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Assessment of Some Physiological and Biochemical Traits for Selection of Tolerant Barley Genotypes (*Hordeum vulgare* L.) in Rainfall Conditions

I. Berkane[1], H. Bendada[1], B. Frih[2], A. Belagrouze[3], N. Louahdi[4], O. Mehanni[4], S. Selloum[4], R. Benniou[5], A. Guendouz[6], M.B. Laouisset[7]

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ABSTRACT

Background: in this study we aim to evaluate some physiological and biochemical important traits for the selection of tolerant Barley genotypes in the rainfall conditions.

Methods: This experiment was conducted during the cropping season (2020-2021) at the experimental field of Technical Institute of Field Crops (ITGC), Setif, Algeria. Ten genotypes of barley were used in this study to evaluate their performances with Chlorophyll contents, relative water contents, drought sensitivity index, proline content, total soluble sugar content, thousand kernels weight and grain yield. The seeds were sown using an experimental drill in 1.2 m × 5 m plots consisting of 6 rows with a 20 cm row space and the seeding rate is about 250 seeds per m^2.

Result: Analysis of variance showed that genotype effect was significatif to very high significatif with all studied parameters, the origin of the genotypes tested was only influenced the drought sensitivity index while the row type had a significatif effect on drought sensitivity index and proline contentsgrain yield was positively correlated with relative water contents however, thousand kernels weight was negatively correlated with relative water contents and Total soluble sugar content. Principal components analysis showed that PC1 was a physio-biochemical axis and the genotypes related to this axis had high values of these traits, while PC2 was a yield and drought tolerance axis and their related genotypes were the most yielding genotypes.

Key words: Barley, Drought, Proline, Rainfed, Sugar, Tolerance.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal grains in terms of quantity produced and area cultivated, is ranked fourth for world cereal crops after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) (FAO, 2016). Barley is used for livestock, malt, drinking and alcohol industries (FAO, 2015).In Algeria, barley is the second cultivated cereal in rainfed conditions after durum wheat (*Triticum durum* Desf.) with 1 million ha harvested areas (Ramla *et al*., 2016). Its area production is mainly located in highland semi-arid agroclimatic zone (300-400 mm rainfall) characterized by variability and severity of climate conditions.

Although drought is one of the most adverse climatic factors that induce dramatic morphological, biochemical, physiological and molecular changes. All these changes severely affect plant growth and barley production (Lesk *et al*., 2016). According to Ahmed *et al*., (2019). Drought stress leads to inhibition of photosynthesis which has been associated with a decrease in chlorophyll content, cell membrane stability, causing loss of membrane permeability and damage to various physiological and biochemical functions that ultimately affect plant growth. The selection of drought-tolerant cultivars has been a major objective of many crop improvement programs and is becoming an important strategy for crop adaptation to climate change (Ghanem *et al*., 2015; Rauf *et al*., 2016).

[1]Department of Agronomy, Environment and Sustainable Development Lab, University of Relizane, Algeria.
[2]Department of Biology and Plant Ecology, VRBN Lab.Setif University, Setif, Algeria.
[3]National School of Foresty, Khenchela, Algeria.
[4]Technical Institute of Field Crops, Setif, Algeria.
[5]Department of Agronomy, University of M'sila, Algeria
[6]National Institute of the Agronomic Research of Algeria, Setif, Algeria.
[7]Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biskra, Algeria.

Corresponding Author: I. Berkane, Department of Agronomy, Environment and Sustainable Development Lab, University of Relizane, Algeria. Email: brahim.berkane@univ-relizane.dz

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Barley is a cereal crop that adapts well to drought stress and it can be studied as a genetic model plant to illustrate drought resistance mechanisms (Baum *et al*., 2007; Baik and Ullrich, 2008; Arshadi *et al*., 2018). Understanding the relationships between yield and yield components may assist breeders to identify key traits that are involved in crop yield under drought stress conditions. Indeed, the selections for the physiological and biochemical characters are very much used in barley breeding programs (Toorchi *et al*., 2020).

This approach requires studying, identifying and verifying the phenological, morpho-physiological and biochemical characteristics related to the yield. Therefore, a good understanding of all of these aspects during the life cycle of the plant and a well comprehension of the main links between the grain yield and these components can be useful in the identification and the selection of the interest traits. The objective of the present study was to evaluate the variability of ten barley genotypes, using some physiological and biochemical traits and to determine the relationships of these traits to select tolerant and adapted genotypes under semi-arid conditions.

