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Estimation of genetic diversity, structure and trait association of winged bean (*Psophocarpus tetragonolobus* (L.) DC.), genotypes through AFLP and ITS markers

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The genetic diversity among ninety five accessions of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) belonging to six countries of African and Asiatic origin was detected by employing amplified fragment length polymorphism (AFLP) and internal transcribed spacer (ITS) of nuclear ribosomal DNA markers. A medium to low-level of genetic diversity exists among the accessions of *P. tetragonolobus*. Association between the AFLP markers and flower, pod and seed traits: days to 50% flowering (DFW), pod length (PDL), pod width (PDW), green pod length (GPL), number of pods per plant (PDSP), number of seeds per pod (SDPD), 100 seed weight (SWT) and seed-oil content (SOC) was carried out by employing the mixed linear model (MLM) based kinship matrix (K-model). Traits like SOC and PDW were found to be strongly associated with AFLP markers. Population structure analysis among the genotypes identified five discrete sub-populations among the studied African and Asiatic lines of *P. tetragonolobus*.

Keywords: Winged bean, *Psophocarpus tetragonolobus*, AFLP markers, ITS, genetic diversity

Introduction

The genus *Psophocarpus* comprises nine species. Nur Fatihah *et al* (2012)[1] placed these nine species into three sub genera. The sub-genera *Psophocarpus* consists of two sections namely section *Psophocarpus* and section *Vignopsis*. Section *Vignopsis* constitutes *Psophocarpus lancifolius* and *Psophocarpus lukafuensis* and the section *Psophocarpus* consists of *Psophocarpus palustris*, *Psophocarpus tetragonolobus* and *Psophocarpus scandens*. Pickersgill (1980)[2] investigated the karyotype morphology of the chromosomes of *P. tetragonolobus* and *P. scandens* and their behavior at meiosis. Both of the species have a common chromosome number of (2n = 2x = 18). Now, this crop *P. tetragonolobus* is gaining importance in terms of product formulation[3-4]. Various schemes are also underway to bring this tropical underutilized legume to the mainstream cultivation and sustainable utilization practices. *P. tetragonolobus*, with an estimated genome size of 1.22 Gbp[5] is now included for various studies related to marker development and transcriptome analysis[6]. Detailed karyotyping of *P. tetragonolobus* was carried out on the basis of chromosome lengths by Chaowen *et al* (2004)[7]. Prasanth and Kumary (2014)[8] estimated the variability, heritability of pod yield and related attributes of twenty one genotypes of *P. tetragonolobus*. The analysis of variance reported significant difference among all the genotypes for yield and associated traits. Mohanty *et al* (2013)[9] conducted the preliminary study of assessing the genetic diversity among the African and Asiatic accessions of winged bean by employing ISSR and RAPD markers and proposed the superiority of the ISSR marker over RAPD markers in the analysis. Chen *et al* (2015)[10] employed the ISSR markers to assess the genetic diversity among forty five *P. tetragonolobus* accessions and reported a narrow genetic diversity. SSR molecular markers have been developed among the various genotypes of *P. tetragonolobus* to detect the level of genetic polymorphism[11-12]. But, all these have their own limitations in terms of number of genotypes and developed markers. Still more efficient

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and reproducible fine molecular markers needed for assessing large number of polymorphic loci in *P. tetragonolobus*. The AFLP method is considered as a highly informative molecular marker for detecting genetic diversity, estimating phylogenetic relationships and identifying variations with high resolution. AFLP markers have been broadly used to characterize the natural populations because of its genome coverage and bi-allelic nature[13]. AFLP markers are considered to be widely distributed across genome and thus are a useful marker-system for genome-wide scanning studies. So, the present study aims at exploring and utilizing the AFLP markers to estimate the genetic diversity among the *P. teragonolobus* accessions from different countries of Africa and Asia and exploring their association with specific flower, pod and seed traits like: days to fifty percent flowering (DFW), pod length (PDL), pod width (PDW), green pod length (GPL), number of pods per plant (PDSP), number of seeds per pod (SDPD), 100 seed weight (SWT) and seed-oil content (SOC).

Materials and Methods

Plant Materials

Ninety-five accessions of *Psophocarpus tetragonolobus* were obtained from ICAR-National Bureau of Plant Genetic Resources (NBPGR), Akola, Maharashtra, India. The list of the accessions are presented in Table 1. These accessions were grown and maintained in the botanic garden of CSIR-National Botanical Research Institute, Lucknow, India for further utilization. These accessions had originated from countries like: Ghana, Papua New Guinea, India, Indonesia, Philippines and Thailand.

