한국식물보호학회지 제 17 권 제 4 호
Korean. J. Pl. Prot. 17(4) : 193~199(1978)

PEROXIDASE ACTIVITY ASSOCIATED WITH *Puccinia sorghi* INFECTION IN MAIZE

Kim, Soon Kwon* • James L. Brewbaker** • and Yoichi Hasegawa**

옥수수에 있어서 녹병감염에 관한 Peroxidase의 활성

김순권 • 제임스 엘 부루베이커 • 요이찌 하세가와

ABSTRACT

Five parents and their 10 F_1 crosses of maize (*Zea mays* L.) were tested by means of diallel analysis for the inheritance of peroxidase activity following *Puccinia sorghi* rust infection. Peroxidase activity was measured at day zero and at 2 days and 8 days after inoculation. Peroxidase activity was increased significantly by *P. sorghi* infection in all 15 genotypes after 8 days, but not after 2 days.

Highly significant differences in peroxidase activity were detected among the 15 genotypes. The highly general resistant inbred, CM105, and its hybrids showed exceptionally high peroxidase activity in both healthy and infected plants. However, another highly resistant inbred, Oh545, showed exceptionally low peroxidase activity.

Significant general combining ability (GCA) and specific combining ability (SCA) means quares were detected for peroxidase activity independent of disease. GCA mean spuares, however, were consistently a major contiribution to the inheritance of peroxidase activity in the infected plants whereas SCA men square contributions were minor.

Rust resistant maize plants controlling mongenic dominant Rp_1^d gene showed stronger peroxidase reroxidase responses than their susceptible counterparts in the gel electrophoresis and densiometric tracings. The increased peroxidase activity occurred in both major leaf peroxidases, Px_3 and Px_7.

INTRODUCTION

Quantitative changes in peroxidase activity have been observed to be induced by pathogenic infection, by senescence, or by wounding. The increases in peroxidase activity after pathogen infection have been reported in maize with *Helminthosporium carbonun*[7] in tomato with *Phytophthora infection*[16] in tobacco with *Collectotrichum destrustivum*[19] in flax with *Melampsora lini*[1], and in beans with bean virus [4,15] Level of peroxidase activity in potato leaves before infection was positively correlated with resistance to *P. infestans*[5,14]. Maize plant resistant to *H. carbonum* showed higher peroxidase activity than healthy counterpart and varied substantially in leaves infected with different pathogen races[17]. Virus infected sweet potato leaf and root and *C. destrustivum* infected tobacco leaf tissues had also significantly

* Crop Experiment Station, Office of Rural Development, Suweon, Korea.
** College of Tropical Agriculture, University of Hawaii Honolulu, HI 96822.

more peroxidase activity than their healthy counterparts, and these increases in the peroxidase activity were associated with the symptom appearance on the tissues and the aging of the leaves[13,14,19]. *M. lini* infected flax leaf and virus infected bean leaf tissues showed more peroxidase isozymes than their healthy leaf tissues[1,4]. Pryor[17] studied phenol oxidase of maize infceted with *Puccinia sorghi* rust and proposed a biochemical system contained the requirement of the hypersensitive response observed in resistant maize plants containing monogenic R_p genes.

The objectives of this study were to assay peroxidase activity of healthy and inoculated plants and to determine gene actions controlling peroxidase activity after *P. sorghi* rust infection in maize.

METERIALS AND METHODS

Plant materials (5×5 *diallel*) *and culture:* Five maize inbreds were selected for this study, including two general, race non-specific rust resistant lines, Oh545 and CM105, and three highly susceptible lines, AA8, B37, and CM104[10,11]. All possible crosses, excluding reciprocals, were made in 1972. The five parents and 10 F_1 crosses were grown under greenhouse conditions in Hawaii in 1973. Each entry consisting of five seedlings in 3 replicated 6-inch plastic pots containing a sterilized soil with nutrients.

