Project ID 368

Competitive Research Grant

Sub-Project Completion Report

on

Morpho-molecular characterization of underutilized indigenous vegetables in Bangladesh

Project Duration

May 2016 to September 2018

Implementing Division/Department with organization
Department of Biotechnology, and Germplasm Centre of the Bangaldesh
Agricultural University, Mymensingh 2202

Submitted to
Project Implementation Unit-BARC, NATP 2
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Project Implementation Unit
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Acronyms

BARI : Bangladesh Agricultural Research Institute
BAU : Bangladesh Agricultural University
BD : Bangladesh
GPC : Germplasm Centre
MO : *Moringaolifera*
PP : Pigeon pea
WTG : Wild teasel gourd
DAS : Days after sowing
CTAB : Cetyltrimethyl ammonium bromide
DNA : Deoxyribonucleic Acid
dNTPs : Deoxynucleotide Triphosphates
ºC : Degree Celsius
PCR : Polymerase Chain Reaction
RAPD : Randomly Amplified Polymorphic DNA
SSR : Simple Sequence Repeat
cm : Centimeter
g : Gram
bp : Base pair
kb : Kilobase
LSD : Latin square design
PIC : Polymorphic information content
% : Percentage
et al. : et alia = and other people

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Executive Summary

There are a number of minor or underutilized vegetables found in Bangladesh. These are the important source of food and nutrition of many tribal and poor people and may have huge undeveloped potential uses. Present research project attempted to collect and characterize four important underutilized vegetables namely Drumstick (*Moringaoleifera* Lam.), Pigeon pea (*Cajanuscajan* L.), Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) and Wild teasle gourd (*Momordicacochinchinensis*Lour). To estimate morphological and molecular variation in drumstick, 19 germplasm were collected and analysed with some morphological descriptors and eight (8) microsatellite loci. Morphological study showed significant variation among the drumstick germplasm. In case of microsatellite analysis, it was found that studied 8 loci were polymorphic and they contained a total of 28 alleles. Loci MO45 had the highest (6) and MO1 had the lowest (2) number of alleles. Differences were found in observed number of alleles, heterozygosities and proportion of polymorphic loci between germplasm. There were 2 private alleles (e.g. $MO12_{258}$ and $MO41_{150}$) found in MO-019, 1 ($MO58_{186}$) in MO-005 and 1 ($MO45_{204}$) MO-008 germplasm. Estimation of overall differentiation (F_{st}) and gene flow (N_m) revealed that a high level of genetic variation existed between germplasm with some exceptions. Genetic distance based UPGMA dendrogram separated 19 germplasm into two clusters where MO-006 and MO-007 are in one cluster and 17 in another cluster.

Standard morphological descriptors and eight microsatellite loci were analyzed to investigate genetic variation in 16 pigeon pea germplasm.Morphological study showed significant variation amonggermplasm. Seven polymorphic microsatellite loci contained a total of 18 alleles. Loci CCB4, CCB7, CCB10 had three (3) alleles; CCB1, CCB2, CCB5, CCB6 had two (2) alleles and CCB9 was monomorphic. A private allele $CCB1_{194}$ was found in PP-9438 germplasm. Genetic distance value was the highest (0.70) between PP-MYM and PP-9438, which indicated theirhigh genetic dissimilarities. Genetic distance-based UPGMA dendrogram separated 16 germplasm into two clusters where 9 are in one cluster and remain 7 in another cluster.

Variation among 4 wild teasel gourd germplasm collected from Dhamrai of Dhaka district and Thailand was determined morphologically through RAPD analysis. Variation was detected in leaf size, shape, fruit size, seed size and shape. Twenty eight decamer RAPD primers produced 139 loci of which 62 (44.60%) were polymorphic. The average highest (97.27) pair-wise band-sharing based similarity value was found between wild teasel gourd Dhamrai1 and Dhamrai2 while the lowest value (71.73) was found between wild teasel gourd Thailand2 and Dhamrai2. The highest genetic distance (0.5400) was found in Thailand2 and Dhamrai2 and the lowest genetic distance (0.0366) was found in Thailand1 and Thailand2. From the difference between the highest and the lowest genetic distance value it revealed that there was a wide variability among four wild teasel gourd genotypes.

Morphological variation was found in sizes of leaves, color of flowers, color and size of fruits in 5 roselle genotypes. Significant differences were observed in fruit weight, number of calyx and epicalyx, weight of calyx and epicalyx, leaf length and plant height. Nine RAPD primers generated a total of 48 loci of which 26 (54.17%) were polymorphic. Nei's gene diversity and Shannon's information index estimated across all loci and roselle genotypes ranged between 0.091 to 0.275 and 0.143 to 0.423, respectively. In the dendrogram, Dark red roselle was separated from other 4 genotypes with relatively high genetic distances. Light red roselle was closer to white roselle with the least genetic distance of 0.073. Gene bank genotype was also closer to Thai roselle with lower genetic distance.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project:** Morpho-molecular characterization of underutilized indigenous vegetables in Bangladesh

2. **Implementing organization:** Department of Biotechnology and Germplasm Centre of the Bangladesh Agricultural University (BAU-GPC), Mymensingh 2202, Bangladesh

3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**Dr. Md. Shahidul Islam, Professor and PI, Department of Biotechnology, BAU, Mymensingh 2202, Cell phone No.: 01738 867466, Email address: shahidj1@yahoo.com

   Dr. Md. Abdur Rahim, Professor and Co-PI, Department of Horticulture, BAU, Mymensingh 2202, Cell phone No.: 01711 854471

4. **Sub-project budget (Tk):**
   4.1 Total: Fifteen Lakh only (15,00,000/-)
   4.2 Revised (if any):

5. **Duration of the sub-project:**
   5.1 **Start date (based on LoA signed):**8 May 2017
   5.2 **End date:**30 September 2018

6. **Justification of undertaking the sub-project:**

Underutilized vegetables refer to those vegetables that are already in cultivation, but are underutilized regionally or globally given their still relatively low global production and market value. In other words, these vegetables are considered underutilized now-a-days because of their reduced consumption as a result of the introduction and commercialization of more improved/favorite vegetables. The vegetables are also known as less utilized or undervalued vegetable species and also branded development opportunity crops. In Bangladesh, there is a number of underutilized vegetable species. These include Drumstick (*Moringa oleifera* Lam.), Pigeon pea (*Cajanus cajan* L.), Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) and Wild teasle gourd (*Momordica cochinchinensis*) (Lour.) etc. Most of these are tropical and subtropical in nature. They are growing without much care largely in the homestead, fallow and forest areas as well as at the roadside of railway lines and paved roads. These underutilized vegetables are used as food, fibre, animal fodder, oil, medicine and have many other undeveloped potential uses. Huge tribal people are also dependant on the underutilized vegetables for nutrition and economy. The contribution of these vegetables to the nutrition of poor people and in alleviating poverty in coastal, flood affected, and hunger prone (*monga*) areas is also recognized. Therefore, their potential role in agriculture is highly promising (*Rahim et al.,* 2013) in remote areas for their economy and nutritional security.

Development of improved varieties of underutilized vegetable species and promotion of planting or farming and consumption could have a great potential for improving food security especially nutritional food security, poverty reduction and socio-economic development of the country. Apart from their commercial, medicinal and cultural value, underutilized vegetables are also considered important for sustainable food production as they reduce the impact of existing production systems of high yielding staple crops on the environment. Most of these vegetables are hardy, adapted to specific marginal soil and climatic conditions (e.g., droughts, salinity etc), and can be grown with minimal external inputs. Significant research, breeding and development efforts are needed for a range of promising crops to convert existing local landraces into competitive varieties with wide adaptation and promising commercial potential. Access to genetic diversity of these selected crops is a pre-condition for success.

The development of underutilized vegetables include identification of potentially useful vegetable species, germplasm collection, evaluation, development of varieties and agronomic testing followed by a series of steps to promote commercialization. The development of any crop needs prior knowledge of its morpho-molecular diversity data together with the assessment of its agronomic performances. The proposed research includes collection of at least 20 germplasms of four underutilized vegetable species from different agro-ecological zones of Bangladesh and documentation of their genetic diversity using both morphological traits and DNA markers

7. **Sub-project goal:** Documentation of genetic diversity in underutilized vegetables for their yield and nutritional improvement

8. **Sub-project objective (s):**

    a) Collection and conservation of important underutilized vegetables;

    b) Documentation of their phenotypic characteristics; and

    c) Documentation of their molecular characteristics using DNA markers.

9. **Implementing location (s):** Department of Biotechnology and Germplasm Centre of the Bangladesh Agricultural University (BAU-GPC), Mymensingh 2202, Bangladesh

10. **Methodology in brief:**

Collection of germplasm of underutilized vegetables:

A total of 44germplasmof 4 minor vegetables were collected. Among them 19 were drumstick, 16 pigeon pea, 5 roselle and 4 wild teasel gourd germplasm (Table 1-4).Some photos of germplasm collection are given in photo 1 and 2. During collection, necessary information about germplasm from farmers or growers was noted. Information were but not limited to type of soil necessary for cultivation, planting time, flowering season, benefits, and popularity etc.

Table 1: Collection of drumstick (*Moringasp*) germplasm

Germplasm	Origin	Reference
1. MO-001	Tanzania	BAU-GPC collection
2. MO-002	Tanzania	BAU-GPC collection
3. MO-003	Dinazpur, BD	BAU-GPC collection
4. MO-004	Dinazpur, BD	BAU-GPC collection
5. MO-005	Dinazpur, BD	BAU-GPC collection
6. MO-006	Keralla, India	BAU-GPC collection
7. MO-007	Keralla, India	BAU-GPC collection
8. MO-008	Keralla, India	BAU-GPC collection
9. MO-009	Natore, BD	This study
10. MO-010	Natore, BD	This study
11. MO-011	Natore, BD	This study
12. MO-012	Rangpur, BD	BAU-GPC collection
13. MO-013	Rangpur, BD	BAU-GPC collection
14. MO-014	Ishwardi, BD	BAU-GPC collection
15. MO-015	Ishwardi, BD	BAU-GPC collection
16. MO-016	Shibganj, Rajshahi, BD	BAU-GPC collection
17. MO-017	Shibganj, Rajshahi, BD	BAU-GPC collection
18. MO-018	Shibganj, Rajshahi, BD	BAU-GPC collection
19. MO-019	Shibganj, Rajshahi, BD	BAU-GPC collection

Table 2: Pigeon pea (*C. cajan*)germplasm collected from different locations

Germplasm	Location of collection	Reference
1. PP-MYM	BAU campus, Mymensingh	This study
2. PP-7181	Gene Bank, BARI, Gazipur	This study
3. PP-7183	Gene Bank, BARI, Gazipur	This study
4. PP-7184	Gene Bank, BARI, Gazipur	This study
5. PP-7185	Gene Bank, BARI, Gazipur	This study
6. PP-7186	Gene Bank, BARI, Gazipur	This study

7. PP-7187	Gene Bank, BARI, Gazipur	This study
8. PP-9436	Gene Bank, BARI, Gazipur	This study
9. PP-9437	Gene Bank, BARI, Gazipur	This study
10. PP-9438	Gene Bank, BARI, Gazipur	This study
11. PP-9439	Gene Bank, BARI, Gazipur	This study
12. PP-9440	Gene Bank, BARI, Gazipur	This study
13. PP-10492	Gene Bank, BARI, Gazipur	This study
14, PP-10493	Gene Bank, BARI, Gazipur	This study
15. PP-10494	Gene Bank, BARI, Gazipur	This study
16. PP-10495	Gene Bank, BARI, Gazipur	This study

Table 3. Collection of roselle (*H. sabdariffa*)germplasm

Germplasm	Location of collection	Reference
1. TR	Thailand roselle	This study
2. GBR	Gene bank, BARI	This study
3. DRR	Dark red roselle, BAU	This study
4. LRR	Light red roselle, BAU	This study
5. WR	White roselle, BAU	This study

Table 4. Collection of wild teasle gourd (*M. cochichinensis*) germplasm

Germplasm	Location of collection	Reference
1. WTG1	Thailand	This study
2. WTG2	Thailand	This study
3. WTG3	Dhamrai, Dhaka	This study
4. WTG2	Dhamrai, Dhaka	This study

Planting of germplasm and sowing of seeds

Seeds of pigeon pea and roselle were sown in the field of BAU-GPC. Collected germplasm of wild teasel gourd and moringa were planted in the BAU-GPC (Photos 3-6).

Photo 1. Collection of wild teasel gourd rhisome from Idilpur, Modhupur, Tangail by PI and Co-PI

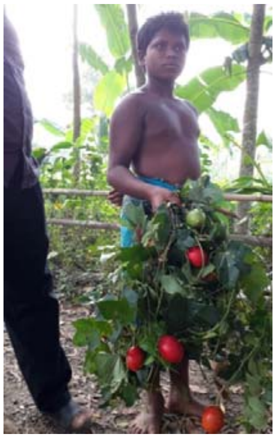


Photo 3.Collected germplasms planted in the BAU-GPC

Photo 4.Collected germplasms of roselle and pegion pea planted in the BAU-GPC

Photo 5. Moringa germplasm planted in the BAU-GPC

Photo6. Wild teasel gourd germplasm planted in the BAU-GPC

Morphometric/phenotypic characterization of collected germplasms:

Differences in morphometric traits were recorded for moringa, pigeon pea, roselle and wild teasel gourd (Table 5). In some cases particularly in moringa and wild teasle gourd, some important descriptors from a number of different germplasmare not provided in the report. It was because flowers and fruits of some germplasm were not available during project implementation period.

Table 5: Morphological descriptors recorded for moringa, pigeon pea, roselle and wild teasel gourd germplasm

Moringa	Pigeon pea	Roselle	Wild teasel gourd
• Type of leaf • Terminal leaflet shape • Terminal leaflet length • Terminal leaflet width • Terminal leaflet size • Pedicel length • Leaf color • Types of flower • Number of petals • Petal color • Flowering pattern • Shape of pod • Pod length • Pod color • Pod shape • Weight of 5 pods • Seeds per pod • Plant height at 30 DAS • Plant height at 45 DAS	• Type of leaf • Terminal leaflet shape • Terminal leaflet length • Terminal leaflet width • Terminal leaflet size • Pedicel length • Leaf color • Types of flower • Number of petals • Petal color • Flowering pattern • Shape of pod • Pod length • Pod color • Pod shape • Weight of 5 pods • Seeds per pod • Plant height at 30 DAS • Plant height at 45 DAS	• Weight of fruit • No. of calyx • No. of epicalyx • Weight of calyx • Weight of epicalyx • Height at 30 DAS • Height at 45 DAS • Height at 60 DAS • leaf length	• Fruit size

Morphological data analysis:

Morphological data had been analyzed by MS Excel 16 and Minitab 17 computer program.

Molecular characterization of germplasms:

Genomic DNA extraction: Fresh young leaves from collected accessions were used to isolate genomic DNA. CTAB method was used to isolate quality genomic DNA from moringa, pigeon pea and wild teasel gourd following Rifat et al. (2019). Genomic DNA extraction kit (Qaigen, USA) was used to isolate DNA from roselle. 0.8% agarose gel was used in electrophoresis to confirm isolation of quality genomic DNA. Yield and purity of DNA was measured using a spectrophotometer.

Selection of DNA markers:Simple sequence repeat (SSR) loci were analyzed for molecular characterization of moringa and pigeon pea and RAPD markers were used in molecular characterization of roselle and wild teasel gourd germplasm. Eleven primer pairs of SSR loci (Wu *et al*., 2010) were screened on two randomly chosen moringa genotypes. The primers were evaluated on the basis of intensity or resolution of bands, repeatability of markers, consistency within individuals and potentiality to different polymorphism. A final subset of eight primers (Table 6) exhibiting better quality banding patterns was selected for the analysis of whole sample set of 19 genotypes of moringa.The volume of each PCR reaction was 10 µl containing template DNA, buffer, 2 primers, dNTPs, Taq DNA polymerase and required amount of double distilled water. The thermal profile was set to 94ºC for predenaturation followed by 35 cycles consisting of 1 min at 94ºC, 1 min at 57ºC for annealing and 2 min at 72ºC for elongation. The last step was followed by 7 min of final extension at 72ºC.

Ten pair of primers (CCB1, CCB2, CCB3, CCB4, CCB5,CCB6, CCB7, CCB8, CCB9 and CCB10) of SSRdeveloped by Burns *et al.*(2001)were screened on randomly chosen two pigeon pea genotypes (PP-7187 and PP-10492). PCR at 57^0C annealing temperature was done with all the primers. To understand the potentiality of the primers PCR was repeated again at 51^0C with CCB2, CCB3, CCB4, CCB5 andCCB8. Like moringa, annealing temperature was set to 57^0C or 51ºC (Table 7). Agarose gel electrophoresis was carried out to observe the intensity of bands and check the referred size of the loci. Finally, 8 loci (CCB1, CCB2, CCB4, CCB5,CCB6, CCB7, CCB9 and CCB10) out of 10 were selected for analysis. After PCR amplification confirmation, PCR products were run on 8% polyacrylamide gel and photographed by gel documentation system.

Initially 30 decamer random primers were tested for PCR amplification of RAPD markers from wild teasel gourd and roselle germplasms. Among them, selected random primers (28) for wild teasel gourd and 9 for roselle genotypes) (Table 8 and 9), which showed good intensity of bands with minimal smearing and sufficient level of polymorphism were used for random amplification of different loci from genomic DNA of each of the collected germplasms of wild teasel gourd and roselle genotype. PCR were performed following a method described by *Williams et al.* (1990) with annealing temperature of 36ºC in a thermal cycler and PCR products were separated in 1.4% agarose gel. A molecular weight marker (100 bp/ 1 kb ladder) were also run to estimate size of each band or locus.

Table 6: Loci and flanking primers developed by Wu *et al*. (2010) used for molecular characterization of moringa germplasm

SSR locus	Motif	Base sequence (5′-3′)	Allele size range (bp)	No. of alleles
MO1	$(TC)_{17}$	TTGTCTGCCTCCTTTTGTCA AACTGTCACCCTCCTATCCA	150-158	2
MO12	$(CT)_{11}$	ACCGAAGATGATAAGGTGG CAAAAGGAAGAACGCAAGAG	260-270	3
MO41	$(GA)_{10}$	TGGGATTAGGGCATTAGAAA TAGTGGGTCCAAGACAAAGC	147-153	4
MO45	$(TC)_5TT(TC)_{10}$	CCTTTGAAGTTGAAAATCTC TTCTAGGGTAGTTGAATCCA	200-208	3
MO48	$(TC)_8C(CT)_{15}A(AC)_7$	AGAAGAACCCAACAGAGGAT CTTTTCACTAACCACCACCC	210-230	3
MO 58	$(CT)_6T(TC)_9$	TGGATTTCTTCTCCTGCTAT CACAGTTCTTATTGTATTGG	193-207	6
MO 62	$(AG)_{11}$	AAACATAGCAACTGTGAGAT CTCCAACAACATACAAAATC	189-193	2
MO68	$(GA)_{12}$	TGCTTCGCTTCCTCTATTCT ACCACAGGCTTGCTTCAGTA	240-250	4

Table 7:SSR primers developed by Burns *et al.* (2001) used in molecular characterization of pigeon pea

SSR locus	Motif	Base sequence(5′-3′)	Allele size range (bp)	Annealing Temp.(°C)
CCB1	$(CA)_{10}$	AAGGGTTGTATCTCCGCGTG GCAAAGCAGCAATCATTTCG	196-204	57
CCB2	$(CA)_{10}$	CCATAATCCAATCCAAATCC AGAAGGCTTTCATGTAACGC	160-166	51
CCB4	$(CA)_{31}$	GGAGCTATGTTGGAGGATGA CCTTTTGCATGGGTTGTAT	220-245	57
CCB5	$(CT)_{22}$	GACAATTTTGCATGCATTGC TTGCAAAAACACTTGGTTGG	190-215	51
CCB6	$(CA)_6$	ACAATGCTAGGGAACACCGC TACCTTAACCCACAATGGCC	202-208	57
CCB7	$(CT)_{16}$	CAACATTTGGACTAAAAACTG AGGTATCCAATATCCAACTTG	150-158	57
CCB9	$(CT)_{22}$	CACTTGGTTGGCTCAAGAAC GCCAATAGGCTCACATCCTTC	155-180	57
CCB10	$(CA)_{15}$	CCTTCTTAAGGTGAAATGCAAGC CATAACAATAAAAGACCTTGAATGC	244-250	57

Table 8: RAPD primers used for molecular characterization of wild teasel gourd germplasm

Primer code	Base sequence (5´-3´)	G+C content (%)
A01	CAGGCCCTTC	70
G10	AGGGCCGTCT	70
M16	GTAACCAGCC	60
N05	ACTGAACGCC	60
OPA02	TGCCGAGCTG	70
OPA10	GTGATCGCAG	60
OPA16	AGCCAGCGAA	60
OPAB O6	GTGGCTTGGA	60
OPAB09	GGGCGACTAC	70
OPAB20	CTTCTCGGAC	60
OPB05	TGCGCCCTTC	70
OPB08	GTCCACACGG	70
OPB12	CCTTGACGCA	60
OPB13	TTCCCCCGCT	70
OPB19	ACCCCCGAAG	70
OPD05	TGAGCGGACA	60
OPD07	TTGGCACGGG	70
OPF07	CCGATATCCC	60
OPH19	CTGACCAGCC	70
OPW19	CAAAGCGCTC	60
PAB04	GGCACGCGTT	70
S106	GGTCCTACCA	60
S1184	GACGGCTATC	50
S1189	AGTCCCCCTC	70
S1234	TCGCAGCGTT	60
S1239	TGACAGCCCC	70
S1265	GAGCTACCGT	60
S1313	CTACGATGCC	60

Table 9: RAPD primers used for molecular characterization of rosellegermplasm

Primer Code	Base sequence (5´-3´)	G+C content (%)
A01	CAGGCCCTTC	70
N05	ACTGAACGCC	60
OPAB20	CTTCTCGGAC	60
OPB05	TGCGCCCTTC	70
OPB08	GTCCACACGG	70
OPB13	TTCCCCCGCT	70
OPD07	TTGGCACGGG	70
OPF07	CCGATATCCC	60
PAB04	GGCACGCGTT	70

Microsatellite and RAPD data analyses

For analyses of both RAPD and microsatellite DNA data, software programs such as AlphaEaseFC 4.0, NTSYSpc software programme (Rohlf, 1997), POPGENE 32 (*Yeh et al.,* 1999) and Power Marker V3.0 (*Liu,* 2004) wereused. Size of each allele in base pair,observed and expected number of alleles, private alleles, frequency of alleles, genetic distance and cluster analysis between different germplasms of a species, gene diversity and polymorphic loci, principal component analysis (PCA) were estimated for RAPD data using POPGENE program and for SSR data using both POPGENE and PowerMarker programs. Band sharing based similarity index of RAPD analysis was estimated using formula Similarity index (SI) = 2Nxy/ Nx+Ny, Where, Nxy is the number of RAPD bands shared by individuals x and y respectively, and Nx and Ny are the number of bands in individual x and y, respectively (Lynch, 1990). This SI value ranges from 0 to 1. When SI=1.0 the two DNA profiles are identical and when SI is 0.0, there are no common bands between the two profiles. Within population similarity (Si) was calculated as the average of SI across all possible comparisons between individuals within a population.

11. Results and discussion

Characterization of moringa germplasm

Morphological characterization

Leaf characteristics

The leaf characteristics at vegetative stage (Figure 1) like type of leaf, shape of leaflet, length of the leaf, length of petiole, breadth of the leaflet, color of the leaf and color of main rachis are presented below:

Type of leaf

The type of leaf was compound and it was tripinnate. There were main, primary and secondary rachis in the leaf. Leaflet was present in the secondary rachis (Table 10, Figure. 2).

Shape of leaflet

Some leaflets were oblong in shape (Table 10, Figure3).

Length of the leaf

The length of leaf ranged from 24.47 - 61.97 cm among moringa germplasm. The longest leaf was 61.97 cm in MO-016 and the shortest leaf was 24.47 cm in MO-001 and were significantly different from each other (Table 11).

Length of petiole

The longest petiole was 14.27 cm in MO-006 and shortest was 4.63 cm in MO-001 and significantly different from each other (Table 11).

Length of rachis

The length of primary and secondary rachis was highest in MO-016 (23.94 cm) and in MO-005 (8.45 cm) and lowest of both was in MO-013 (8.12 cm, 2.71 cm) and significantly different from each other (Table 11).

Breadth of leaflet

The highest leaf breadth was 1.762 in MO-008 and the lowest was in MO-011 is 0.800 cm and they are significantly different from each other (Table 11).

Color of leaf

The color of the leaf was green (Table 10).

Color of main rachis

Color of the main rachis varies from green to reddish green (Table 10).

