



SPECIAL ISSUE: ADAPTING AGRICULTURE TO CLIMATE CHANGE:
A WALK ON THE WILD SIDE

Traits discovery in *Hordeum vulgare* sbsp. *spontaneum* accessions and in lines derived from interspecific crosses with wild *Hordeum* species for enhancing barley breeding efforts

Sajid Rehman¹  | Mariam Amouzoune^{1,2} | Houda Hiddar^{1,3} | Hafid Aberkane¹  |
 Rachid Benkirane² | Abdelkarim Filali-Maltouf³ | Muamar Al-Jaboobi¹ |
 Leila Acqbouch⁴ | Athanasios Tsivelikas¹ | Ramesh Pal Singh Verma^{1,5} | Zakaria Kehel¹ |
 Ahmed Birouk⁴ | Ahmed Amri¹

¹ International Center for Agricultural Research in the Dry Areas (ICARDA), ICARDA-AI-Irfane, Rabat, Morocco

² Faculté des Sciences, Université Ibn Tofail (UIT-Kénitra), Kenitra, Morocco

³ Microbiology and Molecular Biology Laboratory, Faculty of Sciences, Mohammed V University (UM5), Rabat, Morocco

⁴ Institut Agronomique et Vétérinaire Hassan II (IAV HASSAN II), Rabat, Morocco

⁵ ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India

Correspondence

Sajid Rehman, International Center for Agricultural Research in the Dry Areas, ICARDA-AI-Irfane, Rabat, Morocco.
Email: srehman@perennia.ca

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Abstract

Barley is a crop of global significance with multiple uses (feed, food and beverages), but its productivity worldwide is limited by several abiotic and biotic stresses. Crop wild relatives, exclusively, *Hordeum vulgare* ssp. *spontaneum* and *H. bulbosum*, are important genetic resources needed to develop new adapted varieties. A total of 117 accessions of *H. spontaneum* from different origins and 145 lines derived from crosses between barley and *H. bulbosum* supplied by NordGen were evaluated for field reactions to four major diseases. In addition, a set of 45 lines derived from interspecific crosses with *H. spontaneum* and *H. bulbosum* along with 10 checks were evaluated for agronomic traits and yield performance under four different environmental conditions and for quality attributes. The results showed that 37.7%, 71.6%, 15.1%, and 79.5% of *H. spontaneum* accessions were resistant to moderately resistant to net form net blotch, scald, leaf rust and powdery mildew, respectively, while the respective percentages in case of *H. bulbosum* derivatives were 31%, 20.4%, 17.9% and 70.6%. Only three accessions of *H. spontaneum* showed high resistance levels to the four diseases while 23 other accessions and 16 *H. bulbosum* derived lines showed resistance to a combination of two to four diseases. When *H. bulbosum* and *H. spontaneum* derived lines were evaluated under different environments, none of them gave higher grain yield and higher thousand kernel weight than the best checks. However, a few *H. bulbosum* derived lines showed higher straw yield, and lines combining tall stature, and lodging resistance were identified. Pre-breeding efforts need to be strengthened further by evaluating more wild *Hordeum* accessions, their crossing with the best available parents and selection of elite germplasm to be made available to barley breeding programs for the development of new adapted and high yielding varieties.

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1 | INTRODUCTION

Barley (*Hordeum vulgare* L.) is originated from the Fertile Crescent from which its cultivation has expanded over the past 10,000 years to all continents to become now the fourth most important cereal crop worldwide (Nevo et al., 2012; Salamini et al., 2002; Zohary & Hopf, 1993). It is a crop adapted to a wide range of environments including high altitudes and high latitudes regions and to saline and dry conditions. Barley has multiple uses including animal feed, food, and various beverages (Ullrich, 2010; Von Bothmer, Sato, Kamatsuda, Yasuda, & Fischbeck, 2003). In regions with a Mediterranean climate such as West Asia and North Africa (WANA), with more than 70% of barley acreage in the arid and semi-arid regions, where abiotic stress like drought and heat limit productivity. The International Center for Agricultural Research in the Dry Areas (ICARDA) has the global mandate for barley improvement and holds in-trust one of the largest collections of barley which includes unique and rich collections of landraces (18,935) and wild *Hordeum* (2,392) species (ICARDA, GENESYS, accessed April 2020).

The quest for improved yield and quality traits has resulted in a narrow genetic base of modern barley cultivars. Genetically uniform barley cultivars have been planted on large areas which rendered them sensitive to rapidly evolving pathogen populations. Unfortunately crop plants are not evolving in farmer's field unlike their pathogen counterparts (Jarvis, Lane, & Hijmans, 2008; Lenne & Wood, 1991). This has led to an increased use of pesticides to curb their economic losses which have economic and environmental costs. Hence, the identification and deployment of resistant cultivars remains an economical and environmentally safe strategy. Maintaining favorable gene combinations in commercial cultivars with the introgression of desired donor germplasm is a good strategy which can lead to improved and stable yields (Peel & Rasmussen, 2000).

The development of highly productive and adapted varieties is key for sustaining genetic gains but is dependent on continued access to novel diversity and efficient use of genetic resources conserved in various genebanks (Hoisington et al., 1999). Hence the crop wild relatives hold a large genetic base which can supply breeders with useful sources of genes for complex and challenging traits (Feuillet, Langridge, & Waugh, 2008). The gene pool of cultivated barley includes a total of 32 species, with the wild progenitor species *H. vulgare* ssp. *spontaneum* included in the primary gene pool and *H. bulbosum* as the only member of the secondary gene pool (Vincent et al., 2019; Von Bothmer, Jacobsen, Baden, Jørgensen, & Linde-Laursen, 1995). From diverse attempts of interspecific crosses using wild *Hordeum* species, and because of crossability barriers, the only successful crosses were with *H.*

chilense to produce the new species *Tritordeum* when crossed with durum wheat (Martín-Sánchez et al., 2003); *H. bulbosum* mainly used in double haploid production in wheat and barley and to a limited extent to transfer useful genes (Pickering et al., 1998; Pickering & Johnston, 2005; Von Bothmer et al., 2003), and *H. spontaneum* used extensively by many barley breeding programs (Ellis et al., 2000; Tombuloglu, Ozcan, Tombuloglu, Sakcali, & Unver, 2015; Vincent et al., 2013).

