GENETIC DIVERSITY ANALYSIS FOR PHYSIOLOGICAL TRAITS IN RADISH (*Raphanus sativus* L.)

BY

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CERTIFICATE

*This is to certify that thesis entitled, "**GENETIC DIVERSITY ANALYSIS FOR PHYSIOLOGICAL TRAITS IN RADISH (Raphanus sativus 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 & PLANT BREEDING,** embodies the result of a piece of bona fide research work carried out by **MD. MONIRUL ALAM,** Registration No. **11-4676** 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: December, 2012
Place: SAU, Dhaka.

Prof. Abu Akbar Mia
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DEDICATED

TO

MY BELOVED PARENTS

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ABSTRACT

A field experiment was conducted at the Agricultural Farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh during the rabi season of November 2011 to February 2012 to characterize and genetic diversity analysis of 21 radish varieties based on some physiological characters. Four distinct clusters were observed among the radish varieties on the basis of total dry matter (TDM), relative growth rate (RGR), crop growth rate (CGR), leaf area index (LAI), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), leaf number per plant, harvest index (HI), days to harvest and root yield per plant. Cluster IV consisted of the highest number of varieties, viz., Mino Early Long White (Ufsi Mula), BARI Radish-3 (Druti), Mino Long White, Early White-35, Chamak, Nong Woo Bio (F_1 hybrid), Radish Royal-40, White Prince (F_1 hybrid radish). Cluster II composed of Lucky (Early 35 Days), Paira-40, Bright White 40, Rupsa, Tasakisan (China), Rocky-45. Cluster III composed of Snow White (F_1 hybrid), BARI Radish-2 (Pinky), BARI Radish-4, Barisal Local, Ramboo-40. BARI Radish-1 (Tasakisan) and Ivory White formed cluster I. The highest root yield was observed in cluster I (380.06) which also produced the highest TDM and LAI. The longest cluster distance was observed between cluster I and cluster II. Clustering pattern was not influenced by the genotypic origin. Ivory white produced the highest root yield, TDM, and LAI which was followed by BARI radish-1. Ivory white did not flower in growing season. BARI radish-2 produced the highest RGR and NAR. Rupsa produced the highest harvest index. Leaf area index, LAR, LWR and leaf number per plant were the major components of genetic divergence in the radish varieties. Considering genetic diversity and other performances, BARI radish-1, BARI radish-2 and Rupsa appear to be promising parents for future hybridization program.

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LIST OF ABBREVIATION

AEZ	Agro-Ecological Zone
Anon.	Anonymous
BARC	Bangladesh Agricultural Research Council
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BRAC	Bangladesh Rural Advancement Committee
BRRI	Bangladesh Rice Research Institute
CARE	Co-operations for American Relief Everywhere
CGR	Crop Growth Rate
CLSA	Clustering Analysis
CVA	Canonical Vector Analysis
CVSRC	Citrus and Vegetable Seed Research Centre
CV %	Percent Coefficient of Variance
cv.	Cultivar (s)
DAE	Department of Agricultural Extension
DAE	Days After Emergence
DAS	Days After Sowing
DAT	Days After Transplanting
e.g.	*exempli gratia* (L), for example
et al.	And others
etc.	Etcetera
FAO	Food and Agricultural Organization
GA	Genetic advance
GCV	Genotypic Coefficient of Variation
HI	Harvest Index
h^2b	Heritability in Broad Sense
i.e.	that is
IRRI	International Rice Research Institute
Kg	Kilogram (s)
LAI	Leaf Area Index
LAR	Leaf Area Ratio
LWR	Leaf Weight Ratio

LSD	Least Significant Difference
M.S.	Master of Science
m^2	Meter squares
MP	Muriate of Potash
NAR	Net Assimilation Rate
NS	Non significant
PCA	Principal Component Analysis
PCO	Principal Coordinate Analysis
PCV	Phenotypic Coefficient of Variation
RGR	Relative Growth Rate
RCBD	Randomized Complete Block Design
SAU	Sher-e-Bangla Agricultural University
SRDI	Soil Resource and Development Institute
t ha^{-1}	Ton per hectare
TDM	Dry Matter
TSP	Triple Super Phosphate
UNDP	United Nations Development Programme
var.	Variety

CHAPTER I
INTRODUCTION

Radish (*Raphanus sativus* L.) is a popular and widely grown pungent root vegetable; its tender leaves and shoots are used as green. It is roughly divided into two types - one is European origin and the other Far Eastern origin, Shinohara (1984). Kumazawa (1956) divided the world diversity of radish into five groups: European type (having three groups), and Far Eastern or Asiatic type (having two groups)- North China type and South China type. The South China type had spread into South East Asia and developed into innumerable local genotypes. General characteristics of this type are: (i) wide adaptability from temperate to warm temperate, and tropics to sub-tropics with various ecotypes, (ii) medium to late maturity- 70-100 days from sowing, (iii) leaves are deeply lobed and have least or no pubescent, and (iv) large, solid, juicy, fleshy root.

Radish is a popular vegetable in Bangladesh. It's fleshy edible root is rich in Ca, K, P and vitamin C; Srinivas and Naik (1990). Tender leaves of radish is a good vegetable which contain high vitamin A ;Saha (1990). Radish seeds are potential source of a non-drying fatty oil used in soap, illumination and edible purpose Sadu (1986). It is grown mainly for its swollen hypocotyl, i.e., young tender swollen roots which are eaten raw as salad or cooked as vegetable. Roots are also rich in carbohydrate and protein (Gopalan and Balasubramanium, (1966) Anon. 1980). It is widely cultivated in the cool season of Bangladesh covering an area of 57,695 ha and producing 21,0895 t edible roots. It ranks second both in acreage and production among the major vegetables crops of Bangladesh (BBS, 2011).

Radish, as a quick growing vegetable crop, helps in increasing cropping intensity. Most of the high yielding radish varieties are hybrids. The quantity of hybrid seeds being imported annually in Bangladesh is 45.45 tones (Ahmed and Hossain, 1985) at the cost of valuable foreign currency. Thus, this crop reasonably gained priority for improvement in Bangladesh condition.

Evaluation of genetic divergence is important in order to know the source of genes for a particular trait within the available germplasm. Genetic divergence is a useful tool for an efficient choice of parents for hybridization to develop varieties with high yield potential and better quality.

For the development of suitable varieties of radish, it is essential to evaluate the characters of the available germplasm properly and conserve the collected materials for future use. Variability is a touch-stone to the breeders to evolve high yielding varieties through selection, either from the existing genotypes or from the segregates of a cross. Hence, the genetic information on yield and yield contributing characters of the crop species need to be properly assessed for its improvement. This is difficult to know what proportion of the observed variability is for genetic effect. So, it is important to study the genetic parameters, like the genotypic and phenotypic coefficient of variations. On the other hand, duplication of germplasm should be avoided for mid or long term conservation of the collected germplasm. For a rapid and efficient plant breeding programme, knowledge of relationship among the yield and yield contributing characters is necessary.

The qualitative and quantitative improvement of a crop depends on the available gene pool and its manipulation. The assessment of genetic diversity of plant population may be carried out in different ways, such as, characterization and evaluation of physiological traits and molecular markers. The utility of multivariate analysis for measuring the degree of divergence and for assessing the relative contribution of different plant characters to the total divergence in cross-pollinated vegetable crops has been established by sveral workers; Anand and Rawat, (1984) Balasch *et al.*, (1984) Ariyo, (1987) and Patil *et al.*, (1987).

Research on varietal improvement in Bangladesh and elsewhere are mainly concerned with physiological characters. Thurling (1974), Nasayrov (1978), Chauhan and Bhargava (1984) and Whan *et al.*,(1991) suggested that the value of any physiological characters should be assessed in breeding populations therefore it is utilized by the plant breeders. Moreover, being a short duration and quick growing crop growth, parameters and physiological characters are of great importance for radish ; Krug *et al.*, (1989) and Lederle (1989).

Correlation coefficients between characters, which help in construction of selection indices is very important. Correlation between characters may be misleading due to complex interrelations and may not provide the exact picture of influence of one character over others. Splitting correlation into direct and indirect effects, therefore, would provide more meaningful interpretation. Thus correlation in combination with path coefficient analysis is required to assess quantitatively direct and indirect influences of characters attributing fruit yield. Selection of superior genotypes is mostly made on phenotypic values, which are subject to environmental factors. Phenotypic values are correlated with the genotypic values of the characters. Such correlations may vary due to agro-ecological variation from year to year.

Through genetic diversity analysis, several workers have recommended the physiological characters as selection criteria in selecting parents for breeding programme [Yadav and Hari SIngh (1988) in Indian mustard; Lysenko and Shevtsov (1988) in sugarbeet; Agarkova *et al.*, (1988) in pea; Bartual *et al.*, (1985) in soybean].

Genetic variability can offer opportunity for the effective selection for a high yielding desirable plant. The plant breeders are, therefore, always interested to know the genetic divergence of varieties. Considering the above facts, the present study was under taken to

a) Characterize the radish germplasm and to study the genetic diversity,

b) Assess the contribution of different traits towards divergence and

c) Screen out the suitable varieties for future hybridization programme.

