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The Use of Imaging as Non-destructive Tool for Water Stress Tolerance in Spring Barley (*Hordeum vulgare* L.)

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ABSTRACT

Recent trends show a reduction in crop productivity worldwide due to severe climatic change. Water stress is a serious problem for barley production, because it affects simultaneously many morphological, physiological and biochemical traits. The present work attempts to provide comprehensive information related to barley plant response and adaptation to drought stress by using a high throughput phenotyping approach. Morpho-physiological parameters of barley plants were collected using the Scanalyzer 3D high-throughput phenotyping platform (LemnaTec, GmbH), associated to more conventional phenotyping methods. With this approach, we could study the effects of water deficit on barley plant development in terms of early detection of plant physiological stress responses. Our results showed that some barley genotypes cultivated under water stress were comparable to the control in terms of productivity, as well as in digital indices. The doubled haploid line 'DH2' and the genotype 'Safra' were the most tolerant to water stress.

Key words: *Hordeum vulgare; Biovolume; Doubled haploid; Genotype; Phenotyping; Water stress; Yield*

Novelty Statements

- A set of nine barley genotypes and three doubled haploid lines were used.
- Use of plant morphological development through the acquisition and processing of digital images in the visible (RGB) and infrared (NIR) spectra, in a three-dimensional manner.
- The RGB images were used to evaluate High Throughput Phenotyping (HTP) data which are: digital biovolume, Green index and Health index. This method permits an early detection of physiological plant stress response.
- Some genotypes have shown a good tolerance to water stress.

Introduction

Different approaches can be deployed to deal with climate variability and change, water is an essential input for agricultural production; the latter is the largest user of water in the world, accounting for 70% of the total freshwater supply on the planet. Thus, the observed decrease in rainfall can seriously hamper food security [1].

The Mediterranean region has been referenced as one of the most important hot and vulnerable regions a "hot spot" with regard to the impacts of global warming, such as on agricultural production [2]. For more than 30 years, the IPCC (Intergovernmental Panel on Climate Change) has assessed the state of knowledge on climate change, producing five assessment reports whose growing evidence indicates that crop yields will decline.

Thus, a real challenge, for global agriculture has been faced to ensure food security, such as meeting the needs of a growing world population, in a sustainable manner and adapted to the future climate and the urgency of more

sustainable agricultural systems based on reduced inputs including water usage [3]. The complexity of drought adaptive traits explains the progress in improving yields in drought-affected environments.

In the last decade, crop physiology and genomics have provided new insights in drought tolerance and provide breeders with new tools and knowledge for plants improvement [4].

A wide range of plant responses to drought stress could be generalized due to the dynamic nature of its development with different intensities, and it adaptive strategies which may differ according to species, genotypes, type of drought and combination with other stresses.

Breeding programs leading to the adaptation of cropping systems to climate change offer continuous optimization of quantitatively inherited trait complexes is provided, with constant and rapid change in gene frequencies in elite populations [5].

In terms of adaptation strategies of plants to the challenges linked to drought stress, different approaches have been studied such as physiological processes investigations and phenotyping of plants in greenhouses with controlled irrigation systems [6-10].

Currently Phenotyping constitutes a major bottleneck in basic research limiting the power of genetic analysis [11,12]. Plant phenotyping consists of identifying the interaction of genotypes with their environment, which is characterized in several morphological parameters of plants, in their biomass and their accumulated yield [13]. In recent years we observe the lack of quantitative High Throughput (HT) plant phenotyping methods due to the increasing demand for the development of a more precise selection of stressful crops resistant plants, for a productive and sustainable agriculture [14-18]. Nowadays, it has been argued that imaging and software solutions have paved the way for many high throughput phenotyping studies [10,19-23].

Barley is one of the earliest cultivated grain crops in a wide range of climatic areas across many geographical regions of the Mediterranean [24]. It had an enormous importance for the Egyptians, the Greeks and the Romans. Nowadays, barley is generally considered a crop apt to dry climate agriculture, and has a regional importance in North Africa, West Asia, and Latin America [25]. Unfortunately, net barley production is projected to fall due to temperature and water stresses associated to climatic change.

Different studies show that barley is characterized by an enormous drought tolerance potential [26,27]. Currently, new insights have been identified to study water stress tolerant traits such as plant genomics, morphology, physiology and biochemistry [28]. Plant phenomics presents a series of new technologies that lead to facilitate, target and accelerate progress in the understanding of gene function and environmental responses.

Plant phenotypes are, in fact, the result of the interaction between the genotype and the environment Therefore, studying the genotype helps bridging our genetic knowledge to crop field performance. Moreover, phenomics offers the possibility to study plants in a non-destructive way, which implies the possibility to study one and the same individual over its entire life span [1].

In this contribution, we report on the use of a Scanalyzer 3D (LemnaTec GmbH, Aachen, Germany) high throughput phenotyping platform to analyze different barley genotypes under controlled and drought conditions aiming to the early detection of physiological plant stress response. This technology opens new perspectives, for the quantitative and non-destructive analysis of different crops or model plants. Each plant can be digitally imaged with the Scanalyzer 3D platform, which yields an unprecedented number of reproducible and meaningful data points on all aspects of plant development [29]. The aim of the present study is to identify changes in morphological traits measured by high throughput imaging in twelve barley genotypes under water deficit and well-watered conditions, which allows us to select to select the tolerant genotypes to water stress.

