DNA FINGERPRINTING AND MOLECULAR DIVERSITY OF MUNG BEAN (*Vigna radiata* (L.) R. Wilczek) GERMPLASM

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DHAKA-1207

JUNE, 2022

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A Thesis

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

BIOTECHNOLOGY

SEMESTER: JANUARY- JUNE, 2022

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CERTIFICATE

This is to certify that thesis entitled, **"DNA FINGERPRINTING AND MOLECULAR DIVERSITY OF MUNG BEAN (*Vigna radiata* (L.) R. Wilczek) GERMPLASM"** 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 BIOTECHNOLOGY,** embodies the result of a piece of bona fide research work carried out by **TAMANNA TAWFIQ** Registration No.**15-06822** under my supervision and my 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.

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Dedicated To

My Beloved Parents

for their endless love, support and encouragement.

ABBREVIATIONS

FULL WORD(S)	ABBREVIATION
Amplified Fragment length Polymorphism	AFLP
American	*Am.*
And others (at elli)	*et al.*
As for example	e.g.
Bangladesh Bureau of Statistics	B.B.S
Base pair	Bp
Biology	*Biol.*
Biotechnology	*Biotechnol.*
Botany	*Bot.*
Cetyl Trimethyl Ammonium Bromide	CTAB
Current	*Curr.*
Continued	Cont.
Degree celcius	°C
Deoxyribonucleic acid	DNA
Distilled Deionized water	dd.H2O
Ecology	*Ecol.*
Etcetera	Etc.

FULL WORD(S)	ABBREVIATION
Ethidium Bromide	Et-Br
Ethelene Diamine Tetra Acetic Acid	EDTA
Evolution	*Evol*.
Genetic Distance	GD
Genetics	*Genet*.
Gram per liter	g/L
Hectare	ha
Horticulture	*hort*
Human	*Hum.*
International	*Int*.
Inter simple sequence repeat	ISSR
Journal	*j*.
Marker assisted breeding	MAS
Micro liter	µl
Mili liter	mL
Mili metre	mm
Molecular	*Mol.*
Namely	viz
National	*Natl.*
percent	%
proceeding	*Proc.*

FULL WORD(S)	**ABBREVIATION**
Polymerase chain reaction	PCR
Random Amplified Polymorphic DNA	RAPD
Restriction Fragment Length Polymorphism	RFLP
Research	*Res*.
Reports	*Rep.*
Ribonucleic Acid	RNA
Rotation per minute	rpm
Regulation	*Regul.*
Science	*Sci*.
Single nucleotide polymorphism	SNP
Simple Sequence Repeat	SSR
Sodium Chloride	NaCl
Theoretical	*Theor*.
Unweighted Pair Group Method of Arithmetic Mean	UPGMA
Ultraviolet	UV

ACKNOWLEDGEMENTS

All praises are due to Almighty Allah who enable me to complete this thesis. It is a great pleasure to express profound gratitude to my respected parents, who entitled much hardship inspiring for prosecuting my studies, thereby receiving proper education.

I would like to express my heartfelt respect, deepest sense of gratitude, profound appreciation and ever indebtedness to my supervisor ***Dr. Md. Ekramul Hoque****, Professor, Department of Biotechnology, Sher-e-Bangla Agricultural University, (SAU), Dhaka-1207, for his sincere guidance, scholastic supervision, constructive criticism, and constant inspiration throughout the course and in preparation of the manuscript of the thesis.*

I express my sincere respect to my Co-supervisor ***Dr. Md. Rafiqul Islam****, Associate Professor and Chairman, Department of Biotechnology, Sher-e-Bangla Agricultural University, (SAU), Dhaka-1207, for his utmost co-operation, constructive suggestion to conduct the research work, as well as preparation of the manuscript of the thesis.*

I would like to express my heartful indebtedness and profound appreciation to all of my respectable teachers, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka for their nice co–operation and suggestion during the course of MS program.

I would like to thank all of my close friends, lab attendants and well wishers to co- operate and help me during working in the lab.

More diction is not enough to express my profound gratitude and deepest appreciation to my parents for their never ending prayer, encouragement, sacrifice and dedicated efforts to educate me to this level.

I am also grateful to Ministry of Science and Technology, People's Republic of Bangladesh for selecting me National Science and Technology (NST) Fellow and funding of my research work.

I express my immense gratefulness to all of them who assisted and inspired me to achieve higher education and regret for my inability for not to mention everyone by name.

Dated: June, 2022

SAU, Dhaka ***The Author***

LIST OF CONTENTS

CHAPTER	TITLES	PAGE NO
	ABBREVIATIONS	**i-iii**
	ACKNOWLEDGEMENTS	**iv**
	LIST OF CONTENTS	**v-vii**
	LIST OF TABLES	**viii-ix**
	LIST OF FIGURES	**ix-x**
	LIST OF PLATES	**x**
	LIST OF APPENDICES	**xi**
	ABSTRACT	**xii**
CHAPTER I	**INTRODUCTION**	**1-3**
CHAPTER II	**REVIEW OF LITERATURE**	**4-11**
	2.1 Molecular markers	4-6
	2.2 Idea of RAPD primer	6-7
	2.3 RAPD markers for genetic diversity of mung bean	7-11
CHAPTER III	**MATERIALS AND METHODS**	**12-29**
	3.1 Experimental location and duration	12
	3.2 Source of study materials	12
	3.3 Seed germination and collection of leaf sample	13
	3.4 Extraction of genomic DNA	13

LIST OF CONTENTS (Cont'd)

CHAPTER	LIST OF CONTENTS	PAGE NO
	3.4.1 Equipments and small instruments used for genomic DNA isolation	14
	3.4.2 Reagents Used	15
	3.4.3 Reagents preparation	16-18
	3.5 Sequential steps of DNA extraction	18-19
	3.6 Confirmation of DNA preparation	20
	3.6.1 Preparation of 1% agarose gel	20
	3.6.2 Preparation of DNA samples for electrophoresis	21
	3.7 Working solution of DNA samples preparation	21
	3.8 Amplification of RAPD markers by PCR	22
	3.8.1 Principle of RAPD primer amplification	22
	3.8.2 Selection of primers	23
	3.8.3 PCR amplification	23-24
	3.8.4 Electrophoresis of the amplified products	25
	3.9 Data analysis	25-26
	3.10 Precaution	26-27
CHAPTER IV	**RESULT AND DISCUSSION**	**30-50**
	4.1Extraction of DNA and Confirmation	30

LIST OF CONTENTS (Cont'd)

CHAPTER	LIST OF CONTENTS	PAGE NO
	4.2 Analysis of genetic diversity among Mung bean germplasm using RAPD marker	31
	4.2.1 Optimization of PCR amplification conditions and selection of primers for RAPD analysis	31-32
	4.2.2 RAPD profiles of Mung bean germplasm	32
	4.2.2.1 Primer OPBC-O1	33-34
	4.2.2.2 Primer OPA -20	35-36
	4.2.2.3 Primer OPP-03	38
	4.2.2.4 Primer OPT-20	39-40
	4.2.2.5 Primer OPBC-18	42
	4.3 DNA banding profile of all primer	44
	4.4 DNA band scoring and diversity analysis	45
	4.5 Nei's genetic distance and genetic identity	46-47
	4.6 UPGMA dendrogram	48-50
CHAPTER V	**SUMMARY AND CONCLUSION**	**51-52**
	RECOMMENDATIONS	**53**
	REFERENCES	**54-56**
	APPENDICES	**57-60**

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
01	List of mung bean germplasms and their sources	12-13
02	Name of RAPD primers with GC content and sequence information	23
03	PCR mixture composition for each mung bean genotype	24
04	Thermal profile for PCR	24
05	RAPD profile of the primer OPBC-01 in 15 mung bean germplasm	33-34
06	RAPD profile of the primer OPA- 20 in 15 mung bean germplasm	35-36
07	RAPD profile of the primer OPP- 03 in 15 mung bean germplasm	38
08	RAPD profile of the primer OPT- 20 in 15 mung bean germplasm	39-40
09	RAPD profile of the primer OPBC-18 in 15 mung bean germplasm	42
10	Total number of bands, total number of polymorphic bands, range of DNA bands, percentage of polymorphism found among 15 mung bean germplasm for 5 RAPD primers	44
11	Major loci frequency, gene diversity and PIC value of different Mung bean germplasm	45

LIST OF TABLES(Cont'd)

TABLE NO	TITLE	PAGE NO
12	Summary of nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among 15 mung bean germplasms	47
13	List of germplasm with their cluster based UPGMA dendrogram	49

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO
01	Seed germination in a petri dish	28
02	Fresh, young and tender leaf was used for DNA extraction	28
03 (a,b)	Preparing samples for DNA extraction under the guidance of honorable supervisor	28
04	Centrifugation of the samples	29
05	Preparing samples for Gel loading	29
06	Loading DNA samples in gel electrophoresis unit	29

LIST OF FIGURES (Cont'd)

FIGURE NO.	TITLE	PAGE NO
07	Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1983) genetic distance, summarizing the data on differentiation among 15 mung bean germplasm according to microsatellite	50

LIST OF PLATES

PLATE NO	TITLE	PAGE NO
1	Isolation of genomic DNA in 15 mung bean genotypes	30
2	PCR amplification with RAPD primer OPBC-01	37
3	PCR amplification with RAPD primer OPA-20	37
4	PCR amplification with RAPD primer OPP-03	41
5	PCR amplification with RAPD primer OPT-20	41
6	PCR amplification with RAPD primer OPBC-18	43

LIST OF APPENDICES

SL. NO	TITLE	PAGE NO
I	Chemical preparation for DNA extraction and PCR work	57-59
II	RAPD marker analysis related data	60

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ABSTRACT

Mung bean (*Vigna radiata* (L.) R. Wilczek) has been widely used as a pulse crop. The study was carried out at the Biotechnology laboratory of Sher-e-Bangla Agricultural University, during the period of July 2021 to June 2022 to investigate molecular diversity and fingerprinting of fifteen mung bean germplasm. RAPD primers were selected and synthesized for molecular diversity analysis. All of the primers were able to produce polymorphic bands. The highest number of bands were observed in RAPD primer OPT-20. The polymorphic Information Content (PIC) of RAPD markers ranged from 0.24 to 0.32 with an average value of PIC =0.27. Gene diversity ranges from 0.41 to 0.28 with an average value of 0.33. Primer showed OPBC-1, OPA-20, OPP-03, OPT-20 moderate diversity and primer OPBC 18 showed low diversity on the basis of PIC value. The value of pair -wise comparisons of Nei's (1983) genetic distance (D) among 15 genotypes was computed from and it was ranged from 0.1034 to 0.6207 with an average of 0.3621. Dendrogram based on Nei's genetic distance using unweighted Pair Group Method Of Arithmetic Mean (UPGMA) indicated the segregation of 15 genotypes into four main clusters. This result can be conveniently used for molecular diversity analysis to identify diverse parent for mung bean improvement program.

CHAPTER I

INTRODUCTION

Mung bean (*Vigna radiata* (L.) R. Wilczek) is a fast-growing warm-season legume species belonging to the Fabaceae family and has a diploid chromosome number of 2n=2x=22. It is a self-pollinated plant with the estimated genome size 494 to 579 Mb. It is an annual vine with yellow flowers and fuzzy brown pods. It is thought to have originated in South and Southeast Asian regions. It is widely grown in India, Pakistan, Bangladesh, Myanmar, Thailand, the Philippines, China and Indonesia. It is also grown in parts of East and Central Africa, the West Indies, USA and Australia. In Bangladesh, mung bean is traditionally cultivated in the winter months, in about 44,234 hectares of land and about 41189.26 m tons of grains are produced. (BBS, 2020-21).

It is suitable for being planted in temperate, sub-tropical and tropical regions. The most suitable temperature for mung bean's germination and growth is 15-18 °C. It has high adaptability to various soil types, while the best pH of the soil is between 6.2 and 7.2. Mung bean is a short-day plant and long days will delay its flowering and podding. It can be cultivated on sandy loam, laterites, alluvial and heavy clay (black cotton) types of soils. The soil should be well drained since it is sensitive to waterlogging.

