GENETIC DIVERSITY OF TWENTY GERMPLASM OF RADISH
(*Raphanus sativus L.*)

BY

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CERTIFICATE

This is to certify that thesis entitled "Genetic diversity of twenty germplasm of radish (Raphanus sativus L.)" submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree ***MASTER OF SCIENCE in GENETICS AND PLANT BREEDING****, embodies the result of a piece of bona fide research work carried out by* ***Md. Abdulla-Al-Noman Roll No. 6938 Registration No. 15-06938*** *under my supervision and guidance. No part of the thesis has been submitted for any 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 been duly acknowledged by him.

Dated: June, 2016

Prof. Dr. Md. Sarowar Hossain
Supervisor

Dedicated

To

My beloved parents

SOME COMMONLY USED ABBREVIATIONS

Full word	*Abbreviations*	*Full word*	*Abbreviations*
Agricultural	*Agril.*	Milli mole	mM
Agriculture	*Agric.*	Nitrate	NO_3
And others	*et al.*	Number	No.
Applied	*Appl.*	Murashige and Skoog	MS
Bangladesh Agricultural Research Council	BARC	Nanometre	nm
Bangladesh Agricultural Research Institute	BARI	Negative logarithm of hydrogen ion concentration (-log[H+])	pH
Bangladesh Bureau of Statistics	BBS	Nitric Acid	HNO_3
Biology	*Biol.*	Nutrition	*Nutr.*
Calcium ion	Ca^{2+}	Perchloric Acid	$HClO_4$
Centimeter	cm	Percentage	%
Chlorine ion	Cl^-	Plant Genetic Resource Centre	PGRC
Chlorophyll	Chl	Potassium ion	K^+
Days after sowing	DAS	Potassium Chloride	KCl
Decisiemens per meter	dS/m	Parts per million	ppm
Environment	*Environ.*	Review	*Rev.*
Etcetera	etc.	Physiology	*Physiol.*
Food and Agricultural Organization	FAO	Research and Resource	*Res.*
Gram	g	Serial	Sl.
Gram per liter	g/L	Science	*Sci.*
Hectare	ha.	Soil Resource Development Institute	SRDI
Horticulture	*Hort.*	Sodium ion	Na^+
International	*Intl.*	Technology	*Technol.*
Journal	J.	That is	i.e.
Kilogram	Kg	Ton	T
Liter	L	Videlicet (namely)	viz.
Milligram per liter	mg/L	United States of America	U.S.A.
Milligram(s)	*mg*	*Ultraviolet*	UV
Milliliter	mL		

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ABSTRACT

Genetic diversity of 20 radish genotypes was studied for some morphological trait and yield. For this a field experiment was accomplished at experimental site of Sher-e-Bangla Agricultural University during November 2016 to January 2017. A high variation was observed for root color, leaf shape, presence of awn in the leaf, plant height, root length, root weight, shoot length, shoot weight, and first flowering DAS. Among these genotypes, the genetic variation was apparent for most of the character like plant height, root length, root weight, shoot length, shoot weight, root dry weight, shoot dry weight and first flowering DAS that indicated the potential for crop improvement in these traits through selection. On the basis of plant height, number of leaf per plant, root length, root breadth, root weight, shoot length, shoot weight, root dry weight, shoot dry weight, first flowering DAS, Days to maturity four distinct cluster were formed. Cluster IV (G1, G2, G3, G5, G7, G8, G9, G14, G15, G16, G17, G18, G19) contain the highest number of genotypes followed by cluster III (G10, G11, G12, and G13) and cluster I (G4, G6). The lowest genotype presents in the cluster I only one. The highest cluster distance was between cluster I and cluster III which was 16.695 followed by cluster II and cluster III which was 15.747, cluster I and cluster II(15.496), cluster II and cluster IV(13.538), cluster I and cluster IV(13.075). The lowest distant was observed between cluster III and cluster IV (4.311). No clustering was found on the basis of origin. The highest root weight was observed in G4 (BD-4289) which was under cluster II. The highest shoot weight was observed in G20 (BD-10435) possessed by cluster I. Among the cluster the long distance was observed between I and cluster III (16.695) and short distance was between cluster III and cluster IV (4.311). Taking into account the genetic diversity and other performance G4, G6, G7, G19, G20 were considered to be hopeful parents for potential hybridization effort.

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Introduction

CHEPTER I

INTRODUCTION

Radish (*Raphanus sativus L.;* 2n=2x=18) is a tender tuberous edible root vegetable crops belong to the Brassicaceae family that was domesticated in Europe in pre-Roman times. It is suitable for both tropical and temperate climate. This root vegetable crop first domesticated in eastern Mediterranean area (Wang and Ning, 2008) and then subsequently spread to East Asia and Europe. It may be indigenous to china and middle Asia. It possess much importance due to of it's contains such as Ca, K, P and vitamin C. The tender leaves of radish also contain high amount of vitamin C, vitamin A and other minerals (Dias, 2012). The fleshy root of radish eaten as salad and tender leaves as fry vegetables (in local of Bangladesh as "sak").

Radish is a worldwide favorable vegetable crop having characteristic pungency test. The pungency is due to presence of 4-methylthio-3-trans butenyl isothiocyanate (Coogan *et al.*, 2001). This vegetable crop is also favorable in Bangladesh and possesses the third position of major vegetable crop near to brinjal and potato both in area and production. Introduction of improved varieties and attempts at seed production utilizing such varieties as mother plants may lead to loss of the diversity of landraces (Jatoi *et al.*, 2011). In Bangladesh Radish is cultivated in area of 64091 acre and produce 270965 MT of edible root vegetable (BBS-2015). It is a short duration vegetable crop which is helpful for increasing cropping intensity. Maximum of the high yielding verities (HYV) of radish are hybrids. But hybrid seeds are not available in Bangladesh that's why need to import from abroad the hybrid seed at cost of valuable foreign currency. We can reduce this cost through producing hybrid seed in our environment.

For the improvement of a crop hybridization is an important tool. Before using this tool we should have know the genetic diversity of the existing verities. Broad based plant genetic resources are imperative for sound and successful crop improvement program. The diversity

of cultivated plant species depends upon mutation and hybridization, range of dispersal and processes of cultivation and domestication. The knowledge of genetic diversity is required for finding the better hybrid recombinant. More genetic diversity the more chance of getting a better hybrid variety recombination than existing one. For a plant breeding program genetic diversity is the elementary which used as a major tool for selecting better parent. In acute sense genetic diversity is significant to find out the resource of gene for a fastidious trait among the existing available germplasm (Rafiul, 1993).

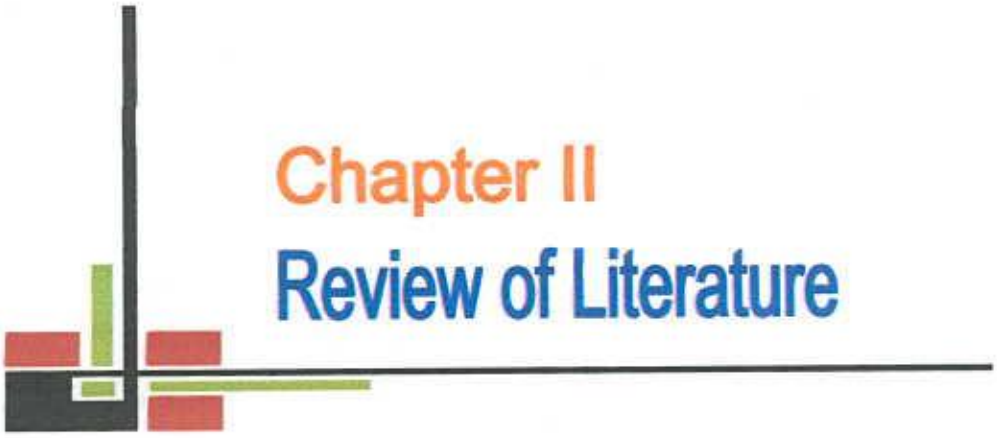
Genetic variability of radish germplasm is not much investigated and only a few studies have been reported on phenotypic diversity. As compared to other crucifer species the diversity of radish for morphological trait and root yield is not yet characterized at morphological levels despite its world-wide economic importance (Jatoi *et al.*, 2011). Multivariate analysis is used for measuring the degree of divergence for determining the relative input of different plant traits to the total divergence in cross pollinated vegetable crops, and it is established by several researchers. In Bangladesh or in another place varietal improvement research is mainly apprehensive with morphological traits. Several researchers suggested that before utilizing the value of a physiological character should be assess in breeding population by the plant breeders. However Radish is a quick growing and short duration crop and the value of growth parameters as well as physiological character are of enormous importance (Rafiul, 1993).

However, the selection indices for production breeding of this crop are not yet perfected and the available information is meager and inadequate. Genetic variability plays an important role in a crop in selecting the best genotypes for making rapid improvement in yield and other desirable characters as well as to select the potential parent of hybridization programs. Heritability is an index for calculating the relative influence of environment of expression of genotypes. It become very difficult to judge how much of the variability is heritable and how

much is non-heritable. Therefore, the present investigation was carried out to study the genetic variability of radish for morphological trait and root yield in radish germplasm collected form BARI germplasm center.

Considering the above idea the present study was undertaken

- To study the genetic variability among the genotypes
- To assess the contribution of different traits towards divergence
- To screen out the suitable varieties for future hybridization program.



Chapter II
Review of Literature

CHEPTER II

REVIEW OF LITERATURE

Radish is a most important prehistoric and well known vegetable crop for both tropical and temperate regions and seems more economic due to its short life cycle followed by increasing cropping intensity. In Bangladesh it is widely grown crop but its production is not bed but quality is low. Some radish are highly pungent in test some are deformed shaped, few verities roots are crack during consumption stage. To remove these problems we have to screen the better one and accumulate the good character into a specific variety through using breeding approach and modern biotechnological tools. Radish yield is greatly influenced by edaphic and agro-climatic and some other morphological traits. To increase the yield and improve the other qualities of radish need diversity knowledge which help to find out the best one. In our country and in another place in the world research exertion on genetic diversity analysis of radish seems to be uncommon. Therefore study on variability for yield trait and other morphological trait require investigate its employment in plant breeding. This section represents some work results which are related to the present study.

2.1 Genetic diversity of radish:

Genetic diversity means genetic distance which is the function of heterosis. In radish genetic diversity was studied based on allozyme variation. Genetic divergence study revealed that the cultivars of different color and similar root shape or those of the same color and different root shape did not cluster.

A field experiment was conducted with 14 genotypes of Radish to study the genetic diversity based on some morphological and physiological characters. Three distinct clusters were formed on the basis of TDM, RGR, CGR, NAI, LAI, leaf number per plant, harvest index,

days to harvest, and root yield per plant. Leaf area index, LAR, LWR, and leaf number per plant were the major components of genetic divergence in the radish verities (Rafiul, 1993)

Genetic variation of forty-nine local and exotic radish genotypes including two checks was studied for morphological traits and seed storage protein electrophoresis using sodium dodecylsulphate polyacrylamide gelelectrophoresis (SDS-PAGE) markers. A high variation in germplasm for root shape, root length, root color (internal and external), flesh texture and root type was observed. Among these genotypes, the genetic variation was apparent for most of the characters like plant biomass, root weight, leaf length, root length and root diameter that indicated the potential for crop improvement in these traits through simple selection. Exotic germplasm exhibited higher variation for plant biomass, root weight and root length which could be utilized through breeding program. Cluster analysis on the basis of genetic diversity for seven quantitative traits resulted into four clusters. No clustering was found on the basis of origin. Low level of variance was observed for SDS-PAGE electrophoresis that suggested acquisition of more germplasm. On the basis of high yield and crispy root texture some genotypes (10076, 10362, 10429, 10658, 10662 and 10667) were identified for further testing under wide range of agro-ecological conditions (Jatoi *et al.*, 2011)

Agarkova *et al.*, (1988) conduct a experiment to analyze the importance of morphological and physiological characters in pea by PCA (principal component analysis). They suggested the use of some indices of photosynthetic activity in breeding program. In another study Banger *et al.* (2003); reported that phenotypic co-efficient of variation (PCV) was higher than genotypic co-efficient of variation. The GCV and PCV were highest for root weight.

