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Evaluation of seven barley genotypes under water stress conditions

C. Vasilaki[1], A. Katsileros[2], D. Doulfi[1,*], A. Karamanos[1] and G. Economou[1]

[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Agronomy, Iera Odos 75, GR11855 Athens, Greece
[2]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Breeding and Biometry, Iera Odos 75, GR11855 Athens, Greece
[*]Correspondence: dimdoulfi@yahoo.gr

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Abstract. The evaluation of seven barley genotypes under water stress conditions using drought tolerance indices was investigated during two agronomical seasons in the experimental field of Agricultural University of Athens in Greece. The experimental design was a split-plot layout in four blocks. Four different levels of irrigation were implemented, with the method of escalated distance from the source of water (drip irrigation line). The experimental plots were protected from rain since the experiment was conducted under a rainout shelter. Measurements of water potential index of the plants were conducted, as well as stomatal resistance and stomatal resistance index of leaves, and grain yield of genotypes. A decrease in water potential index (15–25%) and grain yield (35–54%) was observed in all genotypes as soil moisture decreased. On the other hand, stomatal resistance and stomatal resistance index (26–69%) of leaves increased. Grain yield had a strong relationship with the indices of water potential and stomatal resistance of leaves. Grain yield of all genotypes is affected under water stress conditions, with the six-rowed genotypes being more adaptive than the two-rowed ones. It can be concluded that indices of water potential and stomatal resistance of leaves can be effectively used in the evaluation of genotypes under water stress conditions.

Key words: drought tolerance, stomatal resistance index, water potential index, grain yield.

INTRODUCTION

Barley *(Hordeum vulgare* L.) is an important cereal crop grown in arid and semi-arid regions. However, extreme drought and high temperatures can adversely affect plant growthand yield (Panfilova et al., 2019; Goher & Akmal, 2021; Sánchez-Díaz et al., 2002; Samarah, 2005; Mostipan et al., 2021). In these extreme conditions, plants are unable to adequately replace the water they lose due to increased transpiration and therefore, activate mechanisms to response to water stress (Chaves et al., 2009; González & Agerbe, 2010; Bresta et al., 2011, Karabourniotis et al., 2012, Schmid et al., 2015). The identification of morphological, physiological and metabolic parameters as indices of drought tolerance, and their use for the evaluation ofgenotypes is crucial for breeders and has been the subject of many research works (Jamshidi & Javanmard, 2018; Cai et al., 2020; Feiziasl et al., 2022).

Two important indices of drought tolerance are water potential and stomatal resistance of leaves. Water potential as a concept that holds a very important position in plants physiology. On the one hand, it defines the course of movement of water between neighbouring cells or tissues and the environment and on the other hand, it constitutes a measure of plant's water status. Some of the characteristics which form the values of the water potential and consequently the Water Potential Index are: a) the conductance of the stomata and the ability to absorb water from roots (Sibounheuang et al., 2006; Széles et al., 2021), b) the ability to transfer water from roots or sprout to leaves. According to Turner et al. (1984), low hydraulic conductance of the leaves, leads to a decrease in water potential and c) plants' size, either by the size of its leaves or by the number of its secondary stems or even yet by its height. More particularly, Boonjung & Fukai (1996), found that genotypes with large leaf surface had a lesser ability to hold water because of high demands in transpiration. Furthermore, Jongdee et al. (2002) admit that leaf water potential and osmotic adjustment are traits that may be useful as selection criteria for improving drought tolerance.

According to del Moral et al. (2003) and Flohr et al. (2017), cereals grain yield is sensitive to the intensity of dryness during flowering. Karamanos (1981) also refers, stomata respond to a number of environmental factors (Schulze et al., 1972) such as light, relative moisture, CO_2 concentration and temperature of leaves and aquatic status. More particularly the stomatal resistance of leaves1) is decreased by the increasing tension of light 2) is increased with the increase of CO_2 concentration of the surrounding space 3) it is increased with the increase of atmospheric dryness 4) it is reduced with the increase of temperature until an optimum value beyond of which the stomata begin to close and 5) stomata close when leaves reach into a critical dehydration point. From the aforementioned factors, the concentration of CO_2 is considered to have the most important effect on stomatal's movements, whereas light does not necessarily open the stomata (Raschke, 1976), provided that leaf's potential is above a threshold value. If the dehydration continues and the threshold value is reached, then stomata close regardless of the CO_2 concentration.

Considering this background, a two-factorial experiment was designed to investigate the impact of water stress on water potential index, stomatal resistance index and grain yield and their relationship, in seven barley genotypes.

MATERIALS AND METHODS

Site description and field preparations

Two experiments were conducted during 2013–2014 and 2014–2015 agronomical seasons under a rainout shelter with surface of 300 m^2 (30 m × 10 m) and height 2.80 m (minimum) and 3.80 m (maximum), in the experimental field of the Agricultural University of Athens (AUA) in Greece. Soil was clay-loam (34.7% sand, 29.8% clay, 35.5% silt), with a pH of 7.95 and 16% of $CaCO_3$. Sowing was conducted on 14 December 2013 and 12 December 2014, the first and the second agronomical seasons respectively. Before sowing but also during experiments weed control was conducted manually.

