Identification and Characterization of Storage Proteins in Zygotic and Somatic Embryos of Geranium (*Pelargonium ×hortorum*)

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ABSTRACT. Storage proteins in zygotic and somatic embryos of 'Scarlet Orbit Improved', zonal geranium (*Pelargonium ×hortorum* L.H. Bail.) were identified and characterized using gel electrophoresis. The major seed storage proteins in zygotic embryos were an 11S globulin and two low molecular weight (LMW_{1-2}) proteins. The 11S globulin consisted of four distinct subunits (53-74 ku), with each subunit being composed of an acidic polypeptide (A_1-A_4; 28-44 ku) linked via disulphide bonds to a basic polypeptide (B_1-B_4; 20-25 ku) and was named pelargin. The LMW (15.5 and 12,5 ku) albumins were not linked with disulphide bonds. Mature somatic embryos contained 80% of the proteins in zygotic embryos. Although protein profiles were more distinct in mature somatic embryos compared to nonmature, none of the zygotic embryo storage protein was present in the somatic embryos, indicating lack of complete maturity of somatic embryos. This study identified zygotic embryo proteins and demonstrated that maturation of somatic embryos improves protein content and types of proteins.

Synthesis and accumulation of storage proteins are integral events in seed development. Storage proteins are synthesized in large quantities, sequestered in protein bodies, and hydrolyzed during germination and subsequent seedling development. Any proteins present in amounts >5% probably have a storage function in seeds (Derbyshire et al., 1976).

The major dicot seed storage proteins are albumins (30% to 40% of the extractable protein) (Murray, 1979) and globulins (as much as 70% of the extractable protein) (Derbyshire et al., 1976; Nielsen, 1985). In monocots such as maize (*Zea mays* L.), the major storage proteins are usually prolamins (54% of extractable protein) and glutelins (40% of extractable protein) (Bewley and Black, 1994). Seed storage proteins are a source of carbon skeletons and reduced nitrogen for the developing seedling.

Somatic embryos also synthesize and accumulate seed-specific storage proteins (Crouch, 1982; Shoemaker et al., 1987; Stuart and Nichol, 1988). This accumulation of storage proteins is related directly to the degree of maturation and vigor of the resulting plantlets (Lai, 1994). Attempts at developing artificial seeds of geranium (*Pelargonium ×hortorum*) through desiccation (Marsolais et al., 1991) and gel-encapsulation (Gill et al., 1994) of somatic embryos have not been successful due to poor embryo quality. Poor embryo quality was hypothesized to be due to lack of maturation of somatic embryos (Marsolais et al., 1991). Furthermore, no studies have been reported on storage proteins in both zygotic and somatic embryos of geranium. Therefore, the objectives of this study were to identify and characterize zygotic embryo proteins of geranium and to compare these to somatic embryo proteins.

Materials and Methods

SOMATIC EMBRYO PRODUCTION. Seeds of diploid 'Scarlet Orbit Improved' geranium were obtained from Stokes Seed Co., St. Catherines, Ontario, Canada. Seeds were surface sterilized by immersing first in 70% (v/v) ethanol for 30 s followed by agitation for 20 min in a 1.6% solution of sodium hypochlorite containing one drop (per 200 mL) of Tween-20. Seeds were then rinsed five times with sterile distilled water. Seeds were germinated in 100×15-mm petri dishes (10 seeds per dish) containing 25 mL of a 0.8% water–agar medium (Sigma, Mississauga, Ontario, Canada). Petri dishes were sealed with parafilm, and incubated in the dark at 24 °C for 6 d.

Hypocotyls from 6-d-old etiolated seedlings were excised into 0.8 to 1 cm long explants. Nine explants per petri dish were cultured on an induction medium containing Murashige and Skoog (MS) salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1962), sucrose at 30 g·L^{-1}, sucrose, 20 μM N-phenyl-N'-1,2,3-thidiazol-5-ylurea (thidiazuron, TDZ) (Hutchinson and Saxena, 1995), and gelrite at 3 g·L^{-1} (Scott Laboratories, Carson, California). The pH of the medium was adjusted to 5.5 before autoclaving at 120 °C for 20 min at kPa. The petri dishes were sealed with parafilm and incubated at 24 °C under a 16-h photoperiod (20 to 25 μmol·m^{-2}·s^{-1}) provided by cool-white fluorescent lamps (Phillips Canada, Scarborough, Ontario, Canada). Hypocotyls were then subcultured on basal medium containing MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1968), 3% sucrose, 0.3% gelrite after 3 d. Half of all hypocotyls were subcultured on basal medium (nonmature somatic embryos) and the other half on maturation medium containing basal medium plus 3 mM cysteine, 50 mM glutamine, 3% sucrose, 0.5% casein hydrolysate, 1 μM abscisic acid (ABA), 1 μM napthaleneacetic acid (NAA) and 0.25% polyethylene glycol (PEG) at 21 d from the start of culture. All somatic embryos at the cotyledonary stage were removed carefully with a scalpel from the hypocotyl explant 35 d from the start of induction, placed in microcentrifuge tubes, and freeze dried for protein analysis.

