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The endosperm seed protein Solin: biochemical characterization, induction by ABA and species-specific subunits*

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Summary. Comparison of endosperm storage protein subunits from single seeds of 19 *Solanum* species was done by isoelectric focusing. Species-specific profiles of the subunits were evident and species relationships within a taxonomic series could be delineated. The identification of both intraspecific and interspecific hybrid seed may be possible from Solin IEF subunit profiles. The glutelin nature of the protein was unusual for a dicotyledon genus. We named this major endosperm protein complex "Solin". Solin was found in developing seeds with embryos at the heart stage, 16 to 18 days after pollination. Seeds excised at the globular stage responded to the addition of ABA and synthesized Solin. This is the first report of the induction of seed protein in endosperm cells of *Solanum*.

Key words: Seed protein – *Solanum* endosperm – Solin – ABA

Introduction

Seed protein research has concentrated on cereals and legumes (Boulter and Parthier 1982). The major storage proteins in starchy endosperm of cereals are insoluble prolamins and glutelins; legume cotyledons contain soluble globulins. These seed proteins have served as indicators of taxonomic and genetic relationships among species and cultivars. Equally important, normal seed development and maturation can be traced by the synthesis and accumulation of seed proteins.

Variation among endosperm protein subunits in *Solanum* species provides an opportunity to study the inheritance of seed protein in this genus. Identification of both interspecific and intraspecific hybrids may be possible if the subunits are inherited in an additive manner. Endosperm protein subunits may also be useful probes for gene expression during seed development.

Our objectives were (1) to describe the biochemical nature of the endosperm proteins; (2 a) to establish the stage of embryo development associated with protein production in the endosperm of *Solanum* seeds; (2 b) to investigate whether ABA had an effect on Solin production and (3) to determine whether *Solanum* seed proteins are useful in genetic and taxonomic comparisons within this genus.

Numerous examples of electrophoretic analyses of seed proteins could be cited. Most of these employ gel systems to examine native or dissociated protein patterns. Isoelectric focusing (IEF) has been used because of its greater resolving capacity. For example, zein IEF patterns of maize inbreds are genotype specific. Motto et al. (1979) have used them as a purity test for single crosses. Also, 12 varieties of winged bean were found to have unique seed protein IEF patterns (Westermeir et al. 1985).

Seed proteins represent a major metabolic product and their production is synchronized with the developmental events of the embryo. Zeins are produced in protein bodies of corn endosperm 10 to 12 days after fertilization (Duvick 1961). Storage proteins of the common bean are found in the cotyledons 14 days after fertilization (Sun et al. 1978). The glutelin storage protein complex, Solin, was studied in developing *Solanum* seeds and was detected 16 to 18 days after pollination. The production of Solin by globular stage

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seeds after the addition of ABA was significant. This observation was unique because Solin is a glutelin complex of a dicotyledon. The action of ABA has previously been reported to stimulate amino acid incorporation into storage proteins localized in embryonic tissue (Crouch and Sussex 1981) or enhance synthesis of several proteins including wheat germ agglutinin (Triplett and Quatrano 1982). The synthesis of seed protein has proven useful to the investigation of trnascriptional and translational gene control in higher plants (Ingverson 1983).

Materials and methods

Plant material

Seeds of *Solanum* species were obtained from the IR-1 collection at Sturgeon Bay, WI (Table I), USA. *S. tuberosum* Group Phureja seeds 1837.76 and 1839.76 were from Dr. F. Haynes, North Carolina State University, Raleigh, NC, USA. The Phureja seeds were germinated and seedlings grown in the greenhouse at St Paul, MN. Vigorous plants were grown in the field at Grand Rapids, MN, USA. Timed pollinations were made on flower buds which had been emasculated 1 day prior to opening. Fruits were collected at various stages and weighed.

Extraction of protein fractions

Phureja seeds were counted into lots of 100 and weighed. Each seed lot was ground in a mortar and pestle with sand and 1 ml of acetone. The seed meal was scraped into 1.5 ml microfuge tubes and centrifuged 15 min at 10,000 *g*. The acetone supernatant was removed. This extraction was repeated twice and the defatted seed meal was dried with air at room temperature. The meal was then extracted 3 times with 1 ml volumes of water, 0.5 *M* NaCl, 70% ethanol and b-mercaptoethanol or 0.1 N NH_4OH; these fractions correspond to those of Osborne and Campbell (1898).

