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Assessment of the Phenotypic Variation Within Two- And Six-rowed Barley (*Hordeum Vulgare* L.) Breeding Lines Grown Under Semi-Arid Conditions.

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ABSTRACT

Variation and relationships between eight barley agronomic and morphologic traits were evaluated in 306 two-rowed and 144 six-rowed barley lines. Significant differences were detected within and among group of lines for most of the measured traits. In both row types, number of grains per unit area exerted a significant and positive direct effect and with 1000-kernel weight, explained almost 99.0% of total yield variation. Two-rowed type showed high 1000-seed weight and six-rowed had better seed per spike. Principal component analysis indicated that spike number, 1000-seed weight, harvest index and seed per spike are strongly influenced by row type. Under the growth conditions experienced by the studied breeding material, six-rowed lines out yielding two-rowed entries, suggesting that selection should be directed toward this row type.

Key words: *Hordeum vulgare*, traits, variation, relationships, row type, cluster.

Introduction

Barley (*Hordeum vulgare* L.) is the second important crop grown in Algeria after durum wheat (*Triticum durum* Desf.). It is mainly used as fodder crop, with a small portion, 15 kg/person/year, utilized as human food [1]. Because of its relatively short vegetative cycle which enables it to escape terminal heat and drought stresses, barley is grown in the arid and semi-arid highland areas where durum and bread wheat could not be sown without risk and where it represents along with sheep rearing the main agricultural activity [2]. In these areas, rainfall distribution pattern and frost hazards are important factors determining observed grain yield [3,4]. Grain yield is low due to management factors, adverse

growing conditions and low yield potential of cultivars and landraces adopted. To improve grain yield and sustain the development of these marginal areas, selection of stress resistant varieties, with high yield potential, is a promising alternative. In fact, the adoption of a suitable genotype can change dramatically the production pattern of a given region. Illustrative examples are given by the adoption of the recently released bread wheat cultivar HD₁₂₂₀ and the durum wheat cultivars Waha and Boussalam in the middle plains and the northern parts of the high plateaus [5,6,7]. Even though stress tolerance is a multivariate problem, affected by several morpho-physiological characteristics interacting simultaneously, selecting stress resistant cultivars, especially with good performance under late season

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heat and drought stresses is a major objective of barley breeding program. National barley breeders select, preferably, six-rowed barley lines with large kernel size and good above ground biomass production [8,9,10]. Yield stability and crop adaptation are sought through accurate matching of life cycle duration to moisture availability and frost avoidance [3,4,9]. Most agronomic traits related to yield potential and yield stability vary greatly according to growing conditions and source of germplasm [3,11]. Knowledge about the amount of genetic variation and relationships between traits are of important considerations for efficient utilization of germplasm sources [12,13]. National breeding program is receiving much segregating and advanced barley breeding material from Icarda. This material is made of two- and six- rowed entries. A better understanding of grain yield potential and adaptation of two- and six-rowed genotypes and of the ways their yield is obtained help in planning breeding strategies, by choosing either one or the other type and by choosing the characteristics which are to be selected within each type. The objective of the present study was to assess the phenotypic variation of some agro morphological traits within and among two and six- rowed barley breeding lines grown under semi- arid conditions.

Material and methods

Experimental Design:

450 random fertile tillers, 306 two-rowed and 144 six-rowed lines, were drawn from 10 samples harvested, each from one row 1 m long, from a segregating population grown at the National Institute of Agricultural Research (INRAA), Agricultural Research Station (ARS) of Sidi Bel Abbes (35°02'N and 00°3'W) during the 2008/09 cropping season. The original population consists of a mixture of 1600 F₂ lines, derived from a hierarchical crossing scheme made by Icarda [14]. Seeds of the 450 sampled fertile tillers were grown, the following year (2009/2010) at the Technical Institute of Field Crop (ITGC) ARS of Setif (36°12'N and 05°24'E), in an augmented design with five checks repeated three times. Each line, originating from one spike, was sown in a single 1 m long row, with 0.2 m spacing between two consecutive rows. Two local landraces, Tichedrett, Saïda, and three commercial cultivars, Barberousse, Rihane₀₃ and Fouara were included as checks. Barberousse and Rihane₀₃ are, respectively, French and Tunisian 6-rowed barley varieties. Fouara is a recently released six-rowed cultivar by ITGC, ARS of Setif. Its pedigree is Deir alla 106/Strain 205//Gerbel. ICB85. 1376. 0AP. 1AP. 2AP [15]. Data were recorded on plant height, spike weight and spike number per m², above ground biomass, grain

