Antioxidant potential of barley genotypes inoculated with five different pathotypes of *Puccinia striiformis* f. sp. *hordei*

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Abstract The stripe rust caused by the fungal pathogen, *Puccinia striiformis* f. sp. *hordei* in barley (*Hordeum vulgare* L.) is a global problem that threatens the production of barley. The present study examined the disease reaction, free radical scavenging potential, non-enzymatic antioxidants like total phenols, o-dihydroxy phenols, flavonoids along with total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids of the four barley genotypes viz. Jyoti (susceptible), RD2900, RD2901 and RD2552 (resistant) infected with five different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei*. The disease reaction showing RD2901 in the category of immune to very resistant genotype followed by RD2552 in immune to resistant and RD2900 as moderately resistant and Jyoti as susceptible, which was well correlated with biochemical studies. RD2901 possessed higher antioxidant potential in terms of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity, ferric reducing antioxidant power, reducing power and nitric oxide scavenging activity under control conditions and were maintained sufficiently high on inoculation with different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei*. Further, these free radical scavenging activities showed the positive correlation with total phenols, o-dihydroxy phenols, flavonoids which in turn might be contributing in tolerance behaviour of this genotype. However, Jyoti with sensitive behaviour towards M, G, and

24 pathotypes depicted minimum DPPH activity and reducing power under control conditions.

Keywords *Hordeum vulgare* L. · *Puccinia striiformis* f. sp. *hordei* · Free radicals · Antioxidants

Abbreviations

ROS	Reactive oxygen species
¹ O ₂	Singlet oxygen
H ₂ O ₂	Hydrogen peroxide
O ₂ ^{•−}	Superoxide anion radical
OH [•]	Hydroxyl radical
NO	Nitric oxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
TPTZ	2,4,6-Tri-(2-pyridyl)5-triazine
TCA	Trichloroacetic acid
ANOVA	Analysis of variance

Introduction

Barley (*Hordeum vulgare* L.), a member of the *Poaceae* family is the fourth most-produced cereal after wheat, rice, and maize with a total production of 144 million metric tonnes (FAO 2014). Barley is used as a constituent of different health foods, animal feed and also acts as a fermentable material for the production of beer and other distilled beverages. Barley is greatly influenced by a number of fungal diseases, out of which the stripe rust caused by *Puccinia striiformis* f. sp. *hordei*, poses serious threats to barley producing regions worldwide (Pei et al. 2015). Stripe rust in barley occurs in Asia, North, Central and South America, Central Africa and Europe (Wellings 2011). Financial losses due to stripe rust are usually limited

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to cereal crops like wheat and barley (Savary et al. 2012). The maximum grain yield loss of a vulnerable barley cultivar due to stripe rust was reported to be 72% in the United States (Hovmoller et al. 2016). The most favorable temperature for the development of this infection is 18–22 °C, with disease succession ceasing at a temperature above 26 °C (Waqar et al. 2018). Stripe rust in barley is identified by the occurrence of light yellow, straight-sided pustules in stripes on leaves and head which when get matured, then yellow-orange spores are formed (Chen 2013). As the disease progresses, the discoloration of leaves can be quite obvious with both stripe and leaf rust and therefore field with plants showing severe indications may be simply noticeable from a distance (Chen et al. 2015).

Plants require oxygen to carry out basic cellular metabolic activities for their growth and development. Under normal conditions, consumption of O₂ leads to reactive oxygen species (ROS) production in plant tissues. When exposed to hostile environments like pathogen attack, plant experiences a rapid and significant increase in ROS level (Dietz 2015). These ROS can exist in two forms i.e. non radical forms viz. hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂) and free radical forms such as superoxide anion radical (O₂^{•−}) and hydroxyl radical (OH[•]) (Boyd et al. 2014). Accumulation of high level of ROS may cause photooxidative damage to cell structure and various biomolecules (Xie et al. 2016; Mittler 2017). Despite their detrimental effects, ROS may act as the secondary messenger in a variety of cellular responses including tolerance to environmental constraints (Mignolet-Spruyt et al. 2016). Able (2003) observed an early oxidative burst specifically in the epidermal cells of susceptible and resistant barley genotypes against the *Rhynchosporium secalis*. It was coupled by the phenolic browning and cell death which indicate the role of ROS in the pathogenicity of necrotrophs.