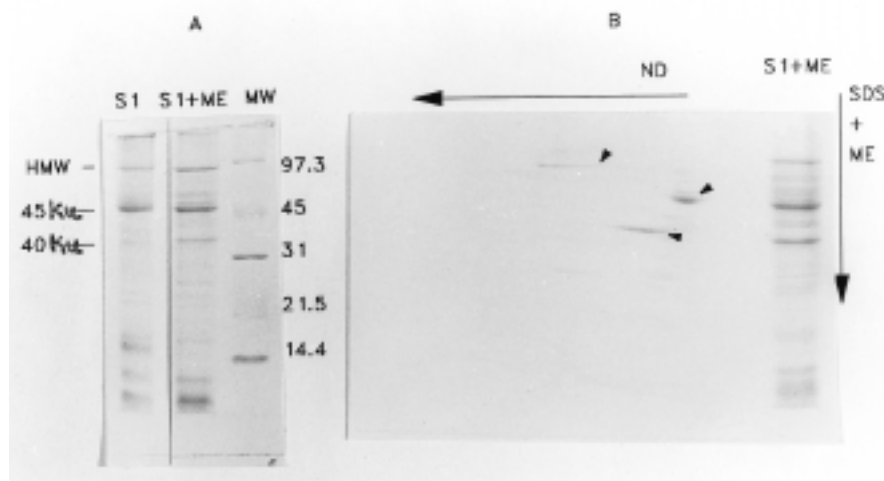SAMPLES. Seed coats were removed from mature dry seeds of

Fig. 1. (**A**) Effect of 5% ß-mercaptoethanol on S1 fraction proteins of geranium seeds. Reduced and nonreduced samples are adjacent to each other (SDS + ME; 16% T). Ten microliters of sample (5 µg protein) were loaded per lane. Molecular weight markers (MW) are included and marked. (**B**) Subunit analysis of S1 fraction proteins by 2-dimensional gel electrophoresis. First dimension (not shown): nondenaturing gel electrophoresis (ND-PAGE, 7% T) placed from top to bottom in the direction of the arrow. Second dimension: electrophoresis under denaturing conditions with a reducing agent (SDS-PAGE + ME, 16% T). The polypeptides are indicated as high molecular weight (HMW), 45 and 40 ku. Arrows indicate the position of the HMW, 45 ku and 40 ku polypeptides in the second-dimensional gel.

'Scarlet Orbit Improved' geranium. Freeze-dried zygotic embryos (60 mg) constituted a sample. A sample of the somatic embryos contained ≈50 to 60 mg dry weight of the cotyledon stage. A zygotic embryo weighed ≈3.5 to 4 mg whereas a somatic embryo weighed only 50 to 65 µg.

PROTEIN ANALYSIS. Zygotic embryo proteins were analyzed first before a comparison was made with somatic embryo proteins. Proteins were extracted from zygotic embryos using three sequential steps with minor modifications according to Krochko et al., (1992). Each sample was ground in 1 mL of buffer [NaCl at 0.05 $mol \cdot L^{-1}$ in 25 mM potassium phosphate buffer, pH 7.0 with protease inhibitors, 1 mM phenylmethylsulphonyl fluoride (PMSF), and 10 µM leupeptin] using a Duall ground glass homogenizer (Bio-Rad, Missisauga, Ontario, Canada). The supernatant (S1 proteins) was collected after centrifugation at 14,000 g_n for 5 min. The remaining pel-leted material was resuspended vigorously in 1 mL of buffer 2 (NaCl at 1.0 mol·L⁻¹ in 25 mM potassium phosphate buffer, pH 7.0, 1 mM PMSF, and 10 µM leupeptin) and the supernatant (S2 protein) was collected after centrifugation. The pellet was resuspended in Laem-mli's buffer (2% SDS, 10% glycerol, 62.5 mM Tris-HCL, pH 6.8) (Laemmli, 1970), centrifuged under the same conditions as above and the supernatant (S3 protein) was collected. Preliminary extractions and gels showed better separation of the

groups of proteins using these buffers. Protein quantification was carried out on all fractions using the bicinchoninic acid assay (BCA) (Smith et al., 1985). Somatic embryos contained small quantities of protein and necessitated extraction by smaller quantities of buffer. Extractions of protein for comparison was therefore carried out by 350 µL of buffer 2 for 60 mg samples of embryos. Hence, buffer 2 alone would extract proteins of buffer 1 plus buffer 2 for the zygotic system.

