Pak. J. Bot., 46(3): 1101-1107, 2014.

PHENYLALANINE AMMONIA-LYASE (PAL) AND PEROXIDASE ACTIVITY IN BROWN RUST INFECTED TISSUES OF PAKISTANI WHEAT CULTIVARS

ADNAN RIAZ[1]*, ABID RIAZ[1], ATIQ UR REHMAN RATTU[2], MUHAMMAD IBRAHIM TAHIR[1] AND MUHAMMAD AZEEM[3]

[1]*Department of Plant Pathology, Pir Mehr Ali shah Arid Agriculture University Rawalpindi, 46300, Pakistan.*
[2]*Crop Disease Research Programme, Institute of Plant and Environment Protection, National Agricultural Research Center, Islamabad, 44000, Pakistan.*
[3]*Department of Soil Science, Pir Mehr Ali shah Arid Agriculture University Rawalpindi, 46300, Pakistan.*
[*]*Corresponding author e-mail: adnandani22@gmail.com; Tel: 92-347-5202574*

Abstract

Besides other factors resistance and susceptibility is the outcome of biochemical processes such as activities of defense-related enzymes. So in this study, Phenylalanine ammonia-lyase (PAL) and Peroxidase activity of resistant (Inqilab-91) and susceptible (Kirin-95) wheat cultivars were determined through spectrophotometer to address the biochemical aspect related to the disease after 8 hours, 24 hours, 48 hours and 72 hours of leaf rust inoculation. The results have shown that these enzymes were present in both the resistant and susceptible cultivars but the activity was more pronounced in the resistant one. The effect of PAL and peroxidase activity was also investigated among inoculated and uninoculated plants within the same cultivar. The activity of both PAL and peroxidase were more significant in inoculated ones. The results have shown that the after 72 hours of inoculation Inqilab-91 had more PAL activity i.e., 5.47 IU/ml/min than in Kirin-95 i.e., 2.08 IU/ml/min at 270 nm. While peroxidase activity in Inqilab-91 was 6.41 IU/ml/min and in Kirin-95, 3.66 IU/ml/min after 72 hours of inoculation, observed under 470 nm wavelength. Increase in one's activity increases the other enzyme's activity. The activity was more prominent after 72 hours of infection as pathogen had successfully established itself in the host plant tissue. The activities of these enzymes act as plants active defense mechanism against the attack of pathogen.

Introduction

Wheat (*Triticum aestivum* L. em. Thell) is a rich source of carbohydrates, fats, proteins and dietary fibers; production is 681.9 million metric tons in the world (Anon., 2010). Wheat is affected by both abiotic and biotic stresses (Singh *et al*., 2008). The wheat rusts (leaf, stripe and stem rust) are important diseases of wheat, frequency of epidemics and damage caused by rusts varies from country to country (Manninger, 2002). Among these, the Brown rust is the most widespread and causes significant yield and quality losses (Kolmer, 2005; Everts *et al.,* 2001). The development of leaf rust highly dependents upon prevailing environmental conditions which play a key role in disease development in a multi-colinear interaction (Adnan Riaz *et al.*, 2013). In the present circumstances, host resistance is the preferred method to reduce losses from leaf rust (Bolton *et al*., 2008). Rust fungi are highly specific obligate parasites that interact with resistance genes in wheat in a gene-for-gene relationship (Flor, 1956; Kolmer, 2005; Zhao *et al*., 2005). In Pakistan, eleven leaf rust resistance genes are present either singly or in combinations. These genes include *Lr1, Lr3, Lr9, Lr10, Lr13, Lr14a, Lr16, Lr17, Lr23, Lr26, Lr27+31*. However, *Lr*26 is the most frequent while *Lr*9 is the least frequent in the Pakistani cultivar (Rattu *et al*., 2010).

Plants respond to pathogen attack by activating both active and passive plant protective mechanisms (Garcia *et al*., 2003). In passive barriers, antimicrobial compounds are involved while in active defense hypersensitive response, phytoalexins, pathogenesis-related proteins, ion fluxes across the plasma membrane, the production of reactive oxygen species and reactive nitrogen species, lignification and the reinforcement of the cell wall through both the cross-linking of cell wall structural proteins and callose deposition are involved (Almagrol *et al*., 2009). The susceptible or resistance reaction to pathogenic infection is often determined by the efficacy of these defense responses produced by the plants, when attacked by the pathogen.