Protein quantification

Alcohol and basic extracts were divided into 1 ml volumes and lypholized to complete dryness. Then 1 ml samples of the water and salt extractions were adjusted to 10% concentration of trichloroacetic acid, and the resulting precipitate was centrifuged 10 min at 12,000 *g*. The precipitates were resuspended twice in acetone and dried before storage at −20 °C. The Lowry et al. (1951) method was used for protein quantification.

Seed protein for electrophoreses

For slab gel electrophoresis, extracts with 100 µg protein were suspended in SDS sample buffer according to Laemmli (1970) and a linear acrylamide gradient of 10%–15% was used. The protein standards were bovine serum albumin 66 kdalton, ovalbumin 45 kdalton, gamma globulin 25 kdalton and myoglobulin 17.8 kdalton. Isoelectric focusing was done in tubes with Servalytes pH 5–7 following the technique of O'Farrell and O'Farrell (1977). The gels were stained by placing them in 12.5% trichloroacetic acid and gently shaken for 1 h, two distilled water rinses of 1 h each with shaking were done and then 0.001% Coomassie Brilliant Blue R for 10 to 20 min. The gels were observed with the aid of a light box until protein bands were apparent. The destain solution was 1 part methanol: 1.5 part glacial acetic acid: 17.5 part distilled water. A total of 132 single seed extracts were compared (Table 1). Rf values were measured as the ratio of the subunit migration from the top of the gel to total gel length.

Table 1. List of 19 species seed sources representing 8 series. Abbreviations are from Huaman and Ross (1985). The number of seeds are the individual seed extracts for IEF analyses

Series and species	Abbrev.	P.I. no.	Seed no.
Pinnatisecta			
S. cardiophyllum	CPH	283063	6
S. pinnatisectum	PNT	275236	6
S. trifidum	TRF	255547	6
Megistracroloba			
S. megistracrolobum	MGA	265873	6
S. boliviense	BLV	283071 × 310975	6
S. sogarandinum	SGR	230510	3
S. raphanifolium	RAP	210048	6
S. sanctae-rosae	SCT	218221	6
Tuberosa			
S. canasense	CAN	210035	6
S. multidessectum	MLT	210043 and 210044	10
S. kurtzianum	KTZ	175434 × 175435	4
S. spegazzinii	SPG	275144	6
S. tuberosum Group Andigena	ADG	Parda Pastusa and Cajica	12
Group Phureja	PHU	Ubilla and Yema de Huevo	7
Group Stenotomum	STN	234008	5
S. sparsipilum	SPL	246536 and 233961	8
Demissa			
S. demissum	DMS	161179 and 210850	5
Acaulia			
S. acaule	ACL	275129 and 435071	6
Commersonii			
S. commersonii	CMM	243503	6
Piurana			
S. chomatophilum	CHM	366387	6
Polyadenia			
S. polyadenium	PLD	320342	6

Indices of similarity (SI) were calculated according to the procedure of Sokal and Sneath (1963). The unweighted pair-group using arithmetic averages was the clustering strategy (Edwards and Cavalli-Sforza 1965).

Amino acid analyses

Protein from whole seeds was extracted with 0.1 N NH_4OH and lypholized. The pellet was suspended twice in DDW to remove residual NH_4OH. A standard method of acid hydrolysis and quantification of amino acids was done with the Dionex analyser.

Seed dissection

Mature Phureja seeds were dissected to separate the embryonic axis, cotyledon and endosperm. Five samples of each

tissue were pooled and extracted with 100 µl of SDS sample buffer.

Culture media

Basal media were the standard formulae of Murashige and Skoog (1962). ABA (mixed isomer) was added from a 1 m*M* stock dissolved in 50% ethanol to give final ABA concentrations of 10^{-5} *M* and 10^{-6} *M*. All media and instruments were autoclaved.

Excision of seeds

Fruits collected 15 to 20 days after pollination were surface sterilized for 15 min in a 10% bleach solution. Seeds were excised and placed on agar medium. About one fourth of the seeds were dissected and the stage of embryonic development recorded. These seeds were frozen for later electrophoretic analyses. The remainder of the seeds were placed onto media with or without ABA. The seeds were grown for 3 to 7 days and then dissected to determine the stage of the embryo.

