Abscisic acid and osmoticum prevent germination of developing alfalfa embryos, but only osmoticum maintains the synthesis of developmental proteins

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Abstract. Developing seeds of alfalfa (*Medicago sativa* L.) acquire the ability to germinate during the latter stages of development, the maturation drying phase. Isolated embryos placed on Murashige and Skoog medium germinate well during early and late development, but poorly during mid-development; however, when placed on water they germinate well only during the latter stage of development. Germination of isolated embryos is very slow and poor when they are incubated in the presence of surrounding seed structures (the endosperm or seed coat) taken from the mid-development stages. This inhibitory effect is also achieved by incubating embryos in $10^{-5} \text{M}$ abscisic acid (ABA). Endogenous ABA attains a high level during mid-development, especially in the endosperm. Seeds developing in pods treated with fluridone (1-methyl-3-phenyl-5-[3-(triftuoromethyl)-phenyl]-4(1H)-pyridinone) contain low levels of ABA during mid-development, and the endosperm and seed coat only weakly inhibit the germination of isolated embryos. However, intact seeds from fluridone-treated pods do not germinate viviparously, which is indicative that ABA alone is not responsible for maintaining seeds in a developing state. Application of osmoticum (e.g. 0.35 M sucrose) to isolated developing embryos prevents their germination. Also, in the developing seed in situ the osmotic potential is high. Thus internal levels of osmoticum may play a role in preventing germination of the embryo and maintaining development. Abscisic acid and osmoticum impart distinctly different metabolic responses on developing embryos, as demonstrated by their protein-synthetic capacity. Only in the presence of osmoticum do embryos synthesize proteins which are distinctly recognizable as those synthesized by developing embryos in situ, i.e. when inside the pod. Abscisic acid induces the synthesis of a few unique proteins, but these arise even in mature embryos treated with ABA.

Thus while both osmoticum and ABA prevent precocious germination, their effects on the synthetic capacity of the developing embryo are quite distinct. Since seeds with low endogenous ABA do not germinate, osmotic regulation may be the more important of these two factors in controlling seed development.

Key words: Embryogenesis – Abscisic acid and seed germination – Osmotic stress – *Medicago* (seed development) – Protein synthesis in embryos – Seed germination

Introduction

Embryogenesis, the developmental process beginning with the formation of the zygote and ending at embryo maturity, is controlled both by the schedule programmed within the genetic information of the embryo itself, and by the influence of its surrounding environment. Developing embryos usually acquire the capacity to germinate long before reaching maturity, and when removed from the surrounding seed structures they complete germination readily, either on an artificial medium or on water (reviewed in Kermode et al. 1989; Kermode 1990). On the other hand, when the intact seed is removed from the mother plant during early- or mid-development, the embryo within fails to germinate.

The restraints that the surrounding tissues impose on the embryo may be the result of a combination of physical, nutritional, hormonal and osmotic effects. The most widely studied of these are the hormonal effects, and abscisic acid (ABA) has been shown to prevent germination of isolated developing embryos in several species (Black 1983; Ackerson 1984b; Obendorf and Wettlaufer 1984; Quatrano 1986). However, Fischer et al. (1988) have argued that the water relations of seeds are a critical element in the control of maturation and germination. This is borne out by the observation that developing seeds or embryos will not germinate when placed onto high osmoticum, but will germinate precociously.
on solutions of low osmoticum or on water (Crouch 1979; Finkelstein and Crouch 1986; Cook et al. 1988). A more recent study by Barratt et al. (1989) has shown that precocious germination of young pea embryos in culture cannot be inhibited fully by $10^{-4}$ M ABA on 2% sucrose medium; however, increasing the sucrose concentration to 5% caused nearly complete inhibition even if the ABA concentration was reduced to $10^{-5}$ M. This result is consistent with the possibility that both ABA and osmoticum are involved in the control of precocious germination, but a combination of both is more effective.

