



Article

Supplemental Irrigation with Brackish Water Improves Carbon Assimilation and Water Use Efficiency in Maize under Tropical Dryland Conditions

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Abstract: Dry spells in rainfed agriculture lead to a significant reduction in crop yield or to total loss. Supplemental irrigation (SI) with brackish water can reduce the negative impacts of dry spells on net CO₂ assimilation in rainfed farming in semi-arid tropical regions and maintain crop productivity. Thus, the objective of this study was to evaluate the net carbon assimilation rates, indexes for water use efficiency, and indicators of salt and water stress in maize plants under different water scenarios, with and without supplemental irrigation with brackish water. The experiment followed a randomized block design in a split-plot design with four replications. The main plots simulated four water scenarios found in the Brazilian semi-arid region (Rainy, Normal, Drought, and Severe Drought), while the subplots were with or without supplemental irrigation using brackish water with an electrical conductivity of 4.5 dS m⁻¹. The dry spells reduced the photosynthetic capacity of maize, especially under the Drought (70% reduction) and Severe Drought scenarios (79% reduction), due to stomatal and nonstomatal effects. Supplemental irrigation with brackish water reduced plant water stress, averted the excessive accumulation of salts in the soil and sodium in the leaves, and improved CO₂ assimilation rates. The supplemental irrigation with brackish water also promoted an increase in the physical water productivity, reaching values 1.34, 1.91, and 3.03 times higher than treatment without SI for Normal, Drought, and Severe Drought scenarios, respectively. Thus, the use of brackish water represents an important strategy that can be employed in biosaline agriculture for tropical semi-arid regions, which are increasingly impacted by water shortage. Future studies are required to evaluate this strategy in other important crop systems under nonsimulated conditions, as well as the long-term effects of salts on different soil types in this region.

Keywords: tropical semi-arid; saline water; biosaline agriculture; complementary irrigation; water stress; photosynthesis



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1. Introduction

Irrigated agriculture is essential for crop production for human and animal consumption in semi-arid regions. However, the expansion of irrigation in these regions is generally limited by the scarcity of water resources, especially in years of drought [1]. On the other hand, rainfed agriculture is a high-risk activity in semi-arid environments, as observed in Northeastern Brazil, due to the high interannual variability and poor distribution of rainfall in space and time [2]. These risks can be minimized with the use of supplemental irrigation [3], a promising climate-smart practice for dryland agriculture [4] even when saline water sources are used [5–7].

The salinity of water and soil is a problem present on all continents, impacting ecosystems and agricultural activities, notably in arid and semi-arid regions [8]. However, the growing demand for food, the scarcity of water resources, and the overuse of groundwater under the ongoing scenario of global climate change have created the need to tap into saltwater resources to maintain food production and generate jobs and income for farmers in drylands. Therefore, it is necessary to use appropriate management techniques and salt-tolerant species, both aspects being part of biosaline agriculture [9,10].

Many studies have been carried out on the use of brackish water applied in a mixed or cyclic way as supplemental irrigation in rainfed farming [5,6,11,12]. The use of brackish water in irrigation can be beneficial for the Brazilian semi-arid region, given the large number of wells containing water with a moderate salt concentration [13]. Under such conditions, supplemental irrigation with brackish water may increase the possibilities of plant cultivation, especially on small farms [7], which predominate in the Brazilian semi-arid region.

From the point of view of plant physiology, it is known that leaf gas exchange, i.e., the loss of water vapor during the transpiration process and the CO_2 intake in the photosynthetic pathways, plays a fundamental role in crop yield. The reduction of leaf gas exchange under water shortages limits plant growth and contributes to low maize yields under rainfed farming in tropical semi-arid regions. In these cropping systems, photosynthesis decreases at different rates depending on the duration of dry spells, with the effects being more intense in years of drought and severe drought [14].

Supplemental irrigation can reduce the negative effects of dry spells on leaf gas exchange. However, the use of brackish water as supplemental irrigation can also impact photosynthetic rates, given both the osmotic and the toxic effects of salts accumulated in the soil [15]. These effects can be minimized by the fact that the salts applied during dry spells tend to be leached down during the rainy season, especially in soils with good natural drainage, thus having a negligible impact even on moderately salt-sensitive crops such as maize [7]. Thus, supplemental irrigation can also increase water productivity and allow the use of an alternative source of water (brackish water) with improved efficiency of irrigation management.

In this context, this research applied the hypothesis that the water stress associated with dry spells is more deleterious to the physiology and water use efficiency of maize plants than the salt stress associated with the use of brackish water in supplemental irrigation. Thus, the objective of the present study was to evaluate the net carbon assimilation rates and the indexes for water use efficiency in maize cultivated under different water scenarios in the Brazilian semi-arid region, with and without supplemental irrigation employing brackish water.

2. Material and Methods

The experiment was conducted in Fortaleza ($3^{\circ}74'$ S, $38^{\circ}58'$ W and altitude of 19 m), Ceará, Brazil in two cycles during the dry season: from 31 August to 21 November 2018, and from 28 September to 18 December 2019. During the experiment, the average, minimum and maximum air temperatures were, respectively, 27.6, 22.7, 30.6 °C (2018) and 27.2, 25.3, 30.1 °C (2019). The average air relative humidity was 70.7% for 2018 and 68.3% for 2019. The soil in the area is classified as a Ultisol, with a sandy loam texture in the 0–20 cm

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layer, pH 6.3, electrical conductivity of the saturation extract of soil 0.20 dS m⁻¹, and an exchangeable sodium percentage of 4.4.

The experiment followed a randomized block design in a split-plot arrangement with four replications. The main plots were designed to simulate the water supply in the soil corresponding to four rainfall scenarios—Rainy, Normal, Drought, and Severe Drought (simulations based on historical series of precipitation data for the rainfed cropping season in the Brazilian semi-arid region). The subplots were assigned to the use or lack of supplemental irrigation with brackish water (electrical conductivity of water—ECw = $4.5 \, \mathrm{dS \ m^{-1}}$). The sub-subplots were assigned to the sampling dates (27, 47, 49, 56, 60, and 67 days after planting). Each experimental plot consisted of six 10 m long rows and each subplot had three 10 m long rows, with a spacing of $0.80 \times 0.20 \, \mathrm{m}$ between rows and plants, respectively.