Foliar diseases, mainly powdery mildew (PM), net blotch (NB), scald (SC), yellow and leaf rusts, and barley yellow dwarf virus (BYDV), are causing significant reductions in yield and quality all over the barley growing areas (Savary, Ficke, Aubertot, & Hollier, 2012). Powdery mildew (*Blumeria graminis* f. sp. *hordei*) is a disease of economic importance of barley and its infection can reduce the yield up to 30% in addition to damaging grain quality (Czembor, 2000). Likewise, barley leaf scald (*Rhynchosporium secalis*) is a dynamic pathogen capable of overcoming any deployed resistance in a short period of time (McDonald, Zhan, & Burdon, 1999). Barley leaf rust (*Puccinia hordei*) can cause yield losses of up to 32% on susceptible barley cultivars (Griffey, Das, Baldwin, & Waldenmaier, 1994). Disease resistance in crop plants is a result of an interaction between host plant and pathogen genotypes. Disease resistance in plants can be classified into race specific (qualitative) and race non-specific (quantitative) types. Race specific resistance is conditioned by dominant *R* genes and is considered non-durable because it imposes strong selection pressure for increased virulence on the pathogen which eventually overcomes it within a short period of time. Race non-specific resistance, on the other hand, involves a number of minor genes and is considered durable (Hill, Becker, & Tigerstedt, 1998). The dynamic host-pathogen interaction requires active monitoring of changes in pathogen virulence and stability of resistance in their host plants in different agro-ecologies.

Many studies have reported the richness of *H. spontaneum* accessions with respect to genes for resistance to major diseases (Abbott, Brown, & Burdon, 1991; Brian, Steffenson, & Bockelman, 1995; Fetch, Steffenson, & Nevo, 2003; Oğuz, Karakaya, & İsmail, 2018; Sato & Takeda, 1997; Tombuloglu et al., 2015), tolerance to major drought and salinity stresses (Barati et al., 2018; Chen et al., 2009; Ebrahim, Arzani, Rahimmalek, Sun, & Peng, 2019; Nevo & Chen, 2010; Wang et al., 2018; Yan, Chen, Cheng, Nevo, & Gutterman, 2008) and end-use quality traits (Cai et al., 2013; Yan et al., 2008). This diversity is extensively used in breeding programs (Nevo et al., 1992) and it is estimated that 40% of the alleles are common between *H. spontaneum* and cultivated barley (Ellis et al., 2000; Tombuloglu et al., 2015). Resistance has been introgressed from *H. spontaneum* into barley to many diseases and pests including, leaf rust (Ivandić, Walther, & Graner, 1998;

Steffenson et al., 2007), powdery mildew (Jørgensen, 1992; Moseman, Nevo, & Zohary, 1983), scald (Abbott et al., 1991), and the aphid species *Rhopalosiphum padi* (Åhman & Bengtsson, 2019).

Extensive evaluation of the limited number of lines derived from interspecific crosses with *H. bulbosum* allowed the identification of resistances to several diseases including, leaf rust (Pickering, Steffenson, Hill, & Borovkova, 1998; Yu et al., 2018), powdery mildew (Czembor, Pietrusińska, Piechota, & Mańkowski, 2019; Hoseinzadeh, Ruge-Wehling, Schweizer, Stein, & Pidon, 2020; Jørgensen, 1992; Pickering, Hill, Michel, & Timmerman – Vaughan, 1995, 1998; Shtaya, Sillero, Flath, Pickering, & Rubiales, 2007; Xu & Kasha, 1992), stem rust (Fetch, Johnston, & Pickering, 2009), septoria speckled leaf blotch (Toubia-Rahme, Johnston, Pickering, Steffenson, & Graner, 2003), barley yellow dwarf virus (Scholz et al., 2009), barley mild mosaic virus (BaMMV) and barley yellow mosaic virus (BaYMV) (Ruge-Wehling, Linz, Habekuß, & Wehling, 2006). This germplasm has not been evaluated extensively for yield performance. Furthermore, molecular markers and agronomic traits associated with drought tolerance were reported for the derived germplasm (Baum et al., 2007; Hajjar & Hodgkin, 2007; Lakew et al., 2013; Schmalenbach, Léon, & Pillen, 2009).

The present study aims at strengthening the pre-breeding efforts through identification of new sources of resistance to four major diseases and important agronomic traits in a set of *Hordeum spontaneum* accessions and the lines derived from interspecific crosses with *H. spontaneum* and *H. bulbosum*.

2 | MATERIALS AND METHODS

2.1 | Plant materials

A total of 145 introgression lines (*Hv/Hb* ILs) (Supplementary Table S1) issued from interspecific crosses of cultivated barley (*Hv*) parents (Vada, Morex, Emir, Golden Promise), with *Hordeum bulbosum* (*Hb*) clones (2920/4, 2032, 2929/1, A10, A17, A17/1, S1, 3811/5, 2920/4 × 2929/1, 2920/4 × Tinos16/1, Emir × Crete6/2, PI 20644 3/3, PI 204579) were obtained from the Nordic Genetic Resource Centre (NordGen), Alnarp, Sweden (Wendler et al., 2015). All the ILs were multiplied from a single spike and were used for field phenotyping. Another 117 *Hordeum spontaneum* (*Hs*) accessions (Supplementary Table S2), originating from 22 countries, were obtained from the gene bank of the International Center for Agriculture Research in the Dry Areas (ICARDA), Rabat, Morocco. These *H. spontaneum* accessions were chosen to represent the geographical distribution of this species as they are collected from 22 countries.

Core Ideas

- Hemi-biotrophic pathogens are major biotic stresses for sustainable barley production in dry areas.
- Barley wild relatives offer a reservoir untapped of disease resistance sources for barley breeding.
- Pre-breeding efforts need to be strengthened to develop new adapted and high yielding barley varieties.

2.2 | Screening for net blotch, leaf scald, leaf rust, and powdery mildew resistance

Resistance at adult plant stage against barley net form net blotch (NFNB), leaf scald (SC), leaf rust (LR), and powdery mildew (PM) was assessed in six field trials for two years in total, at three different locations, and across three growing seasons. To screen for NFNB and SC in 2017–2018 growing season, 145 *Hv/Hb* ILs and 117 *Hs* accessions were sown during the first week of December 2017 at the experimental research station of Marchouch (MCH-18), 33°56' N, 6°63' W, INRA, Morocco. For the screening with LR, the same set of genotypes (*Hv/Hb* ILs, *Hs* acc.) were planted in 2016–2017 and 2018–2019 at the research station of Sidi Allal Tazi (SAT-17, SAT-19), 34° 52' N, 6°32' W, INRA, Morocco. To screen for PM, a subset of 40 selected *Hv/Hb* ILs and 117 *Hs* acc. were planted at the experimental research station of Marchouch (MCH-17), 33°56' N, 6°63' W, and in Annoceur (ANN-18 and ANN-19), 33°41' N 4°51' W, INRA, Morocco, for the growing season of 2017–2018 and 2018–2019. For each trial, the genotypes were sown as a paired row of one-meter using an augmented block design with 20-test genotypes in each block, and a highly susceptible check for each disease was repeated after an interval of 10-test genotypes. For NFNB, Rabat 071 and Annoceur, for SC, Tocada and Tissa, for LR and PM, Rihan-03 and VMorales were used as susceptible checks, respectively. Further, each block was surrounded by a spreader row, consisting of a mixture of susceptible genotypes of each disease as mentioned above.

