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Short communication

Population structure analysis and determination of neurotoxin content in a set of grass pea (*Lathyrus sativus* L.) accessions of Bangladesh origin

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ABSTRACT

Grass pea (*Lathyrus sativus* L.) is a crop that is considered one of the more resilient to climate change. With protein-rich seeds and leaves, it has strong potential as human food as well as animal feed and fodder. However, genetic improvement in this crop remains stagnant owing to the poor characterization of its genetic resources. In this study, we characterized 118 accessions of grass pea with 18 EST-SSR markers. A total of 118 accessions, 101 of *L. sativus* (100 cultivated accessions from Bangladesh and one wild accession) and 17 wild accessions of other *Lathyrus* species, were used. A total of 67 alleles were detected, with an average of 3.72 alleles per locus and average polymorphism information content of 0.52. A dissimilarity matrix was formed and hierarchical cluster analysis performed using the UPGMA method grouped genotypes into four main clusters. Cluster analysis based on the genetic dissimilarity revealed a clear grouping of the 100 cultivated and 18 wild accessions into four main groups. Group I consisted of 20 accessions with high β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) concentration. Of these 20 accessions, 17 were wild accessions. Only one wild accession (*L. cicera*) was clustered in group II, which contained 35 accessions. Most of the group II accessions contained low β-ODAP. Group III was represented by 34 accessions, many of them with high β-ODAP. Group IV consisted of 29 accessions, a few of which had very high β-ODAP concentrations. Analysis of molecular variance of the microsatellite data showed significantly higher values of molecular variance between (83%) than within (17%) populations. These characterized accessions will be useful in grass pea breeding programs.

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1. Introduction

Grass pea (*Lathyrus sativus* L.) is an excellent candidate food crop to provide protein, micronutrients and pre-biotics for human diets and animal feed. Globally, 1.2 Mt is produced from a ~1.5-Mha area [1]. India is the largest producer with 384,800 t, followed by Bangladesh (232,500 t) and Ethiopia (202,126 t). Belonging to the plant family of Fabaceae, the genus *Lathyrus* contains 160 species, with taxonomic relationships detailed by Patto Vaz and Rubiales [2]. Efforts are being made in many countries including Australia, Spain, Italy, and Canada to expand its cultivation as a break crop between cereals and as a bonus crop in fallow land because of its biological nitrogen fixation ability [3,4]. Grass pea contains a neurotoxic non-protein amino acid, β-N-oxalyl-L-α,β diaminopropionic acid (β-ODAP), in health-endangering concentrations, and applied breeding is necessary to reduce the concentration. The narrow range of genetic variation and lack of genomic tools for this crop species has slowed progress in such efforts [5–8]. To make directed improvement in grass pea for ODAP concentration, application of genomic tools such as molecular markers is important.

