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The Effect Soaking Pea Seeds With or Without Seedcoats has on Seedling Growth

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Abstract. **Pea seeds (*Pisum sativum* L. 'Alaska') with intact seedcoats (WC) and with seedcoats removed (WOC) were soaked in distilled water for 24 hours at 20°. The water, containing the pea diffusate, was decanted after the second, fourth, sixth, eighth, twelfth, and twenty-fourth hour and analyzed for total nitrogen, α-amino nitrogen, carbohydrate, and total solute dry weight. The seeds were germinated at 20° in a 16 hour photoperiod of 300 foot candles. Stem lengths and dry weights of roots, shoots and cotyledons were determined after 4, 11, and 18 days of growth. WOC seeds imbibed more water than WC seeds during the 24 hour imbibition period. Diffusates from WOC seeds always contained more solute than diffusates from WC seeds. Maltose, glucose, and fructose were not detected in the early diffusates from WOC seeds but were found in WC seed diffusates at all times. Seedlings from WC seeds had longer stems than those from WOC seeds. The dry weight of stems and roots of WC seedlings was greater than those from WOC seedlings. The dry weight of cotyledons from 18 day-old WC seedlings was less than from WOC seedlings. Water absorption by WC seeds was slower than by WOC seeds. Removal of the seedcoat allowed rapid imbibition resulting in seed injury presumably because of the loss of solutes which included monosaccharides, disaccharides, amino acids, and other nitrogen containing compounds. These results are consistent with the hypothesis that rapid imbibition disrupts membrane organization leading to reduction of seedling growth.**

Before seed germination can proceed a certain amount of water must be absorbed by the hydrophilic components of the seed. How much water depends largely upon the seed in question. Hunter and Erickson (4) report a minimum moisture requirement for germination of 30 %, 26 %, 50 %, and 31 % for corn, rice, soybeans, and sugar beets, respectively. Similar data from Peters (8) (converted to read in percent water of total weight of imbibed seed and therefore comparable with the data of Hunter and Erickson) gave 32 %, 60 %, 58 %, and 41 % for corn, pea, bean, and wheat, respectively. Seeds absorbing these amounts of water could at the same time lose water soluble materials by diffusion. Presoaking of peas in distilled water will within limits accelerate germination. Such factors as length of soaking time, water temperature, aeration during soaking, and amount of water must be considered in evaluating the effect of soaking on germination and growth. Pea seeds do not exhibit dormancy and are more likely to be injured by prolonged soaking than seeds exhibiting some kind of dormancy. Soaking injury could result from a lack of oxygen during imbibition, bacterial action, harmful effect of pure water on imbibing tissues, leaching out of essential compounds or any combination of these. Kidd and West (5) found that submerging *Phaseolus, Pisum,* and *Helianthus* in water at 17° for 24 hours decreased germination and retarded subsequent growth. On the other hand Eyster (2) has shown that soaking bean and pea seeds does not affect germination and growth if the seeds are first allowed to become fully imbibed before they are submerged in water.

In this study a partial analysis of the diffusate produced over a 24 hour imbibition period from peas completely submerged with and without seedcoats is reported as well as the effect soaking the seeds has on seedling growth.

Materials and Methods

Pea seeds (*Pisum sativum* L. 'Alaska') used in this study were obtained from the Burpee Seed Company, Clinton, Iowa. The seedcoat of 1 batch of seeds was carefully removed using a X-acto blade No. 28. By carefully cutting the seedcoat from the dry seed around the area of the radicle, the seedcoat could be removed in 2 or 3 pieces. This method usually scored the cotyledons slightly. Special care was taken not to separate the 2 cotyledons or to damage the radicle and plumule. All damaged seeds were discarded. Three hundred and fifty seeds with seedcoats (WC seeds) and 350 seeds without seedcoats (WOC seeds) were placed in individual 400 ml beakers; the seeds were covered with 200 ml of distilled water and left to imbibe without aeration at 20°. The excised seedcoats (SC) were also covered with water and left to