CHAPTER II

REVIEW OF LITERATURE

Radish is one of the most popular vegetable crops in both tropical and temperate regions. However, as stated earlier a very little research works have been done in Bangladesh on characterization of germplasm and genetic diversity. In Bangladesh and elsewhere in the world research effort on genetic diversity analysis of radish seems to be rare. Therefore, information available in the literature pertaining to the genetic diversity analysis of radish and some other open pollinated vegetable and root crops are reviewed in this chapter under the following sub-headings:

2.1 Genetic diversity in radish

Genetic diversity means genetic distance which is the function of heterosis; Falconar (1960). In radish, genetic diversity was studied based on allozyme variation; Ellustrand and Marshall (1985). Genetic divergence study revealed that the cultivars of different color and similar root shape or those of the same colour and different root shape did not cluster.

2.1.1 Genetic diversity for morphological and physiological characters

Genetic diversity based on different morphological and physiological characters in some crops are reviewed in the following paragraphs.

Agarkova *et al*., (1988) analyzed the importance of morphological and physiological characters in pea by principal component analysis (PCA). They recommended the use of particular indices of photosynthetic activity in breeding programme.

Bartual *et al*., (1985) grouped 125 soybean lines using PCA, maximum likelihood factor analysis and cluster analysis based on physiological and morphological characters. The identified groups were quite stable in their performance observed in changed environments. Some lines were identified as parents for future use in a breeding programme to improve agronomic traits.

Lynsenko and Shevtsov (1988) reported the physiological study of quantitative characters in sugar beet using PCA. Four important components were identified such as leaf number, leaf area, leaf orientation and growth rate. They have emphasized the components for selection criteria in sugar beet.

2.1. 2 Relationship between genetic and geographic diversity in crops

In self-pollinated crops, genetic diversity is not influenced by their geographical distribution [Hussaini *et al*., (1977) Dani and Murthy (1985) Prasad *et al*., (1988) and Kumar (1989)]. But there were some contradictory results in case of open pollinated crops.

Anand and Rawat (1984) studied the genetic diversity, combining ability and heterosis in brown mustard in a set of 50 geographically diverse (including 10 genotypically diverse) lines. The clustering pattern suggested that geographical diversity of a line is not necessarily an index of its genetic diversity.

Ariyo (1987) reported the importance of multivariate analysis in choosing parents for hybridization in okra with 30 genotypes. The genotypes were grouped into five clusters. But there was no relation between clustering pattern and eco-geographical distribution.

Juned *et al*., (1988) investigated genetic diversity in 22 accessions of wild potato from Paragua and Argentina. They observed a close relationship between the geographical groups using PCA, cluster analysis and genetic diversity indices.

2.1.3 Genetic diversity and multivariate techniques

Genetic diversity analysis is mainly based on different multivariate techniques. During last decade different multivariate techniques are developed which may be due to the improvement of computer. However, literature related to efficient multivariate techniques for genetic diversity analysis are reviewed in the following paragraphs.

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 distance. Three methods gave similar results. But factorial discriminant and Mahalanobis's D^2 distances method 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 adoption in chickpea using Mahalanobis's D^2 values, canonical analysis and PCA. The results obtained from Mahalanobis's D^2 and canonical analysis were confirmed by PCA.

They also reported that it may be simpler to represent multivariate analysis in a two-dimensional chart (Z_1-Z_2) 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 method. 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 *et al.*, (1989) 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 al all clear. Therefore, they have suggested non-hirarchical classification, as an alternative approach to optimize some suitabilities choosing criteria directly from the data matrix. They also reported that the squared distance between means are Mahalanobis's D2 statistics when all the dimensions are used, can be computed using principal co-ordinate analysis (PCO). They also recommended the Canonocal Vector Analysis (CVA) for discriminatory purpose.

Digby *et al.*, (1989) reported that the co-ordinates obtained from the PCA is used as input of PCO analysis to calculate the 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.

2.1. 4 Morphological and physiological characters and their relationship

Relationship between different morphological and physiological characters are important as they are used as selection criteria in crop improvement. Some related relationships between morphological and physiological characters are reviewed here.

Ling *et al.*, (1985) studied heterosis and combining ability in radish. Among the 12 hybrids, only one hybrids showed positive heterosis over better parent for root length but nine hybrids showed positive heterosis over better parent for leaf area which was positively correlated with root weight.

Yadav and Hari Singh (1988) reported selection indices based on the importance of crop growth rate, net assimilation rate (NAR), leaf area index (LAI) and leaf area duration (LAD) in Indian mustard. Leaf area index and LAD exhibited superiority over straight selection for yield. The physiological basis of seasonal variation in growth and yield of potato. The cultivar producing greater total dry matter (TDM) gave the highest yield. Higher tuber yield in summer was attributed to longer crop duration, higher harvest index, LAI and NAR. The difference in TDM among the cultivars was due to differences in leaf area.

Mankyu and Ohnishi (2001) reported that *R. sativus* var. hortensis f. raphanistroides (wild radish: Brassicaceae) is an insect-pollinated wild plant that grows mainly on beaches in East Asia. Starch gel electrophoresis was used to investigate the alloenzyme diversity and genetic structure of 25 Japanese and 9 Korean populations of this plant. Although the Korean populations were small, isolated, and patchily distributed, they maintained a high level of genetic diversity; the average percentage of polymorphic loci was 63.1%, the mean number of alleles per locus was 2.27, and the average heterozygosity was 0.278. The corresponding estimates for these parameters in the Japanese populations were 53.3, 2.26 and 0.278%. These estimates are considerably higher than those from species with similar life history and ecological characteristics, but they are lower than those from R. raphanistrum, the wild radish that grows in Europe and the USA. The combination of an insect-pollinated, outcrossing breeding system, large population sizes, gene flow from cultivated radish population, and a propensity for high fecundity may explain the high level of genetic diversity within wild populations.

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Singh and Kumar (2000) conducted an experiment with 21 collections of radish from different regions was conducted during the winter season of 1997-98, in Pithoragarh, Uttaranchal, India. Observation on 13 morphological traits were recorded. The 21 accessions were grouped into 6 clusters. The strength of the cluster varied from one each in cluster III and 5 to 10 in cluster I. Estimates of cluster means and standard deviation for each trait showed that cluster II consisted of accessions having maximum root per leaf ratio, root yield per plot and minimum days taken in maturity. The maximum number of leaves per plant, the broadest leaves and the maximum root diameter characterized cluster III that contained only one accession. Accessions with maximum leaf length and root weight per plant were grouped in cluster IV whereas those with minimum leaves per plant, leaf weight per plant and maximum root length, root length and diameter ratio, number of roots per plot and biological yield per plot were group into cluster VI. The intracluster distance varied from maximum of 2.73 in cluster I to 0.00 in each of clusters III and V. Intercluster distance values were found maximum between clusters III and VI (7.60) followed by clusters III and V (6.88), clusters IV and V (6.80), clusters II and III (6.50) and clusters I and III (6.04). Fairly high intercluster distance was also observed between clusters III and IV, IV and IV, and V and VI.

Huh and Ohnishi (2002) studied genetic diversity and genetic structure of East Asian wild radish (*Raphanus sativus* var. *hortensis f. raphanistroides*), 13 natural populations from Japan and Korea were analysed 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 H< sub>O</ sub>=3.486 and genetic diversity H< sub>EP</ sub>=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 alloenzymes 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*.

Fayaz *et al*., (2003) conducted at the Agricultural Research Institute for Northern Areas Juglote, Gilgit, Pakistan during 2002-03 to evaluate the performance of 7 cultivars of radish i.e. Green Neck, 40-Days, Minowase, Chinese Red, Mino Early, Green Meat and Red Round. The experiment was laid out in Randomized Complete Block design with three replications. Significant variations were found in days to maturity, root length, root weight per plant, root yield, while the differences in root diameter were non-significant. The minimum number of days to edible maturity was obtained from 40-days (48.35), while the maximum number of days to maturity (68.45) was recorded in Minowase. Mino Early showed the highest root length (31.72 cm), root weight (476.50 g plant-1) and root yield (42.23 t ha-1), followed by Green Neck with a root length of 27.50 cm, root weight of 458.30 g plant-1 and a root yield of 40.37 t ha-1. Red Round produced the highest root diameter (5.75 cm). Based on these findings, Mino Early is recommended for the growers in the Northern Areas of Pakistan.

Kumar and Singh (2003) studied sixty-two radish genotypes were screened for resistance to Alternaria blight, caused by A. brassicae, in field experiments in Kanpur, Uttar Pradesh, India. Disease incidence was graded at 60 days after sowing. The genotypes in field experiments were further tested in pot trials under artificial epiphytotic conditions in 1998-99. Potted plants were inoculated using 15-day-old fungal cultures, and disease incidence was scored at 30 days after inoculation. None of the 62 genotypes was completely free from disease, but seven genotypes (Accessions 7401, 6802, 8801, 8803, 8805, Pusa Desi and Jaunpuri) were rated as resistant to Alternaria blight. Eight genotypes were moderately resistant (7212, 7110, 7108, 7208, 7218, 8804, S-271 and IHR-1), while the others were susceptible and highly susceptible.

Li (2006) reported the inheritance of leaf and root morphology were studied using of five radish cultivars. The genetics of leaf morphology is controlled by one pair of genes in the nucleus, the platy leaf is dominant on the deep lobated leaf. Red leafstalk is partly dominant on green one, meanwhile purple flesh gene showed influence on the inheritance of leafstalk colour. The root length and width are controlled by partly-dominant genes in the nucleus. When Xinlimei, which has green skin and purple flesh, was crossed with cultivars with green or white skin, the skin colour of F1 were purple or light purple. It is indicated that purple flesh gene can influence the inheritance of skin colour. When white flesh cultivar was crossed with green one, the flesh colour of F1 was light green. When purple flesh cultivar was crossed with the white one, the flesh colour of F1 was white with purple core.

