

Synopsis of:

PHENOTYPIC AND GENOTYPIC STUDIES OF RED SEED COAT COLOR IN WHEAT (*Triticum aestivum* L.) By CALEB CARBINE

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Background

- Red seed coat color in wheat is controlled by dominant *R* loci, three homoeologous genes.
- Color variation has been found to be dependent on the number of *R1* homoeoalleles, and also on environmental factors.
- The six major classes of wheat grown in the U.S. are hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), soft white (SW), and hard white (HW). Also included as a class is durum, (*Triticum durum*), a tetraploid relative of *T. aestivum*.
- The quality of seed planted has a drastic effect on the quality and yield of a crop (Brick, 1995). Therefore, state seed certification programs even more closely monitor variation and quality in order to meet high quality expectations and to ensure federal limits are met.
- Phenotypic expression of traits can therefore be influenced by both genotypic and environmental factors, as well as interactions between the two.
- **Genetic Basis for Seed Color**
 - Wheat is an allohexaploid, containing three distinct, but similar, sets of seven chromosomes.
 - Red seed coat is controlled primarily by dominant *R* loci, three homoeologous genes located on the long arms of chromosomes 3A
 - A wheat seed may inherit 0 or up to 6 *R-1* alleles, although as a result of common variety development strategies most cultivars are thought to be homozygous for either red or white alleles at each of the 3 *R* loci.
 - Color variation has been found to be directly dependent on the number of *R1* homoeoalleles (Baker, 1981), on the additional minor genes which influence seed coat color (Matus-Cádiz et al., 2003), and on environmental factors (Wu et al., 1999).
 - Each red gene also may not be equal in expression as they came from different ancestral sources.
 - Due to these variables, and the additive nature of red genes, the result is continuous phenotypic variation in color.
 - Before widespread use of genetic markers, this involved crossing red cultivars with white cultivars, and comparing progeny with expected segregation ratios. This process was complicated by the fact that seed coat color is maternally inherited. It is determined by the genotype of the pericarp, a maternal tissue

(Baker, 1981). Therefore any F1 generation will display coloring based on the genetics of the female parent. A 2nd generation is necessary to determine segregation ratios in order to identify the genetic makeup of the preceding generation. After a cross between red and white wheat is made, there is essentially a one generation lag for a seed's physical color to match its genotypic color.

- **NaOH test**

- The maximum color difference observed from this type of treatment peaks at 2 hours of submersion in a 1 M NaOH solution.
- DePauw & McCaig (1988) reported that, with the aid of NaOH, visual sorting could be split into more distinct groups of white, very light red, light red, red, and dark red.
- An optimized protocol was reported which suggests soaking seeds in a 5M (20.0% w/v) NaOH solution at 60°C for 10 min (Ram et al., 2002). The volume of solution was also optimized at 2ml of NaOH solution per gram of seed.
- FGIS now uses NaOH, or the equivalent KOH test, as an optional part of their standard test procedure (USDA GIPSA, 2004).
- Although more helpful, these NaOH assisted classifications are still subjective and 7 rely heavily on human judgment.

- **Environmental influence on seed color**

- Problems have occurred in Canada where the same cultivar has been grown in different locations, and had drastic differences in grain color (Williams, 2006).
- Grain color stability has also been found to be variable in Kansas (Peterson et al., 2001).
- Such variation within varieties is worrisome as certification officials may reject seed because of minor color differences, when the variety is really genetically pure.
- Research suggests that environmental stresses such as salinity, drought, UV radiation, heavy metal contamination and pathogen attack could all affect the coloration of plant tissues (Chalker-Scott, 1999).
- Wheat grown in areas of high solar UV light has been found to have a significantly redder kernel color (Lukow et al., 2012), than the same variety grown in a low UV light environment.
- Water stress has been found to influence grain color in wheat, with a marked difference noted between irrigated and non-irrigated areas (Ozturk & Aydin, 2004). Water stressed plants had significantly darker kernel color than did the irrigated plants.
- Drought conditions have also been identified as causing a more red kernel color in specific varieties (Konopka et al., 2007).

- **Chapter 2: Sources of Seed Coat Color Variation in Certified Wheat**

- **Abstract:**

- Color variation can be due to contamination. This was the first, and major source of color variation found in this study.

