

Water Stress and Storage-protein Degradation during Germination of *Impatiens* Seed

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Abstract. Water uptake by *impatiens* (*Impatiens wallerana* Hook. f. cv. Super Elfin Coral) seeds was measured as an increase in fresh weight every 24 hours during 144 hours of germination. Seeds absorbed most of the water required for germination within 3 hours of imbibition and germinated at 60% to 67% moisture on a dry-weight basis. Germination started at 48 hours and was complete by 96 hours at 25°C. Water stress of -0.1, -0.2, -0.4, and -0.6 MPa, induced by polyethylene glycol 8000, reduced germination by 13%, 49%, 91%, and 100%, respectively, at 96 hours. Under the same water-stress conditions, increases in fresh weight were inhibited by 53%, 89%, 107%, and 106%, respectively. Three distinct groups of storage proteins were present in dry seed; their estimated molecular weights were 1) 35, 33, and 31 kDa; 2) 26, 23, and 21 kDa; and 3) two bands <14 kDa. Major depletion of storage proteins coincided with the completion of germination. Water potentials that inhibited germination also inhibited degradation of storage proteins. During germination under optimum conditions, the soluble protein fraction increased, coinciding with a decrease in the insoluble fraction.

The major portion of most bedding plants is produced in plugs. Plug production is a single-cell plant-production system in which seeds are placed on the surface of a germination medium by a mechanical seeder. Koranski (1987, 1988) has shown that the optimum method of germinating *impatiens* is to leave the seed uncovered. One of the major problems of germinating uncovered seeds in the plug system is water stress.

The uptake of water by seeds is an essential initiating step that activates a number of metabolic processes necessary for germination (Keller and Hadas, 1982). Water uptake by seeds is considered to be triphasic. Phase I, or imbibition, is a rapid water uptake as a consequence of matric potential; Phase II is the lag phase, or water plateau, dependent on osmotic potential; and Phase III is a second rapid uptake due to germination (Bewley and Black, 1985; Bradford, 1986). Mild water stress will increase the length of the lag phase and delay or prevent germination (Hegarty, 1977; McIntyre, 1987).

Water deficits have a profound effect on plant metabolism, including cell-wall synthesis and cell expansion (Bewley, 1981; Bradford and Hsiao, 1982; Hsiao, 1973). This effect is proposed to occur through rapid reduction of protein synthesis by affecting the process of translation, rather than the production of a specific set of proteins (Armstrong and Jones, 1973; Bewley et al., 1983). Limited growth of osmotically stressed plants possibly is the result of a reduced capacity for protein synthesis and is not related to turgor loss (Mason and Matsuda, 1985).

Major storage proteins of legumes and other dicotyledonous plants are believed to be globulins, water-insoluble but salt-soluble proteins (Higgins, 1984). During seed germination, storage proteins in the cotyledons are degraded to produce amino acids and amides. Most of these amino acids are translocated to the growing parts of the developing seedling where they are

used for the synthesis of new functional and structural proteins (Davis and Chapman, 1980; Mitsuhashi et al., 1984; Murray, 1979; Pusztai and Duncan, 1971). Degradation of storage proteins has received little study as a factor affecting radicle protrusion. Very little is known about the effect of water stress on the degradation of seed storage proteins. This investigation was undertaken to study the relationship of qualitative and quantitative changes in storage proteins to the germination of *impatiens* seed under optimum and water-stress conditions.

Materials and Methods

Water uptake. Samples of 50 mg (≈ 100 seeds) of *impatiens* seeds (George J. Ball, W. Chicago, Ill.) were placed in acrylic germination boxes (12 × 12 × 3 cm) with two layers of blue blotter paper (Anchor Paper, St. Paul, Minn.). Blotters were presoaked in deionized (DI) water and drained to remove excess water. Another 10 ml of DI water was then added to each box. Boxes were sealed with parafilm and kept in a growth chamber at 25 ± 0.5°C with continuous irradiance at 4 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ photosynthetic photon flux. At 1-hr intervals (for the first 8 hr) and every 24 hr (for the remaining 136 hr) seeds were collected and surface-dried, and the fresh weight of samples was recorded (Hegarty, 1977).