Acquisition of Phenotypic Data

Seven phenotypic traits of the 95 accessions of *P. tetragonolobus* were recorded for three consecutive cropping seasons and the mean value is represented in Figure 1. These plants were grown in an augmented design with 3.0 metre row length with spacing of 90 cm between rows and 30 cm between the plants. Standard method of cultivation and agronomic practices were followed. The descriptors prepared by International Board for Plant Genetic Resources (IBPGR) were used for evaluation of the selected phenotypic traits. Observations on seven quantitative traits viz. days to 50% flowering (number of days from sowing to 50% flower opening), pod length (measured in centimeters as a mean of 10 dry pods), pod width (mm), days to maturity, green pod weight (g), number of pods per plant (measured as a mean of 10 dry pods), number of seeds per pod (measured as a mean seeds of 10 dry pods) and 100 seed weight (g) were recorded manually as per the standard guidelines mentioned in (www.biodiversityinternational.org).

Seed-Oil Estimation

The estimation of seed-oil content of 95 accessions of *P. tetragonolobus* was carried out by TD-NMR (Bruker-make). TD-NMR is a non-destructive method for oil-content determination of the seeds. The experiment was designed by selecting randomly five seeds (~ 5 g) from each accession. The oil-content of all accessions was determined in the same way and was repeated thrice. The mean value was reported for further analysis.

DNA Extraction

Total genomic DNA from all the 95 accessions were extracted from fresh, young leaves of *P. tetragonolobus* separately following DNeasy plant mini kit (Qiagen). The quality and quantity of the DNA was checked on 1% agarose gel and nanodrop at $OD_{260/280}$ respectively.

AFLP Analysis

AFLP templates were prepared by standard method followed by Vos *et al* (1995)[14] with slight modification. The control module provided by the AFLP kit (AFLP® plant mapping kit, Applied Biosystems) was used to check the experimental quality. About 3 µg of genomic DNA was subjected to digest with 20U *Eco*RI and 10U *Mse*I restriction enzymes (New England Biolabs, USA) for 3 h at 37°C and then deactivated at 70°C for 15 min. Adapters (*Eco*RI (5 pmol) and *Mse*I (25 pmol)] were ligated to the restricted-DNA fragment in ligation buffer (1X T4 DNA ligase buffer), 0.5 M NaCl, 5 µl BSA (1 mg/ml), 0.25 U (0.25 µl) of T4 DNA ligase and incubated at 37°C for 2 h. The restriction-ligated DNA was then diluted to 20-fold with TE buffer (20 mM Tris-HCl, 0.1 mM EDTA at pH 8.0) and performed pre-selective amplification with primer (complimentary to the *Eco*RI and *Mse*I adapters with one selective nucleotide at the end) in the thermal cycler (BioRad). The amplification was confirmed by running the PCR-product on 1.5% agarose gel.

The pre-selective amplified products were further diluted to 10-fold with TE buffer and was used as template for selective amplification. The amplification was carried out with three selective primer-pairs in

Table 1 — List of winged bean accessions with their country of origin

S. No.	Code	NBPGR code	Origin	Oil content (%)
1	NBPT01	IC 26942	India	16.64
2	NBPT02	IC 26944	India	17.15
3	NBPT03	IC 26944-1	India	19.06
4	NBPT04	IC 26945	India	19.67
5	NBPT05	IC 26946	India	23.02
6	NBPT06	IC 26949	India	15.66
7	NBPT07	IC 26949-1	India	18.15
8	NBPT08	IC 31981	India	20.31
9	NBPT09	IC 34861	India	21.93
10	NBPT10	IC 41980	India	16.77
11	NBPT11	IC 95228	India	25.73
12	NBPT12	IC 95229	India	17.25
13	NBPT13	IC 95230	India	19.97
14	NBPT14	IC 95231	India	20.18
15	NBPT15	IC 95232	India	16.07
16	NBPT16	IC 95235	India	16.29
17	NBPT17	IC 95236	India	18.32
18	NBPT18	IC 95237	India	13.48
19	NBPT19	IC 95237-1	India	16.24
20	NBPT20	IC 95238	India	17.96
21	NBPT21	IC 95239	India	15.26
22	NBPT22	IC 95241	India	17.05
23	NBPT23	IC 95242	India	15.16
24	NBPT24	IC 112416	India	19.98
25	NBPT25	EC 11885	India	22.10
26	NBPT26	EC 21904	Ghana	17.02
27	NBPT27	EC 27885-1	Ghana	15.81
28	NBPT28	EC 27885-2	Ghana	14.23
29	NBPT29	EC 27886-A	Ghana	19.60
30	NBPT30	EC 38154	Papua New Guinea	18.73
31	NBPT31	EC 38821	Papua New Guinea	14.89
32	NBPT32	EC 38821 PA	Papua New Guinea	17.54
33	NBPT33	EC 38821P-1	Papua New Guinea	18.75
34	NBPT34	EC 38821 P2-1	Papua New Guinea	16.37
35	NBPT35	EC 38821-B	Papua New Guinea	15.60
36	NBPT36	EC 38821-1	Papua New Guinea	15.56
37	NBPT37	EC 38821-2	Papua New Guinea	16.16
38	NBPT38	EC 38824-2	Papua New Guinea	20.53
39	NBPT39	EC 38825	Papua New Guinea	16.16
40	NBPT40	EC 38954-A	Papua New Guinea	13.70
41	NBPT41	EC 38955-B	Papua New Guinea	12.71
42	NBPT42	EC 38956	Papua New Guinea	9.52
43	NBPT43	EC 38956-1	Papua New Guinea	12.98
44	NBPT44	EC 38956-2	Papua New Guinea	12.82
45	NBPT45	EC 38956-3	Papua New Guinea	11.91
46	NBPT46	EC 38957	Papua New Guinea	10.38
47	NBPT47	EC 38957-A	Papua New Guinea	14.24
48	NBPT48	EC 38957-B	Papua New Guinea	13.69
49	NBPT49	EC 38958	Papua New Guinea	14.85
50	NBPT50	EC 38959	Papua New Guinea	12.98
51	NBPT51	EC 130184	Indonesia	15.06
52	NBPT52	EC 130184-2	Indonesia	13.58

