Performance of Barley Lines Selected under Drought Stressed Conditions and Ultra-Low Density

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ABSTRACT

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Rainfall and temperature are unpredictable in Mediterranean environments, which result in irregular environmental conditions for crop growth and a critical source of uncertainty for farmers. In this study, selected barley lines for grain yield stability under drought stressed conditions and ultra-low plant density (Honeycomb design), were evaluated for agronomic performance in semi-arid areas (Kef and Mornag) compared to the source material. Results showed a significant effect of genotype and genotype×environment (G×E) interaction which indicate the existence of differences among genotypes for plasticity. Biological and grain yield ranged from 3.72 to 7.13 t/ha and 1.46 to 2.66 t/ha across environments with higher values in Kef compared to Mornag. Five high yielding selected lines outyield the original populations (IH17 and IH4-H4 from Imen, AH10-H2 and AH10-H3 from Ardhaoui and MH18 from Manel). The first cycle low yielding lines showed a performance that ranked below the source material. Second cycle high yielding lines did not differ from the first cycle high yielding ones. In conclusion, selection under ultra-low density has been proven an efficient tool to select for lines with high agronomic performance and improved adaptation under the Tunisian dry conditions.

Keywords: Barley, selection, performance, semi-arid, ultra-low plant density

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Among all cereal crops, barley (*Hordeum vulgare*) comes after maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum* spp.) in terms of total production (Schulte et al. 2009). About two-thirds of global barley crop is used for animal feed, while the remaining third serves for the malting, brewing and distilling industries (Schulte et al. 2009). In several areas of the globe, such as in the semi-arid regions of North Africa, in Middle East, in the highlands of Nepal, Tibet and Ethiopia, in the Andean countries of South America and also the Himalayas, it is considered as a food crop (Lakshmi et al. 2016). Furthermore, barley has the widest range of production environments in the world (Horlsey et al. 2009). Barley is adaptable to stress conditions with early flowering, seed development and maturation occur in an optimum time period (Gürel et al. 2016). These attributes render barley as a well-suited crop for cultivation from boreal to equatorial regions (Schulte et al. 2009). The natural tolerance of barley to the different kind of adversities has led to an increasing interest for the identification of stress responsive genes using over expression of some of these resistance/tolerance genes by genetic transformation (Contreras-Moreira et al. 2017; Gürel et al. 2016). Barley production in 70% of the cultivated land in Tunisia depends on rainfall conditions, where drought stress occurs most often.

Breeding for abiotic stresses is a high priority for many barley breeders. A non-exhaustive list of these stresses includes drought and flooding, high and low temperatures, mineral deficiencies and toxicities, poor soil tilth and many others (Horsley et al. 2009). Frequently, more than one kind of stress occur in combination, such as the case of drought and heat, acting in a synergistic manner rather than in a simple additive way (Liu et al. 2015) or in succession (e.g. flooding followed by drought). This trend would maintain environments requiring the enhancement of multiple mechanisms (Mickelbart et al. 2015). However, among all of abiotic adversities, drought is already prominent at several major agricultural areas throughout the world (Luck et al. 2015). The effects are predicted to worsen due to growing water demand, shrinking water supply, and increased seasonal variability (Barnabas et al. 2008; Luck et al., 2015).

In Mediterranean regions, where barley is sown in large scale (Ceccarelli 1994; Ryan et al. 2009), drought occurs several times during the life cycle of crops, especially in the terminal growth stages (Turner 2004). The agronomic traits of grain yield could be strongly influenced (Araus et al. 2002; Fischer and Murner 1978; Saini and Westgate 1999). Breeding for drought tolerance has generated improved cultivars for drought prone environments, but progress has been slow (Nguyen 2000). Functional genomic technologies are used to gain a better understanding of how plants respond to drought and to different abiotic stresses (Langridge et al. 2006). However, establishment of innovative and effective breeding strategies at the field level to tackle biotic adversities is still an imperative process that needs to be employed.

An appropriate strategy to achieve this goal is the exploitation of genetic diversity not yet incorporated into the elite cultivars (Dwivedi et al. 2016). As in other crops, current barley cultivars exhibit a narrower genetic basis than wild progenitors (*Hordeum vulgare* ssp. *spontaneum*) and landraces, which are the primary source of useful genes for breeding programs (Dawson et al. 2015). Moreover, there are plenty of cases well documented that in low-input environments, landraces can perform equally or even better than modern cultivars (Ceccarelli et al. 1998; Pswarayi et al. 2008; Yahiaoui et al. 2014). Therefore, harnessing unexploitable genetic diversity of barley crop can be one of the pillars towards resilience to abiotic adversities.

Physiological responses of plants to drought stress are complex and vary with plant species and the degree or time of the exposure to drought (Bodner et al. 2015). One method to assess tolerance to stresses is by determining plant chlorophyll fluorescence (ChlF) (Sayed 2003). The physiological state of the photosynthetic apparatus is highly sensitive to different stresses, thus ChlF is considered to be a very reliable method for assessing the plant tolerance to different stresses (Ren et al. 2018). High chlorophyll content under stress conditions is also a good index as physiological trait. This trait indicates a low degree of photo inhibition of the photosynthetic apparatus (Talebi 2011). A canopy temperature measurement is another index effective method to assess for stress tolerance. This could be seen as relationship between leaf temperature and transpirational cooling (Jackson 1982).

Tailored to the above tools for developing stress tolerant cultivars, honeycomb methodology has been suggested as a breeding procedure to develop cultivars that entirely confront with the challenges of modern agriculture (Fasoula and Tokatlidis 2012). The primary principles that distinguish this method from the other conventional breeding schemes and experimentation designs include the evaluation and selection under ultra-low plant densities, and the systematic entry arrangement to cope with the soil heterogeneity. Selection in the absence of competition maximizes the phenotypic expression of genetic differences among individuals. This design is facilitating the detection of desirable genotypes (Fasoula and Fasoula 2002; Tokatlidis et al. 2010), while eliminates the confounding effects induced by the negative relationship between yielding and competitive ability (Chatzoglou and Tokatlidis 2012; Kyriakou and Fasoulas 1985; Ninou et al. 2014). In the honeycomb layouts (Fasoulas and Fasoula 1995), entries are always allocated evenly across the experimental area, in such a way that every plant of a given entry is consistently surrounded by plants of the remaining entries forming a complete circular replicate; this systematic instead of a randomized entry arrangement ensures the objective comparison of the entries (Papadopoulos and Tokatlidis 2011).

Improvement of drought tolerant barley genotypes is crucial task for breeders. Thus, the objective of the present study was to (i) investigate stability and drought tolerance of barley lines derived from three commercially released cultivars and two Tunisian landraces, using single-plant selection at ultra-low density under Tunisian drought stress conditions for two consecutive cycles, according to the honeycomb methodology; (ii) determine the efficiency of physiological traits to group barley lines into drought susceptible and drought tolerant; and (iii) investigate the relationships between physiological traits and yield parameters.

MATERIALS AND METHODS

Plant material and field experimentation.

A total of 50 first and second cycle selection lines along with the 5 original populations used as checks, were evaluated in this study. The original populations comprised three commercially released cultivars in Tunisia, i.e. Imen, Manel and Rihane and two Tunisian landraces, i.e. Ardhaoui and Djebali. Lines were selected for two consecutive years by applying intra-cultivar single plant selection for high and low yield, under ultra-low density, according to the honeycomb field layout (Fasoulas and Fasoula 1995). Selection was carried out at Kef experimental station of the National Agricultural Research Institute of Tunisia

(INRAT) during 2013/14 and 2014/15 cropping seasons, under rainfed conditions. Besides the five checks used, the entries included 12 first cycle lines (8 selected for high yield and 4 selected for low yield) and 38 second cycle lines (30 selected for high yield and 8 selected for low yield, the latter subjected for high grain yield selection during the first cycle). A detailed description on the selection process regarding these lines is previously given by Ben Ghanem et al. (2018).

Evaluation of agronomic performance traits and records of physiological parameters was carried out at two research stations in Tunisia, at Kef (36º 14' N; 8º 27' E; 518m) and Mornag (36º 37' N; 10º 17' E; 54m) during 2015/16 cropping season. These two research stations represent two distinct production environments for Tunisia. Mornag is characterized by clay soil and average annual precipitation of 450 mm. Kef is characterized by clay loam soil and barley being the most common rainfed crop for the region. Annual rainfall in Kef and Mornag stations during 2015/16 growing season was 325 and 295 mm, respectively.

