Pol. J. Environ. Stud. Vol. 34, No. 6 (2025), 6751-6761
DOI: *10.15244/pjoes/192580* ONLINE PUBLICATION DATE: 2024-10-14

Original Research

Characterization of Physio-Biochemical Properties for Leaf Rust in Wheat Associated with Yield Losses and Disease Resistance Patterns

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Received: 2 May 2024
Accepted: 23 August 2024

Abstract

Leaf rust of wheat is an important biotic plant stress influence in yield reduction of wheat. Host resistance is a feasible approach for dealing with disease management in wheat. Germplasm screening with resistance reactions will be helpful in the development of resistant lines; on the other hand, plant cellular responses during the host pathogen interaction may vary depending on the resistance and susceptibility of the cultivar. Knowledge of cell membrane stability and stress protein during the pathogen interaction helps in quantifying disease and the effect of these parameters on yield losses. 48 germplasm has been screened with two susceptible germplasm, and two biochemical parameters such as proline and membrane stability were calculated among germplasm. Agra local and PBW343 show the lowest proline content at 0.505 and 0.405 µMoles/g, with the lowest membrane stability of 12.34 and 115.32 with susceptible reactions. Cluster analysis of the germplasm based on quantitative disease scoring and qualitative disease parameters divides the germplasm into four clusters, such as germplasm viz., cluster 1 with 16 germplasm *viz.,* JSW 3, 5, 9, 8, 10, 11, 15, 16, 18, 19, 26, 28, 29, 32, 34, 41. This germplasm was observed with disease severity from 0.75 to 6, and the disease reaction of

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the germplasm ranges from R, RMR, and MR and was recorded as 1.85 to 2.475 µMoles/g, and EC or membrane stability was between 10.32 and 42. Observing the experimental studies, it was identified that the categorization of this germplasm into slow rust and race-specific based on the disease scoring and physio-biochemical parameters helps in the breeding of durable varieties.

Keywords: germplasm, membrane stability, EC, leaf rust

Introduction

Wheat is the second most important cereal crop after rice, which provides nutritional security to 60 percent of the world population [1], and a marginal reduction of wheat yield occurs due to the effects of abiotic and biotic crop stress [2, 3]. The drastic climate change patterns are adding an adverse effect on crop health due to the evolution of new races of pathogens for different fungal genera [3, 4]. Leaf rust, or brown rust, is a major constraint of wheat production targeting yield losses due to the increasing pressure of virulent strains [4]. Prevalent pathotypes of leaf rust (12-5, 77-1, 77-5, 77-9, 104-2) in the Indian subcontinent are creating epidemics in the northwestern parts of the country [5]. Breeding is the most important strategy for the management of triple rust in wheat with exploitation of host resistance, categorized in two ways, such as race-specific and non-race-specific resistance [6-8]. In the present day, nearly 76 resistance race-specific breeding genes with several quantitive loci are identified and exploited in breeding programs for the development of resistance varieties [9-11]. The race-specific resistance is the most manipulated genetic resistance, which is overcome easily due to the evolution of new races challenging the durability of the cultivars, but the cultivars with broad, non-race-specific resistance have shown longer durability [11, 12]. The gene expression is generally based on providing defense techniques in combating plant diseases, altering the changes in the physiochemical structures of the plant cell [13]. The resistance action is provided by active and passive barriers to defense-related actions in plants, Active barriers of the plant cell, such as ion influxes across the plasma membrane alter the changes of the cell membrane and cross-linkages of the cell membrane with callose deposition [14], and membrane leakage due to pathogen infection affects photosynthetic activity associated with high transpiration, which changes the water levels in plants [15, 16]. However, the little-known information on the effect of the physiological and biochemical alternations in wheat plant cells and their impacts on yield reduction was less understood. The present research was conducted to learn about the effect of the physio-biochemical properties in yield reduction and the effect of the resistance patterns of cultivars on physio-biochemical parameters in response to leaf rust. Physical parameters such as disease severity at the flag leaf stage, membrane stability of wheat plant cells, proline concentrations, and test weight of seed samples were important parameters recorded during the cropping seasons 2021-22 and 2022-23.