Rust inoculation: Inoculation was made two weeks after planting when the seedlings were in the 4 to 5 leaf stage with the second leaf fully expanded. All experimental plants were sprayed with *P. sorghi* urediospore suspensions, approximately 30,000 spores /ml. The urediospores were collected from fresh corn leaf tissues at the Waimanalo Farm Univ. of Hawaii, Honolulu. Control plants were sprayed with water. All plants were incubated in moist chambers consisting of inverted plastic garbage cans for 24 hours and then moved to a greenhouse bench.

Enzyme preparation: Six leaves per entry were taken immediately before inoculation (zero day) and at two and eight days after inoculation. One disc (1 cm diameter) was taken from each of the six leaves, and two disc samples combined to provide three samples for peroxidase assey. Samples were ground in 5 ml phosphate buffer, pH 6.0. The macerated suspensions were centrifuged at 10,000 rpm for 10 min and the supernatant was used as enzyme source. Preparations such as these include largely the cytoplasmic fraction; the wall-bound fraction of young leaves, estimated to represent 30% of the total activity, was not included.

Enzyme assay: Peroxidase activity was measured on a Baush & Lomb colorimeter at room temperature. The change in absorbancy at 470 nm of guaiacol substrate in the presence of hydrogen peroxide and enzyme was recorded for 3 min. The guaiacol test solution consisted of 5.4×10^{-4} ml guaiacol and 3.5×10^{-5} ml hydrogen peroxide in 0.02 M phosphate buffer (pH 6.0) made up to 3.0 ml final volume. The Lowry method was used for protein determinations, with crystalline bovine serum albumin as the standard. Peroxidase activity was expressed as enzyme activity in O.D. unit/min./protein content (mg/ml), and analyzed by Griffing's method 2, model I to estimate general and specific combining ability.

Standard electrophoretic horizontal gels were used and were of 7% polyacrylamide and were electrophoresced using lithium-borate buffer at pH 8.1 for approximately 10 hours at 8 v/cm at 4°C. Benzidine dihydrochlroide and o-dianisidine were used for staining gels and these gels were rinsed in running water [8,16].

Densitometric analysis were carried out after staining the electrophoresced gels with o-dianisidine and rinsing with 50% ethanol and running water. The gels was transferred to a Model 542 Densicord to trace the peroxidase bands using a 0.1×6 nm slit aperture and a 465 nm filter.

RESULTS AND DISCUSSION

Peroxidase activity: Average peroxidase activities of 15 genotypes involving 5 maize inbred lines and their hybrids are summarized in Table 1. Data were taken prior to inoculation, and at 2 and 8 days there after. Wide differences were found among the 15 genotypes for average peroxidase activity, ranging from 1.06 for Oh545 to 4.22 for CM105. Inbred CM 105 and Oh545 were identified as high levels of general resistance type in genetic studies[10,11]. Inbred CM105 itself and all crosses involving CM105 had greater peroxidase activity than average activity of 15 genotypes(2.07). Peroxidase activities of CM105

Table 1. Average peroxidase activity for 5 parents and their 10 F_1 hybrids

Entry	Peroxidase activity in A/min/mg protein					Average
	Day 0	Day 2		Day 8		
		C+	I	C	I	
AA8	1.78	2.47	2.12	1.92	3.19	2.30
B37	0.73	1.56	1.26	1.29	1.34	1.24
CM104	1.63	3.42	3.02	1.53	2.38	2.40
CM105	2.51	2.66	3.07	6.33	6.54	4.22
Oh545	0.78	0.80	1.01	1.21	1.50	1.06
AA8×B37	0.58	1.64	1.49	1.47	2.26	1.54
AA8×CM104	1.38	2.11	2.10	1.39	2.08	1.81
AA8×CM105	2.42	2.21	2.85	2.62	3.32	2.74
AA8×Oh545	1.38	1.47	1.30	1.12	2.36	1.53
B37×CM104	1.46	1.31	1.71	1.40	1.89	1.55
B37×CM105	2.30	2.37	2.50	1.52	1.81	2.10
B37×Oh545	0.94	2.43	1.06	1.10	2.52	1.61
CM104×CM105	2.61	3.37	2.85	2.49	4.15	3.09
CM104×Oh545	1.30	1.88	1.87	1.96	2.09	1.82
CM105×Oh545	1.47	1.98	2.32	1.76	3.53	2.21
Avg.	1.57	2.11	2.03	1.96	2.66	2.07

*C=Control. I=Inoculated

were almost always the highest among the 15 genotypes, its hybrids were commonly low in activity.