Considerable progress has been made in recent years in the development of genomic resources in other food legumes (chickpea, pigeonpea, lentil, field pea) as well as in model legumes (*Medicago truncatula* and *Lotus japonicus*) [8,9]. Against this progress, in grass pea only a few reports on genomic resource development are available [4,10–12] owing mainly to the large genome size and poorly characterized germplasm used for such studies. Molecular markers such as ISSR (inter simple sequence repeat), RAPD (random amplified polymorphic DNA), STS (sequence tagged site), RFLP (restriction fragment length polymorphism), and AFLP (amplified fragment length polymorphism) have been used to examine genetic variation and phylogenetic relationships in the genus *Lathyrus* [13–19]. Simple Sequence Repeat (SSR), or microsatellite, markers have emerged as the breeders' choice for marker-assisted selection because of locus specificity, multiple allelism, co-dominance, and high repeatability [20]. The non-availability of species-specific primers limits their use for genetic diversity studies. In *Lathyrus*, only 15 SSR primers have been reported, by Lioi et al. [4] and Shiferaw et al. [10]. Recently [21], 300 expressed sequence tag-simple sequence repeat (EST–SSR) primer pairs were developed and characterized for size polymorphism among 24 grass pea accessions. Among them only 44 EST-SSRs were polymorphic. Lioi and Galasso [22] reported enriching the EMBL-ENA (European Molecular Biology Laboratory-European Nucleotide Archive) database with 119 new SSR containing sequences. Among 10 SSRs tested, seven primer pairs produced clearly distinguishable DNA banding patterns. Successively, SSR primer pairs were successfully tested to identify polymorphism in a set of four different grass pea germplasm accessions. The transferability of SSR markers was high among three related species of *Lathyrus*, namely *L. cicero*, *L. ochrus*, and *L. tingitanus*, and the legume species *Pisum sativum*. Yang et al. [11], using 454 FLX Titanium pyrosequencing, identified 651,827 SSR loci in grass pea. Among 50,144 primer pairs designed, 288 were tested in a set of 24 grass pea accessions (23 of *L. sativus* and one *L. cicera*). Of these, 74 pairs were polymorphic. Very recently, Hao et al. [23] developed a set of polymorphic EST-SSR markers and a panel of SNP derived kompetitive allele-specific PCR (KASP) markers in *Lathyrus*. Gene molecular markers have been used very little in genetic diversity studies in *Lathyrus* and there is a need to use them routinely to characterize *Lathyrus* accessions. In the present study, we used 18 previously reported EST-SSR markers to characterize 100 cultivated and 18 wild accessions of the genus *Lathyrus*.

2. Materials and methods

2.1. Plant materials

A total of 118 accessions, 101 of *L. sativus* (100 cultivated accessions from Bangladesh and one wild accession) and 17 wild accessions of other *Lathyrus* species, were used. In total, these accessions represented 17 species of the genus *Lathyrus*. Details of the accessions are presented in Table 1.

2.2. DNA extraction

Young leaves were harvested from two-week old seedlings. DNA extraction was performed with the modified CTAB (cetyltrimethylammonium bromide) method [24]. Quality and quantity of the isolated DNA were determined on 1.2% (w/v) agarose gel by comparison of bands to those of a known concentration of lambda DNA.

2.3. PCR reaction

SSR markers used in this study are listed in Table 2. PCR reactions were performed in total volumes of 10 μL, containing 1× PCR buffer (1.5 mmol L^{-1} $MgCl_2$), 200 μmol L^{-1} of each dNTP, 10 pmol of each primer, 0.5 U of *Taq* DNA polymerase and approximately 50 ng of genomic DNA. Amplification was performed in a master cycler with an initial denaturation for 5 min at 94 °C, then 35 cycles including 30 s of denaturation at 94 °C, 30 s of annealing at 59 °C, and a 45-s extension at 72 °C. Final extension was performed at 72 °C for 5 min. Amplified products were separated on 6% (w/v) polyacrylamide gels. Gels were stained with ethidium bromide and photographed under ultraviolet light using a gel-documentation system. The size of each band was estimated simultaneously against a 100-bp DNA ladder [21].

2.4. Statistical analysis

Polymorphism information content (PIC) and major allele frequency were calculated for all of the EST-SSRs using PowerMarker 3.25 [25]. The number of polymorphic EST-SSRs and polymorphism rate were also computed for the wild and cultivated germplasm. The unweighted pair-group method with arithmetic mean (UPGMA) method of clustering [26] was applied to the marker data for cluster analysis using DARwin 5.0.158 [27]. The dissimilarity matrix obtained for the germplasm was considered as an indicator of the diversity of the genetic base.

Table 1 – Details of the *Lathyrus* accessions used in the study.

