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Original Research

Characterization of Barley Genotypes and Their Biochemical Responses against Leaf Rust (*Puccinia hordei*) Disease under Cold Arid Environment

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Abstract

Cereal rust is one of the most damaging diseases of small-grain cereals. The fungus *Puccinia hordei* causes leaf rust in barley and other small grain crops. *Puccinia hordei* causes serious yield losses in the cultivating areas where susceptible and late-maturing barley varieties are cultivated. Therefore, rust-resistant barley cultivar is highly demandable for sustainable small-grain crop production. Improving barley yields and quality is one of the major objectives of barley breeding programs in our

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country. Exotic and indigenous germplasm is one of the best sources of resistance to biotic stresses in barley particularly leaf rust caused by *Puccinia hordei*. Hence, the present investigation was carried out to identify the resistance sources to *P. hordei* and incorporate them into the breeding programs for higher barley yields under changing climatic scenarios. The study aimed to identify new resistant cultivars in barley and other small grain crops. In this study, 100 barley genotypes (*Hordeum vulgare* L.) were considered for screening susceptibility to *P. hordei* causing rust disease. Several biochemical responses were analyzed in *P. hordei* infected barley genotypes. However, the variable response was observed among the 100 barley genotypes while those were screened against leaf rust disease under high altitude cold arid conditions of Ladakh, India. The efficiency of the 100 barley genotypes were categorized into different classes including high resistance (4 genotypes)>resistance (14 genotypes)> moderately resistance (20 genotypes)> moderately susceptible (33 genotypes)>moderately susceptible to susceptible (19 genotypes)> and susceptible (10 genotypes) based on plant response to *P. hordei*. Among the total genotypes, SHEIKH/KP-706, SHEIKH-B1, SHEIKH-636, and IC-062190 showed high resistance (8.07-8.63) as per the international leaf rust scale, while EC-667381, EC-667390, EC-667392, EC667396, EC-667417, Jyoti, EC-667434, EC-667442, EC-667445, and EC-667446 were found as susceptible (3.13-3.97) to *P. hordei*. The highly resistant genotypes accumulated a high level of phenols and flavonoids and cooperated with susceptible and other rest of the genotypes in response to *P. hordei* rust. The efficiency of plant immune response and or fitness to *P. hordei* was correlated to the disease susceptibility index of particular genotypes. This provides a new insight and the mechanistic basis of genotype-specific rust disease susceptibility against *P. hordei*. A large number of genotype-based studies at the field level could be useful to plant breeders and farmers for improving rust resistance in barley and other small-grain cereals.

Keywords: leaf rust, genotype, climate change, biotic stress, disease susceptibility

Introduction

Barley (*Hordeum vulgare*) an annual cereal grown on about 70 million hectares is the fourth largest grain crop in the world, with a global production of 160 million tons. Barley has excellent characteristic features such as cold resistance, strong adaptability, short growth period, high-stress resistance, and stable yield [1]. Developing countries account for about 18% (26 million tons) of total barley production and 25% (18.5 million hectares) of the total harvested area in the world. Barley is grown in an area falling in cooler, semi-arid, and arid regions of the world. It is the predominant cereal at high altitudes [2]. It is an important cereal in India and is grown chiefly in the higher Himalayas. Barley is used mainly as feed for livestock, poultry, and malt barley for the manufacture of beer and other liquors [3,4]. Half of the country's requirement of barley is met from Uttar Pradesh. Barley flour can be used for making chapattis either alone or mixed with wheat flour. Grains may be roasted, ground, and used as Sattu. On average barley grain contains 12.5% moisture, 11.5% albuminoids, 74% carbohydrates, 1.3% fat, 3.9% fiber, and 1.5% ash [5]. It was reported that naked barley is a good source of phenolic compounds, such as cinnamic acid derivatives and benzoic, proanthocyanidins, flavonols, flavanones, and flavones, which can benefit the risk of antioxidative, antitumor, decreasing blood lipids and hypoglycemic [1]. Barley has remained a successful cereal crop because of its short growing time and ability to survive in poor conditions. Barley is adaptable to a greater range of climates than any other cereal, with varieties suited to temperate, subarctic, or subtropical areas. Barley production is decreasing due to the damage caused by insect pests and certain diseases. Rust diseases of barley are among the oldest plant diseases known to man.