Materials and Methods

Experimental site description

The experiment was carried out at the Research Center Metapontun Agrobios of ALSIA (Bernalda, Italy, 40°23'31.7"N - 16°47'14.2"E, 16 m. a.s.l.) in a controlled environment structure hosting a High Throughput Phenotyping (HTP) platform LemnaTec Scanalyser 3D. This platform is the location of the Italian Plant Phenotyping Consortium "PhenItaly" and coordinated along with the Italian National Research Council (CNR). This platform analyzes plant morphological development through the acquisition and processing of digital images in the visible (RGB) and infrared (NIR) spectra, in a three-dimensional manner. In fact, each observation is the result of different images taken along the

three main spatial axes X, Y, and Z projections. The process is fully automated, following a standardized policy, and in the absence of operational interferences.

Plant material and growing conditions

A set of nine barley genotypes, (Rihane, Manel, Momtez, Roho, Tej, Kounouz, Lamsi, Ardhaoui, Safr) and three doubled haploid lines (DH1, DH2, DH3) produced from the crosses Roho/Ardhaoaui and Momtez /Roho. The barley material was provided by the barley breeding program of the National Institute of Agronomic Research of Tunisia (INRAT). It was grown in the greenhouse hosting the HTP platform under natural ambient light conditions. The greenhouse is equipped with a multipoint sensor that regulates ventilation to avoid the rise of local micro-climatic conditions. Origin and pedigree of the genotypes are shown in **Table 1**.

Seeds were germinated in Petri dishes on wet filter paper for 4 days at room temperature then transplanted into polystyrene cellular containers filled with peat (50%) and river sand (50%). For synchronizing seedlings growth the trays were stored for 2 weeks at 4ºC. Individual plants were then transferred in pots (6cm in diameter, 20 cm in height, 4 L volume) filled with 3.5 L of peat (50%) and river sand (50%), for a total weight of 1200 g per pot.

Three replicates per genotype, for both stressed and control plants, were randomly distributed in the greenhouse, waiting for being loaded in the automated conveyor for 3D scanning at appropriate timing. To allow the automatic individual plant identification in the platform, a barcode was applied at convenient position on the pots. All plants were manually kept fully irrigated up to the booting stage up to 45 Days After Sowing (DAS). The duration of experiment was from 45 DAS to 90 DAS. The control was kept fully irrigated (100% of field capacity), while treated plants were stressed by reducing irrigation to 50% of the field capacity (FC) through manual irrigation following pot weighting. After 90 DAS, irrigation was stopped for all plants until complete maturity.

High Throughput Phenotyping (HTP) and Traditional Phenotyping

Images in the visible spectrum were used for automated phenotyping. These images are composed of three layers each corresponding to the three principal colors Red, Green, and Blue (RGB). In the platform, three RGB images were taken, one from above the plant and two laterally at an orthogonal angle. Starting from 45DAS and up to 90 DAS, 3D RGB images, involving three mutually orthogonal vantage points, were captured at intervals of 60, 75, 80, 87, and 90 DAS according to Petrozza et al. [30]. The closer interval between 80 and 90 DAS was used to better monitor plant senescence. The RGB images were used to evaluate HTP data which are: digital biovolume, Green index and Health index. Image analysis was performed by using specific pipelines aimed at measuring specific digital characters [1,30]. A complete list of the traditional and digital studied characters is reported in **Table 2**.

The Digital Biovolume (DB) was calculated from the three orthogonal images of the same plant. Green index was calculated starting from the RGB images by applying the function (R – B)/(R + B) where R and B are the red and blue image components, respectively [31].

Health index was calculated by transforming images from the RGB color space into Hue, Saturation and Intensity (HSI) color space, and deriving from these data an index using an appropriate procedure described by Pydipati et al. [32].

Statistical Analysis

A two-way ANOVA test ($P<0.01$) was performed for the measured values of the parameters. Mean comparisons were carried out to estimate the differences between treatments and genotypes using the Tuckey test.

Results

Effect of water stress on yield and its components

Water stress caused the reduction of kernel yield and yield components (**Table 3**). Some yield components were more affected by drought than others. The Number of Spikelets Per Spike (NSPS), the number of kernels per spike (NKPS), the Kernel Yield Per Spike (KYPS) and the Total Kernel Yield (TKY) were more sensitive to water stress than the other yield components as they accused a reduction, compared to the control, of 31.94, 43.49, 47.32 and 38.16%, respectively. However, barley genotypes responded differently to water deficit. The genotypes 'Manel', 'Momtez', 'Roho', 'Tej' and 'Kounouz' were more affected by water stress since they had significant a yield reduction, compared to the control, ranging from 52.4 to 59.2% (**Table 4**). The genotypes 'Rihane', 'Ardhaoui' and 'Safra' appeared to be more tolerant to water stress and had a yield reduction, compared to the control, of 15.6, 19.03 and 22.9%, respectively. The

Table 1: Origin and pedigree of the twelve barley genotypes (G) used in the experiment.