Genotypes	Accession number	IG number	ODAP concentration (% weight)	ODAP concentration
Cultivated types				
1	Bang-1	114481	0.25	Low
2	Bang-2	114487	0.53	High
3	Bang-6	114495	0.27	Low
4	Bang-8	114505	0.30	Low
5	Bang-9	114506	0.34	Low
6	Bang-11	114509	0.32	Low
7	Bang-12	114510	0.52	High
8	Bang-14	114514	0.25	Low
9	Bang-16	114585	0.25	Low
10	Bang-34	116678	0.45	Low
11	Bang-35	116681	0.40	Low
12	Bang-39	116689	0.36	Low
13	Bang-40	116701	0.21	Low
14	Bang-42	116703	0.18	Low
15	Bang-48	116720	0.35	Low
16	Bang-51	116726	0.25	Low
17	Bang-61	116750	0.34	Low
18	Bang-62	116753	0.22	Low
19	Bang-67	116773	0.18	Low
20	Bang-70	116780	0.25	Low
21	Bang-71	116783	0.27	Low
22	Bang-75	116820	0.22	Low
23	Bang-77	116822	0.35	Low
24	Bang-78	116823	0.28	Low
25	Bang-79	116824	0.28	Low
26	Bang-81	116826	0.20	Low
27	Bang-86	116838	0.51	High
28	Bang-87	116839	0.51	High
29	Bang-89	116845	0.37	Low
30	Bang-90	116849	0.24	Low
31	Bang-95	116859	0.32	Low
32	Bang-96	116860	0.28	Low
33	Bang-101	116867	0.22	Low
34	Bang-103	116872	0.21	Low
35	Bang-106	116880	0.51	High
36	Bang-110	116892	0.25	Low
37	Bang-131	116963	0.26	Low
38	Bang-132	116964	0.41	Low
39	Bang-133	116965	0.34	Low
40	Bang-135	116974	0.46	High
41	Bang-136	116977	0.23	Low
42	Bang-140	116890	0.29	Low
43	Bang-144	116999	0.19	Low
44	Bang-148	117030	0.18	Low
45	Bang-149	117031	0.27	Low
46	Bang-150	117049	0.23	Low
47	Bang-151	117062	0.29	Low
48	Bang-152	117063	0.27	Low
49	Bang-154	117067	0.28	Low
50	Bang-155	117068	0.23	Low
51	Bang-156	117072	0.24	Low
52	Bang-157	117075	0.17	Low
53	Bang-158	117076	0.56	High
54	Bang-161	117098	0.20	Low
55	Bang-162	117099	0.48	High
56	Bang-168	117112	0.24	Low
57	Bang-171	117130	0.27	Low
58	Bang-173	117136	0.46	High
59	Bang-174	117138	0.25	Low
60	Bang-181	117200	0.20	Low
61	Bang-189	117249	0.28	Low
62	Bang-190	117250	0.20	Low

(continued on next page)

Table 1
(*continued*)

Genotypes	Accession number	IG number	ODAP concentration (% weight)	ODAP concentration
63	Bang-192	117257	0.20	Low
64	Bang-193	117261	0.23	Low
65	Bang-194	117264	0.32	Low
66	Bang-197	117271	0.36	Low
67	Bang-199	117278	0.13	Low
68	Bang-200	117280	0.47	High
69	Bang-201	117281	0.31	Low
70	Bang-203	117288	0.21	Low
71	Bang-208	117313	0.34	Low
72	Bang-211	117317	0.48	High
73	Bang-212	117319	0.36	Low
74	Bang-214	117326	0.20	Low
75	Bang-215	117332	0.14	Low
76	Bang-226	117361	0.38	Low
77	Bang-227	117362	0.23	Low
78	Bang-231	117372	0.37	Low
79	Bang-235	117381	0.43	Low
80	Bang-238	117389	0.51	High
81	Bang-244	117403	0.39	Low
82	Bang-246	117405	0.41	Low
83	Bang-251	117412	0.19	Low
84	Bang-252	117413	0.29	Low
85	Bang-253	117414	0.21	Low
86	Bang-254	117422	0.42	Low
87	Bang-257	117430	0.23	Low
88	Bang-258	117432	0.30	Low
89	Bang-259	117433	0.57	High
90	Bang-274	117471	0.36	Low
91	Bang-276	117474	0.28	Low
92	Bang-279	117484	0.20	Low
93	Bang-287	117511	0.19	Low
94	Bang-288	117519	0.32	Low
95	Bang-291	117528	0.28	Low
96	Bang-292	117529	0.29	Low
97	Bang-301	117552	0.22	Low
98	Bang-305	117558	0.29	Low
99	Bang-307	117580	0.29	Low
100	Bang-311	117595	0.26	Low
Range			0.13–0.57	
Mean			0.30	
SD			0.10	
Wild types				
101	*L. annuus*	IGWG01		
102	*L. sativus*	IGWG02		
103	*L. clymenum*	IGWG03		
104	*L. sylvestris*	IGWG04		
105	*L. pratensis*	IGWG05		
106	*L. latifolius*	IGWG06		
107	*L. japonicas*	IGWG07		
108	*L. hirsutus*	IGWG08		
109	*L. tuberosus*	IGWG09		
110	*L. inconspicuous*	IGWG10		
111	*L. cicera*	IGWG11		
112	*L. pratensis*	IGWG12		
113	*L. gorgoni*	IGWG13		
114	*L. ochrus*	IGWG14		
115	*L. hierosolymitanus*	IGWG15		
116	*L. boissieri*	IGWG16		
117	*L. chloranthus*	IGWG17		
118	*L. cirrhosis*	IGWG18		