(*Contd*.)

Table 1 — List of winged bean accessions with their country of origin (Contd.)

S. No.	Code	NBPGR code	Origin	Oil content (%)
53	NBPT53	EC 142600	Philippines	13.67
54	NBPT54	EC 178267	Thailand	18.72
55	NBPT55	EC 178268	Thailand	11.80
56	NBPT56	EC 178269	Thailand	13.30
57	NBPT57	EC 178271	Thailand	14.59
58	NBPT58	EC 178272	Thailand	14.08
59	NBPT59	EC 178274	Thailand	12.24
60	NBPT60	EC 178275-1	Thailand	17.25
61	NBPT61	EC 178277	Thailand	10.72
62	NBPT62	EC 178278	Thailand	11.86
63	NBPT63	EC 178279	Thailand	14.91
64	NBPT64	EC 178282	Thailand	13.57
65	NBPT65	EC 178283	Thailand	12.41
66	NBPT66	EC 178284	Thailand	15.30
67	NBPT67	EC 178286	Thailand	13.58
68	NBPT68	EC 178287	Thailand	10.46
69	NBPT69	EC 178288	Thailand	13.79
70	NBPT70	EC 178289	Thailand	10.55
71	NBPT71	EC 178289-1	Thailand	11.55
72	NBPT72	EC 178291	Thailand	12.10
73	NBPT73	EC 178292	Thailand	11.62
74	NBPT74	EC 178293	Thailand	11.78
75	NBPT75	EC 178295	Thailand	14.98
76	NBPT76	EC 178296	Thailand	11.20
77	NBPT77	EC 178297	Thailand	11.57
78	NBPT78	EC 178298	Thailand	13.40
79	NBPT79	EC 178299	Thailand	11.01
80	NBPT80	EC 178301	Thailand	13.13
81	NBPT81	EC 178302	Thailand	15.31
82	NBPT82	EC 178303	Thailand	18.02
83	NBPT83	EC 178304	Thailand	14.30
84	NBPT84	EC 178305	Thailand	16.39
85	NBPT85	EC 178306	Thailand	21.95
86	NBPT86	EC 178307	Thailand	13.21
87	NBPT87	EC 178308	Thailand	20.04
88	NBPT88	EC 178309	Thailand	15.87
89	NBPT89	EC 178310	Thailand	15.29
90	NBPT90	EC 178311	Thailand	13.61
91	NBPT91	EC 178312	Thailand	16.42
92	NBPT92	EC 178313	Thailand	18.00
93	NBPT93	EC 178314	Thailand	20.24
94	NBPT94	EC 178318	Thailand	16.57
95	NBPT95	EC 178319	Thailand	16.58

total volume of 10 µl. The PCR products of the selective amplifications were separated by ABI 3730 XL DNA analyzer and detected by fluorescence as the *Eco*RI site-specific primers were labeled with yellow (NED), blue (FAM) or green (JOE) fluorescent dyes. An internal size marker, Genescan ROX-500 of (35-500) bp was added allowing the co-loading of three different reactions. The criteria for selection of primers were to use the primer combinations that produce the maximum number of markers. The *Eco*RI selected primers were: *Eco*RI+ACA, *Eco*RI+ACG, *Eco*RI+ACT, *Eco*RI+AGG and *Mse*I selected primers with *Mse*I+CAA, *Mse*I+CTT, *Mse*I+CAA, *Mse*I+CTG (Table 2). Data from the selectively amplified DNA fragments were collected and Gene Mapper program was used to evaluate the results (threshold 200 relative fluorescence unit (rfu) for quantification and detection of fragments as followed by Gupta *et al* (2013)[15].

