GENETIC STUDIES OF OIL CONTENT IN MUTANT POPULATION OF RAPESEED (*Brassica Napus* L.) USING MOLECULAR MARKERS

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This dissertation is submitted by **Sher Mohammad** as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biotechnology and Microbiology

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AUTHOR'S DECLARATION

I solemnly declare that the research work presented in this thesis was carried out in accordance with the requirements of the University of Peshawar's regulations for Research Degree Programs. The author has not been submitted this work for any other academic award. The work is original and of author's own data. While work done in collaboration with, or with the assistance of, others, is indicated as such. The views expressed in the thesis, belongs to the authors.

Date_____ Signature_____

DEDICATION

I DEDICATE THIS HUMBLE WORK TO

MY PARENTS AND TO COMMEMORATE

TO MY ELDER BROTHER

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Sher Mohammad

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LIST OF ABBREVIATIONS

NIFA	=	Nuclear Institute for Food and Agriculture
KPK	=	Khyber Pakhtunkhwa
UV	=	Ultra violet
l	=	liter
*m*l	=	millimeter
μl	=	micro liter
DF	=	Days to 50% flowering
PH	=	Plant height
SW	=	Seed weight
SY	=	Seed yield
OY	=	Oil yield
OC	=	Oil content
PC	=	Protein content
GSL	=	Glucosinolates
OA	=	Oleic acid
LA	=	Linolenic acid
EA	=	Erucic acid
σ^2g	=	Genotypic variance
σ^2p	=	Phenotypic variance
PCV	=	Phenotypic coefficient of variation
GCV	=	Genotypic coefficient of variation
PCR	=	Polymerase chain reaction
NIRS	=	Near Infra red Reluctance spectroscopic System
PCA	=	Principal Component Analysis

h^2 = Broad sense heritability

ANOVA= Analysis of variance

MSg = Mean square of genotype

MSe = Mean square of error

LIST OF PUBLICATIONS

1 **Bashir Ahmad, Sher Mohammad,** Farroq-i-Azam, Iftikhar Ali, Javed Ali and Saeed ur Rehman. 2013. Studies of Genetic Variability, Heritability and Phenotypic Correlations of Some Qualitative Traits in Advance Mutant Lines of Winter Rapeseed (*Brassica* napus L.). American Eurasian J. of Agric. & Environ. Sci. 13(4): 531-538. (ISI Index)

1. **Bashir Ahmad, Sher Mohammad,** Javed Ali, Iftikhar Ali. 2013. Genetic variability studies of some quantitative traits in advance mutant lines of winter rapeseed (*brassica napus* L.). Accepted. Life Science Journal. (IF = 0.165)

ABSTRACT

The current study titled "genetic studies of oil content in mutant population of rapeseed (*Brassica napus* L.) using molecular markers" was partially conducted at Nuclear Institute for Food and Agriculture (NIFA), Peshawar in 2008-13. Main objective of the study were to intimate and study the influence of induce mutation on the genetics of oil and oil related components in advance induce mutant line of oilseed rape (Brassica napus). Two contrast mutant lines with high and low oil contents were crossed with each other and F_1 hybrids were developed. These F_1 hybrids were grown in the cropping season 2009-10 and BCF_1 were developed by crossing back the F_1 with their parents. These BCF_1 hybrids were grown in the next cropping season 2010-11 and selfed to develop BCF_2. The parent and the BCF_2 hybrids were used for molecular assessment to confirm mutation in the genetics of the advance mutant lines.

Thirty five advance mutant lines were utilized for agronomical and biochemical study. Five plants were randomly selected from each mutant line and the parent. Data on days to 50% flowering (DF), plant height (PH), 1000- seed weight (SW), seed yield (SY) and oil yield (OY) were recorded. Seeds were analyzed at for biomechanical parameter viz. Oil content (OC), protein content (PC), glucosinolates (GSL), oleic acid (OA), linolenic acid (LA), and erucic acid (EA).

Analysis of variance (ANOVA) for both agronomical and biochemical traits showed highly significant differences among the mutant lines and their respective parental line for all traits.

Broad sense heritability estimates were calculated for all of the agronomical and biomechanical traits. High heritability estimates were observed for DF, OY, GSL,

EA, and OA, moderate to high heritability was recorded for OC, PC, PH, 1000-SW and OA.

Multivariate taxonomic techniques were used to check the mutation pattern. Cluster analysis and principal component analysis (PCA) was done for all agronomical and biochemical traits. Mean of the parental line and individual mutant plants data were standardized prior to cluster and PC analysis. The results of cluster analysis for agronomical traits authenticated the selection. All of the advance mutant lines were distinct from the parental genotypes. Most of the mutant populations fell in separates cluster or isolated than the parental genotype if present in the same cluster. Genetic distances range from 0.00 to 5.6. Two types of scattering patterns were set as standards. In the first type, the parent fills in a separate cluster or was distinct from the mutant progenies. In the second case, some mutants showed deviation towards parent. Regarding biochemical data, three mutants viz OA5, EA4 and G1 showed deviation towards parents while rest of the mutants fell in separate cluster than the parent. The mutant individuals with desirable combination of traits were identified.

The PCA also confirmed the results for the cluster analysis with minor differences. Scatter plots of the PCs that had an Eigen value > 1 were produced to provide graphical representation of pattern of variation among the genotypes i.e. parent and advance mutant lines. Hence both of the analysis was used to check the mutation patterns. The traits DF and PH contributed positively while 1000-SW, SY and OY contributed negatively towards the variability. Regarding biochemical traits, OA and LA contributed negatively, while GSL and EA contributed positively towards the total variability in most of the populations. OC and PC showed mixed divergence pattern. DF along with GSL, OA and EA had remarkable impact on the variations.

In order to confirm the maternal effects and role of the B. napus in induce mutation, molecular assessment of two mutant lines was conducted. These populations were analyzed through simple sequence repeats (SSR) markers. Facilities at oilseed Laboratory of Nuclear Institute for Food and Agriculture (NIFA), Peshawar were utilized for this part of research. DNA was extracted using protocols of Doyle and Doyle (1987) and specific recipe and conditions were followed for Polymerase Chain Reaction (PCR) steps. Out of 25 SSR primers 19 gave positive results during initial screening. Out of 19 SSR primers, 15 gave consistent, bright and highly polymorphic bands. The product size of each primer set was compared with that of expected size given on Brassica Database Domain. Out of 99 amplified alleles detected, 69 were polymorphic. The proportion of polymorphic loci was 69.75. The number of amplified products ranged from 1-5 polymorphism information content (PIC) of the primer sets ranged from 0.24 to 0.75.

1.0 INTRODUCTION

Pakistan needs up to 75% edible oil to meet its domestic use successfully and in a better way. Whereas the tragedy of the situation is that the eatable oil yield is 25%. Keeping in view such a dire need, the country on the one hand, must devise a comprehensive strategy to expedite and increase oilseed produce, and, on the other, improve their quality. Only and only by following such an ambitious but undoubtedly practicable plan it can save its neck from the yoke of crushing import bill. Having been squeezed for extraction of oil, the Brassica seed contains 40% protein with a good source of essential amino acids. So by increasing the oil as well as meal protein not only save billion of import bill but also provide nutrient rich feed supplement for live stock and poultry industry.

Rapeseed and mustard consist of five species of the genus Brassica. Rapeseeds include *Brassica* napus and *Brassica* rapa whereas mustard consists of *Brassica* juncea, *Brassica* carinata and *Brassica* nigra. Rapeseed (*Brassica napus*) is mainly utilized as edible oil in many countries including Pakistan and produced about 21 percent of the total edible oil production all over the world [1]. There is enormous scarcity of edible oil in Pakistan mainly due to rapid increase in population. This increases the demand for oil in the country by 3-4% per year [2].

In Khyber Pakhtunkhwa (KPK) almost 3% of total cropped area is covered with rapeseed. The average yield of rapeseed in KPK is 472 kg ha^{-1}. This yield is even less than our national average yield. The oil content in cottonseed ranged from 10-12 percent, canola 42, rape and mustard 36-41, groundnut 48-50, sunflower 32-36, safflower 17-32, linseed 35-45, sesame 46-48, and in coconut the oil content ranged from 47-50 percent [3].

The oil contents in these plants are under the control of multiple genes and can be altered through induce mutation. About twenty-two hundred different mutant varieties with improved quality and other morphological characters in various crops have been established and distributed to the farmers [4, 5]. Knowledge of the relative level of various genetic parameters of oil yield, seed yield and quality characters are important for an excellent breeding program. A trait having the wider range of genetic variability, relatively high heritability and moderate to high genetic advance would be an effective mean to get better seed yield 6. Efforts are in progress to create rapeseed genotypes with improved quality characters. Genetic variability was estimated in some studies [7]. In order to make the selection more effective, both heritability and genetic advance will be considered instead of heritability alone [8].

Besides increasing yield, improving quality of the oil seed crop is also very essential. The oil seed will be considered to be of good quality if it has high protein, oil, oleic acid and low linolenic acid, glucosinolates and erucic acid [9]. The oil content ranged from 30 to 45% in different *Brassica* species. The oil of *B. campestris* contains high levels of glucosinolates (80-160 μMg^{-1}) and erucic acid (40-50%) in total fatty acids which make it undesirable for human and animal use [10]. The *B. napus* on the other is consider as a good source of edible oil with a low content of glucosinolates and erucic acid and a high content of polyunsaturated fatty acids [11]. The oilseed meal also produces about 40% will balanced protein [12]. Erucic acid is an important mono-unsaturated fatty acid ($C_{22}H_{42}O_2$) and is found in edible oil. High amount of erucic acid increases blood cholesterol level. It accumulates triglycerides in heart resulting in the development of heart lesions [13]. Glucosinolates, a group of organic compounds that contain sulfur and nitrogen, are found in rapeseed and mustard oil and meal. They produce characteristic pungent smell in the meal which

reduces palatability of feed. Glucosinolates also produces toxic effects because they contain goitrogenic and other antinutritional properties [14]. Linolenic acid is one of the few polyunsaturated fatty acid present in rapeseed oil. Linolenic acid disturbs the oxidation stability of oil as it is readily oxidized reducing shelf life and frying stability of the oil [15]. Winter oilseed rape, with low linolenic acid content and high oleic acid content is of great interest for industrial as well as for nutritional purposes because of enhanced frying stability of oil [16].

There is an urgent need to develop new varieties, containing high oil and oleic acid contents and low levels of erucic acid and glucosinolate. Induced mutation is one of the few major techniques which have been broadly used for producing new genetic changes in different crop plants. About twenty-two hundred different mutant varieties with improved quality and other morphological characters in various crops have been established and distributed to the farmers. Mutagenesis technique has also been effectively employed in oilseed *Brassica* for modifying the genetic makeup of plants and to isolate the possible mutants with desired qualitative and quantitative components such as oil content, glucosinolates, grain yield, plant height, 1000- grain weight and disease resistance [17].

There is great need to screen out the mutant lines to the desire level of qualitative and quantitative components. Near infrared reflectance (NIR) spectroscopy, has been extensively applied as an alternative technique to gas chromatography for the analysis of fatty acid profile in many oilseed crops. The main species studied to date are *B. napus*, *H. annus*, and *Brassica carinata* [18, 19].

The oil content in rapeseed is controlled by multiple genes and shows high heritability and negatively correlated with protein content, which means an increase in the amount one component will decrease the other component [20]. The reason is that

they utilize same precursors and are controlled by same genes [21]. These are quantitative traits and controlled by multiple genes with additive and epistatic gene action [22]. The recent advancements in molecular marker techniques provide the possibilities to trace gene behavior at individual gene loci, which greatly helps to understand the complex genetics of quantitative traits [23]. QTLs identification for oil seed and meal protein will help to understand the genetic control of these traits and their relationship with other seed qualities such as glucosinolates, seed color etc [24].

Molecular markers are nowadays efficiently used for exact estimation of genetic diversity and determination of uniqueness of crop genotype [25, 26] and enhance traditional breeding programs to improve crops [27, 28]. A number of molecular markers have been developed to assess genetic diversity and discriminate between genotypes for many different crops. The study of genetic variation, genetic mapping and marker assisted breeding have previously utilized many molecular markers such as RFLP (Restriction Fragment Length Polymorphism) [29], RAPD (Random Amplified Polymorphic DNA) [30], AFLP (Amplified Fragment Length Polymorphism (AFLP) [31] and SSR (microsatellites or Simple Sequence Repeats (SSR) [32].

The extent of required genetic polymorphism, the analytical or statistical approaches available for the techniques application and the cost of materials are the factors that account for the selection for proper molecular techniques [33].

The member of genus *Brassica* is characterized by an increased level of phenotypic as well as genetic variations [34]. The abundance characterizations the usefulness of simple sequence repeats (SSR) markers in *Brassica* species is already known. SSRs were studied in several crops including *Brassica* [36, 37]. These studies

shows that SSRs are a valuable tool for characterizing germplasm and MAS (marker assisted selection) in *Brassica* species because they are highly informative, robust, numerous, technically simple and suitable for automatic allele detection.

The present study was there fore conducted to address the induce mutation in advance mutant line of rapeseed Brassica. A combination of agronomical, biochemical and molecular techniques were employed to estimate the extent of variation regarding oil and oil related components in the mutant population.

1.1 AIMS AND OBJECTIVES

The aims and objectives of the present studies were to:

1 Determine the genetic diversity of oil and oil related components in advance mutant lines.

2 Evaluate the parent and thirty five advance mutant lines for agronomical and biochemical assessment to confirm genetic variation through induced mutations.

3 Estimate heritability for agronomical and biochemical traits in advance mutant lines.

4 Study of genetics of oil content in BCF_2 hybrids of advance mutant lines using SSR molecular marker.

2.0 REVIEW OF LITERATURE

Plant breeder is always interested in the development of such crop plants that have desirable characteristics. The desirable outcomes are either an increased yield or quality crops. In order to achieve the target, plant breeder adopts many strategies to combine desirable traits from two or more parental species to superior genotypes than its parents in many aspects.

Estimation of genetic parameters in the context of traits characterization is an essential component of future targeted crop improvement programs. Collection of knowledge about behavior such as genetic variability, and heritability etc of the germplasm is the step for initiation of any breeding program. Morphological traits, seed proteins, enzymes and several types of DNA markers are various available techniques, which allow of the genetic variability of crop germplasm. Many methods are used for estimation genetic diversity and relationship in germplasm which rely on pedigree, morphological, economic, biochemical and most recently molecular data [37].

2.1 HERITABILITY

Heritability of any trait depends upon genetic properties of breeding material and environmental conditions in which the experiment are carried out [38]. Heritability studies serves as a useful tool in the assessment of transfer of parental characters to the progeny. Hence selection of the parameter that is more under genetic control may reduce the time span of the breeding program.

Total variability is divided into genotypic and phenotypic variability. The evaluation of genetic variability for yield and its components is a necessary for

enhancement of the crop to the desired level. Genotypic variability consists of two types of variances viz additive and non-additive variances. Heritability in broad sense was described as the ratio of genetic variability to the total variability, while describes heritability in narrow sense as the ratio between the total genetic variance to the additive variance [39, 40].

The research work of Burton and Devane expressed the genetic gain for a specific character as the product of its heritability, phenotypic standard deviation and selection differential [41]. Although, heritability value described the comparative effectiveness of selection based on phenotypic expression of a trait, the genetic advance proved to be more useful in describing the actual values for selection [42].

High narrow sense heritability were reported for oil content (58.15 %), while studying the genetic components of variance of oil content, protein and minerals. High heritability as well as high genetic advance was reported for many characters in mustard [43]. Harisingh described that highest heritability was recorded for secondary branches followed by seed yield, 1000 seed weight and seliquae per plant [43]. Low to medium heritability was recorded for primary branches per plant and high heritability for secondary branches per plant and siliquae per plant in mustard [44].

High heritability (64.3 and 93.7%) for many traits and low variability for only seed yield (20.21%) were observed earlier. Similarly the secondary branches and siliquae per plant gave maximum genetic advance (94.99%). In another study different genotypes of rapeseed and mustard were evaluated for genetic studies and concluded that, number of siliquae per plant gave higher values for both PCV and GCV. Similarly days to flowering, plant height, days to maturity, number of siliquae per plant and oil content showed high broad sense heritability [45, 46].

Four species of Brassica viz, *Brassica* juncea, camopestris, napus and *Brassica* carinata were evaluated for two years for plant type traits including basal branching [47]. In B. juncea there was greater variation for plant height but not for seed yield. It was only next to *B. campestris* followed by *B. napus* for variation in basal branching trait. While, in *B. campestris* there was no genetic variation for basal branching trait, but it showed greater variation for days to 50% flowering, total number of branches (primary and secondary) [47].

Twenty five winter rapeseed varieties were evaluated for genetic studies, path analysis and correlation and of yield related traits. Almost all the traits showed significant variability. Low to high broad sense heritability was estimated. Seed yield, seed weight and flower duration produced high heritability and high genetic advance values indicating the improvement of these characters through mass selection [48].

The values for broad sense heritability, variability and genetic advance were estimated for primary branches per plant, selique per plant, plant height and seed yield per plant in four single crosses of B. juncea. Among all of the traits, number of selique per plant had high heritability, genetic advance values. Coefficient of variability was also high for the said trait (49).

The genetic variability, heritability and genetic advance were studied in marketable and agronomic yield related traits in twenty nine lines of cabbage (B. oleracea var. capitata L.). Narrow difference between GCV and PCV proved genetic make up to be the main reason of genetic variability. High heritability was estimated for head size, compactness, marketable yield and weight per plant while marketable maturity showed low heritability values. High heritability indicated that trait were less influence by the environment. High heritability andhigh genetic advance was

observed for marketable yield which indicated that these traits likely to respond better to selection [50].

Aytac and Gulcal evaluated ten winter rapeseed genotypes for genetic variations, genotypic and phenotypic correlations and broad sense heritability for yields related parameters for two years. Significant differences were observed for all of the traits. Genetic variability, broad sense heritability and genetic advance values were maximum for oil yield, seed yield followed protein yield. Strong correlation was observed among these traits. They concluded that plant height, pod length, oil yield, protein yield may serve to be efficient characters for selection [51].

The observations of Inayt evaluated five genotypes for morphological parameters, yield, oil quality and quantity. They observed significant difference for all of these studied parameters except oil and protein percentages. They recommended the genotypes for general cultivation in the area under study because it performs well for most of the desirable character [52].

Three varieties of rapeseed viz Option 500, Galiath and Foseto and their F2 and F3 generation were evaluated for their estimation of heritability, genetic variability and genetic advance values. Maximum variation was observed in number of pod per plant and seed yield. The heritability values for theses two traits in F2 population were 94 and 83 having genetic advance of 33.81 and 40.5 while in f3 population, the values of heritability were 89 and 96 with genetic of 45.69 and 53.7 percent respectively. They concluded that high heritability values are controlled by additive effect of genes hence selection in early segregate generations may be effective [53].

2.2 SEED QUALITY TRAITS

Seed quality traits refer biochemical traits of the germplasm. These are also used in the variability studies and provide reliable data on the fatty acid composition, oil and protein content.

