GENETIC DIVERSITY AND FATTY ACID COMPOSITION ANALYSIS OF MUSTARD (*Brassica rapa* L.)

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CERTIFICATE

This is to certify that thesis entitled, " **GENETIC DIVERSITY AND FATTY ACID COMPOSITION ANALYSIS OF MUSTARD (Brassica rapa L.)** " submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN GENETICS AND PLANT BREEDING**, embodies the result of a piece of bona fide research work carried out by **FAHMIDA SULTANA**, Registration No. **11-04270** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2017
Place: Dhaka, Bangladesh

(Dr. Jamilur Rahman)
Professor
Supervisor

DEDICATED
TO
MY BELOVED PARENTS

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ABSTRACT

An experiment on *Brassica rapa* L. was conducted to determine the genetic variability, character association, genetic diversity and analysis of fatty acid composition of 14 mustard varieties. The research was conducted during the rabi season of 2016-2017 in research farm of Sher-e-Bangla Agricultural University, Dhaka. The analysis of variance showed significant differences among the varieties for all traits except number of primary branches/plant. The phenotypic variances were higher than the genotypic variances for all the traits. High phenotypic variance and high genotypic variance were found for number of secondary branches per plant, number of siliqua per plant, number of seeds per siliqua and seed yield per plant. High heritability coupled with high genetic advance as percent of mean were noticed for days to first flowering, days to 50% flowering, number of secondary branches per plant, number of siliqua per plant and number of seeds per siliqua indicating the effect of additive genes in controlling the traits. Significant positively correlation at both phenotypic and genotypic levels was observed in number of siliqua/plant with number of secondary branches, days to maturity with siliqua length, number of seeds per siliqua with days to first flowering, days to 50% flowering and thousand seed weight. The path analysis revealed days to first flowering, number of secondary branches per plant and 1000 seed weight had direct positive effect on seed yield/plant indicating these were the main contributors to yield/plant. The varieties were grouped into five diverse clusters found in PCA and D^2 analyses. The highest inter cluster distance was found between cluster II and cluster V (20.41) indicating hybridization among these varieties may produce a wide spectrum of segregating population. Significant variations were present in the fatty acid components analysis extracted from oil of six selected varieties. Higher oil content (42.42%) was observed in BARI sarisha-14 variety. The saturated fatty acids viz. myristic, stearic and palmitic acids were less than 7%, was observed in oil of Sonali sarisha and BARI sarisha-14. The total mono-unsaturated fatty acids were ranged from 62.74% in BARI sarisha-15 to 69.51% in Sonali sarisha. The highest oleic acid and eicosenoic acid were obtained in Maghi (21.06%) and Improved Tori (9.08%) respectively. The anti-nutritional factor, erucic acid content was found lowest in Maghi (35.53%) and BARI sarisha-15 (36.98%) varieties. The essential fatty acids, linoleic and linolenic were found highest in the seed oil of BARI sarisha-15 (17.10%). As a whole, the results suggest that for further improvement of the crop hybridization among BARI sarisha-14, BARI sarisha-15, Maghi, Sonali sarisha and Improved Tori would create noble segregates with low erucic acid and yield potential recombinant lines for further breeding program.

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SOME COMMONLY USED ABREVIATIONS

Full word	Abbreviation
At the rate	@
Agro Ecological Zone	AEZ
Agriculture	Agric.
Agricultural	Agril.
Agronomy	Agron.
Analysis of variance	ANOVA
And others	*et al.*
Bangladesh Agricultural Research Institute	BARI
Bangladesh Bureau of Statistics	BBS
Bangladesh	BD
Bangladesh institute of Nuclear Agriculture	BINA
By way of	Via
Cultivars	cv.
Centimeter	cm
Canonical Variate Analysis	CVA
Degree Celsius	^{0}C
Degrees of Freedom	df
Days to 50% flowering	D50%F
Days after sowing	DAS
Days to maturity	DM
Duncan`s Multiple Range Test	DMRT
Etcetera	etc.
Environmental variance	σ^2_e
Food and Agricultural Organization	FAO
Genotypic variance	σ^2_g
Gram	gm
Genotype	G
Genetic Advance	GA
Genotypic coefficient of variation	GCV
Heritability in broad sense	h^2_b
Journal	J.

SOME COMMONLY USED ABREVIATIONS (*Continued*...)

Full word	Abbreviation
Kilogram	Kg
Meter	M
Mean sum of square	MS
Murate of Potash	MP
Ministry of Agriculture	MOA
Number	No.
Namely	Viz.
Principal Component Analysis	PCA
Principal Coordinate Analysis	PCO
Phenotypic coefficient of variation	PCV
Percent	%
Phenotypic variance	σ^2_p
Percentage of Coefficient of Variation	CV%
Residual Effect	R
Randomized Complete Block Design	RCBD
Science	Sci.
Standard error	SE
Siliqua length	SL
Seeds per siliqua	SPS
Seed yield per plant	SYP
Square meter	m^2
Sher-e-Bnagla Agicultural University	SAU
Saturated fatty acid	SFA
Mono unsaturated fatty acids	MUFA
Poly unsaturated fatty acids	PUFA
Triple Super Phosphate	TSP
University	Uni.
Variety	var.

CHAPTER I
INTRODUCTION

Rapeseed is a major oilseed crop and contributes a lion share to the total edible oil production in Bangladesh. *Brassica rapa* is belonging to the family Brassicaceae and commonly known as field mustard, turnip mustard or yellow sarson. Seed contains 42% oil and 25% protein (Khaleque, 1985). Oil is used for healthy balanced diet, hair dressing, body massing, preparing different types of pickles and many industrial purposes. It is second most important oil crop providing 13% of the world's edible oil after soybean. Total area of mustard and rapeseed in the world is 34.33 million hectares (FAO, 2013). In 2016-2017, the edible oil production from major oilseed crops in the world is 563.44 million tons where rapeseed contributes 68.52 million tons. (www.statistica.com).

Brassica rapa is the most popular and major oil seed crop in Bangladesh and covers about 70% of the total oil crops acreage of Bangladesh. It occupies the first position in oil crops in Bangladesh with cultivated area 7,87,025 acres which produced 3,61,909 metric tons oil seed during 2015-2016 (BBS, 2016). Though the variety of *Brassica juncea* and *Brassica napus* are high yielding, they are not short durable. *Brassica rapa* is well suited with the cropping pattern in Bangladesh so, farmers practiced to cultivate it. *Brassica rapa* is a short durated crop compared to other *Brassica* spp. (*Brassica napus* and *Brassica juncea*) hence fits well into the cropping pattern (e.g. Rice (T. Aman) - Mustard – Rice (Boro) or (T. Aman) - Mustard – Maize) (Islam, 2013). About 0.933 million tons of edible oil produced in Bangladesh which is very low against the requirement (BBS, 2016). To fulfill this lacking the country imports 0.246 million tons of mustard oil that costs 301.847 million Tk. (BBS, 2016).

The yield of oil seed is now 1370 kg/ha only whereas the target yield in 2020-2025 and 2025-2030 is 1730 kg/ha, 2141 kg/ha and 2572 kg/ha in Bangladesh (Islam, 2013). There is limited scope to increase acreage due to pressure of other crops. And also there is limited scope to increase yield because farmers usually cultivate the existing low yielding varieties with low input and management. Short duration variety like Tori-7 of *Brassica rapa* is still popular in Bangladesh because it can fit well into the T. Aman-Mustard-Boro cropping pattern. There is no improved short duration variety of *Brassca rapa* which is available to replace Tori-7. However, farmers are being advised to cultivate the improved Tori and BARI shorisha 14 and BARI shorisha 15 varieties. The above scenario indicates there should be an attempt to develop short duration and high yielding varieties with more oil percentage in seed, tolerant to biotic and abiotic stress to fulfill the requirement of edible oils of the country by increasing the production. The improved variety also should well fit into cropping pattern viz. T. Aman-Mustard-Boro.

Edible vegetable oils are the main source of nutritionally required fatty acids in human diet. Mustard, soybean, sunflower and groundnut oil are among the edible vegetable oils mostly consumed in Bangladesh. However, none of these oils alone provide many of the lipid soluble nutrients as per the recommendation of health agencies. Rapeseed oil contains a high amount of selenium and magnesium, which gives anti-inflammatory properties. It also helps stimulating sweat glands and helps lowering body temperature. In traditional to the medicinal value, it is used to relieve the pain associated with arthritis, muscle sprains and strains (Sood *et al.*, 2010).

In a balanced diet 20-25% of calories should come from fats and oils and the average need of fats and oil is about 37g per day (Rahman, 1981). Rapeseed oil (RSO) is the most useful of all cooking oils and it contains a significant amount of ω-3 and ω-6 fatty acids. RSO contains fatty acid such as oleic, linoleic, linolenic, palmitic and strearic acid (Gunstone *et al*., 1994 and Hui, 1996).

RSO consists 95% of tri-acyl glycerols (TAG) and 5% non-tri-acyl glycerols, known as minor components like free fatty acids, mono and di-acyl glycerols, phospholipids, tocopherols, tocotrienols, flavonoids, other phenolic compounds, pigments (chlorophylls), sterols etc (Shahidi and Shukla, 1996).
Mustard oil makes up about 24-40% of the mustard seeds, which is characterized by the presence of higher level of erucic acid and it has the lowest saturated fatty acids content among all the edible vegetable oils. Rapeseed-mustard consists of saturated fatty acid such as palmitic (C16:0), stearic (C18:0) and monounsaturated fatty acids such as oleic (18:1) eicosenoic (C 20:1) and erucic acid (C 22:1) and polyunsaturated fatty acids such as linoleic (C 18:2) and linolenic acid (C 18:3), known as essential fatty acids (USDA, 2000).

The presence of high erucic acid in oil is considered anti-nutritional, as it has been reported to cause lipidosis in children (Ackman, 1977). Erucic acid (EA) is the common name for cis-13-docosenoic acid, a typical example of a very-long chain mono-unsaturated fatty acid. EA has 22 carbon atoms in its straight-chain molecule and one double bond between C13 and C14. The major source of EA is the seed oils of the Crucifereae family, which includes rapeseed, mustard, crambe and wallflower, all containing about 45% to 60% EA (Sonntag, 1991). Higher consumption of erucic acid may increase the concentration of adrenal cholesterol causing fibrotic changes in myocardium, liver weight and cholesterol (Beare-Rogers *et al.*, 1972). High levels of erucic acid is not suitable for human food in developed countries since erucic acid showed serious pathological changes in the heart and skeletal in animals (Technical Report, 2003). In spite of the concern about its safety for human consumption, EA is a very important raw material in the oleo chemical industry and its derivatives possess varieties of superior properties in slipping, softening, antifoaming, emulsifying, and corrosion inhibiting. All these properties offer EA and its derivatives wide applications in the production of

pharmaceuticals, soaps, detergents, cosmetics, plastics, lubricants, rubbers, coatings, etc (Carlson and Van Dyne, 1992; Erickson and Bassin, 1997).

Breeding program should be maintained to produce high-yielding and better-quality lines for release as cultivars to farmers. Analysis of variability and the association among the traits contributing to yield of a crop would be of great importance for a successful breeding program (Mary and Gopalan, 2006). Development of high-yielding cultivars requires knowledge of the existing genetic variation (genetic and environmental) for yield and its components and quality traits. However, estimates of heritability in conjunction with genetic advance, the change in mean value among successive generations should be considered (Shukla *et al*., 2006). Seed yield is a complex character that can be determined by several components reflecting positive or negative effects upon this trait (Marjanovic-Jeromela *et al.*, 2007). Determination of correlation coefficients is an important statistical procedure to evaluate breeding programs for yield (Ali *et al*., 2003). Path coefficient technique splits the correlation coefficients into direct and indirect effects via alternative characters or pathways (Sabaghnia *et al*., 2010).

The present study was undertaken for genetic diversity and fatty acid composition analysis of the varieties of *Brassica rapa* in Bangladesh. The goal of the investigation is to select the early maturating yield potential mustard varieties with low erucic acid containing mustard varieties for hybridization program. To meet the goal the following objectives were addressed.

Objectives

1. To evaluate the varietal performances of fourteen mustard varieties in Bangladesh.
2. To determine the nature of association and direct-indirect relationship between yield and yield contributing characters of the varieties.
3. To analyze the genetic diversity of among the varieties.
4. To analyze the fatty acids content of selected *Brassica rapa* varities.

CHAPTER II
REVIEW OF LITERATURE

Brassica species has received much attention by a large number of researchers on various aspects of its production and utilization. Brassicaceae species is the most important oil crop in Bangladesh and many other countries of the world. Many studies on the genetic variability, interrelationship, path co-efficient analysis, genetic diversity and fatty acid composition of *Brassica* spp have been carried out in many countries of the world. The review of literature concerning the studies presented under the following heads:

2.1 Genotypic and phenotypic variability
2.2 Heritability and genetic advance
2.3 Correlation among different characters
2.4 Path co-efficient analysis
2.5 Genetic diversity
2.6 Fatty acid content in *Brassica* spp

2.1 Genotypic and phenotypic variability

Katiyar *et al.* (1974) studied in *Brassica rapa* L. var. sarson grain on ten characters in 54 plants from each of 40 varieties; seed yield per plant showed a high genotypic coefficient of variation. While working with 65 strains of *B. rapa* by Nanda *et al.* (1995) and reported that days to first flowering varied both by genotypes and date of sowing. In another study, Lekh *et al.* (1998) reported that secondary branches showed highest genotypic co- efficient of variation. High genotypic and phenotypic co-efficient of variation was recorded for days to 50% flowering.

Thousand seed weight is also an important trait of *Brassica* oil crops, where highest consideration is on the seed yield. This trait has been found to vary

widely from genotype to genotype and from environment to environment including macro and micro environments. The coefficient of variation was high for thousand seed weight, pod length and number of seed per pod for both genotypic and phenotypic variability (Masood *et al.* 1999).

An experiment was conducted by Shalini *et al.* (2000) to study variability in *Brassica juncea* L. Different genetic parameters was estimated to assess the magnitude of genetic variation in 81 diverse Indian mustard genotypes. The analysis of variance indicated the prevalence of sufficient genetic variation among the genotypes for all 10 characters studied. Genotypic coefficient of variation, estimates of variability were moderate to high for 1000 seed weight, number of siliquae per plant and number of secondary branches per plant, indicating that the response to selection would be very high for these yield components. For the other characters, low coefficient of variation were observed.

Tyagi *et al.* (2001) evaluated forty-five hybrids of Indian mustard obtained from crossing 10 cultivars for seed yield and yield components. Highest variation for plant height of parents and their hybrids was reported. The seed yield per plant exhibited the highest coefficient of variation (41.1%). Genetic variability for nine traits in 25 genotypes study by Pant and Singh (2001). Analysis of variance revealed highly significant genotypic differences for all traits studied, except for days to flowering, number of primary branches and oil content. Seed yield per plant had the highest coefficient of genotypic and phenotypic variability. The genotypic coefficient of variation estimates for oil content and days to flowering suggest that these traits cannot be improved effectively merely by selection.

Ghosh and Gulati (2001) studied genetic variability in Indian mustard among 12 yield components for 36 genotypes selected from different geographical regions. The genotypic and phenotypic coefficients of variability (GCV and

PCV, respectively) were high in magnitude for all the characters except plant height. The differences between the PCV and GCV were narrow for all the characters studied except plant height, indicating the usefulness of phenotypic selection in improving these traits. Shen *et al.* (2002) tested 66 F_1 hybrids of *Brassica rapa* and significant differences were found between F_1s and their parents for yield per plant and seed oil content.

Choudhary *et al.* (2003) studied variability in Indian mustard for 10 characters during rabi season in India. A wide range of variability was observed for all characters, except for primary branches per plant, siliqua length, number of seeds per siliqua and thousand seed weight. Genotypic and phenotypic coefficient of variability was recorded high for secondary branches per plant, seed yield per plant and number of siliqua per plant. Afroz *et al.* (2004) studied genetic variability of 14 genotypes of mustard and rape. The highest genetic advance was observed in percent of pollen sterility.

Mahak *et al.* (2004) conducted an experiment on genetic variability for eight quantitative characters. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all characters. Niraj and Srivastava (2004), studied on variability in Indian mustard of 21 genotypes of *Brassica juncea*. RH-9704 and IGM-21 recorded the highest seed yield. Phenotypic coefficient of variation was high for oil yield per plant, seed yield per plant and seed weight.

Akbar *et al.* (2007) evaluated eight advanced lines of *Brassica junea* in Pakistan and studied variability of different yield components that were under experiment. The highest GCV was found in seed yield per plant followed by plant height, siliqua per plant and thousand grain weight while lowest GCV was in number of primary branches per plant. Rashid (2007) studied variability of forty oleiferous *Brassica* species. Result revealed that genotypes showed wider variation for morphological characteristics and thus were categorized

under three cultivated species - *B. rapa, B. napus and B. juncea* considering genetic parameters. High GCV (Genotypic Co-efficient of Variation) value was observed for days to 50% flowering, days to maturity, plant height and number of siliqua per plant.

Parveen (2007) studied variability in F_2 progenies of the inter-varietal crosses of 17 *Brassica rapa* genotypes. The result revealed that there were significant variations among the different genotypes used in the experiment. A study was conducted by Hosen (2008) using five parental genotypes of *Brassica rapa* and their ten F_3 progenies including reciprocals. The result revealed that there were large variations present among all the genotypes used in the experiment. Number of primary branches per plant, number of secondary branches per plant, days to 50% flowering, length of siliqua, number of seeds per siliquae, thousand seed weight and yield per plant showed least difference between phenotypic and genotypic variances. The values of GCV and PCV indicated that there was considerable variation among the all characters except days to maturity. The plant height, days to 50% flowering and number of siliquae per plant showed high heritability with high genetic advance and genetic advance in percentage of mean.

A field experiment was conducted by Jahan (2008) to study on inter-genotypic variability in 10 F_4 lines obtained through intervarietal crosses along with eight released varieties of *Brassica rapa* L. Significant variation was observed among all genotypes for all the characters studied. Considering genetic parameters i.e. high genotypic coefficient of variation (GCV) was observed for number of secondary branches/plant, siliquae/plant, yield/plant whereas days to maturity showed very low GCV. An experiment was carried out by Mahmud (2008) with 58 genotypes of *Brassica rapa* L. to study inter-genotypic variability. Significant variation was observed among all the genotypes for all the characters studied except thousand seed weight. High GCV value was observed for number of secondary branches per plant.

Singh *et al.* (2010) studied sixty two F_1 and twenty four parental lines of *Brassica juncea* and observed that higher genotypic variation were found in seed per plant, secondary branches per plant, primary branches per plant, thousand seed weight and seed per siliqua. Roy *et al.* (2011) conducted an experiment on rapeseed mustard (*Brassica* spp.) and studied variability. The result revealed that significant varietal difference except the number of siliqua on main recyme. The PCV and the GCV was high in secondary branches per plant and number of siliqua per plant.

Ali *et al.* (2013) conducted an experiment with thirty lines of *Brassica carinata* and reported that PCV and GCV ranged from 4.92-48.24% and 3.2-38.1%, respectively. Ahmad *et al.* (2013) studied thirty live advanced mutant lines along with a cheek variety of *Brassica napus* called Abasin-95 for variability analysis and reported that seed yield and days to flowering showed high genetic variability. The mutant lines 0A5, G1 and 06 showed their superiority in high seed yield, thousand seed weight and earliness in flowering.

Khan *et al.* (2013) evaluated thirty F_7 segregating lines and two parents of *Brassica rapa* to study variability. The result revealed that except thousand seed weight, significant variation was presented among all the genotypes for all the characters. Highest genotypic, phenotypic and environmental variances were observed in plant height while lowest one was in length of siliquae followed by thousand grain weight. Considering important performances, the genotypes G-15, G-19, G-1, G-3, G-4, G-10, G-18, G21, and G-24 were found suitable for future breeding program. Abideen *et al.* (2013) studied with eight genotypes of *Brassica napus* and observed that there were highly significant variations among the genotypes for most of the traits studied. Non-significant differences were in primary branches per plant and pods per plant among the genotypes.

Walle *et al.* (2014) carried out a study with thirty six genotypes of Ethiopian mustard (*Brassica carinata*) and result revealed that there were significant difference in days to 50% flowering, plant height and primary branches per plant. GCV was lower than the PCV for all yield related characters studied. Mekonnen *et al.* (2014) evaluated thirty six genotypes of Ethiopian mustard, *Brassica carinata* to study variability. The GCV ranged from 4.3% to 44.14% and PCV from 8.3% to 91.7%. Comparatively high GCV estimates were observed for number of pods per plant, primary and secondary braches per plant, seed yield per plot, and seed yield per hectare. The highest PCV was in primary branches per plant. Higher GCV and PCV for seed yield, number of pods per plant, primary and secondary branches which indicated that, it might provide better scope for improvement through selection.

Muhammad *et al.* (2014) studied with four parental genotype along with twelve F_2 generation of *Brassica napus* and reported that days to 50% flowering were significantly different at 5% level of significance. Plant height and pod length showed high heritability and days to 50% flowering showed moderate heritability. lqbal *et al*. (2014) conducted an experiment with ten indigenous variety associated with eight important yield contributing characters of *Brassica rapa* in Pakistan to study variability. The trails showed highly significant differences in almost all traits. It was observed that indigenous accessions had great proportion of genetic variability.

Yared and Misteru (2016) studied on sixty four *Brassica* breeding lines for investigated of some morphological characters to identify the extent and nature of genetic variability during 2014 cropping season. Analysis of variance showed the existence of considerable genetic variation among the lines for further selection and hybridization efforts. The maximum number of secondary branches/plant was observed by the breeding line code≠64. The highest yield/plot was recorded by the breeding line code≠48 followed by the breeding

line code≠25 and code≠64. Breeding line code≠53 exhibited the maximum 1000 seed weight.

2.2 Heritability and genetic advance

Katiyar *et al.* (1974) reported that heritability in the broad sense was associated with high genetic advance for number of siliquae on the main shoot and for seed yield per plant was found. Shalini *et al.* (2000); conducted an experiment to study the heritability and genetypic gain and found that heritability values and genetic gain were moderate to high for 1000 seed weight, number of siliquae per plant and number of secondary branches per plant, indicating that the response to selection would be very high for these yield components. For the other characters, medium to low heritability and low genetic gain were observed.

An experiment was conducted by Khulbe *et al.* (2000) to estimates of heritability and genetic advance for yield and its components in Indian mustard revealed maximum variation for seed yield. All the characters except oil content exhibited high heritability with high or moderate genetic advance, suggesting the role of additive gene action in conditioning the traits. Non-additive gene action appeared to influence the expression of days to maturity, while environment had a major influence on oil content. The use of pedigree selection or biparental mating in advanced generations was advocated to achieve substantial gains. Pant and Singh (2001) studied in experiment with nine traits in 25 genotypes. All traits showed high heritability with the highest value estimated for seed yield per plant. The estimates of genetic advance were comparatively low for oil content and days to flowering. The heritability estimates for oil content and days to flowering suggest that these traits cannot be improved effectively merely by selection.

Heritability studied of yield components in Indian mustard among 12 yield components for 36 genotypes selected from different geographical regions by Ghosh and Gulati (2001). All the characters studied estimates of high heritability except plant height. High heritability, coupled with high genetic advance was observed for oil content, harvest index, number of primary branches, number of siliquae on main shoot, main shoot length and number of seeds per siliqua. This result suggests the importance of additive gene action for their inheritance and improvement could be brought about by phenotypic selection. In a study of heritability in Indian mustard for 10 characters during rabi season in India by Choudhary *et al.* (2003). High heritability coupled with high genetic advance as percentage of mean was observed for secondary branches per plant, seed yield per plant and number of siliquae per plant, indicating preponderance of additive gene action.

An experiment was conducted by Mahak *et al.* (2004) on heritability and genetic advance for eight quantitative characters. High heritability coupled with high genetic advance in percentage of mean was observed for days to flowering, followed by thousand seed weight, days to maturity and plant height. Niraj and Srivastava (2004), studied on heritability in Indian mustard of 21 genotypes of *Brassica juncea*. Heritability was high for test weight, days to flowering, days to maturity and plant height.

An experiment was conducted with eight advanced lines of *Brassica junea* in Pakistan and studied heritability and genetic advance of different yield components by Akbar *et al.* (2007). Highest heritability was found yield per plant followed by plant height, thousand grain weight, siliqua per plant and number of primary branches per plant. The maximum genetic advance was found in seed yield per plant followed by siliqua per plant, plant height, thousand grain weight and minimum in primary branches per plant. Parveen (2007) studied heritability in F_2 progenies of the inter-varietal crosses of 17 *Brassica rapa* genotypes. The result revealed that number of primary branches

per plant and secondary branches per plant showed high heritability coupled with high genetic advance and very high genetic advance in percentage.

Hosen (2008) studied heritability using five parental genotypes of *Brassica rapa* and their ten F_3 progenies including reciprocals. The result revealed that plant height, days to 50% flowering and number of siliquae per plant showed high heritability with high genetic advance and genetic advance in percentage of mean. Jahan (2008) conduced field experiment to study heritability in 10 F_4 lines obtained through intervarietal crosses along with eight released varieties of *Brassica rapa* L. Significant variation was observed among all genotypes for all the characters studied. High heritability with low genetic advance in percent of mean was observed for days to maturity which indicated that non-additive gene effects were involved for the expression of this character and selection for such trait might not be rewarding. High heritability with moderate genetic advance in percent of mean was observed for plant height and days to 50% flowering indicating that this trait was under additive gene control and selection for genetic improvement for this trait would be effective.

Mahmud (2008) carried out an experiment with 58 genotypes of *Brassica rapa* L. to study heritability. Significant variation was observed among all the genotypes for all the characters studied except thousand seed weight. High heritability values along with high genetic advance in percentage of mean were obtained for days to 50% flowering, number of secondary branches per plant, seeds per siliqua, and siliqua length. Singh *et al.* (2010) studied sixty two F_1 and twenty four parental lines of *Brassica juncea* and observed that high heritability and high genetic advance were found in seed per plant, secondary branches per plant, primary branches per plant, thousand seed weight and seed per siliqua.

Alam (2010) conducted an experiment by using twenty six F_4 populations of some inter-varietal crosses of *Brassica rapa* L. to study the variation among

them. Higher phenotypic variation was present than the genotypic variation. High heritability with high genetic advance was found plant height, number of primary branches per plant, number of secondary branches per plant and number of siliquae per plant. Afrin *et al.* (2011) conducted an experiment in *Brassica napus* and studied heritability. The plant height showed highest value of broad sense heritability while the number of primary branches per plant, number of secondary branches per plant, siliqua length, number of seed per siliquae, number of siliqua per plant, thousand seed weight and seed yield per plant showed moderate broad sense heritability. Days to 80% maturity showed lowest heritability.

Roy *et al.* (2011) conducted an experiment on rapeseed mustard (*Brassica* spp.) and studied heritability. High heritability along with high genetic advance as percent of mean was reported in plant height, seed yield, secondary branches per plant, siliqua per plant and seeds per siliqua. Tahira *et al.* (2011) conducted an experiment with ten wide genetic ranged variety of *Brassica juncea* to study heritability in broad sense and showed siliqua length, plant height and seed yield had high values. Patel and Vyas (2011), experimented with three high yielding varieties and two very low quality varieties and their six generation cross product of *Brassica napus*. The result showed that the heritability in broad sence with high to moderate genetic advance was found in thousand grain weights, seed yield per plant. Moderate to high heritability associated with low genetic advance was recorded in days to maturity and days to flowering

Ali *et al.* (2013) conducted an experiment with thirty lines of *Brassica carinata* and reported that the highest heritability values were recorded for pod length (0.83) followed by pods on main raceme and the genetic advance as percent of mean was the highest for seed yield per plant and pods on main raceme. Ahmad *et al.* (2013) studied thirty live advanced mutant lines along with a

check variety of *Brassica napus* called Abasin-95 for heritability. High heritability and advance was recorded for seed yield.

Khan *et al.* (2013) evaluated thirty F_7 segregating lines and two parents of *Brassica rapa* to study heritability and genetic advance. Thousand seed weight, number of secondary branches per plant, seeds per siliquae, and siliquae length showed high heritability along with low genetic advance in percent of mean. Walle *et al.* (2014) carried out a study with thirty six genotypes of Ethiopian mustard (*Brassica carinata*). High heritability with high genetic advance was observed in plant height, number of secondary branches per plant and days to 80% maturity.

Mekonnen *et al.* (2014) evaluated thirty six genotypes of Ethiopian mustard, *Brassica carinata* to study heritability. Higher heritability along with higher genetic advance was observed in days to maturity, days to flowering, grain-filling period, number of pods per plant, secondary branches per plant, plant height, seed yield/plot and hectare and lowest one was in primary branches per plant. Muhammad *et al.* (2014) studied four parental genotype along with twelve F_2 generation of *Brassica napus* and reported that plant height and pod length showed high heritability and days to 50% flowering showed moderate heritability.

lqbal *et al.* (2014) conducted an experiment with ten indigenous variety associated with eight important yield contributing characters of *Brassica rapa* in Pakistan to study heritability. The highest heritability with higher genetic advance was reported in plant height while the seed per siliqua was found medium heritability along with tower genetic advance. It was observed that indigenous accessions had great proportion of genetic heritance. Ejaz-Ul-Hasan *et al.* (2014) studied on heritability of *Brassica napus* and the result stated that

plant height, yield per plant and days to 50% flowering showed high heritability.