Two U.S. corn belt inbreds B37 (rust susceptible), and Oh545 (resistant) showed relatively low peroxidase activities.

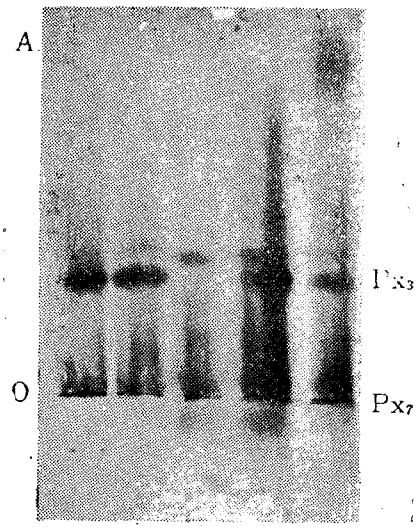
Mean squares of the 15 genotypes at two treatments (control vs incoulated) are highly singificant and summarized in Table 2. Mean squares of the average peroxidase activity of the 15 genotypes at 8 days following inoculation was significantly different at 1% level than that of control. No significant differences were, however, observed at 2 days following inoculation.

Table 2. Mean squares differences of peroxidase activity in A/min/mg protein at 2 and 8 days after inoculation, respectively.

Source	df	Mean square	
		Day 2	Day 8
Genotype	14	0.92**	3.27**
Treatment	1	0.05	4.45
Error	14	0.12	0.16

** Significant at P=0.01 level.

Monogenic rust resistant plants controlled by dominant gene, R_{p1^d} showed stronger peroxidase responses than their susceptible counterparts. Zymogams of



AA8 B37 CM CM105 Oh545

Fig. 1. Anodal peroxidases of five maize inbreds.

peroxidases from five maize inbreds are shown in Figure 1. Highly general resistant inbred, CM105, (Cuban flint) showed the strongest response and highly susceptible inbred, CM104: CM showed the weakest response. Brewbaker and Hasegawa (2) found at least 12 major peroxidases in maize. Among the 12 peroxidase, P×3 and P×7 peroxidases appeared almost exclusively in maize leaf tissues. Two major peroxidases, P×3 and P×7 also dominate in the leaf tissues used in this study. The major evidence of the peroxidase increase is in the "daughter band" or "metazymes" generated from the P×3 band and of increasing mobility on gels, best seen for inoculated hybrid AA8×CM104 shown in Figure 2. In wheat, Seevers et al. (18) detected at least 14 peroxidases

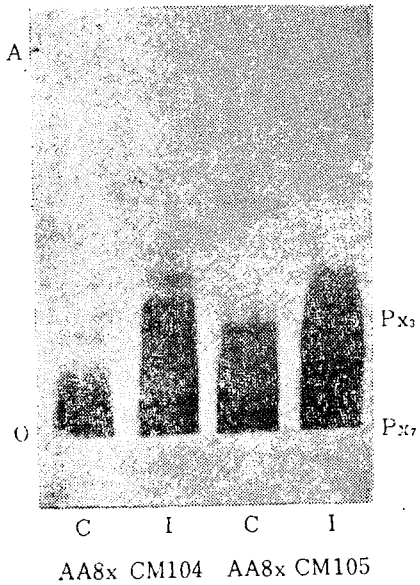


Fig. 2. Peroxidases response of two F_1 crosses at 8 days after inoculation (D 8). C samples were controls, I samples were infected.

in both health and infected leaf tissues. Aspects of rust resistance in wheat were reviewed thoroughly by Daly(3).