1475 genotypes from 21 Brassica species were evaluated for fatty acid composition of seed oil. 358 entries were analyzed by gas liquid chromatography (GLC) and the remaining 1117 entries were analyzed by near infrared reflectance spectroscopy (NIRS), they identified NIRS to be an effective technique in the variability assessment for fatty acids in the intact seed samples of Brassica species. They used some fatty acid ratios to estimate the efficiency of the different biosynthetic pathways. Two patterns were observed. High elongation efficiency and accumulation of high levels of erucic acid were the attributes of the first pattern. The second pattern resulted in the accumulation of high levels of the polyunsaturated linolenic acid because of having desaturation efficiency. They suggested the observed variability as a valuable tool in future plant breeding [54].

In another study 1708 entries of the 20 Brassica species were evaluated for seed glucosinolates (GSL) by near-infrared Reflectance Spectroscopy [55]. One hundred and fifty entries having comparatively high GS content were further analyzed by high performance liquid chromatography (HPLC). Great varability for GS content was observed in B. Montana, B. Oleracea and B. Nigra. Six contrasting GS profiles were identified within B. oleracea depicting the greatest interspecific variability. They discussed the utility of their study in the future breeding programs.

Similarly fatty acid composition of seed oil of four interspecific hybrids, resulting from crosses between zero erucic acid B. Rapa (AA), and high erucic B.

oleracea (CC) and B. carinata (BBCC) were analyzed by Rahman. These resynthesized B. napus (AACC) lines showed half of erucic acid content from the high erucic acid CC genome parents, which indicated that both genomes contributed equally. The differences in oleic acid synthesis between the parents were also evident in the resynthesized B. napus plants. Hexapliod Brassica plants of the genomic constitution AABBCC had lower erucic acid contents than the B. carrinata (BBCC) parent, as AA genome was incapable of erucic acid synthesis. It was concluded that the zero erucic acid AA genome contributes to oil synthesis in hexaploids and reduces erucic acid content [56].

Six cultivars of Canola (B. napus) oilseed (Bulbul, Dunkled Rainbow, Oscar, Range and local were studied for fatty acids and total glucosinolates contents by near infrared Reflection (NIR) spectroscopy, while oil and protein contents were measured by traditional procedures. Significant variations in all the chemical constituents were observed among the six cultivars. Glucosinolates content of the six cultivars tested was less than 30 (uM g-1), which characterized these cultivars to be canola type Brassica. The cultivars Bulbul and Rainbow were considered to be best cultivars because of having more oil content [57].

In another research experiment cluster Ethiopian mustard genotypes were classify on the basis of their fatty acid composition. Principal component and cluster analysis were applied over the data set. First principal component accounted for 39.28% of the total variation and was associated with monosaturated fatty acids, desaturation ratio, elongation ratio, oleic desaturation ratio and vaccinic acid. Second principal component explained 30.97% of the total variation. In all, five principal components explained 96.01% of the total variation. Cluster analysis grouped B. carinata genotypes into 11 distinct clusters. It was concluded that the results of his

study can be useful for the planning of crosses and for maximizing the use of genetic diversity and expression of heterosis [58].

Biochemical parameters of five F_2 lines along with their nine parental lines were assessed by Abbas using near infrared Reflectance Spectroscopy (NIRS). Parental lines had more oil content while the F_2 were rich in protein. Glucosinolates and fatty acids contents were high in both sets population. Genetic relationship was estimated by using Insulin growth like Factor (IGF) primer sets which amplified 29 alleles. A high level of genetic dissimilarity was observed among all genotypes. Dendogram were constructed using cluster analysis. The information derived from these dendogram was used to identify most diverse genotypes for use in future breeding programs [59].

Six $F_{3:4}$ derived interspecific Brassica populations together with three checks were evaluated for their genetic variability and correlation among quality traits. Oil content, oleic acid, glucosinolates, linolenic acid and erucic acid contents were observed to have highly significant genetic variation whereas protein content had no significant variation. Low environmental variance indicated significant genetic control over the expression of quality traits. Linolenic acid, glucosinolates, oleic acid and erucic acid contents had high heritability values while protein content was observed to be low heritable trait. Out of six, four populations had outstanding performance for most of the quality traits.

2.3 CORRELATION

Correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. It resolves the complex relations

between the events into simple form of association. But the two variables cannot be dependant on one another. The association between the various characters in a rapeseed and mustard and the effect of a direct and indirect variable over the dependent variable has been studied by a number of investigators are reviewed here.

Several research studies revealed that, the seed yield posses significantly positive correlation with primary and secondary branches and siliquae per plant. Similarly seed yield significantly and positively associated all other characters except for length of seliquae and days to 50 % flowering [61, 62]. Some results attributed that the basal branching were positively associated among them selves and with the seed yield. There were desirable and negative associations of basal branching with flowering time and plant height [63].

A study was conducted by some researchers to assess the type and extent of variability of some yield related traits of five mustard genotypes (B9, Swarna, Kesari-100, Sarasi Swarna, and Agrani (B-54). Phenotypic correlation studies indicated that seed yield was had significantly positive related with husk weight, plant height and total dry matter. The number of siliquae per plant, crop growth rate within 60-75 days after sowing, 1000-seed weigh tend number of branches per plant was also correlated positively with yield [64].

While in another study a group of scientists compared normal environmental condition with saline and observed significant positive correlation between seed yield and oil content and seed yield and siliquae per plant in both environments. Significant positive correlation was also observed for the secondary branches and number of siliquae per plant in both the environments [65].

2.3 MULTIVARIATE TAXONOMIC TECHNIQUES

Among the multivariate taxonomic techniques, cluster and principal component analysis have been widely used to classify and measure the pattern of genetic variability observed in both qualitative and quantitative traits in germplasm of many crop species [66, 67]. Cluster analysis generally allows precise description of distance between close genotypes, but the results might be confusing for large clusters. In contrast, principal component analysis provides good information of distance between major clusters but exact distance between relatively similar accessories cannot be measured [68]. PCA is less sensitive to data where mixtures are observed to have occurred. Therefore, the combined use of both techniques may give a complete knowledge of the germplasm [69].

To determine the degree of genetic diversity among the Brassica juncea germplasm from Pakistan, total of 52 accessions comprising of locally collected germplasm and commercial varieties were studied in Japan for two years. Thirty five agro morphological characters were recorded from seeding emergence till crop maturity. These characters were analyzed by cluster and principal component analyzed. Cluster analysis distinguished the genotypes into six groups. Oilseed cultivars and vegetable forms were genetically distinct. The evaluated germplasm appeared to have narrow genetic base, as the variability was quite low. The portable reason of genetic erosion was suggested to be improper selection in breeding programs, changes in agricultural land use and replacement by major crops [70].

Thirteen seed and morphological characters in six B Carinata accessions at three locations were tested for principal component and cluster analysis in 2002. High amount of divergence was observed in all characters. Both analysis depicted complex relationships among the studied characters and accessions. Accessions having

potential genes for the improvement of characters like earliness, yield components, oil and proteins contents were identified. Divergent clustering patterns were observed for length of growing period and yield components. Oil, glucosinolates and protein contents also had medium divergence. It was concluded that accessions having more proteins content and high glucosinolates reduce the oil content [71].

A study was conducted to determined similarities and differences regarding morphological variation among locally collected white head cabbage (B. oleracea var. capitata subva alba). The morphological data were analyzed by multiple variance analysis. Cluster analysis based on ten qualitative and twelve quantitative variables identified 10 groups. High morphological variability was observed among the white head cabbage genotypes [72].

In another study fifteen kale land races in two locations with two planting dates i.e. early and late were evaluated [73]. This study was aimed to estimate genetic diversity and relationships and to evaluate their morphological traits. Significant genetic diversity was found for all traits while genotype x environment interaction was non significant for most of the traits. Cluster and principal components analysis were used to determine relationships among landraces. The cluster analysis showed five groups. These groups were distinct from each other on the basis of geographical origin and difference in morphological characters. North and early planted land races fell in first cluster while second cluster was comprised of south and late planted accessions. They noticed a considerable proportion of genetic diversity which could be used to select and combine important traits in order to obtain improved varsities.

The genetic diversity and morphological resemblance of three *B.* rapa subsp. Rapa L, including turnips, turnip greens and turnip tops were determined. 120

landraces were evaluated for 34 agronomic traits. Cluster analysis divided the land races into five groups. The first cluster included landraces having worst agronomic performance. Turnip population with rosette growth habit constituted the second cluster. Occupants of the third cluster were isolated than the second cluster because of having more vigorous growth. Fourth cluster was characterized by having varsities with more secondary branches per plant and fresh matter content per leaf while the last cluster included land races with earliness, large flowering period and more seed weight. In short, high proportion of variability was observed [74].

A research study was conducted by Mahmud et al., (2008) to describe the genetic diversity of 22 rapeseeds (B. napus) advanced genotypes using principal component analysis, canonical vector analysis and non hierarchical clustering. The genotypes were distinguished into four clusters. Second cluster was the largest having nine genotypes and third cluster contained only two advanced genotyped. First cluster had the highest mean values for selique length and thousands seed weight while third cluster had the lowest mean values for half flowering and the number of days to maturity. They suggested that crossing between genotypes belonging to second cluster with genotypes from cluster 1 and cluster IV might produce high heterosis in yield and earliness [75].

Thirteen different quantitative characters were studied in 98 germplasm of mustard along with two checks (Kranti and Varuna). The principal component analysis was performed. Thirteen principal components having eigen value more than 1 were produced. The first principal components had high eigen root of 3.31, followed by 2.12, 1.32, 1.07, 1.02, 0.82, 0.73, 0.65, 0.47, 0.42, 0.31 and 0.21. The first principal component accounted for 25.47% of the total variation. Second up to sixth components had 16.29, 10.17, 8.21, 7.82 and 6.29% of the total variation respectively.

The Proportion of variation explained by 7th to 13th principal components were 5.61, 5.02, 4.33 3.21, 2.34 and 0.63. The cumulative percent of variation was 96.03%. Days to flowering initiation, selique on main raceme, seeds per selique length, seed yield per plant, number of secondary branches per plant and 1000-seed weight proved to be the most important variables as these were having high positive and negative eigen values for different principal components [76].

In another study one hundred fourteen accessions of rapeseed (B. campestris L) were evaluated for two years [77]. Cluster and principal component analysis were used to analysis fifteen agro-morphological and six quality traits. Cluster analysis divided the studied genotypes into six and five clusters during two consecutive years respectively. The first seven and five principal component (PCs) having Eigen values less than one contributed 74.09% and 66.08%of the variability amongst accession, respectively. Nine traits contributed positively to first two PCs during both the year.

2.4 SIMPLE SEQUENCE REPEATS (SSR)

Over the last few decades plant genomics has brought a revolution in the area of biological sciences. Molecular markers, useful for plant genome analysis, have now become an important tool in many fields like taxonomy, physiology, embryology and genetics engineering. Regarding their use in plant breeding, these markers have proved themselves to be a useful tool in the development of marker based gene tags, variability studies, map based cloning of agronomical important genes, marker assisted selection of desirable genotypes etc. each marker differ from other in principle, application, cost and time requirement, type and amount of polymorphism detected. Microsatellite markers also known as SSR provide an efficient mean of

detecting genetic diversity, as they can detect high number of alleles [77]. It has been reported that SSRs can detect genetic diversity better than other molecular markers [78, 79, 80].

The efficiency of microsatellites primers in the plant genome analysis had been addressed by many researchers. They considered perimeter specificity to be the main hinder in the applicability of these perimeters across closely related species. It was proposed that majority of microsatellites identified in B. Napus (AC genome) correspond to the loci from A and C genome. They studied 63 microsatellites pairs, in which 54% detected the loci from both genomes, while 25% and 21% were A and C genome specific respectively. Genetic map of B, oleraceae L, was used to investigate the distribution of rapeseed microsatellites in C genome. Using ninety two markers, level of polymorphism was found to be 49.2% in the mapping population. Their results showed that markers derived from B. napus may serve as a valuable tool for genetic studies in B. Oleraceae [81, 36].

Twenty four Chinese winter, Swedish winter and spring B. napus accessions were compared for genetic diversity by inter-simple sequence repeats (ISSRs) [82]. 125 polymorphic bands were amplified with 20 primers. Cluster analysis (UPGMA) divided the 24 accessions into three groups. Eight Chinese winter and six Swedish winter lines and were in first group, while second cluster comprised of two Chinese winter lines. Eight Swedish spring lines were forming the third cluster. Principal co-ordinates analysis (PCO) showed similar findings as that of cluster analysis. Their result showed that Chinese winter accessions had more genetic diversity as compared to Swedish accessions. Their study indicated the effectiveness of ISSR for the evaluation of genetic diversity in rapeseed germplasm.

In another study the researchers used thirteen SSRs to estimate genetic similarity between 54 B. oleracea cultivars including cauliflower, cabbage and broccoli. Cluster analysis extinguished the cabbage cultivars into two groups. Broccoli and cauliflower fell in same cluster. Narrowest genetic variation was observed in the cauliflower cultivars (B. Oleracea var. botrytis) followed by broccoli (B. oleracea var, italiea) and cabbage (B. oleracea var. capitata) groups. Polymorphism information content (PIC) values ranged between 0.25 to 0.86, while the number of alleles produced per marker was 1 to 8, respectively [83].

The researcher had examined the genetic diversity in the entire rapeseed germplasm by using molecular information illustrated by simple sequence repeats (SSR) loci [84]. Plant material was selected in such a way as to cover maximum diversity available within species. 30 SSR primer sets were used to characterize 96 genotypes. These primers provided unique genetic fingerprints by amplifying 220 alleles at 51 polymorphic loci in the studies genotypes. Cluster analysis enabled identification of four general groups. The said groups were spring oilseed and fodder winter oilseed, winter fodder and vegetable genotypes. They supported the use of molecular information for the identification of genetic variability in rapeseed breeding programs.

Similarly eleven Chinese and twelve Swedish rapeseed (B. Napus) genotypes were analyzed in another research experiment by using 41 microsatellite primers which generated 50 loci. The number of alleles ranged from 1 to 14 for these 50 loci while the average number of alleles per loci was 2.7 [85]. It was observed that a single SSR marker could distinguish 14 different DNA profiles. The cluster analysis (UPGMA) distinguished the genotypes into three clusters, a cluster having only Swedish genotypes while Chinese genotypes were divided in two clusters. Chinese

genotypes were more divers compared to the Swedish material. They considered their results to be helpful in the establishment of a set of microsatellite primer that can be used for the selection of appropriate parents for B. napus hybrids and for screening of hybrids level.

Microsatellite markers were proved to be a powerful tool for cultivars identification and differentiation. In a study fifty nine B. oleracea cultivars, belonging to five botanical varieties were evaluated using 11 microsatellite primers. Five different groups of cultivars were used during this study. In total, 47 fragments were produced which differentiated 51 cultivars. The remaining eight cultivars were isolated from the rest. Polymorphic information content (PIC value) of 0.5 or above was observed in all SSR markers except one. The average diversity for all markers was 0.64, Minor genetic diversity was observed within botanical varieties and groups. Their study substantiated the use of microsatellite markers as a powerful too for cultivars identification and differentiation [86].

Yuan and Chao assessed the genetic diversity of 30 *B. oleracea* samples by using five SSR primer sets. A total of 21 alleles were detected. The number of alleles ranged from 2 to 5 having average of 4.2 cultivars from Japan were totally isolated than the rest, while other were distinguished in four groups [87].

Two quantitative trait loci (QTL) in *B. juncea* (L.) and three in *B. napus* (L.) had been identified for oil content respectively. Similarly six more QTL had been identified for oil content using the same population but the study was made in multiple environments. Two of these QTLs show a close linkage with the two erucic acid genes. This means that the erucic acid has a positive effect on oil content [87, 88].

A research study was made to investigate the effect of quality improvement on the genetic diversity of European winter B. rapa germplasm by comparing 3 open pollinated cultivars, having different breeding period. 32 plants per cultivar were studied with 16 simple sequence repeat (SSR) markers [89]. No significant loss of genetic diversity was observed in the 3 cultivars. Analysis of molecular variance (AMOVA) revealed that 83% of the total variation was attributed to within cultivar variation and the remaining 17% to between cultivars variation. Principal coordinate analysis (PCoA) separated the individual plants into the 3 cultivars. They recorded high genetic diversity within cultivars and hence it was confirmed that there is no decline in performance of *B*.rapa germplasm because of quality improvement.

High level of genetic diversity in 14 genotypes of Brassica species had been estimated. Mean genetic distance estimates ranged from 26-89% and 5-61% while 45.8 and 25.8 alleles were amplified using RAPD and Brassica specific SSR primer sets, respectively. Cluster analysis helped in the identification of most diverts genotypes [90].

3.0 MATERIAL AND METHODS

The research work was conducted to check the effect of induced mutations on the genetics of oil and other oil related components in advance mutant lines of M5 generation. Estimation of mutation was checked on:

1. Agronomical traits

2. Biochemical traits (through Near Infra red Spectroscopy (NIR) System

3. Molecular analysis by Simple Sequence Repeats (SSR)

Thirty five advance mutant lines of M5 generation and one check were employed for the estimation of mutation based on agronomical and biochemical traits, while two mutant lines one with comparatively high oil content and the other with low oil content were used for the development of material for molecular analysis to estimate the genetics of oil and other oil related components.

3.1 AGRONOMICAL AND BIOCHEMICAL STUDY

3.1.1 EXPERIMENTAL MATERIAL

The material used in the study comprised of thirty five advance mutant lines of M5 generation and one check viz Abasin-95. The general view of the experimental material is given in table A.

3.1.2 EXPERIMENTAL SITE

The present field experiment was conducted during rabi 2009 at the Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar.

3.1.3 FIELD PLOT TECHNIQUE

Thirty five advance mutant lines of M5 generation and one check were sown in four replications using Randomized Complete Block Design (RCBD). The materials were sown on a well-prepared seed bed in October 2008, using 10 kg/ha seed. The sowing plot consists of 6 rows each 5m long with 30 cm distance between the rows and 5-10 cm distance between the plants. The experimental plot was irrigated three times during the entire period growth and development. The thinning was performed once to retain 10 cm space among the plants. All the experimental lines were grown under natural conditions (neither fertilizer nor pesticides were applied) in order to assess the full strength of the population under natural conditions. At maturity, seeds were harvested from each plot of each replication and data were analyzed statistically for variability, heritability and correlation of different traits.

Table A **Genotypes and entry number of the thirty five advance mutant lines and the parent used in this study.**

S.No	Genotypes	Entry No.
1	O1	26-4
2	O2	26-7
3	O3	29-3
4	O4	30-3
5	O5	31-2
6	O6	32-5
7	O7	35-6
8	O8	36-4
9	O9	36-5
10	G1	37-1
11	G2	38-9
12	G3	40-5
13	G4	41-7
14	G5	45-5
15	G6	47-8
16	G7	48-9
17	G8	49-1
18	G9	52-7
19	OA1	53-3
20	OA2	54-8
21	OA3	58-6
22	OA4	59-2
23	OA5	61-2
24	OA6	65-4
25	OA7	69-3
26	OA8	71-4
27	OA9	72-5
28	EA1	73-1
29	EA2	81-7
30	EA3	84-4
31	EA4	85-9
32	EA5	90-4
33	EA6	90-7
34	EA7	93-2
35	EA8	97-1
36	Control	Abasin-95

3.1.4 RECORD OF OBSERVATIONS

Observations were recorded on five randomly selected plants for different characters in each genotypes and replication. Traits selection and measurement techniques were based on International Board of Plant Genetic Resources (IBPGR) descriptors for *Brassica* and *Raphanus* (1990). Data were recorded for the following yield and yield related components.

