THE EFFECT OF DESICCATION ON VIABILITY AND MEMBRANE LIPID COMPOSITION OF *ACER PSEUDOPLATANUS* SEEDS

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ABSTRACT

The viability of desiccation-intolerant sycamore (*Acer pseudoplatanus* L.) seeds during desiccation was investigated by tetrazolium and by a germinability test, together with membrane permeability and membrane phospholipid composition. Loss of viability was associated with an increase of solute leakage, reduced content of all phospholipid groups, decrease of unsaturated fatty acids and the unsaturated/saturated fatty acids ratio. Growth of malondialdehyde content was also observed. Some results were compared with those for tolerant to desiccation Norway maple (*Acer platanoides* L.) seeds. The results indicate active participation of membranes in the desiccation process in tolerant seeds and their decomposition in intolerant ones. The destruction of membranes was the result of lipid peroxidation, probably due to the free radical effect.

KEY WORDS: *Acer pseudoplatanus*, *Acer platanoides*, dehydration, membranes, electrolyte leakage, fatty acids, phospholipid, lipid peroxidation, malondialdehyde.

INTRODUCTION

In contrast to other plant tissues, seeds of many plant species possess the ability to withstand considerable desiccation. Roberts (1973) introduced the terms "orthodox and recalcitrant" to distinguish seed storage behaviour. Orthodox seeds are tolerant to desiccation and their longevity increases with the decrease of moisture content and storage temperature. On the other hand, recalcitrant seeds are characterized by inability to withstand desiccation below a relatively high water content in seed tissues. Such seeds cannot be maintained in nothing but short term storage. Cellular membranes are among the primary sites of injury during desiccation of intolerant seeds or other plant organs. This is mediated by an oxidative attack which promotes phospholipid degradation and loss of membrane organisation (Leprince et al. 1993, Pukacka 1989, 1998). Pukacka (1993) found that besides free radicals, phospholipase D was also involved in membrane deterioration of recalcitrant silver maple seeds during desiccation.

Within genus *Acer* there are species with seeds tolerant to desiccation (*A. platanoides*, *A. saccharum*, *A. campestre*) as well as species with recalcitrant seeds (*A. saccharinum*, *A. pseudoplatanus*). The experiments of Hong and Ellis (1990) and Dickie et al. (1991) confirmed the sensitivity of sycamore (*A. pseudoplatanus*) seeds to desiccation. After desiccation to 30% water content, they can be stored at -3°C no longer than two years (Tylkowski 1998). On the other hand, Norway maple (*A. platanoides*) seeds can be dried even to 7% of water content without loss their viability. With regard to maturation time and dormancy after dispersal, sycamore and Norway maple seeds are similar. However, during maturation

sycamore seeds remain hydrated, while Norway maple seeds can dry on tree even to 25% of water content (Pukacka, unpublished data).

In the present study, the effect of desiccation on viability of sycamore seeds, was measured according to ISTA rules with a TTC test and as germination capacity. Simultaneously electrolyte leakage and membrane lipid components were determined. The level of the lipid peroxidation product, malondialdehyde (MDA), in desiccated seeds was also established. Some results were compared to those received for Norway maple seeds, which are tolerant to desiccation. The mechanisms of membrane deterioration are discussed.

MATERIAL AND METHODS

Plant material

Mature fruits of sycamore (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.) were collected on 15th October 1997 from trees grown in the Kórnik Arboretum. Seeds of sycamore contained 60.1% of water content, whereas Norway maple seeds had 27.1% of water content.

Sycamore seeds were deprived of samaras and desiccated on paper rings placed on silica gel in Petri dishes at room temperature. Desiccated samples included seeds for analyses and seeds for determination of water content.

Whole Norway maple seeds were desiccated at room temperature, placed in a monolayer on a table for 5 days.

Water content was determined after drying seed samples at 90°C for 24 hours.

Viability tests

Viability of desiccated seeds was measured by tetrazolium (TTC) test according to ISTA rules (Anonymous 1976) and by the germinability test, subjecting seed samples after desiccation to cold stratification at 3°C and full moisture, according to Pukacka and Pukacki (1997).

Electrolyte leakage

Three samples of 10 seeds were placed in 10 ml bidistilled water for 24 hours at room temperature. The conductance was measured as described by Pukacka (1989). Percentage electrolyte leakage was expressed as $[Co/(Co+C_1)] \times 100$, where Co is the conductance after 24 h incubation and C₁ the conductance after boiling seeds at 100°C.