The water scenarios were defined according to rainfall and dry spell data for the semi-arid region of Vale do Curu, Ceará, Brazil, for February to May (the period of rainfed farming in the region). The climate of this region is very hot and semi-arid according to the Köppen classification, with annual rainfall and potential evapotranspiration of 800 mm and 1700 mm, respectively. For definition of the scenarios, historical series data for 30 years (1989 to 2019) of precipitation in this region were provided by the Foundation for Meteorology and Water Resources of Ceará (FUNCEME). Based on rainfall data during the rainy season (February to May) and the characterization of rainfall patterns in the region [16], the following scenarios were defined: Rainy (803 to 1040 mm), Normal (456 to 590 mm), Drought (383 to 443 mm), and Severe Drought (158 to 338 mm).

Supplemental applications of brackish water were estimated for dry spell periods, adding a leaching fraction of 0.20 in each irrigation event. The applied water depths were estimated based on the values of crop evapotranspiration [17]. In the periods without dry spells, irrigations were carried out using well water of low salinity ((pH 7.1, ECw = 0.9 dS m⁻¹, (SAR 3.9 mmol L⁻¹)^{0.5}). The brackish water (ECw = 4.5 dS m⁻¹) was prepared by adding NaCl, CaCl₂.2H₂O, and MgCl₂.6H₂O to the well water in the equivalent proportion of 7:2:1. This salt ratio is representative of the chemical composition of brackish waters in the Brazilian semi-arid region [18].

For each crop cycle, the total water depths applied, without and with supplemental irrigation, were as follows: 745 and 796 mm (Rainy), 465 and 567 mm (Normal), 345 and 517 mm (Drought), and 240 and 500 mm (Severe Drought). The dry spells were distributed in the water scenarios and their quantity and duration varied according to the rainfall patterns. Dry spells were defined as periods of at least five continuous days without rain [14]. Detailed information on water scenarios and supplemental irrigation was reported previously [7].

We used seeds of Hybrid BRS 2022 maize, a double hybrid with moderate resistance to diseases. To represent the reality of traditional family farming in the northeastern region of Brazil, sowing was carried out after applying a 30 mm water depth of low-salinity water. Fertilization with nitrogen (70 kg ha $^{-1}$), phosphorus (40 kg ha $^{-1}$ of P_2O_5), and potassium (20 kg ha $^{-1}$ of K_2O) was carried out according to the recommendations for rainfed maize cultivation in the State of Ceará [19]. The phosphorus dose (as simple superphosphate) was applied at planting and the nitrogen (as urea) and potassium (as potassium chloride) doses were split into three applications: one at planting and two as topdressing. Irrigation was performed by drip, using drip tapes with a flow rate of 2.7 L h $^{-1}$, with self-compensating emitters spaced 0.4 m apart.

Soil moisture was determined using the gravimetric method [20], with soil samples from the 0–20 cm layer collected at 47, 56, 60, and 67 days after planting in both cycles. For the determination of soil salinity, samples were collected from each subplot at the end of each crop cycle, from the 0–20 cm layer at three points of the central row: beginning, middle, and end. Soil electrical conductivity was measured in a soil:water suspension of $1:1 \ (v/v)$ and expressed in dS m⁻¹.

Measurements of leaf gas exchange were performed at 27, 47, 49, 56, 60, and 67 days after planting (Figure 1) using the third fully expanded leaf from the apex of the plant. The

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net photosynthesis rate A (µmol CO_2 m⁻² s⁻¹), stomatal conductance gs (mol m⁻² s⁻¹), transpiration rate E (mmol m⁻² s⁻¹), and internal CO_2 concentration Ci (µmol mol⁻¹) were measured using an infrared gas analyzer (Li–6400XT, LICOR, USA) under the following conditions: ambient air temperature, CO_2 concentration of 400 ppm, and photosynthetically active radiation of 1800 µmol m⁻² s⁻¹. The instantaneous water use efficiency (WUEi) was estimated using the photosynthesis and transpiration rate data.

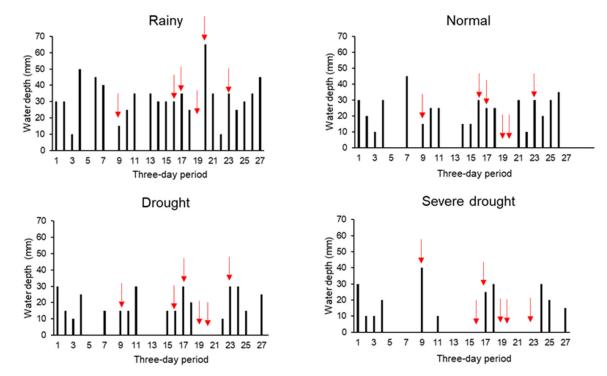


Figure 1. Water depths applied at three-day intervals in two maize crop cycles for different simulated water scenarios, without supplemental irrigation. The red arrows indicate the moments of leaf gas exchange measurements.

Samples of fully expanded leaf blades were collected at 47, 56, and 60 days after planting in both cycles for the determination of sodium and proline concentrations. The material was lyophilized and ground to obtain the extract, according to the method described by [21]. Sodium concentration was determined using a flame photometer. Free proline levels were determined according to a previously established method [22]. Proline readings were performed using a spectrophotometer (model UV—1650PC, Shimadzu, Japan).

At 82 days after planting, 15 plants were collected per subplot, and the production of dry ear biomass and total biomass was determined. Dry biomass productivity per hectare (ears and total) was estimated, taking into account the planting density and final stand. The physical water productivity (PWP, kg m $^{-3}$) was estimated using the relationship between the production of ears (PWP $_{\rm ear}$) or total dry biomass (PWP $_{\rm biomass}$) and the total volume of water applied (simulated rainfall plus supplementary irrigation), according to Equations (1) and (2) [1]:

$$PWP_{ear} = \frac{Biomass of ears (kg ha^{-1})}{Total water appied (m3 ha^{-1})}$$
(1)

$$PWP_{biomass} = \frac{\text{Total biomass of plants } \left(\text{kg ha}^{-1} \right)}{\text{Total water applied } (\text{m}^3 \text{ ha}^{-1})}$$
 (2)

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The efficiency of supplemental irrigation (WUE $_{SI}$) was estimated using the ratio between the increment of biomass (ears and total) and the volume of supplemental water applied, according to Equation (3):

$$WUE_{SI} = \frac{Y_{SI} - Y \left(kg \ ha^{-1} \right)}{Suplemental \ irrigation \ (m^3 \ ha^{-1})} \tag{3}$$

where Y_{SI} and Y represent the yields of plots with and without supplemental irrigation, respectively.

