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Immunochemical properties of seed proteins as systematic markers in Cactaceae

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ABSTRACT

The purpose of this study was to compare seed protein patterns of 10 species of Cactaceae, a native New World plant family distributed from Canada to Argentina, in an attempt to assess their usefulness for systematic studies. Particular attention was paid to analysing antigenic patterns derived from western blotting carried out with different antisera. Similarity (S_J) and distance (D_{BC}) indices were further used to carry out Cluster Analysis (UPGMA) and Principal Coordinate Analysis. Antigenic patterns of species of Opuntioideae and Cactoideae were obtained using anti-*Cereus aethiops*. Between both subfamilies S_J varied from 0.412 to 0.697 while D_{BC} varied from 0.172 to 0.387. On the other hand, between the two species of *Cereus* S_J was 0.971 and D_{BC} was 0.091. Also, antigenic patterns of species of Cactoideae and *Opuntia* were obtained using anti-*Opuntia elata* var. *cardiosperma*. Between both subfamilies S_J ranged between 0.537 and 0.738 while D_{BC} ranged between 0.128 and 0.247. Among species of *Opuntia* S_J varied from 0.744 to 0.946 while D_{BC} varied from 0.082 to 0.168. Thus, findings from our study demonstrate that variability is lowest at the intraspecific level while it increases when different species of the same genus, subfamily, family or class are considered. This indicates that differences in antigenic patterns are tightly related to systematic affinities. It can thus be concluded that western blotting could help bring consensus to the current different points of view on systematic relationships at low taxonomic levels.

1. Introduction

Cactaceae, a native New World plant family, are typically found in a vast territory which extends from British Columbia and Alberta (56°15′ N) in Canada to Patagonia (50° S) in Argentina (Anderson, 2001). Four subfamilies of the Cactaceae have been identified by Anderson (2001), namely Cactoideae, Opuntioideae, Pereskioideae and Maihuenioideae. The main characteristic of species belonging to the subfamily Cactoideae, which is, in fact, the largest subfamily of the Cactaceae, is their diversity. Anderson (2001) observed that Cactoideae subfamily include 9 tribes and 108 genera which agrees, though with minor differences, with Buxbaum's tribal classification of Cactoideae (Buxbaum, 1958; Endler and Buxbaum, 1974). As to the subfamily Opuntioideae, previous research has demonstrated that systematic relationships within this subfamily have not

Abbreviations: Caet, *Cereus aethiops*; D_{BC}, Bray–Curtis's Index; MW, Molecular Weight; *Oela*, *Opuntia elata* var. *cardiosperma*; PCO, Principal Coordinate Analysis; SDS-PAGE, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis; S_J, Jaccard's Index; TBS, Tris Buffered Saline; UPGMA, Unweighted Pair-Group Method using Arithmetic Averages.

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only been but also still are a matter of controversy (Griffith and Porter, 2009). Opuntioideae includes 15 genera, the majority of whose species is included in *Opuntia* s.s Mill. Cactaceae in South America, in particular, is composed of 328 species and 39 genera of Cactoideae and of 66 species and 11 genera of Opuntioideae (Kiesling et al., 2008). Representative species of these taxa were therefore selected for this study.

Since the late twentieth century large plantations of Cactaceae have been established and crops of several of its species have become of importance for economy enterprises (Shedbalkar et al., 2010). Kiesling (2001) recorded 12–15 genera and 50–80 species with agricultural aptitude, the majority of which belong to the subfamily Cactoideae. Cactus fruits are at present merchandized for human and animal consumption and cactus plants are cultivated as ornamentals (Shedbalkar et al., 2010). Furthermore, as some species have either antioxidative effects (Huang et al., 2009) or anti-hyperglycemic properties (Andrade-Cetto and Wiedenfeld, 2011), they have also become of relevance for the pharmaceutical industry.