3.1.4.1 RECORD OF OBSERVATIONS FOR AGRONOMICAL TRAITS

For selection of mutant plants, data on the following agronomical traits were recorded according to the *Brassica* descriptor (1990).

3.1.4.1.1 DAYS TO 50% FLOWERING (DF)

Data were recorded in days from sowing of seed till 50% of plants started flowering.

3.1.4.1.2 PLANT HEIGHT (PH)

At maturity height (cm) of the selected plants were recorded on meter rod by measuring the distance from the ground level to the top of main stem.

3.1.4.1.3 1000-SEED WEIGHT (SW)

It was computed by counting 1000 randomly selected seeds from plants in each line of replication dried to approximately 6% moisture content. The weight in grams was recorded.

3.1.4.1.4 SEED YIELD (SY)

Seed yield was calculated in kg/ha by taking the plants from the central three rows of the plot.

3.1.4.1.4 OIL YIELD (OY)

Oil yield was calculated in kg/ha by the following formula

Oil yield = (oil content/seed yield) 100

3.1.4.2 RECORD OF OBSERVATIONS FOR BIOCHEMICAL TRAITS

Biochemical analysis of the parent and each of the advance mutant lines were conducted at the oilseed Lab, Crop Breeding Section of Nuclear Institute for Food and Agriculture (NIFA), Peshawar, using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) according to the manufacturers protocol through non destructive technique.

Data was recorded on the following biochemical parameters:

3.1.4.2.1 OIL CONTENT (OC)

The oil content (%) was determined by scanning the sample on a monochromator using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) with sample auto changer. About 5g of seed volume was used in a standard ring cup.

3.1.4.2.2 PROTEIN CONTENT (PC)

Theproteinl content (%) was determined by scanning the sample on a monochromator using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) with sample auto changer. About 5g of seed volume was used in a standard ring cup.

3.1.4.2.3 GLUCOSINOLATES (GSL)

The glucosinolates (uMol/g) was determined by scanning the sample on a monochromator using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) with sample auto changer. About 5g of seed volume was used in a standard ring cup.

3.1.4.2.4 OLEIC ACID (OA)

The oleic acid (%) was determined by scanning the sample on a monochromator using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) with sample auto changer. About 5g of seed volume was used in a standard ring cup.

3.1.4.2.5 LINOLENIC ACID (LA)

The linolenic acid (%) was determined by scanning the sample on a monochromator using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) with sample auto changer. About 5g of seed volume was used in a standard ring cup.

3.1.4.2.6 ERUCIC ACID (EA)

The oleic acid (%) was determined by scanning the sample on a monochromator using Near Infra red Reflectance (NIR) Spectroscopy System (FOSS 6500 equipped with ISI version 1.02, Infra Soft International) with sample auto changer. About 5g of seed volume was used in a standard ring cup.

3.1.5 STATISTICAL ANALYSIS

Agronomical and biochemical data for each of the advance mutant line and the parent was recorded. Mean and variance were calculated for these characters.

3.1.5.1 ANALYSIS OF VARIANCE (ANOVA)

The data for all these attributes were subjected to analysis of variance. The analysis of variance was done according to the M-STAT C software and the mean squares were evaluated on the basis of the following formula [88].

3.1.5.2 COMPONENT OF VARIANCE

The genotypic and phenotypic components of variance were calculated according to formulae given below [91].

$$\text{Genotypic variance (Vg)} = \sigma_g^2 = \frac{\left(MS_g - MS_e\right)}{r}$$

$$\text{Genotypic variance (Vg)} = \sigma_g^2 = \frac{\left(MS_g - MS_e\right)}{r}$$

Where, σ^2p (phenotypic variance), σ^2g (genotypic variance), MS_g (mean square of genotype), MS_e (mean square of error), r (number of replication)

3.1.5.3 COEFFICIENT OF VARIATION

Genotypic and phenotypic coefficient of variation were computed according to Burton

and Devane [41].

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100$$

Where

X is the mean of trait considered

The PCV and GCV values are ranked as low, medium and high and are mentioned below [90].

0-10% - Low

10-20% - Moderate

> 20% - High

3.1.5.4 HERITABILITY ESTIMATES

The heritability estimates provide information on transmission of trait (s) from parents to their off springs. Broad-sense heritability was estimated as:

$$h2 = \frac{\mathrm{VM} - \sqrt{\mathrm{VP}}}{\mathrm{VM}}$$

where

h^2 (B.S) = Broad Sense Heritability

VM = Variance of mutant population

VP = Variance of parent

Heritability values were categorized as low, moderate and high as below.

0-0.30- Low

0.31-0.60 – Moderate

> 0.60- High

3.1.5.6 GENETIC ADVANCE

Genetic advance (GA) was computed according to the following formula.

$$\text{Genetic Advance (GA)} = k \times \sigma_p \times H$$

Where

GA (expected genetic advance), k (selection intensity), $\sigma^2 p$ (phenotypic variance), H (heritability in broad sense)

The genetic advance as percent of means (GAM) of each trait was thus estimated by dividing the expected genetic advance of the trait to the mean of the trait considered and multiplies by 100.

3.1.5.7 CLUSTER ANALYSIS

Agronomical and biochemical data recorded on thirty five advance mutant lines along with the parent grouped into clusters using cluster analysis [92].

Single dendogram was constructed for agronomical and biochemical data for all the mutant lines for confirmation of mutation. Prior to cluster analysis, data of individual mutant lines and parental line were standardized so that the effect caused by scaling difference could be avoided. Euclidean distance co-efficient were calculated after standardization of data. The Euclidean dissimilarity coefficient matrices were employed to evaluate the relationship between the mutant lines and the

parent by using cluster analysis through complete linkage method. For this purpose, the software NTSysPC 2.1 was employed. Genetic distances were also calculated.

3.1.5.8 PRINCIPAL COMPONENT ANALYSIS

Scattered diagrams of data variables and individuals based on agronomical and biochemical traits were constructed through principal component analysis (PCA) using statistica software (version 7). Data were standardized prior to analysis. Eigen values and Eigen vectors were calculated and only those factors were retained whose Eigen values were greater than one. The Eigen vector with the highest Eigen value was taken to be the principal components of the data set. It was taken as 1st principal components (PC1) on X-axis and was plotted with the second highest Eigen value holding variable (PC2). The second principal component (PC2) was plotted on Y-axis. Scatter plots were produced to provide a graphical representation of pattern of variation among the genotype (Statistica, Version 7.0).

3.2 MOLECULAR STUDY

3.2.1 EXPERIMENTAL MATERIAL

Two mutant lines i.e. mutant 1 with high oil content (31-1) and mutant 2 with low oil content (60-4) were used for the development of material for molecular analysis to estimate the mutation in the genetics of oil and other oil related components.

Concerned parents i.e. mutant1 and mutant2 and their hybrid ($BCF_{2)}$ population were analyzed through Simple Sequence Repeats (SSR) markers to study the genetic differences among these genotypes. Facilities at the Nuclear Institute for Food and Agriculture, Peshawar were utilized.

Step wise procedure for molecular study is as followed.

3.2.2 PLANT MATERIAL

Leaf tissues of the parental lines and BCF_2 hybrids were collected. Small leaves were collected as these contain comparatively less proportion of polysaccharides and were stored at -80^0c for future use.

3.2.3 DNA EXTRACTION

Genomic DNA extraction was carried out using Cetyltrimethlyl ammonium Bromide (CTAB) DNA extraction protocol (Doyle and Doyle, 1987). About 100 mg of frozen leaf tissues were ground to fine powder with the help of pestle and mortar using liquid nitrogen, and was shifted to 1.5 ml eppendorf tubes. The grinded leaf tissue was homogenized with 500 µl of CTAB buffer (2% Cetyltrimethlyl ammonium bromide, 100 mM Tris HCl, 1.4 M NaCl, 20 mM EDTA , 0.5% SDS) with added 0.2% marceptoethanol. Incubation was done for one hour at 60^0c in water bath

occasionally mixing by gentle swirling. 300 µl of chloroform: Isoamylalcohol (24:1) was added to the mixture and tubes were inverted for 15 minutes. Aqueous phase was recovered by centrifugation for 10 minutes at 15000 rpm and was transferred to another eppendorf tube. The DNA was precipitated by adding 300 µl of isopropanol. Tubes were kept at -20c to enhance precipitation. DNA was pelleted by centrifugation at 12000 rpm for 10 minutes, washed twice with ice cold 70% ethanol, dried at 37^0c and dissolved in 50 µl of TE buffer (10mM Tris-HCl, 1mM Ethylenediamine tetra Acetic acid (EDTA), pH 8.0) containing 40 µg/ml. RNase A. The concentration of DNA was tested by comparing its intensity with that of DNA of known concentration on a 0.8% agarose gel Tris Borate EDTA (TBE) buffer.

Table B. Genotypes and entry number numbers of the two mutant lines used in the present study.

S.No	Genotypes	Entry No.
1	Mutant 1	31-3
2	Mutant 2	60-4

3.2.4 SELECTION OF PRIMERS

A total of 25 SSR primers were tested on five samples, of which 19 primer sets gave positive results during initial screening. These primers were used for polymerase chain reaction (PCR) analysis. Different annealing temperatures (52-58^{0}c) were tested during the screening process to select the best annealing temperature for a specific primer set. These primer sets were selected from the published data of Suwabe et al, (2002), Lowe et al. (2003), Burgess (2006), Batley (2007), Hopkins (2007), and were tested on various genomes of *Brassica* to confirm their cross amplification across Brassica species.

3.2.5 PROTOCOL FOR MOLECULAR MARKER ANALYSIS

Polymerase Chain Reaction (PCR) was performed in PCR tubes containing 20 µl reaction mixtures having 1µl of genomic DNA, 15.5µl of sterile autoclaved and demonized H_2O, 2 µl of 10 x Dream Tag buffer (with added 20 mM of $MgCl_2$), 0.4µl dNTPs (10 mM each), 0.5µl of SSR primer and 0.1µl Dream Tag DNA polymerase.DNA was amplified under the following thermal cycling conditions, 4 minutes (min)denaturation at 94^{o}C, 35 cycles of annealing while each cycle having one minute denaturation at 94^{o}C, one minutes in annealing at 52-58^{o}C depending on the primer set used, 2 minutes extension at 72^{o}C, and a final extension step of 72^{o}C for 7 minutes.

3.2.6 ELECTROPHORESIS OF AMPLIFIED PRODUCTS

After the completion of PCR, 3 µl of 6x loading dye (10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% of xylene cynol FF, 60% glycerol, 60 mM EDTA) was

added to each PCR tube. They were spun in microfuge for few seconds, 10 µl aliquot of PCR products mixed with loading dye was loaded in 3% agarose. 4 µl 20 bp gene ruler (fermentas). DNA ladder was used as a standard fragment size and was loaded in the first and last well of gel. Electrophoresis was carried out in 1X TBE buffer (10 mM Tris-Borate, 1mM EDTA) at 100 volts for 90 min. PCR products were separated by electrophoresis using 3% agarose gels and were visualized under UV light after staining with ethidium bromide.

3.2.7 DATA COLLECTIOON AND ANALYSIS

Photograph of the gels were taken under UV light by gel documentation system. These were used to score the bands of SSR primers. Each band (amplified allele) was considered as unit character and was scored as 1 for presence and 0 for absence for each sample primer combination. Low glowing bands were considered to the result of minimum representation of the fragment; therefore these were not taken into account. Only clearly visible and distinguishable bands were consider for scoring. Allele size was determined in gel by comparing the band with known size of DNA marker. Data for band scoring was entered into binary data matrix using MS excel 2007 sheet.

Cluster analysis was performed for the bivariate (1, 0) dat. Similarity coefficients were generated using absence-presence pattern base pair wise comparison of genotypes both for shred and dissimilar bands (alleles). Genetic similarity estimates (F) were calculated between all pair of genotypes by the DICE algorithm according to Nei and Li (1979) as per following equation [93]:

Similarity (F) = 2 Nab/ (Na + Nb)

Where,

Na= the number of score fragments of individual “a”

Nb= number of score fragments of individual “b”

Nab= number of shared fragments between “a” and “b”

These similarity coefficients were used to determine the relationship among the genotypes under study through cluster analysis using unweighed pair-group method (UPGMA) and dendogram were generated. All these calculations were carried out using softare packages NTSysPC version 2.1 (Applied Biosystems Inc, USA, MS-Excel 2007.

3.2.8 POLYMORPHISM INFORMATION CONTENT (PIC)

The important point in assessing the genetic diversity within and among population is to determine through genetic dissimilarity between population. The polymorphism information content (PIC) values give a proper measure for co dominant marker as it can be applied to binary data mentioning banding profile of population. It uses both the number of alleles at a specific locus and their relative frequencies in a population. PIC value for each SSR primer set was used to measure allele diversity at each locus. It was calculated by the formula proposed by Tonguc and Griffith (2004).

4.0 RESULTS

Extent of mutation at the agronomical level and biochemical level in each of thirty five advance mutant lines were studied. Most of the mutant lines indicating the presence of considerable amount of genetic variability. The main objectives of the present study was to estimate the induce mutation in advance mutant population of M5 generation for oil and oil related components. Estimation of mutation was determined based on agronomical traits, biochemical traits and molecular analysis. For agronomical and biochemical estimation, thirty five advance *Brassica* mutant lines and one check were evaluated for the traits days to 50% flowering, plant height (DF), 1000 seed weight (SW), seed yield (SY) and oil yield (OY) during 2008-13. Seed were analyzed at NIFA, Peshawar for biochemical parameters viz oil content (OA), protein content (PC), glucosinolates (GSL), oleic acid (OA), linolenic acid (LA) and erucic acid (EA).

To understand the extent to which the observed variation was due to genetic factors, the value of genotypic and phenotypic variance, phenotypic and genotypic coefficients of variability, heritability (broad sense) and genetic advance as percent of means, cluster and principal component analysis for different characters were estimated.

4.1 ANALYSIS OF VARIANCE (ANOVA)

4.1.1 ANALYSIS OF VARIANCE (ANOVA) FOR AGRONOMICAL TRAITS

Five agronomic traits i.e. Days to flowering, Plant height, 1000 seed weight, Seed yield (kg ha^{-1}) and Oil yield (kg ha^{-1}) were observed in the parent (Abasin-95) and thirty five advance mutant lines of rapeseed Brassica. The data obtained were

processed with Microsoft excel 2007 software and statistically analyzed by MSTATC program for Randomized Complete Block Design (RCBD). The LSD (0.05) was used for mean comparisons. All the experimental populations showed highly significant differences ($P<0.01$) for all the observed agronomic traits. The co-efficient of variation ranged from 15.54 to 5.26% for various characters (Table 3).

4.1.1.1 DAYS TO 50% FLOWERING (DF)

The results obtained from the analysis of variance (ANOVA) for all the traits are presented in (Table-1). According to this table significant differences were observed for number of GF among 35 tested genotypes against the check. The early flowering was recorded in 25 mutant lines. However, OA5 (68.75), OA6 (68.75), G8 (69.0) and G1 (69.25) flowered earliest compared to check (Table1). Delayed flowering was observed in one mutant line i.e. O9 (91.50) compared to check. On average, the tested genotypes were earlier in flowering than the check.

4.1.1.2 PLANT HEIGHT (PH)

The final plant height reflects the growth behavior of a crop. Both genetic and environmental factors play a vital role in determining the plant height of a plant. The data revealed that the PH of different *Brassica* mutant lines under study was significant (Table-1). Thirty three mutant lines were shorter than the check (177.6) while the mutant lines O3 (145.5), O5 (149.9), O6 (157.3), O8 (157.3) and G8 (153.8) were shortest and only two mutant lines O6 (197.9) and O9 (199.0) were taller than the check (Table-1).

4.1.1.3 1000-SEED WEIGHT (SW)

The data revealed that significant differences were observed for 1000 seed weight among 35 tested genotypes against the check (Table-1). Thirty mutant lines showed low 1000 seed weight compared to check (table-1). However EA3 (4.1), EA2 (4.2), G6 (4.3), O4 (4.3), O6 (4.4) and G7 (4.5) shows the lowest values for SW. Only two mutant lines OA5 (5.65) and G1 (5.60) showed a higher value for 1000 seed weight compare to check.

4.1.1.4 SEED YIELD (SY)

The data revealed that significant differences were observed for seed yield among 35 tested genotypes against the check (Table-1). Nineteen mutant lines showed low values for SY compared to check and four mutant lines produced higher values. However G9 (1083), O7 (1333), O3 (1417), EA5 (1417) and EA6 (1417) showed the lowest values and OA5 (2708) showed the highest value for seed yield.

4.1.1.5 OIL YIELD (OY)

Significant differences were observed for oil yield among thirty five tested genotypes against the check (Table-1). Twelve mutant lines give low OY while six mutant lines give higher oil yield compared to check. However G9 (570.2), O7 (690.9), EA7 (702.3), EA5 (720.3), EA6 (730.5) and EA8 (757.6) showed the lowest values and OA5 (1410) and G1 (1299) showed the highest values for OY compared to check.

Table 1. Means, range and LSD values of some of the agronomical traits of advance Brassica mutant lines.