The *Puccinia hordei* is the most destructive and devastating disease-causing fungus, it causes leaf rust disease in barley and small-grain cereals globally [6]. Barley and other cereals can be damaged by a wide range of insect pests and other arthropod pests including blue oat mites (*Penthaleus* spp.), red-legged earth mites (*Halotydeus destructor*), Bryobia mites (*Bryobia* spp.), Balaustium mites, cutworms, aphids, earwigs, armyworms, Helicoverpa spp., pasture webworm, pasture cockchafers, grass anthelids, lucerne flea (*Sminthurus viridis*), leaf hoppers, slugs, snails, millipedes, slaters and locusts [7]. Barley leaf rust occurs in years with high humidity and low spring temperatures. It is especially prevalent in crops that were planted late. The symptoms are small orange masses with a lighter halo on leaf surfaces. These masses are spores, which are windblown to other plants. The optimal temperatures for spores to grow are 60 to 72ºF (16 to 22ºC.). The spores can cause secondary infections during this time at 7-to-10-day intervals. When severely affected, plant leaves will display lesions and plants will die. The economic importance of rust depends on the region in the world and varies from year to year. The most obvious alternative to fungicide treatment is the use of resistant cultivars. Resistance in barley to P. hordei is widely available.

Barley is the major crop of a cold arid region of Ladakh and occupies the maximum acreage covering all three agricultural zones of the region. A large part of the acreage is under naked barley known as “Grim” while husked barley is also cultivated on limited scales. Local cultivars are widely cultivated. These are heterogeneous populations differing in maturity, less responsive to modern management practices, and highly susceptible to prevalent diseases and insect pests. As the barley cultivation in Ladakh extends from 8,500 ft to 14,000 ft ASL, so a lot of variation for adaptation and different plant characters exists in the present-day landraces. Being in the center of the diversity the collection and evaluation of naked barley from the Ladakh region may offer opportunities for further selection and improvement. Barley improvement efforts initiated at High Mountain Arid Agriculture Research Institute, Leh SKUAST Kashmir have resulted in the development of varieties possessing moderate resistance to yellow rust and responsiveness to modern agricultural practices namely Sindhu, SBL-4, and SBL-8 in naked barley and VLB-1 and SBL-5 in husked barley. Barley leaf rust disease is one of the most important diseases of barley. Hence there is a need to develop resistant cultivars. The development of genetic resistance is the best way to control this deadly disease. Hence, the present study was carried out with the objectives to screen a set of barley germplasm for leaf rust disease resistance and to further identify barley genotypes having genetic resistance for their subsequent use in barley breeding programs to introgress resistance in high-yielding and adapted barley cultivars that are susceptible to barley leaf rust disease.

Experimental

Location of Experimental Area

The present study was carried out at High Mountain Arid Agriculture Research Institute Leh, Ladakh, of SKUAST-K, India. This region is known as the coldest region in India, where the dust bowl of earth, the land of Lamas (monks), the cold desert, and the land of high passes. This area is one of the most elevated regions of the world. More than 85% of the area lies in the range of 5000 m or above from the mean sea level. It is the western extension of the Tibetan plateau and is situated between 34°08′ to 77°33′N and 34°14′ to 77°55′E with an area of 96,701 sq. km.

Experimental Material

The 100 barley genotypes were considered as experimental materials, which were received from the National Plant Genetic Recourse, India from their regional Centre located at Central Institute of Temperature Horticulture, Srinagar. The test materials were sown with randomized complete block design (RCBD) in three independent replications during 2018. Each test genotype was sown in rows of 2.0 m in length, with a plant-to-plant spacing of 10 cm and a row-to-row spacing of 75 cm. Thinning was carried out 7 days after seedling emergence. Normal agronomic practices were followed for raising the crop. Weeding was carried out when necessary. Earthling up and top dressing with urea was done at 15 days of life for healthy growth.

Observations of Rust Susceptibility and Data Collection

Plant susceptibility to rust disease was screened through the evaluation of plant response, measurement of the affected area, leaf rust damage scoring, and biochemical analysis. In the following sections, we described the whole investigation procedures as follows:

Estimating the Leaf Area of Barley Genotypes

A total of one hundred genotypes grown at HMAARI, Satakna farm, Leh under field conditions were selected for the study during Kharif 2018. The leaf area of all the genotypes was quantified by the destructive method in the High altitude and Entomology Laboratory located at HMAARI, Satakna, Leh. The 15 cm flag leaves of all the test genotypes were screened for yellow rust with 500 leaf samples during the full foliage period. Leaves were sampled from five different plants (mid canopy) of each genotype infected from the rust. The leaf area of each leaf was measured using a leaf area meter (SYSTRONICS, Leaf Area Meter-211) with a read-out unit along with a sensor.