Genotype	Origin	Pedigree
Rihane (G1)	INRAT (Tunisia)/ICARDA(Syria)	Atlas 46/Arrivat//Athenais
Manel (G2)	INRAT (Tunisia)/ICARDA(Syria)	L572/5/As54/Tra//2*Cer/Toll/3/Avt/Toll//Bz/4/Vt/Pro/Toll
Momtez (G3)	ICARDA/Alep(Syria)	M126/CM67/As/Pro/3/Arizona 5908/ths//Lignee 640
DH1 (G4)	INRAT (Tunisia)	Momtez/Roho
Roho (G5)	INRAT (Tunisia)/Laboratoire Riso (Denmark)	Roho 03573
Tej (G6)	INRAT (Tunisia)/ ICARDA (Syria)	Bonus/C13576 (W12198-Australia)
Kounouz (G7)	INRAT (Tunisia)/ICARDA (Syria)	Alanda/5/Aths/4/Pro/Toll//Cer*2/Toll/3/5106/6/24569
Lamsi (G8)	--	Rapidan, USA
Ardhaoui (G9)	--	Local landrace
Safra (G10)	--	Local landrace
DH2 (G11)	INRAT (Tunisia)	Roho/Ardhaoui
DH3 (G12)	INRAT (Tunisia)	Roho/Ardhaoui
DH: Doubled haploid.		

Table 2: Description of the traditional and digital characters.

Character code	Traditional (T) or Digital (D) Phenotyping	Period	Description
NSPP	T	Complete maturity	Number of Spikes per Plant
NSPS	T	Complete maturity	Number of Spikelets per Spike
NKPS	T	Complete maturity	Number of Kernels per Spike
KYPS	T	Complete maturity	Kernel Yield per Spike (in grams)
TKW	T	Complete maturity	One Thousand Kernel Weigth (in grams)
TKY	T	Complete maturity	Kernel Yield Total meaning Kernel Yield per Plant(in grams)
DB	D	45,60,75,80,87,90 DAS	Digital biovolume based on 3D imaging
Green index	D	45,60,75,80,87,90 DAS	Color index based on 3D imaging indicative of chlorophyll content
Health index	D	45,60,75,80,87,90 DAS	Color index based on 3D imaging indicative of health status of the plants and of senescence

genotypes 'Rihane' and 'Ardhaoui' come from the southern Tunisia characterized by a desert climate. Their tolerance to water stress would be acquired by their culture for several years in a desert climate. The doubled haploid lines ‘DH2’and ‘DH3’ seemed to be the most tolerant to water deficit with a yield reduction, compared to the control, of 20.32 and 8.10%, respectively. The line ‘DH3’ transgressed his parent ‘Ardhaoui’ tolerant to drought.

Effect of water stress on digital biovolume, green index and health index

Digital biovolume: The Digital Biovolume (DB) was calculated for individual plants at each single measurement point, and analyzed for each genotype by comparing plants cultivated under water stress (treated plants) to the control. Initially (45 days after sowing (DAS)), the DB increased gradually in all genotypes and treated plants were comparable to the control (Fig. 1). From 60 DAS and for the majority of genotypes (Rihane (G1), Manel (G2), Momtez (G3), DH1 (G4), Lamsi (G8), Ardhaoui (G9), Safra (G10)), treated plants were separated from the control which always remained superior to stressed plants until the end of the experiment (90 DAS). The DB of stressed plants represented 74% to 90% of the control for the genotypes Rihane (G1) and Safra (G10), respectively. For the other genotypes (Roho (G5), Tej (G6), Kounouz (G7), DH2 (G11), DH3 (G12)), stressed plants were not separated from the control at 60 DAS and differences between them were less important until the end of the experiment. Thus, this parameter represented for plants cultivated under drought conditions 86 to 98%of the control for the genotypesRoho (G5) and DH2 (G11), respectively. The genotype DH2 seemed less affected by water stress (**Figure 1**).

Green index: This index is used to provide an estimate of leaf chlorophyll content and it is obtained from RGB images by color image. This parameter was stable for the majority of genotypes (Rihane (G1), Manel (G2), Momtez (G3), DH1 (G4), Roho (G5), Tej (G6), Kounouz (G7), Ardhaoui (G9), DH3 (G12)) from 45 to 80 DAS (**Figure 2**). After this date, green index decreased more or less rapidly until the end of the experiment, depending on the genotype and the treatment. The most affected genotypes by water stress were Roho (G5) and Momtez (G3) which showed a decrease of 6.4 and 3.6% compared to the control, respectively.

For the other genotypes Lamsi (G8), Safra (G10) and DH2 (G11), the green index was remarkably stable throughout the experiment and little (Lamsi) or not affected (Safra, DH2) by water stress.

Table 3: Barley growth and kernel yield parameters under water stress. (NSPP) Number of spike per plant, (NSPS) Number of spikelets per spike, (NKPS) Number of kernels per spike, (KYPS) Kernel yield per spike, (TKW) Thousand kernels weight, (TKY) Total kernel yield.