Premathilake (2001) reviews sweet potato germplasm collection and crop improvement programmes conducted in Sri Lanka. The current status on sweet potato germplasm research is also discussed with emphasis on the current number of accessions with passport data, morphological characterization and evaluation data, number of accessions with farmer's knowledge, establishment of a germplasm collection, and problems and constraints of field gene bank management. Future concepts are also briefly mentioned.

Huang (2005) studied Differential Scanning Calorimetry (DSC) and Rapid Visco-Analyzer (RVA) were used to determine the starch gelatinization characteristics during the growth of three sweet potato cultivars. The results showed that the starch contents of three sweet potato cultivars all decreased as growth progressed. Changes of the amylose content at different harvesting periods could be discriminated according to the cultivars. At the early harvests amylose contents of Xushu18 and Zheda9201 were relatively high, but those of Zhe3449 were low. As the growth duration prolonged, the peaks of DSC thermograms tended to occur at a low temperature and not to be so obtrusive with the increased width of the peak. Obvious decreases in values of onset, peak and conclusion temperatures, as well as enthalpy of phase transitions, were observed as growth time lengthened. The peak viscosities, as determined through RVA, showed a rising tendency as growth progressed. In addition, statistical analysis revealed that there were correlations between the amylose content and gelatinization characteristics to some extent, which were affected by genotypes evidently.

Teshome (2003) conducted in 2000-01 in Tamil Nadu, India to investigate the genetic diversity for yield and yield contributing traits in 15 sweet potato clones. The clones were grouped into 5 clusters: cluster I contained Sree Rathna and RNSP-1; cluster II contained S-1086, Co-3, CO2-160 and NDSP-10; cluster III contained BCSP-7, S-1221, CO2-117 and Pol-21-1; cluster IV contained CO2-179 and CO2-156; and cluster V contained Pol-13-4, IGSP-8 and IGSP-9. The most genetically divergent clusters were clusters III and V. Results indicate that hybridization involving the genotypes from cluster I, III and V may produce highly heterotic hybrids. Geographical origin was not correlated with genetic divergence.

Ahmed (1998) studied the genetic divergence of 90 accessions of sweet potato was investigated. Multivariate analysis of divergence among the accessions for 12 characters (plant type, petiole length, vine growth rate, vine pigmentation, mature leaf shape, foliage colour, total vine length, storage root skin and flesh colour, yield of storage root at 115 and 140 days after planting (DAP), and dry matter content) led to their grouping into 6 clusters. No relationship was found between the genetic divergence and geographic distribution of the accessions. The highest contributions towards total divergence were made by the yield of storage root at 115 DAP (32.57%) and at 140 DAP (19.42%), which were the most important characters responsible for the grouping of the accession on a genetic basis. The next important characters were dry matter content (13.07%) and petiole length (9.72%). The inter-cluster D2 values varied from 6.40 (between clusters III and V) to 830.59 (between clusters II and VI); the intra-cluster D^2 values varied from 1.77 (cluster IV) to 3.60 (cluster VI). Based on inter-cluster distance, their contribution to D^2 values and agronomic performances, accessions SP-484 and SP-527 from cluster I; SP-108, SP-161 and SP-161 from cluster II; SP-161 and SP-161 from cluster III; SP-281, SP-296 and SP-396 from cluster IV; SP-436 and SP-495 from cluster V; and SP-013 and SP-395 from cluster VI were recommended for use as parents for future hybridization programmes.

Oliveira (2000) conducted to evaluate the genetic divergence among 51 sweet potato clones from different regions in Brazil. A randomized complete block design with 3 replications was used. Each plot consisted of 3 rows of 3.0 m with the plants in the spacing of 1.00 x 0.25 m. Five competitive plants located within an area of 2.0 m2 in the central row were evaluated for 25 characteristics. Genetic divergence was estimated using Mahalanobis' generalized distance and 2 grouping methods, the Tocher's grouping and the hierarchical nearest neighbour. Large genetic variability was detected among the clones, which were then grouped into 7 heterotic clusters by the 2 grouping methods. There was a good agreement in the formation of the groups between the 2 methods. The occurrence of small genetic distances among some clones and the inclusion of more than one clone into a specific group indicates the possibility of duplications among the evaluated genotypes.

Biswas (2010) selected divergent parents genetic diversity was estimated among twenty genotypes. Thirty F_1 progenies developed by line-tester mating were studied from seedling generation to first clonal generation for five important agronomic traits. Cluster analysis reveals that the parents could be grouped into seven different clusters. Cluster means showed wide range of variation for several traits among singles as well as multi genotypic clusters. Considering diversity pattern, parents should select from cluster I, III, IV and V for the improvement of potato. Analysis of variance revealed that all most all the sources of variation were highly significant for all the studied traits in both generations. Parents Challisha, Lalpakri, Patnai, Chamak, Sadagoti, TPS-67 and TPS-364 were found to be good general combiners for tuber yield and yield contribution traits due to their gca effects. The sca effects showed that out of 30 hybrids 12 were found to have specific combining ability for tuber yield and those hybrids also exhibited considerable heterosis for tuber yield and yield contributing traits.

Mareen and Radhakrishnan (2004) studied sixty Coleus (Solenostemon rotundifolius) accessions collected from different eco-geographical regions of Kerala and neighbouring states indicated genetic diversity among themselves. High heritability was observed for tuber yield, harvest index, biological yield per plant, tuber volume and tuber weight. The sixty accessions were grouped into ten clusters which indicated that factors other than geographical distribution are responsible for clustering and that there is no parallelism between genetic diversity and geographical distribution. EMS [ethyl methansulfonate]-induced genetic variability in Coleus was evaluated based on establishment of mutants under field conditions. The response of Coleus to the mutagen varied with concentration and genotype. EMS changed the size and number of tubers and plant height, and induced photo insensitivity. From the field performance of the selected mutants, M 131 and M 61 were selected for year round cultivation.

CHAPTER III

MATERIALS AND METHODS

This chapter presents materials and methods which were used in carrying out the experiments under investigation. In this chapter the details of different materials used and methodologies followed during the experimental period are presented under the following heads:

3.1 Experimental period

The experiment was conducted at the Agronomy Farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh during the rabi season of November 2011 to February 2012. Details of materials and methods used in this experiment are given below.

3.2 Description of the experimental site

The experimental site is geographically situated at $23^{0}41'$ N latitude and $90^{0}22'$ E longitude at an altitude of 8.6 meter above sea level. For better understanding about the experimental site has been shown in the Map of AEZ of Bangladesh in appendix I.

3.3 Climate

The experimental site under the sub-tropical climate that is characterized by cold temperature and minimum rainfall are the main features of the rabi season. The weather conditions during experimentation such as monthly rainfall (mm), mean temperature (^{0}C), sunshine hours and humidity (%) are presented in appendix II

3.4 Soil

The soil of the experimental field belongs to the general soil type, Shallow Red Brown Terrace Soils under Tejgoan Series. The land was above flood level and sufficient sunshine was available during the experimental period. Soil samples from 0-15 cm depth were collected from the experimental field. The soil analyses were done at Soil Resource and Development Institute (SRDI), Dhaka. The physicochemical properties of the soil are presented in appendix III.

3.5 Land Preparation

The main land selected for conducting the experiments was opened in mid september with a disc plough. The ploughing was followed by harrowing and laddering to obtain a good *tilth*. The surface was leveled and finally irrigation and drainage channels were prepared around the plots. The unit plots were prepared as raised beds. Two adjacent unit plots and blocks were separated by 50 cm and 1.0 m distance respectively.

3.6 Radish varieties

Radish varieties used in this experiment are being cultivated throughout the country. Probable origin and observed morphological features of the varieties are summarized in table 1.

3.7 Source of seeds

The seeds of the 21 radish varieties were collected from different places of Bangladesh. Breeder seeds of the variety pinky and Tasaki San were collected from Citrus and Vegetable Seed Research Centre (CVSRC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The seeds of the Barisal local were collected from Barisal. The seeds of the rest varieties were collected from different markets of the country.

3.8 Design and Layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The unit plot size was $27m^2$. Row to row and plant to plant distances were 75cm and 45cm, respectively. The varieties were randomly distributed to the experimental plots.

Table1 Probable origin and distinct characteristics of 21 radish varieties

Sl. No.	Varieties	Origin	Distinct characteristics	
1	BARI Radish-1 (Tasakisan)	Bangladesh	Leaf-erect, smooth and non-serrated, root color-whitish.	
2	Radish:Lucky (Early 35 Days)	China	Leaf-flat, spiny, rough, non-serrated with whitish vein, root color-whitish, and oval shaped root.	
3	Snow White (F_1 hybrid)	Bangladesh	Leaf-rough, spiny, rough, serrated with whitish vein, root color-whitish, root shape-tapering towards upper side and swollen towards lower side.	
4	BARI Radish-2 (Pinky)	Bangladesh	Leaf-erect, non-serrated with pink midrib; root color-pink.	
5	Mino Early Long White (Ufsi Mula)	Korea	Leaf-flat,spiny,rough,non-serrated with whitish vein, root color-whitish,	

6	Paira-40	India	Leaf-flat,smooth and non-serrated,root color whitish.	
7	BARI Radish-3 (Druti)	Bangladesh	Leaf-flat,spiny and non-serrated, root color-whitish and oval shaped root.	
8	Bright White 40	India	Leaf-rough, spineless and non- serrated with whitish vein, root color-whitish.	
9	Mino Long White (Japanese White)	India	Leaf-rough, spiny and serrated, root color-whitish and oval shaped root.	
10	BARI Radish-4	Bangladesh	Leaf-rough, spiny and non-serrated with whitish vein, root color-whitish.	
11	Early White-35	Japan	Leaf rough, spiny, rough, serrated with whitish vein, root color-whitish, root shape-tapering towards upper side and swollen towards lower side.	