Combining ability effects:General (GCA) and specific combining ability(SCA) mean squares of the 15 genotypes for the peroxidase activity are calculated and summerized in Table 3. GCA variances were major contribution to the inheritance of peroxidase activity following *P. sorghi* infection. GCA mean squares were highly significant, expressed of average GCA value by 54.8%. SCA mean squares were generally small although significant at the 5% level.

The minor importance of the SCA value was also expressed by the 23.0% total variance components. Estimates of GCA effects of the 5 individual parental inbreds for peroxidase sctivity are summarized in Table 4. Wide differences in the GCA effects among the parental inbreds were detected. The GCA effects of inbreds ranged from 0.83 for CM105 to −0.45 for both B37 and Oh545. GCA effects for CM105 were the the greatest in the individual treatments, except those for day 2 control plant samples.

Average SCA effects in inoculated plant samples at day 2 and day 8 following inoculation were negative and were higher than those of control plant samples. SCA effects at day 2 were −0.04 for control and −0.02 for inoculated and those at day 8 were −0.25 for control and −0.07 for inoculated (8). The physiology of host resistance against pathogens has been noted as several defense mechanisms.

Some mechanisms are active before infection ("passive type") and expressed in the forms of nutritional and physically states. Others were active after infection and expressed as active type(6). In case of *P. sorghi* resistance, both passive and active types

Table 3. Combining ability mean square for peroxidase activity.

Source	df	Mean Square					Average variance component (%)
		Day 0	Day 2		Day 8		
			C	I	C	I	
GCA	4	1.18**	1.04**	1.68**	4.30**	3.32**	54.8
SCA	10	0.09	0.31*	0.05	0.69**	0.41*	23.0
Error	28	0.06	0.10	0.11	0.05	0.16	22.0

Table 4. Estimates of general combining ability effects for peroxidase activity.

Parent	General combining ability effects					Average
	Day 0	Day 2		Day 8		
		C	I	C	I	
AA8	0.02	−0.04	−0.03	−0.15	0.16	−0.03
A37	−0.34	−0.26	−0.42	−0.52	−0.69	−0.45
CM104	0.09	0.41	0.34	−0.21	−0.14	0.10
CM105	0.63	0.37	0.63	1.37	1.13	0.83
Oh545	−0.40	−0.47	−0.52	−0.49	−0.35	−0.45
se_d	0.13	0.17	0.18	0.12	0.22	0.16

of enzyme activity appeared to be involved.

Peroxidase reactions of specific resistant types: Seven paired double cross hybrids genetically similar except for monogenic gene $R_{p1}{}^d$ conferring monogenic resistance to *P. sorghi* were also tested in this study for their peroxidase activity. Materials tested in this study were same materials used in the previous study made in Hawaii [9]. These hybrids were grown in paired rows under severe natural rust epiphytotics prevailing at the Waimanalo Farm during April and August in 1973. Disc samples of 2 cm diameter were taken from hypersensitive specific resistant and susceptible plants at flowering time. Both susceptible and resistant plants were heavily infected. Electrophoretic peroxidase responses of five paired double cross hybrid leaves to infection by *P. sorghi* are presented in Figure 3. Leaf samples controlling specific monogenic genes showed strong peroxidase responses in both P×3 and P×7. Infected resistant samples

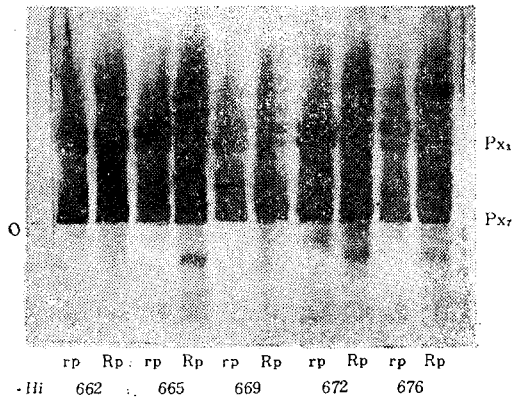


Fig. 3. Peroxidase response of five double-cross hybrid leaves to infection by *P. sorghi*; ip represents susceptible and R_p represents resistant plant.

had several more daughter bands than their susceptible counterparts.