Treatment	NSPP	NSPS	NKPS	KYPS (g)	TKW (g)	TKY (g)
Control	13.62^{a}	27.88^{a}	19.68^{a}	1.12^{a}	61.11^{a}	4.14^{a}
Water stress	10.80^{b}	19.0^{b}	11.12^{b}	0.59^{b}	52.33^{b}	2.65^{b}
Reduction (%)	20.70	31.94	43.49	47.32	14.36	38.16
Averages followed by the same letter are not significantly different						

Table 4: Effect of drought stress on barley growth of twelve barley genotypes. (NSPS) Number of spikelets per spike, (KYPS) Kernel yield per spike, (TKW) Thousand kernels weight, (TKY) Total kernel yield. Data represent the measure of four replicates.

Treatment	Genotype	NSPS	KYPS (g)	TKW (g)	TKY (g)
Control	Rihane	29.0^{bcd}	0.87^{def}	59.60^{cde}	1.66^{hi}
	Manel	31.40^{bc}	1.21^{bcd}	64.23^{bcd}	2.43^{fghi}
	Momtez	28.50^{bcde}	1.21^{bcd}	68.26^{a}	6.11^{bcd}
	DH1	25.66^{cdef}	0.51^{fgh}	51.60^{def}	4.03^{defg}
	Roho	25.50^{cdefg}	1.32^{bc}	50.81^{cde}	4.38^{cdef}
	Tej	25.40^{cdefg}	1.20^{bcde}	51.33^{bcd}	3.24^{efgh}
	Kounouz	23.33^{defg}	1.10^{bcde}	61.23^{bcd}	5.08^{cde}
	Lamsi	27.60^{cde}	1.04^{bcde}	50.25^{def}	2.10^{fghi}
	Ardhaoui	37.80^{a}	1.01^{cde}	56.95^{cde}	2.28^{fghi}
	Safra	22.20^{efgh}	0.79^{defg}	53.29^{cdef}	2.54^{efghi}
	DH2	35.0^{ab}	2.09^{a}	67.90^{ab}	8.497^{b}
	DH3	21.66^{efghi}	1.39^{bc}	68.02^{b}	13.32^{a}
Water stress	Rihane	13.30^{j}	0.58^{fgh}	50.90^{def}	1.41^{hi}
	Manel	21.0^{fgh}	0.41^{gh}	55.85^{cde}	0.99^{hi}
	Momtez	20.0^{ghi}	0.50^{fgh}	55.09^{cdef}	2.63^{fgh}
	DH1	14.0^{j}	0.18^{h}	45.01^{ef}	2.56^{fgh}
	Roho	17.0^{hij}	0.50^{fgh}	36.21^{fg}	1.92^{ghi}
	Tej	14.50^{ij}	0.31^{h}	40.83^{efg}	1.51^{hi}
	Kounouz	16.60^{hij}	0.36^{gh}	48.53^{defg}	2.42^{fgh}
	Lamsi	22.60^{efg}	0.37^{gh}	38.17^{f}	1.37^{hi}
	Ardhaoui	21.0^{fghi}	0.76^{efg}	49.14^{def}	1.84^{ghi}
	Safra	18.33^{ghij}	0.59^{fgh}	46.21^{cdef}	1.95^{fghi}
	DH2	29.80^{bc}	1.45^{b}	53.84^{cde}	6.78^{bc}
	DH3	21.0^{fghi}	1.23^{bcde}	60.61^{cd}	12.24^{a}
Reduction (%)	Rihane	54.13	33.30	14.59	15.06
	Manel	33.12	66.10	13.04	59.20
	Momtez	29.82	58.60	19.29	56.94
	DH1	45.44	63.10	12.77	36.50
	Roho	33.33	62.10	28.73	56.16
	Tej	42.90	74.30	20.45	53.48
	Kounouz	28.80	66.80	20.74	52.40
	Lamsi	18.11	64.40	24.03	34.70
	Ardhaoui	44.40	24.50	13.70	19.03
	Safra	17.34	22.40	13.28	22.90
	DH2	14.80	30.50	20.70	20.32
	DH3	3.04	11.29	10.80	8.10
Averages followed by the same letter are not significantly different					

Health index: Initially (45 DAS), health index increased up to 60 DAS for some genotypes (Rihane (G1), Manel (G2), Momtez (G3), DH1 (G4), Roho (G5), Tej (G6), Kounouz (G7)) or up to 75 DAS for the rest of genotypes (Lamsi (G8), Ardhaoui (G9), Safra (G10), DH2 (G11), DH3 (G12)) (**Figure 3**). Subsequently, health index stabilized perfectly for bothstressed plants and the control (genotypes DH1 (G4), Roho (G5), Tej (G6), Lamsi (G8), Ardhaoui (G9), Safra (G10), DH2 (G11), and DH3 (G12)) or it stabilized perfectly for the control but undergoes certain fluctuations for plants subjected to water stress for the genotypes Rihane (G1), Manel (G2), Momtez (G3), and Kounouz (G7). This parameter was comparable between stressed plants and the control for the genotypes Rihane (G1), DH1 (G4), Roho (G5), Tej (G6), Lamsi (G8), Ardhaoui (G9), Safra (G10), DH2 (G11), and DH3 (G12). On the other hand, health index was better for the control for the genotypes Manel (G2), Momtez (G3), and Kounouz (G7). The genotype Momtez (G3) was the most affected by water stress and its health index was reduced by 32% compared to the control.

