

# The role of peroxidase and polyphenol oxidase isozymes in wheat resistance to *Alternaria triticina*

M. TYAGI\*, ARVIND M. KAYASTHA\*<sup>1</sup> and B. SINHA\*\*

*School of Biotechnology, Faculty of Science\*,  
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences\*\*,  
Banaras Hindu University, Varanasi - 221 005, India*

## Abstract

Polyphenol oxidase activity was higher in resistant wheat cultivar ACC-8226 than in susceptible cultivar MP-845 in control sets and after inoculation of *Alternaria triticina*. However, similar polyphenol oxidase isozyme pattern was found in control and inoculated sets of both the cultivars, but the band intensity was higher after inoculation. Three and four peroxidase isozymes were found in ACC-8226 and MP-845, respectively. An extra peroxidase isozyme band was observed in both the cultivars after inoculation. The results suggest an active role of peroxidase and polyphenol oxidase in defence mechanism of wheat plants.

*Additional key words:* phenolics, susceptibility, *Triticum aestivum*.

## Introduction

Leaf blight has emerged as an economically important disease owing to the heavy losses caused by *Alternaria triticina* in wheat yield. Fungal-induced increase in peroxidase (PO) (EC 1.11.1.7) activity in plants is well documented and has also been reported recently in wheat-*A. triticina* system by us (Tyagi *et al.* 1998). Such increases appear to be caused by the specific isozymes. Role of peroxidase has been implicated in the last enzymatic step of lignin synthesis induced by biotic and abiotic stresses (Vance *et al.* 1976). It also plays an important role in one of the earliest observable aspects of a plants' defence strategy, *i.e.* oxidative burst (Wojtaszek 1997). A positive correlation between polyphenol oxidase (PPO) (EC 1.10.3.2) activity and disease resistance has been suggested in many reports (Retig 1974, Bashan

*et al.* 1987). Rapid changes in its activity after infection of resistant plants indicate their possible participation in the defence mechanism. Differences in the banding pattern and intensity of these enzymes could also be correlated with resistance and susceptibility (Mayer and Harel 1979). However, despite much experimental work directed to establish such a disease-based function for PPO, this idea is still questioned by many workers.

Present investigations are carried out to determine if the trend of changes in the activity of polyphenol oxidase over a period of time could be correlated with resistance and susceptibility to *A. triticina*. Differences in isozyme pattern of PO and PPO in susceptible and resistant reactions have also been evaluated.

## Materials and methods

**Plants:** Two wheat (*Triticum aestivum* L.) cultivars, namely ACC-8226 (resistant) and MP-845 (susceptible)

were selected with contrasting disease scores under field trials conducted earlier at the agricultural farm of Institute

Received 1 September 1999, accepted 22 May 2000.

**Abbreviations:** PO - peroxidase; PPO - polyphenol oxidase.

**Acknowledgements:** B. Sinha (PI) and Arvind M. Kayastha (Co-PI) are grateful to the University Grants Commission (UGC), New Delhi for financial assistance. M. Tyagi is grateful to UGC for a Junior Research Fellowship and Council of Scientific and Industrial Research, New Delhi for a Senior Research Fellowship.

\*<sup>1</sup>Corresponding author, fax: (+91) 542 316693, e-mail: amkayastha@hotmail.com

of Agricultural Sciences, Banaras Hindu University, Varanasi, India. Seeds were germinated in Petri plates at 25 °C. 4-d-old seedlings were transferred to earthen pots containing a sterilised mixture of sandy loam soil and farmyard manure (2:1). Plants were regularly irrigated with distilled water.

Pure culture of *A. triticina* was maintained on Potato Dextrose Agar (PDA) medium. 5-d-old seedlings were sprayed with the aqueous conidial suspension by an atomiser at the end of the light period. Pots were irrigated and covered with plastic bags for 48 h. Suitable controls were maintained. This process was repeated at 21-d-old stage of seedlings. Sampling was done on required days by cutting entire leaf from its base and immediately extracted.

