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Applying Biostimulants to Combat Water Deficit in Crop Plants: Research and Debate

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Abstract: Climate change has increased the severity of drought episodes by further reducing precipitation in vulnerable zones. Drought induces a substantial decrease in agricultural water, reducing crop yields. Consequently, addressing water consumption can increase farmers' profits. This work describes lab-to-field research in *Zea mays*, using two biostimulants: glycine betaine (GB) and L-pyroglutamic acid (PG). The biostimulant optimal dosages were selected using a hydroponic system with 20% polyethylene glycol and nursery experiments under water-deficit irrigation. The established dosages were evaluated in field trials in which irrigation was reduced by 20%. Laboratory biostimulant optimisation showed in stressed treated seedlings (GB 0.1 mM; PG 1 mM) an increased dry weight, relative growth rate and water use efficiency, reducing seedling growth loss between 65 and 85%, respectively. Field trials using a GB-optimised dosage showed an increase in plants' growth, grain yield and flour Ca content. In addition, grain flour carbohydrate content and protein remained similar to control well-watered plants. Finally, the economic aspects of biostimulant treatments, water consumption, water sources (ground vs. desalinated) and grain biomass were addressed. Overall, GB treatment demonstrated to be a valuable tool to reduce water consumption and improve farmers' earnings.

Keywords: water deficit; biostimulants; pyroglutamic acid; glycine betaine; climate change

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1. Introduction

Drought is considered the greatest threat to farmers growing field crops, the frequency and severity of which has increased worldwide [1]. Agriculture and water resources in an overwarming scenario have been the subject of research [2,3], with direct losses suffered by 1.5 billion people being estimated at US $124 billion from 1998 to 2017 [4]. Therefore, water scarcity is a critical concern in agriculture due to its direct impact on crop yield, which directly affects the worldwide economy [5]. Water supply can be achieved using new technologies—for example, desalination facilities [6], particularly in the Mediterranean region [7]. Beyond some other disadvantages amplified by the public, environmentalists and opinion [8], water acquisition via desalination technology is twice as expensive as from groundwater [9]. This higher cost is an impediment for farmers, who resultantly cannot gain their deserved profits from the activity. Crop water management is therefore one of the most important objectives to achieve in the present high drought-risk scenario.

Biostimulants are presented as a potential new way to aid in water management [10–12], reducing field productivity losses [13]. They are compatible with the European eco-friendly philosophy [14] because most of the compounds used are from natural sources [15] and easily degraded in soil [16]. This has doubtlessly encouraged interest from the agrochemical industry in the biostimulants field. Indeed, it is an important investment hotspot with tremendous economic potential as is evident from a global market estimation expected to reach US $4.14 billion by 2025 [17]. The application of biostimulants in stress mitigation is widely reviewed in the literature, but their effects on production figures are not usually assayed quantitatively [10].

Glycine betaine (GB) is a compatible solute that is accumulated in many plants. It is normally used to increase tolerance against abiotic stresses, such as freezing, salinity and drought stress [18]. Foliar application of GB results in rapid uptake, translocating to different plant organs [18], enhancing antioxidant defence [19,20], leaf gas-exchange attributes [21,22] and growth under stress conditions [22]. A recent publication shows how a foliar application of 11.5 g L^{-1} can increase yield [23]. L-pyroglutamic acid (PG) is another interesting biostimulant. It is a non-proteinogenic amino acid [24], and this group has recently shown potential as a source of new biostimulants [10]. To date, PG has been scarcely studied as a biostimulant but is at least able to alleviate a water deficit in lettuce using a root treatment [13].

The focus of the present study is to evaluate the suitability of using PG and GB to reduce economic water deficit losses in maize. Doses were optimised for root treatment and physiological and productive traits analysed. After testing if biostimulants can be used to reduce water consumption, the suitability of applying them as a tool is discussed to thus improve farmers' earnings from their harvest.

2. Materials and Methods

2.1. Plant Material

A local forage variety of maize from Gran Canaria Island (*Zea mays* L. c.v. Lechucilla) was provided by a local plant nursery in a 150-socket nursery tray. One week after sowing, plants were placed in a growth chamber in controlled conditions at 22 °C, 16 h light (300–400 µmol m^{-1} s^{-1}) and 60–70% relative humidity. Plants in the V1 stage were used for dose optimisation experiments, and those in the V2 stage were transplanted out for field experiments [25].

2.2. Dosage Optimisation

L-pyroglutamic acid (CAs number: 98-79-3) and glycine betaine (CAs number: 590-46-5) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). Doses of GB and PG were optimised using two approaches—hydroponic culture and direct application under two watering regimes in the nursery. Both experiments were repeated twice.

