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

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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 sativum L. dried in the absence of O2 were totally impermeable 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 (7, 11) 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.

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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.

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.

<table>
<thead>
<tr>
<th>Breeding line</th>
<th>Seed coat color</th>
<th>Seed coat dry wt (mg)</th>
<th>Mean</th>
<th>Seed coat thickness (mm)</th>
<th>Mean</th>
<th>Osmosis of water (μl/4 hr)</th>
<th>Mean</th>
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<tr>
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<td>48a</td>
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<td>129bcd</td>
<td>122b</td>
<td>.097bcd</td>
<td>.094b</td>
<td>46a</td>
<td>50a</td>
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<td>.090d</td>
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Seed coat pigment

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<tr>
<th></th>
<th>Colored</th>
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<th>White</th>
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<tbody>
<tr>
<td>Colored</td>
<td>135a</td>
<td>.114a</td>
<td>43a</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White</td>
<td>118b</td>
<td>.101b</td>
<td>53b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

²Mean separation within columns by Duncan's multiple range test, 5% level.
be removed intact. The seed coats adhered tightly to the cotyle­
dons 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 $H_2O$ absorption among lines is unclear because the line that
imbibed the most $H_2O$ (B4169) did not differ in either seed
coil 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 mechan­
isms may also influence $H_2O$ 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 agree­
ment 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), suggest­
ing 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 $H_2O$ 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 $H_2O$ should
be investigated for use as another tool in selecting cultivars
having superior germination and seedling vigor.

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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.

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$^{2}$We 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.

$^{3}$Mention 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 in­
clude retention of chlorophyll (4), alteration of respiration
rates (7, 12), inhibition of protein degradation (16), and in­
creased 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