Treatments and experimental design

The design of the experiments included a general factorial structure with two treatment factors. The trials followed a split-plot layout in four blocks, in which each of the seven barley genotypes was assigned to the main plots and the four treatments were allocated to the subplots. Each main plot had a surface of 1.8 m^2 (1.5 m × 1.2 m), whereas each subplot was 0.45 m^2 (1.2 m × 0.375 m). Each experimental plot included six rows, 1.2 m long and 20 cm apart and the planting distance within the rows was 3 cm. The irrigation levels were differentiated according to their distance from the source of water (drippers). Irrigation level A (without water stress) was the closest to water source and irrigation level D (high water stress), was in the longest distance from the drippers. The intermediate levels B (low water stress) and C (medium water stress) were found in between the two extremes (A, D). The genotypes consisted of two populations (ANP-233/07, F-002/06) and five varieties (Elassona, Kos, Athinais, Cha-Cha, Grace). The six-row population ANP-233/07 and the two-row F-002/06 are local populations, a remarkable genetic material for study, preserved in the Bank of Genetic Material (NAGREF). Six-row Elassona, two-row Kos and six-row Athinais are Greek varieties adjusted to Greek conditions and they come from the Institute of Cereals (NAGREF). The two-row Cha-Cha and Grace are modern, early, short varieties, of high efficiency, with excellent malting characteristics, breeding achievements of the company Athenian Brewery SA. The irrigation water which was used was supplied with a drip irrigation line system (1–1.5 bar operating pressure, 5 dripper per plot, 10 L per 1 h drippers flow rate and 24 cm distance between drippers). The frequency of irrigation was determined by laboratory measurements of soil moisture as a percentage of its oven-dried weight taken from plot samples. Irrigation was applied when soil water content falls below 30% of field water capacity. The duration of irrigation ranged from 1.5 to 2.5 hours, and the corresponding volume of water from 11 to 13.75 mm. The total volume of water supplied, and the frequency of irrigation is presented in Fig. 1.

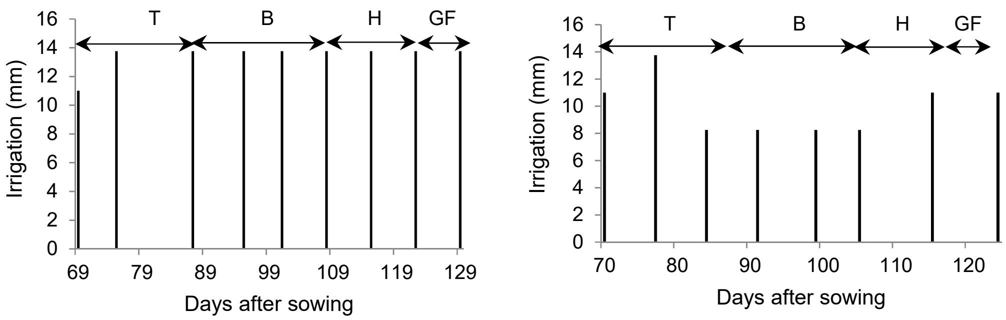

Figure 1. Water treatment after the application of drip irrigation (left) during the first season (2013–2014) and (right) during the second season (2014–2015). T: tillering; B: booting; H: heading; GF: grain-filling.

Plant water status

Two leaves per subplot (irrigation level) and eight leaves in total per main plot were collected as samples at 12 p.m. when the value of water potential reaches its minimum daily value. The youngest fully expanded leaf was sampled until spike emergence (Zadoks stage 58). From that point until maturity, flag leaf was sampled. Leaves were sealed in plastic bags before cutting and transported to the laboratory in closed insulated vessels to avoid water loss. Water potential (*Ψ)* was determined by the Pressure Bomb Technique (Scholander et al., 1964). The pressure chamber was set according to Waring & Cleary (1967). From the time course of *Ψ*, water potential index (WPI) was calculated according to Karamanos & Papatheohari (1999). Water potential index represents plants' water stress history during any period of their growth cycle.

$$WPI = \int_{t=1}^{t} \Psi_t dt/n \qquad (1)$$

where Ψ_t is the water potential at Day *t* within the observation period and *n* is the length of a period in days.

Grain yield

On the first year, harvest was conducted on May 16, 2014 (154 days after sowing) and on the second, on May 10, 2015 (150 days after sowing). From each subplot, 3 plants from the internal lines were chosen as well as 12 plants per main plot in total, in which grain yield per plant (g plant^{-1}) was studied. Grain yield effect of barley's genotypes in water stress was assessed by comparing linear regression's coefficients between grain yield and WPI (Karamanos & Papatheohari 1999; Rizza et al., 2004).

Stomatal resistance and stomatal resistance index ($r_{st}I$).

Measurements of stomatal resistance of the lower surface of leaf's margin were conducted. In every replication two leaves per subplot were sampled (irrigation level) and eight leaves in total per main plot. Before cutting the leaves to be transported to the laboratory and to be measured for their water potential, there was a measurement of stomatal resistance by using a porometer (Porometer AP4, Delta-T Devices-Cambridge-U.K). From the time course of stomatal resistance, stomatal resistance index was calculated. The $r_{st}I$ represents leaf's stomatal resistance history during any period of the biological cycle.

$$r_{st}I = \int_{t=1}^{t} r_{st} dt/n \qquad (2)$$

where r_{st} is the stomatal resistance at Day *t* within the observation.

Statistical Analysis

Statistical analysis was performed separately for each agronomical season. There was an initial check for normal distribution with the Shapiro-Wilk test and for homogeneity of variance with the Levene test as, as well as a check for outliers with the Dixon test. The data were subjected to ANOVA and the results are presented as the means ± standard errors. The comparisons of the means were performed using the Tukey HSD criteria with a level of significance of $\alpha = 0.05$. In order to examine and reveal the relationships between grain yield and the indices of water potential and stomatal resistance, bivariate analysis were used.The statistical analysis was done in R 4.1.

Weather conditions

In Fig. 2, weather data from outside of the rainout shelter for the two agronomical seasons are presented respectively. Data include average monthly maximum, mean and minimum temperature, average relative humidity, and finally average intensity of solar radiation.