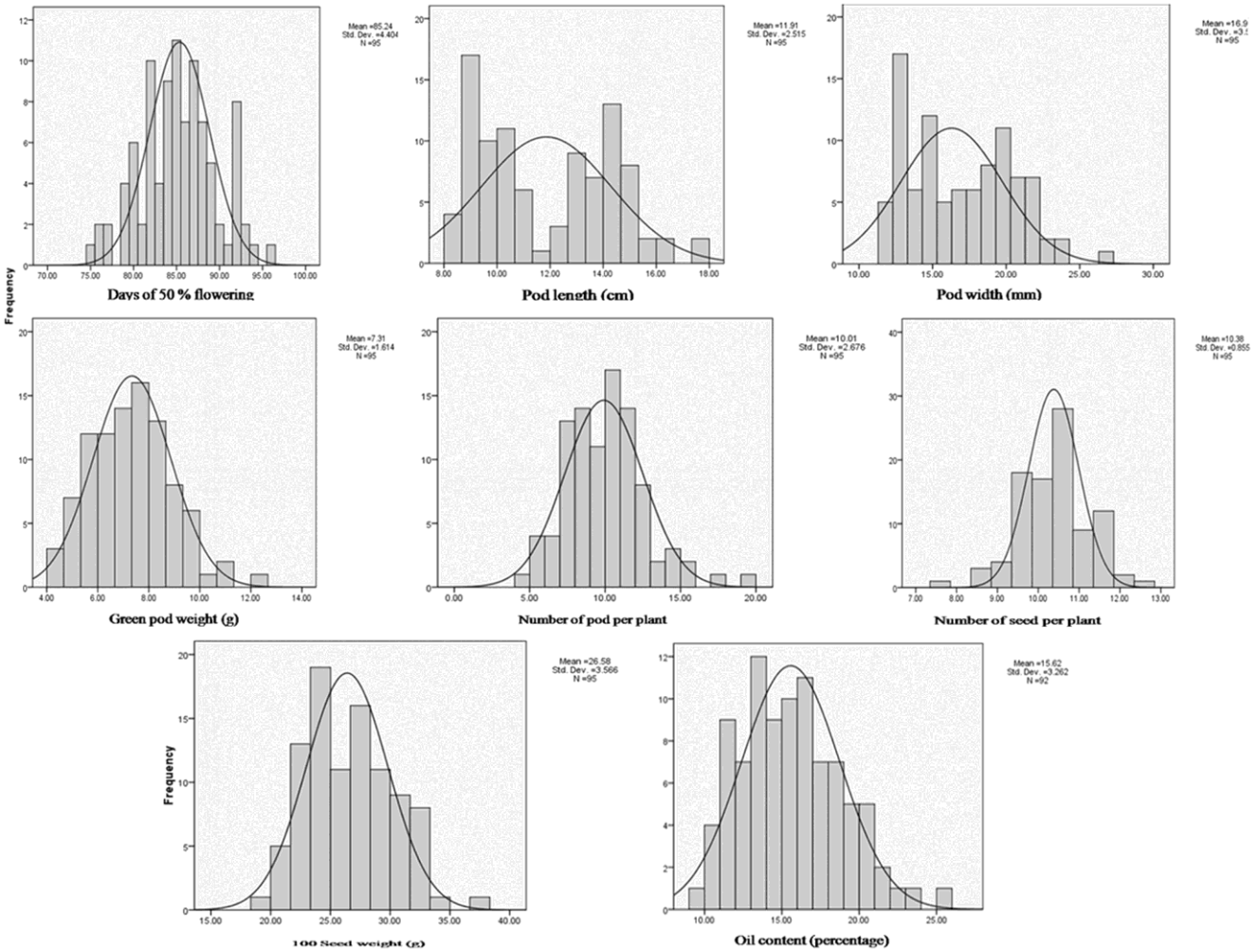

Fig. 1 — Frequency distribution curves on different phenotypic variation of 95 winged bean accessions from Africa and Asia.

Table 2 — Primer combinations used in AFLP and nrITS analysis winged bean accessions from Asia and Africa

S. No.	Primer combinations	Total fragments	Polymorphic fragments
1	E-ACA/M-CAA	87	72
2	E-ACG/M-CTT	9	9
3	E-ACT/M-CAA	96	87
4	E-AGG/M-CTG	13	13
5	Total	205	181
6	Minimum	9	9
7	Maximum	96	96
	nrITS	Primers	Tm (^{0}C)
8	ITS4	TCCTCCGCT TAT TGA TAT GC	56
9	ITS5	TCC GTA GGT GAA CCT GCG G	56

(E= *Eco*RI, M=*Mse*I)

AFLP Data Analysis

The AFLP data was digitally scored (0/1) on GeneMapper with threshold value of 200 relative fluorescence unit (rfu). The genetic similarity (GS) between the pairs of accessions was calculated through Jaccard's similarity coefficient by employing DARwin ver.5.0 software[16]. The generated similarity matrix was used to construct dendrogram as unweighted pair group method with arithmetic average (UPGMA). Model-based clustering program, structure ver.2.2[17] was employed for estimating the number of sub-populations in the *P. tetragonolobus* accessions. The membership of each accession was tested for the range from K = 2 to K = 10. Each run consisted of 1,00,000 burn-in periods and 2,00,000 iterations. Three independent runs were assessed for each fixed K value[18].