Hydroponic culture was employed as previously described but using maize with 20% polyethylene glycol (PEG) as a stressor instead of tomato with salt [26]. The treatment was applied for 24 h directly to the roots using 0.1, 1, 2.5, 5 and 10 mM GB or PG dissolved in distilled water. Then, the plants were placed again in the hydroponic buckets for the next 24 h. After this time, the medium was changed, and by adding 20% PEG, the onset of the stress was triggered. A control group without any treatment or PEG was used as a reference of normal maize growth. Ten plants were weighed at the beginning of the experiment to set up T_0, and the others submitted to different conditions were weighed after one week of growth. Plants were oven-dried at 65 °C for two days and used to calculate the RGR [27]. The concentrations that achieved the best result for this parameter and the consecutive lower doses were used in the followed experiment.

The nursery experiment ensured the suitability of the plant for root treatment. Using the nursery mentioned above, 20 plants were used for each condition. Treatments were applied directly to the roots of V1 plants and consisted of 5 mL of a half-concentration Hoagland solution [28] containing the biostimulants at two concentrations: GB at 0.1 and 0.05 mM and PG at 2.5 and 1 mM. The water-deficit experiment consisted of watering all treated plants and a control without biostimulants with half the amount of water necessary to reach field soil capacity. To compare normal growth rates, another 20 untreated plants were watered to full soil capacity. The parameters measured were as follows. (1) Dried plant weight after 48 h in the oven. (2) Relative growth rate (RGR) [27], RGR = (ln DW 2 − ln DW 1)/(t2 − t1), where DW 1 and DW 2 were seedling dry weights at times t1 and t2 (t1 was the beginning of water deficit and t2 the end of the water deficit). Plant water-use efficiency (WUE) considering all the water used over the experiment timespan, WUE = plant biomass/water used [29] and the weight reduction with respect to control were each calculated using the well-watered untreated plants.

2.3. Field Experiment

Maize plants at the V2 stage were used in this study. The field trial was conducted at Escuela de Capacitación Agraria de Tacoronte, Tenerife, Canary Islands (28°29′47.0″ N 16°25′12.0″ W) during the months of June to August. The hydroponic experiment was in a greenhouse sectored into blocks equipped with a drip irrigation system. During the experimental periods, average daily maximum and minimum temperatures were 30 °C and 22 °C, respectively, with an average relative humidity of 80%. The soil at the site was classified as clay-loam (35% clay, 27% silt, 38% sand). The experiments were performed in randomised 20 m^2 blocks with three replications, with each block containing 80 plants. Irrigation volumes were calculated according to the FAO [30], taking into account the evapotranspiration rate (ET_o) provided by a nearby meteorological station, property of the island council, Cabildo de Tenerife. Soil humidity was measured near the roots of the plants within the wet bulb (TEROS 12 sensor from PESSL INSTRUMENTS GmbH, Weiz, Austria). Two irrigation regimes were established 30 days after transplanting: control (WW, 100% field capacity) and deficit irrigation (WD, 20% less than the control) and separated into two different blocks. Treatments consisted of 20mL of 1 mM PG (CAS number: 149-87-1) or 20 mL of 0.05 mM GB (CAS number: 590-46-5) purchased from Aldrich Chemical Co. (St. Louis, MO, USA), applied directly to the root system (Table 1). The treatment was two weeks after transplanting and was repeated two weeks later at the start of the water deficit regime. Irrigation restriction was continued until harvesting the cobs 45 days later.

Table 1. Treatment and water conditions summary.

Treatment	Water Regime	
	100%	**80%**
None	WW	WD
L-pyroglutamic acid 1 mM	WW-PG	WD-PG
Glycine betaine 0.1 mM	WW-GB	WD-GB

2.4. Growth, Yield and Water Status Measures

After forty-five days, the number of maize cobs per plant was counted for each condition; ten random plants from each condition were selected to measure the length and width of the last fully developed leaf. From those plants, the cobs were selected and harvested for yield measurements and dried in an oven at 65 °C for 1 week in order to avoid bias due to different moisture contents. Yield parameters measured were cob weight, weights of 100 grains (in triplicate from each cob) and weight of all grains. The average weight of grains per cob and the average number of cobs (Table 2) were used to calculate the total grain mass per ha, using as a reference 50,000 maize plants per hectare (Table 3). The result obtained was used to calculate the grain water-use efficiency (WUEg) as the ratio of the mass of grain produced to the water use throughout the growing period [31].

Table 2. Growth and yield parameters in greenhouse experiment.

Treatment	Leaf Lenght (cm)	Leaf Width (cm)	Grain Number	100 Grain Weight (g)	All Grain Weight (g)
WW	77.6 ± 19.6 [a]	7.8 ± 1.1 [a]	1.2 ± 0.4 [a]	10.3 ± 0.5 [a]	37.9 ± 5.5 [a]
WD	66.4 ± 17.3 [b]	6.9 ± 1.2 [b]	0.8 ± 0.6 [b]	8.3 ± 1.4 [b]	29.2 ± 8.7 [b]
WW-GB	73.1 ± 13.1 [ab]	7.9 ± 1.2 [a]	1.2 ± 0.3 [a]	11.2 ± 1.3 [c]	42.2 ± 8.5 [a]
WD-GB	69.2 ± 11.8 [ab]	7.5 ± 1 [a]	1.1 ± 0.1 [a]	10.5 ± 1.1 [ac]	37.9 ± 11.2 [a]
WW-PG	70.2 ± 13.5 [ab]	7.5 ± 1.3 [a]	1.1 ± 0.3 [a]	9.8 ± 1 [a]	35.2 ± 2.1 [a]
WD-PG	69.6 ± 13.3 [ab]	7.2 ± 1.1 [ab]	1.1 ± 0.1 [a]	9.7 ± 1 [a]	35.1 ± 2.7 [a]

Values followed by the same letter means no significant differences at $p < 0.05$.

