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Genetic evaluation of hulless barley (*Hordeum vulgare*) genotypes for morpho-physiological traits and stripe rust resistance

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ABSTRACT

Nowadays, hulless barley (*Hordeum vulgare* L.) is gaining importance as a raw material for food industry due to its highly nutritive value. Non-availability of high yield and disease resistant varieties has restricted its production in marginal areas over the last two decades. Therefore, an experiment was conducted during 2017–18 and 2018–19 at the Seed and Research Farm of ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana to characterized 11 exotic hulless barley genotypes and 4 Indian barley cultivars for morpho-physiological characters and stripe rust resistance. Seedling resistant test for stripe rust was done at Regional Station (ICAR-Indian Institute of Wheat and Barley Research), Flowerdale, Shimla, Himachal Pradesh. Highly significant genotypic differences were found for morpho-physiological traits during analysis of variance. Genotype DWRNB28 showed a good combination of desirable traits, viz. early maturity (118 days), dwarf plant height (64.4 cm) and high 1000-grain weight (55.2 g). Maximum number of tillers per meter (149) was observed in DWRNB17. Genotype DWRNB25 was found resistant to maximum pathotypes (6S0, 7S0, and Q) at the seedling stage. DWRNB23 and DWRNB25 with pooled ACI=0 and ACI=0.04 respectively, were found immune to stripe rust in field conditions. These promising genotypes can be utilized as donor parents for the development of high-yielding and stripe-rust-resistant hulless barley varieties.

Keywords: Hulless barley, Morpho-physiological traits, Resistance, Stripe rust

Barley (*Hordeum vulgare* L.) is an ancient cereal crop that is currently the fourth most important cereal crop in the world after maize, rice and wheat (Yirgu *et al.* 2022). It is a multipurpose crop that has been used for brewing malt, food, animal feed and ethanol production (Meints *et al.* 2021, Diaz *et al.* 2022). In India, barley is cultivated on nearly 0.62 million hectares of land, with 1.69 million metric tonnes of production and a productivity of 2733 kg/ha (Anonymous 2023). In India, hulless barley is cultivated in the Himalayan region, where it is consumed as food by tribal people (Yadav *et al.* 2018).

Hulless barley is enriched with nutrients and has medicinal properties. The grain of hulless barley consists of an ample quantity of ß-glucan, which plays a critical role in lowering the sugar levels of diabetic patients and maintaining an optimal and stable blood sugar level (Wong and Jimmy 2016). In addition, consumption of barley products enriched with ß-glucan may decrease the risk of coronary heart disease (Shimizu *et al.* 2008, Bozbulut and Samlier 2019). Due to health reasons, consumption of hulless barley is now being given more emphasis by modern consumers, which may help restore the status of barley as a significant component in human diets.

The increase demand of food barley in Indian food industry has created a potential need of high yielding and disease resistant hulless barley variety in Indian agriculture sector. Therefore, it has become necessary for barley researchers to develop high-yielding and disease resistant varieties. Among diseases, stripe rust is a major foliar disease that causes severe damage to the crop resulting in a significant yield reduction up to 25% and affect grain quality under severe disease incidence (Verma *et al.* 2005). Thus, present study was carried out with the aim of identifying new rust-resistant hulless genotypes with desirable morpho-physiological traits.

MATERIALS AND METHODS

Evaluation of genotypes for morpho-physiological traits: An experiment was conducted during 2017–18 and 2018–19 at the Seed and Research Farm of ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana. Fifteen genotypes of hulless barley including 11 exotic lines

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and 4 prevalent cultivars were evaluated for 8 morpho-physiological characters in a randomized complete block design (RCBD) with 3 replications. The exotic lines were procured from International Centre for Agricultural Research in the Dry Areas (ICARDA) and seed of 4 Indian cultivars were taken from mid-term storage, ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana. Sowing of selected genotypes was done in 6 rows of 5.0 m length with 23 cm row to row spacing and 10 cm plant to plant spacing and recommended agronomic practices were followed. Data on phenological characters were recorded on the plot basis while 8 morpho-physiological characters were recorded on 5 plants, selected randomly from each replication. The mean values of each replication were subjected to the statistical analysis by using online statistical software SAS 9.3.