Yared and Misteru (2016) studied on sixty four *Brassica* breeding lines for investigated of some morphological characters to identify the extent and nature of genetic heritability during 2014 cropping season. Number of secondary branches/plant and yield/plot were among the major positive contributor while 1000 seed weight recorded high heritability values in broad sense along with high genetic advance as percent of mean for which early generation selection would be effective in improvement program.

2.3 Correlation among different characters

Analysis of correlation among different traits is important in breeding program. A good number of literatures are available on correlation among characters of *Brassica* sp. Some of these literatures are reviewed here:

Days to maturity showed insignificant correlation with seed yield at both genotypic and phenotypic levels. The number of branches per plant and number of siliquae per plant showed significant negative correlation with number of seeds per siliqua and 1000 seed weight was reported by Malek *et al.* (2000) while studied correlation analysis. Badsra and Chaudhary (2001) studied correlation on 14 traits of 16 Indian mustard genotypes. Seed yield was positively correlated with stem diameter, number of siliquae per plant and oil content, while oil content was positively correlated with harvest index only. Among the characters only three characters positively correlated with seed yield.

Association of yield components in Indian mustard among 12 yield components were studied in 36 genotypes selected from different geographical regions by Ghosh and Gulati (2001). Seed yield exhibited significant positive

association with yield contributing traits like days to 50% flowering, days to maturity, plant height, number of secondary branches, number of siliquae on main shoot and oil content. Pankaj *et al.* (2002) studied four parental cultivars and the 174 progenies of resultant crosses for correlation between yield and yield component traits. The genetic correlation was higher than the phenotypic correlation for the majority of the characters. The number of siliqua per plant, which had the strongest positive and significant correlation with yield per plant at both levels, was positively associated with the number of seeds per siliquae and test weight at both levels. The number seeds per siliquae were positively associated with siliqua length and yield per plant at both levels.

Afroz *et al.* (2004) studied correlation and found seed yield per plant had significant and positive correlation with number of primary branches per plant and number of siliqua per plant. Path coefficient revealed maximum direct positive effects on plant height followed by number of siliqua per plant, seed yield per plant, number of primary branches per plant, 1000-seed weight and number of siliqua shattering per plant. Mahak *et al*. (2004) conducted an experiment and studied correlation for eight quantitative characters. Seed yield per plant showed positive correlation with number of primary branches, length of main raceme, 1000-seed weight and oil content. Selection should be applied on these traits to improve seed yield in Indian mustard.

An experiment conducted by Niraj and Srivastava (2004) on character association studies in Indian mustard of 21 genotypes of *Brassica juncea*. Seed and oil yields were positively and significantly correlated with plant height and primary branches but negatively correlated with test weight. An experiment on oleiferous *Brassica campestris* L. was conducted by Siddikee (2006) to study the correlation analysis. The results revealed that yield per plant highest significant positive correlation with number of siliqua per plant.

Tusar *et al.* (2006) studied phenotypic correlation and observed that seed yield per plant was positively and significantly associated with plant height, total dry matter production. The number of siliqua per plant, 1000-seed weight, crop growth rate during 60-75 days after sowing and number of branches per plant were also positively associated with seed yield. Zahan (2006) studied correlation and reported that yield/plant had highly significant positive association with plant height, length of siliqua, siliquae/plant and seed/siliquae but insignificant negative association with days to 50% flowering, days to maturity.

Akbar *et al.* (2007) evaluated eight advanced lines and two check variety of *Brassica junea* in Pakistan and reported that siliqua per plant had strong positive correlation with the seed yield followed by plant height while non-significantly negative correlation with thousand grain weight. But significantly negative correlation was present in siliqua per plant and primary branches per plant. Rashid (2007) carried out an experiment with 40 oleiferous *Brassica* species to estimate correlation and observed that, highly significant positive association of yield per plant with number of primary branches per plant , number of secondary branches per plant, number of seeds per siliqua and number of siliquae per plant.

Parveen (2007) conducted an experiment with F_2 population of *Brassica rapa* to study the correlation and observed that yield per plant had non-significant positive association with plant height, number of secondary branches per plant, number of seeds per siliquae and number of siliquae per plant, days to 50% flowering and length of siliqua. A study was conducted by Hosen (2008) using five parental genotypes of *Brassica rapa* and their ten F_3 progenies including reciprocals. He found yield per plant showed highest significant and positive correlation with days to maturity followed by number of seeds per siliquae, number of secondary branches per plant, length of siliqua and number of siliqua per plant.

In an experiment Mahmud (2008) found highly significant positive association of seed yield per plant with number of primary branches per plant, number of secondary branches per plant, number of siliqua per plant. Singh *et al.* (2010) studied sixty two F_1 and twenty four parental lines of *Brassica juncea* and observed that positive correlation was present in plant height, primary branches per plant, secondary branches per plant, seed per siliquae, thousand grain weight with seed yield.

Afrin *et al.* (2011) studied on *Brassica napus* and found positive correlation with seed yield per plant in plant height, number of primary branches per plant and number of siliqua per plant. Highest significant positive correlation was found between days to 50% flowering and plant height. Maurya *et al.* (2012) carried out an experiment with one hundred genotypes of *Brassica juncea* and observed that a high positive correlation was presented between length of siliqua, seed yield, thousand grain weight and days to 50% flowering.

Uddin *et al.* (2013) conducted an experiment with seven parental and twenty one F_2 progenies of *Brassica rapa* to study correlation among different yield component and found that yield per plant had high significant positive correlation with number of primary branches per plant, number of secondary branches per plant and siliqua per plant at both phenotypically and genotypically and significant positive correlation at genotypically in days to flowering and days to maturity. Ali *et al.* (2013) conducted an experiment with thirty lines of *Brassica cwinai* and observed that highly positive phenotypic correlation for seed yield per plant with plant height and primary branches per plant which was the indication that the traits were the most important contributors to seed yield per plant.

Abideen *et al.* (2013) studied with eight genotypes of Brassica napus and the resulted that positive phenotypically correlation was observed in plant height, pod length and seed yield. Significant positive correlation was also found in

seed yield per plant and pods per plant. Ejaz-Ul-Hasan *et al*. (2014) studied correlation between different traits of *Brassica napus* and found high and positively significant phenotypic correlation between plant height and seeds per plant. Mokonnen *et al*. (2014) studied *Brassica carinata* and found that seed yield per plant were positively correlated with plant height, days to maturity, secondary branches per plant and thousand seed weight at both genotypic and phenotypic level. There were also found that plant height was strongly and positively correlated with number of pods per plant

2.4 Path co-efficient analysis

The path analysis helps to determine the direct and indirect contribution of traits towards the yield. Direct contribution of each component to the yield and the indirect effects it has through its association with other components cannot be differentiated from mere correlation studies. Path coefficient analysis fulfills this study. It was first developed and described by Wright (1921) as a tool in genetic analysis which partition the association of the components on yield and indirect effects of the characters on yield through other components. The association between the various characters in a rapeseed and mustard and the direct and indirect effects of a variable over the dependent variable has been studied by a number of investigators are reviewed here.

The number of siliquae per plant had the highest positive direct effect on seed yield was observed by Yadava *et al.* (1996) when studied path co-efficient analysis of six yield components of 25 diverse varieties of Indian mustard. The number of siliquae per plant had the highest direct effect on seed yield followed by 1000 seed weight, number of primary branches per plant and plant height. Most of the characters had an indirect effect on seed yield was observed by Shalini *et al.* (2000) while studied path analysis of Indian mustard germplasm.

Srivastava and Singh (2002) reported that number of primary branches per plant, number of secondary branches per plant and 1000 seed weight had strong direct effect on seed yield while working with Indian mustard (*B. juncea* L. Czern and Coss). Results suggested that number of primary branches and 1000 seed weight were vital selection criteria for improvement-in productivity of Indian mustard. Afroz *et al.* (2004) studied path analysis of 14 genotypes of mustard and observed that maximum direct positive effects on plant height followed by number of siliqua per plant, seed yield per plant, number of primary branches per plant, 1000-seed weight and number of siliqua shattering per plant.

By path analysis, Zahan (2006) reported that siliquae/plant had positive direct effect on yield/plant. And days to 50% flowering had negative direct effect on yield/plant. Khan *et al.* (2006) studied correlation for some quantitative traits relating to yield and quality. The results indicated that a wide range of genetic variation existed among all the characters under study except 1000-grain weight. Correlation analysis revealed that seed yield per plant was positively and significantly correlated with number of primary branches (0.4015), siliqua per plant (0.505), seeds per siliqua (0.79648), siliqua length (0.37037) and seed yield per plot (0.40931). However, it was negatively and non-significantly associated with number of secondary branches (-0.36663) and protein contents (-0.1372) at genotypic level. It was also found that indirect selection for number of seeds per siliqua would be effective in improving the seed yield per plant in present breeding material.

A study was conducted by Tusar *et al.* (2006) to assess the nature and extent of variability of 11 yield related characters of five mustard genotypes. Phenotypic correlation studies indicated that seed yield per hectare was positively and significantly associated with plant height, total dry matter production and husk weight. The number of siliqua per plant, 1000-seed weight, crop growth rate during 60-75 days after sowing and number of branches per plant were also

positively associated with seed yield. Path coefficient analysis revealed that the number of siliqua per plant had the greatest direct contribution on seed yield followed by the number of seeds per siliqua and 1000-seed weight while indirect via number of siliqua per plant and 1000-seed weight. Although plant height and husk weight had a total positive correlation with seed yield, their direct effect on yield was negative. The number of seeds per siliqua showed very high positive direct effect on yield, but its correlation with yield was non-significant and negative.

Rashid (2007) carried out an experiment with 40 oleiferous *Brassica* species to estimate path analysis and observed that yield per plant had the highest direct effect on days to maturity, number of seeds per siliqua, number of siliquae per plant and number of primary and secondary branches per plant. Parveen (2007) conducted an experiment with F2 population of *Brassica rapa* to study the path analysis and observed that number of seeds per siliqua showed highest direct effect on yield per plant.

The path co-efficient analysis by Hosen (2008) exhibited that thousand seed weight had the highest positive direct effect followed by days to 50% flowering, length of siliqua, number of primary branches per plant, number of secondary branches per plant, days to maturity and number of seeds per siliqua while working with five parental genotypes of *Brassica rapa* and their ten F_3 progenies including reciprocals. An experiment was carried out by Mahmud (2008) with 58 genotypes of *Brassica rapa*. Path analysis showed that yield per plant had the highest direct effect on number of primary branches per plant, number of siliquae per plant, number of secondary branches per plant and number of seeds per siliqua.

Aytac *et al.* (2008) evaluated on six genotypes of spring rape seed and studied path coefficient and the result stated that plant height, number of siliqua per plant, seeds per siliquae had highest and positive direct effect on yield per plant

for all cultivars except cv. Star. Alam (2010) studied path co-efficient analysis that revealed that plant height, number of primary branches per plant, number of siliqua per plant, seeds per siliquae and siliqua length had the direct positive effect on yield per plant while days to 50% flowering, number of secondary branches per plant and thousand seed weight had the negative direct effect on yield per plant.

Afrin *et al.* (2011) studied with *Brassica napus* to identify the path co-efficiant among the characters. The plant height was found the highest positive and direct effect on seed yield per plant followed by number of siliqua per plant and siliqua length. Uddin *et al.* (2013) conducted an experiment with seven parental and twenty one F_2 progenies of *Brassica rapa* to study path coefficient and reported that days to 50% flowering, number of primary branches per plant, number of secondary branches per plant, number of siliqua per plant, siliquae length, seed per siliquae and thousand seed weight showed direct positive association with seed yield per plant while the plant height and days to maturity had direct negative association.

Mekonnen *et al.* (2014) conducted an experiment to study path co-efficient in *Brassica carinata* and founded that days to maturity and secondary braches per plant had positive and direct genotypic correlation with seed yield. Ejaz-Ul-Hasan *et al.* (2014) conducted an experiment on *Brassica napus* and studied path coefficient. The result revealed that the highest direct positive effect of seeds per plant on yield and followed by days to maturity, days to flowering, seeds per siliquae, siliqua length and thousand seed weight while plant height had direct negative effect on the yield per plant.

2.5 Genetic divergence among mustard genotypes

Evaluation of germplasm through genetic divergence which quantifies variation among genotypes on the basis of a group of characters (yield and yield contributing) helps in identification of promising parental materials for crop

improvement. Germplasm collections are also valuable gene pools providing diverse genetic material that may be applied for the improvement of cultivars and advanced agronomic productivity. An assessment of genetic diversity within these collections can be used to assign lines and populations to diverse groups. D^2 statistic developed by Mahalanobis (1936) provides a measure of magnitude of divergence between biological populations and relative contribution of each component character to the total divergence (Nair and Mukherjee, 1960). Mahalanobis D^2 statistic is more reliable in selection of potential parent for hybridization programme using these D^2 values cluster are formed. A summary of literature reviewed on mustard and other allied species are in presented below.

Peter and Rai (1995) studied genetic divergence using the D^2 statistics and canonical analysis among 25 genotypes of *Brassica napus*. They reported that genetic and geographical divergence was highly related with the genotypes. The genotypes were grouped into six clusters of which cluster I was the largest accommodating among these genotypes. The cluster VI had large genetic distance from the remaining clusters. Singh *et al.* (1997) studied genetic divergence through D^2 statistic with 50 genotypes of *B. napus* growing in 12 environments based on 13 characters. They searched the clustering pattern and their inter and intra cluster distances. On the basis of stability, high yield and divergence among the genotypes, nine crosses were recommended as suitable for use in breeding programme.

Khulbe and Pan (1999) reported that siliqua per plant, siliqua length, seeds per siliqua, 1000 seed weight were positively associated with grain yield. Analysis of variance revealed that siliqua per plant, siliqua length, 1000 seed weight and seeds per siliqua were the major characters influencing grain yield. Jagadev *et al*. (1999) studied on some 19 genotypes of rapeseed (*B. napus*).

Aunwinithul *et al.* (2004) studied 33 genetically diverse genotypes of Indian mustard for diversity. The genotypes were grouped into eight different clusters. The cluster III was the biggest with 11 genotypes followed by cluster-I with 9 genotypes, cluster V and VI consisted of 4 and 3 genotypes respectively. The cluster II and VII both had two genotypes each and similarly, cluster IV and VIII included one genotype each. Yadava *et al.* (2004) studied 50 lines of *B. napus* and reported that the lines were grouped into twelve clusters with maximum inter cluster distances between the clusters XII and IX (35.51), II and III (33.03) and XI and IX (31.21). The characters contributing to the maximum divergence were in descending order, oil content days to flowering, plant height, siliqua length and siliqua number on the main raceme.

Goswami *et al.* (2005) conducted experiment on variability studies for number of secondary branches per plant, siliquae on main shoot, seed per siliqua, 1000-seed weight and seed-yield per plant. Results showed that the coefficient of variation of pods per plant. Kardam and Singh (2005) noted that the nature and magnitude of variability for 10 characters in 200 progenies of Indian mustard *(B. juncea)* obtained from six crosses were studied during Rabi 2002-03 in Jobner, Rajasthan, India. Phenotypic coefficients of variation were higher in magnitude compared to genotypic coefficients of variation for most of the characters. Seed yield per plant was significantly associated with plant height, primary branches per plant, and number of siliquae per plant, number of seeds per siliqua and 1000-seed weight. The number of siliquae per plant had the highest direct contribution to seed yield, followed by primary branches per plant, 1000-seed weight, number of siliquae on main shoot and number of seeds per siliqua.

Goswami and Behl (2006) studied 43 genotypes of Indian mustard using D^2 statistics. They recorded data for plant height, primary branches, secondary branches, main shoot length, number of siliquae on main shoot, siliqua length, seeds per siliqua, 1000-seed weight, seed yield per plant and oil content. The

genotypes were grouped into six clusters. The intra cluster distances were almost equal and relatively lower than the inter-cluster distances. Vivek *et al.* (2007) studied the genetic diversity in 81 true breeding advanced generation cultivars of Indian mustard based on yield and yield components. They are followed by cluster analysis and showed that out cluster XII, which was most diverse, had very high seed yield and number of siliquae per plant. Cluster VII also represented entries with high seed yield, number of siliquae per plant and highest number of seed per siliqua. Cluster XI with the lowest number of days to maturity could be considered as a good source for earliness.

Hossain *et al.* (2008) studied the genetic divergence using D^2 statistic in 40 genotypes of rapeseed. The genotypes differed significantly for 10 yield and yield contributing characters, and they grouped then into 9 clusters. They observed that there was no close correspondence between geographical distribution and genetic divergence. A Number of siliqua on the main raceme, seeds per siliqua and harvest index were the major contribution to genetic divergence and cluster IV and these genotypes were suggested for use in heterosis breeding.

Zaman *et al.* (2010) conducted a field experiment for estimation of divergence among 45 advanced lines of mustard. The genotypes were grouped into four clusters. Cluster I contained the highest number of genotypes (six) and the cluster III contained the lowest (three). The highest intra cluster distance was observed in cluster II and the lowest in cluster I. The highest inter cluster distance was observed between the cluster III and II followed by III and I and the lowest between clusters IV and III. Days to 50% flowering (81.94%), days to maturity (8.24%), plant height (5.82 %), branches per plant (1.91%) and siliquae per plant (1.17%) contributed maximum towards the total divergence which suggested that these characters were highly responsible for genetic divergence in the present materials.

Pandey *et al.* (2013) conducted an experiment with 45 Indian mustard genotypes of different origin from India for evaluated for the extent of diversity for utilization in breeding program. D^2 analysis was conducted to measure the genetic diversity among the genotypes. The 45 genotypes were grouped in 8 clusters using Tocher's method. Intra cluster distance was maximum for cluster VI followed by cluster III. The maximum inter-cluster distance was found between cluster II and III indicating high genetic divergence among genotypes of these groups. Maximum contribution towards the divergence was accountable to 1000-grain weight (46.87%) followed by seed yield per plant (20.91%) and number of silique on main raceme (8.38%).

Yared and Misteru (2016) studied on sixty four *Brassica* breeding lines for investigated of some morphological characters to identify the extent and nature of genetic diversity during 2014 cropping season. Cluster analysis categorized the breeding lines into nine clusters. All lines were grouped regardless of their pedigree record which might be the result of selection pressure applied on different morphological characters. 71.1% of the variation was explained by the first three principal components as described by the principal component analysis. Number of secondary branches/plant and yield/plot were among the major positive contributor while 1000 seed weight had negative contribution in the first principal component in which 32% of the variation was explained.

2.6 Fatty acid content in *Brassica* spp

Velasco *et al.* (1998) reported on collecting of 1475 entries from 21 species of *Brassica* was evaluated for the fatty acid composition of the seed oil. A total of 358 entries representing the taxonomic variability in the collection were selected and analysed by gas-liquid chromatography (GLC). Some fatty acid ratios were used to estimate the efficiency of the different biosynthetic pathways. Two well-defined patterns were observed. The first one was characterized by high elongation efficiency and accumulation of high levels of

erucic acid. The highest erucic acid content (>55% of the total fatty acids) was found in the cultivated species *B. napus* L., *B. oleracea* L., and *B. rapa* L., and in the wild species *B. incana Tenore*, *B. rupestris* Raf., and *B. villosa Bivona-Bernardi*, the three latter belonging to the *B. oleracea* group (n=9). The second pattern was characterized by high desaturation efficiency, resulting in the accumulation of high levels of the polyunsaturated linoleic and linolenic acid (up to more than 55%). The highest levels of these fatty acids were found in samples of *B. elongata* Ehrh., especially of the var. *integrifolia Boiss*. The utility of the reported variability for plant breeding is discussed.

Kumar (2013) studied with 24 parents and 80 F_1 crosses of Indian mustard to assess the fatty acid profile and oil content. Analysis of variance indicated significant differences for all the quality characters investigated. The environmental effects were significant for erucic and oleic acid content and the influence of environmental factors appeared to be less on other characters. The genotype × environment interactions were non-significant for all the characters, hence, the data were pooled over the years and discussed on the basis of mean of two years. The coefficients of variation at phenotypic level varied from 4.6% for oil content to 50.9% for oleic acid. The genotypic coefficients of variability were high for oleic, palmitic + stearic, erucic and linolenic acid, erucic acid and palmitic acid + stearic acid had the least genotypic variation (GCV: 16.3 to 16.9%). The heritability in broad-sense was relatively high for oleic (61.5%) and erucic acid (56.3%). The high heritability was associated with high genetic advance only for oleic acid suggesting the role of additive gene action in the inheritance of this character. Erucic acid negatively and significantly correlated with the rest of the fatty acids except linolenic acid and significant correlation with oleic (r = -0.536) and eicosenoic acid (r = -0.260). Although, oil content had very low direct effect (-0.011) on erucic acid but its positive association was the result of its strong positive indirect effect through oleic acid (0.435), which was partially neutralized by negative indirect effects (-0.112) through

linolenic acid. The implications of these results in the quality-breeding programme were discussed in this paper.

Sharafi, *et al.* (2015) studied on 20 accessions of six *Brassica* species including cultivated and five wild relatives for oil and fatty acid composition. The results showed that oil content varied from 21 (*B. nigra*) to 46% (*B. napus*). Among wild species, *B. rapa* and *B. oleracea* had highest oil content (31 and 28%, respectively). The main fatty acids of oleic, linoleic, linolenic, erucic, palmitic, and stearic acids accounted for 89–94% of the total fatty acids in all species. Cultivated species of *B. napus* had highest oleic acid (61%) and lowest erucic acid (1%) content compared to other studied species. *Brassica rapa* and *B. oleracea* had the highest content of erucic acid (41% and 46%, respectively). The highest content of linolenic (20%) and linoleic (19%) acid was observed for *B. juncea*. The results showed that there was high genetic variation among the studied species for oil content and fatty acids composition. This indicates that seed oil of these species is possibly suitable for both human consumption and industrial purposes

Nath *et al.* (2016) reported on rapeseed oil is being utilized from early civilization, but its popularity being declined from the mid-nineteenth century due to presence of erucic acid (C22:1) and glucosinolates. Thereby, several attempts have been made to develop cultivars free from those toxins. In the past 20 years, breeders got success in developing '00'- quality rapeseed, known as 'Canola'. The target mutagenesis of *fae-1* and *fae-2* of *Brassica napus* ensured such success. Thereafter, 'canola' regains its market as a healthy vegetable oil. Moreover, high oleic acid rapeseed lines, with 86% oleic acid, have been developed by using chemical mutagenesis of FAD2 alleles responsible for desaturation of oleic acid (C18:1) to linoleic acid (C18:2). Recently, high erucic acid rapeseed oil regained interest for biodegradable plastic, cosmetic, emollient industries and for biodiesel.

CHAPTER III
MATERIALS AND METHODS

This chapter deals with the information on the subject of materials and methods that were used in conducting the experiment. It consists of a short explanation of locations of the experimental site, soil characteristics, climate, materials used in the experiment, layout and design of the experiment, land preparation, manuring and fertilizing, seed sowing, intercultural practices, harvesting, data recording procedure and statistical analysis etc., which are presented as follows:

3.1 Experimental site

The experiment was conducted at the experimental field of Sher-e-Bangla Agricultural University, Dhaka-1207 during November 2016 to February 2017. The location of the experimental site was situated at $23^0$74' N latitude and $90^0$35' E longitude with an elevation of 8.6 meter from the sea level. Photograph showing the experimental site (Appendix I).

3.2 Soil characteristics

The soil of the experimental site lies in Agro ecological region of Madhupur Tract. Soil of the experimental site belongs to the general soil type, Shallow red brown terrace soils under Tejgaon Series. The soil was loam in texture. The experimental site was medium high land and the pH was 5.6 to 5.8 and organic carbon content was 0.82%. Experimental area was flat which facilitated irrigation and drainage system easily. Physicochemical properties of the soil are presented in Appendix II.

3.3 Climate

The experimental site was situated under the subtropical climatic zone, characterized by three distinct seasons**,** the monsoon or rainy season from November to February and the pre-monsoon period or hot season from March to April and monsoon period from May to October (Edris *et al.* 1979) and also

characterized by heavy precipitation during the month of May to August and scanty precipitation from October to March. The mean of air, temperature, humidity and rainfall during the period of experiment were recorded from the Bangladesh Metrological Department, Agargaon, Dhaka (Appendix III).

3.4 Design and layout

The trial field was laid out in a Randomized Complete Block Design (RCBD) with three replications. The total experimental area was 200 m^2. Per replication plot area was 19 m x 3 m = 57 m^2 and the distance between replications was 0.5 m. The genotypes were randomly distributed to each replication. A pictorial view of experimental field at flowering stage is presented in plate 1**.**

3.5 Plant materials

The healthy seeds of twelve varieties were collected from BARI, Joydebpur, Gazipur, one from local areas and other from BINA, Mymensing. which were used as experimental materials. The plant materials used in that experiment is shown in Table 1.

3.6 Operational practices

3.6.1 Plot preparation

The experimental plot was prepared by several ploughing and cross ploughing followed by laddering and harrowing with tractor and power tiller to bring about good tilt. Weeds and other stubbles were removed carefully from the experimental plot and leveled properly. The final land preparation was done on 16th November 2016. The final land preparation is shown in Plate 2.

3.6.2 Fertilizer application

Organic and inorganic fertilizer viz. cow dung, Urea, TSP and MP fertilizers are required for rapeseed cultivation. The field was fertilized as the rate shown in Table 2. The area was fertilized with 10 ton cow dung per ha on 15 November 2016. The entire amount of cow dung was applied seven days

Table 1. Materials used for the experiment

Name	Varieties	Source
G1	Tori-7	BARI
G2	BARI sarisha-3	BARI
G3	Sonali sarisha	BARI
G4	BARI sarisha-6	BARI
G5	BARI sarisha-9	BARI
G6	BARI sarisha-12	BARI
G7	BARI sarisha-14	BARI
G8	BARI sarisha-15	BARI
G9	BARI sarisha-17	BARI
G10	Improved Tori	BARI
G11	Sonali Tori	BARI
G12	Torsha	BARI
G13	Maghi	Local (Manikgonj)
G14	BINA-10	BINA

Table 2. List of fertilizer with doses and application procedures

Sl No.	Fertilizer	Doses	application Procedure
01	Cowdung	10 ton/ha	as basal
02	Urea	270 Kg/ha	50% basal and 50% at 25 DAS
03	TSP	170 Kg/ha	as basal
04	MP	100 Kg/ha	as basal
05	Gypsum	150 Kg/ha	as basal
06	Zinc oxide	5 Kg/ha	as basal
07	Boron	3 Kg/ha	as basal

Plate 1: A view of experimental plot

Plate 2: Photograph showing final land preparation

before sowing. Half amount of urea, total TSP. MP, Gypsum and Boron were applied during final land preparation and incorporated into the soil. The rest amount of urea was applied as top dressing after 25 days of sowing.

3.6.3 Seed sowing

The spacing of row to row was 30 cm and plant to plant in row was 8 cm. Variety to variety distance in each replication was 60 cm. Distance between replication was 50 cm. Seeds were sown in line in the experimentl plot on 17 November 2016. The seeds were placed at about 1.5 cm depth in the soil. After sowing the seeds were covered with soil carefully so that no clods were on the seeds.

3.6.4 Intercultural operations

Irrigation was given with cane after sowing of seeds to bring proper moisture condition of the soil to ensure uniform germination of the seeds. A good drainage system was maintained for immediate release of rainwater from the experimental plot during the growing period. The weeding was done after 15 days of sowing. At the same time, 1st thinning was done and another after 7days of 1st thinning for maintaining a distance of 8 cm from plant to plant in rows of 30 cm apart. Total experimental plot was tagging on 2 December 2016 by bamboo stick by maintaining variety code and replication number (Plate 3). Second weeding was done after 30 days of sowing. Aphid and disease Alternaria leaf spot infection was found in the crop during the siliqua development stage. To control pest Malathion-57 EC @ 2ml/liter with Rovral 50WP under Iprodione group @ 2gm/lit of water was applied on 13 January 2017 and second time on 17 January 2107. The pesticide was applied in the afternoon.

Plate 3: Tagging of all varieties in all three replications with bamboo stick

3.6.5 Crop harvesting

The crop was harvested in different dates according to maturity. Harvesting was started from 2nd week of February 2016 and continued to 3rd week of February 2016 depending upon the maturity. When 80% of the plants showed maturity symptoms like straw color of siliqua, leaves, stem and desirable seed color in the matured siliqua, the crop was assessed to attain maturity. For harvesting, 10 plants were selected randomly from each variety in each replication. The plants were harvested by uprooting and then they were tagged properly. Data were recorded on different parameters from these plants.

3.7 Parameter recorded

Eleven yield and yield components traits were taken into consideration for studying different genetic parameters, association and genetic diversity. Data were recorded on ten randomly selected plants for each genotype. Recording of data at field stage is shown in Plate 4. Plate 5 showing different plant growth stages of the genotypes..

3.7.1 Days to first flowering

Days to 1st flowering were recorded from sowing date to the date of 5% flowering of every entry.

3.7.2 Days to 50% flowering

Days to 50% flowering were recorded from sowing date to the date of 50% flowering of every entry.

3.7.3 Days to maturity

The data were recorded from the date of sowing to siliquae maturity of 80% plants of each entry.

3.7.4 Plant height (cm)

It was measured in centimeter (cm) from the base of the plant to the tip of the longest inflorescence. Data were taken after harvesting.