For the artificial inoculation of NFNB, inoculum composed of a mixture of 10-diverse isolates, collected from different growing regions of Morocco, was used. All the isolates were maintained as lyophilized agar plugs at –80 °C and the inoculum was prepared from V8PDA plates as described by Friesen, Faris, Lai, and Steffenson (2006) with a spore concentration adjusted to 10³ per milliliter. For SC, pure leaf scald isolates from different regions of Morocco were maintained on lima bean agar (LBA) at 15 °C and a mixture of isolates was

used as an inoculum with a spore density of 10^5 per milliliter as described by Salamati and Tronsmo (1997). A surfactant (Tween 20) was added to a final concentration of 0.01% and a knapsack sprayer was used for the inoculations of NFNB and SC. For LR, two isolates from MCH and SAT were multiplied on susceptible barley cultivars (Aglou, Baudin, Bowman) and the urediniospores were stored at -80°C . About 1 gram of dried spores of each isolate was suspended in 200 ml of mineral oil (Novec 7100, Sigma Aldrich) and sprayed on plants using an airbrush (Revell, Munchen, Germany). The PM trial was assessed under natural disease pressure as Marchouch and Annoceur are considered hot spot locations.

Field inoculations with NFNB, SC, and LR were performed three times with an interval of 10–15 days starting from Zadoks scale GS 30. The infection process and disease establishment were further favored by overhead sprinklers. Disease severity was recorded from 5–10 randomly chosen plants of each genotype. NFNB and SC disease severity was evaluated at GS 73–75 using the Saari and Prescott scale (0–9) where scores of 1–3 were considered resistant (R), 4 as moderately resistant (MR), 5–6 as moderately susceptible (MS), and 7–9 as susceptible (S) (Saari & Prescott, 1975). PM disease severity was evaluated on a modified 0–5 scale developed by Niewoehner and Leath (1998) where 0–1 was considered R, 2 as MR, 3 as MS, and 4–5 as S. Further, LR disease severity and infection type were recorded according to the modified Cobb scale (James, 1971; Melchers & Parker 1922). The coefficient of infection (CI) was calculated by multiplying the infection response value ($R = 0.2$, $MR = 0.4$, $MS = 0.8$, $S = 1$) with the percent disease severity on a scale of 0–100 as described by Stubbs, Prescott, Saari, and Dubin (1986). The genotypes with CI of 0–8 were considered R, 9–16 as MR, 17–24 as MS, and >25 as S.

2.3 | Evaluation of pre-breeding elite germplasm

Elite lines selected from the germplasm acquired from ICARDA barley breeding (5 lines issued from crosses with *H. spontaneum* (*Hv/Hs*)) and 40 *Hv/Hb* ILs from NordGen along with 10 checks (4 Australian varieties, 3 Moroccan varieties and 3 ICARDA elite lines) were included in an alpha lattice trial with two replications (Supplementary Table S3). The trial was conducted during 2018–2019 cropping season at four experiment stations in Morocco representing major agro-ecological regions: Marchouch (high rainfall, $33^\circ36' \text{ N } 6^\circ42' \text{ W}$), Tessaout (under supplementary irrigation, $31^\circ49' \text{ N } 7^\circ25' \text{ W}$), Jemaa Shaim (arid environment, $32^\circ24' \text{ N } 8^\circ46' \text{ W}$), Annoceur (mountainous areas, $33^\circ41' \text{ N } 4^\circ51' \text{ W}$). All trials were planted between 15–25 Nov. 2018 except for the trial at Annoceur station which was planted on 5 Jan. 2019. Each entry was sown in a plot of six rows of 2.0 m length and

0.25 m row spacing. Recommended agricultural practices for each region were applied with manual weeding done when needed. Grain yield (GY) and total biomass were estimated from the four central rows (2.5 m^2) and converted to kilograms per hectare. In addition, days to heading (days from sowing to 50% of the spikes out), plant height (PHT) in centimeter, number of spikes per square meter (SPKM2), and 1000 kernel weight (TKW) were assessed on all plots. Days to maturity (DMA), and lodging (in percent of plot affected) were assessed at Marchouch and Annoceur stations only.

2.4 | Data analysis

For yield and agronomic traits, analysis of variance was performed using the meta-R software (Vargas et al., 2013) to assess the genotype by environment interaction and to estimate the variance components. Heritability and the Best Linear Unbiased Estimations (BLUEs) for each genotype were estimated across environments and within each environment. The BLUEs in each environment were used to construct the Genotype Plus Genotype-by-Environment (GGE) biplot using the package GGE (Wright & Laffont 2018) in R software (R Core Team, 2020). The boxplots were constructed using the ggplot2 (Wickham, 2016). Microsoft Office 2019 was used to analyze and visualize the disease evaluation data.

3 | RESULTS

3.1 | Reaction to diseases

A whole range of disease severity (1–9) was observed in NFNB trial in MCH-18 (Table 1). Both susceptible checks, Rabat 071 and Annoceur, were found susceptible with a disease score of 8. Of the 106 accessions of *Hs* tested at MCH-18, 18 (17%) and 22 (20.7%) accessions were found R and MR, respectively. Similarly of the 132 *Hv/Hb* ILs tested, 14 (10.5%) and 27 (20.5%) of ILs were found R and MR, respectively.

We observed a range of disease severity (0–8) in the SC trial in MCH-18 with the susceptible checks displaying a disease severity of 8 (Tocada) and 7 (Tissa) (Table 1). The *Hs* collection had a higher frequency of leaf scald with 66 (58.4%) of its accessions being R, and 15 (13.2%) accession being MR. *Hv/Hb* collection had lower frequency of resistance as 18 (13.6%) ILs were R, and 9 (6.8%) ILs were MR (Table 1). Additionally, about 50% of ILs were susceptible as compared to only 3.6% of the *Hs* accessions.