yield, seed number per m², and seeds per spike, 1000-kernel weight and harvest index. Plant height was measured in centimeters from the soil surface to the tip of the inflorescence of the most typical plant, per plot, excluding awns. Harvest index was calculated as the ratio between plot grain yield and plot above ground biomass. 1000-seed weight was estimated from the count and weight of an average sample of 250 kernels. Seed number produced per square meter was derived from plot grain yield and 1000-seed weight. Seed per spike was calculated as seed number divided by spike number per square meter.

Data Analysis:

The data were analyzed for basic statistics following the methods implemented in the descriptive analysis subroutine of the free statistical software Openstat [16]. Phenotypic correlation coefficients were calculated and *t*-tested according to the procedure described in Quin and Keough [17]. Stepwise regression analysis was employed to determine the best combination of variables that determinate grain yield in each row type germplasm. Variables are chosen to enter or leave the model according to the significance level of the *F* test and to the size of residual mean square of the model [18]. The path coefficient analysis was performed using phenotypic correlations to assess direct and indirect effect of the measured traits on grain yield [19, 20]. A principal component analysis (PCA) was carried out on scaled agronomic data, followed by a multivariate hierarchical clustering analysis based on the first four PCA components whose eigenvalue was greater than 1. A dendrogram was generated using Ward's minimum variance method implemented in the Past free software package [21].

Results and discussion

Phenotypic Variability Within and Between Two and Six-row Types:

A large level of phenotypic variation was observed for most of the measured traits (Table 1). Hence plant height varied from 40.0 cm to 114.0 cm in both row type barley lines. Above ground biomass ranged from 31.2 to 1260.1 g m², spike number per m² from 20.0 to 420 spikes, and grain yield from 12.0 to 680.6 g m² (Table 1). Thousand -kernel weight ranged from 30.6 to 58.4 g, the number of seeds m² from 260.0 to 15880.7 grains m², while the number of kernels per spike varied from 9.7 to 89.0 and the harvest index from 0.11 to 0.56 (Table 1). Difference between mean values of two- and six-rowed lines was not significant for plant height, number of spike m², 1000-kernel weight and harvest

Table 1: Basic statistics for eight quantitative traits of two-rowed (n= 306) and six- rowed (n= 144) barley lines.

Traits	RT	Mean value	Mean SE	Min. value	Max.value	LDS _{5%} *
PHT	2	85.5	0.61	40.0	114.0	7.48
	6	89.3	0.77	66.0	111.0	
BIO	2	573.1	14.5	31.2	1250.5	175.01
	6	843.7	26.1	97.4	1260.1	
SN	2	192.4	4.4	20.0	420.0	66.72
	6	172.2	5.7	25.0	345.0	
GY	2	211.9	5.3	12.0	512.3	65.36
	6	368.3	11.9	40.3	680.6	
TKW	2	49.9	0.28	30.6	58.4	5.34
	6	45.9	0.45	33.3	55.6	
SNM ²	2	4242.0	104.8	264.0	9572.8	1543.6
	6	8053.5	259.1	967.2	15880.7	
SS	2	22.2	0.31	9.7	55.5	7.85
	6	47.8	0.69	23.0	89.0	
HI	2	0.37	0.004	0.11	0.56	0.08
	6	0.43	0.005	0.24	0.55	

* based on the analysis of variance of the augmented design. PHT= plant height, BIO= above ground biomass, SN= spike number per m², GY= grain yield, TKW= 1000-seed weight, SNM²= seed number per m², SS= seed per spike, HI= harvest index.

index. The difference was significant for above ground biomass, grain yield, seed number per m² and seed number per spike (Table 1). The results revealed considerable variation among row types for yield, yield components, and related agronomic traits, even though the overall means per row type hide the large variability observed, which suggested that selection within each group could lead to the identification of potentially useful breeding lines. Le Gouis [12] reported significant differences between two and six-row type lines for the number of shoots which was higher for the two-row, and for the number of grains per spike, with the advantage of six-row type lines. This advantage of the six-rowed type compensate for their lower spike number per m² resulting in a higher seed number per m². According to Kolodinska-Brantestam *et al.*, [22] two-rowed cultivars had, on average, significantly higher thousand-kernel weight and volumetric weight, and later heading and maturity than six-row.

