 F_2 plants clustered in two major groups at the similarity coefficient of 0.53. Two parental varieties RB 50 and Kranti had low similarity coefficient. Genetic relationship was also assessed by PCA analysis. 2 dimensional and 3 dimensional PCA scaling exhibited that two parental genotypes were quite distinct whereas all 157 F_2 plants interspersed between the two parental lines with distribution of most plants towards RB 50.

5.5.3 Gene based CAPS markers for screening of erucic acid contents of Indian mustard

Two gene based CAPS markers for erucic acid were used to screening of mustard germplasm lines. Maximum major allele frequency was found 0.50 for CAPS237 and minimum 0.31 for CAPS1265 with an average of 0.41. Highest genotype number was 5.00 of CAPS237 and lowest 4.00 of CAPS1265 with mean

4.50. A total of 9 alleles were identified with an average of 4.5 alleles per locus for polymorphic SSR markers. Allele number was obtained in maximum 5.00 of CAPS237 and in minimum 4.00 of CAPS1265 with mean 4.50. Maximum gene diversity was consisted 0.74 of CAPS1265 and minimum was 0.66 of CAPS237 with an average of 0.70. Highest polymorphic information content was found 0.69 of CAPS1265 and lowest 0.61 of CAPS237 with mean 0.65. The primer which showed highest gene diversity (0.74) and PIC value (0.69) was observed in CAPS1265, which is representing 74% polymorphism probability of the marker. Lowest gene diversity and PIC value (0.61) was observed for the primer CAPS237, indicating 61% polyporphic nature of the marker. Both primers showing PIC value more than 50%, so these primers can further be used in molecular breeding.

Based on dendogram, genetic relationships among Indian mustard genotypes were found. Major three clusters were formed *viz.,* C-I, C-II and C-III. Cluster I had 2 genotypes. Cluster II was found 42 genotypes. C-III consisted of 4 genotypes. Most interesting group was formed in cluster II (a), including 41 indigenous and exotic genotypes RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1. RH749, Maya, PM25, PM29, PM24, PM22, JM-2, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30 and RVM-1 showed release varieties having suitable for irrigated and rainfed condition, white rust resistance, high temperature tolerant, suitable for early sown, low erucic acid, timely sown, non-traditional areas, bold seeded, high oil content and high yielding variety, early maturity and tolerant to powdery mildew &*A. blight,* so that these germplasm lines falling alongwith these varieties may be contained these characteristics features as well.

Bhatia and Alok (2014), Gupta *et al*.(2004) and Yan *et al*.(2015) documented that erucic acid trait is governed by two independent genes FAE1.1 and FAE1.2 in allotetraploids *B. napus and B. juncea*. Several earlier studies Pandey *et al*. (2013), Singh *et al*.(2015), Cao *et al*.(2010) and Lühs *et al*.(1999) have also reported the continuous variation in erucic acid content in segregating populations of *B. juncea* and *B. napus*, respectively. Related to our study, Saini *et al*. (2016) have also studied that two SNPs in *FAE1.1* at position 591 and 1265 and one in *FAE1.2* at 237 were found polymorphic among low and high erucic acid genotypes. These SNPs either create or change the recognition site of restriction enzymes. Transition of a single nucleotide at position 591 and 1265 in FAE1.1 and at position 237 in FAE1.2 leads to a change in the recognition site of Hpy99I, BglIIand MnlIrestriction enzymes, respectively. Two CAPS markers for *FAE1.1* and one for *FAE1.2* were developed to separate low and high erucic acid genotypes.

5.6 *In vitro* selection for low erucic acid

5.6.1 Establishment and maintenance of callus and cell suspension cultures followed by plantlet regeneration

5.6.1.1 Callus culture

Under appropriate and defined conditions of nutrient media, type(s) and concentration (s) of plant growth regulators, it is possible to induce callus from any explants derived from any living part of plants. However, the frequency of callus induction varied with type(s) and concentration(s) of plant growth regulators and nature of explants.

In order to select genotype(s), explant (s) types and concentration(s) and combination (s) of plant growth regulators, responding genotype(s) were selected on the basis of their plant growth pattern, higher yield and low and high erucic acid containing abilities were considered for raising callus and cell suspension cultures followed by plantlet regeneration and selection of desired somaclone(s).

Six genotypes *viz*: CS-54, GM-2, RB50, NRCDR-2, Rohini, PM30 and LES-39 were selected for *in vitro* selection research work on the basis of their abilities to have euric acid content weather higher or low. similarly in regard to selection of responding explants, Thakur *et al*. (2013) established plant regeneration protocol in higher frequencies in *Brassica juncea* var. NRCDR-2from cotyledonary petiole explants as the selection of suitable explants is important for applying effective *in vitro* selection techniques, As such different explants type *viz*., mature and immature cotyledons, hypocotyls and seeds were attempted to establish successful *in vitro* regeneration system. These explants were subjected to establish culture system with applying MS as basal medium supplemented with different concentration(s) of plant growth regulators in diverse combinationst5o achieve maximum *in vitro* response.

During present investigations, by using hypocotyls as an explantsfor callus induction with various genotypes, calli of very small size was evidenced. However, Dubey and Gupta (2014) were found callus induction whenhypocotyls explants was cultured on medium containing 0.5mgl^{-1} 2, 4-D in combination with 0.5mgl^{-1} NAA. Subsequently Lone *et al*. (2017) reported formation of callus in higher frequencies fromcultured hypocotyls with ammendation of culture medium with 2,4-D at varying concentrations*i.e*. 0.5, 1.0, 1.5, 2.0 and 2.5 mgl^{-1}.

For initiation of callus cultures by using cotyledons explants, mature cotyledons explants were cultured on MS media fortified with different concentrations of 2, 4-D *i. e*.1.0, 2.0, 3.0 and 4.0mgl^{-1}. Callus induction was started usually from the cut ends of cultured mature cotyledons sections. Higher *in vitro* response in terms of callus induction was observed from diverse Indian mustard genotypes inoculated on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D. Among the six genotypes, *Brassica juncea* var., GM-2 was found. It be most responsive genotype for callus induction (%) resulting with white brown in colour and friable in texture followed by genotypes RB50, CS-54, PM-30, Rohini and LES-39. Earlier Akmal *et al*. (2011) reported highest callusing frequency from cultured cotyledons on medium supplemented with 2.0 mgl^{-1} 2, 4-D. Lone *et al*. (2016) documented maximum callus induction with application of 2,4-D at the concentration in range of 2-2.5 mgl^{-1} from cotyledonary explants. Alam *et al*. (2014) and Nasrin *et al*. (2017) have documented that nature and color of callus was greatly influenced by concentrations of exogenous levels of 2, 4-D could be an accordance of present investigations.

During present investigation, seeds of various genotypes were cultured on MS media amended with different concentrations of 2, 4-D (1.0, 2.0, 3.0 and 4.0 mgl^{-1}) showed varied responsive. Higher *in vitro* response in terms of callus induction was achieved on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D. Among various genotypes NRCDR-2 was found the most responsive for callus induction resulted white cream and light in color and compact in texture followed by genotypes GM-2, CS-54, RB50, Rohini, LES-39 and PM-30. Similar to present observation, Dubey and Gupta (2014) reported callus induction and shoot proliferation using seedling explants.

Response to callus induction using 2, 4-D was more pronounced in terms of type, nature and colour of callus proliferated. The comparative genotypic efficiency in relation to shoot regeneration from callus cultures were followed the order of CS-54>GM2>RB50>NRCDR-2>Rohini>PM30>LES-39. Seed explants of various cultured genotypes on MS media supplemented with 0.5 mgl^{-1} BAP showed differential response in terms of callus and shoot development..Genotype RB50 was proved to be the most responsive for callus induction among seven genotypes where white and light in color and compact in texture callus was induced. Callus initiation frequency was recorded in order of PM-30, Rohini, GM-2, NRCDR-2, LES-39 and CS-54.The present observations are supported by earlier observations, where use of BAP evoked shoot differentiation. Munir *et al*. (2008) reported callus induction followed by shoot differentiation with application of1.0mgl^{-1} BAP, Trivedi and Dubey (2014) with 0.5, mgl^{-1}BAP Ahmad *et al*. (2016) with0.4 mgl^{-1} BAP, Khan *et al*. (2010) and Biswas *et al*. (2017) with 2.0 mgl^{-1}BAP and Kishore*et al*. (2016) and Nasrin *et al*. (2017) with 1.0 mgl^{-1} BAP.

The present results supported by earlier reports clearly point towards role of BAP concentration for initiation of callus and further shoot differentiation. Failure to induce organogenesis from callus tissue derived from hypocotyls and mature cotyledons explants is quite evident. Shoot organogenesis was only observed from callus mass proliferated from seed explants. The inclusion of BAP was found the most effective for inducing organogenesis. This clearly shows that cytokinin BAP utmost required to induce shoot differentiation from callus cultures. Callus having 1-2 green shoot primordia showed series of morphogenetic changes during subsequent growth and development. The green shoot primordia appeared on surface of callus and transferred to fresh media resulted 1-4 shoots after 4 weeks. The removal of these shoots and subsequent transferring on MS media supplemented with BAP resulted multiple shoot formation, elongation and development. The best result for organogenetic callus was observed on MS medium amended with 0.5 mgl^{-1}BAP. Genotype CS-54 was evidenced themost responsive for resulting morphogenic callus induction among seven genotypes. The degree of morphogenetic response was followed by genotypes GM-2, RB50, Rohini, PM-30, NRCDR-2 and LES-39. The best result for shoot induction was also obtained by using MS medium with 0.5 mgl^{-1} BAP. Genotype NRCDR-2 was produced maximum shootlets. The shoot differentiation among six genotypes was in order of RB50,> LES-39, >GM-2, >Rohini, >CS-54 and PM-30. The present results are supported by earlier results of Thakur *et al*. (2013). These workers reported highest average number of shoots per explant with application of 1.0 mgl^{-1} BAP in combination with 0.1 mgl^{-1} NAA and 10 mgl^{-1} $AgNO_3$,Gerszberg *et al*. (2015) reported shoot regeneration in higher frequencies with supplementation of 8.88 µM BAP in combination with 0.53 µM NAA. Kumar and Srivastava (2015) recovered shootlets with application of 3.5 mg^{-1} BAP and 0.019 mgl^{-1}NAA. Ahmad *et al*. (2016) found maximum shoot induction with 3.0 mgl^{-1} BAP. Kishore *et al*. (2016) recorded the highest frequency of callus initiation as well as shoot regeneration on basal medium amended with 1.0 mgl^{-1} BAP and 0.5 mgl^{-1}NAA and Lone *et al*. (2016) reported shoot regeneration with 5.0 mgl^{-1}BAP in combination with0.5 mgl^{-1}2, 4-D.

Among genotypes, genotype PM-30 was proved more responsive in terms of shoots/explants following by genotypes NRCDR-2, CS-54, GM-2, LES-39, RB-50 and Rohini. Shoot proliferation recorded was in order of PM-30, CS-54, LES-39, NRCDR-2, GM-2, RB50 and Rohini. Mean shoot length was recorded highest in genotype PM-30intimately followed byLES-39, CS-54, RB50, GM-2, NRCDR-2 and Rohini. The present results regarding BAP influenced shoot differentiation are supported by according earlier reports of Thakur *et al*. (2013) and Kumar and Shrivastava (2015) for shoots per explants, Dubey and Gupta (2014), Dhania *et al*. (2016) for shoots per explant and shoot length and Abrha *et al*. (2013) for shoot length where BAP has influenced better shoot regeneration.

On transferring well developed shoots on rooting medium, an efficient root formation was observed on MS medium supplemented with 0.5 $mg1^{-1}$IBAfrom genotypesCS-54 and GM-2 with maximum followed byRB50,NRCDR-2,Rohini,PM-30 andLES-39. Alam *et al*. (2008; 2009) supported the present observations for root induction in 1.0 mgl^{-1} IBA and 0.5mgl^{-1} NAA. Earlier reporters Alam *et al*. (2014) for root induction with application of NAA. Biswas *et al*. (2017) reported highest percentage of root induction with2.0 mgl-1 IBA and 0.5 mgl^{-1}NAA whereas Nasrin *et al*. (2017) observed efficient rooting on medium supplemented with 1.0 mgl^{-1} IBA.

Shoot transferred to MS media with IBA rooted. The frequency of root formation was recorded in order ofCS-54, GM-2, RB50, NRCDR-2, Rohini, PM-30 and LES-39. Average no. of roots/explants was recorded in CS-54, LES-39, GM-2, PM-30, RB50, NRCDR-2 and Rohini. Root proliferation/shootlet was recorded highest in CS-54 followed by GM-2, LES-39, RB50, NRCDR-2, Rohini and PM-30. Mean root length was noted maximum in RB50 followed by PM30, GM-2, LES-39, NRCDR-2, CS-54 and Rohini. Earlier observations have been observed by Khan *et al*. (2010) for number(s) of roots per shoot and Abrha *et al*. (2013) and Thakur *et al*. (2013) for number(s) of roots per shoot and root length supported present results.

5.6.1.2Cell suspension culture

Embryogenic callus (2.0 g) having good, friable nature and white brown in colour on transferring to liquid media (50ml) resulted embryogenic cell suspension cultures showing embryoids of two stages (globular and heart shaped) of RB50 variety after 35 days transferring to liquid media. However embryoid developmental stages were not observed in remaining genotypes. After 45 days, effect of 3.0mgl^{-1} 2, 4-D on cell growth was recorded. Maximum relative growth rate was attained on GM-2, followed by RB50, Rohini, NRCHB101, NRCDR-2, CS-54 and PM-30. The present results are in line with earlier researchers observations. Akmal *et al*.(2011) observed globular and heart shaped embryo after ten days of culture on embryo development medium. Patidar *et al*. (2017) recorded highest growth rate of embryogenic tissues with application of 2.0-3.0 mgl-1 2,4-D in combination with 0.5 mgl-1 BAP.

On transferring embryonic clumps on different concentrations and combinations of auxins and cytokinins, plantlet regeneration was achieved on MS media combined with BAP+NAA in PM-30 genotype. Efficient plantlet regeneration was observed in PM-30 followed by Rohini, RB-50, CS-54, GM-2, NRCDR2 and lowest in NRCHB101 using MS supplemented with BAP+NAA combination. On combining of 2, 4-D + BAP, Maximum plantlet regeneration was recorded in GM-2 followed by PM-30, CS-54, RB-50, NRCHB101, NRCDR 2 and Rohini. Earlier to present study Patidar *et al*. (2017) also reported efficient plantlet regeneration form embryogenic culture with amendation of 0.5 mgl^{-1}of BAP, TDZ and NAA.

Summing up the present results best callus induction was obtained when MS media was supplemented with 2, 4-D. Further regeneration *via* shoot organogenesis was achieved with application of BAP in combination with small amount of auxins. The supeority of cotyledon and seed as observed during present study clearly points towards that these two explants had higher organogenetic potential. Further, effectiveness of regeneration *via* callus was quite significantly correlated with genotype, explants type and PGRs. Further it was noticed during present investigations type and concludes that quantity of callus produced was positively correlated with the subsequent shoot induction.

5.6.2 *In vitro* selection and regeneration of somaclones

The potential and limitations of the technique of tissue culture for production of new genotype with desired characteristics for agriculture use having different status of yield parameters have been reported in considerable number(s) of plant species including *Brassica species.* Attempts to select low and high erucic acid lines are based on the assumption that cultured cells represent metabolically at least in part, the cells of the whole plant, metabolic pathways for pathway for particular metabolite (*i.e*. erucic acid) production also operate in cells and cell lines selected on the basis of particular metabolite yield and these lines derive regenerates with altered desired metabolite of interest.

During present experimentations, attempts were made to select the cell lines, estimate the erucic acid and select out low and high erucic acid containing line(s). The procedure and conditions for callus induction, shoot differentiation followed by plantlet regeneration optimized during present studies for various genotypes were utilized for *in vitro* selection studies *via* callus intervention and regeneration of ‘somaclones’ in two genotypes (CS54 and PM30)..Various factors evaluated including genotypes, explants (seed, hypocotyl and cotyledon) and plant growth regulators: 2, 4-D and BAP concentrations and combinations. BAP influenced shoot regeneration from callus. Genotypes (CS54 and PM30) showed efficient shoot differentiation from callus. Explants type effect is quite clearly evident seed explants showed highest shoot regeneration.

