Changes in the permeability of cellular membranes in winter wheat grain (cv. Grana) at different stages of ripeness

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Abstract

The state of cellular membranes is strictly dependent on grain ripeness. The electroconductivity of cell exudates is greatest in milk-ripe grain, lower during wax ripeness and smallest in fully-ripe grain. This decrease in conductivity of the cell exudates as the grain matures is accompanied by an increase in the level of phospholipids in the embryonic mitochondria. The permeability of the cell membranes is also considerably affected by the method of grain harvesting. The exudates from grain threshed immediately after collection demonstrated a greater electroconductivity than those from grain kept in ears for two weeks prior to threshing, whereby these differences were particularly distinct in wax-ripe grain, but less so in fully-ripe grain. The reasons for these effects are discussed.

Key words: membranes permeability, wheat grain, different ripeness

INTRODUCTION

Phospholipids are an important constituent of cytomembrane protoplasm though their content in seeds is fairly low and makes up 0.1-3% d.w. Phospholipids have been found to accumulate mainly in the embryo (Grzesiuk and Kulka 1981).

The permeability of membranes, and hence changes in their selectivity, are due to phospholipid oxidation (Kooststra and Harrington 1969, Abdul-Baki and Baker 1973, Kooststra 1973, Grzesiuk and Kulka 1981). The ensuing degradation of cell membranes facilitates the migration of sugars, amino acids, inorganic salts and other compounds out of the cell and tissues (Takayanagi and Murakami 1969, Ching 1973). The influence of tissue electrolytes affords the possibility of measuring the electroconductivity of these exudates. This may give some indication of

the permeability of cell membranes (Perry 1967, Takayanagi and Murakami 1969, Koostra 1973, Agrawal 1977, Basu and Dhar 1979, Mullet and Wilkinson 1979) and indirectly, an indication of the valuation of seeds as sowing material. The object of the present study was to investigate the relations between the permeability of cell membranes and the level of phospholipids in mitochondria isolated from embryos obtained from grain of varying ripeness, and the influence of the harvesting method on this permeability.

MATERIAL AND METHODS

The experiments were performed on "Grana" winter wheat grain. The grain was collected at three different times — during the milk ripeness phase (ca. 20 days after pollination), the wax ripeness phase (ca. 42 days after pollination) and the full ripeness phase (ca. 56 days after pollination).

Each sample of grain was divided into two series. The first consisted of grain hand-threshed directly after harvesting, then dried in the laboratory (series A). In the second series, the grain was harvested according to the usual two-phase system in which, after being cut, the ears of wheat were dried for two weeks before being threshed (series B). Both series of grain were kept in airy bags in a cool place. After the grain had rested for the requisite period of three months, the phospholipid content in the mitochondrial membranes isolated from embryos was estimated, and the electroconductivity of the cellular exudates from the seeds was determined. Analysis was carried out on imbibing and germinating grain — whole seeds or isolated embryos were used.

For phospholipid analysis the seeds were placed in Petri dishes containing filter paper soaked in water and allowed to germinate in a thermostat at 20-21°C for 24, 48 and 72 h. Electroconductivity was determined on seeds after 24 h imbibition.

The electroconductivity of grain exudates was determined as follows. One hundred grains were rinsed several times in bidistilled water to remove surface contamination. They were then put in a beaker and covered with 100 cm³ of bidistilled water. Several such beakers with their contents were placed in a thermostat at 20-21°C for 24 h. The grains were stirred periodically during imbibition. The samples were then immersed in a water bath at 20°C and the electroconductivity of the solutions containing the grain exudates was measured using a Radelkis OK-102/1 conductometer. Measurements were repeated 16 times, and the electroconductivity of the water used was subtracted each time from the results obtained. These were expressed in $\mu\text{s}\cdot\text{cm}^{-1}$.

To determine the phospholipids in the embryonic mitochondrial membranes of the germinating grain, the mitochondrial fraction isolated from the seeds after 1, 2 and 3 days of germination was purified by the differential centrifuging methods as described by Pomeroy (1974). The phospholipid content was then determined by Schmidt and Thannhauser's method (1945) as modified by Holden (1952) and adapted for the investigations of mitochondria by Grzesiuk et al. (1970). Inorganic phosphorus was estimated by the Fiske-Subbarow method (1925). Analysis of the phospholipid content was repeated four times.

RESULTS AND DISCUSSION

The changes in cell membrane permeability based on the electroconductivity measurements of the exudates obtained from grain of different ripeness are illustrated in Fig. 1.