Relationships Between Traits Within Two and Six-row Types:

It is an established fact that greater the variability among the breeding material better will be the chance for further grain yield improvement. Yield is influenced by a number of characters; their influence is highly variable in direction and magnitude. Correlation coefficient analysis is useful in the identification of suitable traits to target in selection and elimination of those with undesirable correlated changes. Within the two-rowed lines, grain yield showed significant correlations with the measured traits, even though above ground biomass, spike number and seed number per m² were the most yield determinant traits (Table 2). Similar trend was observed within the six-rowed barley lines, except that the seed per spike showed no significant relationship with grain yield (Table 2). 1000-seed weight of the two-rowed group of barley lines

showed no significant relationships with plant height, harvest index and with the yield components: spike number, seed per spike and seed per m² (Table 2). In the six-rowed group of lines, thousand-kernel weight showed no significant correlation with spike number and seed number per m², but exhibited a significant and negative correlation with seed per spike. Spike fertility exhibited no significant relationship with plant height and with seed number per m² (Table 2). Le Gouis [12] reported a negative and significant correlation between 1000-seed weight and seed number per m², in both two and six-rowed barley genotypes. Under the present growing conditions, the results suggested that is, somewhat, difficult to achieve simultaneously high seed number per m² and high 1000-kernel weight in a single genotype within both groups, due to compensating effects. Forward stepwise multiple regression analysis was utilized to highlight the contribution of the measured traits to grain yield variation within each group of lines (Table 3). The first trait to be entered was seed number per m², which explained 94.3 and 89.9% of yield variation in two and six-rowed lines, respectively. With 1000-seed weight, as the second trait entering the model, nearly 99.0% of grain yield variation was explained (Table 3).

Shahinnia *et al.*, [23] reported that harvest index and biological yield explained 96.8% of the total yield variation of barley. The coefficient of regression of the seed number per m², of the two-rowed group of lines, was relatively higher than the one of the six-rowed group of lines, suggesting a stronger effect of this trait on yield variation within the two-rowed group of lines. The reverse was noted for 1000-seed weight, above ground biomass, harvest index, and seed number per spike whose coefficients of regression were relatively higher in the six-rowed barley lines (Table 3). Even though the correlation coefficient between seed number per m² and 1000-seed weight was not significant, the stepwise regression analysis showed the positive synergic

Table 2: Correlation coefficients between 8 quantitative traits of two-rowed (n= 306) and six-rowed (n= 144) barley lines.

	PHT	BIO	SN	GY	TKW	SNM ²	SS	HI
PHT		0.389**	0.219*	0.315**	0.102 ^{ns}	0.302**	0.201*	-0.182*
BIO	0.557**		0.777**	0.894**	0.206*	0.862**	0.244*	-0.223*
SN	0.416**	0.873**		0.856**	0.011 ^{ns}	0.873**	-0.083 ^{ns}	0.116*
GY	0.484**	0.923**	0.922**		0.212*	0.971**	0.337*	0.190*
TKW	0.319**	0.297**	0.106 ^{ns}	0.227*		-0.003 ^{ns}	-0.036 ^{ns}	0.014 ^{ns}
SNM ²	0.405**	0.852**	0.910**	0.948**	-0.072 ^{ns}		0.361**	0.191*
SS	-0.099 ^{ns}	-0.170*	-0.331**	-0.080 ^{ns}	-0.382**	0.051 ^{ns}		0.266*
HI	-0.07 ^{ns}	-0.059 ^{ns}	0.232*	0.301**	-0.188*	0.357**	0.262*	

Correlations of 2r lines above diagonal and of 6r lines below diagonal, ns,* and **= correlation coefficient non significant and significant at 5% level according to the t-test. . PHT= plant height, BIO= above ground biomass, SN= spike number per m², GY= grain yield, TKW= 1000-seed weight, SNM²= seed number per m², SS= seed per spike, HI= harvest index.