Table 10: Qualitative characteristics of leaves of moringa germplasm

Germplasm	Type of leaf	Shape of leaf	Color of leaf	Color of main rachis
MO-01	Compound, tripinnate	Oblong	Green	Green
MO-02	Compound, tripinnate	Oblong	Green	Green
MO-03	Compound, tripinnate	Oblong	Green	Green
MO-04	Compound, tripinnate	Oblong	Green	Green
MO-05	Compound, tripinnate	Oblong	Green	Green
MO-06	Compound, tripinnate	Oblong	Green	Green
MO-07	Compound, tripinnate	Oblong	Green	Green
MO-08	Compound, tripinnate	Oblong	Green	Green
MO-09	Compound, tripinnate	Oblong	Green	Green
MO-10	Compound, tripinnate	Oblong	Green	Green
MO-11	Compound, tripinnate	Oblong	Green	Green
MO-12	Compound, tripinnate	Oblong	Green	Reddish green
MO-13	Compound, tripinnate	Oblong	Green	Reddish green
MO-14	Compound, tripinnate	Oblong	Green	Reddish green
MO-15	Compound, tripinnate	Oblong	Green	Green
MO-16	Compound, tripinnate	Oblong	Green	Reddish green
MO-17	Compound, tripinnate	Oblong	Green	Green
MO-18	Compound, tripinnate	Oblong	Green	Green
MO-19	Compound, tripinnate	Oblong	Green	Reddish green

Table 11. Effect of different genotypes on leaf characteristics of 19 moringa germplasm

	Length (cm) of the characters									
Genotypes	Leaf	Petiole	Primary rachis	Primary rachis	Secondary rachis	Secondary rachis	Stalk of the leaflet	# Primary rachis/ main rachis	# Secondary rachis/ primary rachis	Leaflet Breadth (cm)
MO-01	24.47	4.63	9.57	1.62	3.29	0.35	0.20	7.67	7.67	1.19
MO-02	44.80	10.37	17.49	2.79	5.53	0.52	0.18	8.47	8.47	1.20
MO-03	60.13	9.03	13.81	1.63	3.85	0.47	0.13	10.73	10.73	1.32
MO-04	45.50	8.37	16.53	1.97	3.79	0.46	0.25	10.33	10.33	1.11
MO-05	55.63	11.13	19.17	2.61	8.45	1.19	0.23	9.00	9.00	1.29
MO-06	50.33	14.27	20.59	3.16	7.49	0.92	0.30	7.67	7.67	1.57
MO-07	48.23	6.87	14.00	1.63	4.32	0.49	0.28	7.13	7.13	1.61
MO-08	46.50	7.87	15.69	2.45	5.08	0.71	0.26	7.27	7.27	1.76
MO-09	26.00	5.50	8.59	1.43	3.07	0.43	0.10	9.00	9.00	0.87
MO-10	33.17	7.80	14.00	1.83	4.40	0.50	0.13	9.00	9.00	0.90
MO-11	35.50	7.80	14.17	1.93	3.50	0.43	0.10	9.00	9.00	0.80
MO-12	28.03	6.50	8.51	1.29	4.55	0.41	0.17	11.00	6.80	1.41
MO-13	25.57	6.27	8.12	1.15	2.71	0.31	0.14	9.67	7.13	1.12
MO-14	49.43	11.57	15.43	1.94	4.69	0.56	0.19	13.67	12.20	1.36
MO-15	45.30	10.03	13.29	1.77	5.79	1.04	0.27	14.33	11.40	1.35
MO-16	61.97	11.43	23.94	2.85	6.85	0.85	0.27	9.00	8.73	1.43
MO-17	60.57	12.13	21.71	1.99	4.75	0.49	0.21	9.00	9.27	1.24
MO-18	44.67	7.00	14.91	1.84	4.60	0.55	0.18	9.00	8.20	1.09
MO-19	45.23	10.30	14.93	1.87	4.82	0.51	0.19	9.00	8.33	1.17
LSD (0.01)	13.55	3.92	4.906	0.763	3.916	0.72	0.172	2.555	2.463	0.29
LSD (0.05)	10.11	2.924	3.658	0.569	2.921	0.537	0.128	1.905	1.837	0.216
Level of significance	*	*	*	*	*	**	**	*	*	**

*, **= Significant at 5, 1%, respectively level of probability and ns= not significant.

m

MO-01 MO-02 MO-03 MO-04

MO-05 MO-06 MO-07 MO-08

Figure 2. Different size and shape of leaves of moringa germplasm (Continued)

MO-09 MO-10 MO-11

MO-12 MO-13 MO-14 MO-15

MO-16 MO-17 MO-18 MO-19

Figure 2. Different size and shape of leaves of moringa germplasm

Figure 3. Difference in breadth of leaflet of moringa germplasm

Flower characteristics

We found flowers in only four germplasm during the project period. The flower characteristics like types of flowering, color of petal, color of anther, number of petal per flower and time of flower were recorded.

Types of flower

Flowers were bisexual (Table 12, Figure 4).

Number of petal

The flower had five unequal, thinly veined petals (Table 12, Figure 4).

Color of petal

The color of petal was yellowish white (Table 12, Figure 4).

Color of anther

Color of anther was yellow (Table 12, Figure 4).

Time of flowering

Flowering the test varieties varied from mid-February to mid-March (Table 12).

Table 12. Qualitative characteristics of flowers of moringagermplasm

Germplasm	Type of flower	Number of petal	Color of petal	Color of anther	Time of flowering
MO-05	Bisexual	Five	Yellowish white	Yellow	Mid February - mid March
MO-06	Bisexual	Five	Yellowish white	Yellow	Mid February - mid March
MO-12	Bisexual	Five	Yellowish white	Yellow	Mid February - mid March
MO-16	Bisexual	Five	Yellowish white	Yellow	Mid February - mid March

Figure4. Flowers of fourmoringa germplasm

Fruit characteristics

The fruit characteristics include shape of fruits, length and diameter of fruits, skin color, weight of fruits and number of seeds per fruit.

Shape of fruit

The shape of fruit was pendant (Table 13, Figure 5).

Length of fruit

The length of fruit was 43.5 cm in MO-10 and 45.5 cm in MO-11, 33.6 cm in MO-12 and 25.5cm in MO-13 (Table 13, Figure 5).

Diameter of fruit

The diameter of was 1.4 cm in MO-10 and 1.5 cm in MO-11, 3.9 cm in MO-12 and 2.5 cm in MO-13 (Table 13, Figure 5).

Color of fruit skin

The skin color of fruit was green to brown (Table 13, Figure 5).

Weight of fruits

The weight fruit was 31.457 g in MO-10 and 42.165 g in MO-11, 25.035 g in MO-12 and 21.013 g in MO-13 (Table 13, Figure 5).

Number of seeds per fruit

The no. of seeds per fruit varied from 9-21 according to its length and weight (Table 13, Figure 5).

Table 13: Characteristics of fruit of fourmoringa germplasm

Fruit variety	Shape	Length (cm)	Diameter (cm)	Color	Weight (gm)	Seeds/fruit
MO-10	Pendant	43.5	1.4	Green	31.457	21
MO-11	Pendant	45.5	1.5	Green	42.165	17
MO-12	Pendant	33.6	3.9	Brown	25.035	13
MO-13	Pendant	25.5	2.5	Brown	21.013	9

Figure5. Fruits of fourmoringa germplasm

Molecular characterization moringa germplasm

Intra germplasm allelic variation

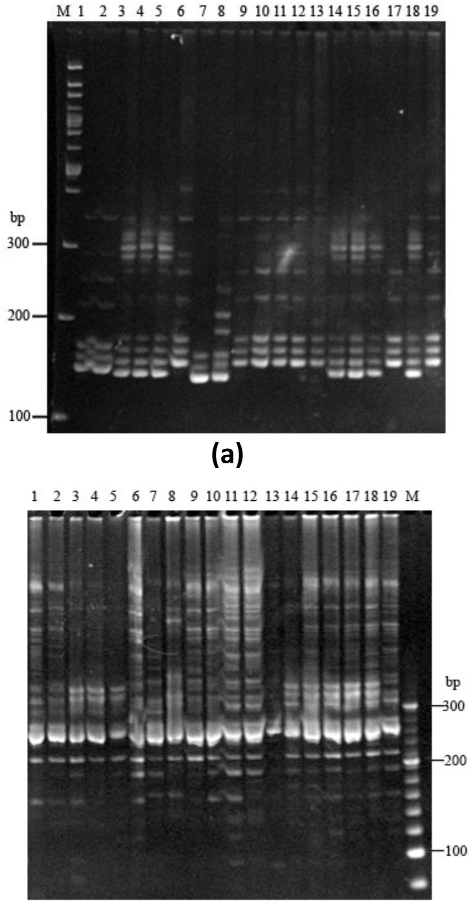
SSR profiles of moringa germplasm at different loci are mentioned in Figure 6 (a-h). There were a total of 28 alleles amplified from eight loci of moringa ranging from 2 to 6 (Table 14). The average number of alleles per locus was 3.5 (Table 14). Two alleles from MO1, 4 alleles from MO12, 3 alleles from MO41, 3 alleles from MO45, 3 alleles from MO 48, 6 alleles from MO58, 3 alleles from MO62 and 4 alleles from MO68 locus were recorded. The allele size ranged from 142 to 258bp. The loci MO58 was found to contain the highest number of alleles (6) while the locus MO1 had the lowest number of alleles (2) (Table 14). There were 2 private alleles (e.g. $MO12_{258}$ and $MO41_{150}$) found in MO-19 and 1 private allele ($MO58_{186}$) was found in MO-05 and 1 private allele ($MO45_{204}$) was found in MO-08 (Table 15). The number of observed alleles and effective no. of alleles were same for each germplasm and was found to be the highest (1.375) in MO-03, MO-04, MO-05 (Table 15). The observed heterozygosity (Ho) value was found to be the highest (0.375) in MO-03, MO-04, MO-05 (Table 16). The overall observed heterozygosity was found to be same to its expected heterozygosity (Table 16). Total no. of loci analyzed were 8 which were polymorphic in nature. The overall percentage of polymorphic loci scored 100 (Table 15). Allelic variations were observed among moringa germplasm. At locus MO1, 2 alleles were found. Homozygous genotypes (142/142 bp) and (152/152 bp) were found in MO-07, MO-08 and in MO-06, MO-09, MO-10, MO-11, MO-12, MO-13, MO-17, MO-19 germplasm respectively, whereas heterozygous genotypes (142/152 bp) were found in MO-01, MO-02, MO-03, MO-04, MO-05, MO-15, MO-16 and MO-18 germplasm (Figure6-a, Table 15).

At locus MO12, 4 alleles were found. Homozygous genotypes (242/242 bp) and (244/244 bp) were found in MO-01, MO-02, MO-03, MO-04, MO-06, MO-07, MO-08, MO-09, MO-10, MO-11, MO-12 and in MO-05, MO-15, MO-16, MO-17, MO-18 germplasm respectively. Homozygous genotypes (252/252 bp) were found in MO-13, MO-14 and (258/258 bp) were found in MO-19 germplasm (Figure 6-b, Table 15).At locus MO41, 3 alleles were found. Homozygous genotypes (144/144 bp) and (147/147 bp) were found in MO-01, MO-02, MO-03, MO-04, MO-05, MO-06, MO-07 and in MO-08, MO-09, MO-10, MO-11, MO-12, MO-13, MO-14, MO-15, MO-16, MO-17, MO-18 germplasm respectively, and homozygous genotypes (150/150 bp) were found in MO-19 germplasm (Figure 6-c, Table 15).

At locus MO45, a total no. of 3 alleles were found. Homozygous genotypes (198/198 bp) and (204/204 bp) were found in MO-01, MO-02, MO-03, MO-04, MO-05, MO-09, MO-10, MO-11, MO-12, MO-13, MO-14, MO-15, MO-16, MO-17, MO-18, MO-19 and in MO-06, MO-08 germplasm respectively, whereas heterozygous genotypes (198/210 bp) were found in MO-07 (Figure 6-d, Table 15).At locus MO48, 3 alleles were found. Homozygous genotypes (202/202 bp) and (206/206 bp) were found in MO-10, MO-11, MO-17 and in MO-06, MO-07, MO-19 germplasm respectively, Homozygous genotypes (218/218 bp) were found in MO-01, MO-02, MO-18 germplasm. Heterozygous genotypes (202/218 bp) were found in MO-03, MO-04, MO-05, MO-09, MO-12, MO-13, MO-14, MO-15, MO-16 germplasm (Figure 6-e, Table 15).

At locus MO58, 6 alleles were found. Homozygous genotypes (191/191 bp) and (193/193 bp) were found in MO-08 and in MO-01, MO-02 germplasm respectively. Homozygous genotypes (199/199 bp) were found in MO-09, MO-10, MO-11, MO-12, MO-13, MO-17, MO-19. Heterozygous genotypes (181/199 bp) and (183/199) were found in MO-15, MO-16, MO-18 and MO-03, MO-04, MO-07, MO-14 germplasm respectively. Heterozygous genotypes (186/199 bp) were found in MO-05 germplasm (Figure 6-f, Table 15).

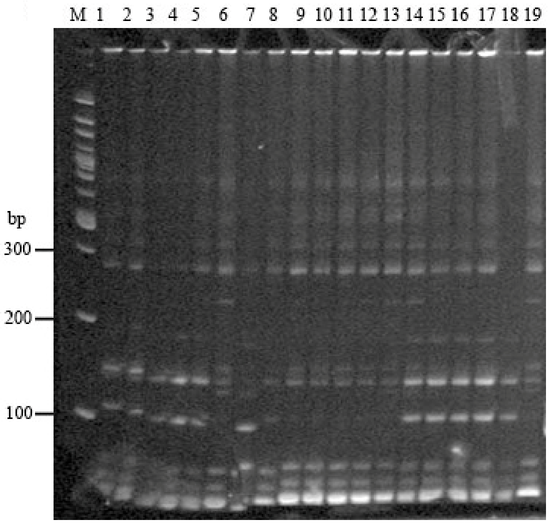
At locus MO62, 3 alleles were found. Homozygous genotypes (183/183 bp) and (187/187 bp) were found in MO-01, MO-02, MO-03, MO-04, MO-05 and in MO-07, MO-11, MO-19 germplasm respectively, homozygous genotypes (193/193 bp) were found in MO-06, MO-09, MO-12, MO-13, MO-17 germplasm (Figure 6-g, Table 15).At locus MO68, 4 alleles were found. Homozygous genotypes (224/224 bp) and (234/234 bp) were found in MO-07, MO-10, MO-11, and in MO-03, MO-04, MO-05, MO-06 germplasm respectively. Again homozygous genotypes (238/238 bp) and (242/242 bp) were found in MO-02, MO-09, MO-15, MO-17, MO-18, MO-19 and in MO-01, MO-12, MO-13, MO-14 germplasm respectively (Figure6-h, Table 15).



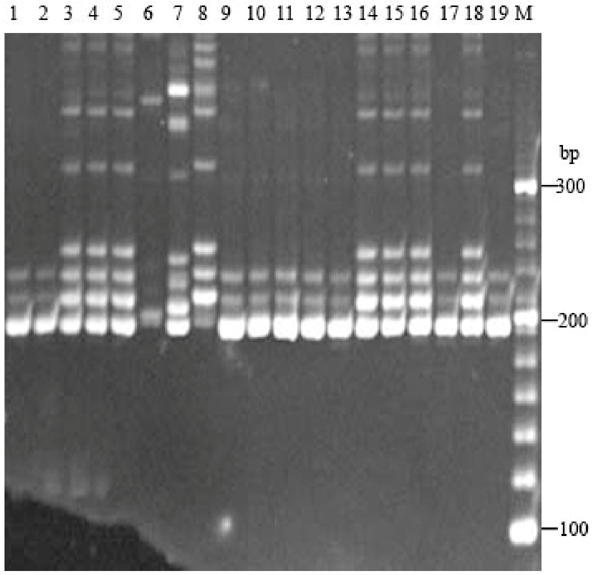
(a)

(b)

Fig. 6. SSR profiles of 19 moringa germplasm (continued)

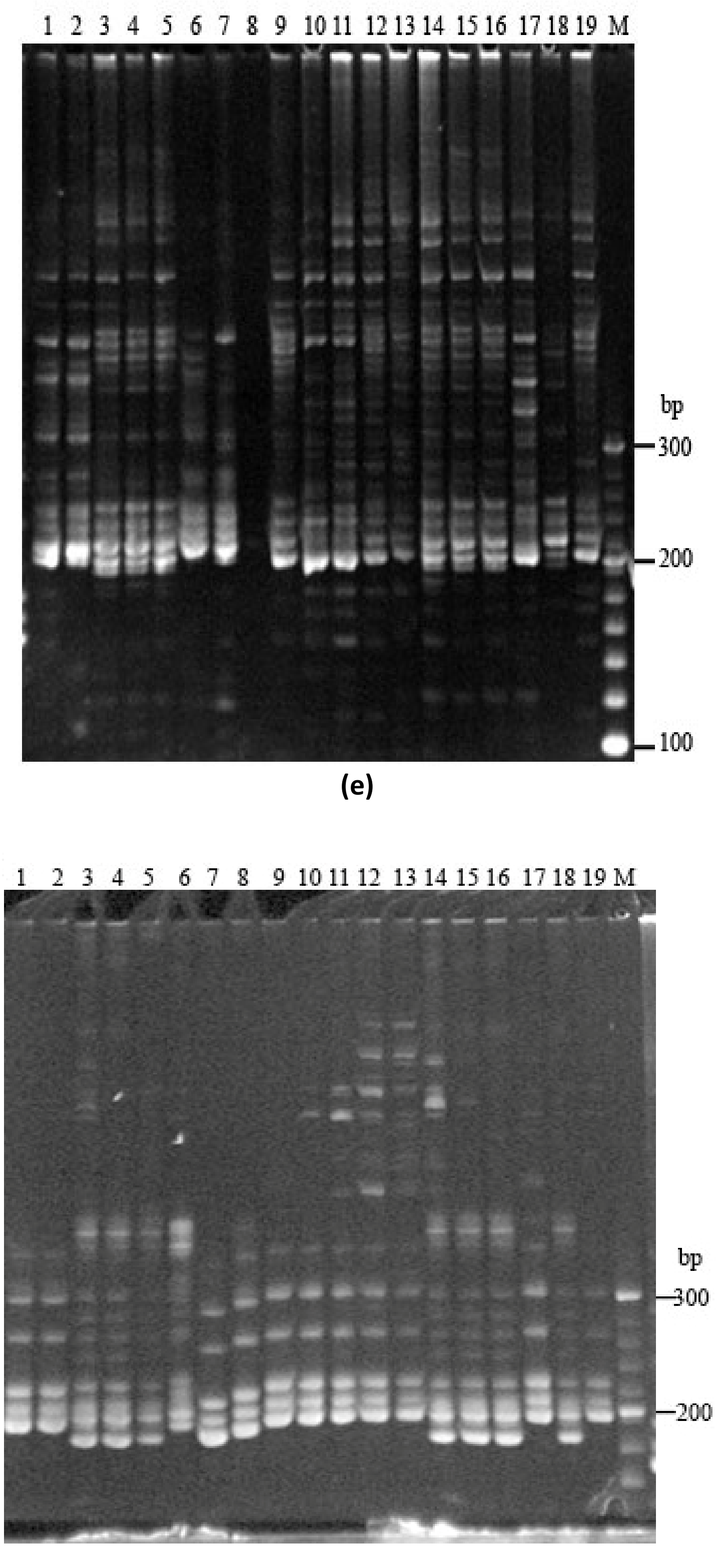


(c)



(d)

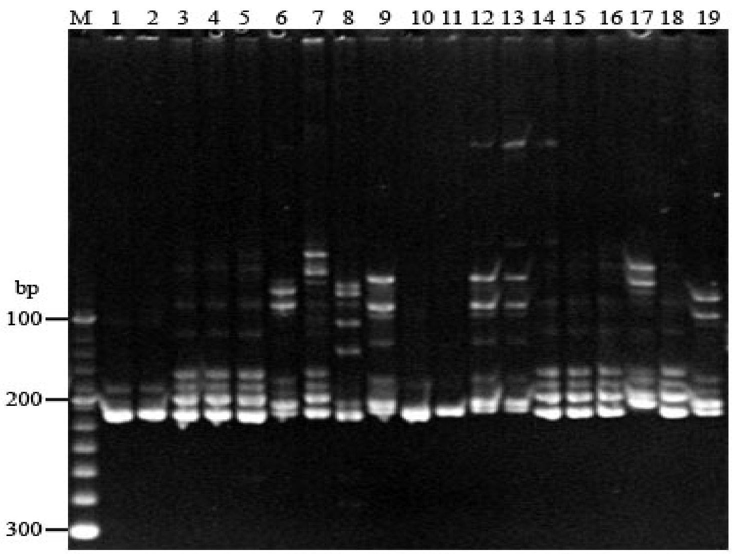
Figure 6. SSR profiles of 19 moringa germplasm (continued)



(e)

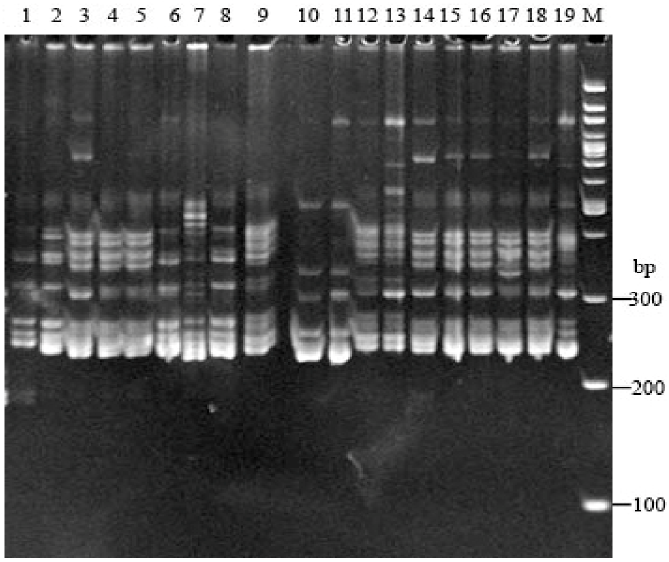
(f)

Figure 6.SSR profiles of 19 moringa germplasm (continued)



(g)

Figure 6. SSR profiles of 19 moringa germplasm (continued)



(h)

Figure 6. SSR profiles of 19 moringa germplasm at loci a) MO1, b) MO12, c) MO41, d) MO45, e) MO48, f) MO58, g) MO62, h) MO68. Lane 1: MO-001, 2: MO-002, 3: MO-003, 4: MO-004, 5: MO-005, 6: MO-006, 7: MO-007, 8: MO-008,9: MO-009, 10: MO-010, 11: MO-011, 12: MO-012, 13: MO-013, 14: MO-014, 15: MO-015, 16: MO-016, 17: MO-017, 18: MO-018, 19: MO-019. M: Molecular weight marker (100 or 20 bp DNA ladder).

Table 14: Overall genetic diversity in 19 moringa germplasm across 8 microsatellite loci

Locus	No.of alleles	Size (bp)	Number of genotypes	Heterozygosity	Nei's gene diversity	PIC	Fst	Nm
MO1	2	142-152	3	0.474	0.450	0.349	0.474	0.278
MO12	4	242-258	4	0.000	0.582	0.524	1.000	0.000
MO41	3	144-150	3	0.000	0.526	0.433	1.000	0.000
MO45	3	198-210	3	0.053	0.234	0.216	0.888	0.032
MO48	3	202-218	4	0.500	0.625	0.565	0.643	0.139
MO58	6	181-199	6	0.421	0.619	0.587	0.660	0.129
MO62	3	183-193	3	0.000	0.571	0.504	1.000	0.000
MO68	4	224-242	4	0.000	0.739	0.688	1.000	0.000
Mean	3.50		3.75	0.181	0.543	0.483	0.840	0.048

Inter germplasm genetic identity and genetic distance

Genetic identity and genetic distances between the respective samples are shown in (Table 17). The estimated genetic identity value (1.00) between MO-03 and MO-04 was observed the highest whereas its lowest value (0.067) was observed between MO-06 and MO-18. The genetic distances and genetic identity were computed by Nei's (1972). Results showed that the highest genetic distance (2.706) was observed between MO-06 and MO-18 germplasm, while the lowest genetic distance was observed between MO-15 and MO-18 (0.037). Many comparisons showed genetic identity and genetic distance value (0).

UPGMA dendrogram

A dendrogram (Figure 7) based on Nei's (1972) genetic distance grouped nineteen moringa germplasm into two major clusters a, and b. Cluster 'a' comprised of MO-06 and MO-07. Cluster 'b'with 17 germplasm were again divided into two groups (c and d). Cluster 'c' is comprised of MO-08 and MO-19. Cluster 'd' is sub-divided into two group 'e' and 'f'. Cluster 'e' is subdivided into 'e1' and 'e2' and f is subdivided into 'f1' and 'f2'. Cluster 'e1' comprised of MO-14, MO-15, M0-18 and M0-16. Cluster 'e2' consisted of MO-09, 12, 13, 17, 10, and 11. Cluster f_1 comprised of MO-03, 04 and 05 and f2 consisted of MO-01 and 02.

