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Short Communication

Genetic relationship among accessions of *Stylosanthes hamata* based on seed proteins profile

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For improvement of any plant character through hybridization, it is necessary to understand the genetic similarity/dissimilarity among the accessions. In the present study an attempt was made to determine the genetic relationship in 61 accessions *of S. hamata* using seed-SDS-proteins. The genus *Stylosanthes* (Fabaceae) consisting of approximately 40 diploid and polyploid species has been classified on the basis of morphological characteristics [1]. Five species of this genus, namely *Stylosanthes hamata, S. scabra, S. viscosa, S. guianensis* and *S. humilis* are widely used as tropical forage legumes. Among these, *S. scabra* and *S. hamata* are allotetraploids (2n = 4x = 40) and rest three species are diploid (2n = 2x = 20). These five species are predominantly self-pollinating with a low but variable degree of outcrossing. Close relationship between tetraploid *S. hamata* and *S. humilis* has been reported by Stace and Cameron [2]. Results of seed-protein analysis [3], rhizobial affinities [4] and morphological and agronomic characters [5] also yielded similar observations. Introduction of a new diploid species, *i.e.,* S. *seabrana,* in 1998 from Australia was visualized another potential species of stylo in India [6]. Among the species introduced in India from Australia and South America, *S. hamata* has been most commonly grown and naturalized but no emphasis has been given on characterization of the species and accessions in past. New accessions and species have been regularly introduced from other countries to fortify the germplasm holding as well as for their proper evaluation and utilization. In the work reported here, the genetic relationship among accessions *of S. hamata* has been studied based on seed-sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) protein profile, and grouping based on un-weighted pair group method with arithmetic mean algorithm (UPGMA) analysis has been made. Such information would be useful in systematic exploitation of the existing collections and identifying accessions of high divergence that could be used in future crossing programmes.

Electrophoresis analysis of seed proteins has been reported for identification of species and many cultivars [7, 8]. Robinson and Megarrity [3] have characterized 14 lines of *S. hamata* based on seed-protein patterns. Seeds of sixty-one accessions of *S. hamata,* as presented in Table 1 have been used in the present investigation. The genotypes were subjected to seed-SDS-protein analysis using vertical polyacrylamide gel electrophoresis system. Following the procedure of Gardiner et al. [9] seed-protein samples were prepared by grinding 40 mg pooled seed of each samples in 500 µl extraction buffer containing 0.50 M Tris-PO_4 (pH 6.8), 2% sodium-dodecyl sulphate (SDS), 20% glycerol, 0.008% bromophenol blue and 5% b-mercaptoethanol (added fresh). Discontinuous SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a system based on Laemmli [10] on a 12% resolving gel. The gel was stained with 0.02% Coomassie Blue R in 5% absolute ethanol, 6% TCA (Trichloric acetic acid) and 25% methanol in water. Only clear and unambiguous bands were recorded based on their molecular weight and the bands were numbered starting from the well, *i.e.,* the slowest band numbered as one. Binary data matrix was generated taking '1' for presence and '0' for absence of a band. The genetic similarity were estimated using Dice's similarity co-efficient and further analyzed by SAHN clustering and UPGMA analysis. The statistical calculations were made using NTSYS programme.

Table 1. Seed SDS-protein pattern in 61 accessions of S. *hamata;* +,++,+++ represents intensities of bands

Band no.	CPI-110123	Check	CPI-61670	IG-542	IG-525	IG-518	IG-59	1G-579	IG-55	IG-579	IG-552	IG-498	IG-586	IG-66	IG-13-4-A	IG-13-4B	IG-8-5	IG-49	IG-50	IG-580	IG-48	IG-540
1	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2	-	-	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	--	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	-	-	-	-	-	-	+	-	++	++	++	++	+	+	+	+	+	=	=	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++
7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+
13	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
16	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+	+	+	+	+
17	-	++	-	-	+	-	-	+	+++	+++	+++	+++	+	+	+	+	+	+	+	+	+	+
18	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+	+	+	+	+

Band no.	IG-71	IG-551	IG-581	IG-589	IG-584	IG-65	IG-543	IG-599	IG-62	IG-571	IG-519	IG-618	IG-7-4	IG-5-4	IG-6-7	IG-445	IG-570	IG-6-3	IG-528	CPI-11013	IG-73	IG-533
1	-	-	+	+	-	-	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+
2	-	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
3	-	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	+++
4	+	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++
5	-	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	+++
6	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
17	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
18	+	+	+	+	+	+	+	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	-

Band no.	IG-591	IG-69	IG-605	IG-560	IG-557	IG-565	IG-620	IG-612	IG-13-4	IG-13-13	IG-608	IG-526	1G-601	IG-595	IG-596	IG-64	IG-57
1	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
2	+	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+
3	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	++
4	++	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+
5	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	++
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The seed-protein banding patterns of 61 accessions of *S. hamata* revealed a total of 18 electrophoretic bands (Table 1) of which 5 bands [5-7, 15, 16] were common in all the accessions. The highest number of bands *i.e.,* 18 was observed in three accessions (IG-55, IG-552 and IG-498) and lowest number *i.e.,* 8 in three accessions namely IG-599, IG-7-4 and IG-560. The molecular weight marker was used to estimate the size of the bands. The number of intense bands (+++) varied from 1 to 4 in molecular weight range of 4000 to 97000 Daltons in different accessions (Table 1). The five prominent protein bands present in all 61 accessions were in the range of 14000 and 64000 Daltons. The variation in terms of absence or presence of bands was largely concentrated to the range of 14000 to 40000 Daltons.