Experimental

Plant Material

Plant material for the evaluation of leaf rust resistance was obtained through AICRP Wheat and Barley, IIWBR-Karnal, Haryana, India. 100g of seed material of wheat germplasm was used as the testing material; forty-eight germplasms of seed material were tested in two replications along with susceptible checks Lalbahadur, and Agra local from the Division of Plant Breeding and Genetics, SKUAST-Jammu. The prominent leaf rust pathotypes were collected from the infected seed samples and stored at 4^0C, and the samples were soaked in sterile distilled water, and the inoculum was multiplied on the susceptible cultivar of Agra local in the controlled growth chamber. Spacing of 10 cm between plant to plant and 22 cm between row to row was maintained, to ensure severe disease infection fields were irrigated adequately for the proper spread of the disease without any abiotic stress conditions.

Adult Plant Screening

Disease severity of leaf rust was recorded from the February 1st week to the end of the crop period, and disease scoring was recorded from the third flag leaf stage to final harvesting until the green leaf stage for two cropping seasons during 2021-2023. A Modified Cobbs scale was used for disease severity scoring, and visual observations were important in recording the disease severity. Below 2% disease severity recorded as a trace, more than 5% severity intervals were used for disease severity recordings, and 5-10% and 20% intervals were used for higher scores [17]. The slow rusting parameters that were used in categorizing germplasm are based on the following disease severity parameters, such as FRS and CI.

Final Rust Severity Values (FRS)

FRS value at the adult plant stage refers to the degree of resistance among screened germplasm, with different types of disease severity response and the classification of screened germplasm based on different values of FRS, such as 1-20%, 20-40%, 40-60%, and > 60%, refers to high, moderate, and low levels of slow rusting genes [18].

Coefficient of Infection

The data from the field observation was converted to disease severity by using the Modified Cobbs scale, combining the disease severity with the host infection reaction [19]. The disease score was recorded as a modified cobs scale with IT values 0-immune; Resistant R-0.2; Resistant to Moderately Resistant RMR-0.3; Moderately Resistant MR-0.4; Moderately Resistant to Moderately Susceptible MRMS-0.6; Moderately Susceptible-0.8; Moderately Susceptible to Susceptible-0.9; and Susceptible-1 [20]. The categorization of the slow rust lines by using the CI lines, such as values 1-20, 21-40, and 41-60 shows high, medium, and low levels of slow rusting genes, which are used in estimating the partial resistance in wheat cultivars [21]. The effect of disease severity on different physio chemical parameters was represented in Fig. 1. through a scatter plot.

Membrane Stability

The membrane stability of plant cells indicates the extent of plant resistance under different biotic and abiotic stress. For this, we have to take 20 ml tubes containing deionized distilled water. Leaf bits of wheat were cleaned and placed in the test tubes, rotated under the vortex machine, and EC was measured as the initial electrical conductivity of sample E0. Samples were refrigerated at 4^0C for 24 hrs, and electrical conductivity was assessed as EC1, and the same samples were autoclaved at 120^0C for 20 min, and EC2 was counted. The membrane stability index was calculated as [22].

$$EC1 - EC0/EC2 - EC0 \text{ X } 1000$$

Proline Content

Proline content in the leaves of the screened germplasm was estimated to know the effect of the proline concentration due to leaf rust on different germplasm leaves, and it was calculated by the procedure [23].

Proline content in leaf tissue was calculated by using the formula given below:

$$\mu\text{mole proline/g tissue} = \frac{(\mu \text{ proline/ml}) \times \text{ml toluene}}{115.5} \text{ X } \frac{5}{(g \text{ sample})}$$

Test Weight of the Seed

The test weight of 1000 grain seed weight was calculated from the screened germplasm lines for two cropping seasons [24].

