Journal of Experimental Botany, Vol. 43, No. 252, pp. 969–974, July 1992

Phytochrome-induced Germination, Endosperm Softening and Embryo Growth Potential in *Datura ferox* Seeds: Sensitivity to Low Water Potential and Time to Escape to FR Reversal

L. DE MIGUEL and R. A. SANCHEZ[1]

IFEVA, Depto de Ecologia, Facultad de Agronomia, U.B.A. Avda San Martin 4453, 1417 Buenos Aires, Argentina

Received 7 October 1991; Accepted 3 February 1992

ABSTRACT

In *Datura ferox* seeds, the far-red absorbing form of phytochrome (Pfr) induces endosperm softening, larger embryo growth potential, and germination. We investigated the effect of exposing the seeds to a range of water potentials in the presence of Pfr on its induction of these responses. In addition, the escape time to far-red-light (FR) reversal of the three responses was determined.

Low water potential inhibited Pfr action on endosperm softening and germination in a similar way. In both cases, a 50% reduction in the response to a saturating red-light (R) irradiation was observed at a water potential of *c.* −0·5 MPa and there was very good correlation between the percentage number of seeds with softened endosperm at 45 h after R and germination at 72 h after R ($R2 = 0{\cdot}95$). In contrast, the effect of decreasing the external water potential on Pfr induction of a larger embryo growth potential was more complex. Moderate decreases in water potential (−0·3 to −0·5 MPa) enhanced Pfr action and the growth potential of the embryos was larger (20–25%) than the water controls; water potentials below −0·7 MPa inhibited the Pfr stimulus.

The escape time to FR reversal of the R effect was shorter for the increase in embryo growth potential than for endosperm softening. Twenty-four h after R, the embryo response had escaped in more than 80% of the population whereas endosperm softening and germination were susceptible to FR inhibition in 100% of the seeds.

These results indicate that in *D. ferox* seeds the increase in embryo growth potential is not sufficient for germination and that endosperm softening is a necessary condition.

Key words: Germination, dormancy, phytochrome, endosperm softening, water potential.

INTRODUCTION

For a long period after ripening, the seeds of *Datura ferox* are in a state of dormancy imposed by the tissues surrounding the embryo, particularly the endosperm (Soriano, Sánchez, and Eilberg, 1964; Sánchez and de Miguel, 1985). Germination of these seeds can be induced by light, through phytochrome, when the seeds are incubated in an alternating temperature regime (Soriano *et al.*, 1964; Sánchez, de Miguel, and Mercuri, 1986; Reisman-Berman, Kiegel, and Rubin, 1990). Pfr can terminate the dormancy imposed by the covering tissues by shifting the balance between the expansive force of the embryo and the resistance opposed to it by the covering tissues (Bewley and Black, 1982). This can be the result of an increase in the expansive force of the embryo (Carpita, Nabors, Ross, and Peteric, 1979; Takeba, 1980) and/or a reduction in the constraints imposed by the tissues surrounding it (Pavlista and Haber, 1970; Pavlista and Valdovinos, 1978; Psaras, Georghiu, and Mitrakos, 1981; Sánchez, Sunell, Labavitch, and Bonner, 1987, 1990). In different species, or in response to different factors, the changes in endosperm and embryo may have a different relative importance. In *Datura ferox* seeds incubated at alternating temperatures, Pfr can induce increases in embryo growth capacity and endosperm softening (Sánchez and de Miguel, 1985; Sanchez *et al.*, 1986). However whether either, or both of these processes are essential for germination

in this species has not been established. It is necessary to examine the relative importance to the induction of germination of changes in the mechanical resistance of the endosperm and the growth potential of the embryo. Moreover, it is useful to compare the sensitivity of these processes to decreases in water availability and also to their requirements for the presence of Pfr. Low water potential can block Pfr-dependent processes leading to germination in several species (Karssen, 1970; Hsiao and Vidaver, 1971; Berrie, Paterson, and West, 1974; Duke, 1978). However, whether changes in the endosperm strength or embryo growth capacity were affected has not, as far as we know, been investigated.

