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Water Stress Effects on the Morphological, Physiological Characteristics of Maize (*Zea mays* L.), and on Environmental Cost

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Abstract: Water stress is one of the most important yield constraints on crop productivity for many crops, and especially for maize, worldwide. In addition, climate change creates new challenges for crop adaptation as water stress appears even in areas where, until recently, there was an adequate water supply. The objective of the present study was to determine the effect of water availability on the morphological and physiological characteristics of maize, and also on the environmental cost under field conditions. The lowest water treatment (ET_{50}) reduced leaf area index, plant height, chlorophyll content, assimilation rate and gas exchange parameters, photosynthetic efficiency, and silage yield. Furthermore, mild water stress (ET_{70}) affected the characteristics that were studied but maintained a high crop yield. Moreover, the outputs/inputs ratio and energy efficiency showed similar trends, with the highest values under ET_{100} treatment and the lowest under ET_{50} treatment in two consecutive years. Therefore, the results of this study can be used by farmers in the Mediterranean area, who can maintain or improve their crop yield using a lower amount of water when the water supply is limited, thereby contributing to reducing the impact of global climate change and maintaining crop productivity.

Keywords: drought tolerance; leaf area index; chlorophyll content; chlorophyll fluorescence; photosynthesis

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1. Introduction

Drought is a major environmental stress that limits plant growth, productivity, and consequently, crop yield worldwide, and especially in the Mediterranean area [1,2]. In addition, recent years brought extensive drought periods and extremely high temperatures, causing widespread economic losses in agriculture; this impact is more likely to worsen with climate change [2–5]. The problem is getting worse as the availability of fresh water and land for agricultural use continues to decline at an unsustainable rate [6]. It is estimated that by 2050, arable land will decline by 8–20% [7]. Consequently, global agricultural production will face the new challenges of adverse environmental conditions, as well as water scarcity, suggesting the need for integrated approaches to sustain and enhance agricultural productivity in the future [8]. The increasing worldwide shortage of water and costs of irrigation are leading to an emphasis on developing methods of irrigation that minimize water use and maximize water-use efficiency [9]. Irrigation scheduling is the decision of when and how much water should be applied to a field in order to maximize production. It was proposed in order to maximize irrigation efficiency and involves applying the precise amount of water needed to replenish the soil moisture to the desired level, thus saving water and energy. It also reduces environmental costs through the reduced loss of fertilizers (resulting from decreased NO_3 leaching [10]) and reduced energy use (lower CO_2 levels, increased biodiversity, and reduced pollution) [11]. It is, therefore, important to use water resources more efficiently as this will help preserve water resources. One way to conserve water is by using the appropriate amount of water, together with appropriate crop species and cultivars with low water requirements [12–14].

Water stress is an extremely important limiting factor in maize production worldwide [1–5,12]. Economic losses in maize production due to water stress are quite significant; accordingly, breeding for drought tolerance is one of the most important challenges that maize breeders currently confront [12,15,16]. In addition, maize has high water requirements, which are required to achieve maximum yields. According to one study [17], water requirements range from 740 to 900 mm, while more recent studies estimated that maize crops have water requirements ranging from 500 to 800 mm [18]. More specifically, the lack of available water in the soil limits the metabolic activity of maize, reduces its biomass and leaf area, and decreases its photosynthetic rate by reducing the chlorophyll content in leaves, ultimately leading to a reduction in maize yield [19]. However, the timing and intensity of water stress also have significant effects, which are important for maize growth [20]. According to another study [21], an adequate water supply is required at all stages of crop growth, but especially after the emergence of the tassels. In addition, it is necessary to maintain an adequate water supply for the formation of the ears, as the plant has special water needs at these stages. The above-mentioned stages are critical as soil moisture must be maintained above 50% of field water capacity [22]. In contrast, maize under mild water stress during the early stages of vegetative development, and the late grain-filling stages, exhibits a certain level of tolerance to water stress due to the low water requirements at these stages [23].

Furthermore, agriculture is a major producer of greenhouse gas (GHG) emissions, contributing to climate change with emissions of CH_4, CO_2, and N_2O, and also to direct losses of soil organic carbon (SOC), and nitrogen forms in the atmosphere [24,25]. It is, therefore, important to use agricultural practices that release fewer GHGs, thereby decreasing the carbon footprint, as this will ultimately lead to a slowing down of climate change [26]. The inputs with a high carbon footprint used in agricultural practices are fertilizers, fuel, and machinery: the entire agricultural sector should implement practices to reduce their effects [26]. GHG emissions released from maize production increased from 3633.7 kg CO_2-eq ha^{-1} in 2004 to 4043.3 kg CO_2-eq ha^{-1} in 2013 [24–27]. A very important source of GHG emissions are fertilizers, especially the N fertilizers used extensively in maize production; together with the soil N_2O emissions and irrigation, these contribute more than 85% of total GHG emissions. On the other hand, the reduction in GHG emissions from maize production is a quite complex and multifaceted challenge. Moreover, the measures to reduce GHG emissions are limited, and most of them are strongly connected to management practices. It was proposed that GHG emissions can be reduced by using sustainable practices, such as crop rotation, reduced or no tillage, use of renewable energy sources, organic cultivation and integrated crop management, reduction in nitrogen fertilizers, the use of alternative organic N fertilization, the use of more sustainable water resources, and, this latter, according to the needs of the crop [28–30].

Understanding the water requirements of a crop, therefore, leads to better water-use efficiency and, according to another study [31], using the reference evapotranspiration (ET_o) of the crop, it is possible to determine the potential water demand of a crop. The water deficit in soil is considered as the main limiting factor affecting maize production in semi-arid regions, so it is, therefore, necessary to improve agricultural practices for water conservation for agriculture. Therefore, practices that improve energy productivity and save water, such as conservation tillage and deficit irrigation to provide sustainable and cleaner crop production, must be promoted. In addition, there is a limited number of studies on reducing water use and improving energy saving for maize silage production. The aim of the present study was, therefore, to study the effect of different irrigation levels on the morphological and physiological characteristics, and silage yield, of maize, and to determine the environmental cost of the crop under different water regimes.

