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# Seed Physiology, Production & Technology: Germination, Emergence, and Yield of 20 Plant-Color, Seed-Color Near-Isogenic Lines of Grain Sorghum

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# SEED PHYSIOLOGY, PRODUCTION & TECHNOLOGY

## Germination, Emergence, and Yield of 20 Plant–Color, Seed–Color Near-Isogenic Lines of Grain Sorghum

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### ABSTRACT

**Although there is growing demand for sorghum [*Sorghum bicolor* (L.) Moench] with white seed and tan plant color, there is limited information on the overall agronomic fitness of sorghum with these characters. A set of experiments was conducted to evaluate the combined effects of plant color and seed color on sorghum germination, emergence, and agronomic performance. Twenty near-isogenic lines with red seed/tan plant (RT), red seed/purple plant (RP), white seed/tan plant (WT), white seed/purple plant (WP) phenotypes were tested under field and laboratory conditions. Plant color × seed color interactions were not significant. Purple plant color phenotypes had higher cold germination, higher germination after accelerated aging, and greater seedling elongation at 10 d than tan plant color phenotypes. Plant color did not influence standard warm germination. No differences in standard warm germination or seed vigor test results were attributable to seed color. Seedling emergence under field conditions was higher for the red seed than the white seed phenotype. Grain yield was higher for the white seed than the red seed phenotype, and higher for the purple plant color than the tan plant color phenotype. Grain test weights from purple plant color lines were higher than those from tan plant color lines. All four phenotypes included relatively high yielding lines. There was considerable overlap between WT, WP, RT, RP lines in yield and other indicators of agronomic performance leading to the conclusion that white seed and tan plant color lines with comparable performance to red seed and purple plant color lines can be selected from segregating breeding populations.**


Grain sorghum markets, such as the poultry and food industries, prefer white grain free of plant pigment stains. The sorghum seed industry responded to that need by developing white-seeded (clear pericarp) genotypes with tan plant color (tan plants lack purple coloration of necrotic leaf or stem tissues); however, there is no agreement on the overall agronomic fitness of WT genotypes compared to RP genotypes.

The role of pigments found in sorghum seed tissues has been researched in several labs. Resistance to various diseases and pests are associated with pigmented testa, but are not the subject of this paper. Red pericarp has been associated with grain mold (caused by various fungi) resistance (Bandyopadhyay et al., 1988; Esele et al., 1993; Jambunathan and Kherdekar, 1990). Melake-Berhan et al. (1996) reported higher concentrations of several phenolic compounds in grain mold resistant genotypes than in susceptible genotypes. Flavan-4-ols are one of the key components of mold resistance in red-seeded genotypes (Jambunathan and Kherdekar, 1990; Melake-Berhan et al., 1996; Mukuru, 1992; Waniska and Rooney, 1992).

Nicholson et al. (1987) suggested that pathogen resistance is associated with phytoalexin accumulation in sorghum plant tissues in response to pathogen infection. Snyder et al. (1991) indicated that phytoalexins in sorghum are pigments, accumulate in the cell undergoing attack, and accumulate in substantially higher amounts than needed for expression of fungitoxicity. Tenkouano et al. (1993) proposed screening mesocotyl tissue in seedlings for phytoalexins as a potential tool for identifying resistance to anthracnose [caused by *Colletotrichum graminicola* (Ces.) G.W. Wils.]; however, Bupe et al. (1993) were unable to detect any difference in susceptibility to anthracnose in the field between lines isogenic for tan and red plant color, even though the two phenotypes differed in composition of phenolic compounds.

A recent report on the effects of plant color on agronomic traits of sorghum showed lower grain yields from a group of tan hybrids compared to pigmented hybrids (Williams-Alanis et al., 1995). We observed that WT lines appeared to exhibit poor germination and emergence in our winter greenhouse and hypothesized that the combined effects of plant color and seed color may impact sorghum germination and agronomic performance. Controlled germination experiments and a subsequent field experiment were therefore conducted to determine the effects of seed and plant color on sorghum germination and yield.