A mini-gel electrophoresis apparatus (Bio-Rad, Mississauga, Ontario, Canada) was used for all electrophoretic techniques. Protein extracts were analyzed by sodium dodecyl sulphate-poly-acrylamide gel electrophoresis (16% SDS-PAGE) with and without 5% ß-mercaptoethanol (ME) according to Krochko and Bewley (1988). Fifteen microliters of sample (≈7.5 µg protein) were loaded per lane. Two-dimensional (2-D) gel electrophoresis for S1 fraction protein (ND(nondenaturing)/SDS + ME) and for S2 fraction protein (SDS/SDS + ME) was also performed according to Krochko and Bewley (1988). After completion of electrophoresis on a first dimensional gel, a section of that gel was incubated in sample buffer containing SDS and ME. This was then electrophoresed into a second dimension gel with the direction of migration of polypeptides on this gel at right angles to the initial separation.

Proteins were stained with 0.1% Coomassie Brilliant Blue R250 in 5 methanol : 4 water : 1 acetic acid (by volume) and destained with 67 water : 25 ethanol : 8 acetic acid (v/v). After destaining, the gels were stored in 88 water : 7 acetic acid : 5 methanol (v/v) and photographed. Molecular weights of polypeptides were determined by comparing their migration distances with those of known marker proteins.

WESTERN BLOTTING. Antibodies raised in rabbits against the alfalfa (*Medicago sativa* L.) 11S storage protein and in chicken

Fig. 2. (**A**) One-dimensional gel electrophoresis of S2 fraction proteins (SDS-PAGE ± ME). Molecular mass markers (MW) are indicated. Acrylamide concentration used was 16% T. (**B**) Two-dimensional gel electrophoresis of S2 fraction proteins. First dimension: nonreduced S2 fraction protein (SDS only, 16% T) and second dimension: first dimension gel reduced in situ by ME. Direction of arrows indicate the dimensions of the gel. The reduced polypeptides of the 11S storage protein are shown.
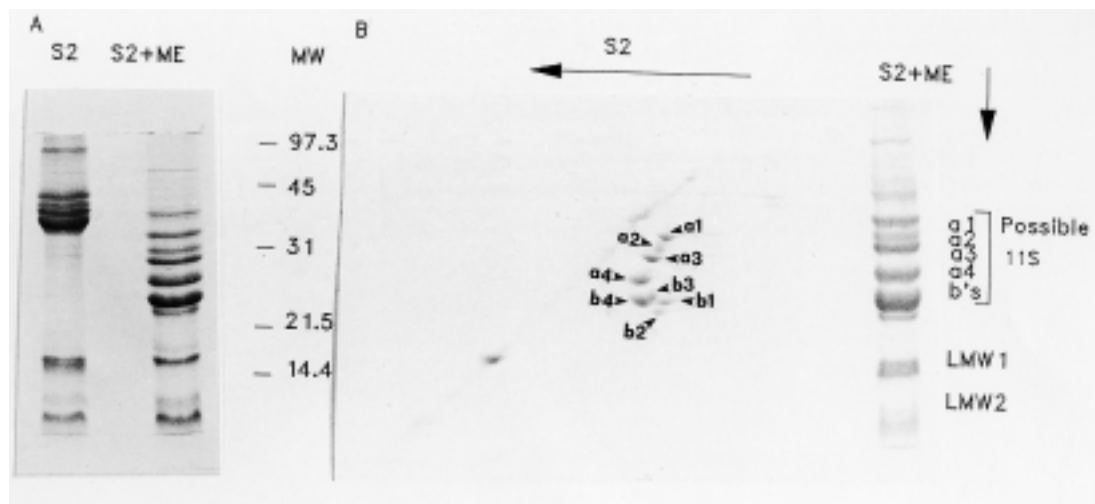
Table 1. Approximate subunit sizes of the putative 11S storage proteins in zygotic embryos of geranium expressed as ku.