2. Materials and Methods

2.1. Experimental Site

The experiments were conducted for two years, 2019 and 2020, in a commercial field in the area of Thessaloniki, (40°34′11.4′′ N 22°59′16.0′′ E, 30 m), in North Greece. The soil type of the field where the experiments took place was clay loam with a pH of 7.8 (1:2 water) and an EC_{se} of 0.673 dSm^{-1}; it contained the following: organic matter 23 g kg^{-1}, N-NO_3 23.8 mg kg^{-1}, P (Olsen) 29.6 mg kg^{-1}, and exchangeable K 800 mg kg^{-1}. The weather conditions were recorded daily with an automated weather station, which was located on site, and the weather data are presented as monthly means for both years (Figure 1).

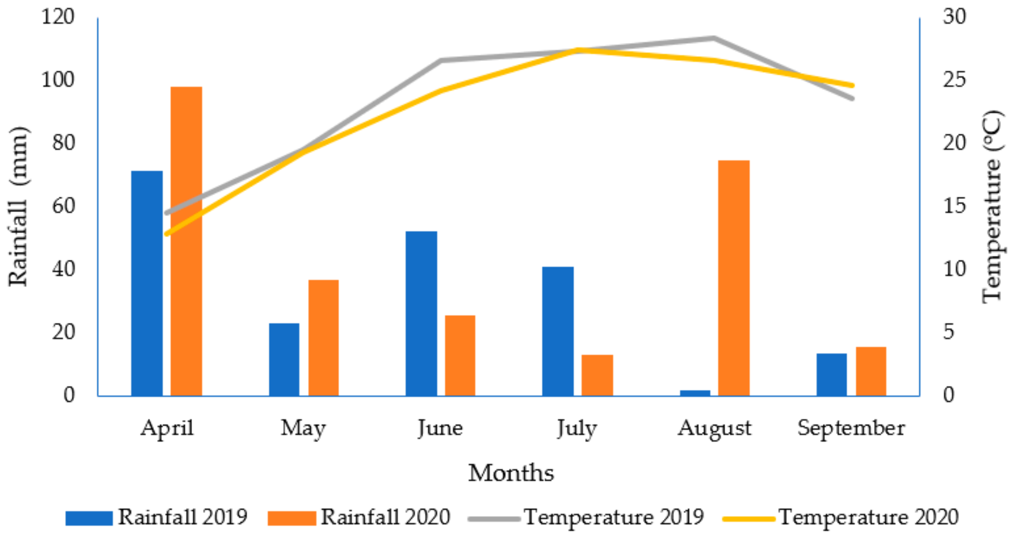

Figure 1. The main weather factors (average temperature and rainfall) for both years, 2019 and 2020, of the experiment in a commercial field crop in the area of Thessaloniki. The weather data were recorded with a weather station on site.

2.2. Crop Management and Experimental Design

The experimental design was the completely randomized block design (RCBD) with four replications (blocks). The treatments were the following: (1) control (100% evapotranspiration (ET_c), (2) 70% of ET_c and (3) 40% of ET_c. The maize hybrid Pioneer 1291 (FAO 700) was used; this is widely used in Greece for silage production. On 2 April 2019 and 5 May 2020, the soil was tilled with a disc harrow to prepare it for sowing. The sowing was conducted on 4 April 2019 and 8 May 2020 with a 4-row pneumatic seeding machine, at a seeding rate of 80.000 plants/ha. The experimental area used was 2345 m^2. Each plot was 5.6 × 20 m, covering a total area of 112 m^2. The emergence of the maize plants was recorded on 17 April 2019 during the first year and 26 May 2020 during the second year, while harvesting took place on 10 August 2019 and 14 September 2020. A drip-irrigation system was used, with a drip spacing of 50 cm and a water flow per drip of 4 L h^{-1}. Drip-irrigation pipes were placed every other plant row. A hydrometer was installed at the beginning of the irrigation system to measure the amount of water that its plot received. Specifically, the amount of water applied in each treatment was: 300 m^3/ha in the control (100% ET_c), 210 m^3/ha in the 70% ET_c treatment, and 150 m^3/ha in the 50% ET_c treatment. Irrigation was applied when soil water losses due to crop evapotranspiration (ET_c) reached 50 mm, while rainfall was taken into account only when it exceeded 4 mm/day. Crop evapotranspiration (ET_c) was calculated by the following equation: $ET_c = k_c \times ET_o$, where k_c is the crop coefficient. The reference evapotranspiration (ET_o) was calculated using the Penman–Monteith method based on meteorological data. Using the Penman–Monteith formula with the evapotranspiration calculation method, the values of ET_o (1) were derived from the meteorological parameters [32]:

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

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 vapor pressure (kP_a), e_a is actual vapor pressure (kP_a), $e_s - e_a$ is saturation vapor pressure deficit (kP_a), Δ is the slope vapor pressure curve (kPa °C^{-1}), and γ is the psychrometric constant (kP_a °C^{-1}). The evapotranspiration rate (ET_c), which is the product of ET_o and the crop coefficient (K_c), was calculated using K_c coefficient values for maize adapted to Greek conditions (K_{cini} = 0.50, K_{cmid} = 1.05, K_{cend} = 0.15) for the 30/40/50-day growth stages from seed germination [33,34].

Weed control was achieved with Terbuthylazine 594 g a.i. ha^{-1}, Mesotrione 126 g a.i. ha^{-1}, and Nicosulfuron 116 g a.i. ha^{-1}. Additional mechanical weeding was performed to control escaped weeds in both years. No other pesticides were used. There were 8 rows in each plot; representative plants were used from the two center rows of each plot and were measured for physiological and morphological characteristics, and silage yield. Representative plants are considered plants with healthy and uninfected leaves, with full exposure to sunlight, and include plants in the same growth stage. Two measurements of the morphological and physiological characteristics were taken during the months June–August in both years, the first at the stage of anthesis and the second 20 days later. Specific details of measurements are given below.

2.3. Morphological Characteristics

2.3.1. Plant Height

Plant height was determined using a measuring tape. Five plants from each plot, located in the central rows, were selected. The plant height was determined by calculating the average value of the five measurements of the plant height.