Table 15: Allelic variationat 8 microsatellite loci in 19 moringagermplasm

Germplasm	Loci								Overall polymorphic loci
	M01	M012	M041	M045	M048	M058	M062	M068	
MO-001	142/152	242/242	144/144	198/198	218/218	193/193	183/183	242/242	0
MO-002	142/152	242/242	144/144	198/198	218/218	193/193	183/183	238/238	1
MO-003	142/152	242/242	144/144	198/198	202/218	183/199	183/183	234/234	3
MO-004	142/152	242/242	144/144	198/198	202/218	183/199	183/183	234/234	3
MO-005	142/152	244/244	144/144	198/198	202/218	186/199	183/183	234/234	3
MO-006	152/152	242/242	144/144	204/204	206/206	193/193	193/193	234/234	0
MO-007	142/142	242/242	144/144	198/210	206/206	183/199	187/187	224/224	2
MO-008	142/142	242/242	147/147	204/204	-	191/191	183/183	234/234	0
MO-009	152/152	242/242	147/147	198/198	202/218	199/199	193/193	238/238	1
MO-010	152/152	242/242	147/147	198/198	202/202	199/199	183/183	224/224	0
MO-011	152/152	242/242	147/147	198/198	202/202	199/199	187/187	224/224	0
MO-012	152/152	242/242	147/147	198/198	202/218	199/199	193/193	242/242	1
MO-013	152/152	252/252	147/147	198/198	202/218	199/199	193/193	242/242	1
MO-014	142/152	252/252	147/147	198/198	202/218	183/199	183/183	242/242	3
MO-015	142/152	244/244	147/147	198/198	202/218	181/199	183/183	238/238	3
MO-016	142/152	244/244	147/147	198/198	202/218	181/199	183/183	242/242	1
MO-017	152/152	244/244	147/147	198/198	202/202	199/199	193/193	238/238	0
MO-018	142/152	244/244	147/147	198/198	218/218	181/199	183/183	238/238	2
MO-019	152/152	258/258	150/150	198/198	206/206	199/199	187/187	238/238	0
No. of Allele	2	4	3	3	3	6	3	4	
Private Allele	0	1	1	1	0	1	0	0	
Allele size Range (bp)	142-152	242-258	144-150	198-210	202-218	181-199	183-193	224-242	
Total no. of loci:	8								
Total no. of polymorphic loci:	8								
Overall percentage of polymorphic loci :	100								

Table 16: Allelic and genetic variation at 8 microsatellite loci of 19 moringa germplasm

Germplasm	*na**	*ne**	*Ho*	*He*	Average heterozygosity	No. and (%) of polymorphic loci
MO-01	1.125	1.125	0.125	0.125	0.088	1(12.50)
MO-02	1.125	1.125	0.125	0.125	0.088	1(12.50)
MO-03	1.375	1.375	0.375	0.375	0.088	3(37.50)
MO-04	1.375	1.375	0.375	0.375	0.088	3(37.50)
MO-05	1.375	1.375	0.375	0.375	0.088	3(37.50)
MO-06	1.001	1.000	0.000	0.000	0.088	0(0.00)
MO-07	1.251	1.250	0.250	0.250	0.882	2(25.00)
MO-08	1.000	1.000	1.000	0.000	0.068	0(0.00)
MO-09	1.125	1.125	1.125	0.125	0.088	1(12.50)
MO-10	1.000	1.000	0.000	0.000	0.088	0(0.00)
MO-11	1.000	1.000	0.000	0.000	0.088	0(0.00)
MO-12	1.125	1.125	0.125	0.125	0.088	1(12.50)
MO-13	1.125	1.125	0.125	0.125	0.088	1(12.50)
MO-14	1.375	1.375	0.375	0.375	0.088	3(37.50)
MO-15	1.375	1.375	0.375	0.375	0.088	3(37.50)
MO-16	1.375	1.375	0.375	0.375	0.088	3(37.50)
MO-17	1.000	1.000	0.000	0.000	0.088	0(0.00)
MO-18	1.250	1.250	0.250	0.250	0.088	2(25.00)
MO-19	1.000	1.000	0.250	0.250	0.088	2(25.00)

*na** = Observed no. of alleles, *ne** = Effective no. of alleles, *Ho* = Observed Heterozygosity, *He* = Expected Heterozygosity

Table 17: Summary of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) in 19 moringa germplasm

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	****	0.867	0.716	0.716	0.573	0.452	0.414	0.345	0.400	0.452	0.323	0.533	0.400	0.573	0.430	0.573	0.194	0.483	0.194
2	0.143	****	0.716	0.716	0.5723	0.452	0.414	0.345	0.533	0.452	0.323	0.400	0.267	0.430	0.5729	0.430	0.323	0.621	0.323
3	0.334	0.334	****	1.000	0.808	0.485	0.519	0.519	0.501	0.624	0.485	0.501	0.358	0.539	0.500	0.500	0.347	0.482	0.277
4	0.334	0.334	0.000	****	0.808	0.485	0.519	0.519	0.501	0.624	0.485	0.501	0.358	0.539	0.500	0.500	0.347	0.482	0.277
5	0.557	0.557	0.214	0.214	****	0.347	0.334	0.371	0.358	0.485	0.347	0.358	0.358	0.500	0.654	0.654	0.485	0.630	0.277
6	0.794	0.794	0.723	0.723	1.059	****	0.401	0.401	0.387	0.250	0.250	0.387	0.258	0.069	0.069	0.069	0.250	0.067	0.250
7	0.882	0.882	0.656	0.656	1.098	0.914	****	0.286	0.276	0.401	0.535	0.276	0.138	0.222	0. 185	0. 185	0.134	0.179	0.401
8	1.064	1.064	0.656	0.656	0.993	0.914	1.253	****	0.276	0.401	0.267	0.276	0.138	0.371	0.371	0.371	0.134	0.357	0.000
9	0.916	0.629	0.691	0.691	1.027	0.949	1.287	1.287	****	0.710	0.710	0.867	0.733	0.501	0.645	0.501	0.839	0.621	0.516
10	0.794	0.794	0.472	0.472	0.730	1.386	0.914	0.914	0.342	****	0.875	0.710	0.581	0.624	0.624	0.624	0.625	0.535	0.375
11	1.131	1.131	0.723	0.723	1.059	1.386	0.626	1.320	0.342	0.134	****	0.710	0.581	0.485	0.485	0.485	0.625	0.401	0.500
12	0.629	0.916	0.691	0.691	1.027	0.949	1.287	1.287	0.143	0.342	0.342	****	0.867	0.645	0.501	0.645	0.710	0.483	0.387
13	0.916	1.322	1.027	1.027	1.027	1.354	1.980	1.980	0.310	0.543	0.543	0.143	****	0.788	0.501	0.645	0.710	0.483	0.387
14	0.557	0.845	0.619	0.619	0.693	2.669	1.503	0.993	0.691	0.472	0.723	0.439	0.239	****	0.654	0.808	0.485	0.630	0.277
15	0.846	0.557	0.693	0.693	0.425	2.669	1.686	0.993	0.439	0. 472	0. 723	0.691	0.691	0.425	****	0.846	0.763	0.964	0.416
16	0.557	0.845	0.693	0.693	0.425	2.669	1.686	0.993	0.691	0. 472	0. 723	0.439	0.439	0.214	0.167	****	0.624	0.815	0.277
17	1.642	1.131	1.059	1.059	0.723	1.386	2.013	2.013	0.175	0.470	0.470	0.342	0.342	0.723	0.271	0.472	****	0.668	0.500
18	0.728	0.476	0.730	0.730	0.462	2.706	1.723	1.030	0.476	0.626	0.914	0.728	0.728	0.462	0.037	0.204	0.403	****	0.401
19	1.642	1. 131	1.283	1 283	1.283	1.386	0.914	0.000	0.661	0.981	0.693	0.949	0.949	1.283	0.877	1.283	0.693	0.914	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Here, pop ID : 1 = MO-01, 2 = MO-02, 3=MO-03, 4 = MO-04, 5 = MO-05, 6 = MO-06, 7 = MO-07, 8=MO-08,9=MO-09, 10 = MO-10, 11 = MO-11, 12 = MO-12, 13 = MO-13, 14 = MO-14, 15 = MO-15, 16 = MO-16, 17 = MO-17, 18 = MO-18, 19 = MO-19

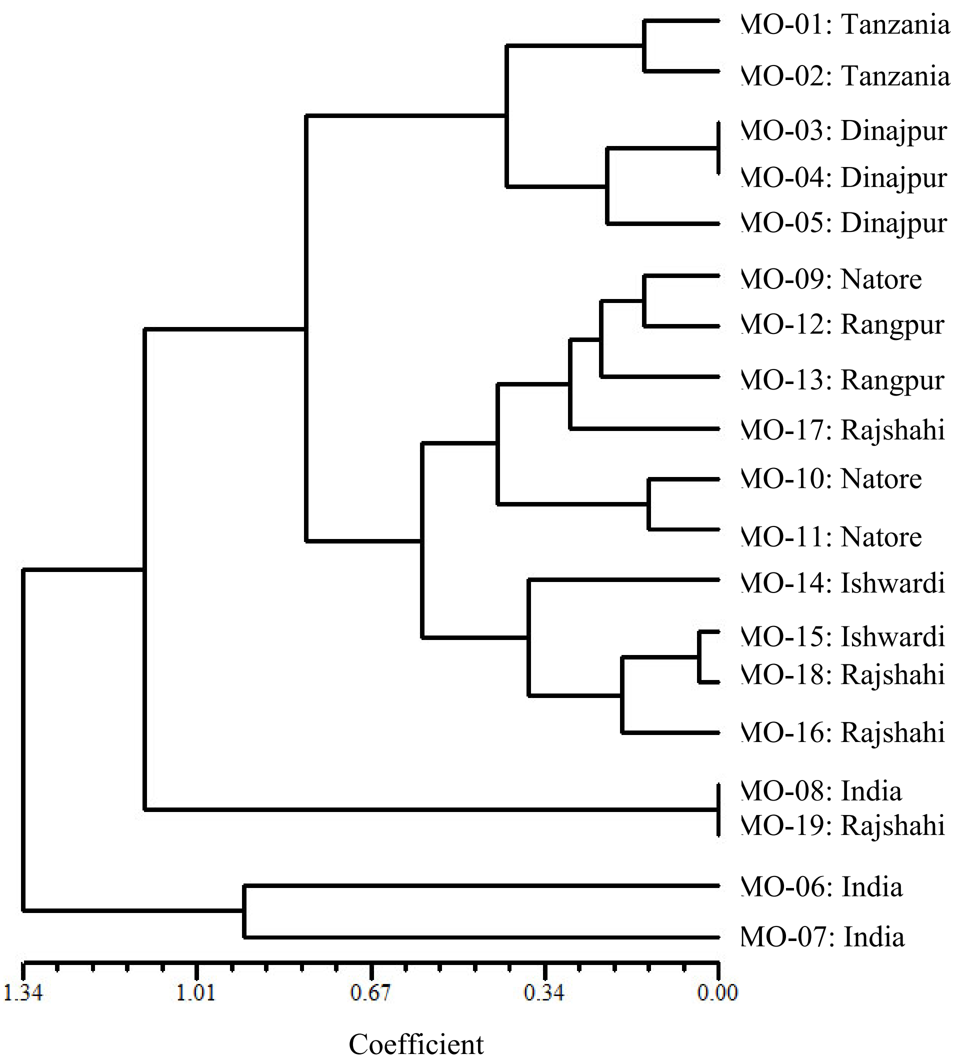


Figure 7.Cluster analysis based on Nei's (1972) genetic distance between moringa germplasm

Characterization of pigeon pea germplasm

Morphological characterization

Leaf characteristics

Leaf characteristics

The leaf characteristics like type of leaf, shape of leaflet, length of the leaf, petiole length, leaflet breadth, leaf color and color of main rachis observed at vegetative stage (Figure 8)are presented below:

Type of leaf

The type of leaf of all the test genotypes was compound and it was trifoliate. There is main and primary rachis in the leaf. Leaflet was present in the primary rachis (Figure 8).

Shape of terminal leaflet

Terminal leaflets were lanceolateshape in PP-7187, PP-10492, PP-10493, PP-10494 genotypes and oval shape in other 12 genotypes(Table 18, Figure 8).

Length ofthe terminal leaflet

The length of terminal leafletwas significantly different among genotypes. It was ranged from 5.43 - 10.50 cm. The longest leaflet was 10.50 cm in PP-10494 and the shortest leaflet was 5.43 cm in PP-9436 and were significantly different from each other (Table 19).

Breadth of terminal leaflet

Breadth of terminal leafletwas significantly different among 16 genotypes. The highest leaflet breadth was 3.33 cm in PP-MYM and the lowest was in PP-9336 is 1.13 cm and they are significantly different from each other (Table 19).

Size of terminal leaflet

Size of terminal leafletwas significantly different among 16 pigeonpea genotypes. The highest leafletsize was 31.56 cm^2 in PP-10495 and the lowest was in PP-9337 is 10.58 cm^2(Table 19)

Length of pedicel

There was significantly different in length of pedicel. The longest pedicel was 6.60 cm in PP-MYM and the shortest was 2.07 cm in PP-9440 (Table 19).

Color of leaf

The color of the leaf was green. The color of the test germplasm was green

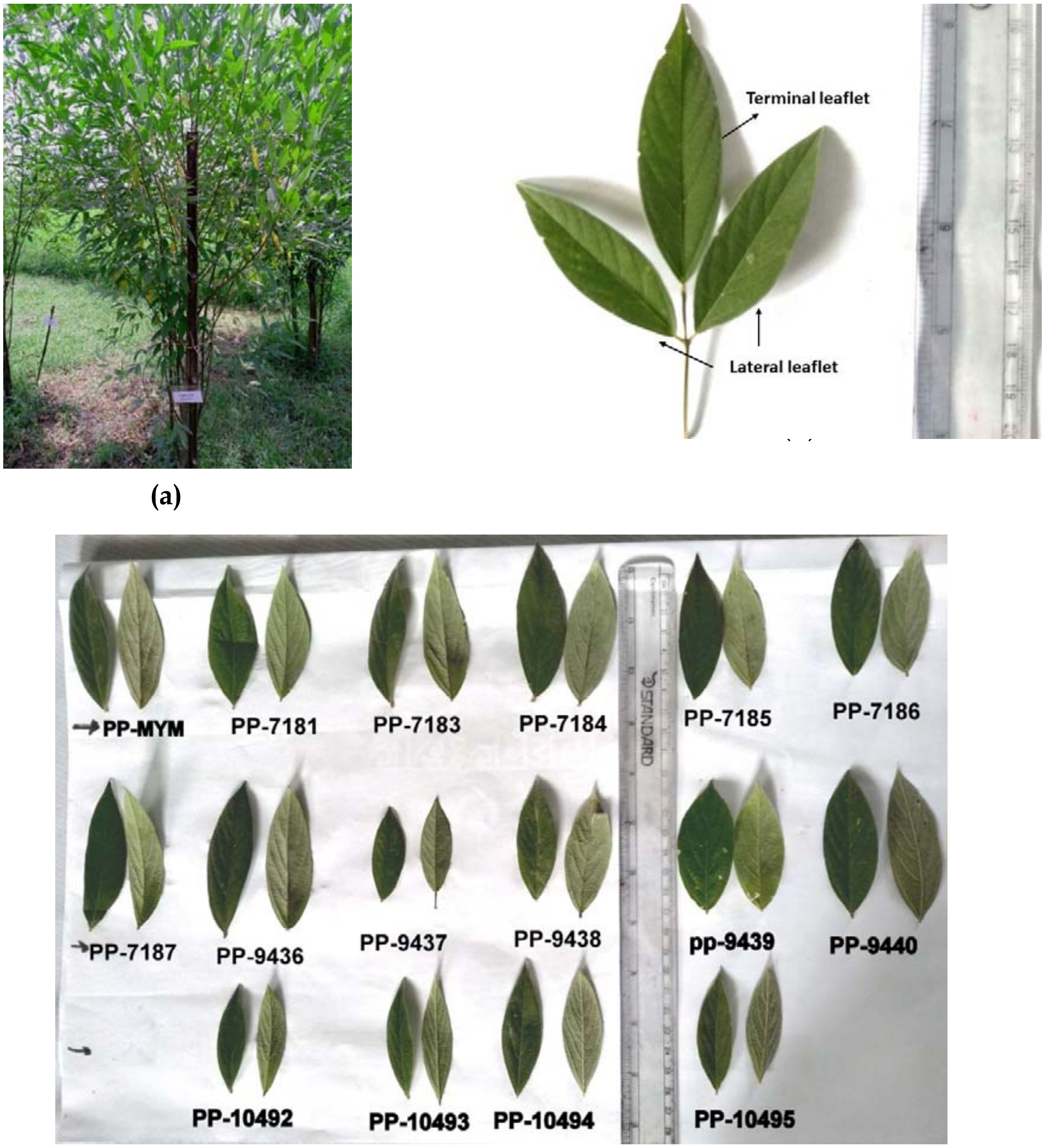
Table 18: Qualitative characteristics of leaves of *C. cajan*germplasm

Germplasm	Type of leaf	Shape of terminal leaflet	Color of leaf
PP-MYM	Compound, trifoliate	Oval	Green
PP-7181	Compound, trifoliate	Oval	Green
PP-7183	Compound, trifoliate	Oval	Green
PP-7184	Compound, trifoliate	Oval	Green
PP-7185	Compound, trifoliate	Oval	Green
PP-7186	Compound, trifoliate	Oval	Green
PP-7187	Compound, trifoliate	Lanceolate	Green
PP-9436	Compound, trifoliate	Oval	Green
PP-9437	Compound, trifoliate	Oval	Green
PP-9438	Compound, trifoliate	Oval	Green
PP-9439	Compound, trifoliate	Oval	Green
PP-9440	Compound, trifoliate	Oval	Green
PP-10492	Compound, trifoliate	Lanceolate	Green
PP-10493	Compound, trifoliate	Lanceolate	Green
PP-10494	Compound, trifoliate	Lanceolate	Green
PP-10495	Compound, trifoliate	Oval	Green

Table 19: Morphological characteristics of 16 germplasm of *C. cajan*

Germplasm	Terminal leaflet length (cm)	Terminal leaflet Breadth (cm)	Size of terminal leaflet (cm^2)	length of pedicel (cm)	Plant Hight at 30 DAS (cm)	Plant Hight at 45 DAS (cm)	Length of pod (cm)	Weight of 5 pod (gm)	No. of seed/ pod
PP-MYM	8.73	3.33	29.16	6.60	25.67	38.00	5.97	06.41	5
PP-7181	8.80	3.13	27.60	3.93	26.00	34.00	6.00	06.45	4
PP-7183	9.03	2.47	22.36	3.53	33.33	29.67	6.00	07.07	4
PP-7184	7.30	2.67	19.51	3.33	34.00	39.00	6.67	05.27	4
PP-7185	5.93	2.40	16.86	4.13	33.67	40.33	7.00	06.33	5
PP-7186	8.30	1.97	11.66	4.27	32.67	40.00	6.33	06.57	4
PP-7187	5.83	2.43	20.20	2.53	32.67	36.67	6.87	07.57	5
PP-9436	5.43	1.97	11.45	3.20	28.33	36.83	5.60	06.45	4
PP-9437	9.37	1.13	10.58	2.27	26.00	18.00	6.40	05.50	4
PP-9438	6.60	3.13	29.38	4.27	25.33	34.33	6.60	05.53	4
PP-9439	7.40	2.27	14.97	3.50	31.33	38.33	6.83	07.60	4
PP-9440	7.33	2.43	18.00	2.07	28.67	37.83	6.17	06.33	4
PP-10492	8.40	2.37	17.33	2.30	31.33	38.00	6.80	06.87	4
PP-10493	9.53	2.77	23.29	3.87	14.33	17.67	7.50	09.70	5
PP-10494	10.50	2.97	28.39	5.33	25.00	35.67	7.80	11.60	5
PP-10495	8.73	3.00	31.56	4.63	26.67	35.00	7.33	11.30	5
LSD(0.01)	**0.52**	**0.19**	**2.69**	**0.44**	**2.02**	**2.33**	**0.27**	**0.66**	**0.18**
LSD(0.05)	**0.36**	**0.13**	**1.86**	**0.30**	**1.39**	**1.61**	**0.19**	**0.45**	**0.12**
Level of significance	**	**	**	**	**	**	**	**	**

*, **= Significant at 5, 1%, respectively level of probability and ns= not significant.



(c)

Figure 8: Leaf characteristic of pigeonpea at vegetative stage
a. *C. cajan*vegetative stage
b.Leaf of *C. cajan*
c. Different size and shape of terminal leaflet of 16 *C. cajan*germplasm

Flower characteristics

The flower characteristics like types of flower, color of banner petal and number of petals per flower were recorded.

Types of flower

In respect of types of flower, the flowers are bisexual (Table 20, Figure 9).

Number of petals

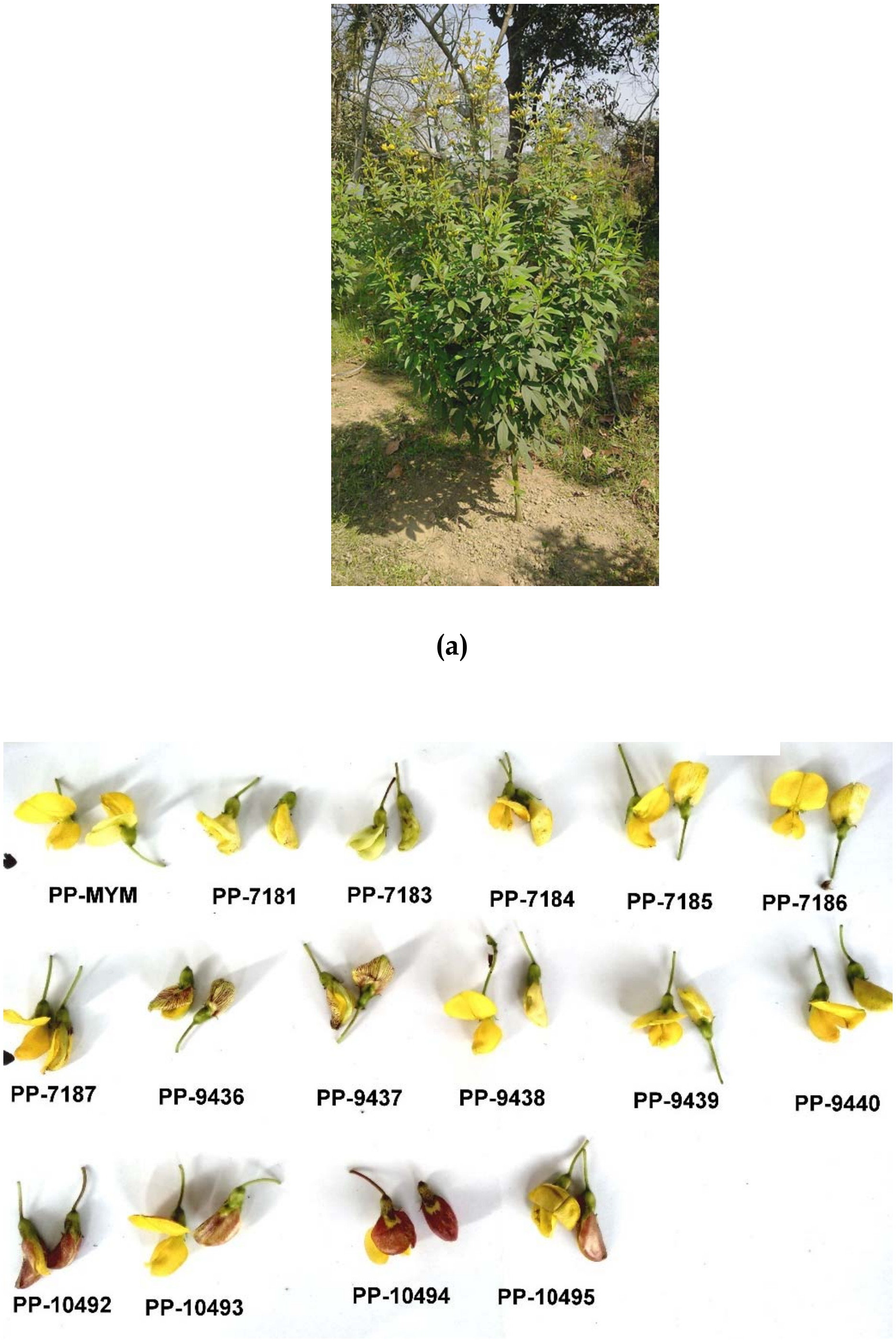
The flower had five unequal petals. There were 3 kinds of petals Standard/banner (1), wings (2), keels (2 joined). (Table 20, Figure 9).

Color of petals

In 16 *C. cajan* the color of wing and keel petals was pale yellowfor PP-7183 but yellow for rest of the germplasm. There was difference in banner/standard petal color. PP-7183 was pale yellow; PP-9436 & PP-9437 were mixed red-yellow; PP-10492, PP-10493, PP-10494 were red in color. (Table 20, Figure 9).

Table 20: Qualitative characteristics of flowers of 16*C. cajan*germplasm

Germplasm	Type of flower	Number of petals	Color of petal		Flowering pattern
			Banner/standard	Wings, keels	
PP-MYM	bisexual	five	Yellow	Yellow	Indeterminate
PP-7181	bisexual	five	Yellow	Yellow	Indeterminate
PP-7183	bisexual	five	Pale yellow	Pale yellow	Indeterminate
PP-7184	bisexual	five	Yellow	Yellow	Indeterminate
PP-7185	bisexual	five	Yellow	Yellow	Indeterminate
PP-7186	bisexual	five	Yellow	Yellow	Indeterminate
PP-7187	bisexual	five	Yellow	Yellow	Indeterminate
PP-9436	bisexual	five	Red -Yellow streaks	Yellow	Indeterminate
PP-9437	bisexual	five	Red -Yellow streaks	Yellow	Indeterminate
PP-9438	bisexual	five	Yellow	Yellow	Indeterminate
PP-9439	bisexual	five	Yellow	Yellow	Indeterminate
PP-9440	bisexual	five	Yellow	Yellow	Indeterminate
PP-10492	bisexual	five	Red	Yellow	Indeterminate
PP-10493	bisexual	five	Red	Yellow	Indeterminate
PP-10494	bisexual	five	Red	Yellow	Indeterminate
PP-10495	bisexual	five	Red	Yellow	Indeterminate



(a)

(b)

Figure 9: Flower characteristics of *C. cajan*at flowering stage
a. *C. cajan* plant at flowering stage
b. Flower of 16 *C. cajan*germplasm

Fruit (pod) characteristics

The fruit is usually called pod. The pod characteristics include shape of pod, length, color, weight of 5 podsand number of seeds per pod.

Shape of pod

The shape of fruit wasflat(Table 21, Figure 10).

Length of pod

Length of pod was significantly different from each other. The highest length of pod of PP-10494 was 7.8 cm and the lowest was 5.60 cmof PP-9436(Table 21, Figure 10).

Color of pod

Variation was found in pod color. PP-MYM, PP-10495 were green; PP-7181, PP-7183, PP-7184, PP-9436, PP-9437, PP-9438 were dense black-green mixed color; PP-7185, PP-10493 were less black-green mixed color; PP-7185, PP-7187 were violet; PP-9439, PP-9440, PP-10492 were dense violet-green mixed color and PP-10494 was less violet-green mixed color(Table 21,Figure 10).

Weight of 5 pods

Weight of 5 of pod was significantly variedin 16 genotypes.The highestweight of 5 pods of PP-10494 was 11.6 gm and the lowest was 5.27 gm of PP-7184(Table 21).

Number of seeds per pod

Significant variation found in number of seeds per pod. The number of seeds per pod varied from 4-5 according to its length and weight (Table 21).

Table 21: Characteristics of fruit of 16 pigeon pea germplasm

Germplasm	Color of pod	Fruit shape	Number of seeds per pod
PP-MYM	Green	Flat	Five
PP-7181	Dense black-green streak	Flat	Four
PP-7183	Dense black-green streak	Flat	Four
PP-7184	Dense black-green streak	Flat	Four
PP-7185	Less black-green streak	Flat	Five
PP-7186	Violet	Flat	Four
PP-7187	Violet	Flat	Five
PP-9436	Dense black-green streak	Flat	Four
PP-9437	Dense black-green streak	Flat	Four
PP-9438	Dense black-green streak	Flat	Five
PP-9439	Dense violet-green streak	Flat	Four
PP-9440	Dense violet-green streak	Flat	Four
PP-10492	Dense violet-green streak	Flat	Five
PP-10493	Less black-green streak	Flat	Five
PP-10494	Less violet-green streak	Flat	Five
PP-10495	Green	Flat	Five

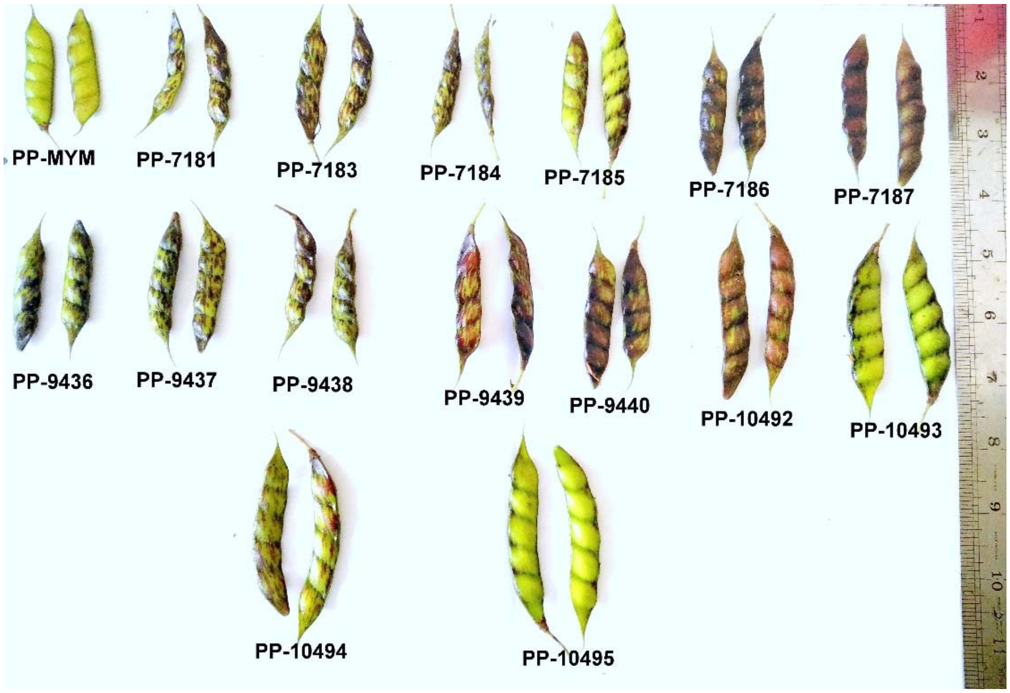


Figure 10. Characteristics of fruits of 16 pigeon pea germplasm

Molecular characterization of pigeon pea germplasm

Intra-germplasm allelic variation

Eight *C. cajan*microsatellite loci specific primers pair were applied to screen the 16 pigeonpea germplasm for genetic identification. Microsatellite profiles of 16 pigeonpea are shown in Figures11-18. Significant level of genetic variation was detected in 16 germplasm. Total 18 alleles amplified from 8 loci of *C. cajan*where 7werepolymorphic in nature. The average number of alleles per locus was 2.25(Table 22). The allele size ranged from 128 to 257 bp. The number of observed alleles (na) was found to be highest (1.71) in PP-9440, PP-10492(Table 22). Highest heterozygosity (0.28)was found in PP-9437, PP-9440, PP-10492. The highest polymorphism (62.50%) was found in PP-7185, PP-9440, PP-10492, PP-10493 and PP-10494. The overall polymorphism was 87.5%(Table 22). One private allele ($CCB1_{194}$) was found in PP-9438 (Table 23).