Screening for Barley Genotypes Against Rust

Leaf rust Severity of leaf rust on barley genotypes was calculated by visual observation as the percentage of leaf area affected with the slightly modified integrated scale (1 to 9) for rust evaluation as adopted by Goel and Saini (2001) [8]. The international rust scale as proposed by Johnston and Browder [9] was followed as detailed in Table 1.

Biochemical Analysis of Barely Genotypes

Leaf samples (500 mg) were ground in a pre-chilled mortar and pestle in 10 mL of ice-cold (80%) methanol. The extracts after processing were transferred to a 15 mL tube and centrifuged (10,000 g, 10 min) and the supernatants were collected for spectrophotometric analysis. The pellet left after collecting the supernatant in a tube was again added with 5 mL of (80%) methanol and kept for 24 hours in a dark cold room. All the samples in the tubes were again centrifuged and the total supernatants were again raised to 12 mL with (80%) methanol. The following metabolites including total phenol and flavonoids were analyzed as follows:

Table 1. International rust scale as per Johnston and Browder, 1964.

Scale	Leaf rust (%)	Score	Host categorization
1	0	9	Very highly resistant (VHR)
2	0-5	8	Highly resistant (HR)
3	5-10	7	Resistant (R)
4	10-15	6	Moderately resistant (MR)
5	15-25	5	Moderately susceptible (MS)
6	25-40	4	Moderately susceptible to susceptible (MSS)
7	40-70	3	Susceptible (S)
8	70-90	2	Highly susceptible (HS)
9	90-100	1	Very highly susceptible (VHS)

Estimation of Total Phenols

Total phenol content was determined as per the Folin-Ciocalteu Reagent (FCR) procedure was adopted with slight modifications [10]. Leaf extract (0.5 mL) was added with 0.5 mL of FCR reagent. Five minutes after, 1.2 mL of sodium carbonate (7.5 w/v) was added. Then the solution was vortexed and allowed to stand for 30 minutes. The absorbance was read at 760 nm. The Gallic acid diluted in (80%) methanol was used for calibrating the standard curve and the results were expressed as µg gallic acid equivalents g^{-1} DW (µg GAE g^{-1} DW) on a dry weight basis.

Estimation of Total Flavonoids

The total flavonoid content was determined by the aluminum chloride assay. 0.5 mL of sample extract was added with 0.3 mL of $NaNO_2$ (5 % w/v), and after six minutes 0.3 mL $AlCl_3$ (10%) was added with slight modifications [11]. After five minutes, 2 mL 1M solution of NaOH was added and the volume was made up to 10 mL with distilled water. The solution was vortexed and the absorbance was read at 510 nm. Quercetin was used as a standard for the total flavonoid content in various barley genotypes. The total flavonoid content was expressed as µg Quercetin equivalents g^{-1} DW (µg QE g^{-1} DW) on a dry weight basis.

Statistical Analysis

The pathological and metabolic data were statistically analyzed using analysis of variance (ANOVA) following GenStat 14th edition [12]. The significant difference in physiological and metabolic traits between the genotypes was tested by using the F-test, respectively. Whereas the genotypic means were compared by least significance difference (LSD) at $p \leq 0.05$. Simple correlations were calculated using Microsoft Excel.