The data were submitted to the analysis of variance (F-test) after passing the Kolmogorov–Smirnov normality test. When the F-test determined statistical significance, the means were compared using the Tukey test (p < 0.05). Statistical analyses were performed using the Sisvar software version 5.6 [23].

3. Results and Discussion

3.1. Soil Moisture and Salinity

Soil moisture content was high throughout the crop cycle in the Rainy scenario, with a small difference between treatments with and without supplemental irrigation only on the last sampling date (Figure 2A). Moisture contents were close to the soil field capacity value (7.21%) on most sampling dates, regardless of supplemental irrigation. The excess water supplied to the soil in the Rainy scenario resulted in water accumulation in the soil profile, with part lost to runoff and part percolated [24–26]. The storage of water in the soil in this scenario favored the maintenance of moisture with minimal need for supplementation, since torrential rains are usually interspersed with medium and low-intensity rains under tropical semi-arid conditions.

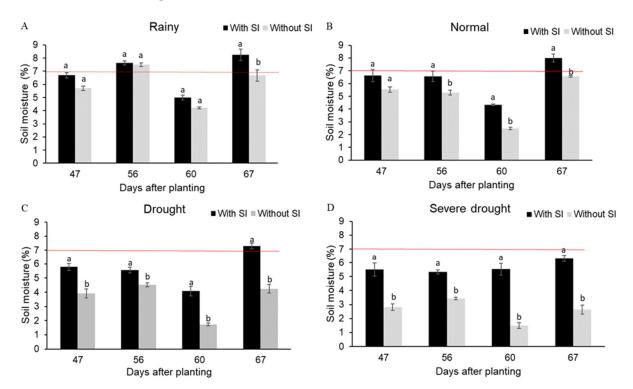


Figure 2. Soil moisture (layer from 0 to 20 cm) in areas cultivated with maize for the Rainy (**A**), Normal (**B**), Drought (**C**) and Severe drought (**D**) scenarios, showing sampling date and presence or absence of supplemental irrigation (SI) with brackish water (ECw = 4.5 dS m^{-1}). For each sampling date, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4). The red line in each figure represents the field capacity of the soil.

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For the Normal scenario, differences in soil moisture were observed on almost all sampling dates except for the first one, with higher values always recorded in the treatment with supplementation (Figure 2B). However, these differences were intensified in the Drought and Severe Drought scenarios (Figure 2C,D), taking as a reference the value of the soil field capacity. In the Severe Drought treatment, soil moisture was always above 5.0% with supplemental irrigation, while the values without supplementation ranged from 1.0 to 3.0%. Supplementation with brackish water in this scenario practically doubled the soil moisture content at 60 and 67 days after planting compared to the treatment without supplementation. These differences reflect the distribution of soil water application during maize crop cycles (Figure 1), which indicate situations of increasing water shortage for both Drought and Severe Drought scenarios.

The mean soil electrical conductivity (EC_{1:1}) increased significantly only with supplemental irrigation with brackish water for the Severe Drought scenario, reaching a mean value of 1.3 dS m⁻¹ towards the ends of the crop cycles (Figure 3). However, the values were relatively low compared to those obtained with continuous irrigation with similar water salinity [27–30]. These differences occurred because in supplemental irrigation with brackish water, part of the salt content was leached after the application of water of lower salinity, simulating rain events. These results also reflect the weighted salinity values of the irrigation water, which were 1.1, 1.5, 2.1, and 2.8 dS m⁻¹, respectively, for the Rainy, Normal, Drought, and Severe Drought scenarios. According to [28], only irrigation water with electrical conductivities higher than 2.2 dS m⁻¹ impacts the biomass production of maize plants under tropical conditions, indicating that brackish water supplementation may have little or no effect on maize crops.

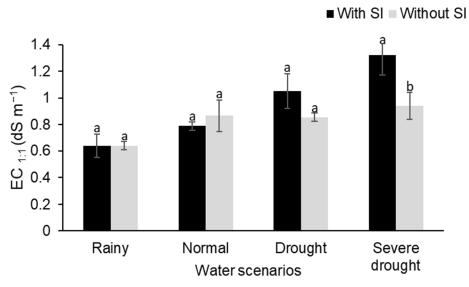


Figure 3. Soil electrical conductivity (soil:water extract 1:1) cultivated with maize under different water scenarios, with or without supplemental irrigation (SI) with brackish water (ECw = 4.5 dS m^{-1}). For each water scenario, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4).

It is worth noting that the well water used in this study had an electrical conductivity of $0.9~dS~m^{-1}$, much higher than the salinity of rainwater (less than $0.1~dS~m^{-1}$). This indicates that under real conditions, the effects of supplemental irrigation on the accumulation of salts in the soil may be even smaller than those observed in the present study. These results suggest that brackish water supplementation presents a small risk of soil salinization if the soil has good natural drainage. The adoption of practices such as leaching fractions and the application of amendments may enable the mitigation of negative effects of the application of brackish water on the soil under Severe Drought scenarios, thus preventing soil degradation [26,31–33].

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3.2. Leaf Gas Exchange

Maize photosynthetic rates reached values above $40~\mu mol~m^{-2}~s^{-1}$ (Figure 4), which are compatible with the photosynthetic metabolism of C4 species [34–36]. In the Rainy scenario, no difference was observed in photosynthetic rates between treatments with and without supplementation in the six evaluations, indicating the absence of water deficit and that supplemental irrigation would not be necessary in this scenario (Figure 4A). However, in the Normal scenario (Figure 4B), the need for supplemental irrigation during the dry periods, and in the reproductive stage (56 and 60 days after planting), was evident, based on the significant increase (2 to 3.5 times) observed for the rate of photosynthesis when compared to the treatment without supplementation.

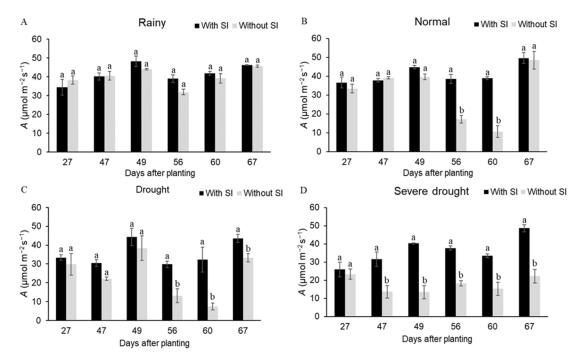


Figure 4. Net photosynthetic rate of maize leaves under Rainy (**A**), Normal (**B**), Drought (**C**) and Severe drought (**D**) scenarios, showing sampling date and presence or absence of supplemental irrigation (SI) with brackish water. For each sampling date, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4).