The LR infection was quite homogenous in SAT-17 and SAT-19 with CI ranging from 0–80 (Table 1). Both checks, Rihan-03, VMorales scored a disease severity of 80S and 60S in SAT-17 and in SAT-19, respectively. Among the 114 *Hs*

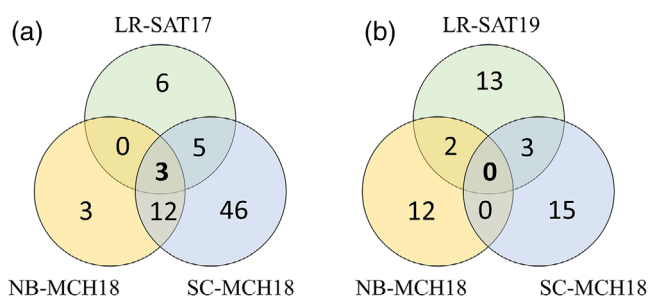
TABLE 1 Number and percent of *Hordeum spontaneum* accessions and *H. bulbosum* derived lines within each disease reaction class for Net blotch (NB), Scald (SC), Leaf rust (LR), and Powdery mildew evaluated at Marchouch (MCH), Allal Tazi (SAT), and Annoucer (ANN) experiment stations in Morocco during 2017, 2018 and 2019

	<i>H. bulbosum</i> derivatives				<i>H. spontaneum</i>				
	NB-MCH-18	SC-MCH-18	LR-SAT-19	PM-MCH-18	PM-ANN-18	NB-MCH-18	SC-MCH-18	LR-SAT-17	PM-MCH-17
Number of R genotypes	14	18	18	21	20	18	66	14	46
Percentage of R genotypes	10.5%	13.6%	12.9%	61.8%	58.8%	17%	58.40%	12.40%	39.50%
Number of MR genotypes	27	9	7	3	4	22	15	3	47
Percentage of MR genotypes	20.5%	6.8%	5.0%	8.8%	11.8%	20.70%	13.20%	2.7%	40%
Number of MS genotypes	64	36	17	4	4	50	28	4	10
Percentage of MS genotypes	48.5%	27.4%	12.1%	11.8%	11.8%	47.20%	24.80%	35.0%	8.50%
Number of S genotypes	27	69	98	6	6	16	4	93	14
Percentage of S genotypes	20.5%	52.2%	70.0%	17.6%	17.6%	15%	3.60%	81.40%	12%
Range of severity	1–9	0–8	0–60	0–5	0–5	1–9	0–7	0–80	0–5
No germination	10	10	2	6	6	11	4	3	4
Genotypes evaluated	132	132	140	34	34	106	113	114	113

R: Resistant; MR: Moderately Resistant; MS: Moderately susceptible; S: Susceptible.

TABLE 2 *Hordeum spontaneum* accessions with combined resistance to Net blotch, Scald, Leaf rust and powdery mildew

IG #	DOI	Origin	NB-MCH18	SC-MCH-18	LR-SAT-17	PM-MCH-17
144933	10.18730/9T9ZX	LBY	R	R	R	R
144113	10.18730/9RXSN	SYR	R	R	R	MR
144112	10.18730/9RXPJ	JOR	MR	R	R	R
145556	10.18730/9VAM1	TJK	R	R	–	R
144123	10.18730/9RY4*	JOR	R	R	–	MR
145088	10.18730/9TJG6	SYR	R	R	–	MR
146811	10.18730/9WPR7	TJK	R	R	–	R
135624	10.18730/9E4J0	TKM	R	R	–	MR
131620	10.18730/9AEDY	KAZ	R	R	–	MR
116104	10.18730/8X02Q	TUR	R	R	–	MR
40009	10.18730/6TFQ~	JOR	R	R	–	MR
39935	10.18730/6TDH0	LBY	R	R	–	R
38821	10.18730/6SC66	TKM	R	R	–	R
39885	10.18730/6TC2V	CYP	R	R	–	MR
144117	10.18730/9RXYT	ISR	MR	–	R	R
38673	10.18730/6S7J6	TUR	MR	–	R	R
144119	10.18730/9RY0W	ISR	MR	R	–	R
145504	10.18730/9V7YT	TJK	MR	R	–	R
38943	10.18730/6SG0H	ISR	–	R	R	R
119937	10.18730/90CSR	MAR	–	R	R	R
40056	10.18730/6TH55	JOR	–	R	R	MR
144120	10.18730/9RY1X	ISR	–	R	R	MR
144127	10.18730/9RY8U	JOR	MR	R	MR	R

**FIGURE 1** Number of accessions and lines with resistant reactions to the three diseases Leaf Rust (LR), Net Blotch (NB) and Scald (SC) in case of (a) *H. spontaneum* accessions, and (b) *H. bulbosum* derived lines

accessions tested, 14 (12.4%) and 3 (2.7%) were found R and MR in SAT-17, respectively. Whereas of the 140 *Hv/Hb* ILs tested, 18 (12.9%) and 7 (5.0%) ILs were found R and MR in SAT-19, respectively.

We observed a range of disease severities (0-5) in the PM trial in MCH-17, MCH-18, and in ANN-18 with both susceptible checks, VMorales and Rihan03, found to be susceptible with a disease severity of 4 and 5, respectively. The *Hv/Hb* ILs were accessions were found to be more effective in resistance to PM with 21 (61.8%) and 20 (58.8%) of ILs being R in

MCH-18 and ANN-18, as compared to 46 (39.5%) *Hs* accession being MR in MCH-17 (Table 1).

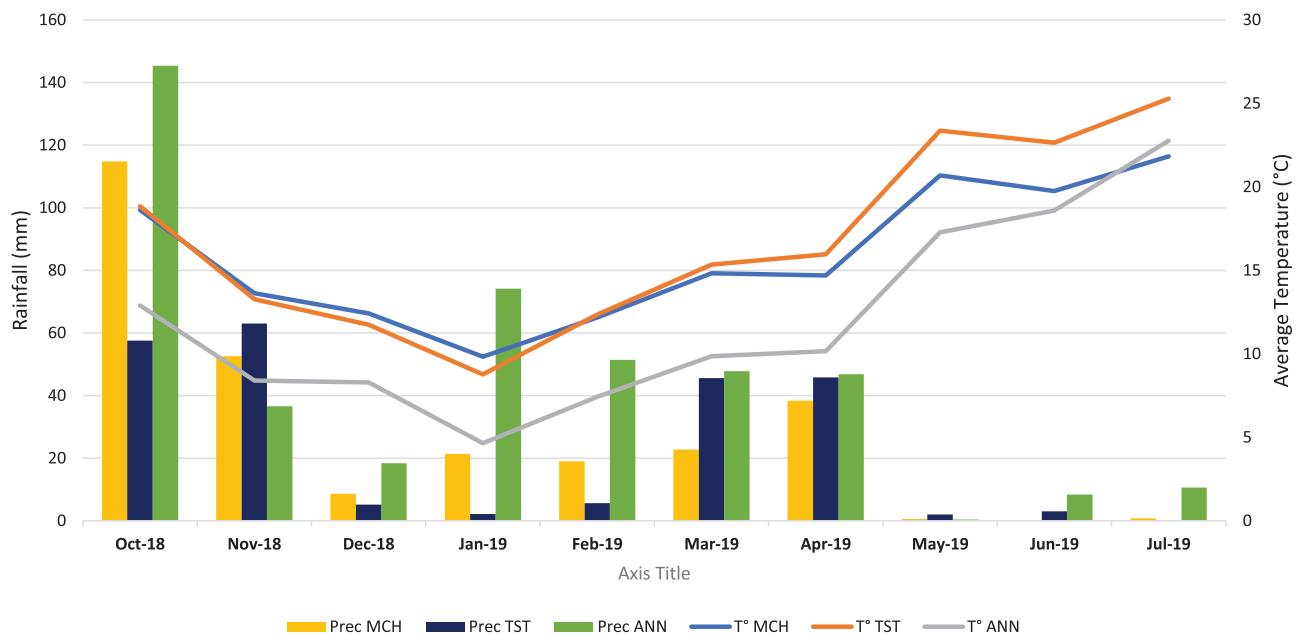
The potential of *Hs* accessions and *Hv/Hb* ILs as combined sources of resistance to hemi-biotrophic and biotrophic pathogens was explored further (Figure 1). Three *Hs* accessions (IG# 144113, 144112, and 144933) collected from Syria, Jordan, and Libya, respectively, were found R to NFNB, SC, LR, and PM (Table 2, Figure 1a). Furthermore, 12 accessions were found R to NFNB and SC, whereas 5 accessions were found R to SC and LR (Figure 1a, Table 2). In contrast, no *Hv/Hb* IL was found to be resistant to all the three pathogens tested (Figure 1b). Only 2 ILs were found R to NFNB and LR, and 3 ILs were found R to SC and LR (Table 3).