Mung bean is an important pulse crop in Asia. It can be harvested 2 months after sowing, which makes it an ideal fit for fallow periods in rice and wheat production systems. Grown between two cereal crops, mung bean provides additional income for farmers and nutritious food for people. As a legume crop, mung bean associates with nitrogen-fixing bacteria and improves soil fertility, lowering the need for nitrogen fertilizers and increasing yield and quality of subsequent cereal harvests.

Mung bean is a popular pulse in the diet because it is easily digestible, free from flatulence and easy to cook. They are high in nutrients and antioxidants, which may provide health benefits. In fact, they may protect against heat stroke, aid digestive health, promote weight loss and lower “bad” LDL cholesterol, blood pressure and blood sugar levels. According to the United States Department of Agriculture (USDA), 100 grams (g) of boiled mung

beans contain: 7.02 g of protein, 19.15 g carbohydrate, 2 g sugar, 7.60 g dietary fiber. They are also an excellent source of fiber, potassium, copper, and B vitamins.

Mung beans are known for having potential health benefits. Researchers have found several antioxidants in mung beans, including linoleic acid, palmitic acid, oleic acid, and more (Anwar *et al.,* 2007). These antioxidants help neutralize free radical activity, which can reduce the risk of disease. Free radical damage has been linked to heart disease, cancer, chronic inflammation, and other diseases. High blood sugar is a main characteristic of diabetes and has been linked to other chronic diseases. Some lifestyle changes, including adopting a balanced diet, can help lower blood pressure. Some of the nutrients in mung beans, including potassium, magnesium, and fiber, have been linked to a lower risk of high blood pressure (Ascherio *et al.,* 1992).

DNA fingerprinting and molecular diversity analysis can help to improve the varieties and to make the varieties more compatible. Diversity in plant genetic resources provides chances for plant breeders to develop new and upgraded cultivars with desirable features, which include both farmer – preferred traits (large seed, High yielding potentials etc.). Information on molecular diversity among the plant materials is essential to a plant breeder for a systematic choice of parents for hybridization. Molecular diversity analysis also remarkably help in varietal selection for breeding purposes as well as for molecular characterization of different released cultivars in Bangladesh.

Several markers may be used to recognize and evaluate the genetic diversity and phylogenetic relationships in plant genetic resources. The traditional method based on morphological traits requires extensive observation of mature plants but cannot serve as unambiguous markers because of environmental influences (Beckmann *et al.,* 1983) Protein and isozyme electrophoresis may be used, but the major constraint of these techniques is deficient polymorphism among the closely related cultivars. Proteins are the product of gene expression and their amounts vary in different tissues, developmental stages and environments (Williams *et al.,* 1990*).* As an alternative, randomly amplified polymorphic DNA (RAPD) technique developed by Williams et al. (Ahmed, 1999) is reliable, faster and easier for exploiting genetic polymorphism within and among species and populations (Wrigley *et al.,*1987). RAPD markers have been already profitably used

on many other crops. RAPD developed by Welsh and McClelland (1990), which is a PCR based simplest marker technique. It can also be used to make relationships among varieties and in the construction of genetic map.

RAPD analysis is often endorsed because of reduced complexity. RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligonucleotide sequences of arbitrary nature as primers (Yifeng & Chen, 2008). RAPD does not need any preceding knowledge of DNA sequence. RAPD- PCR is currently used as a tool for the estimation of genetic variability between genotypes in breeding programs. The RAPD technique used for comparative analysis is quick, easy to use, free from environmental influences, unlimited in number, random but wide coverage of genome, and has a relatively high level of polymorphism (Newbury and Ford -Lloyd, 1993). RAPD are dominant markers that can inspect several loci in a single assay ranging from 0.5 to 5 kb. Fortunately, DNA markers made possible a rapid survey of a species' genetic diversity and genetic structure and RAPD technique is an uncomplicated and powerful DNA marker tool, though the technique has also some limitations.

Keeping in view the role of RAPD markers in the determination of genetic diversity, the present study was carried out to determine genetic diversity among different genotypes of mung bean using RAPD markers and selection of genetically diverse genotypes for future breeding programs. RAPD analysis also showed promise as an effective tool in estimating genetic polymorphism in different mung bean genotypes.

The aim of this work to provide genetic variation and relatedness of mung bean cultivars by PCR based RAPD technique as it is important particularly for variety selection for breeding purpose, hybridization, evaluation and conservation of their genetic pool. To attain this aim, the present study was carried out with the following objectives:

- To analyse molecular diversity analysis of different mung bean germplasm.
- DNA fingerprinting of different mung bean germplasm.
- To study Polymorphism among different mung bean germplasm.
- To establish dendrogram and phylogenic relationship among the genotypes.

CHAPTER II

REVIEW OF LITERATURE

Mung bean (*Vigna radiata* L. Wilczek) is cost-effectively most important crops of the pulse group. The mung bean is also known as green gram, golden gram and moong. It belongs to fabacean family, a diploid species with 2x=2n=22 chromosomes. Several researchers throughout the world have performed research activities on mung bean genetic diversity and relationship, phylogenic study and characterization through molecular markers like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) etc. Some of the research works have also done in Bangladesh. So, in order to improve the genetic status of this crop, conducting more researches should be encouraged. The most relevant literatures about the present study have been reviewed and some of the relevant literatures are cited below.

2.1 Molecular Markers:

Molecular markers are influential tools to determine genetic diversity and genetic relationship. Genetic markers can be grouped into three broad categories i.e. Morphological marker, Biochemical marker and Molecular marker. According to oxford advanced dictionary, marker is a distinctive feature or characteristics indicative of a particular quality or condition. Markers are any trait of an organism that can be identified with confidence and relatively easy way (Bhat *et al.,* 2010).

Datta *et al.* (2011) defined that genetic marker as a readily identifiable genetic trait, gene, DNA segment, or gene product used for identification purposes especially when closely linked to a trait or to genetic material that is difficult to identify.

Morphological markers are precise and distinct. These are connected to color, shape, size and surface of numerous plant parts. For the varietal recognition, such characters are used. Morphological markers vary among varieties, genus and species of plants and animals (Jiang *et al.,* 2013 and Bagali *et al.,* 2010).

The use of biochemical markers implies the evaluation of isozymes and seed storage proteins. Isozymes are alternative forms or systemic variants of an enzyme that have different molecular weights and electrophoretic mobility but have the same catalytic activity or function. Isozymes consider the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution (Xu, 2010). Isozymes can be mapped onto chromosomes and then used as genetic markers to mapped onto chromosomes and then used as genetic markers to mapped other genes. They are also used in seed purity test and occasionally in plant breeding (Jiang, 2013). Major advantages of biochemical markers consist in assessing co-dominance, absence of epistatic and pleiotropic effect, ease of use and low costs (Mondini *et al.,* 2009). But there are only a small number of isozymes in most crop species and some of them can be identified only with a specific strain. Therefore, the use of enzyme markers is limited (Jiang, 2013). Isozymes have limitations in protein they also lack specificity and sensitivity to detect some genomic changes (Jiang, 2013 and Bagali *et al.,* 2010).

Molecular markers are effective because they identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. A molecular marker is a DNA sequence that can be readily detected and whose inheritance can easily be monitored. Amin *et al.,* (2010) stated that, the use of molecular markers is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purposes.

There are mainly two types of molecular markers i.e. (i) Hybridization based or Non-PCR based for example Restriction Fragment Length Polymorphism (RFLP) (Botstein *et al.,*1980) and (ii) Polymerase chain reaction (PCR) based markers, e.g. Random Amplified Polymorphic DNA (RAPD) (Williams *et al.,* 1990), Sequence characterized amplified regions (SCARs) (Michelmore *et al.,*1991) and Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.,* 1995).

Molecular markers can be used for molecular characterization and detecting genetic variation and relationship of plants. These markers can detect the variation that arises for deletion, duplication, inversion and/or insertion in the chromosomes. Such markers

themselves do not affect the phenotype of the traits of interest because they are located only near or linked to genes controlling the traits (Mondini *et al.,* 2009).

2.2 Idea of RAPD primer:

Random amplified polymorphic DNA (RAPD) is a PCR-based marker system. RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD is widely used approach for characterization of DNA from plants and other species. Short oligo-nucleotide primers of arbitrary (random) sequence are used in PCR reaction to produce amplified products. RAPD-PCR primers are not planned to amplify a specific target sequence, the amplified loci are anonymous and probably scattered throughout the genome (William *et al.,* 1990).

The RAPD-PCR technique has provided a comparatively easy and inexpensive method for examining genetic divergence in the total genome (Hadrys *et al*., 1992). RAPD analysis is commanding over isozyme electrophoresis because it produces greater numbers of loci needed for genetic diversity analysis (Kimbeling *et al*., 1996). RAPD markers can be used as presumably unbiased and neutral markers for genetic mapping applications (Chapco *et al.,* 1992) as well as for genetic distinguishing DNA-based markers provide powerful tools for discriminating variations within crop germplasm and for studying evolutionary relationships (Gepts *et al.,* 1993). RAPD has been used to build genetic maps of various agronomic traits in various agronomic traits in various crop species (O'brein, 1990; William *et al.,*1993). It allows the identification of texa and the determination of phylogenic relationship and intra-specific diversity at a molecular genetic level (William *et al*., 1990). The amplified products are visualized by separation on agarose gel and stained with ethidium bromide. They usually bring about DNA fragment patterns that are polymorphic between genotypes, there by detecting diversity within them (Tommercup *et al.,* 1998).

Vierling and Nguyen (1992) identified that, the polymorphism detected between amplification products of different individuals using the random, short, single primers

made RAPD marker studies good for genetic relationships, genetic diversity, genetic mapping, plant breeding, DNA fingerprinting and population genetics.

Despite having many usefulness of RAPD marker it has some barriers. Because of random nature of genome sampling, the RAPD analysis is not a proper technique when the difference between the two genomes is basing compared is limited to a highly small genetic fraction. The most inevitable problem is dominance of RAPD band does not differentiate whether its respect locus is homozygous or heterozygous or co-dominance which is possible when SSR is used (Rahman *et al*., 2006).

In spite of having such problems, the relative ease and agility the high degree of polymorphisms and virtually inexhaustible pool of possible genetic marker makes the RAPD technique advantageous over other molecular technique (Clark and Langian, 1993). RAPD markers, in particular, have been successfully used for determination of intra-species diversity in numeral plants, whereas fewer reports are available on determination of inter-species diversity (Goswami and Ranade, 1999).

2.3 RAPD markers for genetic diversity of mung bean:

Varma *et al*., (2018) studied the genetic diversity of twelve Indian mung bean [*Vigna radiata* (L.) Wilczek] germplasm by using Random Amplified Polymorphic DNA (RAPD) markers. In this research, 17 random primers were used and 11 of them generated reproducible distinct RAPD patterns. This 11 RAPD primers were used for PCR amplification.11 RAPD primers generated 152 fragments, of which 108 were polymorphic, thus with average number of bands amplified per primer was 13.8 and with an average of 9.8 polymorphic fragments per primer. The number of amplified fragments with random primers ranged from 8 (OPA 14) to 22 (OPA11). Polymorphism Percentage ranged from minimum 23.08% (OPA09) to a maximum of 100% (OPN 05 and OPN 016), with an average of 71.05%. The Jaccard's similarity coefficients matrix based on RAPD profiles were subjected to UPGMA cluster analysis. Cluster analysis of data using UPGMA segregated the 12 mung bean germplasm into two major clusters which assured the genetic diversity among the germplasm.

Bhuyan *et al*., (2014) observed the genetic diversity and relationship in 10 selected mung bean germplasm by RAPD molecular marker. In this study, 3 primers (OPA01, OPB06 and OPB07) were used to analyse 7 exotic and 3 advance germplasms. 6 amplified products/primer were formed on an average with 78.33% of overall polymorphisms. AVRDC-3 and AVRDC-4 showed the highest (0.93) similarity co-efficient, indicating less divergence and was least (0.39) between AVRDC-5 and AVRDC-6 which indicated more divergence. Genotypes AVRDC-5, AVRDC-6 and AVRDC-7 were observed to be quite distinct on the basis of UPGMA dendrogram and the simple matching coefficient differed from 0.1824 to 0.8109.