In the Drought scenario, supplemental irrigation was important in maintaining the photosynthetic rate on three (56, 60, and 67 days after planting) out of six sampling dates (Figure 4C), while in the Severe Drought scenario this was observed for five dates (47, 49, 56, 60, and 67 days after planting) (Figure 4D), reflecting the distribution of dry spells (Figure 1) and soil moisture (Figure 2). It should be noted that a reduction in the rate of photosynthesis can be caused by several biotic and abiotic constraints. From the physiological point of view, this reduction can be explained by stomatal limitations, reduction in chlorophyll concentration, photochemical damage, and inhibition of enzymatic activities [37–39].

The water deficit during the dry spells caused a significant reduction in stomatal conductance (Figure 5). Such a reduction in stomatal opening limits the influx of CO₂ during the photosynthetic process [37], decreasing the net carbon assimilation capacity, as observed in the present study (Figure 4). However, on some sampling dates, there was a concomitant reduction in the photosynthetic rate (Figure 4) and an increase in the internal CO₂ concentration (Figure 6), especially for treatments without supplemental irrigation (Drought and Severe Drought scenarios). This indicates that longer dry spells induced more severe water stress, which affected the photosynthetic metabolism. According to [33], even if there was enough substrate (CO₂) in the mesophyll in a situation of severe water shortage,

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there was a high degree of photosynthetic restriction in response to the deterioration of the photochemical and/or biochemical apparatus of the carbon assimilation process in chloroplasts.

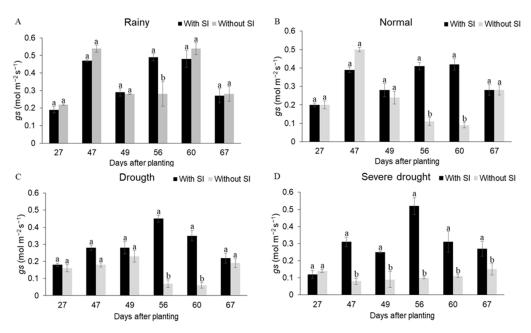


Figure 5. Stomatal conductance of maize leaves under Rainy (**A**), Normal (**B**), Drought (**C**) and Severe drought (**D**) scenarios, showing sampling date and presence or absence of supplemental irrigation (SI) with brackish water (ECw = 4.5 dS m^{-1}). For each sampling date, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4).

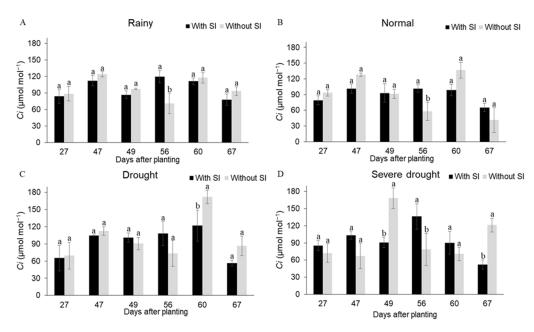


Figure 6. Internal CO₂ concentration of maize leaves under Rainy (**A**), Normal (**B**), Drought (**C**) and Severe drought (**D**) scenarios, showing sampling date and presence or absence of supplemental irrigation (SI) with brackish water (ECw = 4.5 dS m^{-1}). For each sampling date, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4).

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The transpiration rate data (Figure 7) show, in most cases, a similar behavior for the net photosynthetic rate (Figure 4), demonstrating strong stomatal limitations associated mainly with water deficit during dry spells. In the Rainy scenario, no difference was observed between treatments with and without brackish water supplementation and the values varied over time, possibly as a function of environmental changes such as air temperature and relative humidity. In the Severe Drought scenario, there were reductions on all sampling dates except for the one 27 days after planting, reflecting the response of the plants to soil moisture conditions (Figure 2).

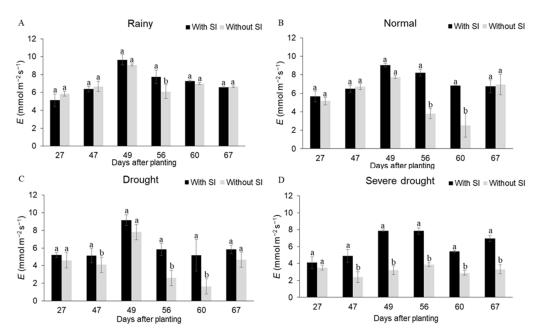


Figure 7. Transpiration rate of maize leaves under Rainy (**A**), Normal (**B**), Drought (**C**) and Severe drought (**D**) scenarios, showing sampling date and presence or absence of supplemental irrigation (SI) with brackish water of ECw = 4.5 dS m⁻¹. For each sampling date, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4).

The values of transpiration and net photosynthetic rates also showed a rapid recovery capacity for leaf gas exchange after a dry spell, as observed in the treatment without supplementation for both Normal and Drought scenarios. This ability to recover leaf gas exchange at the end of a dry spell has also been reported in cowpea grown under rainfed farming in tropical dryland even after low-intensity rainfall [14]. However, severe water deficits associated with long dry spells can cause permanent cellular damage, making a full recovery of physiological processes and plant growth impossible [33,37].

3.3. Indicators of Salt and Water Stress

Regression analysis relating soil moisture and salinity to plant response variables also demonstrated that supplemental irrigation with brackish water reduced the deleterious effects of water stress during dry spells without causing damage associated with salt stress. The reduction of soil moisture from 7 to 2% resulted in a 58% reduction in net photosynthetic rate (Figure 8A) and an 88% reduction in biomass production (Figure 8B), considering treatments with and without supplemental irrigation. In comparison, increasing the weighted salinity of irrigation water from 1.1 dS m $^{-1}$ (Rainy) to 2.8 dS m $^{-1}$ (Severe Drought) reduced the photosynthetic rate and the production of total biomass by only 14 and 11%, respectively (Figure 8C,D). These reductions are expected to be even smaller or nonexistent under field conditions, because if using rainwater (ECw = 0.1 dS m $^{-1}$) instead of low-salinity water (ECw = 0.9 dS m $^{-1}$), the weighted salinity associated with

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supplemental brackish water would be even smaller and would promote greater leaching of salts in the soil profile, as discussed earlier.