In the present study, 188 genotypes were assessed for fatty acid content and profiling. Erucic acid is mono-unsaturated omega-9 fatty acid denoted C22:1ῲ9. It is potent inhibitor of saturated long chain fatty acids. The Indian cultivars of Indian mustard have high amount of undesirable fatty acid and glucosinolates. As such any attempt to develop new varieties having low levels of erucic acid is highly desired for commercial exploitation. The present strategy where low erucic variants could be regenerated *via* callus cultures clearly indicates improvement of *Brassica juncea* could be possible by exploitation of cell line selection for recovery of somaclones having low erucic acid.

The present study is a first of its kind is interesting in regard of regeneration of low erucic acid variants which extend method to use *in vitro* selection study as an approach for *Brassica juncea* improvement. Since conventional breeding scheme have met with limited success to get low erucic acid content variants. Earlier to present attempt Roy and Saha (2006) has reported isolation of low erucic acid containing genotypes of Indian mustard through F_1 hybrid anther culture. According to them three plant in which erucic acid content was lower than parent cultivar of A_2 generation were identified. Further the environment and nutrient conditions can be controlled uniformity and precisely under tissue culture conditions and at a given time large number of somatic cell lines could be screened rapidly and regenerate variants. However earlier to present attempt application of *in vitro* selection methodology was in *Brassica* for salt tolerance (Shafiq, 1996).

The present investigation outcome extend various genotype lines and *in vitro* selection of callus lines using CS54 and PM30 and develop variants with low erucic acid through somaclonal variation provide good germplasm resource. This could be used in *Brassica juncea* breeding programme aiming towards enhancing the oil quality for nutritional and industrial applications.

CHAPTER - VI

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Development of cultivar with desirable quality traits is of priority area of agricultural research. In past, the research approaches have developed from selection to elaborate breeding methods but now today researcher is going through biotechnological interventions. Biotechnology gave virtually limitless resource for crop improvement.

In Indian mustard, the occurrence of erucic acid is considered as anti-nutritional factor for human consumption that cause toxic effects on the heart at high enough doses. As such there is need to minimize the erucic acid content and by employing biotechnological approaches like *in vitro* selection at cell level could solve this problem.

Indian mustard (*Brassica juncea* L.) displays a great polymorphism and is a source of different types of vegetables, condiment and oilseeds. It is major oilseed crop in India and M.P. The mustard seed is nutritionally rich having carbohydrate, fat, protein and also contains vitamins, minerals and water. Thus it is widely used oil in medicinal, cosmetic and leather industry. By these reasons, it is very demandable oilseed in demand is showing increasing trend by fulfilling demand and supply with good quality traits.

The present investigation entitled **"Genetic diversity analysis and characterization of low and high erucic acid genotypes using phenotyping, genotyping and *in vitro* selection in Indian mustard (*Brassica juncea* L.) Czern and Coss"** undertaken with an objective to identify low and high erucic acid containing genotype(s) and to study diversity at molecular level in Indian mustard genotypes. Study also included to estimate genetic variability, correlation and path coefficients analysis, genetic diversity analysis of morphological and biochemical traits and *in vitro* selection. The experiment was conducted with 196 genotypes of Indian mustard in Randomized Block Design with 2 replications in two different years at research farm of Genetics Plant Breeding, College of Agriculture, RVSKVV, Gwalior (M.P.).

The observations were recorded on five randomly selected plants in each entry and replication for morpho-physiological analysis and their mean values were used for statistical analysis. The morpho-physiological traits including days to 50 % flowering, days to maturity, plant height, number(s) of primary and secondary branches, length of main raceme, number(s) of silique per main raceme and per plant, silique length, number(s) of seeds per silique, 1000 seed weight, biological yield per plot, seed yield per plot and per plant and harvest index were studied. One-eighty eight genotypes were used for biochemical screening *viz*., palmitic acid, oleic acid, linoleic acid, linolenic acid, erucic acid and oil percentage. Biochemical analysis of 188 genotypes except oil percentage was conducted at Quality Lab, Genetics division, IARI, New Delhi. Oil extraction was conducted at department ofSoil Science& Agricultural ChemistryCollege of Agriculture, RVSKVV, Gwalior (M.P.). Forty-eight genotypes were considered for molecular diversity analysis and seven genotypes for establishment of callus and cell suspension cultures leading to *in vitro* selection for low erucic had at Plant Molecular Biology & Plant Tissue Culture Labs, Department of Plant Molecular Biology& Biotechnology, College of Agriculture, RVSKVV, Gwalior (M.P.). Furthermore, two putative somaclones obtained from callus cultures were further analyzed for biochemical assessment at Central Instrumental Laboratory, Department of Botany, BHU, Banaras (U.P.).

SUMMARY

Morpho-physiological characterization

1. Pooled analysis of variance over 2 years revealed the presence of considerable amount of variation among 196 genotypes for all the 15 morpho-physiological characters *viz*., plant height, number(s) of primary and secondary branches, length of main raceme, days to 50% flowering, days to maturity, number(s) of silique per main raceme and silique per plant, silique length, number(s) of seeds per silique, 1000 seed weight, biological yield per plot, seed yield per plot, and per plant and harvest index.
2. The highest genotypic and phenotypic coefficient of variation was observed for seed yield per plant followed by harvest index and number(s) of silique per plant.
3. High heritability and genetic advance as percentage of mean had possessed for seed yield per plot, harvest index, biological yield per plot, days to 50 %

flowering, length of main raceme, number(s) of silique per plant, seed yield per plant, number(s) of seeds per silique, 1000 seed weight and number(s) of silique per main raceme.

4. Genotypic and phenotypic correlation coefficient revealed that seed yield per plant had significant and positive correlation with number(s) of secondary branches and number(s) of silique per plant.
5. Genotypic and phenotypic path coefficient analysis had the maximum positive direct effect on seed yield per plant followed by number(s) of silique per plant, days to 50% flowering, harvest index, plant height, biological yield per plot, number(s) of silique per main raceme and number(s) of seeds per silique.
6. Fifteen principal components were found. Among them, first principal component had maximum total variance for the 15 morpho-physiological traits. Euclidean distance cluster analysis was used for diversity analysis among 196 genotypes which were grouped in 16 diverse clusters. The maximum inter-cluster distance was estimated between cluster 9 and cluster 7.The largest numbers of genotypes consisted of 40 in cluster 5. The cluster 8 had the highest mean value for days to maturity, number(s) of primary branches and number(s) of seeds per silique. Similarly; genotypes included in cluster 9 recorded the highest mean value for silique length and biological yield per plot. The cluster 11 revealed maximum values for length of main raceme, silique per main raceme and 1000 seed weight. The cluster 12 had high mean values for plant height, number(s) of secondary branches, seed yield per plot and harvest index whereas, the cluster 14 exhibited highest mean value for days to 50 % flowering, silique per plant and seed yield per plant. Therefore, based on the maximum genetic distance, it is advisable to attempt crossing of the genotypes from clusters 9 and 16 which may lead to broad spectrum of favorable genetic variability for yield improvement in Indian mustard.

Biochemical assessment

1. Analysis of variance for all the 6 biochemical parameters has been carried, where 188 genotypes differed highly significantly for all the characters including palmitic acid, oleic acid, linoleic acid, linolenic acid, erucic acid and oil percentage.
2. The highest genotypic and phenotypic coefficient of variation was observed for oleic acid followed by erucic acid and palmitic acid.
3. Heritability and genetic advance expressed as percentage of mean had possessed maximum for erucic acid, oleic acid, palmitic acid, linoleic acid and linolenic acid.
4. Genotypic and phenotypic correlation coefficient explained that erucic acid showed significant negative correlation with palmitic acid, oleic acid, linoleic acid and linolenic acid.
5. Genotypic and phenotypic path coefficient analysis had the maximum positive direct effect of palmitic acid on erucic acid,whereas highest negative direct effect on erucic acid was evidenced by linoleic acid, oleic acid, linolenic acid and oil percentage.
6. Six principal components were obtained for 6 biochemical characters.Among them, first principal component showed maximum total variance. Genetic divergence among 188 Indian mustard genotypes was studied using Euclidean distance cluster analysis, that was further grouped into 18 different clusters. The highest numbers of genotypes appeared in cluster 2, that consist 82 genotypes. Maximum inter-cluster distance was found between cluster 18 and cluster 12. The cluster mean for palmitic acid and oleic acid was highest in cluster 8 and 13, respectively. Similarly, cluster 13 had highest cluster mean value for oleic acid and cluster 16 had maximum cluster mean value for linoleic acid. The highest cluster mean were recorded for linolenic acid, oil pencentage and erucic acid by cluster 18, 10 and 12, respectively.
7. Characterization of low, moderate and high erucic acid among 188 genotypes of Indian mustard was showed that 9 genotypes showed low, 57 moderate and 122 high erucic acid content

Molecular characterization

1. Molecular diversity analysis using SSR markers revealed that total 50 primers screened in 2 randomly selected genotypes of Indian mustard. Out of 50 SSR primers 27 were found monomorphic and 23 were polymorphic. These 23 polymorphic primers were amplified in selected 48 Indian mustard genotypes. Polymorphic SSR markers produced 109 alleles with an average of 4.47 alleles per locus. Genetic diversity varied from minimum for SSR Na10-D07 to maximum for BRMS- 098.Polymorphism information content (PIC) value of the primers varied from minimum SSR Na10-D07 to maximum for primer BRMS- 098.The primers which showed highest gene diversity and PIC value was observed in BRMS- 098, which is representing 77% polymorphism probability of the marker. All 23 primers representing PIC value more than 50%, representing higher capability of diversity and polymorphic nature of the primers in these genotypes. The genetic relationships among 48 Indian mustard genotypes using SSR primers were classified into major 3 clusters comprising 4, 7 and 37 genotypes. Cluster 3 showed maximum 37 genotypes.
2. Screening of 48 *Brassica juncea* genotypes for erucic acid using 2 gene based SSR markers were also analyzed. A total of 10 alleles were found with an average of 5 alleles per locus for polymorphic SSR markers. Maximum major allele frequency was for Sal-SRK-I and minimum forFAE1 with an average of 0.40. Highest gene diversity was for Sal-SRK-I and minimum for FAE1. Maximum polymorphic information content (PIC) was 0.66 of Sal-SRK-I and lowest 0.64 of FAE1 with mean of 0.65.The primer which showed highest gene diversity and PIC value was observed in Sal-SRK-I, representing 71% polymorphism probability of the marker. Both primers representing PIC value more than 50%, representing highly polymorphic nature of primers, can further be used for screening purpose of these traits. The Phylogenetic tree was made based on scoring of 2 gene specific SSR primers. Major 3 clusters were formed containing 4, 4 and 40 genotypes, respectively. Cluster number 3 had maximum genotypes.
3. Molecular characterization through gene based 2 CAPS markers for erucic acid of 48 Indian mustard genotypes also performed. A total of 9 alleles were obtained with an average of 4.5 alleles per locus for polymorphic CAPS markers. Maximum major allele frequency was found for CAPS237 and minimum for

CAPS1265. Highest gene diversity was consisted for CAPS1265 and lowest gene diversity for marker CAPS237. Highest polymorphic information content (PIC) was found for marker CAPS1265 and the lowest for CAPS237 with mean PIC value 0.65. These primers which showed highest gene diversity and PIC value was CAPS1265, which is representing 74% polymorphism. Both primers showing PIC value more than 50%, so these primers having maximum polymorphic capacity and can be used in Indian mustard molecular breeding for this selected trait. Dendogram was done for the genetic relationships among 48 Indian mustard genotypes. Major three clusters were found *i.e.*, CI, CII and CIII. Cluster I were having 2 genotypes. Cluster II contained 42 genotypes whichhaving maximum numbers of genotypes and Cluster III had 4 genotypes.

4. The population structure of the 48 *Brassica juncea* genotypes was estimated for both SSR and CAPS primers. It was pooled analysis. The optimum K value was determined by using Structure Harvester sofware, where the highest peak was observed at delta K = 3. The number of sub populations (K) was identified based on maximum likelihood and delta K values, with into three groups using a membership probability threshold of 0.80. First population showed by green colour containing 10 genotypes. Second population (red) represented 8 genotypes and third (blue) of 11 genotypes. Admixture genome was presented by subpopulation of 5 genotypes green and red, 5 other genotypes represented green and blue, 3 genotypes showed all three population *i.e.*, green, red and blue and 4 genotypes red and blue subpopulation.

In vitro selection

1. Seven genotypes *namely*: CS-54, GM-2, RB50, NRCDR-2, Rohini, PM30 and LES-39 based on their plant growth, high yield and low and high erucic acid containing abilities were used for initiation of tissue cultures. Appropriate explants which could be used effectively for callus initiation and subsequent regeneration, varioussuitable explants *viz.,* hypocotyl, cotyledon and seed were utilized. Hypocotyl explants were used for callus induction in various genotypes on MS medium supplemented with 3.0mgl^{-1} 2, 4-D, but very small size callus and late response was found and eventually died.
2. Higher *in vitro* response and medium size of callus induction was resulted from cultured cotyledon explants inoculated on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D. Among the seven genotypes var., GM-2 was found the most responsive in terms of callus induction with white brown in colour and friable in texture callus followed by RB50 white cream in colour and friable and granular in texture, CS-54 cream and light in colour and compact in texture, PM-30 white in colour and loose in texture, Rohini white cream in colour and friable in texture

and LES-39 white brown in colour and of loose texture. These calluses were transferred in shooting media. But no response was recorded.

3. Higher *in vitro* response and largest size of callus induction was achieved using seed explants cultured on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D. Genotype NRCDR-2 was found to be the most responsive for callus induction among seven different *Brassica juncea* genotypes with white cream and light in colour and compact in texture callus followed by GM-2 cream in colour and friable in texture, CS-54 dark brown in colour and friable in texture, RB50 white cream in colour and friable in texture, Rohini brown in colour and friable and granular in texture, LES-39 brown in colour and friable in texture and PM-30 cream and light in colour and of compact texture.
4. Callus induction from cultured seed explants also observed on MS medium supplemented with 0.5 mgl^{-1}BAP. Genotype RB50 was proved to be the most responsive for callus induction among seven different genotypes resulting white and light in colour and compact in texture, followed by PM-30 cream light in colour with compact texture, Rohini light green in colour and of compact texture, GM-2 white in colourand compact in texture, NRCDR-2 white in colour and compactin texture LES-39 of cream, light colour and of compact texture and CS-54 of cream colour and compact texture callus.
5. Seed explants showed the best response for morphogenic callus induction.BAP was found stimulatory for morphogenic callus induction followed by shootlet formation by 2, 4-D + BAP and BAP + NAA.The best result for morphogenic callus induction was obtained using MS medium supplemented with 0.5 mgl^{-1} BAP. Among seven genotypes, CS-54 showed the most morphogenic callus induction followed by GM-2, RB50, Rohini, PM-30, NRCDR-2 and LES-39. The best result for shoot regeneration was also evident from MS medium amended with 0.5 mgl^{-1}BAP. Genotype NRCDR-2 proliferated maximum shoot regeneration followed by RB50, LES-39, GM-2, Rohini, CS-54 and PM-30. PM-30 was found to be the most responsive for average number(s) of shoots/explants followed by genotypes NRCDR-2, CS-54, GM-2, LES-39, RB-50 and Rohini. Shoot proliferation/shootlet had maximum in PM-30 followed by CS-54, LES-39, NRCDR-2, GM-2, RB50 and Rohini. Mean shoot length was recorded highest in PM-30 followed by genotypes LES-39, CS-54, RB50, GM-2, NRCDR-2 and Rohini.
6. The best result for root formation was recorded on MS medium supplemented with 0.5 mgl^{-1}IBA. Genotypes CS-54 and GM-2 were recorded maximum root formationfollowed by RB50, NRCDR-2, Rohini, PM-30 and LES-39 with *at par* performance. Average number of roots/explants was estimated in order of CS-54, LES-39, GM-2, PM-30, RB50, NRCDR-2 and Rohini. Root proliferation/shootlet was recorded highest in CS-54 and GM-2, similarly in LES-39, RB50, NRCDR-2, Rohini and PM-30. Mean root length was noted in order of RB50, PM30, GM-2, LES-39, NRCDR-2, CS-54 and Rohini.
7. Friable callus was found suitable for initiation of cell suspension cultures. Friable embrogenic callus (2.0 g) of various genotypes was placed in fresh 50 mlliquid

media containing 3.0mgl^{-1} 2, 4-D. Maximum relative growth rate was attained on genotype GM-2 followed by RB50, Rohini, NRCHB101, NRCDR-2, CS-54 and PM-30. Only two stages globular and heart shape stagesembroyids were observed from RB50 variety. These stages were not evident in remaining genotypes.