Genotype	Number of Days 50% to flowering	Plant height (cm)	1000seed weight (gm)	Seed yield (kg ha^{-1})	Oil yield (kg ha^{-1})
O1	3.25 BCDEFG	164.2 DEFGH	4.550 GHIJKL	1750 FGHIJKL	953.9 EFGHIJKL
O2	83.00 BCDEFG	188.6 AB	4.900 CDEFGH	2250 ABCDE	1177. ABCDEF
O3	72.25 KLM	145.5 I	4.650 FGHIJK	1417. JKLM	749.8 JKLMN
O4	83.50 BCDEFG	188.8 AB	4.300 KLM	2417 ABC	1288. ABC
O5	71.00 LM	149.9 HI	5.300 ABC	1708. FGHIJKL	915.9 GHIJKLM
O6	88.75 ABCD	197.9 A	4.400 JKLM	2500. AB	1327. AB
O7	88.00 ABCD	157.3 FGHI	4.800 DEFGHIJ	1333. LM	690.9 MN
O8	78.50 EFGHIJK	157.3 FGHI	5.000 CDEF	1750. FGHIJKL	908.8 GHIJKLM
O9	91.50 A	199.0 A	4.500 HIJKLM	2333. ABCD	1230. ABCD
G1	69.25 M	168.1 CDEFGH	5.600 A	2500. AB	1299. AB
G2	72.00 KLM	169.1 CDEFG	5.100 BCDE	1708. FGHIJKL	919.8 GHIJKLM
G3	79.75 EFGHIJ	172.9 BCDEFG	4.700 EFGHIJK	1875. DEFGHIJ	1002. DEFGHIJ
G4	76.25 GHIJKLM	169.4 CDEFG	4.850 DEFGHI	2042. BCDEFG	1103. BCDEFGH
G5	71.75 KLM	166.4 CDEFGH	4.650 FGHIJK	1917. DEFGHI	1007. DEFGHIJ
G6	71.50 KLM	164.0 DEFGH	4.300 KLM	1958. CDEFGH	1018. DEFGHI
G7	71.50 KLM	164.6 DEFGH	4.550 GHIJKL	1833. EFGHIJK	967.9 EFGHIJK
G8	69.00 M	153.8 GHI	4.950 CDEFG	2000. CDEFGH	1046. CDEFGHI
G9	73.25 IJKLM	159.3 EFGHI	4.950 CDEFG	1083. M	570.2 N
OA1	77.75 FGHIJKL	169.4 CDEFG	4.800 DEFGHIJ	1583. GHIJKL	838.8 IJKLM
OA2	69.50 M	171.4 BCDEFG	4.800 DEFGHIJ	1917. DEFGHI	1003. DEFGHIJ
OA3	80.00 EFGHIJ	160.4 DEFGHI	4.550 GHIJKL	1542. HIJKLM	815.1 IJKLMN
OA4	89.50 ABC	168.1 CDEFGH	4.700 EFGHIJK	1583. GHIJKL	814.9 IJKLMN
OA5	68.75 M	171.4 BCDEFG	5.650 A	2708. A	1410. A
OA6	68.75 M	164.9 CDEFGH	4.950 CDEFG	2167. BCDEF	1138. BCDEFG
OA7	72.75 JKLM	179.4 BCD	5.150 BCD	1750. FGHIJKL	923.5 FGHIJKLM
OA8	82.00 DEFGH	184.0 ABC	4.550 GHIJKL	1708. FGHIJKL	882.0 HIJKLM
OA9	80.25 EFGHI	168.2 CDEFGH	4.750 DEFGHIJ	2292. ABCDE	1205. ABCDE
EA1	82.25 CDEFGH	173.1 BCDEF	4.650 FGHIJK	1833. EFGHIJK	937.0 FGHIJKLM
EA2	73.25 IJKLM	173.3 BCDEF	4.200 LM	1667. GHIJKL	877.8 HIJKLM
EA3	85.75 ABCDE	171.4 BCDEFG	4.100 M	1958. CDEFGH	1031. DEFGHI
EA4	84.75 ABCDEF	175.4 BCDEF	5.050 CDEF	1583. GHIJKL	796.5 IJKLMN
EA5	80.25 EFGHI	165.8 CDEFGH	4.400 JKLM	1417. JKLM	720.3 KLMN
EA6	75.50 HIJKLM	159.9 EFGHI	4.300 KLM	1417. JKLM	730.5 KLMN
EA7	80.50 EFGHI	170.8 BCDEFG	4.500 HIJKLM	1375. KLM	702.3 LMN
EA8	89.75 AB	166.3 CDEFGH	4.450 IJKLM	1458. IJKLM	757.6 JKLMN
Control	89.75 AB	177.6 BCDE	5.450 AB	2250. ABCDE	1157. BCDEFG
Range	68.75-91.50	145.5-199.0	4.10-5.45	325.0-812.5	570-1410
LSD	6.21	15.42	0.17	120.9	106.08

DMR test (0.05): Means followed by same letters are not significantly different from each other.

Table 2. Mean squares and CV values of some agronomic traits of advance Brassica mutant lines.

Parameters	Mean squares	CV (%)
Days to flowering	202.2**	5.64
Plant height (cm)	548.8**	6.48
1000 seed weight	0.6**	5.26
Seed yield (kg/ha^{-1})	579878.2 **	15.54
Oil yield (kg ha^{-1})	164493.9**	15.39

**Significant at 1% level

4.1.2 ANALYSIS OF VARIANCE (ANOVA) FOR BIOCHEMICAL TRAITS

All the experimental populations showed highly significant differences ($P<0.01$) for oil, protein, glucosinolates, oleic acid, linolenic acid and erucic acid (Table 2). The co-efficient of variation ranged from 18.95 to 1.69 % for various characters (Table 2).

4.1.2.1 OIL CONTENT (OY)

An increase in oil content is the ultimate goal of a grower. The quality of oilseed *Brassica* is determined from its oil content. Highly significant difference ($P<0.01$) was recorded for oil content among all the genotypes and check. However, mean values in respect to oil content percentage displayed significance differences at 5% level of probability for all the genotypes. Data for oil percentage indicated the range of 50.28 to 54.40% (Table 2), in which the lowest was exhibited by the mutant line EA4 while highest by the mutant line O1, with the mean value of 52.43%.

4.1.2.2 PROTEIN CONTENT (PC)

Protein is the major requirement for all living organisms for their growth and development. Highly significant difference ($P<0.01$) was recorded for protein content among all the genotypes and check. The protein percentage ranged from 16.70 to 20.70, in which the lowest was recorded by the mutant line G4 while highest by the mutant line O2 with the mean value of 18.43% (Table 2).

4.1.2.3 GLUCOSINOLATES (GSL)

Glucosinolates is one of the main undesirable elements of oil seed *Brassica,* causing various problems in human beings and animals. Glucosinolate content showed highly significant differences ($P<0.01$) among the experimental populations. The data related to the glucosinolate contents ranged from 11.90 to 113.3 μMg^{-1} in which the lowest was observed for the mutant line EA3 and highest for mutant line O2 with the mean value of 34.31 μMg^{-1} (Table 2).

4.1.2.4 OLEIC ACID CONTENT (OA)

Oleic acid is one of the main mono unsaturated fatty acids which are required for the stability of oil. Highly significant differences ($P<0.01$) were recorded for oleic acid profile among the tested genotypes (Table 2). The oleic acid contents ranged from 35.45 to 60.35 % in which lowest content was observed for the mutant line O9 and highest for EA2 with the mean value of 54.33%. Most of the mutant lines show higher values of oleic acid percentages than the check.

4.1.2.5 LINOLENIC ACID CONTENT (LA)

Linolenic acid is a polyunsaturated fatty acid and like glucosinolates and erucic acid low linolenic acid content is also desirable in the oilseed because its higher value disturbs the stability of oil during storage and frying. The statistical analysis describes highly significant differences ($P<0.01$) for linolenic acid content among genotypes. Linolenic acid content ranged from 10.15 to 13.25% % in which lowest value was observed for the check and highest for mutant line EA4 with the mean value of 12.21% (Table 2). All the mutant lines show higher values of linolenic acid percentages than the check.

4.1.2.6 ERUCIC ACID CONTENT (EA)

Erucic acid is one of the main undesirable components of the oil. Analysis of variance revealed highly significant differences ($P<0.01$) for erucic acid content among the evaluated genotypes (Table 2). Erucic acid content ranged from 1.60 to 59.15 % with the lowest for mutant line G2 and highest for O9 with the mean value of 16.57%. Most of the tested mutant lines obtained lower values of erucic acid compared to check.

Table 3. Means, range and LSD values of some of the biochemical traits of advance Brassica mutant lines.

Genotype	Oil (%)	Protein (%)	glucosinolates (uMg^{-1})	Oleic acid (%)	Linolenic acid (%)	Erucic acid (%)
O1	54.40 A	17.95 HIJKLM	14.48 HI	52.90 IJ	11.13 MN	15.20 FG
O2	52.35 CDEFGHIJ	20.70 A	113.3 A	38.03 MN	11.23 LMN	53.20 BC
O3	52.72 BCDEFGH	17.75 JKLMN	15.40 GHI	58.18 ABCDEFG	11.57 JKLMN	4.275 LMNOP
O4	53.33 ABCDEF	19.10 CDEFGH	87.60 B	39.90 LM	11.05 N	54.85 ABC
O5	53.63 ABCD	17.38 LMN	25.85 EF	53.75 HIJ	11.73 IJKLMN	10.20 GHIJK
O6	53.13 ABCDEF	19.67 ABCDE	107.4 A	37.38 MN	11.20 LMN	56.85 AB
O7	51.90 FGHIJK	18.80 DEFGHIJK	45.03 D	51.40 J	11.43 KLMN	23.40 E
O8	51.93 FGHIJK	18.48 FGHIJKL	24.92 EF	55.40 GHI	11.88 HIJKLM	8.250 IJKLM
O9	52.90 BCDEFGH	20.30 AB	112.7 A	35.45 N	11.63 IJKLMN	59.15 A
G1	52.15 DEFGHIJK	17.90 IJKLM	48.70 D	47.55 K	12.68 ABCDEFG	21.15 E
G2	53.83 ABC	17.45 LMN	22.80 EFGH	58.75 ABCDE	12.38 BCDEFGHI	1.600 P
G3	53.47 ABCDE	17.23 MN	18.25 FGHI	59.10 ABCD	11.68 IJKLMN	2.250 P
G4	54.13 AB	16.70 N	24.83 EF	56.85 CDEFG	12.57 ABCDEFGH	2.625 OP
G5	52.60 CDEFGHIJ	17.45 LMN	29.60 E	55.63 FGH	12.70 ABCDEF	3.300 MNOP
G6	51.97 EFGHIJK	17.92 HIJKLM	25.70 EF	56.70 CDEFG	12.67 ABCDEFG	2.975 NOP
G7	52.83 BCDEFGH	17.15 MN	20.58 FGHI	58.33 ABCDEFG	12.32 CDEFGHIJ	4.125 LMNOP
G8	52.30 CDEFGHIJK	18.58 FGHIJKL	22.33 EFGH	57.17 BCDEFG	13.18 AB	3.725 LMNOP
G9	52.63 BCDEFGHIJ	17.77 JKLMN	19.45 FGHI	58.03 ABCDEFG	11.95 FGHIJKL	6.275 JKLMNOP
OA1	53.00 ABCDEFG	18.27 FGHIJKLM	19.58 FGHI	56.03 EFGH	12.13 EFGHIJK	15.65 F
OA2	52.30 CDEFGHIJK	17.63 KLMN	25.88 EF	57.90 ABCDEFG	13.10 ABC	8.950 IJKL
OA3	52.88 BCDEFGH	17.75 JKLMN	24.08 EFG	56.72 CDEFG	13.07 ABC	6.425 JKLMNOP
OA4	51.55 GHIJKL	19.08 CDEFGHI	20.55 FGHI	60.35 A	11.90 GHIJKLM	11.95 FGHI
OA5	52.13 DEFGHIJK	18.53 FGHIJKL	86.05 B	41.28 L	11.15 MN	52.05 C
OA6	52.55 CDEFGHIJ	18.08 GHIJKLM	26.05 EF	59.17 ABC	12.95 ABCD	8.125 IJKLMN
OA7	52.85 BCDEFGH	17.80 JKLMN	19.48 FGHI	59.45 ABC	12.65 ABCDEFGH	5.975 KLMNOP
OA8	51.83 FGHIJK	18.50 FGHIJKL	23.65 EFG	57.03 CDEFG	12.20 DEFGHIJ	12.07 FGHI
OA9	52.63 BCDEFGHIJ	18.05 GHIJKLM	19.98 FGHI	60.17 AB	12.60 ABCDEFGH	7.825 IJKLMNO
EA1	51.17 IJKL	19.30 BCDEF	12.75 I	56.10 DEFGH	12.95 ABCD	14.55 FGH
EA2	52.67 BCDEFGHI	17.55 LMN	14.15 HI	60.35 A	12.65 ABCDEFGH	8.475 IJKLM
EA3	52.58 CDEFGHIJ	18.83 DEFGHIJ	11.90 I	57.08 CDEFG	12.77 ABCDE	15.80 F
EA4	50.28 L	20.10 ABC	18.77 FGHI	58.30 ABCDEFG	13.25 A	10.75 FGHIJK
EA5	50.83 KL	19.77 ABCD	19.88 FGHI	58.55 ABCDEF	12.95 ABCD	11.43 FGHIJ
EA6	51.50 GHIJKL	18.58 EFGHIJKL	19.02 FGHI	57.63 ABCDEFG	12.82 ABCDE	9.625 HIJK
EA7	51.10 JKL	19.23 BCDEFG	18.05 FGHI	59.47 ABC	12.57 ABCDEFGH	11.02 FGHIJK
EA8	51.95 EFGHIJK	18.33 FGHIJKLM	12.75 I	57.88 ABCDEFG	12.75 ABCDE	10.65 FGHIJK
Control	51.40 HIJKL	19.75 ABCD	63.95 C	51.92 J	10.15 O	41.67 D
Range	50.3 - 54.4	16.7 - 20.7	11.9 - 113.3	35.4 - 60.4	10.2 - 13.2	1.6 - 59.2
LSD	1.239	0.9704	7.158	2.477	0.6516	4.401

DMR test (0.05): Means followed by same letters are not significantly different from each other.

Table 4. Mean squares and CV values of some biochemical traits of advance Brassica mutant lines.

Parameters	Mean squares	CV (%)
Oil (%)	3.28**	1.69
Protein (%)	3.72**	3.76
Glucosinolates (uMg^{-1})	3481**	14.88
Oleic acid (%)	198.84**	3.25
Linolenic acid (%)	2.25**	3.80
Erucic acid (%)	1209.21**	18.95

**Significant at 1% level

4.2 GENETIC VARIABILITY

4.2.1 GENETIC VARIABILITY FOR AGRONOMICAL TRAITS

4.2.1.1 DAYS TO FLOWERING (DF)

For days to flowering phenotypic variance (σ^2p) was 50.54 and genotypic variance (σ^2g) was 45.65 and the phenotypic coefficient of variations (PCV) and the genotypic coefficient of variations (GCV) were 9.06 and 8.61 respectively (Table 5). This indicates that the σ^2p and the PCV give larger values than the σ^2g and the GCV for this trait. Slightly higher value of PCV than GCV and similarly higher value of σ^2p compared to σ^2g shows environmental effect on this character. The coefficient of variation shows only the extent of total variability and does not separate the variability into heritable and non-heritable portion.

4.2.1.2 PLANT HEIGHT (PH)

For Plant height slightly higher σ^2p (137.19) than the σ^2g (106.98) and similarly higher PCV (9.90) compared to GCV (6.10) indicated the environmental effects (Table 5). Ali (1985) also found high genotypic and phenotypic variances for plant height and pods per plant in Brassica juncea.

4.2.1.3 1000 SEED WEIGHT (SW)

For this trait phenotypic variance was 0.14 and genotypic variance was 0.12 and the phenotypic and the genotypic coefficient of variations were 7.88 and 7.43 respectively (Table 3). On average the check showed higher value of 1000 seed weight than the mutant line.

4.2.1.4 SEED YIELD (SY)

High phenotypic variance (144969.56) and high genotypic variance (123563.75) were observed for seed yield. The phenotypic and the genotypic coefficient of variations for seed yield were 20.59 and 19.01 respectively (Table 5). High GCV, PCV were stated for seed yield and number of pods per plant. Higher σ^2g and σ^2p were recorded for seed yield.

4.2.1.5 OIL YIELD (OY)

The phenotypic variance for oil yield was 41123.48 and genotypic variance was 40550.88 and the phenotypic and the genotypic coefficient of variations were 20.91 and 20.76 respectively (Table 5).The value of σ^2p was high compared to σ^2g and the value of PCV was recorded higher compared to GCV for oil yield. This suggests the effect of environment for the inheritance of this character. The coefficient of variation shows only the extent of total variability present for specific characters and does not demarcate the variability into heritable and non-heritable portion.

4.2.2 GENETIC VARIABILITY FOR BIOCHEMICAL TRAITS

4.2.2.1 PHENOTYPIC AND GENOTYPIC VARIANCE

The glucosinolates and erucic acid recorded the highest phenotypic and genotypic variances showing the values 870.4, 863.9 and 302.3, 299.8 respectively. The oleic acid (49.71, 48.9) exhibited moderate values of phenotypic and genotypic variances. The level of phenotypic and genotypic variances were low for the protein (0.93, 0.81), oil (0.82, 0.62) and linolenic acid 0.57, 0.51) respectively.

4.2.2.2 PHENOTYPIC AND GENOTYPIC COEFFICIENT OF VARIATIONNS

The phenotypic coefficient of variation was maximum for erucic acid content (106.9) followed by glucosinolates (86.0). Moderate phenotypic coefficient of variation values (13.0) was obtained for oleic acid. Low phenotypic coefficient of variation values were recorded by linolenic acid (6.2), protein (5.2) and oil (1.7) respectively.

Genotypic coefficient of variation was highest for erucic acid (106.4) followed by glucosinolates (85.6). Moderate phenotypic coefficient of variation values (12.9) was obtained for oleic acid. Low genotypic coefficient of variation value was recorded for linolenic acid (5.8), protein (4.9) and oil (1.5) respectively.

Phenotypic variances (σ^2p) were higher than genotypic variances (σ^2g) and phenotypic coefficient of variations (PCV) were higher than the genotypic coefficient of variations (GCV) for all the observed characters (Table 6). Highest σ^2p, σ^2g, PCV and GCV were observed for glucosinolate and erucic acid. For all components slightly higher PCV than GCV and similarly higher σ^2p compared to σ^2g indicating the influence of environment for the expression of these characters. The coefficient of

variation describes only the degree of total variability present for characters and does not segregate the variability into inherited and non-inherited portion.

4.3 HERITABILITY AND GENETIC ADVANCE

4.3.1 HERITABILITY AND GENETIC ADVANCE FOR AGRONOMICAL TRAITS

4.3.1.1 DAYS TO FLOWERING

Estimates of heritability (broad sense) and genetic advance as percent of means were presented in Table 5. High value of heritability (90.3) and moderate value of genetic advance (16.88) were observed for days to flowering (Table 5) suggesting non-additive (dominance or epistatic) gene effect. This indicated that a trait having high heritability did not essentially produce high genetic advance. Thus only heritability did not give the indication for genetic advancement that could be maintained through selection.

4.3.1.2 PLANT HEIGHT

The trait plant height (Table 5) exhibited high heritability (78.0) and moderate genetic advance (11.11). This suggests the involvement of non-additive (dominance or epistatic) gene effect for this trait.

4.3.1.3 1000 SEED WEIGHT

The trait 1000 seed weight (Table 5) exhibited high heritability (88.9) and moderate genetic advance (14.46). Slightly higher phenotypic variance than the

genotypic variance and high heritability along with moderate genetic advance for this trait was probably due to non-additive (dominance or epistatic) gene effects.

4.3.1.4 SEED YIELD

The seed yield (Table 5) displayed high heritability (85.2) and high genetic advance (36.20). Both heritability and genetic advance showed higher values for this trait which suggests the effect of additive gene for the inheritance and selection in the early generation in this trait could be productive in improving this character.

4.3.1.5 OIL YIELD

The oil yield (Table 5) gives highest heritability (98.6) and highest genetic advance (42.54). A trait having high heritability and high genetic advance is considered under the control of additive genes for inheritance which highlights the usefulness of plant selection based on phenotypic performance and selection in the early generation could be fruitful in improving this character.

4.3.2 HERITABILITY AND GENETIC ADVANCE FOR BIOCHEMICAL TRAITS

4.3.2.1 HERITABILITY

Estimates of heritability (broad sense) and genetic advance as percent of means were presented in Table 6. The experimental populations showed high heritability values for all the characters studied. The highest heritability was recorded by glucosinolates (99) and erucic acid (99) followed by oleic acid (98), linolenic acid (90) and protein (87). Oil content (76) showed the lowest heritability values among all the traits. Broad sense heritability estimates ranged from 78.0% to 98.6% for all characters. The highest genetic advance as percent of mean was recorded for erucic acid (218.7) followed by glucosinolate (176). The characters that had recorded moderate genetic advance as per cent of mean were oleic acid (26.4), linolenic acid and protein (9.4). Among all the traits the lowest genetic advance as percent of mean value was recorded for oil (2.7).

