

Genetic analysis of hulless barley (*Hordeum vulgare*) genotypes for resistance to stripe rust and morpho-physiological characteristics

Dr. Gayathri, M. Triveni

Department of Rural Development and Agricultural Extension,

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ABSTRACT

Due to its high nutritional content, hulless barley (*Hordeum vulgare* L.) is becoming more and more important as a raw material for the food sector. Over the last 20 years, its production in marginal regions has been limited because to the lack of high yield and disease-resistant types. In order to characterize 11 exotic hulless barley genotypes and 4 Indian barley cultivars for morpho-physiological traits and stripe rust resistance, an experiment was carried out in 2017–18 and 2018–19 at the Seed and Research Farm of ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana. At the ICAR-Indian Institute of Wheat and Barley Research Regional Station at Flowerdale, Shimla, Himachal Pradesh, a test for seedling resistance to stripe rust was conducted. Analysis of variance revealed very substantial genotypic differences for morpho-physiological characteristics. Early maturity (118 days), dwarf plant height (64.4 cm), and high 1000-grain weight (55.2 g) were all favorable characteristics of genotype DWRNB28. DWRNB17 has the highest number of tillers per meter (149). At the seedling stage, genotype DWRNB25 was shown to be resistant to maximal pathotypes (6S0, 7S0, and Q). In field settings, DWRNB23 and DWRNB25, with pooled ACI=0 and ACI=0.04, respectively, were proven to be resistant to stripe rust. These promising genotypes may be used as donor parents to create hulless barley varieties with high yields and resistance to stripe rust.

Keywords: Stripe rust, resistance, morphophysiological characteristics, hulless barley

Introduction

After maize, rice, and wheat, barley (*Hordeum vulgare* L.), an ancient cereal crop, is today the fourth most significant cereal crop worldwide (Yirgu et al. 2022). It is a versatile crop that has been used to produce ethanol, food, animal feed, and malt (Meints et al. 2021, Diaz et al. 2022). Barley is produced on around 0.62 million hectares of land in India, yielding 1.69 million metric tons with a productivity of 2733 kg/ha (Anonymous 2023). Hulless barley is grown in the Himalayan area of India, where tribal people eat it (Yadav et al. 2018).

Hulless barley has therapeutic qualities and is rich in nutrients. Hulless barley has a large amount of β -glucan, which is essential for decreasing blood sugar levels in diabetics and keeping blood sugar levels steady and optimum (Wong and Jimmy 2016). Furthermore, eating barley products enhanced with β -glucan may reduce the risk.

1 ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana; 2 ICAR-Indian Institute of Wheat and Barley Research Regional Station, Flowerdale, Shimla, Himachal Pradesh. *Jogendra.singh2@icar.gov.in is the author's email address.

of cardiac disease (Shimizu et al. 2008, Bozbulut and Samlier 2019). Modern consumers are placing greater attention on hulless barley intake because to health concerns, which might help barley regain its position as an important part of human diets.

The Indian agricultural sector may need a high-yielding, disease-resistant hulless barley variety due to the growing demand for food barley in the country's food business. Barley researchers now need to create high-yielding and disease-resistant variants. Stripe rust is a prominent foliar disease that damages crops severely, reducing yields by up to 25% and affecting grain quality when the illness is severe (Verma et al. 2005). In order to find novel rust-resistant hulless genotypes with desired morpho-physiological properties, the current research was conducted.