Allele frequencies at 8 loci in all populations are shown in Table 23. In case of CCB1 locus, 2 alleles were found.The size of the allele ranged from 194 to 198 bp.Allele $CCB1_{194}$ was found only in PP-9438 with a frequency of 0.063and allele $CCB1_{198}$ found in rest of the 15 genotypes (PP-MYM, PP-7181, PP-7183, PP-7184, PP-7185, PP-7186, PP-7187, PP-9436, PP-9437, PP-9439, PP-9440, PP-10492, PP-10493, PP-10494, PP-10495) with a frequency of 0.937 (Table 23).

At CCB2 locus, 2 alleles were found. Allele size ranged from 128 to 130 bp. Allele $CCB2_{128}$ was found in PP-7181, PP-7183, PP-7184, PP-7185, PP-7186, PP-7187, PP-9436 and allele$CCB2_{130}$ was found in PP-MYM, PP-9439, PP-10493, PP-10494 where frequencies were 0.563 & 0.437 respectively (Table 23).

At CCB4 locus, total 3 alleles were found and allele size was in range of 230 to 246 bp. Allele $CCB4_{230}$ was found in PP-MYM, PP-7183, PP-7185, PP-7186, PP-7187, PP-9436, PP-9437, PP-9438, PP-9439, PP-9440, PP-10492, PP-10493, PP-10494 ; allele $CCB4_{242}$ was in PP-MYM, PP-7181, PP-7183, PP-7184, PP-7185, PP-7186, PP-7187, PP-9436, PP-9437, PP-9438, PP-10495 and allele $CCB4_{246}$was in PP-9440, PP-10492, PP-10493, PP-10494 where frequencies ofalleles were 0.406, 0.438, 0.156, respectively(Table 23).

In case of CCB5 locus, 2 alleles were found here. Allele size ranged between 200 to 212 bp. Allele $CCB5_{200}$ and allele $CCB5_{212}$ was found in all genotypes with a frequency of 0.478 and 0.522 (Table 23).

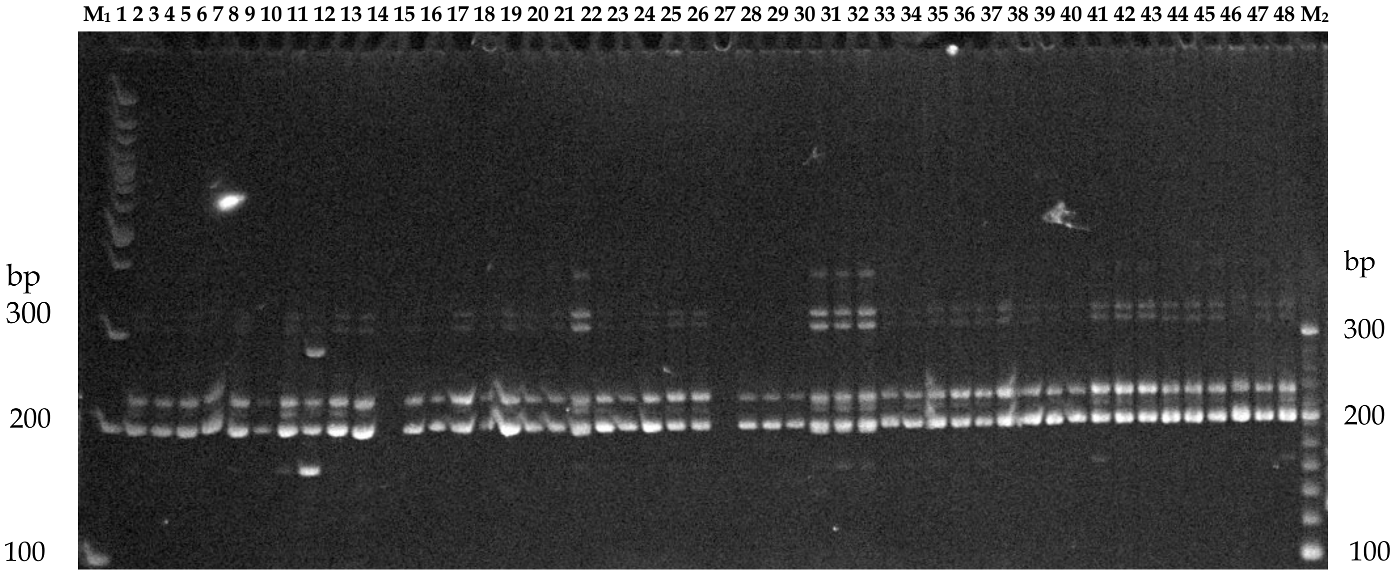


Figure 11. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at loci CCB1
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185 Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 100 bp DNA ladder, M_2: 20 bp DNA ladder

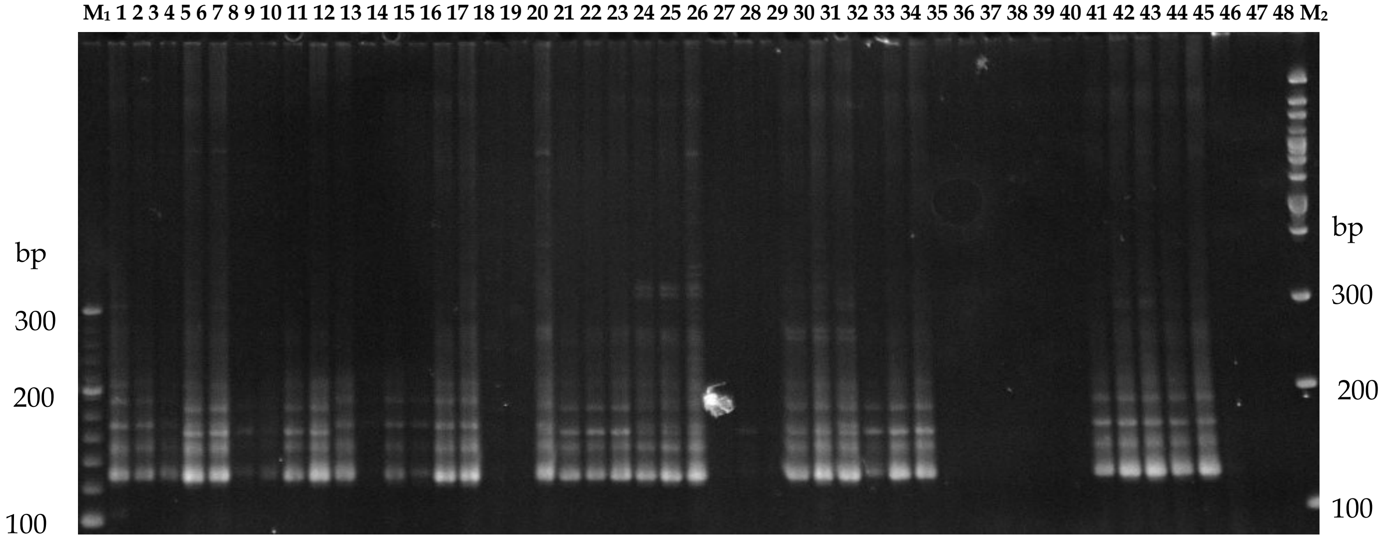


Figure 12. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at loci CCB2
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185 Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 20 bp DNA ladder, M_2: 100 bp DNA ladder.

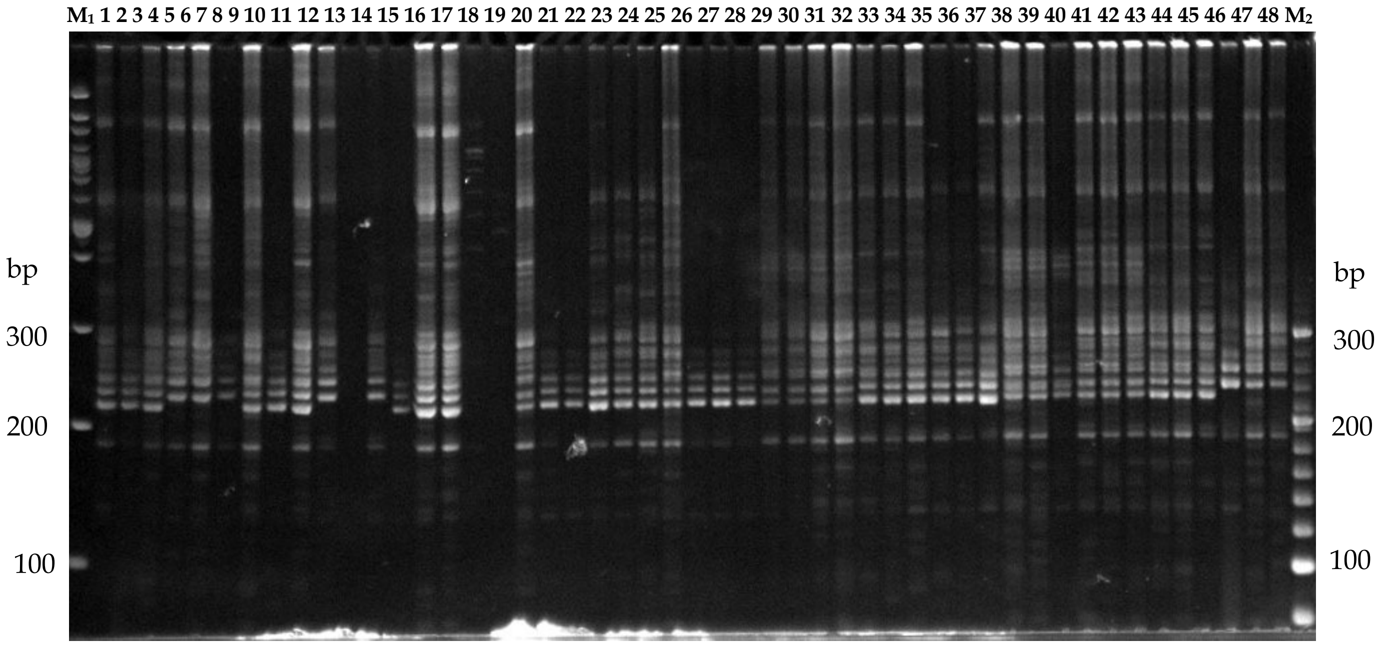


Figure 13. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at loci CCB4
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185, Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494. Lane 46-48:PP-10495. M_1: 100 bp DNA ladder. M_2: 20 bp DNA ladder.

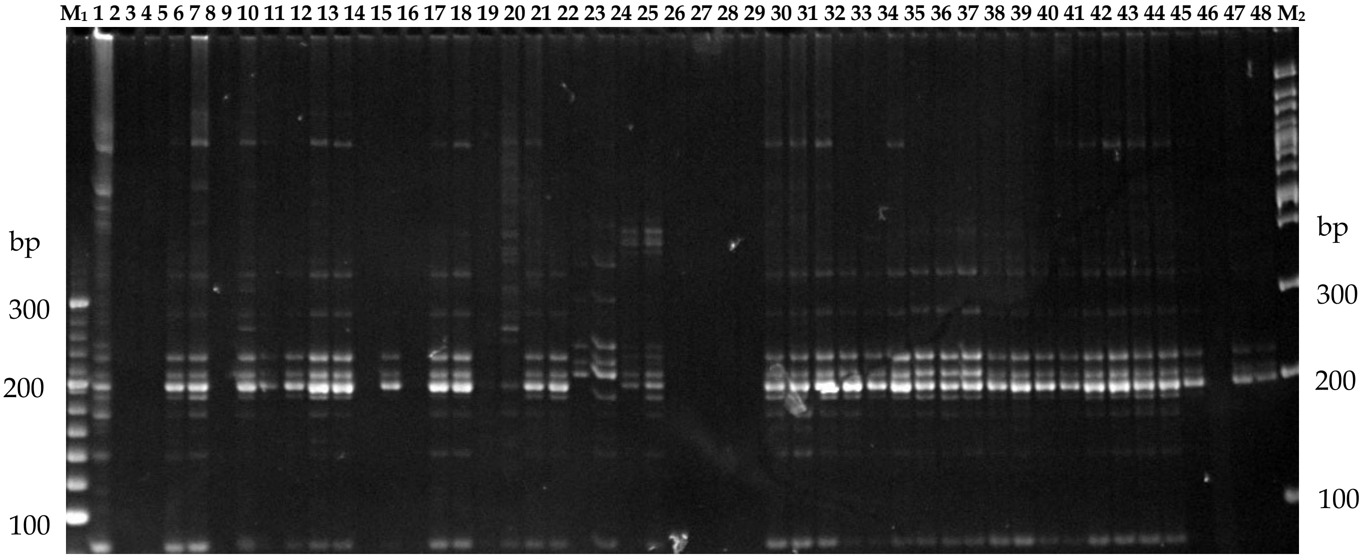


Figure 14. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at locus CCB5
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185 Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 20 bp DNA ladder, M_2: 100 bp DNA ladder.

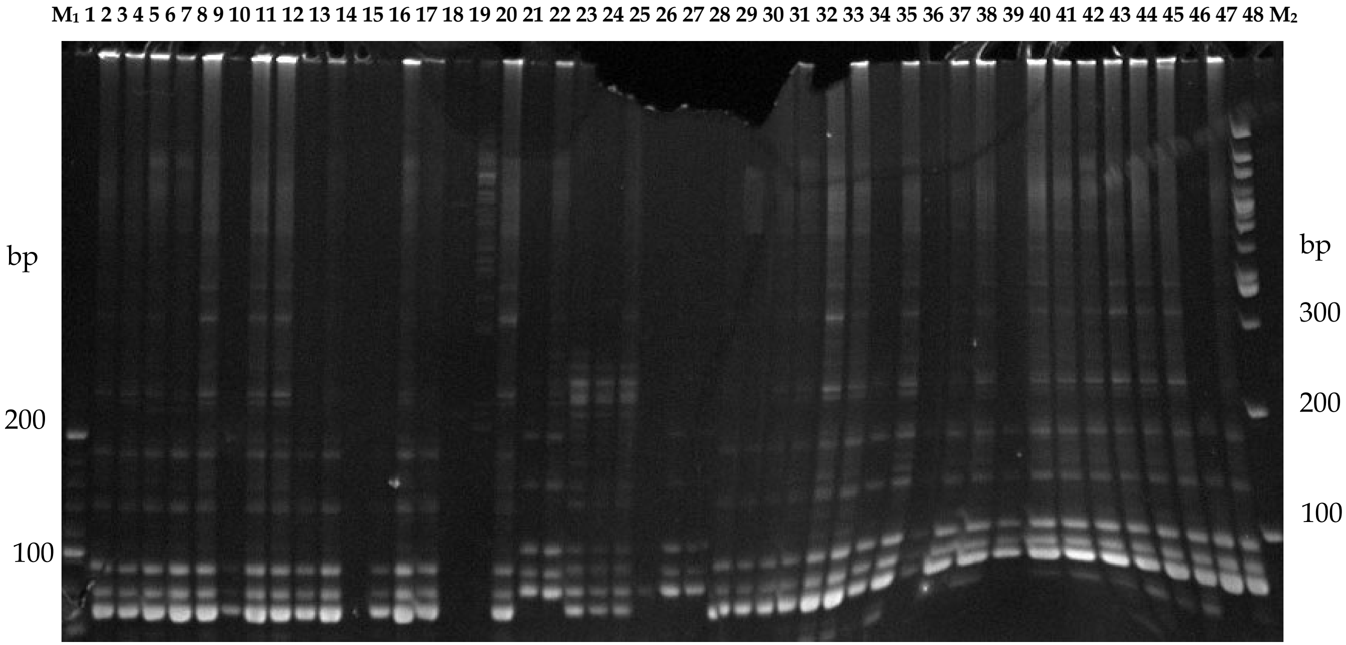


Figure 15. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at locus CCB6
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185 Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 20 bp DNA ladder, M_2: 100 bp DNA ladder.

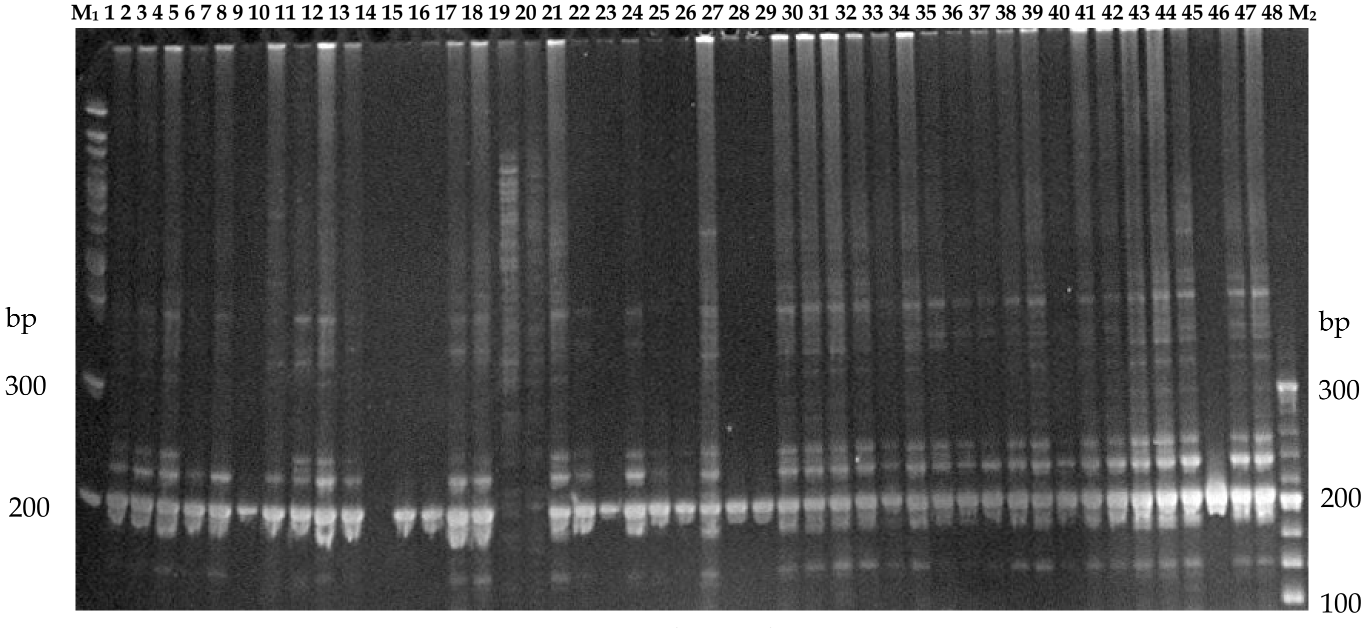


Figure 16. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at locus CCB7
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185, Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 100 bp DNA ladder, M_2: 20 bp DNA ladder.

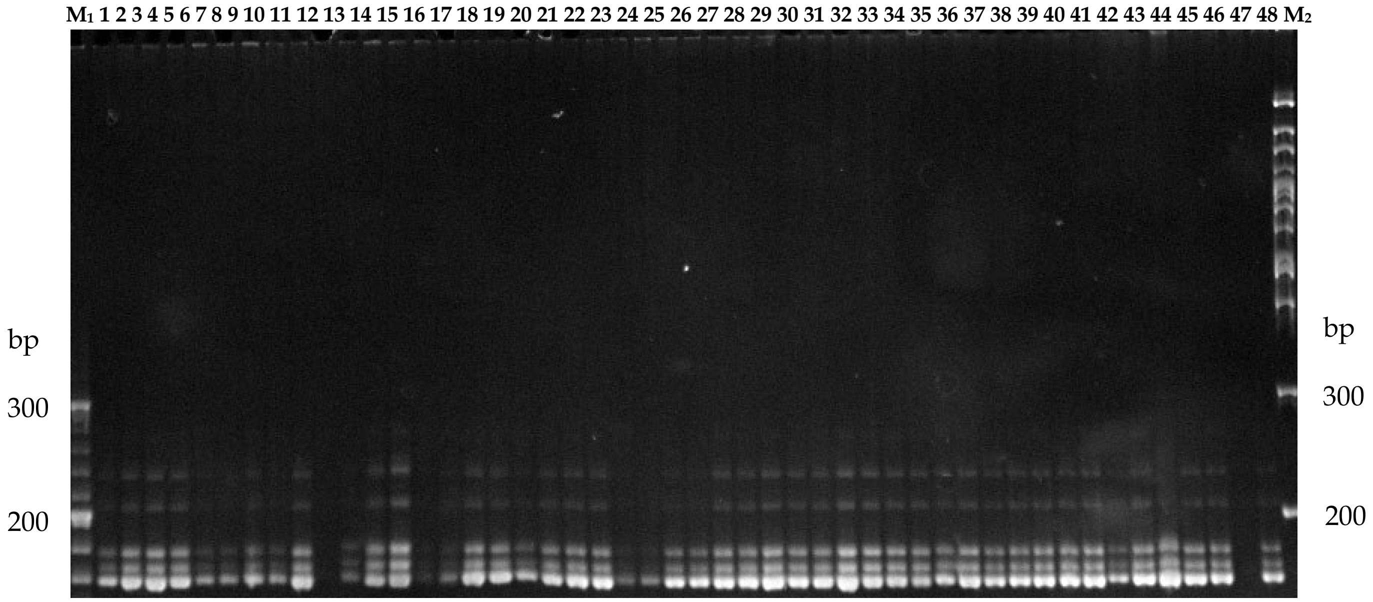


Figure 17. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at locus CCB9
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185 Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 20 bp DNA ladder, M_2: 100 bp DNA ladder.

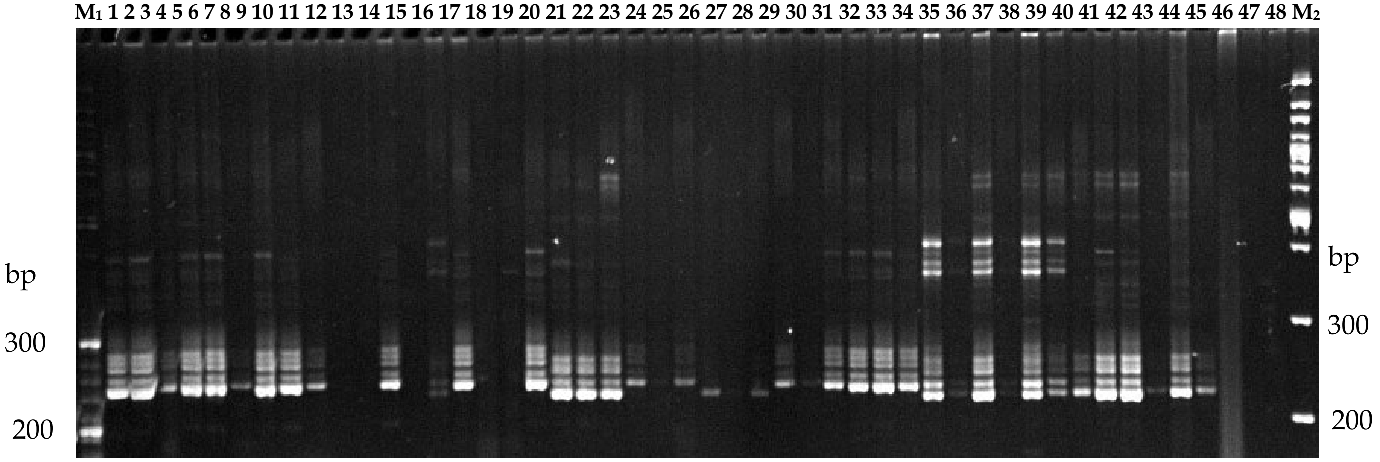


Figure 18. Microsatellite profiles of 16 pigeon pea (*C. cajan*) germplasm at locus CCB10
Lane 1-3:PP-MYM,Lane 4-6:PP-7181, Lane 7-9:PP-7183,Lane 10-12:PP-7184, Lane 13-15:PP-7185 Lane 16-18:PP-7186, Lane 19-21:PP-7187, Lane 22-24:PP-9436, Lane 25-27:PP-9437, Lane 28-30:PP-9438, Lane 31-33:PP-9439, Lane 34-36:PP-9440, Lane 37-39:PP-10492, Lane 40-42:PP-10493, Lane 43-45:PP-10494, Lane 46-48:PP-10495, M_1: 20 bp DNA ladder, M_2: 100 bp DNA ladder.