2.3.2. Leaf Area Index

The LAI was determined using an AccuPAR, LP–80 (Decagon Devices, Inc., Pullman, WA, USA). The device comprises an external sensor, a microprocessor, and a data recorder. The sensors record the photosynthetically active radiation, in the 400–700 nm waveband, in units of micromols per meter squared per second (μmol m^{-2}s^{-1}). The measurements took place during the hours between 11 a.m. and 1 p.m. During this time three measurements were made within the canopy. The mean value of these measurements was used as the value of LAI.

2.4. Physiological Characteristics

2.4.1. Leaf Greenness Index (SPAD Index)

The leaf greenness index was determined using a handheld dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Tokyo, Japan) [35]. This meter calculates the intensity of the green color on the leaves of a plant, according to the light absorbance in two wavelengths (650 and 940 nm). A total of 16 plants from the central rows of each plot were selected. The measurements were taken in the middle of the leaf from the main cob [36].

2.4.2. Photosynthetic Efficiency

Minimum chlorophyll fluorescence (F_0) and maximum chlorophyll fluorescence (F_m) were measured with a portable FluorPen PAR (Qubit Biology Inc., Kingston, ON, Canada). For each plot, 16 young fully expanded leaves were used before each sampling. Photosynthetic efficiency was determined as the maximum quantum efficiency of photosystem (PS) II, which was calculated as F_v/F_m ($F_v = F_m - F_0$).

2.4.3. Gas Exchange Measurements

Gas exchange parameters were determined with a portable photosynthesis system (LCi-SD, ADC BioScientific Ltd., Herts, England); this was equipped with a square (6.25 cm^2) chamber used to measure CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance to water vapor (g_s), and intercellular CO_2 concentration (C_i) at flowering and 20 days later [37]. Measurements were performed on 16 plants in the central rows from each plot and from 09:00 to 12:00 in the morning to avoid high vapor pressure deficit and photoinhibition at midday. The measurements were taken in the middle of the main cob leaf.

2.5. Energy Equivalent

Agricultural practices use a significant amount of energy, and it is important to take into consideration the energy efficiency of the agricultural practices so that low input management can be implemented, and the negative environmental effects can be reduced [38,39]. The energy approach is based on the conversion of all production factors, and every product that is used in the production process, into energy units. Table 1 shows the energy equivalents used in agricultural production. The amount of input in this study was calculated per hectare and these data were multiplied by the coefficient of the energy equivalent. The energy equivalents were conveyed in Megajoules (MJ). To determine the output/input ratio [1] and the efficiency of the energy used [2] in maize production, the following formulas were used as previously described [39,40].

$$\text{Output/input ratio} = \frac{\text{The amount of energy (Output)(MJ/ha)}}{\text{The amount of energy (Input)(MJ/ha)}} \tag{2}$$

$$\text{Energy efficiency} = \frac{\text{Maize Production } \left(\text{kg ha}^{-1}\right)}{\text{The amount of energy (Input)(MJ/ha)}} \tag{3}$$

Table 1. Energy equivalents of inputs and outputs in agricultural production.

Inputs	Unit	Energy Equivalent Coefficient (MJ/Unit)	Reference
Pesticides, Fungicides	kg	120	[41]
Labor	hour	1.96	[41]
Machinery	hour	64.8	[40]
Nitrogen (N)	kg	66.14	[42]
Phosphorus (P)	kg	12.44	[42]
Potassium (K)	kg	11.15	[42]
Manure	ton	303.1	[40]
Diesel	L	56.31	[43]
Electricity	kWh	3.6	[44]
Irrigation water	m^3	0.63	[44]
Seed for vetch	kg	10	[45]
Seed for maize	kg	14.7	[41]

2.6. Carbon Footprint

In the present study, carbon (C) emissions were calculated taking into account the C emissions derived directly from crop management practices, materials, and machinery inputs. The total sum of the maize C footprint for both years was calculated using the following formula [46]:

$$\text{Carbon footprint} = \text{SUM (IR} \times \text{CE)} \quad (4)$$

where IR is the input ratio and CE is the coefficient of greenhouse gas emissions for each input (kg CO_2-eq kg^{-1}) (Table 2).

Table 2. Emission coefficient for each input used in the present study.

Inputs	Emission Factor	Reference
Nitrogen (N)	8.30 kg CO_2-eq kg^{-1} N	[47]
Phosphorus (P)	0.61 kg CO_2-eq kg^{-1} P_2O_5	[48]
Potassium (K)	0.44 kg CO_2-eq kg^{-1} K_2O	[48]
Seeds	3.85 kg CO_2-eq kg^{-1}	[48]
Electricity	0.80 kg CO_2-eq kW h^{-1}	[49]
Pesticides, Fungicides	18 kg CO_2-eq kg^{-1}	[48]
Diesel	2.63 kg CO_2-eq L^{-1}	[50]

2.7. Statistical Analysis

Data for plant height, leaf area index, leaf greenness index (SPAD index), photosynthetic efficiency, and CO_2 assimilation rate (A) were analyzed according to a 2 × 3 × 2 experiment based on the Randomized Complete Block Design. The experiment involved three factors, in a split-split plot arrangement [51,52], with 4 replications (blocks) per combination of factor levels: the “growing season”, “irrigation treatment”, and “growth stage”. The two growing seasons were considered as the main plots, the three irrigation treatments were the sub-plots, and the two growth stages were the sub-sub plots. Data for energy output/input ratio, energy efficiency, and silage yield were analyzed according to a 2 × 3 experiment based on the Randomized Complete Block Design. The experiment involved two factors, in a split plot arrangement [51,52], with four replications (blocks) per combination of factor levels: the “year” and “irrigation treatment”. The two years were considered as the main plots and the three irrigations treatments were the sub-plots. In all cases, data were analyzed within the methodological frame of Mixed Linear Models, using ANOVA [51,52]. The ANOVA method was used mainly for computing the correct standard errors of the differences among all factor level combination mean values. Mean values were compared using the “protected” Least Significant Difference (LSD) criterion. The combined analysis over the two years facilitated the calculation of a common LSD value for conducting all interesting comparisons among mean values. In all hypothesis testing procedures, the significance level was predetermined at a = 0.05 ($p \leq 0.05$). Statistical analyzes were accomplished with the SPSS v.26.0 statistical software (IBM, New York, NY, USA).

