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Testing the Effects of Moisture on Seedcoat Color of Pinto Dry Beans

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Abstract. **Seedcoat color is an important trait, as it affects marketing and consumer acceptance of pinto beans (*Phaseolus vulgaris* L.). Pinto breeding line NE 94-4 showed seedcoat yellowing in on-farm field trials in Nebraska in 1996 and 1997. Hail, sprinkler irrigation, and fall rainfall appeared to be involved in increasing seedcoat yellowing, based on analysis of field and weather data of on-farm trial sites. The objective of this study was to determine the effect of moisture on seedcoat yellowing of pinto line NE 94-4 (susceptible) and pinto 'UI-114' (highly resistant). Two greenhouse experiments were conducted involving misting of bean plants near maturity and injecting water into maturing bean pods. Another experiment evaluated the response of seeds of these two bean entries to moisture by placing them on moist filter paper in petri dishes in the laboratory. Results showed that both genotype and moisture content are involved in seedcoat yellowing. This simple, cheap, and effective filter paper test was then used to evaluate seedcoat yellowing of nine pinto genotypes in response to moisture. Pinto NE 94-4 and 'Kodiak' showed the greatest change, while 'Bill Z' showed the least change, in seedcoat color.**

Food legumes are grown and consumed in nearly all parts of the world. In many developing countries, 20% of the available protein is provided by legumes. Legumes are also an integral part of dietary protein for ≈50% of the world's population (Deshpande et al., 1984). Dry beans are produced in large quantities in South, Central, and North America; Mexico and east Africa (Singh, 1999). Pinto, great northern, and light red kidney beans are the main market classes produced in the central high plain states of Colorado, Nebraska, and Wyoming. Pinto ranks number one of all market classes of dry bean produced in the United States (Schwartz et al., 1996).

Seedcoat color is an important trait affecting marketing of pinto dry bean. Consumers prefer pinto cultivars with bright, shiny, mottled-colored seedcoat s and a bright yellow hilum. Smith (1961) proposed the genotype for the pinto seedcoat color, now corresponding to the genic nomenclature of Prakken (1972), as *PCDJ (G) B (v) RK*. *G* and *v* gene symbols in the above brackets were also recently assigned by M.J. Bassett, Florida State Univ., Gainesville (personal communication to D.P. Coyne, Feb. 2000). The pattern characteristic of the pinto seedcoat is controlled by a particular allele of the *C* locus (not yet named) in place of the M symbol for this trait, formerly designated by Prakken (1974). Symbols for some genes controlling seedcoat color in common bean are: *P*, the basic gene for color to develop; *T*, necessary for a uniformly colored seedcoat ; *C*, a complex locus with different alleles and for constant mottling; *D*, the hilum ring factor; *J*, the shine factor; *G*, the yellow-brown factor; *B*, the gray-greenish brown factor; *V*, the violet factor; and *Rk*, for reddish brown (Prakken, 1974).

Environmental conditions and diseases can cause seedcoat discoloration. Dry bean cultivars need to have the typical color of a particular market class and be free of off-type seeds and seed discoloration. In 1996, the pinto breeding line NE 94-4 was scheduled to be released due to a favorable combination of multiple disease resistance, upright plant habit, high yield, and seed quality. Nevertheless, NE 94-4 was not released because of yellowing of the seedcoat. This problem was not observed in lines selected in earlier generations under dry fall conditions.

Analysis of weather and field data (not presented) from the on-farm field trials indicated that hail, high temperature, rain, and sprinkler irrigation may have influenced the development of bean seedcoat yellowing. Studies were therefore designed to determine the influence of moisture and physical damage to pods on the degree of seedcoat yellowing of pinto NE 94-4 (susceptible) and pinto 'UI-114' (resistant). The latter is recognized in the trade as a standard for pinto seedcoat quality.