12	Chamak	India	Leaf-flat, spiny, rough, non-serrated with whitish vein, root color-whitish.	
13	Nong Woo Bio (F_1 hybrid)	India	Leaf-rough, spiny, rough, serrated with whitish vein, root color-whitish, root shape-tapering towards upper side and swollen towards lower side.	
14	Barisal Local	Bangladesh	Leaf-rough and non-serrated with whitish vein, root color-whitish.	
15	Ramboo-40	India	Leaf-smooth, spiny, rough, serrated with whitish vein, root color-whitish.	
16	Rupsa	Bangladesh	Leaf-erect, rough and non-serrated, root color-whitish.	
17	Radish:Tasakisan (China)	China	Leaf-rough, spiny, rough, non-serrated with whitish vein, root color-yellowish-white.	

18	Ivory White	Ivorycost	Leaf-rough, spiny, serrated with whitish vein, root color-whitish, root shape-tapering towards upper side and swollen towards lower side.	
19	Rocky-45	China	Leaf-smooth and non-serrated with whitish vein, root color-whitish.	
20	Radish Royal-40	China	Leaf-flat, spiny, rough, non-serrated with whitish vein, root color-whitish and cylindrical shaped root.	
21	White Prince (F_1 hybrid radish)	Korea	Leaf-erect, rough, spiny, serrated with whitish vein, root color-whitish and cylindrical shaped.	

3.9 Application of Manure and Fertilizers

Well-decomposed cow dung and NPKS as recommended ; Anon (1980) were applied in Urea, triple super phosphate (TSP), muriate of potash (MP) and gypsum were used as the sources of NPKS. The dates of NPKS were 150, 100, 120 and 20 kg/ha, respectively. Cow dung was applied @ 10 t/ha. The entire quantity of cow dung, TSP and gypsum were applied during final land preparation. Urea and MP were applied as top dressing in two equal splits at three and five weeks after sowing of seeds. Each top dressing was followed by irrigation. Boron (in the form of borax) was applied during final preparation of bed.

3.10 Sowing of Seed

Good quality radish seeds were sown in well prepared raised beds as per treatments. Three to four seeds were sown per hill maintaining spacing of 30 cm x 30 cm for raising roots. Insecticides namely, Nogos or Savin were applied around each plot just after sowing of seed to protect the seeds from ants.

3.11 Intercultural Operations

3.11.1 Thinning of the seedlings : The seedlings were thinned twice, leaving only the best healthy seedling per hill. Thinning was completed within 10-12 days after seed sowing.

3.11.2 Weeding and mulching: Weeding and mulching were accomplished as and when required to keep the crop free from weeds and to keep the soil loose for proper aeration and development of roots.

3.11.3 Irrigation: Irrigations were given as and when needed. Generally, irrigations were given at 7-10 days interval. However, irrigations were given to the stecklings at 12-15 days from planting depending on soil moisture condition. Mulching was done after each irrigation at proper soil condition by breaking the soil crust.

3.11.4 Pest and disease control: The plants were attacked by aphids at bolting stage. It was controlled to some extent by using of aluminum fail (insect repellent ribbon) over the experimental plots, as well as by spraying Melathion at the rate of 2 ml/ liter at weekly interval starting from bolting.

3.13 Data Collection

3.13.1 Collection of data

At 30 days after emergence (DAE), five plants from each unit plot were randomly sampled destructively. Leaf area measured by automatic leaf area meter. Weight on total dry matter (TDM) was recorded after drying the plants in an oven at 70^0C for 72 hours. At harvesting stage, all measurements as of 30 DAE were taken, are presented in appendix III. In addition, leaf number per plant, days to harvest and edible root yield per plant were also taken. Spacing of each plant was considered as ground area covered by plant which was used in case of secondary data generation; Abdussalam *et* *al.*, (1987). Relative growth rate (RGR), crop growth rate (CGR), leaf area index (LAI), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR) and harvest index (HI) were derived according to Radford (1967.).

3.13.1.1 Leaf area per plant

Leaf area per plant was measured by an automatic leaf area meter (Model: LICOR 2000).

3.13.1.2 Leaf area index

It is the ratio of leaf area to land area.

$$LAI = \frac{\text{Leaf area}}{\text{Unit land area}}$$

3.13.1.3 Total dry matter

The total dry matter was recorded by drying parts (80 $^0C \pm 2$) for 72 hours and calculated from summation of leaves, stem, tuber and roots weights was taken in an electronic balance.

3.13.1.4 Crop growth rate (CGR)

Rate of dry matter production per unit of time per area.

$$\text{i.e. } CGR = \frac{1}{A} \times \frac{W_2-W_1}{(T_2-T_1)} \quad g\ m^{-2}\ day^{-1}$$

Where, W_2 and W_1 are the DM at time T_2 and T_1, respectively.

A= Unit area (m^2)

3.13.1.5 Relative growth rate (RGR)

Rate of dry matter production per unit of dry matter per unit of time.

$$\text{i.e. } RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \quad g\ g^{-1}\ day^{-1}$$

Where W_2 and W_1 are the DM at time T_2 and T_1, respectively.

3.13.1.6 Net assimilation rate (NAR)

Rate of DM production per unit of leaf area per unit of time.

$$\text{i.e. NAR} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\ln LA_2 - \ln LA_1}{LA_2 - LA_1} \quad g\ cm^{-2}\ day^{-1}$$

where LA_2 and LA_1 are the leaf area at time T_2 and T_1, respectively

Where,

w_2= Dry weight of the individual plant at harvesting stage.
w_1= Dry weight of the individual plant at 30 DAE.
L_2=Total leaf area of the individual plant at harvesting stage.
L_1= Total leaf area of the individual plant at 30 DAE.
t_2=Number of days at harvesting stage.
t_1= Number of days at 30 DAE.

According to Mahalanobis (1936), multivariate analyses, viz Principal Component Analysis (PCA), Principal Coordinate Analysis (PCO), Canonical Vector Analysis (CVA), and Cluster Analysis (CLSA) were performed using the mean data for each character following the GENSTAT[513] program.

3.13.1.8 Principal Component Analysis (PCA)

Principal Component Analysis, one of the multivariate techniques, is used to examine the interrelationship among several characters and can be done from the sum of squares and also products matrix for the characters. The PCA displays most of the original variability of a set of variable in a smaller number of dimensions. Principal components were computed from the correlation matrix and genotypic scores obtained for the first components and succeeding components with latent roots greater than unity.

3.13.1.9 Principal Coordinate Analysis (PCO)

Principal Coordinate Analysis is equivalent to PCA, but it is used to calculate inter genotypic distances. Through the use of all dimensions of P, it gives the minimum distance between each pair of the N points using similarity matrix.

3.13.1.10 Clustering Analysis (CLSA)

Clustering Analysis divides the genotypes into some numbers of mutually exclusive groups on the basis of a data set. The clustering was done using non-hierarchical classification. In Genstat, the algorithm is used to search for optimal values of the chosen criterion. Starting from some initial classification of the genotypes into required number of groups, the algorithm repeatedly transfers genotypes from one group to another so long as such transfer improves the value of the criterion. When no further transfer can be found to improve the criterion, the algorithm switches to a second stage which examine the effect of swooping two genotypes of different classes, and so on.

3.13.1.11 Canonical Vector Analysis (CVA)

Canonical Vector Analysis finds linear combination of original variabilities that maximize the ratio between groups to within group variation. It provides two-dimensional plots that help in separating different populations involved.

3.13.1.12 Computation of average intra-cluster distance

Average intra-cluster distance for each cluster was computed by taking possible D^2 values within the members of a cluster obtained from the result of the PCO after the clusters were formed. The formula used, $D^2 = \Sigma D_i^{\ 2} / n$ where $\Sigma D_i^{\ 2}$ is the sum of distances between all possible combinations (n) of the genotypes included in a cluster and n is the all possible combinations. The square root of the average D^2 values represents the distance (D) within cluster.

3.13.1.13 Computation of average inter-cluster distance

Inter-cluster distances were calculated between cluster I and II, cluster I and III, cluster I and IV, cluster I and V, cluster II and III, cluster II and IV, and so on. The clusters were taken one by one and their distances from other clusters were calculated.

3.13.1.14 Cluster diagram

Cluster diagram was drawn using the values between and within cluster distances. Cluster diagram presents the pattern of diversity among the genotypes in a cluster.

3.14 Statistical Analysis

The recorded data on different characters were processed and analyzed statistically by MSTAT programme. The means were compared by Duncan's Multiple Range Test for interpretation (Gomez and Gomez, 1984).