Results

Biochemical characterization of Solin

The glutelin of Phureja seed was soluble in basic solution; only trace amounts of protein were present in other fractions. Extracts prepared from embryonic axes or cotyledons had no detectable amounts of glutelin. SDS gradient gels were used to visualize the glutelin subunits; four size classes were observed at about 38, 30, 20 and 10 kdalton (Fig. 1). We propose the name "Solin" for the *Solanum* endosperm glutelin complex. The exact number of Solin proteins is unknown.

The amino acid composition of Solin was determined from seeds of *S. acaule, S. commersonii* and *S. tuberosum* Group Phureja (Table 2). There were minor differences in the amino acids. The recovery of serine and proline was low. The predominant amino acid in Solin was glutamic acid/glutamine.

Three samples of 100 seeds from each species were analysed for protein content. *S. acaule* had 10.6 ± 1.7 µg/seed, *S. commersonii* had 12.0 ± 2.5 µg/seed and Group Phureja had 15.7 ± 2.4 µg/seed. We concluded that a single seed had adequate protein for IEF electrophoretic analysis. Different size seeds did not have significant variation in Solin content.

Relation of Solin to embryo development and ABA treatment

Seed protein production in the endosperm was highly correlated with the developmental stage of the embryo. Seed did not contain Solin prior to the heart stage of embryo formation. Solin was produced at a rapid rate once the cotyledons were initiated (Fig. 1).

If immature seeds with globular embryos were excised from the fruits at 15 days post-pollination and placed onto basal medium without ABA, the development of the embryo stopped and the seed gradually atrophied. None of the 50 seeds with globular, and only 2 of 4 early heart stage embryos germinated. No Solin was produced after they were placed onto basal medium. If seeds were excised when they had a heart or a torpedo stage embryo, the development of the embryo continued and premature germination occurred in about 50% of the seeds, 4 of 22 heart and 27 of 89 torpedo stage embryos.

Seeds containing globular embryos (184 total), which were placed onto basal media with ABA, produced Solin after 3 to 7 days in culture. It was found that 60% of the seeds, 145/241, placed onto media with 10^{-6} *M* ABA and 100% of the seeds (79) produced Solin when placed onto 10^{-5} *M* ABA media. Examination of seeds with globular embryos placed onto media with ABA revealed that, even though the Solin had been induced, the embryo had not progressed in development. Seeds which had embryos at the heart stage and which were placed onto media with ABA continued embryo development but did not germinate.

Phylogenetic relationships

Solanum species have been designated according to morphological characters and geographic distribution. One of our objectives was to establish whether seed protein subunits within this large genus were species-specific. Single seed extracts were compared from 19 *Solanum* species representing 8 different series of the subsection Hyperbasrathrum (Table 1). These species

Table 2. Amino acid composition as percent recovered from Solin and glutelin

	Solin			Glutelin[b]	
	ACL	CMM	PHU	Wheat	Rice
asp	10.0	8.9	12.2	2.7	9.6
thr	3.6	3.1	2.5	2.9	3.4
ser[a]	3.6	3.9	2.1	6.7	7.2
glu	21.8	24.2	24.5	35.7	15.3
pro[a]	3.2	4.1	6.0	12.9	8.9
gly	4.7	4.5	5.5	7.3	7.6
ala	4.0	4.0	5.1	3.4	6.7
val	5.7	5.6	5.5	4.2	6.3
met	2.5	2.4	2.2	1.4	0.9
ile	4.7	4.5	5.1	3.3	4.1
leu	6.4	6.0	8.0	7.0	9.5
tyr	4.2	4.2	ND	2.4	2.0
phe	5.8	6.2	5.7	4.4	4.3
his	2.6	2.7	2.3	1.8	3.2
lys	6.5	5.9	6.5	1.5	4.8
arg	10.6	9.9	6.8	2.4	6.3

[a] Low recovery
[b] Data taken from Payne and Rhodes (1982)

