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Changes in the Strength of Lettuce Endosperm during Germination[1]

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ABSTRACT

The forces required to puncture intact lettuce (*Lactuca sativa*) seed and pericarp, endosperm and embryo were measured by the Instron Universal Testing Machine. It required about 0.6 newton to puncture the endosperm in seeds imbibed in the dark at 6, 12 and 24 hours. Endosperm of seeds imbibed in the light or in dark with gibberellic acid required about 4.2 newtons at 6 and at 12 hours and only about 0.15 newton at 24 hours. Forces required to puncture embryo at all treatments and times remained constant at about 0.3 newton. Changes in the strength of the endosperm do not appear to be related directly to protrusion of the radicle.

The dark dormancy of Grand Rapids lettuce seeds can be broken by light, GA, or fusicoccin (1, 9, 12). Ikuma and Thimann (7) concluded that the integrity of the endosperm determined the photosensitivity of the seed. Experiments involving mechanical removal of various parts of the seed covering and the microscopic observation (8) indicated that the endosperm was responsible for the restriction of embryo growth (4, 6). As injection of hydrolytic enzymes such as pectinase and cellulase promoted dark germination, Ikuma and Thimann (7) proposed that the final step in the germination control process might be the production of such enzymes. Lang and his colleagues (14, 16) further verified that endosperm restricted embryo growth. Nabors and Lang (14) attempted to determine the force required to puncture the seed coats by means of changes in osmotic potential of the red-light-treated embryo. However, these workers used a rather crude means to puncture the empty endosperm seed coat derived from the dark-imbibed seeds.

The Instron Universal Testing Machine[3] has often been used for measuring various textural characteristics of foods, and the puncture force determined by this instrument is regarded as highly objective (2). In the present study this machine was adapted to determine the relative strengths of the coverings (pericarp, endosperm) by measuring the forces required to puncture the intact seed, the seed minus the pericarp, and the excised embryo at various stages of imbibition and growth. We hoped that such a study might reveal the physiological significance of the seed coverings and their involvement in regulation of germination.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa* cv. Grand Rapids) seeds with less than 10% dark germination were obtained from the Ferry Morse Seed Company. Fifty seeds were imbibed in 5 ml of distilled H_2O in the light, in 5 ml of 1 mM GA_3 in the dark (dark + GA_3) and in 5 ml of distilled H_2O in the dark on two layers of filter paper in 9-cm Petri dishes at 25 C. Radicle protrusion began to occur at 13 hr in light. After 6, 12, and 24 hr of imbibition the seeds were transferred to near freezing temperature to stop radicle growth. For the puncture test, pericarp or pericarp and endosperm were carefully removed without visible injury to the embryo and/or endosperm. It is extremely difficult to remove pericarp and/or endosperm from large numbers of seeds which have imbibed for less than 6 hr. Puncture tests were, therefore, performed in seeds imbibed for 6 hr and more.

The Instron Universal Testing Machine (2) was used for the puncture test. The seed, the embryo, or the seed minus the pericarp was placed in the center of an aluminum block directly above a hole which is drilled through the block. A countersink on the block helped to center the seed. A circular flat faced No. 76 drill blank, 0.2 mm in diameter, attached to the inverted load cell, was used to puncture the seed. The load cell was set to 2 newtons (204 g) full scale load. Crosshead and chart speeds were 5 and 10 cm/min, respectively. In each treatment 10 different seeds or seeds minus coverings were punctured individually and the puncture force recorded.

RESULTS

Force peaks from puncture tests on single lettuce seeds imbibed in the light for 12 hr are shown in Figure 1. Although some variation occurred among individual lettuce seeds, these were small compared to that reported for cooked dry beans (2). The force required to puncture an intact seed (pericarp + endosperm + embryo) was consistently higher than that required for puncturing a seed without the pericarp (endosperm + embryo). The difference between the two means gave a good indication of the strength of the lettuce seed pericarp. Similarly, the puncture force for the seed without the pericarp was consistently higher than for the embryo. The strength of the lettuce endosperm can be measured by the difference between the two means. The Instron appears to be an effective means of measuring the strength of the pericarp and the endosperm.