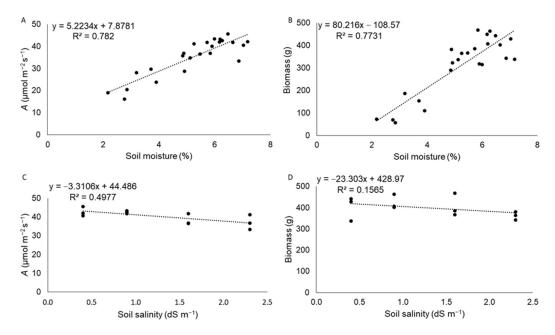


Figure 8. Photosynthetic rate A, and total biomass production per plant of maize as a function of soil moisture (**A**,**B**) and soil salinity (**C**,**D**).

The mineral analysis also suggested that the use of brackish water in supplemental irrigation did not result in an excessive accumulation of sodium in the leaves (Figure 9A). In the plots with supplemental irrigation, there was no increase in the sodium concentration in leaf tissues even when the weighted electrical conductivity of the irrigation water was increased from 1.1 dS m $^{-1}$ (Rainy) to 2.8 dS m $^{-1}$ (Severe Drought), and sodium contents were similar to those in the treatment without supplementation. Sodium contents were less than 2.5 g kg $^{-1}$, which did not result in any damage to the maize crop, as demonstrated by [40]. Irrigations with low-salinity water, simulating the occurrence of rain, reduced the accumulation of salts in the soil and consequently in the plants.

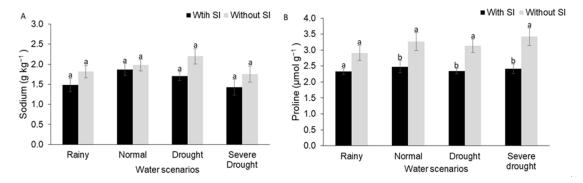


Figure 9. Sodium (**A**) and proline (**B**) concentrations in mature leaves of maize in response to presence or absence of supplemental irrigation (SI) with brackish water of ECw = 4.5 dS m⁻¹. Means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Sodium and proline concentrations represent the average of three sampling dates (47, 56, and 60 days after planting). Error bars represent the standard error of the mean (n = 4).

Proline concentration did not increase in treatments with supplemental irrigation (Figure 9B), suggesting that the salt stress caused by brackish water application (ECw = 4.5 dS m $^{-1}$) was not intense enough to promote proline accumulation. However, leaf concen-

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tration of proline was higher in treatments without supplementation than in treatments with supplementation for scenarios with increasing water shortage (Normal, Drought, and Severe Drought). These results indicate that proline accumulation was more affected by water stress than by salt accumulation in leaves of maize irrigated with brackish water. As for proline, although its accumulation is reported as a common response in plants under water and salt stress, there is controversy regarding the role played by this amino acid during stress. For many authors, the accumulation of proline is an important adaptive response for plants under stress, contributing to osmotic adjustment [41–44] and protecting cellular structures and functions [45–47]. For other authors, proline quantified using the method reported by [21] is an indication of osmoprotection associated with damage caused by abiotic constraints, as demonstrated for barley [48], cotton [49], and cowpea [50] under water deficits, and for rice [51] and sorghum [52,53] under salt stress. Our data presented here (Figure 9B) are in line with those who reported the highest accumulation of proline in response to damage caused by water stress [48,49], as revealed by soil moisture data (Figure 3) and net photosynthetic rates (Figure 4).

3.4. Water Use Efficiency

The similarity of the responses of net photosynthesis (Figure 4) and transpiration (Figure 7) rates resulted in minimal variations in instantaneous water use efficiency (WUEi) (Figure 10). There was also a similarity in the responses of plants under the different scenarios tested. In the present study, the lower values of WUEi in measurements performed 49, 56, and 60 days after planting may have been associated with the increase in transpiration rates in this period (Figure 7). Comparing treatments with and without supplemental irrigation with brackish water, a reduction in WUEi was observed only at 60 days after planting in the Normal and Drought scenarios, a result explained by the dramatic drop in the rate of photosynthesis during a dry spell (Figure 4).

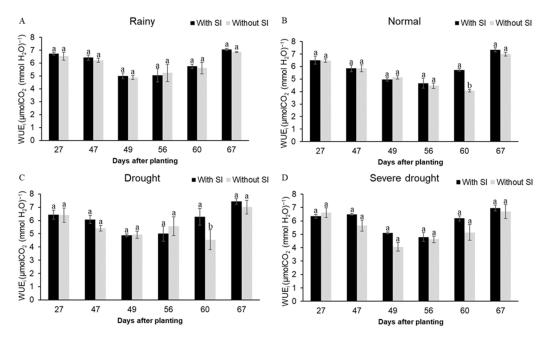


Figure 10. Instantaneous water use efficiency in maize leaves under Rainy (**A**), Normal (**B**), Drought (**C**) and Severe drought (**D**) scenarios, showing sampling dates and presence or absence of supplemental irrigation (SI) with brackish water of ECw = 4.5 dS m⁻¹. For each sampling date, means followed by the same letters do not differ ($p \ge 0.05$) from each other according to the Tukey test. Error bars represent the standard error of the mean (n = 4).

In the scenarios and treatments with and without supplementation, marked differences were observed in the indices of water use efficiency when considering ear or total biomass

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production as a function of the volume of water applied (Table 1). Our results suggest that supplemental irrigation did not significantly increase the physical water productivity (PWP) in the Rainy scenario. However, differences between treatments with and without supplementation were observed in both Drought and Severe Drought scenarios for both crop cycles. Values of PWP_{ear} and PWP_{biomass} were lower without supplemental irrigation compared to supplemental irrigation for Normal, Drought, and Severe Drought scenarios. Additionally, brackish water supplementation homogenized PWP in the different scenarios tested, with no differences between them. This indicates that supplemental irrigation in years of drought and severe drought allows for an improvement in water use efficiency so that it can be compared to the results obtained in the Normal scenario.

Table 1. Physical water productivity (PWP) based on dry ear production (PWP_{ear}), total dry biomass production (PWP_{biomass}), and supplemental irrigation efficiency (WUE_{SI}) in maize as a function of water scenarios with and without supplemental irrigation with brackish water (ECw = 4.5 dS m^{-1}).

