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HISTOCHEMICAL AND PHENOTYPIC STUDIES REVEALED THE DIVERSE NATURE OF WHEAT-YELLOW RUST INTERACTION

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ABSTRACT

Wheat stripe rust disease, produced by *Puccinia striiformis* f. sp. *tritici* (*Pst*), causes severe yield reduction worldwide. With the advent of modern wheat varieties, the sources of rust resistance are eroding. Hence a constant search for resistant genotypes is necessary. Thirty broad-based elite lines and landraces of wheat were characterized for their agronomic traits and assessed for the adult plant resistance against wheat stripe rust disease inside a trap nursery. Furthermore, the chemical response was assessed at cellular level. The landraces and elite lines displayed a diverse nature of host-pathogen interactions. The landrace LLR8 showed a hypersensitive response in the field. Seven genotypes were highly resistant while 07 were moderately resistant at the adult plant stage. The genotype RS1 showed maximum necrosis (2896 µm) indicating moderately resistant (10MR) under field conditions. Among resistant genotypes, the number of hyphae at the infection site were less compared to the susceptible genotypes. The stripe rust fungal colonies were initially larger but with time the fungal colony size decreased, might be the result of the synchronized initiation of defense mechanism. The resistant genotypes also showed higher values for the hypersensitivity index. Multivariate discriminant analysis for agronomic traits divided the genotypes into low and high yielding groups, where nine genotypes were high yielding while twenty-one were low yielding under high yellow rust disease pressure. The genotypes like LLR8, Pirsabak-04 and RS4 displayed higher grain yield per plant and 1000-grain weight. The plant height and biological yield were good discriminators as they helped to discriminate between the 02 groups. The resistance sources such as LLR35, RS13, RS22, RS30, RS55, RS58, RS64, LLR5, LLR17, LLR33, RS1, RS10, RS43, and RS45 could be beneficial for the development of future cultivars with effective resistance. This genetic material should be utilized immediately for the disease management.

Keywords: Histology, landraces, *Triticum aestivum*, *Puccinia striiformis*, resistance.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most widely grown cereal crops of the world. Wheat production must rise along with an ever-increasing human population, but it is hampered by many abiotic and biotic factors (Imran *et al*., 2016; Hafeez *et al*., 2016; Kutlu and Sirel, 2019). Wheat stripe rust disease produced by *Puccinia striiformis* is one of the major biotic factors (Rehman *et al*., 2019). Huge losses are reported in different areas, years and environments (Singh *et al*., 2016; Amil *et al*., 2020). Yield losses as high as 75% have been reported in highly susceptible cultivars. Severe yield losses are caused by the development of new races and the unpredictable nature of this fungus (Wellings, 2011; Sorensen *et al*., 2017; Aboukhaddour *et al*., 2020).

The wheat stripe rust disease has been reported as an increasing problem (Walter *et al*., 2016; Hovmoller *et*

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al., 2016) due to various reasons, such as its migratory nature, higher mutation rate from avirulence to virulence, adaptation to different climatic conditions (Thach *et al*., 2016) and the capacity to form new variants via sexual cycle to overcome resistance. The damaged crop produces seeds with low vigor and reduced germination. To defend against the wheat stripe rust pathogen, the host plants have also devised both active and passive mechanisms. Pathogens capable of overturning the passive system, containing chemical and physical barriers, are confronted by two layers of protection. The first level of active defense is based on molecular structures that result in either the collapse of host defense or pathogen structures during the process of infection. Pathogens have developed specific small effector proteins that act by blocking the initial level of active defense (Toruno *et al*., 2016). The second level of protection is known as the hypersensitive response (HR), whereby the plant cellular receptors identify the effector proteins produced by the pathogen and induce senescence surrounding the place of infection. This HR cuts the pathogen nutrient supply from the host tissues (Niks *et al*., 2015). It is presumed that HR is the result of effector recognition by the second-level active defense leading to the encasement of the pathogen colony (Sorensen *et al*., 2017).

Early seeding and foliar fungicide applications are the main strategies to control wheat rusts (Carmona *et al*., 2020). However, chemical control causes environmental pollution (Gul *et al*., 2019). Wheat stripe rust pathogen can also be controlled by deploying genetic resistance, which is economical and environment-friendly (Singh *et al*., 2016). Production of wheat depends on the development and utilization of well-characterized genetically resistant varieties. The resistance against the wheat stripe rust disease can be assessed at the seedling and adult plant stage. The former is presumed to be the result of a solitary gene; thus, it is usually race-specific. Alternatively, adult plant resistance (APR) develops as plants mature and deliberated as more reliable than race-specific resistance. It is expressed in the seedling stage but shows maximum expression at the adult plant stage (Awan *et al*., 2017).

Adult plant resistance can be assessed by observing the disease at the tissue level known as histochemical studies. These studies have been found useful to obtain knowledge about the pathogen infection biology and the cellular modifications in host and pathogen during infection (Minker *et al*., 2018). The local wheat landraces developed over time while being adapted to their ecological and agricultural environment are the reservoirs of resistance against the biotic and abiotic stress-causing factors that limit the quality and quantity of wheat production. The documentation of these genetic resources is crucial in plant breeding to fight against pest and diseases (Akcura *et al*., 2017). Considering this, a study was initiated to assess the agronomic performance and diversity of wheat genotypes under high wheat stripe rust disease pressure and to estimate their phenotypic and histochemical response against wheat stripe rust disease.

MATERIAL AND METHODS

Field experiment: The field studies were performed in an area characterized by a temperate climate having the widespread population of *Berberis* (Azim *et al*., 2018), the alternate hosts of the wheat stripe rust pathogen (Jin *et al*., 2010). Average rainfall during the stripe rust disease development months of April and May was 66.70mm and 150.55mm with a relative humidity of 52% and 64% during the years 2015-2016 and 2016-2017 respectively.

The genotypes were planted inside a trap nursery during the Rabi seasons of 2015-16 and 2016-17 in the same plot. A total of 30 wheat genotypes of diverse origin were used, included 10 landraces (LLR), 18 elite lines (RS) selected from CIMMYT material and two check varieties (Pirsabak-04 and Nesser). A certified seed of cultivar Morocco (spreader and susceptible check) was obtained from Crop Disease Research Institute, NARC-Islamabad. The experimental units were laid out in an augmented complete block arrangement. The check varieties were subsequently repeated after 10 entries. Two rows of 2-meter length were planted with each genotype while 30cm distance was maintained between the rows. The cultivar Morocco was sown in two rows around the trial. Fertilizers such as urea and di-ammonium phosphate were used as 123.55 kg/ha, while the urea was added in two doses.

Disease response: The natural infection allowed us to record data on stripe rust without artificial

inoculation as Cheng *et al*. (2014). Data collection started when the severity on Morocco was almost 90%, and the grains were at the milk stage (Feekes 10.54-11.1). Ten flag leaves were randomly selected form each variety. Data on disease severity and response to wheat stripe rust pathogen was noted (Leogering, 1959). Modified Cobb's Scale was utilized to observe severity for two consecutive seasons (Peterson *et al*., 1948) the maximum recorded severity and response was considered as final response against wheat stripe rust pathogen.