In fungal plant pathogenesis, attempted penetration may trigger different host cell defense responses during both the pre-penetration and post-penetration stages (Prats *et al*., 2007). The enzymes play a crucial role in plant defense (Lebeda *et al*., 1999). The activity of Phenylalanine Ammonia-Lyase (PAL) and Peroxidase are considered to be important in plant defense mechanism of wheat against rust infections. Changes in PAL and Peroxidase activity occur in wheat at selected stages, which help in the expression of resistance to leaf rust fungus. Southerton & Deverall (1990) reported that PAL and peroxidase activities increased slightly during pre-haustorial stages of development of the leaf rust fungus in each combination of rust strain and wheat line used. Increases in peroxidase activity occurred after the increases in PAL activity and were greater during resistance expression than in leaves infected with virulent strains (Southerton & Deverall, 1990). The objective of the study was to analyse the activity of PAL and Peroxidase in Inqilab-91 and Kirn-95 wheat cultivars against leaf rust through spectrophotometer.

Materials and Methods

Sowing of seedlings: The seeds of Inqilab-91 and Kirn-95 were obtained and grown in trays at glass house in Crop Disease Research Programme (CDRP), Murree station. Each tray containing 120 seedlings was raised and kept in isolation. The seedlings were brought up to two leaf stage when inoculation was carried out. The control was also planted in which mock inoculation was done using air pressure.

Spore viability test: A clean glass slide was taken and drop of water was placed on it. The leaf rust spores were mixed in to a drop of water containing Tween-20. The spores were left for 2 hr and development of germ tube was observed under a compound microscope.

Spore inoculation: Isolate of *Puccinia triticina* was obtained from CDRP, National Agriculture Research Centre, Islamabad. Leaf rust inoculum was prepared by following the method reported by Rattu *et al*., (2010). Leaf rust spores (10 mg) were suspended in petroleum spirit and mineral oil (70: 30). The spore concentration was kept at 6 x 10^5 spores per ml using haemocytometer. Seedlings were inoculated with fresh urediniospores according to the method reported by Southerton & Deverall, (1989). Inoculation was done evenly on both the cultivars. The inoculated plants were kept in dew chamber at 15-20°C for 24 hours in complete darkness. This allowed the fungus to produce germ tube and haustorium to establish itself. After 24 hours, the plants were transferred to glass house and kept in isolation 20-24°C at day and 19-21°C during night.

Preparation of buffers: 0.1 M Sodium borate buffer was prepared and pH value adjusted to 8.8. Similarly, supplemented borate buffer (boric acid 0.421 g, sodium borrate 0.623 g, β-mercaptoethanol 2 µl, EDTA 0.1116 g) was prepared and its pH adjusted to 8.8. 0.1 M phosphate buffer was prepared by dissolving mono-sodium di-hydrogen phosphate (0.0432g), di-sodium mono-hydrogen phosphate (0.2328g), in 100ml of distilled water. Then 10.0µl guaiacol and 10.83µl H_2O_2 added to the solution and pH adjusted to 7.0.

Preparation of cinnamic acid standards: A stock solution of cinnamic acid was prepared by dissolving 2mg in 1000ml of distilled water. Five concentrations of cinnamic acid of 0.2 µl, 0.4 µl, 0.6 µl, 0.8 µl and 1.0µl were prepared from the stock solution. The absorbance was observed at 270 nm wavelength.

Preparation of guaiacol standards: Five concentrations of guaiacol were prepared by dissolving 44.7 µl, 67.11 µl, 89.48 µl, 111.85 µl and 134.22 µl of guaiacol in 100ml distilled water. The absorbance was recorded at 470nm wavelength.

Sampling and processing of samples: The processing of samples was done by following method reported by Southerton & Deverall (1990). The plant samples were taken at 8 hour, 24 hour, 48 hour and 72 hour intervals from both the inoculated and control plants. In each sample, 10 leaves were taken and their tips were cutoff 1cm length. The samples were freezed in liquid nitrogen and the frozen leaves were stored at -80°C until analyzed.

Phenylalanine ammonia-lyase (PAL) assay: PAL activity was determined using the method described by Moerschbacher *et al.,* 1986a and modified by Southerton & Deverall, (1990). The absorbance was measured at 270nm. Cinnamic acid at 0.1-10µg/ml was used as standard.