In plant cells, H₂O₂, O₂^{•−}, OH[•] are generated in the cytosol, chloroplasts, mitochondria and the apoplastic space (Gilroy et al. 2016). Superoxide anion radical is usually the first ROS to be generated and the major site of O₂^{•−} production is the thylakoid membrane-bound primary electron acceptor of photosystem I (Nauseef 2014). Among various ROS, OH[•] is highly reactive which can be formed by the Fenton reaction in the presence of Fe²⁺ and H₂O₂ (Dietz 2016). Hydroxyl radicals that are toxic ROS, interacts with all biological molecules like lipid, protein, nucleic acids etc. Moreover, cells have no enzymatic mechanism to eliminate OH[•] and its overproduction can lead to cell death. Hence, hydroxyl radical scavenging capacity is a crucial antioxidant activity attributed to its high reactivity (Sart et al. 2015). Some previous studies have also reported that the reducing power may serve as an indicator of antioxidant potential (Sowndhararajan and

Kang 2013). Thus, scavenging of OH[•] will be most crucial for imparting tolerance to plants against various biotic and abiotic stresses.

In order to overcome the adverse effects of excessive ROS generated during the host–pathogen interaction, plants have developed non-enzymatic and enzymatic antioxidant mechanisms (Akter et al. 2015). Non-enzymatic antioxidants include total phenols, o-dihydroxy phenols, flavonoids, and total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids. Indeed, barley has been considered as the richest source of phenolic compounds which can be visualized from the fact that in beer, 70–80% of the phenolic compounds were from malted barley whereas the residual 20–30% come from the hops (Gerhauser 2005). Flavonoids are polyphenolic compounds with high antioxidant potential that are oxidised by radicals, leading to the formation of less reactive radicals (Kasote et al. 2015). Carotenoids are lipophilic organic compounds that are able to detoxify various forms of ROS (Das and Roychoudhury 2014). The capability of carotenoids to scavenge or prevent the generation of chlorophyll triplet state is due to the conjugated double bonds that permit easy uptake of energy from excited molecules and dissipation of excess energy as heat (Moucheshi et al. 2014). At present, there is little information on disease reaction, production/scavenging of ROS and antioxidants for stripe rust resistance in barley genotypes. Therefore, this research was formulated with an attempt to investigate the disease reaction, the role of different free radical scavenging activities, antioxidants and photosynthetic capacity (chlorophyll content) in imparting tolerance to barley genotypes against five pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei*.

Materials and methods

Plants, pathogens and inoculation procedure

Many barley genotypes were screened against various stripe rust pathotypes like M, G, 57, Q and 24 in All India Coordinated Research Project (AICRP) trials where RD2900, RD2901, and RD2552 were found to be resistant whereas Jyoti showed a sensitive response (Kharub et al. 2014–15). Moreover, M and 57 are predominant pathotypes frequently reported in Himachal Pradesh, Jammu and Kashmir, Uttarakhand, Rajasthan and Nepal but Q, 24 and G were also reported in these areas. Thus, for the present study, seeds of these barley genotypes were grown in the fields of Plant Breeding and Genetics, PAU Ludhiana. At first leaf stage i.e. 20 days after sowing (DAS), plants were inoculated with different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei* by rubbing leaves with uridiniospores mixed with

talcum powder. Inoculated seedlings were kept in a moist chamber for 48 h at 18–20 °C with high relative humidity (> 90%). The seedlings were then transferred in different polyhouses that were maintained at far off locations so as to prevent cross-contamination. Favourable conditions for germination of uridinospores were provided by maintaining the temperature of 18 ± 2 °C with sufficient humidity (~ 90%). Parallel control of all the four barley genotypes was treated in the same way with tap water in separate polychamber. Three independent biological replications were performed for each treatment. After 22 days of inoculation, seedlings were scored using the scale proposed by McNeal et al. (1971).

Extraction and estimation of free radical scavenging activities

Preparation of methanolic extract

Leaf samples (0.5 g) of control and infected barley genotypes were extracted with 5 ml of methanol by keeping the test tubes in the water bath at 70 °C for 1 h, followed by filtration with whatman No. 1 filter paper. Filtrate was made to 5 ml with methanol which was used for measuring free radical scavenging activities of barley genotypes.