Polyethylene glycol (PEG) treatment. Polyethylene glycol (PEG 8000 Fisher Scientific) solutions of osmotic potentials -0.1, -0.2, -0.4, and -0.6 MPa (50, 75, 100, and 125 g/500 ml, respectively) were prepared. Water potentials were determined at room temperature by using a Wescor 5100 C Vapor Pressure Osmometer (Wescor, Logan, Utah). Germination papers were soaked in the appropriate solution and then used for germination as in the water uptake measurements. For fresh weight measurements, PEG-soaked seeds were washed with distilled water for 2 min, surface-dried, and weighed. One-hundred seeds were placed in each germination box and incubated under the same conditions as in the previous trials. Germinated seeds were counted at 24-hr intervals for a total of 144 hr. Germination was defined as radicle protrusion through the seed coat. DI water was used as the control (0.0 MPa).

Experimental design and analysis. A split-plot arranged in a completely randomized design was used for germination percentages, with PEG at five levels (including control) as the whole-plot factor, and time at six levels as the split-plot factor.

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A factorially arranged, completely randomized design was used for fresh-weight measurements, with PEG and time at five and six levels, respectively. Three replications in time were used for each experiment. Data were analyzed using Statistical Analysis System (SAS) GLM procedure (SAS Institute, Cary, N.C.).

Electrophoresis and protein measurement. Seeds germinated as described above were harvested at 24-hr intervals as in the water uptake experiment and at 96 hr for PEG-treated seeds. Seeds were washed and then dried at 37°C for 48 hr. Roots and hypocotyls were removed, and 50-mg samples of dry cotyledons were used for electrophoresis and protein measurement.

Samples were ground in 400 μ l of extraction buffer (2.5 ml 0.5 M Tris-HCl pH 6.8, 28.5 ml DI H₂O, 6 ml 10% SDS, and 1.0 ml β -mercaptoethanol) over ice by the method of Laemmli (1970). Extracts were centrifuged for 20 min at 11,000 \times g. Six microliters of the supernatant was subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (0.75 mm discontinuous slab gel, 0.1% SDS) by the method of Laemmli (1970).

Dry cotyledons (50-mg samples) were ground in 400 μ l of 5 mM Tris (pH 8.00) to extract soluble protein (Tully and Beevers, 1976). Extracts were centrifuged for 20 min at 11,000 \times g. After collecting the supernatant, the pellet was freeze-dried overnight and then dissolved in a 50:50 solution of 5% SDS : 0.5 N NaOH (Thorn, 1978). After the protein suspension was allowed to stand for 1 hr at room temperature, it was centrifuged and the supernatant was measured for insoluble protein. The soluble and insoluble protein contents of the cotyledons were measured according to Peterson's modification of the micro-Lowry method (Peterson; 1977). Data were analyzed using the SAS analysis of variance procedure.

Results

Water uptake. The increase in fresh weight of seeds during germination (Fig. 1) followed a typical triphasic water uptake curve. A rapid increase in fresh weight was followed by a lag period up to 48 hr. Germination had started at 48 hr and was followed by a second rapid increase in fresh weight. By 96 hr, 94% of the seeds had germinated. Fresh weight measurements every hour for eight consecutive hours (Fig. 1) provided more detailed information about the kinetics of water uptake during the early hours of imbibition. Water uptake reached a narrow

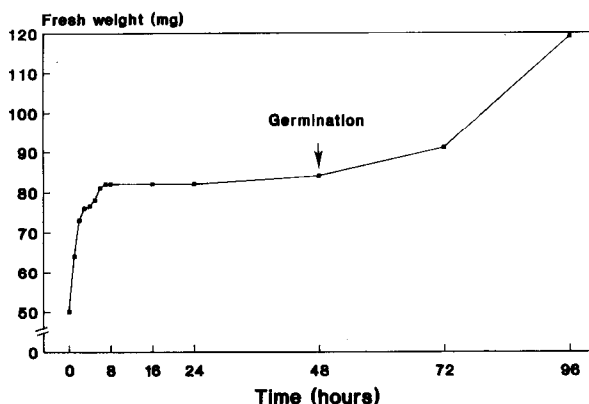


Fig. 1. Water uptake curve for impatiens seeds imbibed in deionized water. Fifty-milligram samples of seeds were imbibed for a designated time at 25°C, dried with absorbent paper, and measured for fresh weight at 24-hr intervals and 1-hr intervals for the first 8 hr. Each point is the mean of three replications.

plateau after 8 hr, but seeds had absorbed most of the water needed for germination in the first 2 to 3 hr.