Peroxidase assay: The peroxidase assay was done by following method reported by Moerschbacher *et al*., (1986b). Absorbance was recorded 470nm for 2 min at 25°C.

The formula used for measuring PAL and Peroxidase activity (Bernfeld, 1959) was

$$\text{Enzyme activity} = \frac{\text{Absorbance of extract solution x Standard factor}}{\text{Time of incubation in minutes}}$$

Statistical analysis: Statistical analysis (ANOVA and LSD) was performed using Statistix 8.1, at $\alpha > 0.05$ following method of Steel & Torrie, (1981).

Results

In the present study, PAL and Peroxidase (POD) activity in wheat varied and also between the inoculated and un-inoculated plants. The level of PAL and peroxidase with the plant variety fluctuated and varied considerably hours after inoculation in both the cultivars. The PAL activity in first 8 hours after inoculation increased due to the development of germ tube, showing the virulence status of pathogen. After 14-20 hours of infection, the formation of haustorium was completed, which exhibited an increase in PAL levels at 24 hours. The peroxidase activity increased after the host cell destruction by the invasion of fungi. At first the level increased more than the PAL but then it was regulated. After 48 hours, the PAL activity was slightly checked in the Inqilab-91 (Fig. 1), similar results were recorded for peroxidase activity (Fig. 2). The intercellular hyphal growth and formation of haustorial mother cells were completed after 48 hours of inoculation. After 48 hours, activity of PAL was increased in a consistent manner. Due to increase in PAL activity there was a reciprocal increase in peroxidase activity as both are involved in a linked reaction.

The difference in activity of PAL between inoculated and un-inoculated plants is quiet visible in Figs. 1, 2, 3 and 4. The same results were observed for the peroxidase in both Inqilab-91 and Kirn-95. It was observed that both the enzymes were present in the cultivars but there activity was nominal. However, when these plants were subjected to inoculum, the response of both cultivars was different. In inqilab-91, there was sudden and abrupt increase in activity of PAL which increased the peroxidase activity and the plants have showed the resistant response against the pathogen. In Kirn-95, both enzymes were present but the activity was less that's why the plants were unable to defend themselves against the pathogen. The activity of both enzymes was more pronounced in the resistant cultivar at all the time intervals. The activity of both PAL and peroxidase was more pronounced in inoculated plants. The results showed that after 72 hours of inoculation, Inqilab-91 had more PAL activity i.e., 5.47 IU/ml/min than Kirin-95 i.e., 2.08 IU/ml/min at 270 nm. When observed under 470 nm wavelength, peroxidase activity in Inqilab-91 was 6.41 IU/ml/min and in Kirin-95 3.66 IU/ml/min after 72 hours of inoculation. Furthermore, enzymes also interacted in a feed forward relationship. So, increase in one's activity increases the other enzyme's activity. The activities of PAL and peroxidase in Inqilab-91 and Kirin-95 were highest after 72 hours of inoculation. Results showed that all treatments were significantly different from each other. The PAL and peroxidase acted as plant active defense mechanism against the attack of pathogen.

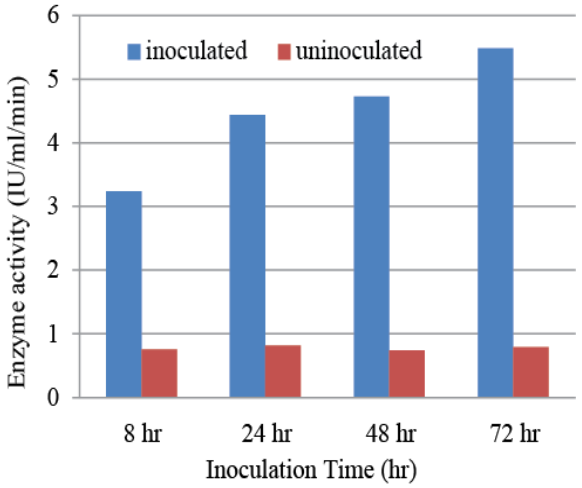

Fig. 1. Phenylalanine Ammonia lyase (PAL) activity in resistant cultivar, Inqilab-91.