3. Results

The weather conditions were quite different in the two years: during 2019, there was a warm and dry summer; during 2020, in contrast, there was quite a mild spring and significant rainfall in both spring and summer (Table 1). The nonhomogeneous variation in the data across years, therefore, reflected climatic fluctuations and prevented a combined analysis.

3.1. Morphological Characteristics

3.1.1. Plant Height

The plant height was affected by the main effects of “year” (Y) ($p < 0.001$), “irrigation” (I) ($p < 0.001$), and “growth stage” (GS) ($p < 0.001$), and also by the two-way interaction “growth stage × year” ($p < 0.001$) (Table 3). According to Table 4, the tallest plants were observed in the second growth stage, with a total mean of 2.54 m. More specifically, in 2020, the plants were taller in both growth stages (2.60 m in the first growth stage and 2.67 m in the second growth stage); in contrast, in 2019, the plants were shorter (2.15 m and 2.41 m in the first and second growth stages, respectively). Moreover, regarding the different irrigation treatments, the ET_{100} treatment showed the tallest plants, with a total mean of 2.55 m, while the shortest plants were observed in the ET_{50} treatment, with a total mean of 2.39 m.

Table 3. Plant height (m) for the two years 2019 and 2020, for two growth stages. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level.

Growth Stage	Year 2019 *	Year 2020 *	Total Mean *
GS1	2.15 ^{c}	2.60 ^{a}	2.37 ^{b}
GS2	2.41 ^{b}	2.67 ^{a}	2.54 ^{a}
Total mean	2.28	2.63	
$LSD_{0.05}$ for interaction GS×Y	0.10		
Significance of main effect of GS (p-value)			<0.001
Significance of main effect of Y (p-value)	<0.001		
Irrigation Treatments	Year 2019	Year 2020	Total mean
50% ET_c	2.21 ^{a}	2.57 ^{a}	2.39 ^{a}
70% ET_c	2.22 ^{a}	2.63 ^{b}	2.42 ^{a}
100% ET_c	2.39 ^{b}	2.71 ^{c}	2.55 ^{b}
$LSD_{0.05}$ for I			0.05

Notes: I: Irrigation; GS: Growth Stage; Y: Year; 50% ET_c: 50% evapotranspiration; 70% ET_c: 70% evapotranspiration; 100% ET_c: 100% evapotranspiration (control); GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days after the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

Table 4. Leaf area index (LAI) for the two years 2019 and 2020, for two growth stages. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level.

Irrigation Treatments	Year 2019 *	Year 2020 *	Total Mean *
50% ET_c	2.72 ^{d}	4.62 ^{b}	3.67 ^{c}
70% ET_c	2.81 ^{d}	4.75 ^{a,b}	3.78 ^{b}
100% ET_c	3.26 ^{c}	4.90 ^{a}	4.08 a
Total mean	2.26	4.75	
$LSD_{0.05}$ for interaction I × Y	0.16		
$LSD_{0.05}$ for I			0.11
Significance of main effect of Y (p-value)	<0.001		
Growth stage	Year 2019	Year 2020	Total mean
GS1	2.97 ^{a}	4.86 ^{a}	3.91 ^{a}
GS2	2.89 ^{b}	4.66 ^{b}	3.77 ^{b}
Significance of main effect of GS (p-value)			0.05

Notes: I: Irrigation; GS: Growth Stage; Y: Year; 50% ET_c: 50% evapotranspiration; 70% ET_c: 70% evapotranspiration; 100% ET_c: 100% evapotranspiration (control); GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days after the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.1.2. Leaf Area Index (LAI)

Leaf area index (LAI) was affected by the main effects of "year" (Y) ($p < 0.001$), "irrigation" (I) ($p < 0.001$), and "growth stage" (GS) ($p = 0.05$), and also by the two-way interaction "irrigation × year" ($p = 0.027$) (Table 4). The lowest LAI values, irrespective of the year, were found in the ET_{50} treatment (with a total mean of 3.67), while the highest values were found in the ET_{100} treatment (with a total mean of 4.08) (Table 4). Increased LAI values for maize crop were also found in the ET_{70} treatment (with a total mean of 3.78). In the year 2019, the highest values of LAI were found in ET_{100} treatment (with a total mean of 3.36), while the lowest values were found in the ET_{50} treatment (with a total mean of 2.72). The same tendency was observed during the second year, with LAI values of 4.75 and 4.62 in the ET_{100} and ET_{50} treatments, respectively. Furthermore, the LAI showed higher values in the first growth stage (with a total mean of 3.91), in contrast to the second growth stage, where the values decreased (with a total mean of 3.77).

3.2. Physiological Characteristics

3.2.1. Leaf Greenness Index (SPAD Index)

The leaf greenness index (SPAD) was affected by the main effects of "year" (Y) ($p < 0.001$), "irrigation" (I) ($p < 0.001$), and "growth stage" (GS) ($p < 0.001$), and also by the two-way interaction "year × growth stage" ($p < 0.001$). The SPAD values were lower in the second growth stage than in the first growth stage, with a total mean of 51.62 and 56.60 for each respective growth stage (Table 5). More specifically, for both years of experimentation, 2019 and 2020, the lowest SPAD index values were found in the second growth stage (57.50 and 45.75, for the years 2019 and 2020, respectively). Between the two different irrigation treatments, the plants in the ET_{100} treatment had the highest SPAD values, with a total mean of 55.05, while the lowest values were found in the plants of the ET_{50} treatment, with a total mean of 49.92. The ET_{70} treatment showed relatively high SPAD values of 55.37.

Table 5. Leaf Greenness Index (SPAD) for the two years 2019 and 2020, for two growth stages. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level.