CHAPTER IV
RESULTS AND DISCUSSION

The results of this experiment showed a distinct variation among the radish varieties in different quantitative and qualitative characters. Characterization is the prerequisites for collection, evaluation, conservation and any genetic improvement program of a specified crop. As per objectives of the research work, the experiment was conducted at the Agronomy Farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The results of the experiment have been presented and discussed in this chapter.

4.1 Studies on the genetic diversity of 21 radish variety using physiological characters

Estimation of genetic diversity by utilizing multivariate technique may help to choose genetically diverse parents. Efficient use of such genetically diverse genotypes has a bright scope to develop high yielding radish variety. Precise information on the nature of genetic divergence of the parent through multivariate analysis is a prerequisite of any crop improvement programme. Moreover, evaluation of genetic diversity is important to know the source of genes for particular trait within the available germplasm; Tomooka (1991). The characters of 21 radish variety related to plant, flower, fruit, seed and yield were used for genetic diversity analysis. Genetic diversity of the collected accessions was studied using Mahalanobis's D^2 statistics; Mahalanobis (1936). Considering the above facts, the present study on genetic diversity using physiological characters in radish was divided into two parts, viz., one is univariate and the other is multivariate technique. The results obtained from the experiment have been presented and discussed under the following headings.

4.2 Genetic diversity through univariate analysis

Range, mean with standard error, genotypic and phenotypic coefficient of variation, heritability in broad sense and genetic advance in means (5%) of different physiological characters of radish have been presented in Table 2. Considerable differences in minimum and maximum values of the above parameters indicated wide diversity among the 21 radish variety.

Table 2. Estimates of genetic parameters for different physiological characters of 21 radish varieties

Characters	Range	Mean± SE	GCV (%)	PCV (%)	h^2b (%)	GA(%)
Total Dry Matter (TDM) (gm)	18.11-47.33	27.45±1.85	30.77	30.96	98.77	63.00
Relative Growth Rate (RGR)	0.02-0.07	0.05±0.003	37.65	43.48	75.00	67.17
Crop Growth Rate (CGR)	3.88-12.27	7.15±0.46	29.29	29.95	95.64	59.01
Leaf Area Index (LAI)	0.82-1.83	1.16±0.08	29.95	30.21	98.34	61.19
Net Assimilation Rate (NAR)	2.49-9.27	5.12±0.41	36.64	37.09	97.59	74.56
Leaf Area Ratio (LAR)	7.30-9.71	8.54±0.16	7.94	9.74	66.39	13.32
Leaf Weight Ratio (LWR)	0.55-0.84	0.69±0.02	10.57	16.71	40.00	13.77
Harvest Index (HI)	22.53-44.24	29.98±1.52	23.14	23.54	96.67	46.87
Leaf Number Per Plant	13.40-24.20	18.46±0.66	16.06	16.96	89.61	31.32
Days to Harvest	40.44-71.06	51.85±2.05	17.90	18.48	93.81	35.72
Root Yield Per Plant (g)	118.40-387.29	213.66±15.82	33.14	35.46	87.35	63.80

(GCV: Genotypic coefficient of variation;

PCV: Phenotypic coefficient of variation

h^2b: Heritability in broad sense;

GA: Genetic advance)

The differences between minimum and maximum values of the above parameters indicated the existence of diversity among the radish variety. In all cases, genotypic coefficient of variation was lower than the corresponding phenotypic one, which indicated the influence of environment. There were considerable differences between genotypic and phenotypic coefficient of variation for almost all characters, which further demonstrated considerable influence of environment for the expression of these traits. Ushakumari (1991) reported higher phenotypic coefficient of variation (PCV) than genotypic ones (GCV) ones for all their studied characters in brinjal. Such findings are in agreement with the present observation.

A considerable GCV (36.64%) and PCV (37.09%) were observed for Net Assimilation Rate. Moreover, the GCV appeared very close to PCV, indicating a minimum environmental influence on this trait. In other words, major portion of the variation for this character was due to genetic effect.

However, high heritability (98.77%) estimates with moderate genetic advance (63.00%) was exhibited in total dry matter content. This suggests that selection for this character would be effective. Singh *et al.* (1974) observed high estimates of heritability together with high genetic advance of this trait. Lal and Pathak (1974), Gill *et al.* (1976) estimated high heritability and genetic advance for TDM. They had the opinion that, additive gene effects were more important in controlling the inheritance. Borilier *et al.* (1981) and Bora and Shadeque, (1993) also reported a high heritability for TDM.

The crop growth rate exhibited moderate GCV (29.29%) and PCV (29.95%) with high heritability (95.64%). The GCV appeared very close to PCV, which indicated a minimum environmental influence on this trait.

The leaf area index showed moderate GCV (29.95%) and PCV (30.21%) with high heritability (98.34%) and the harvest index showed moderate GCV (23.14%) and PCV (23.54%) with high heritability (96.67%), which indicated a considerable role of environment on the expression of this character.

Leaf area ratio showed low GCV (7.94%) and PCV (9.74%), a moderate level of heritability (66.39%) and a low level of genetic advance (13.32%), leaf area ratio is controlled by non-additive genes and has limited scope for improvement.

Days to harvest showed a GCV (17.90 %) and PCV (18.48 %). The GCV was very close to PCV, indicating that the variation was mainly due to genetic effects. Hence, breeding efforts through selection would be effective for the improvement of this character. Days to harvest also showed a high heritability (93.81%) and a moderate genetic advance. Therefore, selection for this character would get a good response even in the early generation.

Higher yield is one of the important goals of any varietal development programme. The trait showed a high GCV (33.14 %) and PCV (35.46 %). The difference between them was very low, indicating a pronounced genetic effect on expression of the character. The character showed a very high heritability (87.35 %), suggesting a possibility of improve this character through selection.

4.1.2 Genetic diversity through multivariate analysis

The results of genetic diversity as estimated through multivariate analysis on different characters of radish variety are presented and discussed under the following heads:

4.1.3 Principal coordinate analysis (PCO)

Principal coordinate analysis was done to get the inter-genotypic distances (D^2) among the 21 radish varieties for all possible combinations between pairs of variety. The highest inter-genotypic distance (113.96) was observed between the variety BARI radish-2 and Ivory white (Table 2), which was followed by the distance (95.64) observed between the variety Tasakisan-China (Which originated from China) and Ivory white. The lowest distance was 10.43, which was observed between the variety Paira 40 and Rocky 45, followed by the distance 11.43 observed between the variety BARI Radish-4 and Ramboo-40 (Table 2). The difference between the highest and the lowest inter-genotypic distance indicated the presence of variability among the radish varieties. Uddin and Chowdhury (1994) reported a range of D^2 values from 0.114 to 1.45 in case of sesame.

Table 3. Inter-genotypic distance (D^2) of 21 radish varieties

Variety	Lucky (Early 35 Days)	Snow White (F_1 hybrid)	BARI Radish-2 (Pinky)	Mino Early Long White (Ufsi Mula)	Paira-40	BARI Radish-3 (Druti)	Bright White 40	Mino Long White (Japanese White)	BARI Radish-4	Early White-35
BARI Radish-1 (Tasakisan)	74.01	28.58	94.30	73.58	69.75	40.36	71.82	57.82	37.77	73.30
Lucky (Early 35 Days)		57.45	76.59	26.17	16.33	40.89	44.50	76.45	39.69	26.13
Snow White (F_1 hybrid)			87.60	56.43	53.95	29.47	62.51	61.83	24.05	54.00
BARI Radish-2 (Pinky)				91.42	70.48	78.06	62.81	73.64	74.95	96.52
Mino Early Long White (Ufsi Mula)					26.34	38.70	46.17	74.70	44.79	17.62
Paira-40						34.37	29.05	64.18	39.12	31.30
BARI Radish-3 (Druti)							39.93	45.53	23.39	43.41
Bright White 40								44.35	52.63	56.23
Mino Long White(Japanese White)									61.89	84.63
BARI Radish-4										42.86

Table 3. Continued

Variety	Chamak	Nong Woo Bio (F_1 hybrid)	Barisal Local	Ramboo -40	Rupsa	Tasakisan (China)	Ivory White	Rocky -45	Radish Royal-40	White Prince (F_1 hybrid radish)
BARI Radish-1 (Tasakisan)	64.16	55.63	48.07	44.66	71.96	79.08	28.72	73.75	53.27	53.17
Radish:Lucky (Early 35 Days)	23.46	26.49	39.76	37.54	28.91	56.06	75.22	21.08	26.84	29.15
Snow White (F_1 hybrid)	47.63	37.91	28.99	29.52	56.64	72.75	34.41	60.46	36.53	41.43
BARI Radish-2 (Pinky)	89.37	69.96	90.96	70.38	83.30	48.73	113.96	69.18	81.19	61.91
Mino Early Long White (Ufsi Mula)	22.57	31.92	38.67	46.77	22.97	64.91	68.30	32.06	24.99	37.47
Paira-40	29.29	21.62	43.16	37.65	19.10	42.81	74.26	10.43	22.70	20.90
BARI Radish-3 (Druti)	37.68	27.94	33.15	30.94	34.43	53.58	46.86	38.14	20.66	20.83
Bright White 40	53.59	38.98	62.68	52.86	30.42	21.92	82.86	26.01	40.45	29.15
Mino Long White (Japanese White)	79.46	63.70	76.54	66.84	61.38	47.15	76.32	62.26	61.84	49.76
BARI Radish-4	32.60	22.76	20.87	11.43	47.55	62.22	43.77	45.44	25.00	25.71
Early White-35	15.59	30.99	32.64	43.60	33.70	73.13	65.76	39.32	24.92	42.29
Chamak		24.68	23.80	32.28	35.73	68.30	58.92	37.09	21.79	35.06
Nong Woo Bio (F_1 hybrid)			28.99	21.35	33.88	51.40	59.97	30.58	15.69	19.19
Barisal Local				23.12	47.96	75.65	41.82	50.97	27.14	38.61
Ramboo-40					48.81	60.21	51.93	44.20	28.84	26.70
Rupsa						48.63	73.60	19.46	27.30	30.88
Tasakisan (China)							95.64	38.03	56.39	39.22
Ivory White								79.86	53.68	63.11
Rocky-45									30.51	24.01
Radish Royal-40										21.73
White Prince (F_1 hybrid radish)										

The intra-cluster distances were computed by using the values of inter-varietal distance from distance matrix. It was observed that the number of variety in a cluster and the intra-cluster distance did not necessarily always match. The magnitudes of the intra-cluster distances were not always proportional to the number of accessions in the clusters (Table 3). In the present study, it was noticed that although the cluster IV composed of the highest number of variety,eight (Table 3), its intra cluster distance was not necessarily the highest. The highest intra cluster distance was observed in cluster III (54.69) which was composed of five variety followed by cluster IV (41.75), cluster II (32.75) with six variety and cluster I (28.72) having only two variety.