A non-replicated augmented design field trial was established, in each of the stations, with five incomplete blocks and 15 entries per block. Plot size was composed of four rows of 2.5 m long each with 0.25 m spacing between rows, resulting in a plot area of 2.5 m^2. Trials planted late November with a seed rate of 120 kg/ha and harvested beginning of June. Distance between plots kept at 2 m; best management practices in terms of chemical applications were applied and weed control performed by hand. All four rows of the plots were harvested.

Data collection for agronomic and physiological traits.

During the growing season a number of agronomic and physiological traits were recorded. For both locations biological yield (BY: t/ha) and grain yield (GY: t/ha) per plot was measured at maturity and, Harvest index (HI) was calculated as the quotient between grain and biological yields. Plant height (PH) was measured at maturity from five randomly selected plants within each plot and recorded as the distance in centimeters from soil level to the tip of spikes excluding the awns. Spike length (SL) recorded in centimeters as the average of ten representative spikes of each plot from the base up to the tip of the spike. Each of these spikes then threshed individually and the average grain weight (SGW) per spike expressed in grams for each of the entries was also recorded. Powdery mildew (PM) reaction was also scored at the seedling stage at both locations based on the prevalence of the disease and entries characterized as resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and very susceptible (VS) (Saari and Prescot 1975).

A number of physiological parameters were also recorded for both locations. SPAD values at the middle of tillering stage (SPAD_TL) were measured on fully expanded leaves of three representative plants of each plot using a MINOLTA SPAD 502 Plus chlorophyll meter. Leaf canopy temperature (LCT) recorded as the average of five representative positions within each plot using an infrared scantemp 440 thermometer. Chlorophyll fluorescence F_0, F_m, F_v, F_m/F_v and F_v/F_o parameters were measured to test the differences of photosystem II (PSII) (Baker 2008) at the fully expanded flag leaves of three representative plants of each plot at heading time stage using an OPTI-SCIENCE 0530+ hand held portable fluorometer. At Kef station, some additional parameters were also recorded, such as days to heading (HD), (the number of days needed from sowing to the time that 50% of the plot reaches Zadoks stage 59 (Zadoks et al. 1974)), number of total and fertile tillers per plant (T/P and FT/P) and SPAD values of five randomly selected plants from each entry/plot at the heading stage (SPAD_FL) (SPAD values per plant is the average from three fully expanded flag leaves).

Data analysis.

Raw data values for agronomic and physiological traits were analyzed using combined analysis of variance (ANOVA) for two locations, augmented design with locations and entries as fixed factors and blocks as random. Best linear unbiased estimates (BLUEs) were derived and appropriate standard errors of means (i.e. between checks, between lines of the same block, between lines of different blocks and between lines and checks) were used to determine significant differences.

To identify best performing lines, a biplot graph was generated using as the reference axes the BLUEs values for grain yield in each location. Those lines that outperformed the best checks jointly in both trials were considered as high yielders for both of the locations (Fig. 1).

Principal component analysis based on pairwise correlations among all agronomic and physiological traits was used to identify the parameters that are contributing mostly to the assessment of total variation and discrimination among lines.

A box plot graph was also generated based on grouping the lines by cycle (first or second cycle) and direction of selection (high or low yield), including also the checks (i.e. original populations) as a separate group to assess simultaneously for grain yield performance at both locations (Fig. 2).

Statistical analysis was performed with JMP statistical package ver. 13.0.0.

RESULTS

Agronomic performance of selected lines.

Combined analysis of variance (ANOVA) for agronomic performance traits revealed significant differences among lines for the biological and grain yield, as well as for the harvest index. No differences among lines were detected for plant height, spike length, and spike grain weight (Table 1). A significant effect of the location was found for biological and grain yield with both of the traits to record higher values in Kef rather than Mornag (average 7.13 t/ha in Kef versus 3.72 t/ha in Mornag for biological yield, and 2.66 t/ha in Kef versus 1.46 t/ha in Mornag for grain yield) (Tables 1 and 3). Significant location effect was also found for plant height (average 72.17 cm in Kef versus 54.19 cm in Mornag) and spike grain weight (average 2.33 g in Kef versus 2.14 g in Mornag) (Tables 1 and 3). A significant G × E (in this case indicated as Entries by Locations (E × L) interaction was observed for the grain yield and harvest index, while no interaction was traced for biological yield, plant height, spike length and spike grain weight (Table 1). Significant entry effects were also highlighted for days to heading, number of tillers and number of fertile tillers per plant, measured at Kef station (Table 2).

Table 1. Combined analysis of variance for biological yield (BY), grain yield (GY), harvest index (HI), plant height (PH), spike length (SL) and spike grain weight (SGW), measured in both locations, (Kef and Mornag)

Source of variation	DF	Mean square					
		BY	GY	HI	PH	SL	SGW
Entries (adj.)	54	3.287*	1.125**	0.01139**	132.44	0.311	0.152
Location (unadj.)	1	358.783**	43.702**	0.00348	10376.85**	0.026	1.074*
Entries x Location	54	1.919	0.469*	0.00394*	99.99	0.138	0.060
Block (unadj.)	4	2.171	0.113	0.00077	208.37	0.049	0.061
Residuals	36	1.109	0.172	0.00092	86.12	0.095	0.115

*Significant differences for α = 0.05; **Significant differences for α = 0.01

Table 2. Analysis of variance for traits measured in Kef location: days to heading (DH), number of total and fertile tillers per plant (T/P and FT/P) and SPAD values flag leaves (SPAD_FL)

Source of variation	DF	Mean square			
		DH	SPAD_FL	T/P	FT/P
Entries (adj.)	54	19.20**	13.12	0.730**	0.276*
• Checks	4	65.34**	8.37	0.654*	0.124
• Checks + Checks.VS.aug.	50	15.51**	13.33	0.723**	0.285*
Block (unadj.)	4	0.54	12.04	0.973**	0.035
Residuals	16	4.94	13.70	0.195	0.117

*Significant differences for α = 0.05; **Significant differences for α = 0.01

For all the agronomic performance traits, a number of superior selected lines were identified at both locations, outperforming the best check. In particular, eleven lines at Kef station outperformed the best check Imen, in terms of biological yield. All of these lines were selected for high yield during the first and second cycle (Table 3). Five of these lines were derived from Djebali (DH12, DH2-H1, DH2-H3, DH2-H4, and DH2-H5), two from Ardhaoui (AH10 and AH10-H1), two from Imen (IH17 and IH4-H1) and one from Manel (MH18-H2) (Table 3). For the same trait in Mornag, Manel was the best performing check while six lines recorded significantly higher BLUEs values (Table 3). These lines were selected as high yield lines, during the first and the second cycles. Three of them coming from Imen (IH17, IH4-H1, and IH4-H4), two from Ardhaoui (AH9-H3 and AH10-H2) and one from Djebali (DH2-H2) (Table 3).

BLUEs values for grain yield at Kef ranged from 0.746 (DH12-L0) up to 5.950 t/ha (IH4-4H) (Table 3). Sixteen of the lines evaluated outperformed the best check Imen; unless two of them, the rest fourteen lines had been selected as high yielding during the first and the second cycles of selection. The two remaining ones had been selected as high yielding lines for the first cycle and as low yielding lines during the second. Nine of these lines had been derived from Imen (IH4, IH17, IH4-H1, IH4-H3, IH4-H4, IH17-H1, IH16-H1, IH16-L0, and IH17-L0), three from Ardhaoui (AH10, AH10-H2, and AH10-H3), two from Manel (MH18 and MH18-H2) and two from Djebali (DH2-H3 and DH2-H5) (Table 3). BLUEs values for grain yield at Mornag ranged from 0.204 (DH-L0) up to 2.858 t/ha (AH10-2H) (Table 3). As happened in Kef, also in Mornag, Imen was the best performing check in terms of grain (Table 3). A total of seven lines, selected from the first and the second cycle as high yielding outperformed significantly the best check in Mornag (Table 2). Four of these lines were coming from Ardhaoui (AH9-H1, AH9-H3, AH10-2, and AH10-H3), two form Imen (IH17 and IH4-H4) and one from Manel (MH18) (Table 3).

To identify simultaneously the high yielding lines at both locations, the biplot graph of Fig. 1 was generated using the BLUEs values of grain yield (in t/ha) at Mornag (x-axis) and the BLUEs values of grain yield (in t/ha) at Kef (y-axis). High yielding lines for each location were considered those outperforming significantly the best check in each location (plotted at the right side of the reference line traced vertically on the x-axis), for Mornag at the cutting point 2.203 t/ha and above the horizontal reference line traced from y-axis for Kef at the cutting point 3.179 t/ha (Fig. 1). Thus, by applying the joint process, five lines all selected as high yielding either at the first either at the second cycle of selection were identified to outperform the best check in both locations (namely IH17 and IH4-H4 from Imen, AH10-H2 and AH10-H3 from Ardhaoui and MH18 from Manel) (Fig. 1). On the other hand, most of the lines selected for low yield, confirmed a low performance at both locations in terms of grain yield, showing a trend to be plotted at the bottom left side of the biplot graph (Fig. 1).