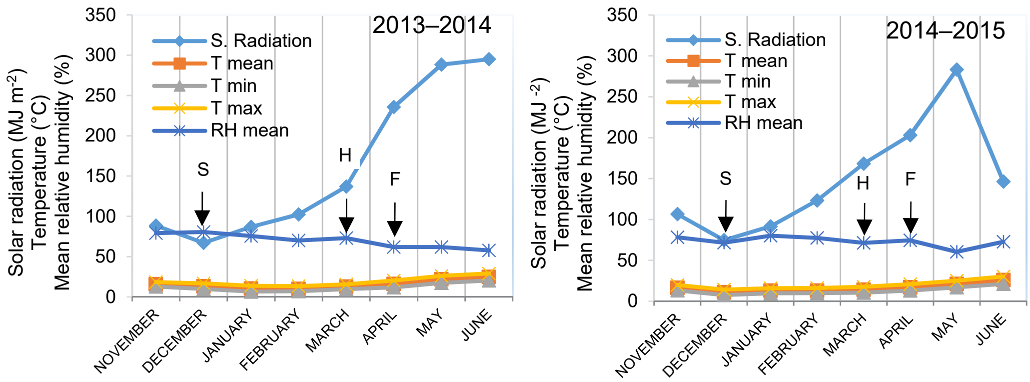

Figure 2. Average monthly maximum, mean and minimum temperature, mean relative humidity and the intensity of the solar radiation, outside of rainout shelter outside of rainout shelter during the first season (2013–2014) (left) and during the second season (2014–2015) (right). The vertical arrows show S: sowing; H: heading; F: flowering. Tmean, Tmin, Tmax: mean, minimum and maximum temperature.

During sowing in the second agronomical season values of minimum, maximum and mean temperature were greater than the first agronomical season by 2.1 °C, 2.5 °C and 2.26 °C, respectively. Both in heading as in flowering, during the first agronomical season values of minimum, maximum and mean temperature, were greater than the ones during the agronomical season. Particularly, during heading, values were greater by 0.95 °C, 2.06 °C and 1.5 °C and in flowering by 1.26 °C, 0.5 °C, and 0.8 °C, respectively. Furthermore, during sowing and heading, on the second season, values of mean relative humidity were greater than the first season by 8.9% and 1.5%, respectively. In flowering, during the first season mean relative humidity was greater by 12.76% from the second one. Also, during sowing and heading on the first season the intensity of the solar radiation was greater than the second season by 6.98 MJ m^{-2} and 31.27 MJ m^{-2}, respectively. On the contrary, during flowering in the second agronomical season, the intensity of solar radiation was greater by 32.64 MJ m^{-2} from the first season. On the first agronomical season during May-June a sharp decline of solar radiation was observed, which was not observed during the second season.

RESULTS

Water Potential Index (WPI)

In both trials, the main effects of genotypes and treatments-irrigation levels were statistically significant while the interaction between the two factors was not significant. The values of WPI for the seven barley genotypes in the four treatments are presented on Table 1. In both trials, all the comparisons of treatments were statistically significant. The genotype Kos has the greatest negative WPI values in high water stress (treatment D), in the first and the second season. The genotypes ANP-233/07 and Grace have the lowest negative WPI values in high water stress, in the first and the second season. The greatest change in WPI values between the extreme water treatments (A–D) during the first season, appeared in genotypes ANP-233/07 (20.2%) and Elassona (17.2%) and in the second season in genotype Elassona (25%). In addition, the smallest change can be observed in genotype Grace, in the first season (15%) and in the second season (15.6%) of the experiments.

Table 1. The means and thetypical errorsof water potential index-WPI (MPa). Means with the same letter are not significantly different from each other (Tukey-HSD test)

Genotypes	Water Treatments				
	A	B	C	D	
	First Season (2013–2014)				Mean Gen.
ANP-233/07	-1.68 ± 0.04	-1.76 ± 0.05	-1.88 ± 0.05	-2.02 ± 0.05	-1.84 a
ATHENAIS	-1.77 ± 0.03	-1.81 ± 0.06	-1.95 ± 0.06	-2.08 ± 0.05	-1.91 ab
GRACE	-1.80 ± 0.02	-1.94 ± 0.02	-1.99 ± 0.02	-2.07 ± 0.02	-1.95 bc
CHA-CHA	-1.79 ± 0.01	-1.94 ± 0.01	-2.02 ± 0.02	-2.08 ± 0.01	-1.96 bcd
F-002/06	-1.86 ± 0.04	-1.95 ± 0.05	-2.06 ± 0.03	-2.18 ± 0.03	-2.01 cde
ELASSONA	-1.86 ± 0.02	-2.00 ± 0.02	-2.07 ± 0.07	-2.20 ± 0.07	-2.03 de
KOS	-1.90 ± 0.03	-2.04 ± 0.03	-2.13 ± 0.04	-2.23 ± 0.04	-2.07 e
Mean W.T.	-1.81 a	-1.92 b	-2.01 c	-2.12 d	G.M[1] = -1.96
	Second Season (2014–2015)				Mean Gen.
ELASSONA	-1.59 ± 0.01	-1.80 ± 0.01	-1.92 ± 0.03	-2.07 ± 0.03	-1.74 a
ANP-233/07	-1.65 ± 0.02	-1.78 ± 0.01	-1.86 ± 0.01	-2.00 ± 0.01	-1.82 b
GRACE	-1.73 ± 0.01	-1.85 ± 0.03	-1.93 ± 0.03	-2.01 ± 0.08	-1.88 c
ATHENAIS	-1.74 ± 0.02	-1.80 ± 0.02	-1.93 ± 0.03	-2.08 ± 0.03	-1.89 cd
F-002/06	-1.71 ± 0.03	-1.84 ± 0.05	-1.93 ± 0.02	-2.08 ± 0.02	-1.89 cd
KOS	-1.76 ± 0.02	-1.84 ± 0.01	-1.95 ± 0.01	-2.13 ± 0.02	-1.92 cd
CHA-CHA	-1.80 ± 0.01	-1.88 ± 0.02	-1.98 ± 0.02	-2.12 ± 0.02	-1.94 d
Mean W.T.	-1.65 a	-1.83 b	-1.93 c	-2.07 d	G.M.= -1.87

[1] G.M. = Grand mean.

Stomatal Resistance (r_{st})

Fig. 3 and Fig. 4 present the changes of stomatal resistance of leaves of the seven barley genotypes over time. The gradually increasing values of stomatal resistance are statistically significant in both trials, for all genotypes and water treatments.