Estimation of free radical scavenging activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was estimated based upon the principle that DPPH[•] (stable free radical) can accept hydrogen or electron donated by the antioxidant present in the extract. It was determined by adding DPPH in 0.5 ml of methanolic extract where the color change was measured against reagent blank at 515 nm (Blois 1958). The antioxidant potential was also measured according to ferric reducing antioxidant power (FRAP) assay given by Benzie and Strain (1996). In this assay, the antioxidant from the sample reduces Fe^{+3} to Fe^{+2} ions in the presence of 2,4,6-Tri-(2-pyridyl)-5-triazine (TPTZ) and the blue color so developed, was read at 593 nm. The FRAP activity was calculated from $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ standards (5–30 µg) that were run simultaneously. Total reducing power was determined by adding FeCl_3 (0.1%, w/v) in the reaction mixture comprising 200 mM sodium phosphate buffer (pH 6.6), potassium ferricyanide (1%, w/v) and trichloroacetic acid (TCA) (Sreeramulu et al. 2009). The intensity of the green color developed, was determined spectrophotometrically at 700 nm against reagent blank. The potential to scavenge $\text{O}_2^{\cdot -}$ activity was estimated by the standard protocol given by Marklund and Marklund (1974), where the inhibition of pyrogallol autoxidation was monitored

spectrophotometrically at 420 nm. The hydroxyl radical scavenging assay is based on the Fenton-type reaction generating hydroxyl radical. To estimate the hydroxyl radical scavenging activity, 1,10 phenanthroline, FeSO_4 , methanolic extract, sodium phosphate buffer and 0.02% hydrogen peroxide were incubated at 37 °C for 1 h and then absorbance of the solution was read at 536 nm against the blank (modified method of Lee et al. 2008). Nitric oxide (NO) radical scavenging activity was measured by the method of Marcocci et al. (1994). This method is based upon the capacity of sodium nitroprusside to generate nitric oxide which interacts with O_2 to form nitrite ions. These nitrite ions were determined by the use of the Griss reagent.

Estimation of non-enzymatic antioxidants

Total phenols can be estimated by evaporating 0.5 ml of methanolic extract in the water bath at 80 °C. Then, 0.5 ml each of distilled water and folin-phenol reagent were added, followed by the addition of 1 ml saturated sodium carbonate. The contents of total phenols were calculated from gallic acid standard (10–50 µg) which was run simultaneously along with the samples (Swain and Hills 1959). Total flavonoids were determined by the standard procedure given by Zhishen et al. (1999). O-dihydroxy phenols were estimated by the standard procedure given by Nair and Vaidyanathan (1964).

Extraction and estimation of photosynthetic pigments

For the estimation of total chlorophyll, chlorophyll a and b and total carotenoids, 0.2 g of fresh leaves were crushed with 80% acetone at room temperature (Porra et al. 1989). After filtration of extracts through whatman filter paper, the filtrate was measured at 480, 645 and 663 nm and contents were calculated using following formulae:

$$\text{Chlorophyll a (mg/g tissue)} = \{12.7(A_{663}) - 2.69(A_{645})\} \times V/1000 \times W$$

$$\text{Chlorophyll b (mg/g tissue)} = \{22.9(A_{645}) - 4.68(A_{663})\} \times V/1000 \times W$$

$$\text{Total chlorophyll (mg/g tissue)} = \{20.2(A_{645}) + 8.02(A_{663})\} \times V/1000 \times W$$

$$\text{Total carotenoids (mg/g tissue)} = \{[(A_{480}) + 0.114(A_{663})] - 0.638(A_{645})\} \times V/1000 \times W$$

Statistical analysis

All analyses were carried out in triplicate and values were presented as Mean $\pm$ SD of three replicates. Data for

various parameters among four barley genotypes were statistically analyzed by one-way analysis of variance (ANOVA) at the probability level of $p < 0.05$ using multiple comparisons (Tukey's post hoc test) by SPSS V16.0 software.

Results and discussion

The stripe rust disease of barley is one of the most destructive diseases leading to the drastic decrease in the productivity when the sensitive genotypes are grown in the field (Chen et al. 2015). The agronomic and chemical methods to control stripe rust are expensive; therefore the use of resistant genotypes is a more desirable strategy to reduce damage caused by stripe rust (Klos et al. 2016). Infected plants respond to these stresses through several morphological, biochemical and molecular mechanisms but the foremost response of plant is the rapid production of ROS that causes oxidative burst. Hence, it activates the plant antioxidant defence machinery to counteract stress induced severe oxidative damages (Nagar et al. 2017). Thus in the present investigation, free radical scavenging potential of barley genotypes at seedling stage in response to different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei* was evaluated and correlated with non-enzymatic antioxidants and photosynthetic capacity in terms of chlorophyll content.