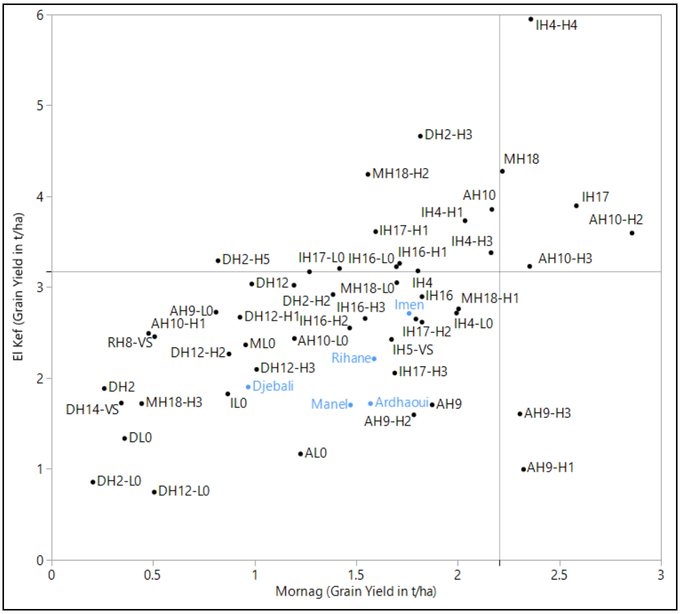

Fig. 1. Biplot graph for grain yield expressed as the best linear unbiased estimates (BLUE) between the two sites of experimentation.

Entries above reference lines indicate significant higher BLUEs values than best check in each of the locations. The entries in the upper right corner indicated superior grain yield for both sites.

First cycle high yielding selected lines outperformed in terms of grain yield performance their original populations (i.e. the checks); while the first cycle low yielding lines showed a performance lower than the checks (Fig. 2). Second cycle high yielding lines did not differ from the first cycle high yielding ones. On the contrary, the second cycle low yielding lines outperformed the ones of the first cycle, as well as the checks, since they have been subjected during the first cycle under selection for high yield (Fig. 2).

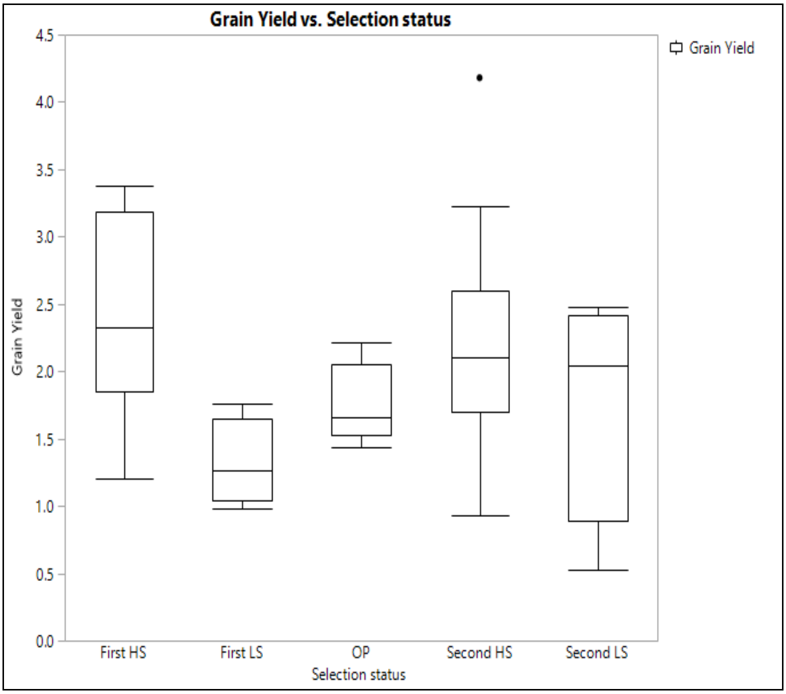

Fig. 2. Box plot graph for grain yield expressed as the best linear unbiased estimates (BLUE) across both sites, based on the selection status of the entries. First HS: First cycle of selection high yielding lines; First LS: First cycle of selection low yielding lines; OP: Original populations; Second HS: Second cycle of selection high yielding lines; Second LS: Second cycle of selection low yielding lines.

Regarding the harvest index, Imen was among all the checks the one with the highest BLUE value at Kef (Table 3). Six lines selected as high yielding at first or second cycles and one selected as low yielding from the second cycle showed higher values for harvest index than the best check. Four of these lines were coming from Imen (IH17, IH4-H4, IH17-H1, and IH17-L0), one from Ardhaoui (AH10-H2) and one from Manel (MH18) (Table 3). For Mornag, Rihane was the check that showed the higher harvest index (Table 3). A total of twelve lines were found to outperform Rihane for harvest index in Mornag (Table 3). All of these lines have been selected for high yield at the first or second cycle, while one has been selected for high yield at the first and low yield at the second cycle of selection. Out of these lines, the nine had been derived from Imen (IH4, IH16, IH17, IH4-H3, IH16-H1, IH16-H2, IH17-H2, IH17-H3, and IH16-L0) and the rest three from Ardhaoui (AH10, AH9-H1, and AH10-H2) (Table 3).

Plant height at Kef ranged from 48.72 (MH18-1H) up to 89.72 cm (AH10-1H) with an average of 72.17 cm (Table 3). Djebali was the tallest check among the five tested (Table 3). On the other hand, Imen was the shortest standing check, ranked as one of the shortest entries within the whole trial in Kef (Table 3). Plant height at Mornag ranged from 38.14 (DH2-L0) up to 68.24 cm (MH18) with an average of 54.19 cm (Table 3). Djebali was the tallest check and Imen the shortest one (Table 3).

Spike length at Kef ranged from 5.76 (DH12-3H) up to 8.96 cm (DH2-1H) with an average of 7.23 cm (Table 3). Manel was the check with the longest spike, while Rihane the check with the shortest one (Table 3). Similar range in terms of spike length was observed also at Mornag with the lower value being 5.99 cm (RH8-VS) and the upper one 8.98 cm (IH5-VS) and average at 7.19 cm (Table 3). At Kef, Manel was again the check with the longest spike, while this time Ardhaoui showed the shortest spike among all the checks of the trial (Table 3).

A wide range in terms of days to heading was recorded at Kef, starting from almost 84 (AH10) days after planting up to 97 days, with an average for the entries of 89 days (Table 4). Djebali, followed by Manel, were the two checks demanding 95 days after planting to reach heading time, while all the three low yielding selected lines derived from Djebali, from the first and the second cycles of selection (DH12-L0, DL0, and DH2-L0) were ranked as the latest entries among all within the trial (Table 4). On the other hand, Rihane was the earliest check, requiring 87 days from planting time to reach heading (Table 4). However, in this case, many of the selected lines ranked as earlier than Rihane, though the differences were not significant (Table 4).

BLUEs values for the number of total tillers per plant ranged from 1.51(DH12-2H) up to 5.85 (AH-10-2) with an average of 3.61 tillers per plant (Table 4). Ardhaoui has the highest number of total tillers per plant. Nine lines were identified showing significantly higher number of total tillers per plant than the best check (Table 4). Out of them, five lines had been selected for high yield during the first or second cycle of selection (IH4-H1 from Imen, DH2-H3 and DH2-H4 from Djebali, AH10-H2 from Ardhaoui, and MH18-H2 from Manel), and the rest four ones had been selected for low yield at the respective cycles (AL0 and AH9-L0 from Ardhaoui, ML0 from Manel, and IL0 from Imen) (Table 4). Among the five checks also tested, Manel and Djebali were the ones recording the lowest value for the number of total tillers per plant (Table 4).