Stomatal Resistance Index ($r_{st}I$)

The values of stomatal resistance index for the seven barley genotypes in the four treatments are presented on Table 2. In first season, the main effects of genotypes and treatments-irrigation levels were statistically significant while the interaction was not significant. All the comparisons of treatments were statistically significant. The genotypes Athinaida, Elassona, ANP-233/07 and Grace have the highest values of $r_{st}I$ in high water stress (treatment D), while the genotypes F-002/06 and Kos have the lowest values. The highest change between the extreme water treatments (A–D) was presented by genotype F-002/06 (55.7%) and the lowest change by the genotype Elassona (48.6%). In second season, the interaction between the two factors was statistically significant. The genotypes Elassona, and Grace have the highest values of $r_{st}I$ in high water stress, wlile the genotypes Athinaida and ANP-233/07 have the lowest values. The highest change between the extreme water treatments (A–D) was presented by genotype F-002/06 (69%) and the lowest change by the genotypes Grace (26.8%) and Cha-Cha (31.1%).

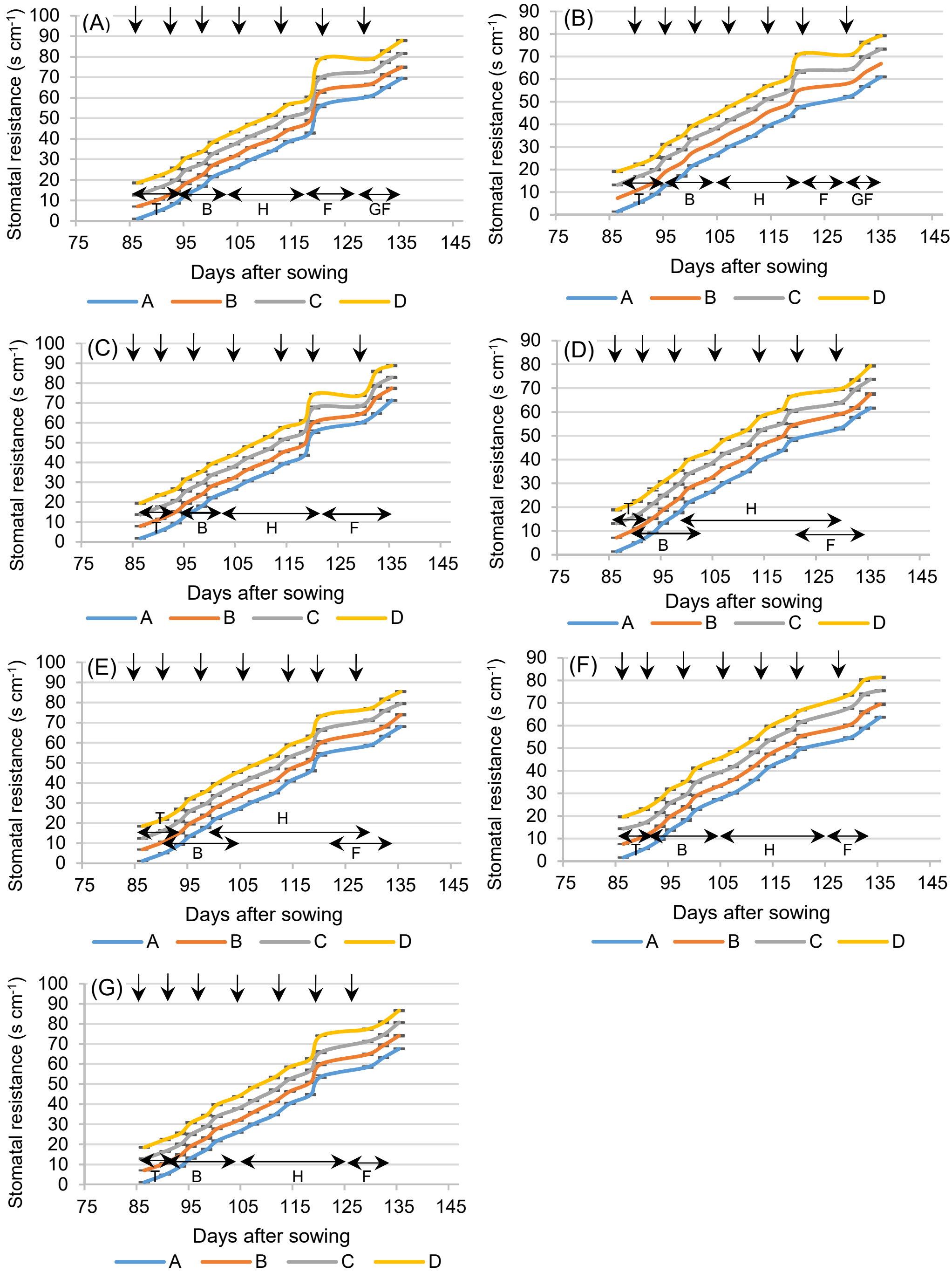

Figure 3. The changes of stomatal resistance of leaves for the barley genotypes during the first season (2013–2014) of experiments. The vertical arrows show each watering point and the horizontal show the growth stage. T: tillering; B: booting; H: heading; F: flowering; GF: grain-filing. A-D: water treatments. (A): ANP-233/-07; (B): F-002/06; (C): Elassona; (D): Kos; (E): Athinais; (F): Cha-Cha; (G): Grace. The vertical bars symbolize the typical error of mean values.