IG numbers are Indigenous Germplasm numbers as maintained in the ICARDA Gene Bank. ODAP denotes β-*N*-oxalyl-L-α,β diaminopropionic acid (β-ODAP). Low ODAP denotes lower ODAP concentration (less than 0.5%) and *vice versa*.

An analysis of molecular variance (AMOVA) was performed using GenAlEx 6.1 [28]. Pairwise population comparisons were made in AMOVA using Genalex 6.1 based on 999 permutations. Variance analysis was performed on three levels: within populations and among populations based on the genotyping data of the 118 *Lathyrus* accessions.

2.5. Determination of ODAP concentration

A field experiment involving 100 grass pea accessions from Bangladesh was grown in in 2013 in Morocco following an alpha lattice design with two replications. These were resolvable incomplete block designs with the number of entries a multiple of the block size [29]. The field experiment had 10 blocks, each with 10 genotypes, thus accommodating all 100 genotypes. The experiment was planted under late-sowing conditions in February to expose the genotypes to terminal water and heat stress during flowering and pod filling stages. For all seed samples, ODAP was determined spectrophotometrically [30]. Means and variances were calculated for seed ODAP content of the tested genotypes.

3. Results

3.1. Molecular analysis of genetic diversity

A set of 100 grass pea accessions from Bangladesh were compared with 18 wild *Lathyrus* accessions using 18 genic SSR markers (Table 1). A total of 67 unambiguous bands were detected, from 16 polymorphic primers (two primer pairs amplified monomorphic sequences). The average major allele frequency was maximum for accession S-103 (0.78) and minimum for S-33 (0.26), while for S-231 and S-177 it was 0.77 and 0.67, respectively (Table 3). Average PIC ranged from 0.30 (S-103) to 0.75 (S-33), while S-6 and S-117 recorded 0.72 and 0.65, respectively (Table 3). Alleles amplified per locus ranged between two to six (Table 3).

Table 2 – Details of 18 EST–SSR markers selected for genotyping (Sun et al. [21]).