Table 3. Entries' BLUEs values for agronomic performance traits (biological yield (BY), grain yield (GY), harvest index (HI), plant height (PH), spike length (SL) and spike grain weight (SGW)) for both locations (Kef and Mornag)

Entries	Block		BY (t/ha)		GY (t/ha)		HI		PH (cm)		SL (cm)		SGW (g)	
	Kef	Mornag	Kef	Mornag	Kef	Mornag	Kef	Mornag	Kef	Mornag	Kef	Mornag	Kef	Mornag
IH4-4H	1	5	7.438	6.347	5.950	2.360	0.824	0.400	50.12	54.24	7.07	6.59	2.33	1.82
DH2-3H	5	3	11.844	4.791	4.662	1.816	0.404	0.420	83.72	60.74	7.44	7.34	3.04	2.53
MH18	2	5	5.824	5.127	4.276	2.220	0.704	0.400	76.12	68.24	6.34	6.57	2.54	2.16
MH18-2H	5	1	10.914	4.243	4.242	1.558	0.404	0.400	98.72	62.14	6.83	6.86	3.08	2.85
IH17	2	2	8.294	5.591	3.896	2.584	0.504	0.500	74.12	49.74	6.75	7.32	2.11	2.13
AH10	3	1	9.618	4.493	3.856	2.168	0.384	0.500	76.72	64.14	6.25	7.39	1.98	2.46
IH4-1H	5	3	9.794	5.491	3.732	2.036	0.404	0.420	88.72	61.74	7.30	6.94	2.99	2.78
IH17-1H	4	1	8.074	4.811	3.612	1.596	0.504	0.320	64.72	51.74	7.33	7.38	2.51	2.04
AH10-2H	5	1	7.818	5.463	3.596	2.858	0.484	0.500	72.72	61.64	6.90	6.76	2.72	2.09
IH4-3H	1	2	7.498	4.491	3.380	2.164	0.424	0.500	68.12	50.24	7.46	7.32	2.74	2.28
DH2-5H	5	5	9.924	2.687	3.292	0.820	0.304	0.300	82.72	53.24	7.72	7.62	2.58	2.29
IH16-1H	5	2	8.044	3.521	3.262	1.714	0.404	0.500	72.72	46.24	6.59	7.55	2.55	2.44
IH16-L0	3	1	7.858	3.463	3.230	1.698	0.384	0.500	68.72	55.14	7.08	6.89	2.60	2.09
AH10-3H	3	1	7.368	4.657	3.226	2.354	0.424	0.480	63.12	57.64	7.11	7.19	2.10	2.19
IH17-L0	4	5	7.284	3.183	3.206	1.418	0.484	0.400	76.32	44.14	6.59	6.76	2.32	1.83
IH4	1	2	6.888	3.531	3.180	1.804	0.424	0.500	67.12	50.24	7.44	7.48	2.67	2.23
DH2-1H	1	5	8.758	4.577	3.170	1.270	0.324	0.300	72.12	57.74	8.96	7.69	2.81	2.17
MH18-L0	1	5	6.668	3.887	3.050	1.700	0.424	0.400	78.12	61.74	6.57	7.01	2.11	2.19
DH12	4	3	9.104	4.601	3.036	0.986	0.284	0.220	76.32	54.74	8.15	7.80	2.45	2.57
DH2-4H	5	4	8.714	3.737	3.022	1.194	0.404	0.280	88.72	57.14	7.31	6.84	2.24	1.67
DH2-2H	1	3	7.378	5.961	2.920	1.386	0.424	0.220	83.12	57.74	7.93	8.62	2.52	2.76
IH16	3	2	6.938	3.911	2.896	1.824	0.384	0.500	71.72	54.74	6.27	6.77	2.08	1.91
MH18-1H	5	4	8.174	4.307	2.762	2.004	0.304	0.480	48.72	65.14	7.05	6.74	2.58	1.93
AH9-L0	4	5	7.614	2.477	2.726	0.810	0.384	0.300	85.32	50.24	8.29	7.08	2.80	2.08
IH4-L0	2	4	6.674	4.417	2.716	1.994	0.404	0.480	52.12	55.14	7.41	6.25	2.51	1.56
Imen	NA	NA	6.864	4.168	2.712	1.760	0.420	0.420	55.80	51.20	7.01	6.86	2.03	2.15
DH12-1H	1	1	7.358	3.413	2.670	0.928	0.324	0.300	77.12	58.14	6.92	6.75	2.52	2.13
IH16-3H	2	2	6.534	3.511	2.656	1.544	0.404	0.400	57.12	47.74	6.70	6.92	2.09	1.89
IH17-2H	5	3	7.818	3.401	2.650	1.794	0.324	0.500	64.12	49.24	7.27	7.33	2.30	2.40
IH4-2H	3	4	7.148	3.847	2.616	1.824	0.384	0.480	78.72	57.14	6.97	6.78	2.59	2.03
IH16-2H	5	1	6.794	3.073	2.552	1.468	0.404	0.500	72.72	55.64	6.23	6.39	2.19	1.58
AH10-1H	4	3	8.234	2.363	2.492	0.478	0.304	0.200	89.72	59.64	8.00	6.89	2.44	2.62

RH8-VS	2	1	5.634	1.593	2.456	0.508	0.404	0.300	71.12	55.64	6.64	5.99	1.39	1.99
AH10-L0	1	4	6.094	2.851	2.436	1.196	0.384	0.420	74.32	48.24	7.94	8.07	2.48	2.10
IH5-VS	3	4	5.728	4.057	2.426	1.674	0.384	0.380	77.72	53.64	7.58	8.98	2.70	3.32
ML0	2	3	7.984	2.851	2.366	0.956	0.304	0.320	76.12	54.74	7.96	8.20	2.33	2.39
DH12-2H	3	4	6.268	3.137	2.266	0.874	0.384	0.280	67.72	58.64	7.27	7.64	2.53	2.34
Rihane	NA	NA	5.966	3.724	2.212	1.588	0.380	0.440	71.40	58.50	6.12	6.38	2.36	2.02
DH12-3H	2	5	7.904	3.237	2.096	1.010	0.304	0.300	72.12	58.74	5.76	7.23	1.93	1.77
IH17-3H	1	2	5.294	3.557	2.056	1.690	0.384	0.500	66.32	54.24	7.54	6.99	2.68	2.33
Djebali	NA	NA	6.792	3.156	1.902	0.968	0.300	0.300	73.80	59.10	7.61	7.15	2.33	1.99
DH2	2	5	6.414	1.447	1.886	0.260	0.304	0.200	64.12	53.74	7.79	7.09	2.26	1.77
IL0	4	1	7.304	2.993	1.826	0.868	0.284	0.300	74.32	49.64	7.71	7.27	2.07	2.67
DH14-VS	4	2	5.274	1.271	1.726	0.344	0.284	0.200	79.32	41.74	8.46	7.41	1.89	1.74
MH18-3H	1	2	5.558	1.371	1.720	0.444	0.324	0.300	71.12	45.24	6.37	7.95	2.50	2.16
Ardhaoui	NA	NA	5.146	3.852	1.720	1.570	0.340	0.400	71.00	57.40	6.68	6.32	2.00	1.69
AH9	3	4	7.028	4.177	1.706	1.874	0.184	0.480	57.72	58.64	6.77	6.97	1.99	1.64
Manel	NA	NA	6.494	4.216	1.704	1.472	0.280	0.340	67.60	55.00	8.65	7.34	2.16	2.68
AH9-3H	2	3	6.024	5.561	1.606	2.306	0.304	0.420	69.12	53.24	7.33	8.06	1.92	2.30
AH9-2H	3	4	6.288	4.367	1.596	1.784	0.284	0.380	67.72	50.14	6.89	6.99	1.84	1.59
DL0	2	5	6.994	1.547	1.336	0.360	0.204	0.300	75.12	47.74	7.21	7.11	2.12	1.96
AL0	4	3	5.234	4.001	1.166	1.226	0.184	0.320	69.32	43.74	7.88	7.74	2.12	1.83
AH9-1H	4	2	4.004	4.411	0.996	2.324	0.284	0.500	79.32	56.24	7.33	8.28	2.02	1.87
DH2-L0	4	4	4.074	1.147	0.856	0.204	0.184	0.180	64.32	38.14	8.00	6.63	1.79	1.58
DH12-L0	3	3	3.598	2.761	0.746	0.506	0.184	0.220	72.72	43.74	7.05	7.11	1.83	2.06
S.E. difference														
Between checks			0.6542	0.5104	0.2531	0.2402	0.0276	0.0265	4.808	4.799	0.320	0.374	0.178	0.273
Between augmented entries (same block)			1.4629	1.1413	0.5660	0.5371	0.0616	0.0592	10.750	10.730	0.716	0.836	0.398	0.610
Between augmented entries (different block)			1.6025	1.2502	0.6200	0.5884	0.0675	0.0648	11.776	11.754	0.785	0.916	0.436	0.668
Between an augmented entry and a check			1.2063	0.9411	0.4668	0.4429	0.0508	0.0488	8.865	8.848	0.591	0.690	0.328	0.503

Blocks are indicated to facilitate comparisons for appropriate S.E.