Singh *et al*., (2014) studied the competency of SSR, ISSR and RAPD markers in molecular characterization of mung bean and other *Vigna* species. By using SSR, ISSR and RAPD markers, genetic diversity among 35 *Vigna* genotypes was analysed. A total of 319 bands were produced by SSR (21), ISSR (17) and RAPD (25) markers, of which 284 exhibited polymorphism. The genotypes were distinguished effectively by all the marker systems. In this study, the similarity coefficients were higher for all the three marker systems, but were lower for SSR compared to ISSR and RAPD markers. The pooled genetic diversity data grouped 35 genotypes into 4 major clusters with the majority of the genotypes showed relationship according to the species abundance. The DNA based markers used in the study were systemic in discerning the studied *Vigna* species.

Sony *et al*., (2012) conducted a research on Genetic diversity analysis of thirteen mung bean (*Vigna radiata* (L.) Wilczek) cultivars using RAPD markers. The genetic diversity analysis was observed by using polymerase chain reaction (PCR) based Random Amplified Polymorphic DNA (RAPD) technique. A total of 20 arbitrary decamer primers were used, 10 primers produced 379 different bands in total with an average of 37.9 bands per primer. All the primers were found to be 100% polymorphic based on the observed banding pattern. Band size ranged from 250 - 5000 bp. 10 unique DNA fragments was amplified in a total from genome of the 13 mung bean cultivars. It was found that the values of pair-wise genetic distances ranged from 0.0700 - 1.0852, which indicated the appearance of wide genetic diversity. BARI Mung-2 and 6 showed the highest genetic distance (1.0852) was found between cultivars while the lowest (0.0700) distance was in between cultivar BINA

Mung-2 and 7. In this study, dendrogram based on Neis genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) segregated the 13 mung bean varieties into two major clusters. Cluster 1 was formed by BARI Mung-1, 2, 3, 4 and 5 and cluster 2 was formed by BARI Mung-6, BINA Mung-1, 2, 7, 6, 4, 5 and 8. For performing mung bean improvement program the genetic variation detected through RAPD analysis which has a lot of importance.

Datta *et al*., (2012) observed the DNA polymorphism in 24 Indian mung-bean cultivars through using Random amplified polymorphic DNA (RAPD) markers. In the study, A total of 60 random primers were used and 33 of them produced reproducible RAPD bands. 249 fragments were yielded through the amplification of genomic DNA with these RAPD primers, among them 224 were polymorphic, 7.0 polymorphic fragments were found on an average per primer. 2 (OPI 9) to 17 (OPD 7) was the range of the number of amplified fragments. It was found that polymorphism percentage ranged from minimum 33% (OPX 5) to a maximum of 100% (OPX 4, OPX 6, OPX 13, OPX 15, OPX 19, OPD 5, OPD 7, OPD 20, OPI 4, OPI 6, OPI 13, OPI 14, OPI 18 and OPF 1), 90% was the average number. The Jaccard's similarity indices based on RAPD profiles were subjected to UPGMA cluster analysis where the genotypes were grouped in two major groups. Cluster I was formed by sixteen out of 24 released cultivars. This pointed the narrow genetic pool in the Indian mung bean cultivars used in the study. The feature of diversity analysis and feasible reasons for narrow genetic base in mung bean cultivars were conferred in this study.

Raturi *et al*., (2012) studied the Molecular characterization of *Vigna radiata* (L.) Wilczek genotypes based on nuclear ribosomal DNA and RAPD polymorphism. To analyse the genetic diversity and phylogenic relationships among forty-four mung bean [*Vigna radiata* (L.) Wilczek] genotypes, random amplified polymorphic DNA (RAPD) technique was followed in this study. Twelve RAPD primers generated Multi locus genotyping by 166 markers and an average of 82% polymorphism in banding patterns was detected. 44 genotypes were segregated into six clusters by a constructive dendrogram. Higher values of Nei's gene diversity (h) and genetic distance analysis confirms the presence of huge genetic variation among mung bean genotypes tested. Besides insertions/deletions (INDELS), single nucleotide polymorphisms (SNPs) and internal transcribed spacer (ITS)

length variations were located at number of places in nuclear rDNA region and the sequences of every sub-cluster and all diverge genotypes had been put forward to NCBI database. Further lineages of distinct genotypes with main RAPD clusters were revealed by multiple sequence alignment with main RAPD clusters. The estimation of relative genetic distances among the genotypes did not completely correspond the topographical places of their development. The phenotypic markers which were homogeneous showed insufficient in exhibiting genetic deviation among mung bean genotypes studied. NDM-56, RMG-62 and RMG-976 had been identified as probable source of parents for crop development. RAPD primers, OPA-2 OPA-9 as polymorphic genetic primers and number of seeds/plant and number of pods/plant as reliable phenotypic markers had been identified for improving yield potentials. This genetic diversity will be of importance in expanding intraspecific crosses in mung bean crop improvement program.

Lavanya *et al*., (2008) observed the molecular assessment of genetic diversity in mung bean germplasm. In this study, RAPD profiles were used to identify the diversity among 54 accessions of mung bean that included both improved and native varieties. Although 40 primers were tested, 174 amplification products was generated by seven primers with an average of 24.85 bands per primer. The RAPD profiles were observed for Jaccard's similarity coefficients which was found to be in the range from 0 to 0.48 and that indicates the presence of huge range of genetic diversity at molecular level. Cluster analysis was performed based on distances (1-similarity coefficient) using neighbour-joining method. The dendrogram involves all the accessions into two major clusters, I (with 11 accessions) and II (with 43 accessions). However, the cluster was further redivided into four subclusters (II A with six, II B with nine, II C with 15 and II D with 13 accessions). The distribution of the accessions in different clusters and subclusters appears to be linked to their performance in field conditions for 10 scored morphological traits. This study indicated that the RAPD profiles provide a simple and easy technique for genetic diversity assessment of mung bean accessions that may reflect morphological trait differences among them.

Lekhanpaul *et al.,* (2000) studied the genetic structure of 32 commercial cultivars of mung bean of India by molecular markers and their genome size. In this study, 21 decamer primers were used in 32 Indian cultivars of mung bean to perform the random amplified polymorphic DNA (RAPD) analysis. 267 amplification products were formed in a total and on an average of 12.71 bands per primer with 64% of overall polymorphism. Polymorphism extent was found medium to low. Jaccard similarity coefficient values ranged from 0.65 to 0.92. The cluster analysis resulted in mainly three clusters revealing higher parity between cultivars which were released from the same source. The outcome of principal components analysis also verified this conclusion. The high degree of prevalence in their pedigrees reveals the close genetic similarity between the cultivars.

CHAPTER III

MATERIALS AND METHODS

The chapter constitutes on the materials and methods of the experiment. The details of materials and methodologies followed for the study have been expressed in this chapter.

3.1 Experimental location and duration

The experiment was carried out at the Biotechnology laboratory of the department of Biotechnology, Sher-e- Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. The period of the experiment was July 2021 to June 2022.

3.2 Source of study materials

Fifteen mung bean germplasms were used in the study. The sources of mung bean germplasm are presented in Table 1.

Table 1: List of mung bean germplasms and their sources

SL.No.	Germplasm name	Source of collection area
1	BU mug1	Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur
2	BU mug 2	Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur
3	BU mug 3	Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur
4	BU mug 4	Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur
5	BU mug 5	Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur
6	BU mug 6	Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur
7	BINA Moog 5	Bangladesh Institute Of Nuclear Agriculture, Mymensingh
8	BINA Moog 8	Bangladesh Institute Of Nuclear Agriculture, Mymensingh

9	BINA Moog 9	Bangladesh Institute Of Nuclear Agriculture, Mymensingh
10	BINA Moog 10	Bangladesh Institute Of Nuclear Agriculture, Mymensingh
11	BARI Mung 1	Pulse Research Centre, BARI, Gazipur
12	BARI Mung 2	Pulse Research Centre, BARI, Gazipur
13	BARI Mung 5	Pulse Research Centre, BARI, Gazipur
14	BARI Mung 6	Pulse Research Centre, BARI, Gazipur
15	BARI Mung 7	Pulse Research Centre, BARI, Gazipur

3.3 Seed germination and collection of leaf sample

Good quality, disease free, healthy mung bean seeds were collected from BSMRAU, BARI and BINA. Seeds were sawn in fifteen different petri dishes at the laboratory of Department Of Biotechnology, Sher-e-Bangla Agricultural university. All management practices were done for raising good quality seedlings from those materials. After raising seedling, fresh and young samples were collected at 3 to 4 leaf stage after 7 days of germination and used as the source of genomic DNA extraction in order to carry out RAPD based PCR amplification of mung bean genome.

3.4 Extraction of genomic DNA

Genomic DNA was extracted from the leaf sample by using appropriate protocol of Phenol-Chloroform-Isoamyl alcohol method described by Islam *et al.,* (2013) with some modifications. The following reagents and methods were used for the isolation of total genomic DNA.

3.4.1 Equipments and small instruments used for genomic DNA isolation:

1. Autoclave machine
2. Digital Balance
3. Centrifuge machine
4. Ice machine
5. Water bath
6. Micropipettes
7. pH meter
8. Refrigerator
9. Water de-ionizer
10. Water distillation plant
11. Fume hood
12. Spin column
13. Incubator machine
14. Beaker
15. Conical Flask
16. Eppendorf tubes
17. Gloves
18. Nuclease free micropipette tips
19. Mortar and pestle
20. Collection tubes
21. Liquid nitrogen

3.4.2 Reagents Used

1. Extraction buffer, pH =8

Composition of extraction buffer as follows:

- 1M tris-HCl (pH=8.0)
- 0.5 M EDTA (Ethelene diamine tetra -acetic Acid) (pH=8.0)
- 5M NaCl
- D.H_2O
- SDS (Sodium Dodecyl Sulphate)
- Marcapto-ethanol
- PVP (polyvinyl pyrrolidone)

2. Phenol: Chloroform: isoamyl Alcohol ratio = 25: 24: 1

3. TE (Tris EDTA) buffer, pH= 8.0

Composition of extraction TE buffer are as follows:

- 1 M Tris – HCl
- 0.5M EDTA
- D.H_2O

4.Isopropanol

5. 0.3 M Sodium Acetate

6. Absolute (100%) ethanol

7. Ethanol (70%)

8. RNase

9. Ethidium Bromide solution

3.4.3 Reagents preparation

Stock Solution for Extraction Buffer for 1000 ml solution preparation

- 100 ml 1 M Tris HCl (pH 8.0) was taken in a measuring cylinder
- Then 40 ml of 0.5 M EDTA was added .
- 100 ml 5 m NaCl was mixed with the mixture.
- Finally sterilized dd. H_2O was added to make the volume upto 1000 ml .
- Then the mixture was mixed well and autoclaved .

Stock solution of 1 M tris -HCl pH 8.0 for 250 ml

- At first 30.28 g Tris was taken in a volumetric flask (500 ml)
- 100 ml dd H_2O was added .
- pH was adjusted to 8.0 by adding HCl.
- Then sterilized dd.H_2O was added to make the volume up to 250 ml.
- The solution was autoclaved.

Stock solution of 0.5 M EDTA pH= 8.0 for 250 ml

- At first 46.53 g EDTA .2H_2O was added in a volumetric flask (500 ml)
- 100 ml dd H_2O was added .
- Then 4.00 g NaOH was added.
- pH was adjusted to 8.0 with NaOH.
- Then sterilized dd.H_2O was added to make the volume up to 50 ml.
- The solution was autoclaved .

Stock solution of 5 M NaCl for 250 ml

- Firstly 73.05 g of NaCl was added in a 250 ml dd H_2O.
- It was then mixed well and autoclaved .
- The solution was autoclaved.

2% SDS Stock solution for 100 ml

- 10 g of SDS was added in a 100 ml of extraction buffer solution in a 250 ml beaker.
- As SDS is hazardous, so the mixture was mixed by a hot magnetic stirrer well but not autoclaved
- 20 ml of 10% that solution added in 80 ml dd H_2O in a 250 ml beaker.

Stock solution of TE Buffer for 100 ml

- 1 ml Tris HCl (pH 8.0) was taken in a volumetric flask.
- Then 0.2 ml EDTA (pH 8.0) was added.
- Sterilized dd H_2O was added to make the volume up to 100 ml.