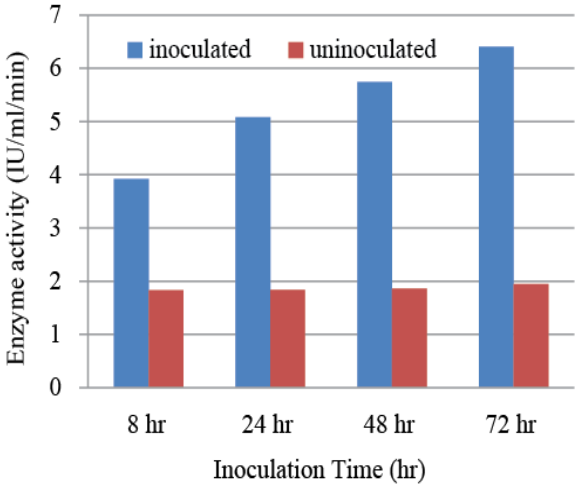

Fig. 2. Peroxidase activity in resistant cultivar, Inqilab-91.

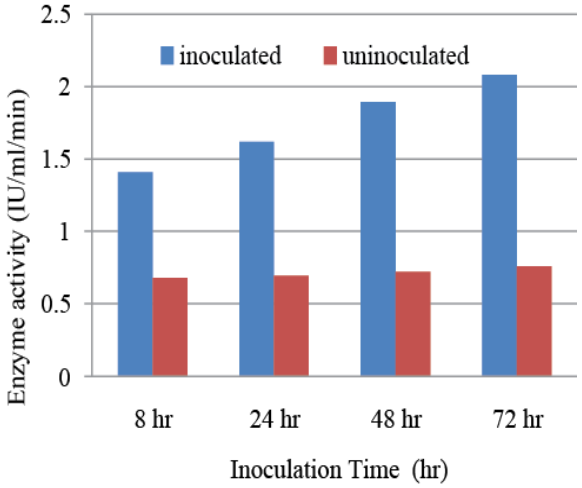

Fig. 3. Phenylalanine Ammonia lyase (PAL) activity in susceptible cultivar, Kirin-95.

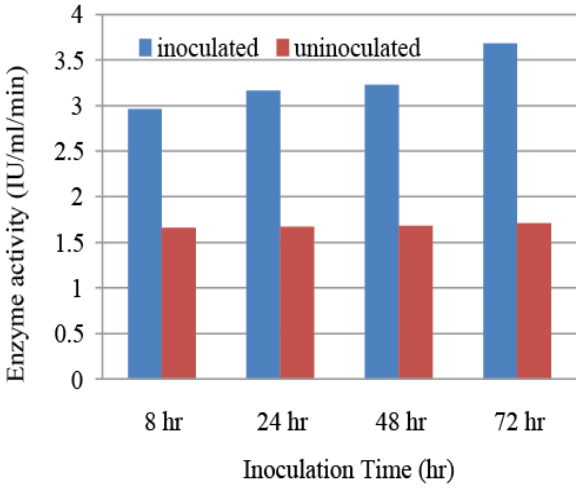

Fig. 4. Peroxidase activity in susceptible cultivar, Kirin-95.

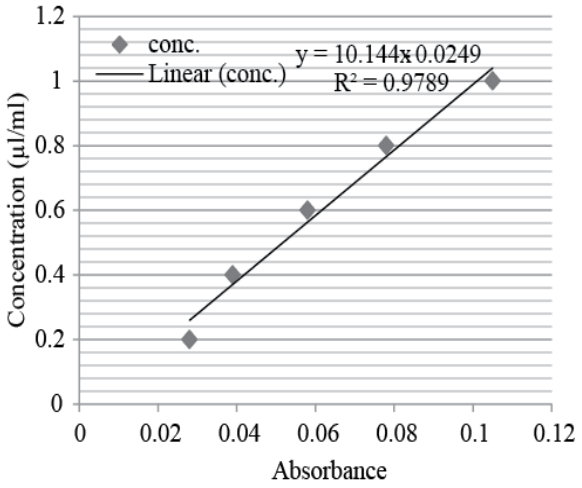

Fig. 5. Absorbance of standards for cinnamic acid used in PAL assay observed at 270 nm wavelength.

