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J. Amer. Soc. Hort. Sci. 102(4):478-480. 1977.

Seed Coat and Water Absorption Properties of Seed of Near-isogenic Snap Bean Lines Differing in Seed Coat Color¹

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Additional index words. *Phaseolus vulgaris*, imbibition

Abstract. Differences in water absorption by intact seeds and in osmotic properties of excised seed coats were measured in 4 near-isogenic breeding lines of snap bean, *Phaseolus vulgaris* L. White seeds absorbed water more rapidly than colored seeds. Excised white seed coats were more permeable to water than colored seed coats in response to an osmotic gradient. Seed coat thickness and seed coat dry weight were negatively correlated with rate of osmosis through the seed coats. Colored seeds had greater seed coat dry weight and thickness than white-seeded isolines.

Seedling emergence, seed yield, and green pod yield (1, 4), and Rhizoctonia root rot resistance (2, 7, 10, 12) and seed germination in cold soils (3) are greater in certain cultivars of snap beans with pigmented seed coats than in cultivars with white seed coats. The reasons for the apparent superiority of lines with pigmented seed coats are not known. The presence of phenolic compounds (2, 10, 12) may cause the greater seed and root rot resistance and seedling vigor (1). However, this is not a universal explanation because some cultivars with pigmented seed coats also exhibit poor germination characteristics (10, 11, 12).

Seeds of *Pisum elatius* Stev. dried in the absence of O₂ were totally permeable to H₂O (6) whereas seeds dried normally were impermeable. Structural changes resulting in impermeability of the seed coats were related to the degree of oxidation of phenolic compounds.

In this study, the initial rate of H₂O imbibition of snap bean seeds differing in seed coat color was investigated and further information was obtained on the apparent superiority of colored-seeded snap beans.

Materials and Methods

Four pairs of snap bean breeding lines were used in this study. Each pair was near-isogenic and consisted of a pigmented and a white seeded counterpart. All lines were derived from crosses made between pigmented- and white-seeded breeding

lines selected at the U. S. Vegetable Laboratory. Line B4061-1X-X was an F₉ with black seed and white seed, line B4073 was an F₉ with brown seed and white seed, line B4163 was an F₆ with purple seed and white seed and line B4169 was an F₆ with brown seed and white seed. Pure lines for each seed coat color were obtained in the F₄ generation by making individual plant selections within each line and progeny testing to determine homozygosity. Seeds used in the study were grown in the snap bean breeding nursery in the fall of 1975.

Single seeds from each lot were weighed to the nearest 0.01 g to obtain uniform samples for imbibition and seed coat studies. Initial seed moisture was 11%. The experimental design was a split-plot with 4 replications; lines were main plots and seed coat colors were sub-plots. Five uniform seeds (by wt) comprised a sub-plot.

After initial wt was determined, seeds were placed in distilled H₂O at approx 24°C. Weights in mg were taken at 15 min intervals for 2 hr, and imbibed H₂O was derived by subtraction.

At the end of the imbibition period, or when the seeds were fully imbibed, the seed coats were removed and dried in an oven for 24 hrs at 100°C and weighed.

Seed coat thickness was measured on a group of 5 seeds comparable to those used in imbibition studies. A section of seed coat was removed from seeds at the midpoint of the long axis. After air drying, the thickness of the section was determined with a micrometer.

Osmotic properties of colored vs. white seed coats were also studied. The micropylar ends of imbibed seeds were excised and the cotyledons carefully removed leaving the remainder of the seed coat intact. Each seed coat was fitted over one end of a

¹Received for publication February 1, 1977.