In this paper, we report the effects of low water potential on the Pfr-dependent germination, endosperm softening, increased embryo growth capacity, and the escape time to far-red light (FR) reversal of the red light (R) induction of these processes in *Datura ferox* seeds.

MATERIALS AND METHODS

Datura ferox L. seeds were collected from plants invading soybean crops in the province of Buenos Aires, Argentina. After harvest, the seeds were stored at 20 °C in dark glass jars until use.

The seeds were incubated in plastic boxes on cotton wool saturated with distilled water or the test solutions. The plastic boxes were wrapped with two black plastic sheets and the seeds were handled under dim green light.

The experimental protocol involved three consecutive phases:

(a) *Imbibition:* during the initial 24 h the seeds were placed on distilled water-saturated cotton wool at 25 °C to allow full imbibition (Soriano *et al.*, 1964).

(b) *Treatment period:* initiated by decoating, replacing the seeds on cotton wool saturated with different PEG solutions (or distilled water for the controls) and then irradiating with saturating R or FR according to the treatments; the temperature during this period was alternating: 30 °C (9 h d^{-1})–20 °C (15 h d^{-1}) to permit Pfr action. Unless otherwise stated the osmotic treatments lasted 45 h.

(c) *Evaluation of the effects:* different samples were tested for endosperm softening, used for measuring embryo growth potential or replaced on distilled water-saturated cotton wool, irradiated with saturating FR and incubated at alternating temperatures during another 48 h for germination counts.

The proportion of seeds with softened micropylar endosperm was evaluated by forcing radicle protrusion. The force-induced protrusion (FIP) was determined by applying a force on the central portion of the seed with a chrome-ended brass shaft driven by a screw. The seed was on a holder placed on the plate of a balance which permitted measurement of the applied force. The force applied was increased slowly until either the radicle protruded out of the endosperm (FIP) at the same place it occurs in normal germination, or until the seed broke up. FIP was observed with forces between 500 and 1200 *g* (Sánchez *et al.*, 1986).

To evaluate embryo growth potential the seeds were first de-tipped to remove mechanical resistance to embryo expansion. Then, all seeds received saturating FR and were placed on distilled water-saturated cotton wool and incubated for 24 h at a constant 25 °C. The increase in length during the 24 h was measured and taken as an expression of embryo growth potential. De-tipping was achieved by excising the conical 0·5 mm micropylar end of the seeds. This removed the portion of the endosperm covering the radicle tip and a small portion of the radicle. The increase in length of de-tipped seeds was linear with time during the 24 h period and was not significantly different from that of embryos of seeds where only the micropylar portion of the endosperm was removed, thus keeping the embryo intact (data not shown).

The R was provided by four Phillips 40/15 40 W red fluorescent lamps with an irradiance at seed level of 800 μW cm^{-2}. The far-red source was an incandescent lamp of 125 W internal reflector filtered through 10 cm of water and a RGN9 Schott glass filter; irradiance at seed level was 1000 μW cm^{-2}.

The osmotic potential of PEG (polyethylene glycol 6000) solutions was measured in psychrometric chambers (Wescor C 51) calibrated with NaCl solutions.

RESULTS

Effect of low water potential on Pfr induction of germination

Radicle emergence in seeds incubated throughout on a water-saturated substratum, began between 48 and 72 h after a saturating pulse of R (Fig. 1; Sanchez *et al.*, 1986, 1990). When the water potential of the incubation medium was lowered for a period of 45 h after R, at the end of which the seeds received FR and were returned to water, germination was inhibited (Fig. 1). On the other hand, when no FR was given at the end of the incubation on PEG, final germination was not affected although germination was delayed by about 24 h with respect to the water controls (Fig. 1). This shows that Pfr action on germination was blocked by low water potential and that this effect was reversible by returning the seeds to incubation on water. Moreover, sufficient Pfr remained 45 h after R to promote germination.