Phospholipid analysis

Three samples of 20 seeds were subjected to lipid extraction using chloroform: methanol (2:1,v/v) containing 0.05% butylated hydroxytoluene (BHT) according to Allen et al. (1966) as described by Pukacka (1983). Aliquots of lipid extracts were separated on SEP-PAK silica cartridge (Waters Associates) according to Juaneda and Rocquelin (1985) and phospholipid fractions were obtained. They were dissolved in chloroform and stored at -20°C until use. Particular phospholipids were determined in aliquots of phospholipid fractions by separation on silica gel TLC plates in the chloroform:methanol: acetic acid: water (85:15:10:3.5, v/v/v/v) solvent (Nicols et al. 1965), using original phospholipid standards. Spots containing phospholipids were detected with iodine vapour and were scraped off for phosphorus analysis. The phosphorus content in spots was estimated according to Ames (1966). Fatty acids were determined in aliquots of phospholipid fractions. Samples with heptadecanoic acid (17:0) as the internal standard were saponified and methylated according to Metcalfe et al. (1966). They were separated using a Hewlett Packard gas chromatograph equipped with a Silar 10C glass column.

MDA determination

The level of lipid peroxidation in desiccated seed tissue was measured as MDA content determined by reaction with thiobarbituric acid (TBA) according to Heath and Packer (1968). Three samples of 10 seeds were homogenized in 5 ml 5% TCA. The homogenate was centrifuged at 10 000 x g for 15 min. To 1 ml aliquot of supernatant 4 ml 20% TCA containing 0.5% TBA was added. Samples were heated at 95°C for 30 min and then cooled in an ice-bath. The absorbance was measured at 532 nm (specific absorption) and 600 nm (non-specific absorption). From the difference of the absorbances and the extinction coefficient of 155 nM⁻¹cm⁻¹, the concentration of MDA was calculated (Heath and Packer 1968).

RESULTS AND DISCUSSION

The pericarp of sycamore seeds after maturation is very thick and renders loss of water inefficient. It must be removed before seed desiccation. Desiccation of sycamore seeds on silica gel, from 60 to 10% of water content lasted 60 hours. The viability of seeds was maintained at a high level till 30% of water content. This was indicated by TTC as well as by germinability tests (Figs 1 and 2). After desiccation below 27% of water content, the viability of seeds declined. The decrease of seed germinability was very sharp (Fig. 2). Decrease of germinability of seeds below 27% of water con-

Fig. 1. Viability of *Acer pseudoplatanus* seeds determined by the tetrazolium test, during desiccation on silica gel. Bars represent SD of five replicates.

Fig. 2. Germinability of *A. pseudoplatanus* seeds, at 3°C and full moisture content, previously desiccated to different water content on silica gel. Bars represent SD of three replicates.

Fig. 3. Electrolyte leakage of *A. pseudoplatanus* seeds after desiccation to different water content. Bars represent SD of three replicates.

Fig. 4. Phospholipid composition of *A. pseudoplatanus* (A) and *A. platanoides* (B) seeds desiccated to different water content (%). PI – phosphatidylinositol, PC – phosphatidylcholine, PG – phosphatidylglycerol, PE – phosphatidylethanolamine, PA – phosphatidic acid. Bars represent SD of three replicates.

tent corresponds with the presence of unfreezable water in tissues (Pukacka and Pukacki 1997). As was stated by these authors using DTA analysis, a low temperature exotherm disappeared in sycamore seeds between 28-26% of moisture content. Increase of electrolyte leakage has been observed when seeds showed 30% moisture content or less (Fig. 3). Correlation between electrolyte leakage and seed viability suggests that destruction of membranes is the probable cause of decrease of seed germinability. The role of lipid peroxidation in degradative and destructive processes of membranes has been discussed in many excellent reviews concerned with the ageing process of seeds (Wilson and McDonald 1986, Hendry 1993). The studies of Senaratna et al. (1985a, b) and Senaratna and McKersie (1986) on the nature of the damage to cell membranes in germinated soybean seeds during desiccation established the important role of free radicals in this process. Free radicals can induce lipid peroxidation (Fujimoto et al. 1984) and phospholipid deesterification (Niehaus 1978, Senaratna et al. 1985a) resulting in the loss of phospholipids and accumulation of free fatty acids. Also the role of oxidative stress as a primary component of desiccation injury in recalcitrant seeds has been established by Hendry et al. (1992) for *Aesculus hippocastanum* L., by Wood et al. (1995) for *Quercus robur* L. and *Quercus rubra* L. and by Pukacka (1989, 1998) for *Acer saccharinum* L. In the present paper, phospholipid analyses showed a marked decrease of all particular phospholipids during desiccation from 60 to 12.3% of water content of sycamore seeds. (Fig. 4A), while in orthodox