Assessment of cellular response: For histochemical studies, clearing and fixing of flag leaf samples were done as described by Carver *et al*. (1991). Since under favourable conditions, the fungus remains spreading within the leaf for approximately 7 to 10 days post infection (dpi) for wheat rust and then the first symptom of uredinial growth becomes obvious (Bolton *et al.,* 2008). Hence, the leaves were collected twice i.e., 10 and 20 dpi. A Trypan blue (0.1%) solution was used to stain the leaves, which were then washed, cleared and observed under a light microscope. The parameters studied include the number of infections per microscopic field, fungal colony area, necrotic area, and hypersensitivity index. The observations for each parameter were taken as triplicate averaged and standardized (Hair *et al*., 2009) to conduct cluster analysis using the Ward's method with the help of a statistical package PAST V. 3.20 (Hammer *et al*., 2001).

Assessment of metric traits: Ten randomly selected guarded plants were selected each year to record data on parameters such as days to 50% heading, flag leaf area, plant height, number of tillers plant^{-1}, peduncle length, spike length, number of spikelets spike^{-1}, thousand grain weight, grain yield plant^{-1}and biological yield plant^{-1}. The data on days to 50% heading was recorded when half of the plants of a particular row reached heading stage while flag leaf area was taken when flag leaf was fully extended and turgid. For rest of the parameters data were recorded at plant maturity.

A combination of cluster analysis and factorial procedures suggested by Lebart *et al*. (2006) was utilized as statistical analysis. The data of 2 seasons were averaged and analysed for analysis of variance following Snedecor and Cochran, (1989). To classify the genotypes into high and low yielding groups and their discriminating factors, multivariate discriminant analysis was performed as Fisher, (1936) using IBM SPSS Statistics, Version 20 (SPSS, 2011).

RESULTS

Disease severity under field conditions against wheat stripe rust pathogen: Wheat stripe rust disease severity was recorded when severity on Morocco was approximately 90S under field conditions. The data indicated that seven genotypes including RS13, RS22, RS30, LLR35, RS55, RS58, and RS64 exhibited complete resistance (Table 1). The genotypes LLR5, LLR17, LLR33, RS10, RS43, and RS45 indicated moderately resistance response ranging from 5MR to 40MR while LLR8, LLR14, LLR41, RS1, RS4, RS23, RS61, RS9, and RS51 showed an intermediate response against the wheat stripe rust pathogen. The genotypes which were found susceptible against the wheat stripe rust pathogen included LLR44 (90S), LLR32 (60S), RS50 (40S) and RS46 (10S). While the check varieties, Nesser and Pirsabak-04 showed a moderate susceptible reaction against wheat stripe rust pathogen (60MS and 10MS respectively).

Histochemical response to wheat stripe rust disease: For histochemical studies, 04 parameters were included.

Number of infections per microscopic field: The number of infections per microscopic field increased from 10 to 20 days post infection (dpi). Mean values for the number of infections per microscopic field ranged from 1 to 11 infections at 10 dpi. However, at 20 dpi the mean values varied between 1 to 16 infections (Figure 1 & 5). At 20 dpi the genotype RS1 (n=16) showed highest value followed by RS9 (n=12), LLR44 (n=11) however, at 10 dpi the number of infections per microscopic field were n=8 in RS1, n=4 in RS61, n=9 in RS9 and LLR44. The check variety, Pirsabak-04 showed least number of infections at 20 dpi (n=1). After 10 days post infection, the highest number of infections were observed in the genotypes LLR44 and RS32 (n=11), showing 90S and 60S respectively under field conditions. The least number of infections were displayed by genotypes RS13, RS58, RS10, RS22, RS46, RS55 and RS30 (n=1), showing resistant response against wheat stripe rust disease under field conditions.

Table 1. Response of 30 broad based bread wheat genotypes against wheat stripe rust disease under natural conditions. The experiment was conducted at Rawalakot, Azad Kashmir during Rabi seasons of 2015-16 and 2016-17

		Severity				Severity	
S. No	Genotypes	2016	2017	S. No	Genotypes	2016	2017
1	LLR11	40MS	50MS	16	RS23	40MRMS	30MRMS
2	LLR14	40MRMS	30MRMS	17	RS61	20MRMS	20MRMS
3	LLR17	20MR	30MR	18	RS10	20MR	30MR
4	LLR35	R	R	19	RS9	20MRMS	10MRMS
5	LLR33	40MR	30MR	20	RS51	20MRMS	20MRMS
6	LLR5	40MR	40MR	21	RS43	5MR	10MR
7	LLR44	90S	80S	22	RS30	R	R
8	LLR8	40MRMS	30MRMS	23	RS13	R	R
9	LLR41	20MRMS	20MRMS	24	RS45	10MR	10MR
10	LLR32	60S	70S	25	RS64	R	R
11	RS58	R	R	26	RS46	10S	20S
12	RS32	60MS	50MS	27	RS22	R	R
13	RS1	10MR	10MR	28	RS55	R	R
14	RS4	40MRMS	40MRMS	29	Pirsabak-04	10MS	10MS
15	RS50	40S	50S	30	Nesser	60MS	50MS

R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible

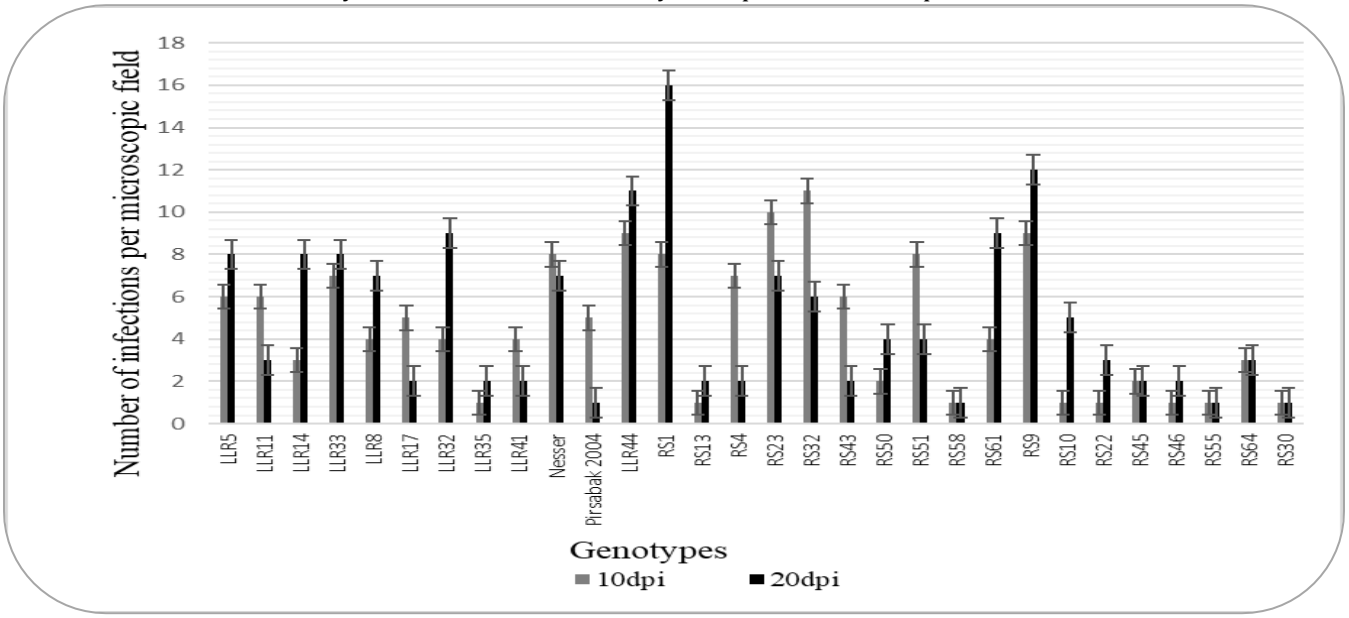


Figure 1. Mean values showing number of infections per microscopic field against wheat stripe rust pathogen at 10 and 20 dpi in 30 broad based bread wheat genotypes

Bars showing standard error at 5% level of significance
DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers Plant^{-1}, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets Spike^{-1}, 1000GW = Thousand Grain Weight, GY = Grain Yield Plant^{-1}, BY = Biological Yield Plant^{-1}

Necrotic area: The mean necrotic area ranged from 36-2467 µm at 10 dpi (Figure 2). The highest value was observed in RS61 (2467 µm) however, at 20 dpi necrotic area of RS61 decreased to 1509 µm. The lowest value was noted in genotype RS 43 (36 µm) at 10 days post infection (dpi). At 20 dpi the necrotic area increased and ranged from 38 to 2896 µm. The necrotic area of RS43 also enlarged to 857 µm at 20 dpi. The advanced line RS1 showed highly necrotic leaf area (2896 µm) while RS45 revealed smallest necrotic area of 38 µm (Figure 5).