### MATERIALS AND METHODS

Development of the RT, RP, WT, and WP near-isogenic lines utilized in these studies was described in the official release notice of the USDA-ARS and University of Nebraska (1999). Briefly, five S8 lines from each of the four phenotypes — WT (N321, N322, N323, N324, N325), RT (N326, N327, N328, N329, N330), WP (N331, N332, N333, N334, N335), RP (N336, N337, N338, N339, N340)—were selected from segregates of a single S3 family from the BC1 generation of the cross (BTx398 ms3 × BTx630) × BT × 630. Given this pedigree, these lines should be ≈97% genetically identical. The genetic stocks resemble BTx630, but have normal endosperm. Seed for laboratory and field experiments were produced at the University of Nebraska Field Laboratory, Ithaca, NE, in 1996. All seed lots were hand harvested after reaching physiological maturity, dried at 35 ± 1°C for 3 d, and stored under controlled conditions (3 ± 1°C, 63 ± 1% relative humidity) until used.

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**Abbreviations:** RP, red seed/purple plant; RT, red seed/tan plant; WP, white seed/purple plant; WT, white seed/tan plant.

### Laboratory Germination and Seed Vigor

Standard warm germination was determined by counting normal seedlings after incubation on a moist blotter at 25 ± 1°C for 5 and 10 d (Association of Official Seed Analysts, 1998). Seed vigor tests included cold germination, accelerated aging germination, and seedling elongation (Association of Official Seed Analysts, 1983). Cold germination was determined by incubating seed on a moist blotter for 7 d at 10 ± 1°C followed by 5 d at 25 ± 1°C before counting normal seedlings. Accelerated aging was accomplished by incubating seed at 48 ± 1°C for 48 h at 100% relative humidity followed by the protocol for standard warm germination. Seedling elongation was determined by aligning seeds on a fold in a blotter (a thin bead of rubber cement was used to hold seeds in place), rolling the blotter keeping the fold at the bottom, and incubating at 25 ± 1°C with the moistened blotter in an upright position. Coleoptile length of each seedling was measured after 10 d.

Each of the above germination and vigor tests was conducted as a separate experiment. The experimental design was a 2 × 2 factorial (2 plant colors, 2 seed colors) with lines nested within the four phenotypes, and replicated four times. For all experiments, the experimental unit was 100 seeds. Data was analyzed by the general linear model procedure of SAS (1990). Least significant differences were used for comparisons among all 20 lines, and orthogonal contrasts were made for tan vs. purple plant color and red vs. white pericarp color.

### Field Performance

Field trials with the 20 lines were conducted at the University of Nebraska Field Laboratory, Ithaca, NE (Sharpsburg silty clay loam; fine smectitic, mesic Typic Agriudoll). Individual plots consisted of two 7.6-m rows spaced 76 cm apart, and each was seeded with a precision vacuum planter calibrated to deliver 240 seed per plot. The seed was not treated with fungicides or insecticides. Planting dates were 22 May 1997 and 18 May 1998. Plots were fertilized with 112 kg ha$^{-1}$ N prior to planting. Propachlor (2-chloro-*N*-osopropylacetanilide) and Atrazine (6-chloro-*N*$^2$-ethyl-*N*$^4$-isopropyl-1,3,5,-triazine-2,4-diamine) were applied at 3.36 and 1.12 kg ha$^{-1}$, respectively, immediately after planting for weed control. No supplemental irrigation was applied.

Field emergence was determined by counting seedlings in each plot 4 wk after planting, and converting to percentage of total seed planted. Notes were taken for anthesis date at least every 3 d during anthesis and days to 50% anthesis was calculated for each plot. Height was measured at maturity. Plots were harvested with a small-plot combine on 21 October 1997 and 14 October 1998, and yield, moisture content, and test weight of the grain were recorded.

The experimental design was a 2 × 2 factorial (2 plant colors, 2 seed colors) with lines nested within the four phenotypes, and replicated five times in each year. Due to large differences in weather and initial field emergence in the two years, data from each year was analyzed separately. Statistical analyses were as described above.

## RESULTS

### Laboratory Germination and Seed Vigor

Although accelerated aging was conducted at 48°C and 100% relative humidity for 48 h with the intention of severely stressing the seeds, reduction in germination was not as great as previously reported by Ibrahim et al. (1993), when sorghum seed was aged at 45°C for 72 h.