8. After 4-6 weeks, cell clumps/ embryoids were placed on different concentrations and combinations on regeneration media supplemented with auxin 2, 4-D and cytokinins (BAP and NAA) for regeneration. Maximum plantlet regeneration was achieved on the media containing BAP+NAA. In BAP+NAA media, highest plantlet regeneration was exhibited from genotype PM-30 followed by Rohini, RB-50, CS-54, GM-2, NRCDR 2 and lowest in NRCHB101. In 2, 4-D + BAP media, maximum plantlet regeneration was recorded in genotype GM-2 followed by PM-30, CS-54, RB-50, NRCHB101, NRCDR-2 and Rohini. Very small size of shoot regenerated from cell clump/embryoid which subsequently dried in due course.
9. Plantlet regeneration from seed explants *via* callus culture on MS supplemented with 0.5 mgl^{-1} BAP were evident from only genotypes CS-54 and PM30. These regenerants were considered as putative somaclones.
10. Putative somaclone derived from Soma clone of CS-54 showed plant height (115cm), primary branches (4), secondary branches (8), 50% flowering in 70 days, days to maturity after transferring in field condition (100 days), siliqua length (5 cm), numbers of seed per siliqua (8), numbers of siliqua per plant (130), biological yield per plant (13 g), seeds per plant (1040) and seed yield per plant (1.90g), In another somaclone obtained from PM30 line showed plant height (90cm), primary branches (2), secondary branches (2), 50% flowering in 68 days, days to maturity after transferring in field conditions (115 days), siliqua length (4.3cm), number of seeds per siliqua (7), numbers of siliqua per plant (140), biological yield per plant (20.86 g), seeds per plant (840) and seed yield per plant (1.6 g).
11. Fatty acid profiling of somaclone (CS54) showed variation of unsaturated fatty acid. Total 39 fatty acid components were detected. Percentage value of fatty acids ranged from 2.88 to 10.90. The highest content of fatty acid (10.90) was recorded for Ethyl 9,12,15-octadecatrienoate,9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and n-Propy1 9,12,15-octadecatrienoate at RT 10.4. The lowest content (2.88%) was recorded for Ethyl 9, 12, 15-octadecatrienoate, 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and n-Propyl 9,12,15-octadecatrienoate at 11.4 RT.
12. Fatty acid analysis of PM 30 somaclone revealed different fatty acid. Total 27 fatty acids were detected. The highest percentage (25.36 %) was observed for Glycidyl oleate, 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester and 9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E) at 21.39 RT. The lowest percentage (1.62%) was recorded for 1-Heptacosanol, Hexacosyl pentafluoropropionate, Hexacosyl heptafluorobutyrate, 17-Pentatriacontene, Oleic acid, 3-(octadecyloxy)propyl ester and Octadecane, 3-ethyl-5-(2-ethylbutyl) at 11.99 RT. Erucic acid content of mother plant and soma clones estimated in

CS54 and PM30 lines. It was record that plants regenerated through *in vitro* selection. Erucic acid content was recorded 5.48 % as compared to erucic acid of mother plants of CS54 (41%).

Conclusions

The following conclusions have been drawn with results of morpho-physiological, biochemical, molecular and *in vitro* selection studies:

Morpho-physiological studies

1. Analysis of variance over 2 years (pooled) data revealed that Indian mustard genotypes lines studied were highly significant for all the morpho-physiological characters showing existence of wide spectrum of sufficient genetic variation in the experimental material.
2. Seed yield per plant showed highest GCV and PCV followed by harvest index and numbers of silique per plant, which clearly points towards importance of these traits through selection.
3. Maximum heritability and genetic advance (as % mean) seed yield per plot, harvest index, biological yield per plot, days to 50 % flowering, length of main raceme, numbers of silique per plant, seed yield per plant, numbers of seeds per silique, 1000 seed weight and numbers of silique per main racemepointed out importance of these traits based on phenotypic performance for development of high yielding varieties.
4. Correlation coefficient (genotypic and phenotypic) showed that seed yield per plant had significant and positive correlation with number(s) of secondary branches and silique per plant. As such importance of these two traits while making selection based on seed yield per plant may be proved quite significant.
5. Path coefficient analysis based on genotypic and phenotypic basis, the maximum positive direct effect on seed yield per plant followed by number(s) of silique per plant, days to 50% flowering, harvest index, plant height, biological yield per plot, number(s) of silique per main raceme and number(s) of seeds per silique, indicating the true relationship. Therefore direct selection through these characters will be effective for yield improvement in seed yield per plant.
6. In principal component analysis, 15 principal components were found. First principal component had maximum total variance for morpho-physiological traits. Thus the results of principal component revealed wide genetic variability exists among these Indian mustard genotypes. Euclidean distance cluster analysis gave 16 clusters among 196 genotypes. The maximum inter-cluster distance was observed between cluster 9 and cluster 16. This suggest that the hybridization programme involving parents from these clusters is expected to give higher frequency of better desirable combination for development of useful genetic stocks.

Biochemical studies

1. Qualitative characters has been analyzed for analysis of variance with 188 diverse genotypes were found highly significant different for all the characters, it means significant mean squares due to erucic acid and attributing characters revealed existence of considerable magnitude of variability in the material studied for the improvement of various traits which suggest better chances for improvement of these traits through selection.
2. The highest genotypic and phenotypic coefficient of variation was observed for oleic acid followed by erucic acid, palmitic acid which clearly showed that variability existed in these characters.
3. Erucic acid, oleic acid, palmitic acid, linoleic acid and linolenic acid had maximum heritability and genetic advance expressed as percentage of mean. High heritability and GA was found for these characters. This pointed towards that heritability is due to additive gene effect and selection of such character(s) may be effective.
4. Correlation coefficient explained that erucic acid showed significant negative correlation with palmitic acid, oleic acid, linoleic acid and linolenic acid at genotypic and phenotypic level. This indicated that there is strong inherent association between these characters.
5. Erucic acid was directly affected by palmitic acid. It was explained by genotypic and phenotypic path coefficient analysis. In order to exercise a suitable selection programme it would be worth to concentrate on characters like palmitic acid.
6. Six principal components were found for qualitative characters in which first principal component showed maximum total variance. Euclidean distance cluster analysis, which was grouped into 18 clusters. Cluster 2 showed maximum numbers of genotypes (82). Maximum inter-cluster distance was observed between cluster 18 and cluster 12. Diversity study suggested that crossing between clustered genotypes may be effective.

Molecular studies

From the SSRs and CAPS study, it was evident that the intra specific dissimilarity was the main reason for genetic diversity because all primers representing PIC value more than 50%.

***In vitro* selection**

1. During present investigation, seven genotypes *namely*:CS-54, GM-2, RB50, NRCDR-2, Rohini, PM30 and LES-39 were considered for *in vitro* studies. Three types of explants *viz*., hypocotyl, cotyledon and seed were used for callus induction and further regeneration.
2. Genotype GM-2 was found the most responsive for callus induction using mature cotyledon explants on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D.

3. Genotype NRCDR-2 was proved the most responsive for callus induction among seven *Brassica juncea*genotypes using seed explants on MS medium supplemented with 3.0 mgl^{-1} 2, 4-D.
4. Callus induction in higher frequencies from seed explants was observed in genotype RB50 inoculated on MS medium supplemented with 0.5 mgl^{-1}BAP.
5. The best result for morphogenic callus induction was found in genotype CS-54 using seed explants on MS medium supplemented with 0.5 $mg1^{-1}$BAP among seven genotypes. Highest mean shoot length was recorded in genotype PM-30.
6. Genotypes CS-54 and GM-2 were recorded maximum root formation on MS medium with amended with 0.5 $mg1^{-1}$IBA. Mean root length was noted higher in genotype RB50.
7. In cell suspension culture, maximum relative growth rate was attained on genotype GM-2. Globular and heart stages of somatic embryos were observed in genotype RB 50
8. Maximum plantlet regeneration was obtained in genotype PM-30 on MS medium supplemented with 1.0 mgl^{-1} BAP in combination with 0.5mgl^{-1} NAA from cell clumps/somatic embryoids obtained from cell suspension cultures.
9. Plantlets wereregenerated from seed explants *via* callus culture on MS medium supplemented with 0.5 mgl^{-1} BAP genotypes CS-54 and PM 30. These regenerated plantlets were considered as putative somaclones as they survived till maturity stage in field conditions.
10. Total 39 fatty acids were detected in somaclone CS 54, among these acids, erucic acid content was recorded 5.48 % as compared to erucic acid of mother plants (41.36%) of CS54 and total 27 fatty acids were found in PM 30 somaclone with no erucic acid content.
11. The present investigation extend various genotypes and low erucic acid containing somaclones regenerated *via* callus, clearly suggest that improvement of *Brassica juncea* could be possible by exploitation of cell line selections for recovery of somaclones having low erucic acid content in *Brassica juncea* breeding programme aiming towards enhancing the oil quality for nutritional and industrial applications

Suggestions for further work

On the basis of present investigation, the following suggestions could be made to plan out future breeding programme in Indian mustard:

1. On the basis of mean performance of morpho-pysiological traits, some germplasm lines *viz.,* MRNJ 37, MRNJ 103, MRNJ108, MRNJ 118, MRNJ 121, MRNJ 133 and varieties *i.e*. RVM 1, JM 1, JM 2 and JM 3 may serve as ideal donors for improving seed yield of Indian mustard.
2. Based on low and moderate erucic acid found in some varieties and germplasm lines like PM 25, PM 2, PM 28, PM 21, PM 22, PM 24, PM 29, 30, LES 39, MRNJ

8, MRNJ 25, MRNJ 30 MRNJ 126, IDM 67 and ISC-12 may be used further in molecular / traditional breeding programmes to develop low erucic acid containg varieties.

3. Further another biochemical assessment can be done to study the chemical compositions.
4. Highest oil containing genotypes were PM 28, CS 54, RB 50, Rohini, JM1 and NRCDR 2. These genotypes can be used in improvement programme for high oil containing Indian mustard genotypes.
5. Based on results of genetic variability, heritability, genetic advance, correlation and path analysis related to morpho-physiological traits, it is suggested that the genotypes with days to 50% flowering, plant height, number(s) of secondary branches, length of main raceme, number(s) of silique per main raceme, number(s) of silique per plant, number(s) of seeds per silique, 1000 seed weight, biological yield per plot, seed yield per plot and harvest index may be utilized to improve the seed yield per plant (g) of Indian mustard genotype through hybridization and selection.
6. On the basis of maximum genetic distance, it is advisable to attempt crossing of the genotypes from cluster 9 with the genotypes of cluster 16. From the study of cluster analysis for morpho-physiological traits those diverse parents belong to cluster 9 *viz.,* MRNJ-26 and cluster 16 *viz.,* GM-2, JD-6 and ISC-23 may be used in mustard breeding programme.
7. Genetic variability, heritability, genetic advance, correlation and path coefficient analysis for qualitative traits, it is applied that the genotypes with palmitic acid, oleic acid, linoleic acid, linolenic acid and oil percentage may be objected to improvement of low and high erucic acid of Indian mustard genotype through selection and/or crossing programmes.
8. In cluster analysis of qualitative traits, maximum inter cluster distance was observed between cluster 18 (Karishma) and cluster 12 (Maya). Thus, it may be used in mustard breeding for improvement of qualitative traits.
9. All 23 random SSR primers and gene based SSR and CAPS primers representing PIC value more than 50%, representing higher capability of diversity and polymorphic nature of the primers in these genotypes may be used for screening of large numbers of germplasm lines.
10. Germplasm lines *viz.,* MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6, MRNJ-26 and MRNJ-25 were grouped with notified varities *viz.,* PM-25, PM-22, PM-21, PM-29, PM-24, LES-39 and PM-30 using random SSR primers and these varieties released for low erucic acid, timely sown and irrigated condition, wider adaptability, high temperature tolerant, early sown and tolerance to white rust. Thus, these germplasm’s genome may be

having these traits. Further these germplasm lines may be used for validation of these traits.

11. Cluster 2 having two germplasm lines *viz*., MRNJ-5, MRNJ-6 was classified through gene based SSR primers with varieties RH-749 and RB-50. These varieties were notified for rainfed, irrigated and salinity conditions. So, these two germplasm lines may serve these characters. In future, these two germplasm lines could be utilized to evident for these characters.
12. In cluster 3, germplasm lines *viz*., MRNJ-17, MRNJ-15, MRNJ-14, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-18, MRNJ-24, MRNJ-23, MRNJ-20, MRNJ-19, MRNJ-1, MRNJ-22, MRNJ-21, MRNJ-2, ISC-3, MRNJ-25, MRNJ-16, MRNJ-11, MRNJ9, MRNJ-4, MRNJ-3, MRNJ-8, MRNJ-7, L-6 and L-4 had combined with varieties PM-21, PM-22, PM-24, PM-25, PM-29, PM-30, LES-39, NRCDR 2, Maya, JD 6, JM 2, Rohini, RVM1 and RVM 2 using gene specific SSR primers. PM series for low erucic acid, suitable for timely sown, irrigated conditions, wider adaptability, high temperature tolerant, early sown and tolerance to white rust, NRCDR-2 for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity, Maya for irrigated condition and white rust resistance,JD-6 for drought (rainfed), suitable for early (September) and late (November) sowing under irrigated situations, Rohini for high oil content, drought tolerant and suitable for irrigated condition, JM-2 for rainfed condition and white rust resistance, RVM1 for early maturity and high oil contentand RVM2 for late sown condition and suitable for rainfed were released. Thus, all these traits could be presented in these germplasm lines. Further these germplasm lines may be helped for justification of these traits and will be used in molecular breeding programme.
13. Germplasm lines *i.e.* MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, MRNJ-23, MRNJ-21, MRNJ-24, ISC-3, L-4 and L6 were clustered through gene specific CAPS primers with RH749, Maya, JM-2, DRMR150-35, DRMRIJ-31, RVM-1, GM-2, PM-21, PM22, PM24, PM25, PM29, PM30 and LES-39 showing release varieties suitable for irrigated and rainfed condition, white rust resistance, high temperature tolerant, suitable for early sown, low erucic acid, timely sown, non-traditional areas, bold seeded, high oil content and high yielding variety, early maturity and tolerant to powdery mildew &*A. blight,* these germplasm lines falling along-with these varieties may be having these characteristics features. These genotypes show that there is great potential in the studied and it may be objected to validation for these traits which can be used in future breeding programmes productive genotypes.

14. Identification of genes responsible for erucic acid and designing of primers can be carried out using advance molecular tools and metabolic engineering.

15. In tissue culture technique, the best explant(s), genotype(s) and plant growth regulator(s)optimized conditions can be used for further mustard tissue culture

and transgenic breeding programme for tailoring low and high erucic acid containing line(s) and another trait(s).

16. Total 39 fatty acids were detected in somaclone CS 54 and 27 fatty acids were detected in PM 30 derived soma clone. Based on spectrum of oil composition variants may be exploited further for developing cultivars for improved plant growth, animal and human health. Alternatively, it could be utilized for characterization of variants for industrial and medicinal purposes.
17. In present study, two soma clones namely: CS 54 and PM 30 regenerated *via* callus cultures. Out of two CS54 showed somaclonal variation and represented erucic acid (5.48 %) as compared to mother plant (41.36 %). Thus, CS54 somaclone may be utilized in molecular breeding for improvement. Further *in vitro* selection strategy could be utilized for exploitation of *in vitro* induced variability for development of low erucic acid containing varieties in Indian mustard in future.