Table 3. Yield and grain water use efficiency.

Treatment	Grain Mass (kg/ha)	WUEg (kg ha/m^3)
WW	2274	0.82
WD	1168	0.52
WW-GB	2321	0.91
WD-GB	2084	0.94
WW-PG	1936	0.70
WD-PG	1930	0.87

After fifteen and thirty days of the water regime, relative water content (RWC) [32] was calculated from twenty excised 1 cm diameter discs for each treatment. We weighed all leaf discs immediately to provide a measure of fresh mass (Wf), then soaked them 24 h in deionised water and re-weighed the resultant turgid mass (Wt). After drying at 85 °C, discs were again weighed to establish the dry-mass (Wd). RWC for each leaf was calculated according to: RWC = (Wf − Wd)/(Wt − Wd).

2.5. Protein, Carbohydrate, Fat and Mineral Determinations from the Maize Flour

Total protein content was determined from total nitrogen by the Kjeldahl Method [33] multiplying by the coefficient 6.25. Total carbohydrates were quantified by the Phenol Sulphuric Acid method using a multiplate protocol as described in [13]. All measurements were repeated four times, and the mean plus the standard deviation was the value used.

From the selected cobs, the grains were ground to a fine powder for the mineral analysis (Ca, Mg, K, P, Na, Cu, Zn and Fe). One gram of this maize flour was taken from each sample, converted to ash in a muffle stove at 480 °C and mineralised by the dry method with 6 N HCl. This extract was determined by ICP OES Avio 500 (Perkin Elmer, Waltham, MA, USA). All measurements were done in triplicate.

2.6. Statistical Procedures

Statistical analyses for growth experiments were performed by a one-way ANOVA ($\alpha = 0.05$). The significance of differences between experimental groups was calculated using a Tamhane post-hoc test.

3. Results

3.1. Glycine Betaine and L-Pyroglutamic Acid Improve Drought Tolerance under Water Deficit Stress

Doses were optimised using two different approaches—first of all using hydroponic culture plus 20% PEG. As shown in Figure 1, plants subjected to stress decreased significantly in growth by 33%. However, a clear dose-dependent reduction in the difference from the control plants was detected after GB treatment, the best treatment concentration being 0.1 mM, which increased tolerance to water deficit by 77%. In contrast, PG needed higher doses to increase tolerance by 80%, with its best treatment at 1 mM.