- Seed coat color is controlled by three genes, and heterozygosity was found in this study to be the second most common source of seed color variation.
- Seed coat color can vary with environmental interactions, and was the least common source of seed color variation.
- The study also suggested that current certification tests and procedures are not completely accurate in determining variety purity.
- **Materials and Methods**
 - In 2011 and 2012, 25 seed lots representing 17 varieties were identified as contaminated based on results of visual 5 M (20% w/v) NaOH soak inspections performed by the Washington State Department of Agriculture Seed Program in Yakima, WA.
 - The NaOH tests 28 for these lots suggested high numbers of contaminant seed from contrasting color classes, both red contaminants in white seed lots, as well as white contaminants in red seed lots.
 - Following identification of problem lots, approximately 0.9 kg of the remnant, unsorted seed from each lot was visually sorted to obtain viable seed.
 - 47 typical and 47 suspected contaminant seeds were selected for analysis from each lot. Plants were then grown out in a greenhouse for phenotypic and genotypic study. Phenotypic notes were taken as the plants developed, for observed differences such as glume color. Plants were harvested by hand so progeny seed color could be noted and compared to parental seeds.
 - Red color genes were determined by PCR, KASP, and SNP.
 - 23 of the 25 seed lots were grouped into one of three categories based on the effects of contamination, environmental interactions, and heterozygosity on one or more loci. The remaining two seed lots exhibited no clear pattern, and color variation was likely due to a mix of reasons.
- **Other Variety** - 16 of the seed lots had the suspected color contaminants that did not match the variety of interest. (70%)
 - The presence of red genes in individuals was consistent with the visual and NaOH color classifications.
 - The red seeds produced red progeny, and the white individuals produced white progeny.
 - Phenotypic observations taken during plant growth verified contamination as the main source of variation in color.
 - Some suspected contaminants, later identified as true contaminants, had darker glumes or different heading dates than the variety of interest.
 - In one case, the suspected contaminants in a spring variety never reached maturity when grown without vernalization.

- **Environmental Variation** – 6 of the seed lots exhibited this result, which was found to only occur in red varieties. (26%)
 - Visual and NaOH examination depicted red and white seed color variation, but marker analysis identified red genes in both color groups.
 - SNP markers demonstrated no significant genetic differences between the seeds visually identified as red and white.
 - The seeds visually identified as white produced red progeny, and progeny color was uniform and consistent with red gene marker results.
 - These seed lots were incorrectly identified as contaminated when the seed lot was actually pure and true to type for the cultivar.
- **Heterozygosity** – 1 white seed lot exhibited this result. (4%)
 - SNP showed no significant differences between the white seed and the suspected red contaminant.
 - Red gene markers identified heterozygosity in the R-B1 locus, and heterogeneity at the R-b1 and R-D1 loci.
 - The progeny of red seeds segregated for seed coat color.
- **Discussion – Contamination**
 - The majority (70%) of the seed coat color variation in the samples appears to have been from direct and clear contamination from a dissimilar seed source.
 - Physical contamination as well as outcrossing are likely and expected causes.
 - If contamination occurs at early stages/generations, the contaminants are a problem in subsequent generations.
 - Retaining samples, and keeping accurate field histories and machinery logs can help identify contamination events.
- **Discussion – Environment**
 - A smaller number of seed lots (26%) had color variation due to environmental sources.
 - Based on previous research, solar radiation, heat, humidity, and water availability can affect seed coat color.
 - 5 of the 6 seed lots with environmental-based color variation were found to only be fixed at on R loci. The one exception was a lot which had two fixed red genes. It is implied that since red genes contribute to color in an additive manner, the more red genes that are present, the darker and more stable it will be in varying environmental conditions.
 - The red and white groups were much more difficult to identify visually than those attributed to contamination.
 - The suspected white contaminants looked different than the red seeds when soaked in NaOH, but no white seed controls were include for comparison, pointing to the possibility that NaOH only identifies seed that looks suspiciously different.
 - Based on this, the NaOH soak is not 100% diagnostic for differentiating color, and does not necessarily diagnose seeds as genetically dissimilar.

○ **Discussion – Heterozygosity**

- As a source of color variation, heterozygosity is not common.
- It could be that while considered genetically pure, this seed lot was segregating for red seed coat color as a result of the original cross, which was possible as the variety was from red and white parent lines.
- A more likely source of heterozygosity may be from outcrossing or contamination with a highly related sister line.