**Tissue extraction and polyphenol oxidase activity assay:** 500 mg leaves were extracted in 0.2 M sodium phosphate buffer, pH 6.0, in a pre-chilled mortar and pestle. Homogenate was filtered through four layered, pre-washed cheesecloth and then centrifuged at 13 200 g at 4 °C for 15 min in *Sigma 2K15* laboratory centrifuge (Osterode, Germany). For routine assay of polyphenol oxidase, method of Retig (1974) was used with suitable modifications. 3 cm<sup>3</sup> reaction mixture containing 50 mM catechol, 20 mM sodium phosphate buffer, pH 6.0, and 0.05 cm<sup>3</sup> of extract was incubated in a water bath at 30 °C for 10 min. Absorbance was read at 420 nm in *Spectronic 1001* spectrophotometer (Rochester, USA).

**Chemicals:** DL-dihydroxyphenylalanine (DL-DOPA), TEMED, and bovine serum albumin (BSA) were purchased from *Sigma Chemical Company*, St. Louis,

USA. Hydrogen peroxide was from *Merck* (Mumbai, India). Catechol was purchased from *Central Drug House* (Mumbai, India). All other chemicals were either from *Glaxo* or *BDH* (Mumbai, India). All solutions were prepared in triple distilled water from an all-quartz assembly.

**Protein estimation** was done by the method of Lowry *et al.* (1951); BSA was used as a standard.

**Peroxidase and polyphenol oxidase isozymes studies:** Isozymes of PO and PPO were studied by native-PAGE at 4 °C. Control and inoculated samples of resistant and susceptible wheat cultivars were run on PAGE simultaneously. Modified method of Seevers *et al.* (1971) was used for peroxidase isozyme studies. The gel was treated with 0.2 M sodium acetate buffer, pH 5.0, containing 5 mM *o*-dianisidine for 1/2 h followed by 13 mM hydrogen peroxide for 15 min. Gel containing reddish brown bands was scanned on *Systonics uP based densitometer 205* (Mumbai, India).

For polyphenol oxidase isozyme studies, gel was soaked in 0.2 M sodium phosphate buffer, pH 6.0, containing 14 mM DL-DOPA for 1 h with intermittent shaking according to the method of Stafford and Galston (1970). Gel with black bands was then subjected to densitometric analysis as described above.

**Statistical analysis:** Statistical analysis was done by *Slide Write Plus* Version 2.0 programme. All experiments were repeated three times with three replicates per experiment.

## Results and discussion

Polyphenol oxidase activity was higher in ACC-8226 than MP-845, both in control and inoculated sets (Table 1). These results are similar to that of phenolic content and peroxidase activity (Tyagi *et al.* 1998) reported previously. A positive correlation was found between control and inoculated seedlings of both the cultivars ( $r_{\text{ACC-8226}} = 0.990$ ,  $r_{\text{MP-845}} = 0.994$ , significant at  $P < 0.001$ ). PPO activity in control sets of MP-845 and ACC-8226 was similar to their corresponding inoculated sets till 25<sup>th</sup> day. After 30<sup>th</sup> day, trend of PPO activity was found to be different in MP-845 and ACC-8226. Inoculated plants of MP-845 showed 11 % higher activity than control in contrast to 20.5 % in ACC-8226. Moreover this higher activity in MP-845 persisted only till 32<sup>nd</sup> day whereas ACC-8226 maintained a higher content in inoculated set till 40<sup>th</sup> day.

The increased PPO activity induced by infection of wheat by *A. triticina* is consistent with the reports of various other workers (Retig 1974, Bashan *et al.* 1987,

Mathur and Vyas 1995). For example, PPO activity has been shown to increase by 300 % in infected water hyacinth leaves compared to that in healthy leaves (Martyn *et al.* 1979). PPO is considered to be the key enzyme associated with the oxidation of plant phenolics. Thus, the increase in its activity may give the plant an enhanced resistance to pathogen invasion by providing increased contents of oxidized quinone derivatives, which impede pathogen growth.

As seen from the results, trend of PPO activity over a period of time is different in susceptible (MP-845) and resistant (ACC-8226) wheat cultivars. Similarly, qualitative difference in PPO activity of susceptible and resistant cultivar has been reported by Ganguly and Dasgupta (1982) in tomato inoculated with root knot nematode. This suggests that these remarkable post-infectional changes in the enzyme be made largely under the influence of pathogen invasion, which subsequently decides the fate of host-parasite relationship.

Table 1. Polyphenol oxidase activity [ $\Delta A_{420} \text{ cm}^{-3}(\text{extract}) \text{ s}^{-1} \times 10^{-3}$ ] in wheat leaves at different seedling stages. Means  $\pm$  SD,  $n = 5$ , n.d. - not detected.