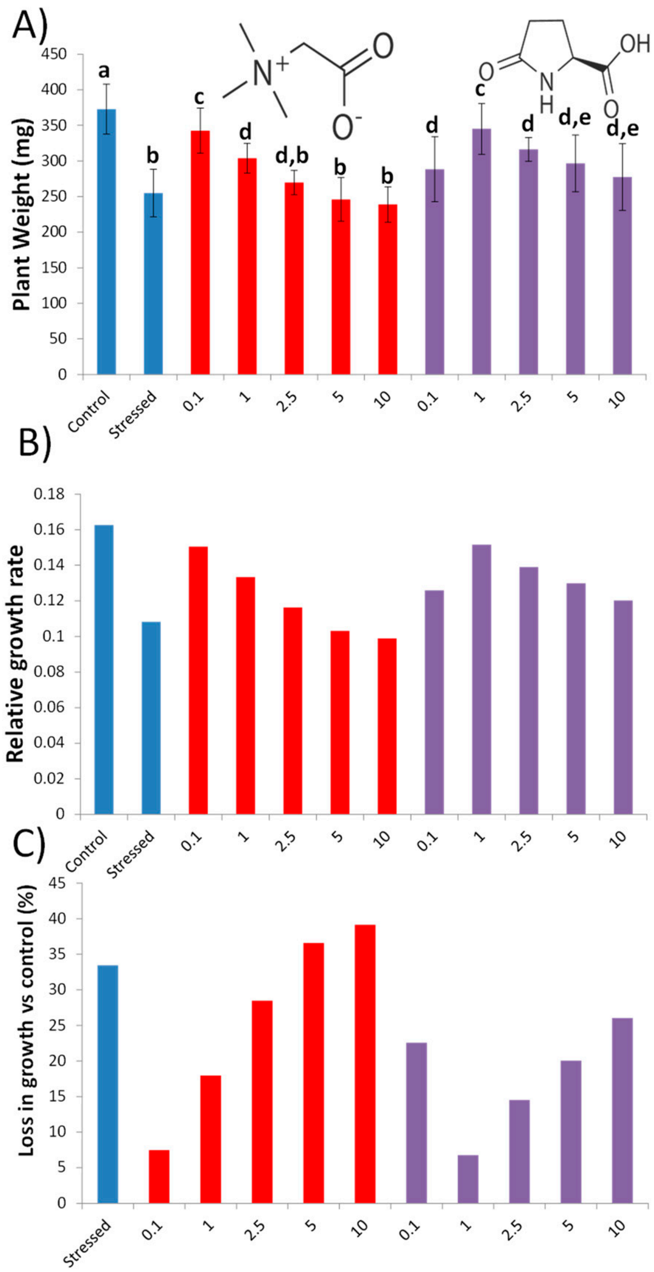

Figure 1. Hydroponic dosage evaluation using PEG 20% as a stressor. Red and violet correspond to GB and PG treatments, respectively. (**A**) Plant weight in mg. (**B**) Relative growth rate. (**C**) Percentage growth loss against control. Bars labelled with the same letter showed no significant differences at $p < 0.05$.

To ensure absorption after root treatment, we applied the compound directly to the soil in nursery trials. Watering with 50% less water for one week decreased maize growth by 14% (Figure 2). This situation was prevented by applying GB and PG, which increased tolerance by 65 and 85% at 0.1 and 1 mM, respectively. Water use efficiency was higher in all plants subjected to this slight water stress. The biostimulants achieved better results, showing more effectiveness in stress adaptation (Figure 2C).

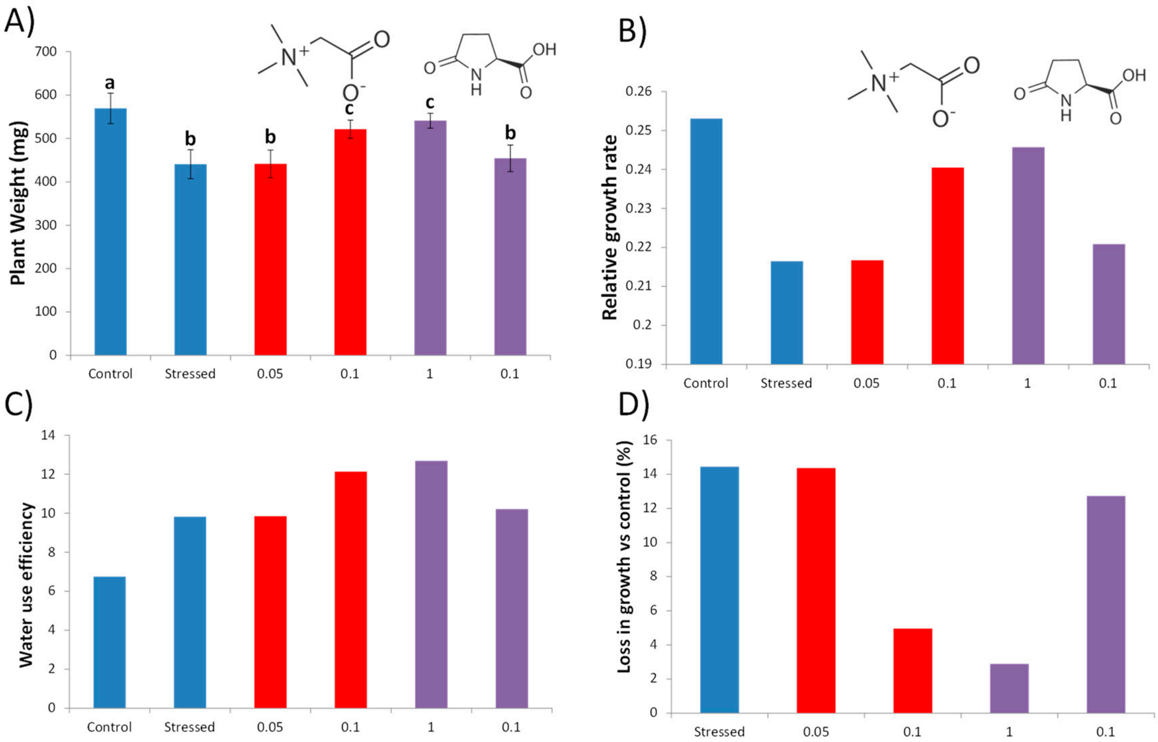

Figure 2. Soil dosage evaluation using 50% less water as a stressor. Red and violet correspond to GB and PG treatment, respectively. Plant weight in mg. (**A**) Relative growth rate. (**B**) Water use efficiency. (**C**) Percentage growth loss against control. (**D**) Bars labelled with the same letter did not show significant differences at $p < 0.05$.