Densitometric tracings of peroxidase activity of one of the paired hybrid gels are presented in Figure 4. Resistant hybrid 681 had higher peroxidase activity and had at least two more daughter bands in P×3 than its susceptible counterparts. Peroxidase activity of P×7 in the resistant hybrid also seemed to be higher than its susceptible counterparts.

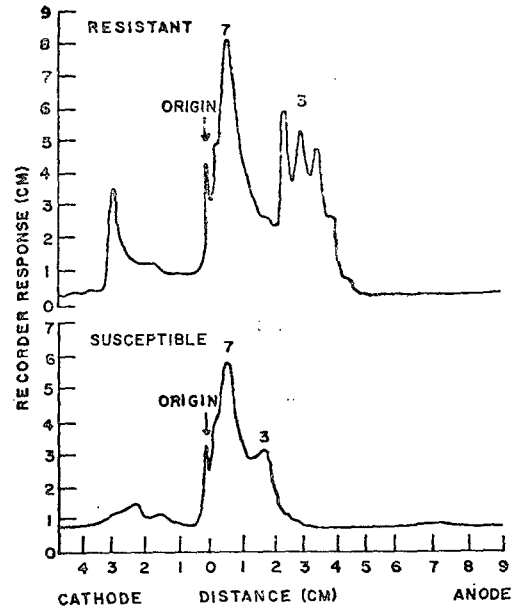


Fig. 4. Densitometric tracings of peroxidase activity of resistant and susceptible hybrid 681.

The results obtained in this study in relation to peroxidase activity with rust resistance are somewhat similar with the results obtained in the peroxidase studies of wheat, potatoes, tobacco, and beans. General resistance appears to be not always directly re-

lated for their stronger peroxidase activity for resistance. Since one highly resistant inbred, Oh545, showed exceptionally low peroxidase activity. Hooker (12) pointed out that common hypersensitive forms of specific resistance to obligate pathogens in maize has not been explained in terms of phytoalexin activity of resistant host plants.

摘　　要

옥수수 植物體에 녹병(*Puccinia sorghi*)이 感染된 狀態와 非感染狀態下에서 Peroxidase(Px) activity(活動)를 좌우하는 遺傳因子의 行動을 規明하기 爲해 5個의 選拔된 自殖系統과 이들사이에 可能한 10個의 F_1(雜種 第一世代)을 갖고 *Px*의 活動에 關한 遺傳分析 試驗을 實施해본 結果 다음의 結論을 얻었다.

1. 利用한 5個 自殖系統과 10個의 F_1 사이의 *Px* 活動은 크게 달랐다.
2. 數個의 遺傳因子에 依해서 좌우되는 一般抵抗性程度와 *Px*活動은 一致되지 않았다. 相加的 遺傳因子에 依해 좌우되는 높은 一般抵抗性 系統 CM105는 높은 *Px*의 活動을 보였으나, 다른 抵抗性系統 Oh545는 낮은 *Px*의 活動을 보였다.
3. 녹병感染과 非感染狀態下에서의 *Px*의 活動은 銹病菌接種後 2日때는 差異가 없었으나, 接種後 8日때는 感染植物體에서 有意差를 보이는 높은 *Px* 活動이 測定되었다.
4. 옥수수에 있어서 *Px*의 活動을 좌우하는 遺傳因子는 相加的作用을 주로 일으키는 GCA(一般 組合能力)効果에 依하고, 非相加遺傳因子에 依한 SCA(特殊組合能力)効果는 比較的 낮았다.
5. 優性 單因子 抵抗性에 依해서 좌우되는 特殊抵抗性 遺傳因子 $R_{p1}{}^d$를 保有한 植物體는 抵抗性 遺傳因子를 保有하지 않는 罹病性 植物體에 比해서 높은 *Px*의 活動을 보였다. 이는 12個의 밝혀진 *Px* 中 主로 Px_3와 Px_7의 增加에 基因한다.
6. 本 試驗에서 얻어진 옥수수의 녹병抵抗性과 關聯한 *Px*의 活動은 但 特殊性抵抗性 遺傳因子인 경우에만 他 作物에서 얻은 試驗結果와 類似한 點이 있다.