Age [d]	ACC-8226 control	ACC-8226 inoculated	MP-845 control	MP-845 inoculated
5	18.88 $\pm$ 0.66	18.71 $\pm$ 0.55	15.06 $\pm$ 0.46	15.05 $\pm$ 0.50
10	15.66 $\pm$ 0.48	15.65 $\pm$ 0.38	13.71 $\pm$ 0.40	13.75 $\pm$ 0.51
15	13.48 $\pm$ 0.51	13.43 $\pm$ 0.46	13.15 $\pm$ 0.33	13.11 $\pm$ 0.31
20	11.91 $\pm$ 0.35	11.95 $\pm$ 0.31	11.21 $\pm$ 0.41	11.25 $\pm$ 0.46
25	10.01 $\pm$ 0.33	10.00 $\pm$ 0.28	9.88 $\pm$ 0.18	9.85 $\pm$ 0.21
30	8.60 $\pm$ 0.43	10.36 $\pm$ 0.85	8.15 $\pm$ 0.15	9.03 $\pm$ 0.18
31	8.16 $\pm$ 0.50	9.91 $\pm$ 0.36	6.30 $\pm$ 0.16	7.56 $\pm$ 0.25
32	6.75 $\pm$ 0.18	8.36 $\pm$ 0.33	4.76 $\pm$ 0.26	6.46 $\pm$ 0.16
33	4.91 $\pm$ 0.20	6.75 $\pm$ 0.38	3.48 $\pm$ 0.16	3.21 $\pm$ 0.23
34	3.26 $\pm$ 0.13	6.25 $\pm$ 0.28	1.91 $\pm$ 0.21	1.68 $\pm$ 0.31
35	2.16 $\pm$ 0.33	3.40 $\pm$ 0.21	0.93 $\pm$ 0.15	0.76 $\pm$ 0.20
36	1.43 $\pm$ 0.58	1.73 $\pm$ 0.23	0.48 $\pm$ 0.10	0.36 $\pm$ 0.15
37	0.95 $\pm$ 0.16	1.46 $\pm$ 0.15	0.33 $\pm$ 0.15	0.30 $\pm$ 0.11
38	0.30 $\pm$ 0.11	0.91 $\pm$ 0.18	0.21 $\pm$ 0.11	0.16 $\pm$ 0.11
39	0.30 $\pm$ 0.13	0.50 $\pm$ 0.11	0.16 $\pm$ 0.10	n.d.
40	0.30 $\pm$ 0.11	0.33 $\pm$ 0.13	n.d.	n.d.

Increased phenol content and the subsequent increase in PPO activity in diseased plants suggests a specific role for this enzyme during pathogenesis.

**Polyphenol oxidase and peroxidase isozymes:** Isozyme pattern of polyphenol oxidase in 30-d-old samples showed the presence of six isozymes in both ACC-8226 and MP-845 (Fig. 1). Densitometric analysis of gel containing control and inoculated samples showed that isozyme pattern was similar in both the wheat cultivars but there was a difference in band intensity of specific isozymes (Fig. 1). Further, there was no change in the isozyme pattern after inoculation in both the wheat lines though there was an increase in the band intensity.

ACC-8226 and MP-845 showed different isozyme pattern of peroxidase in 30-d-old control as well as inoculated plants. The control sample of MP-845 showed the presence of four bands whereas ACC-8226 showed three bands (Fig. 2). Band intensity was higher in ACC-8226 than that of MP-845 in control as well as inoculated sets. This accords with the enzyme activities in both the wheat lines on 30<sup>th</sup> day. In addition, there was an appearance of one extra band after inoculation in both the wheat lines.