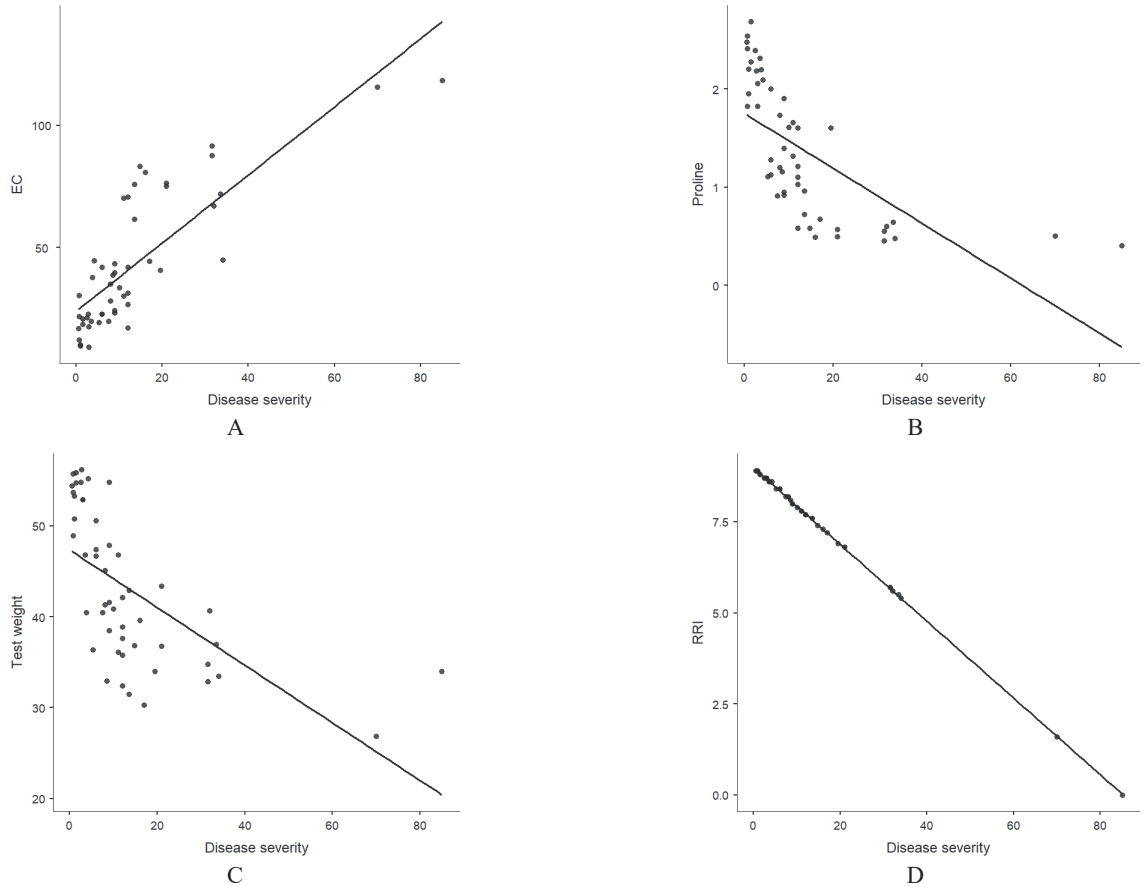

Fig. 1. Scatter Plot representation of impact of disease severity on different physiochemical parameters.

Table 1. Adult Plant reaction and Disease severity values of scrneed germplasm of wheat during rabi 2021-23.

S. No	Germplasm	FRS	FRS	CI	CI	ACI
		2021-22	2022-23	2021-22	2022-23	
1.	JSW1	10MRMS	5RMR	6	1.5	3.75
2.	JSW2	20MRMS	25MRMS	12	15	13.5
3.	JSW3	10MR	15RMR	4	4.5	4.25
4.	JSW4	20MSS	20MS	18	16	17
5.	JSW5	5RMR	TR	1.5	0	0.75
6.	JSW6	20MRMS	25MR	12	10	11
7.	JSW7	20MS	20MS	16	16	16
8.	JSW8	10RMR	10RMR	3	3	3
9.	JSW9	5R	15RMR	1	4.5	2.75
10.	JSW10	10RMR	10R	3	2	2.5
11.	JSW11	5RMR	TR	1.5	0	0.75
12.	JSW12	20MS	15MR	16	6	11
13.	JSW13	15MSS	20MS	13.5	16	14.75
14.	JSW14	20MRMS	30S	12	30	21
15.	JSW15	10RMR	TR	3	0	1.5
16.	JSW16	20MRMS	20MR	12	8	9
17.	JSW17	5MRMS	15MRMS	3	9	6
18.	JSW18	0	5R	0	1	0.5
19.	JSW19	TR	10RMR	0	3	1.5
20.	JSW20	20MSS	50MSS	18	45	31.5
21.	JSW21	15MS	20MR	9	8	8.5
22.	JSW22	10MSS	5RMR	9	1.5	5.25
23.	JSW23	30S	15MS	30	12	21
24.	JSW24	30MSS	40MSS	27	36	31.5
25.	JSW25	20MSS	10MRMS	18	6	12
26.	JSW26	5MR	0	2	0	1
27.	JSW27	20MSS	20MRMS	12	12	12
28.	JSW28	20RMR	5R	6	1	3.5
29.	JSW29	5RMR	0	1.5	0	0.75
30.	JSW30	10MSS	10MRMS	9	6	7.5
31.	JSW31	10MRMS	20MRMS	6	12	9
32.	JSW32	10RMR	1R	2	0	1
33.	JSW33	10MS	10MS	8	8	8
34.	JSW34	20RMR	0	6	0	3
35.	JSW35	20MRMS	20MRMS	12	12	12
36.	JSW36	30MSS	40S	27	40	33.5
37.	JSW37	10MRMS	10MRMS	6	6	6
38.	JSW38	30MRMS	5MR	18	2	10