4.3.2.2 GENETIC ADVANCE

Genetic advance as percent of means was higher for erucic acid and glucosinolate showing that selection based on these traits would be effective. Similar results to high heritability and genetic advance have been recorded by earlier workers for various characters.

In order to guess the selection effects, heritability and genetic advance together is somewhat more useful than heritability alone. Heritability and genetic advance were maximum for erucic acid and glucosinolate. A trait having high heritability and high

genetic advance is considered under control of additive genes which highlights the usefulness of plant selection based on phenotypic performance.

Table 5. Genetic parameters of some quantitative traits of advance Brassica mutant lines

Parameter	σ2p	σ2g	PCV	GCV	h2 (%)	GA (%)
Days to flowering	50.54	45.65	9.06	8.61	90.3	16.88
Plant height (cm)	137.19	106.98	6.90	6.10	78.0	11.11
1000 seed weight (g)	0.14	0.12	7.88	7.43	88.9	14.46
Seed yield kg ha^{-1})	144969.56	123563.75	20.59	19.01	85.2	36.20
Oil yield (kg ha^{-1})	41123.48	40550.88	20.91	20.76	98.6	42.54

σ^2p (phenotypic variance), σ^2g (genotypic variance), PCV (phenotypic coefficient of 'variation), GCV (genotypic coefficient of variation), h^2 (heritability), GA (genetic advance)

Table 6. Genetic parameters of some qualitative traits of advance Brassica mutant lines

Parameter	σ2p	σ2g	PCV	GCV	h2 (%)	GA (%)
Oil (%)	0.82	0.62	1.7	1.5	76	2.7
Protein (%)	o.93	0.81	5.2	4.9	87	9.4
Glucosinolates (uMg^{-1})	870.4	863.9	86.0	85.6	99	176
Oleic acid (%)	49.71	48.9	13.0	12.9	98	26.4
Linolenic acid (%)	0.57	0.51	6.2	5.8	90	11.5
Erucic acid (%)	302.3	299.8	106.9	106.4	99	218.7

σ^2p (phenotypic variance), σ^2g (genotypic variance), PCV (phenotypic coefficient of 'variation), GCV (genotypic coefficient of variation), h^2 (heritability), GA (genetic advance)

4.4 PHENOTYPIC CORRELATION

4.4.1 PHENOTYPIC CORRELATION FOR AGRONOMICAL TRAITS

4.4.1.1 SEED YIELD

The correlation values presented in Tables-7 show significant negative correlation of yield with seed weight (-0.96) and plant height (-0.52) while shows non significant negative correlation with days to flowering (-0.18).

4.4.1.2 DAYS TO FLOWERING

The correlation values presented in Tables-7 indicates that days to flowering had highly significant but negative association with plant height (-0.55) and non significant negative association with yield (-0.18).

4.4.1.3 PLANT HEIGHT

The correlation values presented in Tables-7 show significant negative correlation of plant height with yield (-0.52) and days to flowering (-0.55) respectively, while showed significant positive association with seed weight (0.55).

4.4.1.4 1000 SEED WEIGHT

Similarly according to correlation values presented in Tables-7, seed weight shows significant positive relationship with plant height (0.55) and non significant positive relationship with days to flowering (0.11) while had significant negative association with yield (-0.96).

4.4.2 PHENOTYPIC CORRELATION FOR BIOCHEMICAL TRAITS

4.4.2.1OIL CONTENT (%)

According to the data presented in table-8 the oil showed significant negative correlation with protein (-0.55) and non significant negative association with oleic acid (-0.18) and linolenic acid (-0.24), while showed non significant positive association with glucosinolates (0.11) and erucic acid (0.02).

4.4.2.2 PROTEIN CONTENT

The protein content according to Table-8 had highly significant but positive association with glucosinolates (0.55) and erucic acid (0.65), however protein had significant negative association with oil (-0.55) and non significant negative correlation with linolenic acid (-0.20) and oleic acid (-0.18).

4.4.2.3 GLUCOSINOLATES

The relation of glucosinolates is highly significant but positive with erucic acid (0.94 and protein (0.55) and non significant positive with oil (0.11). Similarly glucosinolates showed highly significant negative association with oleic acid (-0.96) and linolenic acid (-0.56) (Table-8).

4.4.2.4 OLEIC ACID

The oleic acid had highly significant negative correlation with glucosinolates (-0.96), erucic acid (-0.94) and protein (-0.52) while had significant positive correlation with linolenic acid (0.52) while had non significant negative correlation with oil (-0.18).

4.4.2.5 LINOLENIC ACID

Linolenic acid showed significant positive correlation with oleic acid (0.52) and significant negative association with glucosinolates (-0.56) and erucic acid (-0.62), while negative but non significant correlation with oil (-0.24) and protein (-0.20) (table-8).

4.4.2.6 ERUCIC ACID

The correlation values presented in Table-8 indicated that erucic acid had highly significant positive association with protein (0.65) and glucosinolates (0.94 and non-significant positive association with oil (0.02). However, erucic acid was highly significant negative associated with oleic acid (-0.94) and linolenic acid (-0.62) respectively.

Generally, low phenotypic correlation was recorded among different characters; however some of the traits like oil, protein and oleic acid were negatively correlated with each other as compared to the remaining traits which were non-significantly correlated with each other. So selections based on such traits are valuable for quality improvement.

Table-7. Phenotypic Correlations of some agronomical traits of advance Brassica mutant lines.

Parameter	Plant height (Cm)	1000-SW (g)	Seed Yield (Kg/hac)
Days to flowering	-0.55	0.11	-0.18
Plant height (Cm)		0.55	-0.52
1000-seed weight			-0.96

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Table-8. Phenotypic Correlations of some biochemical traits of advance Brassica mutant lines.

Parameter	Protein (%)	glucosinolates (uMg^{-1})	Oleic acid (%)	Linolenic acid (%)	Erucic acid (%)
Oil (%)	-0.55	0.11	-0.18	-0.24	0.02
Protein (%)		0.55	-0.52	-0.20	0.65
glucosinolates (uMol/g)			-0.96	-0.56	0.94
Oleic acid (%)				0.52	-0.94
Linolenic acid (%)					-0.62

4.5 CLUSTER ANALYSIS

Analysis or clustering is the task of assigning a set of objects into groups called clusters so that the objects in the same cluster are more similar to each other than those in the other. This numerical taxonomic technique was used to calculate Euclidean dissimilarity coefficient matrices for the agronomic and biochemical traits.

4.5.1 CLUSTER ANALYSIS FOR AGRONOMICAL TRAITS

Five agronomic traits i.e. Days to flowering, Plant height, 1000 seed weight, Seed yield (kg ha^{-1}) and Oil yield (kg ha^{-1}) were observed in the parent (Abasin-95) and 35 advance mutant lines of rapeseed Brassica. Single dendogram was constructed for all the 35 advance mutant lines to check the performance of the selected material. As expected all the mutant material were distinct from the parent.

4.5.1.1 AGRONOMIC TRAITS

Two main groups were constructed on the bases of agronomic study. The first group had 22 mutant lines, while the second group had 14 mutant lines along with the parent.

Two main clusters were formed in the first group. The first cluster of the first group contains 15 mutant lines and further subdivided into two sub clusters. The first sub cluster contains 13 mutant lines (O1, OA3, EA5, EA7, EA6, O7, OA4, EA8, G3, EA1, OA1 and OA8) and became an ideal group as it had the most desirable range of all the mentioned agronomic traits. The second sub cluster had only two mutant lines

(O3 and G9) and is also the most suitable group on the bases of their agronomic characteristics.

The second cluster of first group is also further subdivided into two sub clusters. The first sub cluster consists of four mutant lines (O5, O8, G8 and OA6), while the second sub cluster contains three mutant lines (G2, OA7 and OA2). All the mutants in this cluster showed high to moderate values for all the agronomic traits under observation.

The second group is also subdivided into two clusters. The first cluster consists of 11 mutant lines and the parent, while the second sub cluster contains only the 2 mutant lines.

The first cluster of the second group is further subdivided into two sub clusters. The first sub cluster contains four mutant lines (O2, O4, O6 and O9) and the parent (Abasin-95), while the second sub cluster had seven mutant lines (G4, OA9, EA3, G5, G7, G6 and EA2). The second cluster of second group had only two advance mutant lines (G1 and OA5). All these mutant lines and the parent showed the lower value for the desired agronomic traits.

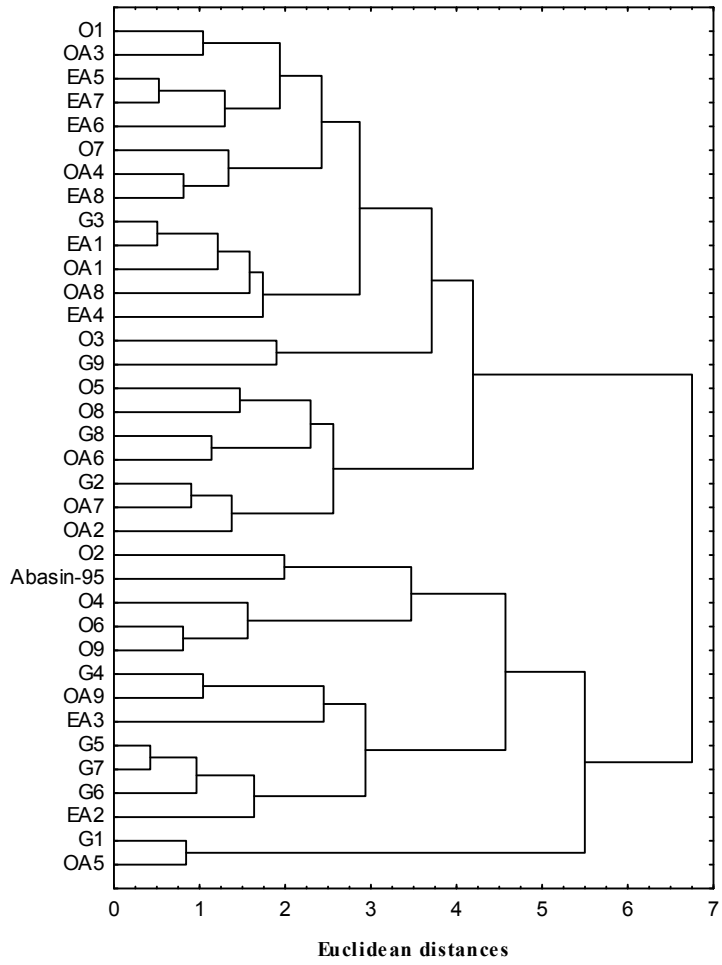

Figure 1. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on some agronomical traits

4.5.1.2 DAYS TO FLOWERING AND PLANT HEIGHT

These traits also produced two groups. The first group had twenty eight mutant lines, while the second group had seven mutant lines along with the parent.

Two main clusters were formed in the first group. The first cluster of the first group was further subdivided into two sub clusters. Sub cluster1 contains eight mutant lines (O1, OA9, EA5, G3, EA7, EA1, G4, and OA1) and became an ideal group as it had the most desirable range for both the mentioned agronomic traits. The second sub cluster had only three mutant lines (O7, OA4, and EA8) and is also the most suitable group on the bases of their agronomic characteristics.

The second cluster of first group is also further subdivided into two sub clusters. The first sub cluster consists of seven mutant lines (O3, O5, G8, O8, OA3, G9 and EA6), while the second sub cluster contains ten mutant lines (G1, OA6, G5, G6, G7, G2, EA2, OA2, OA5 and OA7). All the mutants in this cluster showed high to moderate values for all the agronomic traits under observation.

The second group is also subdivided into two clusters. The first cluster consists of five mutant lines (O2, O4, OA8, EA3 and EA4) and the parent, while the second sub cluster contains only the two mutant lines (O6 and O9).

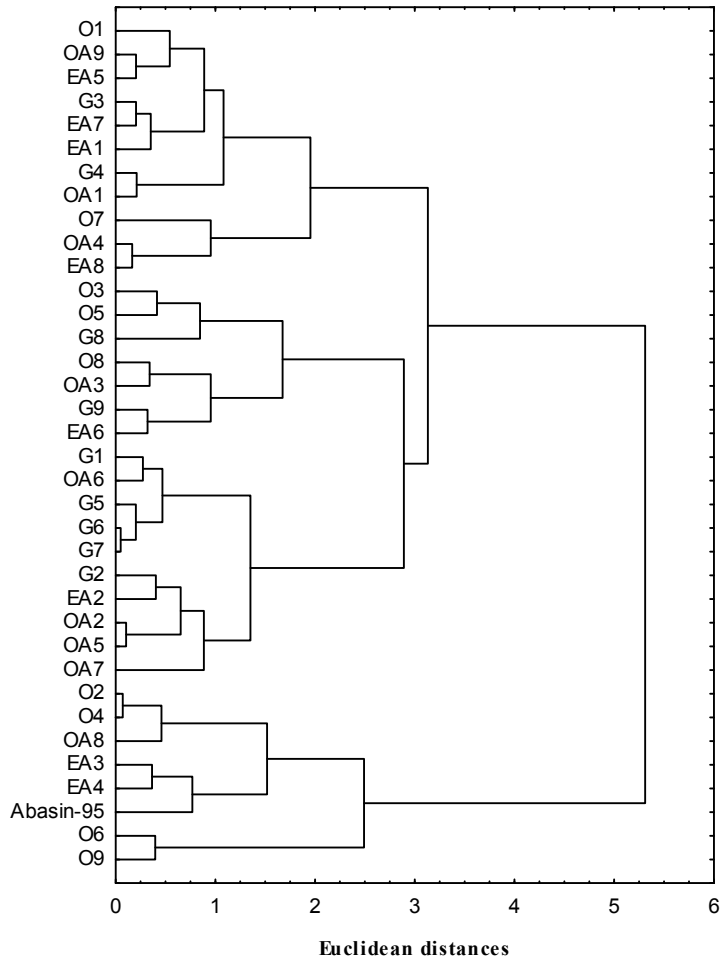

Figure 2. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on days to 50% flowering and plant height

4.5.1.3 SEED YIELD AND SEED WEIGHT

Two main groups were constructed for these characters. The first group had thirty three advance mutant lines, while the second group had only two mutant lines along with the parent.

Two main clusters were formed in the first group. The first cluster of the first group was further subdivided into two sub clusters. The first sub cluster was the largest and contains eighteen mutant lines (O1, OA8, G7, G3, G5, EA1, OA2, O3, OA3, OA1, OA4, G6, EA3, EA2, EA5, EA8, EA7 and EA6) and became an ideal group as it had the most desirable range for both the mentioned agronomic traits. The second sub cluster had eight mutant lines (O2, OA6, OA9, G4, G8, O4, O6 and O9) and is also the most suitable group on the bases of their agronomic characteristics.

The second cluster of first group is also further subdivided into two sub clusters. The first sub cluster consists of five mutant lines (O5, O8, G2, OA7 and EA5), while the second sub cluster contains only two mutant lines (O7 and G9). All the mutants in this cluster showed high to moderate values for all the agronomic traits under observation. The second group is also subdivided into two clusters. The first cluster consists of only two mutant lines (G1 and OA5), while the second cluster had only the parent.

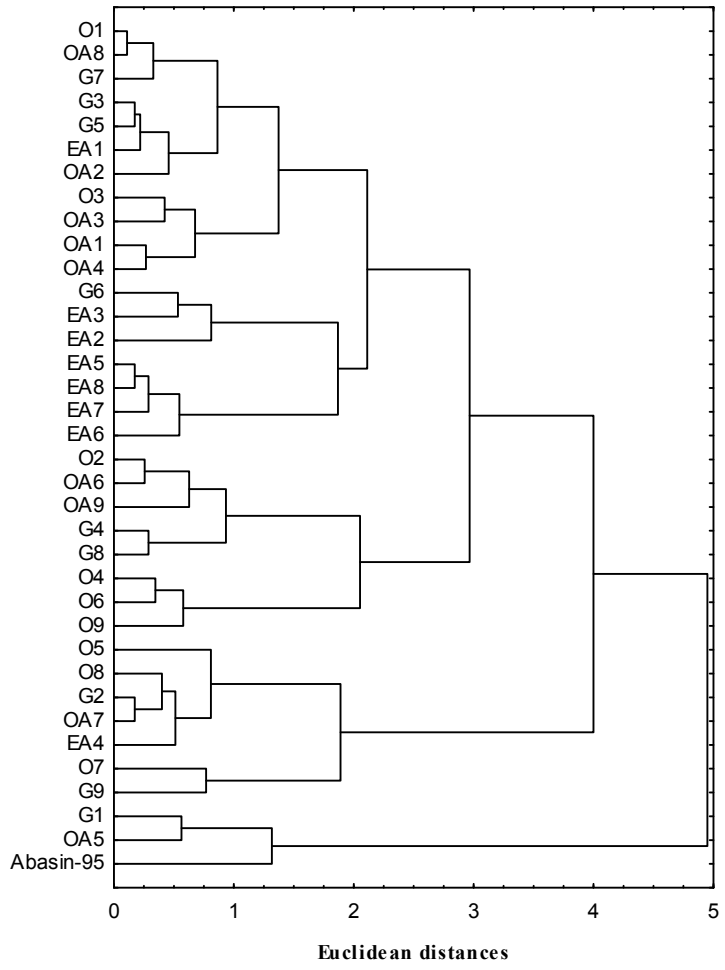

Figure 3. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on seed yield and seed weight

4.5.1.4 OIL CONTENT AND SEED WEIGHT

Two main groups were constructed for these characters. All the mutants except the G1, OA5, EA4 and the parent constitute the first group.

Two main clusters were formed in the first group. The first cluster was further subdivided into two sub clusters. The first sub cluster contains four mutant lines (O1, G4, O5 and G2) and became an ideal group as it had the most desirable range for both the mentioned agronomic traits. The second sub cluster had relatively larger number of mutants (O2, G8, G9, OA6, OA7, O7, OA2, O8, O3, G5, OA9, O9, G7, OA3, G3 and OA1) and is also the most suitable group on the bases of their agronomic characteristics.

The second cluster of first group was also further subdivided into two sub clusters. The first sub cluster consists of four mutant lines (O4, O6, EA2 and EA3), while the second sub cluster contains eight mutant lines (G6, EA6, OA8, EA8, OA4, EA1, EA5 and EA7). All the mutants in this cluster showed high to moderate values for all the agronomic traits under observation. The second group is also subdivided into two clusters. The first cluster consists of two mutant lines (G1 and OA5) along with the parent, while the second cluster had only one mutant line EA4.