Results and Discussion

The results of the analysis of variance (ANOVA) data revealed significant differences between the genotypes for leaf area and leaf rust scale with a significant variance ratio at $p \leq 0.01$. Based on the leaf rust scale the genotypes SHEIKH/KP-706, SHEIKH/B1, SHEIKH-638, SHEIKH-634, SHEIKH-636, IC-062189, IC-062190, EC-667350, EC-663751, EC-667352, EC-667353, EC-667355, EC-667358, EC-667360, EC-667363, EC-667364, EC-667365, EC-667367, EC-667368, EC-667369, EC-667371, EC-667374, EC-667375, EC-667377, EC-667379, EC-667391, EC-667397, EC-667401, EC-667403, EC-667407, EC-667409, EC-667410, EC-667411, EC-667412, EC-667420, EC-667423, EC-667425, EC-667426, EC-667427, EC-667436, EC-667438, DL-36, and EC-667447 exhibited resistance to leaf rust. Four genotypes have been found as highly resistant (HR), fourteen as resistant (R), twenty as moderately resistant (MR), thirty-three as moderately susceptible (MS); nineteen as moderately susceptible to susceptible (MSS), and ten as susceptible (S) among all hundred genotypes analyzed during the study. No genotype has been found to belong to the highly susceptible (HS) and very highly susceptible (VHS) categories (Table 2). Sowing resistant varieties are the most economical way to control the disease. Current malting varieties are susceptible or moderately susceptible (S or MS) but there are some resistant (R) feed varieties also. The highly resistant genotypes *viz*., SHEIKH-B1 have 292 and 93.32 phenol and flavonoid content followed by genotype IC-062190 having phenol and flavonoid content 251.60 and 75.09 respectively. The highly susceptible genotypes viz., EC-667446 and EC-667447 were having phenol (112.50 & 112.40) and flavonoid (40.45 & 84.51) respectively. A minimum leaf area (3.03) has been seen in the EC-667431 genotype and is categorized as moderately susceptible to susceptible (MSS). The genotype EC-667360 has a maximum leaf area (57.73 cm) and belongs to the resistant (R) variety.

Table 2. Evaluation of barley leaf rust resistance in the germplasm lines in Ladakh, 2018.

Entry	Genotype	Leaf area	Leaf rust scale	Host categorization
01	ND		09	Very highly resistant (VHR)
02	SHEIKH/KP-706; SHEIKH-B1; SHEIKH-636; IC-062190;	6.70-22.33	8.07-8.63	Highly resistant (HR)
03	EC-667350; EC-667353; EC-667355; EC-667358; EC-667360; EC-667361; EC-667364; EC-667365; EC-667367; EC-667374; EC-667375; EC-667377; EC-667391; EC-667436	6.87-57.73	7.00-7.90	Resistant (R)
04	SHEIKH-638; SHEIKH-634; IC-062189; EC-663751; EC-667363; EC-667368; EC-667369; EC-667371; EC-667379; EC-667397; EC-667403; EC-667410; EC-667411; EC-667420; EC-667423; EC-667425; EC-667426; EC-667427; EC-667454; EC-667447	3.87-37.87	6.00-6.80	Moderately resistant (MR)
05	EC-667352; EC-667356; EC-667366; EC-667370; EC-667455; EC-667373; EC-667380; EC-667382; EC-667383; EC-667385; EC-667387; EC-667388; EC-667393; EC-667398; EC-667401; EC-667402; EC-667404; EC-667405; EC-667406; EC-667407; EC-667409; EC-667412; EC-667415; EC-667416; EC-667421; EC-667422; EC-667424; EC-667428; EC-667432; EC-667437; EC-667438; EC-667440; EC-667441;	3.33-48.28	5.00-5.87	Moderately susceptible (MS)
06	EC-667372; EC-667384; EC-667386; EC-667389; EC-667394; DL-36; EC-667399; EC-667408; EC-667413; EC-667414; EC-667418; EC-667419; EC-667429; EC-667430; EC-667431; EC-667435; EC-667439; EC-667443; EC-667444	3.03-28.33	4.00-4.93	Moderately susceptible to susceptible (MSS)
07	EC-667381; EC-667390; EC-667392; EC667396; EC-667417; Jyoti; EC-667434; EC-667442; EC-667445; EC-667446	6.30-32.53	3.13-3.97	Susceptible (S)
08	ND		02	Highly susceptible (HS)
09	ND		01	Very highly susceptible (VHS)

ND: not determined during our study

Metabolic pathways in resistant genotypes produce several secondary byproducts and can contribute to the development of crop protection strategies [13]. However, scarce data are available on the barley genotypes and other landraces to show promising resistance against barley rust [14]. In resistant genotypes, high total phenol and flavonoid contents are considered resistant to leaf rust as compared to other test germplasm because of different genetic makeup and biochemical composition [15]. Besides, the occurrence of leaf rust or other diseases on the plants, reduces leaf area, size, and low content of metabolites which are responsible for the low immunity to plants against various defense mechanisms. The present study revealed low phenol and flavonoid content in susceptible germplasm as compared to resistant germplasm.