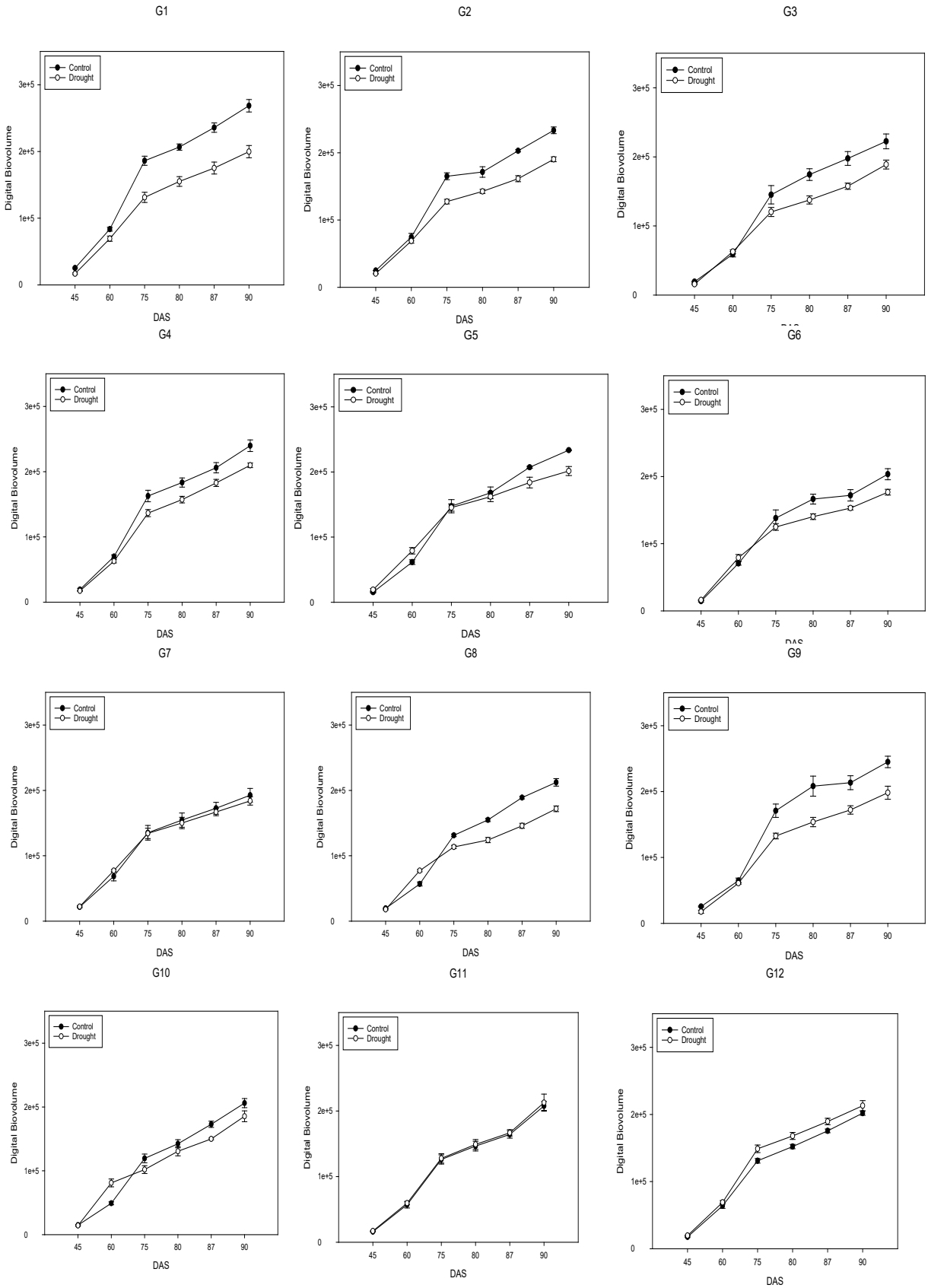


Figure 1: Effect of water deficit on Digital Biovolume of twelve barley genotypes: Rihane (G1), Manel (G2), Momtez (G3), DH1 (G4), Roho (G5), Tej (G6), Kounouz (G7), Lamsi (G8), Ardhaoui (G9), Safra (G10), DH2 (G11), DH3 (G12). DH: doubled haploid.

