Mechanical Resistance of the Seed Coat and Endosperm during Germination of Capsicum annuum at Low Temperature

JAMES T. WATKINS and DANIEL J. CANTLIFFE
Vegetable Crops Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611

Received for publication April 9, 1982 and in revised form December 11, 1982

ABSTRACT

Decoated pepper (Capsicum annuum L. cv Early Calwonder) seeds germinated earlier at 25°C, but not at 15°C, compared to coated seeds. The seed coat did not appear to impose a mechanical restriction on pepper seed germination. Scarification of the endosperm material directly in front of the radicle reduced the time to germination at both 15°C and 25°C.

The amount of mechanical resistance imposed by the endosperm on radicle emergence before germination was measured using the Instron Universal Testing Machine. Endosperm strength decreased as inhibition time increased. The puncture force decreased faster when seeds were imbibed at 25°C than at 15°C. The reduction in puncture force corresponded with the ability of pepper seeds to germinate. Most radicle emergence occurred at 15°C and 25°C after the puncture force was reduced to between 0.3 and 0.4 newtons.

Application of gibberellic acid (100 microliters per liter) resulted in earlier germination at 15°C and 25°C and decreased endosperm strength sooner than in untreated seeds. Similarly, high O₂ concentrations had similar effects on germination earliness and endosperm strength decline as did gibberellic acid, but only at 25°C. At 15°C, high O₂ concentrations slowed germination and endosperm strength decline.

Seed coats and surrounding structures may influence the ability of a seed to germinate through interference with water uptake, gas exchange, diffusion of endogenous inhibitors, or by mechanical restriction of embryo growth. In seeds that do not have hard seed coats or require scarification for water uptake, other seed parts such as the endosperm may mechanically restrict embryo expansion, thus preventing radicle emergence. High O₂ concentrations and high temperatures accelerated germination. This was attributed to increased gas uptake, weakening of the endosperm, and pushing against the endosperm.

Scarification of the endosperm reduced the time to germination. Scarification at 25°C resulted in earlier germination than at 15°C. The puncture force decreased faster when seeds were imbibed at 25°C than at 15°C. The reduction in puncture force corresponded with the ability of pepper seeds to germinate. Most radicle emergence occurred at 15°C and 25°C after the puncture force was reduced to between 0.3 and 0.4 newtons.

Application of gibberellic acid (100 microliters per liter) resulted in earlier germination at 15°C and 25°C and decreased endosperm strength sooner than in untreated seeds. Similarly, high O₂ concentrations had similar effects on germination earliness and endosperm strength decline as did gibberellic acid, but only at 25°C. At 15°C, high O₂ concentrations slowed germination and endosperm strength decline.

Materials and Methods

Decoated Seeds. Air-dried pepper (Capsicum annuum L. cv Early Calwonder) seeds were decoated by inserting a scalpel into the seed cavity and peeling back the seed coat material. Twenty-five decoated or raw (untreated) pepper seeds were placed on a moistened filter paper in a 9-cm Petri dish. The Petri dishes were placed at 15°C or 25°C constant temperature chambers for germination.

Scarified Endosperm Test. Decoated air dry seeds were scarified by removing 0.5 mm of endosperm material directly in front of the radicle tip (Fig. 1). Scarified seed and decoated unscarified (control) seeds were then germinated as described above.

Germination data for both experiments were taken at 24-h intervals and all treatments were replicated four times (25 seeds/replicate). Seeds with visible radicles were counted as germinated. Data taken for each treatment included total percent germination and MDG (as previously described (14)):

\[ \text{MDG} = \frac{\Sigma (\text{days to germination} \times \text{number of seeds germinated})}{\text{total number of seeds germinated}} \]

Endosperm Strength Measurements. The Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA) was used to determine endosperm strength. Pepper seeds (50/
treatment time) were imbibed in 5 ml of distilled H2O, with or without GA4+7, on filter paper in 9-cm Petri dishes at 15°C or 25°C. Seeds treated with 100% O2 were gassed in a continuous flow system at 15°C and 25°C as previously described (15). After the appropriate treatment time, each of the seeds was prepared immediately before measurement on the Instron. The seeds were decoated and approximately one-fourth of the seed, the area of radicle emergence, was excised (see Fig. 1). The radicle was teased out of the surrounding endosperm material with a dissection needle, leaving a clean undamaged radicle cavity in the endosperm. The endosperm section was placed onto a No. 78 drill blank (diameter, 0.4 mm) attached to a basal load cell. The crosshead had a bolt placed in it with a 0.8-mm-diameter counter hole drilled into it. The load cell was set to 100 g (0.98 newton) full scale load. Crosshead and chart speeds were 1.3 and 25.4 cm/mia, respectively. Each treatment consisted of five seeds and all treatments were replicated five times.

**RESULTS**

Decoated seeds germinated earlier than seeds with coats at 25°C but there was no difference in the final germination percentage between the two treatments (Fig. 2A). At 15°C, germination earliness and total germination were unaffected by decoating (Fig. 2B).

When decoated pepper seeds were scarified by removing 0.5 mm of endosperm directly in front of the radicle, germination rates increased dramatically at both 15°C and 25°C (Table I). The scarification treatment did not affect the total germination of pepper seeds at 15°C or 25°C.

The force necessary to puncture the endosperm directly in front of the radicle decreased with increasing incubation time at both 15°C and 25°C (Fig. 3). Endosperm resistance decreased more rapidly when seeds were imbibed at 25°C versus 15°C. Puncture force decreased to 0.3 newton at both 15°C and 25°C before radicle protrusion occurred in a majority of the seeds.