The physiological abnormality and/or significant disruption of plant normal health is broadly referred to as a disease. However, plants respond to disease susceptibility that is different compared to the animal system [13]. The form of a stunning array of structural, chemical, nutritional, and protein-based defensive mechanisms against various disease-causing agents. The first documentation on the status of barley diseases from the high altitude cold arid Trans-Himalayan Ladakh region of India has been explored by Vaish et al., (2011). Yellow rust, powdery mildew, leaf spot blotch/blight, covered smut, loose smut, foot/root rot, and molya disease were reported by Vaish et al., (2011) during the survey [14]. Some barley cultivars show disease brown rust when infected with *Puccinia hordei* Otth. Brown rust is a sporadic but most common disease of barley [15]. Like other barley-grown areas, Ladakh is also susceptible to brown rust. Brown rust shows its presence in all growing areas and is one of the most widely distributed rust than other rusts. According to Nayar et al. [16], wheat rusts shifty pathogens that shows the capability of aerial spread, multiple geometrically and can cause epiphytotics. In this current study, the symptoms of the disease are small orange-brown circular spore masses surrounded by 33 genotypes were placed under moderately susceptible, 19 under moderately susceptible to susceptible, and 10 genotypes under the susceptible category. Generally, brown rust occurs in all the barley-growing areas of India, but this pathogen seldom causes severe epidemics over a wide area. Still, significant yield losses can occur in susceptible cultivars when the inoculum arrives early and levels are high [6]. Das et al., (2007) reported 60% yield losses in highly susceptible

Table 3. Correlation studies of barley leaf rust resistance in the barley germplasm in Ladakh.

Traits	Leaf rust scale	Leaf area	Phenol	Flavonoid
Leaf rust scale	1.00	-	-	-
Leaf area	0.16	1.00	-	-
Phenol	0.27**	-0.03	1.00	-
Flavonoid	0.06	-0.09	0.36**	1.00

barley cultivars under experimental conditions [17]. Vander Plank (1963) stated that high-yielding and well-adapted cultivars when grown extensively over a large area were very much prone to diseases than other traditional cultivars [18].

Every stage of barley is equally susceptible to various diseases and when the appropriate measure will be taken, the spreading of various diseases can be halted. One such measure is fungicide seed dressings or fungicides applied in-furrow with fertilizer can be useful in disease protection or suppression of early seedling infection. Foliar fungicide is used to delay disease development and for the maintenance of the green leaf area, which reduces the impact of diseases on the production and the quality of barley. However foliar fungicide application and its cost-effectiveness are dependent on the disease severity, yield potential of the crop, susceptibility of the genotype, grain quality outlook as well as the abiotic factors surrounding the crop growing areas. According to Gangwar et al., (2018), a temperature ranging from 20-25ºC and prolonged high humidity are pre-requisite for the rapid spread of barley diseases [6].

Such controlling measures are not the permanent solution for the control of rust as well as for other barley diseases. Resistance towards many fungicides in barley to *Puccinia hordei* is widely available. Growing resistant genotypes to many pathogens is result oriented and is not dependent on changing environmental factors. Cultivation of genotypes with resistance against barley leaf rust (*Puccinia hordei*) is appreciable and needs an hour in India as well as in UT of Ladakh. Some barley genotypes are capable of recognizing *Puccinia hordei* Otth and limiting brown rust by showing resistance as an incompatible response. 38 genotypes are categorized under the highly resistant, resistant, and moderately resistant categories. As per Clifford (1985), two types of resistance to rust-causing pathogens are hypersensitivity and non-hypersensitivity resistance which former one governed by Rph genes, which are race-specific and associated with necrosis of plant cells [19]. The latter one also referred to as partial resistance and is not associated with plant cell necrosis [20]. In field trials, the resistant, moderately resistant, and highly resistant genotypes against *Puccinia hordei* are characterized by low levels of infestation despite a high level of infestation. The resistant varieties in the present study can mainly be ascribed to slightly high levels of phenol and flavonoid contents in the evaluated genotypes.