Suppl. Irrigation	Simulated Water Scenarios			
	Rainy	Normal	Drought	Severe Drought
		PWP _{ear} (kg m ⁻³)		
		2018		
With	1.56 ± 0.27 Aa 1	$1.77\pm0.39~\mathrm{Aa}$	$2.16\pm0.58~\mathrm{Aa}$	$1.57\pm0.22~\mathrm{Aa}$
Without	$1.49\pm0.21~\mathrm{Aa}$	$1.38\pm0.26~\mathrm{Ba}$	$1.04\pm0.39~\mathrm{Bab}$	$0.72\pm0.21~\mathrm{Bb}$
		2019		
With	$1.14\pm0.25~\mathrm{Ab}$	$1.57\pm0.15~\mathrm{Aa}$	$1.43\pm0.24~\mathrm{Aa}$	$1.53\pm0.27~\mathrm{Aa}$
Without	$0.98 \pm 0.40~\mathrm{Aab}$	$1.13 \pm 0.55 \text{ Ba}$	$0.73\pm0.28~\mathrm{Bab}$	$0.23\pm0.24~\mathrm{Bb}$
		PWP _{biomass} (kg m ^{−3})		
		2018		
With	$2.78\pm0.36~\text{Ab}$	$3.96 \pm 0.60 \text{ Aa}$	$3.99 \pm 0.49 \text{ Aa}$	$2.87\pm0.61~\text{Ab}$
Without	$2.84\pm0.57~\mathrm{Aa}$	$2.70\pm0.47~\mathrm{Ba}$	$2.19\pm1.17~\mathrm{Bb}$	$1.10\pm0.46~\mathrm{Bc}$
		2019		
With	$2.29 \pm 0.20 \text{ Ab}$	$3.40 \pm 0.16 \text{ Aa}$	$3.31 \pm 0.22 \text{ Aa}$	$3.84\pm0.15~\mathrm{Aa}$
Without	$2.18\pm0.32~\text{Ab}$	$2.77\pm0.18~\mathrm{Aa}$	$1.62\pm0.35~\mathrm{Bb}$	$1.11\pm0.33~\mathrm{Bc}$
		WUE_{SI} (kg m ⁻³) #		
		Ear biomass		
	3.01 ± 0.37	3.53 ± 0.45	3.63 ± 0.11	2.53 ± 0.14
		Total Biomass		
	2.79 ± 0.30	7.99 ± 0.21	7.16 ± 0.19	5.44 ± 0.20

 $[\]overline{}$ Values represent the mean \pm standard error (n = 4). Means followed by the same uppercase letters in columns and lowercase letters in rows do not differ ($p \ge 0.05$) from each other according to Tukey's test. # Values represent the mean of the two maize cycles (n = 4).

When considering only the water use efficiency when supplementary irrigation was applied (WUE $_{\rm SI}$), the highest values were recorded for Normal and Drought scenarios for both ear and total biomass production. In general, the values of WUE $_{\rm SI}$ (Table 1) in the Normal, Drought, and Severe Drought scenarios were quite dramatic and higher than those obtained at the full irrigation depths (350 and 500 mm per cycle of green maize) [54,55], which were much higher than those used in supplemental irrigation in this research (102, 172 and 260 mm for Normal, Drought, and Severe Drought scenarios). However, there was a reduction of about 30% in WUE $_{\rm SI}$ in the Severe Drought scenario compared to the Drought scenario, which was due to the application of a greater depth of brackish water in the supplementation in the first scenario, and also to the small reduction in the biomass production observed in [7], possibly as a result of the accumulation of salts in the soil (Figure 3).

4. Conclusions

Rainfed agriculture in tropical semi-arid regions is limited by irregular rainfall during the rainy season. However, the Brazilian semi-arid region has a large number of wells with brackish water that could be used in supplemental irrigation, thus reducing the water stress in maize and other annual crops. Our results suggest that the water stress associated with dry spells is more deleterious to the carbon assimilation and water use efficiency of maize

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plants than the salt stress associated with the use of supplemental irrigation with brackish water. Our study showed that dry spells compromised the photosynthetic capacity of maize even under the Normal water scenario, but the effects became drastic under both Drought and Severe Drought scenarios due to stomatal and nonstomatal effects. Supplemental irrigation of maize with brackish water with an ECw = $4.5 \, dS \, m^{-1}$ reduced water stress and did not result in excessive salt accumulation in the sandy loam soil used in this study.

The use of brackish water did not lead to sodium accumulation in leaves and improved leaf gas exchange, with a positive impact on the ${\rm CO_2}$ assimilation rate. Thus, supplemental irrigation with brackish water resulted in an increase in water use efficiency in different scenarios under water restriction, constituting an important strategy that can be applied in biosaline agriculture in tropical semi-arid regions to maintain crop yields, especially in years of drought and severe drought. Considering the great spatial variability of rainfall in tropical semi-arid regions and the increase in drought years associated with global climate change scenarios, future studies are required to evaluate this strategy in other important crop systems under nonsimulated conditions, as well as the long-term effects of these salts on different soil types in this region.

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References

- 1. Frizzone, J.A.; Lima, S.C.R.V.; Lacerda, C.F.; Mateos, L. Socio-economic indexes for water use in irrigation in a representative basin of the tropical semiarid region. *Water* **2021**, *13*, 2643. [CrossRef]
- 2. Marengo, J.A.; Torres, R.R.; Alves, L.M. Drought in northeast Brazil: Past, present and future. *Theor. Appl. Climatol.* **2017**, 129, 1189–1200. [CrossRef]
- 3. Ali, A.B.M.; Shuang-En, Y.U.; Panda, S.; Guang-Cheng, S. Water harvesting techniques and supplemental irrigation impact on sorghum production. *J. Sci. Food Agric.* **2015**, *95*, 3107–3116. [CrossRef] [PubMed]
- 4. Nangia, V.; Oweis, T.; Kemeze, F.H.; Schnetzer, J. Supplemental irrigation: A promising climate-smart practice for dryland agriculture. *Wagening*. *CGIAR/CCAFS* 2018. Available online: https://cgspace.cgiar.org/bitstream/handle/10568/92142/GACSA%20Practice%20Brief%20Supplemental%20Irrigation.pdf (accessed on 20 November 2020).
- 5. Hamdy, A.; Sardob, V.; Ghanem, K.A.F. Saline water in supplemental irrigation of wheat and barley under rainfed agriculture. *Agric. Water Manag.* **2005**, *78*, 122–127. [CrossRef]
- 6. Chauhan, C.P.S.; Singh, R.B.; Gupta, S.K. Supplemental irrigation of wheat with saline water. *Agric. Water Manag.* **2008**, 95, 253–258. [CrossRef]
- 7. Cavalcante, E.S.; Lacerda, C.F.; Costa, R.N.T.; Gheyi, H.R.; Pinho, L.L.; Bezerra, F.M.S.; Oliveira, A.C.; Canjá, J.F. Supplemental irrigation using brackish water on maize in tropical semi-arid regions of Brazil: Yield and economic analysis. *Sci. Agric.* **2021**, *78*, 1–9. [CrossRef]
- 8. Food and Agriculture Organization of the United Nations (FAO). 1.5 Billion People, Living with Soil too Salty to be Fertile. 2021. Available online: https://news.un.org/en/story/2021/10/1103532 (accessed on 15 November 2021).