3.2. Biostimulants Are Capable of Ameliorating Negative Effects Caused by 20% Less Watering under Field Conditions

Plants submitted to a watering regime of 20% less than maize plants' optimum water necessities showed significant negative effects in growth and production, as seen in Table 2. With GB treatment, leaf weight and width decreased by 14 and 12%, respectively. These differences were reduced in both parameters by only 5%. Pyroglutamic acid further reduced variations due to the different water regime to less than 1%. Yield parameters showed how the regimen significantly affected the cob number, the weight of 100 grains and total grain weight by reducing them by 33, 19 and 23%, respectively. Again, these differences between well-watered and water-stressed plants were reduced using GB treatment to 8, 6 and 10%, respectively, and further reduced by the use of PG. However, it is worth highlighting that GB increased the grain weight, reaching significantly higher values in the 100 grain parameter and higher, but not statistically different, values in total grain weight.

Using the data obtained in Table 2, we calculated the grain mass in kg/ha and grain water use efficiency (Table 3). Untreated plants stressed by water deprivation had a yield reduction of 1106 kg per hectare in comparison to well-watered. This difference became less with the use of biostimulants: 236 kg with GB but just 6 kg for PG. Water-use efficiency was lower in the water-deficit plants without treatment; this did not happen if the biostimulants were applied, reaching even higher WUE levels with respect to well-watered plants.

Treatments with biostimulants prevented growth losses, as indicated by the RWC value (Figure 3A). Untreated plants subjected to water deficit showed drops in RWC after fifteen days, and this trend continued after 30 days of the two watering regimes; both biostimulant treatments prevented this. The results are consistent with soil water content (Figure 3B); the soil dried to 80% of the humidity level only after 3 days of stress onset. Then, it was more or less stable throughout the experiment, with two periods of more intense water deficit, reaching 65% of the moisture level, which was translated into a lower RWC except for the plants treated with biostimulants.

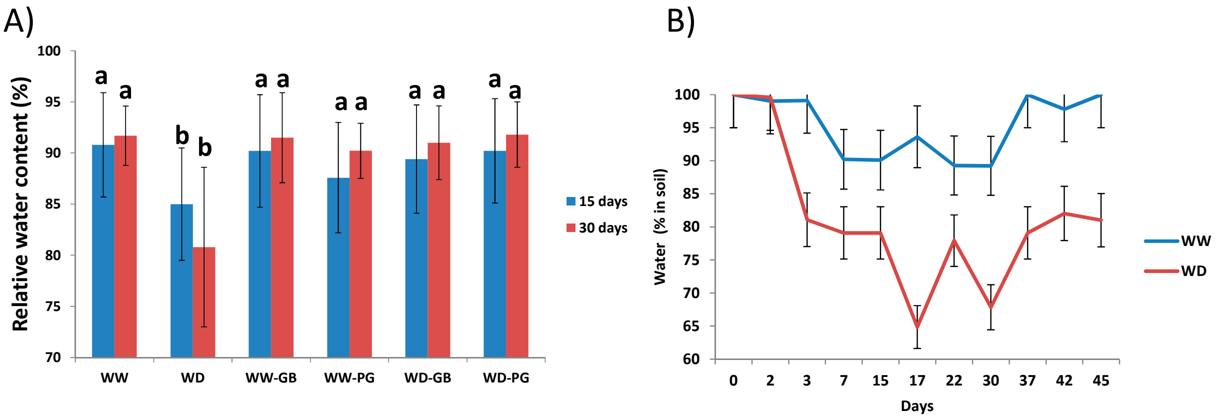

Figure 3. Plant water relations. (**A**) Relative water content. (**B**) Soil water content. Bars labelled with the same letter did not show significant differences at $p < 0.05$.

3.3. Nutritional Quality of Maize Flour Using Biostimulants

Mineral determination revealed how the water deficit significantly decreased the amount of calcium in flour, which was prevented by applying the biostimulants. Moreover, flour from the treated plants contained significantly more calcium than from untreated ones (Table 4). Interestingly, the P and Mg content significantly increased after water deprivation. Biostimulants showed the same trend for P. For Mg, however, all mineral contents were significantly higher in comparison to the untreated well-watered plants, especially in PG-treated plants under water deprivation, which accounted for the significantly higher magnesium levels. Potassium showed similar behaviour; only the latter group showed significantly higher concentrations. Iron, Cu and Zn did not show any difference for both untreated plants; however, plants treated with biostimulants had higher levels of Fe and Cu, and PG significantly increased all three.

Table 4. Flour mineral contents.

