Modulation of germination of embryos isolated from dormant and nondormant barley grains by manipulation of endogenous abscisic acid

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Abstract. Dormant and non-dormant barley (Hordeum distichum L.) grains with identical genetic backgrounds were obtained by maturing grains under different climate conditions. When isolated embryos from dormant grains were incubated in a well containing a fixed volume of water (300 μl), the germination rate and percentage were dependent on the embryo number per well. A higher embryo number per well was correlated with a lower germination rate and percentage. However, this was not the case for the embryos isolated from nondormant grains. During germination, the endogenous cis-abscisic acid (ABA) in isolated embryos from both dormant and non-dormant grains was analyzed. The inhibitory effect on germination of a higher number per well of isolated dormant embryos was due to diffusion of endogenous ABA out of the embryos and accumulation of ABA in the incubation medium. Moreover, there was de-novo synthesis of ABA in embryos isolated from dormant grains during incubation but not in embryos isolated from nondormant grains. The inhibitory effect of ABA on germination of embryos isolated from dormant grains could be mimicked by addition of ABA or the medium in which dormant embryos had been placed. Embryos isolated from nondormant grains were insensitive to addition of ABA and medium from dormant embryos. Our results demonstrate that both ABA content and ABA sensitivity play a role in the control of germination. It is proposed that dormancy-breaking treatments act via changes to these processes.

Key words: Abscisic acid – Dormancy – Embryos – Germination – Hordeum

Introduction

Research on the mechanism of grain dormancy suggests a strong involvement of the phytohormone cis-abscisic acid (ABA); (Robichaud et al. 1980; Fong et al. 1983; Karssen et al. 1983; Koornneef et al. 1984; Kermode 1990). Abscisic acid-deficient or -insensitive mutants of Arabidopsis yield seeds with reduced dormancy (Karssen et al. 1983; Karssen et al. 1987). In these mutants, induction of dormancy is correlated with embryonic ABA content, but not with maternal ABA. In addition, these mutants show precocious germination during grain development (Robichaud et al. 1980). Reduction of ABA by application of the ABA-synthesis inhibitor fluridone to developing maize embryos causes these immature embryos to germinate precociously (Fong et al. 1983). These observations demonstrate that both ABA content and ABA sensitivity influence seed dormancy and germination.

Investigation of the role of ABA in dormancy in cereal grains has mainly been focused on developing grains. The free ABA content is highest in developing seeds and is generally relatively low or even undetectable in mature seeds, though in several species considerable amounts of ABA are detected (Black 1983, 1992). In developing cereal grains, there appears to be no clear correlation between ABA content of the grains and dormancy (Berrie et al. 1979; Dunwell 1981; Walker-Simmons and Sesing 1990). The appearance of ABA peaks during development could be manipulated by different growth conditions (Radley 1976; Quarrie et al. 1988; Weidenhoeft et al. 1988; Walker-Simmons and Sesing 1990). In barley, ABA peaked earlier in high-temperature-grown barley than in low-temperature-grown grains, and cultivars with a lower level of dormancy have less ABA during development (Goldbach and Michael 1976).

Beside these studies on the correlation between physiological state and endogenous ABA in inducing dormancy during seed development, some investigations have focused on the effects of addition of ABA on grain and embryo physiology. Addition of ABA inhibits germination of embryos isolated from various plant species (Robertson et al. 1989; Reid and Walker-Simmons 1990; Corbineau et al. 1991). Embryos isolated from dormant grains are more sensitive to exogenous ABA-induced inhibition of germination than embryos isolated from non-
dormant grains (Walker-Simmons 1987; Reid and Walker-Simmons 1990; Van Beckum et al. 1993; Wang et al. 1994). In addition, ABA-induced proteins in embryos isolated from dormant wheat were expressed over a longer period than in embryos isolated from nondormant grains (Walker-Simmons 1987; Reid and Walker-Simmons 1990).

Although a large amount of information has accumulated about ABA content and sensitivity during grain development, little is known about the amount and physiological role of endogenous ABA during germination. In the present paper, we studied and manipulated the behavior of endogenous ABA during the germination of isolated embryos from grains with high and low dormancy. The mechanism and role of changes in endogenous ABA during embryo germination were investigated.