ITS Amplification, Sequencing and Analysis

The nuclear ribosomal ITS of *P. tetragonolobus* was amplified using universal primer (TCCTCCGCT TATTGATATGC) and (TCCGTAGGTGAACCTGC GG)[19]. The ITS-PCR products were electrophoresed (0.8% agarose gel). The amplified bands were excised and purified using Nucleospin Kit (Machery-Nagel). The purified DNA was quantified using nanodrop spectrophotometre and was sequenced through Applied Biosystems Automated Sequencer (Model 3730 ver. 3.1). The generated sequences were aligned using Clustal W programme[20]. Further sequence alignment was carried using MEGA6 software[21].

Marker-Trait Association

A total of 181 AFLP markers were used to study the marker-trait associations for eight traits like: (DFW, PDL, PDW, GPL, PDSP, SDPD, SWT and SOC) as mentioned in the earlier section. Frequency distribution, including mean and standard deviation for each of the above eight traits were analyzed using SPSS ver.17.0. The analysis was also used to calculate associations among individual markers for each of the eight traits by employing the mixed linear model (MLM) based on the kinship matrix (K-model). The kinship-matrix was generated by TASSEL ver. 3.0 (http://www.maizegenetics.net) through conversion of the distance matrix from TASSEL's cladogram to a similarity matrix, the options of Efficient Mixed Model Association or (EMMA) were chosen for Mixed Linear Model (MLM)[22]. Significance of marker-trait associations was determined at $p \leq 0.05$.

Results

Eight quantitative traits namely: DFW, PDL, PDW, GPL, PDSP, SDPD, SWT and SOC revealed significant variability among the accessions. Frequency distribution with mean and standard deviation for each of the eight traits were represented in Figure 1. High coefficient of variation was observed for number of pods per plant (25.97%) followed by green pod weight (22.02%) and pod length (21.07%). The value was calculated by (SD/Mean) × 100. From the studied accessions more desirable traits identified were higher number of pods per plant in NBPT23, green pod weight in NBPT05, pod length in NBPT01, early flowering in NBPT23 and late flowering in NBPT03.

Among the 64 sets of combinations of *Eco*RI-*Mse*I primers with three nucleotides extension, four most polymorphic combinations were selected for analyzing 95 accessions of *P. tetragonolobus* (25 accessions from India, 42 from Thailand, 21 from Papua New Guinea, 4 from Ghana, 2 from Indonesia and 1 from the Philippines) (Table 1). Each primer set generated (9-87) polymorphic fragments. A total of 181 polymorphic fragments among 205 readable bands were detected by four primer sets. The minimum and maximum percentages of polymorphisms were detected by the primer combinations *Eco*RI-ACT/*Mse*I-ACC and *Eco*RI-ACA/*Mse*I-CAA respectively (Table 2). Pair-wise comparison of genetic similarity among these accessions estimated to be ranged from 0.17 to 0.99. Maximum genetic similarity was reported between the Indian accessions NBPT11 and NBPT10 and the minimum genetic similarity was reported between Papua New Guinea accessions NBPT32, NBPT49 with Indian accessions NBPT04, NBPT11 and NBPT17. Maximum genetic similarity was noticed between accessions NBPT54 (Thailand) with NBPT53 (Philippines) and NBPT55 (Thailand). Accessions from Papua New Guinea had the maximum genetic similarity with NBPT57, NBPT65 and NBPT66, NBPT60 accessions of Thailand.

Cluster Analysis

Cluster analysis of 95 *P. tertragonolobus* accessions provided distinct groupings. The analysis based on Jaccard's genetic distance had grouped the 95 accessions into four clusters with three relatively big clusters (I, II and IV) (Fig. 2). Cluster III is the smallest one accommodating only 12 accessions and majority of the accessions were from Thailand. Only one accession from India (NBPT16) is accommodated in this cluster. Two of the Indonesian accessions grouped together in cluster II. Majority of Indian accessions were grouped in cluster I and IV.

ITS-Sequencing

The length of nuclear-ITS region ranged from 316 bp (NBPT70) to 698 bp (NBPT79). The ITS sequence was rich in GC content which ranged from 52.7% (NBPT8) to 51.0% (NBPT46). The nucleotide frequencies were found to be A (22.6%), C (24.3%), G (27.7%) and T (25.4%). However, the ITS of *P. tetragonolobus* is rich in GC-content (51.8%) (Table 3).

Analysis of Population Structure

The model-based population structure analysis by using STRUCTURE program grouped all the 95 accessions into 5 clusters/ sub-populations (Fig. 3). The result of STRUCTURE provided the number of