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Appendices

Appendices A: Mean performance for yield and its attributes in Indian mustard

Genotypes	ays to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches	No. of secondary branches	ength of main raceme (cm)	No. of silique per main raceme	No. of silique per plant	silique length (cm)	No. of seeds per silique	1000 seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Seed yield per plant (g)	Harvest index (%)
MRNJ-1	37.5	135.5	134.7	5.9	8.7	65.9	47.2	224.6	5.8	16.5	4.1	1118.2	164.9	9.5	14.8
MRNJ-2	36.1	135.1	138.8	5.3	7.6	64.4	52.3	206.1	5.5	13.8	3.7	1215.5	235.4	15.6	19.4
MRNJ-3	39.0	137.6	145.6	5.6	7.6	64.9	54.9	236.2	4.9	12.8	4.1	1261.8	154.9	8.3	12.4
MRNJ-4	32.3	131.2	134.3	5.6	10.3	65.4	56.0	209.5	4.7	16.6	4.2	1036.2	196.4	12.7	19.0
MRNJ-5	40.5	138.9	137.6	5.9	8.6	71.2	49.5	224.3	5.5	11.5	4.1	1390.2	149.4	8.4	10.8
MRNJ-6	36.6	135.7	132.6	5.7	6.7	50.1	52.5	194.6	4.7	14.8	3.5	1101.1	191.3	7.6	17.2
MRNJ-7	34.9	133.8	138.6	5.1	7.7	72.2	53.0	202.3	4.7	10.5	4.5	1145.6	288.3	10.0	25.4
MRNJ-8	34.9	134.1	135.8	5.4	9.2	57.6	55.0	125.9	4.2	12.3	3.5	1317.5	158.1	7.6	12.1
MRNJ-9	33.1	132.9	138.9	5.6	8.6	61.1	55.3	164.2	4.9	10.5	4.1	1288.6	163.1	7.4	12.6
MRNJ-10	30.9	129.7	138.6	5.6	7.1	67.5	47.9	182.1	5.3	12.0	5.5	1090.1	174.3	8.6	16.0
MRNJ-11	29.4	130.9	141.8	5.6	7.7	64.3	44.2	153.6	4.6	10.4	4.6	1234.7	155.2	5.6	12.7
MRNJ-12	27.9	141.9	138.1	5.5	9.3	65.3	42.8	175.6	5.1	12.8	3.6	1187.1	180.8	10.2	15.3
MRNJ-13	38.3	140.8	142.4	5.5	6.9	69.0	43.7	205.6	4.2	15.7	5.5	1354.6	178.3	12.8	13.1
MRNJ-14	33.8	139.6	142.6	5.6	8.9	66.9	44.5	191.6	5.1	12.8	3.7	1286.6	147.6	7.7	11.4
MRNJ-15	30.3	140.0	136.4	5.3	6.1	64.8	48.0	163.0	5.0	20.0	4.4	1357.1	225.4	6.8	16.8
MRNJ-16	32.6	143.9	136.8	5.9	6.9	64.6	51.0	143.4	5.3	17.8	4.2	1298.6	295.8	8.8	22.8
MRNJ-17	30.8	139.6	141.1	5.9	7.9	52.2	51.9	206.7	5.1	12.1	3.8	1339.1	200.5	10.1	15.3
MRNJ-18	32.8	141.4	141.9	6.1	5.9	68.3	50.2	158.1	5.4	14.8	4.6	1102.7	198.1	6.9	18.1

MRNJ-19	30.5	137.4	139.1	5.6	7.5	66.1	48.8	203.0	4.8	12.6	4.2	1399.6	179.8	11.7	12.9
MRNJ-20	29.3	142.0	139.8	5.3	9.2	64.6	50.5	229.3	4.7	13.9	5.3	1301.7	150.1	11.7	11.4
MRNJ-21	30.6	138.6	140.1	5.5	6.8	66.1	44.0	161.7	5.4	15.2	3.5	1182.7	163.5	9.7	13.9
MRNJ-22	27.9	135.9	145.1	5.2	6.6	84.7	42.9	208.2	4.8	14.0	5.0	1410.6	179.3	12.2	12.6
MRNJ-23	30.1	136.8	147.9	5.8	7.1	76.2	38.7	220.1	5.4	12.5	4.2	1423.7	163.1	8.6	11.4
MRNJ-24	29.6	126.2	146.4	5.1	9.1	64.6	38.4	159.4	4.2	12.8	4.4	1423.1	153.4	9.8	10.8
MRNJ-25	28.1	121.0	148.4	5.3	9.4	63.1	41.7	211.1	4.8	14.1	4.3	1147.9	272.8	10.6	23.8
MRNJ-26	32.9	130.4	139.6	5.0	8.4	67.8	47.7	162.3	5.7	14.0	4.5	1819.6	153.5	8.4	8.4
MRNJ-27	32.1	129.3	146.2	5.3	6.5	64.1	56.5	205.0	4.7	15.0	4.8	1483.9	192.8	11.9	13.2
MRNJ-28	32.8	129.9	143.2	5.1	8.4	81.8	49.8	118.6	4.2	13.6	4.6	1269.6	198.3	6.9	15.8
MRNJ-29	32.1	130.5	145.6	6.0	8.9	64.6	50.4	217.5	4.7	14.6	5.0	1271.0	189.9	11.0	15.0
MRNJ-30	30.3	130.6	143.8	4.6	8.5	69.0	41.2	193.2	3.7	15.5	5.3	1316.9	178.5	8.1	13.7
MRNJ-31	34.1	127.4	146.1	5.9	8.5	66.2	45.7	213.1	4.7	11.8	5.8	1351.5	151.3	16.3	11.4
MRNJ-33	27.6	122.8	144.1	4.6	5.9	65.7	43.7	222.4	4.9	13.8	4.6	1104.9	155.4	10.7	14.1
MRNJ-34	35.1	132.6	147.4	5.6	8.3	65.1	47.0	167.1	5.4	16.8	3.5	1576.7	160.0	11.5	10.1
MRNJ-35	29.9	129.4	147.1	5.3	8.4	67.4	62.5	177.6	4.5	12.7	6.5	1267.1	151.5	9.1	12.1
MRNJ-36	33.6	130.0	147.6	5.3	7.7	83.7	53.5	229.9	5.2	13.0	7.3	1364.0	271.5	10.6	20.0
MRNJ-37	39.7	137.8	150.4	5.8	6.0	57.9	54.7	208.2	4.5	14.2	4.3	1331.1	146.3	20.2	11.0
MRNJ-38	39.9	136.9	149.2	5.3	7.0	82.8	59.3	226.3	4.1	11.7	4.5	1274.9	206.9	12.2	16.3
MRNJ-39	33.9	132.6	149.1	5.3	6.9	74.6	50.8	166.0	4.8	14.0	4.4	1144.5	155.2	10.2	13.7
MRNJ-40	38.1	135.1	150.1	5.6	6.5	73.4	53.8	203.8	5.9	12.1	5.4	1184.0	248.5	13.6	21.2
MRNJ-41	31.8	129.6	149.7	5.3	6.8	66.8	52.7	220.1	5.5	13.0	5.9	1283.1	142.4	13.1	11.0
MRNJ-42	34.8	132.8	147.8	5.0	7.2	72.1	50.0	198.7	5.4	10.0	4.3	1373.7	171.1	12.8	12.5
MRNJ-43	39.3	135.8	149.1	5.0	7.9	66.1	51.0	212.1	3.6	14.9	4.6	1235.7	150.3	11.3	12.1
MRNJ-44	45.1	141.8	149.4	5.3	7.8	65.1	46.7	107.6	5.1	13.2	4.4	1088.2	154.9	7.5	14.4
MRNJ-45	39.0	136.2	149.8	5.8	9.0	84.7	45.5	144.0	5.4	11.5	5.1	1456.6	149.6	9.2	10.3
MRNJ-46	38.6	135.6	149.7	4.3	6.7	68.6	54.8	121.7	4.6	11.3	4.3	1282.7	164.3	12.6	12.9

MRNJ-47	40.6	137.7	149.6	5.6	8.3	67.8	49.0	233.1	5.2	11.7	4.4	1304.6	179.7	7.6	13.9
MRNJ-48	38.6	135.3	150.2	5.5	7.4	65.6	48.2	208.1	6.4	13.3	4.9	1412.1	156.5	9.9	11.1
MRNJ-49	34.6	132.4	149.8	5.2	7.0	65.7	56.0	117.8	5.5	13.8	5.4	1044.2	181.5	15.2	17.4
MRNJ-50	33.1	131.8	149.8	5.3	7.3	72.6	52.1	137.1	5.4	13.5	4.5	1119.1	190.8	8.4	16.9
MRNJ-51	32.9	133.4	150.1	4.3	6.8	71.9	46.7	116.2	5.5	13.1	5.3	1318.1	153.4	8.2	11.9
MRNJ-52	32.6	131.1	156.1	4.9	7.1	60.0	47.7	131.7	4.6	15.6	4.2	1188.1	145.7	10.8	12.4
MRNJ-53	8.1	134.1	153.8	5.3	6.8	71.8	40.7	222.3	4.9	10.1	6.1	1150.7	210.1	15.3	18.4
MRNJ-54	3.6	139.4	150.8	4.9	7.5	2.6	45.3	133.1	4.1	11.4	5.5	1028.3	177.6	9.9	17.3
MRNJ-55	9.8	137.1	155.1	5.7	7.7	9.4	50.8	141.2	5.1	13.7	5.2	1325.6	155.5	7.3	11.9
MRNJ-56	0.6	147.9	163.9	5.6	7.5	6.4	56.2	118.0	3.9	10.5	5.4	1350.6	177.2	8.9	13.1
MRNJ-57	9.6	137.1	155.4	5.3	7.0	1.7	60.8	131.9	4.7	10.5	4.9	1418.6	180.0	8.2	12.8
MRNJ-58	7.3	134.3	154.3	5.4	6.5	2.6	51.9	206.9	5.5	12.6	5.2	1323.2	175.1	11.0	13.2
MRNJ-59	4.8	132.8	154.2	5.4	7.8	8.2	55.8	134.3	5.0	14.3	4.7	1143.7	147.9	8.9	13.3
MRNJ-60	0.1	137.3	156.5	5.4	8.8	6.0	53.3	124.1	4.2	12.0	4.4	1388.1	449.4	11.3	32.8
MRNJ-61	0.8	135.8	155.7	5.3	9.0	3.0	52.5	109.6	4.7	12.6	5.2	1326.1	170.1	10.7	12.3
MRNJ-62	0.5	130.9	166.5	5.0	8.3	4.8	55.1	128.0	5.5	13.9	5.8	1378.6	154.5	8.7	11.3
MRNJ-63	5.0	132.6	151.1	5.0	7.3	3.2	46.2	114.9	5.2	14.4	5.4	1538.7	150.7	8.3	9.6
MRNJ-64	5.3	131.9	152.6	5.1	7.2	6.7	44.8	186.8	6.2	13.6	6.4	1290.6	181.0	10.2	14.2
MRNJ-65	6.8	133.6	152.0	5.4	6.6	2.6	42.2	134.7	4.6	12.0	5.8	1296.1	155.6	7.7	12.1
MRNJ-66	4.3	132.1	148.1	4.9	6.5	9.8	45.5	246.8	5.6	13.8	6.0	1316.1	149.1	6.7	11.5
MRNJ-67	8.3	134.9	143.9	5.4	7.6	8.7	40.6	134.5	5.2	13.3	4.7	1382.7	197.4	11.7	14.3

MRNJ-68	50.1	144.3	145.4	5.0	7.0	69.0	38.6	109.3	5.4	13.0	5.5	1367.7	210.2	7.4	15.6
MRNJ-69	6.5	132.6	150.0	5.7	8.6	9.5	42.8	147.6	5.2	12.4	6.1	1609.6	171.9	7.3	10.8
MRNJ-70	3.3	131.6	142.2	5.5	8.3	9.2	46.7	182.4	4.6	12.8	4.6	1422.7	161.9	8.4	11.3
MRNJ-71	2.6	130.8	149.2	5.2	7.7	6.7	60.8	161.9	5.5	12.4	5.1	1360.7	159.6	10.9	11.8
MRNJ-72	6.1	131.4	145.9	5.0	6.2	2.8	38.0	227.7	4.9	11.9	5.6	1146.6	170.4	12.3	15.2
MRNJ-73	0.4	136.3	147.4	3.9	8.8	4.6	42.6	293.4	3.7	13.9	5.8	1480.6	193.4	16.0	13.2
MRNJ-74	4.7	131.9	141.0	4.7	6.6	0.3	44.3	222.6	4.8	12.7	5.2	1541.0	183.3	9.9	12.0
MRNJ-75	4.6	130.8	147.6	5.4	7.5	3.0	48.4	169.6	5.9	13.5	5.6	1610.9	196.1	11.9	12.2
MRNJ-76	5.7	132.7	149.0	5.5	6.8	6.2	50.8	164.9	4.3	12.9	4.4	1101.9	185.5	14.8	17.4
MRNJ-77	0.9	144.7	147.2	5.3	6.2	7.8	55.0	135.6	4.3	12.9	5.7	1083.4	150.6	12.7	13.8
MRNJ-78	8.4	129.4	147.5	5.5	6.5	1.2	54.9	145.5	4.8	12.6	5.9	1254.7	150.0	9.8	12.0
MRNJ-79	6.6	138.1	145.7	5.6	8.0	4.6	58.0	187.3	4.5	12.9	6.6	1126.5	150.8	11.0	13.7
MRNJ-80	1.4	140.8	150.1	5.5	8.1	0.1	60.0	210.5	4.1	11.4	4.2	1315.2	187.9	13.2	14.4
MRNJ-81	7.3	139.1	148.6	5.3	7.2	2.8	55.7	187.6	4.8	12.6	5.9	1241.4	159.9	13.5	12.8
MRNJ-82	2.1	133.8	147.0	5.4	7.7	7.7	48.7	206.9	4.7	16.5	4.8	1462.5	179.5	10.6	12.3
MRNJ-83	7.8	139.1	147.6	5.3	6.5	4.2	49.1	146.4	4.1	13.5	5.9	1437.5	182.3	12.8	12.6
MRNJ-84	6.3	138.6	144.0	5.7	7.0	1.8	50.8	187.9	4.4	11.2	6.3	1489.5	183.9	13.2	12.5
MRNJ-85	0.3	142.1	139.8	5.3	7.7	5.3	50.0	228.6	4.3	12.9	5.4	1114.9	193.8	12.6	17.2
MRNJ-86	9.9	140.6	145.8	5.4	8.5	8.7	49.1	163.6	5.4	13.7	4.7	1201.5	164.6	14.0	13.7

MRNJ-87	5.0	136.4	142.8	5.5	7.0	9.2	57.7	179.9	4.7	11.6	5.2	1668.7	188.7	10.2	11.4
MRNJ-88	6.4	137.8	144.7	5.6	6.8	1.8	44.6	170.1	4.3	15.0	5.1	1206.9	176.5	7.6	14.8
MRNJ-89	3.1	134.7	143.7	5.1	6.1	3.2	52.7	143.3	4.2	13.2	6.5	1458.9	175.9	10.4	12.1
MRNJ-90	2.2	131.6	145.4	5.3	6.5	0.2	58.0	204.4	4.8	11.9	4.5	1135.0	151.0	8.1	13.4
MRNJ-91	4.3	136.9	144.6	6.0	8.1	8.1	46.2	131.6	4.1	12.3	5.0	1264.9	160.8	8.6	12.8
MRNJ-92	3.3	134.4	146.3	5.1	9.5	1.6	60.0	136.1	4.3	11.8	4.6	1062.5	145.6	15.4	13.8
MRNJ-93	4.6	136.3	146.0	5.8	7.6	1.8	47.7	129.4	4.3	12.7	5.4	1227.7	182.4	11.4	15.2
MRNJ-94	5.2	132.6	144.7	6.1	7.6	2.1	42.4	230.8	4.7	14.0	4.4	1308.9	149.4	7.5	11.4
MRNJ-95	4.3	130.5	143.4	4.8	7.8	2.2	39.3	137.7	4.0	15.3	5.3	1374.7	186.4	14.6	13.6
MRNJ-96	8.5	134.4	145.1	5.3	6.2	8.2	34.9	125.3	4.6	12.3	5.7	1216.6	190.5	7.2	15.8
MRNJ-97	6.0	133.8	144.1	5.3	7.1	0.9	42.3	122.1	5.4	12.7	4.2	1279.2	166.4	6.5	13.0
MRNJ-98	4.3	133.1	145.4	5.5	7.6	0.2	43.0	141.7	4.4	13.9	5.7	1109.9	158.3	8.7	14.1
MRNJ-99	7.0	133.2	147.3	5.0	6.8	2.8	39.3	144.4	5.3	17.4	4.2	1361.6	151.1	7.9	11.7
MRNJ-100	5.2	133.7	143.3	5.5	6.7	0.7	39.9	201.0	4.9	14.7	5.9	1434.4	169.4	11.6	12.0
MRNJ-101	4.6	131.1	144.8	5.1	6.7	0.2	39.2	112.3	4.1	15.5	6.1	1098.9	151.6	7.6	13.8
MRNJ-102	6.9	126.4	145.3	5.3	6.4	5.6	42.2	113.6	5.1	13.4	5.1	1190.8	134.7	11.2	11.5
MRNJ-103	9.5	137.4	143.7	4.6	8.5	7.7	33.5	218.9	5.5	12.6	4.3	1307.9	161.0	17.2	12.2
MRNJ-104	5.3	131.6	143.8	4.8	9.7	3.3	38.3	206.2	5.2	13.4	5.9	1185.1	152.6	9.1	13.0
MRNJ-105	4.3	130.6	144.9	5.4	9.5	5.6	36.7	234.9	5.2	16.4	5.1	1078.7	154.4	16.2	14.5

