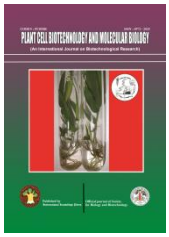

Plant Cell Biotechnology and Molecular Biology

Volume 25, Issue 9-10, Page 47-56, 2024; Article no.PCBMB.12292
ISSN: 0972-2025

Comparative Analysis of Peroxidase and Catalase Isoenzymes via Native-PAGE Electrophoresis and SDS-PAGE Profiling of Leaf Proteins in Rice Varieties under Infection Stress

Vinay Kumar Yadav [a++], N.A. Khan [b#], Bavita Yadav [c†] and Madhurjit Singh Rathore [d*]

[a] CSIR, CIMAP, Lucknow (Uttar Pradesh), India.
[b] N.D. University of Agriculture & AMP; Technology, Kumarganj, Ayodhya (Uttar Pradesh), India.
[c] ICAR-NBAIM, Mau, (Uttar Pradesh), India.
[d] Department of Biotechnology, Dr. YS Parmar University of Horticulture and Forestry, Nauni-Solan (Himachal Pradesh) 173 230, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors VKY and NAK were involved in designing and execution of screening for resistance experiments and contributed in morphological analysis. Author MSR contributed in writing and editing of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/pcbmb/2024/v25i9-108804

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12292

Original Research Article

Received: 25/05/2024
Accepted: 26/07/2024
Published: 05/08/2024

[++] Project Associate;
[#] Associate Professor;
[†] Senior Research Fellow;
**Corresponding author: E-mail: madhur1691946@gmail.com;*

Cite as: *Yadav, Vinay Kumar, N.A. Khan, Bavita Yadav, and Madhurjit Singh Rathore. 2024. "Comparative Analysis of Peroxidase and Catalase Isoenzymes via Native-PAGE Electrophoresis and SDS-PAGE Profiling of Leaf Proteins in Rice Varieties under Infection Stress". PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY 25 (9-10):47-56. https://doi.org/10.56557/pcbmb/2024/v25i9-108804.*

ABSTRACT

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population. However, rice productivity is significantly hindered by various diseases, including sheath blight caused by Rhizoctonia solani. This study aims to evaluate the resistance of different rice varieties and wild rice accessions to sheath blight and to analyze the biochemical responses, specifically peroxidase and catalase activities, under infection stress. The results revealed that Kalanamak and Pusa Basmati were highly susceptible, with high disease indices and visual ratings. Conversely, wild rice accessions such as O. rufipogon and O. australiensis exhibited lower disease indices, indicating moderate resistance. Peroxidase activity varied significantly among the rice leaves, ranging from 148.83 to 182.44 mg/g fresh weight/min. Dhanya 748 showed the highest peroxidase activity, followed by O. australiensis and O. rufipogon. Native-PAGE electrophoresis of peroxidase isoenzymes demonstrated high-intensity bands in O. rufipogon, BPT 5204, and Swarna Sub-1, while minimal or no bands were observed in other genotypes, indicating varying levels of peroxidase activity. Catalase activity also showed significant variation, ranging from 138.18 to 152.14 mg/g fresh weight/min. BPT-5204 exhibited the highest catalase activity, followed by O. australiensis and Pusa Basmati. Isoenzyme analysis via Native-PAGE revealed high-intensity catalase bands in O. australiensis, Dhanya 748, NDR 118, and Pusa Basmati, while no bands were observed in O. rufipogon, CSR 13, Arize 6444, and Swarna Sub-1. SDS-PAGE analysis of leaf proteins showed variability in banding patterns. Non-infected leaves of Swarna Sub-1, Arize 6444, Kalanamak, and Pusa Basmati had minimal bands, while O. australiensis and Dhanya 748 showed maximum bands. Infected leaves of CSR 13, NDR 118, Arize 6444, Kalanamak, Dhanya 748, and Pusa Basmati had minimal bands, whereas O. australiensis exhibited maximum bands. Overall, the study highlights the potential of wild rice species in enhancing disease resistance and provides insights into the biochemical responses of rice varieties under pathogen stress.