The defense response cascades, producing an array of defensive compounds (secondary metabolites and biochemical compounds), and other defensive enzymes that deter the colonization of diseases and inhibit the growth and development of diseases [21]. Plants have developed unique inner mechanisms against insects and pathogens [22]. When pathogens attack mechanical barriers which leads to infection, plant signaling receptors and pathways show the expression of defense response genes. The higher levels of flavonoid in resistant barley germplasm are due to the strong induction of the plant signaling pathways [23]. The higher phenols induced in plants in response to disease occurrence are important plant defensive traits and accumulate in plants when attacked by the diseases, and by some abiotic factors, thus enabling plants to withstand diseases.

After analyzing regression and R-squared analysis of the data, it has been found that there is a positive correlation between the leaf rust scale and the leaf area of *Hordeum vulgare.* As the leaf rust scale increases by one unit, we predict a leaf area to increase by 1.598. Furthermore, 2.78% of the variation in leaf area is accounted for its regression on the leaf rust scale (Fig. 1). While plotting the correlation between the leaf rust scale and phenol, it can be depicted from Fig. 2 that there is a positive linear association. As the leaf rust scale increases by one unit, we predict a phenol content to increase by 14.198. The variation in phenol content (7.05%) is accounted for by its regression on the leaf rust scale. While as the variation in flavonoid content (0.30%) is accounted for its regression on the leaf rust scale and a prediction of flavonoid content to be increased by 1.6412 with a unit change in the leaf rust scale (Fig. 3 and Table 4). The minimum value of phenol (51.20) as well as flavonoid (17.37) content was present in EC-667368 and is characterized as a moderately resistant (MR) variety. Furthermore, the highest value of phenol and flavonoid was present in EC-667366 and EC-667431 genotypes respectively. The lowest leaf area (3.03 cm) as well as the highest flavonoid content was present in EC-667431 and is categorized as per scale as moderately susceptible to susceptible (MSS) category.

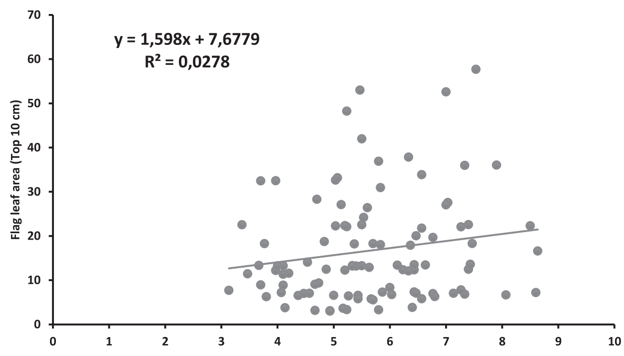

Fig. 1. Leaf rust scale and leaf area of barley genotypes.

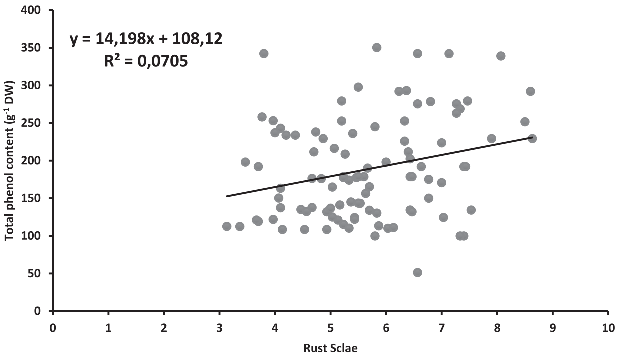

Fig. 2. Leaf rust scale and phenol content of barley genotypes.

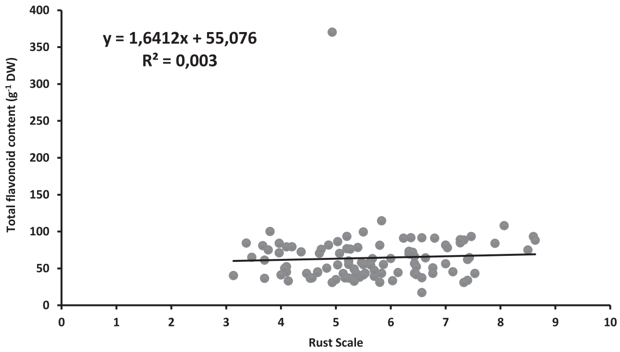

Fig. 3. Leaf rust scale and flavonoid content of barley genotypes.