MRNJ-106	2.5	131.3	142.5	5.5	8.5	7.8	33.4	205.1	3.7	14.3	4.3	1293.0	145.1	13.7	11.1
MRNJ-107	7.3	134.3	145.6	5.5	8.0	3.2	40.5	212.0	4.9	12.5	4.2	1327.0	153.6	10.6	11.6
MRNJ-108	5.5	133.8	145.9	5.1	7.3	4.4	40.9	174.6	3.7	12.4	3.7	1321.9	150.0	17.4	11.5
MRNJ-109	2.0	131.3	143.5	5.2	8.3	3.7	45.8	125.1	4.7	15.3	4.9	1144.0	190.0	7.7	16.5
MRNJ-110	2.3	130.7	148.2	5.5	6.3	2.3	37.2	213.1	4.4	10.3	6.2	1319.0	206.2	9.6	15.6
MRNJ-111	6.2	133.3	145.3	5.0	8.1	3.7	44.0	165.0	3.9	12.2	4.5	1425.7	158.8	13.4	11.2
MRNJ-112	0.6	136.6	146.1	5.6	7.1	4.7	34.7	183.6	4.2	14.5	5.2	1433.0	148.9	16.2	10.3
MRNJ-113	9.8	137.4	144.2	4.7	8.5	8.8	44.4	225.7	4.0	10.9	4.6	1322.7	191.5	15.1	14.6
MRNJ-114	7.7	134.3	145.3	5.3	7.2	8.2	37.8	141.8	5.6	16.1	4.9	1141.7	174.9	8.9	15.3
MRNJ-115	7.2	133.1	142.4	5.2	7.6	7.5	40.5	148.1	5.4	16.3	6.1	1311.5	164.5	12.5	12.4
MRNJ-116	0.4	138.1	152.6	5.2	8.0	3.9	41.7	178.1	4.5	11.3	4.4	1221.9	138.8	8.4	11.5
MRNJ-117	9.6	133.4	151.8	5.5	7.9	2.2	40.7	213.0	5.4	12.1	5.8	1467.1	169.9	11.3	11.9
MRNJ-118	5.4	132.9	148.9	4.7	8.0	7.7	43.0	218.6	5.5	12.3	4.5	1465.4	154.8	20.3	10.6
MRNJ-119	4.4	130.8	146.8	5.1	7.0	5.6	38.3	231.4	5.2	12.4	5.1	1313.1	147.3	16.8	11.3
MRNJ-120	0.4	136.6	149.6	4.8	6.8	9.3	33.0	157.3	5.1	13.1	5.4	1093.5	165.0	9.9	15.1
MRNJ-121	5.5	132.1	147.6	4.7	8.0	5.2	32.9	207.1	5.4	12.8	5.3	1226.4	173.5	17.4	14.3
MRNJ-122	1.3	139.2	147.3	4.8	7.6	9.1	33.3	135.3	4.5	13.4	5.3	1127.6	176.3	6.8	15.5
MRNJ-123	0.3	138.4	149.7	5.1	8.8	5.7	30.0	151.4	5.1	13.7	4.9	1053.0	193.2	11.1	18.4
MRNJ-124	0.7	137.1	147.3	4.7	7.4	5.2	35.9	127.6	4.8	11.9	5.0	1175.8	150.5	8.0	13.1

MRNJ-125	7.5	135.6	149.3	4.2	7.6	9.7	38.4	210.8	4.4	14.4	5.2	1089.4	143.0	12.1	13.2
MRNJ-126	6.4	134.4	165.3	5.9	8.2	8.8	39.3	149.7	4.8	16.0	5.5	1267.8	155.2	8.5	12.5
MRNJ-127	4.2	131.6	172.0	5.2	5.9	2.6	35.7	134.9	4.4	10.0	5.8	1304.9	163.3	6.0	12.4
MRNJ-128	1.5	130.2	167.9	5.1	8.0	2.6	37.4	148.1	4.8	13.6	5.1	1202.4	154.2	11.1	12.8
MRNJ-129	2.1	130.5	165.7	4.4	7.6	1.7	37.0	225.6	4.3	14.0	7.2	1099.4	149.5	10.4	13.6
MRNJ-130	8.0	133.4	170.5	5.5	8.6	3.4	36.7	224.3	4.2	11.7	4.9	1379.4	145.2	10.2	10.6
MRNJ-131	6.5	132.9	161.0	5.2	8.2	5.3	39.7	215.1	4.2	8.9	5.4	1034.0	143.7	11.2	13.7
MRNJ-132	5.6	133.6	150.1	4.7	6.8	3.3	38.2	128.1	4.4	12.5	4.5	1333.4	153.9	9.5	11.6
MRNJ-133	9.9	128.2	151.3	5.6	8.9	1.7	41.0	213.9	4.5	13.5	3.9	1372.4	181.0	18.6	13.1
MRNJ-134	6.6	134.4	151.9	5.1	8.8	3.6	37.7	213.5	4.5	15.6	4.6	1239.9	149.3	16.0	12.2
MRNJ-135	4.1	131.9	153.0	4.7	7.0	5.6	33.4	218.6	3.5	14.5	5.5	1105.4	144.1	12.4	13.2
MRNJ-136	7.9	133.6	149.2	5.2	8.2	5.2	40.2	205.6	3.6	11.7	4.8	1453.4	183.2	11.4	12.7
MRNJ-137	5.5	131.6	153.1	6.0	8.1	3.2	38.5	156.6	4.4	13.1	5.2	1213.4	144.9	9.6	12.1
MRNJ-138	8.5	135.6	145.6	5.4	6.6	2.7	39.5	185.3	3.7	10.6	6.9	1136.9	148.4	7.6	13.1
MRNJ-139	9.0	136.5	154.4	4.6	6.5	3.7	33.3	163.9	5.3	13.7	6.8	1237.2	158.9	7.5	12.8
MRNJ-140	8.5	135.9	153.4	5.7	7.0	2.7	39.4	215.2	3.5	12.5	4.6	1322.4	155.5	16.7	11.7
MRNJ-141	9.0	135.1	155.8	5.4	8.3	1.8	40.9	219.3	4.4	14.1	6.5	1037.4	188.9	15.8	18.3
MRNJ-142	7.0	131.9	151.5	5.4	9.2	5.5	38.9	226.6	5.7	15.4	4.7	1300.4	153.9	15.3	11.9
MRNJ-143	7.0	133.4	148.6	5.3	7.6	6.1	37.0	254.4	5.3	14.8	7.7	1067.4	219.7	16.2	18.6

MRNJ-144	6.9	133.1	149.2	5.1	8.8	5.1	34.5	205.2	4.2	13.3	5.8	1074.3	186.8	14.2	17.8
MRNJ-145	0.2	137.7	150.3	5.0	7.4	6.9	34.3	233.1	4.4	12.8	5.5	1423.5	153.3	11.5	10.8
IDM-2	0.1	136.6	150.3	5.0	8.4	0.2	38.4	161.9	5.4	14.0	6.8	961.8	154.9	13.7	16.0
IDM-8	0.6	135.9	152.8	5.4	6.5	7.9	36.3	195.6	5.3	13.3	5.3	1111.1	173.6	12.8	15.9
IDM-10	2.8	145.3	150.6	5.5	8.8	2.5	31.7	185.5	4.0	12.9	5.3	1048.5	150.6	6.4	14.6
IDM-11	0.5	142.1	149.8	5.2	8.3	4.7	40.7	172.7	4.4	13.3	7.3	1160.1	185.4	10.3	16.3
IDM-12	0.8	135.4	149.8	5.2	7.8	9.6	37.4	212.1	3.8	11.6	6.8	1016.5	157.4	12.2	15.5
IDM-15	2.3	142.8	152.0	4.9	9.1	4.5	33.5	167.0	5.5	12.4	7.4	1170.9	127.5	11.2	10.6
IDM-16	0.3	135.1	149.8	5.5	8.1	2.3	44.0	223.9	4.5	12.4	6.7	1193.0	158.4	11.2	13.4
IDM-25	6.3	131.8	148.0	5.9	9.6	2.2	32.5	227.4	4.6	12.6	5.3	1121.9	187.9	8.8	16.8
IDM-31	9.5	137.2	151.6	5.5	7.4	2.2	32.2	206.9	4.2	12.9	4.8	1029.3	155.5	11.2	15.2
IDM-41	1.1	135.4	155.9	4.8	8.9	4.5	36.5	230.1	4.2	15.1	4.8	980.4	195.9	8.6	20.3
IDM-42	3.0	132.3	150.8	5.0	10.3	4.2	34.8	227.4	5.4	13.0	4.3	1155.9	155.8	12.4	13.4
IDM-53	5.8	134.1	148.1	4.9	9.6	2.6	35.4	243.2	4.2	13.0	6.1	1296.0	152.7	10.8	11.7
IDM-58	5.6	135.3	150.2	4.7	6.9	2.1	34.0	202.6	5.4	14.8	6.2	1057.6	192.1	10.5	18.5
IDM-64	9.6	135.2	153.1	5.7	6.8	4.2	36.5	230.1	4.7	14.4	7.4	988.1	155.1	9.0	17.1
IDM-66	1.0	134.1	152.6	4.9	9.0	0.9	43.0	242.9	4.7	14.1	6.1	1195.9	155.3	10.4	13.0
IDM-67	7.5	136.0	153.8	5.2	8.1	1.7	34.5	124.1	4.9	15.6	5.7	1240.0	163.6	6.4	13.1
IDM-69	7.0	134.1	150.4	5.4	7.1	4.2	36.2	118.1	4.5	13.7	7.3	1426.9	155.5	7.7	10.9

Rohini	5.8	132.2	153.6	5.9	7.8	2.0	39.3	232.0	5.2	12.7	7.1	1135.9	177.7	14.0	15.8
Maya	6.0	132.5	155.1	5.5	9.3	9.1	41.6	234.1	4.9	14.1	5.2	1014.4	180.5	13.9	17.6
RVM-1	0.2	129.9	152.6	4.9	8.2	0.7	41.3	221.6	4.8	13.3	5.5	1192.9	140.5	10.8	11.9
RVM-2	9.5	135.6	154.9	4.9	7.9	5.6	39.2	230.4	5.2	13.6	7.4	1182.6	151.8	17.7	12.9
JM-1	4.5	131.6	153.0	5.4	7.6	9.6	41.6	209.9	5.4	12.6	5.9	1080.9	171.4	17.5	18.1
JM-2	6.7	133.7	156.4	5.3	8.1	5.1	36.8	213.6	4.0	11.8	5.7	1269.9	161.0	17.0	12.9
JM-3	0.6	133.2	158.8	5.1	8.4	0.0	44.3	227.1	4.7	14.4	4.9	1227.2	187.1	19.4	15.4
NRCDR-2	6.0	133.1	158.8	6.0	7.4	5.6	40.7	160.6	4.8	13.1	5.9	1193.5	183.2	12.1	17.8
GM-2	9.6	135.3	157.6	5.1	6.1	3.2	40.5	180.6	4.6	13.0	6.1	817.5	192.2	10.6	25.2
JD-6	9.1	111.4	153.8	5.2	7.7	4.7	43.2	233.3	5.4	12.2	7.1	804.0	166.5	14.8	22.7
CS-54	8.3	129.3	156.7	4.7	7.4	1.1	47.0	215.7	5.7	13.1	5.7	1385.0	181.2	10.9	13.1
PM-26	0.5	128.3	125.9	5.8	7.5	8.0	45.0	232.9	5.1	13.7	6.8	1401.9	140.4	10.5	10.1
PM-25	7.5	129.2	112.0	4.8	6.7	1.1	40.5	233.2	5.2	10.3	4.9	1108.1	157.4	11.0	14.6
DRMR-150-35	5.0	131.0	151.8	6.0	7.6	5.6	51.8	134.4	5.3	14.0	5.5	1431.2	164.2	8.6	11.5
DRMRIJ-31	4.0	138.0	151.9	5.5	7.5	7.7	46.6	144.6	4.5	14.3	5.7	1096.9	173.8	11.2	15.9
RB-50	3.3	142.0	153.0	4.8	5.7	4.6	48.2	124.6	6.0	15.5	5.4	1414.5	175.3	9.4	12.6
NRCHB-101	9.0	132.9	152.0	4.5	7.3	4.2	44.5	134.1	5.0	15.3	4.8	1169.1	166.3	10.0	14.4
PM-28	7.0	130.5	158.7	5.2	6.5	8.6	46.2	155.4	5.1	14.0	6.4	1308.0	174.0	9.6	13.1
RH-74.9	9.0	139.8	152.6	4.8	6.9	7.9	49.8	158.5	5.5	13.3	6.4	1391.9	141.2	11.6	10.3

Kranti	3.8	133.0	145.5	4.3	7.0	1.6	50.6	182.8	5.5	12.7	6.6	1076.9	155.3	9.1	15.0
MC-25	7.6	139.6	155.2	4.8	7.6	67.8	52.9	244.6	5.0	15.1	5.6	1150.9	183.3	1.2	16.2
L-4	7.1	134.7	152.8	4.4	6.5	62.2	50.8	239.7	4.7	12.3	6.5	1006.0	165.0	3.4	16.5
L-6	0.8	134.8	155.4	4.7	7.5	63.1	48.2	175.5	5.6	12.5	8.1	1231.9	164.0	1.4	13.4
ISC-3	9.5	137.9	154.8	5.7	5.9	60.6	48.8	231.1	5.5	11.9	6.5	1323.9	164.7	2.0	12.4
ISC-12	9.7	136.4	150.7	5.3	6.8	64.3	49.3	217.8	4.7	12.3	6.0	1142.7	142.3	8.9	12.5
ISC-17	3.0	143.4	151.5	5.2	8.2	65.1	50.3	176.1	4.8	14.1	5.6	1332.4	153.5	8.0	11.4
ISC-18	4.0	139.4	151.0	5.4	6.8	55.4	41.2	223.2	5.5	13.4	7.6	957.4	145.8	9.3	15.6
ISC-20	5.7	135.3	151.8	5.2	7.8	53.6	47.9	137.3	4.9	12.8	6.2	965.5	179.3	11.0	18.5
ISC-23	6.0	141.1	156.0	6.0	6.2	55.8	50.8	128.1	5.0	13.2	5.8	842.8	147.7	7.9	17.5
PM-21	9.0	141.1	144.0	5.9	6.1	52.1	49.1	138.2	5.2	14.4	7.9	1143.5	150.2	8.1	13.5
PM-22	9.0	143.1	147.1	5.0	8.6	58.6	51.7	163.5	5.0	13.0	5.4	1048.8	181.3	10.3	17.5
PM-24	1.8	134.7	145.2	6.2	6.2	64.7	61.2	211.4	5.0	14.3	6.4	991.5	153.2	9.0	15.9
PM-29	9.6	142.6	136.8	6.2	6.2	55.3	57.6	228.0	6.0	11.3	6.7	1080.0	235.1	10.4	21.7
PM-30	5.3	133.2	152.6	6.4	8.3	64.3	50.3	137.6	5.7	13.8	7.9	1176.1	158.5	9.2	13.9
Karishma	8.5	134.4	152.0	6.5	8.3	83.7	55.4	206.5	5.8	12.1	6.4	1115.6	157.7	12.4	14.2
Mean	36.21	134.66	148.45	5.28	7.61	65.66	45.04	183.16	4.83	13.27	5.35	1242.44	171.53	10.98	14.14
CV	0.017	0.007	0.009	0.04	0.05	0.027	0.040	0.036	0.056	0.044	0.062	0.041	0.021	0.053	0.054