METHODS AND MATERIALS

Genotype evaluation for morpho-physiological characteristics: At the ICAR-Indian Institute of Wheat and Barley Research's Seed and Research Farm in Karnal, Haryana, an experiment was carried out in 2017–18 and 2018–19. Eleven unique lines are among the fifteen genotypes of hulless barley. and eight morpho-physiological traits were assessed for four common cultivars using a randomized complete block design (RCBD) with three replications. The International Center for Agricultural Research in the Dry Areas (ICARDA) provided the exotic lines, while the ICAR-Indian Institute of Wheat and Barley Research in Karnal, Haryana, mid-term storage provided the seed of four Indian varieties. Selected genotypes were sown in six rows, each measuring 5.0 meters in length, with 23 centimeters between rows and 10 centimeters between plants. Recommended agronomic methods were adhered to. Eight morpho-physiological traits were recorded on five randomly chosen plants from each replication, whereas data on phenological traits were collected on a plot basis. SAS 9.3, an online statistical program, was used to statistically analyze the mean values of each replication.

Assessment of genotypes to prevent stripe rust in mature plants: In five locations—Durgapura, Ludhiana, Bajaura, Jammu, and Karnal—the experimental material was tested for stripe rust resistance during the adult plant stage under artificial rust epiphytotic circumstances. Every genotype was planted in a single, one-meter-long row with a 30-centimeter gap between each row. The test genotypes were surrounded by infector rows that included a combination of susceptible cultivars BL2, RD31, RS6, Jyoti, and RD2035. Prior to the crop's early tillering stage (Zadoks GS 10-19), the infector rows were intentionally injected with suspension made from a combination of uredospores of five Psh pathotypes: 24 (OS0-1), 57 (OS0), G (4S0), M (1S0), and Q (5S0). In order to prevent rust epidemics, three to four uredospores were sprayed on infectors in the main field between the tillering and flag leaf stages. Stripe rust observations were made by combining response (infection type) and severity (percentage of leaf surface affected by rust). The modified Cobb's scale was used to score stripe rust when the disease fully manifested on the test lines and infector rows (Peterson et al. 1948). Resistant (R) = no uredia present; Moderately Resistant (MR) = tiny uredia with minor sporulation; Moderately Susceptible (MS) = medium-sized uredia with moderate to heavy sporulation; and Susceptibility (S) = big uredia with plentiful sporulation were the scores assigned to the host reaction in the field. A single figure known as the coefficient of infection (CI) was created by combining data on host response and disease severity. Disease severity (DS) and constant values of infection type (IT) were multiplied to get the coefficient of infection. R=0.2, MR=0.4, MS=0.8, and S=1.0 were used as the constant values for infection types (Stubbs et al. 1986). Five sites' rust ratings were used to compute the average coefficient of infection (ACI). ACI values were used to categorize the genotypes as sensitive or resistant (Sajid-Ali et al. 2009). Genotypes with ACI values between 0 and 5 were thought to have significant resistance, whilst genotypes with ACI values of zero were thought to be immune. ACI values between 5 and 10, however, were regarded as moderately resistant. Genotypes with ACI values between 10 and 20 were classified as moderately susceptible, whilst those with ACI values more than 20 were classified as very susceptible.

Assessment of genotypes to prevent stripe rust in seedlings: At the Regional Station (ICAR-Indian Institute of Wheat and Barley Research), Flowerdale, Shimla, Himachal Pradesh, seedling resistance testing (SRT) of experimental material was conducted in 2017–18 and 2018–19. Stripe rust disease was developed at the seedling stage using seven pathotypes (6S0, 7S0, G, M, 24, 57, and Q) individually under controlled circumstances. Seedlings were raised in aluminum trays (29 cm × 12 cm × 7 cm) filled with a 3:1 combination of fine loam and farm yard manure. In one tray, a total of fifteen genotypes and one susceptible check (Bilara-2) were seeded. For each genotype, four to five seeds were sown per hill. One-week-old seedlings were inoculated using a glass atomizer that contained 10 mg spores of a specific Psh pathotype suspended in 1 ml of light-grade mineral oil (Soltrol 170®). Following a thin mist of water, these inoculated genotypes were stored for 48 hours at 12±2°C in a moist room (RH>80%). Samples were then moved to greenhouse benches under controlled artificial conditions with the proper temperature (16±20°C), relative humidity (60–80%), and light (about 15,000 IX for 12 hours). As recommended by Nayar et al. (1997), observations were made about the genotypes' reactions to each pathotype 16–18 days following inoculation.