Fig. 1. Electroconductivity of solutions obtained after soaking milk- (I), wax- (II) and fully-ripe grain (III) in water for 24 h. a — grain threshed immediately after harvesting (series A), b — grain kept in the ears at ambient temperature for 2 weeks (series B). Vertical bars denote standard error of the mean

The exudates of milk-ripe grain showed the greatest conductivity regardless of the method of collection grain. Fully-ripe grain showed the lowest permeability of electrolytes into the aqueous solution, that is, the greatest cell membrane selectivity and no significant differences between the grain threshed immediately after collection (series A) and grain threshed after two weeks of rest (series B) were observed. Contrary to this, the exudates from the wax-ripe grains of series B showed a lower conductivity than those from series A.

In general, as the grain ripened, the exudation ability of the grain decreased. The greater exudation of electrolytes from the less mature

grain can be explained by the as yet under-developed cell membrane selectivity and the still relatively low content of phospholipids accumulated in the membranes. Such a supposition was confirmed by further experiments, which indicated that the phospholipid content increased substantially during grain ripening (Fig. 2). The possibility of damage to grain during harvesting as a modifying factor in the described phenomena of exudation cannot be excluded.

Fig. 2. Changes in phospholipid content of mitochondrial membranes of embryos in germinating milk- (I), wax- (II) and fully-ripe grains (III). a and b — as in Fig. 1.

Many works (Ching 1972, Pollock and Ross 1972, Woodstock 1973, Simon 1974, Mullet and Wilkinson 1979) also consider that the electroconductivity of grain exudates is an indicator faithfully reflecting grain viability in laboratory studies. A definite relationship between conductivity on the one hand and grain vigor and germination in the field on the other has been established (Takayanagi and Murakami 1969, Ching 1972, Mullet and Wilkinson 1979).

The information gained from the present study shows that the phospholipid content in the mitochondrial membranes of embryos rises as the electroconductivity falls (see Figs. 1 and 2). A fairly low content of phospholipids was observed after only 24-h germination, but it increased after 48-h and again after 72-h germination.

During the entire germination process, the highest phospholipid content was found in the mitochondrial membranes of embryos in fully-ripe grain and the lowest in milk- and wax-ripe grain collected by the one-phase method (series A).

At all times during germination, the collection method affected the differentiation of the phospholipid level in mitochondrial membranes of embryos more in wax-ripe grain than in either fully- or milk-ripe grain.

Wax-ripe grain from the one-phase harvest (series A), having a much greater cell membrane permeability than that from the two-phase harvest (series B), also contained far fewer phospholipids than the wax-ripe grain from series B (Fig. 2).

There exist some data which indicate close relations between the content of phospholipids within mitochondria and the permeability of the cell cytomembranes (Koostra and Harrington 1969, Abdul-Baki and Baker 1973, Koostra 1973, Bewley and Black 1978). The results of the present paper obtained for less ripe grain also confirm these data, showing that the permeability of membranes to various electrolytes and, in consequence, their non-selectivity are the result of too small a quantity of phospholipids having been accumulated in the cell membranes of these seeds.

1. The state of the cell membrane is strictly dependent on grain ripeness. A feature of unripe grains was the high electroconductivity of their cellular exudates, which gradually decreased with grain ripening. At the same time as the grain ripened, the phospholipid level in the mitochondrial membrane of the embryos increased.
2. The method of collection exerts a greater effect on the cell membrane of wax-ripe grain than on milk- or fully-ripe grain. In wax-ripe grain threshed immediately after collection, the exudation of electrolytes was much greater in comparison with grain kept in ears for two weeks after collection. The latter grain showed a higher phospholipid content than that threshed immediately after harvesting.

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Zmiany w przepuszczalności cytomembran w ziarnach pszenicy ozimej odmiany Grana o różnej dojrzałości

Streszczenie

Stan cytomembran komórkowych ściśle zależy od dojrzałości ziarna. Ziarno w fazie dojrzałości młecznnej cechuje największe elektroprowadnictwo wycieków komórkowych, malejące w okresie dojrzałości woskowej i najmniejsze w ziarnie o pełnej dojrzałości. Obniżaniu się elektroprowadnictwa w miarę dojrzewania ziarna towarzyszy wzrost poziomu fosfolipidów w mitochondriach zarodków. Na stan przepuszczalności cytomembran wpływa także w znacznym stopniu sposób zbioru ziarna. Wycieki z ziarna wyluskanego z kłosów bezpośrednio po ich zbiorze wykazują większe elektroprowadnictwo niż wycieki z ziarna przetrzymanego w kłosach przez dwa tygodnie, przy czym różnice te były szczególnie drastyczne w ziarnie o dojrzałości woskowej, mniejsze natomiast w ziarnie o pełnej dojrzałości. Poddano dyskusji przyczyny tych zjawisk.