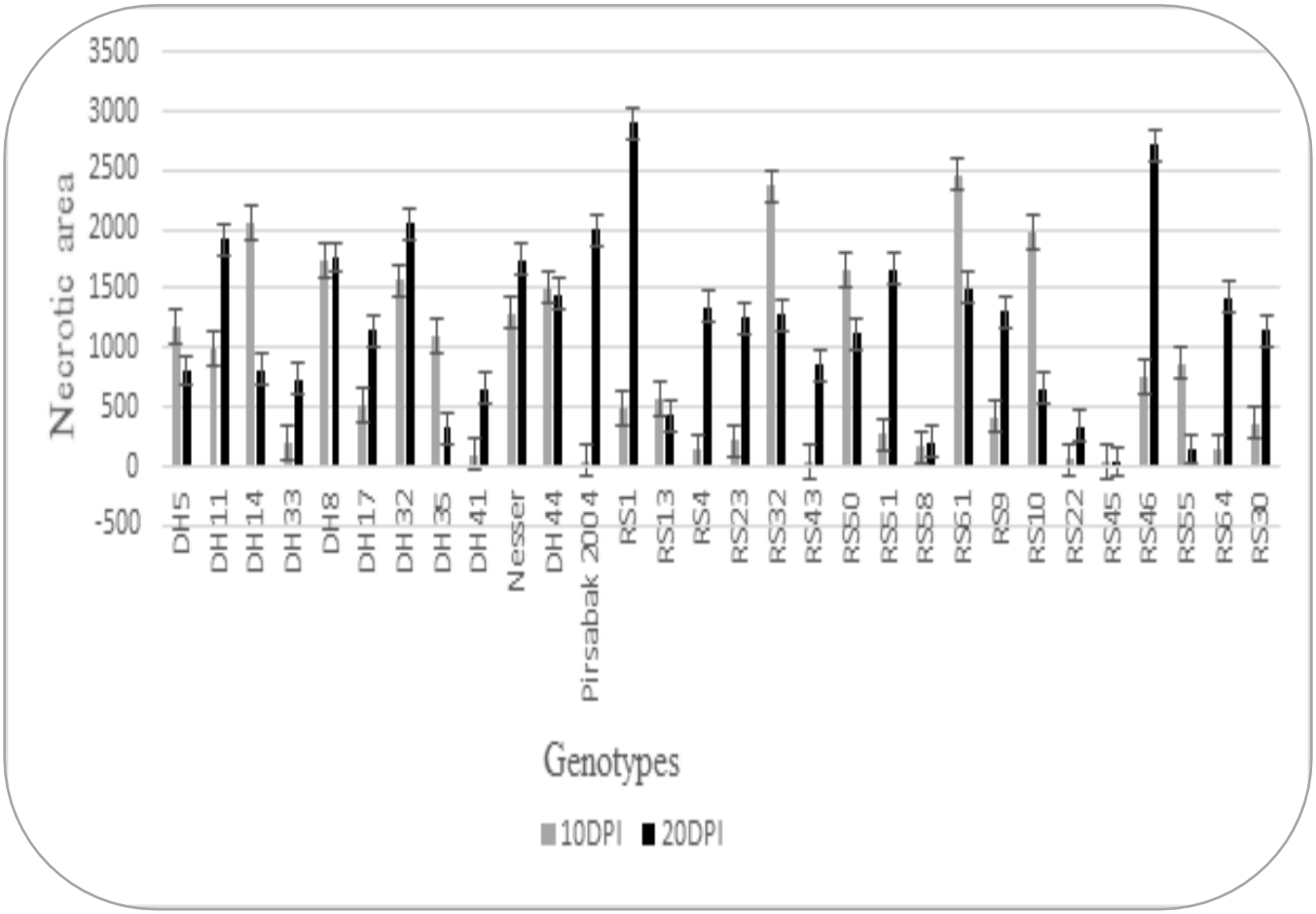


Figure 2. Mean values showing necrotic area against wheat stripe rust pathogen at 10 and 20 dpi in 30 broad based bread wheat genotypes

Bars showing standard error at 5% level of significance
DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers Plant^{-1}, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets Spike^{-1}, 1000GW = Thousand Grain Weight, GY = Grain Yield Plant^{-1}, BY = Biological Yield Plant^{-1}

Fungal colony size: The maximum fungal colony size was observed in the genotype RS46 at 10 dpi (Figure 3 & 5). Wheat stripe rust disease colonies on the leaves of the genotype RS46 were 258 µm in size but at 20 dpi its fungal colony size reduced to 125 µm. No fungal colony was observed in the genotypes LLR14, LLR17, RS55, and RS50 at 10 dpi (Figure 5) but at 20 dpi fungal colonies developed more rapidly, and the situation was reversed as genotypes showed large fungal colony size (119 µm, 99 µm, 105 µm, 197 µm, and 67 µm respectively). The fungal colony size increased at 20 dpi in susceptible wheat genotype like RS50 (197 µm) while the least fungal colony size was observed on landrace LLR41 (67 µm). No fungal colony was recorded on both the genotypes at 10 dpi but at 20 dpi stripe rust colonies of RS50 and LLR41 increased to 197 µm and 67 µm respectively.