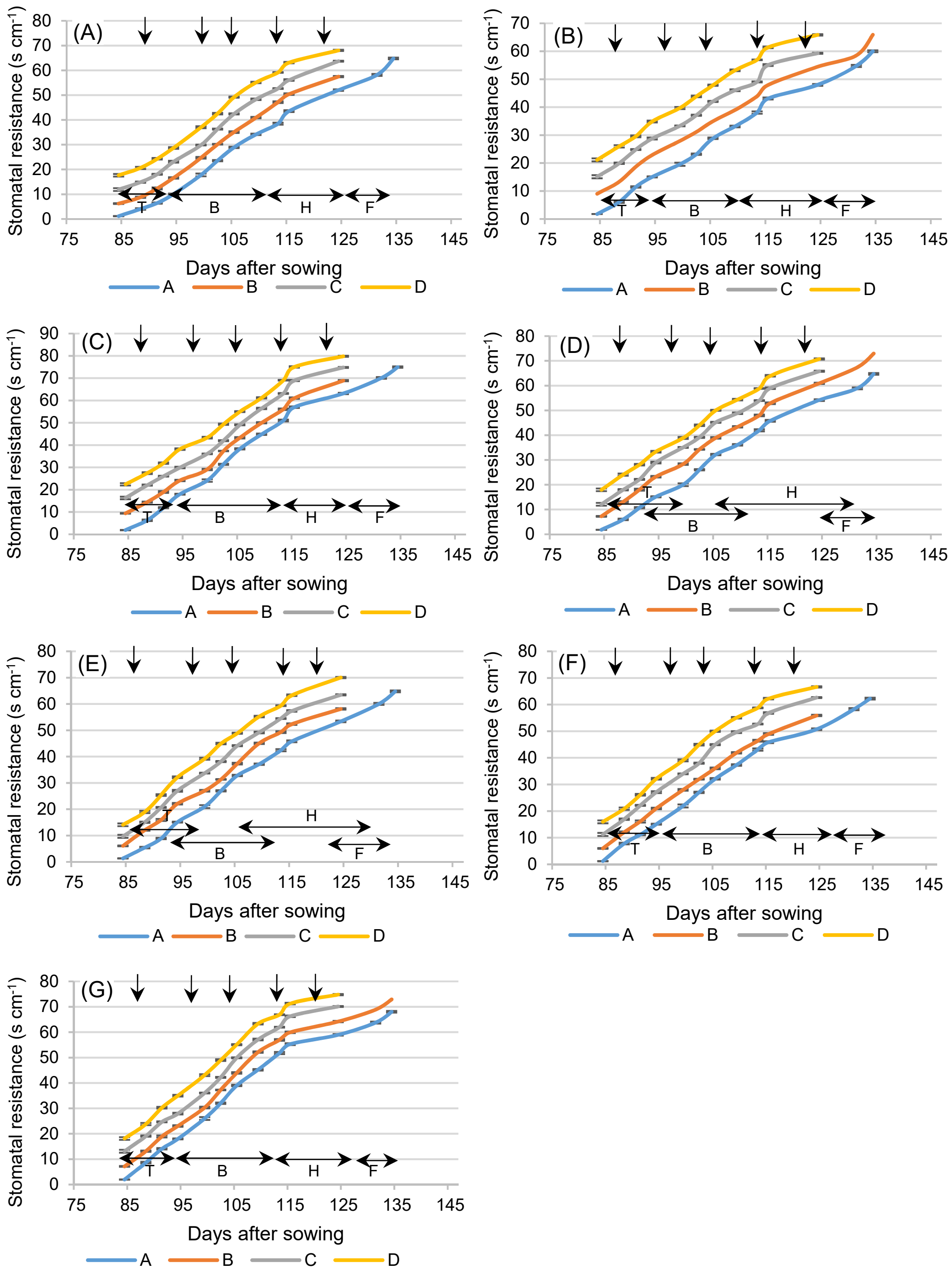

Figure 4. The changes of stomatal resistance of leaves for the barley genotypes during the second season (2014–2015) of experiments. The vertical arrows show each watering point and the horizontal show the growth stage. T: tillering; B: booting; H: heading; F: flowering; GF: grain filing. A–D: water treatments. (A): ANP-233/-07; (B): F-002/06; (C): Elassona; (D): Kos; (E): Athinais; (F): Cha-Cha; (G): Grace. The vertical bars symbolize the typical error of mean values.

Table 2. The means and the typical errors of stomatal resistance index. Means with the same letter are not significantly different from each other (Tukey-HSD test)

Genotypes	Water Treatments				
	A	B	C	D	
	First Season (2013–2014)				Mean Gen.
ATHINAIDA	35.37 ± 0.14	41.52 ± 0.22	47.60 ± 0.21	53.31 ± 0.20	44.45 a
ELASSONA	35.71 ± 0.25	41.43 ± 0.24	47.26 ± 0.27	53.10 ± 0.27	44.37 a
ANP-233/07	35.26 ± 0.19	41.18 ± 0.15	47.17 ± 0.11	53.43 ± 0.12	44.26 a
GRACE	35.01 ± 0.22	41.08 ± 0.18	47.04 ± 0.18	53.25 ± 0.22	44.10 a
CHA-CHA	34.26 ± 0.13	40.17 ± 0.07	46.48 ± 0.18	52.45 ± 0.20	43.34 b
F-002/06	32.82 ± 0.18	38.97 ± 0.18	45.15 ± 0.23	51.11 ± 0.26	42.01 c
KOS	33.23 ± 0.31	39.08 ± 0.27	44.96 ± 0.32	50.72 ± 0.23	42.00 c
Mean W.T.	34.52 a	40.49 b	46.52 c	52.48 d	G.M.[1] = 43.5
	Second Season (2014–2015)				Mean Gen.
GRACE	40.35 ± 0.18 i	45.17 ± 0.31 ef	48.21 ± 0.35 c	51.17 ± 0.20 b	46.23
ELASSONA	36.92 ± 0.29 lm	40.84 ± 0.25 hi	47.01 ± 0.22 cd	53.17 ± 0.27 a	44.49
CHA-CHA	34.52 ± 0.21 n	38.15 ± 0.33 kl	40.62 ± 0.18 hi	45.27 ± 0.16 ef	39.64
KOS	30.06 ± 0.34 q	35.95 ± 0.22 m	41.79 ± 0.35 h	46.21 ± 0.35 de	38.50
F-002/06	27.16 ± 0.31 r	33.12 ± 0.31 op	39.55 ± 0.16 ij	45.92 ± 0.16 def	36.44
ATHINAIDA	28.78 ± 0.12 q	34.09 ± 0.39 no	37.92 ± 0.23 kl	43.37 ± 0.21 g	36.04
ANP-233/07	26.99 ± 0.39 r	32.49 ± 0.23 p	38.65 ± 0.10 jk	44.61 ± 0.19 fg	35.69
Mean W.T.	32.11	37.12	41.97	47.11	G.M. = 39.58

[1] G.M. = Grand mean.