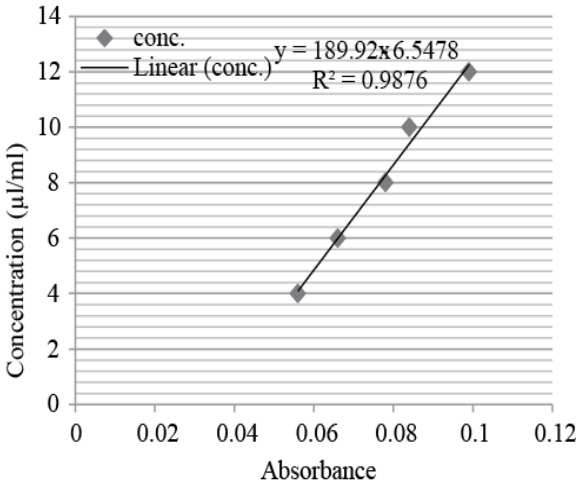

Fig. 6. Absorbance of standards for guaiacol used in peroxidase assay observed at 470 nm wavelength.

The end product of PAL activity was cinnamic acid. So, the standards of cinnamic acid were used and their different concentrations were subjected to absorbance at 270 nm wavelength. As a result a linear pattern was observed (Fig. 5). The standards of guaiacol were used for referring the Peroxidase activity. Its concentrations were subjected to a wavelength of 470 nm and absorbance was recorded. Thus, a linear pattern in absorbance was observed (Fig. 6). All the data obtained during the experiment was subjected to statistical analysis using Statistix 8.1. ANOVA and LSD were applied. The P values and coefficient of variance are represented in Tables 1, 2, 3 and 4.

Discussion

The resistance and susceptibility of crop is a character dependent on enzyme activity controlled by certain genes. Enzymes are produced as a reciprocal action of infection process by the pathogen and play an important role in the active defense mechanism of plant. The wheat seedlings of both cultivars were inoculated with fresh urediniospores of *Puccinia triticina* f.sp. *tritici* to estimate the changes in activity of PAL and POD. These cultivars were selected on the basis of large cultivation area and their reaction to leaf rust inoculum in field conditions in Pakistan as reported by Fayyaz et al., (2008), Rattu *et al*., (2010) and Adnan Riaz *et al.*, (2013). In the present study, the time interval selected for estimation of enzyme activity was very important in the infection process of the pathogen. The level of PAL and POD fluctuated and varied considerably with hours after inoculation in relation to both cultivars (Figs. 1, 2, 3 and 4). This increase in enzyme activity was due to germination of rust spore to form germ tube, appresorium and haustorium. This was in the case of susceptible cultivar Kirn-95, where host and pathogen had shown compatible interaction. While in case of resistant cultivar pathogen fail to develop or develop at a slower rate as in Inqilab-91. It had shown an incompatible host-pathogen interaction. Same results were observed by Southerton & Deverall, (1989) while working on wheat leaf rust on thatcher and dew (varieties).

The PAL and Peroxidase activity varied both in Inqilab-91 and Kirn-95. The plant responded to the pathogen attack and defended itself by active defense mechanism. After first 8 hours of inoculation, seedlings had shown defensive reaction against the pathogen attack. The increase in activity was due to the germination of germ tube from urediniospores that occurred within 4-8 hours after inoculation at 15-20ºC under 100% humidity. Similar results were reported by Zhang & Dickinson, (2001) and Zhang *et al*., (2003). This increase caused the increase in POD activity in turn (Fig. 2). The activity of PAL further increased from 8-24 hours. This resulted in an increased biochemical activity of enzyme in Inqilab-91 as compared to Kirin-95. After 24 hours of inoculation, the pathogen starts forming appresorium, followed by development of haustorium that completes up to 48 hours after inoculation. Similar results were also reported by Hu & Rijkenberg, (1998) and Zhang *et al*., (2003) that pathogen develops haustoria to establish itself in wheat plant host cell wall.

