2. The brown-midrib mutants of maize.

Several years ago here at Purdue we found that the basis of the $\underline{bm_1}/\underline{bm_1}$ and the $\underline{bm_2}/\underline{bm_2}$ phenotypes was the production by these mutants of lignins which are quite different from that produced by normal plants. These altered lignins are responsible for the midrib color in mutant plants. Later we found that Jorgensen, who isolated the $\underline{bm_1}$ mutant, reported that the pigment responsible for the color was either lignin or a pigment indissociably bound to lignin.

Our discovery stimulated a program to attempt to learn something about the biosynthesis and structure of lignin using the mutants as tools as has been done so successfully in Neurospora, E. coli, and other microorganisms. The chemistry of lignin is still poorly understood in spite of much research. Lignin is known to be a polymer of various phenylpropanoid (C6-C3) building blocks (depending on the species). Since this is so, a mutant affecting lignin production could affect either a step in the production of a phenolic building block or a step in the synthesis of the polymer itself.

The current consensus of opinion regarding lignin synthesis (after Freudenburg) is that the only enzymatically mediated step in the synthesis of the polymer itself is a dehydrogenation of the building blocks. The result of this dehydrogenation for a given building block is a radical which can exist in various mesomeric forms. These mesomers can combine at random in all possible combinations to form a disorderly type of polymer. If this view is correct, then the origin of the very different $\underline{bm_1}$ and $\underline{+^{bm}1}$ lignins must be found in different pools of phenolic compounds in which this random polymerization is proceeding. This should be experimentally verifiable, and we are now investigating this point.

The $\underline{bm_1}$ and $\underline{+^{bm}1}$ lignins differ in many ways. In the first place, there is a lower content of Klason lignin in $\underline{bm_1}/\underline{bm_1}$ plants (14 percent) as compared to $\underline{+^{bm}}/\underline{+^{bm}}$ plants of roughly comparable genotype which have 21 percent lignin. Alkali lignin from $\underline{+^{bm}1}$ plants is a light tan amorphous powder which melts at ca 172° C. Alkali $\underline{bm_1}$ lignin is a deep reddish-brown paracrystalline substance which chars at 236° C before melting. Oxidative degradation of native lignins with nitrobenzene in an alkaline medium shows a marked deficiency of p-hydroxy-phenyl residues (p-oH cinnamic acid, p-oH benzoic acid, and p-oH benzaldehyde) in $\underline{bm_1}$ lignin as compared to $\underline{+^{bm}1}$ lignin. This reduction has not been determined quantitatively as yet, but there may be only one fourth as much in $\underline{bm_1}$ lignin.

The grasses are the only group of plants in which p-hydroxy phenylpropanoid building blocks are incorporated into the lignin polymer. The grasses are also the only group in which added tyrosine will serve as a lignin precursor. It was suspected at first that the block in $\underline{bm_1}$ might be in one of the steps between tyrosine and p-oH cinnamyl alcohol

which is apparently the p-oH phenylpropenoid building block. Accordingly tracer experiments were conducted in which both $\underline{bm_1}/\underline{bm_1}$ and $\underline{+bm}/\underline{+bm}$ plants were allowed to take up either UL C^{14} phenylalanine or UL C^{14} tyrosine and then frozen 24 hours later for analysis. Estimation of the specific activity of the isolated alkali lignins indicated that both amino acids were equally good lignin precursors for both $\underline{bm_1}/\underline{bm_1}$ and $\underline{+bm}/\underline{+bm}$ plants. This would appear to rule out the hypothesis suggested above.

If anyone has a brown-midrib mutant which is known not to be 1, 2, 3, or 4, we would be most interested in obtaining it.

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3. The location of the $\underline{Hm_2}/\underline{hm_2}$ locus.

In the 1951 News Letter (25) we reported a second locus affecting susceptibility to Race I, Helminthosporium carbonum. This locus has now been designated as $\underline{Hm_2}/\underline{hm_2}$ and located on chromosome 9. Its location is probably on the long arm of the chromosome since in a 3-point test (CS) the order of the genes is sh wx $\underline{hm_2}$ with recombination between wx and $\underline{hm_2}$ approximating 25 percent.

The allelic constitution at the $\underline{Hm_2}/\underline{hm_2}$ locus can be determined only in the presence of $\underline{hm_1}/\underline{hm_1}$. The double mutant $\underline{hm_1}/\underline{hm_1}$; $\underline{hm_2}/\underline{hm_2}$ is fully susceptible. Such markedly susceptible inbreds as Pr, Mo. 21a, and K61 are of this genotype. Plants which are $\underline{hm_1}/\underline{hm_1}$; $\underline{Hm_2}/\underline{hm_2}$ or $\underline{Hm_2}/\underline{Hm_2}$ display increasing resistance with time. Seedlings are moderately susceptible and quite large lesions are formed on the leaves attacked by the fungus. The leaves last initiated are highly resistant and only chlorotic flecks develop in response to infection. With artificial inoculations at the flowering stage or later, there is no difficulty in distinguishing $\underline{hm_1}/\underline{hm_1}$; $\underline{hm_2}/\underline{hm_2}$ plants from $\underline{hm_1}/\underline{hm_1}$; $\underline{Hm_2}/\underline{hm_2}$ or $\underline{Hm_2}/\underline{Hm_2}$ plants within ten to fourteen days following penetration by the pathogen. Under natural growing conditions $\underline{hm_1}/\underline{hm_1}$; $\underline{Hm_2}/\underline{Hm_2}$ plants are for practical purposes resistant to infection by the fungus.

The data on the location of $\underline{Hm_2}/\underline{hm_2}$ substantiate those collected earlier from a RS progeny. The F_2 progeny investigated in 1960 was derived from $\left(\frac{\underline{hm_1}\ \underline{Sh}\ \underline{Wx}\ \underline{Hm_2}}{\underline{hm_1}\ sh\ wx\ \underline{hm_2}}\right)$ ⊗. The data are given in Table I.