Literature cited

1. Andreev, L. N. and M. Shaw. 1969. A note on the effect of rust infection on peroxidase isozyme in flax. Canad. J. Botany 43:1479-1483.
2. Brewbaker, J. L. and Y. Hasegawa. 1974. Polymorphisms of the 12 major peroxidases of maize. Proceedings, 3rd International Conference on Isozymes. Yale Univ. New Haven. Connecticut. Academic Press, N. Y.
3. Daly, J. M. 1972. The use of near-isogenic lines in biochemical studies of the resistance of wheat to stem rust. Phytopathology 62:392-400.
4. Farkas, G. L. and M. A. Stahmann. 1966. On the nature of changes in peroxidase isozymes in bean leaves infected by southern bean mosaic virus. Phytopathology 56:669-677.
5. Fehrmann, H. and A. E. Diomond 1967. peroxidase activity and *Phytophthora* resistance in different organs of the potato plant. Phytopathology 57:69-72.
6. Hooker, A.L. 1967. The genetics and expression of resistance in plants to rusts of the genus *Puccinia*. Ann. Rev. of Phytopathology 5;163-182.
7. Jennings, P.H., B. L. Brannaman, and F.P. Zscheile, Jr. 1967. Peroxidase and polyphenol oxidase activity associated with *Helminthosporium* leaf spot of maize. Phytopathology 59:963-967.
8. Kim, S. K. 1974. Quantitative genetics of *Puccinia sorghi* resistance and husk number in *Zea mays*. Ph.D. thesis. Univ. of Hawaii 1889.
9. Kim, S. K. and J. L. Brewbaker. 1976. Effects of *Puccinia sorghi* rust on yield and several agronomic traits of maize in Hawaii. Crop Science 16:874-877.
10. Kim, S.K. and J. L. Brewbaker. 1976. Sources of general resistance to *Puccinia sorghi* on maize in Hawaii. Plant Dis. Repty. USDA. 60:551-555.
11. Kim, S. K. and J. L. Brewbaker. 1972. Inheritance of general resistance in maize to *Puccinia sorghi* Schw. Crop Science 17:456-461.
12. Lim, S. M., J. D. Paxton, and A. L. Hooker. 1968. Phytoalexin production in corn resistant to *Helminthosporium turcicum*. Phytopahology 58: 720-721.
13. Loebenstein, G. and N. Linsey. 1961. Peroxidase activity in virus infected sweet potatoes. Phytopathology 5:533-537.
14. Lovrekovich, L., H. Lovrekovich, and M. A. Stahmann. 1968. The importance of peroxidase in the wildfire disease. Phytopathology 58:193-198.
15. Novacky, A. and R. E, Hampton. 1968. Perox-

idase isozymes in virus infected plants. Phytopathology 58:301-305.

16. Peirce, L. C. and J. L. Brewbaker. 1973. Application of isozymes analysis in horticultural science. Hortscience 817-22.
17. Pryor, A. J. 1971. Biochemical basis of rust resistance in maize. CSIRO Report 18.
18. Seevers, P. M. J. M. Daly, and F.F. Cathedral. 1971. Isozymes in resistance to wheat stem rust disease. Plant Physiol. 48:353-360.
19. Yu, L.M. and R.E. Hampton. 1964. Biochemical changes in tobacco infected with *Collectotrichum destructivum* II. Peroxidase. Phytochemistry 3: 499-501.