Keywords: Peroxidase activity; catalase activity; native-PAGE; electrophoresis SDS-PAGE; biochemical responses.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a cornerstone of global agriculture, providing sustenance for over half of the world's population. Its importance is particularly pronounced in Asia, where it forms the dietary staple for millions of people. However, rice productivity is frequently compromised by various diseases, with sheath blight, caused by the fungus Rhizoctonia solani, being one of the most significant. Addressing this challenge requires a deep understanding of the genetic and biochemical mechanisms underlying disease resistance. Wild rice accessions, such as Oryza rufipogon and Oryza australiensis, offer a reservoir of genetic diversity that can be harnessed to enhance disease resistance in cultivated rice varieties. These wild relatives often exhibit traits that are absent in domesticated varieties, including enhanced resistance to pests and diseases. Biochemical responses to pathogen infection are critical indicators of a plant's defence mechanisms. Among these, the activities of peroxidase and catalase enzymes play pivotal roles. Peroxidase activity, which detoxifies reactive oxygen species generated during infection stress, varies significantly among different rice genotypes and can be a marker of resistance. Catalase activity similarly helps protect plant cells from oxidative damage by breaking down hydrogen peroxide, a harmful byproduct of stress responses. To investigate these biochemical responses, advanced techniques such as Native-PAGE electrophoresis and SDS-PAGE are utilized. Native-PAGE electrophoresis separates isoenzymes based on their electrophoretic mobility, allowing the identification and comparison of specific enzyme forms and their activities. This technique is crucial for understanding the presence and intensity of peroxidase and catalase isoenzymes in different rice varieties. SDS-PAGE, on the other hand, profiles leaf proteins, revealing variations in polypeptide banding patterns between infected and non-infected plants. This method provides insights into the changes in protein expression associated with infection stress. By examining these biochemical responses under infection stress, researchers can identify rice varieties and wild rice accessions with enhanced resistance to sheath blight. This knowledge is essential for breeding programs aimed at developing more resilient rice cultivars, ultimately contributing

to sustainable rice production and food security.

2. MATERIALS AND METHODS

2.1 Isoenzyme of Peroxidase by Native-PAGE Electrophoresis

The activity of peroxidase enzyme was determined by the method given by (Vetter, 1959).

Procedure: 200 mg of fresh leaf material was taken and homogenized in 10 ml of phosphate buffer (p^H - 6.0) and centrifuged at 10,000 rpm for 30 min. at room temperature. 2 ml of enzyme extract was taken in a test tube to which 2 ml of phosphate buffer (pH- 6.0), 1 ml pyrogallol and 0.2 ml H_2 O_2 was added. The mixture was shaken and kept in a water bath at 37^0c for 10 min. for the formation of purpurogallin. The intensity of colour was measured at 430 nm by spectronic – 20.

2.2 Peroxidase Isoenzyme Analysis

This procedure involved without denaturation of protein without SDS in any solution. The procedure involved protein electrophoresis without denaturation or the use of SDS in any solution. The apparatus included a vertical slab gel electrophoresis unit with glass plates (18 × 9 × 0.1 cm), a power pack, and micro-pipettes (10-100µl). Solutions prepared for the procedure included a 30% acrylamide bis-acrylamide stock solution (29.2 g acrylamide, 0.8 g bis-acrylamide in 100 ml double-distilled water, stored at 4°C in a dark amber bottle), separating gel buffer (1.875 M Tris, pH 8.8, 22.7 g for 100 ml), and stacking gel buffer (0.6 M Tris-HCl, pH 6.8, 7.26 g for 100 ml). Catalyzing agents used were 10% ammonium persulfate (APS) and 7.5 µl TEMED. The electrode buffer was prepared by dissolving 1.2 g Tris and 5.8 g glycine in 1 L double-distilled water, adjusting the pH to 8.3. The enzyme extraction buffer (0.1 M Tris, pH 6.7) was made by dissolving 1.2 g Tris in 100 ml double-distilled water. The loading dye (pH 6.8) comprised 5 ml Tris-HCl, 7.5 µl β–mercaptoethanol, 100 mg bromophenol blue, and 15 ml glycerol. The gel staining solution was prepared fresh before use and consisted of 6% H2O2, 2 mM O-Guaiacol, 0.2 M phosphate buffer (pH 7), and 100 ml double-distilled water.

For sample extraction, 2 mg of tissue was ground in 0.1 M Tris buffer (pH 6.8) using a mortar and pestle, centrifuged at 5000 rpm for 10 minutes at 4°C, and the supernatant was collected as the sample. The separating gel solution (8%) was prepared by mixing 9.3 ml double-distilled water, 5.3 ml 30% acrylamide bis-acrylamide mix, 5 ml 1.875 M Tris-HCl (pH 8.8), 0.2 ml 10% APS, and 12 µl TEMED. The stacking gel solution (5%) was prepared by mixing 6.8 ml double-distilled water, 1.7 ml 30% acrylamide bis-acrylamide mix, 1.25 ml 0.6 M Tris-HCl (pH 6.8), 0.1 ml 10% APS, and 10 µl TEMED. The separating gel was poured between glass plates and allowed to polymerize for 1 hour, followed by casting the stacking gel with a 10-well comb and allowing polymerization. The plates were assembled into the electrophoresis unit, and 40-50 µl of the sample (containing 75-200 µg protein) was loaded into the wells, ensuring no bubble formation. Electrophoresis was performed at 30 mA and 220 V for 10 minutes through the stacking gel, followed by 20 mA until separation was complete. After separation, the gel was washed 3 times with PBS for 10 minutes each, incubated in 2 mM H2O2 in PBS for 10 minutes, and then in 50 ml of 1 mg/ml Guaiacol solution for 15-20 minutes. Images were recorded within 30 minutes to prevent band disappearance. Similar work was done by (Asgher *et al.* 2023) for the expression of antioxidant enzymes.