Plate 4: Recording the crop field level parameters

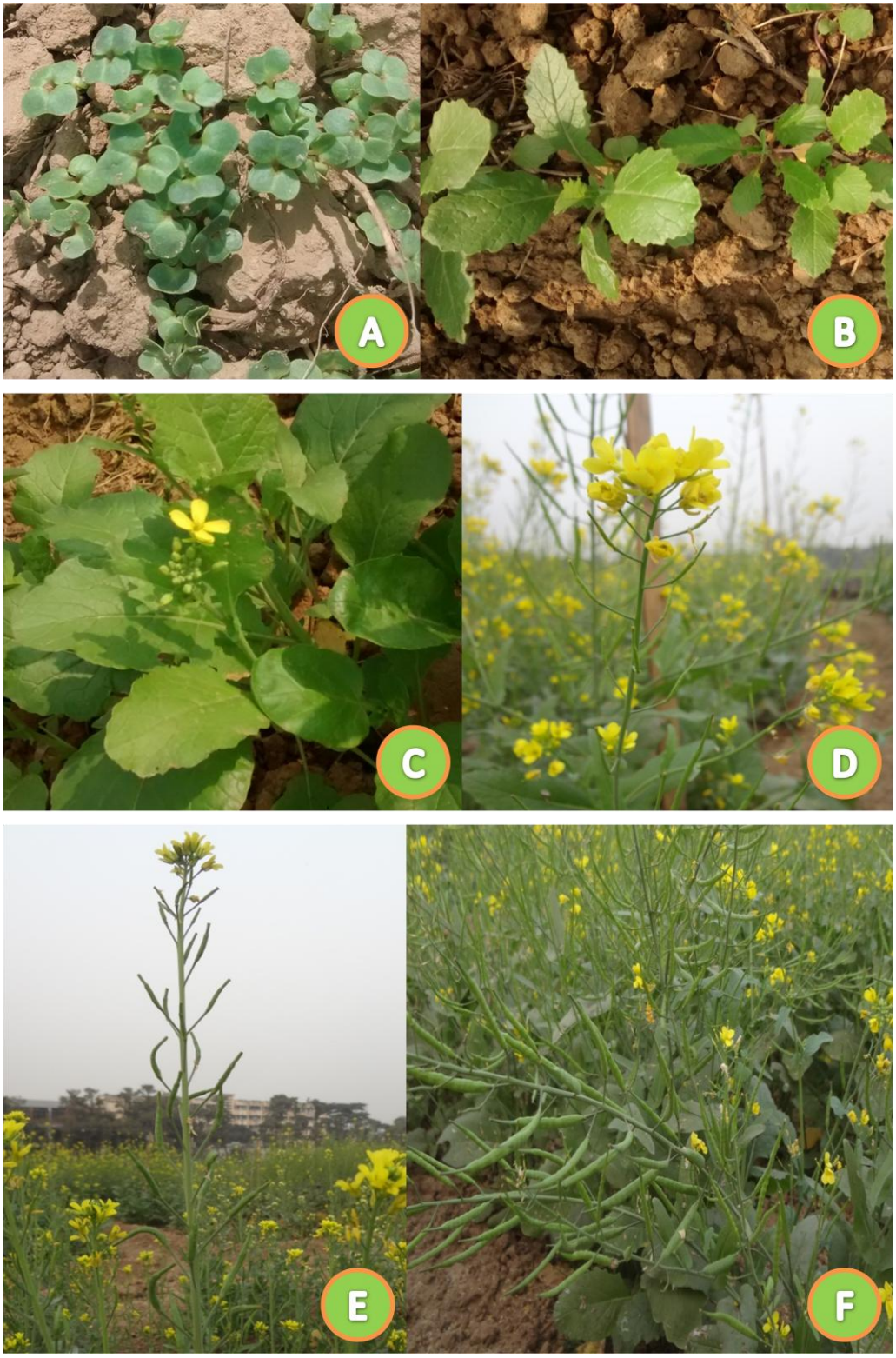

Plate 5: Photograph showing different plant growth stages (A. Seedling after 2 days of germination, B. Seedling stage, C. Flower initiation D. Flowering stage, E. Pod initiation stage, F. Pod maturation stage)

3.7.5 Number of primary branches per plant

The total number of branches arisen from the main stem of a plant was counted as the number of primary branches per plant.

3.7.6 Number of secondary branches per plant

The total number of branches arisen from the primary branch of a plant was counted as the number of secondary branches per plant.

3.7.7 Number of siliquae per plant

Total number of siliquae of each plant was counted and considered as the number of siliquae per plant.

3.7.8 Siliquae length (cm)

This measurement was taken in centimeter (cm) from the base to the tip of a siliqua of the five representative siliquae.

3.7.9 Number of seeds per siliqua

Well filled seeds were counted from five siliquae which was considered as the number of seeds per siliqua.

3.7.10 1000-seed weight (g)

Weight in grams of randomly counted thousand seeds of each entry was recorded.

3.7.11 Seed yield per plant (g)

All the seeds produced by a representative plant was weighed in g and considered as the seed yield per plant.

3.8 Statistical analysis

The mean values of five randomly selected plants used for recording the data. The observed data were computed for each of eleven traits for each genotype in each replication and were subjected to statistical analysis. Duncan's Multiple Range Test (DMRT) was performed for all the characters to test the differences

between the means of the genotypes. Mean, range and co-efficient of variation (CV%) were estimated using MSTAT-C. Multivariate analysis was done by computer using GENSTAT 5.13 and Microsoft Excel 2017 software through four techniques viz., Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Cluster Analysis (CA) and Canonical Vector Analysis (CVA).

3.8.1 Analysis of variance

The analysis of variance for different characters was carried out using mean data in order to assess the genetic variability among genotypes as given by Cochran and Cox (1957). The level of significance was tested at 5% and 1% using F test. The model of ANOVA used is presented in Table 3.

Table 3. Analysis of variance (ANOVA)

Sources of variation	Degrees of freedom (df)	Mean sum of squares (MSS)	Expected MSS
Replication	(r-1)	Mr	$g\sigma^2_r + \sigma^2_e$
Genotypes	(g-1)	Mg	$r\sigma^2_g + \sigma^2_e$
Error	(g-1) (r-1)	Me	σ^2_e
Total	(rg-1)		

Where,

r = number of replications
g = number of treatments (genotypes)
σ^2_r = variance due to replications
σ^2_g = variance due to treatments (genotypes)
σ^2_e = variance due to error

To test significant of the difference between any two-adjusted genotypic mean, the standard error of mean was computed using the formula.

$$\mathbf{S.E} = \sqrt{\frac{2\,Ee}{r}\left(1 + \frac{rqu}{q+1}\right)}$$

Where
S.E = Standars error of mean
Ee = Mean sum of squares for error (Intra block)
r = Number of replications
q = Number of genotypes in each sub-block
u = Weightage factor computed

3.8.2 Genotypic and phenotypic variances

Genotypic and phenotypic variances were estimated according to the formula given by Johnson *et al.* (1955).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{GMS - EMS}{r}$$

Where,

GMS = Genotypic mean sum of square
EMS = Error mean sum of square
r = number of replications

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \sigma^2_e$$

Where,

σ^2_g = Genotypic variance
EMS = Error mean sum of square
σ^2_e = Error variance

3.8.3 Genotypic and phenotypic co-efficient of variation

Genotypic and phenotypic co-efficient of variation were calculated by the formula suggested by Burton (1952):

$$\text{Genotypic co-efficient of variation (GCV \%)} = \sqrt{\frac{\sigma^2_g}{\bar{x}}} \times 100$$

Where,

σ^2_g = Genotypic variance
$\bar{x}$ = Population mean

Similarly, the phenotypic co-efficient of variation was calculated from the following formula.

Phenotypic co-efficient variation (PCV) = $\sqrt{\frac{\sigma^2_{ph}}{\bar{x}}} \times 100$

Where,

σ^2_p = Phenotypic variance

$\bar{x}$ = Population mean

PCV and GCV were classified into three following categories as suggested by Sivasubramanian and Madhamenon (1973).

Categories

Low: Less than 10%; Moderate: 10-20%; High: More than 20%

3.8.4 Heritability

Broad sense heritability was estimated (Lush, 1943) by the following formula, suggested by Johnson *et al.* (1955).

Heritability, $h^2_b = \frac{\sigma^2 g}{\sigma^2 p} \times 100$

Where,

h^2_b = Heritability in broad sense
σ^2_g = Genotypic variance
σ^2_p = Phenotypic variance

Heritability estimates in cultivated plants could be placed in the following categories as suggested by Robinson *et al*. (1966).

Categories

Low: 0-30%; Moderate: 30-60%; High: >60%

3.8.5 Genetic advance

The expected genetic advance for different characters under selection was estimated using the formula suggested by Lush (1943) and Johnson *et al.* (1955).

Genetic advance, GA = K. h^2. σ_p

Or Genetic advance, GA = K. $\frac{\sigma^2 g}{\sigma^2 p} . \sigma_p$

Where,

K = Selection intensity, the value which is 2.06 at 5% selection intensity
σ_p = Phenotypic standard deviation
h^2_b= Heritability in broad sense
σ^2_g = Genotypic variance
σ^2_p = Phenotypic variance

Categories

High (>20%) Moderate (10-20%) Low (<10%)

3.8.6 Genetic advance mean's percentage

Genetic advance as percentage of mean was calculated from the following formula as proposed by Comstock and Robinson (1952):

$$\text{Genetic advance (\% of mean)} = \frac{\text{Genetic Advance}}{\text{Population mean}} \text{ X } 100$$

Genetic advance as per cent mean was categorized into following groups as suggested by Johnson *et al.* (1955).

Categories

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

3.8.7 Genotypic and phenotypic correlation co-efficient

The calculation of genotypic and phenotypic correlation co-efficient for all possible combinations through the formula suggested by Miller *et al.* (1958),

Johnson *et al.* (1955) and Hanson *et al.* (1956) were adopted. The genotypic co-variance component between two traits and have the phenotypic co-variance component were derived in the same way as for the corresponding variance components. The co-variance components were used to compute genotypic and phenotypic correlation between the pairs of characters as follows:

$$\text{Genotypic correlation, } r_{gxy} = \frac{GCOVxy}{\sqrt{GVx.GVy}} = \frac{\sigma_{gxy}}{\sqrt{(\sigma^2_{gx} . \sigma^2_{gy})}}$$

Where,

σ_{gxy} = Genotypic co-variance between the traits x and y
σ^2_{gx} = Genotypic variance of the trait x
σ^2_{gy} = Genotypic variance of the trait y

$$\text{Phenotypic correlation } (r_{pxy}) = \frac{PCOVxy}{\sqrt{PVx.PVy}} = \frac{\sigma_{pxy}}{\sqrt{(\sigma^2_{px} . \sigma^2_{py})}}$$

Where,

σ_{pxy} = Phenotypic covariance between the trait x and y
σ^2_{px} = Phenotypic variance of the trait x
σ^2_{py} = Phenotypic variance of the trait y

3.8.8 Path co-efficient analysis

Path coefficient analysis was carried out using phenotypic correlation values of yield components on yield as suggested by Wright (1921) and illustrated by Dewey and Lu (1959). Standard path coefficients which are the standardized partial regression coefficients were obtained using statistical software packages called OPSTAT. These values were obtained by solving the following set of 'p' simultaneous equation using above package.

$P_{01} + P_{02}\ r_{12} + \ldots\ldots\ldots\ldots\ldots + P_{0p}\ r_{1p} = r_{01}$

$P_{01} + P_{12}\ r_{02} + \ldots\ldots\ldots\ldots\ldots + P_{0p}\ r_{2p} = r_{02}$

$P_{01} + r_{1p} + P_{02}\ r_{2p} + \ldots\ldots\ldots\ldots + P_{0p} = r_{0p}$

Where, P_{01}, P_{02}, ……. P_{0p} are the direct effects of variables 1, 2……P on the dependent variable 0 and r_{12}, r_{13},.....r_{1p}……$r_{p(p+1)}$ are the possible correlation coefficient between various independent variables and r_{01}, r_{02}, r_{03}..... r_{0p} are the correlation between dependent and independent variables. The indirect effects of the i^{th} variable via j^{th} variable was attained as (P_{0j} * r_{ij}). The contribution of remaining unknown factor is measured as the residual factor, which is calculated and given below.

$$P^2_{ox} = 1- (P^2_{01} + 2P_{01}P_{02}r_{12} + 2P_{01}\ P_{03}r_{13} +…...+ P^2_{02} + 2P_{02}\ P_{03}r_{13} +…….. +P^2_{0P})$$

Categories

Negligible - 0.00 to 0.09; Low- 0.10 to 0.19; Moderate 0.20 to 0.29; High – 0.30 to 1.0; Very High- >1.00

After calculating the direct and indirect effect of the characters, residual effect (R) was calculated by using the formula (Singh and Chaudhary, 1985) given below :

$$P^2_{RY} = 1- (r_{1.y}P_{1.y} + r_{2.y}P_{2.y} +……..+ r_{8.y}P_{8.y})$$

Where,

$P^2_{RY} = R^2$

and hence residual effect, $R = (P^2_{RY})^{1/2}$

$P_{1.y}$ = Direct effect of the i th character on yield y.

$r_{1.y}$ = Correlation of the i th character with yield y.

3.9 Multivariate analysis

The genetic diversity among the genotypes was assessed by Mahalanobis's (1936) general distance (D^2) statistic and its auxiliary analyses. The parents selection in hybridization programme based on Mahalanobis's D^2 statistic is more reliable as requisite knowledge of parents in respect of a mass of characteristics is available prior to crossing. Rao (1952) suggested that the quantification of genetic diversity through biometrical procedures had made it possible to choose genetically diverse parents for a hybridization programme.

Multivariate analysis viz. Principal Component analysis, Principal Coordinate analysis, Cluster analysis and Canonical Vector analysis (CVA), which quantify the differences among several quantitative traits, are efficient method of evaluating genetic diversity. These are as follows:

3.9.1 Principal Component analysis (PCA)

Principal Component analysis, one of the multivariate techniques, is used to examine the inter-relationships among several characters and can be done from the sum of squares and products matrix for the characters. Thus, PCA finds linear combinations of a set variate that maximize the variation contained within them, thereby displaying most of the original variability in a smaller number of dimensions. Therefore, Principles components were computed from the correlation matrix and genotypes scores obtained for first components (which has the property of accounting for maximum variance) and succeeding components with latent roots greater than unity. Contribution of the different morphological characters towards divergence is discussed from the latent vectors of the first two principal components.

3.9.2 Principal Coordinate analysis (PCO)

Principal Coordinate analysis is equivalent to PCA but it is used to calculate inter unit distances. Through the use of all dimension of p it gives the minimum distance between each pair of the n points using similarity matrix (Digby *et al.*, 1989).

3.9.3 Cluster analysis (CA)

Cluster analysis divides the genotypes of a data set into some number of mutually exclusive groups. Clustering was done using non-hierarchical classification. In Genstat, the algorithm is used to search for optimal values of chosen criterion proceeds as follows. Starting from some initial classification of the genotypes into required number of groups, the algorithm repeatedly transferred genotypes from one group to another so long as such transfer

improved the value of the criterion. When no further transfer can be found to improve the criterion, the algorithm switches to a second stage which examines the effect of swooping two genotypes of different classes and so on.

3.9.4 Canonical Vector analysis (CVA)

Canonical vector analysis (CVA) finds linear combination of original variabilities that maximize the ratio of between group to within group variation, thereby giving functions of the original variables that can be used to discriminate between the groups. Thus, in this analysis a series of orthogonal transformations sequentially maximizing of the ratio of among groups to the within group variations. The canonical vector are based upon the roots and vectors of WB, where W is the pooled within groups covariance matrix and B is the among groups covariance matrix.

3.9.5 Calculation of D^2 values

The Mahalanobis's distance (D^2) values were calculated from transformed uncorrelated means of characters according to Rao (1952), and Singh and Chaudhury (1985). The D^2 values were estimated for all possible combinations between genotypes. In simpler form D^2 statistic is defined by the formula

$$D^2 = \sum_{i}^{x} d_i^2 = \sum_{i}^{x} (Y_i^j - Y_j^k) \qquad (j \neq k)$$

Where,

Y = Uncorrelated variable (character) which varies from i = 1 ------to x
x = Number of characters.
Superscript j and k to Y = A pair of any two genotypes.

3.9.6 Average intra-cluster distances

Average intra-cluster distances were calculated by the following formula as suggested by Singh and Chuadhury (1985).

Average intra-cluster distance = $\frac{\sum D_i^2}{n}$

Where,

D_i^2 = the sum of distances between all possible combinations (n) of genotypes included in a cluster

n = Number of all possible combinations between the populations in cluster

3.9.7 Average inter-cluster distances

Average inter-cluster distances were calculated by the following formula as suggested by Singh and Chuadhury (1985).

Average inter-cluster distance = $\frac{\sum D_{ij}^2}{n_i \times n_j}$

Where,

$\sum D_{ij}^2$ = The sum of distances between all possible combinations of the populations in cluster i and j

n_i = Number of populations in cluster i

n_j = Number of populations in cluster j

3.9.8 Cluster diagram

Using the values of intra and inter-cluster distances (D = $\sqrt{D^2}$), a cluster diagram was drawn as suggested by Singh and Chuadhury (1985). It gives a brief idea of the pattern of diversity among the genotypes included in a cluster.

3.10 Fatty acids content analysis

Six rapeseed (Brassica rapa) varieties seed oil were used in quality analysis after growing in the field experiment. The chemicals used in fatty acid analysis like sodium chloride acid, Nitrogen gas and Glacial acetic acid and standard fatty acid methyl esters. Determination of fatty Acid Content in Traditional and Commercial Mustard Oils of Bangladesh is done by Gas- Liquid Chromatography (Tanvir, 2014).

3.10.1 Methylation of Fatty Acid

Total lipid (400-600 mg) was taken in a ground joint flask and saponified with 15-30 mL 2M KOH (ethanolic) in water bath at 700 C for 1 hour by joining with a condenser. After cooling, the solution was diluted with equal volume of distilled water and acidified with concentrated HCl to PH<2 as ascertained with a PH meter. The liberated fatty acids (a mixture) were extracted with 30-60 mL of diethyl ether. Small amount of water was also extracted along with free fatty acids. This undesired water was removed by adding anhydrous sodium sulphate. The ether extract devoid of water was collected in another joint flask. The extract was then evaporated to dryness under N_2. Dry methanolic HCl (25-50 mL) prepared as above, was added into the fask containing the fatty acid mixture and the content was heated at 850 C under reflux for 2 hours. After cooling, the fatty acids methyl esters (FAME) were extracted three times with equal volume of petroleum spirit (bp40-600). All extracts were combined and evaporated to a small volume under N_2.

3.10.2 Purification of Fatty Acid Methyl Esters

3.10.2.1 Preparation of TLC Plate

A slurry of silica gel G for thin layer chromatography is made with water (2 mL water per gm silica gel G) in a beaker (500mL capacity) and spread on 2 mm thick glass plates 20×20 cm by a TLC spreader. The silica gel coating is 250 µm. The slurry thus spread is kept on the platform for about 10 minutes, transfer to the metal racks and dried in an oven at 1100 C for about an hour. The plates are now ready for use.

3.10.2.2 Thin Layer Chromatographic (TLC) procedure

Standard fatty acids preparation (~3-5 mL) is now spotted on the plates with a glass capillary taking precaution so that not more than 2-3 µL are spotted on the plates at a distance nearly ¾ for an inch from one edge on the plates. The gaps between two spots should be around half an inch and the spots should be as small as possible for better resolution of the fatty acids. The unknown should

be spotted on the two locations. After air drying the plate is dipped in the solvents (n- hexane: Diethyl ether: glacial acetic acid 70:30:1) in the TLC jar which is pre-equilibrated with the solvent system for about an hour. The solvent rise up the silica gel (ascending chromatography) and is allowed to rise approximately anywhere between 15-18 cm (nearly one hours) at which point the plate is removed from the jar, air dried, placed in the iodine chamber for 5 minutes. The FAME band in the plate was visualized in the iodine chamber. The FAME in the sample can be identified by their Rfvalues when compared to standard. After the yellow color vanished the band was scraped into a centrifuge tube and eluted with methanol. The tube was then centrifuged and the supernatant was transferred into a dry flask. The FAME solution was evaporated to dryness under nitrogen. A small volume of dichloromethane solution was added to re-dissolve the FAME band and a 5-10 mm aliquot and analyzed in Gas-liquid chromatography.

3.10.3 Gas-Liquid Chromatographic (GLC) analysis of fatty acid methyl esters

The fatty acid methyl esters, prepared and purified as above, were analyzed by gas-liquid chromatography (GLC). A 2×4 mm inside diameter column (Preferably glass) packed with 12-15 % (w/w) ethylene glycol succinate liquid phase coated on 100/200 mesh Gas-chrom P was used. The temperature was 1900 C and the detector temperature was 2600 C. The injector temperature of the column was programmed initially at 1700 C for 8 minutes, then it was allowed to rise to 2000 C at a rate of 10 C/min and the isothermal final period was 55 minutes. Thermal conductivity detectors were excellent. Nitrogen was used as a carrier gas at a flow rate of 11.4 mL/min. Hydrogen flow was 10% above nitrogen flow. Standard fatty acid methyl esters were used for the identification of the sample fatty acid peaks. The following Standard fatty acids were used, the methyl esters of $C_{8:0}$ $C_{9:0}$ $C_{10:0}$ $C_{11:0}$ $C_{12:0}$ $C_{14:0}$ $C_{16:0}$ $C_{18:0}$ $C_{18:1}$ $C_{18:2}$ $C_{18:3}$ $C_{20:0}$ $C_{22:0.}$ The peak area of each component was measured automatically by chromatograph machine. It was also measured by the actual physical measurement by the triangulation method (Tanvir et al, 2014). The

total mm of all peak areas were taken as 100% and the percent population of a given fatty acid peak was calculated accordingly. The fatty acids were expressed as weight percentages of total fatty acids.

Brief procedure of determination of Fatty acid composition

Methylation of fatty acids in the oils under study was carried out according to the procedure with some modifications:

- Ten-twelve mustard seeds were crashed and the powdery samples were put in a 50 ml screw capped Pyrex glass tubes having 50 cm length and 1 cm internal diameter.
- Then 2ml of methanolic sulphuric acid added in each tube and glass vials were put in pre heated oven at 80^0C for 1h and shake after 15 min.
- The glass vials taken out, cooled and 2ml of distill water were added in each tube to stop the reaction.
- Then esterifies fatty acids were extracted with 1ml of petroleum ether (40-60^0C) thrice.
- After then the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile.

CHAPTER IV
RESULTS AND DISCUSSIONS

In the present investigation the data was collected from fourteen diverse *Brassica rapa* genotypes on eleven traits related to vegetative, reproductive, yield and nutrient components parameters emphasizing growth, yield, and nutrition. The data were subjected to biometrical and biochemical analysis and results obtained are presented below under the following headings:

4.1 Varietal performance and genetic parameters
4.2 Correlation studies
4.3 Path co-efficient analysis
4.4 Genetic diversity through D^2 statistics
4.5 Fatty acids content analysis

4.1 VARIETAL PERFORMANCE AND GENETIC PARAMETERS

The achievement in any crop improvement program depends on the capability of the breeder to define and accumulate the required genetic variability and to select for yield indirectly through yield associated and highly heritable characters after eliminating the environmental component of phenotypic variation (Mather, 1949). Therefore, it is necessary to have prior information on both Phenotypic coefficient variation and Genotypic coefficient variation, so that the estimate of heritability that helps the breeder to predict the expected GA possibly by selection for a character can be computed.

The results are pertained to analysis of variance (ANOVA), range, grand mean, CV%, mean performance, genotypic and phenotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2_b) and expected genetic advance as per cent of mean (GA) for all the eleven traits are furnished in Table 4 to Table 7. Genotypic and phenotypic coefficient of variation is shown in Figure 1,

heritability and genetic advance as per cent of mean is represented in Figure 2. Out of the eleven traits studied, plant heights, no. of primary branches per plant, no. of secondary branches per plant are considered as growth attributing characters. Days to 1st flowering, days to 50% flowering and days to maturity were regarded as earliness attributes. No. of siliqua per plant, length of siliqua, no. of seeds per siliqua and 1000 seed weight were considered as reproductive traits. Yield per plant was the economic trait. The character wise details of these variability are discussed below of the genotypes evaluated for 11 characters are presented in below**.**

4.1.1 Days to 1st flowering

Days to 1st flowering was ranged from 22.00 to 38.00 DAS with mean value of 28.98 (Table 5). The mean sum of square of days to 1st flowering was 83.40 which showed highly significant differences among the genotypes (Table 4). Highest duration was recorded in Torsha (38.00) which was statistically similar with Sonali sarisha (36.67) and lowest in both Sonali Tori and Maghi (22.00) which were statistically similar with Improved Tori (23.00) and Tori-7 (23.33) (Table 6).

The phenotypic variance (29.32) was higher than genotypic variance (27.15) revealing that the apparent variation was not only due to genotypes but also due to influence of environment. The genotypic co-efficient of variation and phenotypic co-efficient of variation were 17.97 and 18.60 respectively (Table 7). There was a little difference between phenotypic and genotypic co-efficient of variation, indicating minor environmental influence on the expression of this trait. The medium PCV and GCV value for days to 1st flowering indicated considerable scope to selection for this trait. The heritability (82.35%) estimates for this trait was high. Most likely the high heritability is due to additive gene effects. High genetic advance over percentage of mean (35.79) was found for this trait (Table 7). High heritability coupled by high genetic advance percentage of mean was noticed for days to 1st flowering and this was

Table 4. Analysis of variance for different characters in *Brassica rapa* genotypes

Characters	Mean sum of square		
	Replication (r-1) = 2	Genotype (g-1) = 13	Error (r-1)(g-1) = 26
Days to First flowering	0.16	83.40**	1.93
Days to 50% flowering	2.16	91.20**	3.83
Days to maturity	1.78	40.00**	5.17
Plant height (cm)	56.98	325.03**	26.66
No. of primary branches per plant	0.19	1.71ns	0.87
No. of secondary branches per plant	0.62	33.99**	5.16
No. of siliqua per plant	161.92	5636.22**	1098.77
Length of siliqua (cm)	0.17	0.40**	0.09
No. of seeds per siliqua	11.26	147.26**	6.58
1000 seed weight (g)	0.23	0.45**	0.01
Seed yield per plant (g)	1.43	5.99**	1.01

** : Denote Significant at 1% level of probability ns: non significant

Table 5. Range, mean, CV (%) and standard deviation of 14 *Brassica rapa* genotypes

Parameters	Range		Mean	CV (%)	SD	SE
	Min	Max				
Days to First flowering	22.00	38.00	28.98	4.80	1.39	0.53
Days to 50% flowering	26.67	43.00	33.24	5.89	1.96	0.74
Days to maturity	79.00	92.00	85.00	2.68	2.27	0.86
Plant height (cm)	76.03	107.13	91.41	5.65	5.16	1.95
No. of primary branches per plant	3.98	6.57	5.20	17.99	0.94	0.35
No. of secondary branches per plant	1.00	9.20	5.37	42.35	2.27	0.86
No. of siliqua per plant	50.17	181.87	130.32	25.44	33.15	12.53
Length of siliqua (cm)	4.86	6.01	5.22	5.89	0.31	0.12
No. of seeds per siliqua	12.33	34.60	19.45	13.19	2.57	0.97
1000 seed weight (g)	2.20	3.83	2.99	3.51	0.11	0.04
Seed yield per plant (g)	3.90	9.09	6.62	15.22	1.01	0.38

CV (%) = coefficient of variation, SD = standard deviation and SE = standard error

Table 6. Mean performance of different characters of 14 *Brasica rapa* genotypes

Genotype	DFF	D50%F	DM	PH	NPB	NSB	NSP	LS	NSS	**TSW**	**SYP**
Tori-7	23.33f	28.00d	86.00b	76.03e	4.80abc	6.44a	135.1a-d	5.01de	13.60CD	2.20H	3.90D
BARI sarisha 3	29.00cd	33.67b	90.67a	98.50ab	5.30abc	9.20a	153.2ab	6.00a	16.93CD	3.21BC	7.30AB
Sonali sarisha	36.67ab	42.00a	92.00a	104.1ab	3.97c	1.23b	50.17f	5.64a-c	24.00B	3.83A	4.17D
BARI sarisha 6	31.33c	34.67b	86.00b	107.1a	4.43c	7.89a	155.9ab	5.73ab	17.57C	2.93DE	7.40AB
BARI sarisha 9	28.67de	32.00bc	86.00b	97.47ab	5.50abc	8.35a	170.2ab	5.00de	12.33D	3.08CD	6.48BC
BARI sarisha-12	28.67de	32.00bc	86.00b	95.35bc	5.73abc	7.71a	152.5ab	5.54a-d	14.97CD	2.93DE	6.68BC
BARI sarisha-14	30.67cd	33.67b	79.00c	84.86de	6.36ab	1.10b	73.80d-f	4.92e	31.67A	3.32B	7.25AB
BARI sarisha-15	35.00b	41.33a	82.00bc	99.03ab	5.66abc	1.00b	84.53c-f	4.97de	24.23B	3.37B	6.85B
BARI sarisha-17	31.00cd	34.00b	80.67c	86.83cd	4.70bc	1.44b	74.46ef	4.97de	34.60A	3.10CD	9.09A
Improved Tori	23.00f	29.00cd	86.00b	87.00cd	5.06abc	8.71a	180.9a	5.23b-e	16.30CD	2.88E	7.54AB
Sonali Tori	22.00f	27.00d	86.00b	79.37de	4.16c	8.22a	140.7abc	5.09c-e	14.00CD	2.66FG	4.97CD
Torsha	38.00a	43.00a	86.00b	102.8ab	6.56a	1.00b	114.1b-e	5.14c-e	22.80B	2.82EF	7.49AB
Maghi	22.00f	26.67d	81.00c	78.17de	5.36abc	6.05a	157.1ab	4.86e	16.07CD	2.84EF	7.31AB
BINA 10	26.33e	28.33d	82.67bc	83.07de	5.16abc	6.77a	181.9a	4.87e	13.23CD	2.61G	6.27BC

