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Morphological and Biochemical Markers for Varietal Identification in Lucerne

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ABSTRACT An experiment was conducted to establish the varietal differences in lucerne by morphological characters, chemical test and electrophoretic technique. On the basis of morphological characteristics, genotypes Anand-2 and RL-97-2 were distinct from other genotypes by dense stipule pubescence, while Anand-3 and T-9 were distinct from other genotypes by lanceolate leaf shape. The semi-hollow type of pith was useful for identifying Anand-2, RL-88 and RL-97-1. On the basis of electrophoretic profile the presence/ absence of band number 22 having Rm value 0.58 and 26 having Rm value 0.73 were useful for distinguishing genotypes from one another.

Key words : Morphological characters, biochemical markers, lucerne

Lucerne (*Medicago sativa* L.) is an important commercial fodder crop in India and play an important role in dairy business. It is a perennial crop with high nutritive value and there is good demand for seed. Lucerne is allogamous crop with self-incompatability phenomenon. Hence, the population of lucerne is heterozygous and it is very difficult to maintain genetic purity in this crop. Major amount of pollination takes place by insect pollinators. The flower burst open when pollinators visit them, resulting in the dispersal of pollen grains from the host flowers to another flowers of same plant or other plant. The varietal purity is maintained by growing individual genotype in isolation or by enclosing each genotype with fine net. The flower colour, petal spot, nectary, tan-nontan, leaf shape, seed shape, seed colour, stem colour etc. has been the contrasting morphological characters generally useful for varietal identification. Various laboratory techniques have been standardized for establishing varietal differences of which electrophoresis is rapid and accurate technique. The successful exploitation of electrophoresis for plant variety identification is possible as proteins are the product of structural genes and seed proteins are present comparatively in large amount and are readily extracted, hence the electrophoretic examination of seed proteins provides a powerful and convenient way of plant genotype identification.

MATERIALS AND METHODS

Genetically pure seeds of eight genotypes of lucerne namely Anand-2, Anand-3, LLC-3, RL 88, RL 97-1, RL 97-2, T 9 and CO-1 received from IGFRI, Jhansi were grown in RBD with three replications at Seed Technology Research Unit farm during *rabi* 2003-04. The observations on plant height, foliage colour, central leaflet length, central leaflet width, stipule pubescence, leaf shape, stem girth, stem pith and flower colour were recorded at appropriate time as per UPOV guidelines [1]. Part of pure seed of these genotypes were used for electrophoresis by SDS-PAGE method [2]. Number of bands, density of band and Rm value of each band were estimated. Peroxidase test of seed coat of eight genotypes were also carried out as per the procedure [3]. These morphological and biochemical markers were used for making varietal differentiation in lucerne.

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RESULTS AND DISCUSSION

In present study, eight genotypes of lucerne were explored for twelve different morphological characters, some of them are most important for seed production and genetic purity analysis point of view. Out of twelve, five characters were found to be polymorphic and seven monomorphic (Table 1). The polymorphic characters grouped the genotypes into two different categories. Out of eight genotypes, four have dark green foliage, while remaining have green foliage. Anand-3 and T 9 have lanceolate and remaining genotypes have deep ovate leaf shape. Anand-2 and RL-97-2 have dense stipule pubescence, while rest of the genotypes have sparse stipule pubescence (Fig. 1). Semi-hollow type of stem pith was exhibited by Annad-2, RL-88 and RL-97-1, while rest of the genotypes have solid stem pith. Though, the initial plant growth, leaf length and leaf width were contrasting traits but they were less effective for grouping the genotypes as their variations could be attributed to environment factors. The stipule pubescence, leaf shape and stem pith are the key characters useful for distinguishing the lucerne genotypes and rouging of off types in seed production field. The contrasting characters for varietal identification were reported in lucerne [4-6]. Peroxidase test of seed coat of eight genotypes showed negative reaction, which limit its application for identification of these genotypes.