Bartual *et al.*, (1985) clustered soybean lines using Principal component analysis (PCA), maximum likelihood factor analysis and cluster analysis made the basis physiological and morphological character. The performance of the identified group was observed according to

the stability in changed environment. To improve agronomic traits some lines were identified as parents for future use in a breeding program.

Twenty genotypes of radish (*Raphanus sativus* L.) were evaluated to study the genetic variability, heritability and genetic advance in radish at Experimental Farm of Horticultural Research Centre, H.N.B. Garhwal University, Srinagar (Garhwal), Uttarakhand (India) during Rabi season 2010-11. The analysis of variance revealed highly significant differences among genotypes for almost all the traits. Both genotypic as well as phenotypic coefficients of variation were high for number of leaves, leaf weight, total plant weight, root yield/plant, acidity, and ascorbic acid. Heritability in broad sense was high for leaf weight, number of leaves, total plant weight, root weight, total soluble solid and ascorbic acid. Genetic advance in percent of mean was maximum for root yield/plant followed by leaf weight (Naseeruddin *et al.*, 2011).

Anand and Rawat, (1984) conduct a experiment to study the genetic diversity, combining ability and heterosis in brown mustard in a set of fifty geographically diverse (including 10 genotypically diverse) lines. The clustering pattern recommended that geographical diversity of a line is not necessarily an index of its genetic diversity.

Ariyo (1987) suggested the importance of multivariate analysis in selecting parents for hybridization in okra with thirty genotypes. The genotypes were grouped into 5 clusters. But there was no relation between clustering pattern and ecogeographical distribution.

Balasch *et al.*, (1984) reported the use and comparison of different multivariate techniques in classifying an important number of tomato varieties. Principal component analysis, as a simple multivariate technique, was compared with factorial analysis and Mahalanobis's D^2 distances. Three methods gave similar results. But factorial discriminate and Mahalanobis's

D^2 distances methods required collecting data plant by plant, while the PCA method required taking data by plots.

Dani and Murthy (1985) studied the genetic divergence and biology of adaption in chickpea using Mahalanobis's D^2 values, canonical analysis and PCA. The results obtained from Mahalanobis's D^2 and canonical analyses were confirmed by PCA. They also reported that it may be simpler to represent multivariate analysis in a two-dimensional chart if the other Zs do not contribute much to the variation.

Godshalk and Timothy (1988) reported comparisons of index selection with principal factor analysis, maximum likelihood factor analysis and PCA. Multivariate analyses were performed on both simple and genotypic correlation matrices for 3 sets of traits (5 traits per set) in switch grass (*Panicum virgatum*). Comparisons were made by computing Spearman's rank correlations between selection index plant scores and scores computed from multivariate analysis and by determining the number of plants selected in common for the selection methods. Among the multivariate analyses methods, PCA had the highest correlation with index selection. They also suggested that PCA is more economic than the other analyses.

Payne and Singh, (1987) reported that the hierarchical nature of the grouping into various number of classes can impose undue constrains and the statistical properties of the resulting groups are not at all clear. Therefore, they have suggested non-hierarchical classification, as an alternative approach to optimize some suitability's choosing criteria directly from the data matrix. They also reported that the squared distance between means are Mahalanobis's D^2statistics when all the dimensions are used, can be computed using Principal Co-ordinate analysis (PCO). They also recommended the Canonical Vector Analysis (CVA) for discriminatory purposes.

Digby *et al.*, (1989) reported that the co-ordinates obtained from the PCA are used as input of PCO analysis to calculate distances among the points. Thus, PCA is used for graphical representation of the points while PCO is used to calculate the minimum distance in a straight line between each pair of points. They have also suggested that both the analyses should be used to represent the distances of points.

2.2 Morphological and physiological characters and their relationships

For crop improvement through breeding program it is essential to know the relationship among various characters as they are used as selection criteria. The breeders are work with this matter worldwide. Some of the study review related to morphological and physiological character relationship is given below.

An experiment conducted for determining heterosis and combining ability in radish. Along with the twelve hybrids only one hybrids demonstrate +tive heterosis over better parent for root length but 9 hybrids demonstrate positive heterosis over better parent for leaf area with positively correlated with root weight (Ling *et al.*, 1986).

Yadav and Singh (1988) demonstrate some selection catalog on the basis of crop growth rate (CGR), leaf area duration (LAD), net assimilation rate (NAR), leaf area index (LAI), for mustard. LAD and LAI show supremacy over directly selection for yield.

Ezejuek, (1990) conducts a experiment on potato to observe the seasonal variation in growth and yield on the basis of physiology. The varieties which give larger total dry matter give the maximum yield. The summer season give higher yield to longer crop duration, higher harvest index, leaf area index, and net assimilation ret. The variation in total dry matter among the variety was the result of differences in total dry matter.

The radish vegetable is classified as plant primarily in USA and Europe. Maximum countries in Asia favor to grow Chinese radish in classification. The heterosis on the basis of root yield is very prominent. For selecting male sterile lines and hybrid breeding of radish for better root yield have been made in china by using the radish-genetic resources (Quwei *et al.*, 2007)

Verma *et al.*, (1989) observe ecological relations on radish cultivars. Harvest operation done between 28 April and 7 January of five cultivars from eight different sowing. The parameter observed were leaf development pattern in variable environmental parameter conditions, the average number of leaf per plant, root volumes as influenced by various environmental conditions, root shape, leaf length. The cultivars which are sowing in June and subsequently showing the highest production of true to type roots.

Six radish cultivars were cultivated to represent the diversity on cropping and other characteristics in glasshouse trials during the second half of January or between mid February and 10 march. The performance of Durabel and Topsi were best. The mean yield of these two verity is 16.3 and 16.1 bunches respectively per square meter. The suitability of Durable was more for earlier followed by Topsi one (Cools and Jansen, 1986).

Hogendonk *et al.*, (1990) studied on the characteristics and keeping quality of 6 radish cultivars judge against with standard sexa, Mirabelle and Nova from conservatory trials cultivated from mid-may to early August. Hilo was a new cultivar which is considered as best followed by Madeira and Saxa Rafine. The criteria of Hilo is to produced largest roots, with very good color and shape also the better yield.

In order to assess the hybridization rate between oilseed rape and wild radish under normal agronomic conditions, three 1-ha field experiments were performed. In each case, wild radish plants were transplanted at different densities in the middle, the border, or the margin of the herbicide-tolerant oilseed rape field. Among the 189084 seedlings obtained from seeds

harvested on wild radish plants, only one herbicide-tolerant inter-specific hybrid (RrRrAC, 2n = 37) was characterized from seeds harvested on an isolated plant growing in the margin of the field. Thus, for the wild radish total harvest, with a 95% confidence limit, the frequency of inter-specific hybrids was assessed to range from 10–7 to 3.10–5. Inter-specific hybrids were detected in all cases among the smallest seeds with a diameter less than 1.6 mm harvested on oilseed rape, but the highest frequency was obtained from oilseed rape close to wild radish plants growing as clusters in the border or the margin of the field. Most hybrids had the expected triploid genomic structure (ACRr, 2n = 28) except for four amphidiploids (AACCRrRr, 2n = 56) and one hybrid from a wild radish unreduced gamete (ACRrRr, 2n = 37). Among the 73847 seedlings observed on the oilseed rape total harvest, the frequency of inter-specific hybrids was assessed to range from 2.10–5to 5.10–4, with a 95% confidence limit. The results are discussed with regard to the type of oilseed rape variety used and the characteristics of the inter-specific hybrids (Chèvre, *et al.*, 2000).

Twelve amplified fragment length polymorphism (AFLP) primer combinations and 10 inter-simple sequence repeat (ISSR) primers were applied to estimate genetic diversity among 68 varieties of cultivated radish (*Raphanus sativus* L.). The material consisted of open-pollinated varieties, inbred lines, diploid and a few tetraploid hybrid varieties of garden radish (*R. sativus*) and black radish (R. sativus var. niger (Mill.) Pers.). Two accessions of uncultivated relatives of radish that as weeds cause serious contamination during the process of hybrid radish production were added to the analyses. Polymorphic fragments were scored for calculation of Jaccard's coefficient of genetic similarity (GS). Substantial level of genetic variability (average AFLP-based GS = 0.70; average ISSR-based GS = 0.61) was detected in the available germplasm of cultivated radish. Cluster analyses separated two weedy species from the cultivated germplasm. Within cultivated material, black radish and french breakfast radish types formed separate clusters. Based on AFLP data, a principal coordinate analysis

(PCoA) and model-based approach revealed the genetic structure within cultivated radish germplasm and indicated the existence of divergent pools. Although the model-based approach did not separate black radish from French breakfast radish varieties, it offered a clear sub-division within garden radish germplasm. The results of this study may be relevant for hybrid radish breeding (Muminović, *et al.*, 2005)

To assess the genetic diversity and genetic structure of East Asian wild radish *(Raphanus sativus* var. hortensis f. raphanistroides), 13 natural populations from Japan and Korea were analyzed for amplified fragment length polymorphism (AFLP). On the average, 77.4% of the AFLP markers generated by eight primer pairs were polymorphic. Both Japanese and Korean populations of wild radish showed a high within population variation (66.3% polymorphic markers, Shannon's information index HO = 3.486, and genetic diversity HEP = 0.128). The majority of the genetic variation of wild radish (96.7%) was observed within populations. Although no appreciable local differentiation of AFLP markers was detected, AFLP markers were more effective than allozymes in classifying natural populations of East Asian wild radish. AFLP variation showed a very close genetic relationship between R. raphanistrum and *R. sativus*, particularly Kazakhstan *R. sativus*, confirming the assumption that R. raphanistrum might be involved in the origin of *R. sativus* (Huh & Ohmi, 2002)

Randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and sequence-related amplified polymorphism (SRAP) markers were employed for germplasm identification and genetic diversity analysis of 17 radish accessions, which consisted of eleven Chinese varieties, five foreign cultivated varieties and one wild relative. The RAPD marker was the most polymorphic (93.4%) among the three marker types. An average of 23.25 polymorphic fragments amplified with each AFLP primer combination was obtained with a relatively low polymorphic ratio. The dendrogram based on RAPD and SRAP data showed high accordance with the classification according to the morphological

traits. The genetic diversity of radish germplasm was highly associated with the color of fresh root skin and their origin. Moreover, as for revealing the genetic relationship of different radish accessions, the data from multiple marker systems was more reliable than that from only one type of marker analysis (Liu, *et al.*, 2006)

Yamane & Ohmi, 2009 analyzed 25 chloroplast simple sequence repeat (cpSSR) loci in 82 accessions, 59 of cultivated radish and 23 of three wild Raphanus species and identified 7 polymorphic loci and 20 haplotypes. The distribution of haplotypes in different species and different geographical areas was assessed. Minimum-spanning network (MSN) was used to identify phylogenetic relationships in cultivated and wild radish. The MSN provides evidence for at least three independent domestication events, including black Spanish radish and two distinct groups of cpSSR haplotypes. One of these two haplotype groups is restricted geographically to Asia. This led Asian cultivated radish haplotypes to higher cpSSR diversity than Mediterranean cultivated radish or wild radish. These data are consistent with the diversity and distribution of agronomic traits in cultivated radish. At the same time, this implies that Asian cultivated radish is not originated from the diffused descendants of European cultivated radish, probably originated from a wild species that is distinct from the wild ancestor of European cultivated radish. Unfortunately we do not know the wild ancestor of Asian cultivated radish.