Composition of 5x TBE buffer (1litter)

- 54 g Tris – HCl
- 27.5 g of Boric acid
- 4.65 g of EDTA
- pH =8.3
- Added 1000 ml of dd.H_2O and pH was adjusted at 8.3.

Phenol: Chloroform: Isoamyl Alcohol ratio=25:24:1(100ml)

- At first 50 ml Phenol was taken in a volumetric flask (250 ml).
- Then 48 ml Chloroform was added.
- 2 ml Isoamyl Alcohol was also added and mixed well.

- The solution was stored at 4°C.

RNase A/H

- 10 g of RNase was added to 1 ml of dd H_2O.
- Then it was dissolved completely with the help of necessary heat (at 65°C in water bath for 30 minutes)

70% Ethanol (1000 ml)

- 700 ml absolute ethanol was mixed with 300 ml dd.H_2O.

0.3 M Sodium Acetate

- 2.05 gm of Na acetate dissolved in 50 ml sterilized d. H_2O then we get 0.3 M Na acetate.

3.5 Sequential steps of DNA extraction from leaf sample of Mung bean

1. For isolation of genomic DNA, vigorous, young, actively growing fresh leaf tissues were collected from 15 different mung bean germplasm.
2. Initially, leaves were washed thoroughly by tap water followed by de-ionized water. Then the leaves were sterilized by ethanol to get rid of wastes and any source of foreign DNA and leaves were then dried on tissue paper.
3. Approximately 100 mg of young leaves were cut into small pieces and then taken in mortar. 500μl of extraction buffer and 30-40 ml of marcapto-ethanol with near about 30 mg of PVP chemical was added to it. The sample was grinded properly. The grind samples were taken into the 1.5 ml Eppendorf tube and then it was vortexed for 20 second in a vortex mixture and incubated about 65°C for 20 minutes in hot water bath.

4. Equal volume (500 μl) of phenol: Chloroform: Isoamyl Alcohol (25:24:1) was added to the tube. Then it was vortexed for 20 seconds.
5. The solution was then centrifuged for 10 minutes at 13000 rpm. The supernatant was recovered using a micro pipette tip without disturbing the lower portion and transferred into a new eppendorf tube. Approximately 400-500 μl was taken and then equal volume of chloroform: Isoamyl Alcohol (24:1) was added to it. The solution was vortexed for 10 seconds.
6. Again the solution was centrifuged at 13000 rpm for 10 minutes.
7. The supernatant was taken in a separate Eppendorf tube the lower layer was discarded.
8. The amount of the solution was multiplied with 0.6 and then same volume of isopropanol (0.6 volume of the liquid) was added.
9. It was then tapped by finger for 20-30 seconds (the genomic DNA was visible as cotton like structure)
10. After tapping the sample was again centrifuged at 13000 rpm for 15 minutes. The liquid was discarded completely and re-precipitation of DNA solution was done by adding 500μl of absolute (100%) cold ethanol plus 20 μl 0.3 m Sodium acetate.
11. It was shaken gently. Tapping was done to dissolve pellet. The sample was centrifuged at 13000 rpm for 15 minutes. The liquid was removed completely by pouring and blotting the open tube end on fresh tissue paper.
12. The DNA pellet was then air dried for 2-3 hours. It was then dissolved in an appropriate volume (30 to 40μl) of TE buffer and treated with RNAse at 37 ℃ in hot water bath for 15 to 20 minutes.
13. Finally, the DNA samples were stored in freezer at -20℃.

3.6 Confirmation of DNA preparation

The isolated genomic DNA contains large amount of RNA and protein as contaminant and hence purified by treatment with RNase and proteinase further precipitated. To confirm DNA preparation 1% agarose gel used for assessing the quality of genomic DNA.

Reagents :

1. Agarose powder
2. 1x TBE Buffer (pH 8.0)
3. Ethidium Bromide

3.6.1 Preparation of 1% agarose gel

Six hundred mg of agarose powder was taken in a 500ml Erlenmeyer flask containing 60 ml electrophoresis buffer (1x TBE buffer) prepared by adding 20 ml of 5x TBE buffer in 80 mL of sterile deionized water. The flask was covered with aluminum foil paper to prevent excessive evaporation. It was defrosted for about 2 to 3 minutes into a microwave oven with occasional swirling until complete removal of agarose particles to generate homogeneous and crystal clear suspension. Then the agarose gel was cooled to about 45-50℃ (flask was cool enough to hold comfortably with bare hand) and o.75 μl (10 mg/mL) ethidium bromide was added and mixed well by gentle shaking to make the DNA visible under ultraviolet light box (Trans – illuminator). The molten gel was poured immediately on to a clean gel tray (10 x7 x2 cm^3; in size), that was placed on a level bench and appropriate comb was inserted parallel to the plates edge with the bottom of the teeth about 2 mm above the plate. Air bubble were eliminated by pushing away to the side using a disposable tip. After 30-45 minutes gel become completely cooled at room temperature and solidified and the comb was removed gently. The gel was then ready for loading the DNA samples.

3.6.2 Preparation of DNA samples for electrophoresis

Three μl dd. H_2O and 2 μl loading dye (0.25 % xylene ethanol, 0.25% bromophenol blue, 30% glycerol and 1m EDTA) and 3.0 μl of sample DNA was taken in an Eppendorf tube using 0.5-10 μl adjustable micropipette. Loading dye was used for monitoring loading and progress of the electrophoresis and to increase the density of the sample so that it stayed in the well. The sample was then loaded into the well of the gel and allowed them to sink to the bottom of the wells. The gel was placed in the electrophoresis chamber (continental lab product. Inc.) keeping the gel horizontal and submerged in 1x TBE buffer (running buffer). The final level of buffer was about 5 mm above the gel. The gel tank was covered and electrophoresis power supply was connected and turned on to move DNA from negative to positive electrode (Black to Red) through the gel. Electrophoresis was carried out at 80V for about 55 minutes.

3.7 Working solution of DNA samples preparation

DNA concentration were adjusted to 20 -25 ng/μl for PCR amplification. Concentration was measured by using the following formula: $V1 \times S1 = V2 \times S2$

Where,

V1= Initial volume of DNA solution (μl)

S1= Initial DNA concentration (ng/μl)

V2= Final volume of DNA solution (μl)

S2=Final DNA concentration (ng/μl)

Documentation of the DNA sample

The gel was taken from the gel chamber and was placed on an ultraviolet light box (UV transilluminator, Germany) to examine and photographed by a Gel Cam Polaroid camera (Germany). Better quality band showing DNA samples were taken for working solution preparation. Quality bands showing DNA sample were taken for quantification and working solution preparation.

3.8 Amplification of RAPD markers by PCR

3.8.1 Principle of RAPD primer amplification

For amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence is mixed with genomic DNA in the existence of a thermostable Taq DNA polymerase and a suitable buffer and then it is subjected to temperature cycling conditions typical to the Polymerase Chain Reaction (PCR). The products of the reaction depend on the sequence and length of the oligonucleotide, as well as the reaction conditions. At a suitable annealing temperature the single primer binds to the sites on opposite strands of the genomic DNA that are within an amplifiable distance of each other (e.g., within a few thousand nucleotides) and a discrete DNA segment is produced. The presence or absence of this specific product, although amplified with arbitrary primer, will be symptomatic for the oligonucleotide binding sites on the genomic DNA. In practice, the DNA amplification reaction is repeated on a set of DNA samples with several different primers, under conditions that result in several amplified bands from each primer. Often a single primer can be used to identify several polymorphisms, each of which matches to a different locus.

3.8.2 Selection of primers:

Twenty decamer primers were tested. Out of twenty primer which produced in faint or irreproducible DNA fragments were discarded. Among them only five decamer RAPD primers were produced quality DNA amplification. The name and sequence of them along with (G+C) % were given below.

Table 2: Name of RAPD primers with GC content and sequence information

Sl. No.	Primer name	Sequence (5' to 3')	(G+C) %
1	OPA-20	GTTGCGCTCC	70%
2	OPBC -1	CCTTCGGCTC	70%
3	OPP-3	CTGATACGCC	60%
4	OPT-20	GACCAATGCC	60%
5	OPBC-18	GTGAAGGAGG	60%

3.8.3 PCR amplification

PCR reactions were performed on each DNA sample, 2x Taq ready Master Mix was used. DNA amplification was performed in oil- free thermal cycler (Esco Technologies swift^{TM} Mini Thermal Cycler and Q-cycler, (Germany). To prepare a 10.0μl reaction mixture containing ready mix Taq DNA polymerase and other compositions were given in Table 3.

Table 3: PCR mixture composition for each mung bean genotype

Reagents	Amount(μl)
2x Taq Master Mix	5.00
RAPD primer	1.50
De-ionized water	1.50
Sample DNA	2.00
Total Reaction volume	10.00

From frozen stocks of the PCR reagents i.e., 2x Taq Master Mix, Primer and DNA working samples were melt, mixed by vortexing and kept on ice for maintaining standard quality. DNA samples were pipetted first into PCR tubes compatible with the thermos-cycler used (0.2 ml). A pre-mixture was then prepared in the course of the following order: reaction mixture, DNA sample and de-ionized water. Then the mixture was mixed up well and aliquoted into tubes containing primer. Then tubes were then sealed and placed in a thermal cycler and cycling was started immediately.

Table 4: Thermal profile for PCR

Steps	Step name	Temperature	Time
1	Initial denaturation	95℃	4 minutes
2	Denaturation	95℃	45 seconds
3	Primer annealing	35℃	40 seconds
4	Extension	72℃	1 minutes
5	Final extension	72℃	5 minutes
6	Hold	4℃	10 minutes

3.8.4 Electrophoresis of the amplified products

After completion of thermal cycler reaction, each sample of PCR products were confirmed by running 2.0 % agarose gel containing 1.0 μl ethidium bromide in 1x TBE buffer at 90 V for 75 minutes. Loading dye (3.0 μl) was added to the PCR products and loaded in the wells. One molecular weight markers 100 bp (Bio – Basic, Cat No. M-1070-1, Canada) was loaded on the left side of the gel. Under Ultra Violet (UV) light on a trans- illuminator RAPD bands were observed and documented by taking photograph using a Gel Cam Polaroid camera.

3.9 Data analysis

Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single allelic locus (Williams *et al.,*1990). One molecular weight marker, 100 bp (Bio-Basic, Cat. No. M-1070 (Canada) was used to estimate the size of the amplification products by comparing the distance traveled by each fragment with known size fragments of molecular weight markers. all distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The band -size for each of the markers was scored using the Alpha Ease FC 4.0 software.

The scores obtained using all primers in the RAPD analysis were then pooled to create single data matrix. The individual fragments were assigned of the appropriate loci. This was used to estimate polymorphic loci. Using Power marker version 3.25 software. The summery statistics that were determined included the following: the number of the alleles, the major allele size and its frequency, gene diversity and the polymorphism information content (PIC) value. The allele frequency data from POWER MARKER was added to export the data in binary format (presence of allele as “1” and absence of allele as “ 0”). Binary data form of allele frequency used for dendrogram construction by NTSYS-pc software (Rholf, f., 2002). The unweighted pair grouping method, using arithmetic average (UPGMA), was used to determine similarity matrix following Dice co efficient with SAHN subprogram.

Polymorphic Information Content (PIC) value of a primer is calculated as:

$$PIC= 1-\sum pi^2$$

Where *pi* is the frequency of the ith allele. Polymorphic Information Content was used to confirm the suitability of the primers selected for DNA fingerprinting.

Genetic similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel were calculated manually by RAPD markers of the molecular weight on the data matrix according to the following formula:

Similarity Index (SI)= $\frac{2Nxy}{Nx+Ny}$

Where,

Nxy=Number of RAPD bands shared by individuals x and y respectively

Nx and Ny= Number of bands in individuals x and y respectively (Chapco *et al.,*1992; Wilde *et al*.,1992 and Lynch, 1990).

The SI value ranges from 0 to 1. When SI=1.0, the two DNA profiles are identical and when SI 0.0, there are no common bands between the two profiles.