Electrophoretic analysis by SDS-PAGE method revealed that eight genotypes produced total 202 bands (Fig. 2). The number of bands in different varieties ranged from 24 (Anand 3 and LLC 3) to 27 (T 9). The band number 2 corresponding to Rm value 0.07 was present in all the varieties except Anand-2, Anand-3 and LLC-3. Band number 26 corresponding to Rm value 0.73 was present only in varieties RL-88 and T-9. Band number 22 corresponding to Rm value 0.58 was present

Table 1. Morphological diagnostic characteristics of eight genotypes of lucerne

S.No.	Characteristics	Anand-2	Anand-3	CO-1	LLC-3	RL-88	RL-97-1	RL-97-2	T-9
1	Initial growth	Good	Good	Good	Good	Normal	Normal	Normal	Normal
2	Plant height	Long	Long	Long	Long	Long	Long	Long	Long
3	Foliage colour	Dark green	Dark green	Green	Dark green	Green	Green	Green	Dark green
4	Length of central leaflet	Short	Short	Medium	Short	Short	Short	Short	Short
5	Width of central of leaflet	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Medium
6	Days to flower	Late	Late	Late	Late	Late	Late	Late	Late
7	Stipules pubescence	Dense[a]	Sparse	Sparse	Sparse	Sparse	Sparse	Dense[a]	Sparse
8	Stipule length	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
9	Leaf shape	Deep ovate	Lanceo-late[a]	Deep ovate	Deep ovate	Deep ovate	Deep ovate	Deep ovate	Lanceolate[a]
10	Stem girth	Thin	Thin	Thin	Thin	Thin	Thin	Thin	Thin
11	Stem pith	Semi-hollow[a]	Solid	Solid	Solid	Semi-hollow[a]	Semi-hollow[a]	Solid	Solid
12	Flower colour	Purple	Purple	Purple	Purple	Purple	Purple	Purple	Purple

a = indicated contrasting character.

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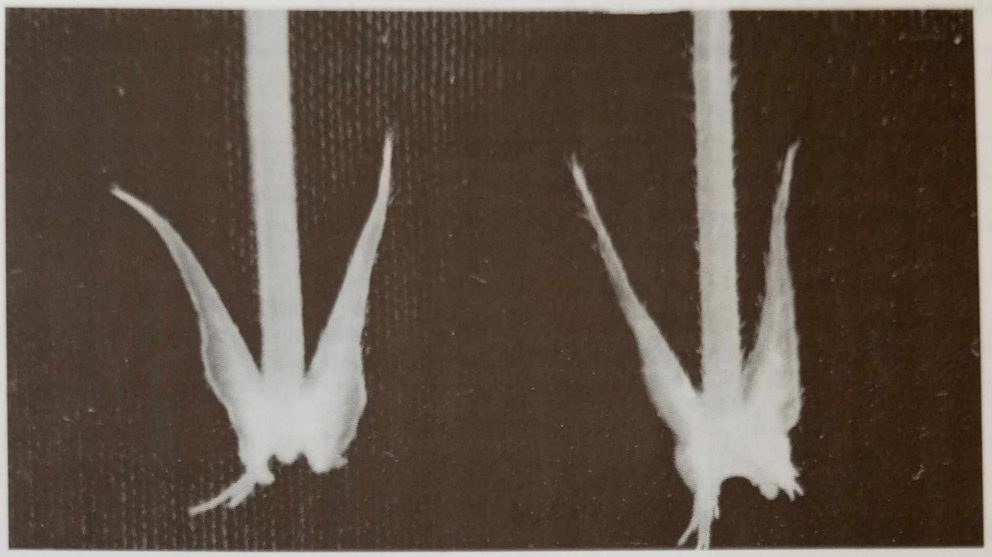

