Effect of the Application of Fluorescent Pseudomonads, Salicylic Acid, Navagavya and Bipolaris and Bipolaris oryzae on Changes in the Enzymatic Contents of Rice Var. ADT 36

B. Vaishali and V. Jaiganesh

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 04

Res Jr of Agril Sci (2021) 12: 1173–1176

CARAS

Res. Jr. of Agril. Sci. (July-Aug 2021) 12(4): 1173–1176
www.rjas.org

Full Length Research Article

Effect of the Application of Fluorescent Pseudomonads, Salicylic Acid, Navagavya and Bipolaris and *Bipolaris oryzae* on Changes in the Enzymatic Contents of Rice Var. ADT 36

B. Vaishali*[1] and V. Jaiganesh[2]

Received: 02 May 2021 | Revised accepted: 18 Jun 2021 | Published online: 12 July 2021

A B S T R A C T

The pot culture studies were undertaken to investigate the changes in the enzymatic contents in rice var. ADT 36 after the application of bioprotectant fluorescent pseudomonads, Resistance inducing chemical Salicylic acid, Organic immunizer Navagavya and inoculation of brown spot pathogen *Bipolaris oryzae*. Among the various treatments undertaken, seed treatment of fluorescent Pseudomonads with sprouted rice seeds at the rate of 10 g/kg of seeds, along with foliar application of Salicylic acid 50 ppm at 15 DAT and Navagavya (5%) at 30 DAT recorded the minimum disease incidence, increased biometrics and yield parameters. Results also revealed that the same treatment increased the activity of β-1,3 glucanase, Peroxidase (PO), Polyphenol oxidase (PPO), Phenylalanine ammonia lyase (PAL). The activity of β-1,3 glucanase, PO, PPO, PAL increased upto 14 days of sampling and then decreased in the test plants.

***Key words*:** *Bipolaris oryzae,* β-1,3 glucanase, Peroxidase, Polyphenol oxidase, Phenylalanine ammonia lyase, Salicylic acid

Rice is an important staple crop and it has shaped the culture, diets and economic of thousands of millions of people and for more than half of the humanity “Rice is life”. The rice plant is a member of *Poaceae* (*Graminae*) family. It has been cultivated in Asia for several thousand years. About fifty per cent of the crop is grown and consumed in Asia and it is the net exporter of rice to the rest of the world. India stands second after China which has developed hybrid rice technology on a commercial scale. India is the home to paddy and the largest paddy growing and second larger paddy producing and consuming country. Rice crop is being affected by several diseases, insects and physiological disorders which accounts several million yield losses. Among them Brown leaf spot disease is the most serious disease of rice [1]. It caused Bengal Famine in 1942, with yield loss of 50-90%, which resulted in death of 2 million people due to starvation. The pathogen can infect both seedlings and mature plants with the coleoptile, leaves, leaf sheath, panicle branches, glumes, and spikelets [2].

Peroxidases are oxido-reductive enzymes that participate in the wall-building processes such as oxidation of Phenols, suberization and lignification of host plant cells during the defence reaction against the pathogenic agents. Accumulation of lignin and phenolic compounds have been correlated with disease resistance in a number of plant–pathogen interactions [3].

Polyphenol oxidases are involved in the oxidation of polyphenols into quinones (antimicrobial compounds) and lignification of plant cells during the microbial invasion. Phenol oxidases generally catalyze the oxidation of phenolic compounds to quinones using molecular oxygen as an electron acceptor [4-5]. A number of studies have indicated that phenol-oxidizing enzymes such as PPO may participate in defense reactions and hypersensitivity in resistant plants to viruses, bacteria and fungi. These enzymes are also involved in reactions culminating in wound-induced tissue browning and erecting physical barriers against parasites [6-7].

Phenylalanine ammonia lyase (PAL) is the first enzyme of phenylpropanoid pathway that catalyzes the conversion of L-phenylalanine to trans-cinnamic acid is the key enzyme in the synthesis of several defense-related secondary compounds like phenols and lignins [8]. The presence of phenolic compounds in plants and their synthesis in response to infection is associated with disease resistance.