Table 4. Average intra and inter cluster D^2 and D values of five clusters by Euclidean method

Cluster	I	II	III	IV
I	**824.673** (28.72)	5945.94 (77.11)	3597.46 (59.98)	3723.53 (61.02)
II		**1072.85** (32.75)	3226.76 (56.80)	1788.19 (42.29)
III			**2990.88** (54.69)	2670.97 (51.68)
IV				**1743.14** (41.75)

4.3 Canonical Vector analysis (CVA)

Canonical vector analysis was performed to compute the cluster distance (Mahalanobis' D^2 value). The intra- and inter-cluster distance or D^2 values are presented in Table 4 and Fig. 1. The inter-cluster distance in the radish variety differed widely. Statistical distances represent the index of genetic diversity among the clusters. The inter-cluster distances were larger than the intra-cluster distances, suggesting a wider genetic diversity among the accessions of different groups. Based on 11 quantitative characters of variety, PCO was carried out to determine the inter-varietal distance (D^2). The intra-cluster distance, estimated by using the values of inter-varietal distance and obtained from CVA, are presented in Table 4.

The highest inter cluster distance was observed between cluster I and cluster II (77.11), indicating a wide range of genetic diversity between these two clusters; which was followed by inter cluster distances between cluster I and cluster IV (61.02), cluster I and cluster III (59.98) and cluster II and cluster III (56.80). Moderate inter cluster distance was observed between Cluster III and IV (51.68). The lowest inter cluster distance was observed between cluster II and IV (42.29), suggesting a close relationship between the variety of these clusters, and that varieties were genetically close. These relationships were also reflected in the intra and inter cluster distances diagrammatically presented in

Fig 1.

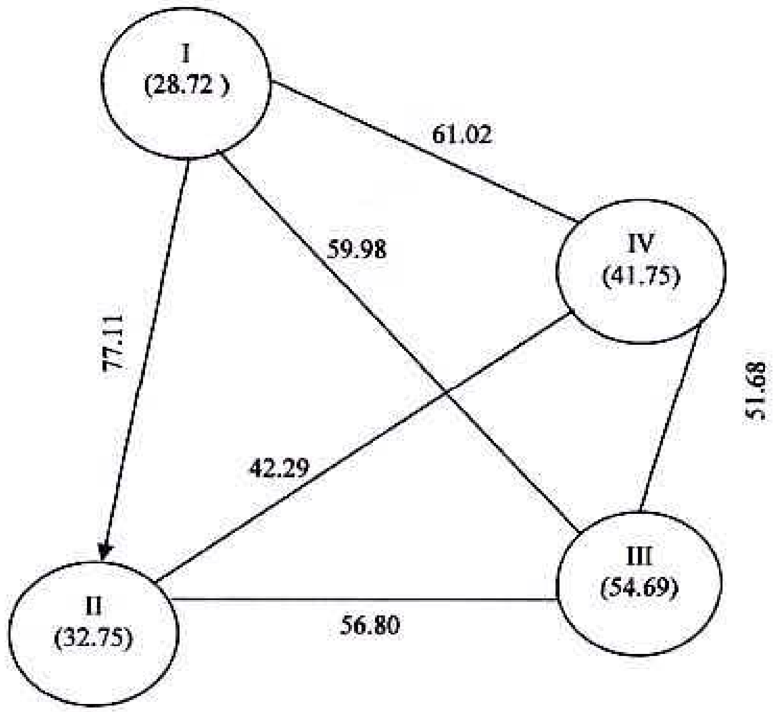

Fig. 1. Cluster diagram showing the average intra and inter cluster distances (D $=\sqrt{D^2}$ values) of 21 radish varieties.

Note: The values along the lines indicate inter cluster distances and the values within the circle indicate intra cluster distances.

4. 5 Non-hierarchical clustering

Non-hierarchical clustering was done by using covariance matrix, where 21 radish varieties were grouped into four different clusters.

Cluster IV showed the highest number of variety (8) followed by cluster II (6), cluster III (5). The lowest number of accessions was observed in cluster I (2). The clustering pattern of the accessions under this study revealed that the accessions collected from the same location were often grouped into different clusters (Table 3), which indicated variation among the variety irrespective of their site of collection. Eight varieties of cluster IV were Mino Early Long White (Ufsi Mula), BARI Radish-3 (Druti), Mino Long White, Early White-35, Chamak, Nong Woo Bio (F_1 hybrid), Radish Royal-40, White Prince (F_1 hybrid radish). All the varieties of this cluster more or less similar originating from India and Bangladesh, also found no variation among the varieties due to geographical distribution; rather genotypes collected from same region were distributed in different clusters randomly. Cluster I composed of two varieties namely, BARI Radish-1 and Ivory white.

In the present study, it was observed that, radish varieties were collected from same location were grouped into different clusters (Table 4). This indicated that factors other than geographical separation are also responsible for divergence, and that the varieties collected from the same place might have different genetic architecture or vice-versa. Mannan *et al.* (1993) and Singh and Singh (1979) which working with pani kachu and okra, respectively reported that falling of materials of same origin into different clusters was an indication of broad diversity. Upadhya and Murty (1970) opined that genetic drift and natural selection in different environment could cause high diversity among genotypes.

Table 5. Distribution of 21 radish varieties collected from different location of in 4 clusters

Cluster number	Number of genotypes	Percent	Name of genotypes
I	2	9.52	BARI Radish-1 (Tasakisan) and Ivory White
II	6	28.57	Lucky (Early 35 Days), Paira-40, Bright White 40, *Rupsa, Tasakisan (China), Rocky-45*
III	5	23.81	Snow White (F_1 hybrid), BARI Radish-2 (Pinky), BARI Radish-4, Barisal Local, Ramboo-40
IV	8	38.10	Mino Early Long White (Ufsi Mula), BARI Radish-3 (Druti), Mino Long White (Japanese White), Early White-35, Chamak, Nong Woo Bio (F_1 hybrid), Radish Royal-40, White Prince (F_1 hybrid radish)

4.6 Cluster means

The cluster means, in respect of 11 different characters, of 21 radish varieties are shown in the Table 5. Cluster IV, comprising 8 variety, had the second highest mean value for harvest index (29.04). The cluster had moderate mean values in rest of the characters. The lowest leaf number/plant, LAR and LWR were found in cluster IV. Thus, materials of the cluster IV, were proper in leaf characters than I and III but produced the highest HI which might be due higher RGR and longer duration of the varieties than the other varieties. Ezekiel (1990) also reported similar relationship between HI, NAR and longer crop duration in potato.

The cluster II had 6 variety, which had the second highest mean value for days to harvest (52.23). This cluster had the maximum mean value in harvest index (29.08) and the other characters had the lowest mean value.

The cluster III had 5 variety, which had the highest mean value for relative growth rate (0.061) and net assimilation rate (7.75) and the second highest yield per plant (237.19g).

Cluster I, consisting of 2 varities, had the highest mean values for total dry matter (46.06g), crop growth rate (11.84), leaf area index (1.83), leaf area ratio (9.33), leaf weight ratio (0.83), leaf number per plant (25.53), days to harvest (52.42) and root yield per plant (380.06g).

From class mean values it was observed that all cluster produced LAI, CGR, TDM and root yield in similar trend and the highest value was observed in the cluster I which was followed by cluster III. Yadav and Hari (1988) and Ezekiel (1990) also reported similar relationship between LAI, TDM and yield in Indian mustard and potato, respectively. Similarly, RGR, NAR, HI and days to harvest showed similar trend and the highest values for the characters were observed in cluster IV.