imbibe at 20°. At the second, fourth, sixth, eighth, twelfth, and twenty-fourth hour, the water containing the pea diffusate was decanted and 200 ml of fresh water added to the peas. The diffusates were evaporated to dryness *in vacuo* at 25° and the residue dissolved in water to make 25 ml of solution. Aliquots from these 25 ml samples were used to determine the dry weight of the diffusates, total soluble nitrogen, α-amino nitrogen and soluble carbohydrate. Total soluble nitrogen was measured by Kjeldahl digestion followed by nesslerization. Alpha-amino-nitrogen was determined using the method of Yemm and Cocking (12). Carbohydrates were estimated by the anthrone method (11). Components of the diffusates were separated using descending paper chromatography with Whatman No. 1 paper and butyl alcohol, propionic acid, and water (26:19:27 by vol). Visualization of carbohydrates was obtained by dipping the chromatogram in aniline-diphenylamine reagent (10) and heating for 1 minute at 100°. Visualization of amino acids was accomplished by dipping the chromatogram in 1 % ninhydrin (in acetone) and waiting several hours for the colors to develop at room temperature.

After the 24 hour imbibitional period, the seeds were placed in trays containing white quartz sand (30 mesh; AFS 54) then put in a 16 hour photoperiod at a light intensity of 300 foot candles. Twenty-five to 44 seedlings were sampled on the fourth, eleventh, and eighteenth day. On sampling days, stem lengths were measured and the dry weight of roots, stems, and cotyledons determined.

Results

Imbibition. Figure 1 shows the rate of imbibition during the 24 hours the seeds were totally submerged in water. WOC seeds demonstrate initially a greater rate of water absorption. Water absorption by WC seeds lags at first but soon equals the rate of WOC seeds. By the eighth hour WC seeds have begun to absorb more water than an equal number of WOC seeds. The greater weight of water absorbed by the WC seeds can be accounted for by the presence of the seed coats.

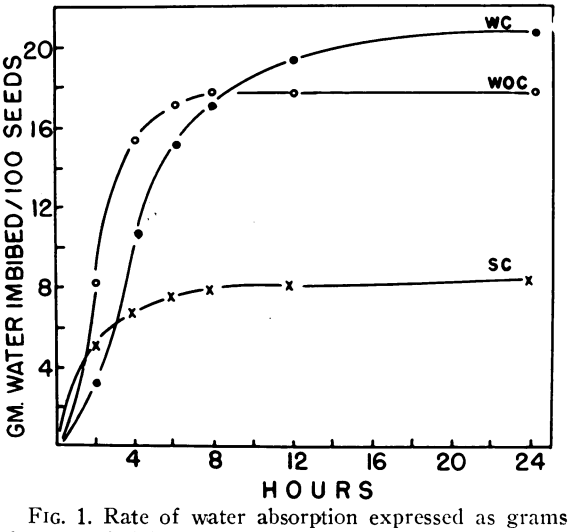

FIG. 1. Rate of water absorption expressed as grams of water imbibed per 100 seeds. ●—●—● seeds with seedcoats (WC); ○—○—○ seeds without seedcoats (WOC); X—X—X seedcoats alone (SC).

Diffusate Analysis. The total dry weight of the solute in the diffusates, and the amounts of carbohydrate, total nitrogen and α-amino nitrogen detected in these diffusates are given in table I.

Table I. *Composition of Diffusates from WC & WOC Seeds*

The dry weight of solute in the diffusate, carbohydrate, total nitrogen, and α-amino-nitrogen from 100 seeds is given. Dry weights are for the time intervals indicated.