Improvements in protein extraction and multiseparative methods (1D and 2D electrophoresis and capillary electrophoresis) have facilitated the exploitation of seed proteins as molecular markers of genetic variability (Cooke, 1995; Reynolds, 2007). Seed protein homology has also been the focus of attention in studies in which cross reactivities are compared using specific antibodies. The usefulness of traditional immunochemical methods has been demonstrated by Petersen and Fairbrothers (1985) and Shneyer et al. (2003) in other plant systematic groups. Also, results from our laboratory demonstrated that in Opuntioideae, western blotting not only broadens the usefulness range of traditional immunological techniques at the specific level but also complements data derived from electrophoretic patterns (Galvez et al., 2009). Compared to traditional techniques, western blotting is also a very useful research tool as it improves both reproducibility and sensitivity.

In view of the above, this study is aimed to analyse and compare seed protein patterns of several species of Cactaceae, paying particular attention to the antigenic properties of seed proteins, in an attempt to assess their usefulness for the systematic study of this family. Immunological relationships with other families are also tested.

2. Materials and methods

2.1. Plant material

Seeds of mature fruits from Argentinean spontaneous populations of the following species were studied: *Harrisia pomanensis* (F.A.C. Weber ex K. Schum.) Britton & Rose subsp. *pomanensis*; *Trichocereus candicans* (Gillies ex Salm-Dyck) Britton & Rose; *Cereus forbesii* Otto ex C.F. Först. and *Cereus aethiops* Haw. –Cactoideae–; *Opuntia elata* Salm-Dyck var. *cardiosperma* (K. Schum.) R. Kiesling; *Opuntia megapotamica* Arechav. (=*Opuntia salagria* A. Cast.); *Opuntia quimilo* K. Schum.; *Opuntia ficus-indica* (L.) Mill.; *Cylindropuntia imbricata* (Haw.) Knuth and *Tephrocactus articulatus* (Pfeiff.) Backeb. var. *articulatus* –Opuntioideae–; *Cucurbita maxima* Duchesne –Cucurbitaceae–; *Amaranthus hybridus* L. subsp. *hybridus* –Amaranthaceae– and *Chenopodium quinoa* Willd. var. *quinoa* –Chenopodiaceae– (Zuloaga et al., 2008). Fruits of *O. ficus-indica* and seeds of *A. hybridus* and *C. quinoa* were purchased. All seeds were stored at 4 °C until required. Voucher specimens were deposited at the herbarium of the *Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Argentina* (BBB).

2.2. Protein extraction

Three grams of mature seeds of each sample of Opuntioideae were ground mechanically (Galvez et al., 2009). One gram of mature seeds corresponding to each sample of Cactoideae was ground in a pestle and mortar and the resulting powder was subsequently deoiled, air dried and kept at 4 °C until required. Five mature seeds of *C. maxima* (0.85 g) and 1.5 g of mature seeds of *A. hybridus* and *C. quinoa* were processed in the same way. Seed proteins were extracted in 0.025 M Tris–0.192 M glycine buffer, pH 8.3 (10 ml/g of dry weight) at room temperature for 1 h. The suspension was centrifuged at 6000*g* during 10 min and the supernatant was stored at −20 °C. Extract protein content was estimated following Bradford (1976) method and using bovine serum albumin as standard.

2.3. Antisera

The seed protein extract obtained in Tris–glycine buffer from a population of *Caet* (*C. aethiops*, Salitral de la Vidriera, Villarino, Buenos Aires, Argentina) was used in order to produce antiserum from New Zealand rabbits (Galvez et al., 2009). Previously prepared anti-*Oela* (anti-*O. elata* var. *cardiosperma*) (Galvez et al., 2009) was also employed.

2.4. SDS-PAGE and western blotting

Tricine-SDS-PAGE was performed in slab gels following the methodology previously described (Galvez et al., 2009). Prior to electrophoresis extracts were added to a denaturing sample buffer (0.125 M Tris–HCl, pH 6.8, 4% SDS, 5% 2-mercaptoethanol, 20% glycerol, 0.02% bromophenol blue) and boiled for 3 min. After the run, gels were stained with 0.5% (w/v) Coomassie Brilliant Blue R-250. Alternatively, the proteins separated by electrophoresis were blotted onto a nitrocellulose membrane (Galvez et al., 2009). The membrane was blocked overnight at 4 °C in 50 mM Tris buffered saline (TBS) solution (pH 7.4)