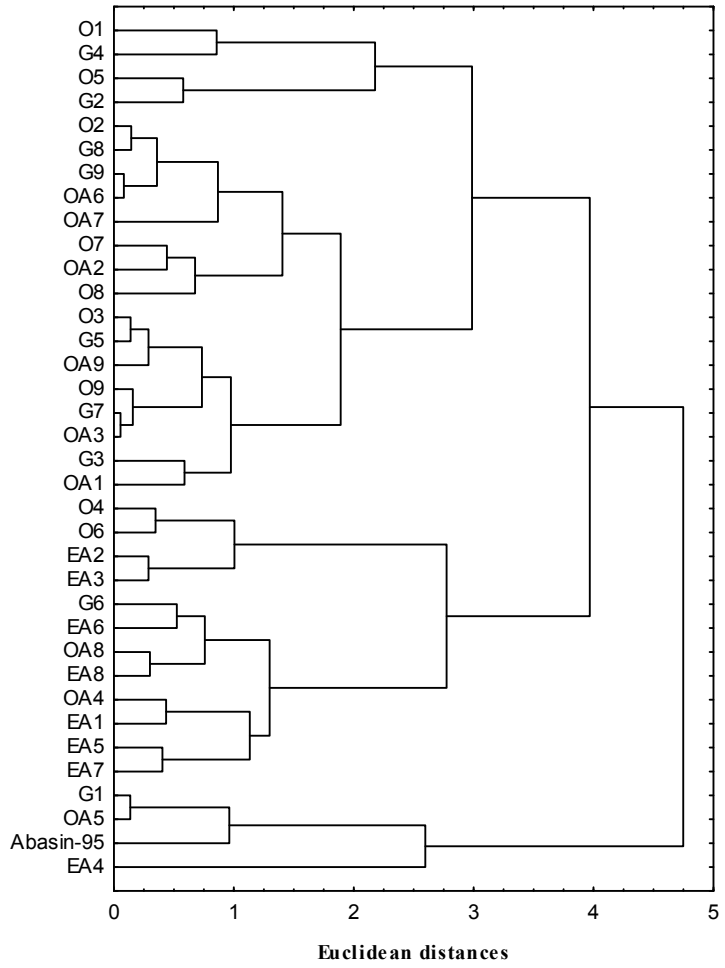

Figure 4. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on oil contents and seed weight

4.5.1.5 SEED WEIGHT AND OIL YIELD

Two main groups were constructed for these characters. All the mutants except the G1, OA5 and the parent constitute the first group.

Two main clusters were formed in the first group. The first cluster of the first group contains twenty five mutant lines and further subdivided into two sub clusters. The first sub cluster was the largest cluster of mutants and contained seventeen mutant lines (O1, G7, EA1, G3, G5, OA2, OA1, OA4, OA3, OA8, EA5, EA8, EA7, EA6, G6, EA3 and EA2) and became an ideal group as it had the most desirable range for both the mentioned agronomic traits. The second sub cluster had eight mutant lines (O2, OA6, G4, OA9, G8, O4, O6 and O9) and is also the most suitable group on the bases of their agronomic characteristics.

The second cluster of first group had eight mutant lines and is also further subdivided into two sub clusters. The first sub cluster consisted of three mutant lines (O3, O7 and G9), while the second sub cluster constituted five mutant lines (O5, O8, G2, OA7 and EA4). All the mutants in this cluster showed high to moderate values for all the agronomic traits under observation.

The second group is also subdivided into two clusters. Cluster 1 had two mutant lines (G1 and OA5), while the second cluster had only the parent (Abasin 95).

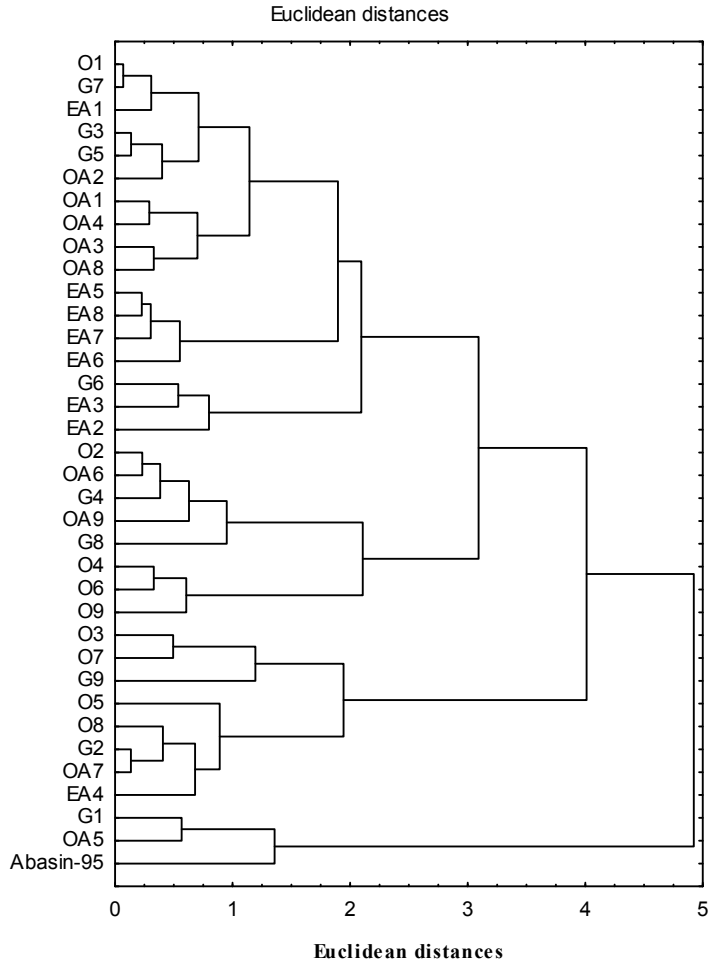

Figure 5. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on seed weight and oil yield

4.5.2 CLUSTER ANALYSIS FOR BIOCHEMICAL TRAITS

The results of cluster analysis were presented in the form of dendogram to estimate the degree of introgression at biochemical level and to check relationships among genotypes using six quality traits i.e. oil content, protein content, glucosinolates and three fatty acids (oleic, linolenic and erucic acid). Dendogram was constructed using complete linkages method.

4.5.2.1 BIOCHEMICAL TRAITS

Two main groups were constructed on the bases of biochemical traits. The first group had thirty mutant lines, while the second group had five mutant lines and the parent.

Two main clusters were formed in the first group. The first cluster of the first group was further subdivided into two sub clusters. The only mutant line O1, was incorporated in the first sub cluster mainly because of high oil (54.4%), low glucosinolate (14.48), high oleic acid (52.90%) and moderate oleic acid (11.13%) and erucic acid (15.20%). The second sub cluster accumulate fifteen advance mutant lines (O3, G9, OA1, G5, G7, OA2, OA6, OA3, OA7, OA9, EA2, O5, G3, G2 and G4) having comparatively high oil (51.1-52.5%) low glucosinolates (15.4-29.6) and erucic acid (3.3-12.0%) and is the most suitable group as it had most of the quality components in the desirable range.

The second cluster is also further subdivided into two sub clusters. The first sub cluster consisted of ten mutant lines (O7, O8, OA8, OA4, G1, G6, EA6, EA8, G8 and EA3) and was clustered together due to relatively moderate quality components. The second sub cluster had four advance mutant lines (EA1, EA7,EA4 and EA5) having comparatively moderate to low erucic acid (10.8-14.6) and moderate to high

glucosinolate (12.8-19.9) The second group is also subdivided into two clusters. The first cluster consists of five mutant lines (O2, O6, O9, O4 and OA5) on the basis of high oil (52.1-53.3%), high glucosinolates (86.1-112.7) and high erucic acid (52.1-59.2%). The parent is totally distinct from the rest of mutants and placed in a separate group as it had the highest value of glucosinolates (64.0).

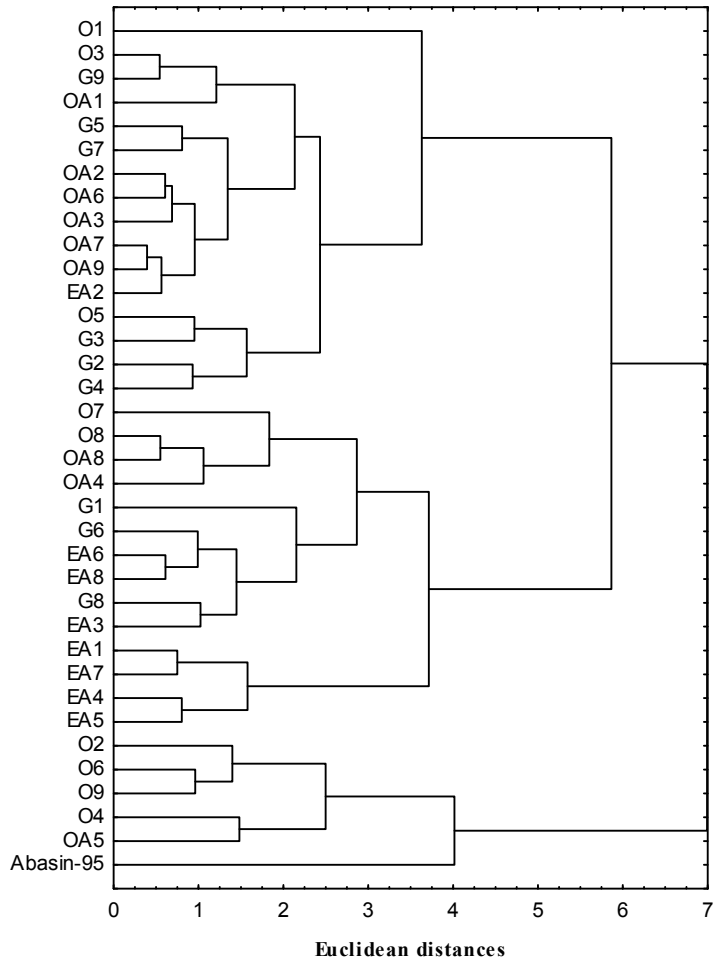

Figure 6. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on some biochemical traits

4.5.2.2 OIL AND PROTEIN

Two main groups were constructed on the bases of biochemical traits; the first group had twenty four mutant lines, while the second group had eleven mutant lines along with the parent.

Two main clusters were formed in the first group. The first cluster contains five mutant lines (O1, O5, G3, G2 and G4), and became an ideal group as it had highest value of oil (54.4-53.6%) and lowest values for protein (16-17.4%). The second cluster is further subdivided into two sub clusters. The first sub cluster consists of ten mutant lines (O3, G9, OA3, OA7, OA6, OA9, G5, EA2, OA2 and G7), while the second sub cluster contains nine mutant lines (O8, OA8, EA8, G8, OA5, G1, G6, OA1 and EA3). Both these sub clusters showed high to moderate values for oil and low to moderate value for protein.

The second group is also subdivided into two clusters. The first cluster had four mutant lines (O2, O9, O4 and O6).this group had relatively high oil (52.9-53.3%) and relatively high protein (19.1 20.7%). The second cluster of group second is also subdivided into two sub clusters. The first sub cluster consists of three mutant lines (O7, OA4 and EA6), while the second sub cluster contains four mutant lines (EA1, EA7, EA5 and EA4) and the parent having relatively low oil (50.3-51.4%) and relatively high protein (19.2-20.1%).

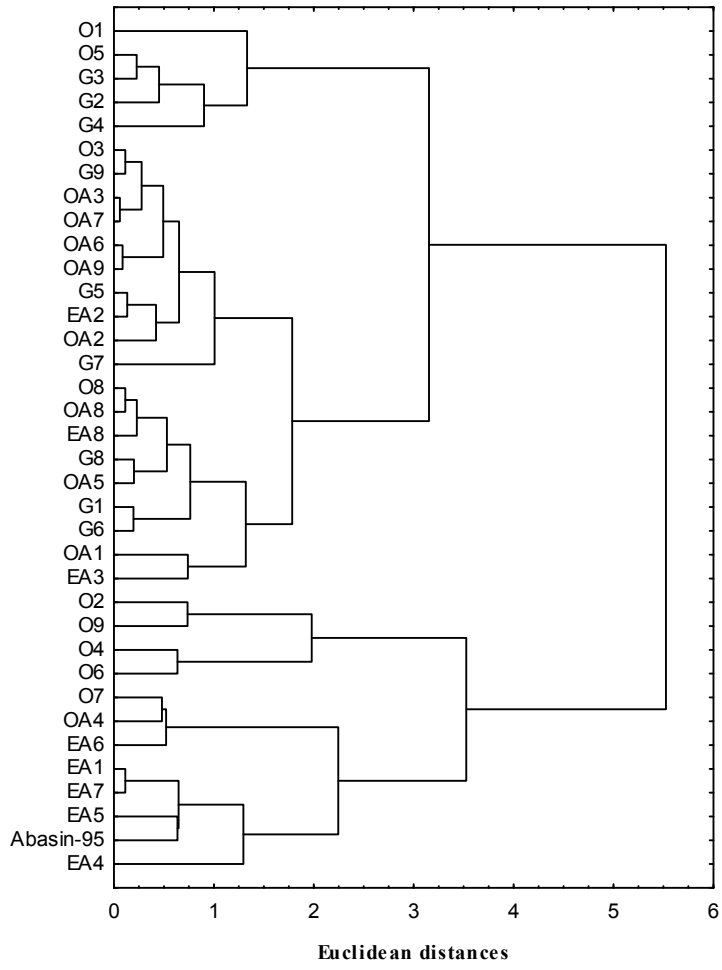

Figure 7. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on oil and protein contents

4.5.2.3 GLUCOSINOLATES AND ERUCIC ACID

Two main groups were constructed for these characters. The first group had thirty mutant lines along with the parent, while the second group had five mutant lines.

Two main clusters were formed in the first group. The first cluster of the first group contains twenty eight mutant lines and further subdivided into two sub clusters. The first sub cluster contains six mutant lines (01, EA1, EA3, OA1, EA2 and EA8), and became an ideal group as it had relatively the most desirable range(Glucosinolates 11.9-19.6, erucic acid 8.5-15.8) for both the mentioned biochemical traits. The second sub cluster clustered larger number of mutants (O3, G3, G9, OA7, OA9, OA3, G2, G4, G6, G7, G8, G5, O5, O8, OA6, OA2, OA4, EA5, OA8, EA4, EA7 and EA6) and had a wide range of both glucosinolates (15.4-64.0) and erucic acid (1.6-41.7).

The second cluster of first group is also further subdivided into two sub clusters. The first sub cluster consists of two mutant lines (O7, G1), while the second sub cluster contains only the parent. All the mutants in this cluster showed high to moderate values for both the mentioned traits (glucosinolates 45.0-48.7 and erucic acid 21.2-23.4).

The second group is also subdivided into two clusters. The first cluster consists of five mutant lines O2, O6, O9, O4 and OA5) and the parent, while the second sub cluster contains only the two mutants (O7 and G1) on the presence of high glucosinolates (86.1-113.4) and high erucic acid (52.1-59.2).

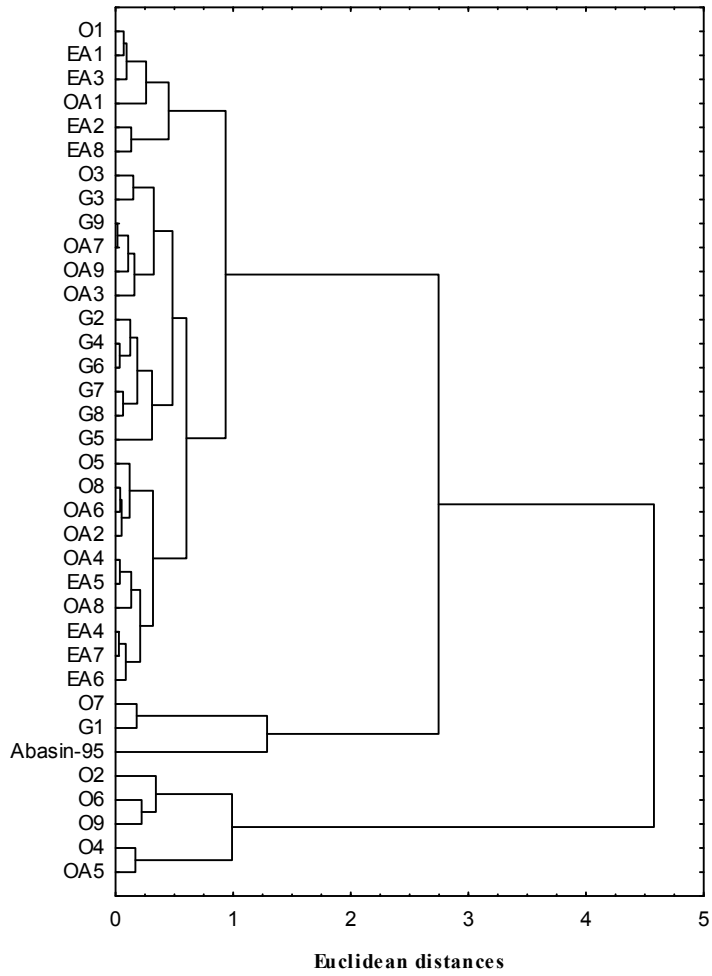

Figure 8. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on glucosinolates and erucic acid

4.5.2.4 FATTY ACIDS

Two main groups were constructed for these characters. The first group had 30 mutant lines while the second group had 5 mutant lines along with the parent. Two main clusters were formed in the first group. The first cluster of the first group contains 10 mutant lines and further subdivided into two sub clusters. The first sub cluster contains 2 mutant lines and became an ideal group as it had the most desirable range for both the mentioned biochemical traits. The second sub cluster had 8 mutant lines and is also the most suitable group on the bases of their fatty acid profile.

The second cluster of first group is also further subdivided into two sub clusters. The first sub cluster consists of only one mutant line, while the second sub cluster contains 19 mutant lines. All the mutants in this cluster showed high to moderate values for all the mentioned traits under observation. The second group is also subdivided into two clusters. The first cluster consists of 5 mutant lines and further subdivided into two sub clusters. The first sub cluster contains 4 mutant lines while the second sub cluster had only one mutant line (O9). The second cluster of second group had only the parent.

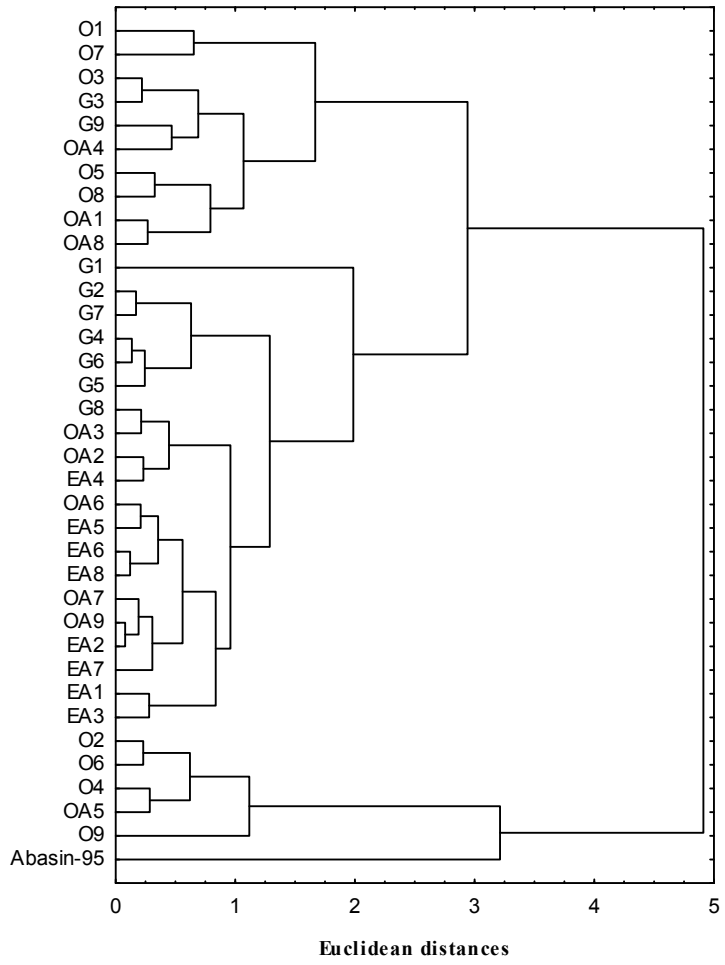

Figure 9. Dendogram depicting the clustering pattern of thirty five advance mutant lines and one check based on fatty acids profile

4.6 PRINCIPAL COMPONENT ANALYSIS (PCA)

PCA involves an arithmetic procedure that converts number of related variables into a number of unrelated variables called principal components. The first principal component describes most of the variability in the data, and each following component suggests for the remaining variability.