Appendices B: Biochemical parameters of Indian mustard genotypes

Genotypes	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid	Oil %
MRNJ1	7.4	13.8	25.3	11.6	36.0	31.5
MRNJ2	6.1	17.3	22.3	10.7	35.2	33.5
MRNJ3	7.1	18.4	25.0	11.7	30.1	34.5
MRNJ4	7.2	19.7	24.7	10.8	29.0	33.5
MRNJ5	7.6	19.6	23.0	10.7	29.2	37.0
MRNJ6	5.8	17.8	16.4	8.9	40.0	36.5
MRNJ7	7.8	14.3	26.0	11.7	33.9	36.5
MRNJ8	8.6	21.5	29.0	11.6	21.3	34.5
MRNJ9	7.4	22.3	26.2	11.0	25.2	34.5
MRNJ10	4.9	15.0	20.8	10.7	37.3	35.0
MRNJ11	4.8	13.9	20.2	10.3	37.6	32.5
MRNJ12	6.1	6.5	23.0	14.3	35.3	32.5
MRNJ13	4.7	18.4	20.5	11.3	33.7	33.0
MRNJ14	4.6	14.7	19.7	12.6	37.1	34.5
MRNJ15	5.8	14.2	19.7	12.0	38.5	33.5
MRNJ16	6.6	8.3	22.4	14.0	34.0	34.0
MRNJ17	5.6	15.8	21.8	11.0	34.0	36.0
MRNJ18	4.2	15.3	17.0	10.3	40.1	36.0
MRNJ19	5.8	10.3	20.3	10.0	39.3	34.0
MRNJ20	5.0	16.1	19.1	10.3	36.2	33.0
MRNJ21	3.9	15.5	19.8	10.4	35.8	34.5
MRNJ-22	4.0	14.6	18.3	9.3	37.9	31.5
MRNJ-23	4.9	8.6	21.1	11.8	40.4	30.5
MRNJ-24	4.4	15.8	18.4	11.8	37.3	32.5
MRNJ-25	5.3	26.8	25.1	11.4	21.5	34.5
MRNJ-26	4.8	16.2	20.3	10.4	35.4	35.0
MRNJ-27	4.7	18.3	23.1	11.4	29.7	37.0
MRNJ-28	4.2	19.1	21.2	10.3	29.9	34.5

MRNJ-29	4.3	20.5	20.4	11.1	32.7	32.5
MRNJ-30	4.2	32.2	23.1	12.0	16.1	33.5
MRNJ-33	4.8	16.3	19.9	10.4	36.2	37.0
MRNJ-34	6.5	14.4	24.9	11.2	30.4	35.5
MRNJ-35	5.5	19.8	21.1	10.2	34.8	36.5
MRNJ-36	5.4	17.5	19.4	9.2	37.4	37.5
MRNJ-37	5.5	19.2	20.2	10.0	32.7	37.5
MRNJ-38	7.4	10.3	23.3	9.9	36.8	34.5
MRNJ-39	6.0	14.7	24.0	9.9	33.5	36.5
MRNJ-40	5.7	19.3	21.1	10.6	31.3	38.5
MRNJ-41	7.0	10.5	24.0	9.2	37.2	34.5
MRNJ-42	6.8	17.5	22.0	8.3	35.1	35.5
MRNJ-43	6.3	19.9	22.6	10.0	30.9	36.0
MRNJ-44	6.1	17.7	20.9	9.8	35.1	37.5
MRNJ-45	7.4	18.4	28.6	10.1	25.9	38.5
MRNJ-46	5.7	18.1	22.8	10.8	30.3	38.5
MRNJ-47	5.7	16.4	21.6	9.4	35.8	36.5
MRNJ-48	5.7	17.4	21.1	10.8	33.8	35.0
MRNJ-49	7.4	17.7	25.6	9.8	30.1	34.0
MRNJ-50	6.2	22.1	23.8	10.0	25.5	35.0
MRNJ-51	5.3	14.7	19.9	8.9	39.6	36.0
MRNJ-52	5.7	15.4	20.4	10.7	35.3	35.5
MRNJ-53	6.2	10.2	21.0	10.1	37.3	34.0
MRNJ-54	6.1	8.8	19.7	9.0	42.4	33.0
MRNJ-55	6.3	15.7	21.9	10.1	33.4	33.5
MRNJ-56	6.8	10.7	20.0	9.2	38.4	35.5
MRNJ-57	6.7	12.5	19.1	9.5	38.0	37.0
MRNJ-58	7.6	16.4	24.2	11.7	29.6	35.5
MRNJ-59	7.5	10.5	27.3	11.8	31.1	32.0

MRNJ-60	5.7	14.3	21.2	11.6	33.6	31.5
MRNJ-61	5.2	12.8	21.9	11.2	35.9	32.0
MRNJ-62	6.2	15.2	24.9	13.0	28.5	35.0
MRNJ-63	5.7	10.5	24.0	14.1	33.7	34.5
MRNJ-64	4.4	14.4	19.3	12.7	36.1	34.0
MRNJ-65	8.1	18.3	27.1	13.2	24.0	34.5
MRNJ-66	6.1	10.6	23.7	13.2	34.1	36.0
MRNJ-67	6.4	10.0	24.9	12.4	32.2	32.5
MRNJ-68	6.1	16.0	23.4	12.1	29.5	30.5
MRNJ-69	5.3	15.8	20.2	10.3	34.2	32.5
MRNJ-70	6.4	8.7	21.2	12.5	37.1	34.5
MRNJ-71	6.8	16.3	23.0	12.3	31.1	34.5
MRNJ-72	5.4	16.7	18.8	9.8	39.1	30.5
MRNJ-73	7.2	18.8	22.9	12.2	29.2	32.5
MRNJ-74	6.3	16.3	21.6	10.4	32.3	32.5
MRNJ-75	4.8	15.3	19.4	11.0	35.1	30.5
MRNJ-76	5.7	16.3	22.4	11.3	31.0	31.5
MRNJ-77	5.6	14.5	20.3	13.0	33.3	33.5
MRNJ-78	4.6	15.3	19.9	11.9	36.3	32.5
MRNJ-79	5.5	15.7	18.7	10.9	38.2	32.5
MRNJ-80	6.3	18.8	22.9	11.0	30.3	33.5
MRNJ-81	7.2	22.5	22.8	10.1	26.5	35.5
MRNJ-82	6.2	17.2	23.9	12.9	28.7	37.5
MRNJ-83	5.4	14.1	18.8	10.6	38.3	35.5
MRNJ-84	6.1	15.2	21.8	11.0	35.4	33.5
MRNJ-85	5.8	16.0	23.6	10.9	32.3	34.5
MRNJ-86	6.7	16.8	22.3	9.8	31.9	31.5
MRNJ-87	7.4	16.2	24.9	10.3	30.9	31.5
MRNJ-88	7.1	17.8	23.6	10.9	30.9	32.5

MRNJ-89	7.4	17.6	26.0	11.8	26.3	33.0
MRNJ-90	8.4	10.9	29.7	13.0	27.1	34.0
MRNJ-91	5.8	20.2	22.0	9.6	29.4	33.5
MRNJ-92	6.7	22.5	24.9	11.9	23.8	31.0
MRNJ-93	7.5	19.0	23.9	11.0	27.1	31.5
MRNJ-94	8.2	13.1	25.5	10.9	31.3	33.0
MRNJ-95	8.3	13.1	24.1	11.1	31.3	31.0
MRNJ-96	7.5	17.4	23.7	10.0	31.1	33.5
MRNJ-97	8.5	12.7	27.7	12.0	29.1	35.0
MRNJ-98	8.8	9.6	27.8	11.3	31.2	20.5
MRNJ-99	6.4	16.3	22.1	11.1	32.8	36.0
MRNJ-100	6.0	14.5	21.6	10.0	37.0	36.0
MRNJ-101	5.3	16.0	20.7	10.2	35.3	33.5
MRNJ-102	6.5	17.8	22.6	10.0	33.0	34.5
MRNJ-103	7.4	18.8	25.9	9.9	28.0	35.0
MRNJ-104	8.1	19.8	26.7	10.0	28.1	33.5
MRNJ-105	8.2	18.9	27.6	10.8	26.3	34.5
MRNJ-106	5.5	18.1	22.0	10.0	33.0	33.5
MRNJ-107	7.0	19.5	27.3	13.3	23.6	32.5
MRNJ-108	7.2	10.8	28.1	14.3	27.2	33.5
MRNJ-109	5.8	18.5	23.5	12.2	28.3	33.5
MRNJ-110	5.4	16.4	20.8	11.6	34.4	35.5
MRNJ-111	7.6	22.0	26.2	10.8	23.9	35.5
MRNJ-113	7.5	21.1	26.9	12.3	23.4	37.5
MRNJ-114	8.3	19.7	28.0	10.8	24.9	38.5
MRNJ-115	7.4	16.1	24.2	11.8	32.8	37.0
MRNJ-116	7.1	18.9	24.2	11.8	27.8	36.5
MRNJ-117	7.3	18.4	25.2	13.7	27.2	35.5
MRNJ-118	6.5	17.1	23.0	11.3	32.8	35.5

MRNJ-119	7.4	18.7	25.0	13.2	25.2	31.5
MRNJ-120	8.8	11.9	29.4	13.2	26.9	32.5
MRNJ-121	7.4	19.1	25.0	10.4	29.1	33.5
MRNJ-122	10.3	11.7	32.6	12.3	27.1	33.5
MRNJ-123	8.1	13.7	25.1	10.0	34.0	33.5
MRNJ-124	7.8	9.7	29.3	10.8	30.7	32.5
MRNJ-125	5.0	9.9	20.0	10.0	41.7	32.5
MRNJ-126	9.7	14.4	32.8	10.2	20.7	33.5
MRNJ-127	5.4	17.1	22.2	12.4	29.8	33.5
MRNJ-128	7.4	10.7	28.0	14.3	26.6	34.5
MRNJ-129	8.3	8.9	27.3	14.0	29.7	35.5
MRNJ-130	7.1	19.6	21.1	12.7	32.1	36.5
MRNJ-131	8.1	22.3	23.7	11.2	27.0	37.5
MRNJ-135	9.7	12.0	28.0	13.4	24.9	32.5
MRNJ-136	9.4	11.8	27.6	12.6	30.7	33.5
MRNJ-137	8.3	20.8	24.0	13.2	26.6	33.0
MRNJ-138	6.4	17.8	23.7	12.3	29.7	35.5
MRNJ-139	8.3	11.0	25.0	12.0	33.9	34.5
MRNJ-140	8.9	12.5	28.6	11.0	28.9	35.0
MRNJ-142	9.4	11.5	28.2	12.3	30.6	36.5
MRNJ-143	7.4	16.8	23.0	13.7	29.4	37.5
MRNJ-144	7.2	17.0	22.9	12.8	30.3	35.5
MRNJ-145	6.1	11.8	18.9	10.0	25.9	36.5
IDM-2	7.3	14.7	25.9	13.9	30.2	35.5
IDM-8	8.3	15.0	26.8	10.6	30.0	33.0
IDM-10	8.9	13.7	24.9	9.0	37.2	34.5
IDM-11	8.6	13.7	25.9	9.6	34.7	33.5
IDM-12	9.0	13.1	28.7	11.0	32.8	36.5
IDM-15	7.6	14.6	27.1	13.9	30.4	35.5

IDM-16	7.2	15.2	23.9	11.8	36.2	34.5
IDM-25	8.2	11.6	22.6	10.7	42.6	32.5
IDM-31	8.5	14.0	27.7	10.1	34.7	35.5
IDM-41	4.6	16.9	19.9	10.9	43.8	36.5
IDM-42	7.7	17.4	28.4	11.0	25.6	33.0
IDM-53	8.1	11.2	27.0	12.1	35.0	34.5
IDM-58	7.2	20.0	22.0	10.9	33.8	31.5
IDM-64	8.5	11.0	26.0	12.2	33.8	33.5
IDM-66	9.2	9.9	26.5	9.4	34.7	35.5
IDM-67	13.3	11.0	35.6	11.2	22.2	34.5
IDM-69	7.6	12.0	25.1	12.9	30.2	32.5
NRCDR-2	7.4	10.8	26.2	13.0	36.7	40.5
NRCHB-101	8.1	9.7	27.3	14.0	36.2	38.5
DRMRIJ-31	7.1	10.9	23.6	12.9	39.9	37.5
DRMR-150-35	6.2	13.7	24.0	12.0	35.0	35.5
RVM-1	7.1	6.1	23.8	11.2	41.7	35.5
RVM-2	4.8	8.3	18.6	11.3	45.1	37.5
JM-1	5.3	12.3	18.9	10.4	46.2	40.5
JM-3	7.5	14.5	19.7	11.2	41.1	38.5
Rohini	4.0	16.0	16.8	9.7	37.8	40.5
Maya	4.5	6.1	15.0	10.1	51.4	38.5
GM-2	5.5	12.0	20.6	12.9	41.0	39.5
L-4	10.7	12.5	31.2	14.0	24.2	33.0
L-6	6.1	11.1	22.7	11.6	36.9	33.5
CS-54	5.9	12.9	21.7	12.1	41.4	40.5
RB-50	7.4	11.3	25.6	13.0	35.8	40.5
RH-74.9	5.1	14.1	23.7	10.0	42.3	39.5
MC-25	7.3	13.4	24.3	11.7	29.9	29.5
ISC-3	9.2	13.4	29.0	11.0	30.2	33.5

ISC-12	12.2	14.8	37.3	10.4	19.6	31.5
ISC-17	11.0	15.1	31.7	11.3	24.8	33.5
ISC-18	7.4	37.1	32.8	11.4	27.0	33.5
ISC-20	7.9	13.3	25.2	9.9	32.7	33.5
ISC-23	8.7	16.0	24.8	9.0	34.0	35.5
JD-6	5.6	9.8	20.3	11.2	31.7	39.5
PM-25	8.6	8.8	28.2	15.3	1.0	38.5
PM-26	6.3	10.1	25.2	9.8	1.6	38.5
PM-28	12.1	9.2	28.8	15.3	1.1	40.5
PM-21	10.0	12.1	30.8	13.1	1.1	35.5
PM-22	6.9	11.8	36.2	11.1	1.1	35.5
PM-24	6.9	22.8	31.0	14.0	1.2	34.5
PM-29	6.2	21.6	32.6	9.6	0.9	36.5
PM-30	5.5	25.6	26.4	13.3	1.1	36.5
Karishma	7.4	23.2	35.9	16.8	1.2	37.5
Mean	6.82	15.36	24.01	11.34	30.99	34.68