3.2 | Evaluation of performance and agronomic traits of pre-breeding lines

The rainfall distribution was characterized by early rains before planting followed by limited rains during the months of December, January, and February at Marchouch and Tessaout except for Annoceur. The months of March and April received around 40 mm except at Marchouch. In addition, the average temperatures were higher at Tessaout starting from the month

TABLE 3 *Hordeum bulbosum* derived lines with combined resistance to Net blotch, Scald, Leaf rust, and powdery mildew

IG #	DOI	NB-MCH18	SC-MCH-18	LR-SAT19	PM-MCH18
167662	10.18730/AXXPJ	R	–	–	R
167706	10.18730/AY04P	R	–	–	R
167651	10.18730/AXX40	R	–	–	R
167687	10.18730/AXZ4V	R	–	–	R
167590	10.18730/AXSQ2	R	–	R	–
167633	10.18730/AXW23	R	–	R	–
167664	10.18730/AXXTP	MR	–	R	R
167692	10.18730/AXZDU	R	MR	–	MR
167692	10.18730/AXZDU	R	MR	–	MR
167600	10.18730/AXT8K	MR	MR	–	R
167671	10.18730/AXY6\$	MR	R	–	R
167727	10.18730/AY1BR	MR	R	–	R
167727	10.18730/AY1BR	MR	R	–	R
167585	10.18730/AXSEY	–	R	R	–
167589	10.18730/AXSN0	–	R	R	–
167682	10.18730/AXYVJ	–	R	R	–

**FIGURE 2** Rainfall (mm) and average temperature at Annoceur (ANN), Marchouch MCH), and Tessaout (TST) experiment stations during 2018–2019 season

of March. Based on the climatic data and performance, the trials were affected by drought at Annoceur, and by terminal heat stress at Tessaout (Figure 2). At Jemaa Shaim, drought combined with terminal heat stress affected the trial as average rainfall of less than 200 mm was reported and the maximum temperature remained higher than 30 °C since March 2019.

The combined analysis of variance showed significant differences between the locations for all agronomic traits except for HI and the number of spikes per m², and highly signifi-

cant differences among genotypes for all traits except for straw yield (SY). The study showed a significant difference between the lines and hence a high heritability for all traits except for SY (Table 4). Genotype by location interactions were significant to highly significant for all studied traits except for TKW and DMA where then no interaction between the lines and the locations were found.

When comparing different types of germplasm, on average the checks showed higher grain yield in the four

TABLE 4 Sources of variance (%) of different measured agronomic traits of diverse barley genotypes grown at Annoceur (ANN), Jemaa Shaim (JS), Marchouch (MCH), and Tessaout (TST) during 2018–2019 cropping season

	PHT	GY	BY	HI	TKW	SPKM2	DMA	SY
Genetic variance (%)	37.65***	9.95***	3.08***	27.32***	44.93***	19.29***	10.61***	2.43 ns
Genotype × location variance (%)	10.85***	4.38*	4.55***	24***	4.62 ns	12.89**	2.3 ns	8.71***
Location variance (%)	37.09**	68.66**	80.27**	9.13 ns	18.4**	27.8*	79.79 ns	72.3**
Heritability	0.89	0.75	0.53	0.71	0.89	0.63	0.78	0.36
Number of locations	4	4	4	4	4	3	2	4

Grain yield (GY), Biological yield (BY), Harvest index (HI), Thousand kernel weight (TKW), Number of spikes per square meter (SPKM2), Days to maturity (DMA), Straw yield (SY), Plant height (PHT).

ns: not significant at 0.05.

*Significant at 5%.

**Significant at 1%.

***Significant at < 1%.

TABLE 5 Grain yield (kg/ha) of different groups of barley germplasm (*HbL*: *Hv/Hb* ILs, *HsL*: *Hordeum spontaneum* derived lines, checks) grown at four different locations during 2018–2019 cropping season

Location	Group	Number	Minimum	Maximum	Mean	Standard Deviation
Annoceur	Checks	10	1147.9	2441.1	1729.3	467.9
	<i>HbL</i>	40	20.3	1463.1	806.1	349.7
	<i>HsL</i>	5	1068.4	1639.7	1337.9	205.4
Jemaa Shaim	Checks	10	982.1	3469.4	2046.3	638.7
	<i>HbL</i>	40	74.1	2442.6	1193.8	563.5
	<i>HsL</i>	5	1437.2	2117.4	1797.5	312.0
Marchouch	Checks	10	3593.3	5599.8	4427.1	737.9
	<i>HbL</i>	40	1615.7	4646.3	3361.3	806.7
	<i>HsL</i>	5	3482.0	3883.4	3713.6	154.3
Tessaout	Checks	10	1676.5	7138.7	4005.7	1458.3
	<i>HbL</i>	40	1232.7	4557.7	3164.8	816.5
	<i>HsL</i>	5	2674.5	4313.8	3374.3	697.3

locations, followed by *Hs* derivatives, while most of the *Hv/Hb* ILs had the lowest yields (Table 5). Only few lines of this later group showed grain yields similar to some of the checks (Figure 3). The locations were clearly different in terms of the grain yield performance, the only exception was the similarity found between JS and ANN (Supplementary Table S4). However, for straw yield, *Hv/Hb* ILs and *Hs* derived lines showed a higher mean performance at Marchouch and Tessaout than the checks, but not at Jemaa Shaim and Annoceur (Table 5).