Table 6. Cluster mean for 11 physiological characters in 21 radish varieties

Characters	**I**	**II**	**III**	**IV**
Total Dry Matter (TDM) (gm)	46.06	19.88	31.71	25.82
Relative Growth Rate (RGR)	0.060	0.030	0.061	0.045
Crop Growth Rate (CGR)	11.84	5.04	8.11	6.96
Leaf Area Index (LAI)	1.83	0.878	1.27	1.13
Net Assimilation Rate (NAR)	6.38	3.20	7.75	4.61
Leaf Area Ratio (LAR)	9.33	8.02	8.15	8.97
Leaf Weight Ratio (LWR)	0.839	0.69	0.75	0.58
Harvest Index (HI)	22.85	29.08	23.41	39.04
Leaf Number Per Plant	23.53	17.72	18.28	17.61
Days to Harvest	52.42	52.23	50.85	52.05
Root Yield Per Plant (g)	380.06	144.03	237.19	209.19

4.7 Selection of varieties

Genotypically distant parents are able to produce higher heterosis (Falconar, 1960; Moll et al., 1962; Ramanujam *et al*., 1974; Chauhan and Singh, 1982; Ghaderi *et al.*, 1984; Mian and Bhal, 1989). *Genetic diversity analysis revealed that* Ivory white, Rocky-45, Ramboo and BARI radish-3 were distantly isolated representing four different cluster. The highest root yield, TDM, CGR,LAI,LAR and LWR were produced by Ivory white which was followed by BARI radish-1 (Tasakisan) (Table 6). However, Ivory white did not flower and BARI radish-1 is well adapted and able to produce seed in Bangladesh conditions (Rashid *et al.*, 1985). (Table 6. BARI radish-2 (pinky) produced the highest RGR (0.068) and NAR (9.26) value (Table 6 and it is attractive in colour. Rupsa produced the highest HI (44.24) in table 6.

Corresponding genetic diversity and other performances, Tasakisan, Pinky and Rupsa might be selected for future hybridization program.

Table 7. Mean performances of 21 radish varieties for eleven physiological characters

Varieties	Total Dry Matter (TDM) (gm)	Relative Growth Rate (RGR)	Crop Growth Rate (CGR)	Leaf Area Index (LAI)	Net Assimilation Rate (NAR)
BARI Radish-1 (Tasakisan)	44.78	0.05567	11.41	1.813	6.453
Radish:Lucky (Early 35 Days)	21.10	0.03467	6.623	0.8500	3.650
Snow White (F_1 hybrid)	35.06	0.06467	7.567	1.633	8.307
BARI Radish-2 (Pinky)	18.68	0.06867	6.357	0.9667	9.267
Mino Early Long White (Ufsi Mula)	22.08	0.03867	6.277	0.9333	4.260
Paira-40	20.94	0.03567	5.470	0.8533	3.710
BARI Radish-3 (Druti)	26.87	0.04767	7.447	1.467	4.497
Bright White 40	19.91	0.03300	4.553	0.9000	3.247
Mino Long White (Japanese White)	23.00	0.03900	6.357	1.717	3.967
BARI Radish-4	33.97	0.06367	9.390	1.353	6.677
Early White-35	24.59	0.04267	6.320	0.8367	4.230
Chamak	31.33	0.04400	7.913	0.8167	4.627
Nong Woo Bio (F_1 hybrid)	28.07	0.05400	7.123	0.9733	6.260
Barisal Local	35.54	0.05567	8.483	1.233	7.173
Ramboo-40	35.32	0.05300	8.740	1.147	7.343
Rupsa	18.11	0.02267	3.880	0.9867	2.767
Radish:Tasakisan (China)	19.98	0.03233	4.260	0.8300	3.320
Ivory White	47.33	0.06500	12.27	1.830	6.287
Rocky-45	19.23	0.02133	5.433	0.8533	2.487
Radish Royal-40	25.91	0.04767	7.200	1.143	4.427
White Prince (F_1 hybrid radish)	24.69	0.04367	7.110	1.140	4.620
$LSD_{0.05}$	1.56	0.0165	0.738	0.074	0.487
Level of significant	**	**	**	**	**
CV%	3.43	34.76	6.26	4.27	5.74

Table 7. contn.

Varieties	Leaf Area Ratio (LAR)	Leaf Weight Ratio (LWR)	Harvest Index (HI)	Leaf Number Per Plant	Days to Harvest	Root Yield Per Plant (g)
BARI Radish-1 (Tasakisan)	8.943	0.8417	23.16	24.20	56.21	372.8
Radish:Lucky (Early 35 Days)	7.303	0.6390	35.00	15.67	40.71	181.1
Snow White (F_1 hybrid)	8.417	0.8267	23.72	20.93	46.06	273.1
BARI Radish-2 (Pinky)	7.373	0.6310	24.18	13.87	70.56	158.4
Mino Early Long White (Ufsi Mula)	9.353	0.6693	34.10	13.40	47.32	197.6
Paira-40	8.190	0.6197	36.17	18.67	48.81	162.2
BARI Radish-3 (Druti)	8.367	0.7313	28.09	17.73	51.80	165.9
Bright White 40	9.287	0.5680	38.07	20.13	64.81	138.1
Mino Long White (Japanese White)	8.550	0.6387	34.62	16.93	71.06	173.7
BARI Radish-4	8.353	0.7543	22.76	19.33	48.96	259.9
Early White-35	9.233	0.6707	28.35	19.20	41.33	205.4
Chamak	9.347	0.7290	24.56	16.67	46.31	250.5
Nong Woo Bio (F_1 hybrid)	9.317	0.6797	26.41	22.27	52.80	241.4
Barisal Local	8.393	0.7640	23.37	15.33	40.44	248.7
Ramboo-40	8.230	0.7623	23.02	18.60	48.22	249.1
Rupsa	7.550	0.5473	44.24	14.53	43.63	127.5
Radish:Tasakisan (China)	8.413	0.5817	38.61	20.07	68.10	136.8
Ivory White	9.707	0.8360	22.53	22.87	48.64	387.3
Rocky-45	7.367	0.5613	42.36	17.27	47.34	118.4
Radish Royal-40	9.310	0.7443	27.92	22.33	50.74	229.3
White Prince (F_1 hybrid radish)	8.300	0.7090	28.30	17.67	55.07	209.6
$LSD_{0.05}$	0.795	0.147	2.13	1.67	3.93	44.47
Level of significant	**	**	**	**	**	**
CV%	5.64	12.72	4.29	5.47	4.60	12.61

CHAPTER V

SUMMARY AND CONCLUSION

A field experiment was conducted at the at the Agricultural Farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh during the *rabi* season of November 2011 to February 2012 to study the genetic diversity for physiological traits of 21 radish varieties based on some physiological characters. The experiment was laid out in RCBD with three replications.

Data on total dry matter, relative growth rate, crop growth rate, leaf area index, net assimilation rate, leaf area ratio, leaf weight ratio, harvest index, leaf number per plant, days to harvest and edible root yield were recorded at harvesting stages for each varieties. To generate secondary data, dry weight of shoot and root and leaf area were recorded at 30 days after seedling emergence as in case of harvesting stage.

Significant differences among the varieties for all the characters were observed. Genetic analysis, principal co-ordinate analysis, cluster analysis using Genstat program. There were four distinct clusters viz., cluster I: BARI Radish-1 (Tasakisan) and Ivory White, cluster II: Lucky (Early 35 Days), Paira-40, Bright White 40, Rupsa, Tasakisan (China), Rocky-45, cluster III: Snow White (F_1 hybrid), BARI Radish-2 (Pinky), BARI Radish-4, Barisal Local, Ramboo-40 and cluster IV: Mino Early Long White (Ufsi Mula), BARI Radish-3 (Druti), Mino Long White (Japanese White), Early White-35, Chamak, Nong Woo Bio (F_1 hybrid), Radish Royal-40, White Prince (F_1 hybrid radish). The longest cluster distance was observed between cluster I and cluster II (77.11) which was followed by inter cluster distances between cluster I and cluster IV (61.02). Inter-genotypic distance between BARi radish-1 and BARI radish-2, BARI radish-1 and Rupsa and BARI radish-2 and Rupsa were 94.30, 71.96 and 83.30, respectively. The highest leaf area index (1.83), total dry matter (46.06) and root yield (380.6) were observed in cluster I. However, clustering pattern was not influenced by origin of the varieties.

Leaf area index, leaf area ratio, leaf weight ratio and leaf number per plant were found major components of genetic diversity in the radish varieties. The highest root yield

(387.3g/plant), total dry matter (47.33g/plant), crop growth rate (12.27) and leaf area index (1.183) were observed in Ivory white which was followed by BARI radish-1 for the characters. Ivory white did not flower. BARI radish-2 produced the highest relative growth rate (0.068g/g/plant) and net assimilation rate (9.26g/m2/day).

The highest harvest index (44.24) was observed in Rupsa. Barisal local was found as the earliest variety (40.44day).Considering genetic diversity and other performances, BARI radish-1, BARI radish-2 and Rupsa were considered to be promising parents for future hybridization program.

