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Impact of Pre-Anthesis Water Deficit on Yield and Yield Components in Barley (*Hordeum vulgare* L.) Plants Grown under Controlled Conditions

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Impact of Pre-Anthesis Water Deficit on Yield and Yield Components in Barley (*Hordeum vulgare* L.) Plants Grown under Controlled Conditions

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Abstract: Drought at pre-anthesis stages can influence barley growth and results in yield losses. Therefore, it is important to understand how drought at pre-anthesis can affect different traits associated with yield reduction in barley. The objective of this study was to understand the relevance of the genetic background of major flowering time genes in barley plants subjected to pre-anthesis drought and its impact on yield and yield components. A glasshouse experiment using a Randomized Complete Block Design was conducted to investigate the effect of drought and its timing on yield and yield components on eleven barley genotypes, which were selected to represent genetic diversity of major flowering time genes (*PPDH1*, *PPDH2*, *HvVrn1*, *HvVrn2* and *HvVrn3*). Barley plants were exposed to three water regimes, non-stressed and stressed, which was applied at two pre-anthesis growth stages, tillering (SS) and stem elongation (SE). Results identified differences among genotypes in all measured traits. Grain yield, grain number and "thousand kernel weight" were reduced in all genotypes due to drought, irrespective of the growth stage. Early flowering genotypes had better performance as reflected in higher yield compared with late flowering genotypes. Results verified the fundamental importance of early flowering to improve productivity in response to pre-anthesis drought. The results of this study can help in selecting barley lines for future breeding purposes with improved resilience to drought conditions in Mediterranean environments.

Keywords: drought; flowering time; barley; yield components

1. Introduction

Barley (*Hordeum vulgare* L.) is a widely cultivated cereal crop in many rainfed areas in the Mediterranean region where drought is considered the main yield-limiting factor [1,2]. In such marginal lands, yield losses is associated with drought conditions resulted from low and inconsistent precipitation during the whole plant growth cycle, either early in the fall or winter (initial drought

conditions) or late during spring (terminal drought) [1]. The eastern part of the Mediterranean basin is also potentially very susceptible to future climate changes, which are predicted to shorten the rainy season and thus increase the frequency of drought, and reduce barley production [3–5].

Barley is characterized by its fast pre-anthesis growth and its ability to form large number of tillers that cover soil surface and subsequently reduce water evaporation. This trait explains why barley is highly successful in dry areas [2,5]. Furthermore, fast pre-anthesis growth enables barley to store more assimilates in their stems since temperature is not hot and soil water is normally available in adequate amounts for its growth and development [2,6]. Drought conditions at early growth stages are known to reduce seed germination percentage and rate and it can affect negatively seedling establishment. The developing plants will have poor tillering capacity leading to fewer plants and tillers per unit area and thus lower yield potential [7]. Additionally, drought at the period of stem elongation causes reduction in number of grains per unit area due to it is negative effect on floret formation and fertility [7,8]. Post-anthesis drought conditions reduce the grain filling rate and duration and this will result in shriveled grains [9]. Knowing that yield has two major components, grain number per unit area and grain weight, with grain number being determined during the pre-anthesis stage, while grain weight is determined at the post-anthesis stage, it is critical to study drought stress in the pre-anthesis and post-anthesis stages. The successful establishment of barley at early growth stages under dry environments is needed for high productivity (*i.e.*, higher grain number production) [10]. During pre-anthesis growth, higher floret fertility is translated into a higher potential grain number per unit area [11], while grain weight depends on the degree to which post-anthesis conditions support grain-filling [12]. In such cases, drought at the pre-anthesis stage can have greater yield reductions than in post-anthesis stages of growth, because it affects yield potential at the sink level via decreasing the number of fertile spikes per unit area at crop establishment and tillering phases, as well as the number of grains per spike [13]. Therefore, increasing drought tolerance at stem elongation and tillering stages must be targeted in breeding programs to produce new varieties for dry areas. The impact of initial drought on barley, especially in the pre-anthesis period, and other related aspects such as reproductive meristem establishment and florets fertility at booting stages is not fully understood. At these stages, barley is considered as a drought sensitive plant but further investigation is needed to dissect its impact on different physiological and developmental processes that are genetically controlled [2,8,14].

Before flowering, the shoot apical meristem in barley progresses through three phases of development: vegetative, inflorescence, and floral. In each phase, the apical meristem produces a different set of organs that are affected by set of genes that establish and maintain meristem identity or transition [15]. Such genes govern different cellular pathways that promote or repress flowering, all by which quantitatively contribute to an activity that switches the shoot apical meristem from producing leaves to form flowers after reaching a threshold level [16]. Historically, breeders improved barley for drought tolerance through targeting yield indirectly depending on the selection for higher yield in unstressed conditions. Further improvement must be directed toward testing other traits or identifying new ones that can minimize the gap between yield potential and harvested yield in water-limiting environment [17]. In this study, the effect of drought imposed at different stages was studied using a diverse set of barley genotypes that differ in major flowering time genes. In cereals, three distinct genetic systems are known to determine flowering time: (i) vernalization (*VRN*) genes; (ii) day-length sensitive photoperiod (*PPD*) genes; and (iii) "earliness *per se*" (*EPS*) genes [18]. Genetic variations in *VRN* genes divide the temperate cereals into winter and spring classes, whereas the differences in the *PPD* genes divide them into photoperiod-sensitive and photoperiod-insensitive classes (Rollins *et al.*, 2013) [18]. Barley is adapted to a wide range of environments due to allelic diversity in *VRN* and *PPD* genes [18].Therefore, the current study examined the response of heading date, yield, yield components and other physiological traits to drought occurred in barley plants at two different pre-anthesis growth stages: tillering stage and stem elongation stage, according to the expected situations of climatic change for the Mediterranean region.