Table 3: Stepwise regression analysis of grain yield and agronomic traits.

Regression coefficients								
Step	Trait	b ₀	b ₁	b ₂	b ₃	b ₄	b ₅	R ²
Two-rowed barley lines (n=306)								
1	SNM ²	1.87	0.049**					0.943
2	TKW	-193.2	0.049**	3.96**				0.989
3	BIO	-184.1	0.046**	3.75**	0.020**			0.990
4	HI	-194.3	0.038**	3.07**	0.008**	116.2**		0.992
5	SS	-191.1	0.038**	3.04**	0.084**	122.3**	-0.225*	0.992
Six-rowed barley lines (n =144)								
1	SNM ²	17.5	0.044**					0.898
2	TKW	-347.6	0.045**	7.79**				0.986
3	BIO	-324.5	0.042**	7.20**	0.028**			0.987
4	HI	-373.1	0.028**	4.98**	0.160**	344.17**		0.991
5	SS	-354.7	0.028**	4.87**	0.160**	348.37**	-0.304*	0.991

*, ** = significant at $p < 0.05$ and $p < 0.01$, respectively. PHT= plant height, BIO= above ground biomass, SN= spike number per m², GY= grain yield, TKW= 1000-seed weight, SNM²= seed number per m², SS= seed per spike, HI= harvest index.

effect of both traits on grain yield variation, in both row types, suggesting that barley grain yield is a complex character, influenced by spike number, seed number per spike, and 1000-seed weight. Seed number per spike had, however, a negative effect on grain yield (Table 3). Path coefficient analysis is a statistical method which permits to identify paths through which causal factors produce an effect on the caused trait. A causal factor may exert a direct effect of its own and also exert indirect effects through other causal factors with which it is correlated. In both row types, seed number per m² exerted a high and positive direct effect on grain yield; followed by above ground biomass, 1000-seed weight and harvest index. These results suggested that proper emphasis should be given on these traits while making selection. Boukerou and Rasmusson [24] proposed to breed for high biomass as a way to enhance barley grain yield. They noted that using harvest index as indirect selection criteria, to improve grain yield, was more efficient than direct selection. Shahinnia *et al.*, [23] observed that two and six-rowed barley lines were separated by the first two functions of the discriminant analysis, with harvest index among the traits having considerable loads in the linear discriminant function; suggesting that harvest index is a discriminating trait between two- and six-rowed lines. The direct effects of plant height, spike number and seed per spike were nil (Table 4). In two-rowed lines, all the measured traits, excepted 1000-seed weight, exerted a positive and sizeable indirect

effects via seed number per m². Similar pattern of effect was observed within six-rowed lines, with the exception that 1000-seed weight and seed number per spike had no indirect effect via seed number per m². Spike number and seed number per m², in two-row lines, and the same traits with plant height and 1000-seed weight, in the six-rowed group of lines, exerted sizeable indirect effects on grain yield via above ground biomass (Table 4). Principal component analysis was conducted with two and six-rowed lines altogether. The first two components separated fairly well the two groups of lines, explaining 70.31% of total variation (Figure 1). Difference in yielding ability among lines is mainly explained by differences in seed number per m² and above ground biomass which are correlated with component 1; but differences in spike number per m², 1000-seed weight, harvest index and seed per spike, which are related to component 2, discriminate between row types (Figure 1). These results indicated that spike number, 1000-seed weight, harvest index and seed per spike are strongly influenced by row type.

The cluster analysis separated the 450 lines into three major groups (Figure 2, Table 5). Cluster one (C₁) is made of six-rowed lines, cluster two (C₂) is made of two-rowed barley lines, but cluster three (C₃) is mixture of two- and six-rowed lines. The most discriminating trait between clusters are above ground biomass, grain yield, seed number per m² and seed per spike (Table 5, Figure 2). The results of the present study indicated high phenotypic variation

Table 4: Direct (diagonal) and indirect effects of different characters on grain yield of two and six-rowed barley lines.