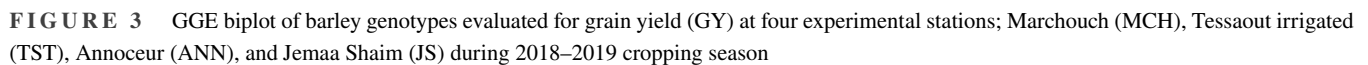
When considering agronomic traits, generally the *HbL* group (*Hv/Hb* ILs) showed large number of lines with higher plant height (Figure 4) at the four locations, and high number of lines with longer cycles in terms of days to heading and days to maturity when evaluated at Marchouch station (Figure 5). *HsL* (*Hv/Hs* ILs) lines showed earliness in terms of heading and maturity similar to the checks. For the grain filling period, the three groups had shown similar averages, but some of *Hv/Hb* ILs had longer period. For thousand ker-

nel weight, *Hv/Hb* ILs had on average lower values compared to the checks and *HsL* across the four sites (Figure 6).

The prevailing environmental conditions at Marchouch allowed the evaluation of the germplasm to lodging. Some of the checks and a large number of *Hv/Hb* ILs showed good levels of lodging (score less than 2) (Figure 7). It should be noted that some of the *Hv/Hb* ILs combined lodging resistance with tallness.

4 | DISCUSSION

Barley breeders are in continuous search for novel diversity of desired traits including sources of durable resistance to major diseases and insects and tolerance to major abiotic stresses. The mobilization of genes from wild relative species will be needed more, calling for intensive and reliable evaluation of accessions of wild relatives. In this research, the use of artificial inoculation (except PM trial) and sprinkler



In general, both *Hs* accessions and *Hv/Hb* derived lines were found to be more resistant to hemi-biotrophic pathogens such as NFNb and SC as compared to biotrophic pathogens such as leaf rust. About 37.7% and 71.6% of *Hs* accessions were found R/MR to NFNb and SC as compared to only

Hordeum spontaneum is confirmed as a rich reservoir of genes for adult plant resistance to scald, net blotch and leaf rust. Most of the previous studies have reported a high percentage of resistant accessions when tested at the seedling stage. While evaluating 116 accessions of wild barley, Fetch et al. (2003) reported diverse sources of resistance to six pathogens of economic importance at seedling stage. A higher frequency of resistance at the seedling stage was observed in collections of wild barley from Jordan and Israel, specifically to leaf rust (70 and 90%), net blotch (72 and 68%), and powdery mildew (58 and 70%). Similarly, Moseman et al. (1983) found 75% of 277 wild barley accessions from Israel to be

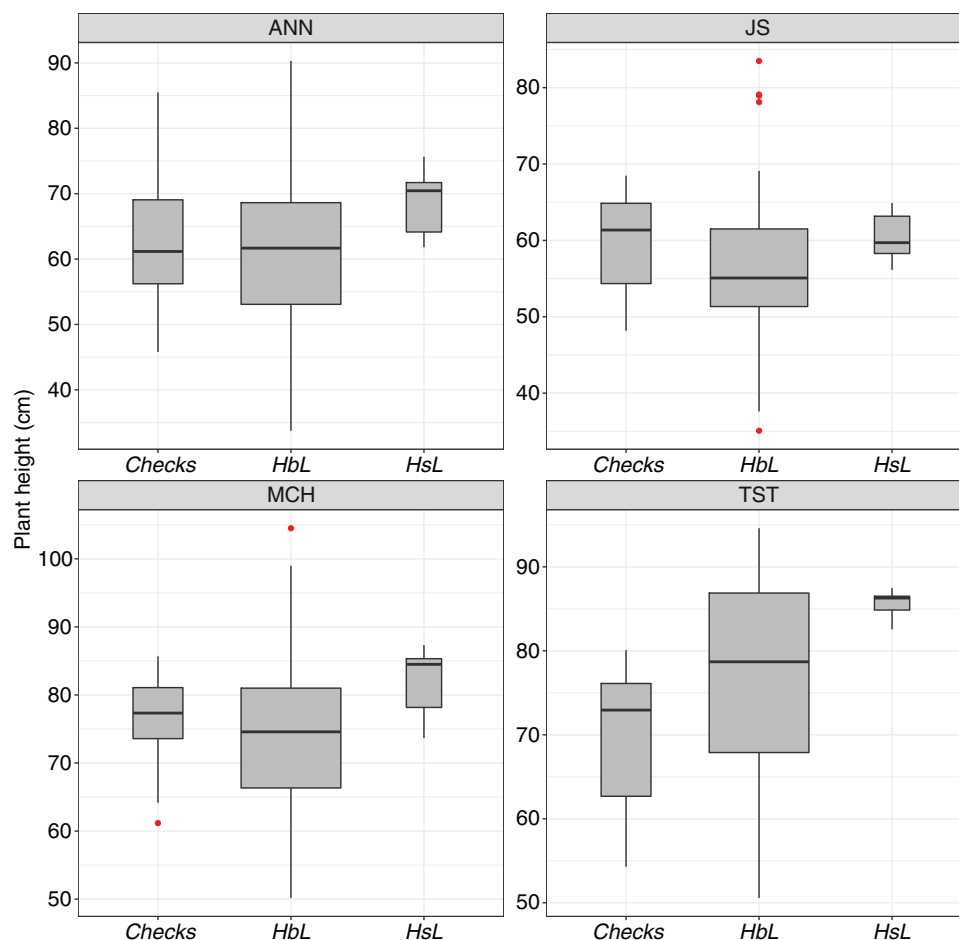


FIGURE 4 Boxplot for plant height of different groups of barley germplasm (HbL: *Hv/Hb* ILs, HsL: *H. spontaneum* derived lines, checks) grown at (a) Annoceur (ANN), (b) Jemaa Shaim (JS), (c) Marchouch (MCH), and (d) Tessaout (TST) during cropping 2018–2019 season