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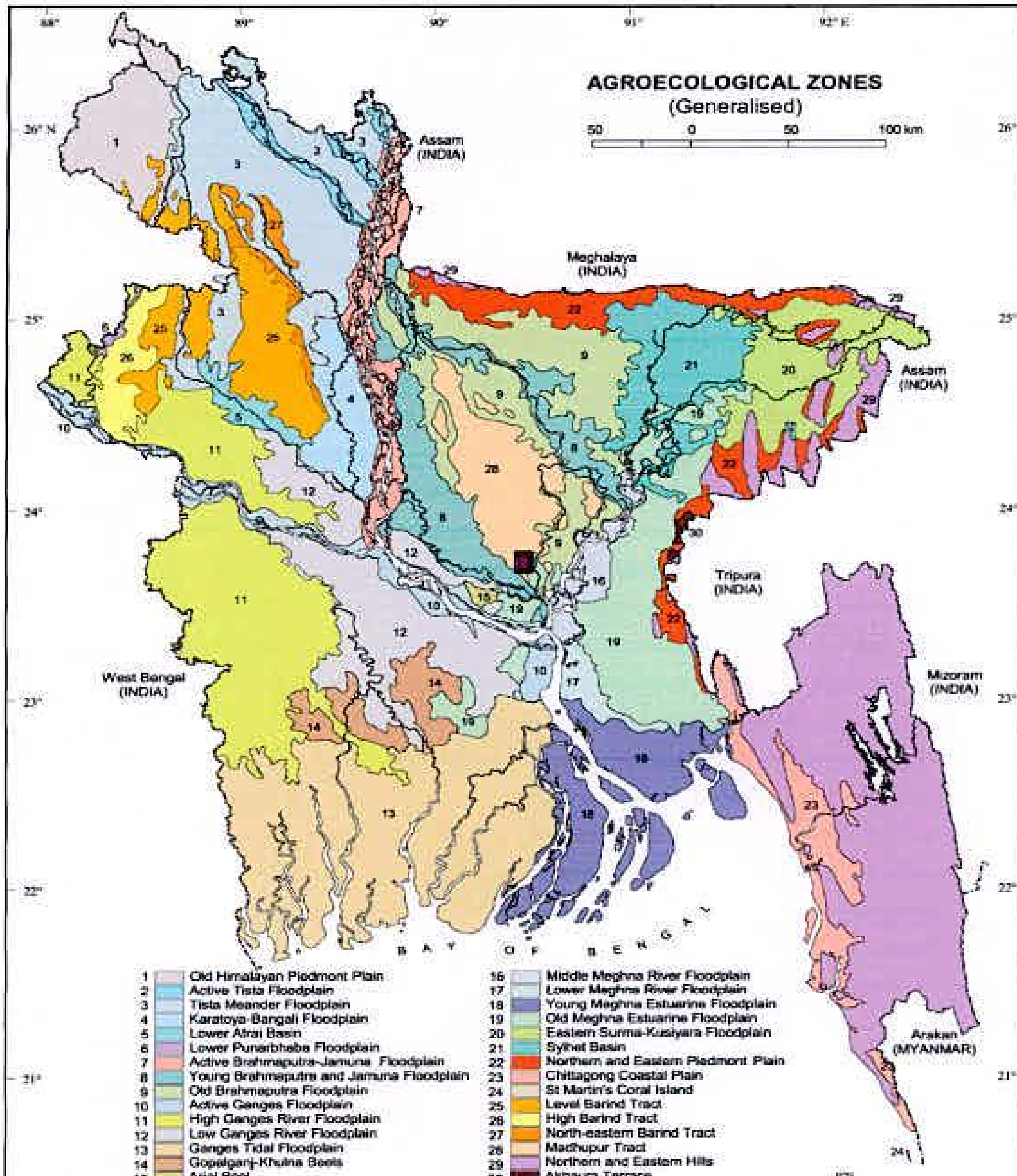
APPENDICES

Appendix I. Map showing the experimental site under the study



The experimental site under study

Appendix II. Monthly average Temperature, Relative Humidity and Total Rainfall and sunshine of the experimental site during the period from October, 2011 to March, 2012

Month	Air temperature (°c)		Relative humidity (%)	Rainfall (mm)(total)	Sunshine (hr)
	Maximum	Minimum			
October, 2011	34.8	18.0	77	227	5.8
November, 2011	32.3	16.3	69	0	7.9
December, 2011	29.0	13.0	79	0	3.9
January, 2012	28.1	11.1	72	1	5.7
February, 2012	33.9	12.2	55	1	8.7
March, 2012	34.6	16.5	67	45	7.3

Source: Bangladesh Meteorological Department (Climate & Weather Division), Agargoan, Dhaka – 1212

Appendix III: Physical Characteristics and chemical composition of soil of the experimental plot

Soil Characteristics	Analytical Results
Agrolofical Zone	Madhupur Tract
PH	6.00 – 6.63
Organic Matter	0.84
Total N (%)	0.46
Available phosphorous	21 ppm
Exchangeble K	0.41meq / 100g soil

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

Appendix IV. Mean values of 21 radish varieties for different characters

Varieties	Root Length Per Plant (cm)		Root weight(gm)		Root weight(gm)-Dry weight		Days to Harvest
	30 DAE	At harvesting stage	30 DAE	At harvesting stage	30 DAE	At harvesting stage	
BARI Radish-1 (Tasakisan)	25.3	37.1	554	847	27.4	45.7	55.38
Radish:Lucky (Early 35 Days)	18.5	21.7	258	467	15.3	20.33	41.17
Snow White (F_1 hybrid)	28.3	31.7	405	635	21.7	38.6	46.62
BARI Radish-2 (Pinky)	14.9	19.2	163	207	10.2	19.53	70.24
Mino Early Long White (Ufsi Mula)	29.7	32.6	213	346	10.8	22.58	46.89
Paira-40	21.1	27.2	183	317	12.7	21.14	49.13
BARI Radish-3 (Druti)	14.9	17.2	227	361	13.2	26.8	52.09
Bright White 40	24.7	30.5	183	304	8.7	19.9	65.38
Mino Long White (Japanese White)	13.2	16.7	271	389	10.3	21.86	71.68
BARI Radish-4	19.6	25.4	179	451	14.8	34.88	49.16
Early White-35	27.3	30.5	268	563	15.3	25.73	40.72
Chamak	28.4	30.9	374	623	19.9	31.89	45.93
Nong Woo Bio (F_1 hybrid)	26.3	32.6	259	445	13.8	27.99	52.68
Barisal Local	18.6	24.8	264	437	18.8	36.61	40.73
Ramboo-40	28.1	31.9	279	438	16.7	35.73	48.36
Rupsa	15.8	22.8	247	383	11.7	17.38	44.17
Tasakisan (China)	29.3	31.6	276	456	13.6	20.66	67.48
Ivory White	22.8	28.6	379	539	22.9	47.02	48.71
Rocky-45	16.4	26.8	231	368	13.7	19.5	46.46
Radish Royal-40	17.3	25.3	257	401	17.4	25.97	50.13
White Prince (F_1 hybrid radish)	14.8	18.7	348	507	13.5	23.49	55.33

Appendix IV. Contd.

Varieties	Leaf Length Per Plant (cm)		Leaf weight(gm)		Leaf weight(gm)-Dry weight		Leaf Area at harvesting stage
	30 DAE	At harvesting stage	30 DAE	At harvesting stage	30 DAE	At harvesting stage	
BARI Radish-1 (Tasakisan)	29.1	39.2	156	210	8.38	11.6	18.05
Radish:Lucky (Early 35 Days)	17.5	24.6	46	69	2.45	3.48	12.13
Snow White (F_1 hybrid)	29.7	38.8	321	446	4.6	7.6	15.83
BARI Radish-2 (Pinky)	29.8	35.2	47	78	2.18	4.24	11.49
Mino Early Long White (Ufsi Mula)	29.9	36.4	77	113	4.52	5.87	13.37
Paira-40	23.6	29.6	48	55	1.87	3.69	13.11
BARI Radish-3 (Druti)	29.3	36.9	131	185	6.89	9.4	14.09
Bright White 40	29.7	39.4	113	176	6.68	11.6	15.95
Mino Long White (Japanese White)	30.1	37.8	140	191	7.69	10.16	17.87
BARI Radish-4	24.8	31.7	159	276	12.98	15.17	11.41
Early White-35	27.1	34.2	38	64	2.09	3.53	15.17
Chamak	33.4	39.6	79	117	4.89	6.87	9.85
Nong Woo Bio (F_1 hybrid)	26.7	31.7	254	378	6.29	10.49	15.71
Barisal Local	35.5	40.6	136	229	10.04	11.86	17.53
Ramboo-40	37.3	43.1	174	288	11.58	13.96	15.13
Rupsa	28.1	33.2	39	56	1.63	2.88	12.15
Tasakisan (China)	25.9	29.3	25	46	1.59	3.16	10.84
Ivory White	27.8	36.2	235	369	17.02	19.96	18.41
Rocky-45	26.3	31.2	23	32	1.24	2.33	10.33
Radish Royal-40	29.7	35.5	47	82	3.11	4.29	15.47
White Prince (F_1 hybrid radish)	31.3	38.3	85	135	5.73	7.59	14.06

Appendix V. Analysis of variance (mean square) for different plant characters in radish

Items	d. f	Total Dry Matter (TDM) (gm)	Relative Growth Rate (RGR)	Crop Growth Rate (CGR)	Leaf Area Index (LAI)	Net Assimilation Rate (NAR)	Leaf Area Ratio (LAR)	Leaf Weight Ratio (LWR)	Harvest Index (HI)	Leaf Number Per Plant	Days to Harvest	Root Yield Per Plant (g)
Replication	2	0.175	0.001	0.186	0.003	0.373	0.308	0.007	5.111	0.012	5.41	710.26
Varieties	21	214.936**	0.001**	13.357**	0.358**	10.645**	1.607**	0.024**	145.977**	27.384**	264.17**	15765.40**
Error	40	0.889	0.0001	0.200	0.002	0.087	0.232	0.008	1.657	1.019	5.68	726.21

** indicates significant at 0.01 probability, respectively