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SHORT COMMUNICATION

DEGRADATION OF ABNORMAL LIGNINS IN THE BROWN-MIDRIB MUTANTS AND DOUBLE MUTANTS OF MAIZE

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Abstract—The lignin content of the single mutants was somewhat lower, and that of the double mutants was considerably lower, than that of normal maize. Alkaline hydrolysis of the stalk of all mutants and double mutants yielded *p*-coumaric and ferulic acids as major products. The yield of *p*-coumaric from bm_1/bm_1 and the double mutants bm_1/bm_1; bm_2/bm_2, bm_1/bm_1; bm_3/bm_3, and bm_1/bm_1; bm_4/bm_4 was considerably less than that from normal maize. The yield of ferulic acid from all double mutants was greater than that from normal maize or the single mutants. Alkaline–nitrobenzene oxidation of maize stalks yielded syringaldehyde, vanillin and *p*-hydroxy-benzaldehyde. The yield of syringaldehyde from all the double mutants and the bm_1/bm_1, bm_2/bm_2, and bm_3/bm_3 mutants was less than that from normal maize. The greatest reduction of syringaldehyde occurred with the bm_1/bm_1 mutant and its double mutants.

INTRODUCTION

PREVIOUS work[1,2] with normal and the *brown-midrib*-1-(bm_1) mutant of maize indicated that *p*-coumaric and ferulic acids were the principal acids esterified onto lignin cores. Alkaline hydrolysis of stalks and solvent-extractable lignins of normal maize liberated at least twice as much *p*-coumaric, but equal quantities of ferulic acid, as that liberated from the mutant. A dimethyformamide (DMF) lignin from the mutant contained more carboxyl groups than a DMF lignin of normal maize[2]. In addition to bm_1, there are three other mutants that are visually similar to bm_1 but genetically different. These are the *brown-midrib*-2 (bm_2), bm_3 and bm_4 mutants. Any two mutant genes can be combined in a single line to constitute a double mutant strain. In this communication, data for the other mutants and all possible double mutants are presented.

RESULTS AND DISCUSSION

Data are presented in Table 1. It was suggested[2] that bm_1/bm_1 incorporates a residue into the lignin core that does not yield syringaldehyde, vanillin or *p*-hydroxybenzaldehyde upon alkaline nitrobenzene oxidation. It was further suggested that this residue contains a carboxyl group and nitrogen. These suggestions are consistent with the data presented in this communication. The genetic block in bm_1 may occur in the synthesis of residues which

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[1] J. KUC' and O. E. NELSON, *Arch. Biochem. Biophys.* **105**, 103 (1964).

[2] MARY SUE GEE, O. E. NELSON and J. KUC', Paper accepted for publication, *Arch. Biochem. Biophys.*

TABLE 1. LIGNIN CONTENT, PHENOLIC ACIDS LIBERATED BY ALKALINE HYDROLYSIS AND PRODUCTS OF ALKALINE NITROBENZENE OXIDATION OF MATURE ETHER AND WATER-WASHED CORN STALKS

		mg/g Estimated lignin				
		Phenolic acids liberated by alkaline hydrolysis		Products of alkaline nitrobenzene oxidation		
Strain	% Lignin	*p*-Coumaric	Ferulic	Syringaldehyde	Vanillin	*p*-Hydroxybenzaldehyde
Normal	24·5	123	34	77	32	16
bm_1/bm_1	21·0	56	34	41	26	11
bm_2/bm_2	22·0	118	41	63	31	14
bm_3/bm_3	21·0	128	42	68	28	21
bm_4/bm_4	21·4	113	34	79	41	32
bm_1/bm_1; bm_2/bm_2	14·1	71	68	22	25	11
bm_1/bm_1; bm_3/bm_3	15·0	63	59	20	25	12
bm_1/bm_1; bm_4/bm_4	14·7	53	79	25	24	16
bm_2/bm_2; bm_3/bm_3	16·9	96	47	30	27	15
bm_2/bm_2; bm_4/bm_4	18·0	113	62	59	34	21
bm_3/bm_3; bm_4/bm_4	13·9	101	91	47	39	18

yield syringaldehyde upon alkaline-nitrobenzene oxidation. The incorporation of an "abnormal" metabolite could account for the reduced yield of aldehydes and markedly reduced ratio of syringaldehyde to vanillin from bm_1/bm_1 and its double mutants as well as the high level of carboxyl groups, and their increase after alkaline hydrolysis, in bm_1/bm_1 DMF lignin.[2] Since there is little difference in the pool of free or esterified *p*-coumaric acid, soluble in 50 per cent methanol, in normal and bm_1/bm_1 maize throughout the growing season,[3] it is possible that the reduced esterification of *p*-coumaric acid on the bm_1/bm_1 core is due to the highly esterified and cross-linked nature of the core arising from the incorporation of an abnormal metabolite.

EXPERIMENTAL

The lignin content and the products of alkaline hydrolysis and nitrobenzene oxidation of mature ether and water-washed stalks were determined as described in earlier manuscripts.[1,2] All six possible double mutant combinations between the four single mutants have been synthesized and proven genetically.

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[3] J. W. HOLLIS, M.S. Thesis, Purdue University (1963).