2.3 Isoenzyme Analysis of Catalase Using Native-PAGE Electrophoresis and Catalase Activity in Rice Leaves

To prepare the dilution series of calibration standards, the same buffer or solvent used for the sample was used to achieve concentrations of 30 to 150 µg/ml in a final assay volume of 5 ml. Then, 1.0 ml of the protein sample, each dilution of calibration standard, or buffer/solvent (reference standard) was added to 0.90 ml of Hartree-Lowry reagent A in separate test tubes and incubated for 10 minutes in a 50°C water bath. The tubes were cooled to room temperature, followed by the addition of 0.1 ml of Hartree-Lowry reagent B, mixed, and incubated at room temperature for 10 minutes. Rapidly, 3 ml of Hartree-Lowry reagent C was added to each tube, mixed thoroughly, and incubated for 10 minutes in a 50°C water bath before cooling to room temperature. The net absorbance of the sample, calibration standards, and reference standard were measured at 650 nm (A650) using 1-cm cuvettes. The net absorbance readings for the standards were graphed against protein concentration (µg protein/5 ml final assay volume) to prepare a calibration plot. The protein

concentration of the sample was determined by interpolation from this plot. The gel was washed three times for 15 minutes each with distilled water, incubated for 10 minutes in a 0.88 mM H2O2 solution, rinsed again with water, and finally incubated with a mixture of equal volumes of 1% (w/v) ferric chloride and 1% (w/v) potassium ferricyanide solution until yellow bands appeared on a green background (Tables 1 and 2).

2.4 Protein Profiling of Rice Leaf

For the extraction of protein from rice leaves, analytical-grade chemicals were used. Sodium phosphate buffer (0.25 M, pH 7.0) containing 0.15N NaCl was prepared along with other materials like rice leaves, pestle and mortar, ice, and centrifuge tubes. The method followed was as described by Laemmli et al. (1970). Fresh rice leaves were cut into small pieces with a razor and crushed in the sodium phosphate buffer. The mixture was mechanically homogenized and centrifuged at 10,000 g at 4°C for 20 minutes. This process was repeated twice. After centrifugation, the supernatant was collected, resulting in crude rice leaf protein.

2.5 Gel Electrophoresis

The gel electrophoresis experiment utilized a vertical slab gel electrophoresis unit measuring 18 x 9 x 0.1 cm, equipped with a power pack. Reagents included a stock acrylamide solution consisting of 30% acrylamide and 0.8% bis-acrylamide dissolved in 100 ml of double distilled water. Separating gel buffer was prepared using 1.875 M Tris HCl (22.7 g in 100 ml, pH 8.8), while stacking gel buffer employed 0.6 M Tris HCl (7.26 g in 100 ml, pH 6.8). Polymerization was catalyzed by 10% ammonium per sulfate and TEMED. The electrode buffer, adjusted to pH 8.2, contained 0.025 M Tris HCl, 0.192 M glycine, and 0.1% SDS in 1 L of double distilled water. A loading dye solution (pH 6.8) was prepared with 1.5 M Tris HCl, 10% SDS, β-mercaptoethanol, bromophenol blue, and glycerol in 100 ml of double distilled water. Gel staining involved Coomassie brilliant blue R-250 in methanol and acetic acid, with a subsequent destaining solution composed of methanol and acetic acid. For sample preparation, rice leaf proteins were extracted in 0.25 M sodium phosphate buffer (pH 7.2) containing 0.15 N NaCl, heated with running dye, and centrifuged. The polyacrylamide gel was then prepared by sequentially mixing the appropriate solutions, ensuring polymerization initiation upon the addition of TEMED and APS.

The separating gel was poured between glass plates and allowed to polymerize for 30-40 minutes. After polymerization, the stacking gel was cast and a Teflon comb (13 wells) was inserted between the plates, followed by waiting for proper gel polymerization. Once polymerized, the Teflon comb was carefully removed, and the plates were assembled into the electrophoresis unit with electrode buffer in both tanks. The unit was connected to a power pack and the gel was run for 8-10 hours at 25 mA and 160 volts. After the complete separation of protein molecules, the power was turned off, and the gel was removed and stained in a tray with staining solution for visualization.