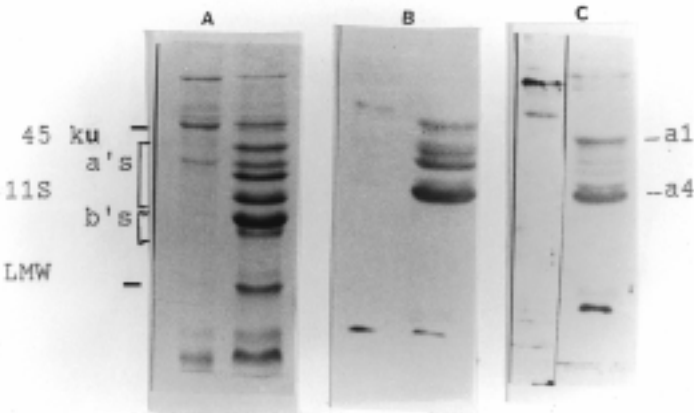| Acidic polypeptides	Basic polypeptides	Subunit A+B M_r
a1 44	b1 24	a1b1 68
a2 40.7	b2 21	a1b2 61.7
a3 40	b3 24	a3b3 64
a4 31	b4 24	a4b4 55

Fig. 3. Western blot analysis of S1 and S2 fraction proteins of zygotic embryos of geranium using the alfalfa 11S antibody raised against alfalfa 11S proteins. (**A**) S1 and S2 proteins reduced with 5% ME. Proteins were extracted sequentially using low salt concentration buffer and high salt concentration buffer 2. (**B**) S1 and S2 proteins detected with the 11S antibody raised against the alfalfa seed 11S storage proteins. (**C**) S1 and S2 fraction protein detected with the 7S antibody. Chemiluminescent detection of reactivity was carried out using the ECL Western Blot Kit.
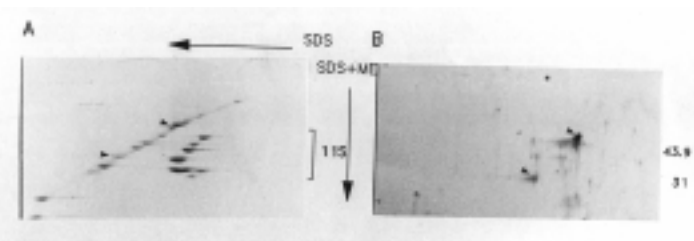

Fig. 4. Western blot analysis of S2 fraction protein in a two dimensional gel to determine the location of the 7S protein. (**A**) Two dimensional gel of S2 fraction protein. First dimension: SDS only, 16% T. Second dimension: SDS+ME, 16% T. (**B**) S2 fraction proteins detected with 7S antibody on a two dimensional gel (SDS/SDS + ME) shown by the markers. Chemiluminescent detection of reactivity was carried out using the ECL Western Blot Kit.

against the alfalfa 7S storage protein were used. After separating by SDS-PAGE, proteins were transferred onto nitrocellulose membranes using a Mini-Trans-Blot electrophoretic gel transfer cell (Bio-Rad) as described by Krochko and Bewley (1988). The dilution of the primary antibody was 1:1000. The secondary antibodies for the 11S and 7S antibodies were peroxidase-labelled antirabbit antibody and antichicken IgG peroxidase conjugate (Sigma), respectively. Chemiluminescent detection of reactivity was carried out using the ECL Western Blotting kit (Amersham, Oakville, Ontario, Canada) according to the manufacturer's instructions.

Results and Discussion

Seed specific storage proteins are synthesized naturally in seeds (Bewley and Black, 1982) and somatic embryos (Crouch, 1982).

Thus, this makes them suitable indicators of somatic embryo maturity.

Analysis of the zygotic S1 fraction revealed the presence of 40, 45 and 97.4 ku (HMW) proteins (Fig. 1A). The positions of the polypeptides on the gel were unchanged after treatment with ME indicating the component polypeptides of the S1 fraction proteins were not joined by disulphide bonds. The 2-D gel (ND/SDS-PAGE + ME) indicated these three major proteins of the S1 fraction from geranium seeds were distinct (Fig. 1B). It is not known whether these polypeptides are seed specific storage proteins.

The nonreduced zygotic embryo S2 fraction protein separated by electrophoresis into discrete bands ranging from 45 to 69 ku (Fig. 2A) and two prominent bands at 15.5 and 12.5 ku. After treatment with ME, the S2 fraction protein migrated as a group of polypeptides of 20 to 44 ku (Fig. 2A). The 15.5 and 12.5 ku storage proteins, appeared at the same positions in reduced and nonreduced gels (Fig. 2A).

The 2-D electrophoresis (SDS/SDS + ME) suggested the presence of reduced polypeptides belonging to a legumin-like storage protein complex (Fig. 2B). Although the charge of specific polypeptides was not determined, a group of four polypeptides ranging in molecular mass from 28 to 44 ku was designated the acidic polypeptides (a1 to a4), and the four polypeptides ranging in molecular mass from 21 to 24 ku were designated the basic polypeptides due to their sizes, specific vertical pairing (a_1b_1, a_2b_2, a_3b_3, and a_4b_4, Fig. 2B), and by analogy to protein bands in other species. The approximate molecular mass of these four subunits are presented in Table 1. The acidic polypeptides of the legumin-like storage protein reacted with the 11S antibody raised against the alfalfa 11S storage proteins (Fig. 3). This confirmed these proteins belong to the 11S class of seed storage proteins. This protein was named pelargin.