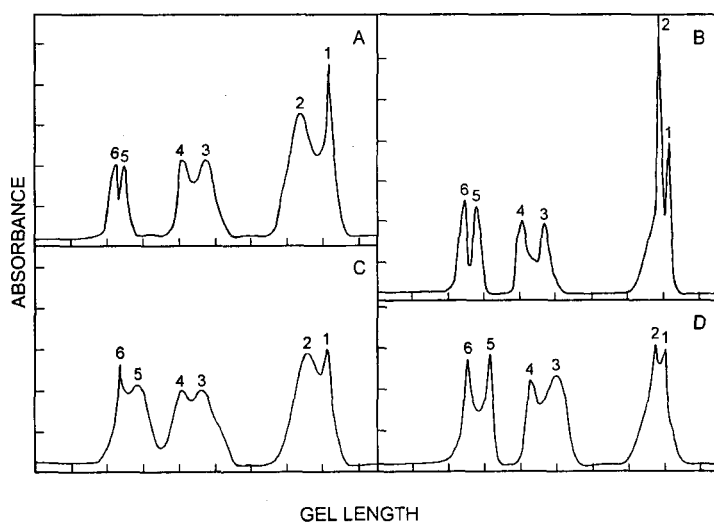


Fig. 1. Polyphenol oxidase isozyme pattern and densitometric scan: A - control ACC-8226, B - inoculated ACC-8226, C - control MP-845, D - inoculated MP-845 (on the left).

Fig. 2. Peroxidase isozyme pattern and densitometric scan: A - control ACC-8226, B - inoculated ACC-8226, C - control MP-845, D - inoculated MP-845 (on the right).

Increased band intensity in both PO and PPO correlates well with the increased content of enzymes due to infection. Appearance of one extra PO band in both the wheat lines accords with suggestions made by several

authors that only some isoenzymic forms of peroxidase are responsible for the increase in its activity in response to infection or elicitation. Specific isozymes are thought to catalyze the polymerization of hydroxycinnamyl

alcohols in the terminal steps of lignin biosynthesis (Gross 1980). Thus, it is reasonable to speculate that higher level or appearance of specific isozymes could lead to a greater accumulation of lignin in the affected

sites. Purification and molecular characterization of infection-related isozymes may provide further insight into the significance of individual isozymes to the defence response in wheat-*A. triticina* system.

## References

- Bashan, Y., Okon, Y., Henis, Y.: Peroxidase, polyphenol oxidase and phenols in relation to resistance against *Pseudomonas syringae* pv. *tomato* in tomato plants. - Can. J. Bot. **65**: 366-372, 1987.
- Ganguly, S., Dasgupta, D.R.: Characterization of polyphenol oxidase from susceptible and resistant varieties of tomato inoculated with the root knot nematode, *Meloidogyne incognita*. - Indian J. Nematol. **12**: 320-329, 1982.
- Gross, G.G.: The biochemistry of lignification. - Adv. Bot. Res. **8**: 25-63, 1980.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the folin-phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.
- Martyn, R.D., Samuelson, D.A., Freeman, T.E.: Ultrastructural localisation of polyphenol oxidase activity in leaves of healthy and diseased water hyacinth. - Phytopathology **69**: 1278-1287, 1979.
- Mathur, N., Vyas, A.: Changes in isozyme patterns of peroxidase and polyphenol oxidase by VAM fungi in roots of *Ziziphus* species. - J. Plant Physiol. **145**: 498-500, 1995.
- Mayer, A.M., Harel, E.: Polyphenol oxidase in plants. - Phytochemistry **18**: 193-215, 1979.
- Retig, N.: Changes in peroxidase and polyphenol oxidase associated with natural and induced resistance of tomato to *Fusarium* wilt. - Physiol. Plant Pathol. **4**: 145-150, 1974.
- SeEVERS, P.M., DALY, J.M., CATEDRAL, F.F.: The role of peroxidase isozymes in resistance to wheat stem rust disease. - Plant Physiol. **48**: 353-360, 1971.
- STAFFORD, H.A., GALSTON, A.W.: Ontogeny and hormonal control of polyphenol oxidase isozymes in tobacco pith. - Plant Physiol. **46**: 763-767, 1970.
- Tyagi, M., Kayastha, A.M., Sinha, B.: The role of phenolics and peroxidase in resistance to *Alternaria triticina* in bread wheat (*Triticum aestivum* L.) - J. Agron. Crop Sci. **181**: 29-34, 1998.
- Vance, C.P., Anderson, J.O., Sherwood, R.T.: Soluble and cell wall peroxidases in reed canarygrass in relation to disease resistance and localized lignin formation. - Plant Physiol. **57**: 920-922, 1976.
- Wojtaszek, P.: Oxidative burst: an early plant response to pathogen infection. - Biochem. J. **322**: 681-692, 1997.