The PAL activity was increased in a consistent manner and achieved highest value after 72 hours of inoculation, in the Inqilab-91 (Fig. 1) that was also visible in POD activity (Fig. 2) because much of the infection processes has completed. These results are in line with those of Stubbs *et al*., (1986) who reported that the latent period for infection is 3-4 days. Maximum sporulation is achieved about four days following initial sporulation at 20°C.The results showed that the activity of PAL and POD was directly proportional. Increase in POD activity occurred after the PAL activity increased in leaves of both Inqilab-91 and Kirn-95 infected with *P. triticina*. The enzyme PAL catalyzed the first reaction from phenylalanine in a pathway leading to substituted cinnamic acid CoA esters. That was used as a reactant to POD synthesis and increased its activity. POD was involved in lignin synthesis from cinnamyl alcohols. A feed-forward relationship between PAL and POD was also reported by Moerschbacher *et al*., (1988). In wheat rust interactions. PAL activity regulated the level of cinnamate derivatives, which was ultimately involved in POD activity during resistance that had been reported by Moerschbacher *et al*., (1988) and Southerton & Deverall, (1990).

The difference in the activation of defense mechanism against *P. triticina* was also observed between inoculated and un-inoculated plants within the same cultivar (Figs. 1, 2, 3 and 4). The activity of both PAL and POD was more pronounced in inoculated ones. Thus confirming that change in activity was a reciprocal reaction to the pathogen attack. The differential behavior of PAL in inoculated and uninoculated wheat plants were found quite similar to that of POD activity. With increase in time after inoculation the enzyme activity in diseased parts significantly increased when compared to that of the uninoculated ones. These results were in accordance with those reported by Southerton & Deverall, 1990 and Anjum *et al*., (2012). The ANOVA and LSD results showed that there was a strong interaction between variety and time after inoculation in relation to activity of both enzymes.

Conclusion

The present study suggested that resistance was a result of involvement of multi component signaling network in plant immunity against leaf rust. Present study reinforced the significant role of Phenylalanine ammonia lyase (PAL) and Peroxidase (POD) in phenylpropanoid (PP) pathway that played an important role in establishing the wheat resistance against leaf rust infection. The infection by the *Pucccinia triticina* effects the enzyme activities, which further increased during expression of resistance. Further studies are needed to investigate the production of enzyme in relation to different inoculum concentrations, crop growth stages and in natural environment. This would lead to apprehension of the molecular mechanism involved in multiorganism and multistress response in natural environment.

Table 1. ANOVA and LSD table for PAL activity in Inqilab-91.

ANOVA Table for inoculated leaves					
Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	7.78676	2.59559	3114705	0.0000
Error	8	0.00001	8.333E-07		
Total	11	7.78677			
Grand Mean 4.4726, CV 0.02					
ANOVA Table for uninouclated leaves					
Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	0.01074	0.00358	3579.00	0.0000
Error	8	0.00001	1.000E-06		
Total	11	0.01075			
Grand Mean 0.7795, CV 0.13					
LSD for inoculated leaves					
Treatment		**Mean***		**Homogeneous Groups**	
72 hr		5.4770		A	
48 hr		4.7320		B	
24 hr		4.4410		C	
8 hr		3.2403		D	
LSD at Alpha = 0.05, Standard Error for Comparison = 7.454E-04 Critical T Value = 2.306, Critical Value for Comparison =1.719E-03					
LSD for uninoculated leaves					
Treatment		**Mean***		**Homogeneous Groups**	
72 hr		0.8210		A	
48 hr		0.7930		B	
24 hr		0.7610		C	
8 hr		0.7430		D	
LSD at alpha = 0.05, Standard Error for Comparison = 8.165E-04 Critical T Value = 2.306, Critical Value for Comparison = 1.883E-03 *All means are significantly different from one another.					

Table 2. ANOVA and LSD table for Peroxidase activity in Inqilab-91.

ANOVA table for inoculated leaves					
Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	9.78382	3.26127	1266.51	0.0000
Error	8	0.02060	0.00258		
Total	11	9.80442			
Grand Mean 5.3025, CV 0.96					
ANOVA table for uninoculated leaves					
Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	0.05220	0.01740	174.00	0.0000
Error	8	0.00080	0.00010		
Total	11	0.05300			
Grand Mean 1.8600, CV 0.54					
LSD for inoculated leaves					
Treatment		**Mean***		**Homogeneous Groups**	
72 hr		6.4100		A	
48 hr		5.7400		B	
24 hr		5.1000		C	
8 hr		3.9600		D	
LSD at alpha = 0.05, Standard error for comparison = 0.0414 Critical T Value = 2.306, Critical value for comparison = 0.0955					
LSD for uninoculated leaves					
Treatment		**Mean***		**Homogeneous Groups**	
72 hr		1.9600		A	
48 hr		1.8700		B	
24 hr		1.8300		C	
8 hr		1.7800		D	
LSD at alpha= 0.05, Standard error for comparison = 8.165E-03 Critical T Value = 2.306, Critical value for comparison = 0.0188 *All means are significantly different from one another					

Table 3. ANOVA and LSD table for PAL activity in Kirin-95.