Materials and Methods

Misting experiment. The treatments, mist vs. control, were designed to simulate different conditions (no rain vs. rain and/or sprinkler irrigation) that can occur in the field during the last 2 weeks of the harvest season. Bean genotypes were planted in clay pots, volume 1450 cm^3, on 22 Jan. 1998, in a horticulture research greenhouse, Lincoln, Nebr. The potting medium consisted of 27% vermiculite, 36% peat, 10% field soil, and 27% sand, by volume. After 3 weeks, half the seedlings were moved to another greenhouse, and placed on benches under a mist system. Plants were fertilized with 300 $mg \cdot L^{-1}$ of 20N–8.8P–16.6K three times per week until pods formed; thereafter, nutrient applications were reduced to one per week. The greenhouse temperatures were 26 °C day/20 °C night. The experimental design was a split-plot with the main plots blocked by both row and column within the greenhouse. The main plot factor was mist. The sub-plot treatments were pinto breeding line NE 94-4 and cultivar UI 114 (control). An individual sub-plot consisted of two plants per treatment combination with nine replications for a total of 72 plants. The treatments were applied 2 weeks before physiological maturity. The mist treatment was applied for 60 min/d (a total of 302.8 L water applied). One sub-plot (two plants) was removed from each replication for misting. The misted plants were separated from the rest of the plants with plastic shields. After every misting, plants were moved back to their original places. Each misted pot was covered with a plastic bag to prevent water leaking into the potting medium. Six seeds were taken from four pods, harvested from the bottom to the upper parts of each plant 2 d after completion of the misting treatments. This technique allowed the samples to reflect variation in pod maturity and moisture. Yellowing was recorded for one to six seeds per pod. For analysis purposes, percentage of yellowing was defined as the average percentage of yellowing for the four plants in a plot.

Injection experiment. The influence of physical damage to the pod, along with the penetration of water into the pod on yellowing of the seedcoat, was determined. The plants were grown at the same time, and under the same conditions as the misting experiment. The experiment design was a randomized

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complete block with 12 replications. Bean genotypes 'UI 114' and NE 94-4 were used as blocks. A total of 72 plants were grown. On 27 Mar. 1998, 15 d before physiological maturity, the treatments were applied to the pods. Three pods were selected from the base, middle, and upper parts of each plant for each of three treatments. For the injury-only treatment, a hypodermic syringe was inserted into each pod near the pedicel. For the injection with water treatment, a hypodermic syringe was used to inject 5 mL of deionized water into the pod through the pod suture near the pedicel. After 15 d, the percentage of yellowing of seeds from each of the three pods per plant was recorded.

Moistened filter paper experiments. This experiment was conducted in the laboratory during Apr. 1998. The design was a randomized complete block with four replications. Pinto 'UI 114' and NE 94-4 were arranged in blocks. The two treatments consisted of dry seeds placed on either moistened or dry (control) filter paper in petri dishes. Pods were randomly selected from control plants in the injection experiment described previously. Each pod was opened in the dark. The seed was extracted and placed immediately in a petri dish. Twenty seeds were placed on either moist or dry filter paper in each dish. The petri dishes were then sealed with tape to prevent air exchange, and kept in the dark during the experiment at 21 °C. The percentage of seedcoat yellowing was recorded 7 d after the initiation of the experiment.

A second moistened filter paper experiment was performed to evaluate diverse pinto bean germplasm for seedcoat yellowing. Eight pinto cultivars and breeding line NE 94-4 were evaluated for the response to seedcoat yellowing in the presence of moisture, using the same procedure developed in the moistened filter paper experiment described above. The experimental design was a randomized complete block with two replications. Twenty seeds of each bean genotype were placed on moistened filter paper per petri dish per replicate, and on dry filter paper for the control treatment. Color of seeds was recorded 7 d after initiation of the experiment.

Measurement of seedcoat yellowing and statistical analysis. Yellowing was recorded visually by determining the percentage of the yellow area on a bean seed in the experiments. A scale from 0% to 100%, measured in 10% intervals, was used. The mixed model procedure (Littel et al., 1996) was used to obtain appropriate statistical estimates and to test data in all three experiments.

Color, value (lightness), and chroma (strength) of the seeds of each of the pinto entries in the moistened filter paper germplasm experiment were recorded as described in the Munsell™ Book of Color (1929–42 pocket edition; Munsell Color Co., Baltimore). The color, value, and chroma are read together to give a description of the color and how it changes. In general, for value, the higher the number, the lighter the shade of color. For chroma, the lower the number, the stronger or deeper the shade.

The data showed no variation within each variety. Thus, the means of the control and moisture treatment as separated by genotypes were the only values examined and presented. No statistical analysis was necessary.