Irrigation Treatments	Year 2019 *	Year 2020 *	Total Mean *
GS1	58.10 ^{a}	55.11 ^{b}	56.60 ^{a}
GS2	57.50 ^{a}	45.75 ^{c}	51.62 ^{b}
Total mean	57.80	50.43	
$LSD_{0.05}$ for interaction GS × Y	1.98		
Significance of main effect of GS (*p*-value)			<0.001
Significance of main effect of Y (*p*-value)	<0.001		
Growth stage	Year 2019	Year 2020	Total mean
50% ETc	53.38 ^{a}	46.47 ^{a}	49.92 ^{a}
70% ETc	59.17 ^{b}	51.57 ^{b}	55.37 ^{b}
100% ETc	60.86 ^{c}	53.25 ^{c}	57.05 ^{c}
$LSD_{0.05}$ for I			1.63

Notes: I: Irrigation; GS: Growth Stage; Y: Year; 50% ET_c: 50% evapotranspiration; 70% ET_c: 70% evapotranspiration; 100% ET_c: 100% evapotranspiration (control); GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days after the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.2.2. Photosynthetic Efficiency

Photosynthetic efficiency was affected by the main effects of "year" (Y) ($p < 0.001$), "irrigation" (I) ($p = 0.003$), "growth stage" (GS) ($p < 0.001$), and by the two-way interaction "year × growth stage" ($p < 0.001$). Values of photosynthetic efficiency, irrespective of the year, were highest in the first growth stage, with a total mean of 0.758 (Table 6). For both years of experimentation, the lowest values were found in the second growth stage (0.762 and 0.706, in the years 2019 and 2020, respectively). Regarding the different treatments, in the ET_{50} treatment, the fluorescence value was the lowest with a total mean of 0.726. On the contrary, the highest values found in the ET_{100} treatment, with a total mean of 0.765. The ET_{70} treatment had an average of 0.747.

Table 6. Photosynthetic efficiency for the two years 2019 and 2020, for two growth stages. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level.

Irrigation Treatments	Year 2019 *		Year 2020 *	Total Mean *
GS1	0.799 ^{a}		0.718 ^{c}	0.758 ^{a}
GS2	0.762 ^{b}		0.706 ^{c}	0.734 ^{b}
Total mean	0.780		0.712	
$LSD_{0.05}$ for interaction GS × Y		0.021		
Significance of main effect of GS (*p*-value)				<0.001
Significance of main effect of Y (*p*-value)		<0.001		
Growth stage	Year 2019		Year 2020	Total mean
50% ET_c	0.770 ^{a}		0.683 ^{a}	0.726 ^{a}
70% ET_c	0.772 ^{a}		0.722 ^{b}	0.747 ^{b}
100% ET_c	0.800 ^{b}		0.732 ^{b}	0.765 ^{c}
$LSD_{0.05}$ for I				0.019

Notes: I: Irrigation; GS: Growth Stage; Y: Year; 50% ET_c: 50% evapotranspiration; 70% ET_c: 70% evapotranspiration; 100% ET_c: 100% evapotranspiration (control); GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days after the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.2.3. CO_2 Assimilation Rate (A)

The CO_2 assimilation rate (A) was affected by the main effects of "irrigation" (I) ($p < 0.001$) and "growth stage" (GS) ($p = 0.034$), and also by the two-way interaction "irrigation × year" ($p < 0.001$). Irrespective of the year, the lowest values were found in the treatment ET_{50} (with a total mean of 4.418), while the highest values were found in treatment ET_{100} (with a total mean of 6.026) (Table 7). In addition, satisfactory values for maize crop were found in treatment ET_{70} (with a total mean of 5.575). Moreover, in the first year, 2019, the highest values of this index were found in the ET_{100} treatment (with a total mean of 5.655), while the lowest were measured in the ET_{50} treatment (with a total mean of 4.772). The same tendency was observed in the second year, 2020, with values of 6.398 and 4.065 in the ET_{100} and ET_{50} treatments, respectively. Furthermore, the CO_2 assimilation rate showed higher values in the first stage of development (with a total mean of 5.421), in contrast to the second stage, where it decreased (with a total mean of 5.095).

Table 7. CO_2 assimilation rate (A) for the two years 2019 and 2020, for two growth stages. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level.

Irrigation Treatments	Year 2019 *		Year 2020 *	Total Mean *
50% ET_c	4.772 ^{c}		4.065 ^{d}	4.418 ^{c}
70% ET_c	5.020 ^{c}		5.731 ^{b}	5.375 ^{b}
100% ET_c	5.655 ^{b}		6.398 ^{a}	6.026 a
Total mean	5.149		5.398	
$LSD_{0.05}$ for interaction I × Y		0.397		
$LSD_{0.05}$ for I				0.281
Significance of main effect of Y (*p*-value)		0.219		
Growth stage	Year 2019		Year 2020	Total mean
GS1	5.385 ^{a}		5.458 ^{a}	5.421 ^{a}
GS2	4.853 ^{b}		5.338 ^{b}	5.095 ^{b}
Significance of main effect of GS (*p*-value)				0.034

Notes: I: Irrigation; GS: Growth Stage; Y: Year; 50% ET_c: 50% evapotranspiration; 70% ET_c: 70% evapotranspiration; 100% ET_c: 100% evapotranspiration (control); GS1: growth stage at the stage of anthesis and GS2: growth stage 20 days after the stage of anthesis. * Means followed by the same letter are not statistically significantly different, at significance level 0.05, according to the LSD criterion.

3.3. Energy Equivalent

The output/input ratio and the energy efficiency input were affected by the main effects of "year" (Y) ($p < 0.001$ for output/input and $p = 0.001$ for energy efficiency, respectively) and "irrigation" (I) ($p < 0.001$ for both treatments); they were also affected by the two-way interactions "irrigation × year" ($p = 0.001$ for output/input and $p = 0.003$ for energy efficiency, respectively). The outputs/inputs ratio and energy efficiency showed similar trends, with the highest values in the ET_{100} treatment and the lowest in the ET_{50} treatment for both years (Figure 2). More specifically, the ratio of outputs/inputs in 2019 was lower in all treatments compared with ratios for the year 2020. The highest values for energy efficiency were calculated in the ET_{100} treatment (1.87 and 1.90 for the years 2019 and 2020, respectively), while the lowest values were calculated for the ET_{50} treatment (1.43 and 1.52 for the years 2019 and 2020, respectively). Moreover, from Figure 2, it can be observed that energy efficiency showed the highest values in 2020 in all treatments. More specifically, the ET_{50} treatment showed the lowest values (0.75 in 2019 and 0.80 in 2020), while the ET_{100} showed the highest values (0.98 in 2019 and 1.00 in 2020).

