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Control of Seed Coat Thickness and Permeability in Soybean[1]

A POSSIBLE ADAPTATION TO STRESS

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L. D. NOODÉN*, K. A. BLAKLEY, AND J. M. GRZYBOWSKI
Botany Department, University of Michigan, Ann Arbor, Michigan 48109-1048

ABSTRACT

Although the seed coat, through its thickness and permeability, often regulates seed germination, very little is known about the control of its development. Using soybean (*Glycine max* [L.] Merrill) explants, pod-bearing cuttings in which defined solutions can be substituted for the roots, we have demonstrated that cytokinin and mineral nutrients moving through the xylem can control soybean seed coat development. Lack of cytokinin and minerals in the culture solution, causes a thicker, less permeable seed coat to develop. The seeds with thickened coats will imbibe water rapidly if scarified; furthermore, these scratched seeds also germinate and produce normal plants. Inasmuch as stress (*e.g.* drought) decreases mineral assimilation and cytokinin production by the roots, the resulting delay in germination could be an adaptive response to stress.

Polymorphy in the seeds produced by an individual plant is fairly common and usually ensures that not all of the seeds germinate at one time or in one habitat (2, 5–7). Often, the seeds which are slow to germinate have thicker and less permeable seed coats. Legumes, in particular, may have highly thickened, hardened, and impermeable seed coats; however, the seeds of leguminous field crops such as soybean normally have relatively thin, permeable coats (3, 8). Environmental factors can influence seed coat development, apparently through effects on the mother plant, and hormone treatments of the parent plant may also alter the thickness and permeability of the coats around the seeds produced (2, 5, 6). Although a mineral nutrient supply is necessary and cytokinins appear to be involved in seed development, very little is known about the endogenous control of seed coat development (2, 10). During our studies on the influence of mineral nutrient and cytokinin supply on senescence and pod development, we noticed large differences in the seed coats, and we decided to investigate these preliminarily. Here, we will show that the flux of root assimilates (minerals and cytokinin) into the shoot system controls seed coat development (thickness and permeability).

MATERIALS AND METHODS

Soybeans (*Glycine max* [L.] Merrill) cv 'Anoka' (inoculated with *Rhizobium* and planted in soil) were grown as described by Lindoo and Noodén (12). Explants (stem cuttings with a single, three-seeded pod and a leaf) were excised at early-midpodfill and cultured on media with or without a complete mineral nutrient mixture (with N and macronutrient levels approximating those in xylem sap) and/or cytokinin (4.6 μM zeatin) (16). These explants were taken from the middle third of the pod-bearing region and carefully selected for uniformity by visual inspection and candling of the pods to check seed size. Thus, the range of variation normally seen on intact plants was greatly narrowed. Within each treatment group (six explants), the explants behaved uniformly in terms of leaf senescence and pod development; moreover, these parameters responded to the treatments exactly as described earlier (4, 16). Each experiment was at least duplicated.

RESULTS AND DISCUSSION

Explants cultured on water show not only earlier foliar senescence compared with similar parts of intact plants, but pod development is also advanced (16). Defined solutions can be substituted for the root system by placing the cut bases in these solutions and allowing the explants to draw the solutions up through the xylem. A mineral nutrient mixture and/or cytokinin delays leaf senescence and pod development, shifting both toward normality (4, 15, 16). The use of explants selected for uniformity as described above and the controlled conditions greatly reduce variability in the seeds produced but, at the same time, this limits the sample size for practical reasons.

The seeds produced by explants cultured on water are smaller than those from intact plants (15, 16); however, germination after 11 d of favorable conditions is greatly reduced compared to seeds from intact plants (Table I). This decrease also reflects a delay in germination (kinetic data not shown). In some experiments, the seeds from water-cultured explants showed even lower germination (greater delay). This difference can be seen by comparing water uptake by seeds from water-cultured explants in Figures 1 and 2. Even visual inspection reveals that the seeds

Table I. *Germination of Seeds from Intact Soybean Plants and Explants Cultured on Water with and without Scarification*

Germination was measured as radicle protrusion after 11 d of incubation in moist but well-aerated vermiculite under the same conditions used for whole plants. Seeds were scarified by rubbing both sides gently with fine carborundum paper until the seed coats showed visible abrasion which did not extend through the seed coat. Each sample consisted of 18 seeds. The variability in these seeds was low, similar to that in Figure 1.