2.6 Destaining of Gel

The destaining solution, composed of 45% methanol and 10% acetic acid in double distilled water (100 ml total volume), was used to destain the gel. The gel was immersed in this solution to remove unbound dye until the background became colorless. Bands were categorized into groups based on their number, presence or absence, and intensity of specific bands. The relative migration (Rm) value of each band was calculated accordingly.

Table 1. Separation gel of (8%)

Solution	8%
Double distilled water	9.3 ml
30% Acrylamide bisacrylamide mix	5.3 ml
1.875 M Tris HCl pH 8.8	5 ml
10 % APS	0.2 ml (200 µl)
TEMED	0.012 ml (16 µl)
Total Volume	20 ml

Table 2. Stacking gel solution (4 %)

Solution	4 %
Distilled water	6.8 ml
30% Acrylamid bisacrylamide mix	1.7 ml
0.6 M Tris (PH 6.8)	1.25 ml
10 % APS	0.1 ml (100 µl)
TEMED	0.01ml (10 µl)
Total Volume	10 ml

3. RESULTS AND DISCUSSION

3.1 Peroxidase Enzyme Activity in Rice Leaves

Peroxidase enzyme activity in rice leaves exhibited significant variation, ranging from 148.83 to 182.44 mg/g fresh weight/min. Dhanya 748 demonstrated the highest peroxidase activity at 182.44 mg/g fresh weight/min, followed closely by O. australiensis (180.10 mg/g fresh weight/min) and O. rufipogon (178.14 mg/g fresh weight/min). In contrast, CSR-14 exhibited the lowest peroxidase activity at 148.83 mg/g fresh weight/min (Table 3 and Fig. 1).

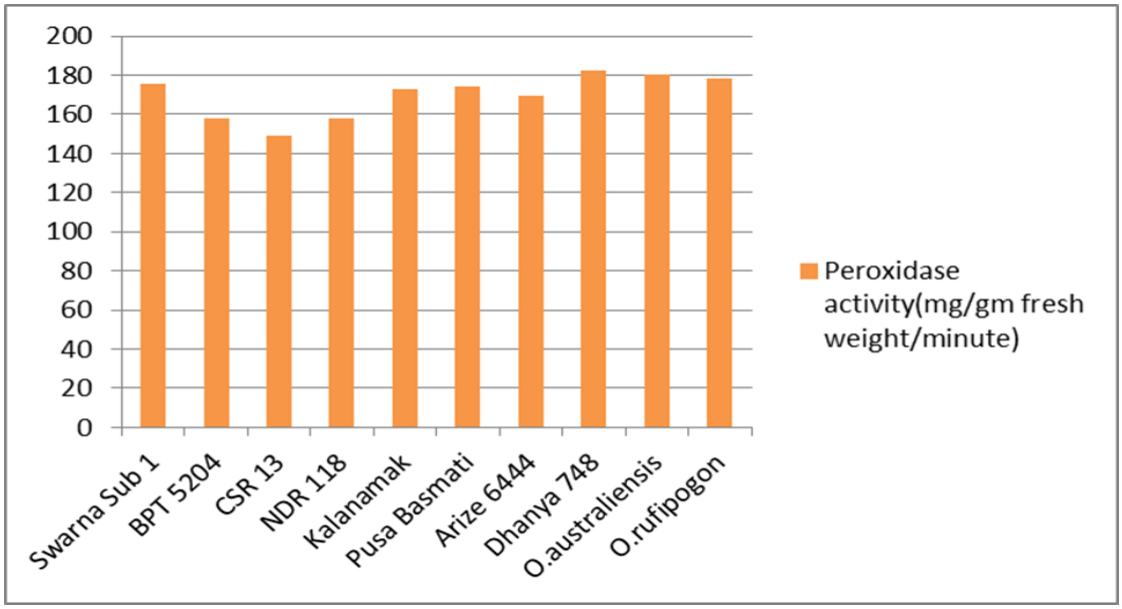

Fig. 1. Graph showing peroxidase activity in rice genotypes (mg/g fresh weight/minute)

Table 3. Peroxidase activity in rice leaves (mg/g fresh weight/minute)

S. No.	Name of Varieties	Peroxidase activity (mg/g fresh weight/minute)
1.	Swarna Sub 1	175.83
2.	BPT 5204	158.11
3.	CSR 13	148.83
4.	NDR 118	157.94
5.	Kalanamak	173.16
6.	Pusa Basmati	174.16
7.	Arize 6444	169.34
8.	Dhanya 748	182.44
9.	*O.australiensis*	180.10
10.	*O.rufipogon*	178.14
SEm ±		**2.25**
CD (at 5%)		**5.36**