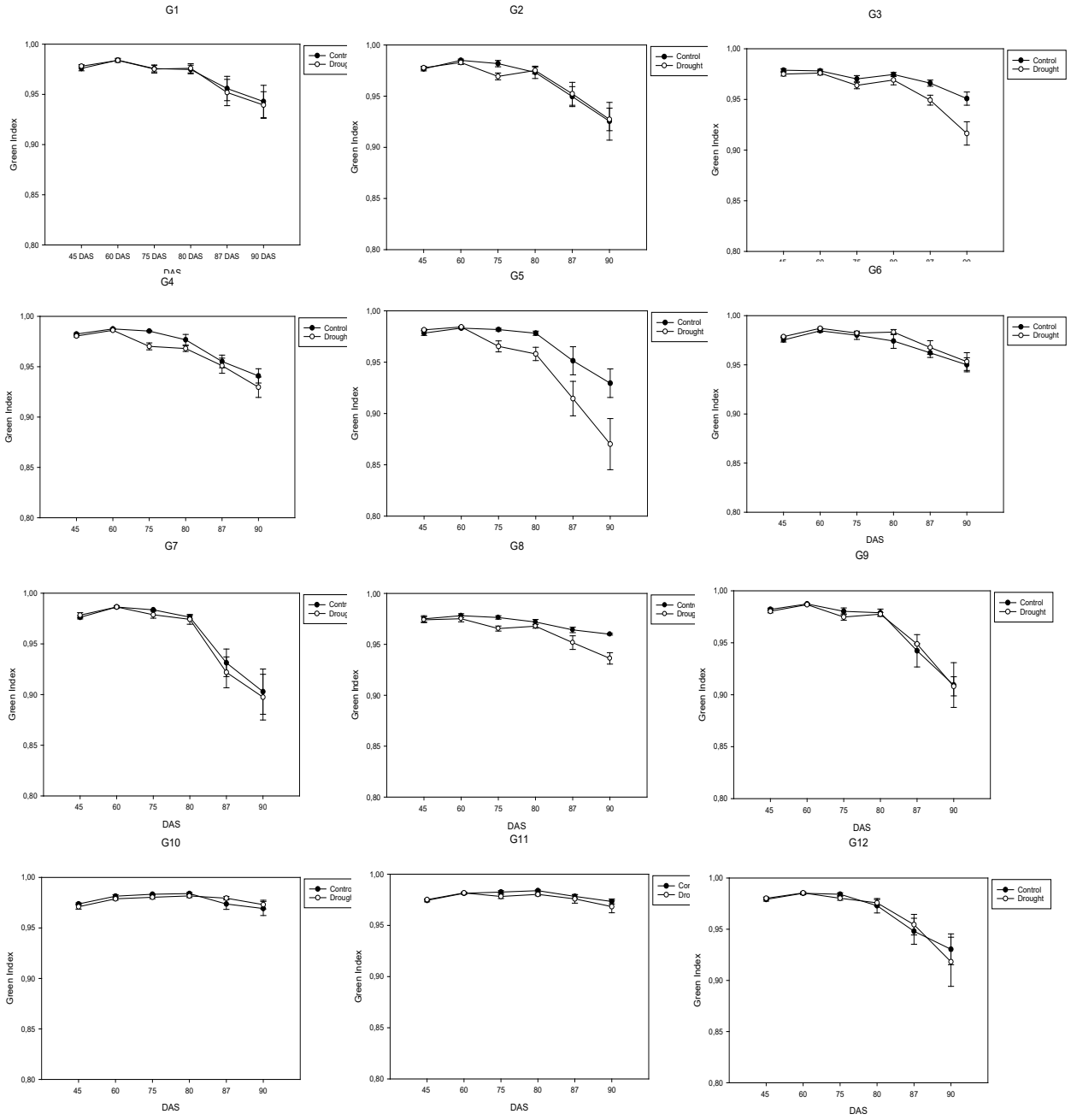
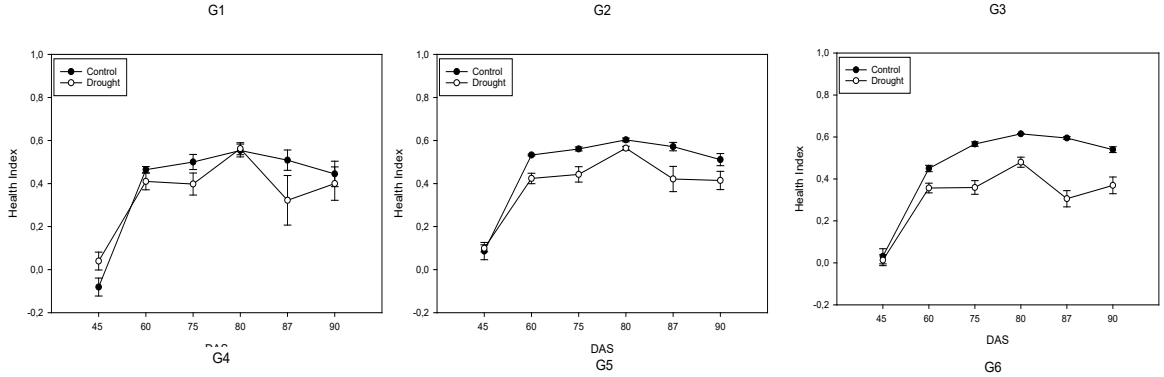


Figure 2: Effect of water deficit on green index of twelve barley genotypes: Rihane (G1), Manel (G2), Momtez (G3), DH1 (G4), Roho (G5), Tej (G6), Kounouz (G7), Lamsi (G8), Ardhaoui (G9), Safra (G10), DH2 (G11), DH3 (G12). DH: doubled haploid.



