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PLANT PATHOLOGY

Biochemical mechanisms of resistance to stripe rust (*Puccinia striiformis* f.sp. *tritici*) in winter wheat (*Triticum aestivum* L.)

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Abstract

Data of the experiments revealed the status of winter wheat genotypes with respect to resistance against stripe rust at adult stage. Among the twenty five genotypes evaluated, China 84-40022, Drina, Drina NS 720, Joss Cambier, Mega, Saptadhara and WW-27 were resistant; Bolal, Centruck, Golden valley, WW-23, WW-24 and WW-25 were moderately resistant and PBW-343 and Agra local were highly susceptible to stripe rust under field conditions. The proline content and total phenols maintained a highly significant negative correlation with final rust severity (FRS) in field across the wheat genotypes. Proline content and total phenols were found maximum in Mega followed by Bolal whereas minimum values for these traits were found in PBW-343 followed by Agra local.

Highlights

- Phenols and proline play important role in the defence mechanism of plants against diseases.
- Higher contents of phenols and proline were found in resistant genotypes compared with the susceptible ones.
- The proline content and total phenols maintained a highly significant negative correlation with final rust severity (FRS) across the wheat genotypes.

Keywords: Wheat, stripe rust, total phenols, proline content, FRS

Wheat (*Triticum aestivum* L.) is the world's most extensively grown cereal crop and is staple food for over 10 billion people. It accounts for 30 per cent in production amongst all cereal crops worldwide followed by rice (27%) and maize (25%). It is a major source of energy, protein and dietary fibre in human nutrition (Kaur *et al.* 2016). Nutritional value of wheat comprises of 11.8 g proteins, 7.2 g carbohydrates, 1.5 g fat, 1.2 g crude fibre, 41 mg calcium and 306 mg of phosphorus per 100 g of wheat (Rai and Mauria 1999). In India, wheat is second important crop next to rice, with a total production of 85.93 million tonnes, grown over an area of 29.25 million hectares and productivity of 29.38 q.ha⁻¹ (Anonymous 2011a), whereas, in Jammu and Kashmir it is grown over an area of 278.72 thousand hectares with a production of 4835.63 thousand quintals and productivity of 17.43 q.ha⁻¹ (Anonymous 2011b).

The major constraints in wheat production are the biotic and abiotic factors that affect the crop yield. The major biotic factors that affect wheat yields are *e.g.*, stem, leaf and stripe rusts, loose smut, karnal bunt, powdery mildew, flag smut and head smut



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diseases. In most wheat producing areas, yield losses caused by stripe rust have ranged from 10 to 70 per cent depending on the susceptibility of the cultivar, earliness of the initial infection, rate of disease development and duration of the disease (Chen 2005).

Stripe rust has traditionally been associated with wheat in the cool, temperate regions of the world, including Asia, Europe, North America, the middleeast and Africa (Line 2002). In India, stripe rust research has not received due attention because this disease is localised only to cooler regions of the North Western Plain Zone and the Northern Hill Zone. This disease develops better at temperatures lower than that required for stem rust and leaf rust and therefore, can appear in epidemic proportions in early winter in the northern states of Punjab, Himachal Pradesh and Uttaranchal. Rapid evolution of stripe rust races and its ability to cause total crop loss demands further research efforts particularly on genetic diversity for resistance against this disease (Wellings et al. 2000). The best, long term, economically and environmentally safe method for sustainable disease control is the use of resistant varieties (Eisa et al. 2013).

Winter wheat (Triticum aestivum L.) offers a potential source of stress resistance because it can withstand freezing temperatures during its early vegetative growth for extended periods of time and it requires a vernalization period (a period of cooler growing conditions) to trigger reproductive growth. The other benefits of winter wheat includes, a higher yield potential than spring wheat, greater profitability as it often requires less inputs than spring wheat. The use of winter wheats as a source of disease resistance has been resorted in a number of breeding programmes, but systematic characterization with respect to stripe rust resistance is lacking. Biochemical parameters play an important role in resistance or susceptibility in plants against diseases. Therefore in this study an attempt has been made to elucidate the role of total phenols and proline as a defence mechanism against the stripe rust.