Treatment	Mineralogical Compositionin mg/100g Flour						
	Ca	P	Mg	K	Fe	Cu	Zn
WW	6.1 ± 0.3 [a]	143.9 ± 13 [a]	54.7 ± 8.6 [a]	309.6 ± 60.7 [a]	0.6 ± 0.2 [a]	0.3 ± 0.1 [a]	0.3 ± 0.1 [a]
WD	5.0 ± 0.3 [b]	195.5 ± 11.7 [b]	86.9 ± 9.7 [b]	356.4 ± 43.2 [a]	0.5 ± 0.1 [a]	0.4 ± 0.4 [a]	0.4 ± 0.1 [a]
WW-GB	9.5 ± 1.4 [c]	147.7 ± 20.4 [a]	71.1 ± 4.7 [b]	325.7 ± 40.7 [a]	0.8 ± 0.2 [ab]	0.2 ± 0.1 [a]	0.5 ± 0.1 [ab]
WD-GB	12.8 ± 1.7 [c]	185.7 ± 6.3 [b]	71.5 ± 88.4 [b]	335.5 ± 41.3 [a]	1.1 ± 0.1 [b]	0.3 ± 0.1 [a]	0.8 ± 0.1 [b]
WW-PG	10.5 ± 2 [c]	131.8 ± 5.8 [a]	71.4 ± 5.4 [b]	317.0 ± 64.3 [a]	0.8 ± 0.1 [ab]	1.2 ± 0.4 [b]	0.5 ± 0.1 [ab]
WD-PG	12.4 ± 1.7 [c]	189.8 ± 15.1 [b]	100.7 ± 4.6 [c]	482.1 ± 37.4 [b]	1.2 ± 0.2 [b]	1.4 ± 0.1 [b]	1.4 ± 0.1 [c]

Same letter means no significant differences at $p < 0.05$.

Total carbohydrate percentage (Figure 4A) was significantly reduced by the reduced irrigation and was prevented with the addition of GB and PG. The same correlation was shown for the protein percentage; the water deficit significantly affected only the plants without treatment and subjected to stress (Figure 4B).

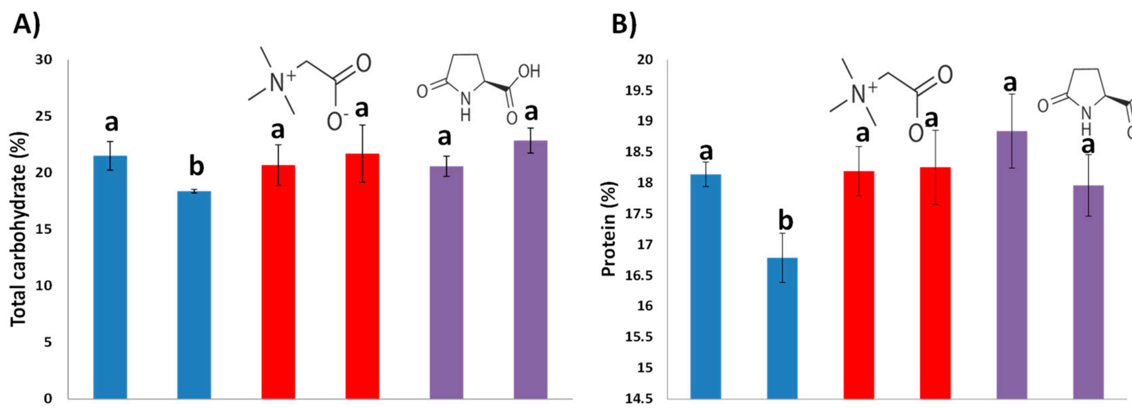

Figure 4. Carbohydrate and protein determination. Red and violet correspond to GB and PG treatment, respectively. Total carbohydrate determination % (**A**); protein % (**B**). Bars labelled with the same letter did not show significant differences at $p < 0.05$.

4. Discussion

Drought periods are expected to become more frequent and intense, especially in the Mediterranean area and western Europe. Moreover, the whole European continent will be affected by more frequent and severe extreme drought episodes [34]. The direct consequence, beyond the climatic and environmental implications, will be increased water scarcity [35] and the resultant difficulty to obtain it. Indeed, it is expected that by 2050, 6 billion people will lack access to a clean water source [36]. In agriculture, water demand will likely be 60% higher [36]. This would result in a rise in water prices for crop irrigation, so that farmers may have to use non-conventional water sources that increase the final production cost [37]. Proper water management is an obvious and promisingly achievable tool to maintain food supply and farmers' earnings, especially when faced with the coming greater uncertainty about its supply [38].

Water deficit stress can be prevented using biostimulants [12], which seems to be both attractive and eco-friendly to prevent losses caused by water shortage [38]. Indeed, the use of these kinds of substances to cope with stress is a prolific field of research that issues a huge number of publications per year. However, yield is not usually evaluated; in [10], an interesting list of biostimulants used against abiotic stress is assessed, but only 14% take it into account. It is possible to find some promising biostimulant applications—for example: a foliar application of mixed chitosan derivatives [39] increased grain yield by 35%. Other interesting work with maize [23] used foliar treatments of GB and salicylic acid to cope with drought stress, increasing grain yield by 40.52% and 60.49%, respectively. Here, we studied the possibility to apply two biostimulants, GB and PG, by fertirrigation, which is probably the easiest and most economical method for farmers to use such treatments [26]. The experiments show how plants subjected to water deprivation had a yield reduced by over 48% (Table 3). Application of the biostimulant GB reduces this difference by 10% and PG by less than 1%. The results are consistent with a previous study [23], although they were obtained through foliar treatment at a much higher concentration (100 mM) than used in our trials. In the case of PG, we here present the first data about the potential of this amino acid for use in maize to prevent losses due to drought, with it being previously reported only in lettuce [13].