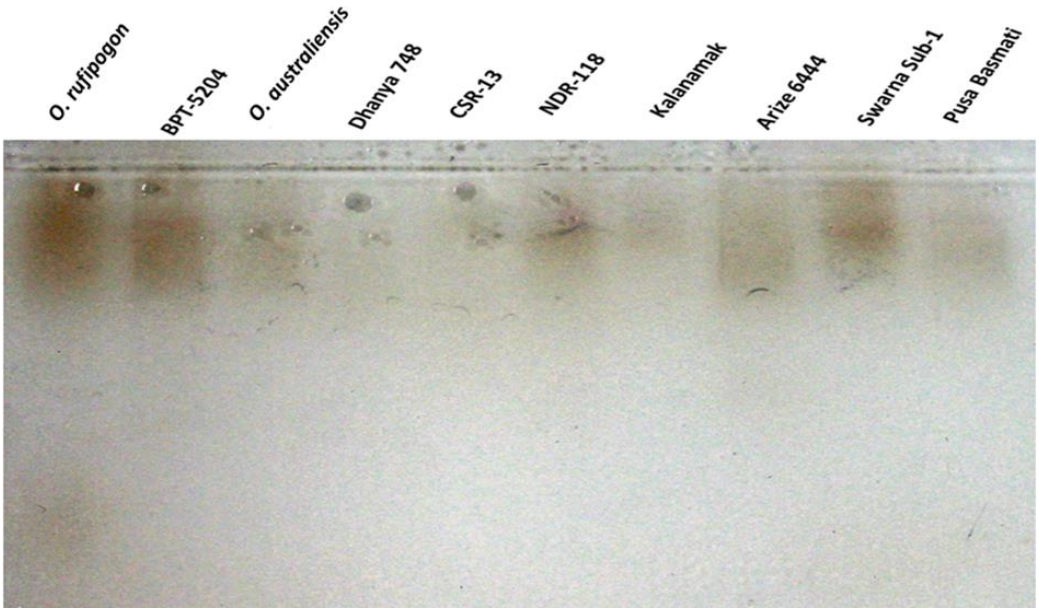

Fig. 2. Appearance of peroxidase of isoenzymes of Rice leaves in native-PAGE (8%) Catalase activity rice leaves

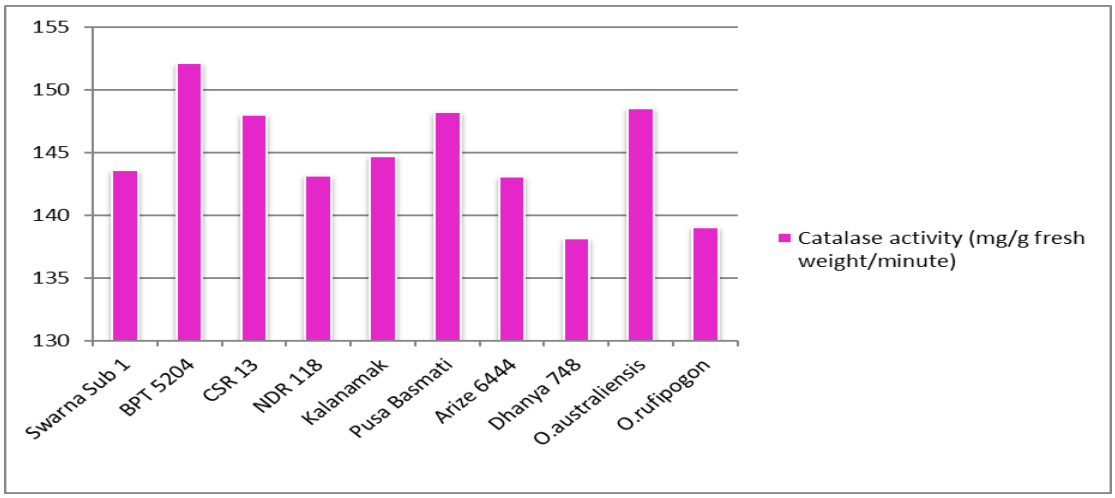

Fig. 3. Graph showing catalase activity in rice genotypes (mg/g fresh weight/minute)

Analysis of peroxidase isoenzymes using native PAGE revealed that O. rufipogon, BPT 5204, and Swarna Sub-1 displayed high-intensity bands, indicating robust peroxidase activity. Conversely, Dhanya 748, CSR 13, and Kalanamak showed no bands, suggesting negligible peroxidase activity. Other genotypes such as Arize 6444, Pusa Basmati, NDR-118, and O. australiensis exhibited minimal band intensities on the gel (Fig. 2).