Table 1. Table continued.

39.	JSW39	20MRMS	20MRMS	12	12	12
40.	JSW40	20MR	20MR	8	8	8
41.	JSW41	20RMR	20RMR	6	6	6
42.	JSW42	20MSS	10MSS	18	9	13.5
43.	JSW43	40MSS	40MS	36	32	34
44.	JSW44	20MRMS	10MRMS	12	6	9
45.	JSW45	40MS	40MS	32	32	32
46.	JSW46	30MSS	20MRMS	27	12	19.5
47.	JSW47	30MR	20RMR	12	6	9
48.	JSW48	20MSS	20MRMS	18	6	12
49.	Agra local	60S	80S	60	80	70
50.	PBW343	80S	90S	80	90	85
CD=0.05%				8.43	6.49	

Note: 0 = Immune, Tr = Trace, R = Resistant, MR = Moderately Resistant, MRMS = Moderately Resistant to Moderately susceptible, MSS = Moderately Susceptible = Susceptible.

Relative Resistance Index

The Relative Resistance Index of the germplasm was calculated to know the effect of resistance patterns among the screened germplasm, in which the Relative Resistance Index of the screened line was calculated by using a 0-9 scale, in which 0 represents the most susceptible and 9 represents highly resistant, and for the stripe rust, the acceptable index of RRI is 6 or 7. The lines with RRI values 6 to 7 are considered the slow rusting lines [25]. The RRI values are calculated by considering the highest CI value as 100, which is the CARPA value of the country's average relative percentage of attack, and the CARPA values of the rest of the lines are calculated accordingly to the highest line.

$$RRI = \frac{100\text{-}CARPA}{100} \text{ X } 9$$

Statistical Analysis

The data of disease scoring parameters, disease severity, and physio-biochemical parameters are subjected to statistical analysis and compared at $p < 0.05$ for significance. SPSS software was used for performing statistical research.

Results and Discussion

Adult Plant Resistance

Average Coefficient of Infection

The ACI value of screened germplasm was calculated based on the final rust severity of the screened germplasm for two successive cropping seasons, and it had been identified that the average coefficient of infection ranged between 0 and 80 percent of the final disease severity. The two susceptible checks of the wheat germplasm Agra local and PBW343 had been noticed with disease severity more than 60 percent with susceptible reaction for two cropping seasons. Among the fifty-germplasm average coefficient of infection value where the disease severity less than 0-10 was observed in twenty-eight germplasm lines such as JSW 1, 3, 5, 8, 9, 10, 11, 15, 16, 17, 18, 19, 21, 22, 26, 28, 29, 30, 31, 32, 33, 34, 37, 38, 40, 41, 44, and 47. The disease severity average coefficient of infection values 11-20 was observed in thirteen germplasm lines such as JSW2, 4, 6, 7, 12, 13, 25, 27, 35, 39, 42, 46, and 48. ACI values between 21 and 40 were observed in seven germplasm lines such as JSW 14, 20, 23, 24, 36, 43, and 45. The susceptible checks PBW343 and Agra local show a susceptible reaction.

R to RMR relative moderate resistance had been noticed in fourteen germplasm lines such as JSW 1, 3, 5, 8, 9, 10, 11, 15, 18, 19, 28, 32, 34, and 41 potential sources of race-specific genes represented in Table 1. FRS values are crucial for the identification of slow rust resistance in germplasm, with the aaverage coefficient of infection values of 1-20%, 20-40%, 40-60%, and > 60% referring to high, moderate, and low levels of slow

rusting genes [25, 26]. Germplasm JSW 3, 5, 9, 8, 10, 11, 15, 16, 18, 19, 26, 28, 29, 32, 34, and 41 and their FRS values are less than 10; these are good sources of monogenic resistance and also recorded the highest 1000 seed weight with RRI values greater than 8, which indicates strong race-specific resistance [27, 28].