Escape of trans genes from genetically modified oilseed rape, *Brassica napus*, into wild radish, *Raphanus raphanistrum*, depends on sexual compatibility. The variation in prezygotic barriers of two different cultivars for interspecific hybridization with a population of wild radish was investigated by hand crossing and fluorescence microscopy of pistils. Significant differences were observed between oil seed rape cultivars in their ability to accept wild radish pollen germinating onto their stigma and the rate of fertilization of ovules. Some differences among the pollen donor plants were also detected. These results suggest that the rate of

interspecific hybridization in the field would depend upon the oilseed rape cultivar and the genotype composition of the local wild populations. The implication of S-related genes, as revealed through identification by pistil tissue prints of class I and II S-types of SLG (S-Locus Glycoprotein) and SLR1 (S-Locus Related),and immuno-IEF, was not significant (Guéritaine, *et al.*, 2003)

The genetic structure of populations is an important determinant of the evolutionary potential of a species. Colonizing plants tend to be characterized by low within and high among-population variability. Genetic differentiation of both floral traits and isozymes was studied in six populations of wild radish (*Raphanus raphanistrum*). Evidence far differentiation in both sets of traits was found, but patterns of differentiation of floral traits did not coincide with isozyme differentiation. Contrary to most colonizing species, wild radish showed high within- and only moderate among-population variability at isozyme loci. In addition, levels of differentiation did not correspond to geographic distance between the populations. These results are likely due at least in part to the self-incompatibility: system of this species, long-distance movement of large numbers of wild radish seeds by humans, and introgression from cultivated radish (Kercher & Jeffrey, 1996)

The present investigation was done to evaluate the effects of ambient air pollutants on physiological and biochemical characteristics of radish (*Raphnus sativa* L. var. Pusa Reshmi) and brinjal (Solanum melongena L. var. Pusa hybrid-6) plants grown in open-top chambers with filtered (FCs) and non-filtered (NFCs) treatments at a suburban site in Varanasi, India. Eight hourly mean concentrations of 11.8, 20.8, and 40.8 ppb for SO_2, NO_2, and O_3, respectively, were recorded. O_3 was the most significant pollutant affecting the plant performance. Photosynthetic rate and stomatal conductance declined in both the test plants in NFCs as compared to FCs. Lipid per-oxidation was higher in NFCs, but the increase was more in radish compared to brinjal. The constitutive levels of the antioxidants as well as their

increments upon O_3 exposure were of higher magnitude in brinjal as compared to radish. Reduction in Fv/Fm ratio of the plants in NFCs was a regulatory mechanism to cope with the inefficiency of Calvin cycle. The data indicate that O_3 triggered the protective mechanisms in plants which resulted in increments in enzymatic and non-enzymatic antioxidants of O_3-exposed plants. The variability of the magnitude of responses in radish and brinjal due to O_3 stress suggests that radish is more susceptible to ambient O_3 injury compared to brinjal (Tiwari & Madhoolika, 2011)

Plants of radish (*Raphanus sativus* L.) were grown under selected light conditions in controlled environmental chambers in order to monitor the role of photoperiod, irradiance level and input light energy in plant development. Results indicated that the daily input of light energy was the most important light factor affecting leaf development while photoperiod and irradiance level had the major influences on storage organ development. Distribution of assimilates to leaves and storage organs varied under different light regimes with long photoperiods and high irradiances producing the largest storage organs. Once initiated, the rate of storage organ growth was similar under all tested light environments (Craker *et al.*, 1983)

Genetic variability, correlation and path coefficient analysis of yield and yield contributing traits in twenty one varieties of radish were studied. Root length, leaf length and root yield showed high genotypic coefficient of variation and heritability with high genetic advance in percentage of mean. The highest genetic advance was observed in root yield. Root yield had significant and positive correlation with days to harvest, root length and root diameter, and showed only positive correlation with plant height and leaf width. Path coefficient analysis revealed that plant height had the maximum positive direct effect on root yield followed by root diameter, leaf width and days to harvest (Ullah, *et al.*, 2010)

Ten Nigerian pumpkin accessions were evaluated during the 2007 and 2008 planting seasons to estimate the magnitude of genetic variability and the character association among some yield characters. The results revealed wide genetic variability among the accessions. The genotypic and heritabilty estimates were high in days to 50% emergence, days to 50% flowering, fruit diameter and number of seeds/fruit in both planting seasons. However, genotypic and heritability estimates were low in number of male and female flowers at both planting seasons. At both plantings, the number of seeds/fruit had a significant ($P< 0.01$) positive correlation with the number of male flowers/plant and fruit diameter. A significant positive correlation was also obtained between the number of female flowers and the number of fruits/plant in both planting seasons, an indication that both traits increased or decreased simultaneously. Thus, increasing the number of female flowers would favor fruiting in pumpkin. In both planting seasons, path analysis revealed that days to 50% flowering had the highest positive direct effect on fruit weight and also, had a high direct contribution to the fruit yield. The significant positive correlation between the weight of harvested fruits and fruit diameter in the 2007 planting season was due to the combination of the direct and indirect effects of fruit diameter to fruit yield. In 2008 planting, the number of female flowers recorded high positive direct effects on the weight of fruits/plant but its influence was nullified by the high negative indirect effects (-0.46) of number of fruits/plant. The results indicated that days to flowering, fruit diameter and number of seeds/fruit can be used as selection criteria to increase fruit yield in Nigerian pumpkins (Aruah *et al.*, 2012)

Archana *et al.*, (1999) reported that shoot length, shoot weight, shoot dry weight, and root dry weight had high genotypic co-efficient of variation and high heritability accompanied by low genetic advance in percent of mean in Radish. He also observed high heritability and genetic advance in percent of mean for maturity DAS and First lowering.

Serological variability of radish mosaic virus (RaMV) isolates from white mustard, winter turnip rape, *Camelina sativa* and Chinese cabbage, collected in the Czech Republic and Russia, was studied using antisera against the Czech isolate RaMV1. In contrast to previous studies, reasonable serological differences were found between isolates from different locations and hosts, and even between neighboring mustard plants in the field. Serological variability of European isolates was confirmed in extended experiments involving an Italian isolate, the American Type Strain (ATS) and its homologous antiserum. The results indicate that, in Europe, RaMV isolates may occur which differ both serologically and in host plant response, but no typical strains can be defined with the methods and isolates employed so far. *Camelina sativa* is reported as a new host of the virus (Špak & Kubelkova, 2000)

Seven crops of carrots and 11 crops of radishes were grown from seed in open-top, clear-plastic-wall, CO_2-enrichment chambers throughout the entire year at Phoenix, AZ. Cumulative dry matter production at weekly intervals was significantly increased by a 300 ppm increase in the CO_2 content of the air at all temperatures encountered, but with progressively greater effects being registered at higher and higher temperatures. At 25°C, the productivity enhancement factor for radish was about 1.5, while for carrot it was approximately 2.0. When regressed upon air temperature, the productivity enhancement factors of both species decreased to a null value of 1.0 in the vicinity of 12°C. The slope of the carrot relationship was nearly 250% greater than that of the radish relationship (Idso & Kimball, 1989)

Genetic diversity of 30 radish (*Raphanus sativus* L.) accessions was investigated at the phenotypic level with morphological characters and at the DNA level using the random amplified polymorphic DNA (RAPD) technique. Thirty six morph-physiological traits were recorded from seedling stage to harvest. The 31 primers used generated 202 RAPD bands, of which 158 (78.2%) were polymorphic. Multivariate procedures were used to classify the

germplasm on the basis of phenotypic traits and RAPD fragments. Dendrograms were generated for the Euclidean distance from the morphological data and the Nei's genetic distance from the RAPD markers. Phenotypically, all the accessions were classified into four major groups corresponding to the different forms of cultivated radish. The morphological diversity existing within each of these groups suggested that they should be discriminated into the three botanical convarieties, sativus T (large-rooted), caudatus (pod-type) and oleifer (oilseed-type). Clustering of the accessions did not show any pattern of association between the morphological characters and the collection sites. Instead, landrace groups were associated with their morphological similarities and horticultural uses. On the other hand, the intra-specific genetic relationships of several accessions based on RAPD analysis were related primarily to their collection sites rather than to their phenotypic affinities. The level of polymorphism exhibited by the various convarieties could be exploited in genetic mapping populations to tag economically important traits. These genotypes also could serve as a useful germplasm source for root, leaf, pod and seed. This preliminary study of traditional radish landraces from Pakistan provides useful information regarding their horticultural potential (Ashiq, *et al.*, 1998)

The genotypic and phenotypic variability present in 30 exotic and indigenous varieties of radish was evaluated utilizing the parameters like phenotypic and genotypic coefficient of variation, heritability and genetic advance. The phenotypic and genotypic coefficient of variation, heritability in board sense, and GA as percentage of mean were all high in three characters *viz.*, weight of root, top and whole plant indicating the predominance of gene effects and less of environmental influence (Arumugam & Muthukiushnan, 1975).

Chapter III

Materials and Methods

CHEPTER III

MATERIALS AND METHODS

This chapter describes information related to methods that was utilized during the implementation of the experiment. It include concise description of the experimental site, soil and climate, planting materials, layout and design of the experimental field, preparation of plot, intercultural operation, harvesting, data collection process and statistical analysis etc. brief description of the mentioned points are given below.

3.1 Experimental site:

To determine the genetic diversity of radish germplasm for morphological trait and root yield a field research work was conducted in the experimental field of Sher-e-Bangla Agricultural University, Dhaka farm during the period of November - 2016 to January - 2017. The experimental site is under Madhurpur Tract (AEZ – 28) agro-ecological zone (AEZ) (Anonymous, 1988) and situated between $23^0 74'$ North latitude and $90^0 35'$ East longitude. The elevation of this location is 8 meter from sea level. The research experimental site is included in the map of AEZ of Bangladesh in (Appendix I).

3.2 Climate and soil:

The experimental place was situated under the subtropical climatic zone (SCZ). The feature of SCZ is plenty of sunshine and remain moderate low temperature during Mid – November to Mid – January. This feature is suitable for radish cultivation. The appendix II and III represents the weather information and physioco-chemical properties of soil respectively. The experimental site soil is belongs to "Modhupur Tract" (AEZ – 28) Agro-ecological Zone. The texture of the soil is clay loam. Also olive gray with common fine to medium separate yellowish brown mottles. Some other information like humidity, rainfall, air temperature

were collected from Bangladesh Metrological Department, Agargaon, and Dhaka and mentioned in Appendix II.

3.3 Planting materials:

Twenty genotypes of Radish were collected from Bangladesh Agriculture Research Institute at Plant Genetic Resource Centre (PGRC). List of the Accession is given below in Table 1.

Table 1. Name and origin of twenty Radish genotypes used in the present study.