Water use efficiency (WUE) is mostly recognised as a key constraint on crop production [31]. The results in Table 3 indicate that plants without biostimulant treatment had lower WUEg values, which indicate that plants are not able to acclimate adequately to the imposed stress [40]. However, the increase in WUEg induced by these biostimulants in water-deprived plants points to an enhanced ability to tolerate this imposed stress. They maintained the yield reached by well-watered plants, maximising acquisition of the available water [41]. Foliar GB application was also demonstrated to be capable of improving yield and water-use efficiency [42], as presented in our results. Leaf relative water content is correlated with maize tolerance against drought [43] and is also correlated with WUE [44]. It is therefore not surprising that plants treated with biostimulants reached higher levels under water deficit (Figure 3), as shown in other studies using GB [42]. In these, treated plants decreased their transpiration rate, which was translated into a better, more efficient yield under stress conditions.

Water deprivation itself has interesting effects on nutritional mineral composition of maize flour and therefore nutrient acquisition by the plants. Phosphorus and Mg increased in concentration, whereas Ca showed a significantly lower concentration under the low-watering regimen, consistent with previous published results [45]. Biostimulants maintain this positive behaviour of the grain in its accumulation of these elements, especially the WD-PG, which reached statistically the highest Mg concentration. This result is very interesting since nutritional health specialists warn us about hypomagnesaemia [46], owing to the fact that two-thirds of the world population does not consume a sufficient amount of Mg daily [47]. One of the reasons is because the concentration of these ions is decreasing in food crops over time [48], with drought events being one of the causes of this phenomenon [49]. Pyroglutamic acid treatment can thus be an interesting way to increase the amount of this nutrient in a food crop. The losses in Ca are prevented by both biostimulants, reaching amounts against WW control plants that are consistent with previous results in lettuce using PG [13]. Glycine betaine seems to behave the same, with it being an interesting point of study regarding biostimulant applications. Water deficit did not significantly change the amounts of the micronutrients studied. However, GB significantly increased the amount of Fe and Zn in plants subjected to stress, while PG increased the concentrations in the mentioned elements and also Cu (Table 4).

It is well-known that drought stress causes changes in cereal grain composition, such as an important decrease in carbohydrate accumulation and an increment in protein content [49,50]. However, our results show a decrease in both parameters (Figure 4). The difference in protein concentration may be due to most maize hybrids being selected according to their drought tolerance, while grain protein content is one of the common selected parameters [51]. Our variety was a local forage variety of maize and not a commercial hybrid. Biostimulant application under drought conditions seems to be conducive to increased total carbohydrate amounts [23,39]. Total carbohydrates can be used as compatible osmolytes by maize [52]. Moreover, for some varieties, the total carbohydrate content can be correlated with a higher tolerance against drought stress [53]. Our results are consistent with such observations—treatments with GB and PG prevented the growth losses caused by drought (Figure 4), in our opinion, by helping to reach the osmotic balance. Again, previous results using PG in lettuce are consistent with carbohydrate loss prevention, which could be because these biostimulants can maintain high rates of photosynthesis in plants undergoing water deficit stress [13]. There are similar results with GB utilisation in the relevant literature [23].

Such biostimulants may result to be an excellent option for farmers, as both GB and PG are able to increase tolerance, and treated plants show no negative differences from untreated well-watered plants. However, is the treatment economically profitable? Using Table 3, the water used in the study, the price in € of a metric ton of maize grains [54], water prices [9] and the biostimulants' price per kg using Sigma Aldrich as the provider (PG @256 € per 500 g and GB @153 € per 1 kg), we calculated Table 5. At a first look, without considering water prices, we can show with our data that the WW-GB treatment could increase profit by 72 € in comparison with WW plants; however, WW-PG decreased it by 272 €. This huge difference between GB- and PG-derived profits is caused by their cost, which is 4.3 € and 171 € per hectare, respectively.

Using the price of conventional water (0.3 € per m^3 [9]), economic losses caused by water deprivation in the WD plants reached 272 €. The WD-GB treatment caused a 1€ profit loss in comparison with WW plants, whereas WD-PG increased profit by 56€ in comparison with WD plants. Using the price of desalinated water (0.6 € per m^3 [8]), profits decrease three-fold in WW; so, under WD or using the PG treatment in both water regimes, the crop remains profitable. However, WW-GB increases profit by 72 € compared to WW, despite a 20% reduction in watering.

Table 5. Economic study of the selected biostimulant application.

Treatment	Grain Mass (kg/ha)	Water Consumption m^3/ha	Profit Using Ground Water €/ha	Profit Using Desalinated Water €/ha
WW	2274		389.5	99.2
WW-GB *	2321	967.9	462.4	172.8
WW-PG+	1936		117.4	−172.9
WD	1168		116.9	−115.4
WD-GB *	2084	774.3	386.7	154.4
WD-PG+	1930		173.9	−58.5

* GB application costs 4.3 €/ha, whereas PG costs 171 €/ha.