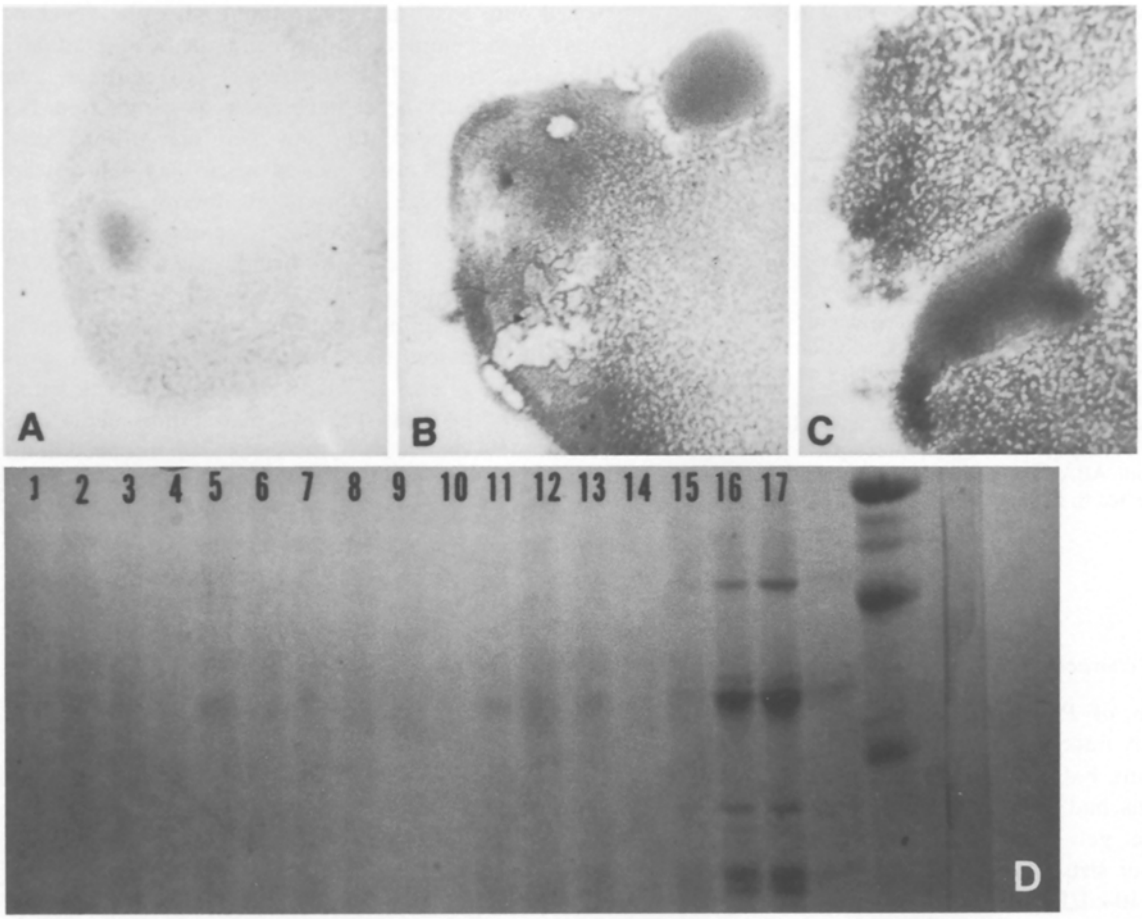

Fig. 1A–D. Stages of embryo development: **A** 14 day globular, **B** 16 day globular and **C** 17 day heart. Solin proteins extracted from single seeds of these Group Phureja stages: lane numbers are days after pollination. Unmarked lane has protein molecular weight standards, see "Materials and methods"

are native to Argentina, Bolivia, Brazil, Mexico and Peru. A total of 132 extracts from single seeds were compared. In general, six seeds of each species were sampled. In two species fewer seeds were used, and in two others, 10 to 12 seeds were extracted. Screening of Solin subunits in gels pH 10 to 3 revealed the major subunits were in the 7 to 5 range. IEF gels with this range for six representative species are shown in Fig. 2. The number of major subunits varied from 5 to 10; this number permitted easy gel to gel comparisons. The R_f values were calculated for subunit migration in each gel. Within the species the standard error was ± 0.5 to 1.0 R_f value. All R_f values were corrected to the nearest integer. Some subunits segregated within a species and some were present as double bands; these were counted as individual subunits. All 19 species had profiles of Solin subunits which were species-specific (Fig. 3).