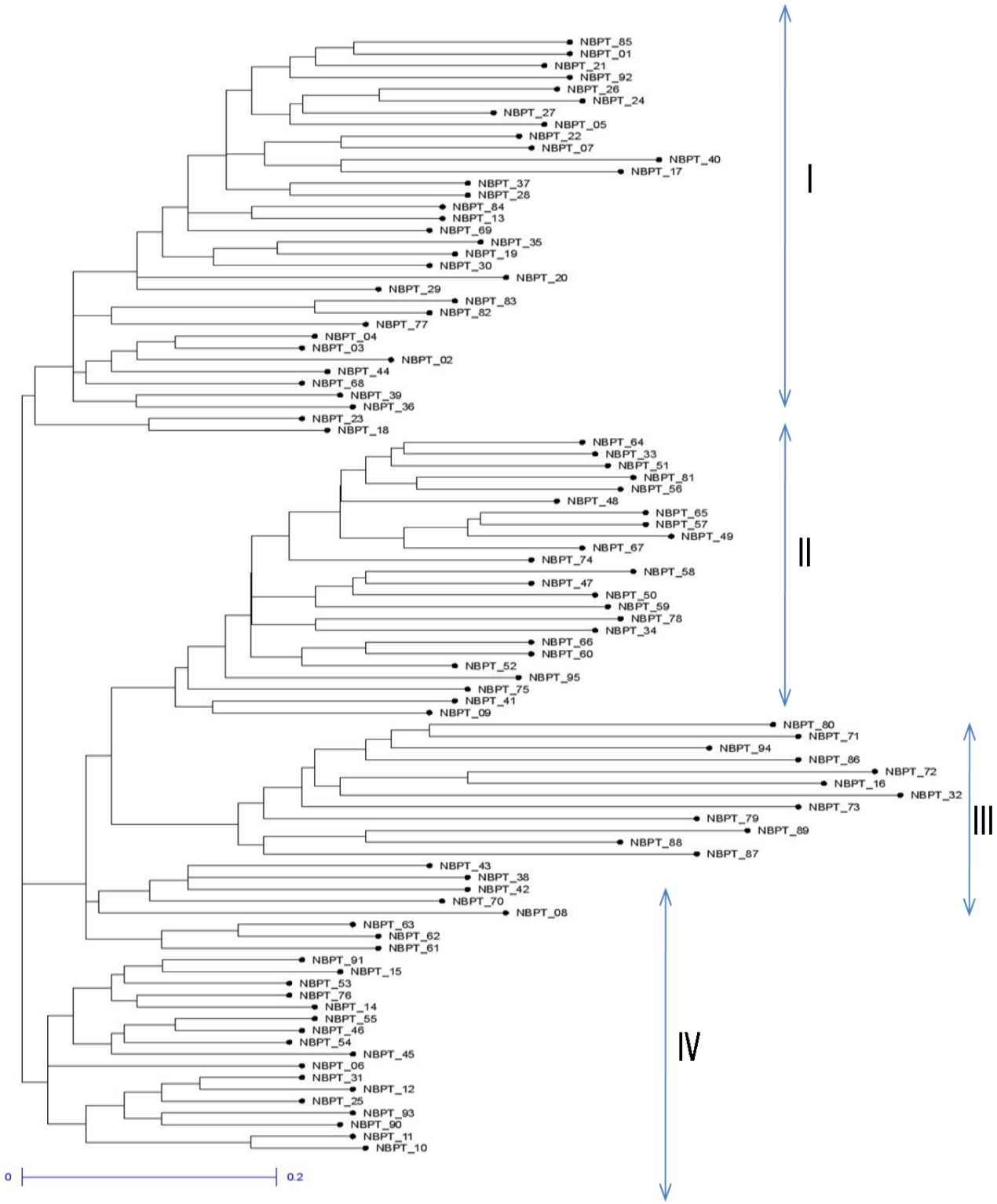

Fig. 2 — UPGMA dendrogram based on Jaccard's coefficient illustrating the genetic similarities and distance among African and Asiatic accessions of *P. tetragonolobus.*

clusters (K) that maximize the ΔK parameter was K=5. All the studied accessions could be split into K=5 groups as per the method of Evanno *et al* (Fig. 4). The five clusters formed in the analysis also genetically paired with different countries. Group I consisted 8 accessions mostly from India and Thailand. Group II and V represented accessions from Indonesia along with accessions from Ghana, India, Philippines and Thailand. Group III included maximum number of accessions from India, Papua New Guinea, Thailand, Philippines except Indonesia.

Marker-Trait Associations (MTAs)

Results of significant MTAs involved in each of the eight different traits were summarized in Table 4. Altogether 35 MTAs were identified involving 26 AFLP markers. A maximum seven AFLP markers were associated with oil content and minimum two AFLP markers were associated with pod-width (Table 4). Out of the 26 AFLP markers, 19 AFLP markers were involved in trait-specific MTAs and seven AFLP markers were involved in multi-trait MTAs which were found associated with up to three traits.

Table 3 — Results of nrITS sequence analysis of *P. tetragonolobus*

Parameters	ITS
Length range	(316 - 698) bp
G + C content range	(52.7 - 51.0)%
G + C content mean	51.8%
Nucleotide frequencies of	
Adenine (A)	22.6%
Thymine (T)	25.4%
Cytosine (C)	24.3%
Guanine (G)	27.7%

Discussion

Mohanty *et al* (2013) estimated the genetic diversity among 24 accessions of *P. tetragonolobus* by employing ISSR and RAPD markers. The comparison of ISSR and RAPD markers indicated the suitability of ISSR markers over RAPD markers