Evaluation of genotypes against stripe rust at adult plant stage: The experimental material was screened for stripe rust resistant for adult plant stage at 5 locations Durgapura, Ludhiana, Bajaura, Jammu and Karnal under the artificial rust epiphytotic conditions. Each genotype was planted in single row of 1.0 m length and spacing was maintained of 30 cm between the rows. The infector rows involving a mixture of susceptible cultivars BL2, RD31, RS6, Jyoti and RD2035 were planted surrounding the test genotypes. The infector rows were artificially injected with suspension prepared with mixture of uredospores of 5 *Psh* pathotypes, i.e. 24 (0S0-1), 57 (0S0), G (4S0), M (1S0) and Q (5S0), before early tillering stage (Zadoks GS 10-19) of the crop. In addition, 3–4 sprays of uredospores were also done on infectors from tillering to flag leaf stage in the main field for developing rust epidemics. Observations were recorded on stripe rust by combining severity (per cent leaf area covered by rust) and response (infection type). The scoring for stripe rust was done when disease appeared completely on the test lines and infector rows using the modified Cobb's scale (Peterson *et al.* 1948). Host response in the field was scored as Resistant (R) = no uredia present; Moderately Resistant (MR) = small uredia with slight sporulation; Moderately Susceptible (MS) = medium sized uredia with moderate to heavy sporulation and Susceptibility (S) = large uredia with abundant sporulation. The disease severity and host response data were combined into a single value called the coefficient of infection (CI). The coefficient of infection was calculated by multiplying disease severity (DS) and constant values of infection type (IT). The constant values for infection types were utilized based on R=0.2, MR=0.4, MS=0.8 and S=1.0 (Stubbs *et al*. 1986). The average coefficient of infection (ACI) was calculated from rust scores of five locations. The genotypes were classified into resistant and susceptible on the basis of ACI values (Sajid-Ali *et al*. 2009). The genotype having ACI value of zero was considered immune while genotype with ACI values of 0–5 were believed to have strong resistance. However, ACI values 5–10 were considered as moderately resistant. The genotype with ACI values between 10–20 were considered as moderately susceptible, while ACI values >20 was recorded as highly susceptible.

Evaluation of genotypes against stripe rust at seedling stage: Seedling resistance testing (SRT) of experimental material was done during 2017–18 and 2018–19 at Regional Station (ICAR-Indian Institute of Wheat and Barley Research), Flowerdale, Shimla, Himachal Pradesh. Seven pathotypes (6S0, 7S0, G, M, 24, 57 and Q) were used separately under controlled conditions to develop stripe rust disease at seedling stage. Aluminium trays (29 cm × 12 cm × 7 cm) containing a mixture of fine loam and farm yard manure (3:1) were used to raise seedlings. Total 15 genotypes and 1 susceptible check (Bilara-2) were sown in single tray. About 4–5 seeds were planted per hill of each genotype. A glass atomizer containing 10 mg spores of an individual *Psh* pathotype suspended in 1 ml light grade mineral oil (Soltrol 170®) was used to inoculate one-week old seedlings. These inoculated genotypes were then sprayed with a fine mist of water and kept in a moist chamber (RH>80%) at 12±2ºC for 48 hours. Thereafter, samples were transferred to the greenhouse benches at an appropriate temperature (16±2^{0}C), relative humidity (60–80%) and illumination (about 15,000 lX for 12 hours) under controlled artificial condition. Observations were recorded on reaction type of these genotypes against each pathotype at 16–18 days after inoculation as suggested by Nayar *et al*. (1997).

RESULTS AND DISCUSSION

Evaluation of hulless barley genotypes for morpho-physiological traits: Eleven exotic lines and four Indian cultivars of hulless barley were evaluated for eight morpho-physiological traits. Analysis of variance showed highly significant genotypic differences for all morpho-physiological characters under study (Table 1). This indicates the presence of inherent genetic variability among genotypes, which offers an opportunity to select the most promising genotypes. Similar findings were reported by Adhikari *et al.* (2018).

Mean performance of yield and its contributing traits: The range for days to 50% heading was registered from 86 to 96 days, with a mean value of 91.3 days. The exotic genotypes DWRNB20 and DWRNB28 showed the shortest days at 50% heading (86 days) and flowered earlier than prevalent hulless cultivars (BHS352, Gitanjali, Karan16, and NDB943). Similar results were reported by Karkee *et al.* (2020). Early maturity is an important trait for adaptation to drought and heat environments. This trait was measured at the physiological maturity of the crop. Maturity ranged from 118 to 130 days, and the mean value was recorded at 124.8. Exotic genotype DWRNB28 showed maturity in 118 days, followed by DWRNB20 and NDB943 (120 days). These genotypes may be utilized as donors for earliness in the development of the variety for drought and heat-tolerant environments. Deniz (2007) found the genotypes to have early maturity along with higher grain yields in his study.