Locus	Primer sequence (5′ to 3′)	Repeat motif	T_a (°C)	Expected size (bp)	Putative function of locus
S5	F: TGTGGGGCTTGTTACACTGA R: AGCTACCATAACAGACAAAACC	$(GT)_8$	58	205	Gonadotropin beta chain
S6	F: CTGCAACAAGAAGCCATTCA R: TATGGGTCCGTCGGAATAAC	$(CT)_9$	58	183	Conserved hypothetical protein
S33	F: TGGTTTTGTTGTGGAAAGTGAG R: ACTGCAAAAGCCTCAAAGGA	$(TTTTG)_3$	58	210	Conserved hypothetical protein
S52	F: CGCCCCTCATCTTATCATTC R: GTTGTTGGGTGAAGGAATCG	$(CTCGCT)_3$	58	204	Inositol phosphate kinase
S70	F: GTGCAACCTTTCATCAATCA R: CGGTGAAGCTAAAGAAGAAGAA	$(GGTTT)_3$	58	250	bZIP transcription factor bZIP11
S102	F: GTTGGGAATCCGTCTTCAAA R: GTCGAGAGAGGTGAGGTTGG	$(CGACGG)_3$	57	207	Leucine-rich repeat, cysteine-containing
S103	F: CCTCCGACATGTCCATCATT R: TCAGCATTGTGTTGGTCGAT	$(CAA)_5$	58	202	Cysteine protease, putative
S114	F: CTAGCATCCCGCCATTTATC R: AACATTTCAGCCCTGACCAC	$(CCT)_5$	57	200	GTP-binding signal recognition particle SRP54, G-domain
S117	F: CATTATCTTCCCCAGCTCCA R: AAGGGATGAGCCACAGAATG	$(CCTCCA)_3$	56	184	Glutathione peroxidase, putative
S137	F: GAGATCATCTTTGTCGGTGGA R: TTCAGTAGGACCCAGCAACC	$(ATTTCC)_3$	58	224	Glycoside transferase, six-hairpin, subgroup
S155	F: CGACGATCTACAACCACCAA R: TGGAAGGAGGGAAGAGAGGT	$(CAC)_6$	55	211	Serine/threonine protein kinase, active site
S159	F: CGATCTCCCACTTTAGAAAACA R: GGTGGCTATAACCAATGATGG	$(AAAC)_4$	56	242	Putative plasma membrane intrinsic protein
S163	F: ACACAGAAACACCACCACCA R: GATTTTCCGTAACGGCTTGA	$(CAT)_5$	57	223	AT2G42690
S165	F: CGCTCTAGAGATAGGAGAGAGAGG R: GCTTCACAAGAACCCATTTCA	$(GA)_8$	58	239	Nucleic acid binding protein, putative
S177	F: GGGAGTGAATCAAAACCAACA R: GAAGTTCTCCGTAGCGGTGT	$(AAAG)_4$	56	245	Gamma-glutamylcysteine synthetase precursor
S194	F: AAGGTTCAACCATTCGCTGT R: CTAGGCCATTCAGCTTCTGG	$(CTT)_5$	57	205	Glutamyl-tRNA reductase precursor
S220	F: AGCTCTTTCTTCCACCACCA R: CAGGTTCCAGCTGAGAGGAG	$(CAGCAA)_3$	57	616	DNA-binding protein, putative
S231	F: GTTAGAAACTCCGGCGAGGT R: CGACCAGACTCCATTTTTCAA	$(GAATTA)_3$	57	618	Transcription factor homebox

3.2. Dissimilarity index and cluster analysis

The genetic dissimilarity between different pairs of genotypes was highest (0.89) between genotypes Bang-194 (IG117264) and Bang-157 (IG117075). The next most dissimilar genotypes were Bang-9 (IG114506), Bang-244 (IG117403), Bang-193 (IG117261), *L. gorgoni*, Bang-42 (IG116703), and *L. chloranthus* at 0.86. Cluster analysis based on the genetic dissimilarity index grouped the 101 cultivated and 17 wild accessions into four main groups (Fig. 1). Group I consisted of 20 accessions with high β-ODAP concentration (dark green in Fig. 1). Of these 20 accessions, 17 were wild. Only one wild accession (*L. cicera*) clustered in group II, which contained 35 accessions in total (dark brown in Fig. 1). Most of the group II accessions had low β-ODAP. Group III (blue in Fig. 1) was represented by 34 accessions, most with high β-ODAP. Group IV (yellow in Fig. 1) consisted of 29 accessions; a few with very high β-ODAP concentrations.

3.3. Analysis of molecular variance

AMOVA (Table 4) was performed to test the significance of the partitioning of genetic variance between and within populations. AMOVA of the microsatellite data showed significantly higher values of molecular variance between (83%) than within populations (17%) (Table 4).

3.4. ODAP concentrations of 100 *Lathyrus* genotypes

Seed ODAP concentration ranged from 0.13%–0.57% with an average value of 0.30% (Table 1). Among the 100 genotypes tested (Table 1) only 14 genotypes had mean seed ODAP concentrations of 0.5% or higher (Bang-106, Bang-12, Bang-135, Bang-158, Bang-162, Bang-173, Bang-2, Bang-200, Bang-211, Bang-238, Bang-259, Bang-34, Bang-86, and Bang-87).