Materials and Methods

The present investigation was carried out at the Research farm, Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu. The experimental site is located between 32°39' N latitude and 74°58' E longitude and has altitude of 332 m above mean sea level.

The experimental material of the study comprised of twenty-five genotypes of wheat of which twentythree genotypes were the test genotypes of winter wheat, while Agra local and PBW-343 are the checks for stripe rust. The winter wheat genotypes were procured from IARI Regional Station, Shimla and maintained in the Division of Plant Breeding and Genetics.

The experiment for recording field observations was laid out in complete randomized block design with three replications. Genotype in each replication was grown in a plot of 3 rows of 2 meter length each with a spacing of 30 cm between rows.

The inoculum of uredospores of stripe rust (spore dust) was sprayed on the germplasm at tillering stage. The spore dust used was a mixture of races occuring naturally. Under Jammu conditions, the two prevalent races occurring naturally causing stripe rust are 78S84 and 46S119. Disease observations were recorded from Ist week of February till the crop attained maturity (FRS). The first and subsequent observations were taken after 15 days interval using Modified Cobb's Scale (Peterson et al. 1948). For below 5 per cent severity, the intervals used are traces to 2 per cent. 5 per cent intervals were used from 5 to 20 per cent severity and 10 per cent intervals between 20 to 100 per cent.

The proline content in the leaf tissue of wheat was estimated as per the method as suggested by Bates *et al.* (1973). Proline content in leaf tissue was calculated by using the formula given below:

	$(\mu g \text{ proline/ml}) \times ml \text{ toluene}$	5
µmole proline/g tissue =		x
	115.5	(g sample)

The total phenol content in the leaf tissue of wheat was estimated as per the method as suggested by Malick and Singh (1980).

Results and Discussion

Adult Plant Resistance

The results in Table 1 reveal that the tested wheat genotypes exhibited differential reactions against infection with stripe rust. The data reveal that, the initial disease severity ranged from 0.00 to 19.67 per cent, with gradual increase in the severity and by the end of

the cropping season the final rust severity (FRS) ranged from 6.40 to 74.00 per cent.

Table 1: Screening of winter wheat germplasm along
with susceptible checks against stripe rust under field
conditions, during 2010-11

S. No.	Germplasm	Initial rust severity (%)	Final rust severity (%)
1.	Alfrog	3.47	52.00
2.	Arkan	2.67	48.00
3.	Beserka	2.53	38.00
4.	Blue boy	2.73	36.67
5.	Bolal	2.73	20.33
6.	Centruck	2.00	24.33
7.	China 84-40022	2.53	8.60
8.	Drina	3.27	6.40
9.	Drina NS 720	3.47	6.60
10.	Golden valley	1.47	19.67
11.	Jose cambier	2.00	9.80
12.	Mega	0.00	6.40
13.	Nordespres	0.53	42.00
14.	Saptadhara	1.60	9.80
15.	Vir-45347	3.53	44.67
16.	WW- 7	3.53	37.33
17.	WW-12	3.33	41.33
18.	WW-21	1.47	38.00
19.	WW-23	3.33	19.67
20.	WW-24	1.47	21.33
21.	WW-25	3.20	23.00
22.	WW-26	3.53	42.00
23.	WW-27	3.40	9.80
24.	Agra local	19.67	68.00
25.	PBW-343	19.33	74.00

With respect to FRS, the wheat genotypes China 84-40022, Drina, Drina NS 720, Jose cambier, Mega, Saptadhara and WW-27 only exhibited resistant reaction. While, wheat genotypes *viz.*, Bolal, Centruck, Golden valley, WW-23, WW-24 and WW-25 showed moderately resistant reaction, Beserka, Blue boy, WW-7 and WW-21 showed moderately susceptible reaction and genotypes *viz.*, Alfrog,

Arkan, Nordespres, Vir-45347, WW-12 and WW-26 exhibited susceptible reaction. The highest disease reaction was recorded with Agra local and PBW-343, which are considered as susceptible checks against stripe rust, while none of the wheat genotypes showed immune or nearly immune reaction (Table 2).