Grain Yield

The values of the grain yield for the seven barley genotypes in the four treatments are presented on Table 3. In both trials, the interaction between genotypes and treatments-irrigation levels were statistically significant. In first season, the highest grain yield in high stress water was observed by genotypes Cha-Cha and Grace and the lowest grain yield by genotypes Elassona and Kos. The highest change between the extreme water treatments (A–D) was observed by genotypes Elassona (46.7%) and Kos (46.7%) and the lowest change by genotypes Cha-Cha (34.5%) and Grace (35.5%). In second season, the highest grain yield in high water stress was observed by genotypes Cha-Cha and Grace and the lowest grain yield by genotypes Elassona and Kos. The highest change in grain yield was observed by genotypes Kos (55.7%) and F-002/06 (54.4%) and the lowest change by genotypes Cha-Cha (38.9%) and ANP-233/07 (36.4%).

Table 3. The means and thetypical errorsof grain yields (g/plant). Means with the same letter are not significantly different from each other (Tukey-HSD test)

Genotypes	Water Treatments				
	A	B	C	D	
	First Crop Season (2013–2014)				Mean Gen.
ANP-233/07	3.51 ± 0.02 d	3.13 ± 0.04 efg	2.75 ± 0.02 ij	2.20 ± 0.04 mn	2.90
ATHINAIDA	3.29 ± 0.03 e	3.02 ± 0.03 g	2.36 ± 0.02 lm	1.95 ± 0.06 o	2.66
CHA-CHA	4.20 ± 0.03 a	4.03 ± 0.03 b	3.22 ± 0.04 ef	2.75 ± 0.03 i	3.56
ELASSONA	2.74 ± 0.03 ij	2.49 ± 0.07 kl	2.03 ± 0.05 no	1.46 ± 0.02 q	2.18
F-002/06	3.12 ± 0.03 efg	2.66 ± 0.02 ijk	2.15 ± 0.02 n	1.68 ± 0.03 p	2.41
GRACE	4.00 ± 0.04 b	3.78 ± 0.03 c	2.99 ± 0.04 gh	2.58 ± 0.01 jk	3.35
KOS	3.10 ± 0.03 fg	2.82 ± 0.03 hi	2.20 ± 0.02 mn	1.65 ± 0.03 p	2.44
Mean W.T.	3.43	3.13	2.53	2.04	G.M.[1] = 2.78

Table 3 (continued)

	Second Crop Season (2014–2015)				Mean Gen.
ANP-233/07	3.20 ± 0.01 d	2.90 ± 0.04 e	2.56 ± 0.02 g	2.04 ± 0.03 j	2.67
ATHINAIDA	2.99 ± 0.02 e	2.72 ± 0.01 f	2.09 ± 0.02 ij	1.66 ± 0.03 l	2.36
CHA-CHA	3.90 ± 0.02 a	3.70 ± 0.02 b	2.92 ± 0.04 e	2.38 ± 0.03 h	3.23
ELASSONA	2.41 ± 0.02 h	2.20 ± 0.03 i	1.78 ± 0.04 kl	1.19 ± 0.02 m	1.89
F-002/06	2.90 ± 0.04 e	2.39 ± 0.03 h	1.74 ± 0.03 kl	1.32 ± 0.02 m	2.09
GRACE	3.75 ± 0.03 b	3.45 ± 0.03 c	2.61 ± 0.02 fg	2.11 ± 0.03 ij	2.98
KOS	2.87 ± 0.03 e	2.66 ± 0.02 fg	1.86 ± 0.03 k	1.27 ± 0.03 m	2.17
Mean W.T.	3.15	2.86	2.22	1.71	G.M. = 2.48

[1] G.M. = Grand mean.

Bivariate Analysis

In both trials the grain yield was significantly positively correlated with the water potential index and significantly negatively correlated with the stomatal resistance index. Linear regression of the response variables of grain yield was performed with the predictor variables of the water potential index and with the stomatal resistance index per genotype and agronomical season (Fig. 5 and Fig. 6). All regression coefficients were significant (Table 4 and Table 5). Also, all regression coefficient comparisons were significant except for the regression coefficients of grain yield and stomatal resistance index during the first season.

Table 4. Regression coefficients between grain yields (g) per plant and water potential index (WPI) during the first and second season

		First Season (2013–2014)				Second Season (2014–2015)			
	Term	Est.	S.E.	Prob>|t|	R^2	Est.	S.E.	Prob>|t|	R^2
ANP-233/07	a[1]	7.64	0.93	<.0001	0.65	8.79	0.30	<.0001	0.96
	b[2]	2.58	0.50	0.0002		3.35	0.16	<.0001	
Athinaida	a	7.78	1.14	<.0001	0.59	9.18	0.57	<.0001	0.96
	b	2.69	0.59	0.0005		3.61	0.30	<.0001	
Cha-Cha	a	12.9	1.33	<.0001	0.78	12.6	0.71	<.0001	0.93
	b	4.79	0.67	<.0001		4.83	0.36	<.0001	
Elassona	a	7.35	1.12	<.0001	0.95	3.88	0.37	<.0001	0.97
	b	2.54	0.55	0.0004		1.14	0.20	<.0001	
F-002/06	a	9.49	0.99	<.0001	0.99	9.17	0.90	<.0001	0.99
	b	3.51	0.49	<.0001		3.75	0.47	<.0001	
Grace	a	13.1	1.40	<.0001	0.77	10.1	1.63	<.0001	0.58
	b	5.01	0.72	<.0001		3.81	0.86	0.0006	
Kos	a	10.0	1.18	<.0001	0.75	10.5	0.55	<.0001	0.94
	b	3.64	0.56	<.0001		4.35	0.28	<.0001	

[1] = intercept; [2]= slope.