For the number of fertile tillers per plant though, Ardhaoui is performing check indicating a different response checks having almost same number of fertile tillers with Manel and Djebali (Table 4). In this case, the best performing check was Imen, showing 2.25 fertile tillers per plant (Table 4). Thirteen lines, out of them the nine lines selected for high yield at the second cycle of selection and the four lines selected for low yield either at the first either at the second cycle, outperformed significantly the best check. Five among these lines has been derived from Ardhaoui (AL0, AH9-L0, AH10-H1, AH10-H2, and AH9-H3), three from Djebali (DH2-H2, DH2-H3, and DH2-H4), three from Imen (IH4-H1, IH16-H1, and IH17-L0) and two from Manel (ML0 and MH18-H2) (Table 4).

Table 4. Entries' BLUEs values for agronomic performance traits and photosynthesis related parameters measured only at Kef location

Entries	Block	HD	T/P	FT/P	SPAD_FL
IH4-4H	1	87.44	2.892	1.957	48.73
DH2-3H	5	87.84	5.298	3.029	41.44
MH18	2	85.24	3.316	2.609	49.35
MH18-2H	5	85.84	5.498	3.849	44.44
IH17	2	86.24	3.546	2.229	56.50
AH10	3	83.84	3.228	2.243	50.70
IH4-1H	5	88.84	4.788	3.119	42.22
IH17-1H	4	85.84	3.638	2.409	54.32
AH10-2H	5	85.84	5.848	2.803	52.23
IH4-3H	1	86.44	2.922	1.827	54.23
DH2-5H	5	90.84	3.978	2.199	45.59
IH16-1H	5	86.84	4.118	2.859	47.49
IH16-L0	3	88.84	2.678	2.213	51.75
AH10-3H	3	88.44	3.122	2.257	48.45
IH17-L0	4	85.64	3.606	2.653	53.12
IH4	1	86.44	3.302	2.327	50.53
DH2-1H	1	93.44	3.572	2.237	46.80
MH18-L0	1	86.44	2.872	2.067	44.93
DH12	4	92.64	3.106	2.193	45.95
DH2-4H	5	92.84	4.958	3.139	51.27
DH2-2H	1	91.44	3.922	2.667	47.53
IH16	3	85.84	2.558	1.743	50.13
MH18-1H	5	84.84	3.928	2.119	47.22
AH9-L0	4	86.64	5.246	3.053	54.75
IH4-L0	2	87.24	3.886	2.319	52.93
Imen	NA	89.00	3.412	2.250	49.70
DH12-1H	1	93.44	3.992	2.187	41.90
IH16-3H	2	87.24	2.986	2.049	49.55
IH17-2H	5	89.44	3.742	2.467	50.15
IH4-2H	3	86.84	2.008	1.313	50.53
IH16-2H	5	86.84	3.788	2.099	46.67
AH10-1H	4	94.84	4.058	3.019	54.02
RH8-VS	2	85.24	2.716	1.389	48.43
AH10-L0	1	86.64	3.976	2.593	51.75
IH5-VS	3	87.84	3.338	1.743	49.78
ML0	2	93.24	4.556	2.939	50.60

DH12-2H	3	94.84	1.508	1.123	49.10
Rihane	NA	87.40	3.384	2.060	49.44
DH12-3H	2	86.24	3.786	2.249	45.53
IH17-3H	1	86.64	4.056	1.993	48.15
Djebali	NA	95.20	2.968	1.816	46.86
DH2	2	93.24	2.696	1.999	45.05
IL0	4	90.64	4.756	2.443	42.50
DH14-VS	4	94.64	2.666	1.653	52.05
MH18-3H	1	87.44	2.332	1.157	56.40
Ardhaoui	NA	89.60	3.984	1.858	49.29
AH9	3	83.84	4.218	2.363	43.38
Manel	NA	95.00	3.032	1.930	46.85
AH9-3H	2	85.24	4.296	2.759	47.78
AH9-2H	3	84.84	3.618	2.643	43.28
DL0	2	96.24	3.206	2.299	49.60
AL0	4	84.64	4.556	2.823	49.70
AH9-1H	4	84.64	2.756	2.173	47.62
DH2-L0	4	96.64	4.226	1.613	48.25
DH12-L0	3	95.84	2.108	1.273	47.78
S.E. difference					
Between checks		1.406	0.2790	0.2166	2.341
Between augmented entries (same block)		3.143	0.6239	0.4843	5.235
Between augmented entries (different block)		3.443	0.6834	0.5306	5.735
Between an augmented entry and a check		2.592	0.5145	0.3994	4.317

Blocks are indicated to facilitate comparisons for appropriate S.E.

Physiological parameters of selected lines.

There were no significant differences for the PSII related parameters among lines (Table 5). Differences were only detected between the locations for F_0, F_v/F_m and F_v/F_0 values (Table 5). For the Kef station, among all the checks, Djebali showed the highest values for the ratios F_v/F_m and F_v/F_0, while Imen showed the lowest ones (Table 6). For Mornag trial, among all the checks, Rihane showed the highest F_v/F_m and F_v/F_0 ratios and Imen showed the lowest ones (Table 6). A number of lines showed also high values of F_v/F_m and F_v/F_0 at both locations. The IH4-H4, second cycle high yielding line derived from Imen, showed high F_v/F_m and F_v/F_0 ratios at Kef station and was ranked second for both of these ratios at Mornag (Table 6). Other lines, scoring high values at both locations for the above-mentioned ratios, were DH2-H5 and DH12-H1 originated from Djebali and AH10-H1 issued from Ardhaoui (Table 6).

Regarding F_0 value, among the checks, Rihane showed the highest value for Kef, while Ardhaoui the highest value for Mornag (Table 6). The latter though was the one among all checks by scoring the lower F_0 value at Kef station, while at Mornag station the lower F_0 value, among all checks, was recorded by Djebali (Table 6). For one more time also, line IH4-H4 showed high values for the PSII related parameters and particularly at Mornag, this line was ranked as first for the F_0 value, among all the entries of the trial (Table 6).

No significant differences were detected among entries for the SPAD values and leaf canopy temperature recorded at the middle of tillering stage, as well as at the heading time (Table 5). However, significant effects were observed among locations for the leaf canopy temperature and the SPAD at the middle of tillering stage (Table 5).

Ranking of lines for SPAD values measured at the two different growth stages (i.e. middle of tillering stage and heading time), did not reveal any significant correlation and the Spearman's rho was not significant (r = 0.115, $P > 0.05$). During the tillering stage, Manel showed among the checks the highest SPAD value. However, during heading time, Manel showed the lowest SPAD value (Table 6).

For Mornag station, Rihane indicated the higher and Djebali the lower SPAD values when assessed during tillering stage (Table 6). Two lines, one selected for high yield from Imen at the first cycle (IH17) and the other selected for low yield from Ardhaui at the second cycle (AH9-L0), recorded high SPAD values at Kef station for both stages (Table 6). These lines were also above the best check Rihane at Mornag trial in terms of SPAD value (Table 6).

Canopy temperature was on average 19.5°C at Kef and 23.7°C at Mornag (Table 6). Among the checks Rihane, showed the highest canopy temperature in both of the locations, while Djebali for Kef and Manel for Mornag recorded the lowest values for this physiological parameter (Table 6). The majority of lines evaluated at Kef (i.e. 30 out of a total of 50 lines), showed lower values for leaf canopy temperature than Djebali, while for Mornag a total of 18 selected lines showed lower leaf canopy temperature than Manel (Table 6).

Principal component analysis and partial correlations between traits.

Principal component analysis (PCA) provided six principal components (PCs) that explained more than 80% of the total variation among the entries tested (Table 7). The first three components accounted together for 59.56% of the total variation (Table 7). For the first PC, growth and yield parameters, such as plant height, biological yield and grain yield were those with high positive loadings along with the PSII related parameter of F_0, while SPAD and leaf canopy temperature, scored at the middle of tillering stage, were the ones with high negative loads (Table 8). The second PC gathered except from F_0, all the other PSII related parameters, all contributing with high positive loads (Table 8). The third PC is mainly associated with agronomic performance traits.

The harvest index followed by grain yield, number of fertile tillers per plant and number of total tillers per plant were the ones with high positive loads, while heading time and spike length the traits with high negative loads (Table 8). The rest of the parameters, such as SPAD measured at the heading time, powdery mildew resistance and spike grain weight, accounted for the other minor PCs and their contribution to the performance assessment and differentiation among the entries tested was considered negligible (Table 8).

Pairwise correlations between phenotypic traits revealed significant coefficients in 74 out of the 324 trait combinations. Correlation coefficient (r) ranged between 0.009 up to 0.977 (Table 9). High positive correlations were revealed among biological yield, grain yield and plant height for both locations (r = 0.484-0.838, $P < 0.01$). A high correlation was also found between harvest index and grain yield (r = 0.484, $P < 0.01$) (Table 9). In addition, a negative correlation was revealed between grain yield and days to heading (r = -0.408, $P < 0.01$), implying that early flowering lines were more productive than the late flowering ones (Table 9).