Plant β-1,3-glucanases are directly involved in defence by hydrolyzing the cell walls of fungal pathogens most commonly in combination with chitinase isozymes. *In*

* B. Vaishali
✉ vaishalisweety704@gmail.com

[1-2] Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India

vitro analysis has shown that β -1,3-glucanases act directly on fungal pathogens by degrading β -1,3/ 1,6-glucans and chitinases act by attacking the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitins in fungal cell wall [9]. Degradation of the hyphal cell wall of the pathogenic fungi not only renders it susceptible to cell lysis but also for the actions of other components of fungal defence responses [10].

MATERIALS AND METHODS

Method of sampling

Samples of plant materials from each treatment were taken at 0, 7, 14 and 21 days after inoculation both in healthy and inoculated plants for estimating the changes in the biochemical constituents viz., starch, ortho dihydroxy phenols, total phenols, amino nitrogen, protein and enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, β 1,3 glucanase and ascorbic acid oxidase.

Enzyme extraction

One g of the leaf material cut into small bits was crushed in chilled 0.1 M sodium phosphate buffer at pH 7.1. The volume was made upto 5 ml with the buffer, centrifuged at 2,100 rpm for 30 min and the supernatant was used as the enzyme source and all the assays *viz*., polyphenol oxidase, peroxidase, phenylalanine ammonia lyase and ascorbic acid oxidase were performed in a UV Spectrophotometer at 28±2°C [11].

Enzymes	References
Polyphenol oxidase (PPO)	[12]
Peroxidase (PO)	[13]
Phenylalanine ammonia lyase (PAL)	[14]

Enzyme activity of PO and PPO was expressed in terms changes in absorbance/ minute /mg of protein. The activity of PAL was expressed as nmol transcinnamic acid min^{-1} mg $protein^{-1}$.

β-1, 3-glucanase (Pan *et al*. [15])

Crude enzyme extract of 62.5 µl was added to 62.5 µl of 4 per cent laminar in and incubated at 40°C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid (DNS) and heated for 5 min on boiling water bath (DNS prepared by adding 300 ml of 4.5 per cent NaOH to 880 ml containing 8.8 g of DNS and 22.5 g potassium sodium tartarate). The resulting-coloured solutions were diluted with distilled water, vortexed and the absorbance was read at 500 µm. The crude extract preparation mixed with laminar in at zero-time incubation served as blank. The enzyme activity was expressed as µg equivalents of glucose min^{-1} g fresh $weight^{-1}$.

Treatment schedule

T_1: SA_1 (15 DAT)
T_2: SA_2 (30 DAT)
T_3: NG_1 (15 DAT)
T_4: NG_2 (30 DAT)
T_5: $T_1 + T_2$
T_6: $T_3 + T_4$
T_7: $T_3 + T_2$
T_8: $T_1 + T_4$
T_9: Mancozeb @ 0.2% (comparison)
T_{10}: Control

RESULTS AND DISCUSSION

The activity of peroxidase was found more in T_8 (FP + SA_1 + NG_2). The minimum peroxidase was recorded in control treatment (29.67 mol transcinnamic acid min^{-1} mg protein $^{-1}$). Generally, the treatments with SA showed increased peroxidase activity when compared to other treatments and control (Table 1). The activity of peroxidase increased up to 14th day of sampling and then decreased in all the treatments. Peroxidases participate into cell-wall reinforcement. They are involved in the final steps of lignin biosynthesis and in the cross-linking of cell wall proteins [16]. They are usually related to local defense responses but they have been associated with systemic resistance in several plant species viz., cucumber [17], potato [18], rice [19] and tobacco [20].

Table 1 Changes in Peroxidase content of rice var. ADT 36 as influenced by application of FP, SA, NG *and B. oryzae inoculation*

Treatments	Peroxidase (changes in absorbance /min/mg of protein)			
	0 (days)	7 (days)	14 (days)	21 (days)
T_1: SA_1	0.79	7.42	83.60	57.97
T_2: SA_2	0.69	7.29	82.56	55.97
T_3: NG_1	0.67	7.12	81.94	55.34
T_4: NG_2	0.41	5.92	73.68	35.87
T_5: $T_1 + T_2$	1.38	19.54	104.38	68.23
T_6: $T_3 + T_4$	1.03	14.67	98.23	64.89
T_7: $T_3 + T_2$	1.23	18.32	103.46	65.08
T_8: $T_1 + T_4$	1.69	20.53	107.85	69.64
T_9: Mancozeb @ 0.2%	0.87	7.86	89.84	63.67
T_{10}: Control	0.35	3.95	55.43	29.67