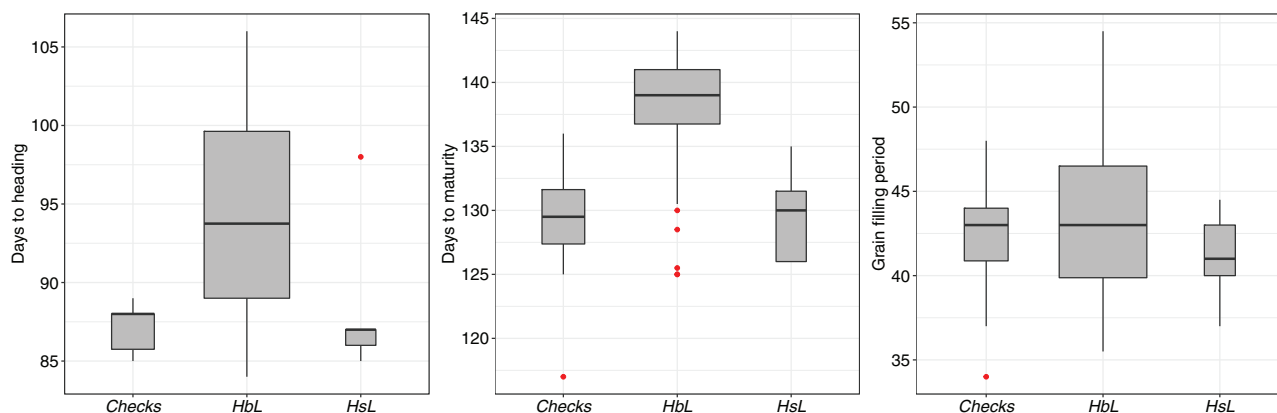


FIGURE 5 Boxplot for days to heading (a), days to maturity (b) and grain filling period (c) of different groups of barley germplasm (HbL: *Hv/Hb* ILs, HsL: *H. spontaneum* derived lines, checks) grown at Marchouch (MCH) station during 2018–2019 cropping season

resistant to two races of powdery mildew. We also found that all of the *H. spontaneum* accessions from Israel were highly resistant to net blotch, scald and powdery mildew at the adult plant stage. In addition, 14 accessions from Jordan and 15

accessions from Syria were found highly resistant to scald (Table 2). Moseman, Nevo, and El-Morshidy (1990) found 52% and 67% of wild barley accessions from Israel to be resistant to two races of leaf rust at the seedling stage. In our

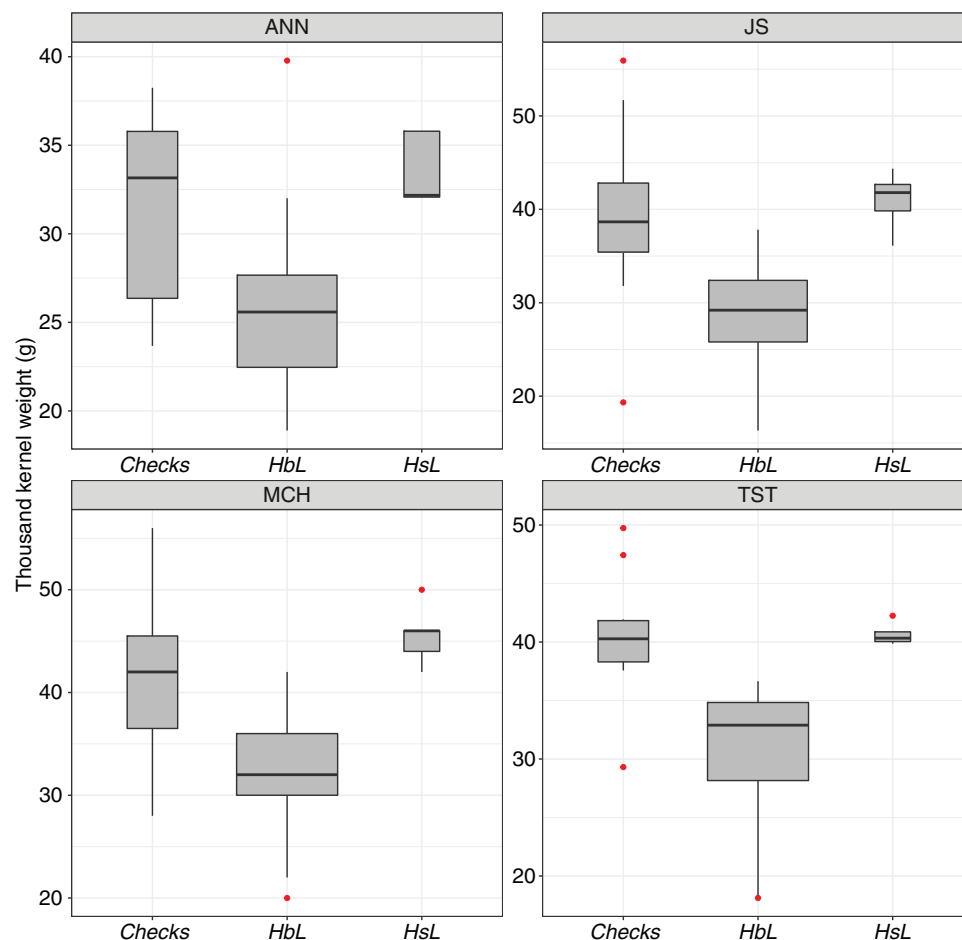


FIGURE 6 Boxplot for thousand kernel weight of different groups of barley germplasm (HbL: *Hv/Hb* ILs, HsL: *H. spontaneum* derived lines, checks) grown at (a) Annoceur (ANN), (b) Jemaa Shaim (JS), (c) Marchouch (MCH), and (d) Tessaout (TST) during 2018–2019 cropping season

screening of 117 *H. spontaneum* accessions from 22 countries, leaf rust resistant accessions from 14 countries were found with 11 out of 14 of these cases being from Turkey, Jordan, Syria, Israel, and Libya (Supplementary Table S2).

Resistance at both seedling and adult plant is important since the effects of major genes are better expressed at seedling stage and field resistance is better expressed at the adult stage. This later stage allows one to combine breeder selections for both agronomic traits and resistance to major diseases. Our study is unique as the screening was performed in the field at the adult plant stage. In addition, considering accessions with moderate resistance might allow the combination of genes with minor and major effects. The efficient use of the sources of desired traits from *H. spontaneum* will require the identification of novel allelic variation using others molecular techniques, along with strengthening pre-breeding efforts through well targeted crosses using wild *Hordeum* species, focusing mainly on wild accessions with a combination of desired traits to be crossed with the best available parental germplasm of cultivated barley.

The *H. spontaneum* accessions and *Hv/Hb* ILs lines were challenged by pathogen isolates with diverse virulence spectra (Supplementary Table S7). In case of LR, all the differential lines were susceptible except one differential line (*Rph2.y*) resistant at the adult plant stage. Furthermore, all the differential lines of net blotch were tested moderately susceptible to susceptible at the adult plant stage. Even the lines with known resistance sources (Harbin, CI5791, Tifang, and Rabat 071) were susceptible. However, in case of powdery mildew 3 and 7 differential lines were resistant and moderately resistant at the adult plant stage. Likewise, 8 scald differential lines were resistant at the adult plant stage. Hence resistant lines reported here could be used in hybridization programs globally.