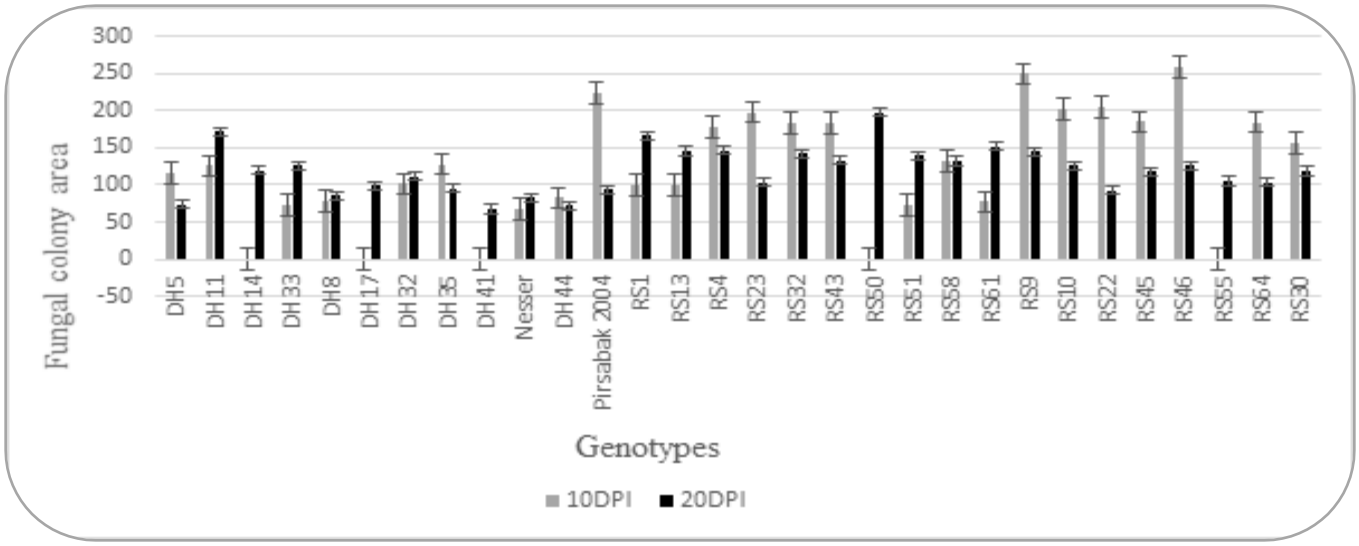


Figure 3. Mean values indicating fungal colony area against wheat stripe rust disease at 10 and 20 dpi in 30 broad based bread wheat genotypes

Bars showing standard error at 5% level of significance

DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers Plant^{-1}, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets Spike^{-1}, 1000GW = Thousand Grain Weight, GY = Grain Yield Plant^{-1}, BY = Biological Yield Plant^{-1}

Hypersensitivity index (HI): At 10 dpi hypersensitivity index ranged from 0 to 31% (Figure 4). The maximum value was observed in RS61 (31%). The minimum value was observed in LLR14, LLR17, LLR41, RS50, RS55, Pirsabak-04, RS4, RS43, RS22 and RS45 (0%). At 20 dpi the genotype RS46 displayed the highest HI (21%); it showed susceptible field response (10S). The lowest HI was shown by the genotypes RS45 and LLR17 (0%) at 20 dpi, displaying a moderately resistant response under field conditions (10MR and 20MR respectively).

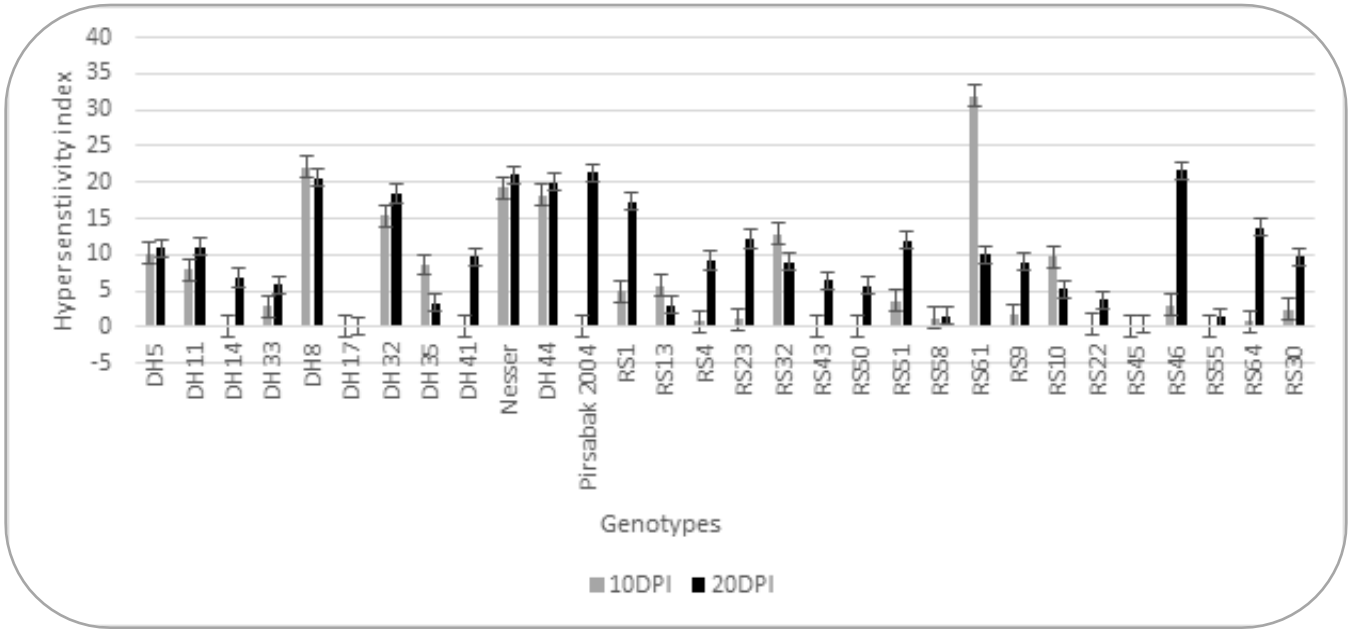


Figure 4. Mean values depicting hypersensitivity index against wheat stripe rust pathogen at 10 and 20 dpi in 30 broad based bread wheat genotypes

Bars showing standard error at 5% level of significance

DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers Plant^{-1}, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets Spike^{-1}, 1000GW = Thousand Grain Weight, GY = Grain Yield Plant^{-1}, BY = Biological Yield Plant^{-1}