Results and Discussion

Misting experiment. Both the cultivar/breeding line effect, (Type III F value = 6.70, $P \leq 0.0125$) and the interaction between cultivar/breeding line and pod were significant (Type III F value = 2.63, $P \leq 0.0512$) such that NE 94-4 seeds showed a higher percentage of seedcoat yellowing (8.4%) than did 'UI-114' seeds (2.6%). If misting affected yellowing, this would be expected because NE 94-4 is susceptible and 'UI-114' is resistant to seedcoat yellowing. This response to misting is analogous to seeds in bean pods lying on moist ground after cutting the plants in the field during a wet fall. Thus, the moisture could cause the seeds to become discolored. In 1997, seedcoat yellowing of NE 94-4 was noted in cut bean plants lying on wet ground, while seeds from adjacent uncut plants of the same line showed little yellowing.

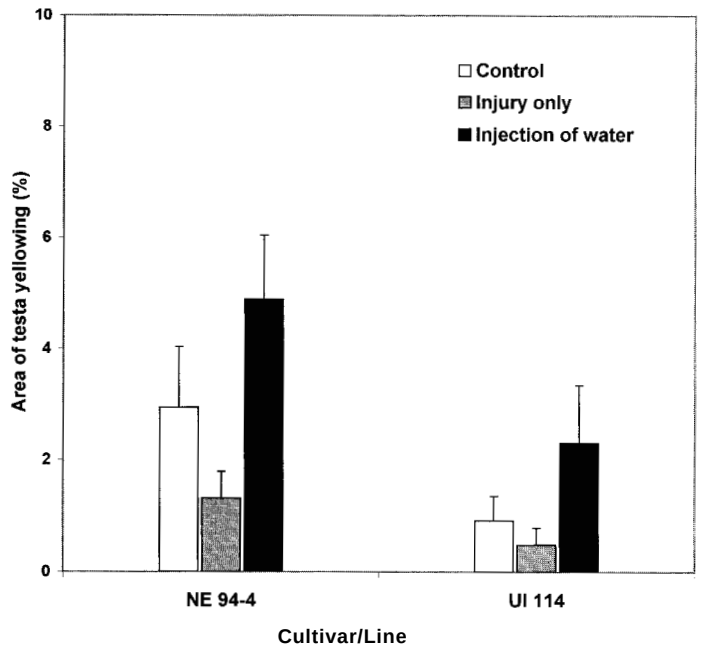

Fig. 1. Effect of injection of water on the percentage of seedcoat yellowing of two bean genotypes under the three different treatments. Means differed significantly within cultivar/lines at $P \leq 0.0238$ and represent 12 replications per treatment. Vertical bars = SE.

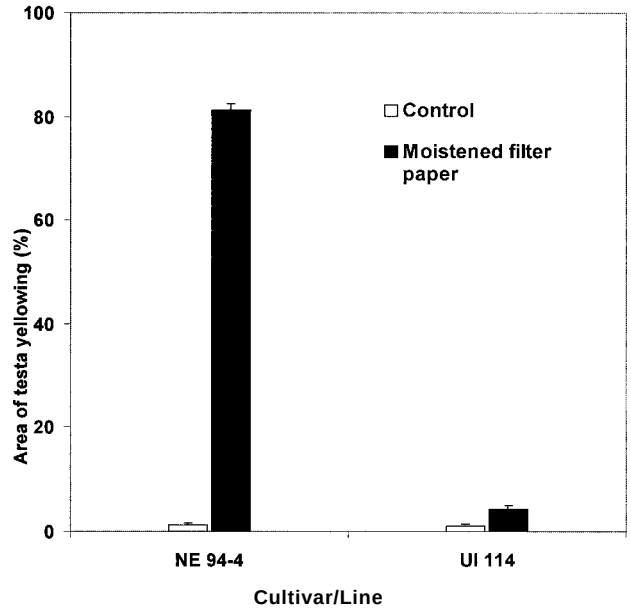

Fig. 2. Percentage of seedcoat yellowing of the two bean entries under two different treatments (control, moistened) in the moistened filter paper experiment. Means differed significantly within cultivars/lines at $P \leq 0.001$ and represent four replications per treatment. Vertical bars = SE.