2. Materials and methods

2.1. Plant Material

Eleven barley genotypes originating from different geographical regions, including Jordan, ICARDA (International Center for Agriculture Research in the Dry Areas), United Kingdom, United States of America, Germany and Australia, were used in the present study (Table 1).

Table 1. Names and origin of genotypes used in this study.

Genotype	Row Type	Pedigree/Source
Acsad176	6	(CM872-189-3Y-1B-2Y-1BX1Y-OB) X (Cr.366/16/2)
Arta	2	A single spike collected from a field of Arabi Abiad in the Hourani plateau. Released as Arabi Abiad Mohassan.
ER/Amp	2	A selected ICARDA line released in Tunisia
GP	2	Gamma-Ray mutant created from Maythorpe cultivar
Keel	2	Clipper/CPI18197 and WI 2645
Morex	6	Derived from Manchurian barley (Cree/Bonanza).
Mutah	2	Roho/Arabi Abiad/6250 developed in ICARDA and certified in NCARE, Jordan
Rum	6	Originated from Harbinger-ArivatX Attiki in CIMMYT (Mexico) and certified in the National Center for Agriculture Research and Extension (NCARE), Jordan.
Steptoe	6	Derived from Coast-type barley originating in North Africa. Washington Selection 3564/Unitan
Tadmor	2	A single spike collected from a field of Arabi Aswad (ICARDA).
Yarmouk	2	Esp/1808-4L//Harmal developed in ICARDA and certified in NCARE, Jordan

2.2. Genotyping of Major Flowering Time Genes

The genotypes were selected to maximize genetic diversity based on the genotyping of major flowering time genes (*PPDH1, PPDH2, HvVrn1, HvVrn2* and *HvVrn3*) and their agronomical performance under field drought conditions.

For genomic DNA extraction, leaf tissue samples from each genotype were harvested from three-week-old seedlings that were grown in a greenhouse at The University of Jordan. Total genomic DNA extraction was performed using the CTAB (cetyl trimethyl ammonium bromide) method as described in [19]. Genotyping of the *PPDH1*, *PPDH2*, *HvVrn2* and *HvVrn3* genes was carried out using gene-specific primers as described in [20]. Furthermore, the nature of allelic variations in *HvVrn1* was analyzed using allele-specific primers as described in [21]. For this purpose, DNA fragments from the targeted genes were amplified using PCR in a 25 µL reaction mixture containing 2.5 µL of gDNA as a template, 2.5 µL of primer (10µM), 12 µL of the master mix (dNTPs (100 µM), 5 µL of 5× PCR buffer, 0.5 µM of each primer and 8 µL of water. The PCR conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 min, 55 °C for 1 min and 72 °C for 1 min, and a final 10 min extension at 72 °C. The amplified PCR products were separated in 1.5% agarose gel and visualized by RedSafe® staining (Intron, Seoul, Korea). The PPDH1 and HvVrn3 PCR products were extracted from the agarose gel using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), and sent for DNA sequencing to Macrogen Inc. (Seoul, Korea).

2.3. Greenhouse Experiment and Stress Treatments

The experiment was conducted in a controlled glasshouse located in the Jubeiha research station/Faculty of Agriculture/The University of Jordan, Amman, Jordan. To ensure the exposure of the plants to low temperatures (vernalization) during the pre-anthesis stages, the tested genotypes were grown from the 1 November to the end of February under a regime that was dependent on

natural weather conditions outside the greenhouse but did not allow the temperature to exceed above 25 °C at day time nor to be below 4 °C at night. Starting 1 March, the glasshouse conditions were adjusted to keep the temperatures around 25/18 °C (day/night) and the relative humidity at ~70% using an automated ventilation system to avoid terminal heat stress conditions. Day length conditions were not adjusted and were dependent on natural day length conditions outside the greenhouse. Seeds of the 11 barley genotypes were grown in pots (40 cm tall and 30 cm in diameter). The pots were filled with a mixture of soil and peat-moss (2:1). Initially and after seed sowing, which took place in 1 November 2012, the soil moisture content in the pots was brought to field capacity by irrigation with tap water. After seedling emergence, plants were subjected to three water regimes: "non-stressed" (NS) and two pre-anthesis stressed treatments imposed at either tillering stage (SS) (Zadoks scale: Z22) or stem elongation stage (SE) (Z34; fourth node was detectable). Soil moisture content for the NS plants was maintained between 80% and 100% of the maximum retention capacity (FC) throughout the entire experiment duration. For the stressed plants, soil moisture content was maintained between 20% and 50% of FC throughout the entire experiment duration after stress. Heading dates were determined visually when 1/2 of inflorescence emerged from the main stem (Z55).

At the end of the experiment, the above-ground biological material in each pot were harvested and several agronomical traits were recorded per plant including: total weight (TW in g), as the total weight of straw and grain weight harvested from each pot; grain weight (GW in g), as the weight of harvested grains from each pot; straw weight (SW in g) was obtained by subtracting GW from TW; grain number (GN), as the total number of grains counted from all harvested spikes in the pot; thousand kernel weight (TKW in g), as GW multiplied by 1000 divided on GW; spike number (SN), as the final number of spikes counted from each pot; tiller number (TN) as the final number of tillers counted from each pot; infertile tiller number (ITN) was obtained by subtracting SN from TN; harvest index (HI as %) calculated by dividing GW by TW multiplied by 100; and Grains/spike, as the number of fertile spikelets/spike counted on 6 selected spikes (except for Steptoe under stress conditions) from each plant.