Table 4. Evaluation of barley leaf rust resistance in the barley germplasm in Ladakh

Entry	Genotype	Leaf rust scale (%)	Leaf area (cm)	Phenol (g^{-1} DW)	Flavonoid (g^{-1} DW)
1	SHEIKH/KP-706	8.07	6.70	339.00	108.12
2	SHEIKH-B1	8.60	7.23	292.00	93.32
3	SHEIKH-638	6.23	12.40	292.00	91.11
4	SHEIKH-634	6.37	17.93	293.00	91.76
5	SHEIKH-636	8.63	16.63	229.20	88.32
6	IC-062189	6.33	37.87	225.80	69.67
7	IC-062190	8.50	22.33	251.60	75.09
8	EC-667350	7.40	22.60	192.00	62.22
9	EC-663751	6.33	12.13	252.60	73.31
10	EC-667352	5.80	36.93	99.80	31.24
11	EC-667353	7.33	6.87	268.80	88.54
12	EC-667355	7.40	12.50	99.80	33.97
13	EC-667356	5.20	12.30	252.60	76.84
14	EC-667358	7.00	52.63	170.60	56.45
15	EC-667360	7.53	57.73	134.24	43.33
16	EC-667361	7.03	27.60	124.40	78.23
17	EC-667363	6.63	13.47	192.00	64.45
18	EC-667364	7.33	36.00	99.80	31.04
19	EC-667365	7.90	36.07	229.20	84.11
20	EC-667366	5.83	18.07	350.20	114.71
21	EC-667367	7.27	7.83	263.00	84.79
22	EC-667368	6.57	5.83	51.20	17.37
23	EC-667369	6.43	7.37	178.60	56.70
24	EC-667370	5.60	26.43	178.60	57.19
25	EC-667371	6.77	19.70	175.00	50.93
26	EC-667372	4.47	7.03	135.00	43.35
27	Jyoti	3.70	6.60	124.30	40.51
28	EC-667373	5.23	22.13	115.20	37.00
29	EC-667374	7.00	27.07	223.60	81.76

Table 4. Continued.

Entry	Genotype	Leaf rust scale (%)	Leaf area (cm)	Phenol (g^{-1} DW)	Flavonoid (g^{-1} DW)
30	EC-667375	7.47	18.33	279.20	93.41
31	EC-667377	7.27	22.10	275.40	89.06
32	EC-667379	6.43	12.37	134.30	43.50
33	EC-667380	5.03	32.67	164.80	54.90
34	EC-667381	3.97	32.53	121.80	71.26
35	EC-667382	5.13	27.13	121.00	43.24
36	EC-667383	5.23	48.27	178.60	60.31
37	EC-667384	4.70	28.33	211.60	70.55
38	EC-667385	5.47	53.03	177.40	58.05
39	EC-667386	4.87	12.50	229.20	81.77
40	EC-667387	5.33	13.23	174.00	49.23
41	EC-667388	5.26	6.47	208.60	76.27
42	EC-667389	4.53	14.10	108.40	36.73
43	EC-667390	3.97	12.23	253.00	84.23
44	EC-667391	7.43	13.63	192.00	64.75
45	EC-667392	3.47	11.47	198.00	65.22
46	EC-667393	5.40	13.23	236.00	78.54
47	EC-667394	4.20	11.60	233.80	79.43
48	EC-667454	6.57	11.37	163.20	52.44
49	EC667396	3.70	32.50	192.00	61.33
50	EC-667397	6.47	20.07	178.60	52.46
51	EC-667398	5.67	5.80	190.00	63.43
52	EC-667399	4.10	13.30	137.30	45.33
53	EC-667401	5.83	30.97	130.20	43.45
54	EC-667402	5.07	33.20	216.20	70.45
55	EC-667403	6.57	21.80	275.40	91.67
56	EC-667404	5.50	22.57	297.60	99.54
57	EC-667405	5.53	24.23	143.20	43.53
58	EC-667406	5.43	5.83	122.00	38.22
59	EC-667407	5.70	18.30	134.00	39.45
60	EC-667408	4.83	18.77	176.00	50.45
61	EC-667409	5.70	5.57	165.20	47.47
62	EC-667410	6.77	7.00	150.00	43.41
63	EC-667411	6.80	6.33	278.40	91.15
64	EC-667412	5.23	3.37	177.40	55.53
65	EC-667413	4.37	6.57	233.80	72.55
66	EC-667414	4.67	3.20	137.60	45.44
67	EC-667415	5.50	13.30	178.60	55.38
68	EC-667416	5.20	22.37	279.20	93.57

Table 4. Continued.