	PHT	BIO	SN	TKW	SNM ²	KS	HI	r _{BDF}
Two-rowed barley lines (n =306)								
PHT	0.000 ^{ns}	0.091	-0.004	0.017	0.234	-0.004	-0.018	0.315**
BIO	0.000	0.233**	-0.013	0.034	0.667	-0.005	-0.022	0.894**
SN	0.000	0.181	-0.017 ^{ns}	0.002	0.676	0.002	0.012	0.856**
TKW	0.000	0.048	0.000	0.164**	-0.002	0.001	0.001	0.212*
SNM ²	0.000	0.201	-0.015	0.000	0.774**	-0.008	0.019	0.971**
SS	0.000	0.057	0.001	-0.006	0.279	-0.021 ^{ns}	0.027	0.337**
HI	0.000	-0.052	-0.002	0.002	0.148	-0.006	0.100**	0.190*
Six-rowed barley lines (n =144)								
PHT	-0.018 ^{ns}	0.211	-0.017	0.059	0.256	0.003	-0.01	0.484**
BIO	-0.01	0.379**	-0.036	0.055	0.538	0.005	-0.009	0.923**
SN	-0.007	0.331	-0.041 ^{ns}	0.02	0.575	0.01	0.035	0.922**
TKW	-0.006	0.113	-0.004	0.186**	-0.046	0.012	-0.028	0.227*
SNM ²	-0.007	0.323	-0.037	-0.013	0.632**	-0.002	0.053	0.948**
SS	0.002	-0.064	0.014	-0.071	0.032	-0.031 ^{ns}	0.039	-0.080 ^{ns}
HI	0.001	-0.022	-0.01	-0.035	0.226	-0.008	0.149**	0.301**

*, ** = significant at $p < 0.05$ and $p < 0.01$, respectively. PHT= plant height, BIO= above ground biomass, SN= spike number per m², GY= grain yield, TKW= 1000-seed weight, SNM²= seed number per m², SS= seed per spike, HI= harvest index.

Table 5: Cluster mean values of the measured traits.

	PHT	BIO	SN	GY	TKW	SNM ²	SS	HI
Cluster ₁	89.6	822.9	169.9	336.6	46.9	7213.4	43.5	0.41
Cluster ₂	85.2	574.8	191.8	227.6	49.5	4635.5	24.7	0.40
Cluster ₃	86.8	666.1	188.2	258.0	48.8	5393.0	29.5	0.38

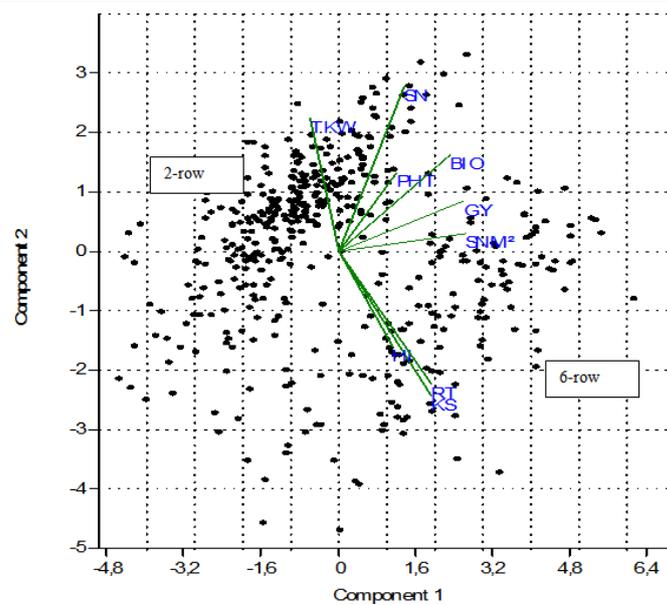


Fig. 1: PCA Biplot showing the relationships between traits within 2 and 6 rowed barley lines (47.62% variation explained by component 1 and 22.69% by component component 2). PHT= plant height, BIO= above ground biomass, SN= spike number per m², GY= grain yield, TKW= 1000-seed weight, SNM²= seed number per m², SS= seed per spike, HI= harvest index.