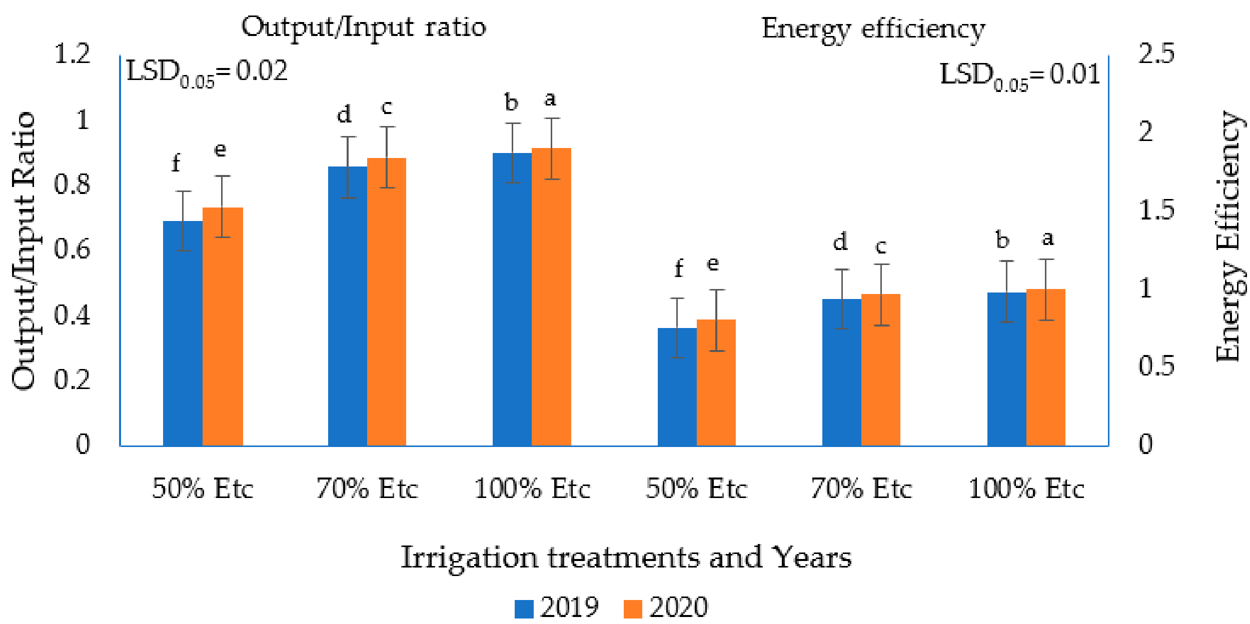

Figure 2. Output/Input ratio and energy efficiency in maize cultivation the two years, 2019 and 2020. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level. Notes: 50% ET_c: 50% evapotranspiration; 70% ET_c: 70% evapotranspiration; 100% ET_c: 100% evapotranspiration (control). Within each year and within each treatment, different letters above the bars correspond to statistically significant difference between the means compared. Error bars correspond to the Standard Errors of the mean values.

3.4. Carbon Footprint

Table 8 shows the different inputs used in maize production, together with the amount of inputs and the amount of CO_2 emissions for each irrigation treatment. In both years, the input with the highest CO_2 emission values was N, followed by fuel (diesel), electricity, maize seeds, phosphorus fertilizers, and pesticides. Electricity, however, showed different CO_2 emissions in each year and in each treatment due to the different amount of water applied. In addition, it can be observed that during the second year, 2020, the CO_2 emissions were higher in all treatments, compared with those during the first year, 2019. In particular, in both years, the lowest emissions occurred in the ET_{50} treatment (176 kg CO_2-eq ha^{-1} and 264 kg CO_2-eq ha^{-1} in the years 2019 and 2020, respectively), while the highest emissions occurred in the treatment with full irrigation (ET_{100}) (352 kg CO_2-eq ha^{-1} in 2019 and 528 kg CO_2-eq ha^{-1} in 2020). Moreover, CO_2 emissions were mainly due to the application of N fertilizers, which made a higher contribution than other management practices. In addition, fuel and electricity also contributed to the carbon footprint, while other inputs made a minimum contribution to the CO_2 emissions.

Table 8. Emission factors for each input used in maize production during the two years.

Inputs	The Amount of Input	50% ET_c	70% ET_c	100% ET_c
Year 2019				
Nitrogen (N)	310 kg ha^{-1}	2573 kg CO_2-eq ha^{-1}	2573 kg CO_2-eq ha^{-1}	2573 kg CO_2-eq ha^{-1}
Phosphorus (P_2O_5)	40 kg ha^{-1}	24.4 kg CO_2-eq ha^{-1}	24.4 kg CO_2-eq ha^{-1}	24.4 kg CO_2-eq ha^{-1}
Electricity	440 kWh ha^{-1}	176 kg CO_2-eq ha^{-1}	246.4 kg CO_2-eq ha^{-1}	352 kg CO_2-eq ha^{-1}
Seeds	20 kg ha^{-1}	77 kg CO_2-eq ha^{-1}	77 kg CO_2-eq ha^{-1}	77 kg CO_2-eq ha^{-1}
Pesticides, Fungicides	1.1 kg ha^{-1}	19.8 kg CO_2-eq ha^{-1}	19.8 kg CO_2-eq ha^{-1}	19.8 kg CO_2-eq ha^{-1}
Diesel	170 L ha^{-1}	447.1 kg CO_2-eq ha^{-1}	447.1 kg CO_2-eq ha^{-1}	447.1 kg CO_2-eq ha^{-1}
Total emissions CO_2		3317 kg CO_2-eq ha^{-1}	3387.4 kg CO_2-eq ha^{-1}	3493 kg CO_2-eq ha^{-1}
Year 2020				
Inputs	**The Amount of Input**	**50% ET_c**	**70% ET_c**	**100% ET_c**
Nitrogen (N)	310 kg ha^{-1}	2573 kg CO_2-eq ha^{-1}	2573 kg CO_2-eq ha^{-1}	2573 kg CO_2-eq ha^{-1}
Phosphorus (P_2O_5)	40 kg ha^{-1}	24.4 kg CO_2-eq ha^{-1}	24.4 kg CO_2-eq ha^{-1}	24.4 kg CO_2-eq ha^{-1}
Electricity	660 kWh ha^{-1}	264 kg CO_2-eq ha^{-1}	369.6 kg CO_2-eq ha^{-1}	528 kg CO_2-eq ha^{-1}
Seeds	20 kg ha^{-1}	77 kg CO_2-eq ha^{-1}	77 kg CO_2-eq ha^{-1}	77 kg CO_2-eq ha^{-1}
Pesticides, Fungicides	1.1 kg ha^{-1}	19.8 kg CO_2-eq ha^{-1}	19.8 kg CO_2-eq ha^{-1}	19.8 kg CO_2-eq ha^{-1}
Diesel	170 L ha^{-1}	447.1 kg CO_2-eq ha^{-1}	447.1 kg CO_2-eq ha^{-1}	447.1 kg CO_2-eq ha^{-1}
Total emissions CO_2		3405 kg CO_2-eq ha^{-1}	3510.6 kg CO_2-eq ha^{-1}	3669 kg CO_2-eq ha^{-1}