The forces required to puncture the intact seed, the pericarp, the endosperm, and the embryo following a 6-hr imbibition under various conditions are shown in Table I. None of the treatments significantly altered the forces required to puncture the intact seed, the pericarp, or the embryo. A significant difference was noted between the forces required to puncture the endosperms in seeds imbibed in the dark and those imbibed in the light or in the dark + GA_3 for 6 hr. This difference was significant at the 1% level (as determined by Duncan's multiple range test). No significant difference was noted between the endosperm strength of light and dark + GA_3-treated seeds at 6 hr. The puncture force for the seed without the pericarp (embryo ± endosperm) was

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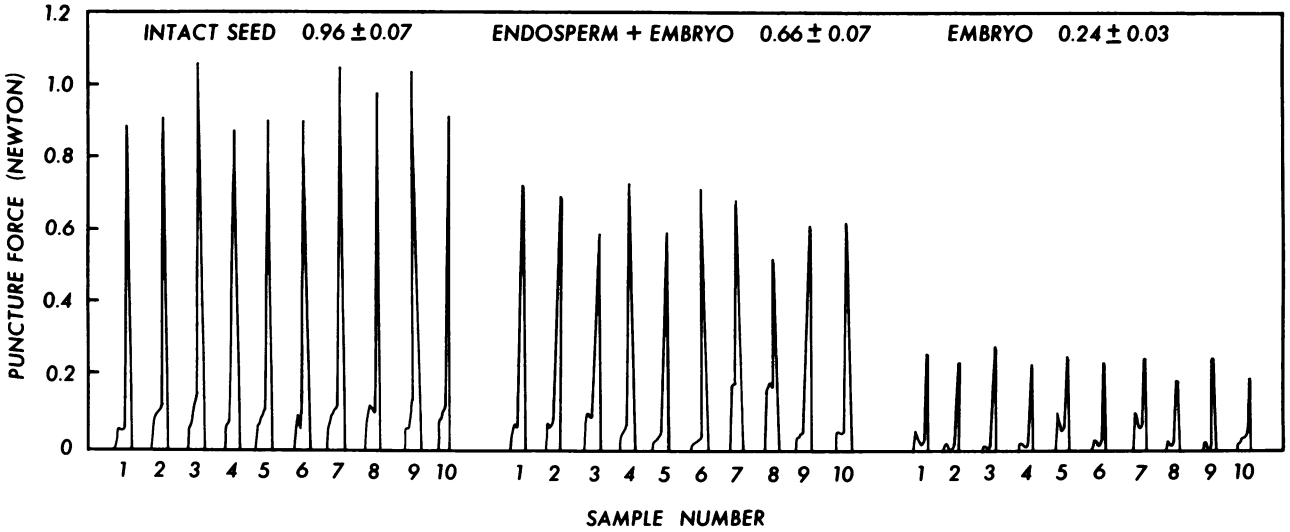

FIG. 1. Force peaks obtained during puncture of intact seed, embryo + endosperm, and embryo by Instron Universal Testing Machine. Seeds were imbibed in light for 12 hr. Full scale load = 2 newtons. Width of base of each peak indicates total puncture time. Chart speed was 5 cm/min.

Table I The forces required to puncture intact lettuce seed and seed parts imbibed for 6 and 24 hr under various conditions

Treatment	Seed parts	Puncture force (Newton) 6 hr Mean[z] ± SE	Puncture force (Newton) 24 hr Mean[z] ± SE
Light	Intact seed	1.06 ± 0.03	---
	Embryo + Endosperm	0.75 ± 0.07	0.52 ± 0.01
	Pericarp	0.31 ± 0.04	---
	Embryo	0.34 ± 0.12	0.33 ± 0.02
	Endosperm	0.41[a] ± 0.06	0.19[a] ± 0.04
Dark + GA_3	Intact seed	1.05 ± 0.01	---
	Embryo + Endosperm	0.74 ± 0.09	0.46 ± 0.02
	Pericarp	0.31 ± 0.10	---
	Embryo	0.30 ± 0.02	0.34 ± 0.03
	Endosperm	0.43[a] ± 0.07	0.12[b] ± 0.02
Dark	Intact seed	1.08 ± 0.05	1.10 ± 0.03
	Embryo + Endosperm	0.84 ± 0.02	0.94 ± 0.07
	Pericarp	0.25 ± 0.04	0.17 ± 0.08
	Embryo	0.24 ± 0.03	0.30 ± 0.03
	Endosperm	0.60[b] ± 0.01	0.63[c] ± 0.05

[z]Mean was calculated from 30 determinations (3 experiments, 10 seeds/treatment). Pericarp and endosperm values were not directly measured, but calculated as differences of measured values. SE for the pericarp and endosperm were calculated as differences of means in each experiment. The endosperm values followed by the same letter in each column are not significantly different at 1% level as analysed by Duncan's multiple-range test.

slightly higher in the dark compared to seeds imbibed in the light or in the dark + GA_3, although the difference in the means was not statistically significant. After 12 hr of imbibition the forces required to puncture the intact seed or various seed parts were not significantly different from those imbibed for 6 hr (data not shown).

