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QUALITATIVE AND QUANTITATIVE CHANGES IN PROTEINS IN *ACER PLATANOIDES* L. SEEDS DURING MATURATION

TOMASZ PAWŁOWSKI[1*] AND ANDRZEJ KALINOWSKI[2]

[1]*Institute of Dendrology, Polish Academy of Sciences, ul. Parkowa 5, 62-035 Kórnik, Poland*
[2]*Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszyńska 34, 61-479 Poznań, Poland*

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Maturation of Norway maple (*Acer platanoides* L.) seeds produces deep physiological dormancy and resistance to desiccation. This study used two-dimensional electrophoresis to investigate the protein products of genes activated during the complex developmental process of maturation. Qualitative and quantitative changes in protein composition during maturation were tracked in this species. The most intensive changes in protein content appeared at the end of seed maturation, in embryo axes and cotyledons. During this time their protein content increased significantly and new proteins appeared. Presumably the proteins Q (15 kDa, pI 8) and X (16 kDa, pI 5) separated from cotyledons are associated with maturation of seeds.

Key words: *Acer platanoides*, Norway maple, desiccation, development, seed dormancy, trees.

INTRODUCTION

Seed development represents a unique transition state in the life cycle of higher plants, providing the physiological link between parental and progeny sporophytic generation. Maturation events prepare the seeds for the germination and subsequent development of the plant. During maturation the developing seed dramatically increases in volume and mass due to cell expansion and concomitant accumulation and storage of proteins, carbohydrates and/or lipids to be used as nitrogen and carbon sources during germination and growth. Maturation also involves physiological adaptations that occur within the seed to ensure embryo dormancy.

Characterization of gene expression during embryo development, maturation and germination has led to the identification of distinct subsets of developmentally regulated genes (Dure, 1985; Goldberg et al., 1989; Kermode et al., 1989; Thomas, 1993; Misra, 1994). Of the many genes activated during maturation only a few are associated with seed development processes (Goldberg et al., 1989; Croissant-Sych and Okita, 1996; Gallardo et al., 2001). The products of these genes can be divided into five major subsets: constitutive proteins, embryo-specific proteins, early embryogenesis proteins, storage proteins and late embryogenesis abundant (LEA) proteins (Dure, 1985). One subclass of LEA proteins consists of dehydrins, whose synthesis is associated with seed resistance to desiccation (Dure et al., 1989). Pelah et al. (1997) isolated from *Populus tremula* seeds two dehydrins: BspA and DSP16. Walker-Simmons (1987) observed that protein kinases are associated with maturation. Cost et al. (1996) isolated from *Lupinus albus* seeds two proteins associated with its development. Colorado et al. (1995) investigated the expression of genes controlled by ABA. They observed proteins similar to LEA proteins, which appeared in the late stage of maturation of

* e-mail: tapawlow@man.poznan.pl

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chick-pea seeds and disappeared after hydration. Dodeman et al. (1998) isolated two proteins associated with maturation from *Daucus carota* seeds: daucin (storage protein) and RAB25 (LEA protein).

Finch-Savage et al. (1994) found dehydrins in seeds of *Acer platanoides* (desiccation-tolerant – orthodox) and *A, pseudoplatanus* (desiccation-sensitive – recalcitrant); they suggested that the presence of dehydrins in these seeds is not associated with the difference in tolerance to desiccation. Gee et al. (1998) made similar findings in two other *Acer* species: *A. saccharinum* (recalcitrant) and *A. rubrum* (orthodox).

Dormant seeds synthesize inhibitory proteins, which disappear after breaking dormancy (Kermode and Bewley, 1985; Misra and Bewley, 1985). Protein SBP65 (LEA protein) isolated by Dehaye et al. (1997) from peas is responsible for keeping the seeds dormant. Other proteins associated with dormancy include a glycine-rich protein isolated from *Fagus sylvatica* seeds (Nicolas et al., 1996, 1997), nuclear protein QP47 isolated from *Pisum sativum* (Chiatante and Onelli, 1993; Baker et al., 1995), proteins isolated from *Acer saccharum* (Hance and Bevington, 1992) and protein from *Pinus taeda* seeds (Schneider and Gifford, 1994). These proteins disappear under conditions that break dormancy. Proteins from the LEA group disappeared after imbibition (Yeoung et al., 1989; Blackman et al., 1995).