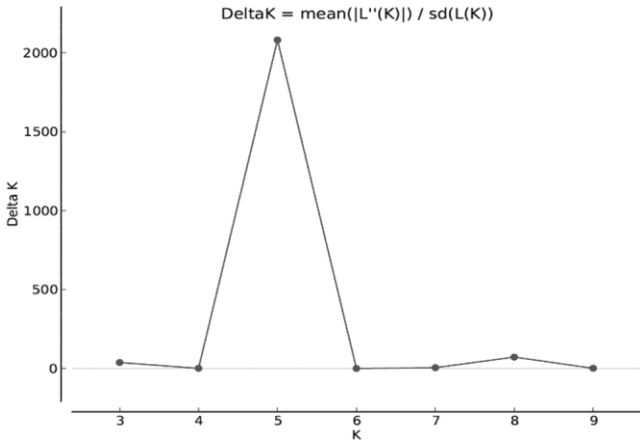

Fig. 4 — Maximum likelihood curve in the genetic analysis of 95 accessions of *P. tetragonolobus.*

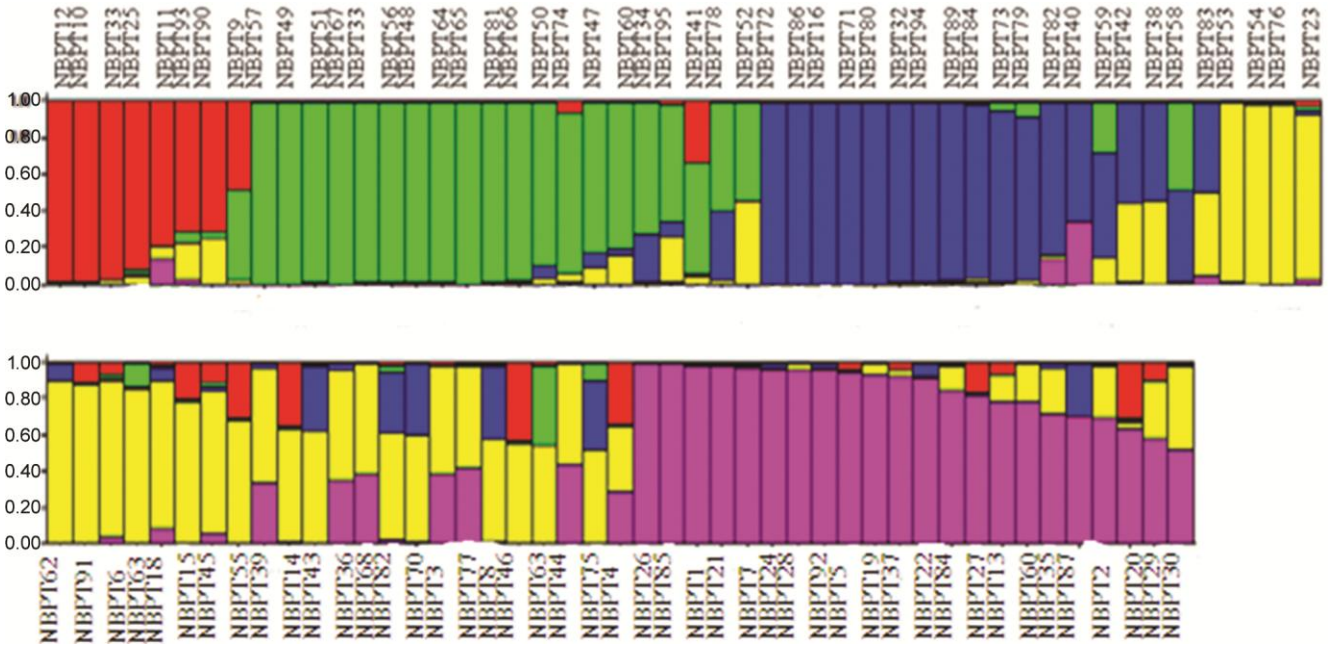

Fig. 3 — Ninety five *P. tetragonolobus* accessions belonging to five sub-populations as calculated by STRUCTURE software.

Table 4 — Marker-trait association ($p \leq 0.05$) for eight important traits of winged bean based on AFLPs and phenotypical characterizations of 95 germplasm accessions from Asia and Africa