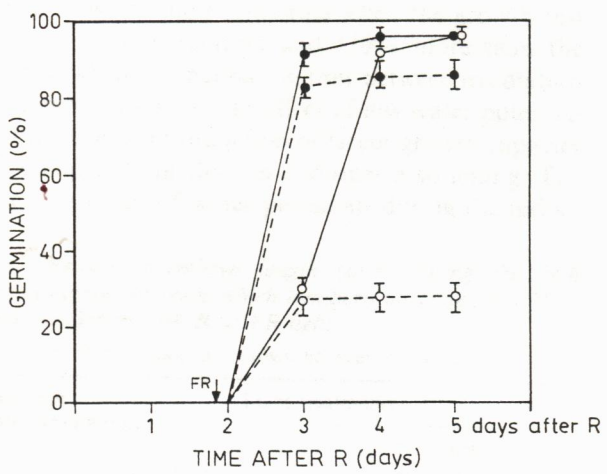

FIG. 1. Time-course of germination of *D. ferox* seeds after a saturating pulse of R. The seeds were incubated on distilled water throughout the experiment (●——) or exposed to a PEG solution (−0·7 MPa) from 0 to 45 h after R (○——). Half of the seeds received FR at 45 h after R (●– – –; ○– – – –). Vertical bars indicate 2 s.e. of the mean.

The effect of a range of water potentials on Pfr induction of germination was tested by incubating the seeds on solutions of different PEG concentrations for 45 h following the R treatment. Afterwards, all seeds received a saturating FR irradiation and were returned to a water-saturated substratum. The inhibition of Pfr induction of germination was proportional to the decrease in the water potential of the medium. A 50% reduction in the response was observed with an external water potential of −0·5 MPa, whereas a complete block of Pfr action required a water potential lower than −0·9 MPa (Fig. 2).

The effect of low water potential on Pfr induction of endosperm softening and larger embryo growth capacity

To test the effect of low water potential on Pfr induction of endosperm softening the seeds were exposed, after a R pulse, to different PEG solutions during a period of 45 h at the end of which the percentage seeds showing FIP was determined. The effect of Pfr on endosperm mechanical resistance was inhibited by low water potential and the sensitivity of this process was similar to that of germination; a reduction of 50% in the response was observed at about −0·5 MPa (Fig. 3A). There was a significant positive correlation between the percentage seeds with softened endosperm at 45 h after R and germination at 72 h after R ($R^2 = 0{\cdot}95$, $P < 0{\cdot}01$) (Fig. 3B).

Embryo growth potential was increased by a pulse of R followed by 24 or 45 h of incubation. This potential was expressed as a faster growth in length, even in the absence of Pfr, once the physical obstacle to embryo expansion was eliminated by de-tipping (Table 1; Sanchez *et al.*, 1987). The embryos of R-treated seeds grew 101% more than those of the FR-treated when the growth test started 24 h after irradiation and 119% more than the FR controls when the incubation time between irradiation and de-tipping was 45 h. The effect of low water potential on this Pfr-dependent induction of larger growth capacity is more complex than that on endosperm softening. The response to a range of water potentials during the induc-