DFF : days to First flowering
D50%F : days to 50% flowering
DM : days to maturity
PH : plant height (cm)
NPB : no. of primary branches per plant
NSB : no. of secondary branches per plan
NSP : no. of siliqua per plant
LS : length of siliqua (cm)
NSS : no. of seeds per siliqua
TSW : 1000 seed weight (g)
SYP : seed yield per plant (g)

Table 7. Estimation of genetic parameters for different characters in *Brassica rapa*

Parameters	$\sigma^2 p$	$\sigma^2 g$	$\sigma^2 e$	PCV	GCV	ECV	h^2	GA (5%)	GA (% mean)
Days to First flowering	29.32	27.15	1.97	18.60	17.97	4.80	82.35	10.37	35.79
Days to 50% flowering	32.96	29.12	3.83	17.27	16.24	5.89	84.32	10.45	31.44
Days to maturity	16.78	11.61	5.17	4.82	4.01	2.68	69.19	5.84	6.87
Plant height (cm)	126.12	99.46	26.66	12.29	10.91	5.65	78.86	18.24	19.96
No. of primary branches per plant	1.16	0.28	0.87	20.67	10.19	17.99	24.29	0.54	10.34
No. of secondary branches per plant	14.78	9.61	5.17	71.61	57.75	42.35	65.03	5.15	95.93
No. of siliqua per plant	2611.26	1512.48	1098.77	39.21	29.84	25.44	57.92	60.97	46.79
Length of siliqua (cm)	0.20	0.10	0.09	8.50	6.13	5.89	51.98	0.47	9.10
No. of seeds per siliqua	53.48	46.89	6.58	37.60	35.21	13.19	87.69	13.21	67.92
1000 seed weight (g)	0.16	0.15	0.01	13.40	12.93	3.51	93.12	0.77	25.70
Seed yield per plant (g)	2.68	1.66	1.02	24.70	19.45	15.22	62.02	2.09	31.55

$\sigma^2 p$: Phenotypic variance
$\sigma^2 g$: Genotypic variance
σ^2 e : Environmental variance

PCV : Phenotypic coefficient of variation
GCV : Genotypic coefficient of variation
ECV : Environmental coefficient of variation

h^2 : Heritability
GA (5%) : Genetic advance (5%)
GA (% mean) : Genetic advance (% mean)

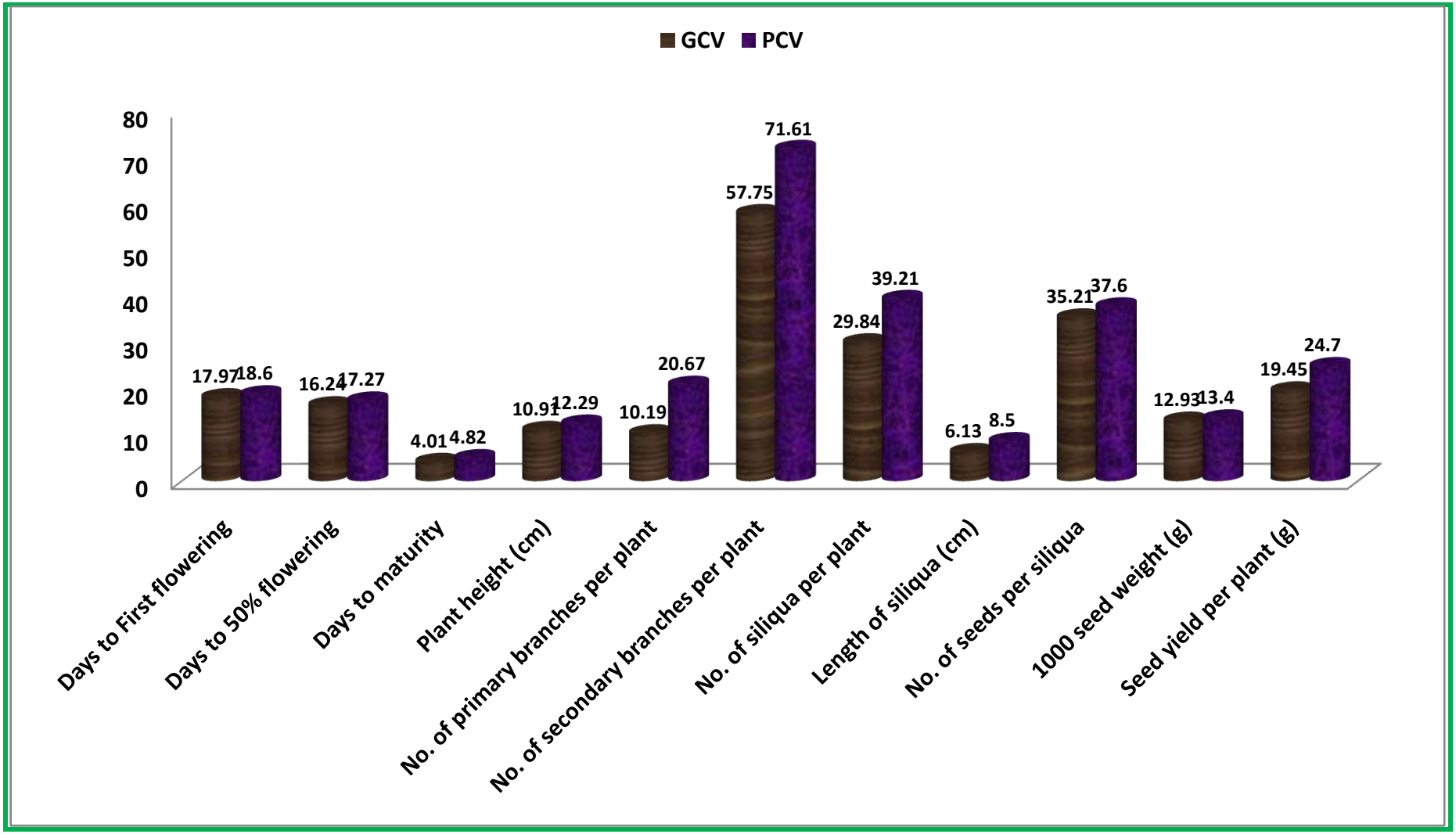

Figure 1: Genotypic and phenotypic variability in *Brassica rapa* L.

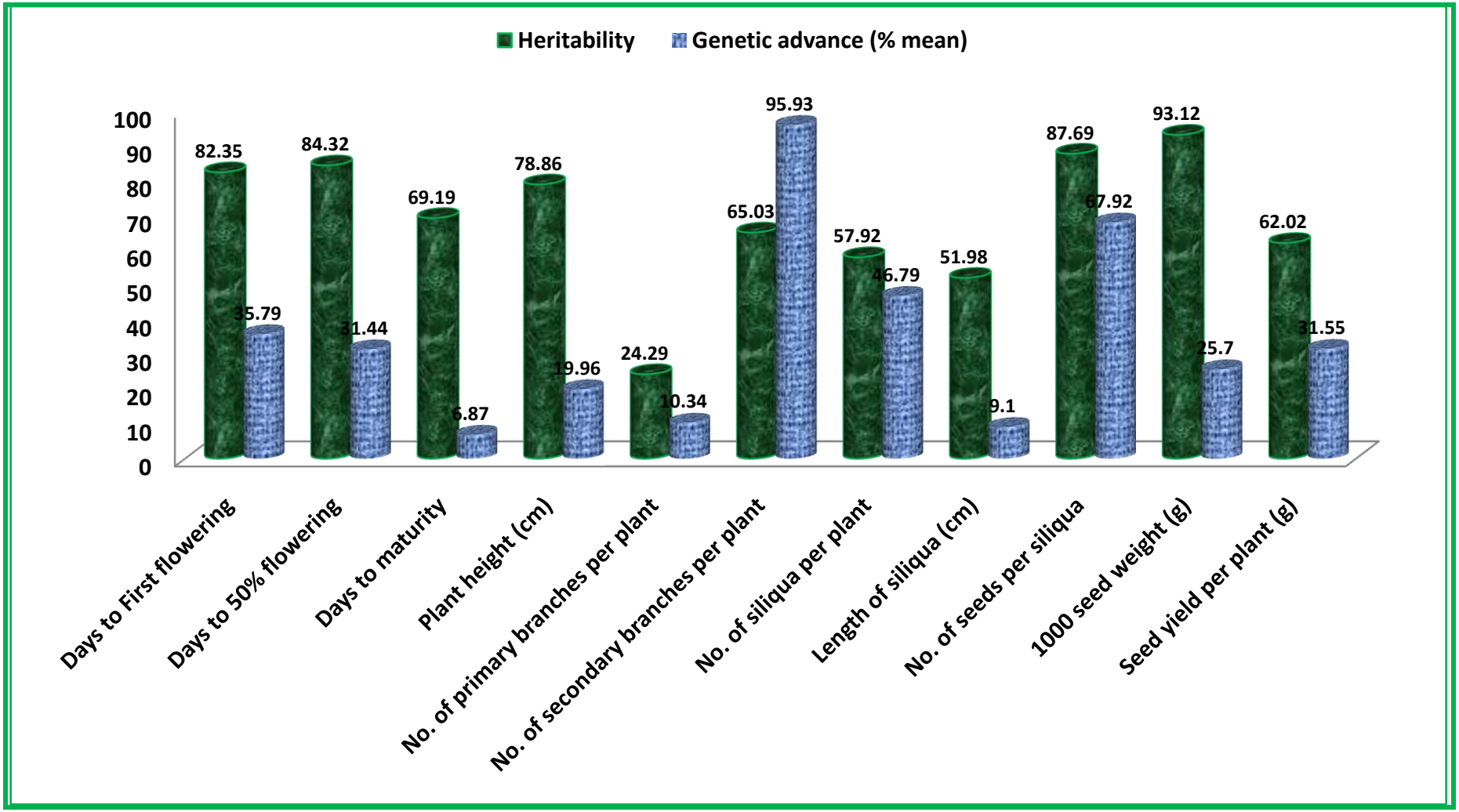

Figure 2: Heritability and genetic advance as percent over mean in *Brassica rapa* L.

supported by Mahak *et al.* (2004) findings. This high value may be due to additive gene action and selection will be beneficial.

4.1.2 Days to 50% flowering

Highly significant mean sum of square was observed in days to 50% flowering with the value of 91.20 (Table 4). The maximum duration to days to 50% flowering was found in Torsha with 43.00 DAS which was statistically similar with Sonali sarisha (42.00) and BARI sarisha-15 (41.33) (Table 6). The minimum days to 50% flowering was observed in Maghi with 26.67 DAS which was in statistically with Sonali Tori (27.00), Tori-7(28.00), BINA 10 (28.33) and Improved Tori (29.00) (Table 6). The mean value of days to 50% flowering was 33.24.

The genotypic variance (29.12) was lower than phenotypic variance (32.96) (Table 7). Thus, genes controlling this trait possessed considerable influence of environment on the expression of the character. The Genotypic co-efficient of variation and Phenotypic co-efficient of variation were moderate with value of 16.24 and 17.27 percent respectively, along with high heritability of 84.32% with high genetic advance as percent of mean 31.44% and moderate genetic advance 10.45% (Table 7). Niraj and Srivastava (2004) and Hosen (2008) reported that days to 50% flowering showed high heritability with genetic advance in percentage of mean. The flowering trait of the plant was moderate sensitive and influenced by the environmental temperature fluctuation which was reflected in the present study. High heritability couple with moderate genetic advance indicating that the traits were being exhibited due to some extends of influence of environment. These findings supported with Jahan (2008) results for this trait. Thus, it was indicating that this trait was under additive gene control and selection for genetic improvement for this trait would be effective. In the present study high heritability along with high genetic advance as percent of mean was observed for this trait. Thus, selection for this trait might be rewarding.

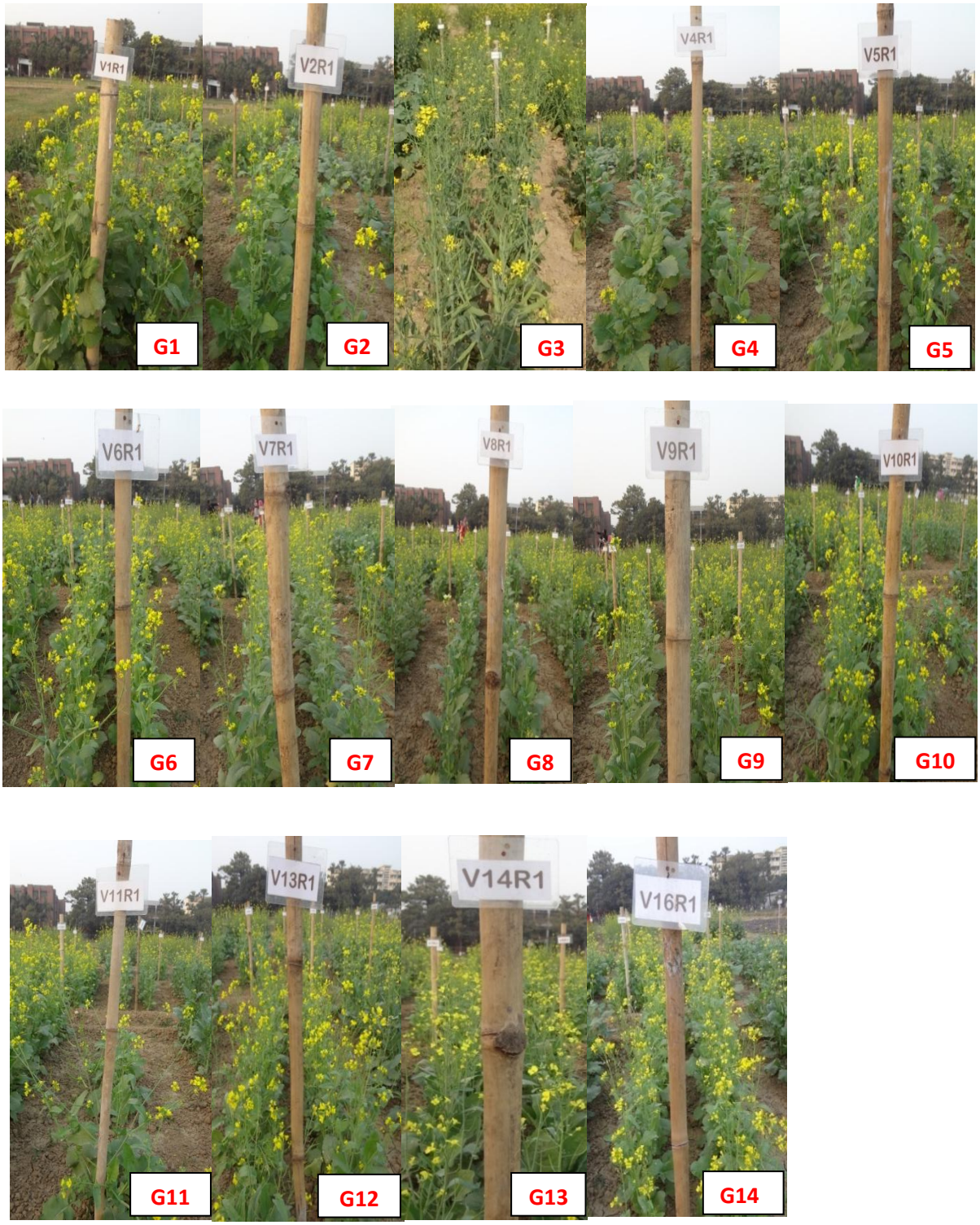

Plate 6: Flowering stage of different varieties at field condition

4.1.3 Days to maturity

The average days to maturity were recorded 85.00 with a range of 79.00 to 92.00 day (Table 4). BARI sarisha-14 required least number of days to maturity (79.00 days) which was statistically similar with BARI sarisha-17 (80.67) and Maghi (81.00) (Table 6). Whereas maximum number of days to maturity was observed in the genotype Sonali sarisha (92.00 days) which was statistically similar with BARI sarisha-3 (90.67) (Table 6).

Days to maturity exhibited low GCV and PCV of 4.01 and 4.82 percent respectively, along with high heritability of 69.19 percent, low genetic advance 5.84 and low genetic advance as percent of mean 6.87 percent (Table 7). Naznin (2013) also found low difference between PCV (22.15) and GCV (19.74) in *B. rapa* L. Heritability was high for days to maturity reported by Niraj and Srivastava (2004). This high heritability with low genetic advance indicates of non-additive gene action. High heritability is being exhibited due to additive gen effect. Jahan (2008) found high heritability with low genetic advance in percent of mean was observed for days to maturity and selection for such trait might not be rewarding. The genotypic and phenotypic variances were recorded as 11.61 and 16.78, respectively. As phenotypic variance is larger than genotypic variance proving that considerable influence of environment is present in the expression of genes for this trait.

4.1.4 Plant height (cm)

Plant height was observed highest in BARI sarisha-6 (107.13 cm) which was statistically similar with Sonali sarisha (104.1 cm), Torsha (102.8 cm) and BARI sarisha-15 (99.03 cm) (Table 6). The lowest plant height was observed in BARI sarisha-1 (76.03 cm) and this was similar in statically with Maghi (78.17 cm) and BINA-10 (83.07 cm). The highest and lowest plant height was showing in Plate 7 and 8 The mean value was recorded as 91.41 cm and mean of sum of square was 40.00 indicating significant differences among the

Plate 7: Highest plant height showing in the variety BARI sarisha-6 (G4) and Sonali sarisha

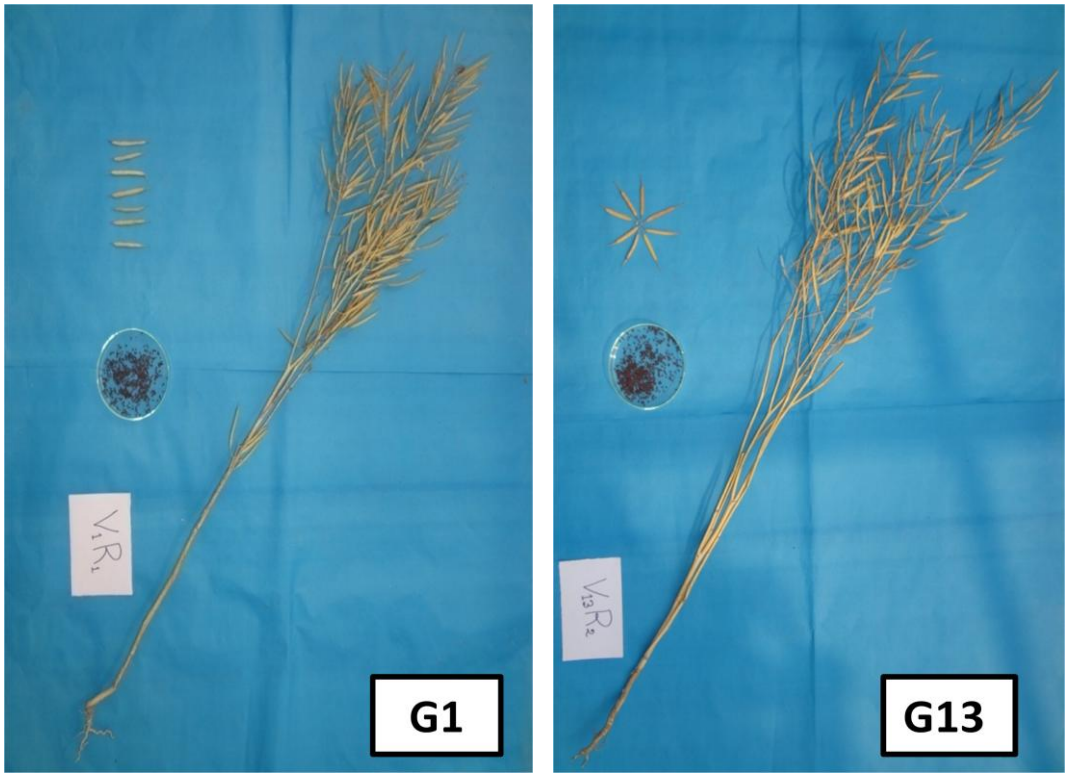

Plate 8: Lowest plant height showing in the variety Tori-7 (G1) and Maghi (G13)

genotypes for this trait (Table 4). It ranged from 76.03 to 107.13 cm. This might be due to genetic configuration and environmental effect.

Genotypic and phenotypic variance was observed 99.46 and 126.12 respectively for plant height with large environmental influence. Highest genotypic, phenotypic and environmental variances were observed in plant height reported by Khan *et al.* (2013). The plant height exhibited moderate genotypic co-efficient of variation (GCV) and phenotypic co-efficient of variation (PCV) of 10.91 and 12.29 percent respectively (Table 7). Low phenotypic coefficient of variation and genotypic coefficient of variation was found by Ghosh and Gulati (2001). High heritability of 78.86 percent, moderate genetic advance 18.24 along with moderate genetic advance as percent of mean (19.96%) was recorded. Afrin *et al.* (2011) found that the plant height showed highest value of broad sense heritability**.** High heritability with moderate genetic advance shows that it is controlled by additive and non-additive gene effects and the selection maybe effective for improvement of *Brasica rapa*. Jahan (2008) also found the same result that high heritability with moderate genetic advance in percent of mean for plant height.

4.1.5 Number of primary branches per plant

Number of primary branches per plant ranged from 3.98 to 6.57 in different genotypes (Table 4). The maximum number of primary branches per plant was recorded in the variety Torsha (6.57) which was similar in statistically with BARI sarisha-14 (6.36). However, minimum number of primary branches per plant exhibited in variety Sonali sarisha (3.98) which was statistically similar with Sonali Tori (4.16), BARI sarisha-6 (4.43) and BARI sarisha-17 (4.70). The mean observed for this trait was 5.20. Highest and lowest primary branching was shown in Plate 9 and Plate 10.

The genotypic variance (0.28) and phenotypic variance (1.16) were least diverse to each other that was supported the result of Hosen (2008). Moderate

Plate 9: Highest primary branching showing in the variety Torsha (G12) and BARI sarisha 14 (G7)

Plate 10: Lowest primary branching showing in the variety Sonali sarisha (G3) and Sonali Tori (G11)

GCV and high PCV were observed as 10.19% and 20.67% respectively (Table 7). This indicates moderate influence of environment upon the character. According to Mekonnen *et al.* (2014) assessed that number of primary branches per plant exhibited comparatively high genotypic and phenotypic coefficient of variation. Whereas, it showed low heritability (24.29%), low genetic advance (0.54) and moderate genetic gain as per cent of mean (10.34%) for this trait. In contrary, Parveen (2007); Ghosh and Gulati (2001) found the high heritability coupled with high genetic advance for this trait. Low heritability with low genetic advance indicates that largely influenced due to environmental effects. Thus, selection is ineffective for the improvement of the crop by this trait.

4.1.6 **No. of secondary branches per plant**

The mean of sum of square for number of secondary branches per plant was significantly recorded as 33.99 (Table 1). Maximum number of secondary branches per plant were found in the variety BARI sarisha-3 (9.20) which was statistically similar with Improved Tori (8.71) and BARI sarisha-9 (8.35). The minimum number of secondary branches per plant were found in variety both BARI sarisha-15 and Torsha (1.00) which were statistically similar with BARI sarisha14 (1.10) and Sonali sarisha (1.23). The mean value of number of secondary branches per plant was 5.37 (Table 6). These variations might be due to difference in genetical constituents as well as environmental effects.

The genotypic and phenotypic variance was recorded as 9.61 and 14.78 respectively. High genotypic co-efficient of variation (GCV) and phenotypic co-efficient of variation (PCV) were observed 57.75 and 71.61 percent respectively (Table 7). High GCV and PCV also observed for number of secondary branches per plant by Choudhary *et al.* (2003); Mahmud (2008). And the variation is caused not only due to genotypes but also the influence of environment. High heritability 65.03% and high genetic advance as per cent mean 95.93% shows that additive gene effects were present, making selection effective for this trait. Findings of Parveen (2007); Shalini *et al.* (2000);

Choudhary *et al.* (2003) were also in agreement with this result. The genetic advance was recorded as 5.15.

4.1.7 No. of siliqua per plant

Number of siliqua per plant ranged from 50.17 to 181.87 with mean value 130.32 in different *Brassica rapa* varieties. The maximum number of siliqua per plant was noticed in the variety BINA-10 (118.9) and it was statistically similar with Improved Tori (180.9) and BARI sarisha-9 (170.2). The variety Sonali sarisha recorded minimum number of siliqua per plant (50.17) and it was insignificantly similar with BARI sarisha-14 (73.80) and BARI sarisha-17 (74.46) (Table 6). The mean sum of square reported significantly for this trait was 5636.22 (Table 5). The phenotypic variance (2611.26) is higher than genotypic variance (1512.48). This indicates high influence of environment on this character. The high phenotypic coefficient of variation (39.21%) and genotypic coefficient of variation (29.84%) (Table 7) indicated presence of considerable variability among the genotypes. Similar result was also reported by Khan *et al*. (2013). Mekonnen *et al.* (2014) observed comparatively high GCV for this trait. The heritability (62.24%) estimates for this trait was high, high genetic advance (60.97) and high genetic advance in percent of mean (46.79) were found (Table 5). So, these traits could be exploited for further improvement through selection procedures. It was also reported by Mekonnen *et al.* (2014), Alam (2010) reported that pods per plant had moderately high GCV and genetic advance and high heritability. Highest and lowest siliqua per plant was showing in Plate 11 and Plate12.

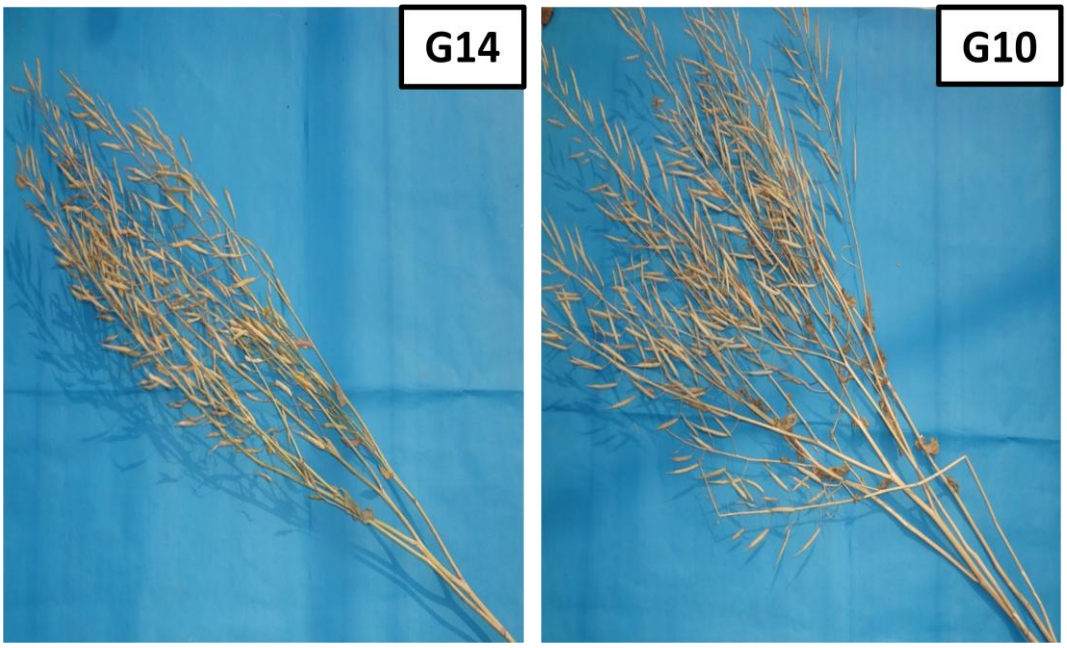

Plate 11: Highest number of siliqua per plant showing in the variety BINA-10(G14) and Improved Tori (G10)

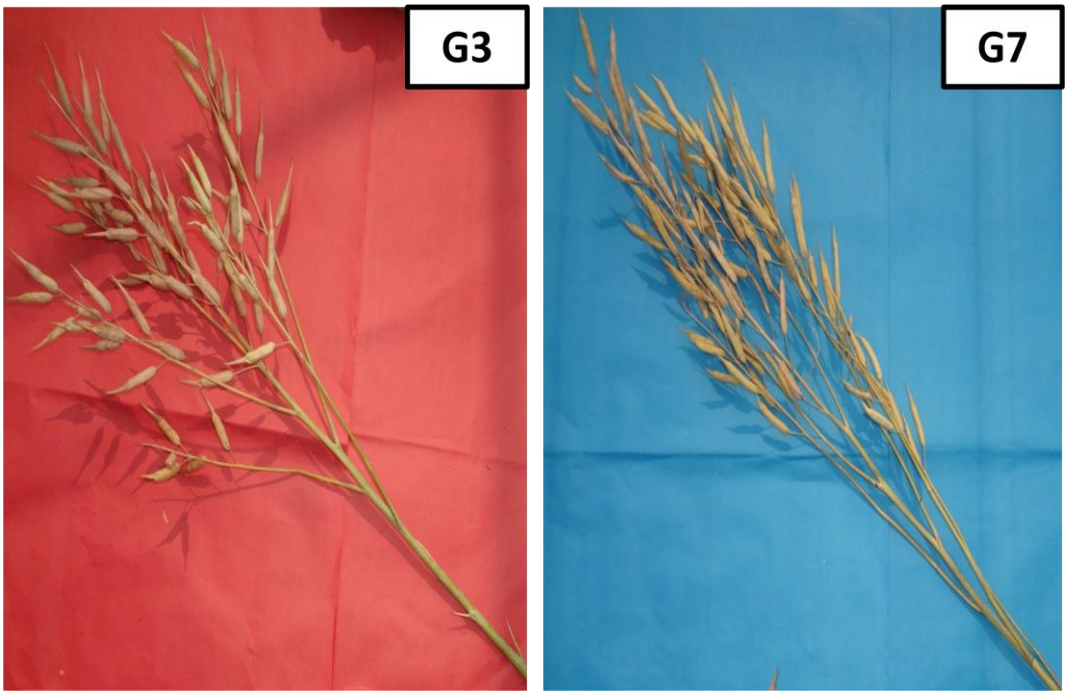

Plate 12: Lowest number of siliqua per plant showing in the variety Sonali sarisha (G3) and BARI sarisha-14 (G7)

4.1.8 Length of siliqua (cm)

The mean of siliqua length was 5.22 cm and ranged from 4.86 to 6.01 cm. The variety BARI sarisha-3 had long siliqua of 6.01 cm which was statistically similar with Sonali sarisha (5.64 cm) and BARI sarisha-6 (5.73 cm). The shortest siliqua was observed in Maghi (4.86 cm) and it was statistically similar with BINA-10 (4.87 cm), BARI sarisha-9 (5.00 cm) and Tori-7 (5.01 cm) (Table 4). The mean sum of square was significant (0.40) which indicated considerable amount of variation for this trait in the varieties (Table 4). The genotypic and phenotypic variance for siliqua length was found as value of 0.10 and 0.20, respectively. Siliqua length exhibited low GCV (6.13%) and PCV (8.50%) values. Similar result was reported by Khan *et al.* (2013). As PCV is higher than GCV thus we can conclude that the trait is not only controlled by genotypes but also controlled by influence of environment. Selection for such traits sometimes may be misleading. A moderate heritability estimates of 51.98%, low genetic advance 0.47 and a low genetic advance as percent of mean 9.10% were observed for the trait. Moderate heritability with combination of low genetic advance as percent of mean allow us to speculate the presence of non-additive gene effects on this trait.