MATERIALS AND METHODS

Field experiment was conducted during the cropping season (2020-2021) at the experimental field of Technical Institute of Field Crops (ITGC), Setif, Algeria (5°20′E, 36°8′N, 958 m above mean sea level). The statistical design employed was based on a completely randomized block design (CRBD) with three replications. Ten (10) genotypes of barley were used in this study. The seeds were sown using an experimental drill in 1.2 m × 5 m plots consisting of 6 rows with a 20 cm row space and the seeding rate is about 250 seeds per m^2. The pedigree and the origin of the genotypes tested during this study are given in Table 1. Chlorophyll content (CHL) of the flag leaf was measured using a digital chlorophyll meter (CCM) with units (cci) used by Frih et *al* (2022), this device allows measuring the absorbance of light in the leaves. Relative water content (RWC) in the leaves obtained by determining leaves fresh weight (FW), dry weight (DW) and weight of turgid leaves (TW) and calculated as follows:

$$RWC\ \% = \frac{(FW\text{-}DW)}{(TW\text{-}DW)} \times 100$$

(Blum, 2010).

The cell integrity test is performed on the last two fully developed leaves, randomized by genotype; these samples were washed in distilled water. The leaves were cut into segments from 1 cm long. A sample of 10 leaf blade segments were placed in a test tube and washed three times with distilled water to remove adhering dust which affects the test results. To each tube we added 10 ml of deionized distilled water. The tubes were periodically stirred manually and left at room temperature in the laboratory. A first reading was taken (EC1) with the conductivity meter 24 hours later. The tubes were then placed in a water bath, the temperature of which is brought to 100°C for 60 minutes. A second conductivity reading was taken 24 hours after passing the samples through the water bath (EC2). The percentage of cells damaged by water stress is estimated, according to the procedure described by Bajji *et al*. (2001), as follows:

$$DSI\,\% = \frac{EC1}{EC2} \times 100$$

Proline was determined according to the method of Monneveux and Nemmar (1986). Samples of 100 mg, taken from themedian part of leaves, are weighed and placed in test tubes. A volume of 2 ml of 40% methanol was added to each tube and the whole was heated for 1 h in a bain Marie at 85°C. After cooling, 1 ml of the extraction solution is added to 1 ml of acetic acid, 25 mg of ninhydrin and 1 ml of the mixture distilled water - acetic acid - ortho phosphoric acid of density 1.7 (120, 300, 80: v/v/v). The unit is carried to boiling during 30 minutes in the water bath, then cooled and added with 5 ml of toluene. After agitation with the vortex, $Na_2\ SO_4$ is added in each tube. Total sugar is measured according to the method of Dubois *et al*. (1956). In a test tube, 1 ml of barley leaf extract is added to 1 ml of 5% phenol solution and 5 ml of sulphuric acid (H_2SO_4) are added to a test tube, after shaking, the solution is left for 20 min in the dark. Spectrophotometer measurements are made at 470 nm. Furthermore, grain yield (GY) and Thousand-kernel weight (TKW) was determined from sub-samples taken from harvested grains of each plot.

Data were analyzed using Costat, version 6.4 (Costat, 1998). The analysis of variance was performed for all agronomical and physiological traits and Fisher's LSD multiple range test was employed for the mean comparisons. Linear correlation analysis was used to determine the relationships between the traits studied. Principal

Table 1: Code, pedigrees, origins and row types of genotypes evaluated in this study.

Code	Variety/Pedigree	Origin	Row type
G1	TARM 92	ICARDA	2 rows
G2	LARENDE	ICARDA	2 rows
G3	Pamir-010/Sahara-3768/3/YEA168.4/YEA605.5//Lignee131/ArabiAbiad	ICARDA	2 rows
G4	Sadik-02*2/3/Narcis//K-281/Skorohod	ICARDA	2 rows
G5	GkOmega/CWB117-5-9-5/5/Excelle/4/Alpha/Durra/3/4679/105//YEA132TH	ICARDA	2 rows
G6	Rahma	Algeria	2 rows
G7	Fouarra	Algeria	6 rows
G8	Saida 183	Algeria	6 rows
G9	Tichedrette	Algeria	6 rows
G10	Rihane 03	Algeria	6 rows

Components Analysis (PCA) was performed using R software version 4.0.2 (R Core Team, 2020).