Discussion

The major seed protein, Solin, was found to occur only in the endosperm as a highly insoluble glutelin complex consisting of four major size classes. The biochemical properties of Solin were similar to the wheat and rice glutelins because reducing agents were required to generate soluble polypeptides of the very high molecular weight glutelin. The amino acid composition of Solin, wheat and rice glutelins were reasonably similar (Table 2). Solin contained glutamic acid/glutamine as the predominant amino acid; a large amount of arginine was also found.

In this study 19 *Solanum* species representing eight series were sampled and single seed extracts compared. When Solin was separated by isoelectric focusing in gels with a pH gradient of 7 to 5, 5 to 10 different

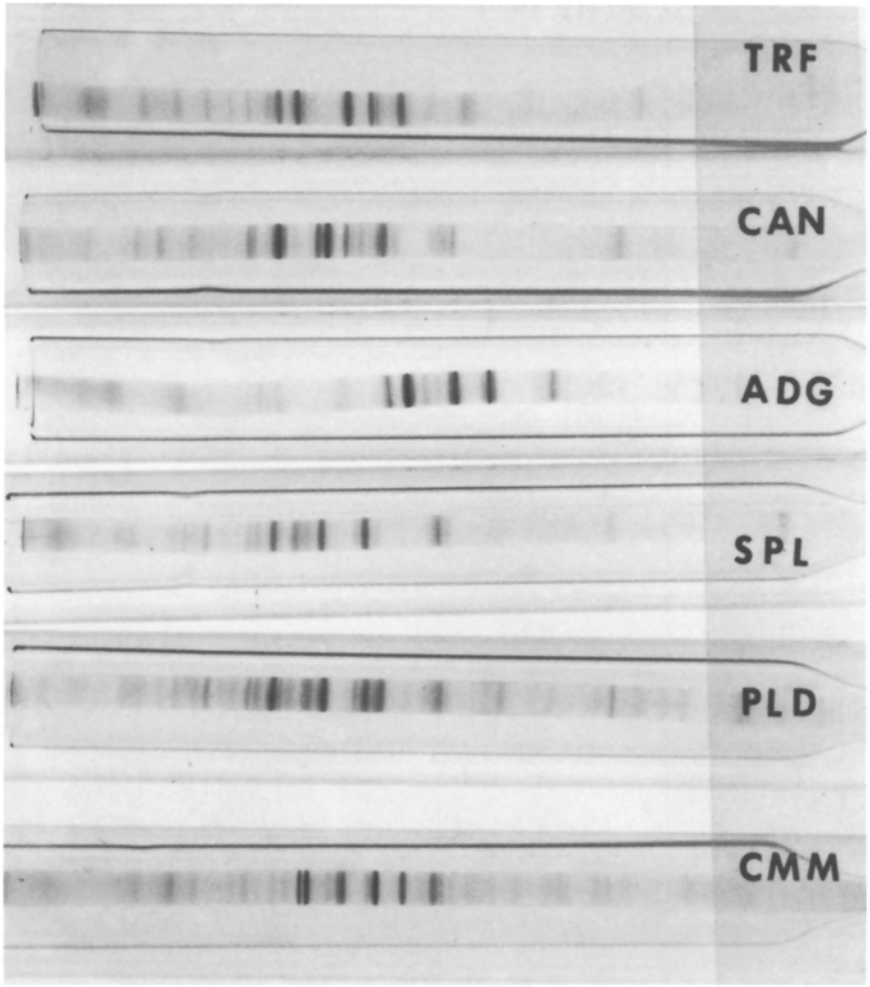

Fig. 2. IEF gels pH 7 to 5 from single seed extracts of the endosperm protein Solin from *Solanum trifidum, S. canasense,* Group Andigena, *S. sparsipilum, S. polyadenium* and *S. commersonnii*

subunits were resolved. This smaller number permitted easier comparisons between the species. It is recognized that these subunits do not constitute all of those from the Solin complex.