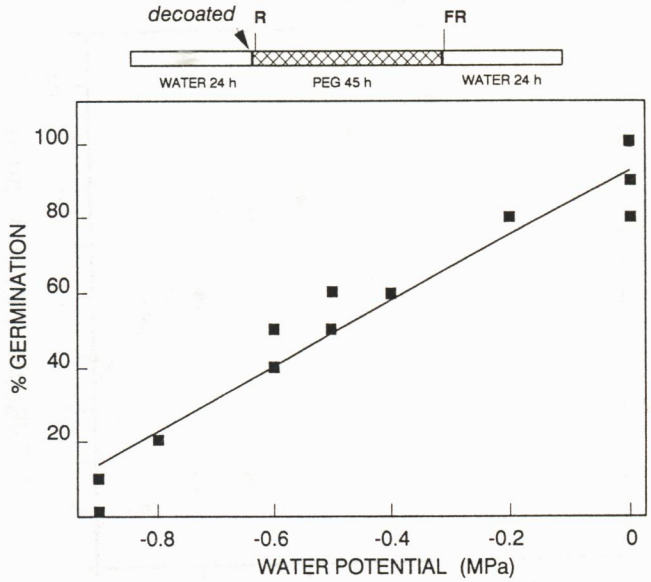

FIG. 2. Effect of low water potential on Pfr induction of germination of *D. ferox* seeds. The seeds were incubated on a series of PEG solutions from 0 to 45 h after R. At the end of the exposure to the osmoticum all seeds received FR and were returned to a water-saturated substrate. Germination was counted 72 h after R. Each point is the average of three replicates of 25 seeds and the regression line is $y = 93{\cdot}05 + 8{\cdot}78x$ ($R^2 = 0{\cdot}93$; $P < 0{\cdot}001$).

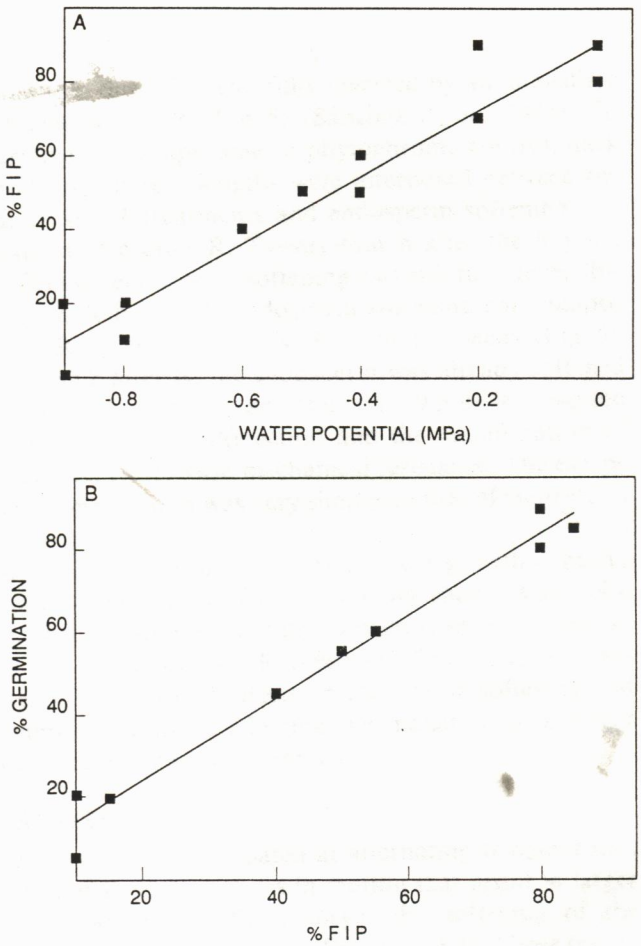

FIG. 3. (A) Effect of low water potential on Pfr induction of endosperm softening. PEG treatments as in Fig. 2. FIP was measured at 45 h after R. The regression line is $y = 90{\cdot}25 + 8{\cdot}99x$ ($R^2 = 0{\cdot}92$; $P < 0{\cdot}001$). (B) Correlation between endosperm softening measured at 45 h after R and germination counted 72 h after R. Seed treatments as in Fig. 2, $y = 3{\cdot}91 + 0{\cdot}99x$ ($R^2 = 0{\cdot}97$; $P < 0{\cdot}001$).

TABLE 1. *Increase in embryo length (mm) during the 24 h following de-tipping of seeds which had been incubated for 24 or 45 h after irradiation with R or FR light*

Standard errors of the means are shown between brackets.

Incubation time (hours after irradiation)	Light treatments	
	R	FR
24	3·34 (0·14)	1·66 (0·04)
45	4·53 (0·16)	2·07 (0·16)

tion phase, i.e. during the time Pfr was present, depended on the duration of the treatment and the concentration of the osmoticum. When the seeds were incubated for 45 h after R in the osmoticum the growth of the embryos (after receiving FR and returned to water) showed a bimodal response (Fig. 4). The embryos of seeds previously exposed to the lower water potentials (−0·8, −1·0 MPa) grew less than the water controls; those of seeds exposed to moderate reductions in water potential (−0·3 to −0·5 MPa) grew more than the controls. No such bimodal response was observed for germination and the correlation between embryo growth capacity and germination was poor. No increase in the growth potential of the embryos resulted after exposing FR-treated seeds to moderate reductions in water potential (data not shown). When the exposure to the osmoticum was for 24 h after R, the response was reduced proportionally to the reduction in water potential (Fig. 4).