3.10 Precautions

To maintain a strategic distance from all types of contaminations and keep DNA pure, all dishes, micropipette tips, Eppendorf tubes, glass pipettes, de-ionized water and buffer solutions were legitimately autoclaved. Metal supplies i.e., scissors, forceps were cleaned with absolute ethanol.

- Hand gloves were utilized taking care of anything that has been presented to Et-Br because Ethidium Bromide (Et-Br) is an extreme mutagen and carcinogenic in nature
- Always power pack was kept turn off and the leads were unplugged before opening the electrophoresis unit to avoid electrical hazard.

- ❖ Eye protector was used while working with trans- illuminator as it produces UV radiation of 254 nm range which can cause eye damage.
- ❖ The common safety measures were kept up at the time of performing PCR responses. All the disposables such as PCR tubes, tips, Eppendorf tubes and reagents used during preparation of PCR reactions were autoclaved. Freezing condition was assured when necessary. Hand gloves were worn amid the treatment of PCR segments. Contamination of PCR segments was maintained a strategic distance form.

Some Pictorial View of molecular work

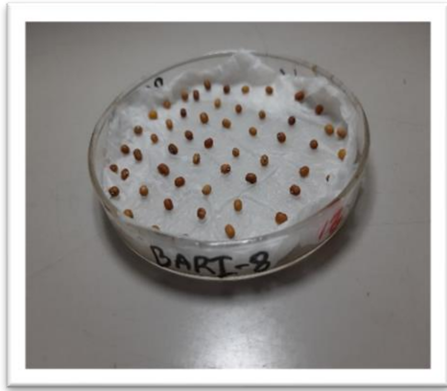
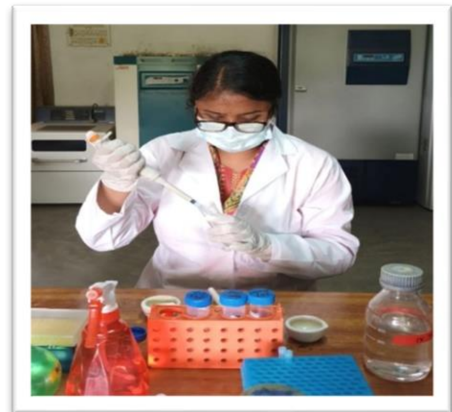


Figure1: Seed germination in a petri dish

Figure 2: Fresh, young and tender Leaf was taken for DNA extraction

3(a)



3(b)

Figure 3(a,b) : Preparing samples for DNA extraction under the guidance of honorable supervisor

Some Pictorial View of molecular work (Continued)

Figure 4: Centrifugation of the samples

Figure 5: Preparing samples for Gel loading

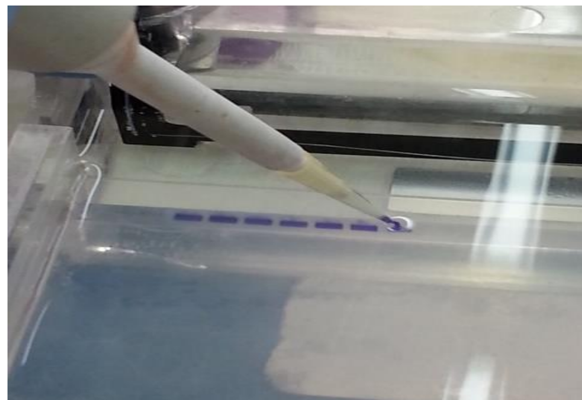


Figure 6: Loading DNA samples in gel electrophoresis unit

CHAPTER IV
RESULT AND DISCUSSION

This chapter comprised the presentation and discussion of the results of the experiment, The results were obtained from 15 genotypes of mung bean using RAPD primers. In the RAPD analysis significant genetic variation and polymorphisms for characterization of different mung bean genotypes were identified.

4.1 Extraction of DNA and Confirmation

The genomic DNA extraction of 15 mung bean genotypes were done by using phenol-chloroform method with minor modification. RNA sharing was removed by applying RNase treatment. The extracted genomic DNA of 15 samples was loaded on 1% agarose gel for confirmation and quantification of DNA sample. It revealed that, all the samples showed clear DNA band in each well (plate 1). Hence, the genomic DNA of each sample was diluted on the basis of concentration. The working DNA sample was prepared for PCR works.

Genomic DNA Confirmation of Mung bean germplasm

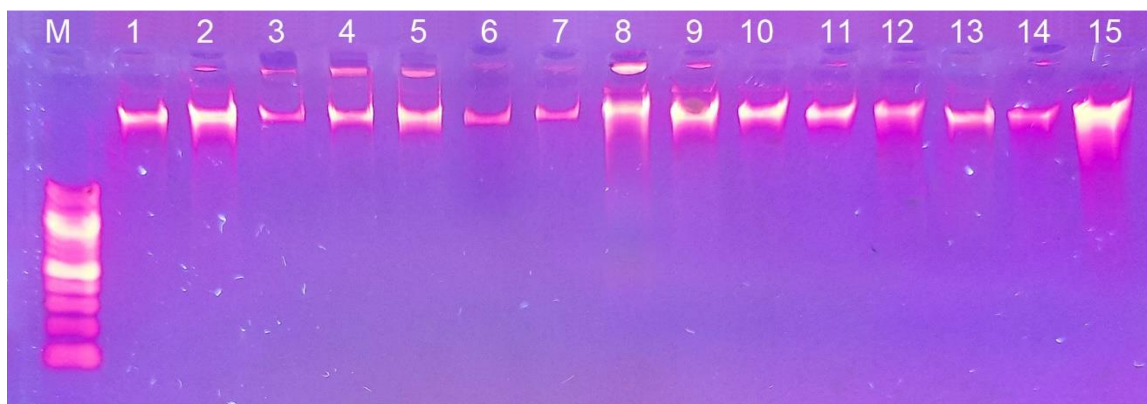


Plate 1: Isolation of genomic DNA in 15 mung bean genotypes

(M=100bp DNA ladder (Bio-Basic,Canada), Lane 1 =BU mug 1, Lane 2= BU mug 2, Lane 3= BU mug 3, Lane 4=BU mug 4, Lane 5=BU mug 5, Lane 6=BU mug 6, Lane 7=BINA Moog 5, Lane 8=BINA Moog 8, Lane 9=BINA Moog 9, Lane10=BINA Moog 10 , Lane 11=BARI Mung1, Lane 12=BARI Mung 2, Lane 13=BARI Mung 5, Lane 14=BARI Mung 6, Lane 15=BARI Mung 7)

4.2 Analysis of genetic diversity among Mung bean germplasm using RAPD marker

Among several molecular marker RAPD (Random Amplified Polymorphic DNA) is widely used molecular marker where DNA fragments are amplified by the Polymerase Chain Reaction (PCR) using short synthetic primers (usually 10 bases in length) of random sequence. In this experiment, 20 primer were used for molecular diversity analysis and among them only 5 primer produced DNA amplification and scorable band in 15 genotypes of mung bean.

4.2.1 Optimization of PCR amplification conditions and selection of primers for RAPD analysis

The technique of RAPD were developed by william *et al*.(1990). This technique is based on the PCR amplification of random site in the genome of the plant with the help of a single decamer primer per reaction. Because this 10 nucleotides long, they have the possibility of annealing at a random number of locations in the genome. However, all the primers do not produce reproducible and polymorhic banding patterns in all plant species.

PCR amplification conditions also play a crucial role in the amplification of DNA. For this reason, various concentrations of the components of the reaction mix as well as variable amplification conditions were tested in obtaining the most reproducible results. Template DNA concentration over the range of 25-50 ng gave a constant banding pattern but higher concentrations resulted in smear in gel in most cases. At lower template concentrations, there was a tendency for the appearance of non- reproducible low molecular weight bands. Template DNA concentrations of 30 ng was found to be suitable for getting reproducible banding pattens.In the case of Taq polymerase, lower concentrations failed to amplify some bands which were consistently produced at higher concentrations. On the other hand, very high concentrations inhibited the reaction.

The major factor affecting the stringency of PCR amplification is annealing temperature.The annealing temperature of 37°C was selected at which bands were reliably

amplified with all primers. Primers were selected carefully as some primers produced non-reproducible bands. The primer which gave reproducible and scorable bands in the investigated germplasm were selected. Mostly bright and prominent bands were scored. The band amplified with RAPD primers were in the range of 100bp-1500bp.

4.2.2 RAPD profiles of Mung bean germplasm

The selected 05 primers produced highly reproducible banding patterns in all the germplasm investigated. The bands were scored visually and a particular band has been described by the primer name by which it was amplified suffuxed with molecular weight of the band (for example, the band OPA -20_{400} denotes that it was amplified with the primer OPA-20 and it's molecular weight was 400 bp). The primer- wise RAPD profiles and banding paterns of 15 collected germplasm are given below-

4.2.2.1 Primer OPBC-O1

The primer OPBC-1 produced 39 RAPD loci (Table 5) within 15 germplasm. The DNA fragments ranged from 200 bp to 900 bp and no monomorphic band was found. The highest number of bands (4 bands) were produced by BU mug 4, BINA Moog 5, BINA Moog 10 and BARI Mung 2 among all germplasm. (Plate-2). The lowest number of bands (1 band) was produced by BU mug 2 & BU mug 5. The amplified bands were comparatively light and visualized clearly in all the germplasm examined.

Table 5: RAPD profile of the primer OPBC-01 in 15 mung bean germplasm

Serial No	Name of germplasm	Total no. of bands with mol. wt.(bp)	No. of light bands with mol. wt (bp)	No. of bright bands with mol. wt (bp)	No. of unique polymorphic bands with mol. Wt.(bp)
1	BU mug 1	2(900,700)	0	2(900,700)	Nil
2	BU mug 2	1(700)	0	1(700)	
3	BU mug 3	2(200,700)	2(200,700)	0	
4	BU mug 4	4(900,800,400, 300)	3(800,400,300)	1(900)	
5	BU mug 5	1(700)	0	1(700)	
6	BU mug 6	2(200,700)	2(200,700)	0	
7	BINA Moog 5	4(900,800,400, 300)	3(800,400.300)	1(900)	
8	BINA Moog 8	3(900,700,400)	3(900,700,400)	0	
9	BINA Moog 9	3(900,700,300)	3(900,700,300)	0	
10	BINA Moog 10	4(900,800,400, 300)	2(400,300)	2(900,800)	
11	BARI Mung 1	2(900,400)	1(400)	1(900)	
12	BARI Mung 2	4(900,800,400, 200)	2(400,200)	2(900,800)	

13	BARI Mung 5	2(900,300)	2(900,300)	0	
14	BARI Mung 6	3(900,700,300)	3(900,700,300)	0	
15	BARI Mung 7	2(900,200)	2(900,200)	0	
--	Total	39 loci	-	-	-

4.2.2.2 Primer OPA -20

The primer OPA-20 produced 28 RAPD loci (Table 6) within the germplasm. The highest number of bands (3 bands) were produced by BU mug 2, BU mug 3, BU mug 4, Bina Moog 8 & Bina Moog 9 among all germplasm. (plate 3). No monomorphic bands was produced and no band was amplified in BARI Mung 2.The lowest number of bands (1 band) was produced by BU mug 1, BU mug 5, BU mug 6, BINA Moog 10 and BARI Mung 6 among all.(plate 3) .The amplified bands were comparatively light and visualized clearly in all the germplasm examined.