Molecular weight size classes, as estimated by SDS-PAGE, were about 38, 30, 20 and 10 kdalton for Solin. These compare more favourably to rice glutelin subunits of 51, 37–34 and 22–21 kdalton (Krishnan and Okita 1986), than to wheat glutelin subunits of 80–140 kdalton (Lawrence and Shepherd 1981). In a study of rice glutelin separated in IEF gels, 12 subunits were observed with a pI range of 6.5 to 7.5 (Wen and Luthe 1985). These results compare with the 5 to 10 subunits we detected in pH 5 to 7 range. They also found 9 rice glutelin subunits with basic pIs. Solin did have additional minor subunits with basic values; we did not attempt to classify them.

The developing seed is a precisely coordinated system of biochemical events. In Group Phureja seeds Solin is normally produced in endosperm cells 16 to 18 days after pollination. The presence of Solin was closely linked to the shift of the embryo from a globular to a heart stage. The failure of seeds with globular embryos to continue development after they were excised from fruits implied that there are essential compounds provided by the maternal tissue in the early stages of seed development. A similar conclusion was arrived at by Haynes (1954). Only embryos which were at the heart stage were able to continue in vitro development.

Abscisic acid is one of the most ubiquitous plant growth regulators. Historically ABA has been assigned the general role of a growth inhibitor. Dure (1977) observed that ABA has a significant role in the inhibition of the translation of total mRNA during germination of the cotton seed. During rape seed maturation, Finkelstein et al. (1985) have found that levels of endogenous ABA and 12S storage protein mRNA peak just prior to desiccation. They suggest increased exogenous ABA is required to suppress germination and continue storage protein accumulation early in seed development, but not during desiccation. Parallel studies with developing wheat embryos have led Quatrano

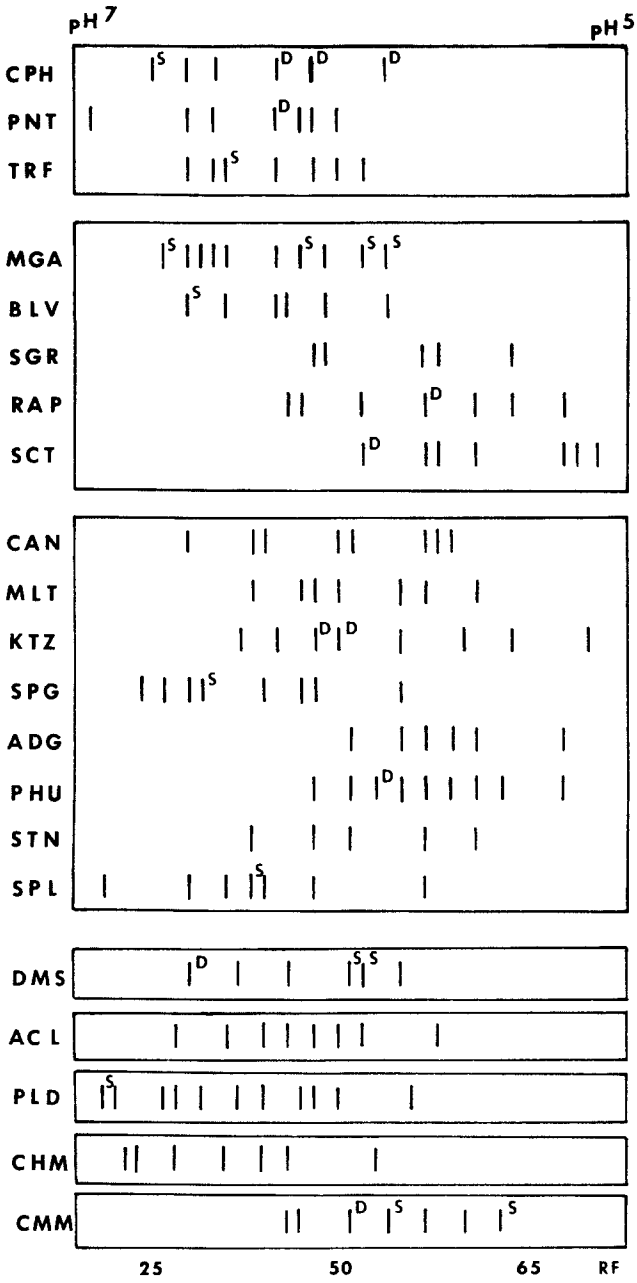

Fig. 3. Diagrams of the R_f values of Solin subunits in IEF gels from 19 *Solanum* species; see Table 1 for abbreviations