Maturation of *Acer platanoides* seeds induces deep physiological dormancy in the embryo. During acquisition of dormancy, *A. platanoides* seeds gain tolerance to desiccation (Pukacka, 1998; Pukacka and Wojkiewicz, 2001) and afterwards can be dehydrated to 7% water content (Hong and Ellis, 1992). To break deep dormancy, *A. platanoides* seeds need to be imbibed and stratified in cold (2–5˚C) for about 12 weeks.

In our earlier publications (Pawłowski and Szczotka, 1997, 2001; Pawłowski et al., 1997) we described qualitative and quantitative changes of protein synthesis in *A. platanoides* seeds during dormancy breaking.

Here we report qualitative and quantitative changes in the protein content of *A. platanoides* seed embryo axes and cotyledons in earlier seed development, that is, during seed maturation.

MATERIALS AND METHODS

Maple tree seeds were collected in the Kórnik Arboretum during development from the 14th week after flowering (embryos fully grown) to late maturation (seeds in deep dormancy) at the 21st week after flowering. After collection, the embryo axes and cotyledons were isolated, frozen and stored in test tubes at -70˚C.

Protein extract for two–dimensional electrophoresis was prepared from 2 g samples of embryo axes or cotyledons homogenized in liquid nitrogen in a mortar. The homogenate was thawed in a centrifuge tube and extracted with 10 ml buffer containing 0.5 M sucrose, 50 mM TRIS-HCl (pH 8.0), 50 mM KCl, 5 mM magnesium acetate, 10 mM 2-mercaptoethanol, 1 mM PMSF (phenylmethylsulfonyl fluoride), 0.1 μM pepstatin and 2.1 μM leupeptin. After centrifugation (15 krpm, 15 min, 4˚C), ammonium sulphate was added to the supernatant (651g/l), and this mixture was dialyzed in buffer containing 9 M urea, 25 mM TRIS-HCl (pH 7.3), 50 mM KCl, 3 mM EDTA, 70 mM DTT (dithiothreitol) and 2% WITAlytes carrier ampholytes (pH 2–11) (WITA, Teltow, Germany). The concentrations of the resulting protein ranged from 0.5 to 1.5 mg/ml as determined by a modification (0.1N HCl was added to remove excess urea) of Bradford's (1976) method.

Two-dimensional electrophoresis was performed by combining isoelectric focusing (IEF; first dimension) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; second dimension), as developed by Klose (1983). The IEF gels contained 4% w/v WITAlytes. Samples containing 10 μg protein were applied to the anodic side of each IEF gel. After IEF, the gels were equilibrated for 10 min in buffer containing 125 mM TRIS-phosphate (pH 6.80), 40% glycerol, 70 mM DTT and 3% SDS. The IEF gel was applied to the SDS-PAGE gel, obtained by polymerization of 15% w/v acrylamide and 0.4% bisacrylamide. The SDS-PAGE system of Laemmli (1970) was used. The separated proteins were later silver-stained according to Heukeshoven and Dernick (1985). The protein patterns were analyzed for qualitative and quantitative changes. The experiments were performed in two replicates.

RESULTS AND DISCUSSION

Flowering of Norway maple (*Acer platanoides* L.) trees usually starts at the end of April and lasts two weeks (Pukacka, 1998). Five weeks later the fruits reach maximum size. Embryo development is relatively slow in Norway maple. The developing embryo (size 0.5–1 mm) can be distinguished with the naked eye only at the 8th week after flowering. Embryo growth lasts to week 14. The embryo acquires the