High negative correlations were found for leaf canopy temperature and SPAD (measured at the middle of tillering stage) with the biological and grain yield (r = -0.417 up to r = -0.628, $P < 0.01$). The SPAD measured at heading time did not reveal any significant correlation with any of the yield related parameters (Table 9). Among the PSII related parameters, F_0 and F_m were highly correlated with grain yield (r = 0.516 and r = 0.389 respectively, $P < 0.01$) (Table 9).

Powdery mildew resistance.

Powdery mildew scores at both locations revealed on average a moderate susceptibility for the entries. None of the entries scored was characterized as resistant to the disease. Among the two locations, 24.67% of the entries were ranked as moderately resistant, 58.00% as moderately susceptible, and 17.35% as susceptible to powdery mildew. Disease symptoms noted at Mornag were more severe than those observed in Kef ($X^2 = 26.714$, $P < 0.01$). Grouping of the entries, based on cycle and direction of selections, showed that within the group of the first cycle high yielding selected lines, no line was scored as susceptible to the disease and all lines were characterized either as moderately resistant or as moderately susceptible (Fig. 3). However, for all the other group of lines as well as for the checks, there were cases of entries that at least in one location were scored as susceptible to the disease (Fig. 3).

Table 5. Combined analysis of variance for physiological parameters measured in both locations (Kef and Mornag) indicating values for degrees of freedom (DF) and mean squares (MS)

Source of variation	DF	MS						
		SPAD_M	LCT	F_0	F_v	F_m	F_v/F_m	F_v/F_0
Entries (adj.)	54	17.50	15.29	254.78	8154.46	12035.67	0.003	0.214
Location (unadj.)	1	2814.73**	573.75**	83986.31**	57783.85	283437.37	0.034**	4.189**
Entries x Location	54	19.43	14.31	291.77	6749.78	10575.96	0.002	0.144
Block (unadj.)	4	10.86	12.08	756.04	3381.00	6303.36	0.004	0.191
Residuals	36	8.78	10.44	783.71	5549.90	9862.88	0.001	0.102

*Significant differences for α = 0.05; **Significant differences for α = 0.01

Table 6. Entries' BLUEs values for photosynthesis related parameters at both locations (Kef and Mornag)

Entries	SPAD_M		LCT		F_0		F_v		F_m	
	El Kef	Mornag	El Kef	Mornag	El Kef	Mornag	El Kef	Mornag	El Kef	Mornag
IH4-4H	46.13	50.64	24.94	23.48	197.30	176.82	591.91	639.12	789.21	815.94
DH2-3H	42.48	50.21	16.95	22.63	223.45	154.12	441.96	307.27	665.41	461.39
MH18	41.82	55.21	18.39	23.01	183.85	163.82	353.91	431.37	537.76	595.19
MH18-2H	40.78	53.17	18.07	25.05	180.20	144.52	392.21	335.52	572.41	480.04
IH17	45.45	55.24	20.11	24.04	189.10	163.42	418.16	310.77	607.26	474.19
AH10	46.31	51.24	15.85	22.43	217.50	164.77	465.31	385.77	682.81	550.54
IH4-1H	41.28	54.16	18.52	23.18	207.95	150.12	418.96	398.52	626.91	548.64
IH17-1H	39.53	53.98	17.97	25.15	217.70	154.37	377.71	284.52	595.41	438.89
AH10-2H	43.84	52.92	15.17	23.83	216.50	154.02	453.56	313.77	670.06	467.79
IH4-3H	41.16	49.46	19.87	24.67	197.80	133.92	364.91	299.27	562.71	433.19
DH2-5H	38.08	47.81	18.05	24.06	214.95	163.82	471.71	465.37	686.66	629.19
IH16-1H	39.83	53.34	18.17	22.79	206.45	145.67	392.46	273.02	598.91	418.69
IH16-L0	45.36	50.19	19.12	24.70	208.75	139.27	497.81	325.02	706.56	464.29
AH10-3H	48.01	53.38	24.47	24.76	196.55	156.12	403.16	355.57	599.71	511.69
IH17-L0	40.84	47.99	22.82	22.13	197.15	152.77	305.41	333.27	502.56	486.04
IH4	37.66	53.69	16.89	22.89	175.55	123.67	451.66	256.77	627.21	380.44
DH2-1H	42.11	49.51	18.94	22.18	196.55	170.82	437.16	350.87	633.71	521.69
MH18-L0	39.81	52.46	15.57	23.43	187.80	140.82	473.16	318.37	660.96	459.19
DH12	45.24	48.08	17.85	24.80	196.40	131.12	359.66	291.52	556.06	422.64
DH2-4H	44.33	47.73	19.25	21.91	209.20	164.12	430.96	413.57	640.16	577.69
DH2-2H	44.23	54.58	14.59	23.25	187.05	160.62	318.66	409.77	505.71	570.39
IH16	47.19	54.49	15.97	23.02	213.50	141.67	432.06	376.27	645.56	517.94
MH18-1H	39.28	51.20	19.37	23.38	216.20	143.62	442.71	336.07	658.91	479.69
AH9-L0	47.01	53.69	18.27	25.73	220.90	152.32	446.41	402.62	667.31	554.94
IH4-L0	42.92	51.98	21.11	20.73	173.85	160.37	325.41	398.32	499.26	558.69
Imen	41.86	52.12	20.77	24.76	194.45	158.65	351.85	354.00	546.30	512.65
DH12-1H	41.13	46.69	21.49	23.05	186.80	161.02	491.66	408.27	678.46	569.29
IH16-3H	43.40	49.29	18.96	23.72	196.85	122.92	398.66	280.52	595.51	403.44
IH17-2H	40.98	52.66	21.54	22.54	190.80	142.67	392.16	290.77	582.96	433.44
IH4-2H	43.51	48.53	18.17	19.71	211.75	141.12	458.81	290.32	670.56	431.44
IH16-2H	42.11	50.54	18.82	23.78	219.95	131.77	411.46	310.77	631.41	442.54
AH10-1H	38.56	46.54	19.35	23.53	180.95	146.52	399.21	380.02	580.16	526.54
RH8-VS	44.92	52.14	23.89	25.08	182.85	141.77	351.41	357.77	534.26	499.54
AH10-L0	44.91	55.16	22.55	25.65	220.15	140.62	406.41	432.02	626.56	572.64

IH5-VS	39.71	54.38	16.70	24.66	210.00	152.37	451.56	363.82	661.56	516.19
ML0	39.87	55.06	22.16	25.50	192.35	147.87	295.16	360.77	487.51	508.64
DH12-2H	38.89	51.95	16.50	21.03	218.25	127.87	455.81	304.07	674.06	431.94
Rihane	44.59	53.62	23.01	24.87	212.45	155.10	393.85	389.45	606.30	544.55
DH12-3H	39.82	52.21	19.71	23.53	204.10	168.32	408.91	436.12	613.01	604.44
IH17-3H	40.71	52.09	19.75	22.76	214.90	176.57	326.66	637.37	541.56	813.94
Djebali	41.65	48.43	19.80	23.72	195.15	149.35	382.65	349.10	577.80	498.45
DH2	41.05	53.04	17.96	25.41	195.35	141.07	357.66	373.37	553.01	514.44
IL0	47.06	48.79	19.27	25.05	221.15	159.27	421.16	393.27	642.31	552.54
DH14-VS	40.26	47.01	23.07	23.67	214.15	154.67	394.41	336.77	608.56	491.44
MH18-3H	43.38	50.31	16.32	24.87	194.80	155.67	359.41	314.27	554.21	469.94
Ardhaoui	39.93	52.99	22.17	24.02	175.00	161.10	334.25	391.65	509.25	552.75
AH9	40.51	54.85	17.30	22.21	221.25	165.37	424.06	348.07	645.31	513.44
Manel	46.68	51.22	22.93	23.09	197.20	154.40	390.70	343.15	587.90	497.55
AH9-3H	38.65	58.33	20.51	23.30	199.35	160.12	361.16	293.52	560.51	453.64
AH9-2H	45.34	51.50	15.80	22.98	217.50	160.37	402.81	406.07	620.31	566.44
DL0	42.20	51.04	22.16	24.46	184.85	163.32	394.66	434.87	579.51	598.19
AL0	42.66	52.53	21.85	22.98	221.15	156.12	423.41	409.02	644.56	565.14
AH9-1H	39.06	50.59	24.00	24.82	242.65	174.67	409.66	431.02	652.31	605.69
DH2-L0	38.64	50.20	22.62	24.68	220.90	156.62	448.16	292.82	669.06	449.44
DH12-L0	44.21	50.61	18.15	24.43	188.00	160.62	354.56	340.27	542.56	500.89
S.E. difference										
Between checks	1.565	1.345	1.944	0.893	11.952	11.068	21.598	36.016	31.091	42.464
Between augmented entries (same block)	3.500	3.007	4.346	1.996	22.038	24.749	48.294	80.578	69.521	94.952
Between augmented entries (different block)	3.834	3.294	4.761	2.187	29.276	27.111	52.903	88.269	76.156	104.014
Between an augmented entry and a check	2.886	2.480	3.584	1.646	22.038	20.408	39.824	66.447	57.328	78.299

Blocks are indicated to facilitate comparisons for appropriate S.E.