Materials and methods

**Materials.** Hordeum distichum L. cv. Triumph (EBC trials 1988 at Carlsberg Plant Breeding, Copenhagen, Denmark) was grown in a phytotron under the growing conditions described previously (Schuurink et al. 1992a). The mature grains were stored at 20°C to preserve dormancy (Schuurink et al. 1992b). Before embryo isolation the husk near the embryo was removed. Embryos were carefully dissected from the grains using a scalpel and no endosperm adhered to the embryos. Volume-displacement measurements showed that the mean volume of both dormant and nondormant embryos was about 0.89 μl. Monoclonal antibody to free (+) ABA was purchased from Idetek, Inc. (San Bruno, Calif., USA). Rabbit anti-mouse alkaline-phosphatase conjugate, (+) ABA and bovine serum albumin (grade suitable for enzyme-linked immunosorbent assay; ELISA) were obtained from Sigma (St. Louis, Mo., USA). Fluridone was kindly provided by the Bulb Research Laboratory, Lisse, The Netherlands.

**Germination tests.** Dissected embryos were placed in 48-well-plates, each well containing 300 μl distilled water with or without (+) ABA. During incubation at 20°C in the dark the plates were sealed with Parafilm to prevent evaporation. Embryos were scored as germinated if the leaf shoots and roots were ≥1 mm.

**Extraction of ABA and ELISA assay.** The extractions of ABA from dissected embryos or from grains and the assay of the amount of ABA was carried out as described by Walker-Simmons (1987). For the assay of ABA in the incubation medium, the medium was directly used for ELISA assay.

The application of ELISA by using monoclonal antibodies to ABA has provided a rapid and sensitive method for ABA measurement (Walker-Simmons 1987; Morris et al. 1988; Harris and Outlaw 1991). In general, yield loss of ABA during extraction should be corrected using [3H]ABA. Since in each extraction the same number of embryos was used, we considered that the relative loss of ABA during extraction should be approximately the same in each sample. Therefore, we did not check the loss of ABA during extraction. In addition, most of our assays were performed with the induction medium for which the ABA extraction procedures were not needed.

Organic acids may interfere with the ABA assay (Belefant and Fong 1989). For example, in the presence of 100 mM malate, the ABA measured by ELISA was about 40% lower than in its absence (Belefant and Fong 1989). However, in our samples the maximum amount of malate measured was less than 2 mM. This means that the effect of organic acids such as malate on the measurement of ABA could be ignored.

The extracellular pH was measured using a standard glass electrode (Pharmacia, Uppsala, Sweden).

**Statistics.** Measured values are expressed as means ± standard deviation for n measurements. Significance of differences between measured values was tested using the Student's t-test (P<0.05).

**Results**

**Correlations between number of embryos per well (E/W) and germination percentage.** Barley grains obtained from plants grown under dormancy-inducing growth conditions were not able to germinate at 20°C (Schuurink et al. 1992a). However, the embryos isolated from these dormant grains were able to germinate, although germination was delayed and the percentage germination was lower than that of embryos isolated from nondormant grains (Van Beckum et al. 1993). When dormant embryos were incubated in a well containing 300 μl water, germination efficiency was dependent on the E/W number (Fig. 1A). In contrast to this, the germination of embryos isolated from nondormant grains was independent of the E/W number (Fig. 1B).

These results show that a factor which is involved in the regulation of dormancy and germination might be diffusing from dormant embryos into the incubation medium. This factor should inhibit germination rate and percentage. At higher E/W numbers (isolated from dormant grains) the following mechanisms may cause inhibition of germination: (i) the concentration of this factor may reach such high levels in the medium that germination is inhibited, or (ii) diffusion out of the embryos may be reduced by high exogenous levels so that the concentration of the factor in the embryos remains too high to allow release from dormancy. Embryos isolated from nondormant grains should not accumulate this factor and/or be insensitive to this factor.

**Involvement of ABA.** Since ABA is involved in the inhibition of germination rate and germination percentage in grains (Walker-Simmons 1987; Van Beckum et al. 1993), the proposed factor may be this phytohormone. Indeed the ABA contents of embryos directly isolated from dormant and nondormant grains were different. The measured ABA content of dry embryos from dormant grains was 1300 ± 180 pg/(mg DW)^{-1} (n=4, 10 embryos per measurement), while in dry embryos isolated from nondormant grains, the ABA content was about 825 ± 100 pg/(mg DW)^{-1} (n=4, 10 embryos per measurement). Therefore, we assayed the amount of ABA in the incubation medium during the germination of embryos isolated from dormant and nondormant grains. When different numbers of dormant embryos were incubated in 300 μl water for 24 h, we observed that a higher number of dormant embryos per well yielded a higher amount of ABA in the incubation medium, while the amount of ABA left in the embryos was relatively independent of the E/W number (Table 1). Nondormant embryos also showed an increase of ABA in the medium with increasing E/W number. However, the concentration of ABA in medium with nondormant embryos was more than ten times lower than in medium which contained dormant embryos (Table 1).