Table 2: Reaction of winter wheat germplasm againststripe rust under field conditions, during 2010-11

1		. 0
Disease score	Disease reaction	Germplasm
1.	Immune (0)	Nil
2.	Nearly immune (1 × to 5%)	Nil
3.	Resistant (6-10%)	China 84-40022, Drina, Drina NS 720, Jose cambier, Mega, Saptadhara and WW-27
4.	Moderately Resistant (11-25%)	Bolal, Centruck, Golden valley, WW-23, WW-24 and WW-25
5.	Moderately Susceptible (26-40%)	Beserka, Blue boy, WW-7 and WW-21
6.	Susceptible (41-65%)	Alfrog, Arkan, Nordespres, Vir- 45347, WW-12 and WW-26
7.	Highly Susceptible (>65%)	Agra local and PBW-343

Thus the genotypes *viz.*, China 84-40022, Drina, Drina NS 720, Jose cambier, Mega, Saptadhara and WW-27 may be considered potential source of resistance against the two prevalent races of stripe rust based on seedling and field reaction. The resistance reaction/pattern may vary over time and environment because of the evolution of new pathotypes and overlapping of stripe rust with leaf rust. These findings got support from the results of earlier studies by Ali *et al.* (2008) and Herrera-Foessel *et al.* (2007).

The data pertaining to mean performance of genotypes for biochemical characters are presented in Table 3.