Table 3 – Diversity statistics for 18 EST-SSR markers studied in 118 grass pea accessions.

Marker	Major allele frequency	No. of alleles	Allele sizes [a]	PIC
S5	0.42	4	120, **160**, 180, 200	0.61
S6	0.36	5	**110**, **120**, 150, 160, 180,	0.72
S33	0.26	6	110, **120**, 150, **160**, 180, 200	0.75
S52	0.40	4	120, 150, **160**, 200	0.67
S70	0.59	2	**180**, 220	0.37
S102	0.58	2	**110**, 120	0.37
S103	0.78	3	**120**, 200	0.30
S114	0.44	4	**110**, **120**, 160, 200	0.54
S117	0.38	4	**110**, 120, 160, 180	0.65
S137	0.64	4	**110**, 120, 150, 200	0.43
S155	0.46	3	**110**, 120, 150	0.53
S159	0.42	4	110, 120, **150**, 180	0.62
S163	0.53	4	110, 120, **150**, 180	0.53
S165	0.56	3	110, 120, **180**	0.42
S177	0.67	3	**110**, 120, 200	0.42
S194	0.57	3	120, **150**, 180	0.51
S220	0.54	4	**120**, 150, 200, **430**	0.49
S231	0.77	5	120, **150**, 200, **330**, 430	0.35
Mean	0.52	3.7		0.51

[a] Major alleles are highlighted in bold.

4. Discussion

4.1. Utility of EST-SSRs in the genus *Lathyrus*

The genomic resources of *Lathyrus* have been little used to characterize genetic diversity [32] and most studies have not included SSR markers [13–19]. Knowledge of genetic diversity is critical for germplasm utilization in grass pea breeding to broaden the genetic base. Therefore, it was necessary to perform a more comprehensive genetic diversity and population structure analysis of *Lathyrus* populations having variable ODAP content.

Previously, SSR markers have been successfully used to study grass pea genotypes [31]. EST-SSR markers were limited to transferability studies across the legumes [31,32]. For example, Shiferaw et al. [10] and Soren et al. [12] used 21 and 35 EST-SSR markers for studying the genetic diversity among Ethiopian and Indian grass pea accessions, and observed 48.57% to 52.38% polymorphic markers, respectively. The PIC value in these studies ranged from 0.35 to 0.42. In our study, EST-SSR markers discriminated accessions belonging to cultivated species (from Bangladesh) from different wild species for which phenotypes for ODAP content were known. Also, number of alleles amplified and PIC value gave evidence for high genetic variation among the various accessions analyzed in this study (Table 3). The primers in the present study seem more efficient than above mentioned studies [13–19] based on their PIC values and number of alleles amplified per locus as well as the ability to discriminate the tested *Lathyrus* genotypes. This efficiency could be due to genetic distinction between cultivated and wild species.

4.2. Cluster analysis reveals relationships among accessions

The groups generated by cluster analysis were distinct, and the grouping of the cultivated accessions differed from that of the wild ones (Fig. 1). Group I consisted of all wild accessions except one *L. cicera* accession. This grouping of *L. cicera* with cultivated types is consistent with its hypothesized [5] role as the progenitor of cultivated *L. sativus*. Group II genotypes included low ODAP-containing genotypes (Fig. 1). Groups III and IV included mostly high ODAP-containing accessions with some exceptions (Fig. 1).