SI.No.	Genotypes No.	Name/ Acc No. (BD)	Source
1	G1	BD-4286	PGRC, BARI
2	G 2	BD-4287	PGRC, BARI
3	G 3	BD-4288	PGRC, BARI
4	G 4	BD-4289	PGRC, BARI
5	G 5	BD-4290	PGRC, BARI
6	G 6	BD-4291	PGRC, BARI
7	G 7	BD-4292	PGRC, BARI
8	G 8	BD-4293	PGRC, BARI
9	G 9	BD-4294	PGRC, BARI
10	G 1 0	BD-7075	PGRC, BARI
11	G 1 1	BD-7076	PGRC, BARI
12	G 1 2	BD-7077	PGRC, BARI
13	G 1 3	BD-7078	PGRC, BARI
14	G 1 4	BD- 7763	PGRC, BARI
15	G 1 5	BD-7064	PGRC, BARI
16	G 16	BD-7065	PGRC, BARI
17	G 17	BD-10438	PGRC, BARI
18	G 18	BD-10437	PGRC, BARI
19	G 19	BD- 10436	PGRC, BARI
20	G 20	BD-10435	PGRC, BARI

** PGRC= Plant Genetic Resource Centre

**BARI= Bangladesh Agricultural Research Institute.

3.4 Design and layout of the experiment:

RCBD (Randomized complete Block Design) with three replication was used in the set up of experimental field. The total land was $80m^2$ (10m X 8m). Total land was divided into three beds each bed is 9m in length and 2m in width. 0.5 m space kept between two beds for the convenience of intercultural operation and data collection. Row to Row distance is 45 cm. A schematic diagram of the experimental field is given below.

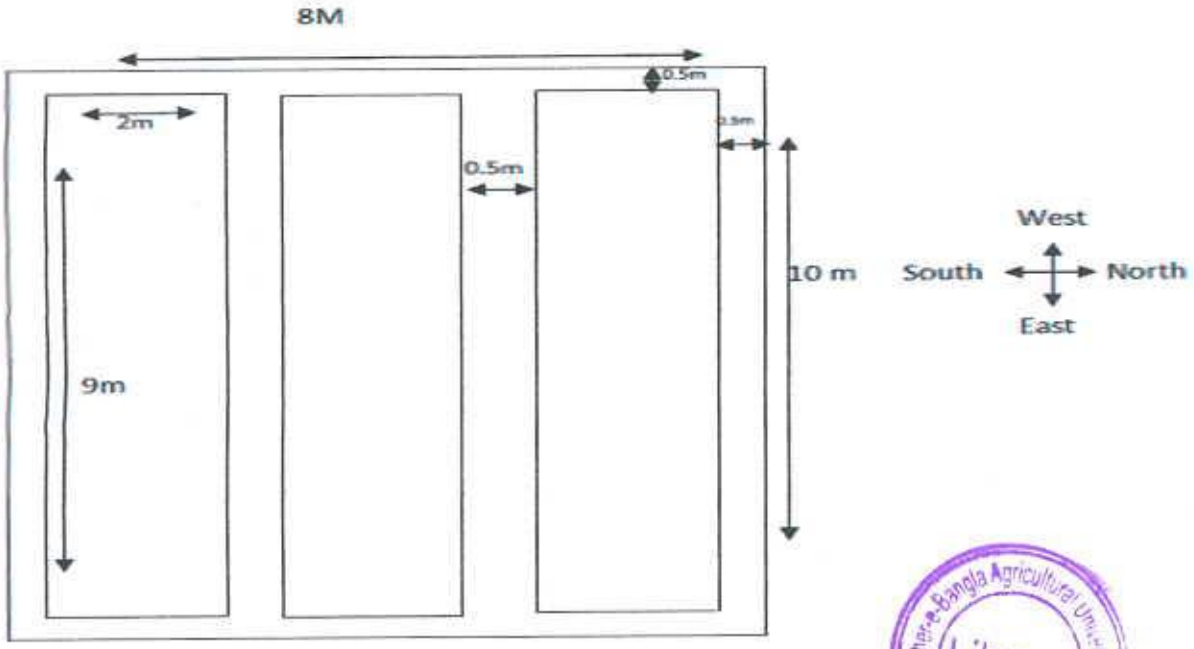


Figure 1. Layout of the Experiment

3.5 Manures and fertilizers:

The requirement of manures and fertilizers are given in the following Table 2.

Table 2. Manures and fertilizers dose for Radish cultivation

Name of the manures and fertilizers	Dose per hector.
Urea	300-350 kg
TSP	250-300 kg
MoP	215-235 kg
Compost	8-10 ton

All compost, TSP, and half of the Urea, MoP was mixed properly after last ploughing. The half of the remaining Urea and MoP were applied in third week of sowing and the rest fertilizers were applied in fifth week after sowing (Mondal *et al.*, 2011).

3.6 Sowing of seeds:

Seeds were sown on 19th November. The seeds were spread on the row. Number of seed in each row is near about 10- 15 and row to row distance is 45 cm. Emergence of seedling were appear three to five days after sowing. Plate 1 Showing vegetative stage of Radish plant.

3.7 Intercultural operation:

Thinning, Weeding followed by fertilizer application and mulching operation done on 14th day after sowing (DAS). The third top dressing were applied in 5th week after sowing in broadcast method. Irrigation were done when needed. The diseases and insect pest attack were minor. Alternaria Blight was appearing but it is negligible and removes through hand picking of affected plant.

3.8 Harvesting:

Harvesting of roots was done after maturity stage. Mature roots were harvested when the roots produce metallic sound on breaking. Different accessions were harvested at different dates as the maturity stage of the accessions was variable.

3.9 Data collection:

Five plants were selected randomly from each row and every replication of the respective accession. Data for different morphological traits associated with yield and its contributing characters were recorded such as plant height, no. of leaf per plant, root length, root breadth, root weight, shoot length, shoot weight, root dry weight, shoot dry weight, first flowering (DAS),

Plate 1. Experimental field at vegetative stage

and days to maturity. The process of data collection or each indices of character are given below. Plate 2 showing data collection in experimental field.

3.9.1 Plant height (cm):

Plant height of each plant at mature stage measured in cm using meter scale and mean was calculated.

3.9.2 No. of leaf per plant:

Number of leaf of selected plant were counted and mean was calculated

3.9.3 Root length (cm):

Length of each selected root was measured from leaf base to tip of the root in centimeter scale and mean was calculated.

3.9.4 Root breadth (cm):

Root breath was taken with slide calipers in three position top, middle and bottom position and make average and finally mean was calculated from these averages.

3.9.5 Root weight (g):

Root weight was taken in electric balance in gram scale and mean was calculated.

3.9.6 Shoot length (cm):

Shoot length was taken with meter scale from base of the root near to root to tip of the leaf and mean was calculated.

3.9.7 Shoot weight (g):

Shoot weight was taken with electric balance and in gram and mean was calculated.

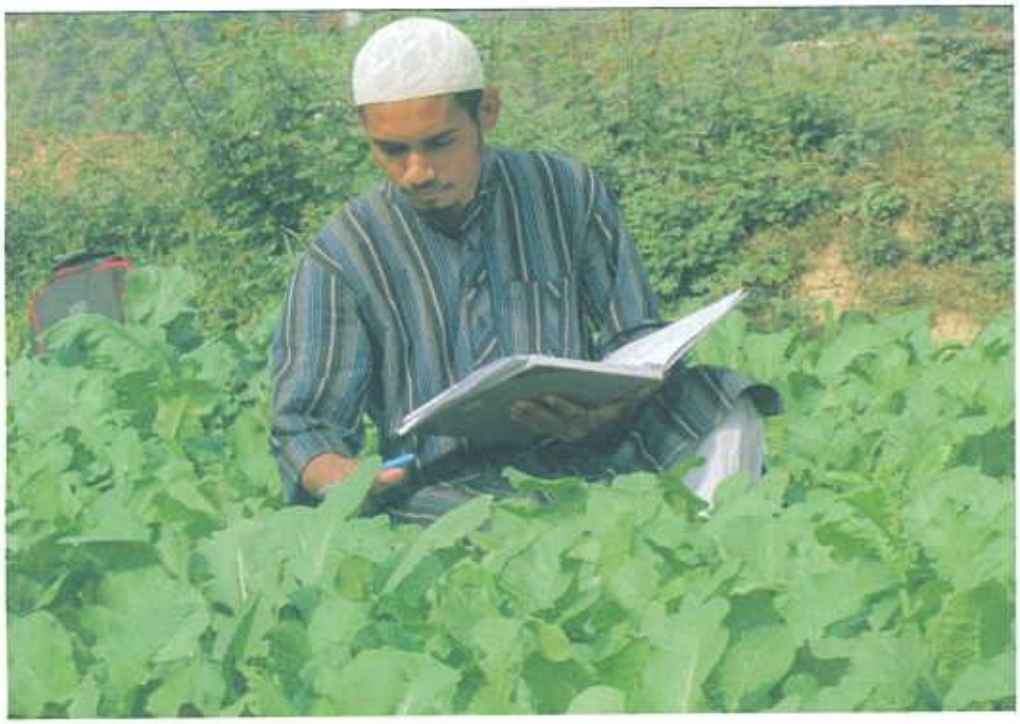


Plate 2. Data collection at experimental field

3.9.8 Root dry weight (g):

The selected plant root was oven dried for 72 hour and weight was taken finally mean was calculated

3.9.9 Shoot dry weight (g):

The selected plant shoot was oven dried for 72 hour and weight was taken finally mean was calculated

3.9.10 Days to first flowering:

First flowering was observed and calculated number of days after sowing.

3.9.11 Days to maturity:

One plant was uprooted and breaks. If it produces metallic sound considered as matured and days to maturity were calculated.

3.10 Statistical analysis:

There are 11 characters were selected for determining the genetic diversity of some radish varieties for morphological trait and root yield. The numerical data were collected for 11 characters from each replication and averaged to make mean data. These mean data require for multivariate analysis. Univariate analysis of the individual characters was done for all characters under study using mean values (Singh and Chaudhury, 1985). Differences between the means of the accessions considering all characters it is essential to perform Duncans Multiple Range Test (DMRT). MSTAT-C computer program were used to estimate mean, range, and co-efficient of variation (CV %).