OUTCOMES AND TALK

Assessment of morpho-physiological characteristics in hulless barley genotypes: Eight morpho-physiological characteristics of four Indian cultivars and eleven foreign lines of hulless barley were assessed. For every morpho-physiological trait under investigation, analysis of variance revealed extremely significant genotypic differences (Table 1). This suggests that genotypes have intrinsic genetic diversity, providing a chance to choose

the most promising genotypes. Adhikari et al. (2018) found similar results.

Average yield performance and the characteristics that contribute to it: Days to 50% heading had a mean of 91.3 days and a range of 86 to 96 days. Compared to common hullless cultivars (BHS352, Gitanjali, Karan16, and NDB943), the exotic genotypes DWRNB20 and DWRNB28 had the shortest days at 50% heading (86 days) and bloomed earlier. Karkee et al. (2020) observed similar findings. An essential characteristic for adapting to heat and dry conditions is early maturity. This characteristic was assessed when the crop reached physiological maturity. The average maturity was 124.8 days, with a range of 118 to 130 days. The exotic genotype DWRNB28 matured in 118 days, whereas DWRNB20 and NDB943 took 120 days. When developing a variety for drought and heat-tolerant settings, these genotypes might be used as donors during early development. In his research, Deniz (2007) discovered that the genotypes had better grain yields and early maturity. Because it is closely related to the lodging of the crop plants, plant height is an important characteristic, particularly in barley. Grain yield may be lowered by up to 20–25% due to lodging. This characteristic in the current investigation demonstrated strongly

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/PETUNIA 1/6/ 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	64.4-	95-149	6.7-10.4	27-75	35-55	17.5-
		-130	111.2					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

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

notable variations between test genotypes. The trial mean plant height was 99.4 cm, with a range of 64.4 to 111.2 cm. Out of all the genotypes, DWRNB28 and DWRNB20 showed the smallest height. Adhikari et al. (2018) found reduced genetic diversity for plant height, which is in contrast to this result. The tillering ability of the test genotypes varied significantly. With a mean of 126.1, the tillers per meter ranged from 95 to 149. The most tillers per meter were generated by the exotic line DWRNB17. These results are consistent with those of Adhikari et al. (2018).

In barley, the two main characteristics that contribute to yield are spike length and grains per spike. The mean spike length was 8.4 cm, with a range of 6.7 to 10.4 cm. DWRNB5 had the longest spike, measuring 10.4 cm. The range of grains per spike was 28 to 75, with a mean of 58.3. The Indian cultivar BHS352 has the most grains per spike, followed by NDB943. One crucial factor in determining grain output is thousand grain weight. DWRNB28 was aggressively seeded with maximal TGW (55 g), followed by DWRNB20 (54 g), and both genotypes are two-rowed. The range for this trait was determined to be 35 to 55.0 g, with a mean value of 42.3 g. barley without a husk. According to Lodhi et al. (2015), TGW is crucial for increasing grain output.

Direct selection for high yield is challenging since grain yield is a complicated trait. Yield and the characteristics that contribute to it are quantitative in nature and rely on conditions and genotypes. Therefore, any alteration in genotype or growth circumstances may result in a change in grain production. The average grain production per plant in this research was 24.4 g, with a range of 17.5 to 29.7 g. There aren't many possibilities for grain yield in exotic material either. The cultivar NDB943 had the maximum grain yield per plant (29.7 g), followed by DWRNB25 (28.3 g) and DWRNB14 (27.0 g).