Catalase enzyme activity in rice leaves also showed significant variation, ranging from 138.18 to 152.14 mg/g fresh weight/min. The highest catalase activity was observed in BPT-5204 (152.14 mg/g fresh weight/min), followed by O. australiensis (148.52 mg/g fresh weight/min) and Pusa Basmati (148.05 mg/g fresh weight/min) (Table 4 and Fig. 3). Dhanya-748 exhibited the lowest catalase activity at 138.18 mg/g fresh weight/min. Analysis of catalase isoenzymes via native PAGE indicated high-intensity bands in O. australiensis, Dhanya 748, NDR 118, and Pusa Basmati, while BPT-5204 and Kalanamak showed minimal band intensities. O. rufipogon, CSR 13, Arize 6444, and Swarna Sub-1 displayed no bands on the gel, indicating the absence of catalase activity [1] (Fig. 4).

Table 4. Catalase activity in Rice leaves (mg/g fresh weight/min)

S. N.	Name of variety	Catalase activity (mg/g fresh weight/minute)
1.	Swarna Sub 1	143.56
2.	BPT 5204	152.14
3.	CSR 13	148.05
4.	NDR 118	143.12
5.	Kalanamak	144.70
6.	Pusa Basmati	148.26
7.	Arize 6444	143.10
8.	Dhanya 748	138.18
9.	*O.australiensis*	148.52
10.	*O.rufipogon*	139.05
SEm±		**2.36**
CD (At 5%)		**9.26**

3.2 Protein Profiling by SDS-PAGE (12%) from Non-Infected Rice Leaves

The comparison between non-infected and infected rice leaves showed notable differences in protein banding patterns. Non-infected leaves generally displayed a greater number of bands compared to infected leaves, indicating a reduction in protein complexity under infection stress. Each variety displayed characteristic RM (relative migration) values, which further differentiated them based on the position of protein bands in the gel. For instance, O. rufipogon exhibited nine distinct RM values in non-infected leaves, highlighting its protein profile complexity.

In infected rice leaves, several varieties including Swarna Sub-1, BPT 5204, and others exhibited consistent RM values, indicating a more uniform protein profile under infection stress. Notably, wild rice O. rufipogon showed no RM values in infected leaves, suggesting a significant alteration or reduction in protein expression under pathogen attack. Li et al. [2] also demonstrated by his findings that potential of SDS-PAGE protein profiling as a tool to assess protein diversity and responses to stress in rice varieties. These findings underscore the potential of SDS-PAGE protein profiling as a tool to assess protein diversity and responses to stress in rice varieties. The correlation between protein profiles and physiological resistance against Rhizoctonia solani reinforces the importance of biochemical analysis in understanding plant-pathogen interactions and identifying resistance sources in rice breeding programs [3] (Sengupta and Chattopadhyay, 2001) (Table 3).

3.3 Protein Profiling by SDS-PAGE (12%) from Non-Infected Rice Leaves

Protein profiling of rice leaf extracts using SDS-PAGE revealed significant diversity among varieties, particularly highlighted by distinct banding patterns in the electropherograms [4]. Wild rice species, particularly O. australiensis, exhibited the highest number of bands, suggesting greater protein diversity compared to cultivated varieties. Specific bands such as 2, 5, 8, 9, 14, 15, and 16 were identified as useful markers for distinguishing between different rice varieties based on their unique mobility and intensity (Fig. 5). The comparison between non-infected and infected rice leaves showed notable differences in protein banding patterns. Non-infected leaves generally displayed a greater number of bands compared to infected leaves, indicating a reduction in protein complexity under infection stress. Similarly, [5] Identified, characterizated a d-cysteine desulfhydrase from rice see. Each variety displayed characteristic RM (relative migration) values, which further differentiated them based on the position of protein bands in the gel. For instance, O. rufipogon exhibited nine distinct RM values in non-infected leaves, highlighting its protein profile complexity.

In infected rice leaves, several varieties including Swarna Sub-1, BPT 5204, and others exhibited consistent RM values, indicating a more uniform protein profile under infection stress. Notably, wild rice O. rufipogon showed no RM values in infected leaves, suggesting a significant alteration or reduction in protein expression under pathogen attack. These findings underscore the potential of SDS-PAGE protein profiling as a tool to assess protein diversity and responses to stress in rice varieties, which is in

accordance with Jiang et al. [6]. The correlation between protein profiles and physiological resistance against Rhizoctonia solani reinforces the importance of biochemical analysis in understanding plant-pathogen interactions and identifying resistance sources in rice breeding programs [3] (Sengupta and Chattopadhyay, 2001) (Table 3) [7-10].