3.10.1 Estimation of genotypic and phenotypic variance:

Genotypic and phenotypic variance were estimated according to the formula of Johnson *et al.* (1955)

Genotypic variance $\delta^2_g = \frac{MSG-MSE}{r}$

Where, MSG = Mean sum of square for genotype

MSE = Mean sum of square for error, and

r = *Number of replication*

Phenotypic Variance $\delta^2_p = \delta^2_g + \delta^2_g$

Where, δ^2_g = Genotypic variance,

δ^2_e = Environmental variance = Mean square of error

3.10.2 Estimation of genotypic and phenotypic co-efficient of variation

Estimation of genotypic and phenotypic co-efficient of variation were calculated using following formula given by Burton in 1952

Genotypic co-efficient of variation (GCV%) = $\frac{\delta g \text{ X } 100}{x}$

Phenotypic co-efficient of variation (PCV%) = $\frac{\delta p \ X \ 100}{x}$

Where,

δg = Genotypic standard deviation

δp = Phenotypic standard deviation

x = *Population mean.*

3.10.3 Estimation of heritability:

Broad sense heritability is the proportion of trait variance that is due to all genetic factors including dominance and gene-gene interactions. This is estimate using following formula given by Johnson *et al.* (1955)

$$h^2_b = \frac{\delta^2 g}{\delta^2 p} X\ 100$$

Where,

h^2_b = Heritability in broad sense

δ^2_g = Genotypic variance

δ^2_p = *Phenotypic variance*

3.10.4 Estimation of genetic advance:

Genetic advance is the improvement in the mean genotypic value of selected plants over the parental population. This is estimated by a formula given by Johnson *et al.* (1955)

$$\text{Genetic Advance (GA)} = \frac{\delta^2 g}{\delta^2 p}\ K.\delta p$$

Where,

δ^2_g = Genotypic variance

δ^2_p = Phenotypic variance

δp = Phenotypic standard deviation

K = Selection differential which is equal to 2.06 at 5% selection intensity

3.10.5 Estimation of genetic advance in percentage of mean:

Genetic advance in percentage of mean was calculated by Comstock, *et al.*, (1952) formula.

$$\text{Genetic Advance in percentage of mean} = \frac{\text{Genetic Advance}}{X} X\ 100$$

Where,

X= Population mean

3.10.6 Genetic diversity analysis for morphological trait and root yield:

The genetic divergence of 20 radish accessions were estimated following Mahalanobis generalized distance (D^2) extended by Rao (1952). Tocher's method (Rao, 1952) was followed for determining the group constellations. Mean data for each character were subjected to multivariate analysis techniques for principal component analysis (PCA), Principal coordinate analysis (PCO). Cluster analyses (CLSA) were done by computer using GENSTAT 5.13 software program.

3.10.7 Principal component analysis (PCA)

To know the interrelationships among several characters principal component analysis (PCA) is the multivariate method. It can be done from the sum of squares and product matrix for the characters. For that reason the principal components were computed from the correlation matrix and genotypic scores obtained for the first components which has the property accounting for the maximum variance and succeeding components with latent roots greater than unity. The first component has the property accounting for maximum variance. The PCA displays most of the original variability in a similar member of dimensions, since it finds linear combinations of a set of variate that maximize the variation contained within them.

Contributions of different characters towards divergence are discussed from the latent vectors of the two principal components.

3.10.8 Principal coordinates analysis (PCA)

Principal Component Analysis (PCA) and principal coordinate analysis are equivalent each other. But principal co-ordinate analysis is used to compute inter unit distances. Through the use of all dimensions of P it gives the minimum distances between each pair of the N points using similarity matrix (Digby *et al.*, 1989). Inter distances between genotypes were studied by PCO.

3.10.9 Cluster analysis (CLSA):

In this experiment there are 20 accessions were used. Some of them have similar values of morphological trait. On the basis of a data set the genotypes were divided into groups. The grouping was done using non-hierarchical classification. In GENSTAT, to search for optimal values of the chosen criterion the algorithm is used. The optimal values of criteria after than some initial arrangement of the genotypes into required number of groups, the genotypes repeatedly transfers from one group to another through algorithm so long as the criterion of the values were improved by such transfer. After completing this transfer the final improvement of the criterion occurs for this no additional transfer can be initiate to improve the criterion. The algorithm switches to second stages that examine the effect swopping two genotypes of different classes, and so on.

3.10.10 Computation of average intra-cluster distances:

Computation of average intra cluster distance for each cluster was calculated by taking possible D^2 values. Within the members of a cluster obtained from the PCO after the clusters are formed. The formula utilized as follows

Average intra cluster distance = $\frac{\Sigma D^2 i}{n}$

Where D^2_i = the sum of distance between all possible combination genotypes included in a cluster.

n = number of all possible combinations between the population in cluster.

3.10.11 Computation of average inter-cluster distances:

Average inter-cluster distance were calculated by the following formula as suggested by Singh and Chaudhury (1985)

$$\text{Average inter-cluster distance} = \frac{\Sigma D^2 ij}{n_j X n_i}$$

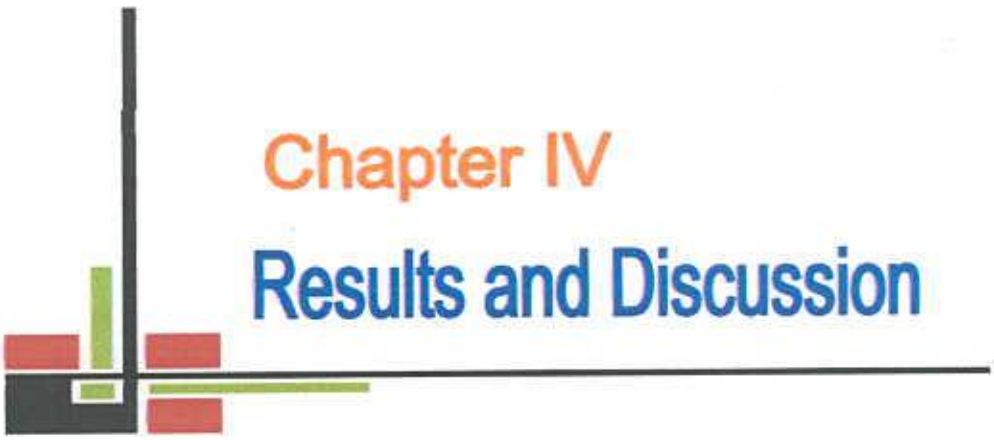
Where; D^2_{ij} =the sum of distances between all possible combinations of the populations in cluster i and j.

n_i = Number of population in cluster i

n_j = Number of populations in cluster j.

Cluster Diagram:

According to Singh and Chaudhury (1985) the value of intra and inter cluster distance (D = $\sqrt{D^2}$) were used to draw a cluster diagram. A concise scheme of the pattern of diversity among the 20 radish variety was obtain.



Chapter IV

Results and Discussion

CHEPTER – IV

RESULTS AND DISCUSSION

Improvement of crop variety, it is essential to use breeding method and to conduct a effective breeding program the exact information about the nature and degree of diversity of the existing parent is the primary requirement. So the parent selection and heterosis is main function of diversity analysis of selected germplasm. The facts related to the genotypic variation among the genotype including morphology, phenology, and yield are make the screening of better genotypes easy. An experiment was conducted on Radish (*Raphanus sativus*) in Rabi season of 2016-17. This chapter represents the result obtained from this study. The data concerning twenty genotypes of Radish with its yield contributing characters were collected, computed and statistically analyzed to represent the present inquiry of variability analysis in the following section.

4.1 Genetic component of variation

The ANOVA (Analysis of Variance) of eleven yield and yield contributing characters of twenty radish genotype is presented in the Appendix V. The ANOVA was pointed out that the significance of variability among the genotypes for all characters which were consider for diversity analysis such as plant height, number of leaf per plant, root length, root breadth, root weight, shoot length, shoot weight, root dry weight, shoot dry weight, Days to first flowering date, and Days to maturity. Hence there was lot of possibility for selection of common of the trait in the genotypes. The mean performance of the genotypes are presented in appendix IV and the MSS (mean sum of square) of all considering 11 characters are presented in the Table 3.

4.2 Genetic variability, heritability and genetic advance:

Degree of genetic variability of the existing genotype is the key point to succeed a breeding program. A statistic used in breeding and genetics works that estimates how much variation in a phenotypic trait in a population is heritability which is an important genetic parameter mostly

helpful for breeders breeding program. Heritability presents the amount of variance resulting for genetic causes. Thus it indicates the consistency of the phenotypic assessment to the genotypic value. Therefore it reveals the assurance of the phenotypic value as a point to the genetic value and takes part in formulas related to the breeding method which is helpful for selection strategies The calculation of mean, range, genotypic co-efficient of variation, phenotypic of co-efficient of variation, genetic advance, percentage of genetic advance and heritability for all characters were focus for study and the outcomes were represent in the Table 3.

4.2.1 Plant height:

The range of plant height remain between G4 (44.60 cm) and G5 (71.80 cm) and the mean value of plant height was 55.23 cm for all the radish genotypes (Table 3). Phenotypic variance was higher than the genotypic variance with 10.39 error variance. Genotypic co-efficient of variance (4.516) and phenotypic co-efficient of variance (5.452) showed that there was negligible environmental effect in expression of this trait. The heritability for this character was 68.609 where genetic advance was 8.132 and percentage of genetic advance was 7.706. Moderately high heritability with low genetic advance and low percentage of mean genetic advance indicated that the expression of this character was controlled by additive gene and thus selection for this character might be worthwhile. Jatoi *et al.*, (2011) reported that a high variation in Radish *(Raphanus sativus)* germplasm for both root length and shoot length also in root shape, as well as some other yield contributing characters.

4.2.2 Number of leaf per plant:

Considering number of leaf per plant the maximum value was 17.60 possesses G20 and minimum value was 9.40 possesses G2. The genotypic variance was 1.1608 whereas phenotypic variance was 2.110 this indicated the influence of environment for controlling the expression of this trait was positive. The difference between genotypic co-efficient of

Table 3. Estimation of Genetic components of variation in 11 characters of 20 genotypes in Radish

Trait	GMS	Min	Max	Mean	CV (%)	δ^2_g	δ^2_e	δ^2_p	GCV	PCV	h^2_b	GA	GA (% mean)
PH	78.5312**	44.60	71.80	55.23	5.84	22.71	10.39	33.105	4.516	5.452	68.609	8.132	7.706
NLPP	4.4318**	9.40	17.60	12.31	7.91	1.1608	0.9493	2.110	0.782	1.055	55.012	1.646	1.195
RL	19.6475**	8.80	28	16.86	13.66	4.78104	5.3044	10.1250	26.155	37.988	47.405	3.101	37.097
RB	0.5395**	1.55	3.80	2.60	10.13	0.1567	0.693	0.8497	1.234	1.482	69.341	0.679	2.116
RW	2200.9650**	33.20	160.8	83.33	15.89	675.218	175.30	850.527	98.316	110.34	79.388	47.694	180.456
SL	52.3260**	26.80	53	38.07	9.86	12.7491	14.079	26.8285	31.598	45.837	47.521	5.070	44.871
SW	2578.8777**	41.20	198	104.75	16.29	762.333	291.08	1053.41	78.297	92.034	72.375	48.396	137.216
RDW	33.7793**	2.10	17.35	7.80	28.24	9.6434	4.8490	14.4924	2.397	2.938	66.541	5.218	4.027
SDW	36.4912**	3.45	21	8.85	23.35	10.741	4.2682	15.0092	14.425	17.052	71.563	5.711	25.138
FF	194.5404**	32	64	43.20	2.13	64.5641	0.8482	65.4123	29.650	29.844	98.703	16.445	60.682
DM	2.0702	41	46	43.33	3.28	0.0183	2.0149	2.0333	0.418	4r.393	0.907	0.027	0.082

PH= Plant height; NLPP= No. of leaf per plant; RL= Root length; RW= Root weight; SL= Shoot length; SW= Shoot weight; RDW= Root dry weight; SDW= Shoot dry weight; FF= Days to First flowering; DM= Days to Maturity

*Significant at 5%, ** Significant at 1%

variance (0.782) and phenotypic co-efficient of variance (1.055) was low indicated minor environmental influence on this character. The heritability of the trait was 55.10, genetic advance was 1.646 and percentage of genetic advance was 1.195 indicated the non additive gene effect, selection might not be effective. According to Rafiul, (1993) number of leaf per plant, leaf area index (LAI) was the major component of genetic divergence in Radish varieties. Naseeruddin *et al.*, (2011) also found both genotypic as well as phenotypic coefficients of variation were high for number of leaves. Plate 3 showing variation of leaf shape and color.