Table 22: Allelic and genetic variation at 8 microsatellite loci of 16 *C. cajan*germplasm

Germplasm	na*	ne*	Ho	He	Ave Het	No. of polymorphic loci	(%)polymorphic loci
PP-MYM	1.50	1.50	0.50	0.35	0.25	4	50.00
PP-7181	1.38	1.35	0.33	0.22	0.25	3	37.50
PP-7183	1.63	1.57	0.50	0.32	0.25	4	50.00
PP-7184	1.38	1.38	0.38	0.28	0.25	3	37.50
PP-7185	1.63	1.58	0.56	0.37	0.25	5	62.50
PP-7186	1.50	1.50	0.50	0.36	0.25	4	50.00
PP-7187	1.50	1.42	0.42	0.26	0.25	4	50.00
PP-9436	1.50	1.42	0.42	0.27	0.25	4	50.00
PP-9437	1.43	1.34	0.33	0.22	0.28	3	37.50
PP-9438	1.50	1.45	0.42	0.29	0.25	4	50.00
PP-9439	1.50	1.50	0.50	0.30	0.25	4	50.00
PP-9440	1.71	1.70	0.67	0.42	0.28	5	62.50
PP-10492	1.71	1.71	0.71	0.43	0.28	5	62.50
PP-10493	1.63	1.63	0.63	0.33	0.25	5	62.50
PP-10494	1.62	1.62	0.63	0.38	0.25	5	62.50
PP-10495	1.50	1.48	0.44	0.30	0.25	3	37.50
Mean	2.25	1.91	0.48	0.42	0.25	7	87.50

*na** = Observed no. of alleles, *ne** = Effective no. of alleles, *Ho* = Observed Heterozygosity, *He* = Expected Heterozygosity

At CCB6 locus, in total 2 alleles were found. The size of the allele ranged from 167 to 181 bp. Allele *$CCB6_{167}$* was found in all genotypes except PP-7187 and PP-9437. Allele *$CCB6_{181}$* was found in all genotypes. Overall frequencies of the 2 alleles were 0.441 and 0.559, respectively (Table 23).

At CCB7 locus total 3 alleles were found and allele size was in range of 184 to 204 bp. Allele *$CCB7_{184}$* was found only in PP-7183; allele $CCB7_{192}$ and $CCB7_{204}$ were found in all the genotypes where frequencies were 0.035, 0.372 and 0.593 respectively (Table 23).

In case of CCB9 locus, only one allele *$CCB9_{158}$*was found that means it was a monomorphic locus (Table 23).

At the last locus CCB10 total 3 alleles were found. Allele size ranged between 241 to 257 bp. Allele *$CCB10_{241}$* was found in PP-MYM, PP-7186, PP-7187, PP-9440, PP-10492, PP-10493; *$CCB10_{245}$* was found in PP-7181, PP-7183, PP-7185, PP-9437, PP-10494 and*$CCB10_{257}$* was found in PP-7184, PP-7185, PP-7187, PP-9436, PP-9438, PP-9439, PP-9440, PP-10492, PP-10493, PP-10494 (Table 23).

Table 23: Frequencies of alleles at 8 microsatellite loci in 16 germplasm of *C. cajan*

Locus Name	Allele Size range (bp)	No. of allele	Alleles	PP-MYM	PP-7181	PP-7183	PP-7184	PP-7186	PP-7187	PP-9436	PP-9437	PP-9438	PP-9439	PP-9440	PP-10492	PP-10493	PP-10494	PP-10495	Overall allele Frequency
CCB1	194-198	2	$CCB1_{194}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.063
			$CCB1_{198}$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.937
CCB2	128-130	2	$CCB2_{128}$	0.00	1.00	1.00	1.00	1.00	1.00	1.00	---	1.00	0.00	---	---	0.00	0.00	---	0.563
			$CCB2_{130}$	1.00	0.00	0.00	0.00	0.00	0.00	0.00	---	0.00	1.00	---	---	1.00	1.00	---	0.437
CCB4	230-246	3	$CCB4_{230}$	0.50	0.00	0.50	0.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.00	0.406
			$CCB4_{242}$	0.50	1.00	0.50	1.00	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	1.00	0.438
			$CCB4_{246}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.00	0.156
CCB5	200-212	2	$CCB5_{200}$	0.50	0.50	0.50	0.50	0.50	0.16	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.478
			$CCB5_{212}$	0.50	0.50	0.50	0.50	0.50	0.83	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.522
CCB6	167-181	2	$CCB6_{167}$	0.50	0.50	0.50	0.50	0.50	0.00	0.50	0.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.441
			$CCB6_{181}$	0.50	0.50	0.50	0.50	0.50	1.00	0.50	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.559
CCB7	184-204	3	$CCB7_{184}$	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.035
			$CCB7_{192}$	0.50	0.33	0.33	0.50	0.50	0.50	0.16	0.16	0.25	0.50	0.33	0.50	0.50	0.50	0.33	0.372
			$CCB7_{204}$	0.50	0.66	0.50	0.50	0.50	0.50	0.83	0.83	0.75	0.50	0.66	0.50	0.50	0.50	0.66	0.593
CCB9	158	1	$CCB9_{158}$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.000
CC10	241-257	3	$CC10_{241}$	1.00	0.00	0.00	0.00	1.00	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.00	---	0.231
			$CC10_{245}$	0.00	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	---	0.256
			$CC10_{257}$	0.00	0.00	0.00	1.00	0.00	0.50	1.00	0.00	1.00	1.00	0.50	0.50	0.50	0.50	---	0.513
	No. of private allele : 2					1 ($CCB7_{184}$)						1 ($CCB1_{194}$)							

Inter-germplasm genetic variation in pigeon pea

Result of pair-wise comparison of different germplasm of *C. cajan*using homogeneity test (Chi-square) is shown in the Table 24. Results showed that pigeonpea germplasm varied each other substantially at different loci. Variation between different germplasm was found to be highest at loci CCB4, CCB2 and CCB10. No significant variation found in any pair-wise comparison at loci CCB6 andCCB7. PP-7181 vs PP-10495, PP-7184 vsPP-10495,PP-7185 vs PP-7186, PP-7185 vs PP-10492, PP-7186 vs PP-9436, PP-9440 vs PP-10492, PP-9440 vs PP-10493, PP-10492 vs PP-10493 germplasm were homogenous at all 8 loci.

Inter-genotype genetic identity and genetic distance

The genetic distances and genetic identity were computed following Nei's (1972). Genetic identity and genetic distances between pigeon pea are shown in (Table 25). Results showed that the highest genetic distance (0.70) was observed between PP-MYM and PP-9438 germplasm, while the lowest genetic distance (0.01) was observed between PP-9440 and PP-10492. The estimated genetic identity value between PP-7185 & PP-9436and PP-7186 & PP-9436 was observed the highest (0.97) whereas the lowest value (0.10) was observed between PP-7184 & PP-7185 and PP-9440&PP-10492 (Table 25).

UPGMA dendrogram

A dendrogram based on Nei's (1972) genetic distance grouped 16 pigeonpea germplasm into two major clusters A & B (Figure 19). Cluster A is composed of PP-MYM, PP-7187, PP-9439, PP-10493, PP-10494, PP-10492 and PP-9440. Cluster A is divided into subgroups $A_1$and A_2. Subgroup A_1consisted of PP-MYM, PP-7187and A_2 composed of PP-9439, PP-10493, PP-10494, PP-10492 and PP-9440 and both werefurther divided into several subgroups. PP-7181, PP-7183, PP-9437, PP-10495, PP-7184, PP-7185, PP-9436, PP-7187, PP-9438 belonged to the Cluster B. B is further divided into two groups B_1 and B_2. PP-7181, PP-7183, PP-9437, PP-10495 were made group B_1. B_2 consisted of PP-7184, PP-7185, PP-9436, PP-7187, PP-9438 and both B_1 and B_2 were further divided into several small sub-groups.

Table 24: Homogeneity test (χ^2 values) between 16 pigeonpea germplasm at 8 SSRloci

Germplasm pairs	Loci							
	CCB1	CCB2	CCB4	CCB5	CCB6	CCB7	CCB9	CCB10
PP-MYM vs PP-7181	00	12***	00	00	00	3.4^{NS}	00	12***
PP-MYM vs PP-7183	00	12***	4*	00	00	1.2^{NS}	00	12***
PP-MYM vs PP-7184	00	10***	4*	00	00	00	00	8***
PP-MYM vs PP-7185	00	12***	00	00	00	00	00	10**
PP-MYM vs PP-7186	00	8**	00	00	00	5^{NS}	00	8**
PP-MYM vs PP-7187	00	00	00	0.88^{NS}	2.85^{NS}	00	00	4*
PP-MYM vs PP-9436	00	12***	00	00	00	1.5^{NS}	00	12***
PP-MYM vs PP-9437	00	00	00	00	4*	1.5^{NS}	00	10***
PP-MYM vs PP-9438	12***	12***	00	00	00	0.62^{NS}	00	12***
PP-MYM vs PP-9439	00	00	6*	00	00	00	00	12***
PP-MYM vs PP-9440	00	00	6*	00	00	0.34^{NS}	00	4*

Germplasm pairs	Loci							
	CCB1	CCB2	CCB4	CCB5	CCB6	CCB7	CCB9	CCB10
PP-MYM vs PP-10492	00	00	6*	00	00	00	00	4*
PP-MYM vs PP-10493	00	00	6*	00	00	00	00	4*
PP-MYM vs PP-10494	00	00	6*	00	00	00	00	12**
PP-MYM vs PP-10495	00	00	4*	00	00	0.34^{NS}	00	00
PP-7181 vs PP-7183	00	00	4*	00	00	1.14^{NS}	00	00
PP-7181 vs PP-7184	00	00	00	00	00	0.34^{NS}	00	8**
PP-7181 vs PP-7185	00	00	4*	00	00	0.27^{NS}	00	6.42**
PP-7181 vs PP-7186	00	00	4*	00	00	4.4^{NS}	00	8**
PP-7181 vs PP-7187	00	12***	4*	1.5^{NS}	2.85^{NS}	0.27^{NS}	00	12**
PP-7181 vs PP-9436	00	00	4*	00	00	0.44^{NS}	00	12***
PP-7181 vs PP-9437	00	00	4*	00	4*	0.44^{NS}	00	00
PP-7181 vs PP-9438	12***	00	4*	00	00	0.07^{NS}	00	12***
PP-7181 vs PP-9439	00	12***	12**	00	00	0.34^{NS}	00	12***
PP-7181 vs PP-9440	00	00	12**	00	00	00	00	12**
PP-7181 vs PP-10492	00	00	12**	00	00	0.34^{NS}	00	12**
PP-7181 vs PP-10493	00	10***	12**	00	00	0.27^{NS}	00	12**
PP-7181 vs PP-10494	00	12***	12**	00	00	0.34^{NS}	00	4*
PP-7181 vs PP-10495	00	00	00	00	00	00	00	00
PP-7183 vs PP-7184	00	00	4*	00	00	1.21^{NS}	00	00
PP-7183 vs PP-7185	00	00	00	00	00	0.83^{NS}	00	6.4**
PP-7183 vs PP-7186	00	00	00	00	00	2.22^{NS}	00	8**
PP-7183 vs PP-7187	00	12***	00	1.5^{NS}	2.8^{NS}	0.83^{NS}	00	12**
PP-7183 vs PP-9436	00	00	00	00	00	1.83^{NS}	00	12***
PP-7183 vs PP-9437	00	00	00	00	4*	1.83^{NS}	00	00
PP-7183 vs PP-9438	12***	00	00	00	00	0.97^{NS}	00	10***
PP-7183 vs PP-9439	00	12***	6*	00	00	1.2^{NS}	00	12***
PP-7183 vs PP-9440	00	00	6*	00	00	1.14^{NS}	00	12**
PP-7183 vs PP-10492	00	00	6*	00	00	1.20^{NS}	00	12**
PP-7183 vs PP-10493	00	10***	6*	00	00	0.84^{NS}	00	12**
PP-7183 vs PP-10494	00	12***	6*	00	00	1.2^{NS}	00	4*
PP-7183 vs PP-10495	00	00	4*	00	00	0.14^{NS}	00	00
PP-7184 vsPP-7185	00	00	4*	00	00	00	00	0.6^{NS}
PP-7184 vsPP-7186	00	00	4*	00	00	0.5^{NS}	00	00
PP-7184 vsPP-7187	00	10***	4*	1.5^{NS}	2.4^{NS}	00	00	1.6^{NS}
PP-7184 vsPP-9436	00	00	4*	00	00	1.5^{NS}	00	00
PP-7184 vsPP-9437	00	00	4*	00	3.42^{NS}	1.5^{NS}	00	6*
PP-7184 vsPP-9438	12***	00	4*	00	00	0.62^{NS}	00	00
PP-7184 vsPP-9439	00	10***	12**	00	00	00	00	00
PP-7184 vsPP-9440	00	00	12**	00	00	0.34^{NS}	00	1.62^{NS}
PP-7184 vsPP-10492	00	00	12**	00	00	00	00	1.61^{NS}
PP-7184 vsPP-10493	00	8**	12**	00	00	00	00	1.60^{NS}
PP-7184 vsPP-10494	00	10**	12**	00	00	00	00	1.60^{NS}
PP-7184 vsPP-10495	00	00	00	00	00	0.34^{NS}	00	00
PP-7185 vs PP-7186	00	00	00	00	00	4^{NS}	00	0.62^{NS}
PP-7185 vs PP-7187	00	12***	00	1.5^{NS}	2.85^{NS}	00	00	3.75^{NS}

Germplasm pairs	Loci							
	CCB1	CCB2	CCB4	CCB5	CCB6	CCB7	CCB9	CCB10
PP-7185 vs PP-9436	00	00	4*	00	00	1.5^{NS}	00	00
PP-7185 vs PP-9437	00	00	00	00	4*	1.26^{NS}	00	4.8^{NS}
PP-7185 vs PP-9438	12***	00	00	00	00	0.53^{NS}	00	1.62^{NS}
PP-7185 vs PP-9439	00	12***	6*	00	00	00	00	1.63^{NS}
PP-7185 vs PP-9440	00	00	6*	00	00	0.27^{NS}	00	3.72^{NS}
PP-7185 vs PP-10492	00	00	6*	00	00	00	00	3.72^{NS}
PP-7185 vs PP-10493	00	10***	6*	00	00	00	00	3.74^{NS}
PP-7185 vs PP-10494	00	12***	6*	00	00	00	00	6.21^{NS}
PP-7185 vs PP-10495	00	00	4*	00	00	2.70^{NS}	00	00
PP-7186 vs PP-7187	00	8**	00	1.5^{NS}	2.4^{NS}	4^{NS}	00	1.6^{NS}
PP-7186 vs PP-9436	00	00	00	00	00	4^{NS}	00	00
PP-7186 vs PP-9437	00	00	00	00	3.4^{NS}	4^{NS}	00	6*
PP-7186 vs PP-9438	12***	00	00	00	00	3.2^{NS}	00	00
PP-7186 vs PP-9439	00	00	6*	1.5^{NS}	2.8^{NS}	00	00	4*
PP-7186 vs PP-9440	00	00	6*	00	00	4.45^{NS}	00	1.61^{NS}
PP-7186 vs PP-10492	00	00	6*	00	00	5^{NS}	00	1.62^{NS}
PP-7186 vs PP-10493	00	6*	6*	00	00	4^{NS}	00	1.61^{NS}
PP-7186 vs PP-10494	00	8*	6*	00	00	5^{NS}	00	1.61^{NS}
PP-7186 vs PP-10495	00	00	4*	00	00	4.4^{NS}	00	00
PP-7187 vsPP-9436	00	12***	00	1.51^{NS}	2.81^{NS}	1.24^{NS}	00	4^{NS}
PP-7187 vsPP-9437	00	00	00	1.51^{NS}	00	1.23^{NS}	00	10**
PP-7187 vsPP-9438	12***	12***	00	1.51^{NS}	2.81^{NS}	0.58^{NS}	00	4^{NS}
PP-7187 vsPP-9439	00	00	6*	1.51^{NS}	2.81^{NS}	00	00	4*
PP-7187 vsPP-9440	00	00	6*	1.51^{NS}	2.81^{NS}	0.27^{NS}	00	00
PP-7187 vsPP-10492	00	00	6*	1.51^{NS}	2.81^{NS}	0.27^{NS}	00	00
PP-7187 vsPP-10493	00	00	6*	1.51^{NS}	2.82^{NS}	00	00	00
PP-7187 vsPP-10494	00	00	6*	1.51^{NS}	2.81^{NS}	00	00	00
PP-7187 vsPP-10495	00	00	4*	1.51^{NS}	2.61^{NS}	0.27^{NS}	00	00
PP-9436 vs PP-9437	00	00	00	00	4*	00	00	10***
PP-9436 vs PP-9438	12***	00	00	00	00	0.10^{NS}	00	00
PP-9436 vs PP-9439	00	12***	00	00	00	1.51^{NS}	00	00
PP-9436 vs PP-9440	00	00	6*	00	00	0.44^{NS}	00	4*
PP-9436 vs PP-10492	00	00	6*	00	00	1.51^{NS}	00	4*
PP-9436 vs PP-10493	00	10***	6*	00	00	1.21^{NS}	00	4*
PP-9436 vs PP-10494	00	12***	6*	00	00	1.51^{NS}	00	4*
PP-9436 vs PP-10495	00	00	4*	00	00	0.44^{NS}	00	00
PP-9437 vsPP-9439	12***	00	00	00	4*	0.10^{NS}	00	10***
PP-9437 vsPP-9440	00	00	6*	00	4*	1.51^{NS}	00	10***
PP-9437 vsPP-10492	00	00	6*	00	4*	0.44^{NS}	00	10**
PP-9437 vsPP-10493	00	00	6*	00	4*	1.51^{NS}	00	10**
PP-9437 vsPP-10494	00	00	6*	00	4*	1.26^{NS}	00	10**
PP-9437 vsPP-10495	00	00	6*	00	4*	1.51^{NS}	00	2.81^{NS}
PP-9438 vsPP-9439	00	00	4*	00	3.5*	$0. 44^{NS}$	00	00
PP-9438 vsPP-9440	12***	12***	6*	00	00	0.62^{NS}	00	4*

Germplasm pairs	Loci							
	CCB1	CCB2	CCB4	CCB5	CCB6	CCB7	CCB9	CCB10
PP-9438 vsPP-10492	12***	00	6*	00	00	0.62^{NS}	00	4*
PP-9438 vsPP-10493	12***	10***	6*	00	00	0.53^{NS}	00	4*
PP-9438 vsPP-10494	12***	12***	6*	00	00	0.62^{NS}	00	4*
PP-9438 vsPP-10495	12***	00	6*	00	00	0.72^{NS}	00	00
PP-9439 vs PP-9440	00	00	00	00	00	0.34^{NS}	00	4*
PP-9439 vs PP-10492	00	00	00	00	00	00	00	4*
PP-9439 vs PP-10493	00	00	00	00	00	00	00	4*
PP-9439 vs PP-10494	00	00	00	00	00	00	00	4*
PP-9439 vs PP-10495	00	00	12**	00	00	0.34^{NS}	00	00
PP-9440 vs PP-10492	00	00	00	00	00	0.34^{NS}	00	00
PP-9440 vs PP-10493	00	00	00	00	00	0.27^{NS}	00	00
PP-9440 vs PP-10494	00	00	00	00	00	0.34^{NS}	00	6*
PP-9440 vs PP-10495	00	00	12**	00	00	00	00	00
PP-10492 vsPP-10493	00	00	00	00	00	00	00	00
PP-10492 vsPP-10494	00	00	00	00	00	00	00	6*
PP-10492 vsPP-10495	00	00	12**	00	00	0.34^{NS}	00	00
PP-10493 vsPP-10494	00	00	00	00	00	00	00	6*
PP-10493vsPP-10495	00	00	12**	00	00	0.27^{NS}	00	00
PP-10494 vsPP-10495	00	00	12**	00	00	0.34^{NS}	00	00

Statistically significant values are marked with asterisks. NS=not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 25: Summary of Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal) in 16*C. cajan*germplasm

POP ID	PP-Mymen	PP-7181	PP-7183	PP-7184	PP-7185	PP-7186	PP-7186	PP-9436	PP-9437	PP-9438	PP-9439	PP-9440	PP-10492	PP-10493	PP-10494	PP-10495
PP-MYM	****	0.63	0.66	0.64	0.69	0.63	0.90	0.65	0.68	0.50	0.79	0.81	0.82	0.92	0.83	0.77
PP-7181	0.50	****	0.96	0.84	0.86	0.77	0.62	0.80	0.83	0.64	0.56	0.65	0.64	0.58	0.67	0.84
PP-7183	0.42	0.04	****	0.79	0.90	0.81	0.65	0.82	0.86	0.66	0.62	0.71	0.71	0.64	0.73	0.76
PP-7184	0.45	0.17	0.23	****	0.10	0.92	0.70	0.94	0.66	0.80	0.72	0.74	0.74	0.67	0.67	0.83
PP-7185	0.37	0.14	0.11	0.05	****	0.95	0.74	0.97	0.75	0.80	0.77	0.81	0.82	0.74	0.76	0.79
PP-7186	0.47	0.26	0.21	0.08	0.05	****	0.70	0.97	0.67	0.80	0.75	0.78	0.77	0.69	0.70	0.73
PP-7187	0.10	0.46	0.44	0.34	0.30	0.36	****	0.72	0.75	0.56	0.86	0.79	0.80	0.90	0.85	0.75
PP-9436	0.42	0.22	0.20	0.05	0.03	0.03	0.32	****	0.71	0.83	0.78	0.82	0.80	0.73	0.73	0.77
PP-9437	0.38	0.18	0.15	0.42	0.29	0.40	0.28	0.35	****	0.53	0.64	0.76	0.74	0.67	0.76	0.81
PP-9438	0.70	0.43	0.42	0.23	0.21	0.21	0.57	0.18	0.63	****	0.62	0.63	0.62	0.56	0.56	0.58
PP-9439	0.23	0.58	0.48	0.33	0.25	0.28	0.15	0.25	0.45	0.48	****	0.86	0.87	0.96	0.96	0.67
PP-9440	0.20	0.42	0.34	0.31	0.20	0.25	0.23	0.20	0.28	0.46	0.15	****	0.10	0.90	0.85	0.78
PP-10492	0.20	0.43	0.34	0.30	0.20	0.26	0.23	0.23	0.30	0.48	0.14	0.01	****	0.90	0.85	0.77
PP-10493	0.09	0.53	0.44	0.40	0.30	0.36	0.11	0.32	0.40	0.58	0.04	0.11	0.10	****	0.95	0.70
PP-10494	0.19	0.40	0.31	0.40	0.27	0.36	0.16	0.32	0.30	0.58	0.04	0.16	0.16	0.05	****	0.70
PP-10495	0.26	0.18	0.27	0.19	0.24	0.31	0.29	0.26	0.22	0.52	0.40	0.25	0.26	0.36	0.36	****

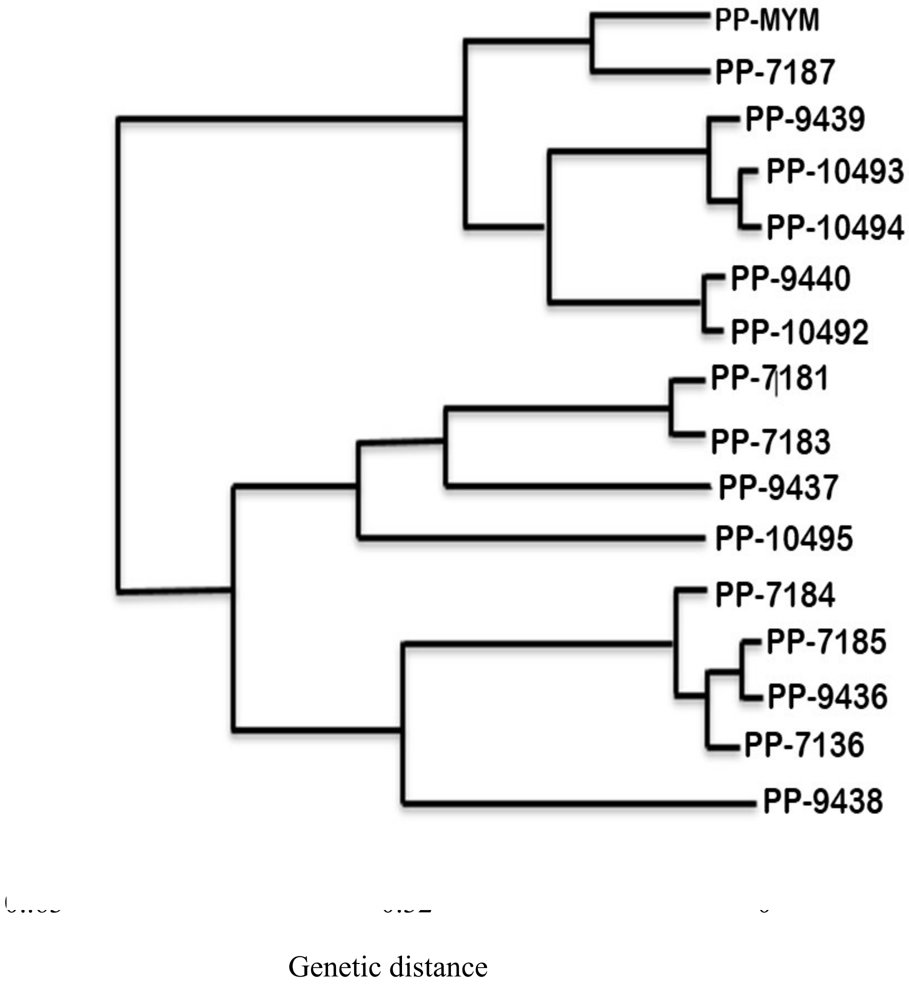


Figure 19. UPGMA dendrogram based on Nei′s (1972) genetic distance, summarizing the data on differentiation among 16*C. cajan*germplasm according to microsatellite DNA analysis

Overall genetic variation

Nei's (1987) overall genetic variation statistics for all loci showed that the average number of alleles per locus was 2.25 and also showed that the average number of expected alleles per locus was 1.91 (Table 26).Expected heterozygosity ranged from 0.00 (CCB9) to 0.62 (CCB4, CCB10) with a mean of 0.42. Average heterozygosity ranged from 0.00 (CCB1, CCB2, CCB9) to 0.49 (CCB5) with a mean of 0.24 (Table 26).

The overall genetic differentiation *(F_{st})* in 16 pigeonpea is showed in Table 26. The highest genetic differentiation (1.00) was found atCCB1 and CCB2 with a mean of 0.42. The average gene flow value in 16 pigeonpea was found to be lower (0.35) with maximum value 9.33 at locus CCB6(Table 26).