At 25°C, GA treatment reduced endosperm resistance and increased earliness of radicle protrusion compared to untreated seeds (Fig. 4). GA treatment had a similar effect on endosperm weakening and radicle protrusion at 15°C, except that the process was slower (Fig. 5). This treatment caused a decrease in endosperm strength by the 2nd d; whereas, endosperm strength reductions occurred by the 4th d from initiation of imbibition in untreated seeds at 15°C.

When seeds were exposed to 100% O2 at 25°C, decreases in endosperm puncture force and radicle protrusion occurred sooner
Table 1. Effect of Removing 0.5 mm of Endosperm (Scarified) Directly in Front of the Radicle on Germination and Germination Rate (MDG) of Decoated Pepper Seed at 15°C and 25°C

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Germination (25°C)</th>
<th>Germination (15°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate %</td>
<td>Total Rate %</td>
</tr>
<tr>
<td>None</td>
<td>3.5</td>
<td>81</td>
</tr>
<tr>
<td>Decoated only</td>
<td>3.1</td>
<td>72</td>
</tr>
<tr>
<td>Decoated and scarified</td>
<td>1.7</td>
<td>78</td>
</tr>
</tbody>
</table>

*NS*, Significantly different; **NS**, not significant at the 5% level.

Fig. 2. Effect of seed coat removal on pepper seed germination at 25°C (A) and 15°C (B). *Mean separation within columns by Duncan's multiple range test, 5% level.

Fig. 3. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by germination temperature (15°C and 25°C).

Fig. 4. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by GA₄+7 (100 µl/l) at 25°C.
radicle and 
(100 µl/l) at 15°C.

FIG. 5. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by GA$_{4+7}$ (100 µl/l) at 15°C.

FIG. 6. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by 100% O$_2$ at 25°C.

FIG. 7. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by 100% O$_2$ at 15°C.

2nd d in seeds at 21% O$_2$ or the 4th d in 100% O$_2$. Endosperm strength in 100% O$_2$-treated seeds was higher at all times compared with the corresponding treatments in 21% O$_2$.

DISCUSSION

Mechanical resistance of the endosperm as measured by the Instron Universal Testing Machine appeared to affect germination of pepper. The resistance to puncture by the endosperm in pepper seeds was dependent on imbibition time, germination temperature, and treatment. Decoated pepper seeds germinated earlier than coated seeds at 25°C. The removal of the seed coat at 25°C may remove a barrier to gas exchange which would allow germination to proceed at a faster rate as was the case in elevated O$_2$ environments. The removal of the seed coat at 15°C did not affect radicle protrusion possibly because there already was a sufficient O$_2$ supply to the embryo for maximal metabolic activities for germination at that temperature. The seed coat and endosperm were found to inhibit O$_2$ uptake at high temperatures but not at low temperatures in Syringa (7). There does not appear to be any mechanical restriction to germination imposed by the seed coat

per se. If this was the case, seed coat removal would be expected to lead to earlier radicle protrusion at 15°C as well as 25°C.

Scarification of the endosperm material directly in front of the radicle markedly reduced the time to germination. Removal of 0.5 mm of endosperm material caused the embryos to be 'pushed' from the endosperm by imbibitional pressure and usually resulted in damage to the radicle. This points to a mechanical resistance imposed by the endosperm; but, the effect of the mechanical restriction was greater at the lower temperature.

A hypothesis was proposed by Pavlica and Haber (10) that germination of lettuce seed requires (a) a mechanical force of the growing embryo pushing against the endosperm and (b) chemical or enzymic weakening of the endosperm. The studies presented here indicate that endosperm strength must be reduced before germination will occur. The reduction in endosperm resistance at 15°C and 25°C when GA$_{4+7}$ was applied offers some evidence as to the mechanics of pepper seed germination. Stimulation of cell division by GA$_{4+7}$ does not appear to be the cause of increased germination rates (14). Conditions which favor germination of Syringa embryos, i.e. high temperature, chilling, and GA$_{4+7}$ favored enhanced radicle elongation (7). GA$_{4+7}$ stimulated earlier radicle protrusion in pepper seeds submerged in osmotic solutions which inhibited cellular expansion (14). GA$_{4+7}$ stimulated enzymic activity in monocot (8, 13) and lettuce seeds after radicle emergence (4). Thus, the possibility exists that GA may regulate pepper seed germination before radicle protrusion by controlling radicle elongation and/or enzymic degradation of endosperm materials. Evidence for this control by GA will be discussed elsewhere (16).

The effect of O$_2$ on endosperm weakening is difficult to interpret. At 25°C, pepper seeds germinated in 100% O$_2$ had a higher respiratory rate and thus possibly a higher metabolism than did seeds germinated in air (15). At 15°C, high O$_2$ did not increase respiration rate. At this temperature, endosperm strength decreased at a slower rate than occurred in air. In excised bean embryos cultured in 100% O$_2$, reductions in growth and enzymic activity were found (11). Eliasson (3) reported that pure O$_2$ inhibited the growth of wheat roots due to inhibition of cell elongation. Albaum et al. (1) suggested that subsequent growth of oxygenated oat seeds was inhibited due to high O$_2$ interfering with proteolytic breakdown of the endosperm, thereby preventing nitrogen transport from the endosperm to embryo, development of enzyme activity, and growth. Yet, high O$_2$ did not affect Cyt oxidase. This may indicate that the reduced endosperm weakening in pepper seeds germinated in 100% O$_2$ at 15°C was due to the inability of embryos to utilize more O$_2$ than that contained in air.
These higher levels of O₂ may then be adversely affecting enzymic breakdown of pepper endosperm, thus slowing germination (16). This reduction in enzyme activity may be directly reflected in the greater endosperm resistance of seeds without GA treatment or which were germinated in 100% O₂ at 15°C.

LITERATURE CITED

6. JONES RL 1974 The structure of the lettuce endosperm. Planta 121: 133–146