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Tolerance of landraces of basil (*Ocimum basilicum*) to water stress under Mediterranean conditions

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Abstract- Basil (*Ocimum basilicum* L.) is an aromatic and medicinal crop that is grown widely for its essential oil, dry leaves, and flowers. Water availability is one of the major issues in modern agriculture and especially in Mediterranean countries. In addition, climate change and the scenarios that are proposed show that water availability will be a serious problem for many countries especially in the Mediterranean area. The objective of the present study was to determine the tolerance of three landraces of basil and a commercial cultivar to water stress under field conditions using agronomic and physiological characteristics. The experiment was conducted at the University farm of Aristotle University of Thessaloniki, Greece during the summer of 2018. The landraces and the commercial cultivar were evaluated using a number of physiological (leaf area index, chlorophyll meter readings, chlorophyll fluorescence, leaf temperature and net assimilation rate) and agronomic traits (dry weight and essential oil content). Two irrigation levels of 40% and 100% of the required evapotranspiration were used for maximum yield (ET_m). The availability of water affected all the characteristics that were studied except from the chlorophyll fluorescence and net assimilation rate. The results of the experiment show that the most efficient use of irrigation water can be done by using appropriate genotypes and by applying deficit irrigation. Consequently, it can be concluded that under these conditions the quality and yield of the basil landraces can be maintained at high levels, which confirms that they have a good adaptability to the dry-land conditions of the Mediterranean area.
Keywords – dry weight, leaf area index, drought, essential oil yield

I. INTRODUCTION

Basil (*Ocimum basilicum* L.) is a widely grown aromatic and medicinal plant that is grown in many different countries such as Egypt, Morocco, France, Greece, Hungary, United States, and Israel [1, 2]. Basil is grown for its essential oil, dry leaves, and flowers and as an ornamental plant [2, 3]. Basil has many different uses such as coughs, headaches, stomach-ache, and kidney malfunctions [1]. Moreover, basil fresh and dry leaves are used in food and spice industries and the essential oil is used in applications such as insect repellent, antibacterial, antifungal, and antioxidant agent [1, 4]. Despite the fact that basil is an underutilized crop species it has a great potential in using it as an alternative crop in many countries because of many different uses. However, there is a lack of commercial cultivars of basil that are well adapted to different environments with high yield and good quality which can be used to replace other more economically profitable crops. Therefore, in many countries farmers use landraces because of the lack of appropriate cultivars. Landraces are locally grown populations which are a collection of many different lines and genotypes, these lines may be different genetically and phenotypically [5]. One important characteristic of many cultivars is the water use efficiency, which differs among the different cultivars in many crop species. In addition, there not enough information about the WUE of the different basil cultivars and also how the different morphological, agronomical and physiological characteristics affect the tolerance of basil to water stress.

Water availability is a major problem in modern agriculture and especially in Mediterranean countries. In addition, climate change and the scenarios that are proposed show that water availability will be a serious problem for many countries especially in the Mediterranean area [6]. Also it is important to use water resources more efficiently because there is an increased need for the use of water in domestic, industrial and agricultural consumption and because of global warming. Water stress is one of the most important limiting factor for crop production worldwide [6]. The economic losses of most crops due to water stress are quite significant and better water management can help to conserve water and to use water more efficiently. Better water management can be achieved by using genotypes more tolerant to water stress and by applying water when there is higher demand for maximum productivity [7-9]. There are no studies that show the effect of water stress on different basil landraces on agronomic and physiological characteristics. The objective of the present study was to determine the effect of water stress on agronomic and physiological characteristics of the different landraces of basil under field condition.

II. MATERIALS AND METHODS

2.1 Study site

A field experiment was conducted in Northern Greece at the University farm of Aristotle University of Thessaloniki (40°32'9"N 22°59'18"E, 0m) in 2018. The soil that was used was a clay loam soil with pH (1:1 H_2O) 7.77, $CaCO_3$ 11.3%, EC (dS m^{-1}) 1.07, organic matter 12.40 g kg^{-1}. Before seeding, the cultivation area was moldboard plowed, harrowed and a cultivator was used. Nitrogen and P fertilizer was applied at planting at the rates of 100 and 50 kg ha^{-1}, respectively. Complete weed control was obtained by tilling and hand weeding. Weather data (rainfall, temperature, relative humidity, solar radiation, and wind speed) were recorded daily with an automatic weather station which was close to the experimental site and are reported as mean monthly data for both years (Figure 1).