2.4. Statistical Analysis

Treatment combinations were set as a factorial arrangement (Plant genotype X 3 water regimes) in a Randomized Complete Block Design (RCBD) with six replications. Each replication was composed from 1 pot with one biological sample per pot. Analysis of variance (ANOVA) for yield and yield-components traits was statistically analyzed using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) to determine the significant differences between treatments. Mean separation was analyzed using least standard error of the differences between means (LSD) test at 0.05 level of probability.

3. Results and Discussion

Allelic variation of five major FTi-genes (*HvVrn1*, *HvVrn2*, *HvVrn3*, *PPDH1* and *PPDH2*) was determined in 11 different barley cultivars using specific functional markers. The genotyping of the *PPDH1* gene was successful in separating the tested lines into two groups: long day photoperiod sensitive and long day photoperiod insensitive (Table 2). The genotyping of the *HvVrn1*, *HvVrn2* and *HvVrn3* genes was successful in discriminating the tested lines into two groups: spring (no vernalization requirements) and winter (vernalization is required). According to [21], Morex and GP carried *HvVrn1-1*, which is known to be found in spring genotypes and shows accelerated flowering behavior in barley. On the other hand, Rum, Acsad, Keel and Mutah possessed the allelic variant *HvVrn1*-7, which is associated with accelerated flowering behavior. Yarmouk and Steptoe carried *HvVrn1-4* allelic variant known to carry the largest deletion in the *HvVrn1* gene and associated with an early flowering behavior. Finally, the *HvVrn1-6* allelic variant was detected in Arta and Tadmor, which is associated with late flowering phenotype in barley. Based on the genotyping results, the expected response to photoperiod and vernalization for each tested line was predicated (Table 2). In temperate cereals, flowering relies primarily on environmental cues such as photoperiod and vernalization to

flower at the proper time of the year to maximize their reproductive success [21]. Barley is divided into spring and winter types where the exposure to cold conditions for a period of time in order to flower, a process known as vernalization [22]. Furthermore, barley can be classified according to their responses to daylight into photoperiod sensitive and insensitive. The broad adaptability of barley to different environments is in part attributed to their genetic makeup of a set of FTi genes [23].

Table 2. Allelic variation of five major *FTi* genes in 11 different barley genotypes as assayed by using diagnostic markers (based on Drosse *et al*. (2014) [20]).

Genotype	*PPDH1*	*PPDH2*	*HvVrn1*	*HvVrn2*	*HvVrn3*	Expected Response to Photoperiod and Vernalization
Rum	Responsive	Responsive	HvVrn1-7	Winter	Winter	Long and short day with no vernalization
Acsad	Responsive	Responsive	HvVrn1-7	Winter	Winter	Long and short day with no vernalization
Morex	Non-Responsive	Responsive	HvVrn1-1	Spring	Spring	Short day with no vernalization
Steptoe	Responsive	Non-responsive	HvVrn1-4	Spring	Winter	Long day with no vernalization
Mutah	Responsive	Responsive	HvVrn1-7	Winter	Winter	Long and short day with no vernalization
Yarmouk	Responsive	Responsive	HvVrn1-4	Winter	Winter	Long and short day with no vernalization
Keel	Responsive	Responsive	HvVrn1-7	Spring	Winter	Long and short day with no vernalization
Arta	Responsive	Responsive	HvVrn1-6	Winter	Spring	Long and short day with vernalization
GP	Non-Responsive	Responsive	HvVrn1-1	Spring	Winter	Short day with no vernalization
Tadmor	Responsive	Responsive	HvVrn1-6	Winter	Winter	Long and short day with vernalization
Er/Amp	Responsive	Responsive	Spring	Winter	Winter	Long and short day with short vernalization

In this study, the tested genotypes were selected to carry different alleles of major FTi governing photoperiod and vernalization requirements. In the current study, Steptoe failed to flower early when compared with other spring genotypes when grown under cold and short-day conditions (Figure 1). This might highlight the importance of the *PPDH2* in promoting flowering under mild winter conditions. This result is supported by the frequency of a functional *PPDH2* allele in winter barley germplasm from the Mediterranean basin [24]. It was proposed that *PPDH2* promotes flowering under short-day conditions irrespective to photoperiod conditions and vernalization [24,25]. Furthermore, PPDH2 was reported to have a pronounced effect on the heading date in a Morex X Steptoe population grown under short-day conditions [26]. The delayed flowering behavior of Steptoe resulted in severe reduction in GY and GN under drought conditions as a negative correlation was found between heading date with GY and GN. On the other hand, the effect of *PPD1* on heading date was not as pronounced in the current study when the two tested genotypes (Morex and GP) carrying a loss of function allele were compared. Nevertheless, Morex was among the fastest genotypes to flower under different treatment indicating a putative additive role of the spring allele of *HvVrn3* in accelerating flowering under SD conditions or may be due to other genetic factors [27]. Finally, the impact of drought stress at SS stage on the flowering behavior of facultative genotypes (Arta and Tadmor) and Mutah (includes Arta in its pedigree) was clear when compared with early flowering genotypes. This might indicate the existence of genetic factors determine the flowering behavior under initial drought conditions.