Finding mature plant resistance to stripe rust in genotypes of hulless barley: When a plant reaches the adult stage in a field setting, it exhibits a more resilient resistant stage known as adult plant resistance (APR). According to Singh and Rajaram (1992), APR is more resilient than seedling resistance and is regulated by many minor genes. In five sites, fifteen genotypes of hulless barley were tested for yellow rust reactivity at the adult plant stage under artificial epiphytotic circumstances (Table 2). Four genotypes—DWRNB17, DWRNB18, DWRNB20, and DWRNB28 with pooled $ACI \leq 5$ —were shown to be very resistant to stripe rust under field circumstances, while DWRNB23 and DWRNB25 with pooled $ACI=0$ and $ACI=0.04$, respectively, were immune as a result of pooled data on adult plant stages. Previous research has shown that advanced breeding lines and genotypes from ICARDA had adult-stage plant resistance against Indian races of the stripe rust pathogen (Verma et al.

2018, Singh and colleagues, 2019). Additionally, Kumar et al. (2020) identified native genotypes in barley that demonstrated resistance to stripe rust. While Gitanjali, Karan 16, and NDB943 had ACI values of 32.0, 38.5, and 72.0, respectively, suggesting that they were very vulnerable to stripe rust, the released cultivar BHS352 showed a modest resistance to the disease with a pooled $ACI < 10$.

Determination of hulless barley genotypes' resistance to stripe rust in seedlings: 15 genotypes of hulless barley were tested for seedling resistance to seven pathotypes of stripe rust pathogens between 2017 and 2019 (Table 3). These include the newly discovered pathotypes 6S0 and 7S0, which can infect both wheat and barley hosts (Gangwar et al. 2019). According to Duplessis et al. (2011), novel pathotypes may spread quickly because of their rapid rate of reproduction and airborne nature. DWRNB17 was resistant to 6S0 and Q out of 15 genotypes, whereas DWRNB21 was resistant to 24 and 57 pathotypes, respectively. Nevertheless, it was discovered that DWRNB25 conveys resistance to the Q pathotype in addition to being resistant to two recently discovered pathotypes (6S0 and 7S0). At the seedling stage, none of the genotypes demonstrated resistance to every pathotype. In a similar vein, 12 barley genotypes were shown to be resistant to pathotypes 24, 57, G, M, and Q, according to Verma et al. (2018).

In conclusion, it is hypothesized that the exotic genotype DWRNB28 demonstrated a favorable mix of desired features, including increased 1000-grain weight, dwarf plant height, and early maturity. There were more tillers per meter in DWRNB17. Resistance was discovered for genotype DWRNB25.

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

Genotype	IBDSN 2018						IBDSN 2019						Pooled ACI
	Durgapura	Ludhiana	Bajaura	Jammu	Karnal	ACI (2017-18)	Durgapura	Ludhiana	Bajaura	Jammu	Karnal	ACI (2018-19)	
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 stands for average coefficient of infection.

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

Genotype	Reaction* to Psh pathotypes													
	6S0		7S0		G		M		24		57		Q	
	2017–2018–		2017–2018–		2017–	2018	2017–	2018	2017–	2018	2017–	2018	2017–2018–	
	18	19	18	19	18	–	18	–	18	–	18	–	18	19
						19		19		19		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		2	3	2		3+	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;	3+	3+	3	3+	3+	3+	3+
							1P3+							
BHS 352(c)	3-	3	3+	3+	3	3	1P0;	2-	3	3	3+	3+	2	3
							1P3+							
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+ denotes susceptibility, 3; 3- denotes moderate susceptibility, 2; 2+- denotes moderate resistance, 2; and 0 denotes resistance, 1.

during the seedling stage to the maximal pathotypes (6S0, 7S0, and Q). In field settings, DWRNB23 and DWRNB25 were found to be resistant to stripe rust, with pooled ACI=0 and ACI=0.04, respectively. In order to boost output and meet the needs of the Indian food industry sector in the near future, these promising exotic genotypes could be a viable donor for the development of high-yielding, stripe-rust-resistant hulless barley cultivars.

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