URBANA, ILLINOIS
USDA/ARS/MWA
Maize Genetics Cooperation • Stock Center

A dominant enhancer of yellow color in *albescent1* mutant endosperms identified in South American orange flint lines.

--Stinard, PS

In the absence of modifiers and in the presence of *Y1*, *al1* (*albescent1*) conditions off-white endosperm kernels that give rise to albescent plants. EA Graner (1945. The yellow-orange endosperm of maize. Am Nat 79:187-192) describes a dominant enhancer of yellow color in *albescent1* mutant endosperms, which he isolated from a deep orange Brazilian maize open pollinated variety "Cateto," and which he named *Y5*. Homozygous *Y1 Y5 al1* kernels have deep yellow endosperms that are easily distinguished from *Y1 y5 al1* kernels. All original stocks carrying *Y5* have been lost. We evaluated Cateto and other deep orange endosperm lines obtained from the North Central Regional Plant Introduction Station and other sources in order to see whether we could re-isolate a *Y5* variant.

The stocks we evaluated are the following: Maiz Colorado Cuarenton (PI 162702), Cristalino Flint Anaranjado (PI 493094), Cargill Temperate Cateto (PI 451691), Ki3 (Ames 27123), Narino 330 (obtained from James Brewbaker), NC356 (Ames 27174), KUI2007 (from Matt Krakowsky and Major Goodman), KYS (MGCSC stock number T940D), and a High Carotenoid Corn line obtained from Torbert Rocheford. The yellow endosperm corn belt dent line M14 (NSL 30867), which is known to not carry any modifiers of *al1* endosperm color, served as a control. All stocks under evaluation were crossed with the *al1-y3* allele (MGCSC stock number 203BB) and with a *y1 bn1* stock (MGCSC stock number 602C) and a *y1 Bn1* stock (MGCSC stock number 713A). The latter crosses were done in order to determine whether the stocks under evaluation carry *Bn1*, an allele that conditions a pale yellow or brown aleurone color, which would interfere with the analysis. All F1 crosses were subsequently self-pollinated in order to generate F2's for analysis.

Evaluation of F2 segregation of crosses with the *y1 bn1* and *y1 Bn1* lines revealed that the High Carotenoid Line obtained from Torbert Rocheford carries *Bn1*, so this line was excluded from further analysis. Evaluation of F2 segregation of crosses with *al1-y3* revealed that the following lines are homozygous *bn1* and carry a strong dominant enhancer of yellow color in *albescent1* mutant endosperms: Maiz Colorado Cuarenton and Cristalino Flint Anaranjado (Figure 1). The following lines are homozygous *bn1* and carry a weak dominant enhancer of yellow color in *albescent1* mutant endosperms: Cargill Temperate Cateto, Narino 330, and KYS. The following lines are homozygous *bn1* and do not carry an enhancer of yellow color in *albescent1* mutant endosperms: NC356, Ki3, KUI2007, and M14.

The strong dominant enhancers of yellow color in *albescent1* mutant endosperms isolated from the Maiz Colorado Cuarenton and Cristalino Flint Anaranjado lines were

carried forward for further analysis. Second generation backcrosses were made of *al1-y3* to these two respective lines, and isolates homozygous for the dominant enhancers and heterozygous for *al1-y3* were obtained. Tests of allelism of these enhancers were performed by crossing the two separate homozygous enhancer lines together and self-pollinating the *All* and *al1-y3* progeny (Figure 2). All *al1-y3* progeny kernels from F2 ears were a deep pale yellow in color (n = 3467, indicating a separation of less than 3.4 centiMorgans), so we conclude that the Maiz Colorado Cuarenton and Cristalino Flint Anaranjado lines most likely carry a dominant allele at the same enhancer locus. Since no authentic *Y5* lines exist and *Y5* hadn't been mapped, we cannot perform a test of allelism of our enhancers with *Y5*, although we strongly suspect that our enhancers are identical to Graner's *Y5* due to their identical interaction with *al1-y3* and their provenance from South American orange endosperm flint lines. We have provisionally named our enhancer locus *eec** (*enhancer of endosperm color**). The two independent dominant *eec** alleles have been named *Eec*-MCC* (from Maiz Colorado Cuarenton) and *Eec*-CFA* (from Cristalino Flint Anaranjado).

We have not determined whether the effect of *Eec** alleles on *al1* is due to a direct interaction of *eec** with the *al1* locus, or whether it is due to an overall enhancement of carotenoid content that is readily visible in an *al1* background. The presence of *Eec** alleles seems to be associated with deep orange endosperm color, although not all orange endosperm lines carry an *Eec** allele. Chandler *et al.* (2013. Genetic analysis of visually scored orange kernel color in maize. Crop Science 53:189-200) analyzed the heritability of visual scores for relative intensity of orange endosperm color in ten NAM mapping sub-populations that segregated for visible differences in endosperm color. Only one of the ten NAM sub-populations (B73 X Ki3) involved a line included in our analysis, and that line, Ki3, did not carry a strong *Eec** allele. However, it is conceivable that the *eec** locus is identical to one of the loci that map close to QTL's for orange endosperm color that were identified in Chandler *et al.*'s study. These loci include *y1*, *zds1*, *lyce1*, *zep1*, *wc1*, and *hyd3*. A more recent study by Owens *et al.* (2014. A foundation for provitamin a biofortification of maize: Genome-wide association and genomic prediction models of carotenoid levels. Genetics 198:1699-1716) adds *cyp14* and *hyd4* to the list of loci associated with QTL's for orange endosperm color. We have *Eec** stocks available at the Maize Genetics Cooperation Stock Center for anyone who wishes to conduct a more thorough analysis.

Figure 1. Ears segregating for *al1-y3* and dominant enhancers of endosperm color (*Eec**). Left: Self-pollinated ear segregating for *al1-y3* and *Eec**-*CFA* (from Cristalino Flint Anaranjado). Right: Self-pollinated ear segregating for *al1-y3* and *Eec**-*MCC* (from Maiz Colorado Cuarenton). Middle: Self-pollinated control ear segregating for *al1-y3*, but carrying no enhancers of endosperm color (M14 background). Note the dark pale yellow color of the mutant *al1-y3* endosperms segregating in the two *Eec** lines (left and right) relative to the control line (center).

Figure 2. F2 ears from mapping population of double heterozygotes for the two *Eec** alleles (*Eec*-CFA* and *Eec*-MCC*) and either segregating (left) or homozygous (right) for *al1-y3*.