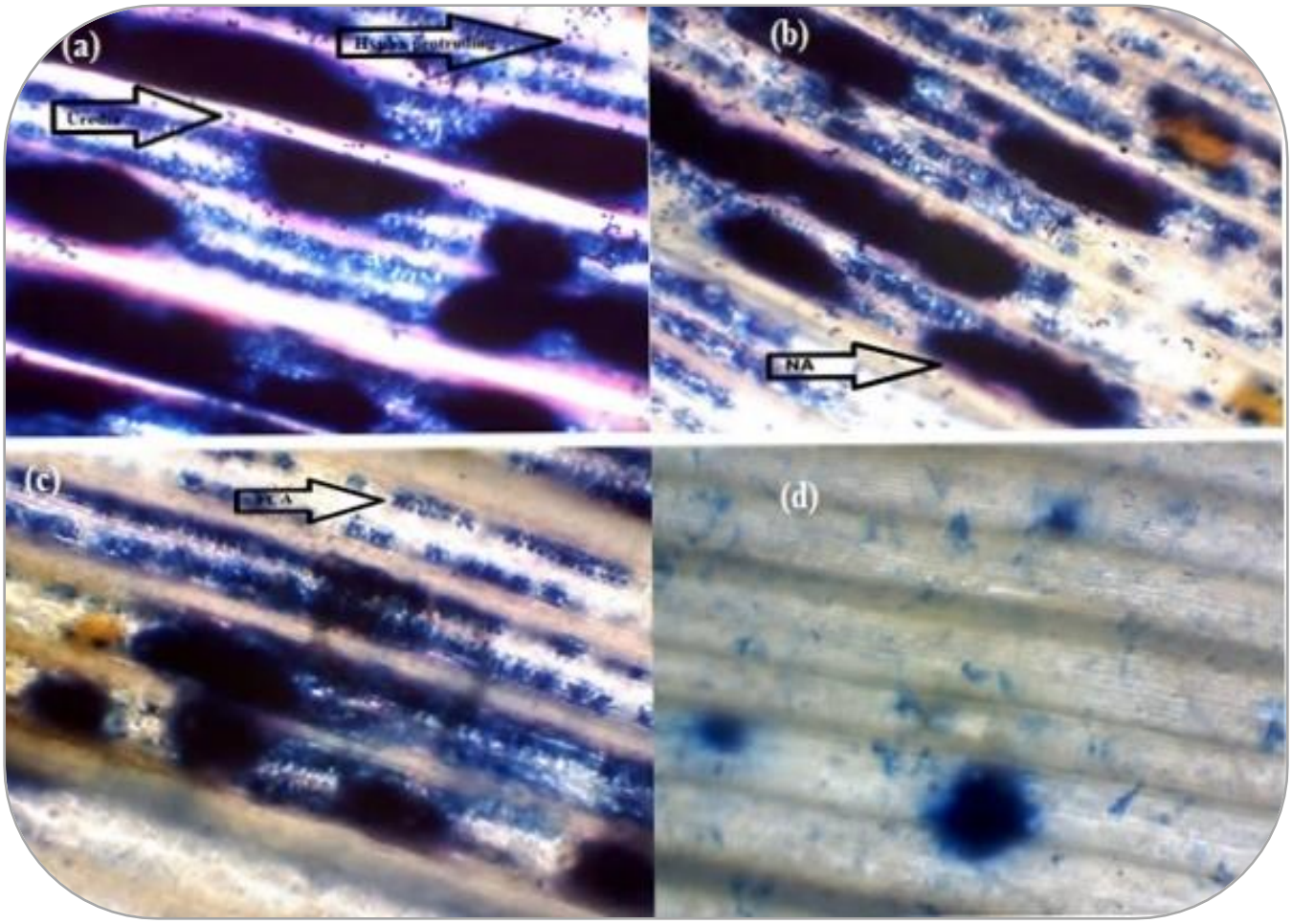
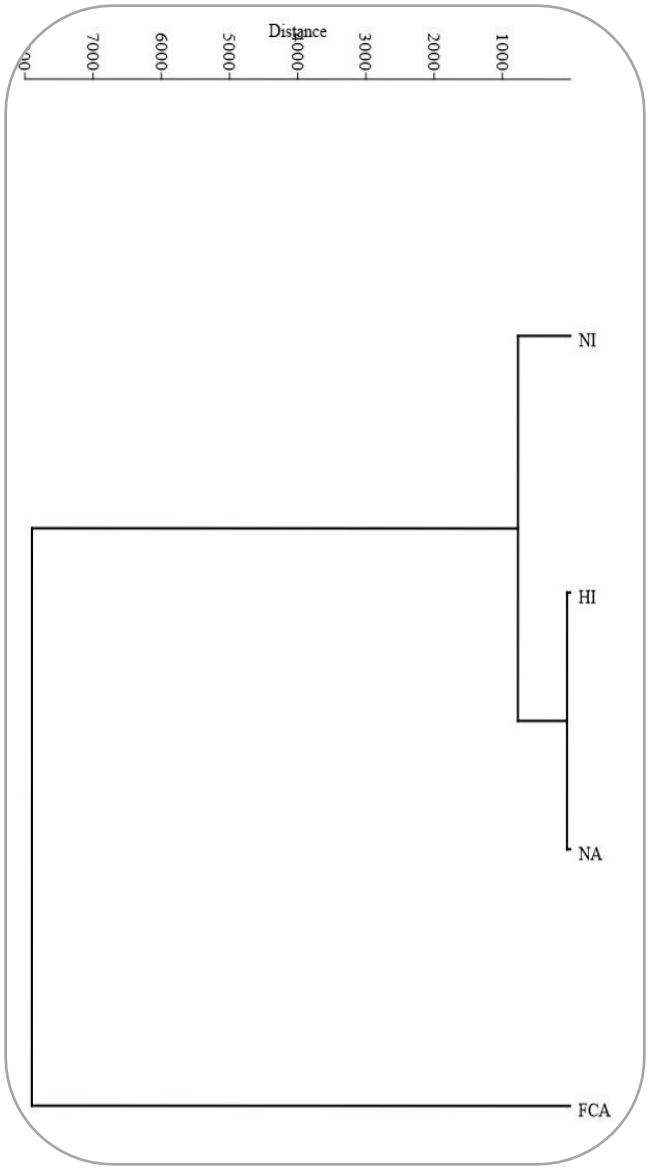


Figure 5. Histochemical response of 30 bread wheat genotypes against wheat stripe rust pathogen
(a) Higher number of infections per microscopic field in the genotype RS32 (b) Large necrotic area detected in the genotype RS1 at 20 dpi (c) The genotype LLR41 showing minimum fungal colony area under light microscopy (d) The genotype LLR17 showing no fungal colony at 10 dpi

The dendrogram constructed for histochemical traits indicated two clusters (Figure 6). The cluster I grouped three traits i.e., necrotic area, number of infections per microscopic field and hypersensitivity index. The lowest linkage distance was observed among the necrotic area and hypersensitivity index specifying that the hypersensitivity index increased with the increase in necrotic area. Similarly, the number of infections per microscopic field also affected the necrotic area and HI. The cluster II terminated to fungal colony size, showing a distant relationship with the other 03 parameters.

Morphological Traits: The days taken to 50% heading were found minimum in the landrace LLR8 (226 days). The mean flag leaf area varied between values of 13 to 35.86cm^2. The genotype RS50 (35.86cm^2) contributed maximum towards flag leaf area followed by Pirsabak-04 (35.53cm^2) and RS4 (35.32cm^2) whereas the least was noted in LLR33 with a value of (13cm^2). The average number of tillers per plant varied between 5 to 13. The highest values for tillers per plant were noted in genotype RS58 and landrace LLR35 (n=13) followed by LLR8 and RS64 (n=12) while minimum value was observed in genotypes LLR14, RS51, RS22 (n=5). The average 1000-grain weight ranged from 29.5-48g. The landrace LLR8 (48g) contributed the maximum for 1000-grain weight followed by Pirsabak-04 (47.5g). The grain yield per plant ranged from 17-234g. For grain yield per plant the maximum values were noted in LLR8 (234g) followed by Pirsabak-04 (212g) and RS4 (208g) while minimum grain yield per plant was shown by landrace LLR5 (17g). The biological yield per plant showed a wide range of variability showing mean values ranging between 47-623g. The highest biological yield per plant was shown by Pirsabak-04 (623g) while the minimum was obtained in RS1 (47g) (Table 2).



NI = Number of infections per microscopic field, FA = Fungal colony area, HI = Hypersensitivity index, NA = Necrotic area

Figure 6. Dendrogram showing association of histochemical traits under wheat stripe rust disease in 30 broad based bread wheat genotypes

Discriminant analysis (DA) for morphological traits: The genotypes were grouped into two distinct sets as low and high yielding (Table 3). The group statistics revealed that the dissimilarity among plant height and biological yield per plant was highest and considered as good discriminators. Both these traits were helpful to discriminate against the genotypes of one yield group from the other. The weighted and unweighted cases showed that out of 30 broad based bread wheat genotypes, 09 were high yielding, and 21 were low yielding under higher wheat stripe rust disease pressure. The scatter plot indicated overlapping among low and high yielding genotypes for all variables except for biological yield per plant and spike length, hence they were the most reliable discriminatory traits (Figure 7).