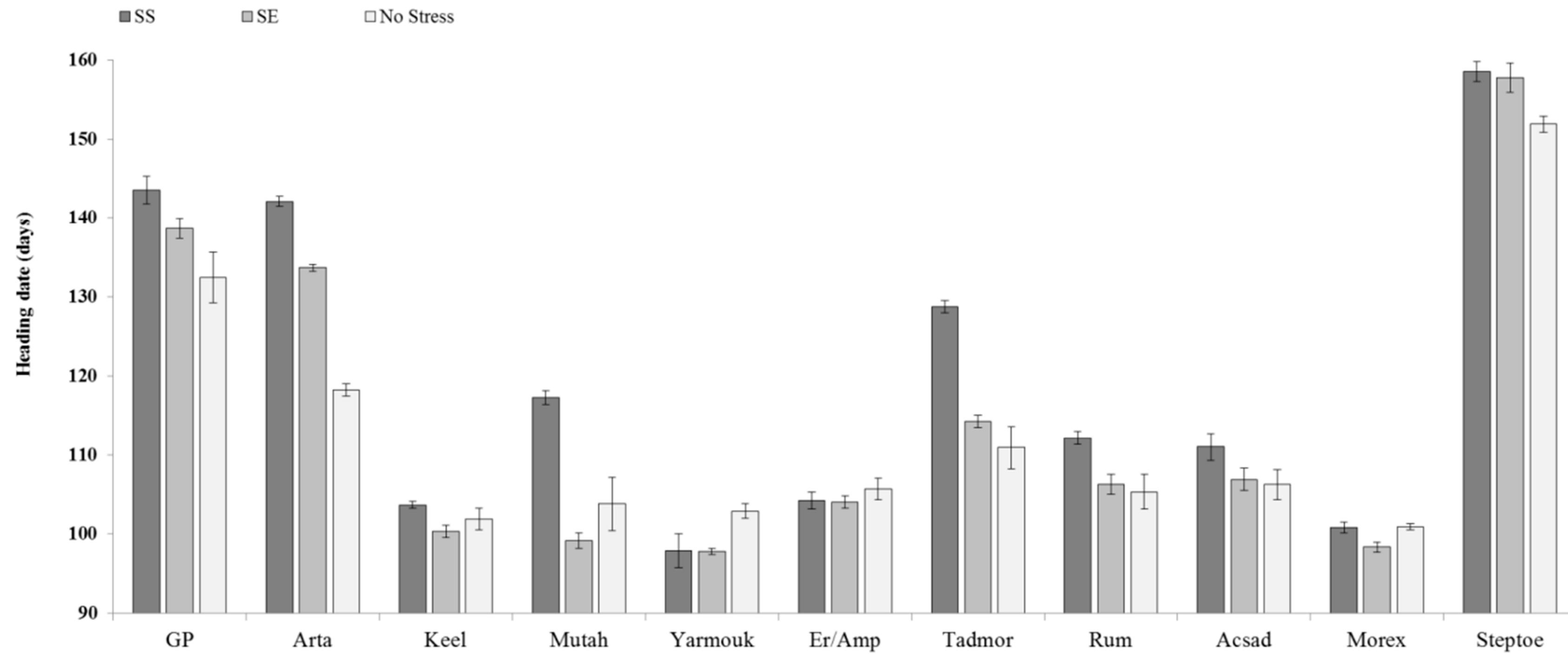

Figure 1. Heading dates of 11 barley genotypes grown under glasshouse conditions and subjected either to well-watered conditions (No stress) or to drought stress at two different growth stages: seedling stage (SS) and stem elongation stage (SE). Bars represent standard errors of means of six replicates.

Combined analysis of variance (Table 3) showed significant genotypic differences ($p < 0.05$) for all measured traits. Significant differences were also observed between drought treatments applied on the 11 genotypes for all measured traits. The interaction between stress treatments and tested genotypes was also significant for all measured traits (Table 3). The phenological characteristics to be used in genotypic selection for yield optimization under water deficit stress conditions confirmed intraspecific variation [11]. Indeed, the current study confirmed this by the presence of variation amongst the studied 11 barley genotypes. The effects of water stress on the yield reduction of barley have been well documented [28,29].The present study showed that yield and yield components were significantly reduced in all tested genotypes due to water deficit during tillering and stem elongation stages (e.g., yield reduced by 66% and 54% at SS and SE, respectively; Table 4). These results indicated that drought stress during the pre-anthesis period reduced grain yield by decreasing the number of fertile spikes and grains per plant (Table 4). Our results were supported by findings of [29] that examined the response of spring barley to pre-anthesis drought and reported a yield reduction due to pre-anthesis water stress on number of grains per plant. This reduction might be linked with reduction in plant growth that resulted in reduction in the capacity of source (photosynthetic rate) and sink size (grain and stem soluble carbohydrates) in drought stressed plants compared to well-watered plants. Decrease in photosynthetic rate due to pre anthesis drought has been shown to be associated with reduction in grain number [29–31]. Even though the number of fertile florets was not verified in the current study, the reduction in grain number in SS and SE plants revealed that significant decline in the final grain number was credited to floret abortion occurred in the pre anthesis phase [29]. Under fluctuated Mediterranean conditions, yield and stability were highly related to the effect of genotype (G), environmental condition (E) and their interaction (G X E) [28]. Main G X E interaction effect can be anticipated when there is a large difference between genotypes in certain physiological traits showing stress resistant and/or large variation between environments for occurrence of the same stress [28]. Present study showed that yield variation was largely explained by variation in the treatment (E; environment, *i.e.*, drought; Tables 3 and 4). Furthermore, the drought treatment mean squares were nearly two times more than variation in both genotype and genotype X environment interaction (G + G X E) (Table 3). Many reports demonstrated that that grain yield is more limited by grain number rather than grain weight in wheat and barley [28,32]. Grain number is determined during the pre-anthesis growth, grain set is affected by the vital task controlled by the timing of flowering in defining the wide adaptation of certain genotype and hence for grain yield determination. Breeders tend to breed for crop adaptation to cope with unfavorable conditions during the most critical stages, and the period before the beginning of grain filling is essential for the determination of grain number [28]. In wheat, this period is from near the end of stem elongation (GS 39) to the beginning of post flowering (GS 70) [33] while in barley it is from near the beginning of stem elongation (GS 31) to anthesis (GS 69) [31,34]. On the other hand, grain weight is determined in the period between anthesis and physiological maturity [28]. The current study found a significant reduction in final grain weight due to pre-anthesis water stress. This was consistent with findings of [12,35] where they indicated that unfavorable growing conditions such as limited water availability or heat stress that occurred during the pre-anthesis growth stage reduced grain weight potential in barley. Voltas *et al*. (1999) [35] found that final grain weight was reliant on carpel weight at anthesis. The expected reason for reduction in grain weight in SS and SE plants might be due to drought influences the emergent florets and lessens the weight of the carpel at pollination [12,36–38]. In the current study, carpel weight at pollination was not assessed, but lower weight carpels might develop in drought stressed plants. Ethylene, Abscisic acid, Cytokinins and their derivatives were either produced or activated by plants under water stress conditions to start self-protective reactions in plants to defend plant's crucial progression in stressed growing conditions [39,40]. In combination with adaptation, these hormones standardize yield potential via controlling floret survival and capacity of grain filling in cereals [41]. Therefore, portion of the drought-caused decline in fertile floret, grain number and final grain weight could be linked to hormonal influence.