The introgression of genes from *H. spontaneum* into cultivated barley are routine as no hybridization barriers exist. In contrast, interspecific crosses with *H. bulbosum* are more difficult due to difficulties in embryo rescue, the fertility of F1 plants, and the low seed setting in successive back crossings (Pickering & Johnston, 2005). The *H. bulbosum* derived lines supplied by NordGen constitute an example of valuable germplasm shared with many users who reported new sources

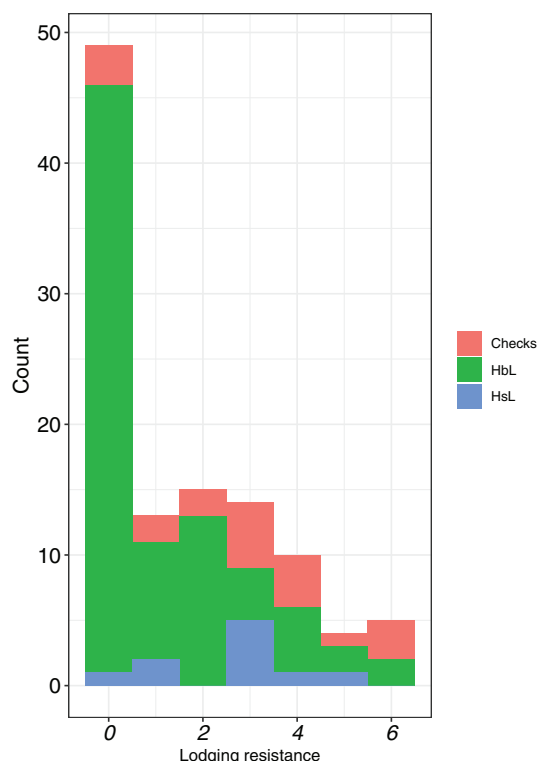


FIGURE 7 Response of HbL (*Hv/Hb* ILs), HsL (*H. spontaneum* derived lines), and checks (percentage) to lodging at Marchouch research station during 2018–2019 cropping season

of resistance to leaf rust (Pickering et al., 1995; Ruge-Wehling et al., 2006; Yu et al., 2018), to powdery mildew (Czembor et al., 2019; Pickering et al., 1995; Shtaya et al., 2007; Xu & Kasha, 1992); scald, stem rust, Septoria leaf blotch and barley yellow dwarf virus (Fetch et al., 2009; Scholz et al., 2009; Shtaya et al., 2007; Toubia-Rahme et al., 2003; Wojacki et al., 2013). This germplasm, when tested in our study showed a high number of lines with resistance to leaf rust, net blotch and scald with only few lines with combined resistances to two or three diseases (Figure 1).

Hs has higher percentage of resistant accessions to NFNB and SC (hemibiotrophic) as compared to the *Hv/Hb* derived lines. For example, 17% of *Hs* accessions were found highly resistant to NFNB as compared to 10.5% of the ILs tested. The most pronounced differences were observed for SC where 58.4% of the *Hs* accessions were found highly resistant compared to only 13.6% of the *Hv/Hb* derived lines. Compared to *H. spontaneum* accessions, a lower percentage of entries of *Hv/Hb* were resistant to scald and none of the lines combined resistances to the three diseases (Tables 2 and 3). However, *Hv/Hb* derived lines exhibited a higher proportion of resistance against biotrophic pathogens. For example, 61.8% (MCH-18) and 58.8% (ANN-18) of ILs were found to be resistant to powdery mildew as compared to only 39.4% of *Hs* accessions (Table 1). Although large number of genes for resistance to diseases were identified in this germplasm, and

because of limited number of *H. bulbosum* and cultivated barley parents used in initial crosses, *H. spontaneum* might include more diversified sources of traits. However, *H. bulbosum* could provide a more durable resistance as in the case of *mlo* gene for resistance to powdery mildew (Jørgensen, 1992; Lyngkjær, Newton, Atzema, & Baker, 2000) and could be a source of non-host type of resistance as in the case of the gene *Rph22* of leaf rust (Johnston, Niks, Meiyalaghan, Blanchet, & Pickering, 2013).

While *Hv/Hb* derived ILs included lines with strong resistance to the three major diseases, their evaluation along with *H. spontaneum* derived lines and the checks did not allow the identification of any derivative lines with higher grain yield or higher thousand kernel weight compared to the best checks in each of the four environments representing various abiotic stresses. However, some of *Hv/Hb* ILs showed higher straw yield and taller plant height with good resistance to lodging. In addition, several *Hv/Hb* ILs had winter growth habit and extended growth cycles which could explain in part the low performances under drought conditions experienced at Annoceur and Jemaa Shaim and under heat stress at Tessaout. The limited grain yield performance of *Hv/Hb* ILs and their poor adaptation to Mediterranean types of environments could be explained by the fact that this germplasm was developed from crosses of a limited number of *H. bulbosum* accessions with a few European barley varieties, some of which are winter type or late. However, most pre-breeding efforts are targeting the development of parental germplasm for use by barley breeding programs for development of elite germplasm which will allow the release of productive varieties adapted to different conditions and end uses.

More intra-specific and interspecific crosses using diverse accessions of *H. spontaneum* and *H. bulbosum*, respectively, are needed to transfer genes for resistance to major diseases and insect pests, genes for tolerance to heat, drought and mineral stresses, and genes for better quality and nutritional value. The value of these wild species will be increased following precision phenotyping and genotyping/sequencing (Brozynska, Furtado, & Henry, 2016). ICARDA, within its efforts to strengthen pre-breeding efforts, is performing intra-specific crosses between selected *H. spontaneum* combining resistance to leaf rust, scald, net blotch and with high beta glucan content using Australian and Moroccan varieties and elite ICARDA germplasm as recurrent parents. In collaboration with partners at the National Institute for Agricultural Research in Morocco (INRA-Morocco), crosses with *H. bulbosum* were successfully performed. Segregating generations are advanced, and all germplasm generated will be shared with partners around the world using the Standard Material Transfer Agreement. The elite pre-breeding germplasm with confirmed sources of breeders sought traits are included in an active collection held in-trust by ICARDA. For *H. bulbosum* derived germplasm, even segregating populations generated

should be kept in the active collection for further evaluation of any other target traits.

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ORCID

Sajid Rehman  <https://orcid.org/0000-0003-1959-5203>

Hafid Aberkane  <https://orcid.org/0000-0003-4092-389X>

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SUPPORTING INFORMATION

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