Fig. 5. Malondialdehyde (MDA) content in *A. pseudoplatanus* seeds during desiccation. Bars represent SD of three replicates.

Norway maple seeds desiccation leads to an increase of main phospholipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and no change the rest (Fig. 4B). Changes were also observed in the saturation of fatty acids of the phospholipid fraction of sycamore seeds. During desicca-

TABLE 1. Fatty acid composition (mg g⁻¹DW) (mean ±SD, n=3) of phospholipid fraction during desiccation of *Acer platanoides* and *Acer pseudoplatanus* seeds

Water content (%)	16:0	18:0	18:1	18:2	18:3	Unsat/Sat
<i>Acer pseudoplatanus</i>						
60.1	1.19±0.06	0.16±0.01	0.81±0.03	3.06±0.3	0.50±0.09	3.2
20.1	1.19±0.19	0.18±0.01	0.70±0.06	2.51±0.16	0.34±0.05	2.6
12.3	1.04±0.18	0.15±0.03	0.57±0.01	2.19±0.24	0.34±0.06	2.6
<i>Acer platanoides</i>						
27.4	1.19±0.06	0.14±0.08	0.80±0.06	2.99±0.16	0.28±0.02	3.1
10.5	1.25±0.08	0.17±0.01	0.92±0.1	3.24±0.4	0.33±0.04	3.2

tion unsaturated fatty acids and the ratio of unsaturated/saturated decreased (Table 1). Norway maple seeds showed an increase of unsaturated fatty acids and unchanged unsaturated/saturated ratio. Changes in membrane lipid composition in Norway maple seeds (Fig. 4B and Table 1) indicate active participation of membrane structures in the desiccation process of orthodox seeds. Peroxidation of fatty acids in sycamore seeds during desiccation was confirmed by the increased level of MDA in tissues (Fig. 5). This increase is sharper after dehydration of seeds to 10% of water content.

The presented results indicate that the decrease of viability of sycamore seeds during desiccation is connected with membrane deterioration, as a result of lipid peroxidation, mediated probably by a free radical mechanism.

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WPLYW ODWODNIENIA NA ŻYWOTNOŚĆ I KOMPOZYCJĘ LIPIDÓW BŁON CYTOPLAZMATYCZNYCH NASION *ACER PSEUDOPLATANUS*

STRESZCZENIE

Podczas odwadniania nasion jaworu (*A. pseudoplatanus* L.) typu rekalcitrant, badano ich żywotność za pomocą testu tetrazolowego (TTC) i zdolności kiełkowania, jak również przepuszczalność i kompozycję lipidów błon cytoplazmatycznych. Utrata żywotności nasion związana była ze zwiększonym wpływem elektrolitu, spadkiem zawartości wszystkich grup fosfolipidów, nienasyconych kwasów tłuszczowych i stosunku nienasyconych do nasyconych kwasów tłuszczowych. Stwierdzono także wzrost zawartości aldehydu malonowego (MDA). Niektóre wyniki porównano z podobnymi dla nasion klonu zwyczajnego (*A. platanoides* L.), odpornych na odwodnienie. Wyniki wskazują na aktywny udział błon cytoplazmatycznych w procesie odwodnienia w nasionach odpornych i destrukcję tychże w nasionach wrażliwych. Destrukcja błon cytoplazmatycznych była prawdopodobnie wynikiem peroksydacji lipidów na skutek działania wolnych rodników.

SŁOWA KLUCZOWE: *Acer pseudoplatanus*, *Acer platanoides*, błony cytoplazmatyczne, wpływ elektrolitu, fosfolipidy, kwasy tłuszczowe, peroksydacja lipidów, aldehyd malonowy.