The similarity matrix calculated using NTSYS programme showed 33 to 100% similarity among the accessions. The number of accessions showing 100% similarity with others was 53, indicating a very high level of similarity among the *S. hamata* accessions. However, SAHN clustering analysis revealed that these 53 accessions having 100% similarities to each other formed 12 groups and placed in between the rest of the accessions in dendrogram developed using NTSYS computer software (Fig. 1). Two major clusters were observed which further subdivided into sub-clusters. These two main clusters joined together at the similarity level of 50%. Cluster 1 embodied 23 accessions which further subdivided into two sub-clusters. Sub-cluster 1 possessed 22 accessions and joined with accession IG-71 at the similarity level of 82%. This indicated that IG-71 accession is the most diverse among the accessions of this cluster. Cluster 2 was further divided into three sub-clusters. In this cluster also one accession namely IG-551 placed alone and joined with the rest of the two sub-clusters at the similarity of 73% (Fig. 1). The two major sub-clusters of cluster 2 embodied highest number of accessions possessing 100% similarity among them. A group of 17 accessions having 100% similarity among them was flanked by other two small groups having 2 and 3 accessions of 100% similarity. Accession IG-64 joined alone with this cluster and showed 93% similarity. The other sub-cluster of cluster 2 possessed two small groups of more than two

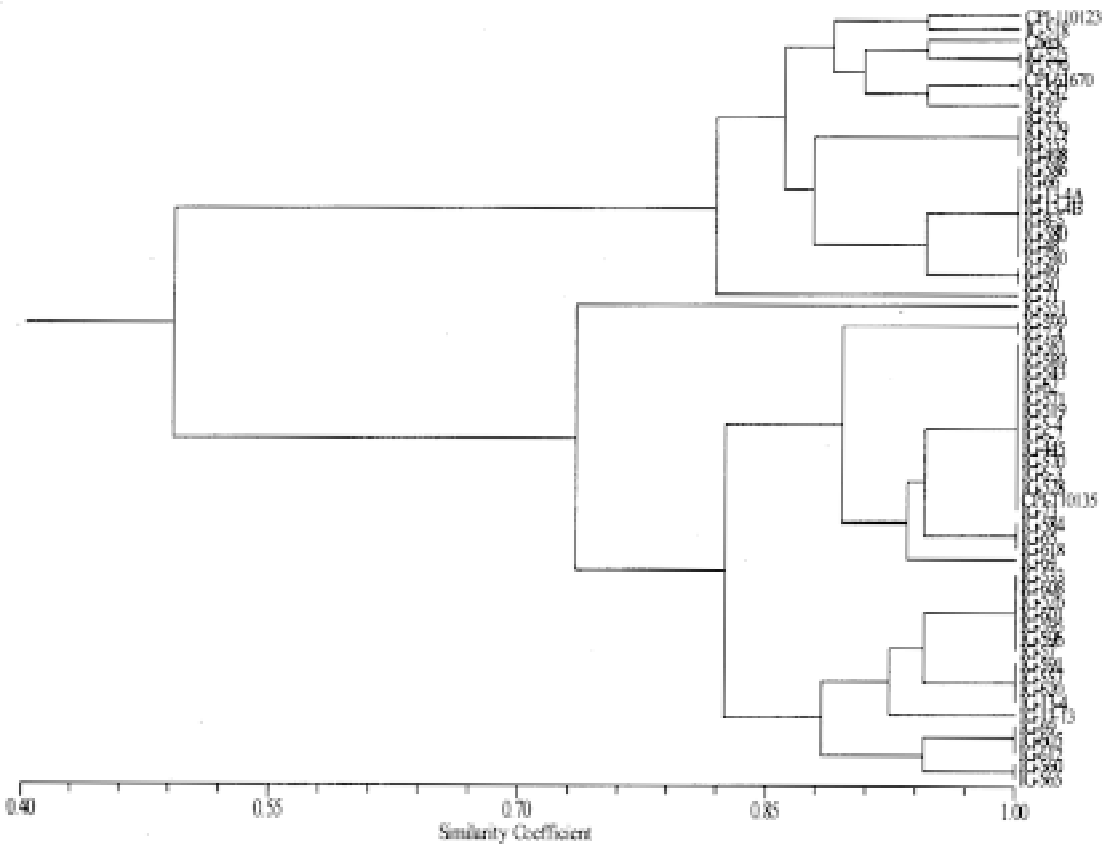

Fig. 1. Dendrogram based on seed SDS-protein bands developed using UPGMA module showing genetic relationships among different accessions of *S. hamata*

accessions except accession IG-13-13 which work as a bridge among them. Interestingly, all the accessions of this sub-cluster showed more than 88% similarity among them. Accessions IG-13-13, IG-64, IG-551 and IG-71 showed more variability as they were placed individually in the dendrogram. Accession IG-518 showed only 33% similarity with seven accessions namely IG-533, IG-608, IG-526, IG-601, IG-595, IG-596 and IG-57 indicating usefulness of these genotypes as parents in breeding programmes. Similarly, accessions IG-64 and IG-13-13 also showed 33 % similarity with *S. hamata* check and CPI-110123 accessions respectively. Earlier work based on seed-protein pattern among accessions of seven species of stylo showed similar observations [3].

Low level of polymorphisms as evidenced from the present studies as well as reports based on RAPD and STS molecular markers [11,12] has been the biggest bottleneck in genetic improvement of this crop. Nevertheless, the present study provides the information about the accessions having a reasonable level of variability, which can be used for inter-specific crosses in the stylo breeding programmes.

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