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9. Masters, D.G.; Benes, S.E.; Norman, H.C. Biosaline agriculture for forage and livestock production. *Agric. Ecosyst. Environ.* **2007**, 119, 234–248. [CrossRef]

- 10. Silva, J.E.S.B.; Matias, J.R.; Guirra, K.S.; Aragão, C.A.; Araujo, G.G.L.; Dantas, B.F. Development of seedlings of watermelon cv. Crimson Sweet irrigated with biosaline water. *Rev. Bras. De Eng. Agrícola E Ambient.* **2015**, *19*, 835–840. [CrossRef]
- 11. Hassanli, M.; Ebrahimian, H. Cyclic use of saline and non-saline water to increase water use efficiency and soil sustainability on drip irrigated maize. *Span. J. Agric. Res.* **2016**, *14*, e1204. [CrossRef]
- 12. Kiani, A.R.; Mosavata, A. Effect of different alternate irrigation strategies using saline and non-saline water on corn yield, salinity and moisture distribution in soil profile. *J. Water Soil* **2016**, *30*, 1595–1606.
- 13. Silva, F.J.A.; Araújo, A.L.; Souza, R.O. Águas subterrâneas no Ceará—Poços instalados e salinidade. Rev. Tecnol. 2007, 28, 136–159.
- 14. Fernandes, F.B.P.; Lacerda, C.F.; Andrade, E.M.; Neves, A.L.R.; Sousa, C.H.C. Efeito de manejos do solo no déficit hídrico, trocas gasosas e rendimento do feijão-de-corda no semiárido. *Rev. Ciência Agronômica* **2015**, *46*, 506–515.
- 15. Munns, R.; Tester, M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 2008, 59, 651–681. [CrossRef] [PubMed]
- 16. Xavier, T.M.B.S. Tempo de Chuva: Estudos Climáticos e de Previsão para o Ceará e Nordeste Setentrional; Editora ABC: Ceará, Brazil, 2001; 478p.
- 17. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. Crop Evapotranspiration Guidelines for Computing Crop Water Requirements. *Rome, FAO—Irrigation and Drainage*; FAO: Rome, Italy, 1998; Volume 300, p. 56.
- 18. Medeiros, J.F. Qualidade da água de irrigação e evolução da salinidade nas propriedades assistidas pelo "GAT" nos Estados do RN, PB e CE. **1992**. 173 f. Dissertação (Mestrado em Engenharia Agrícola)—Universidade Federal da Paraíba. Available online: http://dspace.sti.ufcg.edu.br:8080/jspui/bitstream/riufcg/2896/3/JOS%c3%89%20FRANCISMAR%20DE%20 MEDEIROS%20-%20DISSERTA%c3%87%c3%88O%20PPGEA%201992.pdf (accessed on 15 January 2020).
- 19. Fernandes, F.H.F.; Aquino, A.B.; Aquino, B.F.; Holanda, F.J.M.; Freire, J.M.; Crisostomo, L.A.; Costa, R.I.; Uchoa, S.C.P.; Fernandes, V.L.B. *Recomendações De Adubação E Calagem Para O Estado Do Ceara*; Editora ABC: Fortaleza, Brazil, 1993; 247p.
- Embrapa—Empresa Brasileira de Pesquisa Agropecuária. Manual de métodos de análise de solo. 1997, 212p. Available online: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/173611/1/Pt-2-Cap-20-Sais-soluveis.pdf (accessed on 8 November 2020).
- 21. Cataldo, J.M.; Haroom, M.; Schrader, L.E.; Youngs, V.L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. [CrossRef]
- 22. Bates, L.S.; Waldren, R.P.; Teare, J.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
- 23. Ferreira, D.F. Sisvar: Um sistema computacional de análise estatística. Ciência E Agrotecnologia 2011, 35, 1039–1042. [CrossRef]
- 24. Hillel, D. Fundamentals of Soil Physics; Academic Press: London, UK, 1980; 413p.
- 25. Guerra, H.C. Física Dos Solos; UFPB: Campina Grande, Brazil, 2000; 173p.
- 26. Ngolo, A.O.; Oliveira, M.F.; Assis, I.R.; Rocha, G.C.; Fernandes, R.B.A. Soil physical quality after 21 years of cultivation in a Brazilian Cerrado Latosol. *J. Agric. Sci.* **2019**, *11*, 124–136. [CrossRef]
- 27. Assis Junior, J.O.; Lacerda, C.F.; Silva, F.B.; Silva, F.L.B.; Bezerra, M.A.; Gheyi, H.R. Produtividade do feijão-de-corda e acúmulo de sais no solo em função da fração de lixiviação e da salinidade da água de irrigação. *Eng. Agrícola* **2007**, 27, 702–713. [CrossRef]
- 28. Bezerra, A.K.P.; Lacerda, C.F.; Hernandez, F.F.F.; Silva, F.B.; Gheyi, H.R. Rotação cultural feijão caupi/milho utilizando-se águas de salinidades diferentes. *Ciência Rural* **2010**, *40*, 1075–1082. [CrossRef]
- 29. Lacerda, C.F.; Sousa, G.G.; Silva, F.L.B.; Guimarães, F.V.A.; Silva, G.L.; Cavalcante, L.F. Soil salinization and maize and cowpea yield in the crop rotation system using saline waters. *Eng. Agrícola* **2011**, *31*, 663–675. [CrossRef]
- 30. Neves, A.L.R.; Lacerda, C.F.; Sousa, C.H.C.; Silva, F.L.B.; Gheyi, H.R.; Ferreira, F.J.; Andrade Filho, F.L. Growth and yield of cowpea/sunflower crop rotation under different irrigation management strategies with saline water. *Ciência Rural* **2015**, 45, 814–820. [CrossRef]
- 31. Rhoades, J.D.; Loveday, J. Salinity in irrigated agriculture. In *Irrigation of Agricultural Cropsstewart*; Stewart, D.R., Nielsen, D.R., Eds.; American Society of Agronomy: Madison, WI, USA, 1990; pp. 1089–1142.
- 32. Ayers, R.S.; Westcot, D.W. A Qualidade De Água Na Agricultura. Estudos FAO: Irrigação E Drenagem, 29, 2nd ed.; UFPB: Campina Grande, Brazil, 1999; p. 153.
- 33. Cavalcante, L.F.; Santos, R.V.; Hernandez, F.F.F.; Gheyi, H.R.; Dias, T.J.; Nunes, J.C.; Lima, G.S. Recuperação de solos afetados por sais. In *Manejo Da Salinidade Na Agricultura: Estudos Básicos E Aplicados*; Gheyi, H.R., Dias, N.S., Lacerda, C.F., Gomes Filho, E., Eds.; INCTSal: Fortaleza, Brazil, 2016; Volume 28, pp. 461–477.
- 34. Larcher, W. *Ecofisiologia Vegetal. São Carlos, Rima, Artes E Textos*; Tradução do orignal: Okoplysiologe der, Brazil, 2000; 531 p, ISBN 8586552038.
- 35. Osborne, C.P.; Sack, L. Evolution of C4 plants: A new hypothesis for an interaction of CO₂ and water relations mediated by plant hydraulics. *Philos. Trans. R. Soc.* **2012**, 367, 583–600. [CrossRef] [PubMed]
- 36. Souza, M.L.C.; Silva, A.Z. Starling, C.; Machuca, L.M.R.; Zuñiga, E.A.; Galvão, Í.; Jesus, G.J.; Broetto, F. Biochemical parameters and physiological changes in maize plants submitted to water deficiency. *SN Appl. Sci.* **2020**, *2*, 1–9. [CrossRef]
- 37. Rahnama, A.; James, R.A.; Poustini, K.; Munns, R. Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil. *Funct. Plant Biol.* **2010**, *7*, 255–269. [CrossRef]
- 38. Taiz, L.; Zeiger, E.; Moller, I.M.; Murphy, A. Fisiologia E Desenvolvimento Vegetal; Editora Artmedl: Porto Alegre, Brazil, 2017; 888p.

