

### **Isolation and characterization of a dominant inhibitor of *Bn1*.**

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For several years, the Maize Genetics Stock Center grew a hybrid between Mo20W and 4Co63 as a *pl-ww* standard for purposes of re-extracting pericarp and cob color traits. Both inbreds have white endosperm due to the presence of *yl* and *Wc1*. However, we were surprised to observe segregation for a low frequency of dark pale yellow kernels on selfed ears of this standard. The pale yellow kernel trait was tested against *Bn1* (dominant brown aleurone color) and found to be allelic. Tests of the two inbred lines revealed that 4Co63 is *bn1* and Mo20W is *Bn1*, but Mo20W carries a dominant inhibitor of *Bn1*, which explains why Mo20W has white endosperm despite the presence of *Bn1* (in retrospect, Mo20W has kernels that are slightly off-white in comparison with 4Co63). This also explains the observed segregation for pale yellow kernels in the F2 between the two lines: the F1 is heterozygous for both *Bn1* and the inhibitor; *Bn1* expression is observed in the F2 kernels that carry *Bn1*, but do not carry the inhibitor due to independent segregation.

A second white endosperm *yl Wc1* inbred line, K55, was also found to carry a dominant inhibitor of *Bn1* (although it carries a recessive allele at the *bn1* locus). The inhibitors from both Mo20W and K55 were converted to a B73 inbred background by crossing to a B73 *Ht1* conversion of *yl Bn1* for seven generations, selecting white endosperm kernels carrying the inhibitor each generation. Both conversions were self-pollinated to homozygosity for the inhibitor and curiously, both homozygous conversions were also found to be homozygous for *Wc1*. This would indicate either that the inhibitor of *Bn1* is tightly linked to *Wc1* in both Mo20W and K55, or that the inhibitor is an allele of *Wc1*. Dominant alleles of *Wc1* are known to reduce the colored carotenoid content of *Y1* endosperms due to overexpression of a carotenoid cleavage dioxygenase (Vogel et al. 2008, Journal of Biological Chemistry 283:11364-11373; Tan et al. 2004, Maize Genetics Conference Abstracts 46:T14). The nature of the brown pigment that accumulates in the aleurones of *Bn1* kernels is unknown, other than that it is water soluble (Kulkarni 1927, Mich Acad Sci Arts and Letters Papers 6:253-273). It is conceivable that this pigment may provide a substrate that is degraded by carotenoid cleavage dioxygenase; however, if that is the case, then not all *Wc1* alleles inhibit *Bn1* expression since 4Co63 does not carry an inhibitor of *Bn1* although it carries a dominant *Wc1* allele. In the absence of data supporting the allelism of the inhibitor of *Bn1* with *Wc1*, we have named the locus corresponding to the inhibitor isolated from Mo20W *ibn1* (*inhibitor of Bn1*) and the inhibitor isolated from Mo20W has been named *Ibn1-Mo20W*. Tests of allelism will be made between *Ibn1-Mo20W* and the inhibitor isolated from K55. We are also testing two other isolates of *Wc1* (*Wc1-Wh* and a *Wc1* allele isolated from a PI accession of South American Caragua maize, possibly the source of the dominant white endosperm trait named *Wh* by O. White; 1917, American Journal of Botany 4:396-406) for the presence of linked inhibitors of *Bn1*.