At 24 hr of imbibition of seeds radicles had already protruded in the light and in the dark + GA_3 treatments. During the puncture test of the intact seed the germinated embryo with attached endosperm tended to slide off the pericarp. Hence no puncture test was recorded for the intact seed and the pericarp in these two treatments. In seeds imbibed in the dark only ungerminated intact seeds were used for the puncture test. Significant differences were found in the strengths of the endosperm and endosperm plus embryo in the three treatments, the forces required to puncture them being highest in the dark-imbibed seeds and lowest in the dark + GA_3 with light-germinated seeds showing an intermediate value (Table I).

No significant differences were noted in the force required to puncture the embryo in the three treatments at 6, 12, or 24 hr.

DISCUSSION

Indirect evidence has suggested that the lettuce endosperm layer could control radicle protrusion by its restricting force (4, 6–8, 15, 17). The thick cell wall and the dense cytoplasm of the endosperm cell (7) attest to the strength of the endosperm layer. Our results indicate that a force of approximately 0.6 newton is required to puncture the endosperm layer of seed imbibed in the dark (Table I). The endosperm strength represents about 60% of the total force required to puncture the intact seed. These data are consistent with earlier observations (4, 7, 14) on the greater mechanical strength of the endosperm layer compared to the pericarp.

Nabors and Lang (14), using glass rods with hemispherically shaped tips with diameters of 0.25 and 0.4 mm, reported that the forces required to break the endosperm-pericarp layers obtained

from seeds imbibed for 18 to 20 hr in the dark were 8.5 and 20.7 g, respectively. Using a drill with a flat circular end, 0.2 mm in diameter, the force required to puncture the endosperm-pericarp layers, in the present study, ranged from 0.80 to 0.85 newton or 82 to 87 g during 6 to 24 hr of dark imbibition. Thus, our results are at variance with the data of Nabors and Lang (14). It is possible that their method measured the force in an area of the coverings (radicle end) that offered least resistance to the pressure exerted (14). Their low values could also be due to the following. (*a*) The tension applied to seed covers by hand might be uneven and higher than when intact seeds are used. When a membrane is under high tension, it could be punctured by applying less force. The uneven pressure applied to the seed covers could decrease the contact area between the rod tip and the cover and thus result in easier breakage. (*b*) The hemispherical end of the rod is sharper than the flat circular end. (*c*) The measuring method was rather crude and very subjective. A subjective error is difficult to avoid.

When the seeds were imbibed in darkness for various periods there was no significant change in the strength of the endosperm (Table I). In the light or in the dark + GA_3, on the other hand, the strength of the endosperm at 6 hr decreased noticeably, with no further reduction until 12 hr. By 24 hr considerable reduction in the strength of the endosperm had occurred (Table I). These results affirm Jones' microscopic observations (8) that the breakdown in the endosperm cells occurs at 12 to 15 hr of imbibition in light, whereas dark-imbibed seeds show no such change. Jones (8) also reported that the digestion of the lateral and the inner walls, but not the outer walls, was completed after 24 hr in the light. The forces required to puncture the endosperm layer at 24-hr germination in the light could represent the strength of the outer endosperm wall. Neither the treatments nor the duration of a treatment altered the force required to puncture the embryo (Table I). It is not certain that the Instron can be used to detect changes in the growth potential of the embryo.

What are the nature of the changes which reduce the strength of the endosperm? Is the reduction in strength directly related to germination? Are events in the embryo and endosperm prior to germination independent of each other? These problems remain, essentially, unresolved. The studies of Ikuma and Thimann (7) and that of Halmer *et al.* (5) lend support to the suggestion that degradative enzymes produced during imbibition could digest the endosperm. The time course of light- or GA_3-induced mannanase degradation of mannose-containing polysaccharides (the major constituent of the endosperm) does not correspond with that of radicle protrusion (5), suggesting that changes in endosperm strength may not be directly related to germination, a situation reminiscent of barley grain (3). Fusicoccin, a diterpene glucoside which is more active than GA_3 or light in inducing dark germination of lettuce seeds, is unable to induce α-amylase activity in barly endosperm halves but is able to promote root growth in the intact grain (10 and unpublished observations). That reduction in the strength of the endosperm may not have a direct role in germination is further indicated by the anomalous observation that GA_3, which is slower than light by 3 to 4 hr in inducing dark germination (13), nonetheless decreases to a greater extent the strength of the endosperm layer (Table I). There was no difference in the strength of the endosperm in light and the dark + GA_3 at 12 hr although radicle protrusion occurred, soon thereafter, only in light. It is quite possible that germination, which is essentially a function of the embryo, can be independent of degradative activity in the endosperm of seeds including lettuce.

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