Table 6: RAPD profile of the primer OPA- 20 in 15 mung bean germplasm

Serial No	Name of germplasm	Total no. of bands with mol.wt.(bp)	No. of light bands with mol.wt (bp)	No. of bright bands with mol. wt(bp)	No. of unique polymorphic bands with mol. Wt.(bp)
1	BU mug 1	1(600)	0	1(600)	Nil
2	BU mug 2	3(800,600, 300)	3(800,600,300)	0	
3	BU mug 3	3(800,600, 300)	3(800,600,300)	0	
4	BU mug 4	3(800,600, 300)	3(800,600,300)	0	
5	BU mug 5	1(400)	1(400)	0	
6	BU mug 6	1(400)	1(400)	0	
7	BINA Moog 5	2(900,400)	2(900,400)	0	
8	BINA Moog 8	3(800,600, 300)	3(800,600,300)	0	
9	BINA Moog 9	3(800,600, 300)	3(800,600,300)	0	
10	BINA Moog 10	1(900)	1(900)	0	
11	BARI Mung 1	2(800,200)	0	2(800,200)	
12	BARI Mung 2	0	0	0	

13	BARI Mung 5	2(400,200)	1(200)	1(400)	
14	BARI Mung 6	1(200)	1(200)	0	
15	BARI Mung 7	2(600,200)	2(600,200)	0	
--	Total	28 loci	-	-	-

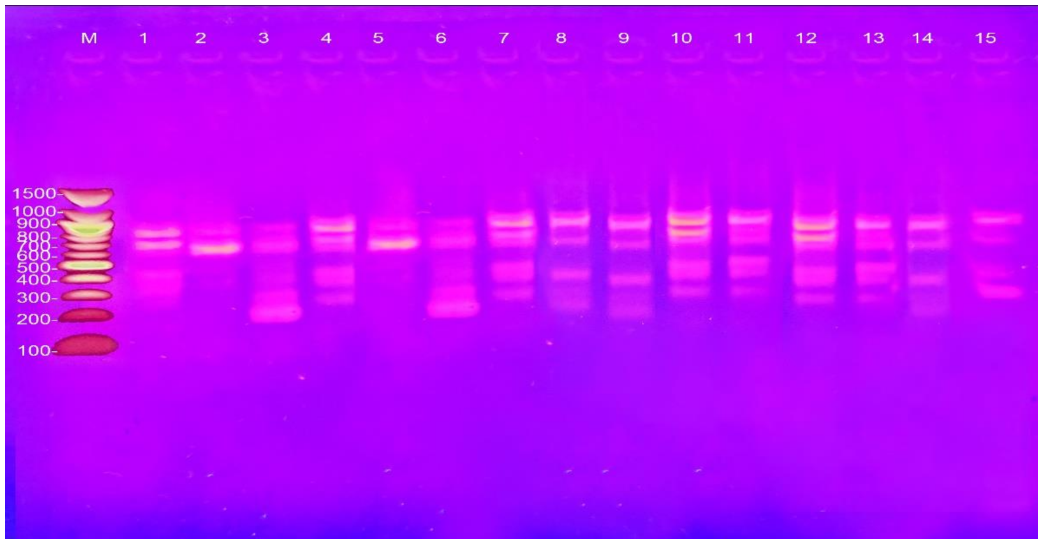


Plate 2: PCR amplification with RAPD primer OPBC- 01

(M=100bp DNA ladder (Bio-Basic, Canada), Lane 1 =BU mug 1, Lane 2= BU mug 2, Lane 3= BU mug 3, Lane 4=BU mug 4, Lane 5=BU mug 5, Lane 6=BU mug 6, Lane 7=BINA Moog 5, Lane 8=BINA Moog 8, Lane 9=BINA Moog 9, Lane 10=BINA Moog 10, Lane 11=BARI Mung1, Lane 12=BARI Mung 2, Lane 13=BARI Mung 5, Lane 14=BARI Mung 6, Lane 15=BARI Mung 7)

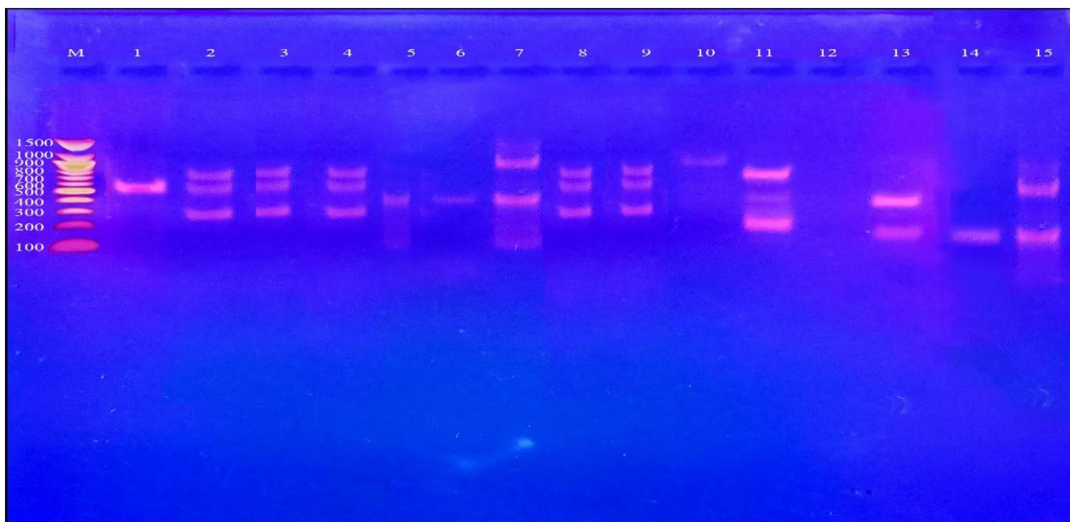


Plate 3: PCR amplification with RAPD primer OPA- 20

(M=100bp DNA ladder (Bio-Basic, Canada), Lane 1 =BU mug 1, Lane 2= BU mug 2, Lane 3= BU mug 3, Lane 4=BU mug 4, Lane 5=BU mug 5, Lane 6=BU mug 6, Lane 7=BINA Moog 5, Lane 8=BINA Moog 8, Lane 9=BINA Moog 9, Lane 10=BINA Moog 10, Lane 11=BARI Mung1, Lane 12=BARI Mung 2, Lane 13=Mung 5, Lane 14=BARI Mung 6, Lane 15=BARI Mung 7)

4.2.2.3 Primer OPP-03

The primer OPP-03 produced 36 RAPD loci (Table 7) within 15 mung bean germplasm. The highest number of bands (5 bands) were produced in germplasm BU mug 1 among all germplasm. (plate 4). No monomorphic bands observed. The lowest number of band (1 band) were produced by germplasm BU mug 2, BINA Moog 5, BARI Mung 1 & BARI Mung 6 among all. The unique polymorphic band was produced by the germplasm BU mug 1 at 400 bp and 150 bp.

Table 7: RAPD profile of the primer OPP- 03 in 15 mung bean germplasm

Serial No	Name of germplasm	Total no. of bands with mol.wt.(bp)	No. of light bands with mol.wt (bp)	No. of bright bands with mol. wt (bp)	No. of unique polymorphic bands with mol. Wt.(bp)
1	BU mug 1	5(1000,850,600,400,150)	4(850,600,400,150)	1(1000)	2(400,150)
2	BU mug 2	1(1000)	1(1000)	0	
3	BU mug 3	2(1000,600)	2(1000,600)	0	
4	BU mug 4	3(1200,850,600)	2(850,600)	1(1200)	
5	BU mug 5	3(1200,850,600)	0	3(1200,850,600)	
6	BU mug 6	3(1200,850,600)	0	3(1200,850,600)	
7	BINA Moog 5	1(600)	1(600)	0	
8	BINA Moog 8	3(1200,850,600)	2(850,600)	1(1200)	
9	BINA Moog 9	2(1200,850)	1(850)	1(1200)	
10	BINA Moog 10	3(1200,850,600)	0	3(1200,850,600)	
11	BARI Mung 1	1(600)	1(600)	0	
12	BARI Mung 2	3(1200,850,600)	0	3(1200,850,600)	
13	BARI Mung 5	2(850,600)	2(850,600)	0	
14	BARI Mung 6	1(1000)	0	1(1000)	
15	BARI Mung 7	3(1000,850,600)	3(1000,850,600)	0	
--	Total	36 loci	-	-	-

4.2.2.4 Primer OPT-20

The primer OPT-20 produced 68 RAPD loci (Table 8) within germplasm. The highest number of bands (7 bands) were produced by germplasm BU mug 3 & BU mug 6 among all germplasm. (plate 5) Monomorphic bands were observed in OPT $_{400}$. The lowest number of bands(1band) was produced by germplasm BARI Mung 5 among all.

Table 8: RAPD profile of the primer OPT- 20 in 15 mung bean germplasm

Serial No	Name of germplasm	Total no. of bands with mol. wt.(bp)	No. of light bands with mol. wt (bp)	No. of bright bands with mol. wt (bp)	No. of unique polymorphic bands with mol. Wt.(bp)
1	BU mug 1	2(400,200)	0	2(400,200)	Nil
2	BU mug 2	5(900,700,400, 200,100)	2(900,100)	3(700,400,200)	
3	BU mug 3	7(900,700,500, 400,300,200,100)	4(900,500,300,100)	3(700,400,200)	
4	BU mug 4	6(700,500,400, 300,200,100)	2(300,100)	4(700,500,400, 200)	
5	BU mug 5	5(900,700,400, 200,100)	2(900,100)	3(700,400,200)	
6	BU mug 6	7(900,700,500, 400,300,200,100)	4(900,500,300,100)	3(700,400,200)	
7	BINA Moog 5	6(700,500,400, 300,200,100)	2(300,100)	4(700,500,400, 200)	
8	BINA Moog 8	4(900,700,400, 200)	2(900,700)	2(400,200)	
9	BINA Moog 9	4(700,400,200, 100)	2(700,100)	2(400,200)	
10	BINA Moog 10	5(900,700,400, 200,100)	1(100)	4(900,700,400, 200)	
11	BARI Mung 1	6(900,700,400, 300,200,100)	2(300,100)	11(900,700,400,200)	
12	BARI Mung 2	2(400,200)	0	2(400,200)	

13	BARI Mung 5	1(400)	1(400)	0	
14	BARI Mung 6	6(900,700,400, 300,200,100)	3(900,300,100)	3(700,400,200)	
15	BARI Mung 7	2(400,200)	0	2(400,200)	
--	Total	68 loci	-	-	-

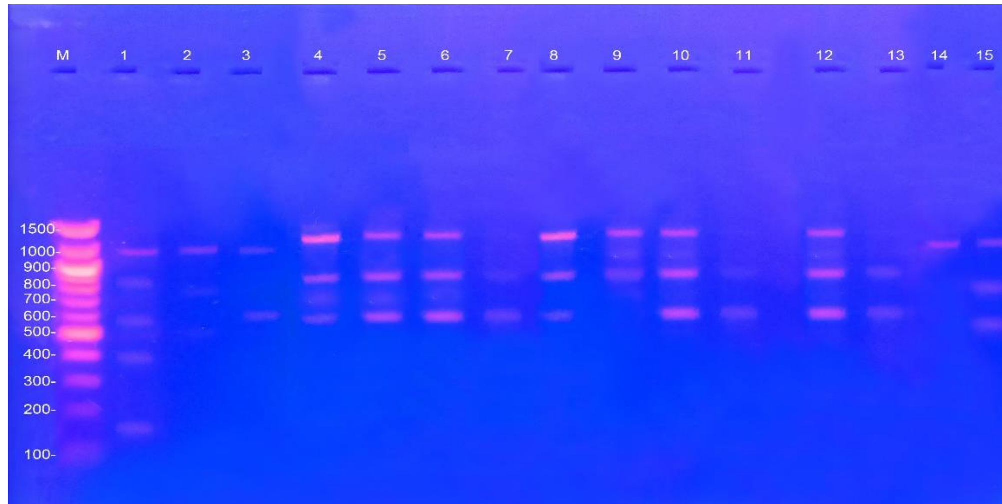


Plate 4: PCR amplification with RAPD primer OPP-03

(M=100bp DNA ladder (Bio-Basic,Canada), Lane 1 =BU mug 1,Lane 2= BU mug 2,Lane 3= BU mug 3,Lane 4=BU mug 4,Lane 5=BU mug 5,Lane 6=BU mug 6,Lane 7=BINA Moog 5,Lane 8=BINA Moog 8,Lane 9=BINA Moog 9,Lane 10=BINA Moog 10 ,Lane 11=BARI Mung1,Lane 12=BARI Mung 2,Lane 13=Mung 5,Lane 14=BARI Mung 6,Lane 15=BARI Mung 7)