The escape time to FR reversal of R effects on endosperm softening, embryo growth capacity and germination

Nearly 85% of the seeds receiving a saturating pulse of R showed endosperm softening when tested 45 h after irradiation; this effect was fully reversed by an immediate treatment with FR (Fig. 5) (Sánchez *et al.*, 1986). To determine the escape time to phytochrome control, dark periods of different lengths were interposed between the R and the FR treatments and endosperm softening was measured 45 h after R. Twenty-four h after the R pulse the effect on endosperm softening was still fully reversible and even 36 h after R endosperm softening had escaped from FR reversion in only 50% of the seeds (Fig. 5). Forty-five h after R, the endosperm was already softened in *c.* 80% of the seeds, and thus only 9 h or less elapsed between the end of Pfr action and the manifestation of its effect on endosperm mechanical resistance. The escape time of germination was very similar to that of endosperm softening (Fig. 5).

In contrast, the induction of a larger growth capacity required the presence of Pfr for a much shorter time; 24 h after R this response had fully escaped from FR reversion (Fig. 5). Therefore, a FR treatment 24 h after R completely blocked the R effects on endosperm softening and germination without affecting the induction of a larger growth capacity in the embryo.

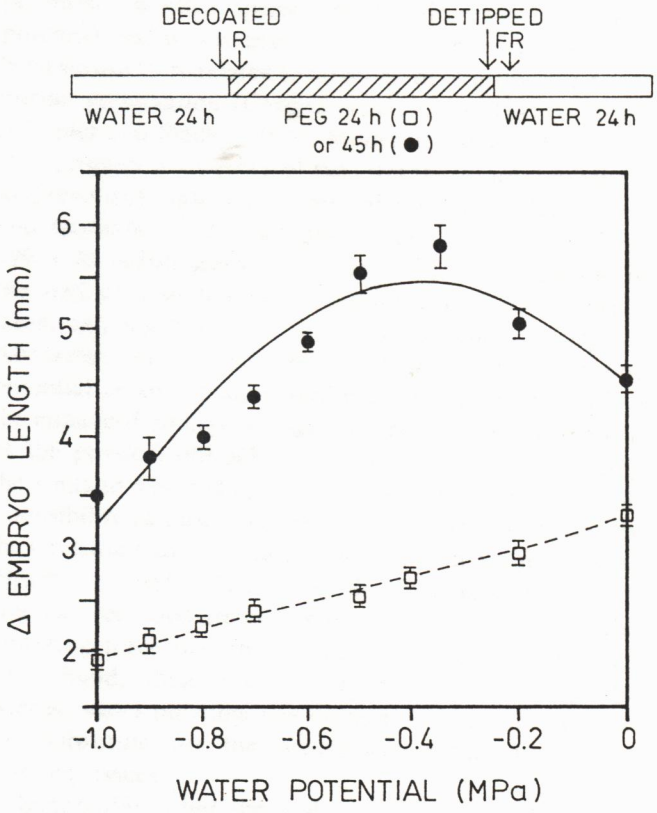

FIG. 4. Effect of low water potential on Pfr induction of increased embryo growth potential in *D. ferox* seeds. The seeds were incubated on PEG solutions for 24 (– – –□– – –) or 45 (—●—) h after R. At the end of exposure to the osmoticum the seeds were de-tipped, transferred to a water-saturated substrate, and irradiated with FR. After further incubation at 25 °C in darkness for 24 h the embryo lengths were measured. Vertical bars indicate 2 s.e. of the mean.

DISCUSSION

In *D. ferox* seeds, incubated at alternating temperatures, a pulse of R sets processes in motion that result in larger growth capacity of the embryo, the softening of the endosperm and germination (Figs 1, 2, 3 4). These results are in agreement with previous work on this species (Sanchez *et al.*, 1986, 1987).