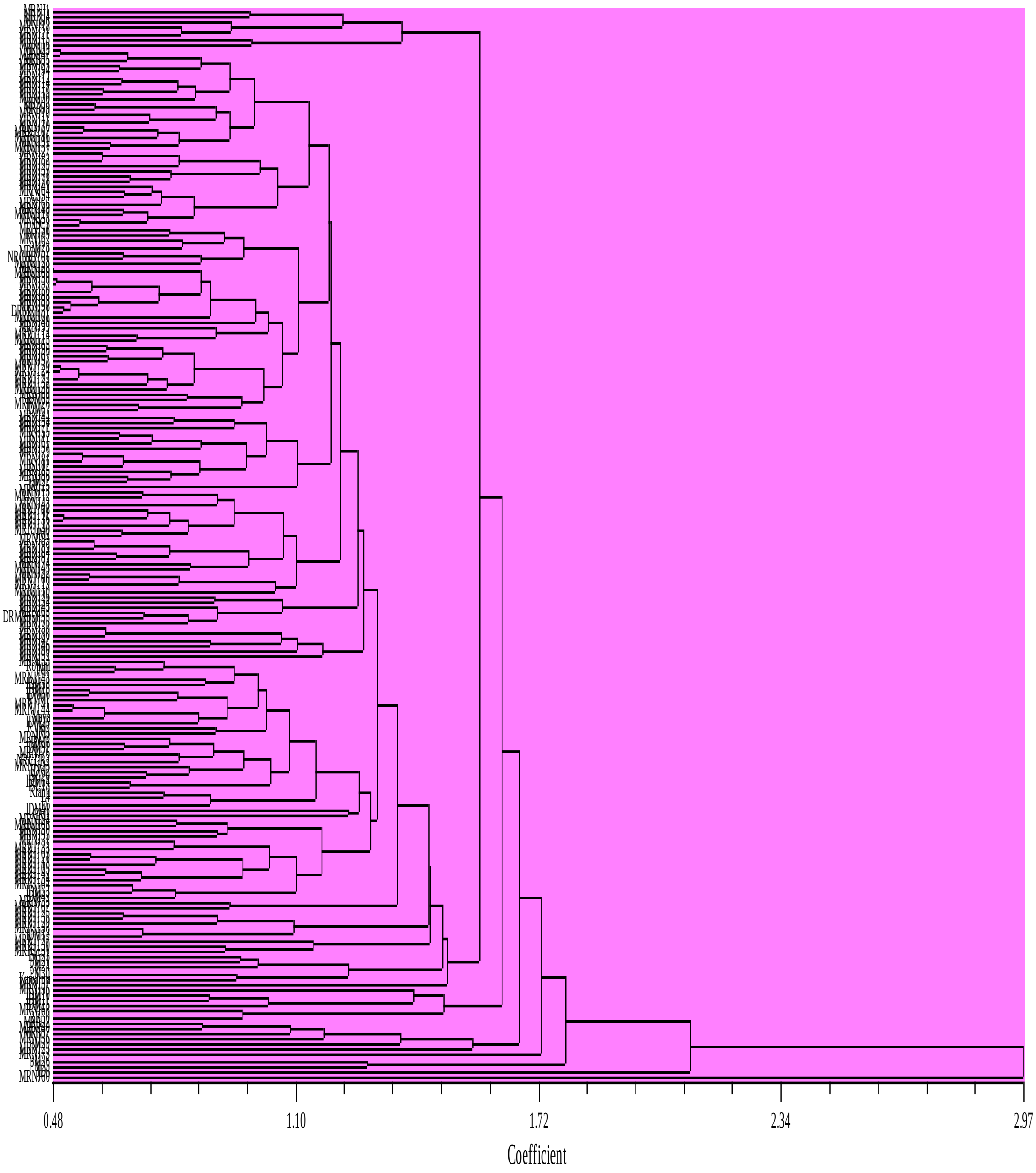
Appendices C: Variety Description of Indian mustard

. N.	Varieties	Specific traits	Release centre & year	Variety notified for State
	JM-2	Suitable for rainfed condition and white rust resistance	ZARS, RVSKVV, Morena (M.P.) 2005	Madhaya Pradesh and Chhattisgarh
	RVM-1	Early maturity and high oil content	RVSKVV, Gwalior (M.P.) 2013	Madhaya Pradesh and Chhattisgarh
	RVM-2	Suitable for rainfed and late sown conditions	RVSKVV, Gwalior (M.P.) 2013	Delhi, Rajasthan, Punjab, Haryana and Jammu
	Rohini	High oil content, drought tolerant and suitable for irrigated condition	CSAU A&T, Kanpur (U.P.)	Madhaya Pradesh, Chhattisgarh and Uttar Pradesh
	Maya	Suitable for irrigated condition and white rust resistance	CSAU A&T, Kanpur (U.P.) 2003	Madhya Pradesh and Uttar Pradesh
	NRCDR-2	Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity	DRMR, Bharatpur (Raj.) 2007	Delhi, Haryana, Jammu Kashmir, Punjab and Rajasthan
	DRMRIJ-31	Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety	DRMR, Bharatpur (Raj.) 2013	Rajasthan, Haryana, Delhi, Jammu Kashmir and Punjab
	DRMR-150-35	Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew & *A. blight*	DRMR, Bharatpur (Raj.) 2015	Bihar, Jharkhand, West Bengal, Orissa, Assam, Chhattisgarh and Manipur

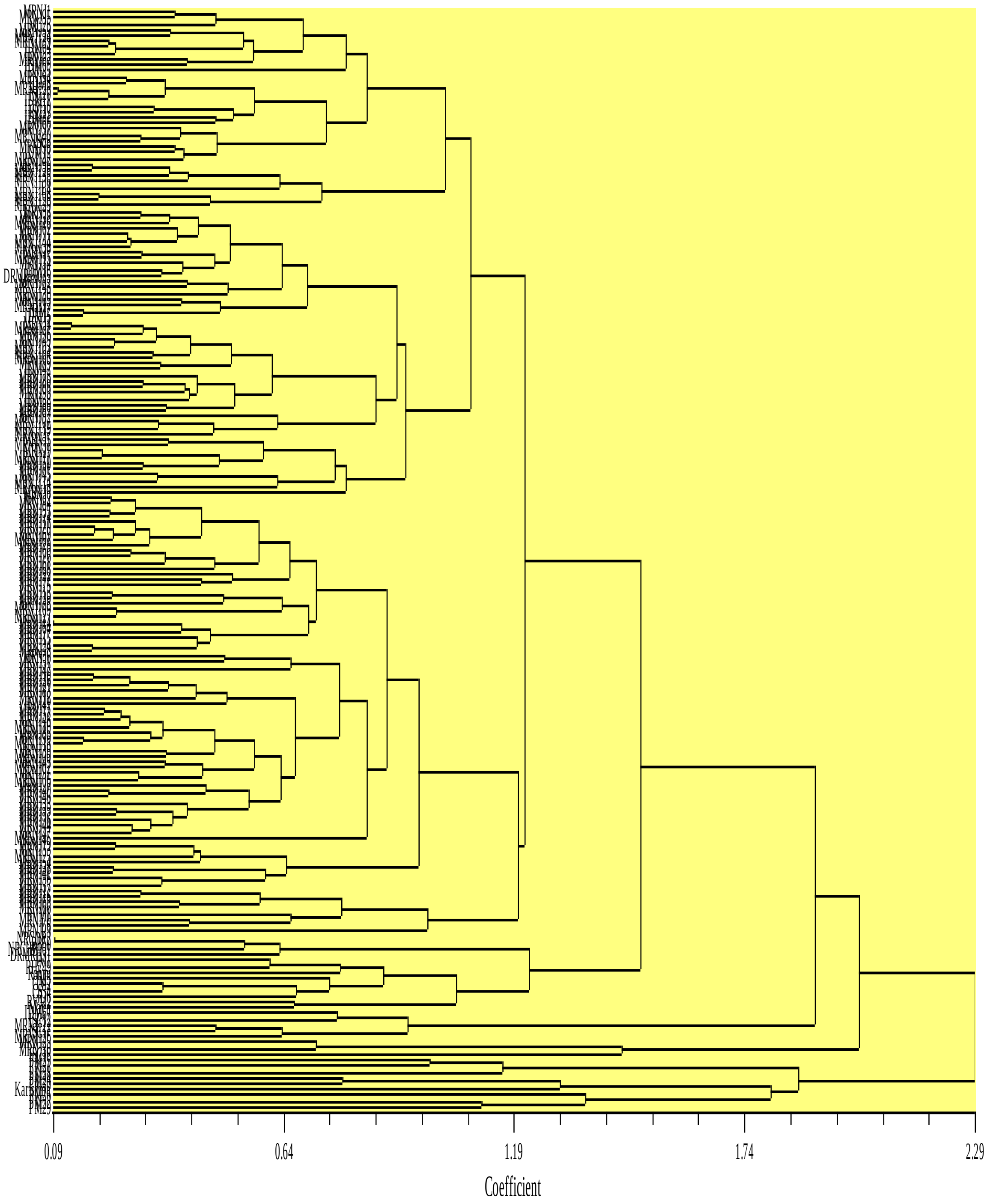
	GM-2	Nontraditional areas	SD *Agricultural University,* Banskantha, (Gujrat) 1995	Gujrat and Rajasthan
0	CS-54	Salinity tolerant	CSSRI, Karnal (Haryana) 2005	Haryana, Madhya Pradesh, Rajasthan, Uttar Pradesh and Gujrat
1	RB-50	Suitable for rainfed and salinity condition	RRS, CCSHAU, Hisar (Haryana) 2009	Delhi, Haryana, Punjab, Jammu Kashmir and Rajasthan
2	RH-749	Suitable for irrigated condition	CCSHAU, Hisar (Haryana) 2013	Jammu Kashmir, Punbaj, Haryana, Delhi and Rajasthan
3	JD-6	Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations	IARI, New Delhi 2005	Bihar, West Bengal, Orissa, Jharkhand, Chhattisgarh,
4	PM-21	Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability	IARI, New Delhi 2007	Rajasthan, Haryana, Punjab, Delhi, Jammu Kashmir, Plain areas of Himachal Pradesh, Uttar Pradesh, Uttarakhand, Madhya Pradesh and Chhattisgarh
5	PM-22	Low erucic acid, suitable for timely sown and irrigated conditions	IARI, New Delhi 2008	Delhi

6	PM-24	Low erucic acid, suitable for timely sown and irrigated conditions	IARI, New Delhi 2009	Rajasthan, Punjab, Haryana, Delhi, Plains areas of Jammu Kashmir and Himachal Pradesh and Western parts of Uttar Pradesh
7	PM-25	High temperature tolerant, suitable for irrigated conditions and early sown	IARI, New Delhi 2010	Rajasthan, Haryana, Punjab, Delhi, Jammu Kashmir and Himachal Pradesh
8	PM-29	Low erucic acid, timely sown and irrigated conditions	IARI, New Delhi 2013	Delhi, East Rajasthan, Punjab, Haryana and Jammu
9	PM-30	Low erucic acid, timely sown and irrigated conditions	IARI, New Delhi 2013	Uttar Pradesh, Uttarakhand, Madhya Pradesh and East Rajasthan
0	LES-39	Low erucic acid, timely sown, irrigated condition and tolerance to white rust	IARI, New Delhi 2005	Delhi

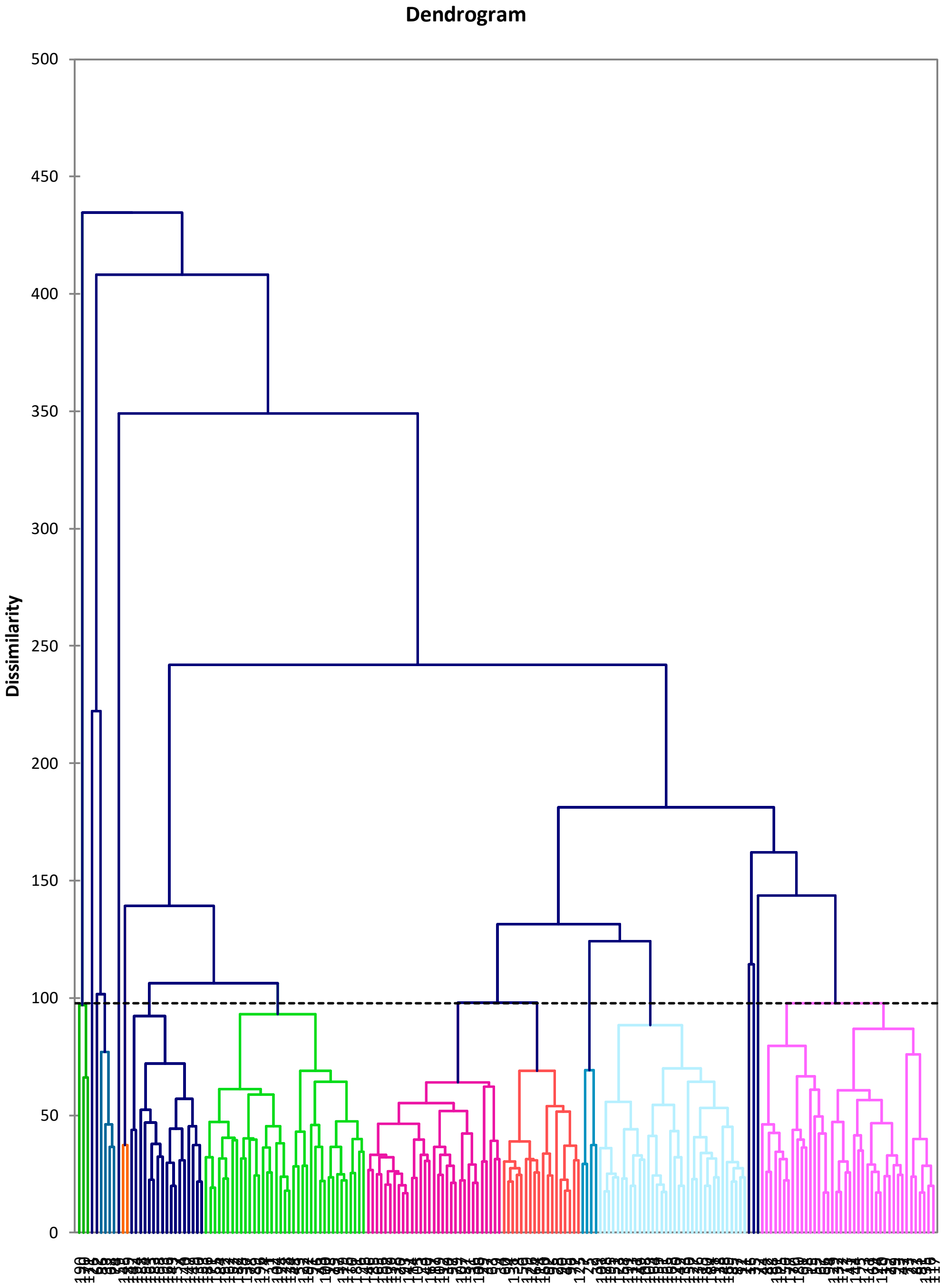
Appendices D: 2D Dendogram based on morpho-physiological