3.5. Silage Yield

Silage yield was affected by the factor "irrigation" (I) ($p < 0.001$) and "year" (Y) ($p = 0.001$). The lowest silage yield was found in the ET_{50} treatment (Figure 3), while the highest silage yield was found in the ET_{100} treatment (4.00 Mg ha^{-1}); a high silage yield was also found in the ET_{70} treatment, with a total mean of 3.78 Mg ha^{-1}.

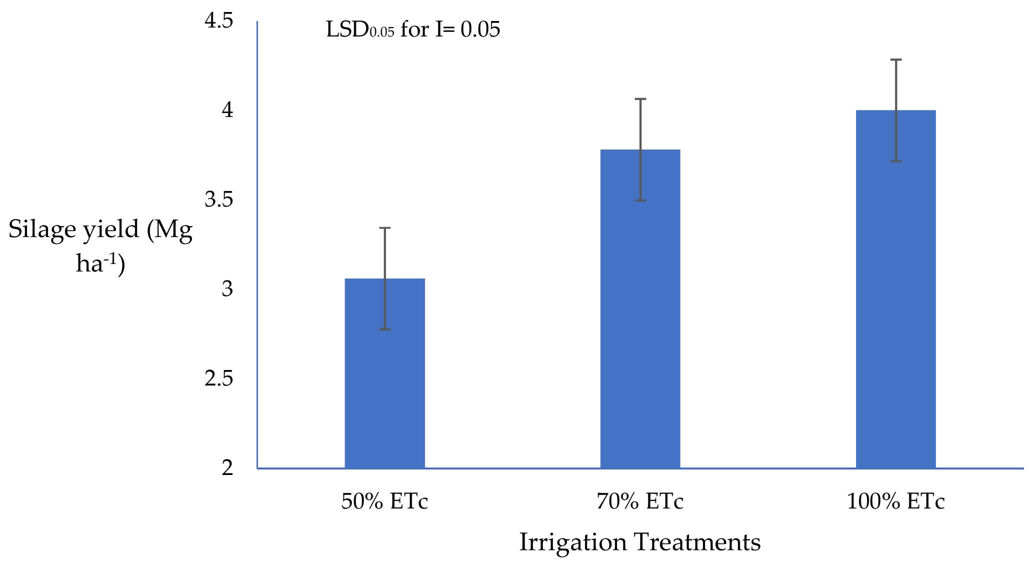

Figure 3. Silage yield during the two years, 2019 and 2020. Data presented are mean values, where $LSD_{0.05}$ is the Least Significant Difference at the 0.05 significance level. Notes: 50% ET_c: 50% of evapotranspiration; 70% ET_c: 70% of evapotranspiration; 100% ET_c: 100% of evapotranspiration (control). Error bars correspond to the Standard Errors of the mean values.

4. Discussion

4.1. Morphological Characteristics

4.1.1. Plant Height

It was found that plants were affected by growth stage, year, and irrigation levels. Growth in height ceases completely as soon as the tassel appears [12,13]. The results of the study showed that the tallest plants appeared in the full irrigation treatment (100% ET_c), while the shortest plants appeared in the lowest irrigation treatment (50% ET_c). Similar results were reported by other researchers who found that this may be due to plants having sufficient moisture at all stages of growth and continuing to grow, compared with water stress treatments where plants were stressed, and the plant cells could not elongate and reach their full size [53–55].

4.1.2. Leaf Area Index (LAI)

It was observed that the LAI remains lower in the treatment with the lowest water availability. The results are in agreement with other studies that applied a drip-irrigation system, and which reported that the highest values of the LAI for maize were obtained under full irrigation conditions [53–56]. In intense water stress treatments, the LAI can decrease because water stress limits canopy development by inhibiting leaf production and leaf growth. Leaf and stem growth are very sensitive to water stress as they are dependent on cell expansion. According to other studies [57,58], similar findings were reported for maize with respect to the LAI under water stress. Dry matter accumulation was linearly related to water availability in maize, and plants in well-watered treatments accumulated more dry matter and had a higher leaf area than plants in severely water-stressed treatments [59].