Plant color by seed color interactions were not significant for any of the traits measured. Plant color effects were significant for several seed vigor characters (Table 1), with purple plant color giving slightly higher cold

**Table 1. Laboratory seed vigor and germination of plant color/seed color near-isolines.**

| Phenotype | Line | Warm germination 5 d | Warm germination 10 d | Cold germination | Accelerated aging 5 d | Accelerated aging 10 d | Elongation |
|---|---|---|---|---|---|---|---|
| | | % | | | | | cm |
| WT† | N321 | 97 | 98 | 87 | 94 | 94 | 1.9 |
| | N322 | 95 | 95 | 91 | 90 | 93 | 2.3 |
| | N323 | 84 | 85 | 80 | 93 | 91 | 2.2 |
| | N324 | 96 | 97 | 91 | 87 | 89 | 2.5 |
| | N325 | 97 | 98 | 94 | 90 | 91 | 2.6 |
| RT | N326 | 97 | 98 | 95 | 95 | 96 | 2.4 |
| | N327 | 96 | 96 | 85 | 93 | 95 | 2.1 |
| | N328 | 91 | 93 | 84 | 92 | 93 | 2.0 |
| | N329 | 94 | 96 | 89 | 88 | 90 | 2.4 |
| | N330 | 97 | 98 | 95 | 91 | 92 | 2.8 |
| WP | N331 | 93 | 94 | 86 | 91 | 90 | 2.3 |
| | N332 | 93 | 95 | 93 | 94 | 95 | 2.1 |
| | N333 | 96 | 97 | 92 | 93 | 95 | 3.3 |
| | N334 | 97 | 98 | 89 | 96 | 96 | 2.6 |
| | N335 | 95 | 97 | 93 | 96 | 97 | 3.6 |
| RP | N336 | 99 | 99 | 95 | 93 | 93 | 2.7 |
| | N337 | 96 | 97 | 93 | 96 | 97 | 2.6 |
| | N338 | 92 | 93 | 89 | 96 | 96 | 2.4 |
| | N339 | 94 | 95 | 95 | 96 | 96 | 3.2 |
| | N340 | 92 | 94 | 91 | 93 | 92 | 2.6 |
| LSD (0.05) within phenotype | | 3 | 3 | 4 | NS‡ | NS | 0.5 |
| **Orthogonal Comparisons** | | | | | | | |
| White seed | | 94 | 95 | 90 | 92 | 93 | 2.5 |
| Red seed | | 95 | 96 | 91 | 93 | 94 | 2.5 |
| Prob F | | 0.42 | 0.42 | 0.18 | 0.16 | 0.46 | 0.88 |
| Tan plant | | 94 | 95 | 89 | 91 | 92 | 2.3 |
| Purple plant | | 95 | 96 | 91 | 94 | 95 | 2.7 |
| Prob F | | 0.48 | 0.16 | 0.05 | 0.01 | 0.04 | 0.03 |

† WT = white seed tan plant color, RT = red seed tan plant color, WP = white seed purple plant color, RP = red seed purple plant color.
‡ NS = no significant differences at $P = 0.05$.