Electric Conductivity

Electric conductivity refers to the membrane stability of wheat germplasm, which indicates the level of resistance membrane stability index ranged from 9.765 to 118.31. The pattern of the stability index for two cropping seasons from 2021-2023 is as follows: from 9 to 19.4 in nine germplasm lines and values between 20.85 to 28.5 in twelve germplasms, 30.3 to 39.75 in seven germplasm lines, and 40.8 to 44.7 observed in eight germplasm. Values of membrane stability from 61.5 to 67.5 were observed in two germplasm lines, such as membrane permeability from 70.25 to 75.95 was observed in six germplasm lines. Values of 80.8 to 87.8 were observed on three germplasm, one germplasm with membrane stability of 91.7, and the highest membrane stability was observed between 115.8 and 118.319 in Agra local and PBW343. Membrane stability, or EC values, are important factors for understanding the host-pathogen interaction. The more resistance of the germplasm, the lesser membrane stability, or EC, is observed, and the higher the susceptibility, the higher the EC. Germplasm with a disease severity response of R and RMR showed lower EC as the infection type and severity increased the electric conductivity; these are similar and supported by the research findings previously [29, 30].

Proline Content

The proline content of wheat under biotic stress was calculated from the leaf tissues; the proline content ranges from 0.503 µMoles/g of leaf samples in Agra Local to 2.685 µMoles/g of leaf samples in JSW15. Proline, which is a stress protein released due to adverse effects of environmental changes; rust susceptibility leads to a strong decrease in the proline content of the plant, but the germplasm that is resistant has a high level of the proline in their leaf tissues [8] found higher proline content in the moderately infested leaves of HD1222 compared with the controls as reported previously. The proline content and disease severity (%) maintained a highly significant negative correlation across the wheat germplasm, indicating wheat genotypes with high proline tend to have low disease severity, and the results were strongly supported by the results of [9, 10].

1000 Seed Weight

1000 seed weights were calculated among different germplasm, with a maximum seed weight of 55.7 g in JSW5 and the lowest seed weight of 26.85 g observed in Agra Local. The results of seed weight were reduced as the rust severity increased.

RRI

The RRI values of the screened germplasm indicate the durability of the germplasm. The highest RRI values were observed in the germplasm, which were above 8 in germplasm, viz., JSW 1, 5, 8, 9, 10, 11, 15, 16, 17, 18, 19, 21, 22, 26, 28, 29, 32, 33, 34, 37, 40, 41, 44, and 47. The germplasm RRI values, which are above 7, are recorded in *viz.,* JSW 2, 4, 6, 7, 13, 25, 27, 35, 38, 39, 42, and 48. The germplasm with an RRI value below 7 was observed in germplasm, *viz.,* GWJ 14, 20, 23, 24, 36, 43, and 45. The lowest RRI values were observed in susceptible checks such as Agra Local and PBW343, with RRI values of 1.6 and 0. The acceptable limits of RRI values for the leaf rust above 6 or 7 are considered to have the best potential, and above 7 is considered to be the best, as mentioned earlier in germplasm for RRI [31, 32].

Correlation Studies

Correlation studies were performed between biochemical parameters and quantitative disease parameters of screened germplasm of wheat to understand the host-pathogen interactions and their corresponding effect on host resistance, which allowed us to understand the physio biochemical parameters related to resistance. Among the different variables, there was a strong correlation between the membrane stability or EC of the wheat with an average coefficient of infection values, which was a significant positive correlation ($r= 0.808^{***}$) and positively correlated between seed weight and proline ($r=0.794^{***}$). There is a negative correlation between the two biochemical parameters such as EC and proline, in which both are negatively correlated ($r= -0.711^{***}$). The Relative Resistance Index, which describes the stability of the cultivars based on the ACI values, is more important, and it is negatively correlated with EC or membrane stability ($r= -806^{***}$) and positively correlated with proline ($r=0.648^{***}$). The calculated values of membrane stability (EC), proline, 1000 seed weight, and RRI are given (Table 2, Fig. 2.). The correlation studies of different variables, mainly EC and disease severity are positively correlated with each other [25, 26], and proline content was negatively correlated with disease severity [27, 28].