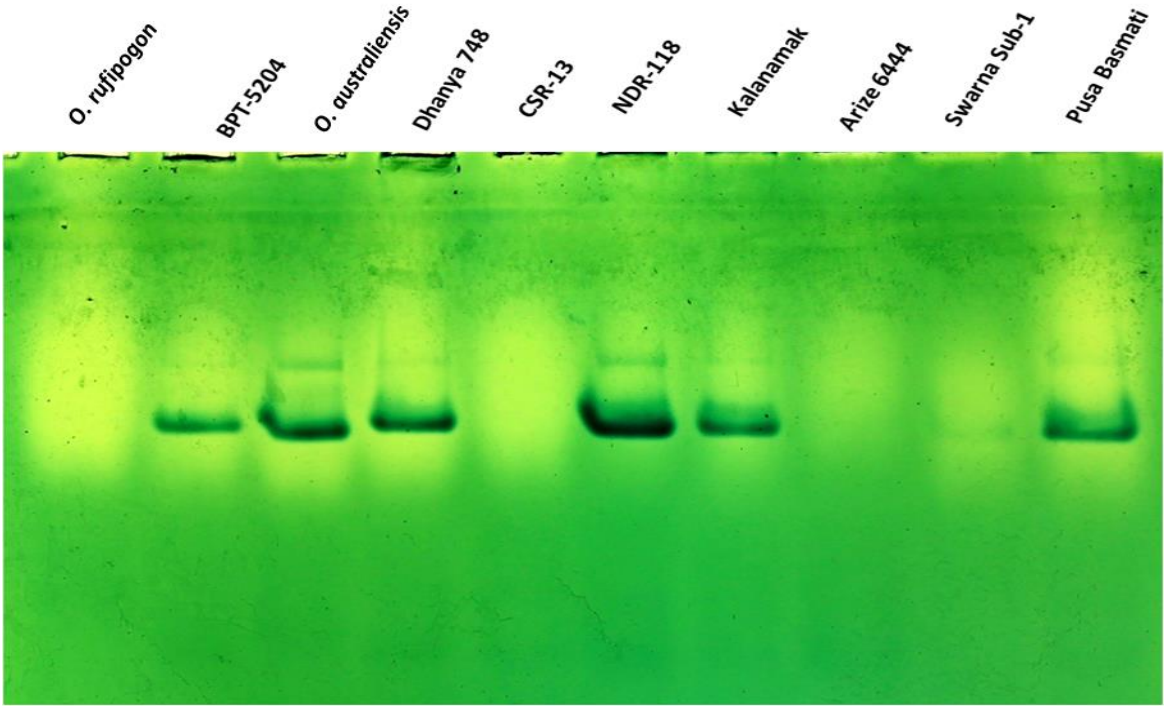

Fig. 4. Appearance of catalase isoenzymes of rice leaves in native-PAGE (8%)