Scoring of disease in barley genotypes infected with five different pathotypes of *P. striiformis* f. sp. *hordei*

In cereals rusts, resistance has been found at two stages i.e. seedling and adult stage. Seedling resistance/all-stage resistance is highly effective against stripe rust pathotypes since it is race specific. Moreover, plants showing seedling resistance will be resistant at the adult stage but vice versa is not true (Park 2008). So, disease reaction at the seedling stage is helpful for understanding the host–pathogen interaction. In susceptible plants, stripe rust penetrates the stoma, ultimately forming haustoria through which a considerable amount of plant nutrients are extracted that are used for sporulation (Waqar et al. 2018). Disease data was taken at seedling stage according to the 0–9 scale of McNeal et al. (1971). All the four barley genotypes showed immune response under control conditions (Fig. 1 and Table 1). The barley genotype RD2901 showed immune response when inoculated with four pathotypes (M, G, 57 and Q) of *P. striiformis* f. sp. *hordei*, whereas it was very resistant showing necrotic flecks when inoculated with 24 pathotype. It has been found that Jyoti showed variable levels of response. It was susceptible showing abundant sporulation without chlorosis, when inoculated with M, G and 24

pathotypes of *P. striiformis* f. sp. *hordei*, but moderately resistant to 57 and Q pathotypes. The barley genotype RD2900 was susceptible, moderately susceptible, moderately resistant, resistant and immune to 24, M, Q, 57 and G pathotypes respectively, revealing a wide range of symptoms. The barley genotype RD2552 showed immune to resistant response when inoculated with five pathotypes of *P. striiformis* f. sp. *hordei*. So, it was concluded that barley genotypes RD2901 and RD2552 showed best host response when inoculated with these pathotypes whereas, Jyoti exhibited most susceptible character. McIntosh et al. (2001) observed that wheat genotypes also respond differentially to stripe rust pathotypes.

Free radical scavenging activities in barley genotypes on inoculation with *P. striiformis* f. sp. *hordei*

Antioxidant capacity, non-enzymatic antioxidants, and photosynthetic capacity could play a vital role in the detoxification of ROS (Gill et al. 2011). Higher DPPH free radical scavenging activity (%) means a higher scavenging activity towards DPPH radical. RD2901 which was a tolerant genotype towards *P. striiformis* f. sp. *hordei*, depicted maximum DPPH free radical scavenging activity under control conditions whereas Jyoti which was the sensitive genotype, showed minimum activity (Fig. 2a). It was higher in RD2900 (65.18%) when infected with pathotype M as compared to control. In comparison to control, DPPH free radical scavenging activity was recorded maximum in RD2901 and RD2552 when infected with pathotype G and 24 respectively. Therefore, it can be inferred that in comparison to Jyoti, RD2900, RD2901 and RD2552 possessed higher DPPH free radical scavenging activity under control conditions and was maintained significantly higher when infected with different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei*.

The free radical scavenging activity based on FRAP assay was found to be maximum in RD2901 and minimum in RD2900 under control conditions (Fig. 2b). All the four barley genotypes (Jyoti, RD2900, RD2901 and RD2552) showed differential behaviour with respect to different pathotypes of *P. striiformis* f. sp. *hordei*. However, maximum FRAP activity was reported in RD2900, RD2901 and RD2552 when infected with pathotype 24. Similar results for DPPH free radical scavenging activity and FRAP were found when leaves, pod wall and developing seeds of chickpea plants were infested by the insect *Helicoverpa armigera* (Kaur et al. 2017). The reducing capacity of a compound may be determined by the conversion of Fe^{3+} to Fe^{2+} ions (Sowndhararajan and Kang 2013). Under control conditions, maximum reducing power was reported in RD2901 and minimum in Jyoti and RD2900. On inoculation with

Fig. 1 Four barley genotypes infected with five different pathotypes (M, G, 57, Q and 24) *P. striiformis* f. sp. *hordei*

pathotype M, the maximum reducing power was found in RD2900 whereas it was higher in RD2552 when infected with pathotype 24 (Fig. 2c). Debona et al. (2012) reported a more proficient antioxidative system in the leaves of the

moderately resistant genotype of wheat during the infection of *Pyricularia oryzae* as compared to susceptible.