Table 3: Mean performance for biochemical traits in	
wheat genotypes	

$(\mu moles/g of leaf sample) (mg/100g of leaf sample)$ 1. Alfrog 0.67 232.18 2. Arkan 0.75 229.08 3. Beserka 1.17 249.38 4. Blue boy 1.85 258.86 5. Bolal 2.39 298.24 6. Centruck 0.82 243.04 7. China 2.05 264.32 84-40022 8 Drina 2.10 291.34 9. Drina NS 2.06 272.42 720 10. Golden 0.81 238.68 valley 11. Joss 1.99 261.06 Cambier 2 243.42 2 12. Mega 2.83 308.14 13. Nord 0.96 243.42 Desprez 2 2 2 14. Saptadhara 2.31 252.76 15. Vir-45347 0.75 236.96 16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 <th></th> <th></th> <th>~ / *</th> <th></th>			~ / *	
1.Alfrog 0.67 232.18 2.Arkan 0.75 229.08 3.Beserka 1.17 249.38 4.Blue boy 1.85 258.86 5.Bolal 2.39 298.24 6.Centruck 0.82 243.04 7.China 2.05 264.32 $84-40022$ 8Drina 2.10 291.34 9.Drina NS 2.06 272.42 720 72072072010.Golden 0.81 238.68 valley11.Joss 1.99 261.06 Cambier12.Mega 2.83 308.14 13.Nord 0.96 243.42 Desprez72 20.71 236.96 14.Saptadhara 2.31 252.76 15.Vir-45347 0.75 236.96 16.WW-7 0.88 238.32 17.WW-12 0.71 234.80 18.WW-21 1.30 250.96 19.WW-23 1.55 254.24 20.WW-24 0.88 238.92 21.WW-25 1.11 249.66 22.WW-26 0.69 220.98 23.WW-27 1.61 257.18 24.Agra local 0.65 213.52	S. No.	Germplasm	(µmoles/g of	
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3. Beserka 1.17 249.38 4. Blue boy 1.85 258.86 5. Bolal 2.39 298.24 6. Centruck 0.82 243.04 7. China 2.05 264.32 84-40022 2 2 2 8. Drina 2.10 291.34 9. Drina NS 2.06 272.42 720 20 2 2 10. Golden 0.81 238.68 valley 1.99 261.06 11. Joss 1.99 261.06 Cambier 1.99 261.06 12. Mega 2.83 308.14 13. Nord 0.96 243.42 Desprez 1.14 Saptadhara 2.31 252.76 14. Saptadhara 2.31 252.76 15. Vir-45347 0.75 236.96 16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 18.	1.	Alfrog	0.67	232.18
4. Blue boy 1.85 258.86 5. Bolal 2.39 298.24 6. Centruck 0.82 243.04 7. China 2.05 264.32 8. Drina 2.10 291.34 9. Drina NS 2.06 272.42 720 720 720 720 10. Golden 0.81 238.68 valley 1. Joss 1.99 261.06 Cambier 1. Joss 1.99 261.06 Cambier 1. Joss 2.83 308.14 13. Nord 0.96 243.42 Desprez 14. Saptadhara 2.31 252.76 15. Vir-45347 0.75 236.96 16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 18. WW-21 1.30 250.96 19. WW-23 1.55 254.24 20. WW-24 0.88 238.92 21. <td>2.</td> <td>Arkan</td> <td>0.75</td> <td>229.08</td>	2.	Arkan	0.75	229.08
5.Bolal 2.39 298.24 6.Centruck 0.82 243.04 7.China 2.05 264.32 $84-40022$ 210 291.34 9.Drina 2.10 291.34 9.Drina NS 2.06 272.42 720 20 212 210 10.Golden 0.81 238.68 valley 1.1 Joss 1.99 261.06 11.Joss 1.99 261.06 Cambier 2.83 308.14 13.Nord 0.96 243.42 Desprez 243.42 2.83 308.14 14.Saptadhara 2.31 252.76 15.Vir-45347 0.75 236.96 16.WW-7 0.88 238.32 17.WW-12 0.71 234.80 18.WW-21 1.30 250.96 19.WW-23 1.55 254.24 20.WW-24 0.88 238.92 21.WW-25 1.11 249.66 22.WW-26 0.69 220.98 23.WW-27 1.61 257.18 24.Agra local 0.65 213.52	3.	Beserka	1.17	249.38
6. Centruck 0.82 243.04 7. China 84-40022 2.05 264.32 8. Drina 2.10 291.34 9. Drina NS 720 2.06 272.42 10. Golden valley 0.81 238.68 11. Joss valley 1.99 261.06 12. Mega 2.83 308.14 13. Nord 0.96 243.42 Desprez 2 2 2 14. Saptadhara 2.31 252.76 15. Vir-45347 0.75 236.96 16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 18. WW-21 1.30 250.96 19. WW-23 1.55 254.24 20. WW-24 0.88 238.92 21. WW-25 1.11 249.66 22. WW-26 0.69 220.98 23. WW-27 1.61 257.18 24. Agra local 0.65 213.	4.	Blue boy	1.85	258.86
7. China 84-40022 2.05 264.32 8. Drina 2.10 291.34 9. Drina NS 720 2.06 272.42 10. Golden valley 0.81 238.68 11. Joss valley 1.99 261.06 12. Mega 2.83 308.14 13. Nord Desprez 0.96 243.42 14. Saptadhara 2.31 252.76 15. Vir-45347 0.75 236.96 16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 18. WW-21 1.30 250.96 19. WW-23 1.55 254.24 20. WW-24 0.88 238.92 21. WW-25 1.11 249.66 22. WW-26 0.69 220.98 23. WW-27 1.61 257.18 24. Agra local 0.65 213.52	5.	Bolal	2.39	298.24
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9. Drina NS 720 2.06 272.42 10. Golden valley 0.81 238.68 11. Joss Cambier 1.99 261.06 12. Mega 2.83 308.14 13. Nord Desprez 0.96 243.42 14. Saptadhara 2.31 252.76 15. Vir-45347 0.75 236.96 16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 18. WW-21 1.30 250.96 19. WW-23 1.55 254.24 20. WW-24 0.88 238.92 21. WW-25 1.11 249.66 22. WW-26 0.69 220.98 23. WW-27 1.61 257.18 24. Agra local 0.65 213.52	7.		2.05	264.32
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Cambier12.Mega2.83308.1413.Nord0.96243.42Desprez14.Saptadhara2.31252.7615.Vir-453470.75236.9616.WW-70.88238.3217.WW-120.71234.8018.WW-211.30250.9619.WW-231.55254.2420.WW-240.88238.9221.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	10.		0.81	238.68
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15.Vir-453470.75236.9616.WW- 70.88238.3217.WW-120.71234.8018.WW-211.30250.9619.WW-231.55254.2420.WW-240.88238.9221.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	13.		0.96	243.42
16. WW-7 0.88 238.32 17. WW-12 0.71 234.80 18. WW-21 1.30 250.96 19. WW-23 1.55 254.24 20. WW-24 0.88 238.92 21. WW-25 1.11 249.66 22. WW-26 0.69 220.98 23. WW-27 1.61 257.18 24. Agra local 0.65 213.52	14.	Saptadhara	2.31	252.76
17.WW-120.71234.8018.WW-211.30250.9619.WW-231.55254.2420.WW-240.88238.9221.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	15.	Vir-45347	0.75	236.96
18.WW-211.30250.9619.WW-231.55254.2420.WW-240.88238.9221.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	16.	WW- 7	0.88	238.32
19.WW-231.55254.2420.WW-240.88238.9221.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	17.	WW-12	0.71	234.80
20.WW-240.88238.9221.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	18.	WW-21	1.30	250.96
21.WW-251.11249.6622.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	19.	WW-23	1.55	254.24
22.WW-260.69220.9823.WW-271.61257.1824.Agra local0.65213.52	20.	WW-24	0.88	238.92
23.WW-271.61257.1824.Agra local0.65213.52	21.	WW-25	1.11	249.66
24. Agra local 0.65 213.52	22.	WW-26	0.69	220.98
0	23.	WW-27	1.61	257.18
25 PBW-343 0.60 205.58	24.	Agra local	0.65	213.52
	25.	PBW-343	0.60	205.58