	Germination
	%
Seeds from intact plants	
Without scarification	100
With scarification	100
Seeds from explants cultured on water	
Without scarification	28
With scarification	67

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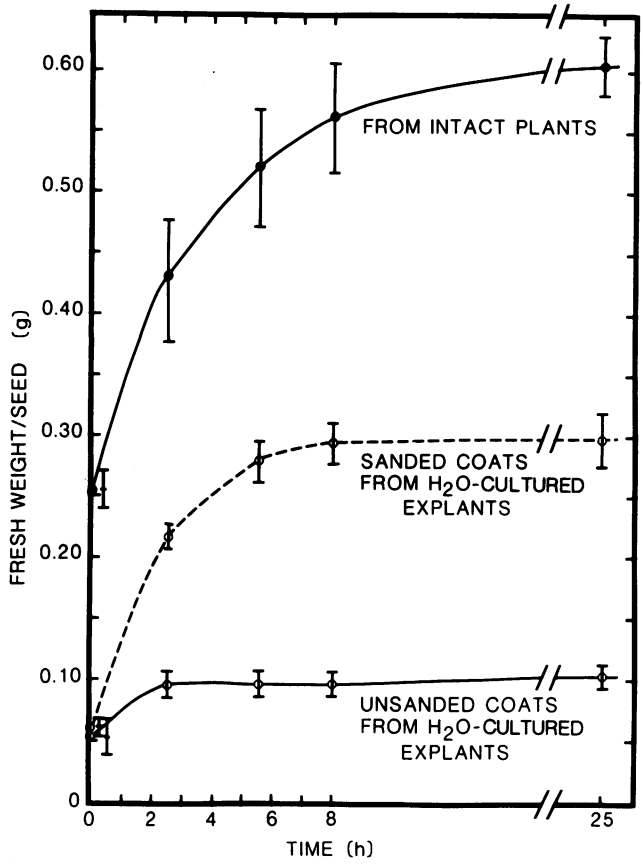

FIG. 1. Time-courses for imbibition of water by seeds from water-cultured explants with or without scarification as compared with seeds from intact plants. The seeds were soaked in distilled H_2O at 25°C. SE are shown.

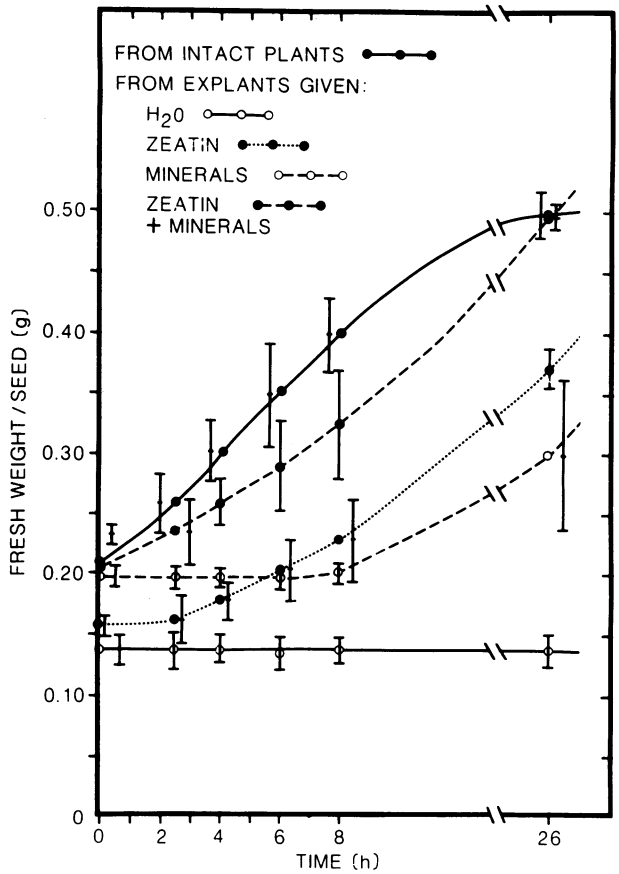

FIG. 2. Time-courses for imbibition of water by seeds from explants cultured in water only, cytokinin, minerals, or minerals plus cytokinin or from intact plants. Conditions similar to those cited for Figure 1.

from water-cultured explants do not swell as much as the seeds from intact plants. The slowness of the water explant seeds to imbibe water and the ability of scarification to overcome this are shown in Figure 1. Since the seeds from water-cultured explants are smaller than those from intact plants, it may be more appropriate to compare the fresh weight gain over 25 h for unsanded seeds from water-cultured explants (0.046 g/seed or 94%) to that for sanded seeds from water-cultured explants (0.24 g/seed or 400%) and for seeds from intact plants (0.35 g/seed or 138%). Thus, the sanded seeds imbibe a substantial amount of water for their size. In addition, the initial rates of water uptake are similar for the sanded seeds and the seeds from intact plants. Returning to Table I, it can be seen that scarification likewise promotes germination of the seeds from water explants. Furthermore, these sanded seeds produce normal plants.