Time interval	Diffusate	Carbohydrate	Kjeldahl N	α-NH_2 N
	WC Seeds			
	Dry. wt	*mg*	*mg*	
Hrs	*mg/100 seeds*	*glucose/100 seeds*	*100 seeds*	*mg/100 seeds*
0–2	30	7.0	0.73	0.25
2–4	42	8.8	0.92	0.30
4–6	30	7.5	0.57	0.20
6–8	20	5.3	0.37	0.14
8–12	24	6.5	0.39	0.11
12–24	30	8.0	0.52	0.16
Total (0–24)	176	43.1 (24 %)[1]	3.50 (2 %)[1]	1.16 (0.7 %)[1]
		WOC seeds		
0–2	82	31.7	1.36	0.54
2–4	67	26.8	1.32	0.36
4–6	67	31.1	1.26	0.39
6–8	43	21.8	1.02	0.27
8–12	34	14.7	1.21	0.32
12–24	43	9.7	1.67	0.64
Total (0–24)	336	135.8 (40 %)[1]	7.84 (2.3 %)[1]	2.52 (0.75 %)[1]

[1] Percent of total diffusate dry weight.

Table II. *Chromatographic Analysis of Carbohydrates in Diffusates of WC and WOC Seeds*

Relative concentrations are indicated by: (0) not present; (+) low concentration; (++) medium concentration; (+++) high concentration.

Carbohydrate	Time interval in hrs 0–2	2–4	4–6	6–8	8–12	12–24
			WC Seeds			
Unknown 1	+	+	+	+	++	+
Unknown 2	+	+	+	+	++	+
Raffinose	0	0	0	0	0	0
Maltose	++	++	++	+	+	+
Sucrose	+	+	++	++	++	++
Glucose	++	++	++	++	++	++
Fructose	++	++	++	++	++	++
			WOC Seeds			
Unknown 1	++	++	+++	++	++	+
Unknown 2	++	++	+++	++	++	+
Raffinose	+	+	+	+	+	0
Maltose	0	0	0	+	+	+
Sucrose	++	++	+++	++	++	++
Glucose	0	0	0	+	+	+
Fructose	0	0	0	+	+	+

By the twenty-fourth hour of imbibition, WOC seeds had lost 3.36 mg and WC seeds 1.76 mg per seed. This represents 2.2% and 1.05% of their respective dry weights. In table I the carbohydrate fraction of the solute is given as glucose equivalents. The WOC diffusate not only contained more carbohydrate than the WC diffusate but the WOC carbohydrate accounts for a greater portion of the WOC diffusate than does the WC carbohydrate of the WC diffusate. Analysis of these diffusates by paper chromatography indicated both a qualitative and quantitative difference existed (table II). The most striking differences are: 1) the high amounts of unknowns 1 and 2 from WOC seeds; 2) the lack of the compound tentatively identified as raffinose from WC seeds and its presence, though in low concentration, from WOC seeds; 3) the disappearance of raffinose by the twelfth hour of diffusion from WOC seeds; 4) the absence of maltose in early diffusates from WOC seeds and its consistent presence in WC seed diffusates; and 5) the absence of glucose and fructose for the first 6 hours in WOC seed diffusates.

As would be expected from the total solute and carbohydrate data, greater loss of nitrogen containing compounds occurred from WOC seeds than from WC seeds during imbibition, even though, in each case the nitrogen fractions each account for about 2% of the total solute lost. Chromatographic analysis of the WC and WOC diffusates indicated at least 12 amino acids were present in each diffusate. No qualitative differences were detected.

Growth. Figure 2 shows stem lengths of the seedlings sampled. On the fourth day the WOC seedlings were 1.4 times longer than WC seedlings.