Irrigation treatments that were applied were 100% and 40% of the net irrigation requirements (IR_n) and are presented as d_{100} and d_{40} respectively. IR_n was calculated from the equation:

$$IR_n = ET_c - P_e - CR + D_p + R_{off} \quad (1)$$

were ET_c was the crop evapotranspiration, P_e was the effective rainfall and was taken into account only when it was higher than 4 mm on any day and entire rainfall was considered as effective rainfall, CR was the capillay rise from the groundwater table, D_p was the deep percolation, and R_{off} was the runoff. In this study, the CR, D_p and R_{off} were negligible because (a) there is no shallow water table problem in the experimental area, thus CR value was assumed to be zero, (b) D_p was not assumed since the amount of irrigation water was equal to the deficit amount in the root zone and (c) irrigation was performed with drip irrigation and there was no runoff.

Reference evapotranspiration (ET_o) was calculated with the FAO Penman-Monteith method with the following equation:

$$ET_o = [0.408\Delta(R_n - G) + \gamma[900/(T+273)]u^2(e_s - e_a)]/[\Delta + \gamma(1+0.34u^2)] \quad (2)$$

where ET_o is the reference evapotranspiration (mm day^{-1}), R_n is net radiation at the crop surface (MJ m^{-2} day^{-1}), G is soil heat flux density (MJ m^{-2} day^{-1}), T is mean daily air temperature at 2 m height (°C), u^2 is wind speed at 2 m height (m s^{-1}), e_s is saturation vapour pressure (kP_a), ea is actual vapour pressure (kPa), e_s - e_a is the saturation vapour pressure deficit (kP_a), Δ is the slope vapour pressure curve (kP_a °C^{-1}), γ is the psychrometric constant (kPa °C^{-1}).

Crop evapotranspiration (ET_c) was calculated with the following equation:

$$ET_c = k_c \times ET_o \quad (3)$$

where k_c is the crop coefficient.

The following values of crop coefficient (k_c) was used: for the beginning of flowering 0.9, for full bloom 1.1 and for the end of flowering 1.0.

Soil moisture was kept at 70% of field capacity which is considered adequate for plant growth in all growth stages at full irrigation (d_{100}). The differentiation of irrigation levels started when the plants were at vegetative stage and 40 days after transplantation and 30 days before anthesis. After transplantation, 30 mm of irrigation water was applied in order to promote the establishment of the newly transplanted plants. The water was applied with a drip irrigation system, after transplanting with the drippers spaced at 50 cm intervals the water supply of the drippers was 4 L h^{-1}. The drip irrigation lines were placed every other row. The same irrigation system was extensively used in other experiments [15].

2.2 Plant cultivars used in the study

During 2016 and 2017 years a number of landraces were evaluated under field conditions for their agronomic characteristics and also for their essential oil yield. From the twenty basil landraces, three different basil landraces were used in this study which had differences in earliness, biomass production, and essential oil content. The landraces were Gigas white spike (GWS), Corymb White (CW), Pink spike (PS), and Sweet (S).

Gigas white spike (GWS) | Corymb white (CW) | Pink spike (PS) | Sweet (S)

Figure 1. The different landraces of basil Gigas White Spike (GWS), Corymb White (CW), and Pink Spike (PS) that were used in the present study together with the commercial cultivar Sweet (S).

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2.3 Crop sampling and essential oil determination

Three crop sampling was made when plants were at the beginning of flowering, full bloom and end of flowering and started from the first week of July until the first week of August. In each plot, a 1 m^2 of inner row was randomly selected, the number of plants that were sampled was eight plants per plot and per sampling and the plants were cut at the ground level weighted to obtain the fresh weight (kg ha^{-1}) and let it dry at room temperature for a week, when a constant weight was reached plants were weighted to obtain the dry weight. The samples (0.5 kg biomass) were dried at 65 ^oC to constant weight to determine the relative water content and the dry weight yield. Following the leaves of the samples were separated from the stems by hand and weighed. Essential oil content was determined using dry leaf materials of 40 g and were subjected to a 3 h water-distillation using a Clevenger apparatus, and the extracted essential oils were stored at -20 ^oC. The essential oil content of the plants was determined by a volumetric method (ml/100 g) [10].