Table 7. Principal components analysis (PCA) based on correlations between all traits.

Number	Eigenvalue	Percent	Cum. %
1	5.1471	28.595	28.595
2	3.2367	17.982	46.577
3	2.3363	12.980	59.556
4	1.7325	9.625	69.182
5	1.3045	7.247	76.429
6	0.8974	4.986	81.415

Table 8. Factor loads for the first 6 PCs accounting for more of the variation revealed among entries

Trait	PC1	PC2	PC3	PC4	PC5	PC6
HD	-0.11943	-0.10394	**-0.48519**	0.20566	0.19535	-0.25555
PH	**0.33671**	0.01153	-0.14204	0.05204	-0.04342	-0.01531
BY	**0.41117**	0.02136	0.02356	0.08919	0.00893	-0.04861
GY	**0.34518**	0.06582	**0.27569**	-0.02128	0.23136	-0.17406
HI	0.04550	0.05050	**0.49269**	-0.07202	0.35104	-0.33976
SPAD M	**-0.33373**	0.07574	0.17597	0.15543	0.08852	-0.06745
LCT	**-0.31044**	0.09885	0.03900	0.12972	-0.06420	-0.24854
SPAD FL	-0.00898	-0.09404	0.27642	-0.13216	0.41756	0.15835
T/P	0.10806	0.01960	**0.20128**	0.48545	-0.40438	-0.10216
FT/P	0.18386	-0.02187	**0.26625**	0.50864	-0.24178	0.05775
PM	-0.15973	0.19673	0.14597	-0.07998	-0.12534	0.71755
Fo	**0.36563**	0.11616	-0.14245	-0.23168	-0.12157	0.02617
Fv	0.13934	**0.51151**	-0.10353	-0.05820	0.02520	-0.04526
Fm	0.22484	**0.44324**	-0.13075	-0.11928	-0.01314	-0.03196
Fv/Fm	-0.17311	**0.46878**	-0.00495	0.15600	0.09249	-0.02538
Fv/Fo	-0.18129	**0.47319**	0.01351	0.14101	0.09769	-0.05604
SL	0.05437	-0.03849	**-0.36626**	0.37705	0.39176	0.13128
SGW	0.18390	-0.03276	0.03239	0.35286	0.41467	0.37622

Table 9. Pearson correlation coefficients analysis for agronomic and physiological related traits and powdery mildew symptoms across both locations (Kef and Mornag)

	HD	PH	BY	GY	HI	SPAD_M	LCT	SPAD _FL	T/P	FT/P	PM	Fo	Fv	Fm
HD	1.000													
PH	0.105	1.000												
BY	-0.156	0.676**	1.000											
GY	-0.408**	0.486**	0.838**	1.000										
HI	-0.442**	-0.037	0.113	0.582**	1.000									
SPAD _M	0.052	-0.594**	-0.628**	-0.417**	0.193*	1.000								
LCT	0.195	-0.551**	-0.609**	-0.462**	0.002	0.573**	1.000							
SPAD _FL	-0.194	-0.139	-0.066	0.161	0.295*	-0.022	-0.032	1.000						
T/P	-0.213	0.143	0.253*	0.169	0.012	-0.120	0.085	-0.026	1.000					
FT/P	-0.290*	0.305**	0.512**	0.374**	0.144	-0.056	-0.236*	-0.050	0.664**	1.000				
PM	-0.225*	-0.226**	-0.286**	-0.204*	-0.003	0.316**	0.221**	0.014	-0.100	-0.075	1.000			
Fo	-0.320**	0.600**	0.710**	0.516**	-0.105	-0.667**	-0.486**	-0.128	0.050	0.058	-0.247**	1.000		
Fv	-0.155	0.249**	0.306**	0.286**	0.013	-0.167*	-0.052	-0.152	0.034	-0.028	0.130	0.537**	1.000	
Fm	-0.208	0.386**	0.466**	0.389**	-0.023	-0.342**	-0.193*	-0.158	0.041	-0.009	0.026	0.740**	0.965**	1.000
Fv/F m	0.057	-0.223**	-0.312**	-0.194*	0.030	0.412	0.361	-0.093	0.003	-0.076	0.350**	-0.293**	0.621**	0.403**
Fv/Fo	0.039	-0.261**	-0.327**	-0.188*	0.079	0.432**	0.386**	-0.097	-0.006	-0.088	0.358**	-0.308**	0.629**	0.405**
SL	0.535**	0.080	0.121	-0.054	-0.271**	-0.089	-0.094	-0.087	-0.004	0.060	-0.199*	0.095	0.053	0.072
SGW	-0.089	0.305**	0.389**	0.363**	0.090	-0.188*	-0.233**	0.087	0.149	0.300**	-0.129	0.153	0.029	0.071

*Significant differences for α = 0.05; **Significant differences for α = 0.01

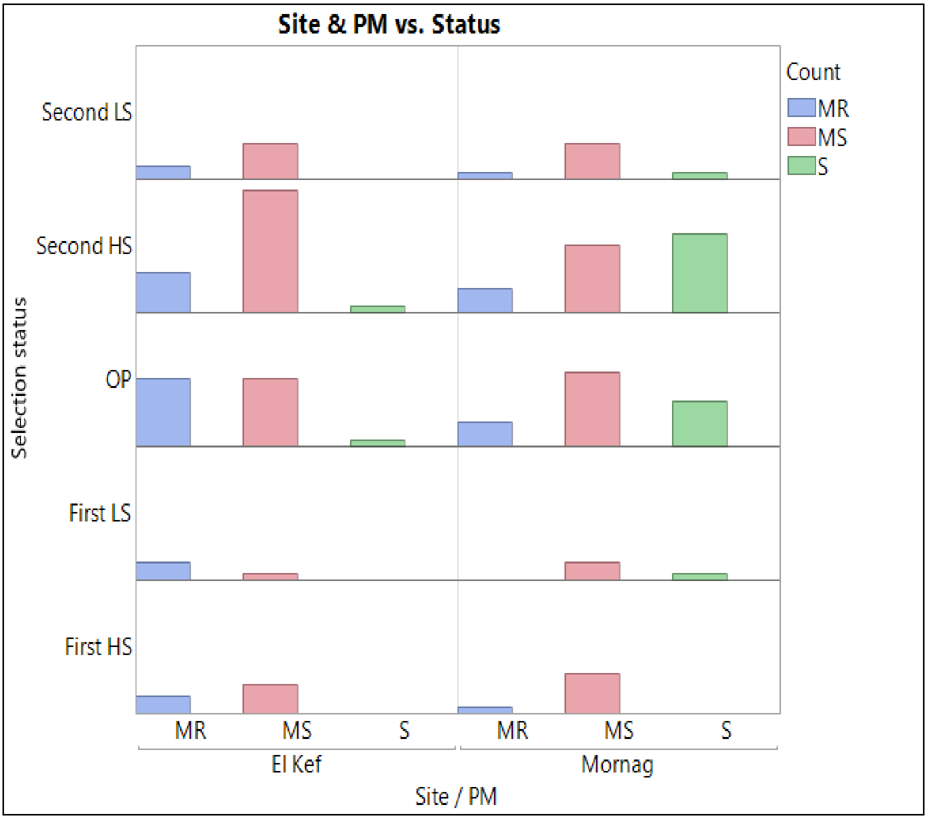

Fig. 3. Entries' response to powdery mildew (PM) at the two sites of experimentation; based on the selection status of the entries. First HS: First cycle of selection high yielding lines; First LS: First cycle of selection low yielding lines; OP: Original populations; Second HS: Second cycle of selection high yielding lines; Second LS: Second cycle of selection low yielding lines.

DISCUSSION

Intra-cultivar heterogeneity has long been recognized in crop species; this phenomenon is often ignored because most researchers assume that elite monogenotypic cultivars are composed of relatively homogeneous genetic pools (Haun et al. 2011). Attributed to genetic homogeneity a priori, only plants obviously of incorrect type are removed in breeder-seed treatment for cultivar maintenance (Parlevliet 2007). Nevertheless, evidence from selection experiments within fairly homogeneous genetic pools suggests that the genome is more flexible and plastic than previously assumed (Yates et al. 2012). Mechanisms that create new variations may accumulate undesirable mutations and gradually contribute to cultivar degradation (Fasoula 1990, 2012; Tokatlidis et al. 2006, 2011).