DISCUSSION

Cereal rust fungi are one of the most serious threats to global agriculture (Fedotova and Bankina, 2018) and resistance against them is limited among germplasm (Rehman *et al*., 2019). The genotypes LLR8, LLR14, LLR41, RS1, RS4, RS9, RS23, RS51 and RS61 showing MRMS reaction indicated horizontal resistance furnished by minor genes which could be exploited to acquire durable resistance. While the genotypes LLR35, RS13, RS22, RS30, RS55, RS58 and RS64 displaying complete resistance maybe utilized in conjugation with other genes to prevent a rapid break down by new races of wheat stripe rust pathogen. The genotype RS1 exhibited the highest necrotic area (2896 µm). The number of necrotic cells per colony and wheat stripe rust resistance is interrelated (Zhang *et al*., 2018), hence fungal development was reduced in the resistant genotypes. A minimum number of hyphae were observed close to the infection sites in resistant genotypes. The necrotic area indicated a programmed cell death in host cells, limiting the nutrient source to the wheat stripe rust fungus (Ma *et al*., 2009). The RS1 was moderately resistant (10MR) at the adult plant stage under field conditions. Similarly, the genotype RS50 showed susceptible reaction (40S) while the landrace LLR41 showed intermediate reaction (20MRMS) under field conditions. The APR found in these landraces is mostly considered durable resistance (Kankwatsa *et al*., 2017; Yuan *et al*., 2018), which could be utilized against new wheat stripe rust races in a breeding program (Long *et al*., 2019).

The wheat stripe rust fungal colonies on the leaves of the genotype RS46 at 10 dpi were 258 µm in size but fungal colony size decreased to 125 µm at 20 dpi. The decrease in fungal colony size might have resulted from the synchronized activation of defense mechanisms (Saleem *et al*., 2019), like biosynthesis of phytoalexin and the production of Pathogenesis Related Proteins (PRP) (Agrios, 2005). The induced lignification might also have avoided the intercellular spread of wheat stripe rust fungus. Lignification of the cell wall has been recognized as a significant method of host resistance in cereal crops to confront wheat rust pathogens. Deposition of lignin results from polymerization of lignin inside the cells causes necrosis in HR. Lesser size of fungal colony in wheat is related to slow rusting genes (Gao *et al*., 2000).

Table 2. Means values and standard deviations for morphological parameters in 30 bread wheat genotypes

Genotype	DTH	FLA (cm^2)	PH (cm)	NT	PL (cm)	SL (cm)	NS	1000GW (g)	GY (g)	BY (g)
LLR5	241 ± 0.14	16.53 ± 1.70	70.00 ± 1.26	9.00 ± 0.31	26.70 ± 0.89	8.40 ± 1.89	17.20 ± 1.40	37.00 ± 0.17	17 ± 1.04	96.43 ± 0.70
LLR8	226 ± 1.09	26.41 ± 0.07	102.30 ± 1.31	12.00 ± 1.00	28.40 ± 0.59	14.00 ± 1.42	24.00 ± 1.60	48.00 ± 2.15	234 ± 2.15	220.46 ± 0.11
LLR11	241 ± 0.14	21.47 ± 0.88	84.10 ± 0.14	10.00 ± 0.13	25.62 ± 1.09	11.08 ± 0.30	18.10 ± 1.00	33.60 ± 0.45	129 ± 0.61	401.08 ± 1.30
LLR14	242 ± 0.07	30.31 ± 0.58	98.50 ± 1.01	5.40 ± 1.89	40.60 ± 1.62	12.74 ± 0.68	22.00 ± 0.72	42.90 ± 1.23	23 ± 0.95	137.33 ± 0.42
LLR17	237 ± 0.39	23.19 ± 0.60	66.30 ± 1.56	10.40 ± 0.30	15.90 ± 2.84	9.00 ± 1.53	18.40 ± 0.87	36.30 ± 0.04	27 ± 0.89	87.93 ± 0.75
LLR32	238 ± 0.33	26.76 ± 0.01	123.10 ± 2.97	11.50 0.78	45.00 ± 2.41	13.16 ± 0.93	22.30 ± 0.85	24.50 ± 2.10	205 ± 1.73	237.98 ± 0.23
LLR33	276 ± 2.09	13.57 ± 2.19	75.50 ± 0.83	8.80 ± 0.40	34.30 ± 0.48	9.76 ± 1.09	18.20 ± 1.00	29.90 ± 1.12	24 ± 0.94	97.70 ± 0.68
LLR35	241 ± 0.14	20.53 ± 1.04	67.50 ± 1.46	13.00 ± 1.44	32.60 ± 0.17	11.80 ± 0.12	21.20 ± 0.36	32.60 ± 0.63	30 ± 0.85	65.64 ± 0.89
LLR41	242 ± 0.07	27.06 ± 0.04	84.30 ± 0.12	10.80 ± 0.48	33.30 ± 0.30	12.50 ± 0.54	22.60 ± 0.98	32.70 ± 0.61	21 ± 0.98	142.40 ± 0.39
LLR44	231 ± 0.77	22.90 ± 0.65	77.30 ± 0.68	8.00 ± 0.75	27.30 ± 0.78	9.60 ± 1.18	17.60 ± 1.22	28.80 ± 1.32	37 ± 0.75	59.93 ± 0.93
RS1	237 ± 0.39	22.36 ± 0.74	79.00 ± 0.55	6.60 ± 1.37	28.80 ± 0.51	10.10 ± 0.88	21.60 ± 0.54	35.90 ± 0.03	39 ± 0.72	47.71 ± 1.01
RS4	241 ± 0.14	35.32 ± 1.41	97.90 ± 0.96	9.50 ± 0.09	31.70 ± 0.01	14.60 ± 1.78	22.40 ± 0.89	38.60 ± 0.45	208 ± 1.77	474.74 ± 1.77
RS9	237 ± 0.39	28.06 ± 0.21	89.45 ± 0.29	11.20 ± 0.65	38.50 ± 1.24	11.85 ± 0.15	21.50 ± 0.50	31.70 ± 0.79	91 ± 0.05	269.68 ± 0.44
RS10	232 ± 0.71	28.49 ± 0.28	75.50 ± 0.83	10.00 ± 0.13	27.04 ± 0.83	12.64 ± 0.62	21.40 ± 0.45	37.10 ± 0.18	65 ± 0.33	150.72 ± 0.34
RS13	241 ± 0.14	31.58 ± 0.79	87.45 ± 0.13	7.90 ± 0.80	36.45 ± 0.87	11.82 ± 0.13	19.70 ± 0.30	37.70 ± 0.29	75 ± 0.19	172.34 ± 0.20
RS22	239 ± 0.26	27.06 ± 0.04	85.70 ± 0.01	5.80 ± 1.72	25.40 ± 1.13	12.00 ± 0.24	20.80 ± 0.19	34.70 ± 0.25	34 ± 0.79	77.12 ± 0.82
RS23	241 ± 0.14	34.56 ± 1.28	88.00 ± 0.17	11.00 ± 0.56	36.60 ± 0.89	13.00 ± 0.83	22.40 ± 0.90	39.30 ± 0.58	79 ± 0.13	125.57 ± 0.50
RS30	233 ± 0.65	25.26 ± 0.26	84.00 ± 0.15	9.10 ± 0.27	34.95 ± 0.60	8.76 ± 1.68	15.20 ± 2.28	38.60 ± 0.45	87 ± 0.01	274.99 ± 0.47
RS32	230 ± 0.84	17.64 ± 1.52	81.40 ± 0.36	10.00 ± 0.13	26.90 ± 0.86	9.50 ± 1.24	16.00 ± 1.93	34.70 ± 0.25	94 ± 0.09	51.25 ± 0.98
RS43	273 ± 1.90	23.54 ± 0.54	83.40 ± 0.20	11.60 ± 0.83	27.75 ± 0.70	13.10 ± 0.89	21.80 ± 0.63	35.50 ± 0.11	116 ± 0.42	275.85 ± 0.48
RS45	276 ± 2.09	28.08 ± 0.21	81.80 ± 0.32	7.10 ± 1.15	32.60 ± 0.17	9.55 ± 1.21	18.40 ± 0.87	40.50 ± 0.80	84 ± 0.05	417.59 ± 1.40
RS46	278 ± 2.22	31.95 ± 0.85	83.20 ± 0.21	6.40 ±1.45	32.00 ± 0.06	11.54 ± 0.03	21.20 ± 0.36	40.00 ± 0.71	45 ± 0.63	123.90 ± 0.51
RS50	233 ± 0.65	35.86 ± 1.50	81.55 ± 0.34	12.40 ± 1.18	32.90 ± 0.23	12.10 ± 0.30	23.20 ± 1.24	30.00 ± 1.10	195 ± 1.58	556.70 ± 2.30
RS51	231 ± 0.77	32.03 ± 0.86	88.00 ± 0.17	5.60 ± 1.80	31.60 ± 0.01	11.24 ± 0.21	19.20 ± 0.52	38.60 ± 0.45	29 ± 0.86	76.10 ± 0.82
RS55	237 ± 0.39	30.28 ± 0.06	88.90 ± 0.24	12.50 ± 1.22	32.80 ± 0.21	12.85 ± 0.74	23.10 ± 1.20	33.70 ± 0.43	182 ± 1.39	285.95 ± 0.54
RS58	241 ± 0.14	24.86 ± 0.32	79.50 ± 0.51	13.00 ± 1.44	27.40 ± 0.77	10.04 ± 0.92	20.20 ± 0.08	30.00 ± 1.10	32 ± 0.82	149.39 ± 0.35
RS61	237 ± 0.39	33.79 ± 1.15	80.20 ± 0.45	9.00 ± 0.31	30.20 ± 0.26	13.48 ± 1.12	22.40 ± 0.89	41.30 ± 0.94	102 ± 0.21	122.06 ± 0.52
RS64	278 ± 2.22	31.99 ± 0.86	79.20 ± 0.53	12.40 ± 1.18	37.00 ± 0.97	11.48 ± 0.07	18.00 ± 1.05	43.00 ± 1.25	26 ± 091	77.99 ± 0.81
Nesser	237 ± 0.39	17.31 ± 1.57	117.00 ± 2.49	11.20 ± 0.65	35.90 ± 0.77	12.01 ± 0.25	21.20 ± 0.36	28.00 ± 1.46	89 ± 0.02	180.48 ± 0.14
Pirsabak-04	227 ± 1.03	35.53 ± 1.44	95.30 ± 0.75	10.20 ± 0.21	33.15 ± 0.27	14.10 ± 1.48	20.00 ± 0.17	47.50 ± 2.06	212 ± 1.83	623.94 ± 2.73

DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers $Plant^{-1}$, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets $Spike^{-1}$, 1000GW = Thousand Grain Weight, GY = Grain Yield $Plant^{-1}$, BY = Biological Yield $Plant^{-1}$

Table 3. Mean values and standard deviation of low and high yielding groups of bread wheat genotypes (21 genotypes included in low yield and 09 in high yield group)

	Low yielding group		High yielding group		Total	
Traits	Mean	SD	Mean	SD	Mean	SD
DTH	244.80	16.46	239.22	13.79	243.13	15.68
FL	25.49	5.89	29.88	5.53	26.81	6.04
PH	82.79	10.94	92.97	13.71	85.84	12.53
NT	9.17	2.41	10.96	1.30	9.71	2.28
PL	31.51	5.67	31.94	5.55	31.64	5.54
SL	10.92	1.44	13.16	1.07	11.59	1.69
NS	19.71	2.14	21.92	1.81	20.37	2.26
GWT	35.71	4.43	36.96	7.76	36.09	5.52
BY	137.24	89.97	355.41	168.28	202.69	154.06

Where, DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers Plant^{-1}, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets Spike^{-1}, 1000GW = Thousand Grain Weight, GY = Grain Yield Plant^{-1}, BY = Biological Yield Plant^{-1}, SD = Standard Deviation

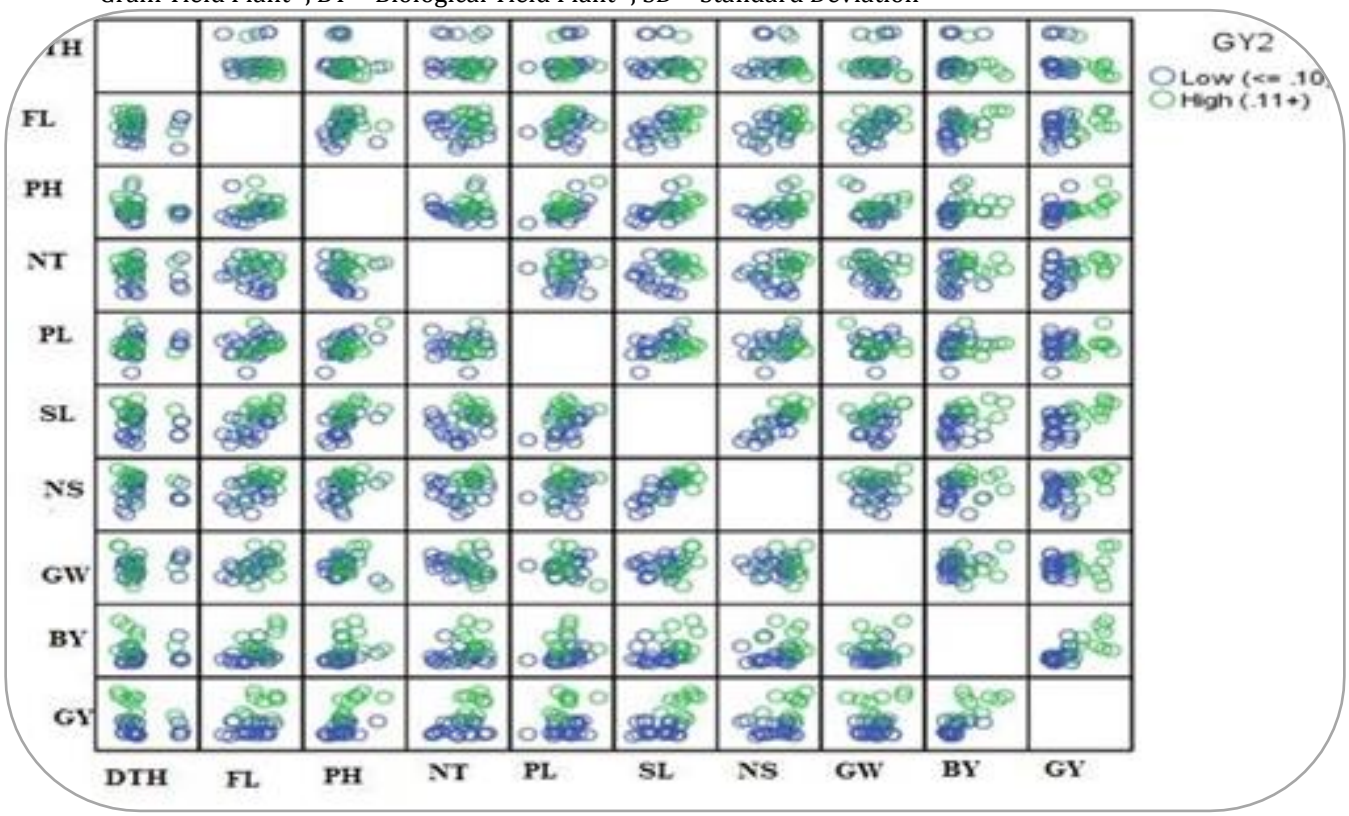


Figure 7. Scatter plot diagram of low and high yielding groups of genotypes based on metric traits in 30 broad based bread wheat genotypes

DTH = Days to 50% Heading, FLA = Flag Leaf Area, PH = Plant Height, NT = Number of Tillers Plant^{-1}, PL = Peduncle Length, SL = Spike Length, NS = Number of Spiklets Spike^{-1}, 1000GW = Thousand Grain Weight, GY = Grain Yield Plant^{-1}, BY = Biological Yield Plant^{-1}

The association among the extent of necrosis and the amount of wheat stripe rust fungal colony size showed that genotypes are exhibiting HI i.e., the necrosis of host tissue increased as compared to the fungal colony size. The average number of pustules formed per unit of the affected leaf area was higher in the susceptible genotypes however in resistant genotypes the pustules were much lesser in the amount, smaller and poor in sporulation as observed by Elahinia, (2008).