The results revealed increased activity of polyphenol oxidase due to treatment with combined application of Bioprotectant resistance inducing chemical, *Navagavya* and pathogen alone inoculated control (Table 2). Among the treatments, T_8 (FP + SA_1 + NG_2) recorded the maximum PPO activity (21.74 changes in absorbance /min/mg of protein) on 21st day after inoculation which was followed by T_5 treatment (SA_1 + SA_2). PPO is a copper containing enzyme, oxidizing phenolics to highly toxic quinines and involved in the terminal oxidation of diseased plant tissues which was attributed for its role in disease resistance [21]. PPOs play a role in plant immunity, and emerging evidence suggested that PPOs might also be involved in other physiological processes [22].

Table 2 Changes in polyphenol oxidase content of rice var. ADT 36 as influenced by application of FP, SA, NG and *B. oryzae* inoculation

Treatments	Polyphenol oxidase (changes in absorbance /min/mg of protein)			
	0 (days)	7 (days)	14 (days)	21 (days)
T_1: SA_1	0.68	2.32	20.52	20.63
T_2: SA_2	0.59	2.10	20.37	20.62
T_3: NG_1	0.47	1.99	19.54	20.53
T_4: NG_2	0.44	1.95	18.27	16.43
T_5: $T_1 + T_2$	0.95	2.98	21.65	21.71
T_6: $T_3 + T_4$	0.87	2.67	21.41	21.64
T_7: $T_3 + T_2$	0.94	2.69	21.53	20.65
T_8: $T_1 + T_4$	1.29	3.67	21.84	21.74
T_9: Mancozeb @ 0.2%	0.75	2.38	20.74	21.59
T_{10}: Control	2.73	4.17	18.12	14.68

The data depicted in (Table 3) showed significant increase in the activity of Phenylalanine ammonia lyase (PAL) in rice plants treated with FP, SA and NG. The induction of Phenylalanine ammonia lyase (PAL) reached the maximum on the 14th day and thereafter gradual decline was observed. Generally, the treatments with SA showed increased Phenylalanine ammonia lyase activity when compared to other treatments and control. Among the different treatments, T_8 recorded the maximum activity with 74.98 n mol transcinnamic acid min^{-1} mg protein^{-1} of PAL on 21st day of sampling. PAL is also a key enzyme for biosynthesis of salicylic acid (SA), a plant hormone required to initiate systemic acquired resistance (SAR) in plants [23]. In rice, Phenylalanine ammonia lyase genes are activated during both Pattern Triggered Immunity (PTI), Effector Triggered Immunity (ETI), PAL mRNA accumulation and enzyme activity is induced by diverse types of rice pathogens [24]. Current research findings are consistent with previous results from plant pathosystems that implicate PAL in induced plant defense responses [25-26].

Table 3 Changes in phenylalanine ammonia lyase content of rice var. ADT 36 as influenced by application of FP, SA, NG and *B. oryzae* inoculation

Treatments	Phenylalanine ammonia lyase (n mol transcinnamic acid min^{-1} mg protein^{-1})			
	0 (days)	7 (days)	14 (days)	21 (days)
T_1: SA_1	20.54	99.87	110.65	71.23
T_2: SA_2	20.23	99.54	110.65	70.67
T_3: NG_1	20.12	98.98	109.11	70.52
T_4: NG_2	18.76	89.04	94.12	65.32
T_5: $T_1 + T_2$	22.75	101.64	120.87	73.54
T_6: $T_3 + T_4$	22.38	101.12	114.76	72.95
T_7: $T_3 + T_2$	22.58	101.34	118.54	72.98
T_8: $T_1 + T_4$	23.32	102.98	122.98	74.98
T_9: Mancozeb @ 0.2%	22.15	100.65	112.33	71.32
T_{10}: Control	13.51	82.98	87.06	54.23