Regarding its reproductive system, barley is an inbred crop and as such, elite barley cultivars are considered to be genetically homogeneous. Nevertheless, even within fairly homogeneous gene pools, an intrinsic amount of latent genetic variation may still occur, whereas mechanisms that generate de novo variation may also be present. Residual heterozygosity, due to segregation of parental loci during the breeding process is presumably one source of genetic variation (Haun et al. 2011; Tokatlidis 2015). On the other hand, additional heterogeneity might stem from de novo generated variation, resulting from spontaneous mutations (Ossowski et al., 2010; Shaw et al. 2000) or via genetic and epigenetic mechanisms, such as intragenic recombination, unequal crossing over, gene duplications or deletions, DNA methylation, excision or insertion of transposable elements, chromatin alterations, etc. (Cavrak et al. 2014; Kim and Zilberman 2014; Rasmusson and Phillips 1997; Sani et al. 2013). The original populations (checks) included in this study comprised three commercially released cultivars in Tunisia, i.e. Imen, Manel and Rihane and two Tunisian landraces, i.e. Ardhaoui and Djebali. The study targeted to investigate the stability of yield and plasticity of agronomic and phenological traits in two diversified environments (Kef and Mornag) in Tunisia. In fact, the mean rainfall recorded at Kef during the whole season (325 mm) was higher than that registered at Mornag (295 mm) especially for January and March.

The results showed that the effects due to environment, genotype and genotype×environment (G×E) interaction were significant, which indicates the existence of differences among genotypes for plasticity. Biological and grain yield ranged from 3.72 to 7.13 t/ha in Kef and from 1.46 to 2.66 t/ha in Mornag.

Based on PCA of the phenotypic traits, these barley lines were mainly grouped with respect to their plant height, biological yield and grain yield and the PSII related parameter of F_0. There was strong variability response among the genotypes. According to Fang and Xiong (2015), to overcome drought stress at the physiological level, plants can adjust their rates of photosynthesis by modifying photosystem II, stomatal closure, and low electron transport, carbohydrate and nitrogen metabolism, nucleic acid and protein activity, and growth as a whole.

High positive correlations were revealed between spike grain weight and grain yield, biological yield and grain yield, for both locations. In dry areas, moisture stress is prevalent at all stages, especially grain filling; thus, breeders tend to select material based on grain weight.

The potential of this novel approach to exploit latent or de novo variation within barley cultivars for the development of high-yielding lines under drought stressed conditions is also discussed. On average, high yielding selected lines outperformed in terms of grain yield performance their original populations (i.e. the checks), while the first cycle low yielding lines showed a performance that ranked them underneath the checks. Similar results were found by Tokatlidis et al. (2010) when a set of seven maize hybrids were grown at a range of four densities, in comparison with the normal density (8.33 plants/m), an eightfold greater seed yield per plant at nil competition (0.74 plants/m) was accompanied by a top-to-bottom genotype gap that was 15 times higher. In another one study, two high-yielding bread wheat families were significantly superior over source material in both generations under either low density and/or typical crop density, as well as averaged across the four densities in the split plot trials (Tokatlidis et al. 2005). Second cycle high yielding lines did not differ from the first cycle high yielding lines. On the contrary, the second cycle low yielding lines outperformed the ones of the first cycle, as well as the checks, since they have been subjected during the first cycle to selection for high yield.

Rasmusson and Phillips (1997) reported that in barley, incremental gains for several traits were made in a very narrow gene pool, attributable to variation present in the original gene pool as well as to de novo variation. The continuous selection within a cultivar is necessary to exploit the potential existence of variation for either cultivar conservation or upgrading, and this target is feasible at the single plant level in the absence of competition (Fasoulas 1993). Christakis and Fasoulas (2002) found exploitable genetic variation for yield in tomato that was uncovered in advanced generations, after the point of achieving theoretical homozygosity (F7 generation). The selection study for modified oil and protein in maize, with selection being practiced effectively for more than 90 generations (Dudley and Lambert 1992), highlights the importance of continuous selection. Fasoula and Fasoula (2000) stated, ‘‘Continuous selection after the release of cultivars is imposed by the need to eliminate deleterious mutations and exploit any positive source of existing and newly derived variation, either genetic or epigenetic”.

Results from multi field evaluation indicated that the selection process applied within each commercial cultivar and landrace succeeded in isolating single-plant progeny lines of high performance. Selection within cultivars, especially for those released earlier, may prove to be a useful technique either to upgrade gradual degeneration of genetic background. Our data fully agrees with the conclusion coming from Tokatlidis (2015) who suggests that selection should be a perpetual process, so that any existing or newly developed variation is exploited and optimal quality of breeder’s seed is secured.

RESUME

Ben Ghanem H., El Felah M., Najar A., Kehel Z., Amri A., Rezgui S. et Tsivelikas A.L. 2018. Performances de lignées d'orge sélectionnées dans des conditions de stress hydrique et à très faible densité. Tunisian Journal of Plant Protection 13 (1): 1-25.

L'imprévisibilité des précipitations et de la température dans la région méditerranéenne est à l'origine d'une irrégularité des conditions environnementales influençant les cultures et engendrant un contexte d'incertitude pour les agriculteurs. Dans cette étude, des lignées d’orge sélectionnées pour la stabilité du rendement en grains en conditions semi-arides et à faible densité de semis "Honeycomb design", ont été évaluées pour leurs performances agronomiques dans des régions semi-arides (Kef et Mornag) suivant le dispositif expérimental "Augmented design" au cours de la campagne 2015/16. La comparaison a été réalisée par rapport aux parents témoins (Manel, Rihane, Imen, Ardhaoui et Djebali). L'analyse de la variance a montré que les rendements, biologique et grainier sont influencés par l’environnement, le génotype et l’interaction génotype × environnement (G × E). En effet, les variations respectives ont été de 3,72 à 7,13 t/ha et de 1,46 à 2,66 t/ha et les valeurs enregistrées au Kef étaient supérieures à celles notées à Mornag. Il est à signaler que cinq lignées-plantes sélectionnées pour leur rendement élevé (IH17 and IH4-H4 provenant de Imen, AH10-H2 et AH10-H3 de Ardhaoui et MH18 de Manel) dépassent les populations d'origine.

Mots clés: Evaluation, faible densité, orge, performances, sélection, semi-aride

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ملخص

بن غانم ، هاجر ومو□دي□فا□ح وأسماء نجار وزكريا كحيل وأحمد عمري وصا□ح رزقي وأثناسيوس ل. تسيفيليكاس، تقييم سلالات شعير منتقاة تحت ظروف الإجهاد□مائي وكثافة ضئيلة.
Tunisian Journal of Plant Protection 13 (1): 1-25.

تتميز مناطق المتوسطية بعدم الانتظام في ظروفها المناخية خاصة فيما يتعلق بالأمطار والحرارة. هذا الاضطراب تؤثر سلبا على الزراعات وتؤدي الى الإحساس بالشك لدى المزارعين بما في ذلك المتخصصين في ميدان الحبوب. في هذا الصدد، اعتمدت الدراسة الحالية على طريقة تجريبية معروفة باسم "تصميم قرص العسل" (Honeycomb design) لاختيار السلالات الأفضل ضمن مجموعة معينة وقع بذرها بكثافة ضئيلة. اثر ذلك تمت عملية تقييم المردودية الزراعية للسلالات المنتخبة طبق مثال تجريبي يسمى "التصميم المعزز" (Augmented design) وذلك خلال الموسم 2016/2015 في جهتين مختلقتين من المناطق □به الجافة (الكاف ومرناق) وقد مكن هذا التقييم بالمقارنة مع الخمس أصناف الأم (إيمان ومنال وريحان وعرضاوي وجبالي) من التأكد من تأثر عناصر الإنتاج بالمناخ والصنف ومدى التفاعل بين هذين العاملين. من ذلك فان المردود البيولوجي قد تراوح بين 3.72 و 7.13 طن/هك بينما تراوح مردود الحب بين 146 و 2.66 طن/هك مع أفضلية لمنطقة الكاف. من ضمن هذه النتائج سجلنا تفوق خمسة سلالات على الأصناف الأصلية منها اثنان وقع انتقا□ هما من ايمان (IH17 و IH4-H4) و اثنان من عرضاوي (AH10-H2 و AH10-H3) وواحدة من منال (MH18).

كلمات مفتاحية: انتقاء، تقييم، □به الجاف، □عير ، كثافة ضئيلة

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