Entry	Genotype	Leaf rust scale (%)	Leaf area (cm)	Phenol (g^{-1} DW)	Flavonoid (g^{-1} DW)
69	EC-667417	3.80	6.30	342.20	100.21
70	EC-667418	4.13	3.80	108.40	33.11
71	EC-667419	4.93	3.13	108.40	31.00
72	EC-667420	6.43	13.53	202.40	66.29
73	EC-667421	5.37	18.23	145.00	43.48
74	EC-667422	5.63	12.93	156.31	54.78
75	EC-667423	6.47	7.10	132.00	41.67
76	EC-667424	5.33	13.33	110.20	32.76
77	EC-667425	6.40	3.87	211.60	71.98
78	EC-667426	6.00	8.37	198.00	63.54
79	EC-667455	6.57	8.97	119.10	36.66
80	EC-667427	6.03	6.77	110.00	33.45
81	EC-667428	5.80	3.33	245.00	81.54
82	EC-667429	4.73	9.40	238.00	76.00
83	EC-667430	4.07	7.23	150.20	50.21
84	EC-667431	4.93	3.03	132.00	370.45
85	EC-667432	5.17	3.67	141.00	37.21
86	EC-667434	3.67	13.37	121.00	81.00
87	EC-667435	4.10	8.90	243.00	79.34
88	EC-667436	7.13	7.07	342.10	45.56
89	EC-667437	5.00	6.60	136.70	34.97
90	EC-667438	5.87	7.33	113.30	55.45
91	EC-667439	4.67	9.10	176.23	44.56
92	EC-667440	5.50	42.00	143.50	41.25
93	EC-667441	5.03	22.30	125.00	86.45
94	EC-667442	3.77	18.30	258.00	75.25
95	EC-667443	4.00	13.27	237.00	41.31
96	EC-667444	4.57	7.07	132.30	37.23
97	EC-667445	3.13	7.73	112.50	40.45
98	EC-667446	3.37	22.57	112.40	84.51
99	DL-36	4.10	33.90	342.20	37.45
100	EC-667447	6.13	13.43	111.00	44.55
	Fpr	<0.001	<0.001	-	-
	Vr	2.90	133.84	-	-
	Mean	5.66	16.72	-	-
	SE±	0.74	1.05	6.74	3.75
	LSD	2.06	2.91	67.44	37.50
	CV%	22.70	10.80	35.79	58.26

Accumulation of phenol and flavonoid contents in barley leaves plays an important role in the rust resistance of *Puccinia hordei*. The study shows dissimilar results with the resistant germplasms of malus with high flavonoid contents [24]. Similarly, higher levels of phenolic compounds have been observed in resistant than in susceptible wheat cultivars [25]. Furthermore, various workers [26, 27], demonstrated a high positive correlation between free phenolic compounds and antioxidant capacity, which leads to enhanced plant immunity and adaptation to stress stimuli [23-28]. Besides the antioxidant properties of many cereals due to anthocyanins, phenolic acids, flavonols, flavans, and/or other phytochemical compounds [29], barley shows a great resistance against barley leaf rust (*Puccinia hordei*) due to their high phenol and flavonoid content.

Conclusions

This study infers novel insights into genotype-specific disease resistance and susceptibility to *Puccinia hordei* causing rust disease in barley. In this study, 100 barley genotypes showed divergence towards leaf rust infection and their biochemical responses against *P. hordei*. Among the genotypes, SHEIKH/KP-706, SHEIKH-B1, SHEIKH-636, and IC-062190 exhibited high resistance reaction to barley leaf rust caused by *Puccinia hordei*, while barley genotypes EC-667381, EC-667390, EC-667392, EC667396, EC-667417, Jyoti, EC-667434, EC-667442, EC-667445, and EC-667446 were found to be susceptible to *P. hordei*. This study further explored the high level of metabolite accumulation in the barley genotypes and its expression was correlated to the disease susceptibility index of a particular genotype. The identified resistant genotypes will be used in the barley-breeding program of SKUAST Kashmir for the introgression of barley leaf rust into the high yielding and adapted barley cultivars.

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Conflict of Interest

The authors declare no conflict of interest.

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