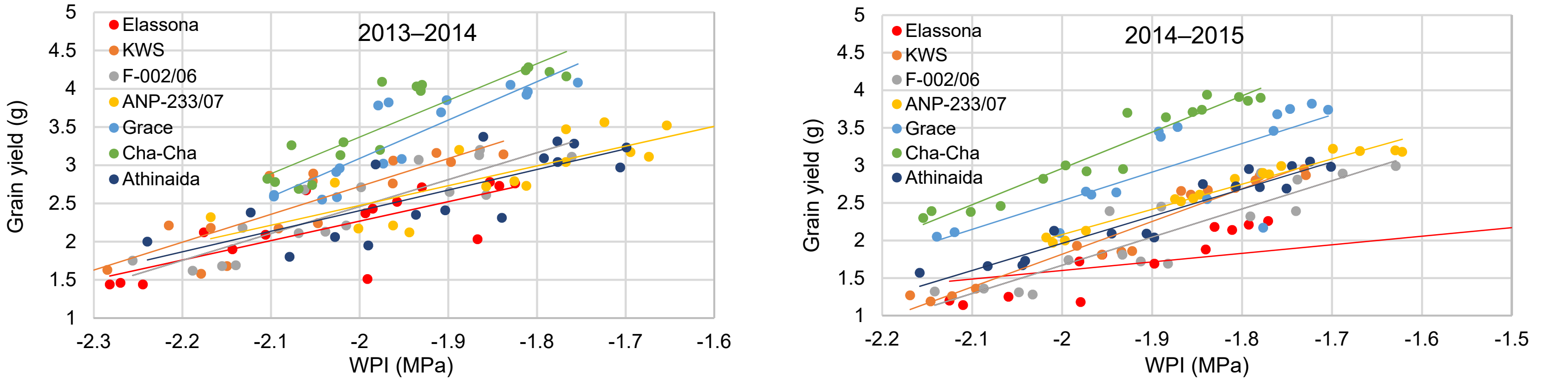

Figure 5. The fitted linear regressions between grain yields (g) per plant and water potential index (WPI) during the first season(left) and second season(right).

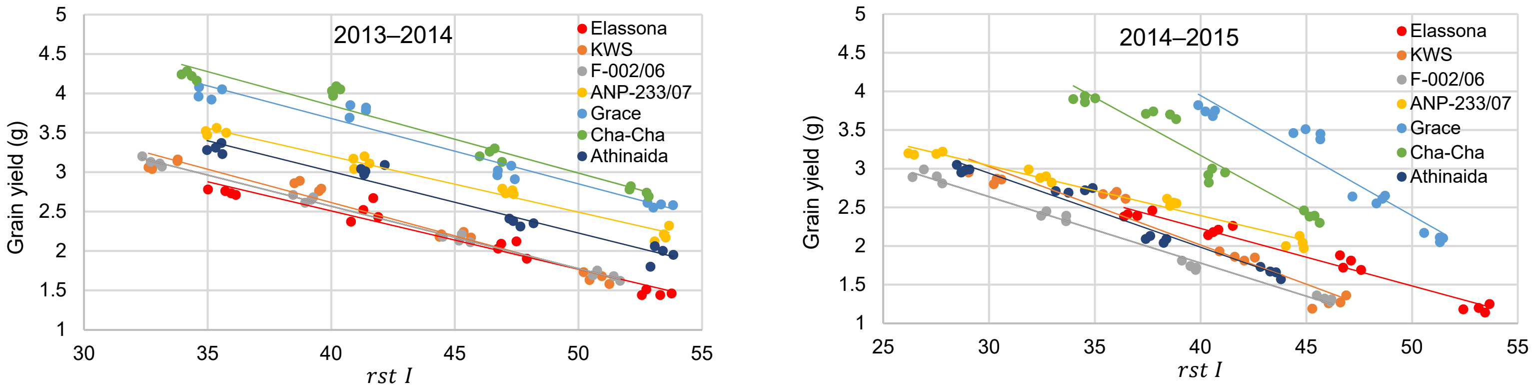

Figure 6. The fitted linear regressions between grain yields (g) per plant and stomatal resistance index ($r_{st}I$) during the first season (left) and second season (right).

Table 5. Regression coefficients between grain yields (g) per plant and stomatal resistance index ($r_{st}I$) during the first and second season

		First Season (2013–2014)				Second Season (2014–2015)			
	Term	Est.	S.E.	Prob>|t|	R^2	Est.	S.E.	Prob >|t|	R^2
ANP-233/07	a[1]	6.03	0.12	<.0001	0.97	4.98	0.11	<.0001	0.97
	b[2]	-0.07	0.01	<.0001		-0.06	0.00	<.0001	
Athinaida	a	6.13	0.19	<.0001	0.96	5.81	0.21	<.0001	0.95
	b	-0.07	0.01	<.0001		-0.10	0.01	<.0001	
Cha-Cha	a	7.28	0.22	<.0001	0.95	9.15	0.45	<.0001	0.97
	b	-0.08	0.01	<.0001		-0.15	0.01	<.0001	
Elassona	a	5.47	0.20	<.0001	0.95	5.20	0.16	<.0001	0.97
	b	-0.07	0.01	<.0001		-0.07	0.00	<.0001	
F-002/06	a	5.73	0.05	<.0001	0.99	5.21	0.08	<.0001	0.99
	b	-0.07	0.01	<.0001		-0.09	0.00	<.0001	
Grace	a	6.99	0.23	<.0001	0.96	10.17	0.59	<.0001	0.91
	b	-0.08	0.01	<.0001		-0.16	0.01	<.0001	
Kos	a	5.99	0.17	<.0001	0.95	6.07	0.28	<.0001	0.93
	b	-0.08	0.01	<.0001		-0.10	0.01	<.0001	

[1] = intercept; [2]= slope.

Discussion

Plants' aqueous status depends on both weather conditions and the irrigation provided. In the first agronomical season the values of the minimum, maximum and mean temperature both in heading and flowering were greater in relation to the second season. Levels of humidity and intensity of the solar radiation did not change dramatically from the one year of experiments to the other (Fig. 2). Moreover, the millimeters of irrigated water were higher during the first season than the second (Fig. 2).