4.2.3 Root length:

For root length the ranged remain between 8.80 cm to 28 cm with mean value 16.86 cm. Genotype G8 showed the lowest where G19 showed the highest length (Table 3). The phenotypic variance and genotypic variance was 10.1250 and 4.7810, respectively suggested that the influence of environment on the expression of the genes was present. The genotypic (26.155) and phenotypic (37.988) coefficient of variation were closely related that showed the minimum environmental effect of the expression of trait. The haritability (4.78104) estimated for this trait moderately high, genetic advance (3.101) was low and percentage of genetic advance (37.097) was moderately high indicated that this trait was governed by additive gene. So selection of this trait was rewarding. Jatoi *et al.*, (2011) showed that high variation was observed in root length and root shape. Ullah *et al.*, (2010) also reported the root length showed high genotypic coefficient of variation and heritability with high genetic advance in percentage of mean.

4.2.4 Root breadth:

The highest and lowest value for this trait was 3.80 cm and 1.55 cm possessed by G5 and G18, respectively with mean value 2.60 cm. The genotypic and phenotypic variance was detected 0.1567 and 0.8497 with low difference 0.693 indicated the environmental effect for this trait was low.

Plate 3. Presence of variation in leaf shape and leaf colour.

The GCV and PCV were 1.234 and 1.428, respectively which were closely related to the minor for expressing this trait. The heritability (69.341) was high with genetic advance (GA) and percentage of genetic advance was 0.679 and 2.116, respectively which were low indicated the trait was governed by non additive gene. So selection for this trait might not be rewarding. Shape of the root depends on the length and breadth of the root. A high variation in Radish (*Raphanus sativus*) germplasm for both root length and shoot length also in root shape, as well as some other yield contributing characters revealed by Jatoi *et al.*, (2011). Plate 4 showing variation of root shape and color.

4.2.5 Root weight:

The highest and lowest value for this trait was 160.80 g and 33.20 g possessed by G4 and G10, respectively with mean value 83.33 g. The genotypic and phenotypic variance was detected 675.218 and 850.527 with high difference 175.30 indicated the environmental effect for this trait was major. The GCV and PCV were 98.316 and 110.34, respectively which had few difference that showed some environmental effect for expression this trait. The haritability (79.388) was high with genetic advance (GA) and percentage of genetic advance was 47.694 and 180.456, respectively which were high indicated the trait was governed by additive gene. So selection for this trait might be rewarding. Bangar *et al.*, (2003) reported that phenotypic co-efficient of variation was higher than genotypic co-efficient of variation. Ullah *et al.*, (2010) also reported the root weight showed high genotypic coefficient of variation and heritability with high genetic advance in percentage of mean.

4.2.6 Shoot length:

For shoot length the ranged remain between 26.80 cm to 53 cm with mean value 38.07 cm. Genotype G11 showed the lowest where genotype G9 showed the highest length. The phenotypic variance and genotypic variance was 26.8285 and 12.7491 respectively suggested that the influence of environment on the expression of the genes was present.

Plate 4. Presence of root color and root shape variation among the varities.

The genotypic (31.598) and phenotypic (45.837) coefficient of variation having some difference showed the environmental effect for expressing the character. The haritability (47.521) estimated for this trait moderately high, genetic advance (5.070) was low and percentage of genetic advance (44.871) was moderately high indicated that this trait was governed by additive gene. So selection of this trait was rewarding. High heritability and low percentage of genetic advance in case of shoot length of Radish which was similar to the earlier findings by Archana *et al.* (1999). Ullah *et al.*, (2010) also reported the shoot length showed high genotypic coefficient of variation and heritability with high genetic advance in percentage of mean.

4.2.7 Shoot weight:

For shoot weight the range remain between 41.90 g to 198 g with mean value 104.75. Genotype G10 showed the lowest where G20 show the highest. The phenotypic variance and genotypic variance was 1053.41 and 762.333, respectively suggested that the influence of environment on the expression of the genes was present. The genotypic (78.297) and phenotypic (92.034) coefficient of variation having differences indicated the environmental effect for expressing the character. The heritability (72.375) estimated for this trait was high, genetic advance (48.396) was moderately high and percentage of genetic advance (137.216) was high indicated that this trait was governed by additive gene. So selection of this trait was rewarding. High heritability and low percentage of genetic advance in case of shoot weight of Radish which was similar to the earlier findings by Archana *et al.* (1999)

4.2.8 Root dry weight:

The range of root dry weight remain between G10 (2.10 g) and G20 (17.35 g) and the mean value of root dry weight was 7.80 g for all the radish genotypes. Phenotypic variance (14.4924) was higher than the genotypic variance (9.6493) with 4.8490 error variance. Genotypic co-efficient of variance (2.397) and phenotypic co-efficient of variance (2.938)

showed that there was negligible environmental effect in expression of this trait. The heritability for this character was 66.541 where genetic advance was 5.218 and percentage of genetic advance was 4.027. High heritability with low genetic advance and low percentage of genetic advance indicated that the expression of this character was controlled by additive gene and thus selection for this character might be valuable. High heritability and low percentage of genetic advance in case of shoot length of Radish which was similar to the earlier findings by Archana *et al.* (1999)

4.2.9 Shoot dry weight:

Considering shoot dry weight the maximum value was 21.00 g possesses G1 and minimum value is 3.45 g possesses G16. The genotypic variance was 10.741 whereas phenotypic variance was 15.0092 this indicated the influence of environment for controlling the expression of this trait was positive. The difference between genotypic co-efficient of variance (14.425) and phenotypic co-efficient of variance (17.052) was low indicated minor environmental influence on this character. The heritability of the trait was 71.563, genetic advance was 5.711 and percentage of genetic advance was 25.138 indicated the additive gene effect, selection might be effectual. High heritability and low percentage of genetic advance in case of shoot length of Radish which was similar to the earlier findings by Archana *et al.* (1999)

4.2.10 First flowering DAS

The mean first flowering days after sowing was 43.20 with a range between 32.00 to 64.00 where the highest values possess G15 and the lowest value possesses G18. The genotypic and phenotypic variance was 65.4123 and 64.5641, respectively with difference 0.8482 which was low indicated the environmental effect in the expression of this trait was low. Considering the GCV and PCV value which were closely related indicated minor environmental effect. The GA (16.445) and percentage of GA (60.682) was moderately high

and high revealed the trait was governed by additive gene and selection was rewarding. Archana *et al.*, (1999) reported high heritability and high genetic advance in first flowering and maturity day after sowing.

4.2.11 Days to maturity:

The mean days to maturity were 43.33 with a range between 41 to 46 where the highest value possess G4 and the lowest value possesses G3. The genotypic and phenotypic variance was 0.0183 and 2.0333; respectively with difference 2.0149 which was low indicated the environmental effect in the expression of this trait was low. Considering the GCV and PCV value which were closely related indicated minor environmental effect. The GA (0.027) and percentage of GA (0.082) was very low which express the character was non additive gene governing so the selection might not be rewarding. Archana *et al.*, (1999) reported high heritability and high genetic advance in first flowering and maturity day after sowing.

4.3 Multivariate Analysis:

4.3.1 Principal component analysis (PCA)

Eleven characters were considered for genetic diversity analysis. Eigen values of eleven principal and percentage of total variation accounting for them obtained from the principal component analysis are presented in Table 4. The result revealed the first principal axis largely accounted for the variation among the genotypes which alone contributed 33.30% of the variation followed by the 2^{nd} axis 16.21% while the first six Eigen values for the principal component axis of genotype accounted for 88.69% of the total variation among 11 characters describing 20 genotypes while the first two accounted 49.51%.

4.3.1 Cluster analysis:

The analysis of variance revealed highly significant differences among the genotypes for all the eleven characters indicating the existence of genetic variability among the experimental genotype.

Table 4. Latent roots (Eigen values) and percent of variation in respect of 11 characters in Radish.

Principal component axis	Latent roots	% of total variation accounted for	Cumulative percent
1. Plant height (cm)	3.6633	33.30	33.30
2.First flowering DAS	1.7835	16.21	49.51
3. Leaf number per plant	1.4407	13.10	62.61
4. Root length (cm)	1.2150	11.05	73.66
5. Root breadth (cm)	0.8709	7.92	81.58
6. Root weight (g)	0.7817	7.11	88.69
7. Days to maturity	0.5934	5.39	94.04
8. Shoot length (cm)	0.2770	2.52	96.6
9. Shoot weight (g)	0.1646	1.50	98.1
10. Root dry weight (g)	0.1474	1.34	99.44
11 Shoot dry weight (g)	0.0624	0.57	100

The twenty genotypes were grouped into four clusters using the non-hierarchical clustering method by Genstat Version 3.11 software programme in such a way that the genotypes within the cluster had smaller D^2 values among themselves than those belonging to different clusters Table 5. Pattern of distribution of genotypes among various clusters reflected the considerable genetic variability present in the inbreds under study. The maximum number of genotyps (20) was comprised into cluster IV indicating overall genetic similarity among them which was followed by cluster III and II contain 4 and 2 accessions respectively whereas cluster I contain only one accession. Rafiul, (1993) suggested three distinct cluster among fourteen radish germplasm.

4.3.2 Construction of scatter diagramme:

From the principal component analysis some value obtained which were principal component scores I and II plotted in a two dimensional (Z_1-Z_2) graphe as X axis and Y axis, respectively represented in the Figure 2. The location of the genotypes in this scatter diagram forms 4 groups. This clearly indicated the considerable diversity among the existing group (Figure 2). Rafiul, (1993) constructed scatter diagrame for fourteen radish germplasm and found three distinct group.

Table 5. Distribution of 20 genotypes of Radish in four different clusters

Cluster	No. of Genotypes	Genotypes in different clusters
I	1	G20
II	2	G4, G6
III	4	G10, G11, G12, G13
IV	13	G1, G2, G3, G5, G7, G8, G9, G14 , G15, G16, G17,G18, G19

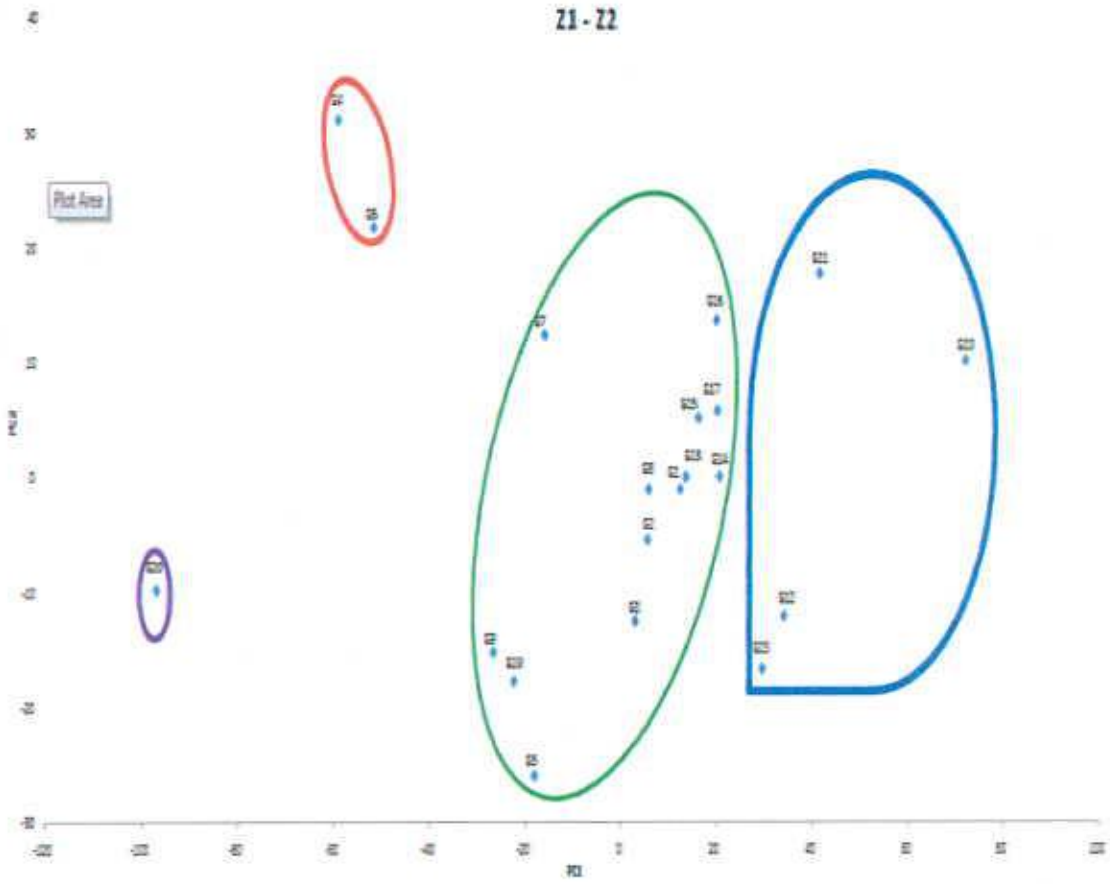


Figure 2. Scatter duagran of 20 Radish genotypes based on their principal component scores superimposed with clustering.