Injection experiment. When pods were injected with water, NE 94-4 again showed more seedcoat yellowing than did 'UI 114' (Fig. 1; Type III F value = 4.01, $P \leq 0.0238$). The water injection treatment was associated with more seedcoat yellowing than occurred in control seeds. The mechanism causing the change in seed color pigmentation in the presence of moisture is unknown.

Moistened filter paper experiments. Seeds placed directly on the moist filter papers showed some yellowing after 1 d. Yellowing became more extensive after 3 d with NE 94-4 developing more seedcoat yellowing than 'UI 114' (Type III F value = 2518.010, $P \leq 0.0001$). The seeds of NE 94-4 on moistened filter paper yellowed more (80%; Fig. 2) than those taken from the pods injected with water (5%; Fig. 1), or from those pods which were exposed to mist in the greenhouse (8.5%; data not shown). The difference in yellowing may be due to the more uniform contact between the seed and moisture in the petri dishes, while in the other two cases the seeds matured in the presence of moisture, and dried down. In the misting experiment and the field, seeds were shielded from moisture by the pods.

Seedcoat discoloration may result from other factors, such as storage. Certain pinto genotypes such as 'Chase' tend to develop a brownish color when stored dry for a long time. Light exposure and temperature may also interact with moisture and genotypes to cause seedcoat discoloration (Hughes and Sandsted, 1975). However, these two factors were held constant in our studies.

A combination of base color, value (lightness), and chroma (strength) in combination was used to describe the color of each bean genotype. Seeds of NE 94-4 and 'Kodiak' showed the most pronounced color changes in the moistened filter paper test (Table 1). NE 94-4 changed from a pale beige and became more yellow with a touch brown as shown by a decrease in lightness (value) and an increase in strength of the color (chroma), while 'Kodiak' became more red with a significant increase in the strength of the color. The cultivar Bill Z showed the least change in seedcoat color in response to moisture, a trait highly desired by bean growers, processors, and consumers. The lightness (value) of seeds of 'Apache', 'Chase', 'Vision', 'Buckskin', and 'UI-114' did not change in the presence of moisture, but all of these genotypes showed an increased in strength of the color (a shift from pale beige to tan). These results again confirmed that the presence of moisture on mature pinto seeds caused seedcoat yellowing on susceptible lines. We recommend that the moistened filter paper test be used by researchers to detect lines that are susceptible to seedcoat yellowing in response to moisture.

Table 1. Differentiation of seedcoat color traits of pinto bean genotypes in response to moisture using the moistened filter paper test. A statistical analysis of data was not carried out because no variation was observed.

Cultivar/breeding line	Treatment	Color description[z]	Value[y]	Chroma[x]
Bill Z[w]	Moisture	Yellowish yellow-red	8	2
	Control	Yellowish yellow-red	8	2
Kodiak [v]	Moisture	Yellow-red	6	10
	Control	Yellow-red	5	8
Apache[u]	Moisture	Yellow-red-yellow	8	4
	Control	Yellowish yellow-red	8	2
Chase[t]	Moisture	Yellow-red-yellow	8	4
	Control	Yellowish yellow-red	8	2
Vision[s]	Moisture	Yellow-red-yellow	8	4
	Control	Yellowish yellow-red	8	2
Rogers 179[r]	Moisture	Yellow-red-yellow	7	2
	Control	Yellowish yellow-red	8	2
NE 94-4[t]	Moisture	Yellow-red-yellow	6	4
	Control	Yellow-red	8	2
Buckskin[r]	Moisture	Yellow-red-yellow	8	4
	Control	Yellowish yellow-red	8	2
UI 114[q]	Moisture	Yellow-red-yellow	8	4
	Control	Yellowish yellow-red	8	2

[z]Munsell™ Book of Color, 1929–42, pocket edition (Munsell Color Co., Baltimore).
[y]The color, value, and chroma are read together to give a description of the color and how it changes. For value, the higher the number, the lighter the shade of color.
[x]For chroma, the lower the number the stronger or deeper the shade.
[w]Colorado State Univ., Fort Collins.
[v]Michigan State Univ., East Lansing.
[u]Idaho Seed Bean, Twin Falls, Idaho.
[t]Univ. of Nebraska, Lincoln.
[s]Seminis–Asgrow, Twin Falls, Idaho.
[r]Novartis–Rogers Bros., Nampa, Idaho.
[q]Univ. of Idaho, Kimberly.

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