Abscisic acid has been reported to promote the synthesis of a number of proteins in developing embryos (Triplett and Quatrano 1982; Stinissen et al. 1984; Ackerson 1984a; Eisenberg and Mascalhanas 1985; Finkelstein et al. 1985; Williamson et al. 1985; Galau et al. 1986; Barratt et al. 1989), and some of these proteins are of the storage type typical of developing seeds. Osmoticum has also been reported to have the same effects on protein synthesis in developing seeds as ABA (Finkelstein and Crouch 1986) although these appear not to be mediated through an increase in the endogenous levels of this growth regulator. In this study, we distinguish between the effects of ABA and osmoticum on protein synthesis, thus demonstrating that they are unrelated.

Although ABA is known to be inhibitory to germination and is present in seed tissues (Black 1983; Bulard and Le Page Degivry 1986; Finkelstein and Crouch 1987; Walker-Simmons 1987; Wang et al. 1987; Lopez et al. 1989), there is no direct evidence that the seed tissues surrounding the embryo are involved in the regulation of development and germination. Also, although high osmoticum has been shown to suppress precocious germination of the embryo, there is little indication that the osmotic potential in the intact developing seed is sufficient to achieve this. Here we demonstrate that both endogenous ABA and osmotic potential are sufficient to restrain the germination of developing alfalfa embryos. However, only the osmoticum is capable of promoting the maintenance of a pattern of protein synthesis which is typical of the developing embryo.

### Material and methods

**Plants and seeds.** Medicago sativa L. cv. Excalibur plants (seed source: United Cooperative of Ontario, Mississauga, Ont., Canada) were grown in a controlled growth chamber (23 °C, 16 h 400 μmol m⁻² s⁻¹ light, 60% RH days; 18°C, 8 h darkness, 85% RH nights) in Turface (Applied Industrial Materials Corp., Deerfield, Ill., USA). Plants were fertilized with 1/10 concentration Hoagland's solution (Arnon and Hoagland 1938) 5 d a week and with 20-20-20 fertilizer (Peters, Fogelsville, Penn., USA) twice a month. To avoid variation in seed development among individual plants, one plant was cloned as a pollen donor. Flowers were hand-pollinated by centrifugation and re-extracted twice. The ABA-containing supernatant was lyophilized and dissolved in TBS (25 mM Tris-2-amino-2-hydroxymethyl-1,3-propanediol-HCl, 100 mM NaCl, 1 mM MgCl₂, pH 8.5). Abscisic acid was determined by an enzyme-linked immunoassay according to instructions of the supplier (Phyto detek-ABA, Idetek, San Bruno, Calif., USA).

**Fluridone treatment.** Fluridone solution was made by dissolving 200 μl 43% fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone; supplied by Dr. Franklin Fong, Texas A&M University, College Station, USA) in 500 ml 1% aqueous acetone. Pods at various stages of development were sprayed or injected with fluridone twice at a 2-d interval. Control pods were treated with 1% aqueous acetone. Following development to stage VI (stage of maximum ABA accumulation) seeds were harvested and assayed for ABA.

To determine the effects of fluridone on germinability, pods were sprayed at stage III (approx. 10 d after pollination, when fluridone was most effective in reducing ABA levels) and seeds were harvested at various stages thereafter.

**Osmotic-potential measurements.** Seeds were dissected into embryo, endosperm and testa in a moist chamber, wrapped in an aluminum-foil bag containing a filter-paper disc (6.5 mm diameter), frozen in liquid nitrogen, and stored at −80°C. The sample bags were thawed and each squeezed with a hammer. The tissue-sap-saturated filter-paper disc was removed and immediately placed in the chamber of a sealed thermocouple psychrometer connected to a microvoltmeter (both built by Dixon Instruments Co., Guelph, Ont., Canada, according to the specifications outlined in Wall schlegel et al. 1988). The psychrometer was placed in a thermostat-chamber at 25°C and allowed to equilibrate for 1.5 h. The osmotic-potential measurement was made after cooling for 15 s.

**Ex-situ [35S]methionine labelling of tissue.** The protein-synthetic pattern was determined for developing and germinating seeds and embryos, and for embryos prevented from germinating by osmoticum and ABA. Thus, the following seed and embryo treatments were used: stage VII seeds on water, stage VII embryos on water,