Agriculture **2022**, 12, 544 15 of 15

39. Lacerda, C.F.; Oliveira, E. Victor.; Neves, A.L.R.; Gheyi, H.R.; Bezerra, M.A.; Costa, C.A.G. Morphophysiological responses and mechanisms of salt tolerance in four ornamental perennial species under tropical climate. *Rev. Bras. De Eng. Agrícola E Ambient.* **2020**, *24*, 656–663. [CrossRef]

- 40. Barbosa, F.S.A.; Lacerda, C.F.; Gheyi, H.R.; Farias, G.C.; Silva Junior, R.J.C.; Lage, Y.A.; Hernandez, F.F.F. Yield and ion content in maize irrigated with saline water in a continuous or alternating system. *Ciência Rural* **2012**, 42, 1731–1737. [CrossRef]
- 41. Verslues, P.E.; Bray, E.A. Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA—Insensitive loci in low water potential-induced ABA and proline accumulation. *J. Exp. Bot.* **2006**, *57*, 201–212. [CrossRef]
- 42. Chen, Z.; Cuin, T.A.; Zhou, M.; Twomey, A.; Naidu, B.P.; Shabala, S. Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *J. Exp. Bot.* **2007**, *58*, 4245–4255. [CrossRef]
- 43. Chun, S.C.; Paramasivan, M.; Chandrasekaran, M. Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Front. Microbiol.* **2018**, *9*, 2525. [CrossRef]
- 44. Li, J.; Ma, J.; Guo, H.; Zong, J.; Chen, J.; Wang, Y.; Li, D.; Li, L.; Wang, J.; Liu, J. Growth and physiological responses of two phenotypically distinct accessions of centipede grass (*Eremochloa ophiuroides* (Munro) Hack.) to salt stress. *Plant Physiol. Biochem.* 2018, 126, 1–10. [CrossRef] [PubMed]
- 45. Kishor, P.B.K.; Hima Kumari, P.; Sunita, M.S.L.; Sreenivasulu, N. Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. *Front. Plant Sci.* **2015**, *6*, 1–17. [CrossRef] [PubMed]
- 46. Wang, H.; Tang, X.; Wang, H.; Shao, H.B. Proline accumulation and metabolism-related genes expression profiles in *Kosteletzkya virginica* seedlings under salt stress. *Front. Plant Sci.* **2015**, *6*, 792. [CrossRef] [PubMed]
- 47. Zhang, L.; Becker, D.F. Connecting proline metabolism and signaling pathways in plant senescence. *Front. Plant Sci.* **2015**, *6*, 552. [CrossRef] [PubMed]
- 48. Hanson, A.; Nelsen, C.E.; Everson, E.H. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. *Crop Sci.* **1977**, 17, 720–726. [CrossRef]
- 49. Ferreira, L.G.R.; Souza, J.G.; Prisco, J.T. Effects of water deficit on proline accumulation and growth of two cotton genotypes of differing drought resistance. *Z. Pflanzenphysiologie*. **1979**, 93, 189–199. [CrossRef]
- 50. Melo, A.S.; Melo, Y.L.; Lacerda, C.F.; Viégas, P.R.A.; Ferraz, R.L.S.; Gheyi, H.R. Water restriction in cowpea plants [Vigna unguiculata (L.) Walp.]: Metabolic changes and tolerance induction. Rev. Bras. De Eng. Agrícola E Ambient. 2022, 26, 190–197. [CrossRef]
- 51. Lutts, S.; Kinet, J.M.; Bouharmont, J. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza Sativa L.*) cultivars differing in salinity resistance. *Plant Growth Regul.* **1996**, *19*, 207–218. [CrossRef]
- 52. Lacerda, C.F.; Cambraia, J.; Cano, M.A.O.; Ruiz, H.A.; Prisco, J.T. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ. Exp. Bot.* **2003**, *49*, 107–120. [CrossRef]
- 53. Lacerda, C.F.; Cambraia, J.; Cano, M.A.O.; Prisco, J.T. Proline accumulation in sorghum leaves is enhanced by salt-induced tissue dehydration. *Rev. Ciência Agronômica* **2006**, *37*, 110–112.
- 54. Embrapa—Empresa Brasileira de Pesquisa Agropecuária. A Cultura Do Milho-Verde. 2008. Available online: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/11921/2/00082390.pdf (accessed on 10 November 2020).
- 55. Pereira Filho, I.A.; Silva, A.R.; Costa, R.V.; Cruz, I. Milho Verde. 2010. Available online: https://www.agencia.cnptia.embrapa.br/gestor/milho/arvore/CONT000fy779fnk02wx5ok0pvo4k3c1v9rbg.html (accessed on 10 November 2020).