4.3.3 Average intra and inter cluster distance:

The intra and inter cluster distance (D^2) values worked out form divergence analysis are presented in Table 6. The magnitude of intra cluster distance indicated the extent of genetic diversity among genotypes within the same cluster. The inter cluster distance in all cases were larger than the intra cluster distance which indicated that wider diversity was present among the genotypes of distant group. The genotypes within a cluster had less diverisity among themselves. The highest inter cluster distance of 16.695 was observed between cluster III and cluster I followed by 15.747 between III and II, 15.496 between II and I suggesting wide diversity between them and the genotypes in the genotypes in these cluster could be used as parent for new hybrid development. The minimum inter cluster distance between III and IV which was 4.311. This finding's were supported by Howlader *et al.*, (1995). The highest intra cluster distance was computed cluster II (1.0997) followed by cluster IV (0.8011). The cluster I showed the least cluster distance due to this cluster only one accession. So no way to judge.

4.3.4 Principal coordinate analysis (PCO)

The result obtained for principal coordinate analysis showed that the highest inter genotypic distance was made with G10 (Table 7). The genotype G10 made three highest distances with G6, G5 and G4. The second one was recorded in between G10 and G19 with 2.2510. The lowest distance was found between G14 and D15 with 0.3573 followed by G12 and G13, G13 and G14, G16 and G17, G12 and G14 with 0.3947, 0.4317, 0.4528, and 0.4934, respectively. By using these inter-genotypic distance intra-cluster genotypic distance were calculated as suggested by Singh and Chaudhury (1985).

Table 6. Intra (bold) and inter-cluster distances of 20 genotypes of Radish

Cluster	I	II	III	IV
I	**0.000**			
II	15.496	**1.0997**		
III	16.695	15.747	**0.7965**	
IV	13.075	13.538	4.311	**0.8011**

Table 7. Five highest and Five lowest inter genotypic distances among the 20 genotypes of radish.

SI. No.	Genotypic combination	Highest distance	SI. No	Genotypic combination	Lowest distance
1	G10 and G20	2.6384	1	G14 and G15	0.3573
2	G10 and G19	2.2510	2	G12 and G13	0.3947
3	G6 and G10	2.1168	3	G13 and G14	0.4317
4	G4 and G10	1.8563	4	G16 and G17	0.4528
5	G5 and g10	1.8015	5	G12 and g14	0.4934

4.3.5 Cluster means analysis:

Mean values of cluster for different characters are presented in the Table 8. It appeared that the dwarf genotype remain in cluster II (48.94) followed by cluster III (49.99). The tallest genotype remain in cluster IV (56.72) where as the second tallest remain in the cluster I (51.80). Considering maturity cluster IV (43.16) showed early maturity followed by cluster I (44.34) cluster III (43.59) and cluster II (43.56). When the leaf of radish consumed as vegetable then we may consider the traits number of leaf and shoot length. In case of number of leaf cluster I (15.34 ~ 15) showed the better performance followed by cluster IV (12.45) and cluster III (11.49). The lowest value for this character was in cluster II(11.34). In case of shoot length cluster II (40.56) contain the highest value whereas cluster IV (38.47) contain the second most highest value followed by cluster I (36.34). The lower most value on cluster III (35.92). Attention on root length, root breadth and root weight, the highest value contained in cluster I (18.80, 140.60) for root breadth and root weight, respectively whereas for root breadth cluster II (3.32) contain the highest value. The second highest value for root breadth, root length and root weight contained the cluster II. These findings were in accordance with Singh and Chauwdhary (1985).

4.3.6 Contribution of characters towards divergence:

Contribution of characters towards divergence was estimated through canonical variate analysis. In this method, vectors of canonical roots were calculated to represent the genotypes in graphical form (Rao. 1964). The co-efficient pertaining to the different characters in the first two canonical roots are presented in the Table 9. The positive absolute values of the two vectors revealed that only shoot dry weight had the greatest contribution to genetic divergence. The negative values for two vectors were for first flowering DAS and root breadth. However the positive absolute value of vector I and negative in vector II for the character like plant height, number of leaf per plant, root length, maturity DAS and shoot weight. Positive in vector II and negative in vector I for root weight, shoot length and root dry weight.

Table 8: Cluster means for 11 different characters of 20 genotypes of Radish

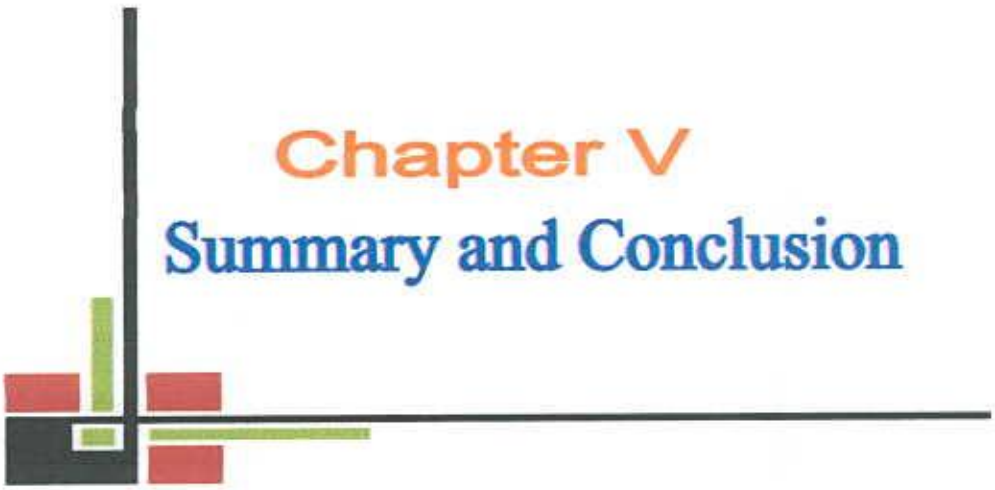
Characters	Cluster			
	I	II	III	IV
1. Plant height (cm)	51.80	48.94	49.99	56.72
2. First flowering DAS	54.00	40.02	41.00	41.77
3. Leaf number per plant	15.34	11.34	11.49	12.45
4. Root length (cm)	18.80	16.12	14.79	17.00
5. Root breadth (cm)	2.90	3.32	2.36	2.52
6. Root weight (g)	140.60	14.10	53.78	80.14
7. Days to maturity	44.34	43.67	43.59	43.16
8. Shoot length (cm)	36.34	40.56	35.92	38.47
9. Shoot weight (g)	183.00	128.83	72.77	104.34
10. Root dry weight (g)	16.20	9.77	4.61	8.08
11. Shoot dry weight (g)	14.00	8.04	6.31	9.37

Table 9. Relative contributions of the 11 characters to the total divergence in Radish.

Sl. No.	Characters	Vector I	Vector II
1	Plant height (cm)	0.3109	-0.3014
2	First flowering DAS	-0.0464	-0.0864
3	Leaf number per plant	0.5560	-1.5841
4	Root length (cm)	0.1149	-0.3841
5	Root breadth (cm)	-0.1568	-2.4990
6	Root weight (g)	-0.1619	0.0616
7	Days to maturity	1.3462	-1.2944
8	Shoot length (cm)	-0.2803	0.7168
9	Shoot weight (g)	0.0255	-0.1116
10	Root dry weight (g)	-0.2798	0.3805
11	Shoot dry weight (g)	0.0046	0.1792

4.3.7 Selection of genotypes as parent for hybridization program.

The main issue in plant breeding is detection and exploitation of various germplasm. According to Chaudhary *et al.*, 1974 there are three factors for selecting parents to conduct a breeding program these are relative contribution of the character to the total divergence, formation and selection of a particular group, and selection of a specific variety from this group. The knowledge related to genetic diversity of a specific crop is essential to choice better parent that help in maximizing genetic improvement (Rahman & Mamunur, 2011). Hiroshi, *et al.*, (2000) mention that more precise and absolute explanation of genotypes and model of genetic diversity could help to establish future breeding approach and facilitate the transfer of genetic information from one species to another of diverse genotype into the present profitable radish genetic base. The information among the group which contains the particular trait obtain from principal component analysis which is so important as it allow breeders to perform particular breeding program (Ellstrand, and Norman C., 1984). The parents which have genetically highest distant parent are usually able highest heterosis. Giving attention on the magnitude of cluster means for different characters, contribution of different characters towards the total divergence, magnitude of genetic distance and field performance the genotype G1, G2, G7, G9, G15, G16, and G19 from cluster IV, G4 and G6 from cluster II, G20 from cluster I and G11 from cluster III would be suitable for highest yield for future hybridization program.



Chapter V

Summary and Conclusion

CHAPTER V

SUMMARRY AND CONCLUSION

Collection of materials having genetic variation is the first step in a plant breeding program. For hybridization program the characters contributing to genetic diversity, estimation of the magnitude of genetic divergence in genotypes and assess the variability in respect of yield and some yield contributing characters are important to identify divergent parent. To study the genetic variability among the genotypes an experiment was conduct using 20 radish varieties at the experimental site of Sher-e-Bangla Agricultural University, Dhaka, during the Rabi (November 2016 to January 2017) season. The summary of the finding are presented here on the basis of character studied.

Data of plant height, root length, root breadth, root weight, shoot length, shoot weight, root dry weight, shoot dry weight, first flowering DAS, and maturity DAS were collected at harvesting stage for each varieties. Highly significant difference among the varieties in each character was pragmatic. Considering mean performance for appendix IV G5 is tall and G4 is dwarf. In case of number of leaf per plant G2 had lowest and G20 had highest. The root length had highest value in G19 and lowest in G8. Considering root breadth the highest value showed G5 and G18 and root weight highest value showed G4 and lowest value in G10. In case of shoot length, shoot weight, root dry weight, shoot dry, first flowering DAS and maturity DAS, the highest value contained the G9, G19, G19, G1 G15, G4, respectively and the lowest value contained G11, G10, G10, G16, G18, G3, respectively.