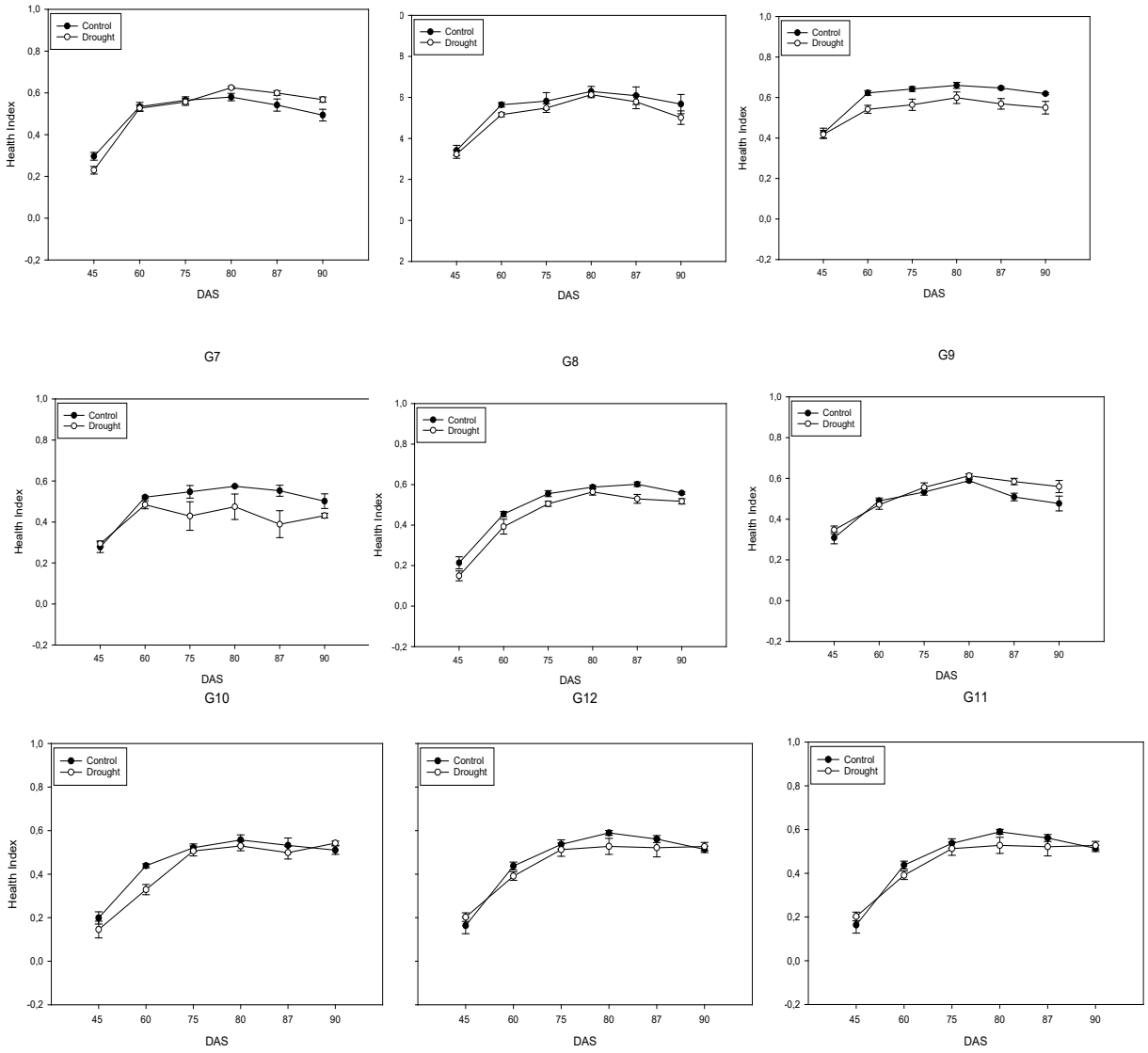


Figure 3: Effect of water deficit on health index of twelve barley genotypes: Rihane (G1), Manel (G2), Momtez (G3), DH1 (G4), Roho (G5), Tej (G6), Kounouz (G7), Lamsi (G8), Ardhaoui (G9), Safra (G10), DH2 (G11), DH3 (G12). DH: doubled haploid.

Discussion

Barley genotypes actively perceived stress conditions and responded to stress signals by modifying the expression of some phenotypic characters. The present experiment was based on the phenotypic effects of water stress that plant developed from the booting stage to maturity. This kind of stress simulates typical spring conditions of the Mediterranean region, especially of the southern regions, with dry warm springs, when water deficit develops gradually and affects plant growth and production.

Water stress at various stages, especially before anthesis, can reduce the number of grains per spike [33,34]. Grain yield is affected by different environmental conditions, including the availability of moisture in the soil, to an extent depending on the interaction of these conditions with genotypes [35]. Individual grains weight yielded under stress conditions showed that responses of different genotypes to drought during grain filling can lead to differences in individual grains weight [36-38].