Plant height is a critical trait, especially in barley, because it is directly associated with the lodging of the crop plants. Lodging reduces the grain yield by up to 20–25%. In the present study, this trait showed highly

Table 1 Mean performance of hullness barley genotypes during 2017–18 and 2018–19

Genotype	Pedigree	DHE	DMA	PH (cm)	T/M	SL (cm)	G/S	TGW (g)	Y/P (g)
DWRNB5	EIBGN(2012-13)	95	127	104.6	139	10.4	28	46.2	18.7
DWRNB14	EIBGN(2009-10)-45	96	130	105.8	105	8.6	67	43.7	27.0
DWRNB17	ZIGZIG/4/TOCTE//HIGO/LINO/3/ PETUNIA1	95	127	111.2	149	8.0	65	35.0	24.4
DWRNB18	PENCO/CHEVRON-BAR/3/LEGACY// PENCO/CHEVRON-BAR	91	125	106.7	100	8.4	70	41.3	26.6
DWRNB20	NACKTA/HJAA33//FNC1	86	120	65.2	107	7.9	27	53.9	26.1
DWRNB21	PENCO/CHEVRON-BAR/3/LEGACY// PENCO/CHEVRON-BAR	94	127	102.7	110	8.0	74	40.4	25.4
DWRNB22	ZIGZIG/3/PENCO/CHEVRON-BAR// PETUNIA1	93	126	107.0	99	8.6	71	37.4	21.6
DWRNB23	P.STO/3/LBIRAN/UNA80// LIGNEE640/4/BLLU/5/PETUNIA1/6/ LEGACY//PENCO/CHEVRON-BAR	95	130	101.1	105	8.5	67	38.5	25.4
DWRNB25	PETUNIA 2/M112	92	127	105.8	113	6.9	72	38.5	28.3
DWRNB26	BICHY2000/PRTL	92	126	102.1	95	9.9	28	48.1	17.5
DWRNB28	NACKTA/HJAA33//FNC1	86	118	64.4	140	8.5	28	55.2	23.4
BHS352	HBL240/BHS504//VLB129	90	123	108.9	98	8.9	75	44.1	23.9
Gitanjali	K12/K572/10/EB410	87	122	95.2	117	6.7	63	43.0	24.9
Karan16	AZAM(DWARF)1/EB7576	90	124	103.4	122	8.2	64	41.7	22.9
NDB943	K1178/Karan748	88	120	108.7	115	8.3	74	40.1	29.7
Genotype (*P* value)	*P* value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Year		<.001	NS	0.01	NS	NS	NS	NS	0.08
Y × G		Ns	0.01	NS	NS	NS	NS	NS	NS
Genotype (LSD 0.05)	LSD 0.05	2.09	2.64	7.99	13.94	0.79	5.75	2.19	3.35
Year		0.76	0.96	2.92	5.09	0.29	2.10	0.80	1.22
Y × G		2.95	3.73	11.29	19.70	1.12	8.13	3.10	4.73
Range		86-96	118 -130	64.4- 111.2	95-149	6.7-10.4	27-75	35-55	17.5- 29.7
Mean		91.3	124.8	99.5	114.2	8.4	58.2	43.2	24.4
CV%		1.9	1.8	6.9	10.6	8.2	8.5	4.4	11.9

DHE, Days to 50% heading; DMA, Days to maturity; PH, Plant height (cm); TM, Tillers/metre; SL, Spike length (cm); G/S, Grains/spike; TGW, 1000-grain weight; GY, Grain yield/plant (g).

significant differences among test genotypes. Plant height ranged from 64.4 to 111.2 cm, with a trial mean of 99.4 cm. The genotypes DWRNB28 and DWRNB20 revealed the shortest height among all the genotypes. This result is contrary to the findings obtained by Adhikari *et al.* (2018) who reported lesser genetic variability for plant height. Significant differences were observed for tillering capacity among test genotypes. The range of tillers per meter was registered from 95 to 149 with a mean value of 126.1. Exotic line DWRNB17 produced the highest tillers per meter. These findings are in concurrence with Adhikari *et al.* (2018).