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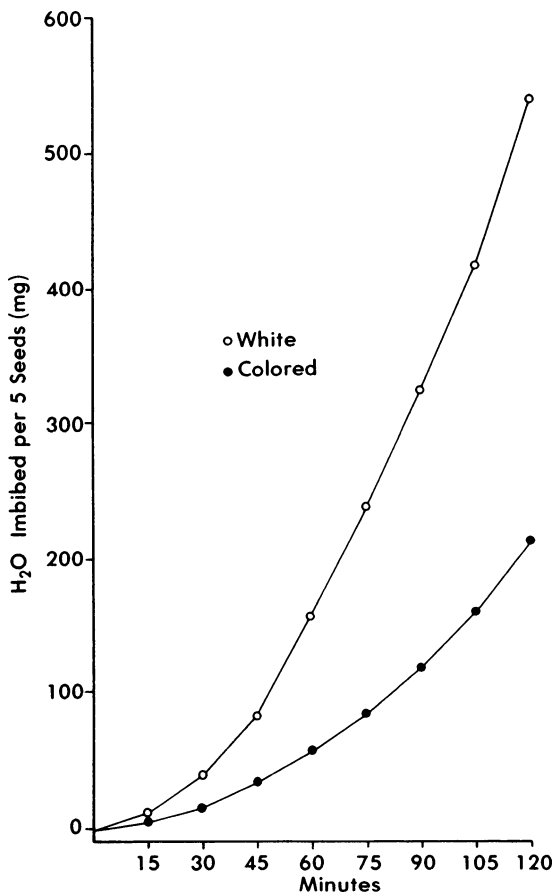


Fig. 1. Water imbibition by colored and white seed of near-isogenic snap bean breeding lines differing in seed coat color. Means represent combined data from four lines for each pigment class.

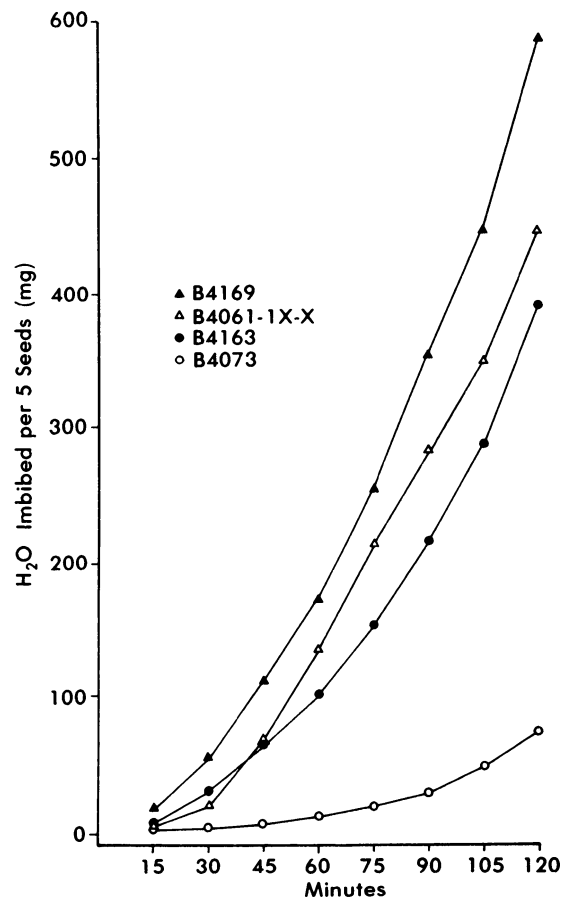


Fig. 2. Water imbibition by seed of four near-isogenic snap bean breeding lines. Means represent combined data of pigmented- and white-seeded isolines.

4-cm section of rubber tubing (inside diam 2mm) and secured with a rubber band. The edges of the seed coat were sealed to the tubing with contact cement. A 0.5 M sucrose soln was introduced into the tubing with a syringe, and the open end of the tubing was attached to a 1-ml pipette with graduations of 0.01 ml. The sucrose level in the pipette was adjusted to an initial reference point by means of a screw-type pinch clamp on the tubing. The system was suspended in distilled H₂O for 4 hr. Osmosis of H₂O through the seed coat was measured by an increase in vol in the pipette.

Results and Discussion

White seeds absorbed more H₂O during the first 2 hr of imbibition than colored seeds (Fig. 1). These differences were significant at all measurement intervals after 15 min. Water absorption by colored seeds did not exceed 40% of that by white seeds after imbibition for 15 min.

A significant difference in H₂O absorption among lines was demonstrated at the 45 min measurement and at subsequent intervals (Fig. 2). Line B4073 imbibed H₂O slowly and usually required soaking for at least 8 hr before the seed coats could

Table 1. Seed coat properties of 4 near-isogenic snap bean breeding lines and their colored and white isolines.