The proline content of the wheat genotypes ranged from 0.60 µmoles/g of leaf sample (PBW-343) to 2.83 µmoles/g of leaf sample (Mega) with a mean of 1.34 µmoles/g of leaf sample. Similarly the total phenol content ranged from 205.58 mg/100g of leaf sample (PBW-343) to 308.14 mg/100g of leaf sample (Mega) with a mean of 249.76 mg/100g of leaf sample.

Correlation studies

The proline content (μ moles/g of leaf sample) and final rust severity (%) in field maintained a highly significant negative correlation (r=-0.760**) across

all the genotypes (Figure 1), indicating that the wheat genotypes with high proline level tended to show greater disease resistance. The total phenols (mg/100g of leaf sample) and final rust severity (%) in field also maintained a highly significant negative correlation (r=- 0.784^{**}) across the germplasm (Figure 2), indicating that phenols do play a role towards the disease resistance in plants.

Understanding the interaction of characters among themselves and with the environment has been of great use in the plant breeding. Correlation between different characters of plant could arise because of linkage, pleiotropy or developmentally influenced functional relationships. Correlation studies provide information on the nature and extent of association between any two pairs of metric traits. Thus, it could be possible to bring about genetic upgradation in one trait by selection of the other trait. The proline content and disease severity (%) maintained a highly significant negative correlation across the wheat genotypes, indicating wheat genotypes with high proline level tended to show greater tolerance to disease. These results were supported by Ahmed and Hasan (2011), who found high proline content in heat tolerant wheat genotypes than heat sensitive ones. Also Sbartai et al. (2015) found higher proline content in the moderately infested leaves of HD 1222 compared with the controls. The data pertaining to the correlation coefficients total phenols and disease severity (%) in winter wheat genotypes revealed that phenol is an important parameter for resistance response in plants. The high content of phenol indicates lower disease severity. There was a highly significant negative correlation between disease severity and total phenolic content. Similar results were reported by Al-Maaroof et al. (2014) and Mishra et al. (2010). The results are also in line with the findings of Malli et al. (2002), who reported that there was more reduction in total phenols in susceptible genotypes than in resistant genotypes of moth bean following yellow mosaic virus infection.

Conclusion

Results of this study indicate the possibility of adopting a practical and eco-friendly method for the control of wheat stripe rust through the use of resistant cultivars with enhanced concentration of phenols and proline in their leaves. Biochemical mechanisms of resistance to stripe rust (Puccinia striiformis f.sp. tritici) in winter wheat...

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