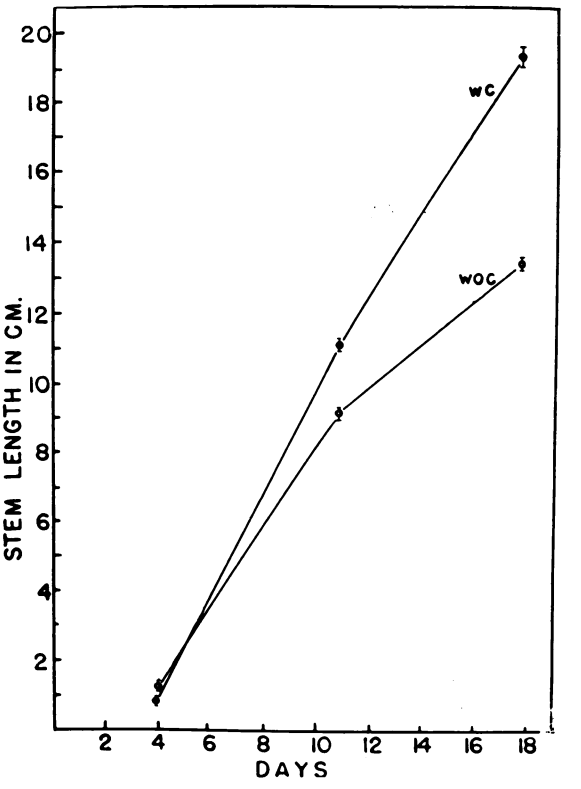

FIG. 2. - Stem-height of pea seedlings 4, 11, and 18 days after planting. ●—●—● stem height of seedlings grown from seeds with seedcoat (WC); ○—○—○ stem height of seedlings grown from seeds without seedcoat (WOC). Vertical bar represents standard error. Each point represents 25 to 44 seedlings.

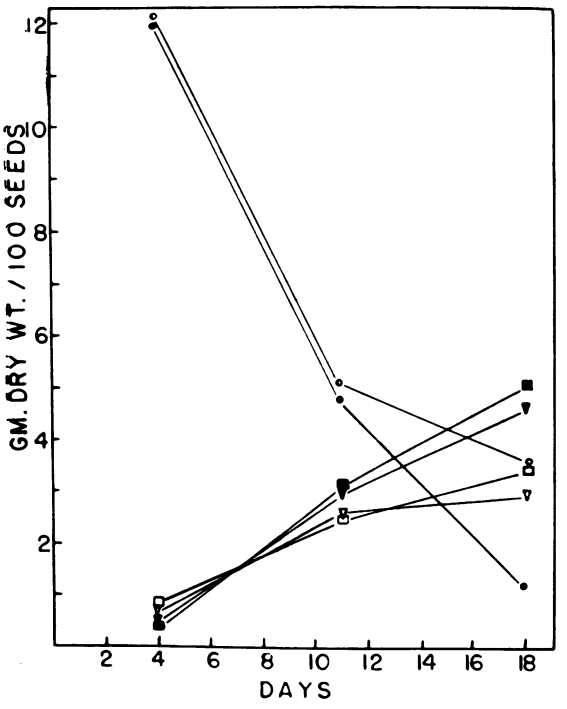

FIG. 3. Dry weight of roots, stems, and cotyledons of seedlings grown from seeds with seedcoats (WC) and from seeds without seedcoats (WOC). —●—●— WC cotyledons; ■—■ WC stems; ▼—▼ WC roots; ○—○ WOC cotyledons; □—□ WOC stems; ▽—▽ WOC roots. Each point represents 25 to 44 seedlings.

By the eleventh day the WOC seedlings were only 0.8 the height of WC seedlings and by the eighteenth day this value had fallen to 0.7. Clearly a significant decrease in stem length was exhibited by these WOC seedlings. Figure 3 shows the dry weights of roots, shoots and cotyledons of WC and WOC seedlings. Little difference in dry weight changes between WC and WOC seedlings was detected until after the eleventh day.