In the intact plant, the roots supply cytokinin (11, 17, 20) and minerals (1, 14) to the shoots via the xylem. We (4, 15, 16) have already demonstrated that a supply of minerals and cytokinin can at least partially replace the root system in normalizing leaf senescence and pod development in the explants. Seeds from explants supplied with minerals in combination with cytokinin show both increased imbibition of water (Fig. 2) and improved germination (Table II). Although the combination of minerals and cytokinin is most effective, cytokinin alone may exert a very substantial effect, more than minerals alone.

Covering the hilum with lanolin does not substantially inhibit imbibition (data not shown). This demonstrates that the hilum is not the main route for water entry in these seeds as it is in some other legumes (9). Therefore, the permeability barrier must reside in the seed coat.

Table II. *Germination of Seeds from Explants Cultured on Media with and without Cytokinin (4.6 μM Zeatin) and/or Mineral Nutrients (16) and from Intact Plants*

Conditions similar to those cited in Table I. Measured after 7 d. The variability in these seeds was low, similar to those in Figure 2.

	Germination
	%
Seeds from intact plants	100
Seeds from explants cultured on	
Water only	0
Minerals only	0
Cytokinin only	20
Minerals plus cytokinin	60

Figure 3 shows that the coats of seeds from water-cultured explants are much thicker than those of intact seeds. The treatments with minerals, cytokinin, or the combination produces seeds with coats similar in thickness to intact plants. While coat thickness may be a major factor governing the permeability and germination of seeds from water explants, this does not account for all the differences in imbibition rates (compare seeds from explants supplied minerals only *versus* zeatin plus minerals in Figs. 2 and 3). Therefore, differences in seed coat composition must also be important.

Clearly, root assimilates, particularly cytokinin, control seed coat maturation (thickening and hardening) in explants. Although a direct (but small) xylem connection with the main vascular system (19) would allow for a direct influence of xylem-carried materials on seed coat development, the minerals and cytokinins could also act indirectly via an effect on the leaves

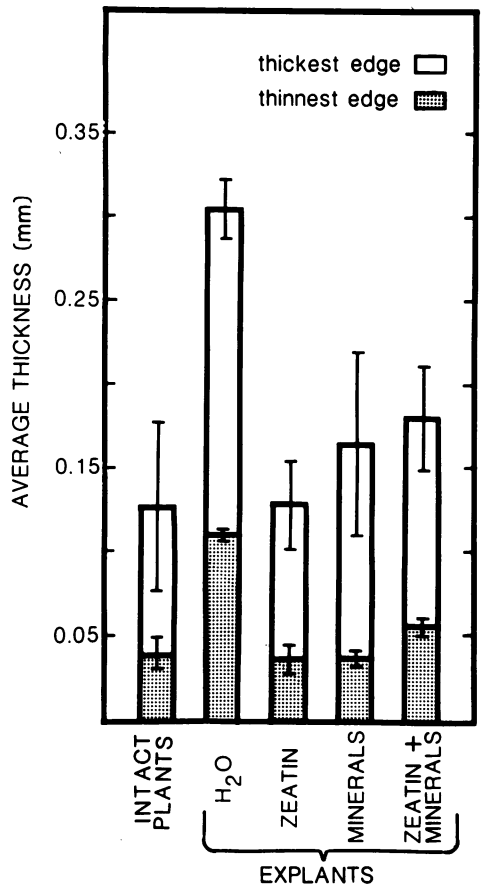

FIG. 3. Thickness of the seed coats from explants cultured in water only, cytokinin, minerals, or minerals plus cytokinin or from intact plants. Each sample included 18 or more seeds. The thickness was determined by cutting out sections of the coat with a sharp razor blade, mounting them in wax (melting point 55°C), and measuring the cut edge of a thin cross-section under a microscope with an ocular micrometer. Because the thickness of the seed coat differs quite a lot from one part of the seed to another, we chose to compare thicknesses at standard locations, namely, 1 mm on either side of the middle of the hilum (thick part) and further down the sides, halfway between the hilum and the opposite edge of the seed (thin part). This allows a precise comparison of the effects of the treatments despite differences within the seed coat.

(16). Inasmuch as stress (*e.g.* drought) depresses root assimilation, especially cytokinin production, stress would decrease cytokinin and also mineral nutrient flux into the shoot (1, 13, 21) which could in turn cause the formation of seeds with greatly delayed germination. Impermeable or otherwise resistant seed coats could serve to carry these propagules past an unfavorable period under natural conditions (7). Of course, stress conditions would probably produce only a partial reduction in the mineral and cytokinin content of xylem sap; however, it is possible that a decreased cytokinin (and mineral nutrient) flux may provide a regulatory signal to induce development of a resistant seed coat, a response that warrants further study. Moreover, hard or impermeable seed coats do occur in field-grown soybeans, and this can be an economic problem (see Rana [17] and literature cited). The data reported here also provide a possible explanation for this phenomenon.

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