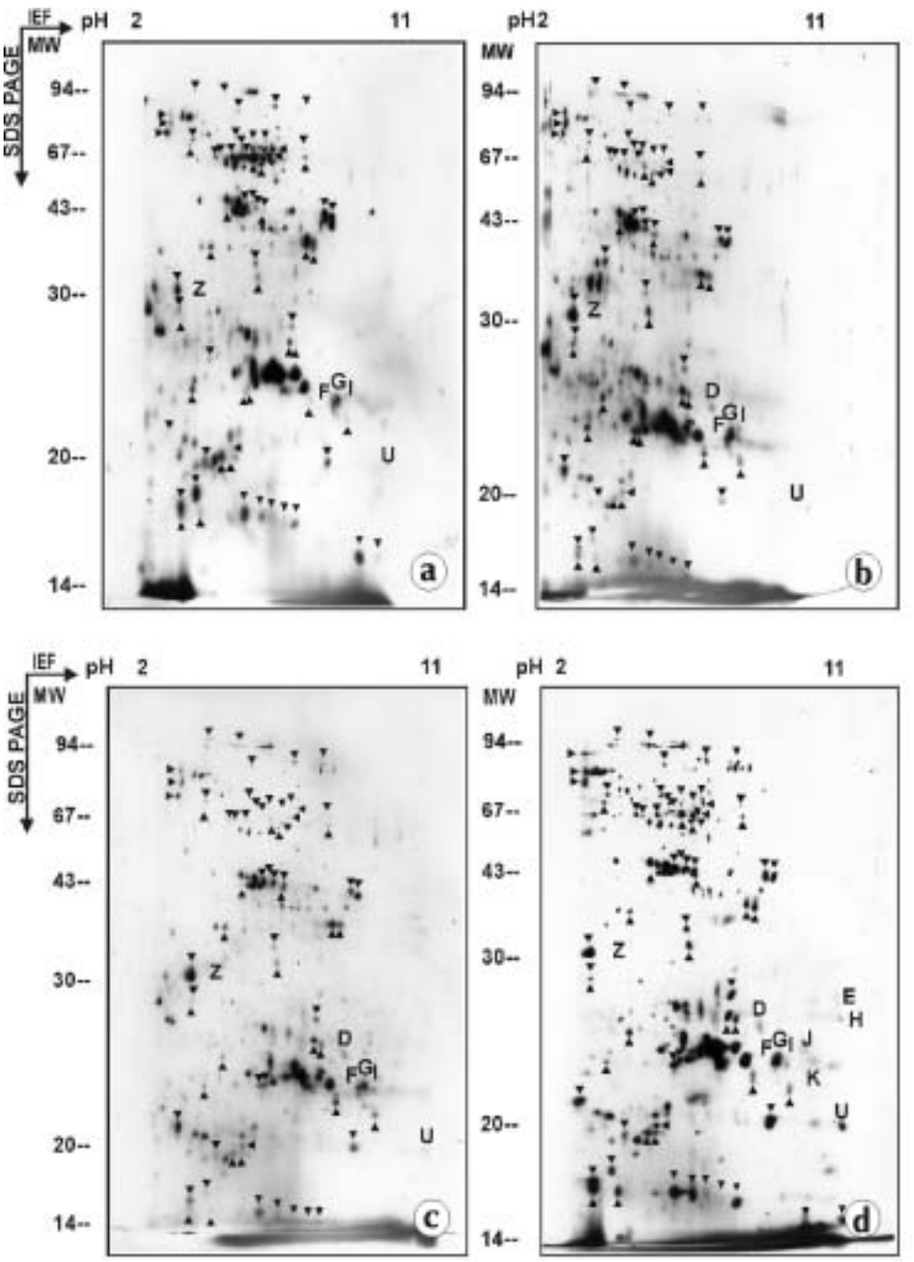

Fig. 1. Comparison of 2-DE protein patterns from embryo axes of *Acer platanoides* seeds at (**a**) 14, (**b**) 16, (**c**) 18 and (**d**) 21 weeks after flowering. Quantitative changes of proteins during maturation are marked with arrowheads, qualitative changes with letters.

maximum fresh and dry weight (maximum reserve deposition) 18 weeks after flowering. Seeds are fully mature and in deep dormancy 3 weeks later.

Qualitative analysis of proteins from embryo axes of Norway maple, referenced to week 14 (Fig. 1), showed changes in the protein pattern during maturation. At week 16 after flowering a new protein appeared (marked D, molecular weight ~26 kDa, pI 8). This protein was also present in the following weeks of maturation (weeks 18 and 21). At week 21