4.1.9 No. of seed per siliqua

Number of seeds per siliqua ranged from 12.33 to 34.60 in different varieties. The maximum number of seeds per siliqua was recorded in variety BARI sarisha-17 (34.60) and it was statically similar with BARI sarisha-14 (31.67). However, minimum number of seeds per siliqua exhibited in variety BARI sarisha-9 (12.33) which were similar in statistically with BINA-10 (13.23) and Tori-7 (13.60) (Table 6). The mean observed for this trait was 19.45. Variation in pod length was shown in Plate 13 and Plate 14.

Plate 13: Photograph showing variation in pod length among the varieties

Plate 14: Photograph showing variation in pod length among the varieties

The genotypic variance (46.89) was lower than the phenotypic variance (53.48). High GCV and PCV were observed as 35.21 and 37.60 respectively (Table 7). This indicates little influence of environment upon the character. It showed high heritability (87.69%), moderate genetic advance (13.21) and high genetic gain as percent of mean (67.92%) for this trait. High heritability with moderate genetic advance and high genetic advance as percent of mean indicates that selection is effective for the improvement of the crop.

4.1.10 1000 seed weight (g)

1000 seed weight of different genotypes ranged from 2.20 g to 3.83 g. The variety Sonali sarisha was exhibited maximum 1000 seed weight (3.83 g) and it was statistically significant differ from BARI sarisha-15 (3.37) and BARI sarisha-14 (3.32). Whereas, the variety Tori-7 was recorded minimum seed weight of (2.20 g) followed by variety BINA-10 (2.61 g) and Sonali Tori (2.66 g). The grand mean found for this trait was 2.99 g (Table 4). The mean sum of square was significant (0.45) in *Brassica rapa* which allows to show the presence of considerable variation for this trait.

1000 seed weight was recorded moderate PCV (13.40%) and GCV (12.93%) (Table 7). As PCV is greater than GCV, there is considerable influence of environment on this trait (Table 7). While it recorded high heritability (93.12%), very low genetic advance (0.77) and high genetic gain as percent of means (25.70%) was found for this trait. High heritability with low genetic advance suggests that the character is governed by the additive and non additive gene actions. Thus, selection may be effective in this trait for the improvement of the crop. Plate 15 was showing different varieties of seed.

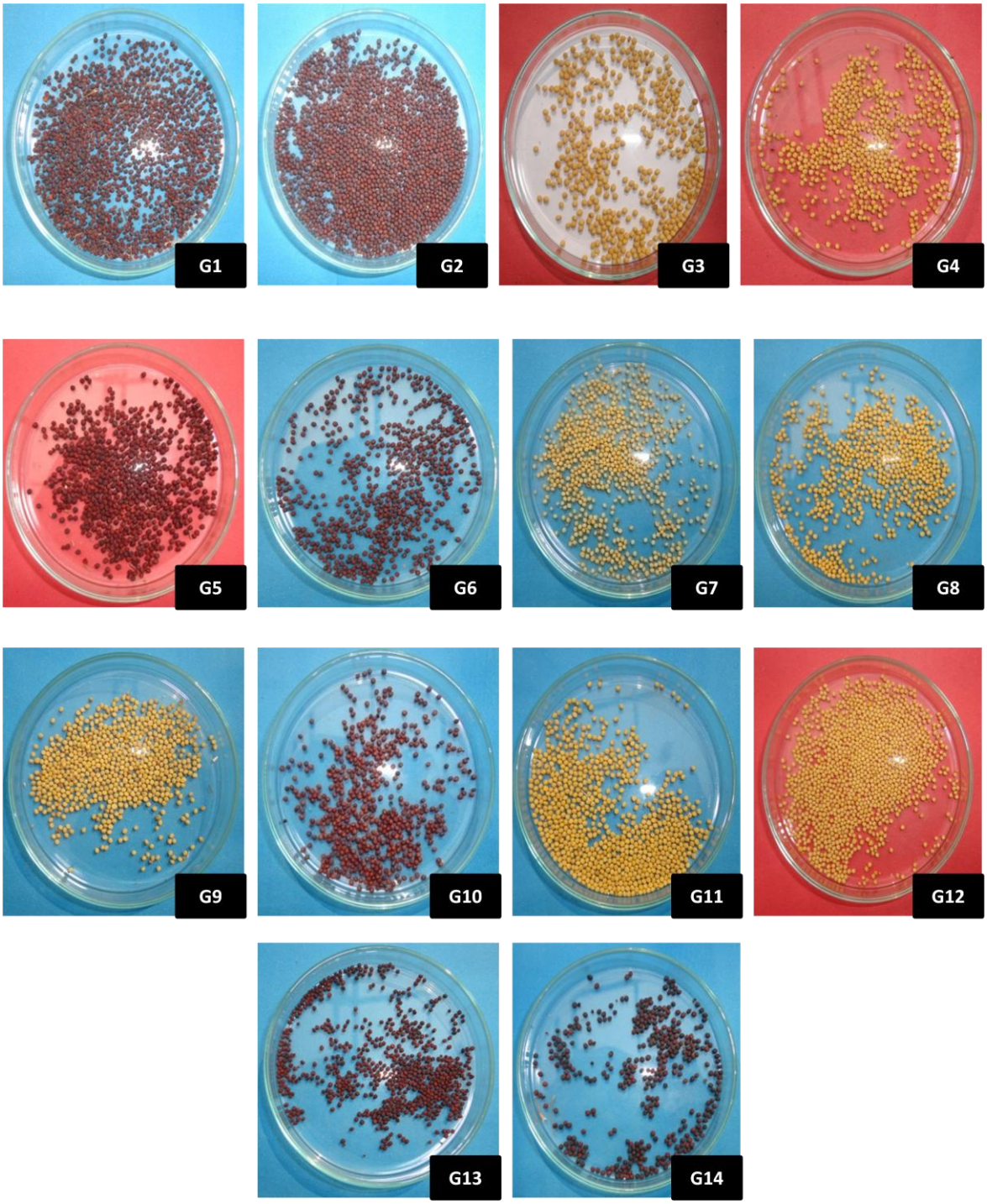

Plate 15: Photograph showing different varieties seed

4.1.11 Seed yield per plant (g)

Seed yield ranged from 3.90g to 9.09g, with a mean value of 6.62g. Maximum yield was recorded by the variety BARI sarisha-17 (9.09g) and it was statistically similar with Improved Tori (7.54g), Torsha (7.49g) and BARI sarisha-6 (7.40 g). The lowest yield was recorded by the genotype Tori-7 (3.90 g) which was statistically similar with Sonali sarisha (4.17 g) (Table 6). The mean sum of square was significant (5.99).

Seed yield per plant exhibited high estimates of PCV (24.70%) and GCV (19.45%) in Table 7. Whereas, it also recorded moderately high heritability (62.02%), couple with low genetic advance (2.09) and high genetic gain as percent of mean (31.55%) for this trait. Selection would be effective for this trait as there are additive gene effects on the gene controlling this trait. Seed yield per plant showed high heritability with high genetic advance as percent of mean.

4.2 CORRELATION ANALYSIS

Improvement of a specific trait in all the breeding programs can be achieved by indirect selection via other characters. This needs a good understanding of the association of different characters with the target character and among the different characters themselves. It is necessary to have the estimates of correlation of yield with other characters for which the genotype could be assessed visually. The phenotypic and genotypic correlation reveals the extent of association between different characters, thus, it helps to base selection procedure to a required balance, when two opposite desirable characters affecting the principal characters are being selected. A positive correlation occurs due to coupling phase of linkage and negative correlation arises due to repulsion phase of linkage of genes controlling different traits. No correlation indicates that genes concerned are located far apart on the same chromosome or they are located on different chromosomes. Yield being a complex character is

governed by a large number of genes. The influence of each character on yield could be known through correlation studies with a view to determine the extent and nature of relationships prevailing among yield and yield attributing characters. So, the genotypic and phenotypic correlation co-efficient values for 11 characters in *Brassica rapa* genotypes studied are presented in Table 8 and Figure 3.

4.2.1 Days to 1st flowering

Days to 1st flowering is highly significant and positively correlated with days to 50% flowering (0.999, 0.927), plant height (0.847, 0.724), number of seeds per siliqua (0.584, 0.548) and thousand seed weight (0.665 and 0.625) at both genotypic and phenotypic levels (Table 8).

There is also positive correlation with days to maturity (0.159, 0.196), number of primary branches per plant (0.333 and 0.233), siliqua length (0.319, 0.206) and seed yield per plant (0.165 and 0.165). However, it showed significant and negative association with number of secondary branches per plant (-0.787, -0.586), number of siliqua per plant (-0.752 and -0.510), at both levels.

4.2.2 Days to 50% flowering

At genotypic and phenotypic levels days to 50% flowering showed highly significant and positive association with days to 1st flowering (0.999, 0.927), plant height (0.839, 0.722), number of seeds per siliqua (0.545, 0.522) and thousand seeds weight (0.684, 0.627). Highest significant positive correlation was found between days to 50% flowering and plant height (Afrin *et al.* (2011). Significant positive correlation between days to 50% flowering and thousand grain weight found by Maurya *et al.* (2012).

Negative and significant association of days to 50% flowering with number of secondary branches per plant (-0.840, -0.498), number of siliqua per plant (-0.793, -0.488). The association with other characters was non-significant.

Table 8. Genotypic and phenotypic correlation coefficients among different pairs of yield and yield contributing characters for different genotype of *Brassica rapa.*

Traits		DFF	D50%F	DM	PH	NPB	NSB	NSP	LS	NSS	TSW
D50%F	G	0.999**									
	P	0.927**									
DM	G	0.159	0.250								
	P	0.196	0.240								
PH	G	0.847**	0.839**	0.524**							
	P	0.724**	0.722**	0.445**							
NPB	G	0.333*	0.290	-0.742**	-0.060						
	P	0.233	0.203	-0.175	0.205						
NSB	G	-0.787**	-0.840**	0.366*	-0.238	-0.576**					
	P	-0.586**	-0.498**	0.289	-0.097	-0.052					
NSP	G	-0.752**	-0.793**	0.077	-0.317*	-0.335*	0.953**				
	P	-0.510**	-0.488**	0.120	-0.070	0.278	0.805**				
LS	G	0.319*	0.314*	1.017**	0.813**	-0.404**	0.393*	0.065			
	P	0.206	0.258	0.492**	0.429**	-0.199	0.219	0.020			
NSS	G	0.584**	0.545**	-0.435**	0.156	0.209	-0.948**	-0.970**	-0.170		
	P	0.548**	0.522**	-0.342*	0.137	0.178	-0.666**	-0.667**	-0.082		
TSW	G	0.665**	0.684**	0.187	0.633**	-0.031	-0.521**	-0.703**	0.418**	0.584**	
	P	0.625**	0.627**	0.198	0.549**	0.019	-0.433**	-0.525**	0.259	0.502**	
SYP	G	0.165	0.074	-0.601**	0.154	0.340*	0.240	-0.109	-0.068	0.468**	0.155
	P	0.165	0.148	-0.325*	0.199	0.531**	0.033	0.256	-0.022	0.419**	0.152

** = Significant at 1%. * = Significant at 5%.

DFF : days to First flowering
D50%F : days to 50% flowering
DM : days to maturity
PH : plant height (cm)
NPB : no. of primary branches per plant
NSB : no. of secondary branches per plan
NSP : no. of siliqua per plant
LS : length of siliqua (cm)
NSS : no. of seeds per siliqua
TSW : 1000 seed weight (g)
SYP : seed yield per plant (g)

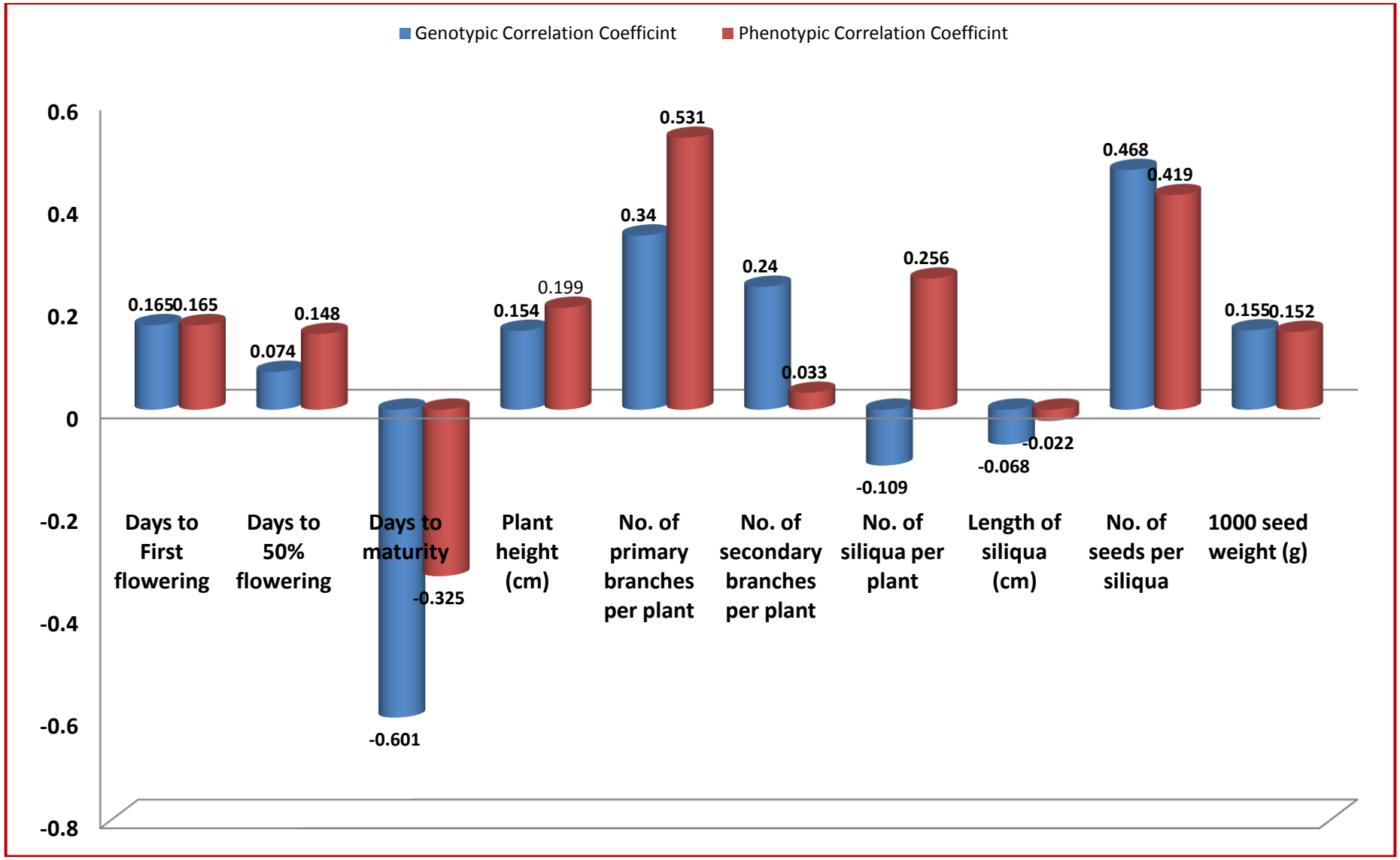

Figure 3: Genotypic and Phenotypic Correlation coefficient of ten characters with yield in *Brassica rapa L.*

It showed positive non significant correlation with days to maturity (0.250, 0.240), number of primary branches per plant (0.290, 0.203) seed yield per plant (0.074, 0.148) (Table 8). Parveen (2007) observed that days to 50% flowering was positively correlated with seed yield per plant.

4.2.3 Days to maturity

At genotypic and phonotypic level days to maturity displayed high significant positive association with plant height (0.524 and 0.445), length of siliqua (0.907 and 0.492) and negative significant association with number of seeds per siliqua (-0.435 and -0.342) and seed yield per plant (-0.601 and -0.325). At genotypic level significant negative correlation was recorded with number of primary branches per plant (-0.742). It showed positive and significant association with number of secondary branches per plant (0.366) at genotypic level (Table 8). It indicated if days to maturity increased then yield per plant decreased. It indicated if days to 80% maturity increased then yield/plant decreased. Zahan (2006) but Parveen (2007) reported insignificant and positive interaction with yield per plant for this trait.

4.2.4 Plant height (cm)

At genotypic and phenotypic level plant height had positive and high significant correlation with days to 1st flowering (0.847 and 0.724), days to 50% flowering (0.839 and 0.722), days to maturity (0.524 and 0.445), length of siliqua (0.813 and 0.429) and 1000 seed weight (0.633 and 0.549). It had negative association with number of secondary branches per plant (-0.238, -0.097) and number of siliqua per plant (-0.317 and -0.070) (Table 8). It was positive insignificant correlation with seed yield per plant and revealed that if plant height increased then the yield also increased. In the contrary, Basalma (2008) reported negative correlation with seed yield for this trait.

4.2.5 Number of primary branches per plant

At genotypic level number of primary branches per plant were positive and significant correlation with days to first flowering (0.333) and negatively significant association with days to maturity (-0.742), number of secondary branches (-0.576), number of siliqua per plant (-0.335) and length of siliqua 9-0.404). Number of primary branches per plant was significant positively correlated with seed yield per plant (0.340 and 0.531) which indicated if number of primary branches per plant increased then yield per plant also increased. As the value of phenotypic correlation co-efficient was greater than genotypic correlation co-efficient, it showed that the apparent association of two characters was not only due to genes but also due to favorable influence of environment. Malek *et al.* (2000) reported similar result for number of primary branches and seed yield both at genotypic and phenotypic level.

4.2.6 Number of secondary branches per plant

At genotypic level number of secondary branches per plant were positive and significant association with days to maturity (0.366) and length of siliqua (0.393) and significant negative correlation with number of primary branches per plant (-0.576). Number of secondary branches per plant was positively and highly significant association with number of siliqua per plant (0.953 and 0.805) which denoted if number of secondary branches per plant increased then number of siliqua per plant increased. However, it showed negative and highly significant correlation with days to first flowering (-0.787 and -0.586), days to 50% flowering (-0.840 and -0.498), number of seeds per siliqua (-0.948 and -0.666), 1000 seed weight (-0.521 and -0.433) at both level (Table 8). Number of secondary braches per plant was positively correlated with seed yield per plant (0.240 and 0.033) which denoted if number of secondary branches per plant increased then seed yield per plant increased. The similar findings by Naznin (2013) found positive significant relation with yield/plant.

4.2.7 Number of siliqua per plant

Number of siliqua per plant displayed high significant and positive correlation with number of secondary branches per plant (0.953 and 0.805) at both levels. But it showed negatively significant association with days to first flowering (-0.752 and -0.510), days to 50% flowering (-0.793 and -0.488), number of seeds per siliqua (-0.970 and -0.667), 1000 seed weight (-0.703 and -0.525) at both level but negative siginificant correlation with plant height (-0.317), number of primary branches per plant (-0.335) at genotypic level. It showed negative and non-significant association with plant height (-0.070) at phenotypic level. It represented positive association with seed yield per plant (0.109 and 0.256) at both genotypic and phenotypic level. It indicated that for the increased of number of siliqua per plant the seed yield per plant must be increased (Table 8). Almost similarly, Uddin *et al.* (2013) conducted an experiment and found that yield had high significant positive correlation with number of siliqua per plant at both phenotypic and genotypic level.

4.2.8 Siliqua length (cm)

Siliqua length also a yield components in *Brassica rapa*. Siliqua length showed significant and insignificant association in both genotypic and phenotypic levels. It was recorded that siliqua length had positive and high significant correlation with days to maturity (0.907, 0.492), plant height (0.813, 0.429) at both levels (Table 8). Siliqua length was positively significant association with days to first flowering (0.319), days to 50% flowering (0.314), number of secondary branches (0.393) and 1000 seed weight (0.418) at genotypic level. It was negatively correlated with number of primary branches (-0.404) at genotypic level. It showed insignificant negative association with yield per plant (-0.068 and -0.022) that denoted, the longer the siliqua length the less seed yield per plant and *vice versa* (Table 8). Results suggests that due to insignificant correlation this character might play least role on seed yield per plant. However, Saifullah (2010) reported positive significant correlation of the trait with yield.

4.2.9 Number of seeds per siliqua

Number of seeds per siliqua was found significant and positive association with days to first flowering (0.584, 0.548), days to 50% flowering (0.545, 0.522) at both level. This also had positive correlation with plant height (0.156, 0.137), number of primary branches (0.209, 0.178). However the trait showed highly significant positive correlation with 1000 seed weight (0.584, 0.502) and seed yield per plant (0.468, 0.419) at both levels. It was revealed that if number of seeds per siliqua increased then seed yields per plant increased. Due to significant value, the association between the traits showed strong association indicating that seed yield per plant could be increased by improving this trait. Significant and negative correlation with days to maturity (-0.435, -0.342), number of secondary branches (-0.948 and -0.666) and number of siliqua per plant (-0.970 and -0.667) at both genotypic and phenotypic level (Table 8). In the contrary, Naznin (2013) found negative significant correlation of number of seeds per siliqua with seed yield.

4.2.10 1000 seed weight (g)

1000 seed weight had positive and significant correlation with days to first flowering (0.665, 0.625), days to 50% flowering (0.684, 0.627), plant height (0.633, 0.549) and number of seeds per siliqua (0.584, 0.502) at genotypic and phenotypic levels. It showed negative and significant association with number of secondary branches per plant (-0.521, -0.433), number of siliqua per plant (-0.703, -0.525) at both levels. 1000 seed weight was positive association with seed yield (0.155 and 0.152) at both levels in the present study, that means if thousand seed weight increased then yield per plant also increased (Table 8). Alam (2010) found insignificant positive correlation of the trait with seed yield. Akter (2010) found positive significant correlation with yield per hectare at genotypic level but negative correlation at phenotypic level while Saifullah (2010) found positive significant correlation at both level.

4.2.11 Seed yield per plant

Seed yield per plant was significant and positively correlated with number of primary branches per plant (0.340, 0.531), number of seeds per plant (0.468, 0.419) at both genotypic and phenotypic levels. It showed positive association with days ti first flowering (0.165, 0.165), days to 50% flowering (0.074, 0.148), plant height (0.154, 0.199), number of secondary branches per plant (0.240, 0.033), number of siliqua per plant (0.109, 0.256) and 1000 seed weight (0.155, 0.152) at both genotypic and phenotypic levels (Table 8). These character association studies suggested that number of primary branches per plant, number of seeds per plant, seeds per siliqua and 1000 seed weight may be the most important yield attributes in *Brassica rapa*. Based on phenotypic and genotypic correlation between yield and yield attributing characters, it was suggested that selection should made for the characters, which are having positive significant association to improve the seed yield per plant in *Brassica rapa*. The inter-correlations estimated for the yield components indicated the probability of simultaneous improvement of these traits by selection. If the correlation existing between the characters was positive, simultaneous improvement of these traits by a single selection program was possible, but when negative association existing, it would be difficult to exercise simultaneous selection of these characters in developing a variety (Newell and Eberhart, 1961). Since the characters were inter correlated among themselves, selection in any one of these traits would result in the improvement of other character thereby, resulting in increasing in seed yield.

4.3 PATH COEFFICIENT ANALYSIS

Simple correlation does not consider the complex relationships between the various traits related to the dependent variable. Correlation coefficients show relationships among independent variables and the linear relationship between these variables. But it is not sufficient to describe these relationships when the causal relationship among variables is needed. A clear picture of the interrelationship between seed yield and others yield contributing characters, direct and indirect effects of them can be worked out by using path analysis. Genotypic path was worked out in the present study considering yield per plant as dependent character and its attributes as independent characters viz, days to First flowering, days to 50% flowering, days to maturity, plant height (cm), no. of primary branches per plant, no. of secondary branches per plant, no. of siliqua per plant, length of siliqua (cm), no. of seeds per siliqua and 1000 seed weight (g). Each component has two path actions viz, direct effect on yield and indirect effect through components which are not revealed by correlation studies. Estimation of direct and indirect effect of path co-efficient analysis for *Brassica rapa* is presented in Table 9. Residual effects of their independent variables, which have influenced on yield been denoted as 'R'.

4.3.1 Days to 1st flowering

Days to 1st flowering recorded highest positive direct effect (6.314) towards yield per plant (Table 9). This relationship indicated that an early flowering genotype usually has higher seed yield than a late flowering genotype. Selection based on this character would be effective.

Further, it showed positive indirect effect towards yield per plant via, number of siliqua per plant (0.696) and 1000 seeds weight (0.11). However, it was negative indirect effect towards yield per plant via, days to 50% flowering (-4.506), days to maturity (-0.065), plant height (-0.623), number of primary branches per plant (-0.066), number of secondary branches per plant (-1.509),

Table 9. Partitioning of genotypic correlations into direct and indirect effects of 11 important characters by path analysis of *Brassica rapa*

	Direct effect	Indirect effect via										r_g with yield
		DFF	D50%F	DM	PH	NPB	NSB	NSP	LS	NSS	**TSW**	
DFF	6.314		-4.506	-0.065	-0.623	-0.066	-1.509	0.696	-0.158	-0.026	0.11	0.165
D50%F	-4.512	6.311		-0.103	-0.618	-0.057	-1.611	0.734	-0.155	-0.024	0.11	0.074
DM	-0.416	1.004	-1.128		-0.386	0.146	0.702	-0.071	-0.503	0.019	0.03	-0.601**
PH	-0.735	5.350	-3.785	-0.215		0.012	-0.456	0.293	-0.402	-0.007	0.10	0.154
NPB	-0.198	2.104	-1.308	0.305	0.044		-1.105	0.310	0.200	-0.009	-0.01	0.340*
NSB	1.916	-4.971	3.789	-0.150	0.175	0.113		-0.882	-0.195	0.042	-0.08	-0.240
NSP	-0.926	-4.750	3.577	-0.032	0.233	0.066	1.828		-0.032	0.043	-0.11	-0.109
LS	-0.494	2.015	-1.416	-0.418	-0.598	0.080	0.754	-0.060		0.007	0.07	-0.068
NSS	-0.047	3.689	-2.458	0.179	-0.115	-0.041	-1.818	0.897	0.084		0.09	0.468**
TSW	0.18	4.20	-3.09	-0.08	-0.47	0.01	-1.00	0.65	-0.21	-0.03		0.155

Residual effect: **0.302** **: Significant at 1%. * : Significant at 5%.

DFF : days to First flowering
D50%F : days to 50% flowering
DM : days to maturity
PH : plant height (cm)
NPB : no. of primary branches per plant
NSB : no. of secondary branches per plan
NSP : no. of siliqua per plant
LS : length of siliqua (cm)
NSS : no. of seeds per siliqua
TSW : 1000 seed weight (g)
SYP : seed yield per plant (g)
r_g : genotypic correlation

length of siliqua (-0.158) and number of seeds per siliqua (-0.026). Day to 1st flowering was positive and non- significantly correlated with yield per plant.