ANOVA table for inoculated leaves

Source	DF	SS	MS	F	P
Treatment	3	0.78964	0.26321	150407	0.0000
Error	8	0.00001	1.750E-06		
Total	11	0.78965			

Grand Mean 1.7492 CV 0.08

ANOVA table for uninoculated leaves

Source	DF	SS	MS	F	P
Treatment	3	0.01408	0.00469	4599.04	0.0000
Error	8	0.00001	1.020E-06		
Total	11	0.01409			

Grand Mean 0.7155, CV 0.14

LSD for inoculated leaves

Treatment	Mean*	Homogeneous Groups
72 hr	2.0790	A
48 hr	1.8920	B
24 hr	1.6190	C
8 hr	1.4070	D

LSD at alpha =0.05, Standard error for comparison = 1.080E-03
Critical T Value = 2.306, Critical value for comparison = 2.491E-03

LSD for uninoculated leaves

Treatment	Mean*	Homogeneous Groups
72 hr	0.7692	A
48 hr	0.7200	B
24 hr	0.6940	C
8 hr	0.6790	D

LSD at alpha = 0.05, Standard error for comparison = 8.250E-04
Critical T Value = 2.306, Critical value for comparison = 1.902E-03
*All means are significantly different from one another

Table 4. ANOVA and LSD table for Peroxidase activity in Kirin-95.

ANOVA table for inoculated leaves

Source	DF	SS	MS	F	P
Treatment	3	0.84189	0.28063	10898.2	0.0000
Error	8	0.00021	0.00003		
Total	11	0.84209			

Grand Mean 3.2477, CV 0.16

ANOVA table for uninoculated leaves

Source	DF	SS	MS	F	P
Treatment	3	0.03855	0.01285	499.03	0.0000
Error	8	0.00021	0.00003		
Total	11	0.03876			

Grand Mean 1.7230 CV 0.29

LSD for inoculated leaves

Treatment	Mean*	Homogeneous Groups
72 hr	3.6580	A
48 hr	3.2610	B
24 hr	3.1420	C
8 hr	2.9300	D

LSD at alpha = 0.05, Standard error for comparison = 1.080E-03
Critical T Value = 2.306, Critical value for comparison = 2.491E-03

LSD for uninoculated leaves

Treatment	Mean*	Homogeneous Groups
72 hr	0.7692	A
48 hr	0.7200	B
24 hr	0.6940	C
8 hr	0.6790	D

LSD at alpha=0.05, Standard error for comparison = 4.143E-03
Critical T Value = 2.306, Critical value for comparison = 9.554E-03
*All means are significantly different from one another

References

Adnan Riaz, A. Riaz, A.R. Rattu, M.I. Tahir and M. Azeem. 2013. Impact of epidemiological factors on development of *Puccinia triticina* sp*. Tritici* on wheat in Pakistan. *J. Biol. Agri. Health.,* 3(19): 50-60.

Almagro, L, L.V. Gomez Ros, S. Belchi-Navarro, R. Bru, A. Ros Barcelo and M.A. Pedren. 2009. Class III peroxidases in plant defence reactions. *J. of Exp. Bot*., 60 (2): 377-390.

Anjum, T., S. Fatima and S. Amjad. 2012. Physiological changes in wheat during development of loose smut. *Trop. Pl. Pathol*., 37(2): 102-107.

Anonymous. 2010. The State of Food and Agriculture-2010. Food and Agriculture Organization of the United Nations. Rome. http://faostat.fao.org/site/444/DesktopDefault.aspx?PageID=444#ancor

Bernfeld, P. 1959. Amylases, a and 1B. *Methods Enzymol*., 1: 149-158.

Bolton, M.D., J.A. Kolmer and D.F. Garvin. 2008. Pathogen profile: Wheat leaf rust caused by *Puccinia triticina*. *Molecular Plant Pathol*., 9(5): 563-575.

Everts, K.L., S. Leath and P.L. Finney. 2001. Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat. *Plant Dis*., 85: 423-429.