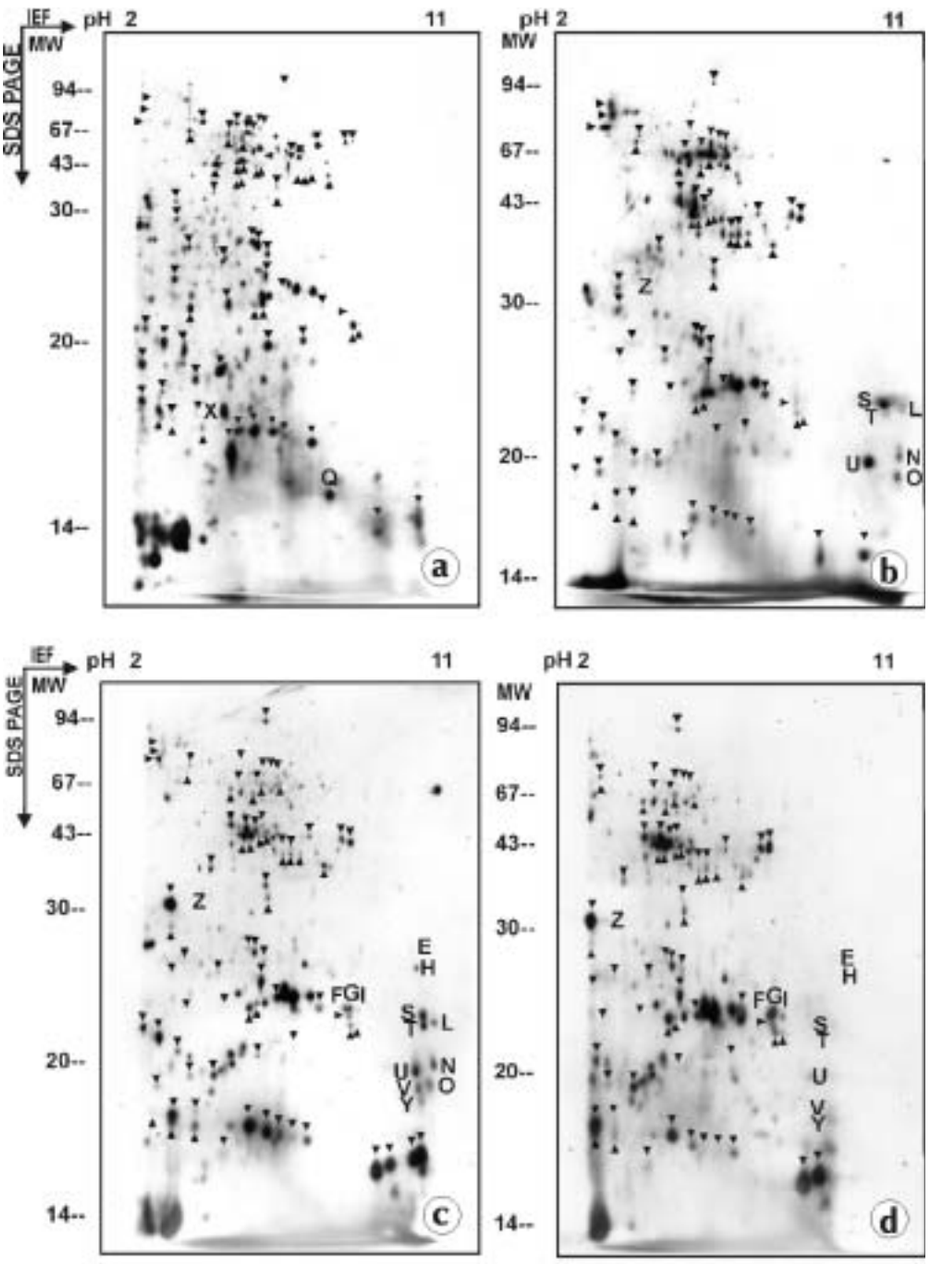

Fig. 2. Comparison of 2-DE protein patterns from cotyledons of *Acer platanoides* seeds at (**a**) 14, (**b**) 16, (**c**) 18 and (**d**) 21 weeks after flowering. Quantitative changes of proteins during maturation are marked with arrowheads, qualitative changes with letters.

a few new proteins appeared: E (28 kDa, pI 10.5), H (27 kDa, pI 10.5), J (24 kDa, pI 10) and K (24 kDa, pI 10).