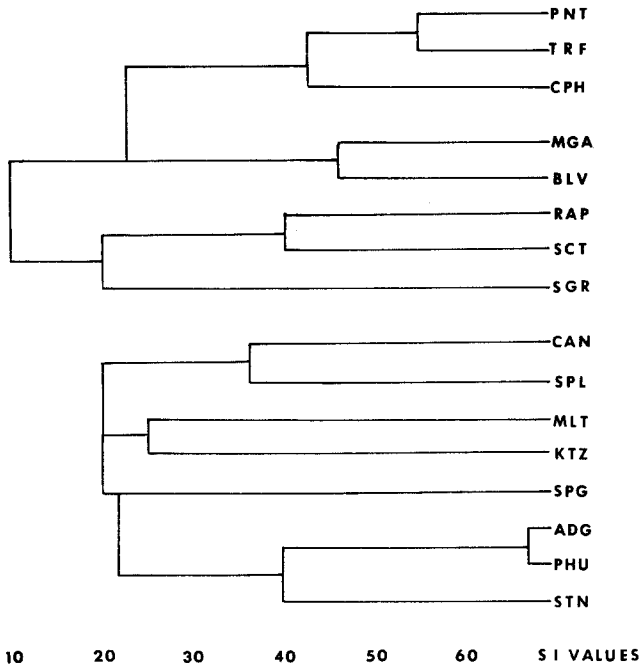

Fig. 4. Cluster analysis of indices of similarity (SI) values calculated from Solin subunits in IEF gels of *Solanum* species from Series Pinnatisecta, Megistracroloba and Tuberosa

et al. (1986a) to a similar conclusion that one of the effects of ABA is at the level of modulating mRNA levels, increasing those for maturation and decreasing those for germination. The proteins expressed in wheat embryos included the lectin wheat germ agglutinin, an abundant mature embryo protein and the globulin storage proteins (Quatrano et al. 1986b). We have demonstrated that the endosperm proteins of immature seeds of Group Phureja were induced with ABA, even though the embryo does not continue normal development.

Each of the *Solanum* species had unique Solin subunit patterns in IEF gels with pH 7 to 5 (Fig. 3). The subunits from Pinnatisecta species had pI values between 6 and 7. Both Megistracroloba and Tuberosa series had some species with subunits toward the pH 7 or pH 5 end of the gel; only *S. multidissectum* and Group Stenotomum have most subunits near the middle of the gel.

Segregation of Solin subunits within *Solanum* species presents the opportunity to determine whether these may be simply inherited. Lawrence and Shepherd (1981) observed 1:1 segregation in F_1 hybrids and found allelic genes at two loci for wheat glutelin subunits. More recently, the copy number of 5 to 10 has been estimated for genes at the *Glul* locus on the long arm of homologous group 1 chromosomes (Thompson et al. 1983).

The species-specific patterns were amenable to cluster analysis; those series represented by only one species were not included. Four clusters are shown in Fig. 4. The first cluster of three Pinnatisecta species had SI values from 44 to 55; the second Megistracoloba cluster of species had SI values from 10 to 46. These two series form one large cluster. The second large cluster contained both wild species and cultivated groups from the Tuberosa series. The cultivated groups had SI values from 40 to 67 to form a cluster. Phureja

Solin subunits compare most closely with Andigena. The groups had an average SI of 22 with the cluster of wild species, and the species themselves had an overall SI of 20. From this analysis, we can conclude that the species seed protein subunits indicate the same relationship established by systematic classification.

In summary, the endosperm protein Solin was glutelin similar in composition to seed protein from rice and wheat. Solin was produced in the seed during the time the embryo is changing from a globular to a heart stage. When seeds with globular staged embryos were excised from the maternal tissue and placed onto agar media containing ABA Solin was induced, but the embryo did not continue to develop. These observations may help define the series of events which occur during seed maturation. Solin subunits analysed from mature seeds have species-specific patterns in IEF gels. They may be useful in identifying true interspecific hybrid seeds. Further studies should include isolation of mRNA for Solin so that gene expression in potato seed can be compared to other seeds which contain glutelin.

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