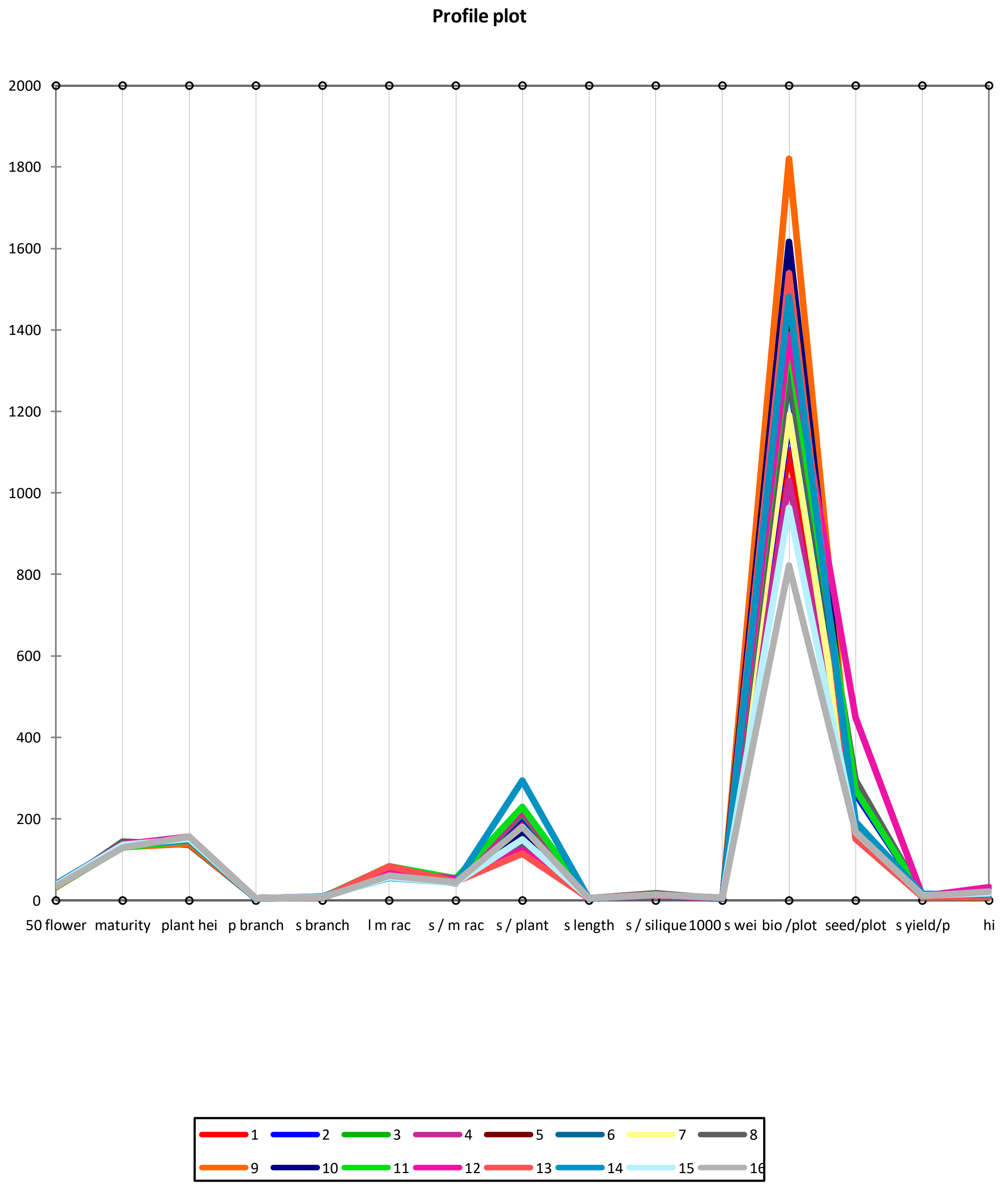
Appendices E: 2D Dendogram based on biochemical traits in Indian mustard using NTSYS-pc v 2.1 software



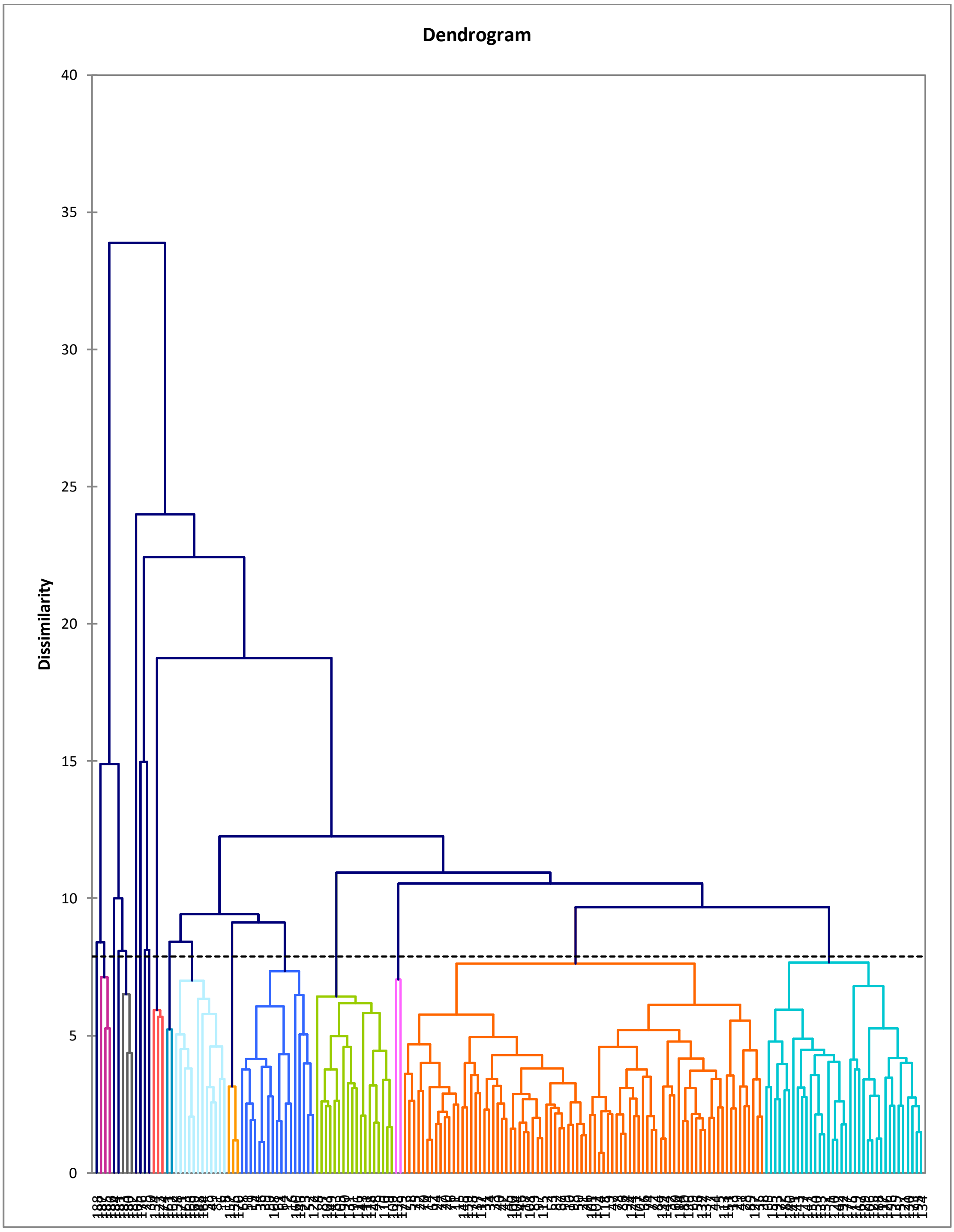
Appendices F: Dendogram of pooled Morpho-physiological traits using XLSTAT software



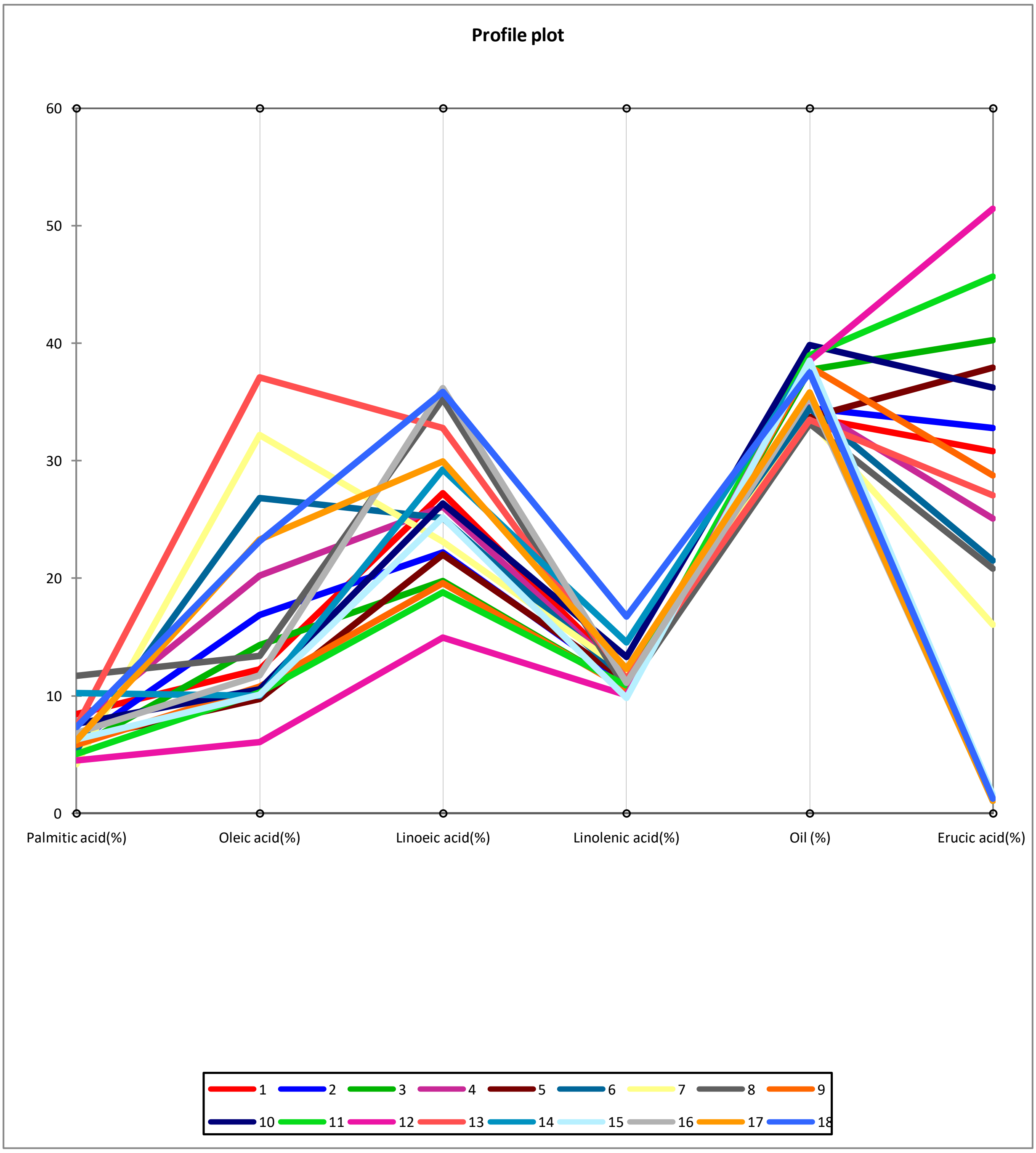
Appendices G: Profile plot of pooled Morpho-physiological data using XLSTAT software



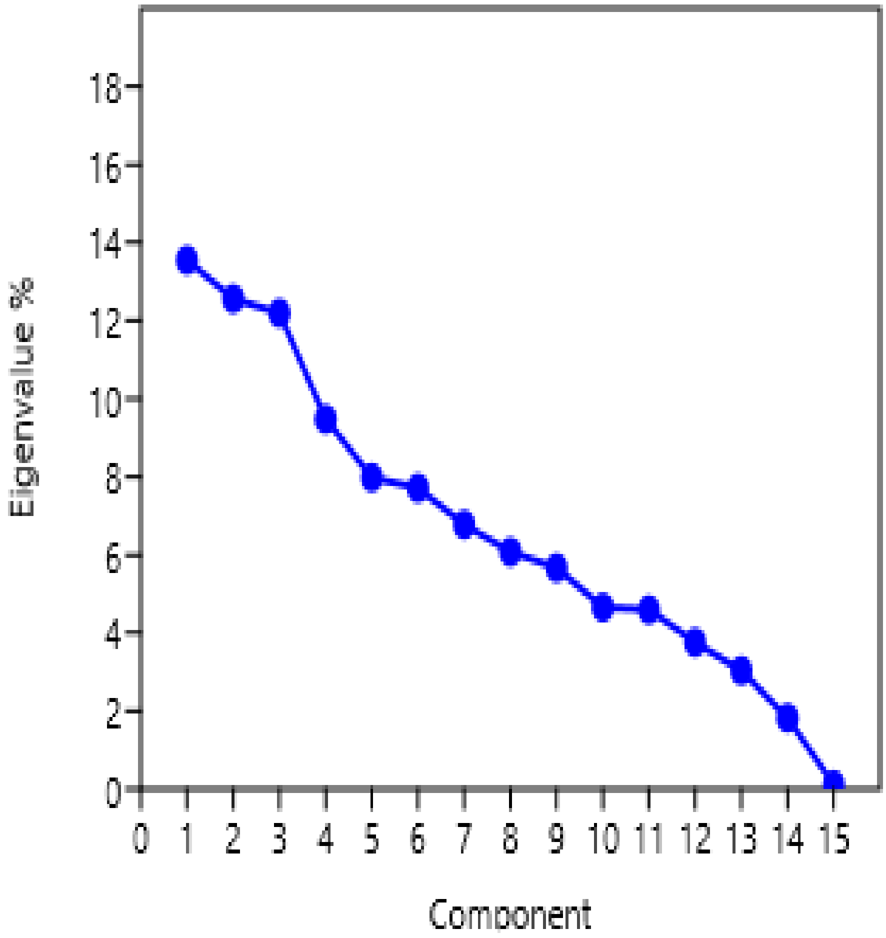
Appendices H: Dendogram of Biochemical traits using XLSTAT



Appendices I: Profile plot of Biochemical traits using XLSTAT software

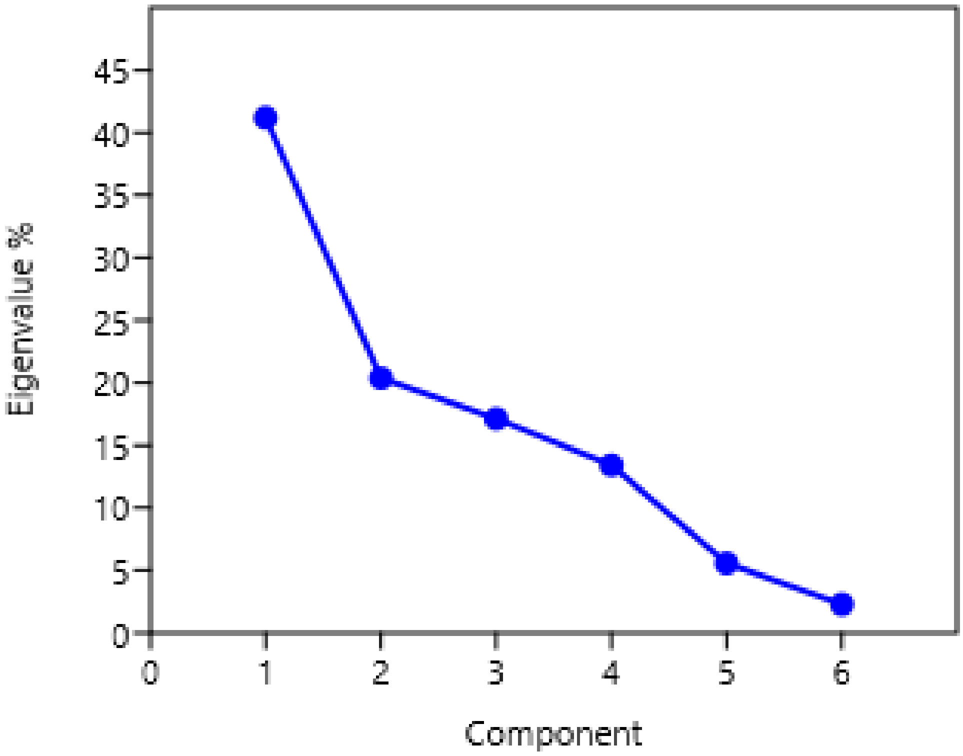


Appendices J: Graphical representation of eigen value % based on morpho-physiological traits in Indian mustard using



PAST v3.14 software

Appendices K: Graphical representation of eigen value % based on biochemical traits in Indian mustard using PAST v3.14 software



VITA

The author of the thesis **Chitralekha Shyam** D/o Shri Dhyan Singh Shyam was born on 05th June, 1991 at Bilaspur (C.G.).

I have passed High School Certificate (10th) in First division with 73.0 % marks during 2006 from Saraswati Shishu Mandir, Marwahi, Chhattisgarh Board of Secodary Education, Raipur (C.G.).

I have passed Higher Secondary School Certificate (10+2) in First division with 60.8 % during 2008 from Government Girls Higher Secondary School, Marwahi, Chhattisgarh Board of Secodary Education, Raipur (C.G.).

After schooling, i got admission for graduation. I have passed B.Sc. (Agriculture) obtaining 67.8 per cent in 2013 from IGKV, Raipur (C.G.).

After completing graduation, i was selected for Post graduation. I have passed M.Sc. (Ag.), Department of Genetics and Plant Breeding achieving 73.4 % during 2015 from IGKV, Raipur (C.G.).

After Post graduation, I admitted Ph.D. in RVSKVV, Gwalior (M.P.). I have passed all required courses in Ph.D. obtaining approximately 71.2 Percent.

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