

6. B<sup>1</sup> effect in the presence of a heterozygous translocation.

Plants from B<sup>1</sup>/B T2-9a x B are indistinguishable from B<sup>1</sup> plants derived from crosses without the translocation. T2-9a has breaks at 2S. 36 and 9L. 58, with the map location in chromosome 2 to the right of sk, according to one small test by Patterson (Newsletter 26:10).

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7. Bronze mutants and their action in anthocyanin synthesis.

The gene action sequence of A<sub>1</sub>, A<sub>2</sub>, C<sub>1</sub>, R, In and Bz<sub>1</sub> in anthocyanin synthesis has been constructed, using complementary interactions between aleurone tissues (Newsletter 35:95). The position of action of bronze genes based on these observations was inconclusive. The bronze testers have anthocyanin pigment and when they are combined with colorless mutants, the colorless mutants develop pigment, leaving the possibility of simple diffusion of pigment rather than the required substrate transfer in anthocyanin synthesis. To eliminate this possibility of pigment diffusion, some further experiments were performed.

The double recessives r bz<sub>1</sub> and r bz<sub>2</sub> paired in the four possible combinations with singly recessive bz<sub>1</sub> and bz<sub>2</sub> were subjected to the previously described standard conditions. When colorless r bz<sub>1</sub> and r bz<sub>2</sub> are combined with bz<sub>1</sub> or bz<sub>2</sub> testers, only r bz<sub>1</sub> in the r bz<sub>1</sub>:bz<sub>2</sub> pair develops pigment, while the others remain colorless, indicating that Bz<sub>1</sub> precedes Bz<sub>2</sub> in sequential action in anthocyanin formation and that the action of Bz<sub>2</sub> follows R and Bz<sub>1</sub> in anthocyanin synthesis. Further, double recessive bz<sub>1</sub> bz<sub>2</sub>, although colorless, causes pigment to develop in a<sub>2</sub> and other testers, indicating that the bronze factors act after the others. These observations clearly show that the simple diffusion of anthocyanin pigment is not involved, at least from bronze mutants. Finally this method, i.e., use of double recessives, allows placement of the modifier genes in the action sequence in anthocyanin synthesis.

The action of C<sub>2</sub> in the sequence has not been established definitely as the c<sub>2</sub>-mutant in some cultures gives pigment by itself. All these observations, including the previous findings, establish the following sequence:

C<sup>I</sup>, C<sub>1</sub>, R, (In), A<sub>1</sub>, A<sub>2</sub>, Bz<sub>1</sub>, Bz<sub>2</sub> ----- anthocyanin

Some of the preliminary studies of extraction of substrates with various solvents, acetone, alcohol, acid-alcohol etc. toward the direct demonstration of gene-enzyme relationship are encouraging but not convincing. It is possible that the characterization of these substrates can be expected to reveal the intermediates and the reaction steps in the biosynthesis of anthocyanin and may lead to further analysis of the mechanism of gene action and interaction in this system.

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