RESULTS AND DISCUSSION

Variance analysis (ANOVA)

Analysis of variance (Table 2) showed that genotype effect was significatif to very high significatif (p<0.05; 0.01; 0.001) with all studied parameters. Chlorophyll content varied from 33.5 cci for Tichedrett (G9) to 52.1 cci for $Rihane_{03}$ (G10) with overall genotypic mean of 40.32 cci. Chlorophyll tends to decline more rapidly than when plants are under stress (Gitelson and Merzlyak,1994), the variations in leaf chlorophyll content have also been shown to be related to leaf development (Carter and Knapp, 2001). Relative water contents arranged from 77.64% for $Saida_{183}$ (G8) to 89.92% for G4 with 85.77% over all genotypic mean. Differences between treatments in leaf water potential and osmotic potential were small and there was no evidence that osmotic adjustment contributed to the drought response (Day, 1981). Relationships between leaf water potential, stomatal resistance and environmental factors are explored and compared for the two cereals (Biscoe *et al.*, 1973). Drought sensitivity index taked the values of 42.32 and 79.91% as min and max for Fouarra (G7) and G2 genotypes consecutively with overall genotypic mean of 63.11%. Proline content varied from 1.36 µmol g^{-1} MF for $Rihane_{03}$ to 2.79 µmol g^{-1} MF for G4 with 2.06 µmol g^{-1} MF as mean of all genotypes. Hellal et *al.* (2020) reported that Tunisian barley varieties (Kebili-3 and Sidi-Bou) under drought stress gained the highest proline content and the lowest proline value was attained by Tombari (at ear emergence). Lower-molecular-weight osmolytes, as proline, glycine, betaine and organic acids and polyols are crucial to maintain cellular functions under the drought stress condition (Farooq *et al.*, 2009). Total soluble sugar content arranged from 1.83 $ug.g^{-1}$ MF for G2 to 7.02 $ug.g^{-1}$ MF for G4 with a genotypic mean of 4.99 $ug.g^{-1}$ MF. Chapin *et al.* (1990); Sperdouli et Moustakas, (2012) observed the increase in the soluble sugars content in plants exposed to drought. Analysis of variance of agronomic traits showed that thousand kernels weight taked the values of 33.42 g for Fouarra to 47.06 g for G2, the genotypic mean of TKW was 39.69 g, in the same time, grain yield was arranged from 34.26 q/ha for $Saida_{183}$ to

Table 2: Genotype, origin and row type effects on all parameters studied.

Parameters	CHL	RWC	DSI	PC	SC	TKW	GY
Genotypes	cci	%	%	umol.g^{-1} MF	ug.g^{-1}MF	g	q/ha
G1	34.56 (cd)	81.37 (bc)	75.19 (ab)	2.06 (bc)	6.37 (ab)	41.95 (bc)	36.16 (d)
G2	38.36 (bcd)	83.96 (ab)	79.91(a)	2.20 (bc)	1.83 (d)	47.06 (a)	48.81 (a)
G3	36.43 (cd)	89.57 (a)	67.51 (abc)	2.04 (bc)	4.19 (bcd)	38.9 (cde)	48.27 (ab)
G4	42.73 (bc)	89.92 (a)	71.51 (abc)	2.79 (a)	7.02 (a)	36.78 (def)	40.45 (bcd)
G5	43.4 (abc)	85.6 (ab)	58.44 (cd)	1.92 (bc)	4.69 (abc)	39.16 (cd)	39.04 (cd)
G6 (Rahma)	47.2 (ab)	89.24 (a)	63.83 (bc)	2.27 (b)	5.21 (abc)	38.13 (cde)	48.23 (ab)
G7 (Fouarra)	35.5 (cd)	84.81 (ab)	42.32 (e)	2.28 (b)	6.04 (abc)	33.42 (f)	45.13 (abc)
G8 ($Saida_{183}$)	39.43 (bcd)	77.64 (c)	45.96 (de)	1.8 (c)	3.97 (cd)	44.88 (ab)	34.26 (d)
G9 (Tichedrett)	33.5 (d)	87.04 (ab)	62.89 (bc)	1.93 (bc)	5.1 (abc)	41.75 (bc)	35.56 (d)
G10 ($Rihane_{03}$)	52.1 (a)	88.54 (a)	63.51 (bc)	1.36 (d)	5.54 (abc)	34.92 (ef)	47.42 (ab)
				Génotype			
Min	33.5	77.64	42.32	1.36	1.83	33.42	34.26
Max	52.1	89.92	79.91	2.79	7.02	47.06	48.81
Total mean	40.32	85.77	63.11	2.06	4.99	39.69	42.33
Genotype effect	**	**	***	***	*	***	**
LSD 5%	8.86	6.10	13.90	0.40	2.36	4.10	8.05
				Origin			
Local G mean	41.54	85.46	55.70	1.93	5.17	38.62	42.12
Introduce G mean	39.1	86.09	70.51	2.20	4.82	40.77	42.55
Origin effect	ns	ns	**	ns	ns	ns	ns
LSD 5%	5.52	3.76	8.50	0.30	1.40	3.49	5.35
				Row type			
2 Rows mean	40.45 (a)	86.61 (a)	69.40 (a)	2.21	4.88	40.33 (a)	43.49 (a)
6 Rows mean	40.13 (a)	84.51 (a)	53.67 (b)	1.84	5.16	38.74 (a)	40.59 (a)
Row type effect	ns	ns	***	*	ns	ns	ns
LSD 5%	6.27	4.11	9.31	0.31	1.57	3.95	5.84