Water Potential is the main and most reliable natural parameter for the estimation of the aqueous status of plants (Karamanos, 1981; Karamanos & Papatheohari, 1999). Values of water potential during the experiments, consequently led to the calculation of the water potential index based on the suggested method of Karamanos & Papatheohari (1999). This particular index provides a fairly reliable indication of the total water stress sustained by a plant during a given season. On the contrary Water Potential provides information for the given time when the sample is taken. The use of Water Potential as an objective index for the estimation of total stress that plants can sustain in a given environment has many advantages and can be used in order to evaluate its genotype drought resistance (Papastavrou et al., 2004). On the first season, WPI index had a greater amount of negative values, meaning that plants were under more intense stress conditions than in the second year. WPI presented an average value of 1.81 MPa, 1.92 MPa, 2.01 MPa and 2.12 MPa for irrigation levels A, B, C, D respectively. On the second year, WPI presented an average value of 1.65 MPa, 1.83 MPa, 1.93 MPa and 2.07 MPa for the four different levels of irrigation, respectively. These results can be easily explained by the alterations in temperature (Vahamidis et al., 2018). Experiments were conducted in a protected environment under the same circumstances so there was no differentiation due to e.g. different wind speeds. Moreover, a common feature of the WPI values of the seven studied barley genotypes in both agronomical seasons was the genotypic differences and clear classification between treatments (Table 1). On the first season, Kos, F-002/06 and Elassona had the greater negative values of WPI. Whereas the less negative value on irrigation level A, was observed for the population ANP-233/07, on irrigation level D, the most negative values appeared on Kos and Elassona and the less negative values on ANP-233/07, Athenais, Cha-Cha and Grace. On the second agronomical season, the most negative values appeared on Cha-Cha and Kos and the less negative value Elassona, on irrigation level A. On irrigation level D, the most negative values appeared on Kos and Cha-Cha and the less negative values on ANP-233/07 and Grace. In conclusion, we ascertain the tendency for more negative values on Kos and less negative on ANP-233/07 and Grace. Intra-genotypic differentiation reflects the corresponding genetic variation they conceal and which is expressed in a many different phenotypic ways (Papastavrou, 2004; Panfilova et al., 2020). The almost complete differentiation of WPI between treatments showed that the seven genotypes during the experimental procedures had different water stress intensities in the four subplots.

The increased stomatal resistance of leaves to water vapor diffusion is one of the immediate and rapid reactions of plants to water scarcity (Reynolds-Henne et al., 2010). The course of stomatal resistance revealed low values during the first growth stages, followed by a rise with the progress of growth stages. Variation in the seven barley genotypes was also observed in the values r_{st} in various irrigation treatments (Figs 3 and 4), with greater stomatal resistance values in treatment D in relation to treatment A. Sezen et al. (2019), ended in similar assumptions in red pepper plants and Nemeskeri et al. (2015), in pea plants. The observed increase in parameter values is attributed to both increased water deficits and growth stage (Karamanos et al., 1983; Gupta et al., 2001). Moreover, according to Teare et al. (1973), Ahmed et al. (2013) and Ghotbi-Ravandi et al. (2014), the closure of stomata on barley plants and the reduction of their conductivity is a general plant reaction to drought in order to prevent dehydration. A similar reaction, namely the closure of the stomata and a decrease in their conductivity was observed in soybean plants (Fenta et al., 2012) in drought susceptible genotypes. The negative effects of drought in the closing of stomata and photosynthesis leadto lower capacity development and reduction of biomass accumulation (Benešová et al., 2012). The effect of water stress was also visible on the stomatal resistance index of leaves in all the seven barley genotypes, with increasingly higher values from treatment A to treatment D (Table 2). Moreover, in the first season plants were more fatigued therefore the values of the stomatal resistance index are higher than in the second season.

In our experiment, in both experimental years, in irrigation levels A and D, the greatest grain yield appeared for the two-rowed varieties Cha-Cha and Grace whereas the smallest for the variety namely Elassona. The reduction on grain yield per plant due to the reduction on soil humidity for both seasons was obvious for all seven of the studied barley varieties (Table 3). The reduction on yield with the reduction of the available water is the most characteristic reaction of all crops (Horváth et al., 2021), barley included. Indicatively, relative reports have been done in the studies of Fischer & Maurer (1978), Brisson & Cassals (2005), Samarah et al. (2009); Hakala et al. (2012); Arshadi et al. (2018) and Zargar et al. (2018), where they tried to explain the effect of different treatments on yield differentiation. Grain yield modification in cereals is a multi-factor dependent process which involves complex procedures. In our case, the intensity of water stress as well as the implemented growth stage play an important role. Three critical stages for the grain yield were defined: from booting till the beginning of the last leaf's sheath swelling, flowering and grain filling. Time before heading has the greatest contribution in the final reduction (Karamanos, 2008). Still, according to del Moral et al. (2003) and Flohr et al. (2017) grain yield is sensitive to water scarcity during flowering.

Moreover, during the experiment it has been observed that the two-row genotypes were more sensitive (higher coefficient b) to water stress. Furthermore, in the first season in which we had more intense water stress (more negative values of WPI), there was a clearer separation of inclination regarding the second one. Additionally, we observed that WPI and $r_{st}I$ presented a strong relationship with the grain yield per plant, which was stronger in the second season. The definition coefficient (R^2) ranged the first season from 0.59 till 0.79 and the second season from 0.59 till 0.96 (Fig. 5). The definition coefficient (R^2) for stomatal resistance index ranged the first season from 0.59 till 0.78 and the second season from 0.91 till 0.99 (Fig. 6).

CONCLUSIONS

For the evaluation of barley's drought resistance, some aqueous status and acclimatization parameters in two populations and five modern varieties were studied. In both populations and varieties, the reduction of the available soil moisture decreased the water potential index (appearance of more negative values). All genotypes showed an increased tendency in stomatal resistance and stomatal resistance index of leaves. In addition, water stress decreased grain yield of all the genotypes. The two-row genotypes Cha-Cha and Grace have statistically significant the highest grain yield in all water stress treatments and the stronger relationship between the grain yield and the variables WPI and $r_{st}I$. Stomatal resistance index has for the first time been introduced in this research and could be applied in order to define plants' water stress. Finally, it can be concluded that indices of water potential and stomatal resistance of leaves can be effectively used in the evaluation of barley genotypes under water stress.

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