Some of the spike characters are associated to the total crop production in cereals [39,40]. In our experiment, we observed that some of these traits were more intensely affected by water stress. The number of spikelets per spike (NSPS), and the Kernel Yield per Spike (KYPS) were significantly reduced under water stress by 31.94 and 47.32%, respectively. The Total Kernel Yield (TKY) showed a decrease in grain yield per plant under drought conditions

averaging 38%. Nevertheless, some genotypes proved to be less affected by water deficit, thus suggesting that they could possibly bear traits for adaptation to water stress. For instance and considering these three parameters, the genotype 'DH3' (G12) proved to be less affected by drought conditions (**Table 4**).

Reduced yields are reported to be attributed mostly to lower grain weight and only minimally to lower grain number [41,42]. The Total Kernel Yield (TKY) was the most affected yield component by drought conditions showing a reduction of 38.16%, while the decrease of Thousand Kernels Weight (TKW) was much lower (14.36%), possibly because the reduction of other spike characters, such as the number of seeds per spike, which allows a better grain filling even in the presence of reduced resources resulting from water stress (**Table 3**).

The present results showed that a solid level of variability exists with respect to all the phenotypic characters. Water stress at various stages, especially before anthesis, can reduce the number of ears and the number of grains per spike [33,34]. In our experiment, the number of spikes per plant (NSPP) which declined by 20.70% did not appear statistically significant, conversely the number of kernels per spike (NKPS) was significantly reduced for stressed plants by 43.49% (**Table 3**).

The plant development was analyzed through the digital biovolume which is a morphometric measurement previously employed in high-throughput (HTP) studies to monitor the influence of abiotic stresses, mainly drought, on plant growth or the positive action of bio-stimulating formulations [1,43]. The curves of this index as a function of time showed that water stress induces a reduction of the plant's total biomass. Nevertheless, the response of genotypes to water stress wasn't the same: some ones showed a sudden fall in the digital biovolume when the stress was applied, but they recover and continue to grow thereafter and at the same rate as the control. Other genotypes tend to fell the stress chronically and reduced their growth rate compared to the control. This may be an indicator that the former ones were better able to resist to a chronically water deficit and the genotypes 'Safra' (G10), 'DH1' (G4), and 'DH3' (G12) appeared to possess this ability.

Our results confirm the efficacy of the Digital Biovolume (DB) as a strong phenomics indicator of the overall health status of the plant in response to external stimuli, both of negative and positive effect. It has the great advantage of being nondestructive, thus allowing following the same plant throughout its development, so reducing the aleatory effect of comparing different individuals, as it happens with traditional destructive methods. The characteristic of being nondestructive, scalable and applicable to many crops generalizes its applicability for both basic and applied researches. It can surely be proposed as a tool for Germplasm selection aimed at pre-breeding and breeding programs or in evaluating the effect of agricultural practices on plant growth [1].

Green Index

The degradation of chlorophyll during abiotic stress or during senescence leads to a reduction of the value of the Green index, based on the reflectance of the green component of the visible spectrum. Of course, this index tends to zero as a consequence of the yellowing of the leaves, independently of its cause, stress, senescence or disease. Leaf yellowing in late developmental stages is the result of remobilization of carbohydrates and assimilated nitrogen from the leaves to developing tissues and or reproductive organs to ensure the reproduction of the plant [44]. For this reason, a drop of the green index along with maturation of the plant is a physiological event.

In the case of water stress, this index tends to drop more rapidly in more sensitive genotypes. The consequent reduced remobilization of nutrients explains the decrease in grain yield components, which is lower in the tolerant genotypes. In fact, in our experiment, the genotypes 'Safra' and 'DH3' which had a small reduction of the number of spikelets per spike (NSPS) and the Kernel Yield Per Spike (KYPS) were characterized by a high green index and biovolume at 90 Days After Sowing (DAS), when plant maturation was initiated.

Health Index

Color information caused by spectral reflectance in the visible spectrum is an important characteristic for phenotypic analysis, especially for reflecting nutritional status. Color indices, which are representative indicators of color characteristics, are effective in reversing growth conditions in the image analysis process and have been shown to be more relevant to many plant monitoring requirements to assess plant health status [45]. With the exception of the genotypes 'Manel', 'Momtez', and 'Kounouz', all the other genotypes had a health index comparable to the control.

The complexity of plant response to drought needs an accurate trait dissection to deepen the understanding of resistance or adaptation to drought. High-Throughput Phenotyping HTP) associated to more traditional indicators provide a significant new opportunity to identify genotypes able to better elucidate the genetic basis of these responses. The tools developed for HTP can be transferred to the field in order to assess the health of species in response to environmental changes, and to changing agricultural techniques employing lower inputs [46-49].

Conclusion

The evaluation of the tolerance of the genotypes to water stress was based on different yield parameters, in particular the Number of Spikelets per Spike (NSPS), the Kernel Yield Per Spike (KYPS), and the Thousand Kernel Weight (TKW), as well as on high-throughput phenotyping (HTP) data which are: Digital biovolume, green index and health index. According to yield parameters, the genotypes 'Rihane', 'Ardhaoui', 'Safra' and the doubled haploid lines 'DH2' and 'DH3' were more tolerant to drought. However, regarding all the HTP data, only the genotypes 'Safra' and 'DH2' showed a tolerance to water stress.

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