CHL: Chlorophyll content, RWC: Relative water contents, DSI: Drought sensitivity index, PC: Proline content, SC: Sugar content, TKW: Thousand kernels weight, GY: Grain yield, [ns]Not significant; *Significant at p<0.05; **Significant at p<0.01; ***Significant at p<0.001.

48.81 q/ha for G2 with 42.33 q/ha as genotypic mean. The origin of the genotypes tested was only influenced the drought sensitivity index (p<0.001) while the row type had a significatif effect on drought sensitivity index and proline contents (p<0.001; 0.05 consecutively).

Correlations study

In our study, only three significatives correlations were observed among all measured parameters (Table 3). Grain yield was positively correlated with relative water contents ($r= 0.37^{*}$; p<0.05). This correlation in good agreement with that obtained by Sassi *et al*. (2012), who reported that genotypes that maintain their high RWC during water stress are probably to be the most tolerant and will be the most productive. In addition, thousand kernels weight was negatively correlated with relative water contents and total soluble sugar content ($r= -0.36^{*}$ at p<0.05; -0.52^{**} at p<0.01 consecutively).

Principal components analysis

Fig 1 visualizes the correlations of the different parameters measured with the three best PCA components as well as the coordinates of the different genotypes tested on the three best PCA components on a triple magnitude scale, the area of the circle shows the importance in absolute values of coordinates and correlations, the colors mark the sign (blue = +; red = -) and the darkest, most significant color gradient. Chlorophyll content, Relative water contents and proline content were positively correlated with the physiologic axis PC1 (first principal component), the genotype Rahma positively connected to this component had high values of these parameters. In addition, thousand kernels weight negatively correlated with PC1 with the genotypes G1, $Saida_{183}$ and Tichedrett negatively related to the same component where they had high values of thousand kernels weight. PC2 positively correlated with grain yield and drought sensitivity index and negatively with total soluble sugar content was yield and drought tolerance axis, the genotypes G2 and G3 positively connected to this component were the most yielding genotypes, Fouarra negatively connected to this component have a high value of total soluble sugar content. PC3 was positively connected with G4 and negatively with G5 and $Rihane_{03}$; this component had no

Table 3: Correlations study of all measured parameters.

	CHL	RWC	DSI	PC	SC	TKW	GY
CHL	1						
RWC	0.18	1					
DSI	-0.05	0.28	1				
PC	-0.25	0.20	0.20	1			
SC	0.09	0.26	-0.12		1		
TKW	-0.25	-0.36*	0.24	-0.03	-0.52**	1	
GY	0.34	0.37*	0.24	-0.05	-0.34	-0.13	1

CHL: Chlorophyll content, RWC: Relative water contents, DSI: Drought sensitivity index, PC: Proline content, SC: Sugar content, TKW: Thousand kernels weight, GY: Grain yield, ^{ns}Not significant; *Significant at p<0.05; **Significant at p<0.01; ***Significant at p<0.001.