Variable	Seed coat color	Seed coat dry wt (mg)	Mean	Seed coat thickness (mm)	Mean	Osmosis of water (μl/4 hr)	Mean
<i>Breeding line</i>							
B4161-1X-X	black	140a ^z	129a	.121a	.116a	38a	46a
	white	118e		.111ab		54a	
B4169	brown	136ab	128a	.118a	.106a	45a	48a
	white	120de		.094cd		51a	
B4073	brown	136abc	127a	.119a	.114a	40a	46a
	white	118e		.109abc		52a	
B4163	purple	129bcd	122b	.097bcd	.094b	46a	50a
	white	114e		.090d		54a	
<i>Seed coat pigment</i>							
	Colored		135a		.114a		43a
	White		118b		.101b		53b

^zMean separation within columns by Duncan's multiple range test, 5% level.

be removed intact. The seed coats adhered tightly to the cotyledons and this factor may have contributed to the slow imbibition of the line. Adherence of the seed coat may also have influenced the differences observed between colored vs. white seeds, but this was not demonstrated.

Significant differences among lines in seed coat dry wt and thickness were demonstrated (Table 1). As expected, these 2 properties are positively correlated ($r = .48^{**}$). Their influence on H₂O absorption among lines is unclear because the line that imbibed the most H₂O (B4169) did not differ in either seed coat wt or thickness from the line that absorbed the least (B4073). The line that had the lightest and thinnest seed coat (B4163) was intermediate in water absorption. Other mechanisms may also influence H₂O absorption. These include seed coat adherence to the cotyledons, elasticity of the seed coat when wet, seed coat porosity or seed coat colloidal properties. These mechanisms may operate independently of the physical barrier to water passage expressed as seed coat dry wt and thickness and may result in the significant differences seen among main plots (breeding lines) and between sub-plots (pigment classes).

Colored seeds had greater seed coat dry wt and thickness than did white seeds, but their coats permitted less osmosis than did their white counterparts (Table 1). This is in agreement with earlier studies (8) on permeability of bean seed coats. Both seed coat thickness and dry wt were negatively correlated with osmosis rate ($r = -.17$ and $-.53^{**}$, respectively), suggesting that osmosis through colored seed coats may be slowed by a physical barrier of greater cell numbers, by differences in cell density, or by some chemical reaction (phenolic oxidation) unique to colored seeds. Lignin content of Lima bean seed coats (5) has been shown to influence both testa thickness and rate of water absorption.

Slower absorption of H₂O by colored seeds may permit more uniform swelling of the cotyledons, thereby reducing seed coat and/or cotyledon cracking, both detrimental factors

in snap bean germination and early seedling growth (9, 12). Differences among lines in their capacity to imbibe H₂O should be investigated for use as another tool in selecting cultivars having superior germination and seedling vigor.

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J. Amer. Soc. Hort. Sci. 102(4):480-484. 1977.

Use of Natural Cytokinins to Extend the Storage Life of Broccoli (*Brassica oleracea*, Italica Group)¹

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Additional index words. zeatin, dihydrozeatin, senescence

Abstract. Two natural cytokinins, zeatin and dihydrozeatin, were effective in preserving broccoli appearance and chlorophyll content. Single treatments with 100 ppm aqueous solutions of the 2 compounds, followed by storage at 13°C, permitted storage life of 5 days for zeatin- and 4 days for dihydrozeatin-treated samples of broccoli. Repeated treatments with these compounds increased broccoli storage life to 6 days at 13°C, approaching the apparently limiting value of 7 days conferred by the synthetic cytokinin, 6-benzylamino purine (25 ppm). Broccoli without cytokinin treatment remained salable for only 2 days at 13°C. Visual scores for color were linearly related to chlorophyll concentration.

¹Received for publication January 10, 1977.

²We acknowledge the assistance of Mrs. Gertrude Stark in preparing samples for panel evaluation. We are also grateful for technical advice from Mr. Dante G. Guadagni, WRRC, and Dr. Werner Lipton, ARS, Fresno, CA. We thank John Inglis Frozen Foods Co. and The Mann Packing Company, Salinas, CA, for broccoli.

³Mention of commercial products is for information only and does not imply endorsement by the U. S. Department of Agriculture.

Pre- and postharvest applications of the cytokinins, 6-benzylamino purine (BA) and kinetin (K), have reportedly delayed senescence of green vegetables. The effects described include retention of chlorophyll (4), alteration of respiration rates (7, 12), inhibition of protein degradation (16), and increased formation of organic acids (8). The magnitude of the effects is reported to vary with species and cultivar (13, 15), state of maturity (2), time and method of application (8), and storage conditions such as temp and illumination (2). Since