

PAGE of storage proteins to identify seeds of *Lotus tenuis* and *Lotus corniculatus*.

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Lotus forage species of major diffusion in the Argentine Republic are *Lotus tenuis* and *Lotus corniculatus*. *L. tenuis* is naturally spread in an extensive calving production area of Buenos Aires province. Each one is adapted to different productive situations.

Their seeds are morphologically very similar. This makes difficult their identification in a purity analysis of a sampled seed lot.

SDS-PAGE was applied with the aim of identifying both species from seed samples.

MATERIALS AND METHODS

Storage proteins were extracted from bulked ungerminated seed samples of three accessions of *Lotus tenuis* and four of *Lotus corniculatus*. Three g of each sample were finely ground using a Tecator mill. Portions of 40 and 80 mg. were weighed from each finely ground bulk. Polypropylene hemolysis tubes were used for extraction of proteins using ISTA method for peas and ryegrass (1992). The ratio of extracting solution was the indicated by Gardiner and Forde (1992). Two modifications were made: 1-Immediately after the addition of the extracting solution all the samples were homogenized with a vortex mixer. 2-The samples of 80 mg were treated with a mechanic homogenizer with teflon pestle. This was an attempt of improving extraction. Samples were incubated for one hour. All the extracts were finally centrifuged in a Sorvall centrifuge, during 20 minutes at 15,000 r.p.m. Five µl of each supernatant and of the standard were loaded using a Hamilton syringe in individual wells of minigels. Gel buffers for main and stacking gels were prepared by ISTA method.

The Mini-Protean II electrophoresis cell and the Power Pac 300 (Bio-Rad) were successfully used. Standard employed was Bio-Rad Nro.:161-0317. Tank buffer was Tris-Glycine pH 8,3. It was used to fill wells, upper and lower reservoirs. Running

conditions were: 200 V (constant voltage setting) and run time approximately 40 minutes. Gels were fixed, stained and destained following ISTA (1992). Coomassie Blue (Bio-Rad) was used.

RESULTS AND DISCUSSION

Electrophoregrams showed 23 bands in both species, varying in position and thickness. Differences among accessions of each specie were not taken into account. Figure resumes the bands founded in each specie. Bands were named D_1, D_2, \dots, D_{23} , considering "D" as the distance from the origin. An ascending scale was designed based on the thickness of bands from T_1 to T_4 .

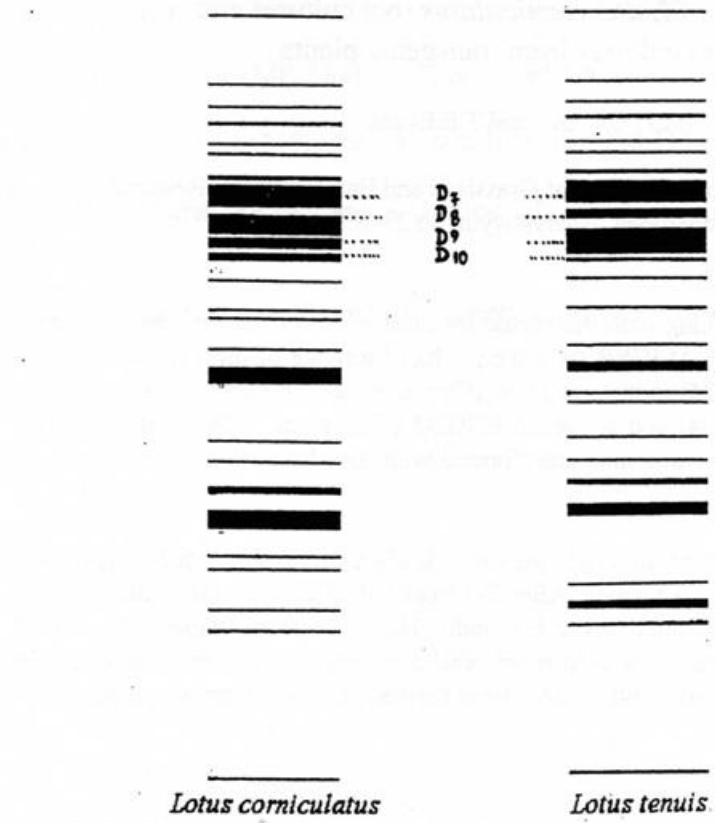
Although there were differences in presence /absence and thickness of bands, the main distinctions between species were clearly displayed in D_7, D_8, D_9 and D_{10} . In the cited sequence the schemes were *Lotus corniculatus*: $T_4 - T_4 - T_2 - T_2$ and *Lotus tenuis*: $T_4 - T_2 - T_4 - T_1$.

Lotus corniculatus and *L. tenuis* can be identified by SDS- PAGE of storage proteins from seed sampled lots using the $D_7 \dots D_{10}$ electrophoregram sequence.

REFERENCES

- Gardiner, S.E. and Forde, M.B.** 1992. Identification of cultivars of grasses and forage legumes by SDS-PAGE of seed proteins. *In*: Seed Analysis. Linskens, H.F. and Jakson, J.F. Ed. Springer-Verlag. pg. 44-45.
- ISTA.** International Seed Testing Association. 1992. Handbook of Variety testing. Proposed ISTA standard reference method for the identification of varieties of peas and ryegrass by SDS-PAGE. pg. 2.7-210.

Figure: SDS- PAGE electrophoregrams of storage proteins in seed bulks of *Lotus tenuis* and *Lotus corniculatus*.



D: distance from the origin.