There were four distinct cluster formed such as cluster I: G20, cluster II: G4, G6, cluster III: G10, G11, G12, and G13, cluster IV: G1,G2,G3, G5, G7, G8, G9, G14, G15, G16, G17, G18, G19. The highest cluster distance was between cluster I and cluster III which was 16.695 determine using D^2 formula. This distance followed by cluster II and cluster III which was 15.747, cluster I and cluster II (15.496), cluster II and cluster IV(13.538), cluster I and cluster

IV(13.075). The lowest distant between cluster III and cluster IV (4.311). The cluster was formed on the basis of similar morphology, and this clustering was not influenced by origin of varieties.

Finally it could be concluded that plant height, number of leaf per plant, root length, root breadth, root weight, shoot length, shoot weight, root dry weight, shoot dry weight, first flowering were found the major component of genetic diversity in the radish varieties. Taking into account the genetic diversity and other performance G1(BD- 4286), G2(BD - 4287), G4(BD-4289), G6(BD - 4291), G7(BD - 4292), G9(BD - 4294), G11(BD - 7076), G15(BD - 7764), G16(BD - 7765), G19(BD - 10436), G20(BD - 10435), were considered to be hopeful parents for potential hybridization effort. The parents those are genetically distant have higher possibility in heterosis production. Due to higher heterosis their progenies which may be scrutinized by crossing and promising varieties of radish.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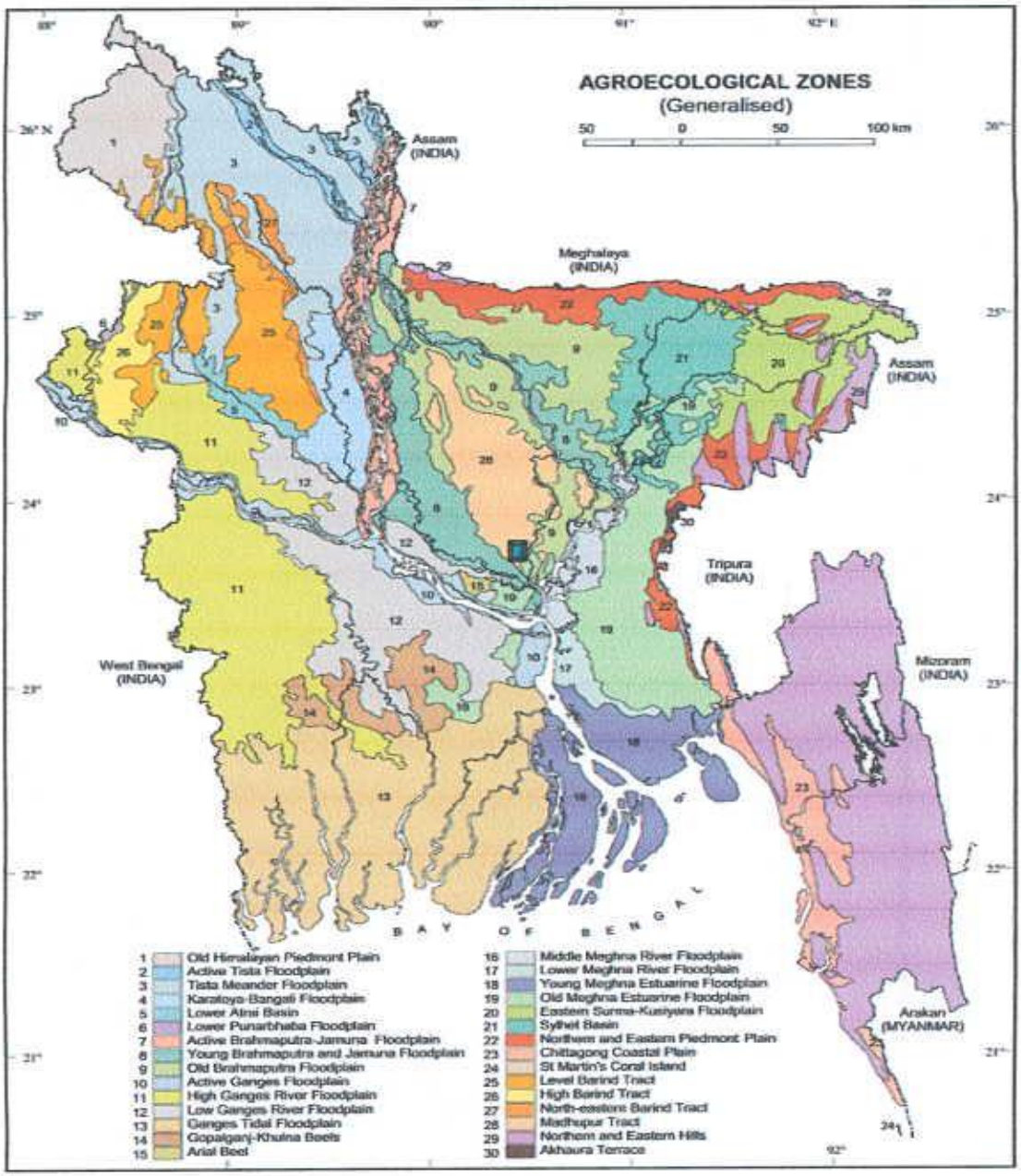
Appendices

APPENDICES

Appendix I. Map showing the experimental site under the study



The experimental site under study

Appendix II. Monthly records of air temperature, relative humidity, rainfall and sunshine hours during the period from November 2016 to January 2017

Month	Year	Monthly average air temperature (°C)			Average relative humidity (%)	Total rainfall (mm)	Total sunshine (hours)
		Maximum	Minimum	Mean			
Oct	2012	29.36	18.54	23.95	74.80	Trace	218.50
Nov	2012	28.52	16.30	22.41	68.92	Trace	216.50
Dec.	2012	27.19	14.91	21.05	70.05	Trace	212.50
Jan.	2013	25.23	18.20	21.80	74.90	4.0	195.00
Feb.	2013	31.35	19.40	25.33	68.78	3.0	225.50
Mar.	2013	32.22	21.25	26.73	72.92	4.0	235.50

Source: Bangladesh Meteorological Department (Climate division), Agargaon Dhaka-1212.

Appendix III. The mechanical and chemical characteristics of soil of the experimental site as observed prior to experimentation (0 - 15 cm depth).

Mechanical composition:

Particle size constitution

Sand	:	40%
Silt	:	40%
Clay	:	20%
Texture	:	Loamy

Chemical composition:

Soil characters	:	Value
Organic matter	:	1.44 %
Potassium	:	0.15 meq/100 g soil
Calcium	:	3.60 meq/100 g soil
Magnesium	:	1.00 meq/100 g soil
Total nitrogen	:	0.072
Phosphorus	:	22.08 µg/g soil
Sulphur	:	25.98 µg/g soil
Boron	:	0.48 µg/g soil
Copper	:	3.54 µg/g soil
Iron	:	262.6 µg/g soil
Manganese	:	164 µg/g soil
Zinc	:	3.32 µg/g soil

Source: Soil Resources Development Institute (SRDI), Khamarbari, Dhaka

Appendix IV: Mean performance of various growth parameter and yield components of 11 characters of 20 genotypes of Radish.

genotypes	PH	NLPP	RL	RB	RW	SL	SW	RDW	SDW	FF	DM
G1	51.97	12.87	16.67	2.745	74.67	36.87	96.27	5.88	16.4	34	43.34
G2	56.6	9.40	18.07	2.695	76.34	38.13	104.13	6.14	9.977	37	43.66
G3	54.54	11	16.87	2.36	72.37	36.14	111.47	5.784	5.82	44	41
G4	44.60	10.87	18	3.295	160.80	41.94	129.2	5.19	9.88	44.33	46
G5	71.80	11.79	20.8	3.80	78.9	50.8	132.14	7.66	10.47	43	42.67
G6	56.75	11.8	14.25	3.35	134.7	39.177	128.47	14.35	6.2	35.7	42
G7	60.8	12.94	16.45	2.315	104.67	42.3	107.4	7.15	7.9	33	44.34
G8	56.53	13.87	8.80	3.155	79.47	38.114	100.87	9.38	7.94	40	43.34
G9	58.87	13	13.89	3.035	90.9	53	133.87	7.03	14.417	39	43
G10	48.93	11.3	12.74	2.71	33.20	34.53	41.20	2.10	4.55	37	43.34
G11	48.87	11.8	19.2	2.135	68.67	26.80	63.4	4.88	6.37	33	43.67
G12	49.67	10.94	13.73	2.45	51.8	36.53	88.14	4.45	6.57	49	43.33
G13	52.47	11.93	13.47	2.145	51.67	40.87	93.94	6.4	7.75	45	44
G14	51.06	12.47	16.53	1.945	70.2	34.34	89.47	5.79	8	50	42
G15	58.67	11.67	14.34	1.914	78.67	35.27	87.34	5.52	6.83	64	43.34
G16	55	11.87	18.14	3.05	81.07	37.4	80.2	9.68	4.917	42	43.34
G17	55.87	13	16.6	2.095	75.27	34.8	84.94	12.7	5.65	50	43.67
G18	49.94	13.74	19.5	1.55	73.8	34.4	96.01	8.285	7.5	32	43.34
G19	57.2	13	28	2.53	85.5	39.4	132.34	14	16	35	43
G20	51.8	17.60	18.8	2.9	140.6	36.34	198	17.35	14	54	44.34
Mean	54.3505	12.29	16.557	2.5902	83.8885	38.05975	104.41	7.95845	8.8570	42.0515	43.353

PH= Plant height; NLPP= No. of leaf per plant; RL= Root length; RW= Root weight; SL= Shoot length; SW= Shoot weight; RDW= Root dry weight; SDW= Shoot dry weight; FF= First flowering; DM=Days to maturity.

Appendix V: Analysis of variance of 11 yield and yield contributing characters of radish

Sources of variation	df	PH	NLPP	RL	RB	RW	SL	SW	RDW	SDW	FF	DM
Genotype	19	78.53 **	4.43 **	19.65 **	0.53 **	2200.97 **	52.33 **	2578.88 **	33.7793 **	36.49 **	194.54 **	2.07
Replication	2	32.30	0.39	7.07	0.0023	189.48	3039	2080.03	2.10	2.86	10.55	1.72
Error	38	10.39	0.94	5.30	0.069	175.30	14.07	291.07	4.84	4.26	0.84	2.01

- * significant at 5% level of probability
- ** significant at 1% level of probability

PH= Plant height; NLPP= No. of leaf per plant; RL= Root length; RW= Root weight; SL= Shoot length; SW= Shoot weight; RDW= Root dry weight; SDW= Shoot dry weight; FF= First flowering; DM= Days to maturity.

Appendix VI: Z_1 - Z_2 scors of 20 genotypes or Radish

Genotype	PC1	PC2
G1	12.18	-1.08
G 2	5.35	-5.45
G 3	2.83	-12.52
G 4	-59.34	31.08
G 5	-18.18	-25.96
G 6	-51.85	21.73
G 7	-16.12	12.37
G 8	5.6	-1.1
G 9	-26.78	-15.21
G 10	71.69	10.13
G 11	41.26	17.7
G 12	33.85	-12.06
G 13	29.31	-16.66
G 14	20.41	0.04
G 15	15.98	5.15
G 16	19.81	13.64
G 17	19.93	5.74
G 18	13.35	-0.01
G 19	-22.47	-17.72
G 20	-96.82	-9.81