Cluster Dendrogram Analysis

The cluster analysis of the germplasm was based on different parameters and classified the germplasm into four different clusters, such as cluster 1 with 16 germplasms, *viz.,* JSW 3, 5, 9, 8, 10, 11, 15, 16, 18, 19, 26, 28, 29, 32, 34, and 41. This germplasm was observed with disease severity from 0.75 to 6, and the disease reaction of the germplasm ranges from R, RMR, and

Table 2. Tabular values of Physiochemical -characters in scrneed germplasm of wheat.

S.NO	Germplasm	EC			Proline			1000 seed weight			RRI
		2021-22	2022-23	2021-23	2021-22	2022-23	2021-23	2021-22	2022-23	2021-23	
1.	JSW1	32.4	43.2	37.8	2.13	2.26	2.195	42.3	38.7	40.5	8.6
2.	JSW2	82.6	69.3	75.95	0.92	1.00	0.96	44.2	41.6	42.9	7.6
3.	JSW3	44.1	45.3	44.7	2	2.18	2.09	54.6	55.8	55.2	8.6
4.	JSW4	42.4	46.3	44.35	0.46	0.89	0.675	32.4	28.2	30.3	7.2
5.	JSW5	32.3	28.4	30.35	2.18	2.64	2.41	57.2	54.3	55.75	8.9
6.	JSW6	66.2	74.3	70.25	1.54	1.09	1.315	32.6	39.6	36.1	7.8
7.	JSW7	78.2	83.4	80.8	0.52	0.46	0.49	43.5	35.7	39.6	7.3
8.	JSW8	20.3	15.7	17.65	1.98	2.13	2.055	53.3	52.4	52.9	8.7
9.	JSW9	24.3	21.3	22.8	2.21	2.16	2.185	57.8	54.6	56.2	8.7
10.	JSW10	20.3	22.4	21.35	2.33	2.45	2.39	55.9	53.7	54.8	8.7
11.	JSW11	18.4	25.5	21.95	2.65	2.43	2.54	43.9	54	48.95	8.9
12.	JSW12	34.3	26.3	30.3	0.89	2.43	1.66	44.9	48.7	46.8	7.8
13.	JSW13	68.3	98.3	83.3	0.54	0.63	0.58	38.4	35.3	36.85	7.4
14.	JSW14	72.4	77.8	75.1	0.45	0.69	0.57	41.4	45.3	43.35	6.8
15.	JSW15	23.3	18.4	20.85	2.83	2.54	2.685	56.4	55.3	55.85	8.8
16.	JSW16	32.4	54.3	43.35	1.53	1.26	1.395	55.4	54.2	54.8	8.0
17.	JSW17	23.4	22.5	22.95	1.32	0.93	1.125	43.3	50.1	46.7	8.4
18.	JSW18	15.4	18.4	16.9	2.43	2.52	2.475	56.3	52.5	54.4	8.9
19.	JSW19	20.3	16.9	18.6	2.21	2.34	2.275	55.3	54.2	54.75	8.8
20.	JSW20	92.4	91	91.7	0.42	0.68	0.55	29.4	36.4	32.9	5.7
21.	JSW21	45.3	32.3	38.8	0.78	1.53	1.155	33.4	32.5	32.95	8.1
22.	JSW22	20.3	18.5	19.4	0.32	1.89	1.105	37.3	35.4	36.35	8.4
23.	JSW23	65.3	87.3	76.3	0.43	0.56	0.495	38.2	35.3	36.75	6.8
24.	JSW24	92.3	83.3	87.8	0.38	0.52	0.45	35.3	34.2	34.8	5.7
25.	JSW25	45.3	38.6	41.95	0.43	0.73	0.58	33.2	38.3	35.8	7.7
26.	JSW26	12.3	8.34	10.32	2.19	2.21	2.20	52.3	54.2	53.3	8.9
27.	JSW27	33.3	29.4	31.35	1.10	1.32	1.21	30.2	34.6	32.4	7.7
28.	JSW28	18.3	21.5	19.9	2.18	2.43	2.31	48.9	44.7	46.8	8.6
29.	JSW29	10.3	14.3	12.3	1.89	1.74	1.82	52.3	55.1	53.7	8.9
30.	JSW30	21.3	18.7	20	0.98	0.83	0.91	38.3	42.7	40.5	8.2
31.	JSW31	26.3	22.1	24.2	1.30	0.54	0.92	37.3	39.6	38.5	8.0
32.	JSW32	8.93	10.6	9.765	1.76	2.13	1.95	52.3	49.2	50.8	8.9
33.	JSW33	28.3	28.1	28.2	1.54	1.92	1.73	44.3	38.2	41.3	8.2
34.	JSW34	10.2	8.63	9.415	1.43	2.21	1.82	53.3	52.4	52.9	8.7
35.	JSW35	14.3	20.1	17.2	1.21	1.98	1.60	42.2	35.6	38.9	7.7
36.	JSW36	67.2	76.9	72.05	0.52	0.76	0.64	38.4	35.6	37.0	5.5
37.	JSW37	23.4	22.1	22.75	1.22	1.34	1.28	45.5	49.3	47.4	8.4
38.	JSW38	35.2	32.2	33.7	1.54	1.68	1.61	39.4	42.3	40.9	7.9