Fig. 1. Stipule pubescence — a morphological marker in lucerne genotypes

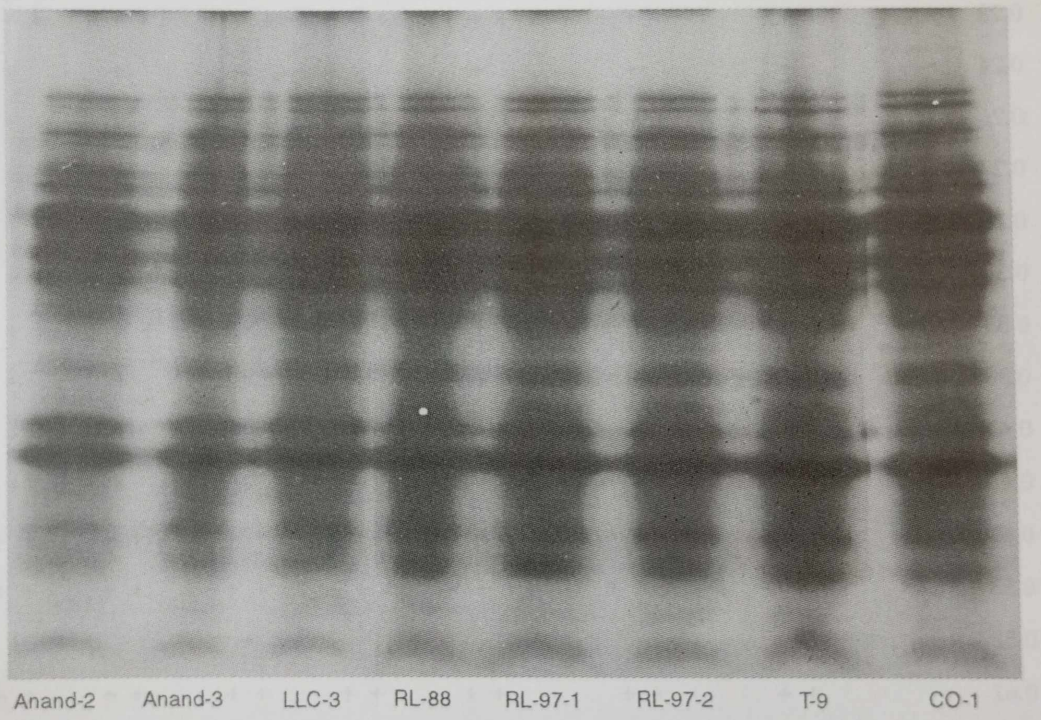

Fig. 2. Electrophoretic profile of seed proteins of eight lucerne genotypes

in Anand-2, RL-97-1 and T-9. On the basis of presence/absence of bands these varieties may be distinguished from rest of the varieties. The minimum number of high, medium and low intense band were found in variety RL-97-1 and RL 97-2 (5 bands), Ananad-2 (6 bands) and LLC-3 (8 bands), respectively (Table 2). Band number 20 and 21 corresponding to Rm value 0.49 and 0.53 were present in all the varieties but their intensity was high in Anand-2, Anand-3, LLC-3 and RL-88 and medium in rest of the genotypes. Thus, the intensity of bands, position of bands and presence or absence of bands could be used as a biochemical marker for distinguishing the lucerne genotypes. Singh *et al.* [7] reported unique banding pattern of 12-21 bands from 6 varieties of mung seeds by SDS PAGE. They also reported that, not any two varieties were having similar banding pattern. The band number 3 and 11 were useful for distinguishing the mung varieties.

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Table 2. Electrophoretic profile of seed proteins (Relative mobility) of eight Lucerne genotypes

Band No.	RM value	Genotypes							
		Anand-2	Anand-3	LLC-3	RL-88	RL-97-1	RL-97-2	T-9	Co-1
1	0.008	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
2	0.07	-	-	-	+	+	+	+	+
3	0.12	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
4	0.13	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +
5	0.15	+	+	+	+	+	+	+	+
6	0.17	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
7	0.18	+	+	+	+	+	+	+	+
8	0.183	+	+	+	+	+	+	+	+
9	0.20	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
10	0.23	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
11	0.24	+	+	+	+	+	+	+	+
12	0.26	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
13	0.27	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
14	0.28	+	+	+	+	+	+	+	+
15	0.30	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
16	0.33	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
17	0.38	+	+	+	+	+	+	+	+
18	0.41	+	+	+	+	+	+	+	+
19	0.43	+	+	+	+	+	+	+	+
20	0.49	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ +
21	0.53	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ +
22	0.58	+	-	-	-	+	-	+	-
23	0.62	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
24	0.66	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
25	0.71	+	+	++	++	+	+	++	+
26	0.73	-	-	-	++	-	-	++	-
27	0.75	+ +	+ +	+ +	+ +	+ +	+	+ +	+ +

+ = Low intensity, ++ = Medium intensity, +++ = High intensity, Distance traveled by tracking dye = 12 cm.

Total no. of bands = 202;

$$\text{Relative mobility (Rm)} = \frac{\text{Distance traveled by protein band (cm)}}{\text{Distance traveled by tracking dye (cm).}}$$

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