In cotyledons (Fig. 2) at week 14 after flowering 2 proteins were observed: Q (15 kDa, pI 8) and X (16 kDa, pI 5). These were not present in cotyledons or embryo axes in the following weeks of maturation, nor during dormancy breaking caused by cold stratification (Pawłowski and Szczotka, 1997, 2001). At week 16 after flowering new proteins were observed:

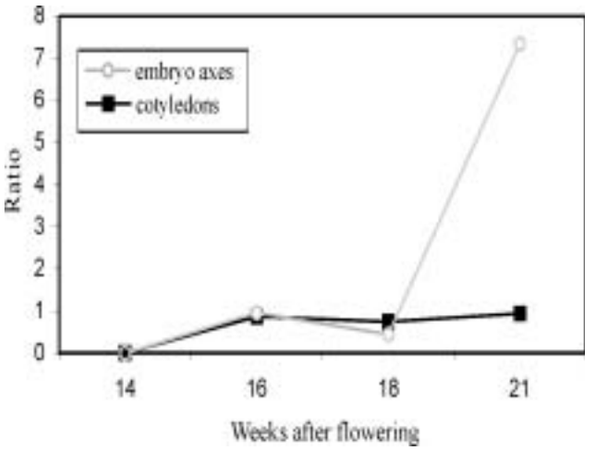

Fig. 3. Dynamics of quantitative changes of proteins in embryo axes and cotyledons during maturation of *Acer platanoides* seeds. Curves show the ratio of the number of proteins whose quantity increased to the number of proteins whose quantity decreased.

group of proteins marked Z (36 kDa, pI 4–5), protein L (22 kDa, pI 11), N (20 kDa, pI 11), O (19 kDa, pI 11), S (22.5 kDa, pI 10.5), T (22 kDa, pI 10.5) and U (20 kDa, pI 10.5). At week 18 these proteins were still present and more new proteins appeared: F, G, I (MW ~25 kDa and pI 8.5–9), V (19 kDa, pI 10.5), Y (18 kDa, pI 10.5), E and H. Proteins E and H appeared in embryo axes only at week 21 (Fig. 1). Proteins F, G, I, U and Z were present in embryo axes throughout maturation. Proteins V and Y were found only in cotyledons. At week 21 after flowering, proteins L, N and O were not found in cotyledons.

Quantitative changes were noted in 68 proteins in embryo axes and 77 in cotyledons (Fig. 3). In embryo axes at week 16 after flowering the number of proteins whose quantity increased was twice the number of proteins whose quantity decreased. At week 18 after flowering this ratio decreased by about half. In mature seeds (21 weeks after flowering) the number of proteins whose quantity increased was several times higher than the number of proteins whose quantity decreased. In cotyledons this ratio remained on the same level during maturation.

The most dynamic quantitative changes were observed in two groups of embryo axis proteins: the first, molecular mass ~67 kDa and pI 4–7, and the second, molecular mass ~43 kDa and pI 3–6 (Fig. 1). More quantitative changes were observed in proteins in cotyledons at week 14 after flowering than in subsequent weeks of seed maturation.

In embryo axes the most dynamic quantitative changes were observed at the end of maturation, when a significantly higher number of proteins increased in amount and new proteins appeared (E, H, J, K). In cotyledons the highest number of new proteins was observed at the end of maturation. At that time the seeds reach their maximum fresh and dry weight and resistance to desiccation (Pukacka, 1998). Such changes testify to intensive synthesis of the proteins accompanying seed maturation.

All new proteins (except Q and X) present in embryo axes and cotyledons of mature seeds were also observed in dormant dry seeds and during cold stratification (Pawłowski and Szczotka, 1997, 2001). Two proteins observed during maturation in cotyledons (V, Y) disappeared after desiccation but appeared again during stratification (Pawłowski and Szczotka, 1997, 2001).

Comparison of the protein patterns from mature and stratified *Acer platanoides* seeds (Pawłowski and Szczotka, 1997, 2001) indicates that some of the proteins found in mature seeds may be involved in maintenance of dormancy. In the literature there are descriptions of this kind of protein, for example glycine-rich protein encoded by *lea* genes isolated from *Fagus sylvatica* seeds (Nicolas et al., 1996, 1997), protein SBP65 (Dehaye et al., 1997) and nuclear protein QP47 isolated from *Pisum sativum* (Chiatante and Onelli, 1993), and unnamed proteins isolated from *Acer saccharum* cotyledons (Hance and Bevington, 1992) and *Pinus taeda* seeds (Schneider and Gifford, 1994). These proteins tend to disappear under conditions breaking seed dormancy.

In this study the most intensive qualitative and quantitative changes in protein content appeared at the end of seed maturation in *Acer platanoides*. Proteins Q (15 kDa, pI 8) and X (16 kDa, pI 5) isolated from cotyledons probably are associated with seed maturation.

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