Superoxide anion radical scavenging activity in Jyoti and RD2901 genotypes did not show a significant

Table 1 Four barley genotypes infected with five different pathotypes (M, G, 57, Q and 24) *P. striiformis* f. sp. *hordei*

Genotypes	Control	M	G	57	Q	24
JYOTI	0	9	9	5–6	5–6	9
RD2900	0	7–8	0	3–4	5–6	9
RD2901	0	0	0	0	0	1
RD2552	0	1	0	2	3–4	0

Infection type classes for stripe rust (McNeal et al. 1971)

Immune (0), very resistant (1), resistant (2), resistant (3–4), moderately resistant (5–6), moderately susceptible (7–8), susceptible (9)

difference on inoculation with different pathotypes of *P. striiformis* f. sp. *hordei* (Fig. 3a). In comparison to control, RD2900 exhibited an increase in superoxide anion radical

scavenging activity when pathotypes 57 and Q were used as inoculum, whereas it declined on infection with pathotype 24. However, in RD2552, superoxide anion radical scavenging activity showed a decrease on inoculation with pathotype Q and 24. Hydroxyl radical scavenging activity was found to be higher in all of four barley genotypes (Jyoti, RD2900, RD2901 and RD2552) when inoculated with pathotype G. From the Fig. 3b, it was clear that hydroxyl radical scavenging activity in tolerant genotypes RD2901 and RD2552 increased significantly in response to different pathotypes as compared to Jyoti and RD2900 which showed sensitive and moderate behaviour respectively in disease data (Fig. 1 and Table 1).

Nitric oxide radical scavenging activity was significantly higher in Jyoti when inoculated with pathotype M as compared to control whereas in RD2900, it did not show

Fig. 2 DPPH free radical scavenging activity (a), Ferric reducing antioxidant power (b) and Reducing power (c) in four barley genotypes infected with five different pathotypes of *P. striiformis* f. sp. *hordei*. Values are mean $\pm$ SD of triplicates. Bars with different letters in a genotype represent significantly different values ($p \leq 0.05$)

Fig. 3 Superoxide free radical scavenging activity (a), Hydroxyl free radical scavenging activity (b) and Nitric Oxide free radical scavenging activity (c) in four barley genotypes infected with five different pathotypes of *P. striiformis* f. sp. *hordei*. Values are mean $\pm$ SD of triplicates. Bars with different letters in a genotype represent significantly different values ($p \leq 0.05$)

any increase on infection with different pathotypes of *P. striiformis* f. sp. *hordei* (Fig. 3c). RD2901 which is a tolerant genotype exhibited an increase in nitric oxide radical scavenging activity when pathotypes M, G, 57 and 24 were used as inoculum, whereas in RD2552, it increased on infection with pathotypes M and Q. So, it can be concluded that RD2901 showed a better response in terms of nitric oxide radical free scavenging activity when infected with different pathotypes of *P. striiformis* f. sp. *hordei*. It appears that NO in RD2901 might be acting as a signalling molecule for the activation of defence related responses as reported by Baudouin and Hancock (2014).

Status of total phenols, o-dihydroxy phenols and flavonoids in barley genotypes in response to *P. striiformis* f. sp. *hordei*

Phenols having low redox potential can play a key role in the cyclic reduction of ROS (Agati et al. 2012). Total phenols varied from 1.01 to 1.87 mg/g when inoculated with different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei* (Fig. 4a). Although among four barley genotypes, maximum total phenolic content was reported in RD2901 under control conditions, however, it did not exhibit any increase on infection with different pathotypes. Shalaby and Horwitz (2015) also reported the higher level

Fig. 4 Total Phenols (a), O-dihydroxy phenols (b) and Flavonoids (c) in four barley genotypes infected with five different pathotypes of *P. striiformis* f. sp. *hordei*. Values are mean $\pm$ SD of triplicates. Bars with different letters in a genotype represent significantly different values ($p \leq 0.05$)

of phenols in resistant cultivars of many crops under control conditions. Total phenols were higher in Jyoti and RD2900 in response to pathotype M as compared to control. In comparison to control, it was significantly higher in RD2552 when inoculated with pathotype 24 of *P. striiformis* f. sp. *hordei*. The contents of o-dihydroxy phenols were significantly higher in RD2900 and RD2901 under control conditions, but in comparison to control, it was maximum in Jyoti and RD2552 when inoculated with pathotypes Q and 24 respectively (Fig. 4b). Although RD2900 and RD2901 had maximum content of o-dihydroxy phenols under control conditions, they showed decrease on infection with different pathotypes of *P. striiformis* f. sp. *hordei*.