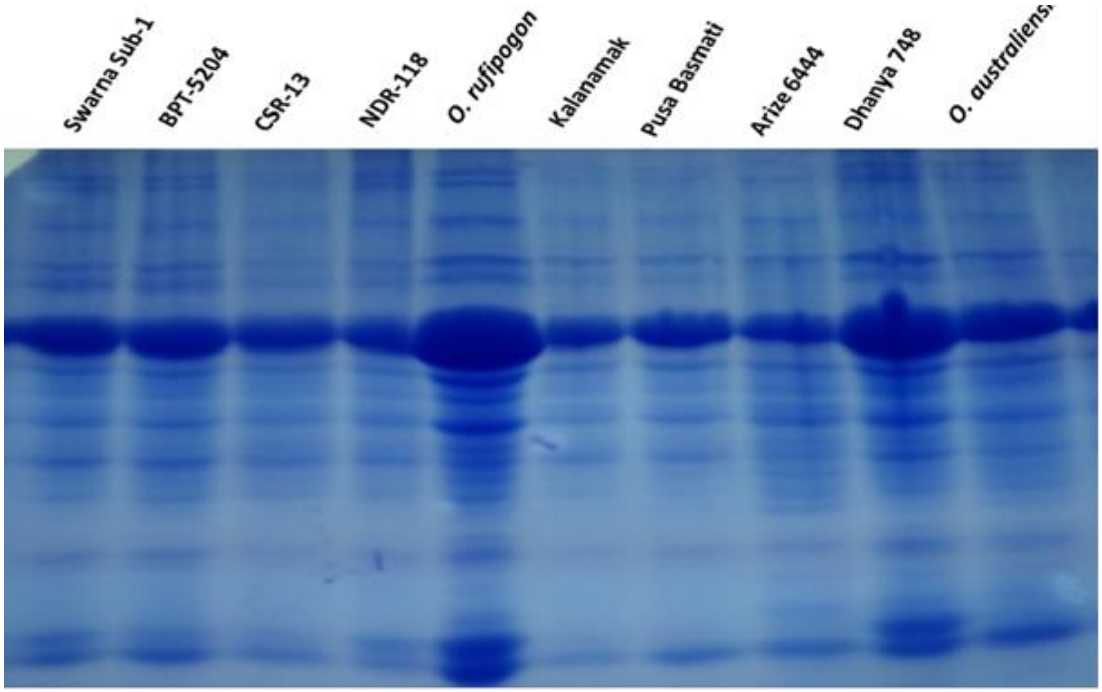

Fig. 5. Appearance of protein bands from non-infected rice leaves on SDS-PAGE (12%)

Table 5. Relative mobility of 12 % SDS-PAGE in non- infected rice leaves

Length of gel (cm)	R.M. Value	Varieties									
		Swarna sub-1	BPT 5204	CSR 13	NDR 118	*O.rufipogon*	Kalanamak	Pusa Basmati	Arize 6444	Dhanya 748	*O.australiensis*
0	-	-	-	-	-	-	-	-	-	-	-
1	0.0909	-	-	-	+	+	-	-	-	+	+
2	0.181	-	-	-	-	+	-	-	-	-	+
3	0.272	-	+	-	-	+	+	+	-	-	+
4	0.363	++	++	++	++	+++	++	++	++	+++	++
5	0.454	-	-	+	-	-	-	-	_	-	+
6	0.545	-	-	+	+	+	+	_	+	+	-
7	0.636	-	-	-	-	+	-	-	-	-	-
8	0.727	-	-	-	-	+	-	-	-	-	-
9	0.818	-	-	-	-	+	-	-	-	+	-
10	0.909	+	+	-	-	++	-	-	-	++	+

4. CONCLUSION

The study highlights significant variations in peroxidase and catalase activities among different rice varieties, influencing their resistance to sheath blight. Wild rice accessions like O. rufipogon exhibited lower disease indices and distinct enzyme activity profiles compared to susceptible varieties like Kalanamak and Pusa Basmati. Protein profiling through SDS-PAGE further elucidated unique banding patterns in both non-infected and infected leaves, emphasizing the potential of biochemical markers in breeding for disease resistance in rice.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

Authors are highly thankful to department of plant molecular biology & genetic engineering. N.D. University of Agriculture & Technology, Kumarganj, Faizabad-224 229 (U.P.) INDIA for maintaining the experimental material to conduct the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sofo A, Scopa A, Nuzzaci M, Vitti A. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses, Int. J. Mol. Sci. 2015;16:13561-13578.
2. Li J, Kong D, Song T, Hu Z, Li Q, Xiao B, Xie G. OsFBN7–OsKAS I module promotes formation of plastoglobules clusters in rice chloroplasts. New Phytologist. 2023; 239(5):1771-1789.
3. Chauhan JS, Nanda JS. Varietal identification of rice (*Oryza sativa* L.) by physico-chemical characteristics of the grain and electophoretic variants of salt soluble seed proteins. Seed Res. 1984;12:78-89.
4. Vighi IL, Seixas Neto A, de Sousa GF, de Carvalho JO, da Silva Pinto L. Standardization of a protein extraction protocol for the proteomic analysis of the leaves of Oryza sativa L. cv. BRS AG. Separation Science Plus. 2021;4(8):305-313.
5. Yamasawa R, Saito H, Yashima Y, Ito H, Hamada S. Identification, characterization, and application of a d-cysteine desulfhydrase from rice seed (*Oryza sativa* L.). Protein Expression and Purification. 2023;211:106341.
6. Jiang D, Zeng Y, Almakas A, Tong X, Gao Z, Chen G. Physiological features and thylakoid membrane proteomic analysis of rice NG46 during natural leaf senescence. Acta Physiologiae Plantarum. 2021;43:1-11.
7. Asgher M, Ahmed S, Sehar Z, Gautam H, Gandhi SG, Khan NA. Hydrogen peroxide modulates activity and expression of antioxidant enzymes and protects photosynthetic activity from arsenic damage in rice (*Oryza sativa* L.). Journal of Hazardous Materials. 2021;401: 123365.
8. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 1970;227:680-685.
9. Sengupta S, Chattopadhyay NC. Rice varietalidentification by SDS-PAGE. Seed Sci. & Technol. 2000;28:871-873.
10. Vetter JL, Steinberg MP, Nelson AI. Enzyme assay, quantitative determination of peroxidase in sweet corn. Journal of Agricultural and Food Chemistry. 1958; 6(1):39-41.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12292