Table 2. Table continued.

39.	JSW39	32.2	21.3	26.75	1.08	0.98	1.03	38.3	36.9	37.6	7.7
40.	JSW40	36.2	34.2	35.2	1.54	0.86	1.20	45.3	44.8	45.1	8.2
41.	JSW41	44.4	39.6	42	1.98	2.01	2.00	52.3	48.9	50.6	8.4
42.	JSW42	65.0	58.1	61.55	0.78	0.65	0.72	32.4	30.5	31.5	7.6
43.	JSW43	45.2	44.8	45	0.43	0.52	0.48	30.5	36.4	33.5	5.4
44.	JSW44	38.2	41.3	39.75	1.11	0.78	0.95	44.3	38.9	41.6	8.0
45.	JSW45	62.3	71.9	67.1	0.44	0.76	0.60	42.4	38.9	40.7	5.6
46.	JSW46	38.4	43.2	40.8	1.21	1.98	1.60	32.5	35.5	34.0	6.9
47.	JSW47	24.3	22.1	23.2	1.87	1.93	1.90	46.5	49.3	47.9	8.0
48.	JSW48	85.3	56.3	70.8	0.76	1.43	1.10	45.3	38.8	42.1	7.7
49.	A. L	110.2	121.4	115.8	0.48	0.53	0.505	24.3	29.4	26.85	1.6
50.	PBW343	121.3	115.32	118.31	0.43	0.38	0.405	38.6	29.4	34	0.0

Note: EC = Electric conductivity, Proline, RRI = Relative resistance Index, AL = Agra local

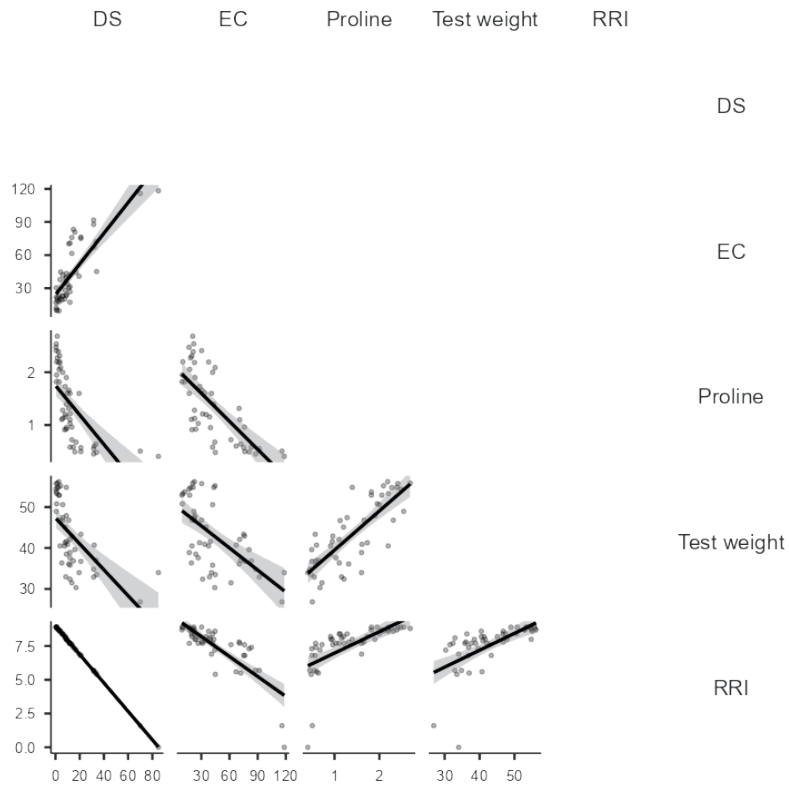

Fig. 2. Persoon Correlation Analysis between disease severity and physio chemical parameter and graphical representation between variables.