PEG treatment. The majority of seeds germinated between 48 and 96 hr from imbibition at 0.0 MPa (control) (Fig. 2). Water potentials of -0.1 and -0.2 MPa delayed germination for 18 and 36 hr, respectively (compared with control) but germination was less inhibited as time passed. Germination percentages at -0.1 and -0.2 MPa were significantly less than the control, but greater than at lower water potentials at each examination. At -0.4 MPa, germination started at 96 hr and reached only 31% by 144 hr. A water potential of -0.6 MPa totally inhibited germination for up to 144 hr. At 96 hr, -0.1, -0.2, -0.4, and -0.6 MPa inhibited germination by 13%, 49%, 91%, and 100%, respectively.

The effect of PEG on seedling growth, as measured by an increase in fresh weight (Fig. 3), was parallel to its effect on germination. Significant increases in fresh weight of seeds at 0.0 MPa (control) began between 48 and 72 hr. With PEG treatment, the increase in fresh weight at -0.1 and -0.2 MPa had occurred by \approx 72 hr, and with -0.4 MPa between 96 and 120 hr, both of which periods correspond to the time of germination under these treatments. But fresh weight at -0.4 MPa was not significantly different from that at -0.6 MPa, under which there was no second increase in fresh weight (seeds do not enter Phase III) and no germination. At 96 hr, -0.1, -0.2,

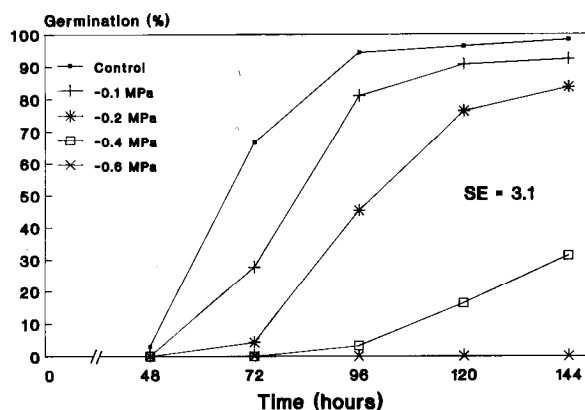


Fig. 2. Germination (radicle protrusion) at 25°C during 144 hr of impatiens seeds in PEG solutions of various water potentials. Each point is the mean of three replications. Deionized water was control (0.0 MPa).

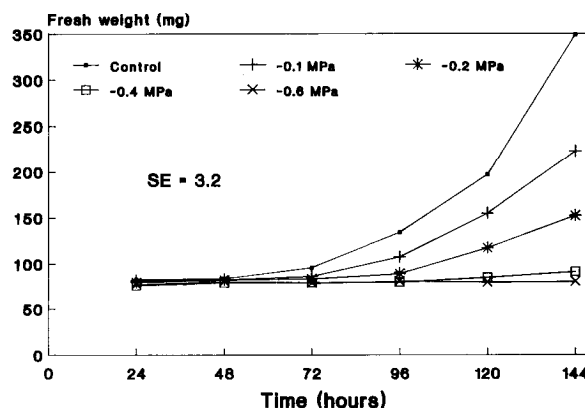


Fig. 3. Fresh weight of 50-mg samples of impatiens seeds germinated at 25°C for 144 hr in PEG solutions of various water potentials. Each point is the mean of three replications. Deionized water was control (0.0 MPa).

-0.4, and -0.6 MPa inhibited increases in fresh weight by 53%, 89%, 107%, and 106%, respectively, relative to the control.

SDS-PAGE and protein content. The SDS-PAGE profile of protein extracts from dry impatiens seeds separated into three distinct groups (Fig. 4, lane 0). The molecular weights of the proteins in these groups were estimated to be 1) 35, 33, and 31 kDa; 2) 26, 23, and 21 kDa; and 3) <14 kDa for two bands. The general patterns of degradation of storage proteins during 144 hr of germination are shown in lanes 2 through 6 (Fig. 4). Degradation started at 72 hr (Fig. 4, lane 3, 31 kDa) and proceeded rapidly through 96 hr, corresponding to the greatest increase in the percentage of germination (Fig. 2, control). By 144 hr, most of the insoluble storage proteins disappeared, and new bands started to appear at 96 hr (lanes 4, 5, and 6; see arrow).