for most traits within and between row types. In both row types, seed number per m² and 1000-seed weight explained almost 99.0% of the total yield variation; and seed number per m² exerted a high and positive direct effect on grain yield, followed by above ground biomass, 1000-seed weight and harvest index. Positive and sizeable indirect effects of most measured traits; were noted via seed number per m² and above ground biomass, in two and six -rowed lines. Principal component analysis indicated that spike number, 1000-seed weight, harvest index and seed per spike are strongly influenced by row type.

Cluster analysis separated the studied entries into three groups, two of which were highly divergent mainly for seed per spike and 1000-kernel weight; while the third one was composed of two and six-rowed entries.

This advantage could be explained by the ability of the six-rowed type to achieve high seed number per unit area as well as high seed number per spike. These results corroborated findings of Le Gouis *et al.*, [12] who reported that, on average, six-rowed lines out yielded two-rows by 4% under favorable growing conditions and by 11% under low yielding

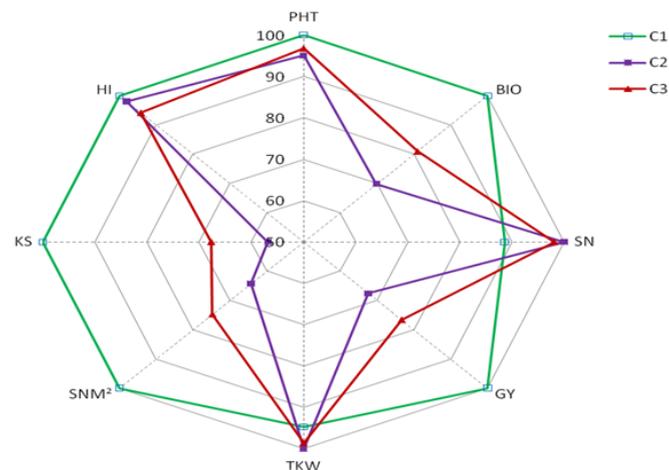


Fig. 2: Relative means of the measured traits of the three clusters formed between two and six- rowed barley.

conditions. This suggested that six-rowed were more adapted to low yielding environment. Garcia Del Moral *et al.*, [11] noted that two-rowed barley lines were more responsive to environmental changes than their six-rowed counterparts, which were more stable. In fact, these authors reported a regression coefficient value less than unity for the six-rowed lines, suggesting their better adaptation to low yield environments.

Blum [25] mentioned that differences between two and six-rowed barley genotypes in carbon exchange rate were mainly associated with differences in ear surface area and amount of awn, which were greater in six-rowed than in two-rowed barley. These adaptive traits give an advantage to the six- rowed over the two-rowed type, under semi-arid drought conditions. Le Gouis [26] suggested that greater translocation of assimilates, previously stored in vegetative parts could explain the higher yield ability of six-row genotypes. These results suggested as mentioned by Marquez-cedillo *et al.*, [27] that two-rowed barley lines are a potentially valuable germplasm source for six-row barley. Two-row x six-row crosses are usually made in an attempt to transfer desirable genes from one type to another; but under semi-arid conditions, such as those under which the present experiment was conducted, selection of six row genotypes will be preferred.

Conclusions:

A combination of statistical procedures was used to study variation and relationships between eight measured traits within and among two and six-rowed barley breeding lines. High phenotypic variation for most traits within and between row types was noted. In both row types, seed number per m² and 1000-seed weight explained almost 99.0% of the total yield variation. Seed number per m² exerted a high and positive direct effect on grain yield, followed by

above ground biomass, 1000-seed weight and harvest index. Principal component analysis indicated that spike number, 1000-seed weight, harvest index and seed per spike are strongly influenced by row type. On average, six-rowed lines out yielded two-rowed lines suggesting that six-rowed were more adapted to the low yielding environment under which the experiment was conducted and that selection should be directed toward six-rowed type.

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