Flor, H.H. 1956. The complementary genetic systems in flax and flax rust. *Adv. Genet*., 8: 29-54.

Hu, G. and F.H.J. Rijkenberg. 1998. Scanning electron microscopy of early infection structure formation by *Puccinia recondita* f. sp. tritici on and in susceptible and resistant wheat lines. *Mycol. Res*., 102: 391-399.

Kolmer, J.A. 2005. Tracking wheat rust on a continental scale. *Curr. Opin. Plant Biol*., 8: 441-449.

Lebeda, A., D. Jancov and L. Luhova. 1999. Enzymes in fungal plant pathogenesis. *Phyton (Horn, Austria)*, 39(3): 51-56.

Garcia, P.C., R.M. Rivero, J.M. Ruiz and L. Romero. 2003. The role of fungicides in the physiology of higher plants: Implications for defense responses. *The Bot. Rev*., 69(2): 162-172.

Manninger, K. 2002. Effective resistance genes as sources of resistance against Hungarian wheat rusts. *Czech J. Genet. Plant Breed*., 38(3-4): 153-154.

Moerschbacher, B., K.H. Kogel, U. Noll and H.J. Reisener. 1986a. An elicitor of the hypersensitive lignifications response in wheat, leaves isolated from the rust fungus *Puccinia graminis* f. sp. *tritici* I. Partial purification and characterization. *Zeits. Fur Naturfor*., 41(c): 830-838.

Moerschbacher, B., B. Heck, K. H. Kogel, O. Obst and H.J. Reisener. 1986b. An elicitor of the hypersensitive lignifications response in wheat, leaves isolated from the rust fungus *Puccinia graminis* f. sp. *tritici* II. Induction of enzymes correlated with biosynthesis of lignin. *Zeits. Fur Naturfor*., 41(c): 839-844.

Moerschbacher, B.M., U.M. Noll, B.E. Flott and H.J. Reisener. 1988. Lignin biosynthetic enzymes in stem rust infected, resistant and susceptible near-isogenic wheat lines. *Physiol. Mol. Plant Pathol*., 33: 33-46.

Northcote, D.H. 1985. Control of cell wall formation during growth. In: *Biochemistry of plant cell walls* (Ed.): C.T. Brett and J.R. Hillman, 177-197. Cambridge University Press, Cambridge.

Prats, E., F. Martínez, M.M. Rojas-Molina and D. Rubiales. 2007. Differential effects of phenylalanine ammonia lyase, cinnamyl alcohol dehydrogenase and energetic metabolism inhibition on resistance of appropriate host and non-host cereal–rust interactions. *Phytopath.,* 97(12): 1578-1583.

Rattu, A.R., I. Ahmad, R.P. Singh, M. Fayyaz, J.I. Mirza, K.A. Khanzada and M.I. Haque. 2010. Resistance to *Puccinia triticinia* in some Pakistani wheats. *Pak. J. Bot*., 42(4): 2719-2735.

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, P. Njau, R. Wanyera, S.A. Herrera-Foessel and R.W. Ward. 2008. Will stem rust destroy the world's wheat crop? *Adv. in Agron*., 98: 272-306.

Southerton, S.G. and B.J. Deverall. 1989. Histological studies of the expression of the Lr9, Lr20 and Lr28 alleles for resistance to leaf rust in wheat. *Plant Pathol*., 38: 190-199.

Southerton, S.G. and B.J. Deverall. 1990. Changes in phenylalanine ammonia lyase and peroxidase activities in wheat cultivars expressing resistance to the leaf- rust fungus. *Plant Pathol*., 39: 223-230.

Steel, R. G. D. and J. H. Torrie. 1981. Principles and procedures of statistics. New York USA. McGraw Hill.

Stubbs, R.W., J.M. Prescott, E.E. Saari and H.J. Dubin. 1986. *Cereal disease methodology manual*. 46. Mexico, DF, CIMMYT.

Zhang, L., H. Meakin and M. Dickinson. 2003. Isolation of genes expressed during compatible interactions between leaf rust (*Puccinia triticina*) and wheat using cDNA-AFLP. *Mol. Pl. Pathol*., 4(6): 469-477.

Zhao, J., L.C. Davis and R. Verpoorte. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotech. Adv*., 23: 283-333.

(Received for publication 7 November 2012)