Discussion

The difference in the structure and composition of the seedcoat and embryo of mature dry pea seeds could account for the variation in imbibition rates exhibited by these 2 tissues. The embryo consists largely of living parenchyma storage tissue containing 30% protein and 45% starch by dry weight (6). Mature pea seedcoats contain 3 distinct layers of dead cells; palisade, column, and a multilayer parenchyma (7). A cuticle is present on the outside of the palisade and would restrict passage of materials into and out of the embryo. Larson (unpublished data) could not detect O_2 uptake, CO_2 evolution or $^{14}CO_2$ fixation by mature pea seedcoats excised after intact seeds had imbibed water for 4 hours. The cells of the pea seedcoat contain large lumens which hold substantial amounts of water. Manohar and Heydecker (7) contend that these seedcoats are differentially permeable, and they suggest that solute absorption occurs through the micropyle, an orifice of about 80 μ by 120 μ. Water uptake is apparently not restricted to the micropyle. The author has measured the rate of imbibition of pea seeds on moist paper towels with the micropyle facing downward, on moist paper towels with the micropyle facing upward and seeds submerged in water, their micropyles plugged with silicon grease. During the first 12 hours, differences could be seen in the degree of seed swelling, but by the twelfth hour all seeds had absorbed approximately the same amount of water based on their increase in fresh weight.

The small amount of material lost by pea seeds totally submerged during imbibition has a pronounced effect on the subsequent growth and development of the seedling. Greater loss of material from WOC seeds can be attributed to the absence of the seedcoat. The removal of this physical barrier allows a greater rate of water uptake by imbibition as well as a greater rate of solute loss by diffusion. Eyster (2) found bean seeds soaked on moist paper towels until fully imbibed will not lose material if later fully submerged, whereas seeds initially imbibed by submerging do lose materials to the bathing solution.

Hydration of WC seeds is slower than of WOC seeds. Also cell membranes of WOC seeds may be disrupted resulting in greater solute loss while membranes of WC seeds remain intact. The diffusate dry weight data of table I are consistent with the fact, that the WC diffusate will not approach the value of WOC diffusate loss; even after 24 hours of imbibition, the rate loss of material from WOC seeds is still greater than from WC seeds. Also by 24 hours, the total amount of diffusate loss by WOC seeds is almost twice that from WC seeds.

Variation in the composition of the diffusates from WC and WOC seeds for any 1 time interval may be explained by the presence or absence of the seedcoat. Compounds present in the WOC seed diffusate but not in the WC diffusate, such as raffinose, were able to diffuse freely into the surrounding solution. If all compounds in the WC seed diffusate had to pass through the micropyle, as suggested earlier (7), a certain amount of time would be required for imbibition and mobilization before these compounds could be detected in the surrounding solution. Yet maltose, glucose, and fructose were detected in WC seed diffusates at all times but were not detected in WOC seed diffusates until after the sixth hour of imbibition. These 3 sugars were not detected in diffusates from the seedcoats alone. Possibly glucose and fructose are quickly catabolized in WOC seeds. WOC seeds, freed of their seedcoats, imbibe water over the entire embryo surface initiating metabolic activity.

Maltose, glucose, and fructose could be utilized and consequently would not be found in the diffusate. Bacterial contamination of WC seeds appears not to play an important role in the variety of carbohydrates detected in the diffusates. WC seeds which had been surface sterilized with 1.0 mM $HgCl_2$ yielded the same carbohydrate pattern in the diffusate as unsterilized WC seed.

Greater loss of total nitrogen and amino nitrogen occurred from WOC seeds even though these nitrogen fractions represent the same percent of the total solute. Similar nitrogen values were reported by Bonner *et al.* (1). Growth promoting substances (3) as well as nucleotides (9) have been reported in diffusates of soaking seeds.

During the 24 hour imbibition time, the WOC seeds lost more solute by diffusion than did WC seeds. These losses represent an insignificant amount of material compared to the dry weight changes of root, shoot, and cotyledon of the seedlings during their 18 days of growth. It is not clear if the changes observed in growth between WC and WOC seedlings was caused by the loss of some factor in the diffusate or to some other factor directly related to the presence or absence of the seedcoat.

Rapid imbibition under high moisture stress can injure seeds (3, 9). A manifestation of this injury is a decrease in the germination rate. The reduction in the WOC seedling growth reported here may have been caused by such stress. WOC seeds imbibe at a greater rate than WC seeds. Presumably, rapid imbibition occurs because the seedcoat, a physical barrier, is absent. A consequence of rapid imbibition may cause cell membrane damage resulting in greater solute loss. This may be offered to explain the findings reported in this paper.

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