4.3.2 Days to 50% flowering

Days to 50% flowering showed high negative direct effect (-4.512) towards yield per plant. Further, it showed high positive indirect effect towards yield per plant via, days to 1st flowering (6.311) and moderate positive indirect effect through number of siliqua per plant (0.734) and 1000 seed weight (0.11). However, it was recorded negligible negative indirect effect to yield per plant via, days to maturity (-0.103), plant height (-0.618), number of primary branches (-0.057), number of secondary branches (-1.611), length of siliqua (-0.155), number of seeds per siliqua (-0.024). It showed positive and non-significant genotypic correlation (0.074) with yield per plant. Zahan (2006) reported that days to 50% flowering had negative direct effect on yield that was support the present study.

4.3.3 Days to maturity

Days to maturity found negative direct effect (-0.416) towards yield per plant. Further, it recorded positive indirect effect towards yield per plant via, days to first flowering (1.004), number of primary branches (0.146), number of secondary branches (0.702), seeds per siliqua (0.019) and 1000 seed weight (.03) (Table 9). However, it was recorded negative indirect effect towards yield per plant via, days to 50% flowering (-1.128) and plant height (-0.386). It showed negative and significant genotypic correlation (-0.601) with yield per plant. Negative direct effects on seed yield was exhibited for days to maturity (-0.015) observed by Ali *et al*. (2003).

4.3.4 Plant height

Plant height recorded negative direct effect (-0.735) towards yield per plant. Whereas, in the present study the correlation was positive and non-significant (0.154) with yield per plant (Table 9). Further, it was recorded positive indirect effect towards yield per plant via, days to first flowering (5.350), number of primary branches (0.012) number of siliqua per plant (0.293) and 1000 seed weight (0.10). On the other hand, it was found negative indirect effect towards yield per plant via, days to 50% flowering (-3.785), days to maturity (-0.215), number of secondary branches (-0.456), length of siliqua (-0.402), number of seeds per siliqua (-0.354). Plant height showed negative direct effect on seed yield but the indirect effects was high through days to first flowering. In the contrary, Han (1990) working with *Brassica napus*, observed positive direct effect of plant height (0.321) on seed yield.

4.3.5 Number of primary branches

Number of primary branches negative direct effect (-0.198) towards yield per plant. In the contrary, positive direct effect of plant height (0.32) on yield was observed by Han (1990). Further, it was recorded positive indirect effect towards yield per plant via, days to first flowering (2.104), days to maturity (0.305), plant height (0.044), number of siliqua per plant (0.310), siliqua length (0.200) (Table 9). However, it was found negative indirect effect towards yield per plant via, days to 50% flowering (-1.308), number of secondary branches (-1.105), number of seeds per siliqua (-0.009) and 1000 seed weight (-0.01). The correlation of number of primary branches was significant (0.340^{*}) with yield per plant.

4.3.6 Number of secondary branches

Number of secondary branches recorded positive direct effect (1.916) towards yield per plant. Further, it was recorded high positive indirect effect towards yield per plant via, days to 50% flowering (3.789), plant height (0.175), number of primary branches (0.113), number of seeds per plant (0.042) (Table

7). However, it was found negative indirect effect towards yield per plant via, days to first flowering (-4.971), days to maturity (-0.150), number of siliqua per plant (-0.882), length of siliqua (-0.195) and 1000 seed weight (-0.08). The correlation of number of secondary branches was negatively non-significant (-0.240) with yield per plant. Therefore yield per plant can be improved through direct selection of no. of secondary branches per plant to reduce undesired indirect effect. Rashid (2007) observed that number of secondary branches per plant had the highest direct effect on seed yield per plant.

4.3.7 Number of siliqua per plant

Number of siliqua per plant recorded negative direct effect (-0.926) towards yield per plant. Further, it was recorded positive indirect effect towards yield per plant via, plant height (0.233), number of primary branches (0.066), number of secondary branches (1.828), number of seeds per siliqua (0.043) and significant, high, positive indirect effect via days to 50% flowering (3.577) (Table 9). However, it was found negative indirect effect towards yield per plant via, days to first flowering (-4.750), days to maturity (-0.032), length of siliqua (-0.032) and 1000 seed weight (-0.11). The genotypic correlation of number of siliqua per plant was negatively non-significant (-0.109) with yield per plant. Therefore yield per plant can be improved through direct selection of no. of secondary branches per plant to reduce undesired indirect effect. In the contrary, Marjanovic-Jeromela *et al.* (2008) worked on *Brassica napus* and found positive direct effect (0.26) for this trait on yield.

4.3.8 Siliqua length

Siliqua length observed negative direct effect (-0.494) towards yield per plant. It was also recorded negative indirect effects to yield per plant via, days to 50% flowering (-1.416), days to maturity (-0.418), plant height (-0.598), number of siliqua per plant (-0.060) (Table 7). On the other hand, it was found negligible positive indirect effect toward yield per plant via, number of primary branches (0.080),, number of seeds per siliqua (0.007) and 1000 seed weight (0.07).and

high positive indirect effect of days to first flowering (2.015) and number of secondary branches (0.754). The genotypic correlation of siliqua length (-0.068) with yield per plant was negative and non-significant. Selection based on this trait would not be wise. In the contrary, Ejaz-Ul-Hasan *et al.* (2014) observed that silique length had direct positive effect (0.241) on yield.

4.3.9 Number of seeds per siliqua

Number of seed per siliqua showed negligible negative direct effect (-0.047) towards yield per plant. Further, it was recorded negligible positive indirect effect towards yield per plant via, days to maturity (0.179), length of siliqua (0.084), 1000 seed weight (0.09) and high positive indirect effect of days to first flowering (3.689) and number of siliqua per plant (0.897) (Table 9). It also found negative indirect effect towards yield per plant via, days to 50% maturity (-2.458), plant height (-0.115), number of primary branches per plant (-0.041), number of secondary branches per plant (-1.818). Tusar *et al.* (2006) concluded that the number of seeds per siliquae had negative direct effect on yield.

4.3.10 1000 Seed Weight

1000 seed weight found positive direct effect (0.18) towards yield per plant. It was found the lowest positive direct effect of 1000 seed weight on yield per plant. Further, it was recorded high positive indirect effect towards yield per plant via, days to first flowering (4.20) and number of siliqua per plant (0.65) (Table 7). It also reported high negative indirect effect towards yield per plant via, days to 50% flowering (-3.09) and number of secondary branches per plant (-1.00). The trait was genotypically positive and non- significantly (0.155) correlated with yield per plant. Again the value of the direct effect of it, is very close to correlation coefficient. This result suggested that selection could be done based upon this trait for yield improvement per plant. Kakroo and Kumar (1991) found that thousand seed weight had positive direct effect (0.784) on yield.

Path analysis revealed that the direct positive effect of different traits were the main contributor for higher yield per plant. Indirect effect of different traits should be given priority along which exerted direct effects.

4.3.11 Residual effect

The magnitude of residual effect (0.302) indicated that traits included in the path analysis explained about 70% of the variation in seed yield. However, the remaining variation in seed yield (30%) can be attained by incorporating other yield related traits in the path analysis as far as studies involving association of traits is concerned.

4.4 GENETIC DIVERSITY ANALYSIS

4.4.1 Mahalanobis' generalized distance (D^2)

Conservation of genetic diversity is an essential prerequisite for developing new cultivars with desirable agronomic traits. Although a large number of germplasm collections have been established worldwide, many of them face major difficulties due to large size and a lack of adequate information about population structure and genetic diversity. The development of new varieties is mainly governed by the magnitude of genetic variability in the base material and extent of variability for the desired characters. Genetically diverse parents are likely to produce high heterotic effects and desirable segregants. D^2 analysis is a useful tool in identifying the best parents and their combinations for generating variability with respect to various traits under study. The results of D^2 analysis may be useful tool in identifying the best parental combination for generating variability with respect to various traits under study. Progenies derived from diverse crosses are expected to show a broad spectrum of genetic variability providing greater scope for isolating high yielding segregants in the succeeding generations. The genetic diversity among 14 genotypes was measured by employing D^2 statistics. The contribution of each character towards total diversity is present in Table 10.

4.4.2 Principal component analysis (PCA)

Principal component analysis was carried out with 14 genotypes of *Brassica rapa*. The first three Eigen values for three principal coordination axes of genotypes accounted for 84.32% variation (Table 11). Out of 11 characters studied, Component I contributed maximum towards the total diversity with the value 61.72%, followed by Component II 13.14%, Component III 9.46%, and Component IV 6.36%.

4.4.3 Clustering of the genotypes

The correlated mean values (X) for all genotypes for 11 characters under consideration were transferred to the uncorrelated standardized value (Y). The D^2 value which being the sum of squares for each (Y) value was calculated for all combinations. Based on D^2 values the genotypes were grouped into five clusters using Tocher's methods given by Rao (1952). Clustering of genotypes is presented in the Table 10. Among five clusters, cluster I was the largest comprising of six genotypes followed by cluster III and Cluster IV with three genotypes, and Cluster I and cluster V are the solitary cluster.

Cluster I was composed of G3 (Sonali sarisha). Cluster II comprised of six varieties from BARI (BARI sarisha-3, BARI sarisha-6, BARI sarisha-9, BARI sarisha-12), BINA (BINA-10) and Improved Tori. Cluster III possesses three varieties from BARI (BARI sarisha-14, BARI sarisha-15 and BARI sarisha-17). Cluster IV consists of three like Tori-7, Sonali Tori and Maghi . Cluster V consist of genotype Torsha. Zaman *et al.* (2010) reported four cluster of 45 genotypes. The 45 genotypes were grouped in eight clusters using Tocher's method found by Pandey *et al.* (2013). Goswami and Behl (2006) reported with 43 genotypes and found six cluster by D^2 statistics. The cluster III was the biggest with 11 genotypes followed by cluster I with 9 genotypes reported by Aunwinithul *et al.* (2004).

Table 10. Distribution of fourteen genotypes of *Brassica rapa* in different clusters

Cluster no.	Given name	Name of genotypes	No. of populations
I	G3	Sonali sarisha	1
II	G2, G4, G5, G6, G10, G14	BARI sarisha-3 BARI sarisha-6 BARI sarisha-9 BARI sarisha-12 Improved Tori BINA-10	6
III	G7, G8, G9	BARI sarisha-14 BARI sarisha-15 BARI sarisha-17	3
IV	G1, G11, G13	Tori-7 Sonali Tori Maghi	3
V	G12	Torsha	1
Total			14

Table 11. Eigen values and yield percent contribution of 11 characters of 14 genotypes

Principle component axis	Eigen values	Percent variation	Cumulative % of Percent variation
I	8.0233	61.72	61.72
II	1.7077	13.14	74.86
III	1.2299	9.46	84.32
IV	0.8265	6.36	90.68
V	0.4293	3.30	93.98
VI	0.3204	2.46	96.44
VII	0.2205	1.70	98.14
VIII	0.1114	0.86	99
IX	0.0828	0.62	99.66
X	0.0288	0.23	99.91
XI	0.0106	0.09	100.00

The present investigation shows that there is no perfect relationship between genetic diversity and geographical diversity. This maybe attributed since the genotypes of the present study were indigenous and released varieties from BARI. The genotypes have overlapped in different clusters with some distinctness.

4.4.4 Principal Coordinate Analysis (PCA)

The results obtained from principal coordinate analysis showed that the highest inter genotypic distance was observed between Sonali sarisha and Improved Tori (2.149) followed by Sonali sarisha and BARI sarisha-9 (2.071), Sonali sarisha and BINA-10 (2.017) and BARI sarisha-3 and Sonali sarisha (2.01) (Table 12). The difference between the highest and the lowest inter genotypic distance indicated the moderate variability among the 14 genotypes of *Brassica rapa*.

4.4.5 Cluster mean analysis

Cluster means were computed for all the 11 characters studied and presented in Table 13. Genotypes grouped within cluster IV were relatively early to days to first flowering (22.44 days) whereas; genotype grouped under cluster V were relatively late (38.00 days). Similarly, genotypes grouped within cluster IV were relatively early to 50% flower (27.22 days) whereas; genotype grouped under cluster V were relatively late to 50% flowering (43.00 days). Cluster I exhibited the highest mean for plant height with 104.13 cm, whereas the cluster IV had the lowest average of plant height 77.86 cm followed by cluster III (90.24 days). Highest number of primary branches per plant was found in cluster V (6.57) and the lowest number of primary branches per plant was observed in cluster I (3.98). In case of secondary branches per plant genotypes of cluster V (1.00) was less and genotypes under cluster II (8.11) was relatively high. Maximum number of siliqua per plant was found in cluster II (165.76) whereas the minimum number of siliqua was found in cluster I (50.17) followed by cluster III (77.60). Cluster I comprised of genotypes with long siliqua (5.65) while cluster III consisted of genotype with shorter siliqua (4.96).

Table 12. Ten highest and ten lowest inter genotypic distance among the 14 genotypes of *Brassica rapa*

Highest distance				**Lowest diatance**			
Sl No.	**Genotype**		**Distance**	**Sl No.**	**Genotype**		**Distance**
01	Sonali sarisha	Improved Tori	2.149	01	BARI sarisha-9	BARI sarisha-12	0.247
02	Sonali sarisha	BARI sarisha- 9	2.071	02	BARI sarisha-3	BARI sarisha-12	0.254
03	Sonali sarisha	BINA-10	2.017	03	BARI sarisha-3	BARI sarisha-6	0.255
04	BARI sarisha-3	Sonali sarisha	2.01	04	BARI sarisha-6	BARI sarisha-12	0.325
05	Sonali sarisha	Sonali Tori	1.982	05	BARI sarisha-9	BINA-10	0.331
06	BARI sarisha-9	BARI sarisha-14	1.944	06	Maghi	BINA-10	0.351
07	BARI sarisha-14	Improved Tori	1.923	07	Tori-7	Sonali Tori	0.353
08	Sonali sarisha	BARI sarisha-12	1.921	08	BARI sarisha-12	BINA-10	0.357
09	Sonali sarisha	Maghi	1.906	09	BARI sarisha-12	Improved Tori	0.366
10	BARI sarisha-14	Sonali Tori	1.904	10	BARI sarisha-14	BARI sarisha-17	0.375

Table 13. Cluster mean values of 11 different characters of 14 genotypes

Characters	**I**	**II**	**III**	**IV**	**V**
Days to First flowering	36.67	27.83	32.22	22.44	38.00
Days to 50% flowering	42.00	31.61	36.33	27.22	43.00
Days to maturity	92.00	86.22	80.56	84.33	86.00
Plant height (cm)	104.13	94.75	90.24	77.86	102.77
No. of primary branches per plant	3.98	5.20	5.58	4.78	6.57
No. of secondary branches per plant	1.23	8.11	1.18	6.91	1.00
No. of siliqua per plant	50.17	165.76	77.60	144.29	114.07
Length of siliqua (cm)	5.65	5.40	4.96	4.99	5.14
No. of seeds per siliqua	24.00	15.22	30.17	14.56	22.80
1000 seed weight (g)	3.83	2.94	3.27	2.57	2.83
Seed yield per plant (g)	4.18	6.95	7.73	5.39	7.50

Cluster I with three genotypes exhibited the highest mean for number of seeds per siliqua (30.17), whereas the cluster II had the lowest average (15.22). Cluster I with three genotypes exhibited the highest mean for 1000 seed weight (3.83g), whereas the cluster IV had the lowest average (2.57 g). Genotypes grouped under cluster III recorded highest mean value for seed yield per plant (7.73 g) and the lowest by the genotypes of cluster I (4.18 g).

4.4.6 Canonical Variate Analysis

4.4.6.1 Inter and intra cluster distances

Genotypes grouped into the same cluster presumably diverge little from one another. Theoretically, crossing of genotypes belonging to the same cluster is not expected to yield superior hybrids or segregants. However theoretically, a general notion exists that the larger is the divergence between the genotypes, higher will be the heterosis (Falconer, 1981).

From Table 14 it is observed that cluster IV showed the maximum inter-cluster distance with the cluster V (20.414), followed by cluster I and cluster IV (16.181); and minimum inter cluster distance between cluster II and cluster IV (9.166). Figure 4 and Figure 5 is shown to present different clusters. All clusters showed intra cluster distances except cluster I and cluster V whose constitute one genotype. Intra cluster distance was highest in the cluster II (2.41) followed by the cluster IV (1.01). Intra cluster distance was maximum for cluster VI followed by cluster III found by Pandey *et al.* (2013). Therefore, it would be desirable to attempt crosses between genotypes belonging to distant clusters for getting highly heterotic crosses.

However, the crosses involving parents from clusters with high inter cluster distance are likely to yield desirable recombinants in the advanced generations which could be developed as traditional homozygous varieties. The lowest intra cluster was recorded in cluster IV (1.01). The highest inter cluster distance (20.414) was observed between cluster IV and cluster V. This high inter-cluster

Table 14. Intra (Bold) and inter cluster distances (D^2) for 14 genotypes

Cluster	I	II	III	IV	V
I	**0.00**	15.404	9.374	16.181	15.628
II		**2.43**	13.640	9.166	15.642
III			**1.43**	13.802	11.761
IV				**1.67**	20.414
V					**0.00**

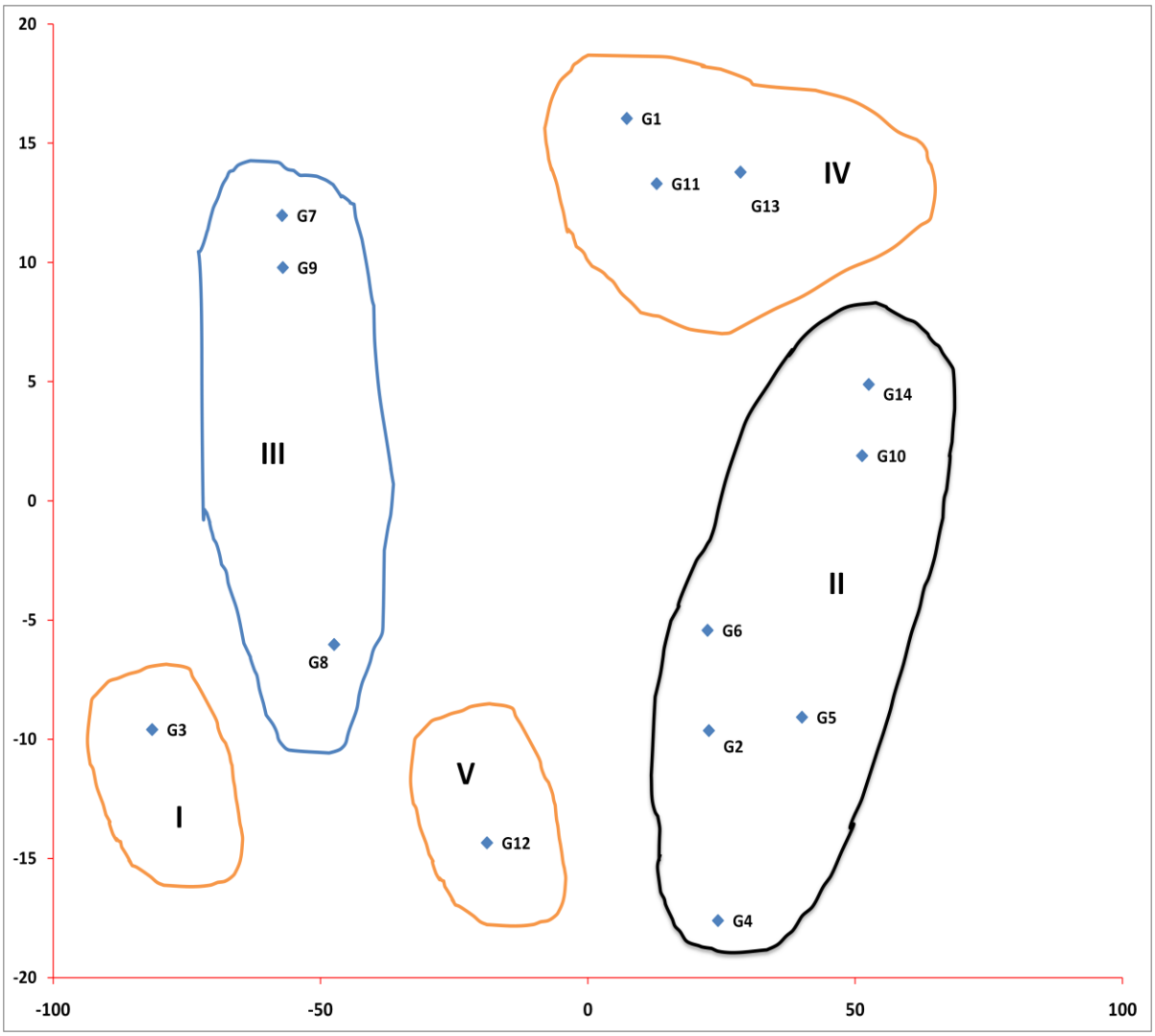

Figure 4: Cluster diagram of *Brassica rapa* genotypes of based on their principal component scores

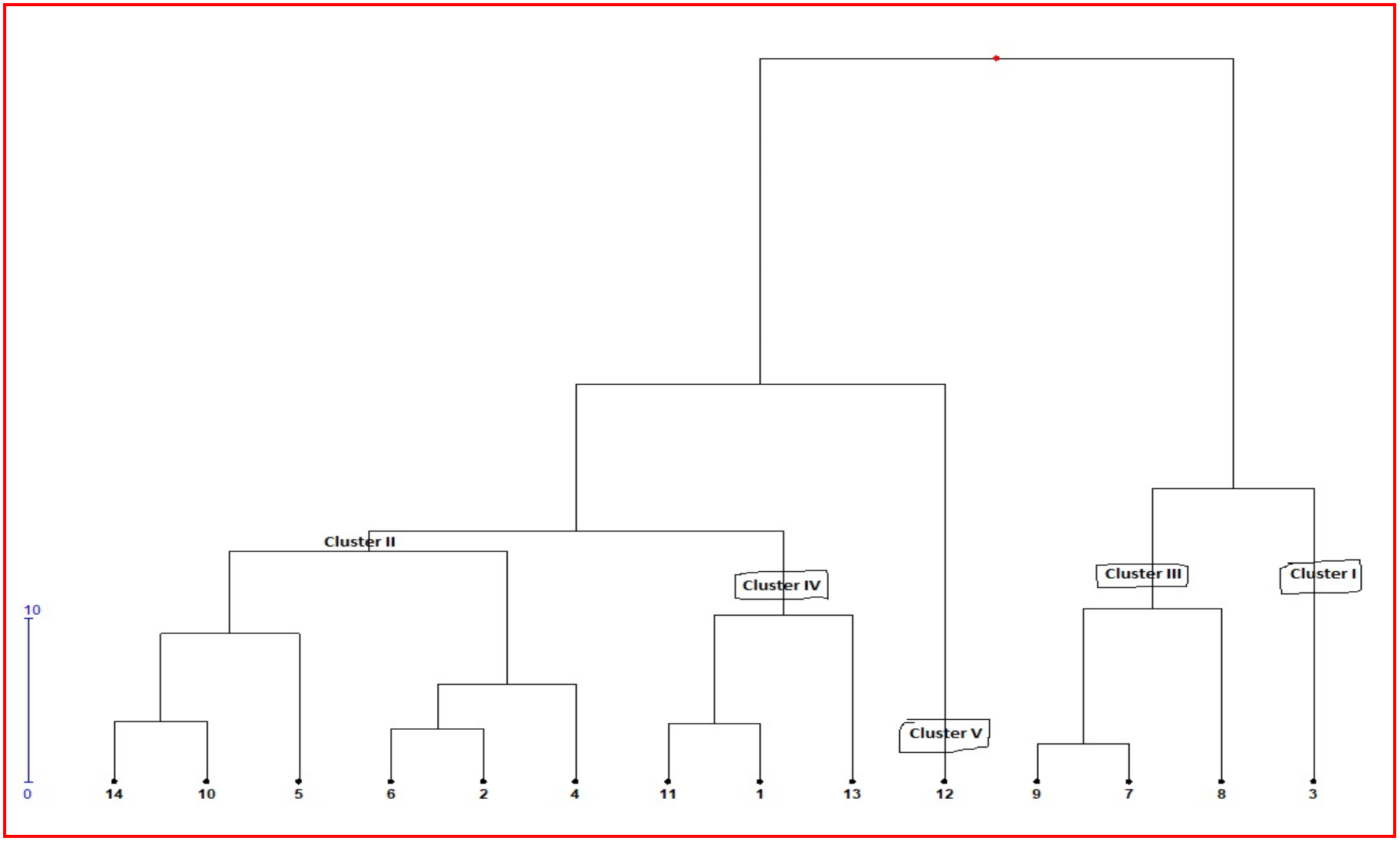

Figure 5: Clustering tree showing different cluster of 14 genotypes

distance indicated the wider genetic diversity among the genotypes, which could be used in yield improvement of *Brassica rapa*. The lowest inter cluster distance (9.166) was seen between cluster II and cluster IV. The genotypes from distant clusters exhibit wide diversity. So, genotypes from divergent clusters (IV and V) can be selected for hybridization program in order to achieve novel recombinants.

4.4.6.2 Nearest and farthest cluster

Cluster I consist of nearest cluster with D^2 values cluster III (9.374) & farthest cluster with D^2 values cluster IV (16.181) (Table 15). Cluster II consist of nearest cluster with D^2 values cluster IV (9.166) and farthest cluster with D^2 values V (15.642). Cluster III consist of nearest cluster with D^2 values cluster I (9.374) and farthest cluster with D^2 values IV (13.802). Cluster IV consist of nearest cluster with D^2 values cluster II (9.166) & farthest cluster with D^2 values V (20.414) (Figure 5). Cluster V consist of nearest cluster with D^2 values cluster III (11.761) & farthest cluster with D^2 values IV (20.414).

4.4.6.3 Contribution of characters towards divergence of the genotypes

The values of Vector I and Vector II are presented in Table 16. Vector I obtained from PCA expressed that Days to 50% flowering (0.1158), No. of secondary branches per plant (0.5753), length of siliqua (3.8963) and no. of siliqua per plant (0.0294) were major characters that contribute to the genetic divergence. It was the reflection of first axis of differentiation. In vector II length of siliqua (2.4547), seed yield per plant (1.5743), 1000 seed weight (0.4918) and number of secondary branches per plant (0.0535) showed their important role toward genetic divergence. The value of Vector I and Vector II revealed that both Vectors had positive values for length of siliqua, no. of secondary branches per plant and 1000 seed weight indicating the highest contribution of these traits towards the divergence among 14 genotypes of *Brasica rapa*. Negative values in both vectors for days to first flowering, days to maturity and no. seeds per siliqua had lower contribution towards the

Table 15. Nearest and farthest cluster distances

Cluster	Nearest cluster distance	Farthest cluster distance
I	III (9.374)	IV (16.181)
II	IV (9.166)	V (15.642)
III	I (9.374)	IV (13.802)
IV	II (9.166)	V (20.414)
V	III (11.761)	IV (20.414)

Table 16. Relative contributions of the eleven characters of 14 genotypes to the total divergence

Characters	Principal Component	
	Vector-1	**Vector-2**
Days to First flowering	-0.5943	-0.4339
Days to 50% flowering	0.1158	-0.1513
Days to maturity	-0.4784	-0.6466
Plant height (cm)	0.0563	-0.0975
No. of primary branches per plant	-0.7671	-1.5532
No. of secondary branches per plant	0.5753	0.0535
No. of siliqua per plant	0.0294	-0.2387
Length of siliqua (cm)	3.8963	2.4547
No. of seeds per siliqua	-0.2358	-1.0806
1000 seed weight (g)	0.0259	0.4918
Seed yield per plant (g)	-0.5764	1.5743

divergence. In the present study, eighteen genotypes of *Brassica rapa* were grouped into five clusters. The magnitude of D^2 values confirmed that there was considerable amount of diversity in the experimental material evaluated. Length of siliqua, no. of secondary branches per plant and 1000 seed weight were the maximum contributors for divergence in the present study should be given utmost importance for selecting the genotypes for crossing program.

Cluster I is highest values of days to maturity, plant height, length of siliqua and 1000 seed weight. Cluster II was performed for high value of secondary branches per plant and no. of siligua per plant. Cluster III represented high value for no. of seeds pere siliqua and seed yield per plant. Cluster V has shown high value of days to first flowering, days to 50% flowering, primary branches per plant.

4.4.6.4 Cluster diagram

The positions of the genotypes in the scatter diagram were apparently distributed into five groups, which indicated that considerable diversity existed among the genotypes (Figure 4).

4.4.6.5 Selection of genotypes as parent for hybridization program

Among the inter cluster distance, distance IV and V (20.414) followed by I and IV (16.181) were the highest and other cluster were more or less intermediate distance. Intermediate diverse parents have the more chance to contribute heterosis in the subsequent generations. To select cluster to obtain more heterotic genotype five pairs of clusters to be considered for this purpose, they are IV and V (20.414), I and IV (16.181), III and V (11.761), II and III (13.640). Cluster II had the highest cluster mean for number of secondary branches plant (8.11), number of siliqua per plant (165.76) were the most important yield contributing character. On the other hand the cluster III comprised the highest cluster mean for no. of seeds per siliqua (30.17), seed yield per plant (7.73) and minimum days to maturity (80.56). Cluster IV had

the early days to flowering. Selection of genotype from cluster III BARI sarisha-14 and BARI sarisha-17, from cluster IV maghi, from cluster V Torsha, from cluster II improved tori and BINA-10 and from cluster I sonali sarisha. Hybridization between the genotypes of cluster V and cluster II may manifest maximum heterosis and creates wide genetic variability.