Objectives of PCA

- To find out or to decrease the dimensionality of the data.
- To recognize new significant primary variables.

4.6.1 PCA FOR AGRONOMICAL TRAITS

Five morphological parameters were analyzed by PC analysis (Sneath and Sokal, 1973). The parameters includes Days to flowering, Plant height, 1000 seed weight, Seed yield (kg ha^{-1}) and Oil yield (kg ha^{-1}).

The mean of parental lines and the advance mutant lines were standardized prior to PC analysis using Z score (Stat view, version 4.02). PCA was performed with the said data matrix. Scatter plots of the PCs that had an Eigen value more than 1 were produced to provide a graphical representation of pattern of variation among the mutants (Statisca version 7.0). These coefficients were scaled, so that they present correlations between observed variables derived components.

It was observed that 3 PCs exhibited 94.29%of the total variability among the 35 advance mutant lines and one parental line. The first PC accounted for 49.36 % of the total variance and contribute negatively for all the parameters i.e. for seed yield (-0.39), oil yield (-0.39) and for plant height (-0.10) and depicted quite different divergence of mutations. Second PC accounted for an additional 32.52% of the total variation and had a positive contribution of 0.52 for days to flowering, and 0.34 for plant height. All the remaining variables i.e. seed weight (-0.45), seed yield (-0.10) and oil yield (-0.10) depicted negative influence. The PC3 contributed 12.39% of the total variations and was a measure of seed weight (0.99), days to flowering (0.72) and plant height (0.09), while the contribution of oil yield (-0.23) and seed yield (-0.20) were inverse.

Table 9. Principal components for some of the agronomical traits in the 35 advance mutants lines and one check

PCA table	PC1	PC2	PC3
Eigen value	2.46	1.62	0.61
Cumulative Eigen value	2.46	4.09	4.71
% Total variance	49.36	32.52	12.39
Cumulative %	49.36	81.89	94.28
Traits		**Eigenvectors**	
Days to flowering	-0.05	0.52	0.71
Plant height	-0.29	0.34	0.08
Seed weight	-0.10	-0.45	0.99
Seed yield	-0.39	-0.10	-0.20
Oil yield	-0.39	-0.10	-0.23

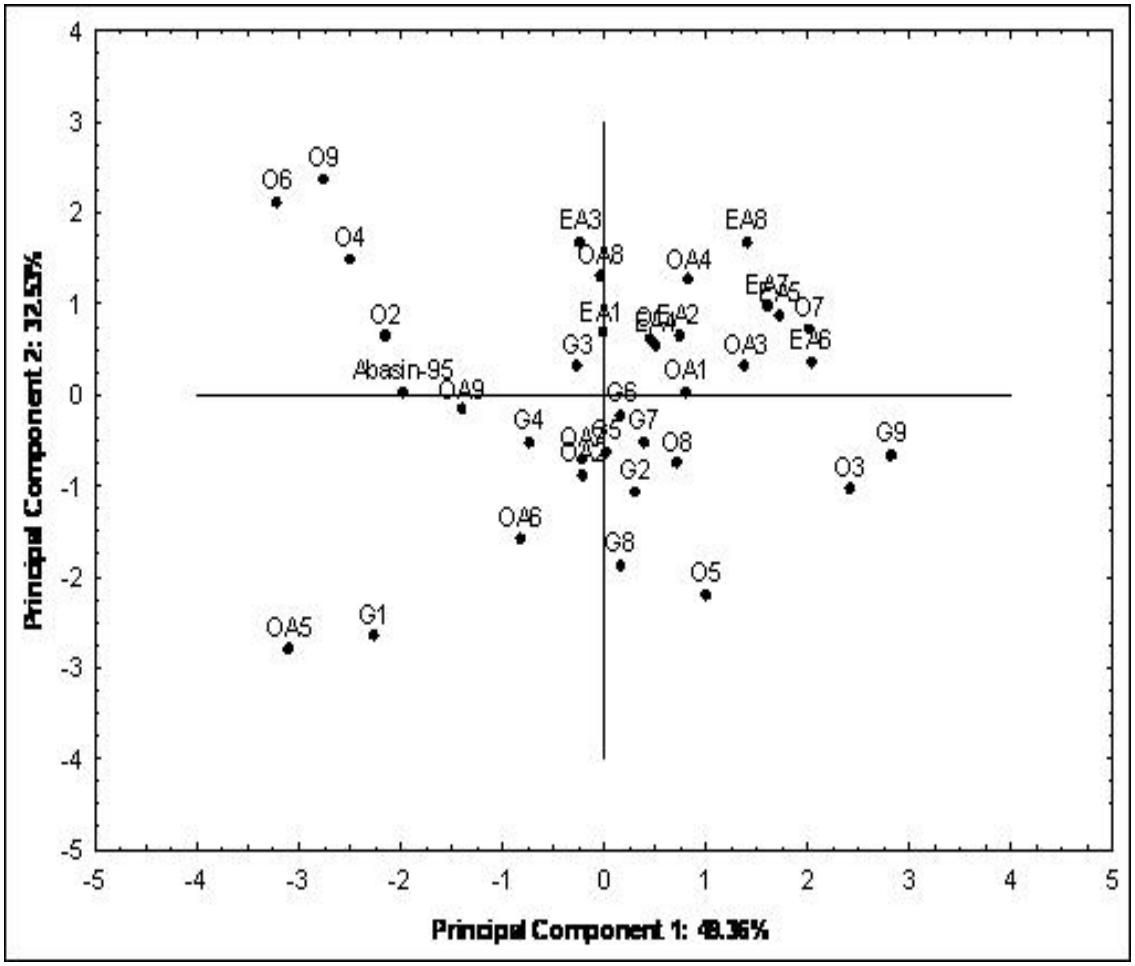

Figure 10. Scatter diagram for the first two PCs for some agronomical traits in thirty five advance mutant lines and one check

4.6.2 PCA FOR BIOCHEMICAL TRAITS

Six morphological parameters were analyzed by PC analysis (Sneath and Sokal, 1973). The parameters include Oil content, protein content, glucosinolates, oleic acid, linolenic acid and erucic acid. It was observed that 3 PCs exhibited 96.11% of the total variability among the thirty five advance mutant lines and one parental line. The first PC accounted for 63.02 % of the total variance. Out of six variables three contributed positively i.e. erucic acid (0.98), glucosinolates (0.96) and protein content (0.71), while the remaining three i.e. oleic acid (-0.93), linolenic acid (-0.72) and oil content (-0.05) contributed negatively.

Second PC accounted for an additional 24.55% of the total variation and was a measure of oil content (0.97) and glucosinolates (0.11). The proportion of erucic acid (0.001) was very small in this PCs, while the variables protein (-0.64), linolenic acid (-0.29) and oleic acid (-0.19) depicted no association with this PCs. The third PC accounted for 8.53% of the total variations. Except oleic acid (-0.26), all other variables contributed positively. Maximum positive contribution was depicted by linolenic acid (0.63). Glucosinolates (0.18) and oil content (0.11) showed moderate contribution, while the contribution of erucic acid (0.04) and protein 0.001) were extremely small.

Table 10. Principal components for some of the biochemical traits in the 35 advance mutants lines and one check

PCA table	PC1	PC2	PC3
Eigen value	3.78	1.47	0.51
Cumulative Eigen value	63.02	24.55	8.53
% Total variance	3.78	5.25	5.77
Cumulative %	63.02	87.58	96.11
Traits		**Eigenvectors**	
Oil	-0.05	0.97	0.11
Protein	0.71	-0.64	0.00
GSL	0.96	0.11	0.18
Oleic Acid	-0.93	-0.19	-0.26
Linolenic Acid	-0.72	-0.29	0.63
Erucic Acid	0.98	0.00	0.04

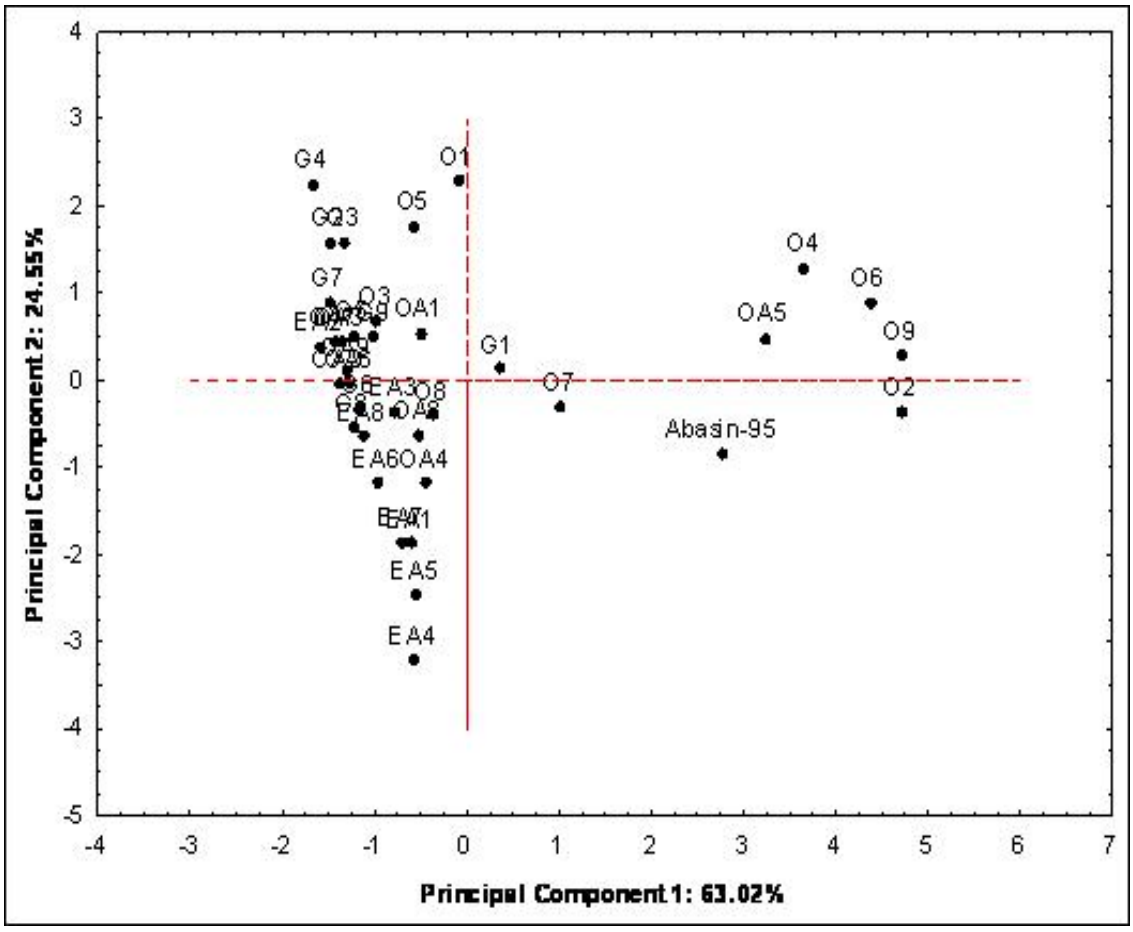

Figure 11. Scatter diagram for the first two PCs for some biochemical components in thirty five advance mutant lines and one check

4.7 MOLECULAR ANALYSIS

In the present study SSR marker were employed to confirm the mutation in the rapeseed Brassica. Two mutant lines 31-3 with comparatively high oil content (53.3 %) and 60-4 with low oil content (39.6 %) were crossed and F_1were developed. The BC F_1 were developed by crossing back the F_1 with the parent mutant lines. The BCF_1 were selfed to produce BCF_2. The parental mutant lines and twelve BCF_2 hybrids had been employed for molecular analysis.

4.7.1 VARIATION IN BANDING PATTERN

The amplification of total genomic DNA with SSR primers produced specific and non specific bands. In order to avoid these differences, only clear and easily detectable bands were scored among the tested crosses. These bands were producible over duplicate runs, with sufficient intensity to determine their presence or absence.

4.7.2 PRIMERS SCREENING AND SELECTION

Initially 25 SSR primers sets were employed to check their ability to produce polymorphic pattern on parental line and their corresponding BCF_2 populations. Out of 25 SSR primers, 19 primers sets gave clear and consistent banding patterns during initial screening and were selected to check the mutation. Each of the selected primer varied greatly in their ability to identify the deviation of F_2 parental lines from the parental genotypes or otherwise. Divergent pattern of polymorphism were observed i.e. 12 primers gave consistent, bright and highly polymorphic bands showing good amplification. One primer was mono morphic while one elicited variable polymorphism. Five primers gave non specific bands; hence they were excluded from

the further analysis. The product size of each primer was compared with that of expected size given on *Brassica* database domain (www.Brassica.info). Out of 99 amplified alleles, 69 were polymorphic. The highest polymorphism was exhibited by the primer sets NA 10-D03 and Na 10-D11 which produced five amplification products while varying polymorphic alleles were specified by many primer sets. The proportion of polymorphic loci was 66.7%.

The numbers of amplified products were ranged from 1-5with an average of 2.49 discrete DNA per primer. Polymorphism information content (PIC) of each *Brassica* primer sets was also calculated. PIC illustrates value of a marker for detecting level of polymorphism within a population [94]. It depends on the number of amplified alleles and their frequency distribution [95]. The PIC value for these polymorphic primer sets ranged from 0.24 to 0.75. Microsatellites primer Ra 2F11 had the highest PIC value. Single F2 individual came in cluster with the parent 31-3. The similarity among F2 population and parent 31-3 parental line was highest. Most of the primers had PIC value more than 0.40. Range of product size and PIC value of each polymorphic primer set are given in the table 9(b).

TABLE C. LIST OF SSR PRIMER SETS USE D IN THE PRESENT STUDY

SSR Primer	Forward Primer (bp)	Reverse Primer (bp)
BRMS-019	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT
BRMS-037	CTGCTCGCATTTTTTATCATAC	TACGCTTGGGAGAGAAAACTAT
Na 10-D03	ATGATTTGCCTTGAAATGCC	GATGAAACAATAACCTGACACAC
Na 10-D011	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG
Na 12-A02	AGCCTTGTTGGCTTTTCAACG	AGTGAATCGATGATCTCGCC
Na 12-A07	TCAAAGCCATAAAGCAGGTG	CATCTTCAACACGCATACCG
Na 14-D07	GCATAACGTCAGCGTCAAAC	CAGCAGCCACAACCTTACG
Ra 2-D04	TGGATTCTCTTTACACACGCC	CAAACCAAAATGTGTGAAGCC
Ra 2-E03	AGGTAGGCCCATCTCTCTCC	CCAAAACTTGCTCAAAACCC
Ra 2-G09	ACAGCAAGGATGTGTTGACG	GATGAGCCTCTGGTTCAAGC
Ra 2-F11	TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC
Ra 3-H10	TAATCGCGATCTGGATTCAC	ATCAGAACAGCGACGAGGTC

TABLE D. PIC VALUES, BAND SIZE AND ANNEALING TEMPERATURE OF SSR PRIMERS

SSR Primer	Polymorphism information content (PIC)	Band size (bp)	Annealing temperature C°)
BRMS-019	0.51	200-250	56
BRMS-037	0.36	250-260	52
Na 10-D03	0.59	160-200	52/54/56
Na 10-D11	0.57	180-200	55
Na 12-A02	0.63	160-200	52/54
Na 12-A07	0.40	150-180	52/54/56
Na 14-D07	0.56	120-140	52/54/56
Ra 2-D04	0.63	160-200	55
Ra 2-E03	0.25	280-300+	54
Ra 2-G09	0.43	240-260	55
Ra 2-F11	0.68	190-260	55
Ra 3-H10	0.66	130-160	52/54/56

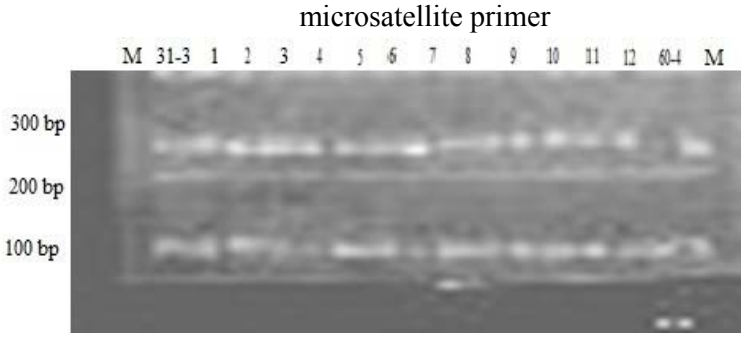

Figure 12. SSR banding pattern of F_2 hybrid population and parental line by BRMS-019

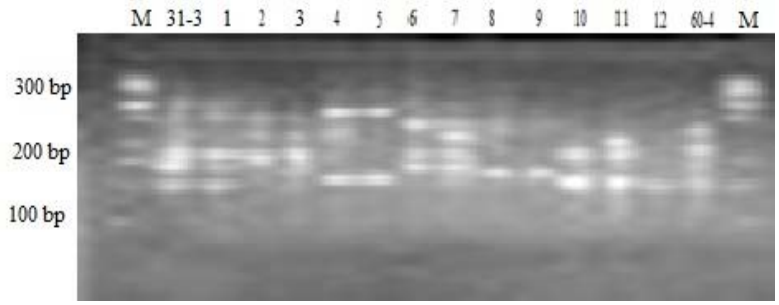

Figure 13. SSR banding pattern of F_2 hybrid populations and parental line by microsatellite primer Na 10-D11

Lane represent

M- marker

31-1- parental line

1-12- 12 F_2 hybrids from the cross 31-3 x 60-4 (high oil x low oil)

60-4- parental line

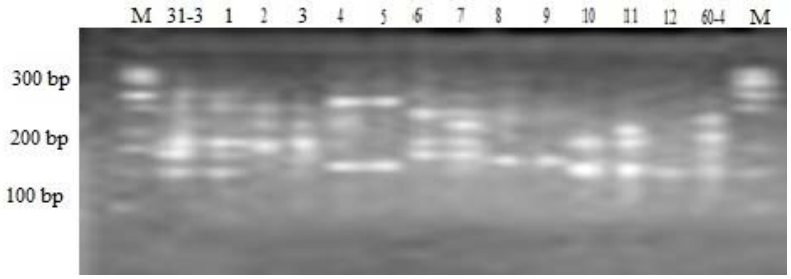

Figure 14. SSR banding pattern of F_2 hybrid populations and parental line by microsatellite primer Na 10-D03

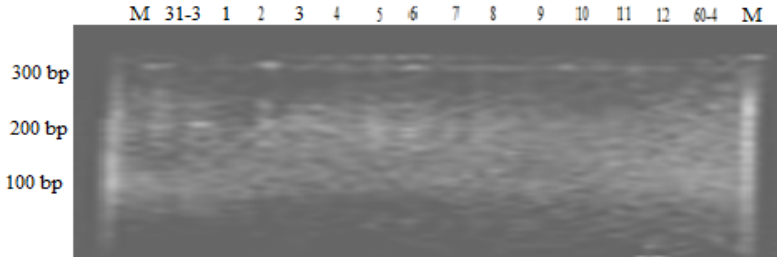

Figure 15. SSR banding pattern of F2 hybrids population and parental line by microsatellite primer Ra 2-F11

Lane represent

M- marker

31-1- parental line

1-12- 12 F_2 hybrids from the cross 31-3 x 60-4 (high oil x low oil)

60-4- parental line

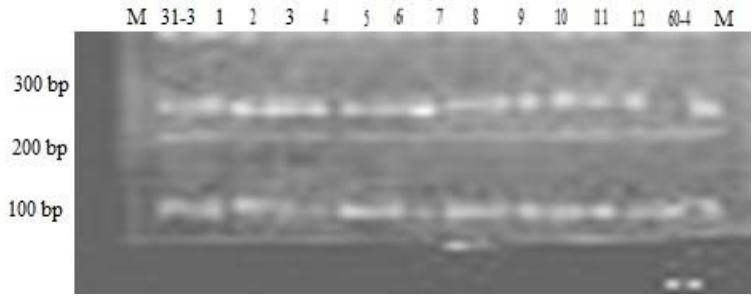

Figure 16. SSR banding pattern of F2 hybrid populations and parental line by microsatellite primer Na 12-A07

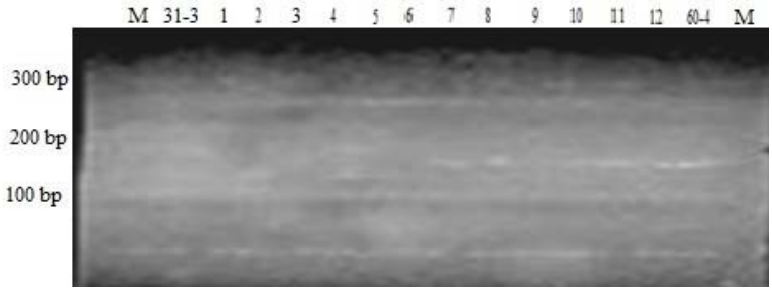

Figure 17. SSR banding pattern of F_2 hybrids population and parental line by microsatellite primer Na 14-D07

Lane represent

M- marker

31-1- parental line

1-12- 12 F_2 hybrids from the cross 31-3 x 60-4 (high oil x low oil)

60-4- parental line

4.7.2 GENETIC SIMILARITY MATRIX

The important point in assessing the clustering and genetic diversity within and among population is to determined through genetic dissimilarity between population. The Dice coefficient is a proper measure for co dominant marker as it can be applied to binary data mentioning banding profile of population.