2.4 Leaf area index

Leaf Area Index (LAI) was recorded by AccuPAR system (model LP-80, PAR/LAI Ceptometer, Decagon Devices, Inc., Pullman, WA), which intercepts light using a 1 m long line quantum sensor connected to a plant canopy analyzer. The probe was used to measure the amount of incident photosynthetically active radiation (PAR) at the top of the canopy (full sunlight) and at soil levels (below the canopy). Readings below the canopy were taken at five randomly selected locations within each plot at even intervals in a diagonal transect among the centre rows. For each plot, the average of the five readings was calculated.

2.5 Photosynthesis measurements

Net assimilation rate (A) was recorded at midday (11:00-13:00 h), using the LCSD portable gas exchange system (ADC BioScientific Ltd, Hoddesdon, UK). Measurements were taken at the full expanded youngest leaf, at photosynthetic photon flux density >1200 $mmolm^{-2}s^{-1}$. Leaf temperature was measured with a handheld infrared thermometer. Measurements were performed on six plants from each plot.

2.6 Chlorophyll fluorescence

The minimum Chl fluorescence (F_0) and the maximum Chl fluorescence (F_m) were measured also in situ with the portable Z995 FluorPen PAR (Qubit Biology Inc. Kingston, Ontario, Canada). The maximum quantum efficiency of photosystem (PS) II was calculated as F_v/F_m ($F_v = F_m – F_0$).

2.7 Chlorophyll content

Chlorophyll readings were taken with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each plot the 20 youngest fully expanded leaves per plot were used when the plants were at anthesis. The instrument stored and automatically averaged these readings to generate one reading per plot.

2.8 Statistical Analysis

The data were analyzed with the ANOVA method according to a split-split-plot design (irrigation levels × cultivars × growth stages) with four replications (blocks) per treatment combination. The irrigation levels were considered as the main plots, cultivars were the sub-plots, and growth stages were the sub-sub plots. It must be noted that the basic experimental design was based on the RCBD in a split plot arrangement, as described previously. The Least Significant Difference (LSD) criterion was used to test the differences between treatment means and the significance level of all hypotheses tested was preset at $P<0.05$. All statistical analyses were performed using the SPSS software package (ver. 17, SPSS Inc., Chicago, USA).

III. RESULTS AND DISCUSSION

The weather conditions are given at figure 2 and rainfall was low during April and June and higher during May and July, however this did not affect the experiment as the harvest was done before the heavy rain that we had in August. In addition, temperature was higher during August and followed by July as the warmest month.

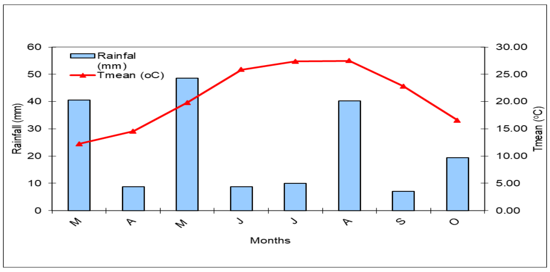

Figure 2. The main weather parameters rainfall and mean temperature (T_{mean}). The weather data were recorded with an automatic weather station close to the experimental site

Table 1. Analysis of variance results (significance of the effects) for testing the effects (main and interactions) of Irrigation (W), Genotype (G), and growth stages (S), on the measured plant characteristics.

Plant characteristic	Irrigation (W)	Genotype (G)	Growth stages (S)	G x S	W x S	G x W	G x W x S
df	1	3	2	6	2	3	6
LAI	**	***	**	***	NS	NS	NS
Dry weight	**	***	***	NS	NS	NS	NS
Chlorophyll fluorescence	NS	**	***	***	*	NS	NS
Chlorophyll content	**	***	***	***	NS	NS	NS
Leaf temperature	***	*	***	***	NS	NS	NS
Net assimilation rate	NS	***	***	***	**	**	NS
Essential oil content	*	***	***	NS	NS	NS	NS
WUEDW	NS	***	***	***	**	NS	NS

* Significant at 0.05 significance level.
** Significant at 0.01 significance level.
*** Significant at 0.001 significance level.
NS, non significant ($p>0.05$)

Most of the characteristics were affected by the main effect of irrigation (W), genotype (G), and growth stages (S) and also some of characteristics were affected by the two way interactions (Table 1). More specifically, the interaction "genotype × growth stages" affected all the characteristics except the dry weight and the essential content oil. The interaction "irrigation × growth stages" had a statistically significant effect only on chlorophyll fluorescence, net assimilation rate and WUE. The interaction "genotype × irrigation" affected only the net assimilation rate. There was no three way interaction of "genotype × growth stage × irrigation".