At water potentials of -0.1 and -0.2 MPa, where some seeds germinated, degradation of storage proteins was detected (Fig. 5, lanes 2 and 3). At -0.4 and -0.6 MPa, conditions under which little or no germination occurred (lanes 4 and 5), the integrity of the major storage proteins was similar to that in dry seed (Fig. 5, lane O). Fully germinated seeds (Fig. 5, Lane 1) showed a considerable degree of storage protein degradation after 96 hr. The protein bands in lanes 4 and 5, compared to lane 0, indicated that very little degradation of the major storage proteins occurred in seeds when germination was prevented by water stress.

The protein content of seeds germinated in water (Table 1) reflected the changes in protein profiles shown in Fig. 4. As germination proceeded, soluble-protein content increased and insoluble storage protein decreased. The imposition of water stress by decreasing the water potential caused decreased germination, which, in turn, was reflected by an increased conservation of protein content (Table 2; Fig. 5). Although there were no significant differences in insoluble-protein content after 96 hr of germination due to water potential, there was a significant

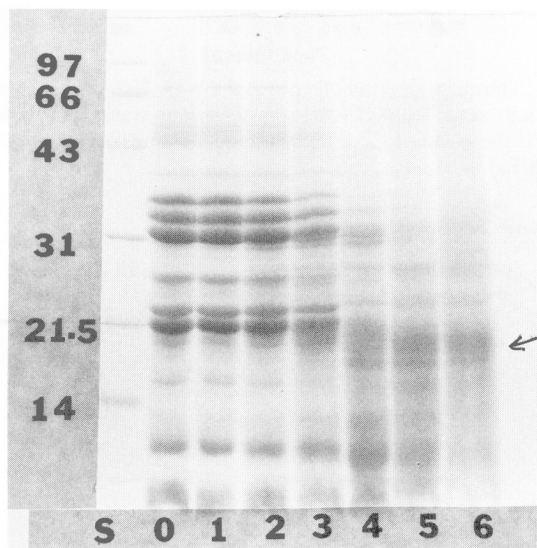


Fig. 4. SDS-PAGE profile of proteins extracted from seeds germinating in water. S, molecular weight markers; lane 0, mature impatiens seeds prior to germination; lanes 1-6, 24 to 144 hr of germination at 24-hr intervals. Arrow shows new bands started to appear at 96 hr. Molecular weights in kilodaltons are indicated on the y-axis.

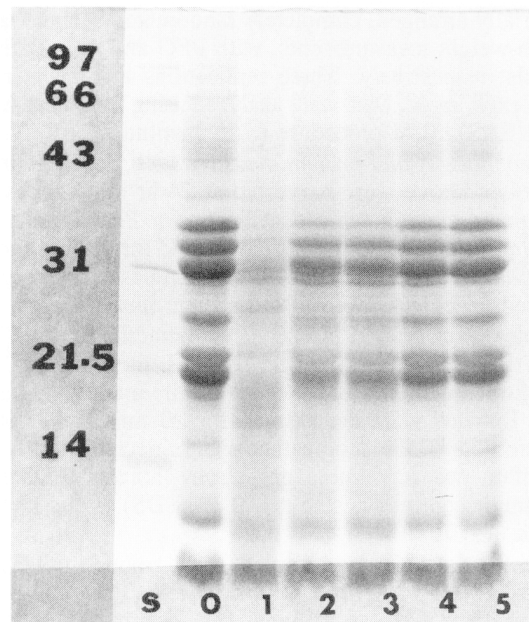


Fig. 5. SDS-PAGE profile of proteins extracted from seeds germinating under PEG-induced water stress conditions. S, molecular weight markers; lane 0, dry seed; lanes 1-5, seeds germinated for 96 hr under water potentials of 0, -0.1, -0.2, -0.4, and -0.6 MPa. Molecular weights in kilodaltons are indicated on the y-axis.

Table 1. Changes in soluble- and insoluble-protein content of cotyledons during 144 hr of germination of impatiens seeds.

Incubation time (hr)	Cotyledon protein content (mg·g ⁻¹)	
	Soluble	Insoluble
0	93	134
24	94	138
48	104	131
72	117	127
96	126	127
120	164	94
144	191	90
Linear	***	***
Quadratic	***	***
Model R ²	0.94	0.86

***Significant at $P = 0.001$ for linear and quadratic regression.

Table 2. Changes in soluble- and insoluble-protein content of impatiens seeds germinated under PEG-induced water stress for 96 hr.