Table 26. Summary of overall heterozygosity statisticsat8 loci of *C. Cajan*germplasm

Locus	*na**	*ne**	Ho	He	Ave hete	F_{st}	*Nm**
CCB1	2.00	1.13	0.00	0.11	0.00	1.00	0.00
CCB2	2.00	1.96	0.00	0.50	0.00	1.00	0.00
CCB4	3.00	2.62	0.81	0.62	0.40	0.34	0.48
CCB5	2.00	1.99	0.96	0.50	0.49	0.02	0.33
CCB6	2.00	1.97	0.88	0.50	0.44	0.11	9.33
CCB7	3.00	2.03	0.81	0.51	0.46	0.10	2.12
CCB9	1.000	1.00	0.00	0.00	0.00	0.00	------
CCB10	3.000	2.01	0.41	0.62	0.17	0.72	0.10
Mean	**2.25**	**1.91**	**0.48**	**0.42**	**0.24**	**0.42**	**0.35**

*na** = Observed no. of alleles; *ne** = Effective no. of alleles; Ho= Observed heterozygosity; He=Expected heterozygosity; F_{st} = Genetic differentiation; Ave hete= Average heterozygosity; **Nm* = Gene flow estimated from F_{st}= 0.25(1- F_{st})/F_{st}

Characterization of wild teasel gourd

Morphological variation in wild teasel gourd germplasm

Among the four (4) wild teasel gourd germplasm, WTG-1 and WTG-2 that were collected from Thailand were male and female, respectively. Two germplasm collected from Dhamrai were male. The longest leaf (18.50 cm) was found in wild teasel gourd collected from Thailand 2 and the shortest leaf (16.00 cm) was found in wild teasel gourd from Dhamrai (Figure 20, Table 27). There was a variation on leaf breadth and length of petiole. Wild teasel gourd collected from Thailand gave fruits. The weight of one fruit was 800 g (Figure 21)

Figure 20. Leaf of wild teasel gourd

Table 27: Leaf characteristics of wild teasel gourd germplasm

Genotype	Length of leaf	breadth of leaf	Length of petiole
WTG1-Thai (Male)	16.40 b	13.17 b	4.833
WTG2-Thai (Female)	18.50 a	17.00 a	6.833
WTG3-Dhamrai (Male)	16.00 b	16.50 a	6.333
LSD(0.05)	1.976	0.887	2.535
Level of sig.	*	**	NS
CV (%)	5.14	2.51	18.63

Figure 21. Fruit of wild teasel gourd collected from Thailand

Molecular characterization of wild teasel gourd germplasm using RAPD marker

RAPD profile of 4 wild teasel gourd germplasm are given in Figures22-25. Twenty eight primers generated a total of 139 loci. Out of 139 loci generated 62 were polymorphic in nature (Table 28) and the highest polymorphic value was found (77.77%) for the primer OPB08 and OPB19 while the lowest polymorphic value (14.28) for the primer OPF07. The highest Nei's (1973) gene diversity value (0.375) was found for OPB19 and OPB13 primers while the lowest Nei's (1973) gene diversity value (0.071) was found for OPFO7.Fourteen alleles found in wild teasel gourd of Thailand were absent in wild teasel gourd Dhamrai. On the other hand, a total 12 alleles found in wild teasel gourd Dhamrai were absent in wild teasel gourd from Thailand (Table 29).

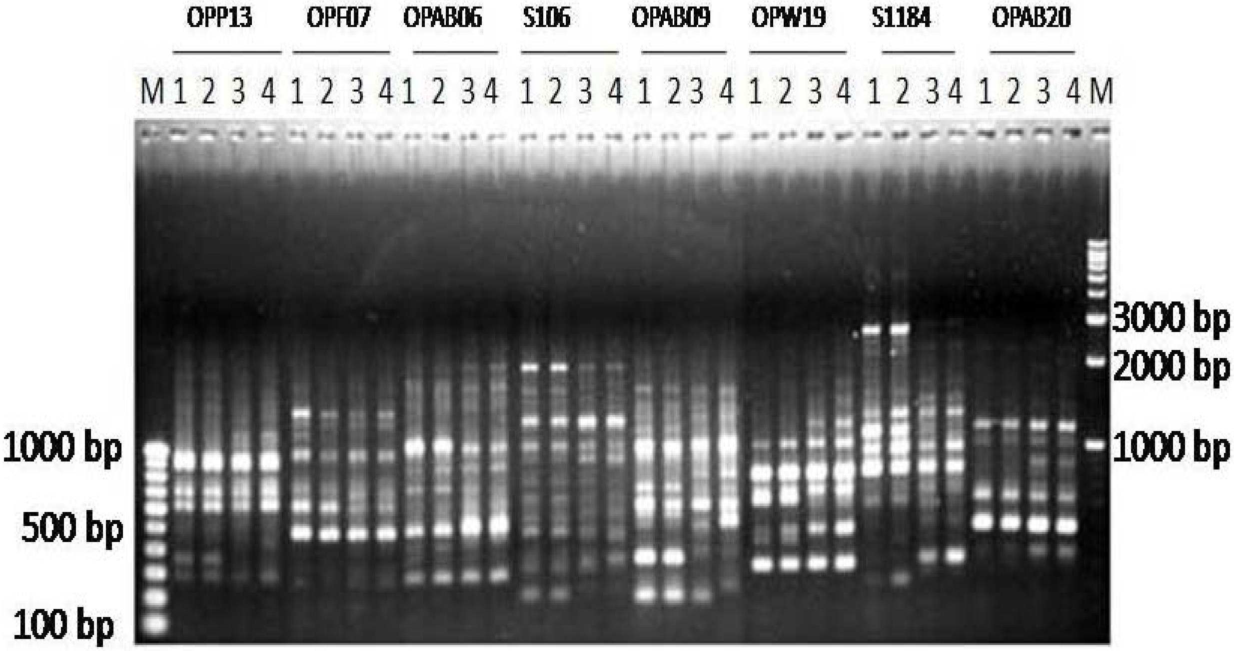


Figure 22. RAPD profile of four wild teasel gourd genotypes using primers OPP13, OPF07, S106, OPW19, S1184 and OPAB20. .1: WTG Thailand1,2: WTG Thailand2,3: WTG Dhamrai1,4: WTG Dhamrai2,M: 100bp/1kb bp DNA ladder.

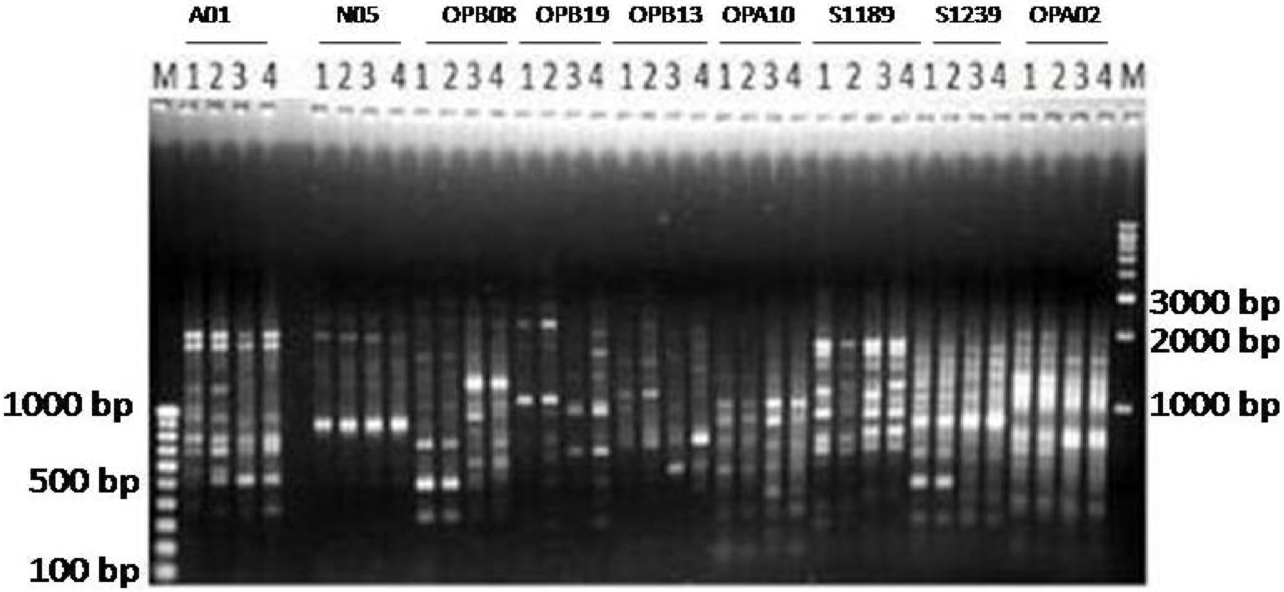


Figure23. RAPD profile of four wild teasel gourd genotypes using primers A01, N05, OPB08, OPB19, OPA10, S1189, S1239 and OPA02.1: WTG Thailand1,2: WTG Thailand2,3: WTG Dhamrai1,4: WTG Dhamrai2.M: 100bp/1kb DNA ladder.

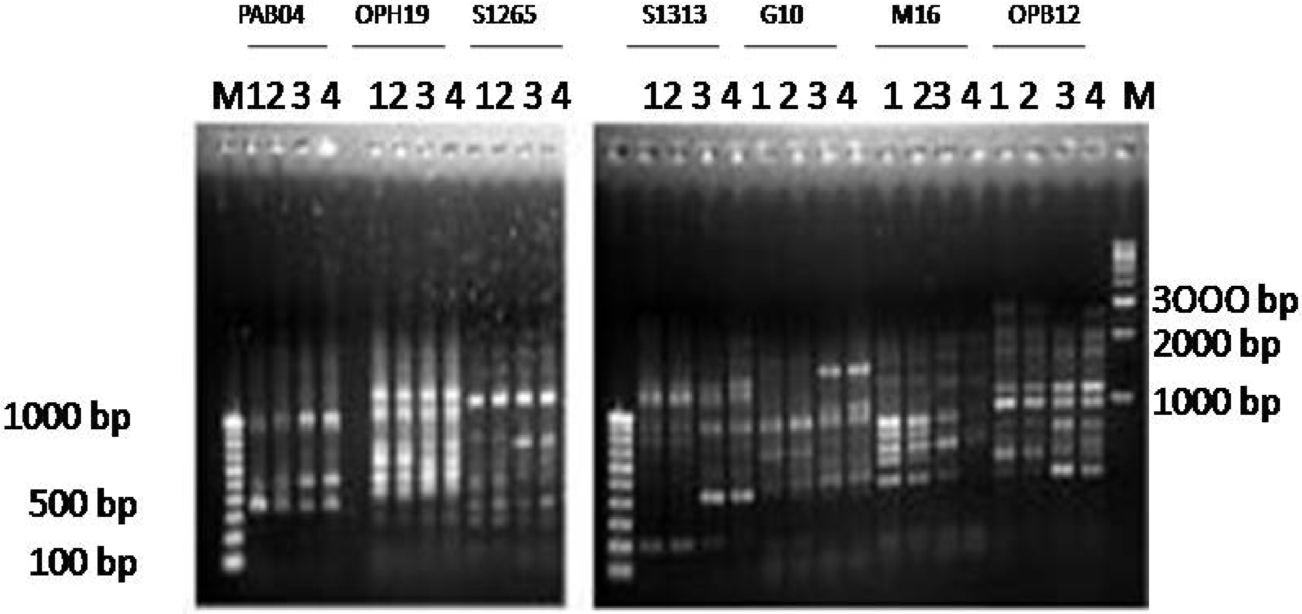


Figure 24. RAPD profile of four wild teasel gourd genotypes using primers PAB04, OPH19, S1265, S1313, G10, M16 and OPB12. 1: WTG Thailand1,2: WTG Thailand2,3: WTG Dhamrai1,4: WTG Dhamrai2. M: 100bp/1kb DNA ladder.

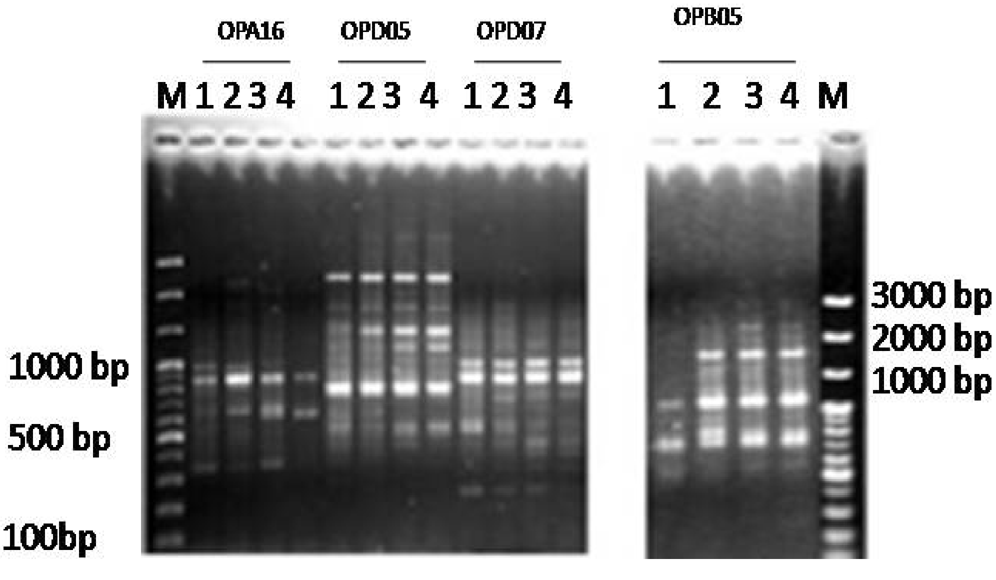


Figure25. RAPD profile of four wild teasel gourd genotypes using primers OPA16, OPD05, OPD07 and OPB05.1: WTG Thailand1,2: WTG Thailand2,3: WTG Dhamrai1,4: WTG Dhamrai2. M: 100bp/1kb DNA ladder.

Table 28: Estimation of genetic variation in wild teasel gourd

Primer code	Total number of loci	Total number of Polymorphic loci	% polymorphic loci	Gene diversity
OPP13	5	1	20.00	0.100
OPF07	7	1	14.28	0.071
OPAB06	7	2	28.58	0.142
S106	8	3	37.50	0.185
OPAB09	9	6	66.66	0.320
OPW19	8	3	37.50	0.187
S1184	10	5	50.00	0.250
OPAB20	6	2	33.33	0.166
A01	9	3	33.33	o.166
OPB08	9	7	77.77	0.361
OPB19	9	7	77.77	0.375
OPB13	4	3	75.00	0.375
OPA10	5	1	20.00	0.100
S1239	6	3	50.00	0.250
OPA02	3	1	33.33	0.166
OPB05	4	2	50.00	0.187
OPD05	6	1	16.66	0.083
S1313	5	3	60.00	0.275
G10	5	3	60.00	0.300
M16	5	2	40.00	0.175
OPA16	3	1	33.33	0.125
OPD07	6	2	33.33	0.145
Overall	139	62	44.60	0.214

Table 29: Specific alleles for Thailand and Dhamrai wild teasel gourd germplasm

Alleles	Genotypes	
	Wild teasel gourd (Thailand)	Wild teasel gourd (Dhamrai)
A01-3	1	0
A01-4	1	0
A01-8	1	0
G10-1	0	1
G10-2	0	1
G10-4	1	0
M16-2	1	0
OP01-3	1	0
OP10-5	0	1
OP05-3	1	0
OPB08-2	0	1
OP0B08-4	0	1
OPB08-5	0	1
OPB08-7	0	1
OPB08-8	1	0
OPB08-9	1	0

Alleles	Genotypes	
	Wild teasel gourd (Thailand)	Wild teasel gourd (Dhamrai)
OPB13-1	1	0
OPB13-2	1	0
OPB13-4	0	1
OPB19-2	1	0
OPB19-3	0	1
OPB19-4	0	1
OPB19-5	1	0
OPB19-8	0	1
OPB19-9	1	0
OPD05-4	0	1
OPD07-3	0	1
OPD07-4	1	0
OPF07-3	1	0
OPP13-4	1	0
OPW19-1	0	1
OPW19-5	1	0
OPW19-7	1	0
OPAB06-1	0	1
OPAB06-5	1	0
OPAB09-2	1	0
OPAB09-4	0	1
OPAB09-5	1	0
OPAB09-7	0	1
OPAB09-8	1	0
OPAB20-3	0	1
OPAB20-6	0	1
S106-2	1	0
S106-5	0	1
S106-8	1	0
S1184-1	1	0
S1184-3	1	0
S1184-8	0	1
S1184-9	0	1
S1184-10	1	0
S1239-3	0	1
S1239-4	1	0
S1239-5	0	1
S1313-3	1	0
S1313-4	0	1

The DNA polymorphisms were detected according to the presence and absence of bands. The detection of DNA polymorphisms was rendered comparing the presence and absence of bands which may be caused by failure of primer to prime a site in some individual's nucleotide sequence or by insertions or deletions between priming sites. Overall gene frequency of 4 wild teasel gourd germplasm is presented in Table 30.The highest valueof gene frequency was obtained 1.0000 and the lowest vale was 0.5000.Genetic diversity values of 4 wild teasel gourds are given in Table 31.The average Nei's gene diversity and Shannon's information index for all loci were estimated to be 0.2140 and 0.2998, respectively. The highest level of gene diversity value was found to be 0.5000 and Shannon's information index value was 0.6931. The lowest level of Nei's gene diversity and Shannon's information index for all loci were estimated 0.3750 and 0.5623, respectively. The overall gene diversity across all primers and genotypes was 0.2140.

Table 30: Overall gene frequency in four wild teasel gourd germplasms

Locus	Alleles	Frequency
A01	A01-1	1.0000
	A01-2	1.0000
	A01-3	0.5000
	A01-4	0.5000
	A01-5	1.0000
	A01-6	1.0000
	A0107	1.0000
	A01-8	0.5000
	A01-9	1.0000
G10	G10-1	0.5000
	G10-2	0.5000
	G10-3	1.0000
	G10-4	0.5000
	G10-5	1.0000
M16	M16-1	1.0000
	M16-2	0.5000
	M16-3	1.0000
	M16-4	1.0000
	M16-5	0.7500
OPA02	OPA02-1	1.0000
	OPA02-2	1.0000
	OPA02-3	0.5000
OPA10	OPA10-1	1.0000
	OPA10-2	1.0000
	OPA10-3	1.0000
	OPA10-4	1.0000
	OPA10-5	0.5000
OPA16	OPA16-1	1.0000
	OPA16-2	1.0000
	OPA16-3	0.7500
OPB05	OPB05-1	0.7500
	OPB05-2	1.0000
	OPB05-3	0.2500
	OPB05-4	1.0000

Locus	Alleles	Frequency
OPB08	OPB08-1	1.0000
	OPB08-2	0.5000
	OPB08-3	1.0000
	OPB08-4	0.5000
	OPB08-5	0.2500
	OPB08-6	0.7500
	OPB08-7	0.5000
	OPB08-8	0.5000
	OPB08-9	0.5000
OPB13	OPB13-1	0.5000
	OPB13-2	0.5000
	OPB13-3	1.0000
	OPB13-4	0.5000
OPB19	OPB19-1	1.0000
	OPB19-2	0.5000
	OPB19-3	0.5000
	OPB19-4	0.5000
	OPB19-5	0.5000
	OPB19-6	1.0000
	OPB19-7	0.5000
	OPB19-8	0.5000
	OPB19-9	0.2500
OPD05	OPD05-1	1.0000
	OPD05-2	1.0000
	OPD05-3	1.0000
	OPD05-4	0.5000
	OPD05-5	1.0000
	OPD05-6	1.0000
OPD07	OPD07-1	1.0000
	OPD07-2	1.0000
	OPD07-3	0.5000
	OPD07-4	0.2500
	OPD07-5	1.0000
	OPD07-6	1.0000
OPF07	OPF07-1	1.0000
	OPF07-2	1.0000
	OPF07-3	0.5000
	OPF07-4	1.0000
	OPF07-5	1.0000
	OPF07-6	1.0000
	OPF07-7	1.0000

Locus	Alleles	Frequency
OPP13	OPP13-1	1.0000
	OPP13-2	1.0000
	OPP13-3	1.0000
	OPP13-4	0.5000
	OPP13-5	1.0000
OPW19	OPW19-1	0.5000
	OPW19-2	1.0000
	OPW19-3	1.0000
	OPW19-4	1.0000
	OPW19-5	0.5000
	OPW19-6	1.0000
	OPW19-7	0.5000
	OPW19-8	1.0000
OPAB06	OPAB06-1	0.5000
	OPAB06-2	1.0000
	OPAB06-3	1.0000
	OPAB06-4	1.0000
	OPAB06-5	0.5000
	OPAB06-6	1.0000
	OPAB06-7	1.0000
OPAB20	OPAB20-1	1.0000
	OPAB20-2	1.0000
	OPAB20-3	0.5000
	OPAB20-4	1.0000
	OPAB20-5	1.0000
	OPAB20-6	0.5000
S106	S106-1	1.0000
	S106-2	0.5000
	S106-3	1.0000
	S106-4	1.0000
	S106-5	0.5000
	S106-6	1.0000
	S106-7	1.0000
	S106-8	0.5000

Locus	Alleles	Frequency
S1184	S1184-1	0.5000
	S1184-2	1.0000
	S1184-3	0.5000
	S1184-4	1.0000
	S1184-5	1.0000
	S1184-6	1.0000
	S1184-7	1.0000
	S1184-8	0.5000
	S1184-9	0.5000
	S1184-10	0.5000
S1239	S1239-1	1.0000
	S1239-2	1.0000
	S1239-3	0.5000
	S1239-4	0.5000
	S1239-5	0.5000
	S1239-6	1.0000
S1313	S1313-1	1.0000
	S1313-2	1.0000
	S1313-3	0.5000
	S1313-4	0.5000
	S1313-5	0.7500

Table 31: Overall genetic variation statistics acrossall loci and wild teasel gourd germplasm

Locus	na*	ne*	h*	I*
A01-1	1.0000	1.0000	0.0000	0.0000
A01-2	1.0000	1.0000	0.0000	0.0000
A01-3	2.0000	2.0000	0.5000	0.6931
A01-4	2.0000	2.0000	0.5000	0.6931
A01-5	1.0000	1.0000	0.0000	0.0000
A01-6	1.0000	1.0000	0.0000	0.0000
A0107	1.0000	1.0000	0.0000	0.0000
A01-8	2.0000	2.0000	0.5000	0.6931
A01-9	1.0000	1.0000	0.0000	0.0000
G10-1	2.0000	2.0000	0.5000	0.6931
G10-2	2.0000	2.0000	0.5000	0.6931
G10-3	1.0000	1.0000	0.0000	0.0000
G10-4	2.0000	2.0000	0.5000	0.6931
G10-5	1.0000	1.0000	0.0000	0.0000
M16-1	1.0000	1.0000	0.0000	0.0000
M16-2	2.0000	2.0000	0.5000	0.6931
M16-3	1.0000	1.0000	0.0000	0.0000

Locus	na*	ne*	h*	I*
M16-4	1.0000	1.0000	0.0000	0.0000
M16-5	2.0000	1.6000	0.3750	0.5623
OPA02-1	1.0000	1.0000	0.0000	0.0000
OPA02-2	1.0000	1.0000	0.0000	0.0000
OPA02-3	2.0000	2.0000	0.5000	0.6931
OPA10-1	1.0000	1.0000	0.0000	0.0000
OPA10-2	1.0000	1.0000	0.0000	0.0000
OPA10-3	1.0000	1.0000	0.0000	0.0000
OPA10-4	1.0000	1.0000	0.0000	0.0000
OPA10-5	2.0000	2.0000	0.5000	0.6931
OPA16-1	1.0000	1.0000	0.0000	0.0000
OPA16-2	1.0000	1.0000	0.0000	0.0000
OPA16-3	2.0000	1.6000	0.3750	0.5623
OPB05-1	2.0000	1.6000	0.3750	0.5623
OPB05-2	1.0000	1.0000	0.0000	0.0000
OPB05-3	2.0000	1.6000	0.3750	0.5623
OPB05-4	1.0000	1.0000	0.0000	0.0000
OPB08-1	1.0000	1.0000	0.0000	0.0000
OPB08-2	2.0000	2.0000	0.5000	0.6931
OPB08-3	1.0000	1.0000	0.0000	0.0000
OPB08-4	2.0000	2.0000	0.5000	0.6931
OPB08-5	2.0000	1.6000	0.3750	0.5623
OPB08-6	2.0000	1.6000	0.3750	0.5623
OPB08-7	2.0000	2.0000	0.5000	0.6931
OPB08-8	2.0000	2.0000	0.5000	0.6931
OPB08-9	2.0000	2.0000	0.5000	0.6931
OPB13-1	2.0000	2.0000	0.5000	0.6931
OPB13-2	2.0000	2.0000	0.5000	0.6931
OPB13-3	1.0000	1.0000	0.0000	0.0000
OPB13-4	2.0000	2.0000	0.5000	0.6931
OPB19-1	1.0000	1.0000	0.0000	0.0000
OPB19-2	2.0000	2.0000	0.5000	0.6931
OPB19-3	2.0000	2.0000	0.5000	0.6931
OPB19-4	2.0000	2.0000	0.5000	0.6931
OPB19-5	2.0000	2.0000	0.5000	0.6931
OPB19-6	1.0000	1.0000	0.0000	0.0000
OPB19-7	2.0000	2.0000	0.5000	0.6931
OPB19-8	2.0000	2.0000	0.5000	0.6931
OPB19-9	2.0000	1.6000	0.3750	0.5623
OPD05-1	1.0000	1.0000	0.0000	0.0000
OPD05-2	1.0000	1.0000	0.0000	0.0000
OPD05-3	1.0000	1.0000	0.0000	0.0000
OPD05-4	2.0000	2.0000	0.5000	0.6931
OPD05-5	1.0000	1.0000	0.0000	0.0000
OPD05-6	1.0000	1.0000	0.0000	0.0000
OPD07-1	1.0000	1.0000	0.0000	0.0000
OPD07-2	1.0000	1.0000	0.0000	0.0000