4.2. Physiological Characteristics

4.2.1. Leaf Greenness Index (SPAD Index)

In plant science, the Leaf Greenness Index was proposed as a good indicator of green color and the stay-green characteristic [60,61]. The leaf greenness index (SPAD) was affected by the main effects of year, irrigation, and growth stage, and also by the two-way interaction "year $\times$ growth stage". The SPAD index values were lower in the second growth stage than in the first growth stage; it was also observed that the highest values of the SPAD index occurred in the ET_{100} treatment, while the lowest values occurred in the ET_{50} treatment. Maize is considered to be relatively tolerant to water stress in the vegetative stage but becomes very sensitive during the tasseling, silking, and pollination periods [62]. However, our results indicate a significant decrease in SPAD values toward the end of the growing season. This agrees with others, who observed a significant decline in the leaf chlorophyll content by withholding irrigation at the reproductive stage of maize [12,63]. A water deficit causes a reduction in the uptake of nutrients, such as N and Mg, leading to a reduction in chlorophyll synthesis and its concentration in the leaves [64,65]. Nevertheless, maize plants under the reduced water availability of ET_{70} maintained their chlorophyll content, which was comparable to the full irrigation treatment, ET_{100}. According to another study [66], a minimal decline in the chlorophyll content index was observed at a mild water stress of 60% of available water compared with a water stress of 45% of available water. In addition, water stress causes leaf senescence and reduces the chlorophyll content and photosynthesis, while any treatment that maintains the green color for a longer period can supply the developing kernels with photoassimilates for a longer time, thereby resulting in higher yields [67,68].

4.2.2. Photosynthetic Efficiency

In the present study, photosynthetic efficiency measured as chlorophyll fluorescence was affected by water availability and had the lowest values under the ET_{50} treatment. It was also affected by the growth stage, giving the highest values in the first growth stage. These results agree with other studies [69] that found that the chlorophyll fluorescence decreased with the decreasing availability of water. A higher chlorophyll fluorescence produced a higher grain yield, and is also thought to increase the sugar content in certain crops. Many reports suggested that using the analysis of chlorophyll 'a' fluorescence is considered a reliable method of determining the changes in the function of PSII under stress conditions [70,71]. Our results report reductions in F_v/F_m, F_v/F_0 and the performance index (PI) under deficit irrigation stress conditions, which were possibly due to the reduction in leaf photosynthetic pigments needed for photosynthesis. These results are in agreement with other studies [72,73]. Water stress may also reduce the photosynthesis rate through a direct influence on the metabolic and photochemical processes in the leaf, or an indirect influence on stomatal closure and the cessation of leaf growth, which results in a decreased leaf area [74].

4.2.3. CO_2 Assimilation Rate (A)

The CO_2 assimilation rate (A) was affected by the main effects of irrigation and growth stage, and also by the two-way interaction "irrigation × year". The lowest 'A' values, irrespective of the year, were observed under the treatment ET_{50}, while the highest values were found in the control treatment (ET_{100}). Similar results were reported by another study [75], in which it was found that the CO_2 assimilation rate was higher in the ET_{100} treatment than in the reduced irrigation treatment. This fact is likely due to the water stress on the plants, resulting in the closure of stomata, which reduces the CO_2 assimilation rate [76].

4.3. Energy Equivalent

The ratio output/input and energy efficiency input were affected by the main effects of "year" and "irrigation", and also by the two-way interaction "irrigation × year". The ratio of outputs/inputs in this study ranged from 1.43 to 1.90 in the different irrigation treatments, indicating that the ratio is low, a fact that shows that the inputs are not used efficiently [40,43,77]. The ratio of energy output/input for maize production in the present study is much lower than the results from another study [78], in which the ratio of energy outputs/inputs was 6.41. In this study, the ratio is low because of high energy consumption due to increased inputs (fertilizer, fuel, machinery, and irrigation water). Farmers, therefore, need to be trained in the efficient use of inputs in maize production, while maintaining high yields.

4.4. Carbon Footprint

During the experiment, the carbon footprint was affected by N fertilizer application, fuel, and electricity. Similar results were already reported for maize cultivation in terms of carbon footprint [46,79]. One study [80] reported that fertilizer application contributed to 60% of CO_2 emissions, and another [50] showed that N fertilizer inputs were the highest source of CO_2 emissions. Moreover, electricity showed different CO_2 emissions in each year, and in each treatment, due to the different amounts of water applied. In addition, it can be observed that during the second year, 2020, the CO_2 emissions were higher in all treatments, compared with those of the first year, 2019, because of the higher amount of water applied. Although chemical fertilizer application has the highest impact on the carbon footprint, fuel and electricity also contribute significantly and attention should, therefore, be paid to improving mechanical efficiency, irrigation as an application of electricity and fuel to the crop, and fertilizer efficiency, to reduce their contribution to the carbon footprint.

4.5. Silage Yield

Silage maize is one of the most important products of maize and is used as a livestock feed because of its positive characteristics, such as dry matter content, high concentration of nutrients, low buffering capacity, and high carbohydrate concentration for lactic acid fermentation [20,22]. The silage yield of maize plants was affected by irrigation treatments, the highest yields being found in the ET_{100} treatment, and the lowest yields being found in the ET_{50} treatment. Several studies evaluated the effect of deficit irrigation on maize by applying the drip-irrigation method [53,54,76]. More specifically, the ET_{100} treatment in all studies resulted in the highest yield, while the ET_{50} treatment produced the lowest, and the intermediate amount of water produced an acceptable yield. Moreover, the ET_{70} treatment produced a good yield, which means that when there is a shortage of water, farmers can apply less water but still obtain an acceptable silage yield. It can, therefore, be concluded that water availability has a significant effect on the silage yield of a crop of maize.

5. Conclusions

Maize is a crop species that requires a high amount of water due to its high production of dry matter and grain yield. In the present study, which was conducted in a commercial field in the area of Thessaloniki, it was found that water availability affects the morphological and physiological characteristics, and the silage yield, of maize plants. The control treatment (ET_{100}) had a positive effect on maize growth and yield, since an increase was found in all the characteristics studied, morphological, physiological, and agronomic. In contrast, however, under the treatments with the greatest water stress (ET_{50}), the lowest values were observed in all characteristics. The energy equivalent was low, suggesting that inputs are not used efficiently; moreover, inputs contribute largely to CO_2 emissions and, thus, to the carbon footprint of maize cultivation. The mild water stress, $ET_{70,}$ produced the best results of all the treatments, for the characteristics evaluated, maintaining the yield of maize. The results of this study can, therefore, be used by farmers in the Mediterranean area as they can maintain or improve their crop yield when water availability is limited. It is sometimes important to make a rational decision about the use of water, to protect water resources, while simultaneously contributing to reducing the impact of global climate change and maintaining crop productivity.

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