Water potential (MPa)	Cotyledon protein content (mg·g ⁻¹)	
	Soluble	Insoluble
0.0	130	125
-0.1	121	127
-0.2	115	130
-0.4	107	130
-0.6	106	131
Linear	***	NS
Quadratic	*	NS
Model R ²	0.94	---

***,*,NSSignificant at $P = 0.001$ or 0.05 or nonsignificant, respectively, for linear or quadratic regression.

decrease in soluble protein with a decreasing water potential (Table 2). The lowest levels of soluble-protein content in cotyledons was a reflection of the lowest levels of germination (-0.4 and -0.6 MPa, Fig. 2).

Discussion

Germination of *impatiens* seeds under the conditions of this study had started by 48 hr after imbibition. Therefore, ≈40 hr of lag phase (water plateau) was required to develop the growth potential necessary for germination. This growth potential is provided by osmotic substances, such as amino acids, probably derived from storage proteins (Takeba, 1980a, 1980b). The increases in fresh weight and soluble-protein content that coincide with the first sign of radicle protrusion in *impatiens* support this conclusion (Table 1).

Low water potentials delay water uptake, increase the length of the water-uptake plateau, and subsequently delay or prevent germination (Figs. 2 and 3). Phase I of water uptake is not dramatically affected by PEG treatment because of the very low water potential of the dry seed (-100 MPa) (Hegarty, 1978). Delay in germination at water potentials of -0.1, -0.2, and -0.4 MPa demonstrates this induced inhibition (Fig. 3). A water potential of -0.6 MPa totally inhibited germination in *impatiens* for up to 12 days, whereas other species required lower water potentials for complete inhibition of germination: carrot, -1.0, calabrese, -1.5 MPa (Hegarty, 1977), jute, -0.8 MPa (Palit, 1987), and tomato, -1.0 MPa (Haigh and Barlow, 1987). This increased sensitivity of *impatiens* seeds could be due to the relatively small size of the seed, high surface : volume ratio, and the origin of this plant (elevated, humid regions of East Africa).

As germination proceeded, the pattern of protein bands changed considerably. Although the extent of degradation was less at water potentials of -0.1 and -0.2 MPa than for the control, the water potential still was high enough to permit germination. In contrast, at water potentials of -0.4 and -0.6 MPa, where no germination took place, very little degradation occurred. Therefore, under PEG-induced water-stress, a gradual decrease in water potential causes a gradual decrease in germination, and this is correlated with the inhibition of storage-protein degradation. It is likely that germination and the breakdown of storage proteins are both affected by low water potentials. On the basis of our data, we conclude that the breakdown of insoluble storage proteins occurs after radicle protrusion in *impatiens* seeds (Table 2).

Some of the metabolic processes that are inhibited under water stress are the cell-wall extension, protein synthesis, and enzyme activity (Hsiao, 1973). Yomo and Srinivasan (1973) and Minamikawa et al. (1983) reported cycloheximide, α -amanitin, and cordycepin inhibited protease formation and the degradation of the globulin (storage protein) in bean and cowpea. Their results indicated that de novo synthesis of some of the proteolytic enzymes was occurring during germination. Therefore, integrity of storage proteins of *impatiens* seed under stress in this experiment may be explained as inhibition of de novo synthesis of some of the proteolytic enzymes responsible for degradation of storage proteins.

Degradation of storage proteins (soluble or insoluble) in the embryo axis and cotyledons after the start of imbibition is complex. New synthesis of proteolytic enzymes in the axis is not a prerequisite for degradation of albumin (soluble proteins) during the initial stages of germination (Minamikawa et al., 1983). But synthesis of proteolytic enzymes is required for degradation of

globulin (insoluble proteins) in the embryo axis of cowpea. It seems that in *impatiens* seed a major part of the increase in soluble proteins up to 96 hr comes from the soluble storage proteins (Table 1). Alternatively, a small amount of insoluble storage-protein degradation may be required for germination but could not be detected by our techniques.

In practice, imposition of any water stress during the three phases of germination is detrimental. The temporal correlation of germination and degradation of storage proteins and the observation that both of these processes are inhibited under PEG-induced water stress imply that storage-protein (soluble or insoluble) breakdown is an important part of germination. The role of water stress in the prevention of degradative processes, along with its role in germination, may be exerted through the inhibition of synthesis or activity of preexisting proteolytic enzymes.

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