β-1,3-glucanase activity was observed in the leaf samples of rice at different day intervals. Among the various treatment, the plants treated with fluorescent Pseudomonads (seed treatment) @ 10 g / kg, SA and NG (foliar spraying at 30 and 45 DAT) by challenge inoculated with *B. oryzae* (T_8) recorded a maximum induction of β -1,3-glucanase activity 127.9 µg of Glucose released/ min/g of fresh tissue on 7th day after pathogen inoculation. The enzyme activity significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Table 4). SA may act as an endogenous signal responsible for activating particular components of resistance to *Phytophthora capsici* and the induction of pathogenesis-related proteins such as β-1,3-glucanase and chitinase [27]. The exogenous application of salicylic acid enhanced the activities of antioxidant enzymes ascorbate peroxidase (APX) and SOD with a concomitant decline in the activity of CAT in maize plants [28].

Table 4 Changes in Beta 1,3 glucanase content of rice var. ADT 36 as influenced by application of FP, SA, NG and *B. oryzae* inoculation

Treatments	Glucose min^{-1} g fresh weight^{-1}			
	0 (days)	7 (days)	14 (days)	21 (days)
T_1: SA_1	22.8	116.9	108.5	82.8
T_2: SA_2	22.2	115.5	107.4	81.7
T_3: NG_1	20.7	114.8	106.6	80.3
T_4: NG_2	20.2	113.7	105.0	79.6
T_5: $T_1 + T_2$	25.2	124.2	115.2	92.1
T_6: $T_3 + T_4$	23.8	121.3	110.3	85.9
T_7: $T_3 + T_2$	24.4	123.5	112.8	89.2
T_8: $T_1 + T_4$	27.5	127.9	120.7	95.2
T_9: Mancozeb @ 0.2%	23.5	118.4	110.0	85.4
T_{10}: Control	14.9	91.5	77.6	59.3

CONCLUSION

Seed treatment of fluorescent pseudomonads with sprouted rice seeds at the rate of 10 g/kg of seeds, along with foliar application of salicylic acid 50 ppm and Navagavya (5%) recorded the minimum disease incidence, increased biometrics and yield parameters also increased the activity of β-1,3 glucanase, PO, PPO, PAL. The activity of β-1,3 glucanase, PO, PPO, PAL increased upto 14 days of sampling and then decreased in the test plants.