**Table 2. Field emergence and agronomic performance of plant color/seed color near-isolines at Ithaca, NE in 1997.**

| Phenotype | Line | Emergence | Days to anthesis | Height | Yield | Moisture | Test weight |
|---|---|---|---|---|---|---|---|
| | | % | d | cm | kg h$^{-1}$ | g kg$^{-1}$ | kg m$^{-3}$ |
| WT† | N321 | 38 | 79 | 146 | 7763 | 243 | 703 |
| | N322 | 32 | 78 | 142 | 6110 | 214 | 723 |
| | N323 | 37 | 75 | 148 | 6083 | 249 | 696 |
| | N324 | 38 | 76 | 159 | 4712 | 227 | 843 |
| | N325 | 45 | 78 | 143 | 5084 | 265 | 697 |
| RT | N326 | 47 | 79 | 149 | 5747 | 333 | 684 |
| | N327 | 37 | 76 | 134 | 4876 | 219 | 709 |
| | N328 | 38 | 74 | 142 | 5902 | 184 | 718 |
| | N329 | 44 | 75 | 142 | 7318 | 152 | 733 |
| | N330 | 54 | 77 | 137 | 4894 | 342 | 692 |
| WP | N331 | 36 | 79 | 151 | 7327 | 299 | 705 |
| | N332 | 45 | 73 | 134 | 6873 | 176 | 736 |
| | N333 | 41 | 73 | 140 | 6583 | 208 | 747 |
| | N334 | 39 | 79 | 143 | 6991 | 196 | 725 |
| | N335 | 38 | 75 | 138 | 7591 | 184 | 738 |
| RP | N336 | 49 | 78 | 155 | 6719 | 302 | 732 |
| | N337 | 51 | 74 | 154 | 6864 | 275 | 760 |
| | N338 | 40 | 79 | 138 | 6274 | 166 | 724 |
| | N339 | 46 | 76 | 151 | 6483 | 232 | 760 |
| | N340 | 44 | 77 | 136 | 6664 | 257 | 737 |
| LSD (0.05) within phenotype | | 7 | 1 | 6 | 791 | 110 | 23 |
| Orthogonal Comparisons | | | | | | | |
| White seed | | 39 | 77 | 144 | 6512 | 226 | 720 |
| Red seed | | 45 | 76 | 144 | 6174 | 246 | 725 |
| Prob F | | 0.01 | 0.04 | 0.65 | 0.06 | 0.22 | 0.50 |
| Tan plant | | 41 | 77 | 144 | 5849 | 243 | 709 |
| Purple plant | | 43 | 77 | 144 | 6837 | 230 | 737 |
| Prob F | | 0.18 | 0.17 | 0.88 | 0.01 | 0.41 | 0.01 |

† WT = white seed tan plant color, RT = red seed tan plant color, WP = white seed purple plant color, RP = red seed purple plant color.

germination (91 vs. 89%), slightly higher germination after accelerated aging at 5 d (94 vs. 91%) and 10 d (95 vs. 92%), and greater seedling elongation at 10 d (2.7 vs. 2.3 cm). No differences in standard warm germination were attributable to plant color ($P > 0.05$). No differences in standard warm germination or seed vigor indicators were attributable to seed color.

Differences among lines within phenotypes were detected for standard warm germination, cold germination, and seedling elongation ($P = 0.05$). One WT line,

**Table 3. Field emergence and agronomic performance of plant color/seed color near-isolines at Ithaca, NE in 1998.**

| Phenotype | Line | Emergence | Days to anthesis | Height | Yield | Moisture | Test weight |
|---|---|---|---|---|---|---|---|
| | | % | d | cm | kg h$^{-1}$ | g kg$^{-1}$ | kg m$^{-3}$ |
| WT† | N321 | 47 | 83 | 151 | 7520 | 142 | 684 |
| | N322 | 42 | 82 | 153 | 6320 | 138 | 687 |
| | N323 | 44 | 80 | 162 | 7280 | 136 | 700 |
| | N324 | 49 | 81 | 171 | 5960 | 156 | 725 |
| | N325 | 58 | 82 | 153 | 6580 | 132 | 665 |
| RT | N326 | 57 | 82 | 153 | 7020 | 147 | 645 |
| | N327 | 46 | 79 | 134 | 5060 | 137 | 675 |
| | N328 | 44 | 78 | 150 | 6280 | 137 | 679 |
| | N329 | 53 | 79 | 144 | 6300 | 132 | 653 |
| | N330 | 59 | 81 | 151 | 5160 | 155 | 652 |
| WP | N331 | 43 | 83 | 163 | 7580 | 156 | 691 |
| | N332 | 52 | 77 | 149 | 7000 | 141 | 739 |
| | N333 | 49 | 82 | 151 | 6700 | 146 | 730 |
| | N334 | 51 | 82 | 154 | 7120 | 137 | 702 |
| | N335 | 49 | 79 | 144 | 7360 | 137 | 706 |
| RP | N336 | 60 | 82 | 164 | 6900 | 152 | 706 |
| | N337 | 58 | 80 | 166 | 7540 | 154 | 725 |
| | N338 | 51 | 82 | 134 | 6080 | 135 | 667 |
| | N339 | 53 | 80 | 165 | 7000 | 161 | 720 |
| | N340 | 49 | 81 | 145 | 6240 | 138 | 693 |
| LSD (0.05) within phenotype | | 6 | 1 | 5 | 655 | 14 | 23 |
| Orthogonal Comparisons | | | | | | | |
| White seed | | 48 | 81 | 155 | 6942 | 142 | 703 |
| Red seed | | 53 | 81 | 151 | 6358 | 145 | 682 |
| Prob F | | 0.01 | 0.03 | 0.01 | 0.01 | 0.33 | 0.08 |
| Tan plant | | 50 | 81 | 152 | 6348 | 141 | 676 |
| Purple plant | | 51 | 81 | 154 | 6952 | 146 | 707 |
| Prob F | | 0.30 | 0.88 | 0.15 | 0.01 | 0.14 | 0.01 |