AFLP marker	Oil content (%)	Days to 50% flowering	Pod length (cm)	Pod width (mm)	Green pod weight (g)	No of pods per plant	No of seeds per pod	100 Seed weight (g)
a10	-	-	-	-	-	-	-	0.04 (4.63)
a100	-	0.02 (5.84)	-	-	-	-	-	-
a104	0.04 (4.98)	-	-	-	-	-	-	-
a108	-	-	-	-	-	-	0.05 (4.52)	-
a109	0.02 (5.75)	-	-	-	-	-	-	-
a124	-	-	0.03 (5.14)	-	-	0.02 (6.52)	-	-
a125	-	-	-	-	-	0.04 (4.49)	-	-
a137	-	-	-	0.04 (4.67)	-	-	-	-
a140	-	-	-	-	-	-	0.03 (5.68)	-
a156	0.03 (5.14)	-	-	-	-	0.03 (5.09)	-	-
a174	-	-	-	-	0.05 (4.36)	-	-	0.02 (5.75)
a180	-	-	-	-	0.00 (11.54)	-	-	0.00 (9.25)
a38	-	-	-	-	-	0.04 (4.62)	0.04 (4.82)	-
a42	0.01 (8.04)	-	-	-	-	-	-	-
a43	-	0.01 (7.23)	-	-	-	-	-	-
a52	0.04 (4.71)	-	-	-	-	-	-	-
a57	-	-	-	-	-	-	-	0.04 (4.84)
a63	-	-	0.01 (7.51)	0.05 (4.34)	-	-	0.04 (4.94)	-
a65	0.04 (4.54)	-	0.01 (7.07)	-	-	-	0.01 (7.53)	-
a7	-	-	-	-	-	-	-	0.01 (7.34)
a80	0.03 (5.54)	-	-	-	-	-	-	-
a84	-	-	-	-	-	0.01 (6.61)	-	-
a86	-	-	-	-	0.02 (5.62)	-	-	-
a87	-	-	-	-	-	0.03 (5.31)	-	-
a90	-	0.02 (6.42)	-	-	-	-	-	-
a92	-	-	-	-	0.02 (6.22)	-	-	-

R square values are given in parentheses

in elucidating the genetic diversity among the *P. tetragonolobus* accessions. Using only ISSR markers, Chen *et al* (2015) estimated the genetic distances among 45 accessions of *P. tetragonolobus*. Their result revealed that the germplasm had a narrow-level of genetic diversity with genetic dissimilarity coefficients ranging from 0.73 to 0.97. The present investigation by AFLP markers detected the genetic similarity to range between 0.17 to 0.99 among the African and Asiatic accessions of winged bean. The maximum genetic similarity among various Indian accessions indicated a probable common ancestral origin. The AFLP primer combinations used in the present study produced DNA profile with an average of 45.2 (92.8%) polymorphic fragments per primer combination. The fragment distribution pattern among the studied accessions showed that the primer combination *Eco*RI-ACT/*Mse*I-CAA generates maximum number of fragments (96) as well as maximum polymorphic fragments (87) followed by *Eco*RI-ACA/*Mse*I-CAA. The primer combination *Eco*RI-ACG/*Mse*I-CTT produces least number of fragments (9) (Table 2). Some primer combinations which produced unique fragments in specific accessions now used to develop sequence tagged site (STS) marker and the similar fragments sharing ones for evolutionary studies.

Genetic Distances and Clustering

Understanding of clustering pattern and estimation of genetic distance among the accessions and between the pair of accessions could be useful in developing mapping populations[23]. As per the Jaccard's genetic distance matrix, highest genetic distance was found to be in between two accessions NBPT10 (India) and NBPT85 (Thailand). Based on the genetic distance and utilization of these lines NBPT10 (India-origin) and NBPT85 (Thailand-origin) may further be used for marker assisted selection and breeding programs. The cluster analysis grouped 95 *P. teragonolobus* accessions into four clusters. Accessions from different geographical origins clustered into different

sub-clusters and no clear pattern of groupings were observed. There exists contrasting opinions regarding the geographic origin of *P. tetragonolobus*[24]. Some botanists[25-26] pointed to Papua New Guinea to be the centre of origin of this plant, whereas, Vavilov (1951)[27] and Burkill (1935)[28] pointed for its Indian and African origin, respectively. The distribution of *P. tetragonolobus* showed that India is in the western limit of natural range of *P. tetragonolobus* and Papua New Guinea is its eastern-most border. In this range, *P. tetragonolobus* grows naturally in Sri Lanka, Bangladesh, Burma, Indonesia, Malaysia, Thailand and Philippines. However, the present estimation of genetic diversity could not able to dissect the origin of these plants on the basis of their origin. This may be due to a common stock of their origin. However, the synonymous trivial name Goa bean to winged bean might have certain-level of significance to the practice of domestication and cultivation of this plant in the Goa province of India.

Model-based clustering of these accessions also suggests that, the estimation of allelic frequencies categorizes the accessions into 5 discrete sub-populations which has a unique set of allelic frequencies. The pair-wise comparisons of genetic similarity among these accessions revealed the existence of a moderate to low level of genetic diversity among the African and Asiatic accessions.

ITS Characterization

The length of ITS sequence of winged bean ranged from 316 bp to 698 bp. Several length-variations in ITS were also reported in several angiosperms[29]. The region is rich in GC-content (51.8%) (Table 3). The higher GC-content of nr-ITS region was also reported in members of Poaceae and among the arid plants from temperate regions[30].

Association Analysis

The phenotypic attributes like: SOC, DFW, PDL, PDW, GPL, PDSP, SDPD, SWT showed strong correlation with the selective markers (Table 3). This may provide a useful target for future breeding programs[31]. Although the marker-trait association identified during the present study provided only the preliminary information but estimated molecular diversity, genomic characterization carried out in the present study will be very useful for selecting the appropriate accessions for further improvement programs.

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Conflict of interest

The authors have no conflict of interest.

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