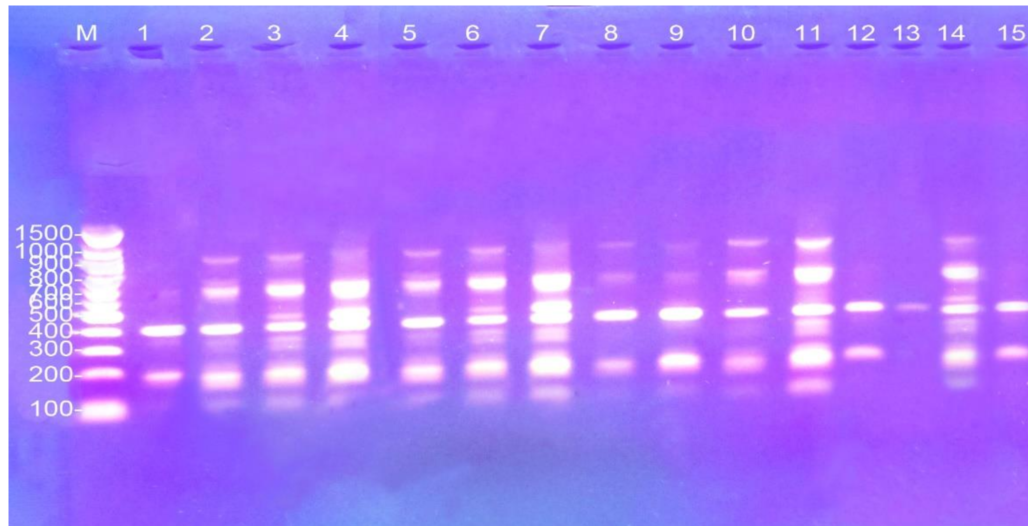


Plate 5: PCR amplification with RAPD primer OPT-20

(M=100bp DNA ladder (Bio-Basic,Canada), Lane 1 =BU mug 1, Lane 2= BU mug 2, Lane 3= BU mug 3, Lane 4=BU mug 4, Lane 5=BU mug 5, Lane 6=BU mug 6, Lane 7=BINA Moog 5, Lane 8=BINA Moog 8, Lane 9=BINA Moog 9, Lane 10=BINA Moog 10, Lane 11=BARI Mung1, Lane 12=BARI Mung 2, Lane 13=Mung 5, Lane 14=BARI Mung 6, Lane 15=BARI Mung 7)

4.2.2.5 Primer OPBC-18

The primer OPBC-18 produced 22 RAPD loci (Table 9) within germplasm. The highest number of bands (3 bands) were produced in germplasm BARI Mung 7 among all germplasm. (plate 6). No monomorphic bands observed and no band was amplified in BINA Moog 8 & 9. The lowest number of band (1band) were produced by germplasm BU mug 2, BU mug 5, BINA Moog 10, BARI Mung 2 & BARI Mung 5. The unique polymorphic band was produced by the germplasm BARI Mung 5 at 500 bp.

Table 9: RAPD profile of the primer OPBC-18 in 15 mung bean germplasm

Serial No	Name of germplasm	Total no. of bands with mol.wt.(bp)	No. of light bands with mol.wt (bp)	No. of bright bands with mol. wt (bp)	No. of unique polymorphic bands with mol. Wt.(bp)
1	BU mug 1	2(800,700)	0	2(800,700)	1(500)
2	BU mug 2	1(700)	0	1(700)	
3	BU mug 3	2(700,200)	2(700,200)	0	
4	BU mug 4	2(800,700)	1(700)	1(800)	
5	BU mug 5	1(700)	0	1(700)	
6	BU mug 6	2(700,200)	2(700,200)	0	
7	BINA Moog 5	2(800,700)	1(700)	1(800)	
8	BINA Moog 8	0	0	0	
9	BINA Moog 9	0	0	0	
10	BINA Moog 10	1(800)	0	1(800)	
11	BARI Mung 1	2(700,100)	2(700,100)	0	
12	BARI Mung 2	1(700)	0	1(700)	
13	BARI Mung 5	1(500)	1(500)	0	
14	BARI Mung 6	2(600,100)	0	2(600,100)	
15	BARI Mung 7	3(700,600,200)	3(700,600,200)	0	
--	Total	22 loci	-	-	-

Plate 6: PCR amplification with RAPD primer OPBC -18

(M=100bp DNA ladder (Bio-Basic, Canada), Lane 1 =BU mug 1, Lane 2= BU mug 2, Lane 3= BU mug 3, Lane 4=BU mug 4, Lane 5=BU mug 5, Lane 6=BU mug 6, Lane 7=BINA Moog 5, Lane 8=BINA Moog 8, Lane 9=BINA Moog 9, Lane 10=BINA Moog 10, Lane 11=BARI Mung1, Lane 12=BARI Mung 2, Lane 13=Mung 5, Lane 14=BARI Mung 6, Lane 15=BARI Mung 7)

4.3 DNA banding profile of all primer:

The 05 primers produced a total of 193 bands in all the germplasm studied with an average of 38.6 RAPD loci per primer. Of the five primers one monomorphic loci was observed at 400 bp in primer OPT-20 among all germplasm. The minimum and maximum size of the bands ranged from 100 bp to 1200 bp. The minimum DNA band size 100 bp was regenerated by the primer OPT-20. The maximum size of DNA amplification 1200 bp was produced by the primer OPP-03. A single unique polymorphic band was observed at 400bp and 150 bp in OPP-03, at 500 bp in OPBC-18.

Table 10: Total number of bands, total number of polymorphic bands, range of DNA bands, percentage of polymorphism found among 15 mung bean germplasm for 05 RAPD primers

Sl.No.	RAPD Primer	Total no. of bands	Total no. of Polymorphic bands	Range of DNA bands (bp)	Polymorphism (%)
1	OPBC-01	39	39	200-900	100%
2	OPA- 20	28	28	200-900	100%
3	OPP -03	36	36	150-1200	100%
4	OPT -20	68	53	100-900	77.94%
5	OPBC -18	22	22	100-800	100%

4.4 DNA band scoring and diversity analysis

The binary matrix representing different alleles of the 05 markers which were scored as binary data whether present (1) or absent (0) was used for estimation of genetic distance and similarity coefficients. The summary statistics including major loci frequency, gene diversity and polymorphism information content (PIC) values are given in Table No. 10.

The frequency of the major loci ranged between 0.69 (OPA-20) to 0.81 (OPBC-18) with an average value of 0.76. Gene diversity range from 0.28 to 0.41 with an average value of 0.33. The highest (0.41) was found in the primer OPA-20 and it was lowest (0.28) in OPBC-18. Polymorphic Information Content (PIC) value for 05 markers ranged between 0.24 to 0.32 and the average PIC value was 0.27. The highest PIC value (0.32) was obtained for OPA-20 and the lowest value PIC value (.24) was obtained for OPBC-18. PIC value revealed that OPA-20 was considered as the best marker in mung bean genotypes followed by OPBC-1 and OPT-20. Gene diversity ranged between 0.28 (OPBC-18) to 0.41 (OPA-20) with an average of 0.33.

The PIC was a good index for genetic diversity evaluation. Botstein *et* al. (1980) reported that PIC value more than 0.5 indicates high diversity and less than 0.25 indicates low diversity. When PIC lies between 0.25 and 0.5 indicates intermediate diversity. Data in that table show all the studied primers produced polymorphic amplification products with intermediate diversity.

Table 11: Major loci frequency, gene diversity and PIC value of different Mung bean germplasm

Marker	Observation No	Loci No	Major Loci Frequency	Genetic Diversity	PIC Values
OPBC-1	15	6	0.78	0.33	0.28
OPA-20	15	6	0.69	0.41	0.32
OPP-03	15	6	0.76	0.32	0.26
OPT-20	15	7	0.74	0.33	0.26
OPBC-18	15	6	0.81	0.28	0.24
Maximum	-	-	0.81	0.41	0.32
Minimum	-	-	0.69	0.28	0.24
Mean	-	-	0.76	0.33	0.27

4.5 Nei's genetic distance and genetic identity

Genetic distance refers to the genetic deviation between species or between populations within a species. It is measured by a variety of parameters like Nei's standard genetic distance. This distance assumes that genetic differences arise due to mutations and genetic drift, but this distance measures is known to give more reliable population trees than other distances particularly for DNA data. Similarity indices measure the amount of closeness between two individuals, the larger the value the more similarity between two individuals. There is a variety of alternative measures for expressing similarity, like Jaccard's coefficient of similarity which can be used for binary data and often is applied in RAPD-based studies. This coefficient is based on positive matches between two individual whereas joint absences are excluded. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. Genetic diversity studies help in formulating proper conservation, preservation and selection of material for breeding program.

The genetic distance of mung bean germplasm were presented in Table 11. The value of pair-wise comparisons Nei's (1983) genetic distances between 15 mung bean genotypes were computed from combined data through 05 primers, ranging from 0.1034 to 0.6207 with an average of 0.3621. The highest genetic distance 0.6207 was observed in BU mug 3 vs BARI Mung 5 varietal pair whereas lowest value 0.1034 was observed in BINA Moog 8 vs BINA Moog 9.

Nei's analysis on mung bean germplasm was also performed by several several scientists. The study conducted by Sony *et al*. (2012) observed highest genetic distance (1.0852) between cultivar BARI Mung 2 and the lowest (0.0700) between BINA Moog 2 and 7.

Table 12: Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among 15 mung bean germplasms

Germ plasm	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
G1	0	0.3793	0.5172	0.4138	0.3793	0.5517	0.4483	0.3793	0.4138	0.4138	0.4828	0.2759	0.3793	0.5172	0.2414
G2	0.3793	0	0.2069	0.3793	0.2759	0.4483	0.4828	0.2759	0.2414	0.4483	0.3103	0.4483	0.5517	0.3448	0.4138
G3	0.5172	0.2069	0	0.3103	0.4138	0.2414	0.4138	0.3448	0.3793	0.5172	0.2414	0.5172	0.6207	0.4138	0.4138
G4	0.4138	0.3719	0.3103	0	0.3793	0.3448	0.2414	0.2414	0.2069	0.2759	0.3448	0.2759	0.5172	0.5172	0.4483
G5	0.3793	0.2759	0.4138	0.3793	0	0.1724	0.3448	0.2759	0.3103	0.2414	0.3103	0.2414	0.3448	0.4138	0.4138
G6	0.5517	0.4483	0.2414	0.3448	0.1724	0	0.3103	0.3793	0.4138	0.3448	0.2759	0.3448	0.4483	0.4483	0.4483
G7	0.4483	0.4828	0.4138	0.2414	0.3448	0.3103	0	0.4828	0.4483	0.2414	0.3103	0.3103	0.4138	0.4138	0.4828
G8	0.3793	0.2759	0.3448	0.2414	0.2759	0.3793	0.4828	0	0.1034	0.2414	0.3103	0.2414	0.3448	0.4138	0.3448
G9	0.4138	0.2414	0.3793	0.2069	0.3103	0.4138	0.4483	**0.1034**	0	0.2759	0.3448	0.2759	0.3793	0.3793	0.3793
G10	0.4138	0.4483	0.5172	0.2759	0.2414	0.3448	0.2414	0.2414	0.2759	0	0.3448	0.2069	0.3793	0.3793	0.4483
G11	0.4828	0.3103	0.2414	0.3448	0.3103	0.2759	0.3103	0.3103	0.3448	0.4828	0	0.3448	0.3793	0.2414	0.3103
G12	0.2759	0.4483	0.5172	0.2759	0.2414	0.3448	0.3103	0.2414	0.2759	0.2069	0.3448	0	0.2414	0.4483	0.2414
G13	0.3793	0.5517	**0.6207**	0.5172	0.3448	0.4483	0.4138	0.3448	0.3793	0.3793	0.3793	0.2414	0	0.4138	0.2759
G14	0.5172	0.3448	0.4138	0.5172	0.4138	0.4483	0.4138	0.4138	0.3793	0.3793	0.2414	0.4483	0.4138	0	0.3448
G15	0.2414	0.4138	0.4138	0.4483	0.4138	0.4483	0.4828	0.3448	0.3793	0.4483	0.3103	0.2414	0.2759	0.3448	0

Legend: G1 (BU mug1), G2 (BU mug 2), G3 (BU mug 3), G4 (BU mug 4), G5 (BU mug 5), G6 (BU mug), G7 (BINA Moog 5), G8 (BINA Moog 8), G9 (BINA Moog 9), G10 (BINA Moog 10), G11 (BARI Mung 1), G12 (BARI Mung 2), G13 (BARI Mung 5), G14 (BARI Mung 6),G15 (BARI Mung 7).