In other species, low external water potential inhibits the Pfr-dependent processes leading to germination (Karssen, 1970; Berrie *et al.*, 1974; Duke, 1978) increasing the escape time to FR reversal of the R effect. We have found a similar effect in *D. ferox* (Figs 1, 2). In addition, our results demonstrate that in *D. ferox* the changes in

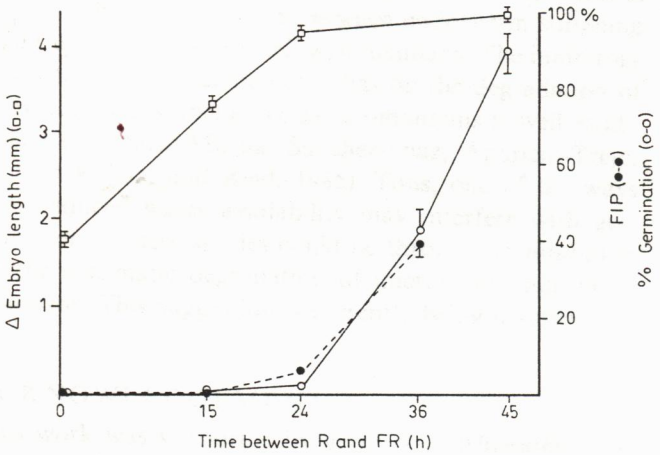

FIG. 5. Escape time to FR reversal of R-induced germination (○—), endosperm softening (●– – –) and embryo growth potential (—□—). Germination and embryo growth potential were recorded 72 h after R and FIP 45 h after R. Vertical bars indicate 2 s.e. of the mean.

both the embryo and the endosperm are affected by low water potential, but in different ways. Pfr induction of endosperm softening is inhibited by low water potential and the sensitivity of this response is similar to that of germination (Fig. 3B). This is expressed in the high correlation between percentage of seeds with endosperm softened at 45 h after R and germination counted 72 h after R; this is observed in seeds exposed to a range of PEG concentrations determining germination values between 20% and 85% (Fig. 3B).

The relationship between Pfr, water potential, and embryo growth potential is more complex. When the seeds were exposed for 45 h to the osmoticum, only the lower water potentials inhibited Pfr action, whereas moderate osmoticum concentrations enhanced the Pfr stimulus of a larger growth potential (Fig. 4). There was no stimulatory effect of moderately low water potentials in the absence of Pfr or when the exposure times to the osmoticum were relatively short (Fig. 4). Therefore, low water potential can either inhibit or enhance the Pfr effect on embryo growth potential. The stimulatory effect takes longer to be detected and is overriden by the inhibitory effect when the decrease in water potential is too large. We have no information about the physiological basis for the enhancement of embryo growth potential by Pfr, alone or in association with moderate decreases in water potential. In other species, both a decrease in osmotic potential and/or increases in cell wall extensibility have been shown to be related to larger embryo growth capacity during germination (Carpita *et al.*, 1979; Takeba, 1980; Schopfer and Plachy, 1985). Moderate osmotic treatment may produce a lowering of the internal osmotic potential as previously observed in several materials (Wyn Jones and Gorham, 1983), including seeds (Ni and Bradford, 1992). In radish seeds the inclusion of an osmoticum in the medium stimulates H^+ extrusion which, it was speculated, can result in wall loosening (Cocucci, Morgutti, Abruzzese, and Alisi, 1990). Either a lower osmotic potential or an increased wall extensibility could explain the enhanced embryo elongation observed upon removal of the physical obstacle to its expansion and returning the seeds to water (Fig. 4). However, an increase in wall extensibility cannot overcome the resistance imposed by the covering tissues when the embryo is in the intact seed (Bradford, 1986). Therefore, the increases in growth potential recorded are not necessarily proportional to the rupturing force that the embryo could develop. On the other hand, these results indicate that lowering the external water potential can do more to the embryo than just substitute for the mechanical constraint of the covering tissues.