† WT = white seed tan plant color, RT = red seed tan plant color, WP = white seed purple plant color, RP = red seed purple plant color.

N323, was the only line to exhibit <90% standard warm germination, and also exhibited the lowest (80%) cold germination. Considerable overlap in warm and cold germination was observed among the four phenotypes. Each of the four phenotypes had lines exhibiting warm germination (10 d) percentages >97% and cold germination percentages >92%. No differences were detected among lines within phenotypes for germination following accelerated aging.

## Field Performance

Seedling emergence under field conditions was higher for phenotypes with red seed than for phenotypes with white seed in 1997 (45 vs. 39%; $P = 0.01$) and 1998 (53 vs. 48%; $P = 0.01$) (Tables 2 and 3); however, grain yield was higher for phenotypes with white seed than red seed in 1997 (6512 vs. 6174 kg ha$^{-1}$ ; $P = 0.06$), and 1998 (6942 vs. 6358 kg ha$^{-1}$ ; $P = 0.01$). No differences in seedling emergence under field conditions were attributable to plant color. Purple plant color phenotypes were higher yielding than tan plant color phenotypes in 1997 (6837 vs. 5849 kg ha$^{-1}$ ; $P = 0.01$), and 1998 (6952 vs. 6348 kg ha$^{-1}$ ; $P = 0.01$), and had higher test weights in both years (737 vs. 709 kg m$^{-3}$ and 707 vs. 676 kg m$^{-3}$, respectively; $P = 0.01$). Although days to anthesis was statistically longer for white seed phenotypes in both years, the mean difference was ≈0.5 d and probably has no biological significance. Similarly, white seed phenotypes were slightly taller than red seed phenotypes in 1998 (155 vs. 151 cm; $P = 0.01$), but this small difference in average height probably has no practical significance.

Differences were detected among lines within phenotypes for all traits measured in the field ($P = 0.05$). Considerable overlap in the range of values was observed among the four phenotypes. Each of the four phenotypes had lines with emergence approaching 50% in both years, and with one exception (RP in 1997) had yields over 7000 kg ha$^{-1}$ in both years.

## DISCUSSION

Since red pericarp has been shown to be associated with grain mold resistance (Bandyopadhyay et al., 1988; Esele et al., 1993; Jambunathan and Kherdekar, 1990), it is not surprising that field emergence was higher for untreated red seed phenotypes than for white seed phenotypes. Soil would be expected to be biologically active and contain numerous molds and other pathogens that could affect seed germination and emergence. The lack of seed color effects on laboratory measurements of seed vigor, the lack of plant color effects on standard warm germination in the lab, and the significant effects of plant color on controlled laboratory measurements of seed vigor were more difficult to interpret. It would appear that purple plant color enhanced seed and seedling vigor under controlled laboratory conditions, but the mechanism of this effect is not apparent from these studies. Plant color effects were not biologically significant enough to cause detectable differences in field emergence under our environmental conditions.

Our observation of higher average grain yield associated with purple (vs. tan) plant color phenotypes is consistent with the observations of Williams-Alanis et al. (1995) in a very different environment; however, our observation of higher average grain yield being associated with white (vs. red) seed color was unexpected. It is important to note that by developing near-isogenic lines to test the effects of pericarp and plant color, we effectively narrowed the experimental population of inference to that set of near-isolines. Within this set of near-isolines, all four phenotypes included relatively high yielding lines. Considerable overlap between WT, WP, RT, RP lines in yield and other indicators of agronomic performance were observed leading to the conclusion that white seed and tan plant color lines with comparable performance to red seed and purple plant color lines can be selected from such segregating breeding populations.

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