The 11S globulin appears to be the dominant protein of zygotic embryos of geranium, forming the bulk of the S2 fraction protein. Pelargin showed obvious similarities to the major 11S seed
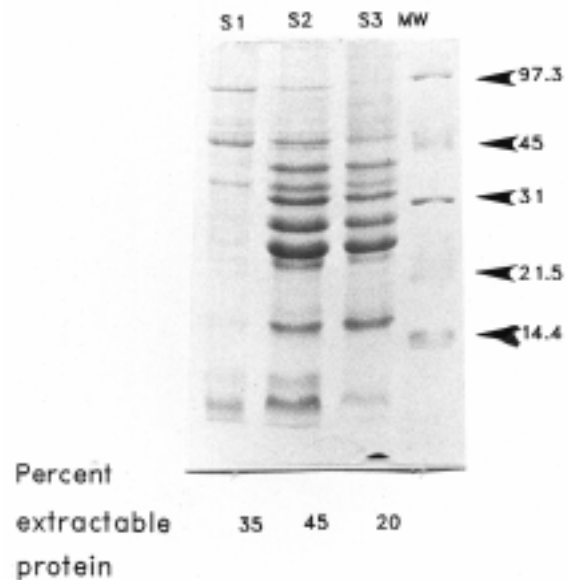

Fig. 5. S1, S2, and S3 proteins of zygotic embryos of geranium. Proteins were extracted from zygotic embryo samples sequentially with 1 mL of low salt concentration buffer followed by high salt concentration buffer and finally in Laemmli's buffer. One 10-µL (5-µg protein) sample was loaded per lane. All samples were reduced with 5% ß-mercaptoethanol. (SDS + ME; 16% T). The protein quantities expressed at the bottom of the gels were determined using the BCA assay. Values presented are means of three samples.
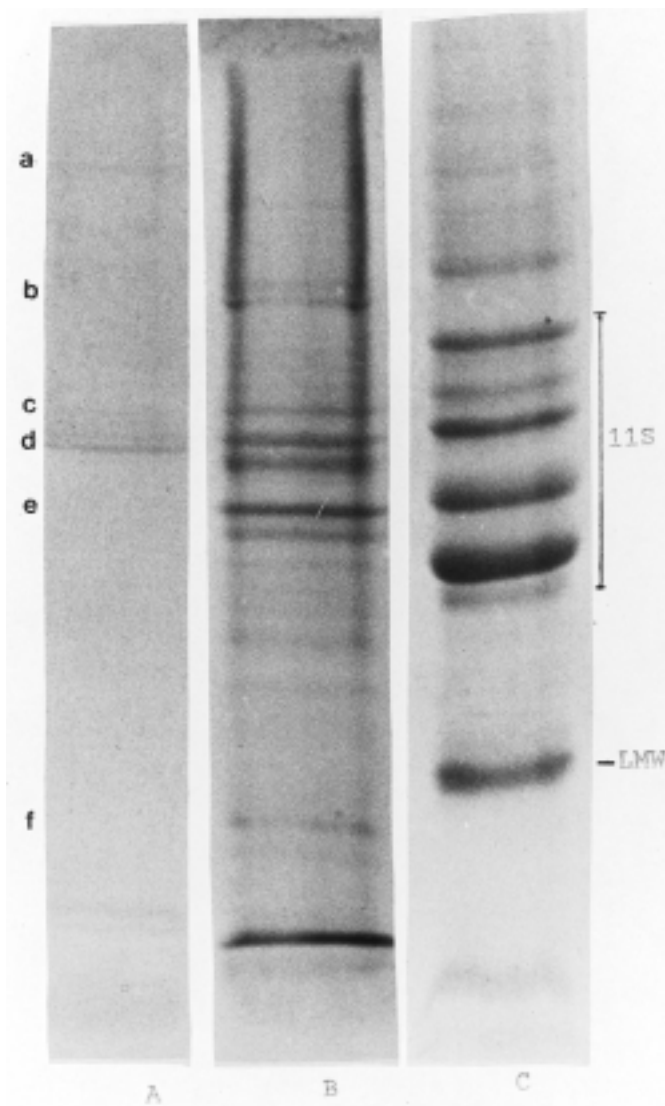
Fig. 6. One dimensional gel electrophoresis of proteins from zygotic and somatic embryos of geranium. (**A**) Protein from nonmatured somatic embryo, (**B**) protein from matured somatic embryo, and (**C**) protein from zygotic embryos. Proteins were extracted with high salt concentration phosphate buffer; 15 μL (7.5 μg protein) were loaded per lane.