Flavonoids were found in the range of 4.16–7.26 mg/g in four barley genotypes when inoculated with five different pathotypes of *P. striiformis* f. sp. *hordei* (Fig. 4c). In comparison to control, there was an increased level of flavonoids in Jyoti when inoculated with pathotypes G and Q whereas it was maintained higher in RD2900 in response to five different pathotypes. Maximum flavonoids were present in RD2901 under control conditions but decreased when infected with different pathotypes. It was higher in RD2552 when infected pathotype 24 as compared to control. Anthony et al. (2017) also observed the increase in total flavonoid content in bananas when infected with *Fusarium oxysporum* as compared to the uninfected samples. The antioxidant potential in terms of DPPH free

radical scavenging activity, FRAP, and reducing power was positively correlated to total phenols, o-dihydroxy phenols, and flavonoids which means that higher the content of these phenolic compounds, the maximum antioxidant potential will be there to quench various free radicals generated under hostile conditions (Table 2).

Effect of different pathotypes on total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids of barley genotypes

Total chlorophyll content varied from 2.69 to 9.65 mg/g among four barley genotypes when inoculated with different pathotypes of *P. striiformis* f. sp. *hordei* (Fig. 5a). In comparison with control, total chlorophyll content decreased in Jyoti in response to different pathotypes, whereas it increased in RD2552. In RD2901, total chlorophyll content was maintained in response to different pathotypes when compared to control, whereas it was significantly decreased in RD2900 when inoculated with pathotype 24 of *P. striiformis* f. sp. *hordei*. In corroboration with the previous study (Ashraf and Harris 2013), the stripe rust infection resulted in a decrease in the chlorophyll content in both the susceptible and the resistant barley genotypes, but more prominently in the susceptible genotype. Chlorophyll a content was found to be significantly higher in Jyoti followed by RD2900 under control conditions. However, it decreased significantly in susceptible genotype on inoculation with five different pathotypes of *P. striiformis* f. sp. *hordei*. (Figure 5b). On the other hand, the content of chlorophyll a was maximum in resistant genotypes RD2901 and RD2552 on infection with five different pathotypes of *P. striiformis* f. sp. *hordei* as compared to control. The content of chlorophyll b was observed to be significantly higher in Jyoti and RD2901 under control conditions (Fig. 5c) but decreased in Jyoti on inoculation with five pathotypes of *P. striiformis* f. sp. *hordei*. RD2900 which is moderately resistant genotype has significantly higher chlorophyll b content on infection with 57 and Q pathotypes whereas it increased in resistant genotype RD2552 when inoculated with five pathotypes of *P. striiformis* f. sp. *hordei* in comparison to control. The stripe rust induced a decrease in chlorophyll content which might be attributed to its low biosynthesis or high degradation as reported by Perveen et al. (2010). Chen et al. (2015) had observed the decrease in chlorophyll content in wheat genotype in response to stripe rust. Total carotenoid content ranged from 1.81 to 5.00 µg/g among four barley genotypes when inoculated with different pathotypes of *P. striiformis* f. sp. *hordei* (Fig. 5d). In comparison with control, it decreased in Jyoti and RD2901 in response to different pathotypes, whereas increased in RD2552. In RD2900, total carotenoid content significantly decreased

when inoculated with pathotypes M, G and 24. These results indicate that the resistant barley RD2901 has an efficient system to cope up with the pigment damage in response to infection.