Table 3. Mean square from the combined analysis of variance of 11 barely genotypes grown under different stress conditions.

Source of Variation	*DF*	Total Weight	Grain Weight	Grain Number	Thousand Kernel Weight	Spikes Number	Tiller Number	Grain/Spike	Harvest Index
Replication	5	16.4 ^{ns}	9.2 ^{ns}	998 ^{ns}	340.9 ^{ns}	37.83 *	8.9 ^{ns}	40.4 ^{ns}	0.0035 ^{ns}
Genotype	10	214 **	144.4 **	483004 **	1050 **	801.7 **	810.6 **	92.3 **	0.0668 **
Stress Treatment	2	6462 **	2398.6 **	583325 **	2806 **	108.6 **	185.9 **	454.1 **	0.371 **
Stress Treatment X Genotype	20	34.7 *	15.1 **	106064 **	183.5 *	43.12 **	8.9*	34.4 **	0.0045 **
Error	160	19.8	4.6	997	107.3	16.35	25.6	10.8	0.0019
Total	197								

* is significant at $p < 0.05$; ** is significant at $p < 0.01$; ^{ns} = non-significant.

Table 4. Total weight, Grain yield, grains number, and 1000 grain weight of 11 barely genotypes grown under well-watered conditions (NS) and subjected to drought stress conditions at different stages (stem elongation (SE) and seedling (SS)).