Remarkably, when the seeds are exposed for 45 h after R to moderate decreases in water potential the embryo growth potential is enhanced by about 30% with respect to the water controls (Fig. 4). In spite of that, the treated intact seeds germinate between 30% and 50% *less* than the water controls (Fig. 2). This shows that the Pfr-dependent increase in embryo growth capacity is not sufficient for germination in *D. ferox* seeds, although the possibility that it may be necessary cannot be ruled out. At the same time the proposal that endosperm softening may be decisive receives strong support.

The same conclusion can be reached by considering the escape time to FR reversal of the R stimulus of each process. For a complete enhancement of embryo growth capacity, the presence of Pfr is required for 24 h, whereas for endosperm softening and germination Pfr must be present for longer. Consequently, a FR irradiation at 24 h after R fully reverted the stimulus on endosperm softening and germination without affecting the growth capacity of the embryo (Fig. 5). Therefore, in *Datura ferox*, the enhancement of embryo expansion capacity may not be sufficient for the exit from dormancy and germination; deciding whether it is necessary requires further research. On the other hand the relevance of endosperm softening is substantiated.

Embryo expansion potential is considered a key factor in the germination of several species such as *Xanthium pennsylvanicum* (Esashi and Leopold, 1968) and particularly in phytochrome-induced lettuce seed germination (Carpita *et al.*, 1979; Takeba, 1980). It should be noted, however, that SHAM (salicylhydroxamic acid) induces germination of lettuce cv. Waldman's Green without changing embryo growth potential (Brooks and Mitchell, 1988) but it stimulates endosperm softening.

A good relationship between endosperm softening and the induction of germination has also been found in responses to factors other than light in various species. Such is the case of temperature stimulus of pepper seeds (Watkins, Cantliffe, Huber, and Nell, 1985) and gibberellin action on tomato (Groot and Karssen, 1987; Groot, Kieliszewska-Rokicka, Vermeer, and Karssen, 1988). In these cases, as in *Datura* (Sánchez *et al.*, 1990), there is support for a relationship between endosperm softening and the mobilization of cell wall mannans. The inhibitory effect that low water potential has on the degradation of polysaccharides, including galactomannans is well established (Gonzalez-Murua, Sanchez-Diaz, Aparicio-Trejo, Munoz-Rueda, and Reid, 1985). Thus, one of the ways that reduced water availability may interfere with germination in these species could be through an inhibition of the enzymatic degradation of micropylar endosperm mannans. This suggestion is currently being investigated.

ACKNOWLEDGEMENT

This work was supported by grant No. 307030088 from the Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina.

LITERATURE CITED

BERRIE AM, PATERSON J, WEST HP. 1974. Water content and responsivity of lettuce seeds to light. *Physiologia Plantarum* **31,** 90–6.

BEWLEY JD, BLACK M. 1982. *Physiology and biochemistry of seeds in relation to germination* Vol. 2. Berlin, Heidelberg, New York: Springer-Verlag, 37.

BRADFORD K. 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Horticultural Science* **21,** 1105–12.

BROOKS CA, MITCHELL CA. 1988. Effect of salicylhydroxamic acid on endosperm strength and embryo growth of *Lactuca sativa* L. cv. Waldman's Green seeds. *Plant Physiology* **86,** 826–9.

CARPITA NC, NABORS MW, ROSS CW, PETERIC NL. 1979. The growth physics and water relations of red light induced germination in lettuce seeds. III. Changes in the osmotic and pressure potential in the embryonic axes of red and far-red-treated seeds. *Planta* **144,** 217–24.

COCUCCI SM, MORGUTTI S, ABRUZZESE A, ALISI C. 1990. Response to osmoticum medium and fusicoccin by seeds of radish (*Raphanus sativus*) in the early phase of germination. *Physiologia Plantarum* **80,** 294–300.

DUKE SO. 1978. Interactions of seed water content with phytochrome-initiated germination of *Rumex crispus* (L.) seeds. *Plant and Cell Physiology* **19,** 1043–9.

