

A genetic test for the involvement of catechol oxidase in hypersensitive resistance to rust

Phenolic compounds and polyphenol oxidases have been implicated frequently in plant resistance mechanisms (Kosuge, T., 1969, Ann. Rev. Phytopath. 7:195). In previous work, we also suggested (Pryor and Schwartz, 1973, Genetics 75:75) that a specific phenol oxidase, catechol oxidase (CX), functioned in the resistance mechanism. We have now been able to demonstrate that this enzyme activity is not involved in the resistance of corn to its rust Puccinia sorghi Sch. Nor does the level of endogenous CX substrate appear to affect the expression of resistance.

The experimental method was to construct a line which carries the gene for resistance and is homozygous for a null mutant of CX (Cx-N). Two different null lines were used, P3 and P4, having respectively high and low levels of endogenous CX substrate. The expression of resistance in these plants will then allow us to determine the involvement of CX activity and endogenous substrate in the hypersensitivity mechanism.

Table 1. Segregation of monogenic resistance to Puccinia sorghi in testcrosses also segregating for a null allele of catechol oxidase.

Gene	Backcross*	Susceptible	Resistant	Total	$\chi^2(1:1)$
<u>Rp-d</u>	3 x 68.3	18	28	46	2.2
	68.3 x 3	27	23	50	0.32
	4 x 68.4	-	-	-	-
	68.4 x 4	26	18	44	1.45
<u>Rp-f</u>	3 x 70.3	22	24	46	0.09
	70.3 x 3	28	22	50	0.72
	4 x 70.4	-	-	-	-
	70.4 x 4	16	17	33	0.03
<u>Rp-d(2)</u>	3 x 77.3	20	25	45	0.56
	77.3 x 3	25	29	54	0.30
	4 x 77.4	-	-	-	-
	77.4 x 4	24	19	43	0.58
<u>Rp-g</u>	3 x 78.3	-	-	-	-
	78.3 x 3	20	25	45	0.56
	4 x 78.4	30	19	49	2.47
	78.4 x 4	62	77	139	1.62
<u>Rp4-a</u>	3 x 85.3	-	-	-	-
	85.4 x 3	24	19	43	0.58
	4 x 85.4	-	-	-	-
	85.4 x 4	25	20	45	0.56
<u>Rp5</u>	3 x 87.3	-	-	-	-
	87.3 x 3	19	26	45	1.09
	4 x 87.4	22	24	46	0.35
	87.4 x 4	16	15	31	0.03

\*68, 70, 77, 78, 85 and 87 are our family numbers given to the monogenic resistant lines carrying the resistance genes listed in column one. Lines 3 and 4 are susceptible, and homozygous for Cx-N.

The resistance genes were derived from samples of the International Rust Monogenic lines developed by Hooker and supplied by Dr. K. S. McWhirter. All lines carrying a gene for resistance were homozygous for the Cx-F allele of catechol oxidase. Six of those lines showing resistance to the local rust strain were crossed and backcrossed reciprocally to the two null lines, P3 and P4. If CX activity is a necessary component of the resistance mechanism then only 25% of the backcross progeny will carry the gene for resistance with an active CX and will show resistance. Conversely, if CX is not involved, 50% of the progeny will be resistant and 50% susceptible. The results are presented in Table 1 and indicate 50% resistant progeny for all alleles and the three loci tested. This result indicates that CX is not involved with the expression of resistance with one reservation. This would arise if the genes for resistance and CX were tightly linked. Rp, Rp5 and Cx are on chromosome 10 but should be some 33 recombination units apart (Rp is at position 0 and Cx is very close (<0.2% recombination) to dull endosperm (du) at position 33). Rp4 is unlinked on chromosome 4. For reasons mentioned previously (Pryor and Schwartz 1973) it was not possible to assay unambiguously for resistance and catechol oxidase in the same plants. Thus, seedlings from the backcross involving the Rp-g gene were grown to maturity and the selfed progeny were scored for Cx genotype:

<u>rp Cx-N</u>	<u>rp Cx-F</u>	<u>Rp-g Cx-N</u>	<u>Rp-g Cx-F</u>	<u>Total</u>
9	3	6	8	26

Recombination between Rp and Cx is estimated at 34%. But, more significantly, 6 of 14 resistant plants were also homozygous for the null gene (Cx-N) for catechol oxidase.

Conclusion: Catechol oxidase activity is not required for the expression of genes for resistance to rust.

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#### Genetics of fertility restoration for male sterile cytoplasm

Crosses were made between homozygous male sterile (rf rf) and homozygous restored (Rf Rf) plants to produce a series of plants heterozygous for the pollen-restoring genes (Rf rf) for each of 26 different sources of cytoplasmic male sterility. These heterozygous F<sub>1</sub> plants were crossed onto their respective cytoplasmic male sterile parent; Table 1 presents the data on the number of fertile versus sterile plants in each backcross. All of the cms-S type cytoplasm and cms-K, -L, -B, and -D (which are similar to cms-S but have shown some fertility restoration differences) gave no sterile plants. Therefore, all of the cms-S type cytoplasm exhibited gametophytic restoration in which the recessive (rf) fertility restoration alleles produced by the Rf rf parent were not functional. The cms-T cytoplasm gave the 1:1 ratio between fertile and sterile plants expected of the sporophytic type of fertility restoration in which both rf as well as Rf pollen grains are functional. The cms-C type cytoplasm also follow the sporophytic pattern of restoration.