globulins of other species, for example legumin in pea (*Pisum sativa* L.) (Thompson et al., 1978), glycinin in soybean (*Glycine max* L.) (Nielsen, 1985), and medicagin in alfalfa (Krochko and Bewley, 1988). Despite these similarities, considerable diversity exists among species in subunit molecular mass and the numbers and proportions of subunit variants (Barton et al., 1982; Matta et al., 1981a, 1981b). The 11S antibody reacted with the acidic polypeptides only, probably due to low transfer efficiency of the basic polypeptides. In

Table 2. Protein content in zygotic and somatic embryos of geranium. Values presented are means ± SE (n = 10).

Tissue	Protein content (μg·mg⁻¹ dry wt)
Zygotic embryo	179 a^z (6.7)
Matured somatic embryo	141 b (6.1)
Nonmatured somatic embryo	124 c (9.6)

^{z}Mean separation by LSD ($P < 0.05$).

alfalfa, the 11S antibody also reacted mainly with the acidic polypeptides (Krochko et al., 1992) possibly for the same reason.

The 7S antibody raised against alfalfa reacted with the 45 kD polypeptide of the S1 fraction proteins and also with two polypeptides at the same positions as the 11S acidic polypeptides (a_1 and a_4) in the S2 fraction (Fig. 3C) indicating either cross-reactivity of the 7S antibody with 11S proteins or that there are 7S proteins also located at the same positions as these 11S polypeptides. The 7S antibody reacted with proteins not on the diagonal (Fig. 4), therefore failing to establish the presence of 7S storage proteins. Generally, the 7S globulins are fashioned from a trimer of morphologically similar subunits (ranging from 50 to 70 kD depending on the species) held together by noncovalent bonds (Derbyshire et al., 1976). However, 7S storage proteins are less abundant compared to the 11S proteins and are more variable (Casey et al., 1986; Derbyshire et al., 1976).

Low molecular mass storage proteins occur in substantial quantities in seeds of other species such as pea, rape (*Brassica napus* L.), radish, and alfalfa (Croy et al., 1984, Ericson et al., 1986, Krochko and Bewley, 1988, Laroche et al., 1984). Unlike the geranium proteins, all the LMW proteins from the above species contain polypeptides joined by disulphide bonds. The LMW protein of geranium (15.5 ku) reacted with both the 11S and 7S antibody suggesting antigenic relatedness with both storage proteins.

The S1 and S2 fraction proteins constitute ≈80% of the total extractable protein from zygotic embryos of geranium and most of the storage proteins are in the S2 and S3 fraction (Fig. 5). The S3 fraction contained similar protein as the S2 fraction so all the work on somatic embryo proteins concentrated on the S2 fraction proteins.

Protein content was significantly increased in mature somatic embryos compared to nonmature controls and was ≈80% of the zygotic embryo protein level (Table 2). The major storage proteins (11S globulins and LMW proteins) found in zygotic embryos were absent in somatic embryos (Fig. 6). Two weak bands (a and d) of molecular weights 49 and 40 were detected from nonmature somatic embryos. The predominant protein was the 40 ku (d) polypeptide (Fig. 6). Maturation of somatic embryos improved the protein profiles with the protein in mature somatic embryos showing several polypeptides (b at ≈45 ku, c and d at 40 ku, e at 39 ku, and f at 24 ku). Two-dimensional gel electrophoresis (SDS/SDS+ME) was carried out on mature somatic embryo protein to determine whether there were disulphide bonded polypeptides and the distinct pairing of polypeptides similar to the 11S zygotic embryo storage proteins. This gel showed that most of the proteins remained on the diagonal except for one small polypeptide (a) (Fig. 7). Western blot analysis of the mature somatic embryo protein confirmed lack of 11S storage proteins (Fig. 8).