ESASHI Y, LEOPOLD AC. 1968. Physical forces in dormancy and germination of *Xanthium* seeds. *Plant Physiology* **43,** 871–6.

GONZALEZ-MURUA C, SANCHEZ-DIAZ M, APARICIO-TREJO P, MUNOZ-RUEDA A, REID JSG. 1985. The effect of NaCl and water stress on germination and α-galactosidase activity in germinated seeds of *Medicago sativa, Trifolium repens* and *T. brachycalcynum. Journal of Plant Physiology* **119,** 317–26.

GROOT SPC, KARSSEN CM. 1987. Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. *Planta* **171,** 525–31.

GROOT SPC, KIELISZEWSKA-ROKICKA B, VERMEER E, KARSSEN CM. 1988. Gibberellin-induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle protrusion. *Planta* **174,** 500–4.

HSIAO AI, VIDAVER W. 1971. Seed water content in relation to phytochrome-mediated germination of lettuce seeds (*Lactuca sativa* L. var. Grand Rapids). *Canadian Journal of Botany* **49,** 111–15.

KARSSEN CM. 1970. The light promoted germination of the seeds of *Chenopodium album* L. IV. Effects of red, far-red and white light on non-photoblastic seeds incubated in mannitol. *Acta Botanica Neerlandica* **19,** 95–108.

NI B-R, BRADFORD K. 1992. Quantitative models characterizing seed germination responses to abscisic acid and osmoticum. *Plant Physiology* **98,** 1057–68.

PAVLISTA AD, HABER AH. 1970. Embryo expansion without protrusion in lettuce seeds. *Plant Physiology* **45,** 636–7.

PAVLISTA A, VALDOVINOS JG. 1978. Changes in the surface appearance of the endosperm during lettuce achene germination. *Botanical Gazette* **139,** 171–9.

PSARAS G, GEORGHIU K, MITRAKOS K. 1981. Red light-induced endosperm preparation for radicle protrusion of lettuce embryos. *Botanical Gazette* **142,** 13–18.

REISMAN-BERMAN O, KIEGEL J, RUBIN K. 1989. Short-soaking in water inhibits germination of *Datura ferox* L. and *D. stramonium* seeds. *Weed Research* **29,** 357–63.

SÁNCHEZ RA, DE MIGUEL LC. 1985. The effect of red light, ABA, and K^+ on the growth rate of *Datura ferox* embryos and its relations with the photocontrol of germination. *Botanical Gazette* **146,** 472–6.

SANCHEZ RA, DE MIGUEL LC, MERCURI, O. 1986. Phytochrome control of cellulase activity in *Datura ferox* L. seeds and its relationship with germination. *Journal of Experimental Botany* **37,** 1574–80.

SANCHEZ RA, SUNELL L, LABAVITCH J, BONNER BA. 1987. Changes in the micropylar region of the endosperm before radicle protrusion in the seeds of two *Datura* species (abstract No. 711). *Plant Physiology* **83,** S-118.

SANCHEZ RA, SURNELL L, LABANTCH J, BONNER BA. 1990. Changes in endosperm cell walls prior to germination in two *Datura* species. *Plant Physiology* **93,** 89–97.

SCHOPFER P, PLACHY C. 1985. Control of seed germination by abscisic acid. III. Effect on embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in *Brassica napus* L. *Plant Physiology* **77,** 676–8.

SORIANO A, SÁNCHEZ RA, EILBERG BA. 1964. Factors and processes in the germination of *Datura ferox* L. *Canadian Journal of Botany* **42,** 1189–1203.

TAKEBA G. 1980. Phytochrome-mediated accumulation of free amino acids in embryonic axes of New York lettuce seeds. *Plant and Cell Physiology* **21,** 1651–6.

WATKINS JT, CANTLIFFE DJ, HUBER DJ, NELL TA. 1985. Gibberellic acid stimulated degradation of endosperm in pepper. *Journal of the American Society of Horticultural Science* **110,** 61–5.

WYN JONES RG, GORHAM J. 1983. Osmoregulation. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological plant ecology* III. Berlin, Heidelberg, New York: Springer-Verlag, 35–58.