A pair-wise similarity matrix based on the population of common SSR fragments was used to measure differences and established the level of introgression among the parental lines and F2 population.

Pair-wise similarity indices ranged from 4.4 to 9.2 (table). Parental line were quite diverse in distant and had less similarity index (5.5). The parental lines showing very less similarity between them were at the extremes and the 12 BCF_2 hybrids populations were adjusted among them. The similarity matrices of BCF_2 and parental line 31-3 were in the range of 6.4 to 8.9. On the whole BCF_2 hybrids 9 and 5 were the closest genotypes having similarity index of 9.20. The BCF_2 hybrid line 9 and 31-3 parental line were more distant having similarity matrix as less as 4.4.

5.0 DISCUSSION

Plant breeder is always interested in the development of such crop plants that have desirable characteristics. The desirable outcomes are either an increased yield or quality crops. In order to achieve the target, plant breeder adopts many strategies to combine desirable traits from two or more parental species to superior genotypes than its parents in many aspects.

Assessment of genetic parameters in the perspective of traits categorization is an important element of future crop enhancement programs. Compilation of knowledge about activities such as genetic parameters of the germplasm is the basic step for the commencement of a specific breeding program. Morphological traits, seed proteins, enzymes and several types of DNA markers are various available techniques, which allow of the genetic variability of crop germplasm. Many methods are used for estimation genetic diversity and relationship in germplasm which rely on pedigree, morphological, economic, biochemical and most recently molecular data.

Both environmental and genetic factors are responsible for variations in a segregating population. The genetic factors have key importance for plant seeders since it can easily be manipulated for the improvement of the population. Environmental factors play vital role for the distribution of total variability. If environmental variability is less as compared to genetic variability, selection would become more efficient. Variability is the determination of the selection of a specific character and an index transmissibility of genes controlling the character [96].

The present study was therefore conducted to estimate the outcomes of induced mutation in rapeseed Brassica. Different morphological and biochemical (quality) traits were used to investigate the genetic variability and heritability of the

advance mutant population. The studied traits were days to 50% flowering, plant height (PH), 100-seed weight (SW), seed yield and oil yield. Seeds were analyzed at NIFA for biochemical parameters viz. oil content (OC), protein content PC), glucosinolates (GSL) , oleic acid (OA), linolenic acid (LA) and erucic acid (EA).

5.1 ANALYSIS OF VARIANCE (ANOVA)

Flowering is the most critical stage having great influence on the yield of oilseed *Brassica*. The onset of flower initiation can have strong influence on flower, siliquae and seed related parameters [97. Various studies on oilseed rape (Brassica napus L) have shown flowering period to be a highly heritable character. In the present project the advance mutant lines took less days on average (84) for the 50% flowering period than that of parental lines (89.75). Our results are in conformity with the findings of some already reported research regarding the said parameter [98, 99, 100], which strengthened our results. On the other hand there are some reverse results are also reported [101, 102], who reported in their studies that the hybrids F_2 population took more days on average for the flowering period than that of parental lines. The plant height (PH) reflects the growth pattern of a crop. Genetic characteristics as well as environmental condition play vital role in the determination of PH of any individual plant. In this study, parental line was observed to be taller than the observed material. Our results are confirmed by various early reports [51, 60]. The weight of seed depicts the magnitude of seed development that determines seed yield of any variety. There was not any major difference among the seed weight (SW) of parent and the mutant population. On average, 1000-SW of parental line was 5.45gm while the mean of the mutant populations for 1000-SW was 4.56gm. Similar finding for the said trait were already reported [51].

Regarding seed quality traits, most of the mutant lines shows higher oil content than the check. All the advance mutant lines were recorded significantly different for oil content profile. These results are in agreement with some already reported results which describes 4% difference between different *Brassica lines* for seed oil content [103]. Significant variations for oil content were also recorded by some other researchers [104, 105].

Regarding protein content most of the mutant lines show lower values of protein content with a few with higher values of protein content than the check. This might be due to the variation in the genetic makeup of the population. All the advance mutant lines were recorded significantly different for protein content profile. Our results find conformity with a reported result who observed significant variation for oil, glucosinolate and protein from a diallel cross of six inbred lines of *B. carinata* [71].

Most of the mutant lines show lower values of glucosinolates content with a few with higher values of glucosinolates than the check. Our results are in showed similarity with various reported results where they described significant differences for glucosinolates [103, 106, 107].

Most of the mutant lines show higher values of fatty acids (OA, LA, EA) percentages than the check. Highly significant difference was found for OA, LA and EA. The result of the present study is in conformity with finding of Khan et al., (1998). They reported highly significant results for OA, LA and EA in $F_{3:4}$ *Brassica* populations [108]. Similar results are reported for GSL, OA, and EA in rapeseed [52, 108, 109]. Significant differences for EA in Indian mustard observed by Chauhan and Tyagi, 2002 also confirmed our results [110].

5.2 GENETIC VARIABILITY

5.2.1 GENETIC VARIABILITY FOR AGRONOMICAL TRAITS

Regarding days to flowering phenotypic variance (σ^2p) and phenotypic coefficient of variations (PCV) give larger values than the genotypic variance (σ^2g) and the genotypic coefficient of variations (GCV) for this trait. Slightly higher value of PCV than GCV and similarly higher value of σ^2p compared to σ^2g shows environmental effect on this character. Our results are also in conformity with the reported observations where they reported higher value of PCV than GCV and similarly higher values of σ^2p compared to σ^2g for days to 50% flowering [7, 111].The coefficient of variation shows only the extent of total variability and does not separate the variability into heritable and non-heritable portion.

Similarly plant height also produced same trend of higher σ^2p (137.19) than the σ^2g (106.98) and similarly higher PCV (9.90) compared to GCV (6.10). Ali (1985) also found high genotypic and phenotypic variances for plant height and pods per plant in Brassica juncea. Which indicated the environmental effects? For this trait phenotypic variance was 0.14 and genotypic variance was 0.12 and the phenotypic and the genotypic coefficient of variations were 7.88 and 7.43 respectively (Table 3). On average the check showed higher value of 1000 seed weight than the mutant line. Our results are similar with some already reported results [111, 112]

High phenotypic variance (144969.56) and high genotypic variance (123563.75) were observed for seed yield. The phenotypic and the genotypic coefficient of variations for seed yield were 20.59 and 19.01 respectively (Table 5). High GCV, PCV were stated for seed yield and number of pods per plant. Higher σ^2g and σ^2p were recorded for seed yield. Similarly for oil yield the phenotypic genotypic

variance were comparatively high suggesting the effect of environment for the inheritance of this character. Kumar and Misra also described similar results while Akbar and Saleem reported reverse results regarding seed yield where they found moderate σ^2g and σ^2p while low values for GCV and PCV [111, 112]. The GCV and PCV of variation developed only the degree of total variation for definite characters but do not differentiate the variability factor into heritable and non-heritable segment.

5.2.2 GENETIC VARIABILITY FOR BIOCHEMICAL TRAITS

Regarding the biochemical traits the glucosinolates and erucic acid recorded the highest values for phenotypic and genotypic variances and for phenotypic and genotypic coefficient of variations while oleic acid gave moderate values and low values were recorded for linolenic acid, protein, and oil content. Phenotypic variances (σ^2p) were higher than genotypic variances (σ^2g) and phenotypic coefficient of variations (PCV) were higher than the genotypic coefficient of variations (GCV) for all the observed characters (Table 6). Highest σ^2p, σ^2g, PCV and GCV were observed for glucosinolate and erucic acid. For all components slightly higher PCV than GCV and similarly higher σ^2p compared to σ^2g indicating the influence of environment for the expression of these characters. High GCV, PCV were stated by earlier workers for various traits [113]. High genotypic and phenotypic variances were found for seed yield [6]. The coefficient of variation describes only the degree of total variability present for characters and does not segregate the variability into inherited and non-inherited portion.

5.3 HERITABILITY AND GENETIC ADVANCE

5.3.2 HERITABILITY FOR AGRONOMICAL TRAITS

Estimates of heritability (broad sense) and genetic advance as percent of means were presented in Table 5. High value of heritability and moderate value of genetic advance were observed for days to flowering, plant height and 1000-seed weight. Slightly higher phenotypic variance than the genotypic variance and high heritability along with moderate genetic advance for this trait was probably due to non-additive (dominance or epistatic) gene effects. Kumar and Misra also reported high heritability for days to flowering [114]. This indicated that a trait having high heritability did not essentially produce high genetic advance. Thus only heritability did not give the indication for genetic advancement that could be maintained through selection.

The seed yield and oil yield displayed high heritability and high genetic advance. Both heritability and genetic advance showed higher values for these traits suggests the effect of additive gene for the inheritance and plant selection based on phenotypic performance and selection in the early generation could be fruitful in improving this character.

5.3.2 HERITABILITY FOR BIOCHEMICAL TRAITS

High heritability (broad sense) and genetic advance as percent of means were recorded for all the biochemical traits. The highest heritability was recorded by glucosinolates and erucic acid followed by oleic acid, linolenic acid and protein. Oil content showed the lowest heritability values among all the traits. Genetic advance as percent of means was higher for erucic acid and glucosinolate showing that selection based on these traits would be effective. Oil content gave the lowest values of

heritability and genetic advance. Similar results have been recorded by earlier workers for various characters.

In order to guess the selection effects, heritability and genetic advance together is somewhat more useful than heritability alone. Singh and Kumar and Misra also reported high heritability and high genetic advance for seed yield, which supports our results [44, 114]. A trait having high heritability and high genetic advance is considered under control of additive genes which highlights the usefulness of plant selection based on phenotypic performance.

5.4 PHENOTYPIC CORRELATION

5.4.1 PHENOTYPIC CORRELATION FOR AGRONOMICAL TRAITS

The correlation values presented show significant positive correlation of yield with plant height (0.54) while shows non significant positive correlation with seed weight (0.26). However, yield had non significant but negative connection with flower duration (-0.03).The results showed relevancy with that of Singh et al., 1987 where positive correlation of seed yield with various traits such as plant height etc were also reported [44]. The results are reversed with the some reported findings where positive correlation of seed yield with days to flowering was reported [107].

Similarly seed weight shows non-significant positive relationship with yield while non significant negative correlation with plant height. The correlation values of days to flowering had highly significant but positive association with plant height, and non significant negative association with seed yield. Similarly plant height showed significant positive correlation with seed yield and days to flowering, while showed non significant negative association with 1000-seed weight. Our results are in

agreement with Ozer *et al*., 1999 who found positive correlation between 1000-seed weight and seed yield [115]. The harvest index was observed to be significantly positive correlated with seed weight, days to flowering and seed yield in rapeseed by Ali et al. (2003) which proved that enhancement in the seed weight will give better harvest index [48].

5.4.2 PHENOTYPIC CORRELATION FOR AGRONOMICAL TRAITS

Regarding the biochemical traits the oil content showed significant negative correlation with protein and non significant negative association with oleic acid and linolenic acid, while showed non significant positive association with glucosinolates and erucic acid.

High significant positive association was observed between protein and glucosinolates, protein and erucic acid, glucosinolates and erucic acid and Linolenic acid and oleic acid, while significant positive association were observe between oleic acid and linolenic acid. Both erucic acid and glucosinolates were highly significantly negative associated with oleic acid and linolenic acid. Significant negative association was depicted between oil and protein, glucosinolate and oleic acid and glucosinolates and erucic acid.

Significant negative correlations were already reported between seed oil and protein content [116], which supports our results. In some other studies negative association between total oil and linolenic acid contents in *Brassica rapa* L were mentioned [117, 71]. However our results are against with some findings where they observed strong negative correlation between linolenic acid and oleic acid [54].

Generally, low phenotypic correlation was recorded among different characters; however some of the traits like oil, protein and oleic acid were negatively correlated

as compared to the other remaining characters which showed non-significant correlation. So selections based on such traits are valuable for quality improvement.

5.5 CLUSTER ANALYSIS

Cluster analysis was performed for agronomical and biochemical data for the thirty five advance mutant lines and the parent. Two types of scattering patterns were observed. In first type the parental line was quite distinct from the mutant lines. In the second type mutant populations show deviation toward the parent. For agronomical parameters, the proportion of variables having Eigen value ≥1 towards the total variability which was above 69% while it was ≥66% for biochemical traits. The trait DF contribute positively toward variation for most of the traits while the traits like 1000-SW and PH had great negative contribution toward the variability because of having negative Eigen value negative in most cases. Regarding biochemical traits, OA and LA for most of the mutant lines while GSL and EA contributed positive contribution toward the total variability, oil and protein contents showed mix divergence pattern. The results were in line with the findings of Pandey et al., (2009) who proposed days to flowering initiation, seed per selique, seed yield per plant and 1000-SW provides to be the most important variables as they had high negative and positive eigen values for different principal components [76].

5.6 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis was performed for agronomical and biochemical data for the thirty five advance mutant lines and the parent. Two types of scattering

patterns were observed. In first type the parental line was quite distinct from the mutant lines. In the second type mutant populations show deviation toward the parent.

Our results are also supported by Houli (1993) who analyzed 10 agronomic and two quality characters of 49 hybrids in Brassica napus by principal component analysis [118]. His result showed that all of the twelve parameters accounted for nearly 90% of the total genetic variation. Another researcher evaluated 114 genotypes of rapeseed for two years. Nine traits showed positive contribution to two PCs for two consecutive years. The first five and four principal components (PCs) having eigen values ≥ 1 contributed 98.65% and 95.04% to the total variability present amongst the accessions respectively [118].

5.7 MOLECULAR ANALYSIS BY SIMPLE SEQUENCE REPEATS (SSR)

The selection of simple sequence repeat SSR primers was done because they are polymorphic and suitable marker for the plant genome analysis. Highly polymorphism and abundance makes the micro satellites a perfect marker for genetic studies in crop plants, including marker assisted selection genetic mapping and population analysis [120, 34]. The application of primers derived from one species of genus Brassica varies in other species as the SSR primers mostly are species specific. Their specificity hinders their applicability between closely related species for comparative studies. Furthermore, these SSRs mostly present in gene-rich genomic regions, which increased their relevance for allele-trait relevance [119].

The PIC of each Brassica primer set was also determined. These values were in the range of 0.24 to 0.75. The results were in consistence with Tonguc and Griffith (2004) who detected 1 to 8 alleles [82]. In their study the PIC value were in the range

of 0.25-0.86 for thirteen SSR primers. Louarn et al, (2007) observed PIC value of 0.5 or above in 11 SSR primer [85]. Our findings were further supported by the research work of Yuan and Chao (2007) who detected a total of 21 alleles after using 5 SSR primers [120]. The number of alleles ranged from 2 to 5. PIC range was 0.25-0.92. Another reported result regarding the use microsatellites further strengthened our findings where they tested twenty five microsatellite-specific primer pairs on seventy five Brassica species and detected 2 to 7 alleles per microsatellite locus while another study reveled 1 to 14 alleles while analyzing 11 Chinese and 12 Swedish rapeseed accessions with 41 SSR primers [85, 122]. The results for number of alleles were also in conformity with Ofori et al, (2008) who detected 2-8 alleles per SSR markers across cultivars [123].

5.8 CONCLUSIONS

The major conclusions drawn from this study are as follows:

1. There are more chances of induce mutation in Brassica
2. Qualitative parameters may also serve as phenotypic markers for the identification of mutation within the population.
3. Presence of sufficient genetic variability for yield and quality characters may be used as indicator for efficient selection.
4. Principal component along with cluster analysis could serve as best tool for the estimation of scattering pattern of population over the factor plane.
5. Divergent of the mutant lines from the parent may help in the widening of the narrow genetic base of Brassica species.
6. Principal component analysis confirmed that flowering related parameter accounted greatly toward the variability present among the mutant populations (a criteria for selection) and hence may be used as tool in early selection.
7. Through cluster analysis groups of individuals having combination of desirable characteristic were identified, and may also be used as a tool in the identification of ideal genotypes.

5.9 RECOMMENDATIONS

1. Traits having high heritability in these selected advance mutant lines can be manipulated in the future breeding programs.
2. Plants with desirable traits (early flowering, higher yield, high oil content, low in glucosinolates and erucic acid) can be used as selection criteria for Varity development.
3. The individuals having desirable characters from both parents can be advanced for fixing desirable genes.
4. SSR served as useful DNA marker for the assessment of genetic variability, hence morphological, biochemical and molecular tools must be used in combination to enhance the efficiency of selection.
5. Induced mutation must be applied among other Brassica species as well in order to broaden the genetic base of the available germplasm.

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