4.5 FATTY ACIDS CONTENT ANALYSIS

Vegetable oils in human diet constitute an important source of energy and have considerable importance in human health. Besides being the gastronomic delights and a source of energy, edible vegetable oils provide fat-soluble micronutrients and essential and nonessential fatty acids, which perform various functions. The Brassicas are served to be one of the most agronomical important oilseeds with a diverse range of species may be used as a variety of oilseed, vegetable, and fodder crops. *Brassica* oil has fatty acids composition in higher genetic variations compared to those contained in other major vegetable oils. The seven major fatty acids were extracted from members of the genus *Brassica* are found to be palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosanoic (C22:0), and erucic (C22:1) acids. Palmitic acid and Stearic acid are saturated fatty acids and oleic, linoleic, linolenic, eicosaoic and erucic are the unsaturated fatty acids. High erucic acid contained oils are useful for industrial applications, but not for human consumption. Therefore, to develop varieties having both low erucic acid as well as those with high erucic acid is a promising objective for breeding programs in *Brassica* oilseed crops. Other important objectives are the increase of oleic acid, linoleic acid and linolenic acid content. Oleic acid is found to be one of the main unsaturated fatty acids playing fundamental role in human nutrition. In the present study, *Brassica napus* showed higher oleic acid content but the most remarkable variation for oleic acid was found in *Brassica rapa*. Other species showed no clear differences among accessions. High oleic acid contained oils are greatly resistant to heating and oxidation and suitable for wide variety of uses. Erucic acid is one of the most important fatty acids in

mostly found within *Brassica* genus. This 22-carbon fatty acid is harmful to the human health. Generally, zero-erucic acid genotypes are belonged to *Brassica napus* and *Brassica rapa* and have been also developed in *Brassica juncea*. The nutritional properties of *Brassica* seed oil, like other fats and oils, are dependent on its fatty acids composition, particularly the amount of oleic, linoleic, linolenic, and erucic acids in turn has great deal of importance in terms of in human nutrition. high oleic acid oil has cholesterol lowering properties; while saturated (palmitic and stearic) fatty acids tend to raise blood cholesterol levels considerably (Sharafi, 2015).

On the basis of morphological features six varieties were selected for fatty acid analysis. The results from ANOVA for fatty acids compositions (Table 17) revealed significant differences among six *Brassica rapa* varieties ($p < 0.01$).

4.5.1 Oil Content

High oil content trait is the most remarkable characteristics of oilseed of *Brassica* crop. In the present study, six varieties of *Brassica rapa* were analyzed. The seed oil content of these varieties ranged between 36.52 to 42.42% (Table 18). The maximum oil content was observed in BARI sarisha-14 (42.42%) and minimum in Maghi (36.52%). These results were in close agreement to the previous studies (Mukherjee and Kiewitt, 1984; Velasco *et al.* 1998; Kumar and Tsunoda, 1980).

4.5.2 Fatty Acid Composition Analysis

In the present study six mustard varieties oils were analyzed by Gas Chromatography (GC) to study their fatty acid profiles. The fatty acid composition of seed oils of six varieties is indicated in Table 19. These calculated fatty acids are categorized into two groups; saturated and unsaturated fatty acids. Myristic, Palmitic, Stearic, Arachidic, Behenic Lignoceric are saturated fatty acids and Palmitoleic, Oleic, Elaidic, Linoleic,

Table 17. Analysis of variance (ANOVA) for fatty acids content

Source of variation	df	SS	Mean square
Treatment	5	41.67	8.33**
Error	6	0.71	0.12
Total	11	42.38	3.85

**Significant at 1%level

Table 18. Oil content percent of *Brassica rapa* genotypes

Code	Variety	Oil content percent
V3	Sonali sarisha (SS 75)	39.85
V4	BARI sarisha-6	38.82
V7	BARI sarisha-14	42.42
V8	BARI sarisha-15	41.3
V10	Improved Tori	39.65
V13	Maghi	36.52
Min		36.52
Max		42.42

Table 19. Percentage allotments of the most important fatty acids in oil of six *Brassica rapa* genotypes detection by gas liquid chromatography

Variety	Fatty acids													
	Saturated fatty acids						Unsaturated fatty acids							
	Myristic	Palmitic	Arachidic	Behenic	Lignoceric	Stearic	Palmitoleic	Oleic	Elaidic	Linoleic	Linolelaidic	Linolenic	Ecosadienoic	Erucic
Sonali sarisha	0.076	2.575	1.668	0.545	0.418	1.413	0.562	19.525	2.055	13.239	1.883	6.058	6.826	43.157
BARI sarisha-6	0.117	5.190	1.526	0.453	0.254	1.194	0.658	18.839	1.983	14.066	1.788	6.898	5.954	41.081
BARI sarisha-14	0.066	2.934	1.415	0.438	0.556	1.329	0.517	17.225	1.602	13.957	1.419	6.794	7.728	44.022
BARI sarisha-15	0.057	4.686	1.498	0.407	0.404	2.023	0.838	20.335	1.799	17.098	2.084	6.366	5.420	36.984
Improved Tori	0.039	3.991	1.338	0.406	0.378	1.139	0.618	20.909	2.165	13.502	1.538	8.163	9.083	36.732
Maghi	0.141	5.181	1.390	0.338	0.349	1.411	0.890	21.058	2.211	14.319	1.882	6.970	8.334	35.525

Linolelaidic, Linolenic, , Ecosadienoic, , Erucic acid are unsaturated fatty acids.

Chromatogram of fourteen fatty acids content of six different varities viz. Sonali sarisha, BARI sarisha-6, BARI sarisha-14, BARI sarisha-15, Improved Tori and Maghi were shown in Figure 6 to Figure 11.

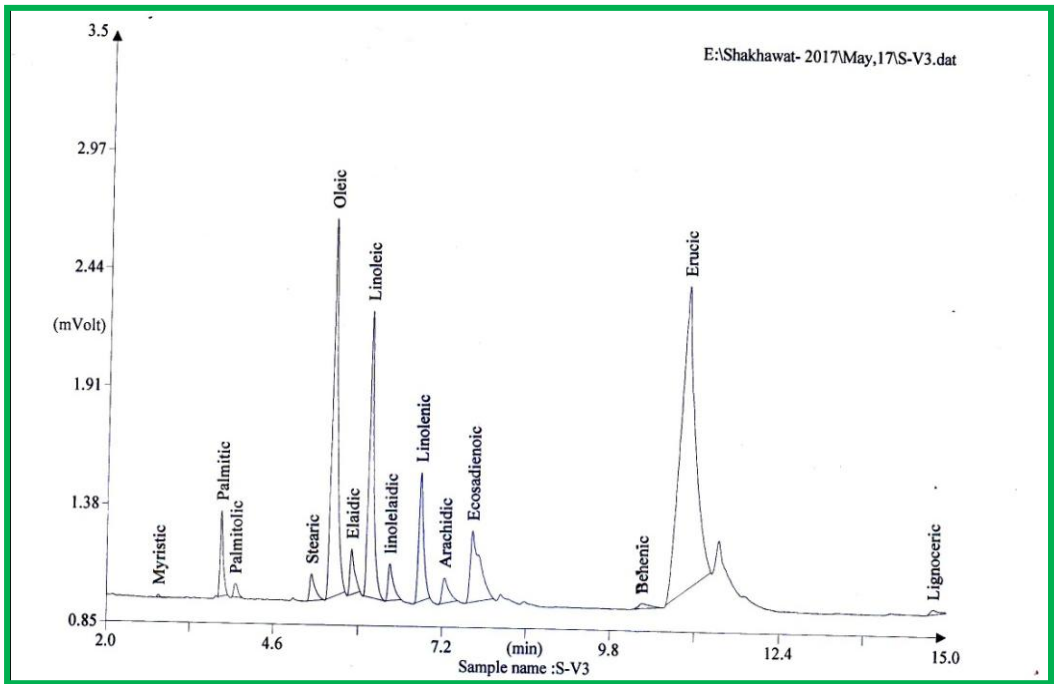

Figure 6: Chromatogram of different fatty acids of Sonali sarisha oil

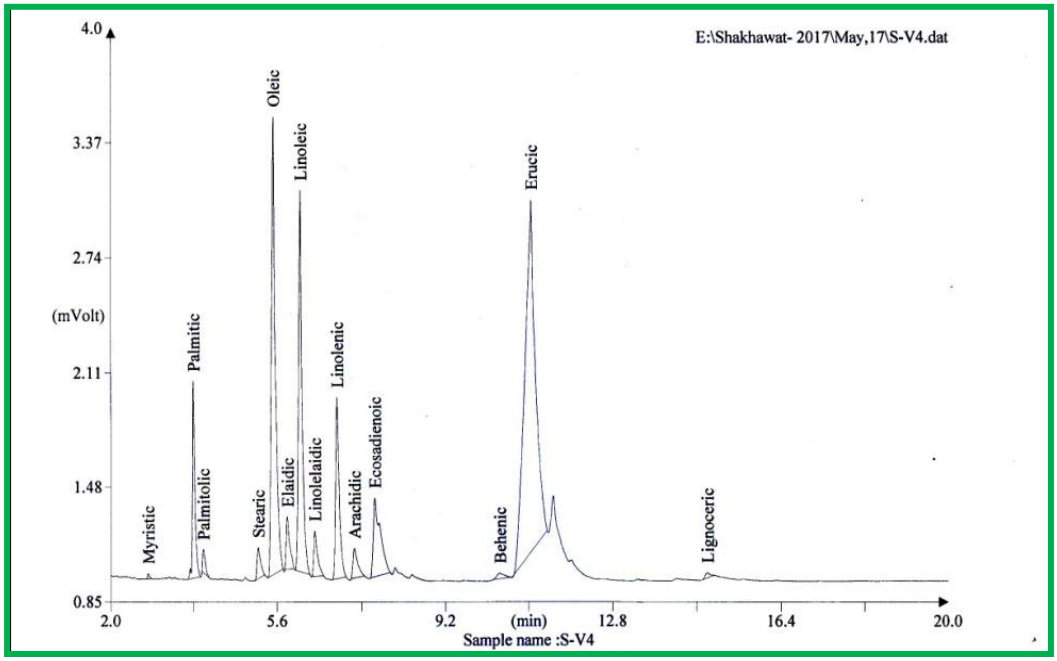

Figure 7: Chromatogram of different fatty acids of BARI sarisha-6 oil

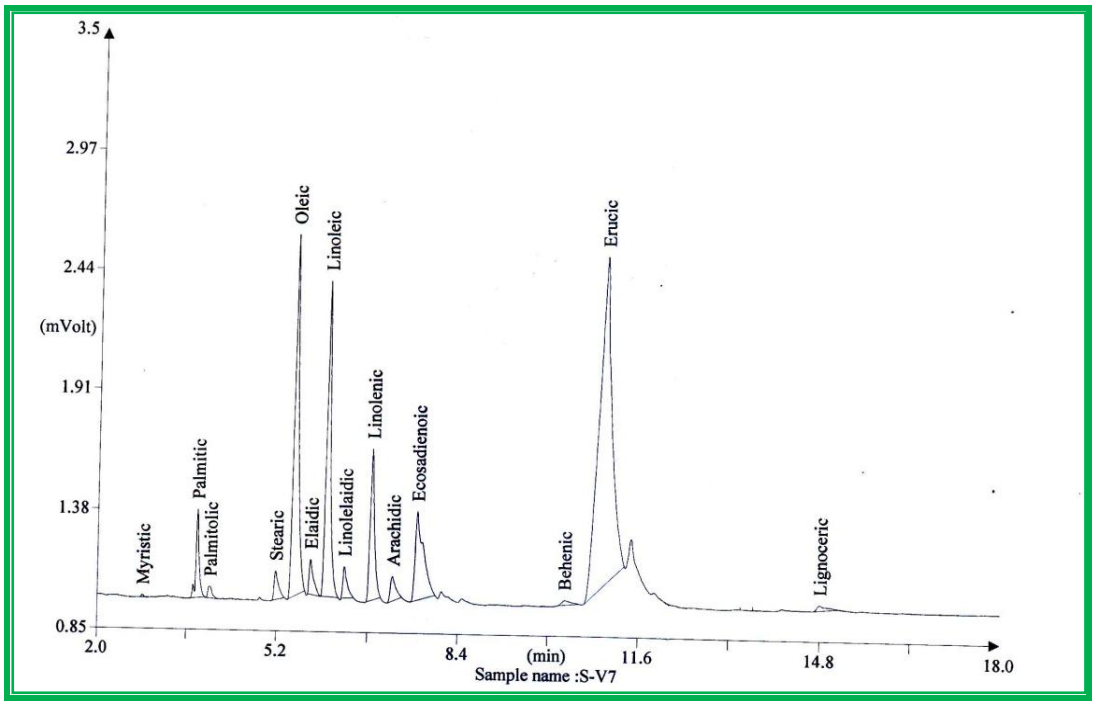

Figure 8: Chromatogram of different fatty acids of BARI sarisha-14 oil

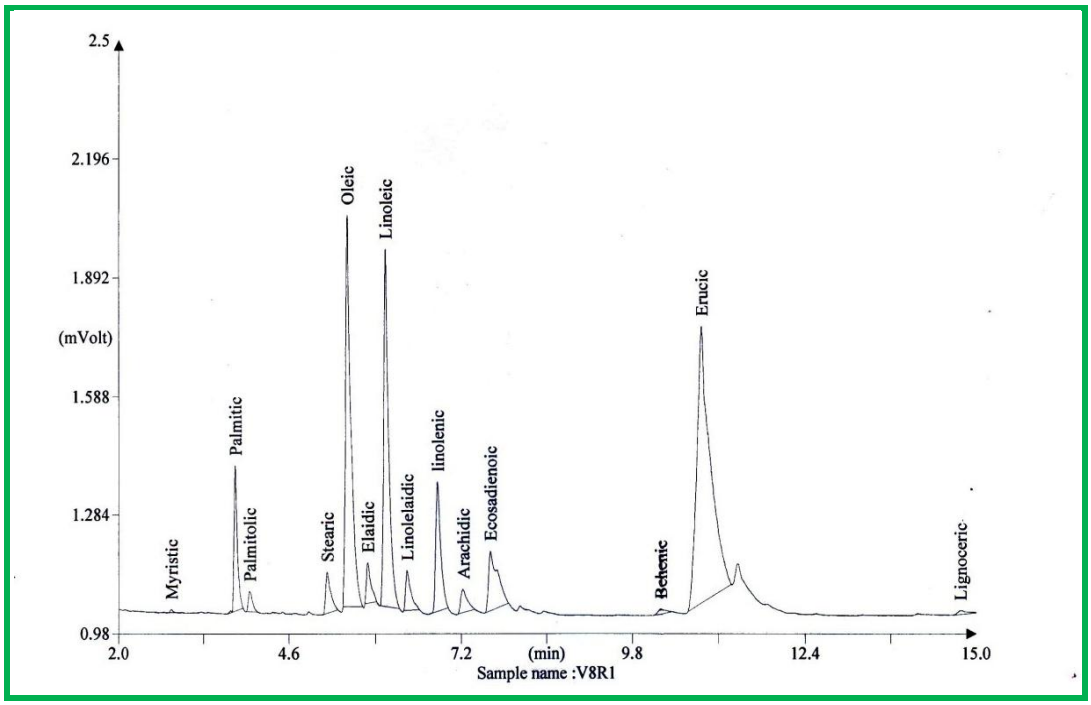

Figure 9: Chromatogram of different fatty acids of BARI sarisha-15 oil

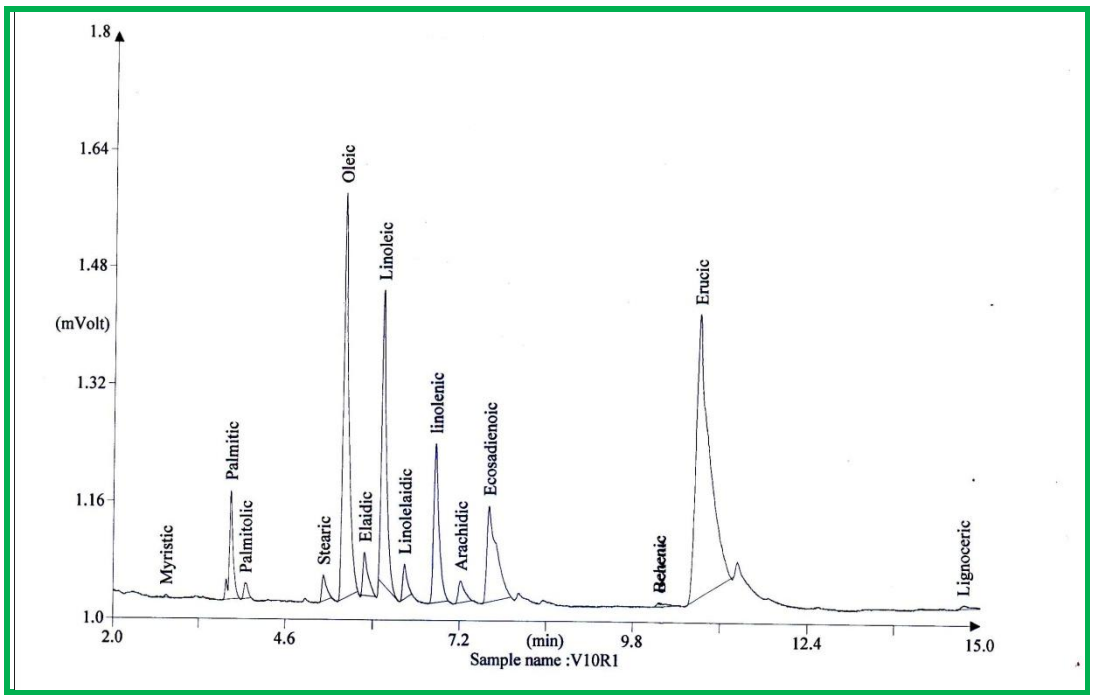

Figure 10: Chromatogram of different fatty acids of Improved Tori oil

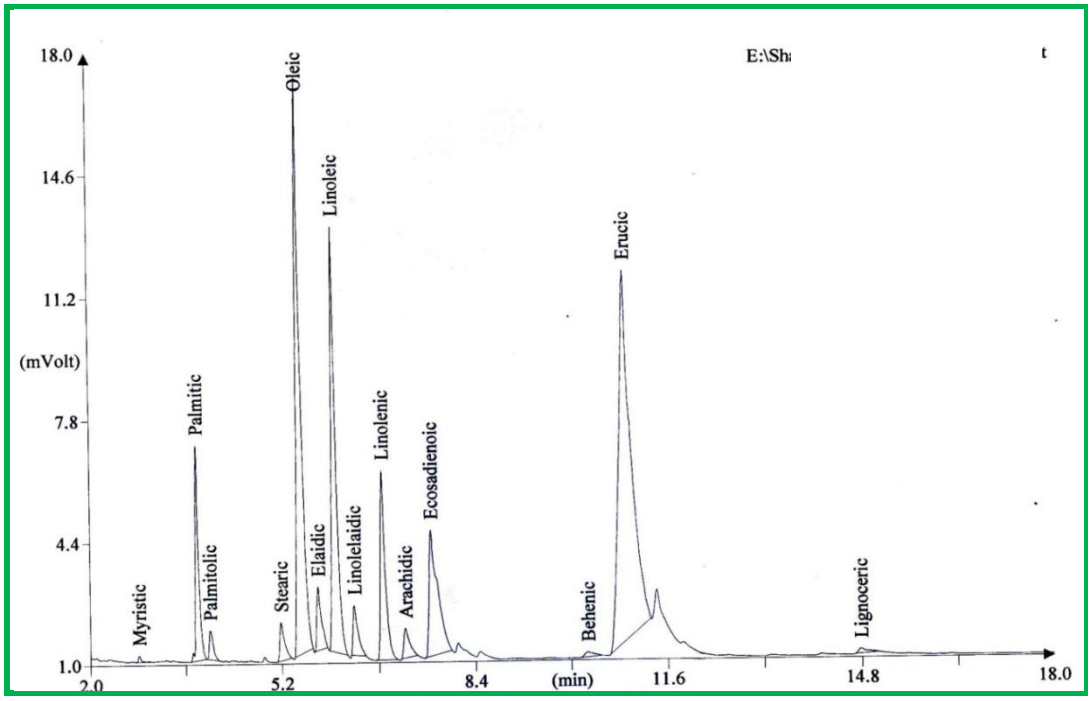

Figure 11: Chromatogram of different fatty acids of Maghi oil

4.5.2.1 Saturated fatty acids

Saturated fatty acids compositions in percentage of selected six popular mustard varieties is presented in Table 20. Here the analyzed saturated fatty acids were Palmitic acid, Stearic acid, Arachidic acid and Behenic. The contents of total saturated fatty acids (SFAs) ranged between 6.12 (BARI sarisha-14) to 8.62% (BARI sarisha-15). SFA percent less than 7% has been considered the maximum threshold acceptable for human consumption (Wilson, 2004). It is particularly important from the perspective of human health as higher proportion of SFAs in oil causes hypercholesterolemia. However, species with high SFAs content are also important and they may find utility in soap and oleo-chemical industries. The highest Palmitic acid was observed in BARI Sarisha 6 (5.19%) followed by Maghi (5.18%) and BARI sarisha-15 (4.69%) and lowest value in Sonali sarisha (2.58%) (Figure 12). In case of Stearic acid highest value was observed in the *Brassica rapa* variety BARI sarisha-15 (2.02%) and lowest in improved tori (1.14%) (Figure 13). Highest Arachidic acid was found in Sonali sarisha (SS 75) (1.67%) and lowest in Improved Tori (1.34%). Highest Behenic acid was represented by Sonali sarisha (SS 75) (0.55%) and lowest value in Maghi (0.34%).

4.5.2.3 Unsaturated fatty acid

4.5.2.3.1 Mono unsaturated fatty acids (MUFAs)

The total MUFAs, represented by the sum total of the contents of oleic (C18:1), eicosenoic (C20:1) and erucic (C22:1) acids in the seed oils, ranged from 62.739% in BARI sarisha-15 to 69.508% in Sonali sarisha (Table 21). The highest content of oleic acid was obtained in Maghi (21.06%) (Figure 14) while that of eicosenoic and erucic acids was observed in Improved Tori (9.08%) and BARI sarisha-14 (44.02%), respectively (Table 21). The minimum percentage of erucic acid was found in the seed oil of Maghi (35.52%) (Figure 15). Seed oils with a high content of oleic acid are of interest for nutritional and industrial

Table 20. Percentage of the saturated fatty acids in oil of six *Brassica rapa* genotypes

Variety	Saturated fatty acids				
	Palmitic (C16:0)	Stearic (C18:0)	Arachidic (C20:0)	Behenic (C22:0)	Total
Sonali sarisha (SS 75)	2.575	1.413	1.668	0.545	6.201
BARI sarisha 6	5.190	1.194	1.526	0.453	8.363
BARI sarisha-14	2.934	1.329	1.415	0.438	6.116
BARI sarisha-15	4.686	2.023	1.498	0.407	8.614
Improved Tori	3.991	1.139	1.338	0.406	6.874
Maghi	5.181	1.411	1.390	0.338	8.32
Min	2.575	1.139	1.338	0.338	6.116
Max	5.19	2.023	1.668	0.545	8.614
Mean	4.093	1.418	1.473	0.431	7.415

Table 21. Percentage allotments of the unsaturated fatty acids in oil of six *Brassica rapa* genotypes

	Mono unsaturated				Poly unsaturated			Oleic/ linoleic ratio	ω-6/ ω-3 ratio
	Oleic (C18:1)	Ecosenoic (C20:1)	Erucic (C22:1)	Total	Linoleic **(**C18:2)	Linolenic **(**C18:3)	Total		
Sonali sarisha (SS 75)	19.525	6.826	43.157	69.508	13.239	6.058	19.297	1.475	2.185
BARI sarisha 6	18.839	5.954	41.081	65.874	14.066	6.898	20.964	1.339	2.039
BARI sarisha-14	17.225	7.728	44.022	68.975	13.957	6.794	20.751	1.234	2.054
BARI sarisha-15	20.335	5.420	36.984	62.739	17.098	6.366	23.464	1.189	2.686
Improved Tori	20.909	9.083	36.732	66.724	13.502	8.163	21.665	1.549	1.654
Maghi	21.058	8.334	35.525	64.917	14.319	6.970	21.289	1.471	2.054
Min	17.225	5.42	35.525	62.739	13.239	6.058	19.297	1.189	1.654
Max	21.058	9.083	44.022	69.508	17.098	8.163	23.464	1.549	2.686
Mean	19.6485	7.224167	39.5835	66.45617	14.3635	6.874833	21.23833	1.376	2.112