LITERATURE CITED

1. Arshad HMI, Khan JA, Jamil FF. 2008. Screening of rice germplasm against blast and brown spot diseases. *Pakistan Journal of Phytopathology* 20(1): 52-57.
2. Webster RK. 1992. *Compendium of Rice Diseases*. The American Phytopathological Society.
3. Chittoor JM, Leach JE, White FF. 1999. Induction of peroxidase during defense against pathogens. *In*: (Eds) Datta SK, Muthukrishnan S. Pathogenesis: Related Proteins in Plants, CRC Press, Boca Raton, FL. pp 291.
4. Mayer AM. 1987. Polyphenol oxidase in plants-recent progress. *Phytochemistry* 26: 11-20.
5. Sommer A, Neeman E, Steffens JC, Mayer AM, Harel E. 1994. Import, targeting and processing of a plant polyphenol oxidase. *Plant Physiology* 105(4): 1301-1311.
6. Goodman RN, Kiraly Z, Wood KR. 1986. *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press, USA. pp 433.
7. Leina MJ, Tan TK, Wong SM. 1996. Resistance of *Hibiscus esculentus* L. and *Vigna sinensis* (L.) Endl. to *Pseudocercospora* and plant peroxidase activity in relation to infection. *Ann. Appl. Biology* 129(2): 197-206.
8. Hemm MR, Rider SD, Ogas J, Murry DJ, Chapple C. 2004. Light induces phenylpropanoid metabolism in *Arabidopsis* roots. *Plant Journal* 38: 765-778.
9. Balasubramanian V, Vashisht D, Cletus J, Sakthivel N. 2012. Plant β-1, 3-glucanases: their biological functions and transgenic expression against phytopathogenic fungi. *Biotechnology Letters* 34(11): 1983-1990.
10. Mohammadi M, Karr AL. 2002. β-1, 3-glucanase and chitinase activities in soybean root nodules. *Journal of Plant Physiology* 159(3): 245-256.
11. Sridhar R, Chandramohan D, Mahadevan A. 1969. The role of parasite and its metabolism in triggering host physiology. *Journal of Phytopathology* 69: 21-27.
12. Matta A, Dimond AE. 1963. Symptoms of *Fusarium* wilt in relation to quantity of fungus and enzyme activity in tomato stems. *Phytopathology* 53: 574-575.
13. Hampton RE. 1963. Activity of some soluble oxidases in carrot slices infected with *Thielaviopsis basicola*. *Phytopathology* 53: 497-499.
14. Southern SG, Deverall BJ. 1990. Changes in phenylalanine ammonia lyase and peroxidase activities in wheat cultivars expressing resistance to the leaf rust fungus. *Plant Pathology* 39: 223-230.
15. Pan SQ, Ye XS, Kuc J. 1991. A technique for detection of chitinase, beta-1, 3- glucanase, and protein patterns after a single separation using polyacrylamide gel electrophoresis or isoelectro focusing. *Phytopathology* 81(9): 970-974.
16. Kombrink E, Somssich IE. 1995. Defense responses of plants to pathogens. *In*: (Eds) Andrews JH and Tommerup IC. Advances in Botanical Research (Advances in Plant Pathology). Academic Press, London. 21: 1-34.
17. Hammerschmidt R, Nuckles EM, Kuć J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology* 20(1): 73-82.
18. Chai HB, Doke N. 1987. Systemic activation of O-2 generating reaction, superoxide dismutase, and peroxidase in potato plants in relation to induction of systemic resistance to *Phytophthora infestans*. *Japanese Journal of Phytopathology* 53(5): 585-590.
19. Dorneles KR, Refatti JP, Pazdiora PC, de Avila LA, Deuner S, Dallagnol LJ. 2020. Biochemical defenses of rice against *B. oryzae* increase with high atmospheric concentration of CO2. *Phy. and Mol. Plant Pathology* 110: 101-484.
20. Ye XS, Pan SQ, Kuc J. 1990. Association of pathogenesis-related proteins and activities of peroxidase, P-1,3-glucanase and chitinase with systemic induced resistance to blue mould of tobacco but not to systemic tobacco mosaic virus. *Physiological and Molecular Plant Pathology* 36(6): 523-531.
21. Kosuge T. 1969. The role of phenolics in host response to infection. *Annu. Rev. Phytopathology* 7: 195-222.
22. Tonnessen BW, Manosalva P, Lang JM, Baraoidan M, Bordeos A, Mauleon R, Leach JE. 2015. Rice phenylalanine ammonia-lyase gene OsPAL4 is associated with broad spectrum disease resistance. *Plant Molecular Biology* 87(3): 273-286.
23. Malamy J, Carr JP, Klessig DF, Raskin I. 2014. Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 80(250): 1002-1004.
24. Cui Y, Magill J, Frederiksen R, Magill C. 1996. Chalcone synthase and phenylalanine ammonia-lyase mRNA levels following exposure of sorghum seedlings to three fungal pathogens. *Physiol. Mol. Plant Pathology* 49: 187-199.
25. Riaz A, Riaz A, Rattu AUR. 2014. Phenylalanine ammonia-lyase (PAL) and peroxidase activity in brown rust infected tissues of Pakistani wheat cultivars. *Pakistan Journal of Botany* 46: 1101-1107.
26. Taranto F, Pasqualone A, Mangini G, Tripodi P, Miazzi MM, Pavan S, Montemurro C. 2017. Polyphenol oxidases in crops: biochemical, physiological and genetic aspects. *International Journal of Molecular Sciences* 18(2): 377.
27. Hwang BK, Sunwoo JY, Kim YJ, Kim BS. 1997. Accumulation of β-1, 3-glucanase and chitinase isoforms, and salicylic acid in the DL-β-amino-n-butyric acid-induced resistance response of pepper stems to *Phytophthora capsici*. *Physiological and Molecular Plant Pathology* 51(5): 305-322.
28. Krantev A, Yordanova R, Janda T, Szalai G, Popova L. 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *Jr. Plant Physiology* 165: 920-931.