Locus	na*	ne*	h*	I*
OPD07-3	2.0000	2.0000	0.5000	0.6931
OPD07-4	2.0000	1.6000	0.3750	0.5623
OPD07-5	1.0000	1.0000	0.0000	0.0000
OPD07-6	1.0000	1.0000	0.0000	0.0000
OPF07-1	1.0000	1.0000	0.0000	0.0000
OPF07-2	1.0000	1.0000	0.0000	0.0000
OPF07-3	2.0000	2.0000	0.5000	0.6931
OPF07-4	1.0000	1.0000	0.0000	0.0000
OPF07-5	1.0000	1.0000	0.0000	0.0000
OPF07-6	1.0000	1.0000	0.0000	0.0000
OPF07-7	1.0000	1.0000	0.0000	0.0000
OPP13-1	1.0000	1.0000	0.0000	0.0000
OPP13-2	1.0000	1.0000	0.0000	0.0000
OPP13-3	1.0000	1.0000	0.0000	0.0000
OPP13-4	2.0000	2.0000	0.5000	0.6931
OPP13-5	1.0000	1.0000	0.0000	0.0000
OPW19-1	2.0000	2.0000	0.5000	0.6931
OPW19-2	1.0000	1.0000	0.0000	0.0000
OPW19-3	1.0000	1.0000	0.0000	0.0000
OPW19-4	1.0000	1.0000	0.0000	0.0000
OPW19-5	2.0000	2.0000	0.5000	0.6931
OPW19-6	1.0000	1.0000	0.0000	0.0000
OPW19-7	2.0000	2.0000	0.5000	0.6931
OPW19-8	1.0000	1.0000	0.0000	0.0000
OPAB06-1	2.0000	2.0000	0.5000	0.6931
OPAB06-2	1.0000	1.0000	0.0000	0.0000
OPAB06-3	1.0000	1.0000	0.0000	0.0000
OPAB06-4	1.0000	1.0000	0.0000	0.0000
OPAB06-5	2.0000	2.0000	0.5000	0.6931
OPAB06-6	1.0000	1.0000	0.0000	0.0000
OPAB06-7	1.0000	1.0000	0.0000	0.0000
OPAB09-1	1.0000	1.0000	0.0000	0.0000
OPAB09-2	2.0000	2.0000	0.0000	0.6931
OPAB09-3	1.0000	1.0000	0.5000	0.0000
OPAB09-4	2.0000	2.0000	0.0000	0.6931
OPAB09-5	2.0000	2.0000	0.5000	0.6931
OPAB09-6	1.0000	1.0000	0.5000	0.0000
OPAB09-7	2.0000	2.0000	0.0000	0.6931
OPAB09-8	2.0000	2.0000	0.5000	0.6931
OPAB09-9	2.0000	1.6000	0.5000	0.5623
OPAB20-1	1.0000	1.0000	0.3750	0.0000
OPAB20-2	1.0000	1.0000	0.0000	0.0000
OPAB20-3	2.0000	2.0000	0.0000	0.6931
OPAB20-4	1.0000	1.0000	0.5000	0.0000
OPAB20-5	1.0000	1.0000	0.0000	0.0000
OPAB20-6	2.0000	2.0000	0.0000	0.6931
S106-1	1.0000	1.0000	0.5000	0.0000

Locus	na*	ne*	h*	I*
S106-2	2.0000	2.0000	0.0000	0.6931
S106-3	1.0000	1.0000	0.5000	0.0000
S106-4	1.0000	1.0000	0.0000	0.0000
S106-5	2.0000	2.0000	0.0000	0.6931
S106-6	1.0000	1.0000	0.5000	0.0000
S106-7	1.0000	1.0000	0.0000	0.0000
S106-8	2.0000	2.0000	0.0000	0.6931
S1184-1	2.0000	2.0000	0.5000	0.6931
S1184-2	1.0000	1.0000	0.5000	0.0000
S1184-3	2.0000	2.0000	0.0000	0.6931
S1184-4	1.0000	1.0000	0.5000	0.0000
S1184-5	1.0000	1.0000	0.0000	0.0000
S1184-6	1.0000	1.0000	0.0000	0.0000
S1184-7	1.0000	1.0000	0.0000	0.0000
S1184-8	2.0000	2.0000	0.0000	0.6931
S1184-9	2.0000	2.0000	0.5000	0.6931
S1184-10	2.0000	2.0000	0.5000	0.6931
S1239-1	1.0000	2.0000	0.5000	0.6931
S1239-2	1.0000	1.0000	0.0000	0.0000
S1239-3	2.0000	1.0000	0.0000	0.0000
S1239-4	2.0000	2.0000	0.5000	0.6931
S1239-5	2.0000	2.0000	0.5000	0.6931
S1239-6	1.0000	2.0000	0.5000	0.6931
S1313-1	1.0000	1.0000	0.0000	0.0000
S1313-2	1.0000	1.0000	0.0000	0.0000
S1313-3	2.0000	1.0000	0.0000	0.0000
S1313-4	2.0000	2.0000	0.5000	0.6931
S1313-5	2.0000	2.0000	0.5000	0.6931
Mean	**1.4460**	**1.4173**	**0.2140**	**0.2998**
St. Dev	**0.4989**	**0.4770**	**0.2414**	**0.3368**

* na = Observed number of alleles; * ne = Effective number of alleles;* h = Nei's (1973) gene diversity
* I = Shannon's Information index

The highest average polymorphism was found in wild teasel gourd Thailand2 vs wild teasel gourd Dhamrai 2 (41.73%).While the lowest polymorphism value was found in wild teasel gourd Thailand1 vs wild teasel gourd Thailand2(3.6%).The highest Nei's (1973) gene diversity was found in wild teasel gourd Thailand2 vs wild teasel gourd Dhamrai 2 (0.209) while the lowest Nei's gene diversity value(0.018) was found in wild teasel gourd Thailand1 vs wild teasel gourd Thailand2 (Table 32).

Table 32: Inter-germplasm genetic variation in wild teasel gourd

Genotype	na*	% polymorphism	h*	I*
WTG1 VS WTG2	1.036	3.6(5)	0.018	0.025
WTG1 VS WTG3	1.389	38.85(54)	0.194	0.264
WTG1 VS WTG4	1.410	41.01(57)	0.205	0.284
WTG2 VS WTG3	1.410	41.01(57)	0.205	0.284
WTG2 VS WTG4	1.417	41.73(58)	0.209	0.290
WTG3 VS WTG4	1.050	5.04(57)	0.025	0.035

WTG1=Wild teasel gourd Thailand1, WTG2= Wild teasel gourd Thailand2, WTG3= Wild teasel gourd Dhamrai1, WTG4= Wild teasel gourd Dhamrai2.
* na = Observed number of alleles; * h = Nei's (1973) gene diversity; * I = Shannon's Information index

The highest pair-wise average band-sharing based similarity index(97.27) was found between WTG Dhamrai1 and WTG Dhamrai2 while the lowest value (71.73) was found between WTG Thailand2 and WTG Dhamrai2.Higher level of similarity was detected by OPP13, OPF07, OPA10, OPD05 and OPA16 primers whereas lower level of band-sharing based similarity indices were generated by S1184, OPB08, OPB19, OPB13, S1239, S1313, and G10 (Table 33).

The values of pair wise Nei’s genetic distance between germplasms were computed from combined data for all of 28 primers. The value ranges from0.0366 to 0.5400 (Table 34).The highest genetic distance (0.5400) was found in WTG Thailand2 and WTG Dhamrai2 and the lowest genetic distance (0.0366) was found between WTG Thailand1 and WTG Thailand2.A dendrogram based on Nei’s(1978) genetic distance grouped four wild teasel gourd germplasm into two clusters: cluster1 consistedWTG Thailand1 and WTG Thailand2whilst cluster 2 comprised WTG Dhamrai1 and WTG Dhamrai2 (Figure 26).

Table 33: Pair-wise band-sharing based similarity indices in 4 wild teasel gourd germplasms

Primers	Germplasm						
	Thai1vs Thai2	Thai1vs Dhamrai1	Thai1 vsDhamrai2	Thai2 vsDhamrai1	Thai2 vs Dhamrai2	Dhamrai1 vs Dhamrai2	Average
OPP13	100	89	89	89	89	100	92.67
OPF 07	100	92	92	92	92	100	94.67
OPAB06	100	83	83	83	83	100	88.67
S106	100	76	76	76	76	100	84.00
OPAB09	100	61	50	61	50	90	68.67
S1184	100	66	66	66	66	100	77.33
OPW19	100	76	76	76	76	100	84.00
OPAB20	100	80	80	80	80	100	86.67
A01	100	80	80	80	80	100	86.67
OPB08	100	36	54	36	54	100	63.33
OPB19	80	44	40	36	33	100	55.50

Primers	Germplasm						
	Thai1vs Thai2	Thai1vs Dhamrai1	Thai1 vsDhamrai2	Thai2 vsDhamrai1	Thai2 vs Dhamrai2	Dhamrai1 vs Dhamrai2	Average
OPB13	100	40	40	40	40	100	60.00
S1239	100	66	66	66	66	100	77.33
OPA10	100	89	89	89	89	100	92.67
OPA02	100	80	80	80	80	100	86.67
OPB05	66	80	80	85	85	100	82.67
S1313	100	75	57	75	57	85	74.83
OPD05	100	90	90	90	90	100	93.33
G10	100	57	57	57	57	100	71.33
M16	100	89	75	89	75	85	85.50
OPD07	89	89	89	80	80	100	87.83
OPA16	100	100	80	100	80	80	90.00
Average	96.58	74.45	72.23	73.91	71.73	97.27	81.03

WTG1=Wild teasel gourd Thailand1, WTG2= Wild teasel gourd Thailand2, WTG3= Wild teasel gourd Dhamrai1, WTG4= Wild teasel gourd Dhamrai2.

Table 34:Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) in four wild teasel gourd germplasm

pop ID	WTG Thailand1	WTG Thailand2	WTG Dhamrai1	WTG Dhamrai2
WTG Thailand1	****	0.9640	0.6115	0.5899
WTG Thailand2	0.0366	****	0.5899	0.5827
WTG Dhamrai1	0.4918	0.5278	****	0.9496
WTG Dhamrai2	0.5278	0.5400	0.0517	****

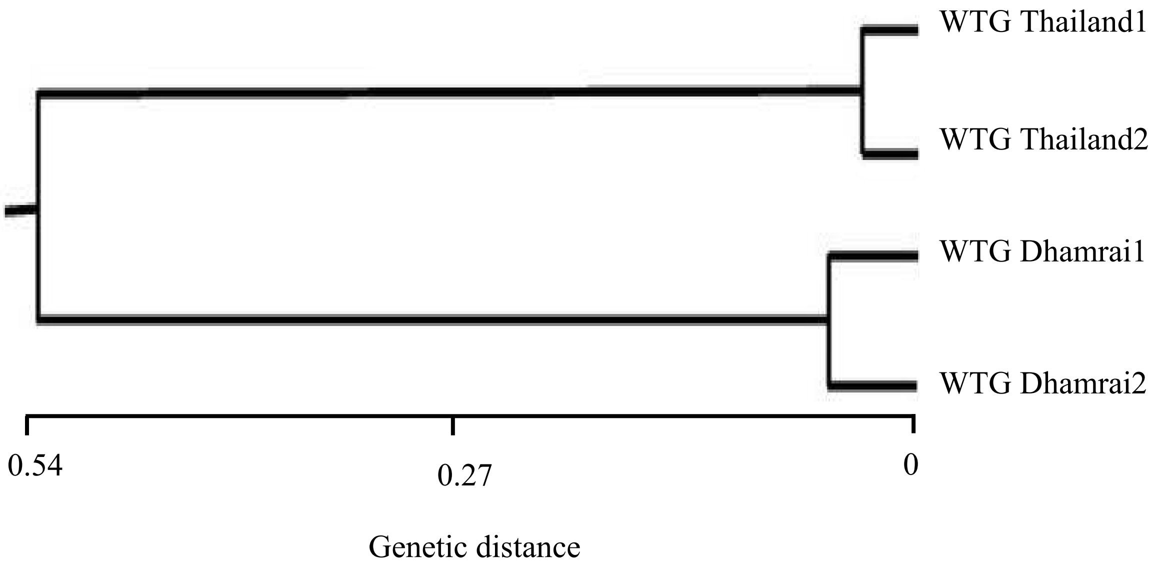


Figure 26: A UPGMA dendrogram constructed on the basis of Nei's(1978) genetic distance that summarizes genetic differentiation in wild teasel gourd germplasm

Characterization of rosellegermplasm

Morphological variation in roselle germplasm

Variation was found in sizes of leaves, color of flowers, color and size of fruits (Figure 27). Significant differences were observed in fruit weight, number of calyx and epicalyx, weight of calyx and epicalyx, leaf length and plant height (Table 35).

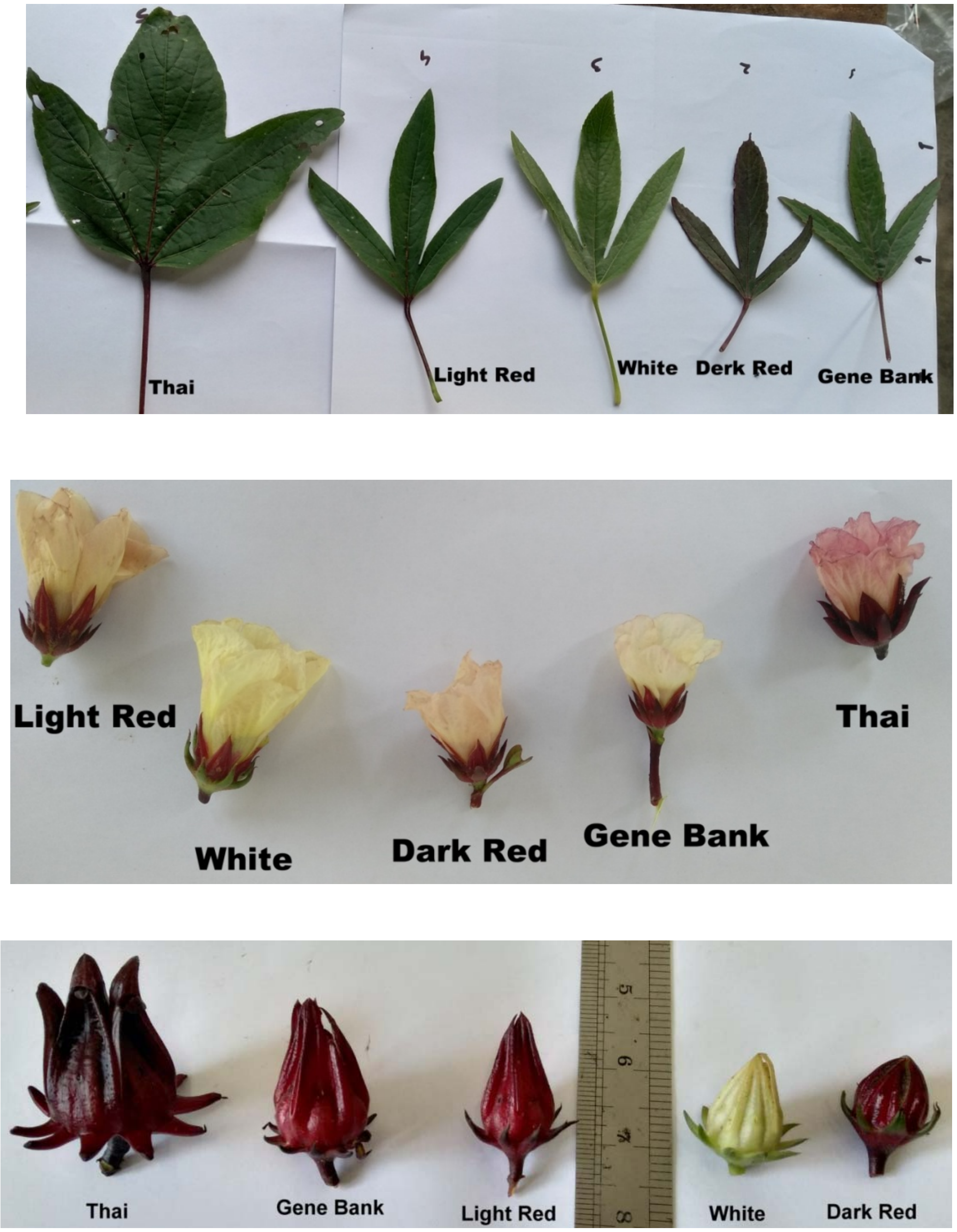


Figure 27. Variation in leaves, flowers and fruits in 5 roselle genotypes

Table 35: Morphological characteristics of 5 roselle germplasm

Genotypes	Weight of fruit (g)	No. of calyx	No. of epicalyx	Weight of calyx (g)	Weight of epicalyx (g)	Height at 30 DAS (cm)	Height at 45 DAS (cm)	Height at 60 DAS (cm)	Leaf length (cm)
Dhamri-001	7.11	5.00	9.40	2.56	0.40	15.00	25.00	31.00	11.00
Dhamri-002	7.47	5.00	9.80	2.67	0.55	17.00	22.00	27.00	11.50
Dhamri-003	6.99	5.00	9.20	2.24	0.56	19.00	23.00	28.00	11.80
Thai-002	12.56	5.00	9.60	3.55	1.41	42.00	74.50	115.00	8.10
Thai-003	8.46	5.00	8.80	2.78	1.10	46.00	87.50	150.00	9.00
Thai-004	9.46	5.00	10.00	2.32	1.00	43.00	87.00	137.00	10.50
GB-001	5.43	5.00	11.00	0.72	0.46	25.00	32.00	100.00	12.50
GB-002	5.42	5.00	11.00	0.82	0.38	25.00	33.00	96.00	12.70
GB-003	6.00	5.00	10.00	0.65	0.39	18.00	23.00	98.00	11.60
D. red-1	2.50	5.00	10.00	0.87	0.15	25.00	95.50	132.00	13.50
D. red-2	3.00	5.00	11.00	1.05	0.18	27.00	100.50	126.00	13.00
D. red-3	2.80	5.00	10.00	0.98	0.17	29.00	104.00	132.00	12.80
L. red-1	5.00	5.00	11.00	1.75	0.30	21.00	35.00	90.00	13.40
L. red-2	5.20	5.00	11.00	1.92	0.31	20.00	37.00	98.00	12.00
L. red-3	4.80	5.00	11.00	1.68	0.29	19.00	35.00	90.00	12.50
White-1	6.00	5.00	10.00	2.10	0.36	22.00	32.00	84.00	11.20
White-2	6.40	5.00	11.00	2.24	0.38	21.00	36.00	90.00	12.00
White-3	6.60	5.00	10.00	2.31	0.40	22.00	33.00	94.00	10.50
Sig. level	**	**AND**	**	**	**	**	**	**	**
LSD(0.05)	**0.643**	**AND**	**0.252**	**0.262**	**0.074**	**1.598**	**1.97**	**2.926**	**0.273**
LSD(0.01)	**0.862**	**AND**	**0.338**	**0.352**	**0.1**	**2.145**	**2.644**	**3.928**	**0.366**
CV (%)	**6.27**	**AND**	**1.48**	**8.55**	**8.6**	**3.8**	**2.34**	**1.85**	**1.4**

Significant at 1% level of probability; AND= Analysis not done

Molecular characterization of roselle germplasm

RAPD markers were used to detect genetic variation in 5 roselle genotypes. A total of 10 genotypes (2 from each genotype) were subjected to RAPD analysis using 9 decamer primers viz., A01, N05, OPAB20, OPB05, OPB08, OPB13, OPD07, OPF07 and PAB04. RAPD profiles of roselle genotypes are shown in Figure 28.Primers amplified a total of 48 loci of which 26 (54.17%) were polymorphic (Table 36). Frequencies of alleles are shown in Table 37. Among the 9 primers, primers A01 and OPB13 produced the highest number of polymorphic bands (6) whereas OPB05 and OPD07 generated the least number of polymorphic bands (1) (Table 36). Nei's gene diversity and Shannon's information index estimated across all loci and roselle genotypes ranged between 0.091 to 0.275 and 0.143 to 0.423, respectively.

Lower level of genetic variation ranging from 0.017 to 0.043 was found within roselle genotypes (Table 38). Genetic distance value was found to be the highest (0.325) between dark red roselle andlight red roselle and the lowest (0.073) between light red roselle and white roselle (Table 39). Dendrogram based on genetic distances separated 5 roselle genotypes into 2 clades. Dark red roselle was separated from other 4 genotypes.

Light red roselle was closer towhite roselle whereas BARI gene bank genotype was closer to Thai roselle (Figure 29).

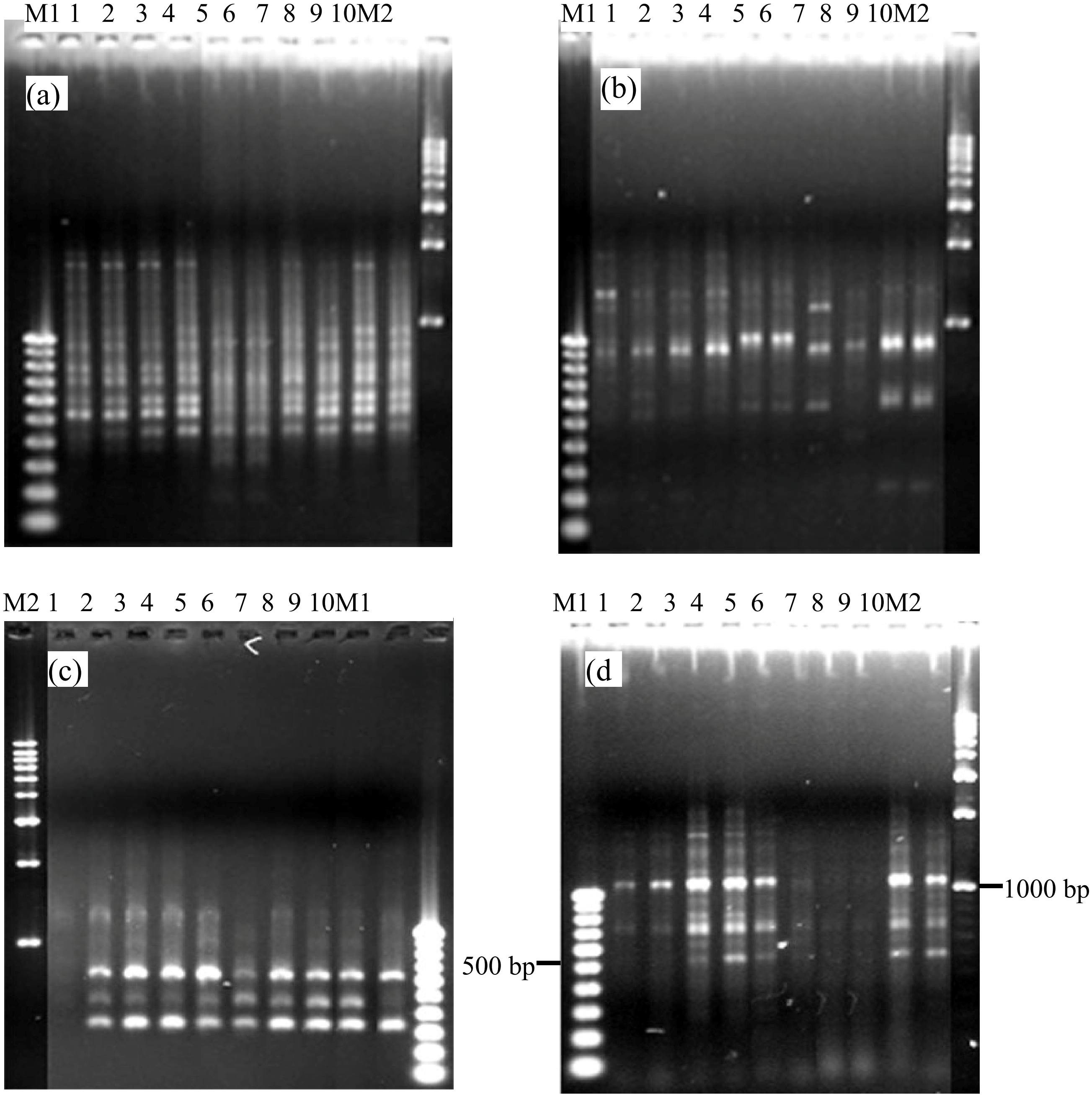


Figure 28. RAPD profiles of 5 roselle genotypes using primers A01 (a), N05 (b), OPAB20 (c) and OPB05 (d). 1-2: Thai roselle; 3-4: BARI roselle; 5-6: BAU roselle-1; 7-8: BAU roselle-2 and 9-10: BAU roselle-3. M1/M2: 100 bp/1 kb DNA ladders (continued).

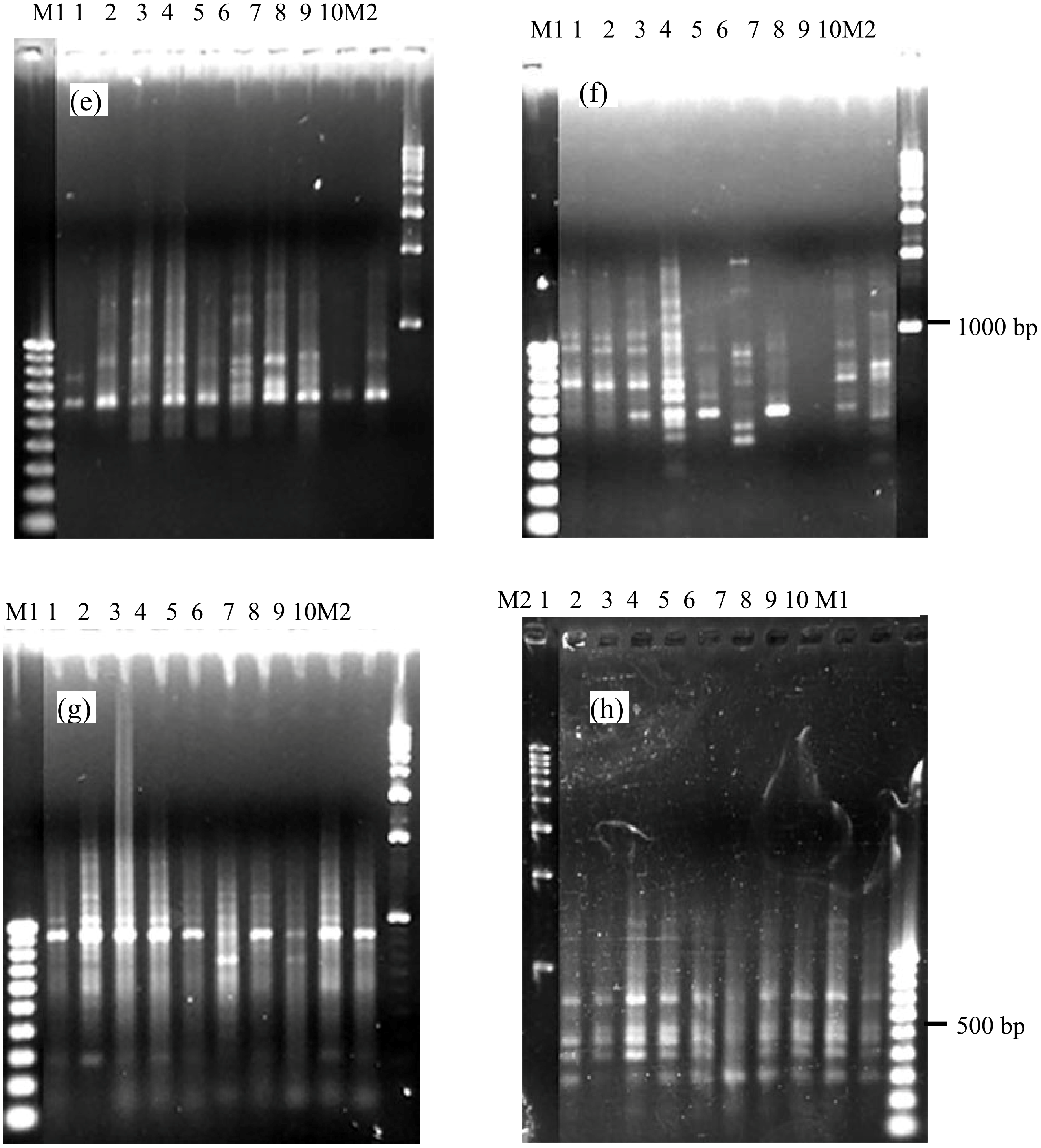


Figure 28. RAPD profiles of 5 roselle genotypes using primers OPB08 (e), OPB13 (f), OPD07 (g) and OPF07 (h). 1-2: Thai roselle; 3-4: BARI roselle; 5-6: BAU roselle-1; 7-8: BAU roselle-2 and 9-10: BAU roselle-3. M1/M2: 100 bp/1 kb DNA ladders (continued).