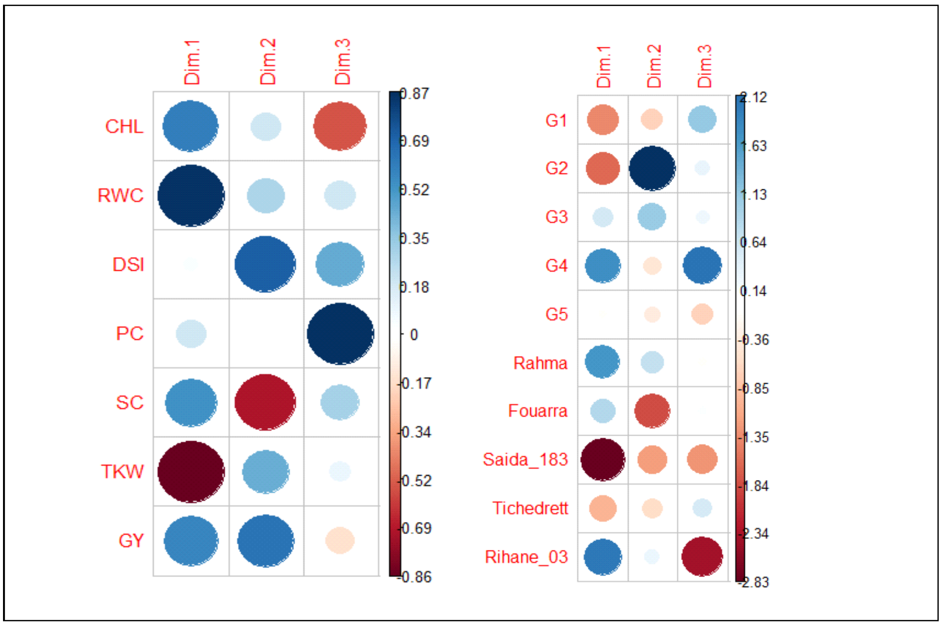

Fig 1: Visualization of the relationships of measured parameters and genotypes tested with the first's three components of the PCA.

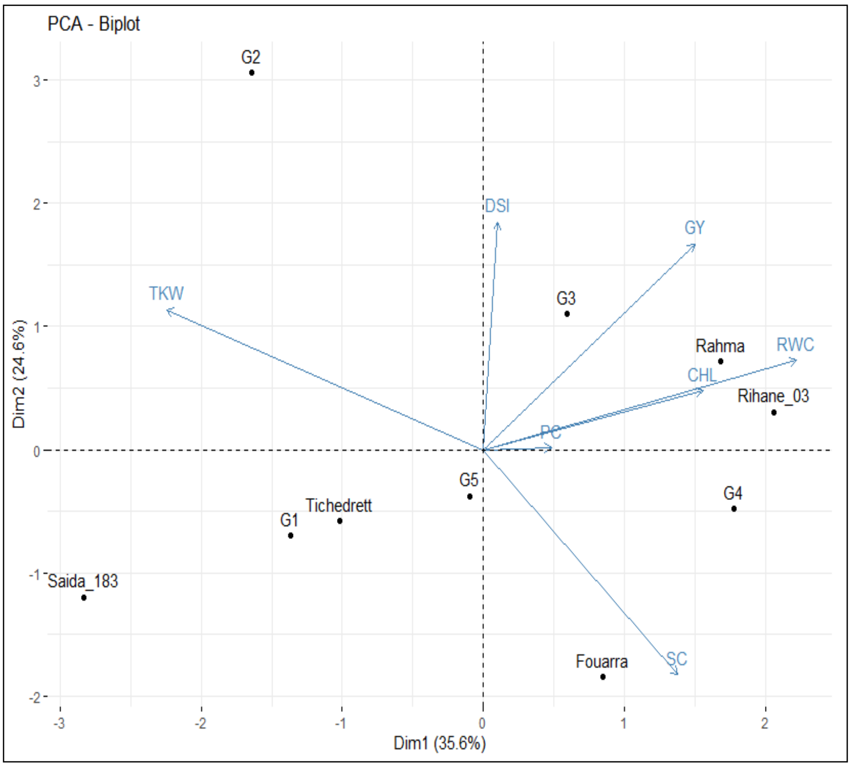

Fig 2: Biplot of the relationships of genotypes and measured parameters with the first's three components of the PCA.

relationship with the studied parameters. Fig 2 summarizes the relationship of the genotypes studied and the parameters measured with the first three components of the PCA.

CONCLUSION

The analysis of variance showed that genotype effect was significatif to very high significatif with all studied parameters, however, origin of the genotypes tested was only influenced the drought sensitivity index while the row type had a significant effect on drought sensitivity index and proline contents. Grain yield was positively correlated with relative water contents. Thousand kernels weight was negatively correlated with relative water contents and Total soluble sugar content. The G2 genotype as most yielding genotype was caracrized by the lowest soluble sugar contents and low proline content. Principal components analysis showed that PC1 was a physio-biochemical axis and the genotypes related to this axis had high values of these traits, while PC2 was a yield and drought tolerance axis and their related genotypes were the most yielding genotypes.

Conflict of interest

The authors have no conflicts of interest to declare.

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