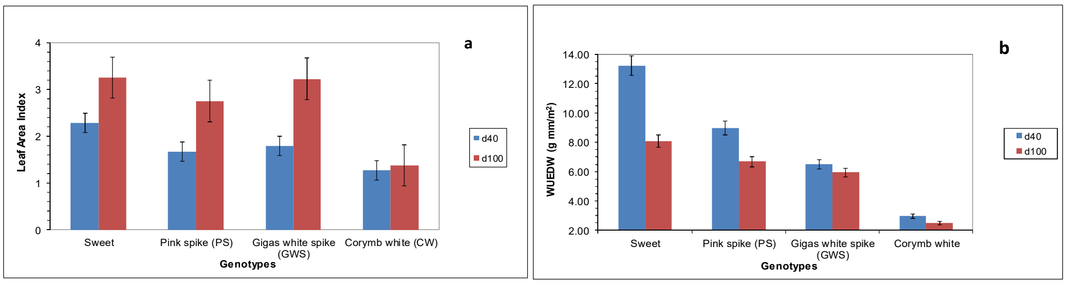

Figure 3. Effect of water availability on leaf area index (LAI) (a) and on chlorophyll fluorescence (b) by the four basil genotypes.

Water availability affected leaf area index when water was limited and the leaf area index was lower in most genotypes and especially in GWS as LAI was much lower in water stress compared with the control (Figure 3a). The landrace CW did not showed significant change in LAI and this is can be because of the landrace was not so sensitive to water stress [10-13]. Chlorophyll fluorescence was not significantly affected in most landraces and in PS landrace was lower in water stress treatment than in the control (Figure 3b). Both LAI and leaf chlorophyll fluorescence are traits contributing to productivity under water stress conditions [14] however in basil seems that chlorophyll fluorescence was not affected by water stress.

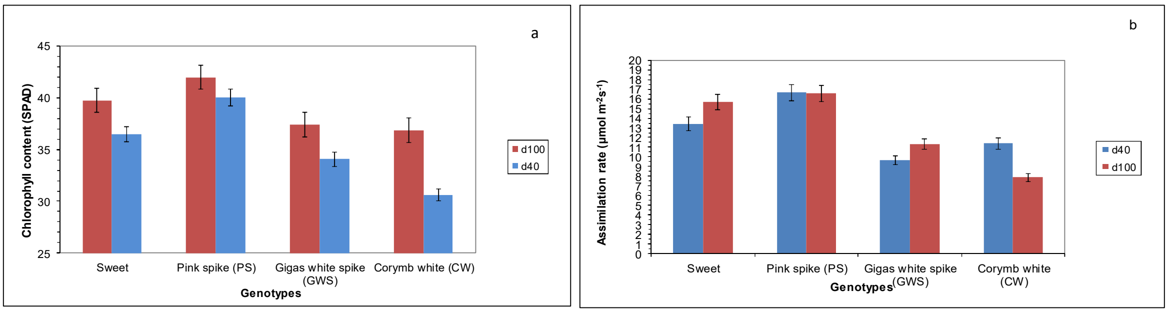

Figure 4. Effect of water availability on chlorophyll content (SPAD) (a) and on assimilation rate (b) of the four basil genotypes.

Chlorophyll content was lower at the control treatments compared with the water limited treatments. Also in CW landrace there was much higher decrease in chlorophyll content in the water limited treatments and this was much higher than the other genotypes (Figure 4a). This can be because some of the landraces can be more tolerant to water stress than the others [15]. Assimilation rate showed a different trend was not affected by the irrigation treatment which can be because some landraces are not very sensitive to water stress [15] (Figure 4b).