Lack of pelargin and LMW proteins in somatic embryos suggests a deficiency in the nutritional and/or regulatory systems necessary for storage protein synthesis. Addition of glutamine or inorganic compounds containing sulphur to the maturation medium of alfalfa improved the synthesis of medicagin and low molecular weight storage proteins (Lai, 1994) indicating that supply of both nitrogen and sulphur improves storage protein synthesis. In white spruce, somatic embryos cultured on low osmoticum lacked several major storage polypeptides while others were underexpressed (Joy et al., 1991; Misra et al., 1993). According to Misra et al., (1993), the crystalloid protein profile was similar to the protein profile of immature zygotic embryos. It is possible with geranium that the storage protein profile in the mature somatic embryos is the same as that in immature zygotic embryos.
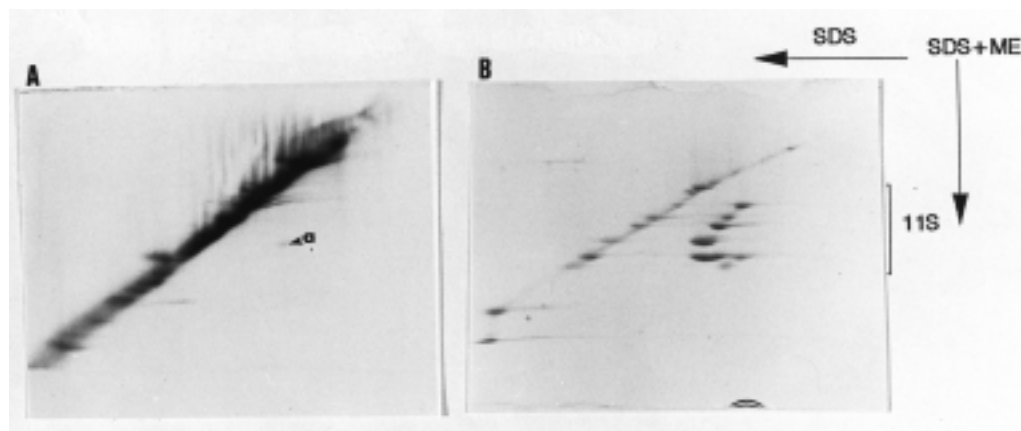
Fig. 7. Two dimensional gel electrophoresis (SDS/SDS+ME) of S2 fraction protein from (**A**) matured somatic embryos and (**B**) from zygotic embryos. First dimension gels were nonreduced protein (SDS, 16%T) and second dimension consisted of first dimension gel reduced in situ by ME (SDS + ME, 16% T). Directions of arrows indicate the dimensions of the gel. Protein labelled (a) in matured somatic embryo sample is a possible 11S polypeptide.
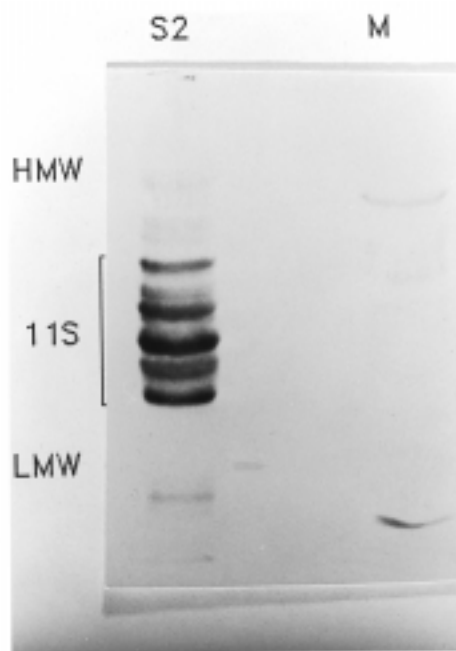

Fig. 8. Western blot analysis of S2 fraction proteins from zygotic embryos (S2), and matured somatic embryos (M) using the alfalfa 11S antibody raised against alfalfa 11S storage proteins. Chemiluminescent detection of reactivity was carried out using the ECL Western Blot Kit from Amersham Scientific.

Another reason for lower quantities of storage proteins in somatic embryos may be morphological because, unlike zygotic embryos, somatic embryos lack well developed cotyledons, and consequently storage proteins in somatic embryos are deposited in the embryonic axis (Xu and Bewley, 1992). Further studies to determine whether regulatory or processing blocks exist in somatic embryos accounting for the lack of seed-specific storage proteins are warranted.

In conclusion, zygotic embryos of geranium synthesized two major types of storage proteins: 11S (pelargin) and two LMW proteins. These proteins are soluble in a high salt concentration phosphate buffer. Somatic embryos did not contain the major storage proteins synthesized in zygotic embryos although maturation improved protein profiles.

Literature Cited

Barton, K.A., J.F. Thompson, J.T. Madison, R. Rosenthall, N.P. Jarvis, and R.N. Beachy. 1982. The biosynthesis and processing of high molecular weight precursors of soybean glycinin subunits. J. Biol. Chem. 257:6089–6095.

Bewley, J.D. and M. Black. 1982. Physiology and biochemistry of seeds. In relation to Germination. vol 2. Viability, dormancy and environmental control. Springer Verlag, New York. p. 224–255.

Bewley, J.D. and M. Black. 1994. Seeds: physiology of development and germination. 2nd ed. Plenum Press, New York.