For the quick adoption of genotypes for cultivation, identification of the genotypes having higher grain yield with improved agronomic traits in addition to the disease resistance is of great importance (Sorrells, 1998; Singh *et al*., 2012; Awan *et al*., 2017). The simultaneous study for both these traits is particularly important in the context that some of the slow rusting genes have a negative association with the grain yield (Singh and Huerta-Espino, 1997; Spielmeyer *et al*., 2005). Chen *et al*. (2016) observed that the isogeneic lines having *Lr34/Yr18* gene complex produced more height, early maturing and low yielding with lesser grain weight as compared to the lines devoid of *Lr34/Yr18*.

One of the main characters necessary for the adaptation of any crop to a particular environment is its heading time. The utilization of early maturing varieties is a valuable tactic to decrease yield losses incurred by rusts because early maturity enables crop to avoid the rust infestation (Gessese, 2019). Days to 50% heading were higher in the genotypes RS64 and RS46 (275 days) while the lowest value was observed in LLR8 (226 days). Flag leaf is at the immediate vicinity of plant and has a major contribution to photosynthesis (Shirdelmoghanloo *et al*., 2016). The maximum flag leaf area was displayed by RS50 (35.86 cm^2). The highest value for the number of tillers per plant was displayed by RS58 and LLR35 (13). A higher number of tillers improves grain yield by producing more spikes and spikelets resulting in improved yield (Xie *et al*., 2015). It was observed that the 1000-grain weight and grain yield per plant decreased with the increase in wheat stripe rust severity (Figure 8). Ahmad *et al*. (2010) also reported that the susceptible genotypes displayed more yield loss when compared to resistant genotypes, the losses reduced in the susceptible to moderate susceptible genotypes and diminished in the genotypes with moderate resistant and moderate susceptible reactions.

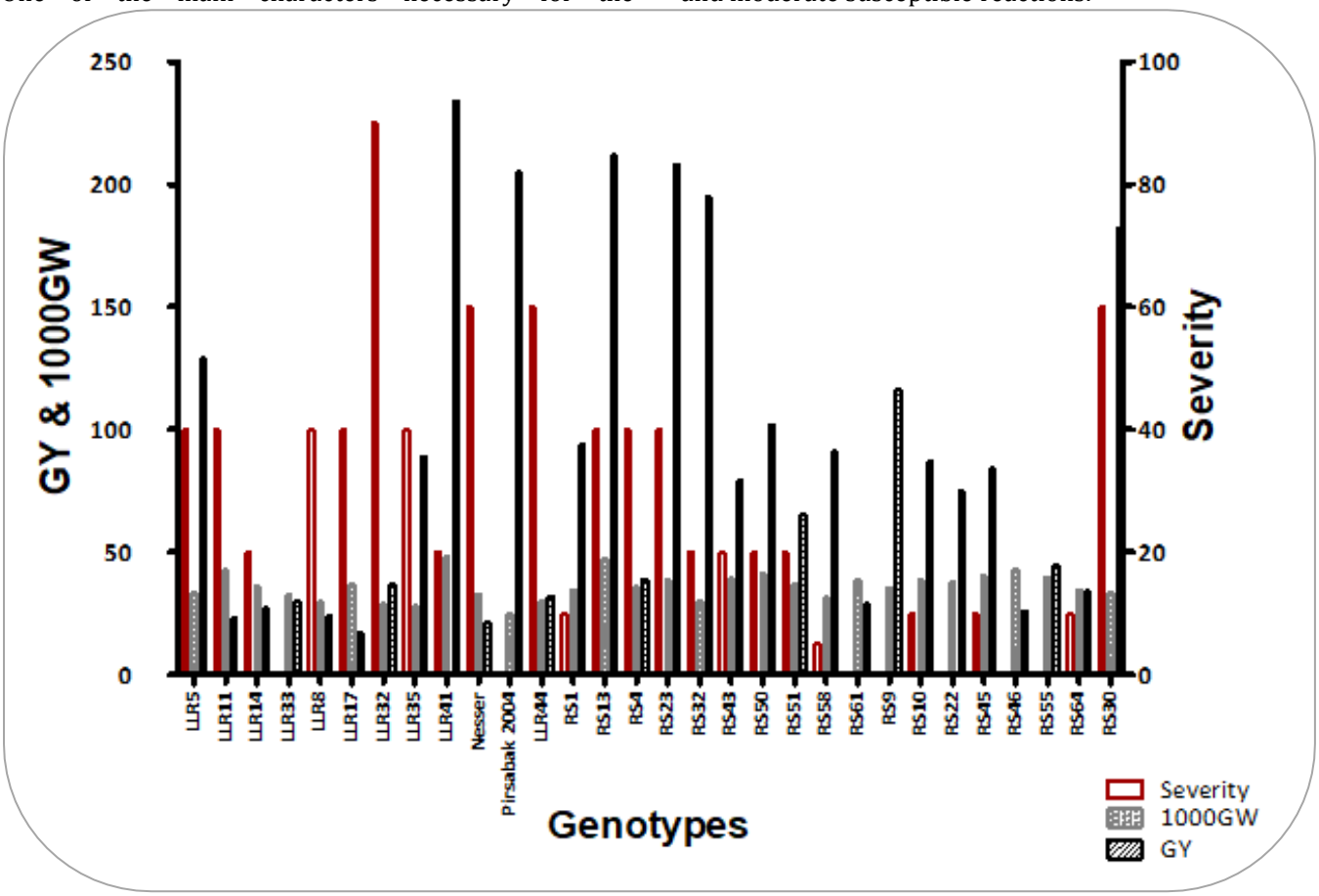


Figure 8. Influence of wheat stripe rust disease severity on 1000-grain weight and grain yield per plant recorded in 30 broad based bread wheat genotypes

The landraces and elite lines displayed the diverse nature of host-pathogen interactions offering several options for resistance breeding. The genotypes LLR35, RS13, RS22, RS30, RS55, RS58, and RS64 were resistant against stripe rust and recommended for resistant breeding. The genotypes LLR5, LLR17, LLR33, RS1, RS10, RS43, and RS45 were moderately resistant and maybe utilized in crossing programs to accumulate genes of durable resistance. The genotypes LLR8, Pirsabak-04 and RS4 were high yielding and could be utilized in breeding high yielding cultivars.

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Muhammad Ilyas	:	Edited the initial and final draft
Adnan Idris	:	Assisted in the data analysis and graphics
Muhammad Rizwan	:	Assisted in the conduct of field trials