4.3. AMOVA discriminates between- and within-population diversity

The high diversity of these test accessions is also reflected in the results of AMOVA (Table 4). AMOVA revealed low but significant genetic differentiation among cultivated and wild

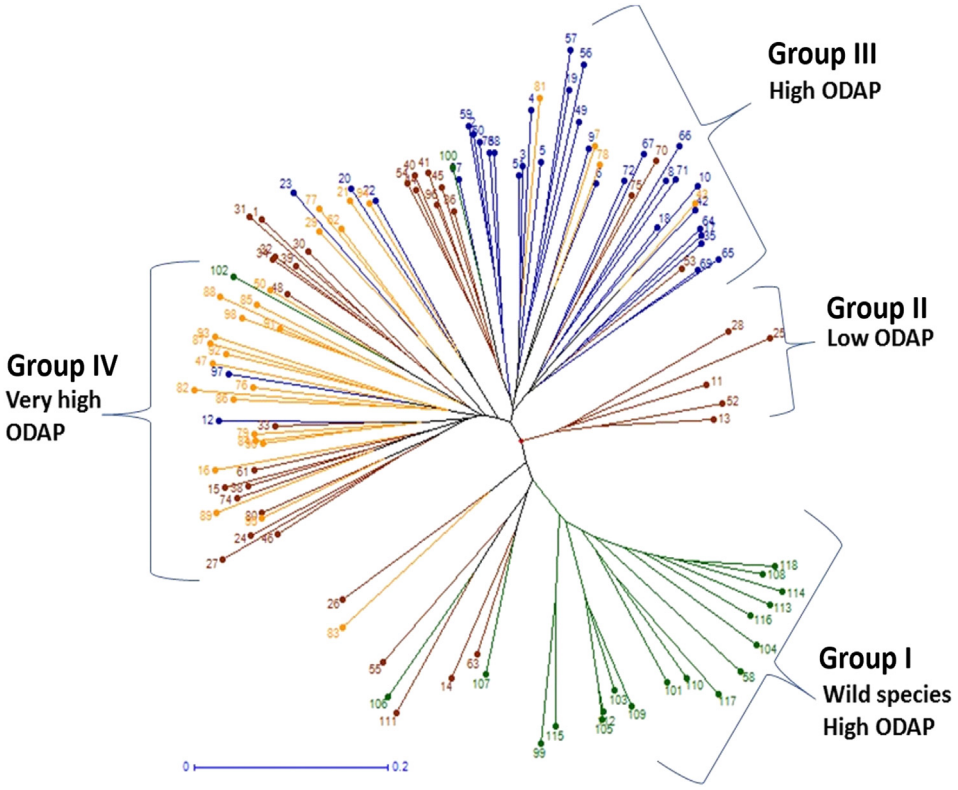

Fig. 1 – Dendrogram depicting the genetic relationships among 100 *L. sativus* accessions and 18 wild accessions based on genotyping with 18 EST-SSR markers. Accessions in group I are *dark green*, group II *dark brown*, group III *blue*, and group IV *yellow*. Details of the accessions are presented in Table 1.

accessions and high and significant diversity between accessions of grass pea accessions.

4.4. *Variation in ODAP concentration among 100 Lathyrus genotypes*

The ODAP concentration was low for the entire set of 100 genotypes, with only 14 genotypes having ODAP content of 0.5% or higher. Most traditional *Lathyrus* cultivars have 0.5%–2.5% ODAP content [1]. This set of genotypes could be an excellent source of alleles for grass pea breeding for low seed ODAP content. However this potential must be verified in multiyear and multilocation testing, given that ODAP concentration in *Lathyrus* seed is affected by environmental conditions [1]. In this experiment, owing to seed insufficiency, ODAP concentrations in wild *Lathyrus* species could not be determined.

Table 4 – Analysis of genetic differentiation among accessions of grass pea by AMOVA.

Source of variation	*df*	SS	MS	Estimated variance	%	P-value
Within populations	1	162	162	5	17	0.0001
Between populations	116	2601	22	22	83	0.0001
Total	117	2763	184	27	100	

5. Conclusions

The results of the present study will be useful in grass pea breeding programs, in particular for selection of parents to cross for high-yielding low-neurotoxin (ODAP) *Lathyrus* cultivars. Crossing between genetically distant accessions, both with low ODAP content, may produce high-yielding, low-ODAP segregants in early breeding generations. Further accessions can be introduced into the program after genotyping with the polymorphic set of markers described here, and still more polymorphic EST-SSRs can be used. Both more number of molecular markers and increased number of accessions' use will add more resolution while studying the genetic relationships among *Lathyrus* accessions.

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