4.6 UPGMA dendrogram

On the basis of Nei's genetic distance calculation of 15 mung bean germplasm, a dendrogram was calculated. Unweighted Pair Group Method of Arithmetic Mean (UPGMA) indicated the segregation of 15 mung bean germplasm into four main clusters: The genotype G2, G3, G5, G6, G11, G14 were grouped in cluster I (Figure7); G8, G9 were grouped in cluster II. G1, G12, G13, G15 were grouped in cluster III and G4, G7, G10 were grouped in cluster IV. Here "G" indicated the mung bean germplasm.

In cluster I, G5 (BU mug 5), G6 (BU mug 6) formed sub-cluster 1; again G2 (BU mug 2), G3 (BU mug 3), G11(BARI Mung1), G14 (BARI Mung 6) formed sub-cluster 2. G8 (BINA Moog 8), G9 (BINA Moog 9) formed cluster II. Here, G13 (BARI Mung 5), G1 (BU mug 1), G15 (BARI Mung 8) formed sub-cluster 1 and G12 (BARI Mung 2) formed sub-cluster 2 in cluster III. Again G7 (BINA Moog 5), G4 (BU mug 4) formed sub-cluster I and G10 formed sub-cluster II in cluster IV. In this dendrogram, mung bean germplasm of more genetic similarity are placed in the same cluster. This dendrogram revealed that the germplasm that derivatives of genetically similar type clustered together.

The genotypes in the same cluster show closeness or lower distance, they are genetically similar such as G8 (BINA Moog 8), G9 (BINA Moog 9). The other germplasm showing more genetically dissimilarity grouped in another cluster due to higher distance. They can be easily used for crop improvement program.

Cluster analysis on mung bean germplasm was also performed bey several scientists. The study conducted by Golam *et al*. (2012) on genetic distance by UPGMA dendrogram segregated the 20 mung bean genotypes into 3 main groups and 1 minor group. Cluster I consisted of 9 genotypes, cluster II of 7, cluster III of 1 and cluster IV of 3 genotypes.

Sony *et al*. (2012) conducted Dendrogram based on using Unweighted Pair Group Method of Arithmetic Means (UPGMA) has segregated the 13 mung bean cultivars into two major clusters. BARI Mung-1, 2, 3, 4 and 5 formed cluster 1 and BARI Mung-6, BINA Mung-1, 2, 7, 6, 4, 5 and 8 have made cluster 2.

Table13: List of germplasm with their cluster based UPGMA dendrogram

Cluster I	Sub cluster	Germplasm
I	1	G5 (BU mug 5), G6 (BU mug 6)
	2	G2 (BU mug 2), G3 (BU mug 3), G11 (BARI Mung1), G14 (BARI Mung 6)

II	1	G8 (BINA Moog 8), G9 (BINA Moog 9)

III	1	G13 (BARI Mung 5), G1 (BU mug 1), G15 (BARI Mung 8)
	2	G12 (BARI Mung 2)

IV	1	G7 (BINA Moog 5), G4 (BU mug 4)
	2	G10 (BINA Moog 10)

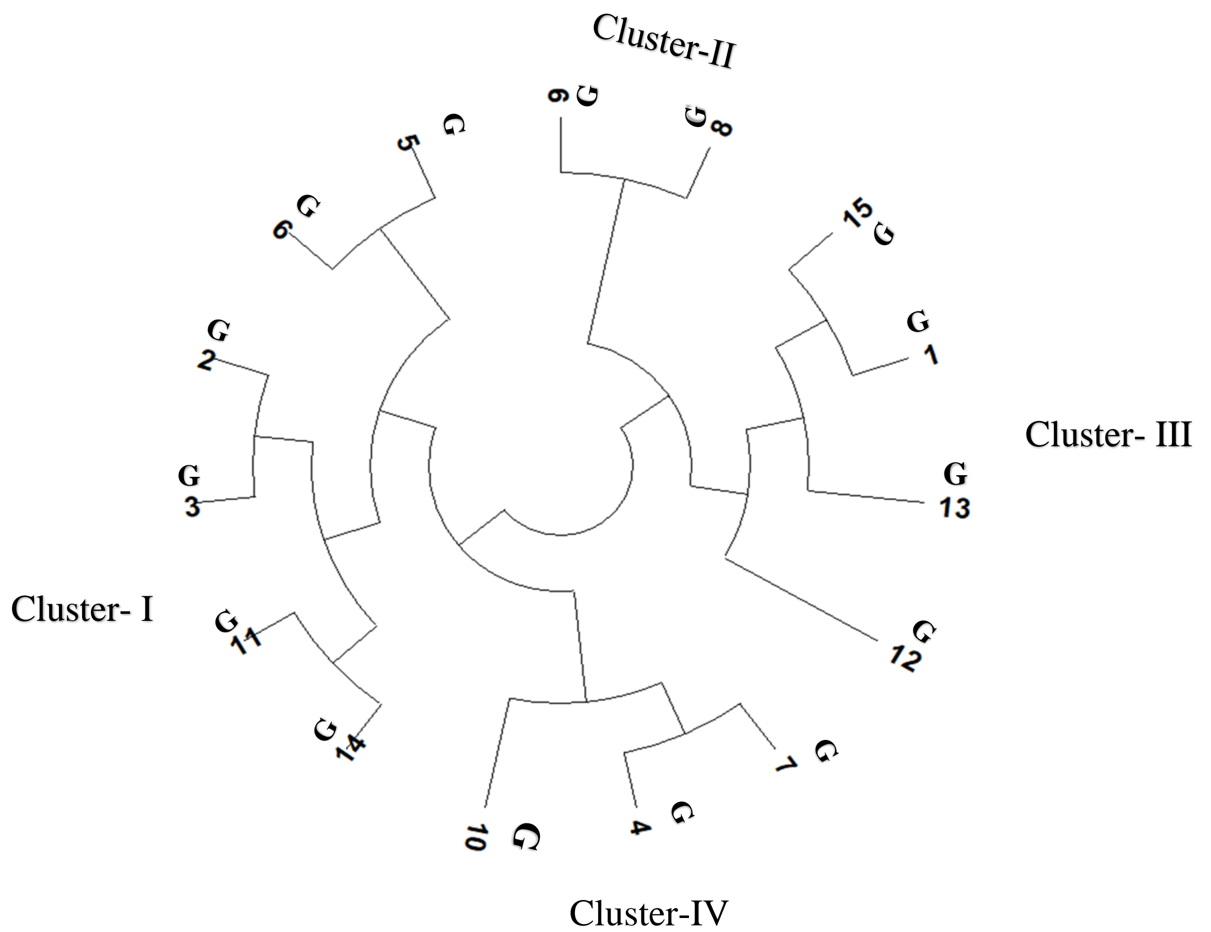


Figure 7: Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1983) genetic distance, summarizing the data on differentiation among 15 mung bean germplasm according to microsatellite

CHAPTER V
SUMMARY AND CONCLUSION

In Bangladesh, Mung bean is one of the most important pulse crop in Bangladesh . It has been persistently contributing to higher pulse production in last successive years. The experiment was conducted to assess the genetic variation ,diversity and genetic relatedness among 15 mung bean germplasm by using five highly polymorphic RAPD markers . The experiment was carried out at the laboratory of the Department of Biotechnology, Sher-e -Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh.

Fifteen Mung bean germplasm were used for PCR amplification. Five primers were applied for the study . The primers were OPBC-1,OPA-20,OPP-03,OPT-20,OPBC-18 showed reproducible and distinct polymorphic amplification.

The primers were produced 193 bands and an average 38.6 were detected. Among them 178 polymorphic bands were also identified. The frequency of the major loci ranged between 0.69 (OPA 20) to 0.81 (OPBC 18). Polymorphic Information Content (PIC)value for five RAPD markers ranged from 0.24 to 0.32 and the average PIC value was 0.27. Genetic diversity ranged between 0.28 (OPBC18) to 0.41 (OPA 20) with an average of 0.33. Primer OPA 20 showed highest gene diversity (0.41) and primer OPBC 18 showed the lowest gene diversty (0.28). PIC value revealed that OPA 20 was considered as the best marker for diversity analysis in 15 mung bean germplasmm followed by others and OPBC-18 was considered as the least powerful marker for those germplasm.

The value of pair-wise comparisons of Nei's (1983) genetic distance among 15 mung bean germplasm were computed from combined data for the 05 primers. Genetic distance obtained with a range between 0.1034 to 0.6207 with an average of 0.3621. The higher distance (0.6207) was observed between BU mug 3 vs BARI Mung 5 varietal pair.

It can be concluded that, RAPD markers are the powerful tools to detect genetic variation and genetic relationship within and among different mung bean germplasm. These markers are unmasking new genes for the improvement of crop varieties assessment of genetic diversity, fingerprinting,determine the genetic structure and for marker-assisted selection

(MAS).The stuudy result can become a guideline for further work on genetic variation and diversitry analysis for mung bean landraces of Bangladesh.

RECOMMENDATIONS

The results obtained from this study on molecular characterization provided some useful implications for establishment of sovereignty of Bangladesh mung bean gene pool. In this study, it is suggested that RAPD markers were effective in the detection of polymorphism. The present study can be used as a guideline for the next researchers who have concern for experimenting mung bean. Following points might be considered for studying the qualities of mung bean in Bangladesh.

- A large number of RAPD markers could be used for obtaining more precise result.
- A large number of germplasm could be included for getting more diverse result.
- Other molecular markers such as SSR, SNP, AFLP, micro-satellite etc. should be developed for mung bean germplasm of Bangladesh.

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APPENDICES

Appendix I: Chemical preparation for DNA extraction and PCR work

I(a): Composition and preparation of DNA extraction buffer

Reagent	250 ml preparation
Tris HCl (1M) (pH=8.0)	25ml
EDTA (0.5M, pH=8.0)	10ml
NaCl (5M)	25ml
D.H_2O	190ml

I(b): Composition & Preparation of 100 ml tris HCl (pH=8.0)

Reagent	100 ml preparation
MW of Tris HCl	121
D.H_2O	100
Tris HCl	12.1gm

I(c): Composition & Preparation of 100 ml EDTA (pH=8.0)

Reagent	100 ml preparation
MW of EDTA	372.24
D.H_2O	100ml
EDTA	37.22 gm

I(d): Composition & Preparation of 250 ml NaCl

Reagent	250 ml preparation
NaCl	73.05 g
dd.H_2O	250 ml

I (e): Composition & Preparation of 200 ml Phenol: Chloroform: IAA (25:24:1)

Reagent	200 ml preparation
Phenol	100 ml
Chloroform	96 ml
IAA	4 ml

I(f): Composition & Preparation of 1L 5X TBE Buffer

Reagent	1L 5X TBE buffer
Tris HCl (pH=8.0)	54 g
Boric Acid	27.5g
EDTA	4.65 g
D.H_2O	Up to 1 L

I(g): Composition & Preparation of the 10X TBE Buffer

Reagent	1L preparation
Tris HCl (pH=8.0)	108g
Boric Acid	55g
EDTA	9.3g
D.H2O	Up to 1 L

I(h): DNA dilution: (Working sample)

From the main stock of DNA 20 µl of DNA was diluted with 180 µl of de-ionized water.

I(i): Primer dilution

Primer was centrifuged at 13000 rpm for 5 min

TE buffer was added (10 times more) according to the concentration labeled on the bottle

Keep overnight at 4°C

Next day, it was centrifuged at 500 rpm for 15 seconds

Stored at -20°C freezer

I(j): PCR cocktail for 96 samples

Reagent	Amount
DNA	288
Primer	48
Master Mix	432
DD H_2O	144

Appendix II: RAPD marker analysis related data

Marker	Observation No	Allele No	Major Allele Frequency	Genetic Diversity	PIC Values
OPBC 1	15	4	0.78	0.33	0.28
OPA 20	15	6	0.69	0.41	0.32
OPP 03	15	6	0.76	0.32	0.26
OPT 20	15	7	0.74	0.33	0.26
OPBC 18	15	6	0.81	0.28	0.24
Maximum	-	-	0.81	0.41	0.32
Minimum	-	-	0.69	0.28	0.24
Mean	-	-	0.76	0.33	0.27