Spike length and grains per spike are the major yield-contributing traits in barley. The spike length showed a range from 6.7 to 10.4 cm, with a mean value of 8.4 cm. The maximum spike length was observed in DWRNB5 (10.4 cm). The grains per spike revealed a range of 28 to 75, with a mean value of 58.3. Maximum grains per spike were found in Indian cultivar BHS352, followed by NDB943. Thousand grain weight is an important trait to determine the grain yield. The range for this trait was found to be 35 to 55.0 g, with a mean value of 42.3 g. DWRNB28 was boldly seeded with maximum TGW (55 g), followed by DWRNB20 (54 g), and both genotypes are two-rowed hulless barley. TGW plays an important role in enhancing grain yield as reported by Lodhi *et al.* (2015).

Grain yield is a complex character hence direct selection for high yield is difficult. Yield and its attributing traits are quantitative nature and depending on genotypes and environments. Hence, any change on genotype or growing conditions is liable to bring the change in grain yield. In the present study, range of grain yield per plant was observed from 17.5 to 29.7 g with mean value 24.4 g. The cultivar NDB943 showed the highest grain yield per plant (29.7 g) followed by DWRNB25 (28.3 g) and DWRNB14 (27.0 g), indicating that few options are available for grain yield in exotic material also.

Identification of adult plant resistance in hulless barley genotypes to stripe rust: Adult plant resistance (APR) is a more durable resistant stage that is observed at the adult plant stage in the field condition. APR is controlled by more than one minor gene and is reported to be more durable than the seedling resistance (Singh and Rajaram 1992). Fifteen genotypes of hulless barley were screened for yellow rust reaction at the adult plant stage (Table 2) at 5 locations, under artificial epiphytotic conditions. As a consequence of pooled data on adult plant stages, DWRNB23 and DWRNB25 with pooled ACI=0 and ACI=0.04, respectively, were immune, and 4 genotypes, viz. DWRNB17, DWRNB18, DWRNB20 and DWRNB28 with pooled ACI≤5, were observed to be highly resistant to stripe rust in the field conditions. In earlier studies, adult-stage plant resistance against Indian races of the stripe rust pathogen has been identified in advanced breeding lines and genotypes from ICARDA (Verma *et al.* 2018, Singh *et al*. 2019). Kumar *et al.* (2020) also registered indigenous genotypes that revealed resistance to stripe rust in barley. The released cultivar, BHS352, with pooled ACI <10, indicated moderate resistance to stripe rust, while Gitanjali, Karan 16 and NDB943 exhibited ACI values of 32.0, 38.5, and 72.0, respectively, which inferred that they were highly susceptible to stripe rust.

Identification of seedling resistance in hulless barley genotypes to stripe rust: A seedling resistance test was performed on 15 genotypes of hulless barley against 7 pathotypes of stripe rust pathogens during 2017–19 (Table 3). Among these, pathotypes 6S0 and 7S0 were identified recently and have the ability to infect both wheat and barley hosts (Gangwar *et al.* 2019). Duplessis *et al.* (2011) reported that new pathotypes may spread fast due to their high multiplication rate and airborne nature. Out of 15 genotypes, DWRNB17 was resistant to 6S0 and Q, and DWRNB21 was resistant to 24 and 57 pathotypes, respectively. However, DWRNB25 was found to be resistant to two newly identified pathotypes (6S0 and 7S0) and also carries resistance to the Q pathotype. None of the genotypes showed resistance to all pathotypes at the seedling stage. Similarly, Verma *et al.* (2018) also reported 12 barley genotypes that were found resistant to pathotypes 24, 57, G, M and Q.