	Total Weight (g)				Grain Weight (g)				Grain Number				1000 Grain Weight			
Genotype	**NS**	**SE**	**SS**	**Mean**	**NS**	**SE**	**SS**	**Mean**	**NS**	**SE**	**SS**	**Mean**	**NS**	**SE**	**SS**	**Mean**
Acsad	62.1 b–d	46.3 ab	45.9 b–d	51.4 b–d	22.8 a	8.4 ab	9.2 bc	13.45 a	441.7 a	191.2 a	199.0 ab	277.3 a	51.6 cd	44.4 b–e	46.2 d	47.4 cd
Arta	61.5 b–e	41.8 d	45.0 b–e	49.4 c–e	19.6 bc	4.4 c	9.1 bc	11.05 cd	298.0 c–e	91.33 d	163.7 cd	184.3 f	66.2 a	47.9 a–d	55.8 a	56.6 ab
ER/Amp	65.0 b	45.9 a–c	46.4 a–c	52.4 b	18.2 b–d	8.6 ab	10.2 ab	12.35 a–c	343.8 bc	181.7 a	203.5 ab	240.8 b	53.0 cd	47.7 a–d	50.3 a–d	50.4 b–d
GP	63.3 bc	42.3 b–d	43.3 c–e	49.6 b–e	16.8 de	5.0 c	5.0 e	9.43 e	357.5 b	144.7 bc	148.8 de	226.1 b–d	47.3 d	37.9 c–f	33.6 e	39.6 ef
Keel	56.1 d–e	44.3 b–d	45.2 b–e	48.5 de	19.4 b–d	8.9 a	11.5 a	13.28 ab	338.5 b–d	147.8 bc	214.3 a	233.6 bc	57.4 bc	60.7 ab	53.8 ab	57.3 a
Morex	54.7 e	37.1 e	40.9 de	44.2 f	14.6 e	5.7 bc	9.5 bc	9.91 de	267.5 e	137.7 c	204.3 ab	203.2 ef	54.9 bc	34.6 d–f	46.3 cd	45.3 de
Mutah	58.3 b–e	41.9 cd	41.9 de	47.3 e	20.0 b	8.8 a	8.4 cd	12.41 a–c	313.7 b–e	142.3 bc	177.3 bc	211.1 de	64.3 a	62.2 a	48.1 b–d	58.2 a
Rum	63.5 bc	45.1 b–d	46.5 a–c	51.6 bc	20.5 ab	6.5 a–c	7.3 bd	12.01 bc	347.5 bc	137.2 c	128.2 e	213.3 c–e	59.9 ab	46.5 a–e	48.1 b–d	51.6 a–d
Steptoe	74.6 a	49.2 a	50.2 a	58.0 a	8.8 f	0.3 d	0.3 f	3.29 f	168.5 f	6.33 e	15.6 f	66.3 g	52.3 cd	36.2 d–f	22.4 f	34.8 f
Tadmor	63.7 bc	44.3 b–d	47.9 ab	51.9 bc	18.8 b–d	8.2 ab	9.8 bc	11.85 c	292.6 c–e	155.6 bc	191.5 ab	208.5 de	64.2 a	52.5 a–c	50.8 a–d	55.8 ab
Yarmouk	57.8 ce	45.1 b–d	49.0 ab	50.6 b–d	17.2 cd	7.2 a–c	10.3 ab	11.53 cd	287.0 fe	159.5 b	192.5 ab	213.0 c–e	59.9 ab	44.4 b–e	53.2 a–c	52.5 a–c
Mean	61.9 a	43.9 c	45.7 b		16.9 a	5.8 c	7.7 b		314.5 a	136.4 c	170 b		54.0 a	43.0 b	44.6 b	
	Spike Number				**Tiller Number**				**Grains/Spike**				**Harvest Index**			
Genotype	**NS**	**SE**	**SS**	**Mean**	**NS**	**SE**	**SS**	**Mean**	**NS**	**SE**	**SS**	**Mean**	**NS**	**SE**	**SS**	**Mean**
Acsad	18.0 cd	15.0 c–d	17.7 de	16.6 cd	18.0 cd	20.2 c–e	20.8 de	19.7 cd	26.9 b	11.4 b–d	12.9 b	17.1 a	0.37 a	0.18 ab	0.22 b	0.25 ab
Arta	23.7 ab	22.3 b	25.3 ab	23.8 ab	23.7 ab	34.5 a	30.7 ab	29.6 a	12.7 b	6.7 e	5.0 de	8.1 gf	0.32 b–d	0.11 c	0.20 b	0.22 c
ER/Amp	23.6 ab	28.5 a	25.3 ab	25.8 a	24.3 ab	33.3 ab	27.2 b–d	28.3 ab	15.4 c	7.5 de	6.5 c–e	9.5 ef	0.28 de	0.19 a	0.22 ab	0.23 bc
GP	26.0 a	16.5 cd	22.7 bc	22.4 b	26.8 a	22.0 c–e	27.5 a–d	25.9 b	13.9 c	6.8 e	10.4 bc	10.4 ef	0.26 e	0.12 bc	0.12 d	0.17 d
Keel	20.7 bc	15.3 cd	16.3 de	17.4 c	21.3 bc	20.2 c–e	19.8 de	20.4 dc	16.1 c	13.5 b	9.9 bc	13.4 cd	0.35 ab	0.20 a	0.25 a	0.27 a
Morex	7.7 e	5.67 e	6.8 f	6.7 e	7.7 f	7.3 f	6.8 f	7.3 f	36.3 a	32.3 a	27.4 a	32.0 a	0.27 e	0.15 a–c	0.23 ab	0.22 c
Mutah	17.3 bc	17.5 b–d	19.7 cd	19.2 c	21.0 bc	18.8 c–e	23.2 c–e	21.0 c	15.3 c	9.5 c–e	8.2 b–d	11.0 de	0.34 ab	0.21 a	0.20 b	0.25 ab
Rum	13.5 d	14.8 d	14.2 e	14.1 d	15.2 de	18.6 de	17.2 e	16.9 de	23.3 b	11.8 bc	9.2 b–d	14.8 bc	0.32 bc	0.14 a–c	0.16 c	0.22 c
Steptoe	13.0 d	3.0 e	2.0 g	6.2 e	12.3 e	15.2 e	17.6 e	14.9 e	13.1 c	1.5 f	2.8 e	5.8 g	0.12 f	0.01 d	0.01 e	0.04 e
Tadmor	20.6 bc	20.5 bc	27.7 a	23.1 b	21.2 bc	26.5 bc	34.5 a	27.8 ab	14.4 c	7.0 e	7.8 cd	9.7 ef	0.29 c–e	0.18 ab	0.20 b	0.23 bc
Yarmouk	24.5 a	23.2 ab	25.3 ab	24.3 ab	26.2 a	25.8 b–d	28.8 a–c	26.9ab	11.9 c	7.7 de	6.9 c–e	8.8 ef	0.30 c–e	0.16 a–c	0.21 b	0.22 c
Mean	18.9 a	16.6 b	18.78 a		19.8 b	22.1 a	23.1 a		18.2 a	10.5 b	9.7 b		0.29 a	0.151 c	0.18 b	

Means followed by the same letter within the column are not significantly different according to LSD test at $p \leqslant 0.05$.

The effects of drought stress on the flowering time irrespective to genotype showed that stress at SS caused a delay in heading time when compared with well-watered conditions. Irrespective of water treatments imposed, Steptoe and GP were among the latest flowering genotype, while Yarmouk, Keel and Morex showed early flowering behavior. The interaction between stress treatments and genotypes on flowering time was highly significant (Table 3). Under NS conditions, no significant differences in flowering time were observed between the spring genotypes except for Steptoe and GP compared with all tested genotypes, which were considerably later than the facultative genotypes (Arta and Tadmor), and between Morex with Acsad, Rum and Er/Amp (Figure 1). With the exception for Steptoe and GP, the facultative genotypes, Arta and Tadmor, flowered later than spring types irrespective of stress treatment highlighting the importance of vernalization to induce flowering. Under SS conditions, Yarmouk followed by Keel were the fastest flowering genotypes, while Steptoe was the latest flowering genotype. The impact of SS treatment on Mutah genotype was severe and included a significant delay in heading time when compared with NS and SE conditions (Figure 1). Similarly, the facultative genotypes, Arta and Tamdor were severely affected by the SS stress conditions when compared with NS and SE, respectively. Under SE stress conditions, Yarmouk, Mutah and Morex were the fastest genotypes to flower, while Steptoe was the slowest (Figure 1). The impact of SE on Arta genotype was severe where a significant delay in flowering time was observed when compared with NS conditions.