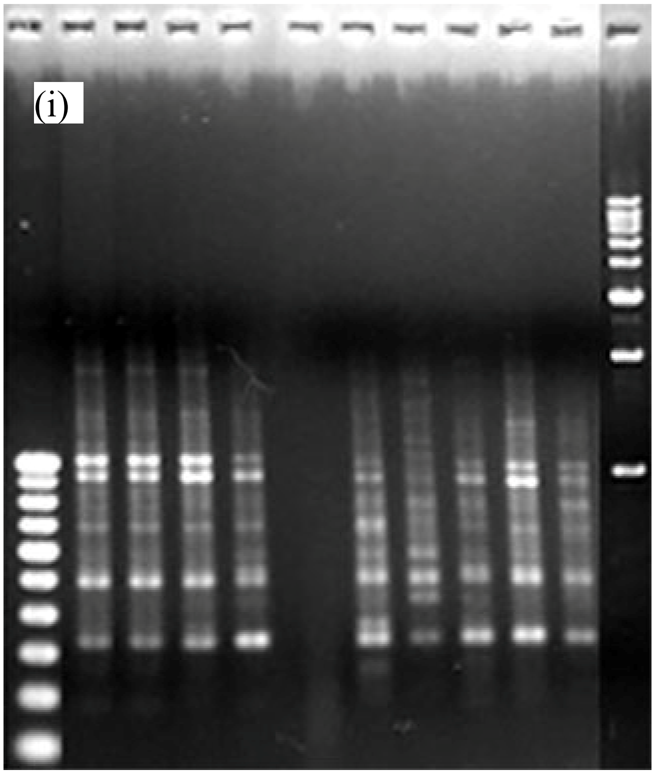


Figure 28. RAPD profiles of 5 roselle genotypes using primers PAB04 (i). 1-2: Thai roselle; 3-4: BARI roselle; 5-6: BAU roselle-1; 7-8: BAU roselle-2 and 9-10: BAU roselle-3. M1/M2: 100 bp/1 kb DNA ladders.

Table 36: Genetic variation in roselle germplasm revealed by 9 RAPD primers

Primer	Total number of loci	Number of Polymorphic loci	% polymorphic loci	Nei's gene diversity	Shannon's informaion index
A01	11	6	54.55	0.186 ± 0.182	0.286 ± 0.277
N05	5	4	80.00	0.267 ± 0.167	0.410 ± 0.244
OPAB20	4	2	50.00	0.121 ± 0.140	0.204 ± 0.235
OPB05	3	1	33.33	0.107 ± 0.185	0.167 ± 0.289
OPB08	3	2	66.67	0.172 ± 0.210	0,273 ± 0.300
OPB13	7	6	85.71	0.275 ± 0.173	0.423 ± 0.232
OPD07	3	1	33.33	0.150 ± 0.260	0.214 ± 0.371
OPF07	5	2	40.00	0.097 ± 0.133	0.163 ± 0.223
PAB04	7	2	28.57	0.091 ± 0.156	0.143 ± 0.244
Overall	48	26	54.17	0.171 ± 0.173	0.266 ± 0.260

Table 37: Frequencies of alleles in different roselle genotypes

Locus	Thai roselle	Gene bank roselle	Dark red roselle	Light red roselle	White roselle	Overall
AO1-1	0.00	0.00	1.00	0.00	0.00	0.20
AO1-2	0.00	0.00	1.00	0.00	0.00	0.20
AO1-3	1.00	1.00	1.00	0.00	0.29	0.66
AO1-4	1.00	1.00	1.00	1.00	1.00	1.00
AO1-5	1.00	1.00	1.00	1.00	1.00	1.00
AO1-6	1.00	1.00	0.00	1.00	1.00	0.80
AO1-7	1.00	1.00	1.00	1.00	1.00	1.00
AO1-8	1.00	1.00	0.00	1.00	1.00	0.80
AO1-9	1.00	1.00	1.00	1.00	1.00	1.00
AO1-10	1.00	1.00	1.00	1.00	1.00	1.00
AO1-11	1.00	1.00	0.00	1.00	1.00	0.80
N05-1	1.00	1.00	1.00	0.29	1.00	0.86
N05-2	1.00	1.00	0.00	1.00	1.00	0.80
N05-3	0.00	0.00	1.00	0.00	0.00	0.20
N05-4	1.00	1.00	1.00	0.29	0.00	0.66
N05-5	1.00	1.00	1.00	1.00	1.00	1.00
OPAB20-1	1.00	1.00	1.00	1.00	1.00	1.00
OPAB20-2	1.00	1.00	1.00	1.00	0.29	0.86
OPAB20-3	1.00	1.00	1.00	1.00	1.00	1.00
OPAB20-4	1.00	1.00	0.29	1.00	1.00	0.86
OPB05-1	0.00	1.00	1.00	1.00	1.00	0.80
OPB05-2	1.00	1.00	1.00	1.00	1.00	1.00
OPB05-3	1.00	1.00	1.00	1.00	1.00	1.00
OPB08-1	1.00	1.00	1.00	1.00	1.00	1.00
OPB08-2	0.29	0.00	0.00	0.00	0.00	0.59
OPB08-3	0.29	1.00	1.00	1.00	0.29	0.71
OPB13-1	0.00	0.29	0.29	0.00	0.00	0.12
OPB13-2	0.00	0.29	0.29	0.00	0.29	0.17
OPB13-3	0.00	1.00	0.29	1.00	0.29	0.52
OPB13-4	0.00	0.29	0.29	0.00	0.00	0.12
OPB13-5	1.00	1.00	1.00	1.00	1.00	1.00
OPB13-6	1.00	1.00	1.00	1.00	0.29	0.86
OPB13-7	1.00	1.00	0.00	0.00	0.00	0.40
OPD07-1	1.00	1.00	1.00	1.00	1.00	1.00
OPD07-2	1.00	1.00	1.00	1.00	1.00	1.00
OPD07-3	1.00	1.00	1.00	0.00	0.29	0.66
OPF07-1	0.29	1.00	1.00	1.00	1.00	0.86

Locus	Thai roselle	Gene bank roselle	Dark red roselle	Light red roselle	White roselle	Overall
OPF07-2	1.00	1.00	1.00	1.00	1.00	1.00
OPF07-3	1.00	1.00	1.00	1.00	1.00	1.00
OPF07-4	0.29	1.00	1.00	1.00	1.00	0.86
OPF07-5	1.00	1.00	1.00	1.00	1.00	1.00
PAB04-1	1.00	1.00	1.00	1.00	1.00	1.00
PAB04-2	0.00	0.00	1.00	0.00	0.00	0.20
PAB04-3	0.00	0.00	0.00	1.00	0.00	0.20
PAB04-4	1.00	1.00	1.00	1.00	1.00	1.00
PAB04-5	1.00	1.00	1.00	1.00	1.00	1.00
PAB04-6	1.00	1.00	1.00	1.00	1.00	1.00
PAB04-7	1.00	1.00	1.00	1.00	1.00	1.00

Table 38: Genetic variation in 5 roselle genotypes

Germplasm	The number of polymorphic loci	The percentage of polymorphic loci	Nei’s Gene diversity	Shanon information index
Thai Roselle	4	8.33	0.035 ± 0.116	0.050 ± 0.169
Gene bank roselle	3	6.25	0.026 ± 0.101	0.038 ± 0.138
Dark red roselle	5	10.42	0.043 ± 0.138	0.063 ± 0.186
Light red roselle	2	4.17	0.017 ± 0.084	0.025 ± 0.122
White roselle	7	14.58	0.060 ± 0.148	0.088 ± 0.216

Table 39:Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) between roselle genotypes

Germplasm	Thai roselle	Gene bank roselle	Dark red roselle	Light red roselle	White roselle
Thai roselle	---	0.927	0.741	0.824	0.878
Gene bank roselle	0.076	---	0.794	0.895	0.901
Dark red roselle	0.300	0.231	---	0.723	0.746
Light Red rosellet	0.194	0.111	0.325	---	0.930
White roselle	0.130	0.104	0.293	0.073	---

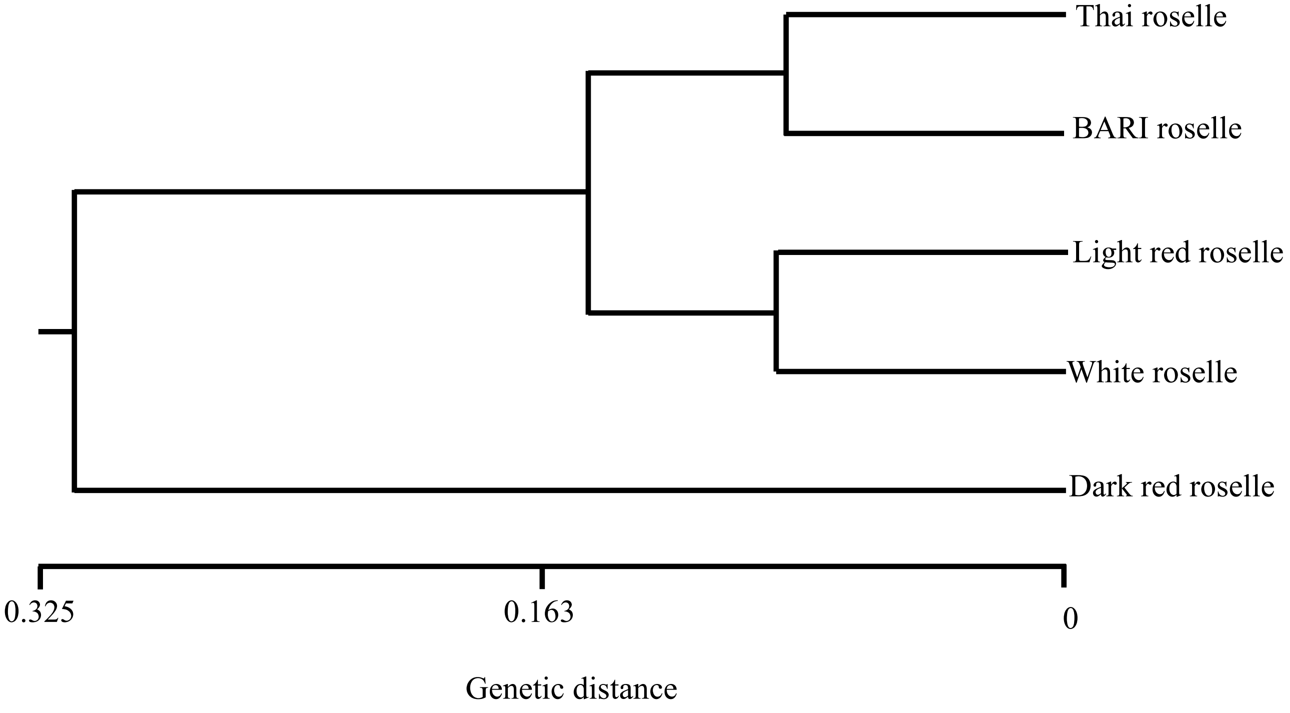


Figure 29. A UPGMA dendrogram constructed on the basis of Nei's (1978) genetic distance that summarizes genetic differentiation in roselle germplasm

Discussion

Morpho-molecular characterization of moringa germplasm

In morphological characteristics of moringa germplasm, significant difference was observed in leaf characters. Germplasm MO-016 showed the highest leaf length (60.13 cm). In the previous study, the leaf length of moringa was 44.3 cm (Shaltout *et al.,* 2017). Length of petiole, length of main, primary and secondary rachis of leaves, breadth of leaflets of moringa also showed significant variation among the germplasm. Color of flower was yellowish-white of the different germplasm whereas it was found white or cream colored in previous work (Morton, 1991).The length of pod in this experiment ranged from 25.5 to 45.5 whereas the pod length of the previous study rangedfrom 24.5 to 59.6cm (*Shaltout et al.,* 2017). The variation between different studies might be due to differences in germplasm used and environmental conditions.

In our study, 28 alleles ranging between 142-258 bp were amplified from 8 loci of moringa genome with an average of 3.5 alleles per locus. The average expected (*He*) and observed (*Ho)* heterozygosities were 0.558 and 0.181, respectively. Wu *et al.* (2010) found 32 alleles while working on the same 8 loci from*M. oleifera*and got 32 alleles having size range between 147 to 270 bp with an average of 4 alleles per locus. The average expected (*He*) and observed (*Ho)* heterozygosities were 0.5455 and 0.4562, respectively. Interestingly we found 4 private alleles from three germplasm. Private alleles can be used as diagnostic marker to identify particular germplasm. Allele $MO58_{186}$ was found for MO-05 germplasm and allele $MO45_{210}$ for MO-06 germplasm. There was only one genotype MO-019 which showed 2 private alleles at the loci of MO12 and MO41 of 258 bp and at 150 bp, respectively. Germplasm having private alleles can play important role in future study for moringa improvement.The overall genetic differentiation (*Fs:* 0.840) and gene flow (N_m: 0.048 reflected that there was a high level of genetic differences in 16 moringa germplasm. The UPGMA dendrogram based on Nei's (1972) genetic distance showed that MO-006 and MO-007 were grouped into one cluster due to their similarity in genetic information and lower genetic distance. Other 17 germplasm formed second cluster. The germplasm pairs MO-001 and MO-002, MO-003 and MO-004, MO-009 and MO-012, MO-010 and MO-011, MO-013 and MO-014, MO-015 and MO-018, MO-008 and MO-019 showed lower genetic distance between them. Higher level of genetic distance was found in MO-006 and MO-018 (2.70) and thus they were found in different clusters. The polymorphism detected among the germplasm could be used in breeding program to maximize the use of genetic resources as well as to develop new moringa varieties. The germplasm pair showing higher genetic distance is very important for future breeding program as there prevails scope of improvement potentials.

Morpho-molecular characterization of pigeon pea germplasm

In morphological characteristics of 16 *C. cajan*germplasm, significant difference was observed in respect of terminal leaflet length, breadth and size in *C. cajan*. Two types of leaf shape were observed. 75% were oval and rest of 25% was lanceolate in shape. Like present study Oinam (2011) found oblong and ovate shaped leaflet of pigeonpea while worked on morphological study of pigeonpeawhich are very close to oval andlanceolate shape. In case of petal color, we found color variation in banner/ standard petal such as yellow, red, pale yellow, red & yellow mixed color. Significant differences were observed in pod color. Green, violet, black and green mixed, violet & green mixed color were observed in pod color and 75% pods were mixed color. Similarly, Oinam (2011) found color variation in 90% germplasm. Number of seeds per pod ranged from 4 to 5 with a majority of 5 seeds/ pod found in the germplasm whereas Oinam (2011) found it in a range of 2 to 4 seeds per pod with a majority of 3 seeds/ pod. Length of pod had significant different too. As there were significant variation in number of seed/ pod and pod length so there could be significant variation in pod weight. It was found while the weight of 5 pods was estimated. Here PP-10494 was the heaviest (11.60 g) and PP-7184 is the lightest (5.27 g). No variation was detected among the genotypes studied at the experiment in some morphological characteristics like type offlower (bisexual), flowering pattern (indeterminate), type of leaf (compound, trifoliate). Variation in these characteristics is unexpected because similarities in these characteristics define the same family, genus and species. In molecular characterization, the purpose was to detect the genetic diversity among 16 germplasm using neutral DNA marker. High genetic diversity was observed among the 16 pigeonpea germplasm using SSR markers. Overall polymorphism was 87.5%. Average observed heterozygosity was 0.48 among the 16 pigeon pea germplasm. Average genetic differentiation was 0.42 and gene flow (Nm) was low (0.30). These results indicate the high genetic variation among the studied 16 pigeon pea germplasm. We found high significant variation in all quantitative characters and most of the important qualitative characters. Variation found in the morphological studies correlated with the variation at DNA level. Some germplasm which showed different flower colors were found in different clusters in the genetic distance-based dendrogram of RAPD analysis. The polymorphism detected among the germplasm could be used in breeding program to maximize the use of genetic resources as well as to develop new pigeonpea varieties. The germplasm pairs which showing higher genetic distance could be considered as potential parents for future breeding program.

Morpho-molecular characterization of wild teasel gourd germplasm

Morphological characteristics of wild teasel gourd germplasm showed differences in leaf and size, shape and fruit shape and sizes. To detect the genetic diversity in four wild teasel gourd germplasm PCR based RAPD markers were used. A total number of bands scored 139 and out of them 62 was polymorphic which was generated from 22 primers out of these 28 primers. The overall percent polymorphic loci obtained in this study was 44.60 % which implies that there is a high level of polymorphism in studied wild teasel gourd genotypes. The average Nei's gene diversity for all loci was estimated 0.214. Toan *et al.* (2017) performed RAPD analysis of 20 wild teasel gourd genotypes using 10 primers. They found total 114 polymorphic bands. The percentage of polymorphic loci of their study was 90.4% which is higher than that of our study (44.60 %). These differences might be due to different experimental set up or the use of different primers and germplasm. Band sharing based inter germplasm similarity indices and pair wise Nei's (1978) genetic distance are important parameters in detecting variations in wild teasel gourd germplasm. The average highest polymorphism value was found in wild teasel gourd Thailand2 vs wild teasel gourd Dhamrai 2 (41.73%) as they were collected from different locations. The lowest polymorphism value was found in wild teasel gourd Thailand1 vs wild teasel gourd Thailand2(3.6%) and this might be due to the fact that they werecollected from same geographical location. The higher the genetic distance the lower the genetic similarity. The highest average pair-wise band sharing-based similarity value was found between wild teasel gourd Dhamrai1 and wild teasel gourd Dhamrai2 and between wild teasel gourd Thai1 and wild teasel gourd Thai 2while the lowest genetic distance was foundbetween wild teasel gourd Thai2 and wild teasel gourd Dhamrai2.The UPGMA Dendrogram showed that wild teasel gourd Thailand1 and wild teasel gourd Thailand2 showed similarity and also wild teasel gourd Dhamrai 1&2 showed similarity. These similarities might be due to the same origin. This suggests that geographical distances might led to genetic distance.. The polymorphism detected between genotypes could be used in breeding program to maximize the use of genetic resources as well as to develop wild teasel gourd varieties. The germplasm pairs which showed higher genetic distance could be used in future breeding program.

Morpho-molecular characterization of roselle germplasm

A total of 5 roselle germplasm were collected. From morphological analysis significant level of variation was detected in weight of fruits, number of epicalyx, weight of calyx and epicalyx, and length of leaves between roselle genotypes. RAPD analysis found moderate level of genetic variation between roselle genotypes. Overall polymorphism considering all loci and genotypeswas 54.17% reflecting that some degree of genetic varation exists among the studied roselle genotypes. The polymorphism in the studied roselle genotypes was higher than the findings of Abou-Ellail et al. (2014). Interestingly, like Abou-Ellail, genotype specific alleles were found in our study. For instance, four BAU roselle-1 specific alleles (e.g., A01-1, A01-2, N05-3 and PAB04-2) and one BAU roselle-2 specific allele (PAB04-3) were detecetd in the present study. These genotype specific markers along with genotype pairs having higher level of genetic distances could be a potential genetic germplasmthat could be used for the improvement of the roselle in the country.

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12. Research highlight/findings (Bullet point – max 10 nos.):

a) A total of 19 moringa, 16 pigeon pea, 5 roselle and4 wild teasel gourd germplasm collected;
b) Morphological differences in 19 moringa germplasm documented;
c) Variation between 19 moringa germplasm at 8 SSR loci of their genome determined;
d) Morphological differences in 16 pigeon pea genotypes documented;
e) Variation at 8 SSR loci in 16 pigeon pea determined;
f) Morphological differences in 4 wild teasel gourd genotypes documented;
g) Variation at 62 RAPD loci between 4 wild teasel gourd genotypes determined;
h) Morphological differences in 5 roselle genotypes documented;
i) Genetic variation in 5 roselle genotypes at 21 RAPD loci estimated; and
j) Some genotype specific alleles or diagnostic alleles for moringa, pigeon pea, wild teasel gourd and roselle identified.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment Laptop	1	60,000	1	59,900	
(b) Lab &field equipment Minispin centrifuge machine	1	1,90,000	1	1,90,000	

(c) Other capital items					

2. Establishment/renovation facilities: Not applicable

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: Not applicable

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Figures in Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	385387	385387	385387	0	100	
B. Field research/lab expenses and supplies	618782	618412	613586	4826	99.22	GoB fund unspent
C. Operating expenses	88600	88529	87865	664	99.25	GoB fund unspent
D. Vehicle hire and fuel, oil & maintenance	44055	44055	44055	0	100	
E. Training/workshop/seminar etc.	0	0	0	0		
F. Publications and printing	75000	24701	0	24701	0	Payment pending for thesis printing
G. Miscellaneous	38276	38276	38276	0	100	
H. Capital expenses	249900	249900	249900	0	100	
Bank charge =	-	-	3095	-3095		
Total Taka =	1500000	1449260	1422164	27096	98.13	Payment for thesis printing pending and some unspent GoB fund

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output(i.e. product obtained, visible, measurable)**	**Outcome(short term effect of the research)**
➢ Collection and conservation of important underutilized	➢ Staff recruitment ➢ Procurement of capital expenses, field & lab supplies ➢ Germplasm collection ➢ Collection of relevant	➢ 19 moringa, 16 pigeon pea, 4 wild teasel gourd and 5 roselle germplasms collected and conserved at BAU-GPC. ➢ Phenotypic variation of 19	➢ Collection and conservation of 19 moringa, 16 pigeon pea, 4 wild teasel gourd and 5 roselle

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
➢ Documentation of their phenotypic characteristics vegetables ➢ Documentation of their molecular characteristics using DNA markers	information from farmers about germplasm ➢ Germplasms conservation ➢ Estimation of intraspecific phenotypic variations ➢ Selection and evaluation of important phenotypic traits of germplasms ➢ Estimation of intraspecific genotypic variation ➢ Genomic DNA isolation from germplasms ➢ Confirmation and quantification of DNA ➢ Amplification and documentation of DNA markers ➢ Data analysis and interpretation ➢ Preparation and submission of reports	moringa, 16 pigeon pea, 4 wild teasel gourd and 5 roselle using different descriptors determined ➢ Molecular characterization of 19 moringa germplasm at 8 SSR loci, 16 pigeon pea at 8 SSR loci, 4 wild teasel gourd germplasm at 139 RAPD loci and 5 roselle germplasm at 48 RAPD loci documented ➢ Three MS student trained on molecular biology research ➢ Molecular biology lab of the Department of Biotechnology, BAU improved	germplasms ➢ Genetic variation in collected moringa, pigeon pea, wild teasel gourd and roselle germplasms determined ➢ Three students completed MS degrees and produced 3 theses ➢ Research facility at the Department of Biotechnology, BAU improved

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication	Under preparation		4 research articles
Information development			
Other publications, if any ➢ Three MS Theses ➢ Abstract		Completed	Thesis titles 1. Morpho-molecular characterization of drumstick (*Moringa oleifera*) 2. Morpho-molecular characterization of the underutilized vegetable, pigeon pea (*Cajanus cajan* L. Millsp.) 3. Morpho-molecular chracterization of wild teasel gourd (*Momordica cochinchinensis*) **Project findings presented in BAURES workshop 2019** Title: Morpho-molecular characterization of underutilized indigenous vegetables in Bangladesh

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

DNA markers are effective for characterization of indigenous minor vegetables

ii. Generation of new knowledge that help in developing more technology in future

Wide range of genetic variation found in germplasm of moringa, pigeon pea, wild teasel gourd and roselle. Information will help to develop new varieties

iii. Technology transferred that help increased agricultural productivity and farmers' income

There is scope for improving minor indigenous vegetables and have potentials to raise vegetable production and livelihoods followed by up scaling

iv. Policy Support

As minor vegetables are potential for raising vegetable production and livelihoods, so up scaling activities may be undertaken

G. Information regarding Desk and Field Monitoring

i) **Desk Monitoring[description & output of consultation meeting, monitoring workshops/seminars etc.):**
A training workshop on ‘implementation procedure of CRG sub-projec’ was attended on the 14 June 2017 at BARC. The purpose of the training was to familiarize the recipients of the CRG grant on the implementation procedure related to technical, financial and procurement aspects.The Principal Investigators of the CRG sub-project were oriented on the procedural details to be followed in execution of the project activities.Fruitful discussion about project management includingprocurement plan for successful implementation was made. A day long research review workshop was attended on the 21 December 2017 at BARC. Project implementation progress workshop was held on the 28 February 2018. In that workshop, project implementation progress was presented successfully. A monitoring workshop on the 15 May 2018 at BARC was attended. In that workshop, research progress was presented successfully. In annual workshop on the 10 March 2019, projectactivities were presented. Molecular part of the project presented in that workshop was incomplete. As per feedback, molecular part of the project has been completed successfully and included in the project completion report.

ii) **Field Monitoring (time& No. of visit, Team visit and output):**
Dr. Mian Sayeed Hassan, Director, PIU, NATP-2, BARC and Dr. Md. Abdul Jalil Bhuyan, Research Management Specialist, PIU, NATP-2, BARC visited project site and laboratory on 7-8 March 2018 at BAU. On the 7 March, project progress was presentedsuccessfully. Monitoring team then visited research field in the BAU-GPC and laboratory in the Department of Biotechnology, BAU. Monitoring team was satisfied by observing research progress.

H. Lesson Learned/Challenges (if any)

i) Huge work load within the stipulated time

ii) Collection of germplasm particularly wild teasel gourd and roselle was difficult

I. Challenges (if any)

Release of fund delayed

Signature of the Principal Investigator

Date:7 May 2019

Seal

Counter signature of the Head of the organization/authorized representative

Date: 7May 2019

Seal