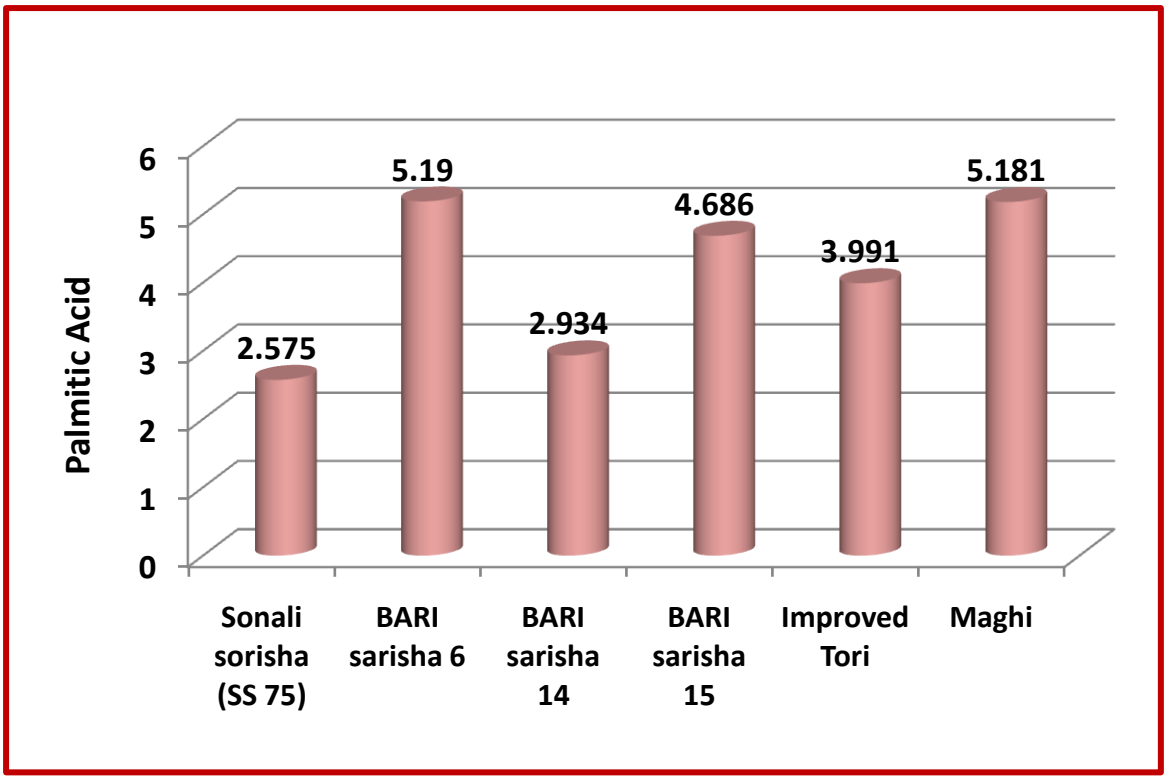

Figure 12: Palmitic acid content (%) in oil of six mustard varieties

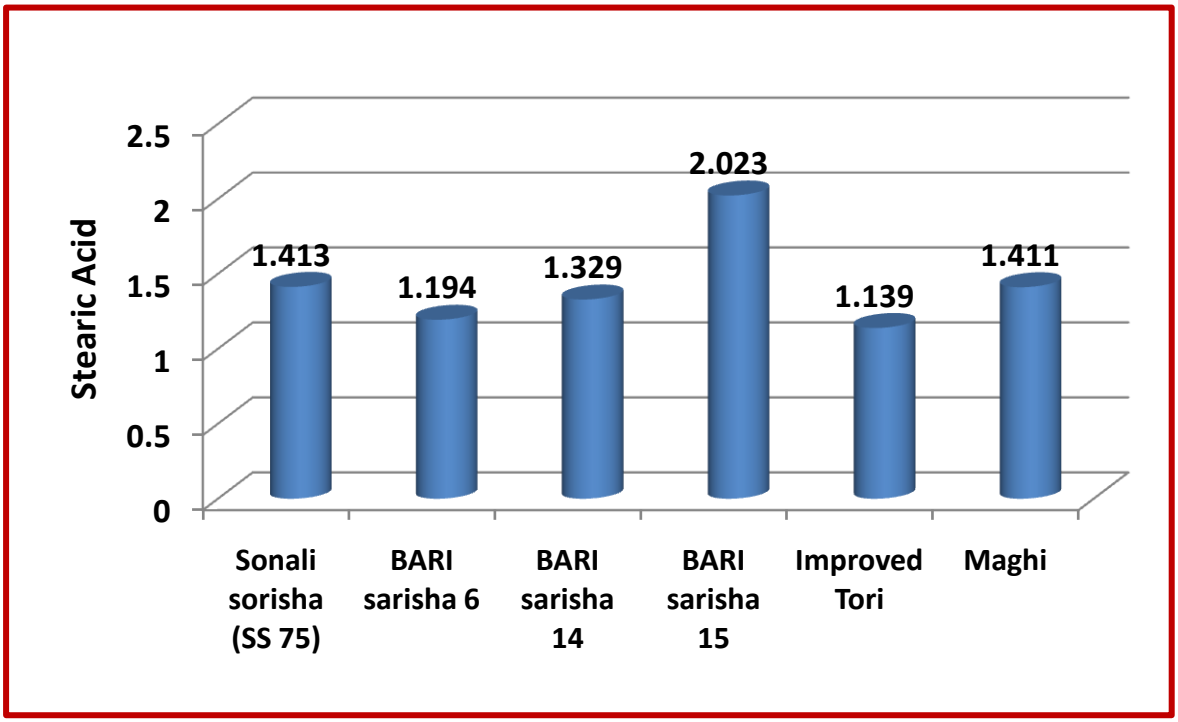

Figure 13: Stearic Acid content (%) in oil of six mustard varieties

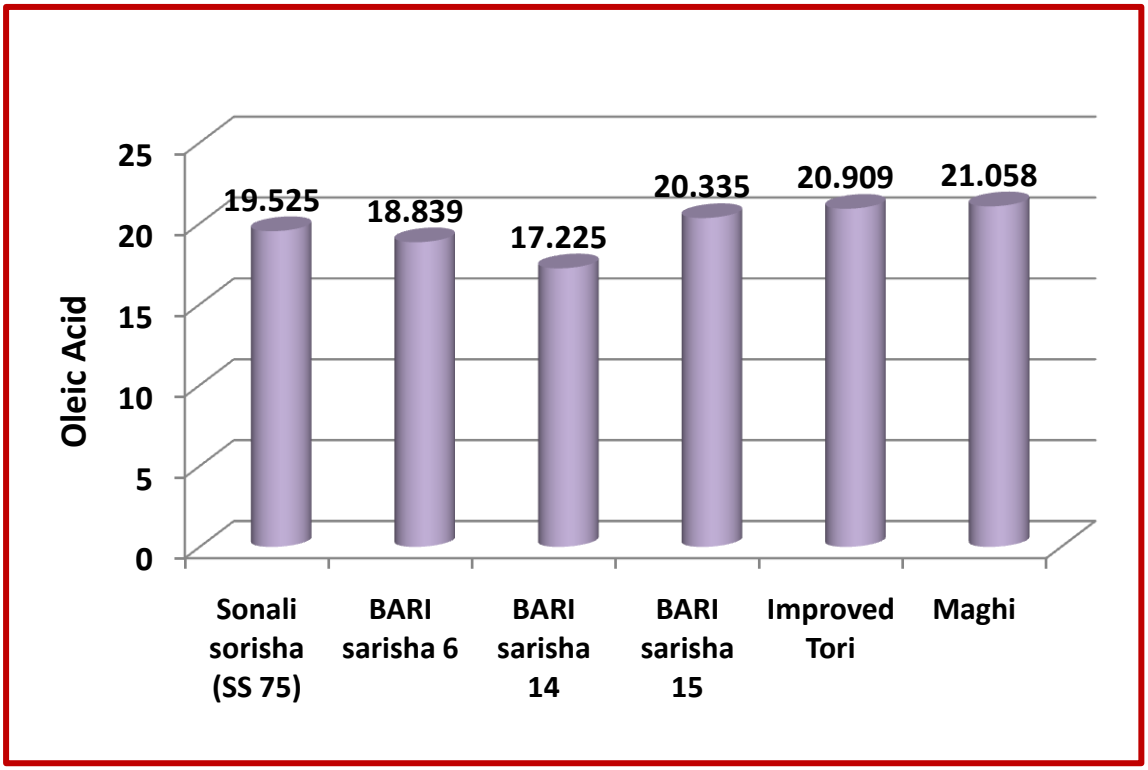

Figure 14: Oleic Acid content (%) in oil of six mustard varieties

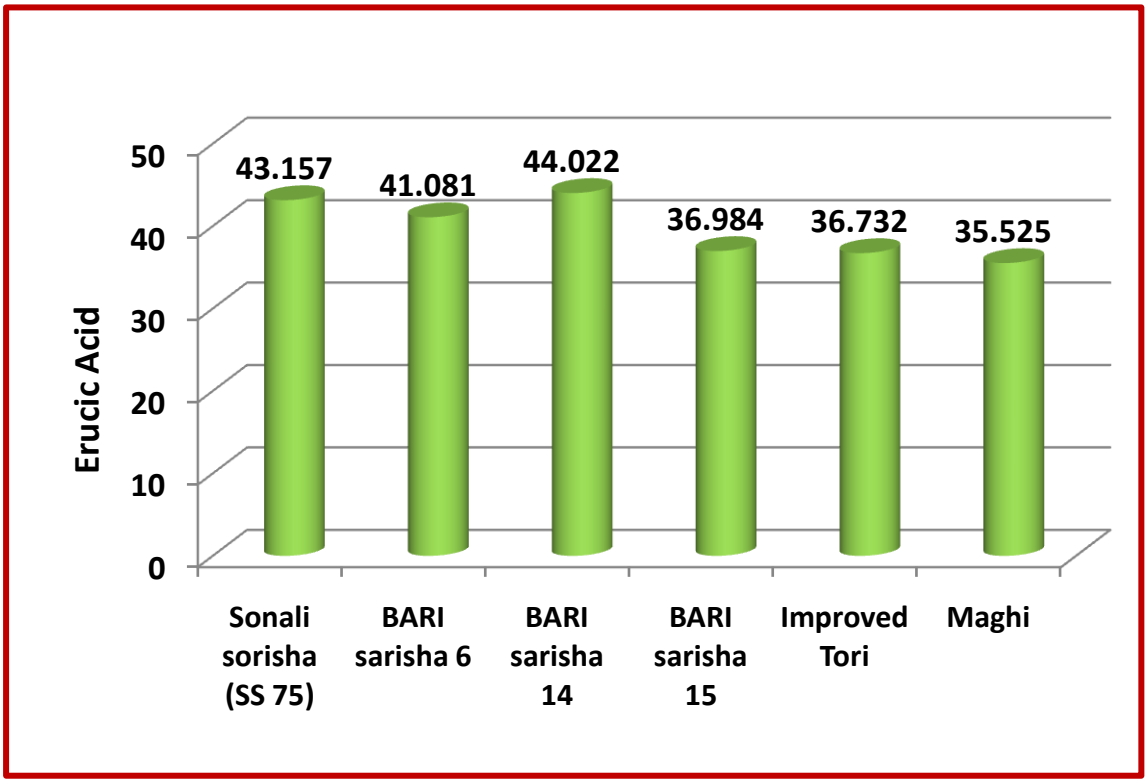

Figure 15: Erucic Acid content (%) in oil of six mustard varieties

purposes (Liu *et al.* 2002). High oleic acid content in the seed oil increases its thermo stability, making it more suitable as cooking oil (Appelqvist, 1968). Further, oils with high oleic acid are considered to be good for human consumption owing to the property of oleic acid to increase the level of high-density lipoproteins (HDLs) and reduce the level of low-density lipoproteins (LDLs) in blood (Chang and Huang, 1998). The presence of high erucic acid in oil is considered anti-nutritional, as it has been reported to cause lipidosis in children and myocardial fibrosis in monkeys (Ackman *et al.* 1977). Oils containing high erucic acid content are not desired for human consumption and they increase blood cholesterol. Nevertheless, erucic acid derivatives can be used as chemical additives in plastic, tannery and cosmetic industries (Bozzini *et al.* 2007).

4.5.2.3.2 Poly unsaturated fatty acids (PUFAs)

The contents of linoleic (C18:2) and linolenic (C18:3) acids, the major polyunsaturated fatty acids (PUFAs) found in seed oils, ranged between 13.24-17.10% and 6.06–8.16%, respectively (Table 21). The highest content of linoleic acid was found in the seed oil of BARI sarisha-15 (17.10%) while minimum was observed in Sonali sarisha (13.24%) (Table 21 and Figure 16). The maximum content of linolenic acid was recorded in the seed oil of Improved Tori (8.16%) and minimum in Sonali sarisha (6.06%) (Figure 17). A rich content of linoleic and linolenic acids in the seed oils of BARI sarisha-15 and Improved Tori and its superiority over other vegetable oils in terms of proportions of PUFAs has already been reported (Peiretti and Meineri, 2007). Linoleic and α-linolenic acids are essential fatty acids and are the precursors of bioactive long-chain (>20-carbon) fatty acids which are reported to have health beneficial effects and involved in many important metabolic functions of human body like synthesis of prostaglandins. Moreover, oils containing about 50.0% total PUFA (this study 23.46% in BARI sarisha-15) may find several industrial applications, especially in the manufacture of oil based paints.

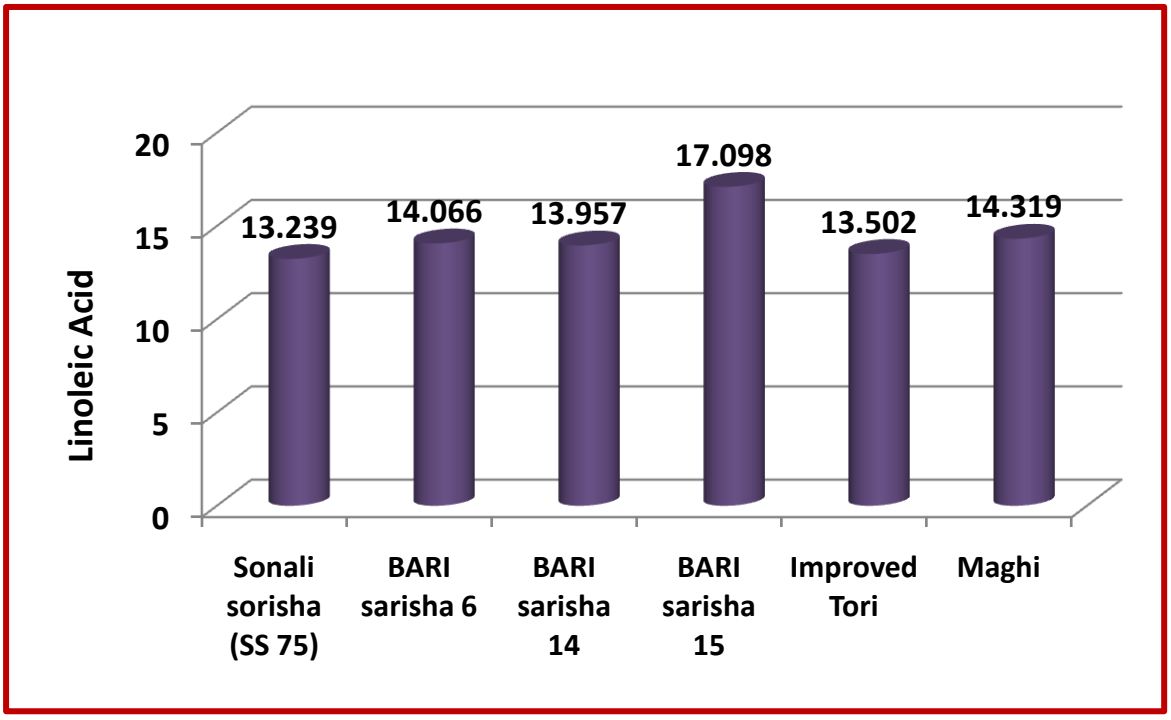

Figure 16: Linoleic Acid content (%) in oil of six mustard varieties

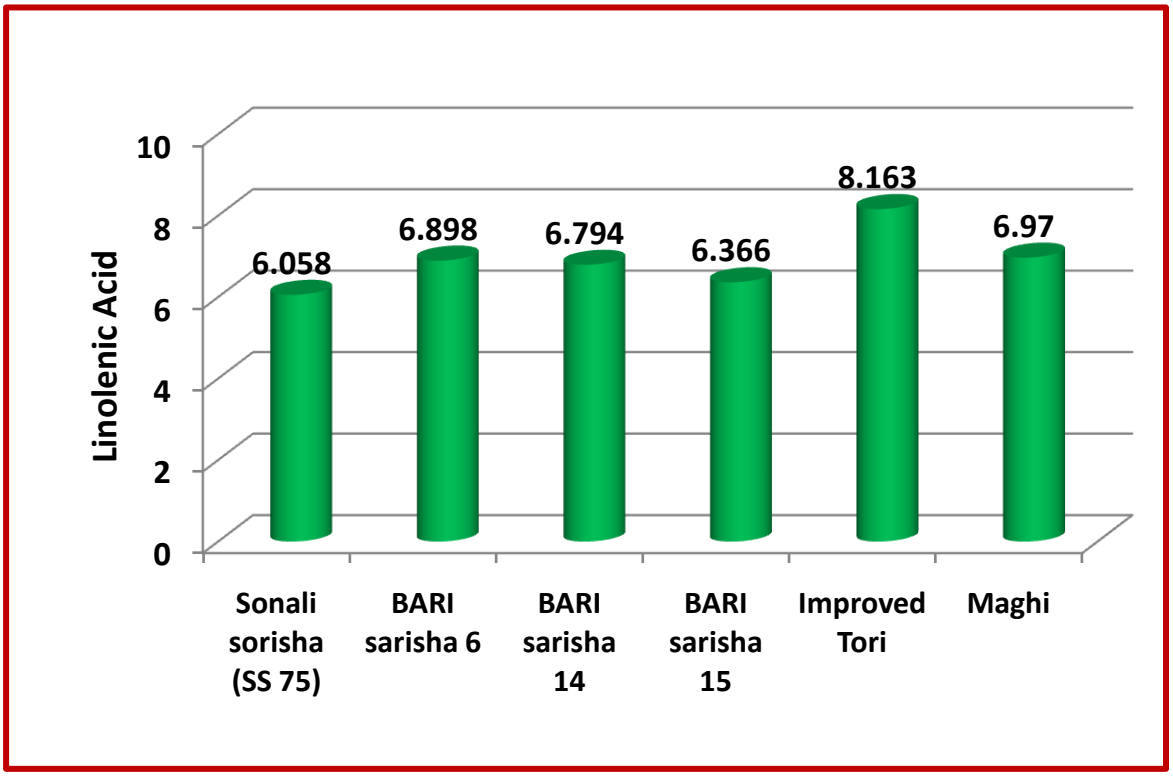

Figure 17: Linolenic Acid content (%) in oil of six mustard varieties

The ratio of oleic to linoleic fatty acids (stability index) in the seed oils ranged from 1.189 in BARI sarisha-15 to 1.549 in Improved Tori, while for the linoleic (ω-6) and linolenic (ω-3) acids it varied from 1.654 in Improved Tori to 2.686 in BARI sarisha-15 (Table 21). These ratios are critical and important for human diet (Zhang *et al.*, 2004). In general, it is recommended that, we should strive for a diet in which oleic/linoleic and ω-6/ ω-3 approaches 2:1 and 4:1, respectively (Yehuda and Carasso, 1993). In the present study seed oil of none of the varieties were found to meet the recommended specification with regards to oleic/linoleic. The varieties exhibited ω-6/ ω-3 and oleic/linoleic ratio less than the recommended value. Since majority of the vegetable seed oils presently in use, fall short of these recommendations, they are marketed in blended forms. Now-a-days consideration is given on the total balance in SFA : MUFA : PUFA approximately 1:1.3:1 ratio for a healthy balanced diet.

CHAPTER V
SUMMARY AND CONCLUSION

The present investigation was envisaged on 14 *Brassica rapa* varieties on the title 'Genetic diversity and fatty acid composition analysis of mustard (*Brassica rapa* L.)'. The objectives of this study were selecting the best genotypes for further research. The genetic variability, heritability, correlation, path analysis and genetic diversity were estimated for fourteen varieties and fatty acid composition was analyzed on six varieties. The wide genetic variability that exists in the available genotypes provides enormous scope for further improvement. Yield is a complex quantitative character, direct selection for yield may not result in successful improvement. Therefore, it is necessary to allocate the observed variability into heritable and non-heritable components by calculating genetic parameters such as genotypic and phenotypic coefficient of variation, heritability and genetic gain. The material for the present study comprised of 14 genotypes collected from BARI, BINA and local regions, were evaluated using RCBD design with three replications for 11 quantitative characters on 2016 at Sher-e-Bangla Agricultural University, Dhaka.

The analysis of variance showed significant differences among the genotypes for all the traits viz. days to first flowering, days to 50% flowering, days to maturity, plant height (cm), no. of primary branches per plant, no. of secondary branches per plant, no. of siliqua per plant, length of siliqua (cm), no. of seeds per siliqua, 1000 seed weight (g) and seed yield per plant (g) except no. of primary branches per plant. Highest days to first flowering were recorded in Torsha (38.00) and lowest in both Sonali Tori and Maghi (22.00). The minimum days to 50% flowering was observed in Maghi with 26.67 DAS while in Torsha was maximum with 43.00 DAS. The range of days to maturity were recorded from 79.00 (Bari sarisha-14) to 92.00 days (Sonali sarisha). The maximum plant height was produced by the genotype Bari sarisha-6 (107.13 cm) and minimum in the genotype Tori-7 (76.03 cm).

The maximum number of primary branches per plant was produced by the genotype Torsha (6.57) and minimum was by the genotypes Sonali sarisha (3.98). The highest secondary branches per plant was produced by the genotype Bari sarisha-3 (9.20) and the lowest was produced by the both genotypes both Bari sarisha-15 and Torsha (1.00). The genotype BINA-10 (30.68) represented the maximum no. siliqua per plant and the minimum was observed by the genotype Sonali sarisha (50.17). The longest siliqua was found in genotype Bari sarisha-3 (6.01 cm) and the shortest was observed in the genotype Maghi (4.86 cm). The genotype Bari sarisha-17 (34.60) represented the highest no. of seeds per siliqua and the lowest was observed by the genotype Bari sarisha 9 (12.33). The maximum 1000 seed weight was produced by the genotype Sonali sarisha (3.83 g) and minimum in the genotype Tori-7 (2.20 g). Genotype Bari sarisha-17 (9.09g) produced the highest yield per plant and genotype Tori-7 (3.90 g) produced the lowest yield per plant.

Phenotypic variance was higher than the genotypic variances for all the traits. Phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the traits. High PCV and GCV were found for no. of secondary branches per plant (71.61 and 57.75), no. of siliqua per plant (39.21 and 29.84), no. of seeds per siliqua (37.60 and 35.21) and seed yield per plant (24.70 and 19.45). High heritability was recorded by days to first flowering (82.35), days to 50% flowering (84.32), days to maturity (69.19), plant height (78.86), no. of secondary branches per plant (65.03), no. of seeds per siliqua (87.69), 1000 seed weight (93.12) and seed yield per plant (62.02). Genetic advance in percent of mean was high for days to first flowering (35.79), days to 50% flowering (31.44), no. of secondary branches per plant (95.93), no. of siliqua per plant (46.79), no. of seeds per siliqua (67.92), 1000 seed weight (25.70) and seed yield per plant (31.55) and lowest for length of siliqua (9.10). High heritability couple with high genetic advance as percent of mean was noticed for the traits, days to first flowering, days to 50% flowering, no. of secondary branches per plant, no. of siliqua per plant,

no. of seeds per siliqua, 1000 seed weight and seed yield per plant provided opportunity for selection of high yielding genotypes.

Genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients in most of the associations which might be due to masking or modifying effect. Very close genotypic and phenotypic correlations were observed in the traits viz days to first flowering with days to 50% flowering; days to first flowering with number of seeds per siliqua, number of seeds per siliqua with seed yield per plant, number of seeds per siliqua with 1000 seed weight, which might be due to reduction in error (environmental) variance, thus selection for higher yield on the basis of above traits would be reliable. Seed yield per plant positively and significantly correlate with number of primary branches per plant (0.340 and 0.531) and number of seeds per plant (0.468 and 0.419) at both genotypic and phenotypic levels. Plant height was correlated positively and significantly in genotypic and phenotypic levels with days to first flowering (0.847 and 0.724), days to 50% flowering (0.839 and 0.722), days to maturity (0.524 and 0.445), length of siliqua (0.813 and 0.429), 1000 seed weight (0.633 and 0.549). Highly significant positive correlations were recorded for number of siliqua per plant with number of secondary branches (0.953 and 0.805); days to maturity with siliqua length (0.907 and 0.492). Highly significant positive correlation of no. of seeds per siliqua with days to first flowering (0.584 and 0.548), days to 50% flowering (0.545 and 0.522) and 1000 seed weight (0.584 and 0.502) at both genotypic and phenotypic levels.

Path analysis revealed days to first flowering (6.314), number of secondary branches (1.916) and 1000 seed weight (0.19) had direct positive effect on seed yield per plant, indicating these were the main contributors to yield per plant. The highest positive indirect effects on seed yield per plant were obtained by days to 50% flowering (6.311), days to maturity (1.004), plant height (5.350), number of primary branches per plant (2.104), length of siliqua (2.015), number of seeds per siliqua (3.689) and 1000 seed weight (4.20) via days to

first flowering. Days to first flowering, days to 50% flowering, plant height, number of primary branches per plant, number of seeds per siliqua and 1000 seeds weight had positive and higher indirect effect on seed yield per plant through number of siliqua per plant.

Genetic diversity among Brassica rapa genotypes was performed through Principal Component Analysis (PCA), Cluster Analysis, Canonical Variate Analysis (CVA) using GENSTAT. The first three components with eigen value were greater than unity contributed a total of 84.32% variation towards the divergence. As per PCA, D2 and Cluster Analysis, the genotypes were grouped into five different clusters. Cluster II, III and IV composed of six, three, three varieties, respectively. The cluster I and V were performed as solitary cluster. The highest inter-cluster distance was observed between clusters IV and V (20.414) indicating diverse genotypes from these two clusters, if involved in hybridization may produce a wide spectrum of segregating population while the lowest inter-cluster distance was observed between cluster II and IV (9.166).

Vegetable oils in human diet constitute an important source of energy and provide fat soluble micronutrients and essentials fatty acids. Out of 14 varieties, on the basis of their mean performance and yield contributing traits six varieties were selected for fatty acid composition analysis. The analysis of variance revealed that fatty acids composition extracted from mustard varieties exhibited significant difference among six *Brassica rapa* varieties. The seed oil content of these studied varieties ranged from 36.52 to 42.42%. More oil content (42.42%) was observed in BARI sarisha-14 variety. Saturated (Palmitic acid, Stearic acid, Arachidic acid, Myristic acid, Lignoceric acid and Behenic acid) and unsaturated fatty acids (Linoleic acid, Linolenic acid, Linoleidic acid, Oleic acid, Elaidic acid, Erucic acid, Ecosadienoic acid and Palmitoleic acid) were analized by GC method. SFA less than 7% are the maximum threshold acceptable for human consumption is observed in Sonali sarisha and BARI sarisha-14. Total mono unsaturated fatty acid (MUFA) viz oleic, eicosenoic

and erucic acids ranged from 62.739% in BARI sarisha-15 to 69.508% in Sonali sarisha. The highest oleic acid, eicosenoic and erucic acids were obtained in Maghi (21.058%), Improved Tori (9.083%) and BARI sarisha-14 (44.022%), respectively. High content of oleic acid has been considered as better for nutritional and industrial purposes because of increases its thermo stability that is suitable as cooking oil and good for health. The minimum percentage of erucic acid was found in the seed oil of Maghi (35.525%). Research revealed that high erucic acid in oil is considered anti-nutritional and not desired for human consumption and they increase blood cholesterol. The highest content of linoleic and linolenic acid was found in the seed oil of BARI sarisha-15 (17.098%) and Improved Tori (8.163%), respectively. Linoleic and α-linolenic acids are essential fatty acids and which have health beneficial effects. In the present study seed oil of none of the varieties were found to meet the recommended specification of oleic/linoleic approaches 2:1 and ω-6/ ω-3 approaches 4:1. The varieties exhibited ω-6/ ω-3 and oleic/linoleic ratio less than the recommended value.

Results of the present studies indicated significant variation among the genotypes for all the characters studied. Number of siliqua per plant, seeds per siliqua, plant height, days to first flowering contributed maximum towards yield improvement. Diversity analysis showed the fourteen genotypes grouped into five different clusters. Considering diversity pattern and other agronomic performance selection of genotype BARI sarisha-14 and BARI sarisha-17 from cluster III as seed yield per plant, Maghi from cluster IV as early flowering, from cluster II improved tori and BINA-10 and from cluster I Sonali sarisha could be considered suitable genotypes for efficient hybridization in future. So, divergent genotypes are recommended to use as parents in future hybridization program. BARI sarisha-14 may be selected for high oil content. Variety selection for safe saturated fatty acids is observed in Sonali sarisha and BARI sarisha-14. Maghi may be selected for MUFA, also it contain the less erucic acid which is highly desirable.

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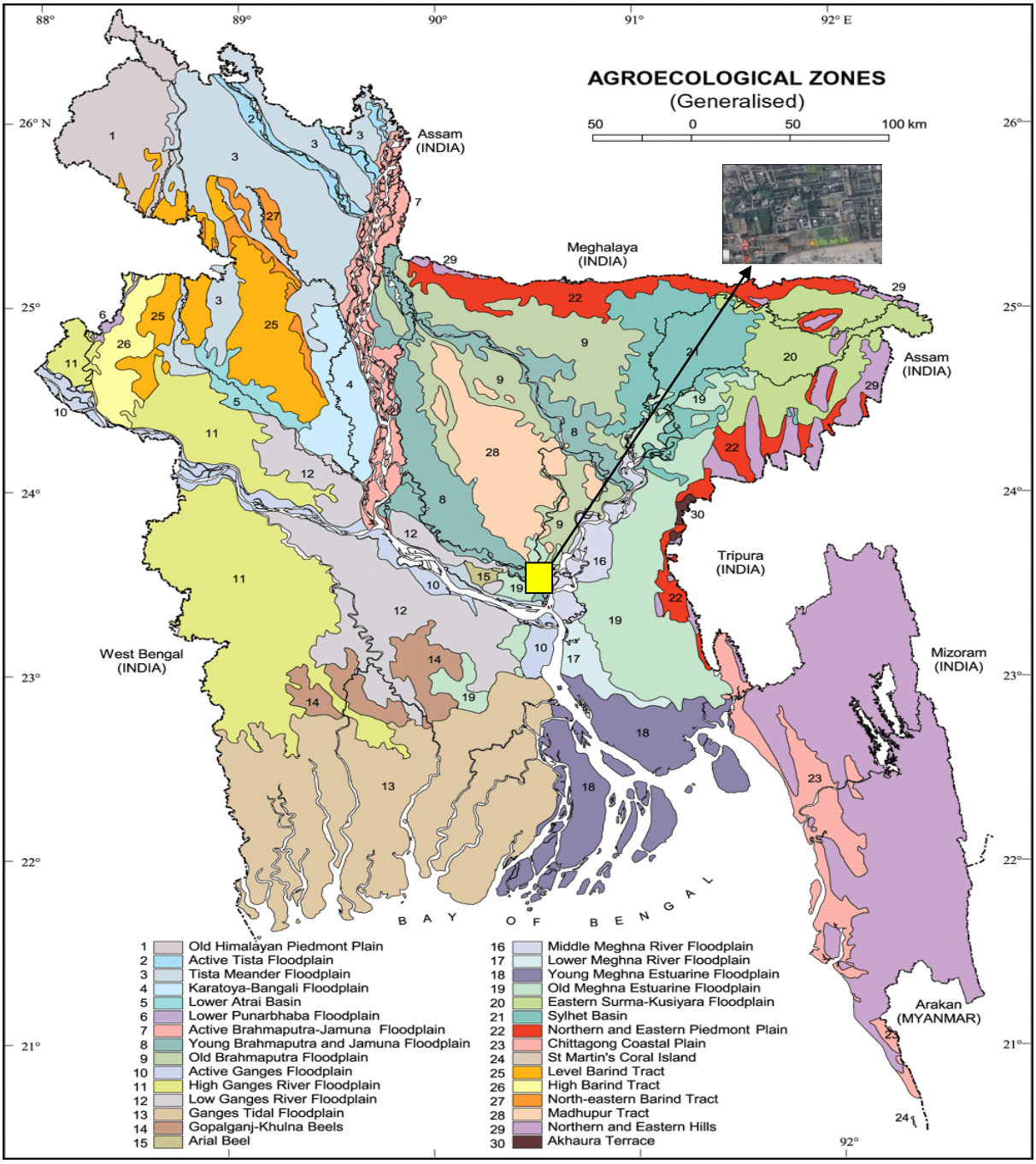
APPENDICES

Appendix I. Map showing the experimental site of the study



Legend showing the research site

Appendix II. Physical characteristics and chemical composition of soil of the experimental plot

Soil Characteristics	Analytical Results
Agrological Zone	Madhupur Tract
p^H	6.00-6.63
Organic matter	0.84
Total (%)	0.46
Available phosphorous	21 ppm
Exchangeable K	0.41meq/ 100 g soil

Source: Soil Research and Development Institute (SRDI), Dhaka

Appendix III. Monthly average Temperature, Relative Humidity, Total Rainfall and Sunshine of the experimental site during the period of November, 2016 to March, 2017

Month	Air temperature (°c)		Relative humidity (%)	Rainfall (mm) (total)	Sunshine (hr)
	Maximum	Minimum			
October, 2016	33.8	25.0	81.81	0	5.8
November, 2016	29.3	18.3	72	0	7.9
December, 2016	27.0	17.0	89	0	3.9
January, 2017	26.2	16.1	67	0	5.7
February, 2017	28.9	23.0	45	0	8.7
March, 2017	29.8	20.5	65	0.1	7.3

Source: Bangladesh Meteorological Department (Climate & Weather Division), Agargaon, Dhaka - 1212