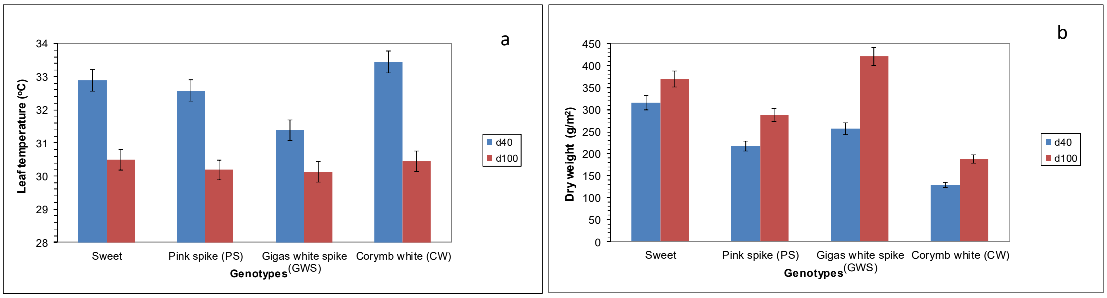

Figure 5. Effect of water availability on leaf temperature (a) and on dry weight (b) of the four basil genotypes.

Leaf temperature was affected by water availability as in CW landrace there was much higher increase of leaf temperature because of the water stress (Figure 5a). This is because stomata close and the leaf temperature rises. However, in other landraces there was much lower increase in leaf temperature such as GWS. In addition, in all genotypes leaf temperature was higher under water stress and this indicates that the different genotypes were affected by the water availability [14]. Dry weight was significantly affected at the GWS landrace much lower decrease was found at the commercial cultivar (Figure 5b) this indicates that GWS is more sensitive to water stress.

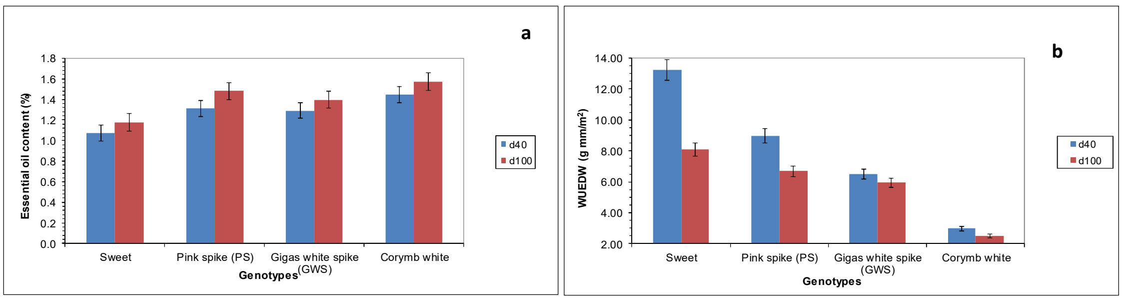

Figure 6. Effect of water availability on essential oil content (a) and WUE (b) of the four basil genotypes.

Water stress did not affected the essential oil content in all genotypes that were tested (Figure 6a). WUE was affected by the water availability and by the cultivar and it was higher at the water stress treatment and there was significant difference at the commercial cultivar (Figure 6b). Also at the GWS and CW there was no statistical significant difference at the two levels of water.

One of the ways that we can confront to water shortages in agriculture is by growing tolerant genotypes [16], which yield better under drought by adjusting their physiological responses like gas exchange physiology and WUE [17-21]. In the present work, some landraces differed significantly in dry weight and in the responses in water stress but this could not be ascribed to physiological responses.

The use of physiological traits in plant breeding can help in the improvement of plant tolerance but has to fulfill several criteria such as the possibility of relatively simple and fast measurements of the respective parameter in many samples, its good correlation with the tolerance/sensitivity to the target stress factor, and an adequate intraspecific genetic variation [22]. The physiological parameters examined in our study certainly satisfy the first condition (particularly the Chl fluorescence measurements). In other studies it was found a good association between maize drought tolerance and Chl fluorescence excitation spectra [23] or Chl content [24]. From this point of view, the measurement of A seems to be the least suitable among the three categories of photosynthetic parameters examined, as it is rather time-consuming and the relationship between A and drought-induced changes in plant morphology and development is not unequivocal [25].

IV.CONCLUSION

The availability of water affected the LAI, dry weight, chlorophyll content, leaf temperature and essential oil content in some of the landraces but not in all the landraces and did not affect chlorophyll fluorescence, net assimilation rate and WUE. The results of the experiment show that the most efficient use of irrigation water can be done by using appropriate genotypes and by applying deficit irrigation. Consequently, it can be concluded that under these conditions the quality and yield of the basil landraces can be maintained at high levels, which confirms that they have a good adaptability to the dry-land conditions of the Mediterranean area.

V. ACKNOWLEDGMENTS

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