Casey, R., C. Domoney, and N. Ellis. 1986. Legume storage proteins and their genes. Oxford Surveys Plant Mol. Cell Biol. 3:1–95.

Crouch, M.L. 1982. Non-zygote embryos of *Brassica napus* L. contain embryo-specific storage proteins. Planta 156:64–74.

Croy, R.R.D., M.S. Hoque, J.A. Gatehouse, and D. Boulter. 1984. The major albumin proteins from pea (*Pisum sativum* L.): Purification and some properties. Biochem. J. 218:795–803.

Derbyshire, E., D.J. Wright, and D. Boulter. 1976. Legumin and vicilin storage proteins of legume seeds. Phytochemistry 15:3–24.

Ericson, M.L., J. Rodin, J. Lenman, K. Glimelius, J.G. Josefsen, and L. Rask. 1986. Structure of the rapeseed 7S storage protein, napin, and its precursor. J. Biol. Chem. 261:14576–14581.

Gill, R., T. Senaratna, and P.K. Saxena. 1994. Thidiazuron-induced somatic embryogenesis enhances viability of hydrogel-encapsulated somatic embryos of geranium. J. Plant Physiol. 143:726–729.

Hutchinson, M.J. and P.K. Saxena. 1995. Acetylsalicylic acid enhances and synchronizes thidiazuron-induced somatic embryogenesis in geranium tissue cultures. Plant Cell Rpt. 15:512–515.

Joy, R.W., I.W. Edward, C. Yeung, L. Kong, and T.A. Thorpe. 1991. Development of white spruce somatic embryos I. Storage product deposition. In Vitro Cellular Dev. Bio. 27P:32–41.

Krochko, J.E. and J.D. Bewley. 1988. Use of electrophoretic techniques in determining the composition of seed storage proteins in alfalfa. Electrophoresis 9:751–763.

Krochko, J.E., S.K. Pramanik, and J.D. Bewley. 1992. Contrasting storage protein synthesis and messenger RNA accumulation during development of zygotic and somatic embryos of alfalfa (*Medicago sativa* L.). Plant Physiol. 99:46–53.

Lai, F.M. 1994. Maturation and conversion of alfalfa somatic embryos. PhD Diss., Univ. of Guelph, Guelph, Ontario, Canada.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680–685.

Laroche, M., L. Aspart, L. Delseny, and P. Penon. 1984. Characterization of radish (*Raphanus sativus*) storage proteins. Plant Physiol. 74:487–493.

Marsolais, A.A., D.P.M. Wilson, M.J. Tsujita, and T. Senaratna. 1991. Somatic embryogenesis and artificial seed production in zonal (*Pelargonium x hortorum* Bailey) and regal (*Pelargonium ×domesticum* Bailey) geraniums. Can. J. Bot. 69:1188–1193.

Matta, N.K., J.A. Gatehouse, and D. Boulter. 1981a. Molecular and subunit heterogeneity of legumin of *Pisum sativum* L.: A multidimensional gel electrophoretic study. Can. J. Bot. 32:1295–1307.

Matta, N.K., J.A. Gatehouse, and D. Boulter. 1981b. The structure of legumin of *Vicia faba* L.: A reappraisal. J. Expt. Bot. 32:183–197.

Misra, S., S.A. Attree, I. Leal, and L.C. Fowke. 1993. Effect of abscisic acid, osmoticum and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. Ann. Bot. 71:11–22.

Murray, D.R. 1979. A storage role for albumins in pea cotyledons. Plant Cell Environ. 2:221–226.

Nielsen, N.C. 1985. The structure and complexity of the 11S polypeptides in soybeans. J. Biol. Chem. 250:4007–4021.

Shoemaker, R.C., C.I. Christofferson, and D.W. Galbraith. 1987. Storage protein accumulation patterns in somatic embryos of cotton (*Gossypium hirsutum* L. Merr). Plant Cell Rpt. 6:12–15.

Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, and D.C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76–85.

Stuart, D.A. and J. Nichol. 1988. Isolation and characterization of alfalfa 7S and 11S seed storage protein. J. Plant Physiol. 132:129–133.

Thompson, J.A., H.E. Schroeder, and W.F. Dudman. 1978. Cotyledonary storage proteins in *Pisum sativum* I: Molecular heterogeneity. Austral. J. Plant Physiol. 5:263–279.

Xu, N. and J.D. Bewley. 1992. Contrasting patterns of somatic and zygotic embryo development in alfalfa as revealed by scanning electron microscopy. Plant Cell Rpt. 11:279–284.

J. Amer. Soc. Hort. Sci. 125(4):525–529. 2000.

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