In conclusion, it is suggested that the exotic genotype DWRNB28 showed a good combination of desirable traits like early maturity, dwarf plant height and higher 1000-grain weight. DWRNB17 had the highest number of tillers per meter. Genotype DWRNB25 was found resistant

Table 2 Adult plant resistance score of hulless barley genotypes to stripe rust

Genotype	IBDSN 2018						IBDSN 2019						
	Durgapura	Ludhiana	Bajaura	Jammu	Karnal	ACI (2017–18)	Durgapura	Ludhiana	Bajaura	Jammu	Karnal	ACI (2018–19)	Pooled ACI
DWRNB5	10MS	5S	20S	TMR	5S	7.7	10S	20S	30S	5S	10S	15	11.4
DWRNB14	20S	0	0	10S	0	6.0	15S	5S	10S	10S	0	8.0	6.0
DWRNB17	0	0	0	10S	0	2.0	5S	0	0	10S	0	3.0	2.5
DWRNB18	0	0	15S	0	0	3.0	10MR	0	20S	0	10S	6.8	4.9
DWRNB20	0	0	10S	0	0	2.0	15MS	0	20S	0	0	6.4	4.2
DWRNB21	0	0	0	0	0	0.0	20S	0	40S	5MS	0	12.8	6.4
DWRNB22	0	0	0	0	0	0.0	30MS	0	30S	20S	30S	20.8	5.4
DWRNB23	0	0	0	0	0	0.0	0	0	0	0	0	0.0	0.0
DWRNB25	0	0	0	0	0	0.0	TMR	0	0	0	0	0.08	0.04
DWRNB26	0	0	0	5S	0	1.0	60S	0	40S	20S	0	24.0	12.5
DWRNB28	0	0	0	0	0	0.0	15MS	0	NG	0	0	3.0	1.5
BHS352(c)	10MS	0	20S	40S	0	13.6	20S	0	0	0	10MS	5.6	9.6
Gitanjali (c)	30S	40S	20S	40S	20S	30.0	60S	10S	40S	20S	40S	34.0	32.0
Karan16(c)	60S	60S	60S	20S	40S	48.0	60S	5S	60S	0	20S	29.0	38.5
NDB943(c)	40S	60S	100S	40S	80S	64.0	100S	60S	80S	80S	80S	80.0	72.0

ACI, Average coefficient of infection.

Table 3 Seedling resistance test of hulless barley genotypes to the individual pathotype of *Puccinia striiformis*f. sp. *hordei* (*Psh*)

Genotype	Reaction* to *Psh* pathotypes													
	6S0		7S0		G		M		24		57		Q	
	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19	2017–18	2018–19
DWRNB5	3	3^+	3	3	3	3^+	3^+	3^+	3^+	3^+	3^+	3^+	2	3^+
DWRNB14	3	3	3	3^+	3+	3^+	3+	3^+	3	3	; 2^-	2^-	0;	0;
DWRNB17	0;	0;	3	3^+	2	3^+	2	3^+	2^-	2^-	2^-	2^-	0;	0;
DWRNB18	2	2	3	3	2	3	3	3^+	2	3	2	3^-	3+	3
DWRNB20	3^+	3^+	3+	3^+	3+	3^+	3+	3^+	1P3+	3^+	1P2	3	1P3+	3
DWRNB21	2	3	1P3	3	$1P2^-$	2^-	2	1P1	0;	0;	0;	0;	3+	3
DWRNB22	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3
DWRNB23	1P3	3^-	1P3	3+	0;	0;	3^-	3	2^-	2^-	2^-	2^-	0;	2^-
DWRNB25	0;	0;	0;	0;	2^-	2^-	2^-	2^-	3^+	3^+	3^+	3^+	0;	0;
DWRNB26	3	3^+	3^+	3^+	3^+	3^+	3^+	3^+	1P3+	3^+	$1P3^+$	3^+	2	3^+
DWRNB28	3^+	3^+	1P3+	3^+	2	3^+	1P0; $1P3^+$	3^+	3^+	3	3^+	3^+	3+	3^+
BHS 352(c)	3^-	3	3+	3^+	3	3	1P0; $1P3^+$	2^-	3	3	3^+	3^+	2	3
Gitanjali (c)	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+
Karan 16(c)	3^+	3^+	3^+	3^+	3^+	3	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3
NDB 943(c)	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+	3^+

3^+ = susceptible, 3; 3^- = moderately susceptible, 2; 2^{+-} = moderately resistant, 2; 0 = resistant, 1.

to maximum pathotypes (6S0, 7S0, and Q) at the seedling stage. DWRNB23 and DWRNB25, with pooled ACI=0 and ACI=0.04, respectively, were found immune to stripe rust in field conditions. These promising exotic genotypes may be a potential donor to develop high-yielding and stripe-rust-resistant hulless barley varieties to strengthen their production and address the demand of the Indian food industry sector in the near future.

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