The flowering behavior of Yarmouk had significantly faster heading time responses under SE and SS when compared with NS conditions. No significant differences were observed in flowering times of Keel plants subjected to NS or SE and between plants subjected to NS and SS, while a significant delay was observed under SS conditions when compared with SE conditions. No significant effects of stress treatments on the flowering behavior of ER/Amp plants were observed when compared with well-watered plants. The GP, Arta, Tadmor, Rum, Acsad, Mutah and Steptoe genotypes were found to flower faster under NS conditions, while a significant delay in their flowering occurred under SS conditions was observed (Figure 1). At the same time, Keel and Morex genotypes had the same response to water treatments by which they accelerated flowering under SE conditions compared with SS conditions (Figure 1).

Genotypic differences (significant at $p < 0.05$) were found in GW, and GN where Steptoe produced the lowest values, while Acsad produced the highest GN compared to all tested genotypes and the highest GW although it was not significant when compared with Keel (Table 4). On the contrary, Steptoe produced the highest total weight when compared to all other tested genotypes. As expected, plant grown under well-watered conditions produced significantly higher GW and GN when compared with stress conditions grown plants (Table 4). Stress at SE stage produced significantly lower GN and GW when compared with SS stage conditions.

Cross-treatment averages showed significant differences ($p < 0.05$) among genotypes in GW, GN and the TKW (Table 4). Acsad and Rum produced the highest grains weight (22.8 and 20.5 g, respectively), while Steptoe produced the lowest grain weight (8.8 g) (Table 4). Similar trends were observed for grain number, where Acsad produced 441.7 grains and was ranked the first, whereas Steptoe produced the lowest number of grains among the tested genotypes (168.5 grains). With regard to TKW, Arta, Mutah and Tadmor were found to have the heaviest grains, while GP had the lightest grains amongst genotypes (Table 4). The TKW was decreased ($p < 0.05$) by 9.4 g and 11 g due to the effect of the water deficit stress in SE and SS, respectively. Drought stress conditions at the seedling stage (SS) and stem elongation stage (SE) reduced the grains weight when compared with NS conditions (Table 4). Stress at the SS stage had a greater yield reduction when compared with SE and NS. There was also a significant water stress X genotype interaction ($p < 0.05$), as shown in Table 3. The above-mentioned results were consistent with previous findings on barley [28], which reported genotypic differences coupled with negative effect on all tested traits in response to drought stress. Acsad had the highest yield (13.4 g) with the highest grain number mean value (277.28) among all tested genotypes (Table 4). On the other hand, Steptoe produced the lowest yield (3.3 g) and number

of grains (66.29). Arta produced the heaviest grains, while Steptoe had the lightest grains although it was not significantly different from GP (Table 4). Significant genotypic differences ($p < 0.05$) were detected in total weight means values (Table 4). Water deficit at SE and SS significantly ($p < 0.05$) reduced total weight when compared with non-stressed conditions. Steptoe produced the highest total weight when compared to the rest of genotypes under all treatments. Treatments means comparison showed that Steptoe was top ranked among the tested genotypes and accumulated 58 g, while Morex was the lowest among tested genotypes and accumulated 44 g of total weight (Table 4).

Arta produced 29.6 tillers per plant and ranked highest among the genotypes (Table 4), while Morex produced the lowest number of 7 tillers (Table 4). Genotypic differences in grain number were accounted for by variation in spikes number and grains/spike (Table 4). Spikes number was significantly affected ($p < 0.05$) by water deficit at stem elongation stage and it seems that drought imposed at this stages affected other yield components traits (Table 4). Under unstressed condition, the overall mean of harvest index (HI) was 28%. The top yielding Acsad genotype transferred 37% of its total dry matter into grains compared to 12% for the lower yielding genotype Steptoe. The same trend was observed under stress treatments, where Acsad was among the top ranking genotypes, whereas Steptoe had the lowest HI among all genotypes (Table 4). Many reports indicated that harvest index is a reasonably steady trait for a given genotype in the lack of harsh stress however HI changes are frequently reported with drought stress [29,42]. In the current study, the average decrease from 0.29 in NS plants to 0.15 and 0.18 in SE and SS plants, respectively, under drought was reliably consistent with previous reports. Differences between genotypes in HI were related to the differences in grain yield responses to drought [29]. The fact that Acsad and Keel had the highest HI under the three treatments (NS, SE and SS) could be explained by their capability in partitioning more dry matter into ears than the lowest HI genotype (Steptoe). Reduction in HI under drought has been reported in barley [29,42]. Great declines in HI are frequent under drought conditions indicated that HI may be stable under the more favorable growing conditions but can decline if a crop is grown under resource-deficient conditions. Larger reduction in yield and yield components under water deficit conditions in the current study might be due to the effect of lower accumulation and/or translocation of water soluble stem carbohydrate remobilization during the pre-anthesis water stress.