Correlation studies

Correlation studies revealed that DPPH free radical scavenging activity, FRAP and reducing power were positively correlated to total phenols, o-dihydroxy phenols, and flavonoids at 1% level of significance (Table 2). FRAP and reducing power were positively correlated to each other and to other biochemical parameters viz. total phenols, o-dihydroxy phenols, and flavonoids at 1% level of significance. Nitric oxide radical scavenging activity was positively correlated with total carotenoids ($r = 0.269$) at 5% level of significance whereas it was negatively correlated to flavonoids at 1% level of significance. Total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids were positively correlated with each other at a 1% level of significance. Therefore, it can be inferred that all the resistant (RD2901 and RD2552) and moderately resistant (RD 2900) barley genotypes had sufficient free radical scavenging activities and antioxidant compounds that can counteract the oxidative burst caused by five pathotypes of *P. striiformis* f. sp. *hordei*. Moreover, constitutive defence responses in RD2901 also play an important role in imparting resistance against different pathotypes of stripe rust. Similar results were observed when maize genotypes i.e. CO441 and CO354 were infected with *Fusarium verticillioides* (Lanubile et al. 2014).

Conclusion

The present study showed that stripe rust infection markedly altered the free radical scavenging potential and non-enzymatic antioxidants in susceptible and resistant barley genotypes. The results of the disease reaction showed that RD2901 was immune to very resistant followed by RD2552 and RD2900 whereas Jyoti was susceptible genotype. We found that resistant barley genotype RD2901 under control conditions possessed higher DPPH free radical scavenging activity, FRAP, reducing power, total phenols, o-dihydroxy phenols, and flavonoids that were maintained in sufficient amounts when infected with different pathotypes (M, G, 57, Q and 24) of *P. striiformis* f. sp. *hordei*. This was further evident from the positive correlation obtained between these phenolic compounds and DPPH free radical scavenging capacity, FRAP and reducing power. However, Jyoti which is the sensitive genotype had lower DPPH free radical scavenging activity

Table 2 Correlation coefficient (r) between different biochemical parameters in barley genotypes when inoculated with five different pathotypes of *P. striiformis* f. sp. *hordei*

	FRAP	Reducing Power	O ₂ ⁻	OH ⁻	NO	Total Phenols	ODHP	Flavonoids	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Total Carotenoids
DPPH	.590 ^{**}	.703 ^{**}	— .223	— .229	.089	.566 ^{**}	.554 ^{**}	.291 [*]	— .331 ^{**}	— .293 [*]	— .298 [*]	— .344 ^{**}
FRAP		.734 ^{**}	— .362 ^{**}	— .377 ^{**}	— .005	.518 ^{**}	.336 ^{**}	.436 ^{**}	— .018	— .076	.024	.022
Reducing Power			— .283 [*]	— .424 ^{**}	.063	.581 ^{**}	.447 ^{**}	.480 ^{**}	— .093	— .198	— .008	— .104
O ₂ ⁻				.333 ^{**}	— .090	.339 ^{**}	— .204	.142	.139	.248 [*]	.043	— .210
OH ⁻					— .094	.342 ^{**}	— .337 ^{**}	.267 [*]	— .153	— .137	— .138	— .230
NO						.213	.205	— .371 ^{**}	.085	.176	.012	.269 [*]
Total Phenols							.555 ^{**}	.290 [*]	— .090	— .069	— .087	.006
ODHP								.250 [*]	— .104	— .176	— .039	.143
Flavonoids									.191	.037	.306 ^{**}	.023
Chlorophyll										.836 ^{**}	.934 ^{**}	.667 ^{**}
Chlorophyll a											.585 ^{**}	.407 ^{**}
Chlorophyll b												.722 ^{**}

ODHP O-dihydroxy phenols

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Fig. 5 Total Chlorophyll content (a), Chlorophyll a content (b), Chlorophyll b content (c) and Total Carotenoid content (d) in four barley genotypes infected with five different pathotypes of *P. striiformis* f. sp. *hordei*. Values are mean $\pm$ SD of triplicates. Bars with different letters in a genotype represent significantly different values ($p \leq 0.05$)

and reducing power under control conditions. Although, it had higher FRAP, nitric oxide radical scavenging activity, total phenols, o-dihydroxyl phenols, flavonoids, total chlorophyll and carotenoids under control conditions but failed to maintain these antioxidants and free radical scavenging potential when infected with different pathotypes of *P. striiformis* f. sp. *hordei*. Therefore, this study might be helpful to select the highly contrasting genotypes for understanding expression studies that may give more insight into the mechanism of stripe rust resistance against virulent pathotypes.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflicts of interest.

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