MR infection type, with proline concentrations of 1.85 to 2.475 µMoles/g and EC or membrane stability between 10.32 and 42. The second cluster consists of 15 germplasms *viz.,* JSW 1, 12, 17, 21, 22, 27, 30, 31, 33, 35, 37, 38, 39, 40, 44, and 47. These germplasm was observed with disease response RMR, MR, MRMS, and MS, and proline concentration recorded as 0.92 to 2.195 µMoles/g, and EC or membrane stability with between 17.2 and 39.5. The third cluster consists of 16 germplasms, *viz.,* JSW 2, 4, 6, 7, 13, 14, 20, 23, 24, 25, 36, 42, 43, 45, 46, and 48. The fourth cluster consists of only two germplasm lines, Agra Local and PBW343, with the lowest proline of 0.53 and 0.38 µMoles/g. A cluster based dendrogram of germplasm is represented

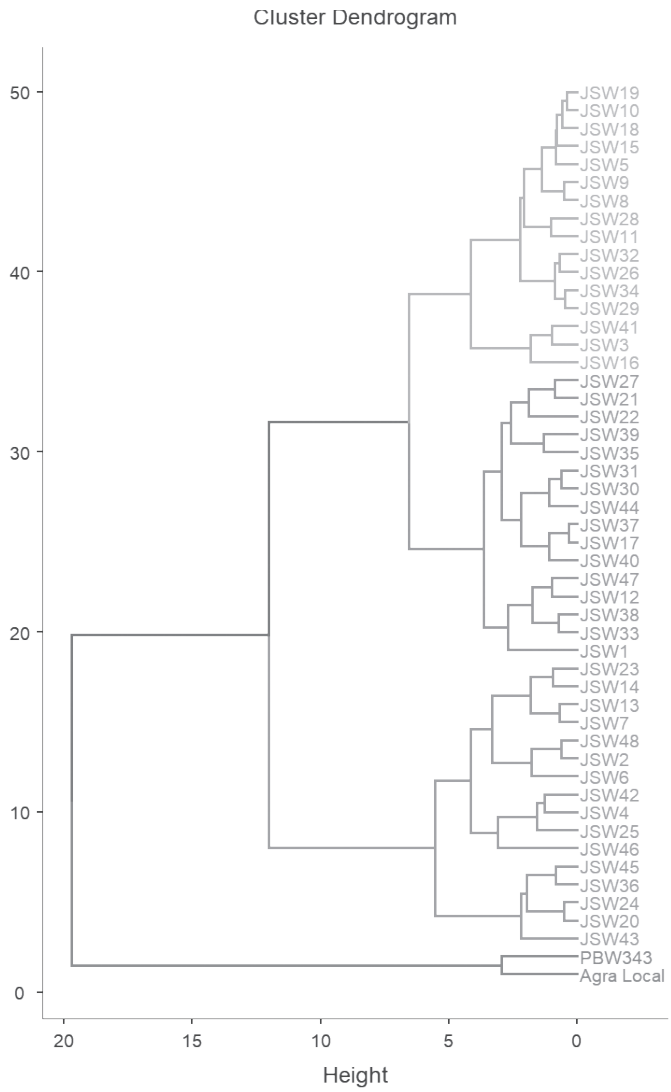

Fig. 3. Cluster dendrogram of scrneed germplasm based on different physiochemical parameters.

in Fig. 3. The cluster analysis approach is the best way to group the screened germplasm based on different parameters; this type of cluster group in germplasm screening was reported previously [29, 30].

Conclusion

The present study reveals that the lines had enough diversity regarding partial resistance, ranging from immunity to partial resistant lines. Most of the tested germplasm exhibited better performance under high disease pressure. The resistance of germplasm and physio-biochemical parameters in accordance with disease severity exhibited high variation in response to disease severity. These lines were supposed to have genes for varying degrees of race-specific to partial resistance, and enough diversity was observed to be used for further genetic manipulations. Further testing for stability over years and locations for leaf rust along with other desirable characteristics must be made before approval.

Funding

This research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-79).

Acknowledgment

The authors would like to acknowledge AICRP -Wheat and Barley, IIWBR (ICAR), Karnal, Haryana-India, for providing research facilities. The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-79).

Conflict of Interest

The authors declare no conflict of interest.

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