Under NS conditions, heading date was negatively correlated ($p < 0.05$) with grain weight (grain yield per plant) and harvest index, and positively correlated with total weight (Table 5). Moreover, grain weight was positively correlated with grain number and harvest index. Also, there was a negative correlation between spike number and grains per spike. Under SE, and SS conditions, heading date was negatively correlated with grain weight, grain number and harvest index, while there was no correlation between heading date and total weight (Table 5). Furthermore, grain weight was positively correlated ($p < 0.05$) with grain number, TKW and harvest index, while there is no correlation between spike number and grains per spike (Table 5). These findings were consistent with [28] who studied the eco-physiological performance of 118 doubled haploid population of barley in a multi-environment trial of 18 site–year combinations in six Mediterranean countries over two years and confirmed a strong positive correlation between water regime and yield and yield components at the level of environmental means. Furthermore, pleiotropic effects of developmental genes on physiological parameters have been described previously. For instance, Mickelson *et al.* (2003) [43] observed negative correlation between heading date and both grain and protein yield. It appears that delayed heading coincided with higher prevailing temperatures, which might have resulted in reduced carbohydrate reallocation in plants leading to fewer or smaller grains.

Table 5. Spearman's coefficients of agronomic traits for the 11 barley genotypes evaluated under in three testing environments (NS, SE, and SS).

NS	TW	GW	GN	TKW	SN	TN	GS	HI
GW	−0.50 ^{ns}							
GN	−0.37 ^{ns}	0.87 **						
TKW	−0.30 ^{ns}	0.34 ^{ns}	−0.18 ^{ns}					
SN	−0.02 ^{ns}	0.34 ^{ns}	0.34 ^{ns}	0.04 ^{ns}				
TN	−0.02 ^{ns}	0.40 ^{ns}	0.37 ^{ns}	0.12 ^{ns}	0.98 **			
GS	−0.40	0.13 ^{ns}	0.22 ^{ns}	−0.15 ^{ns}	−0.78 **	−0.75 **		
HI	−0.72 *	0.95 **	0.80 **	0.37 ^{ns}	0.25 ^{ns}	0.33 ^{ns}	0.23 ^{ns}	
HD	0.82 **	−0.70 *	−0.52 ^{ns}	−0.38 ^{ns}	0.02 ^{ns}	−0.07 ^{ns}	−0.43 ^{ns}	−0.82 **
SE	**TW**	**GW**	**GN**	**TKW**	**SN**	**TN**	**GS**	**HI**
GW	−0.17 ^{ns}							
GN	−0.23 ^{ns}	0.90 **						
TKW	0.03 ^{ns}	0.68 *	0.32 ^{ns}					
SN	0.07 ^{ns}	0.59 *	0.58 *	0.45 ^{ns}				
TN	0.26 ^{ns}	0.23 ^{ns}	0.22 ^{ns}	0.32 ^{ns}	0.88 **			
GS	−0.70 *	0.25 ^{ns}	0.40 ^{ns}	−0.26 ^{ns}	−0.37 ^{ns}	−0.64 *		
HI	−0.31 ^{ns}	0.99 **	0.88 **	0.67 *	0.55 ^{ns}	0.18 ^{ns}	0.33 ^{ns}	
HD	0.34 ^{ns}	−0.86 **	−0.78 **	−0.46 ^{ns}	−0.34 ^{ns}	0.07 ^{ns}	−0.52 ^{ns}	−0.88 **
SS	**TW**	**GW**	**GN**	**TKW**	**SN**	**TN**	**GS**	**HI**
GW	−0.30 ^{ns}							
GN	−0.46 ^{ns}	0.95 **						
TKW	−0.20 ^{ns}	0.94 **	0.81 **					
SN	0.03 ^{ns}	0.57 ^{ns}	0.56 ^{ns}	0.62 *				
TN	0.36 ^{ns}	0.19 ^{ns}	0.16 ^{ns}	0.29 ^{ns}	0.87 **			
GS	−0.57 ^{ns}	0.23 ^{ns}	0.30 ^{ns}	0.07 ^{ns}	−0.54	−0.80 **		
HI	−0.43 ^{ns}	0.98 **	0.97 **	0.90 **	0.48	0.07 ^{ns}	0.34 ^{ns}	
HD	0.24 ^{ns}	−0.79 **	−0.76 **	−0.66 *	−0.16 ^{ns}	0.26 ^{ns}	−0.45 ^{ns}	−0.80 **

^{ns,} * and **: not significant and significantly rank corrected at the 0.05 and 0.01 probability level, respectively; TW, total weight; GW, grain weight; GN, grain number; TKW, thousand kernel weight; SN, spikes number; TN, tiller number; GS, grain/spike; HI, harvest index.

4. Conclusions

According to the prediction of scientists, the world climate will change. Such change will expose barley crops to frequent pre-anthesis water stress. Indeed, the current study showed a huge impact of pre-anthesis water deficit on barley productivity. In general, most of the genotypes were affected by stressed regimes. In addition, the early flowering and maturing genotypes had better enactment, as reflected in higher yield and its components when compared with late flowering ones. These results proved the essential importance of the early flowering behavior in barley to improve productivity in response to pre-anthesis water deficit conditions. The obtained results can be used as a guide for the selection of appropriate lines for future breeding purposes of barley varieties with improved elasticity and resilience to drought conditions under dry environments. Correlation analysis of yield and yield components indicated negative association with heading dates. The presented results might highlight the importance of heading date in assisting breeding of new barley lines with improved adaptation to dry environments.

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P. Stephen Baenziger significantly contributed to the realization of the study, data availability and the interpretation of the results. All authors read and approved the final manuscript.

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