5. Conclusions

Climate change is a concerning situation for agriculture and farmers. Beyond the natural disasters and plagues caused by a rapidly changing environment, water scarcity due to lower precipitation in vulnerable zones is a serious threat to overcome in order to derive a profit from crops. One of the most studied and promising solutions is the application of biostimulants.

Foliar biostimulant treatments are usually applied in field trials; here, we demonstrate that GB and PG can be administered as root treatments. This adds or injects them into the fertirrigation system, reducing operational costs. Furthermore, we optimise the doses, demonstrating that 0.01 mM GB and 1 mM PG reduced yield losses in a situation where 20% less water was provided. In addition, both treatments improve water use efficiency, preventing evapotranspiration losses and maintaining the nutritional benefits of the maize.

Nevertheless, considering the price of the treatment and the yield obtained in this assay, together with the price of water, only GB can be proposed as a viable biostimulant to cultivate maize in a water-deprivation regimen. Its extra cost is 4.3 € per hectare, reaching an additional profit ranging from 154.4 to 386.7 € under those conditions.

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References

1. He, Y.; Fang, J.; Xu, W.; Shi, P. Substantial Increase of Compound Droughts and Heatwaves in Wheat Growing Seasons Worldwide. *Int. J. Climatol.* **2021**, *in press*. [CrossRef]
2. Lopez-Nicolas, A.; Pulido-Velazquez, M.; Macian-Sorribes, H. Economic Risk Assessment of Drought Impacts on Irrigated Agriculture. *J. Hydrol.* **2017**, *550*, 580–589. [CrossRef]
3. Howitt, R.; MacEwan, D.; Medellín-Azuara, J.; Lund, J.; Sumner, D. *Economic Analysis of the 2015 Drought for California Agriculture*; University of California: Davis, CA, USA, 2015.

4. GAR Special Report on Drought. 2021. Available online: https://www.undrr.org/publication/gar-special-report-drought-2021 (accessed on 26 June 2021).
5. Dolan, F.; Lamontagne, J.; Link, R.; Hejazi, M.; Reed, P.; Edmonds, J. Evaluating the Economic Impact of Water Scarcity in a Changing World. *Nat. Commun.* **2021**, *12*, 1915. [CrossRef] [PubMed]
6. Jiménez-Arias, D.; Morales-Sierra, S.; García-Machado, F.J.; García-García, A.L.; Luis, J.C.; Valdés, F.; Sandalio, L.M.; Hernández-Suárez, M.; Borges, A.A. Rejected Brine Recycling in Hydroponic and Thermo-Solar Evaporation Systems for Leisure and Tourist Facilities. Changing Waste into Raw Material. *Desalination* **2020**, *496*, 114443. [CrossRef]
7. March, H.; Saurí, D.; Rico-Amorós, A.M. The End of Scarcity? Water Desalination as the New Cornucopia for Mediterranean Spain. *J. Hydrol.* **2014**, *519*, 2642–2651. [CrossRef]
8. Carson, R.S. "Not in My Backyard" Is Not Sustainable. *INCOSE Int. Symp.* **2017**, *27*, 1749–1766. [CrossRef]
9. López-Serrano, M.J.; Velasco-Muñoz, J.F.; Aznar-Sánchez, J.A.; Román-Sánchez, I.M. Economic Analysis of the Use of Reclaimed Water in Agriculture in Southeastern Spain, A Mediterranean Region. *Agronomy* **2021**, *11*, 2218. [CrossRef]
10. García-García, A.L.; García-Machado, F.J.; Borges, A.A.; Morales-Sierra, S.; Boto, A.; Jiménez-Arias, D. Pure Organic Active Compounds Against Abiotic Stress: A Biostimulant Overview. *Front. Plant Sci.* **2020**, *11*, 1839. [CrossRef]
11. Jiménez-Arias, D.; Morales-Sierra, S.; Borges, A.A.; Díaz, D.D. Biostimulant Nanoencapsulation: The New Keystone to Fight Hunger. *J. Agric. Food Chem.* **2020**, *68*, 7083–7085. [CrossRef]
12. Jiménez-Arias, D.; García-Machado, F.J.; Morales-Sierra, S.; García-García, A.L.; Herrera, A.J.; Valdés, F.; Luis, J.C.; Borges, A.A. A Beginner's Guide to Osmoprotection by Biostimulants. *Plants* **2021**, *10*, 363. [CrossRef]
13. Jiménez-Arias, D.; García-Machado, F.J.; Morales-Sierra, S.; Luis, J.C.; Suarez, E.; Hernández, M.; Valdés, F.; Borges, A.A. Lettuce Plants Treated with L-Pyroglutamic Acid Increase Yield under Water Deficit Stress. *Environ. Exp. Bot.* **2019**, *158*, 215–222. [CrossRef]
14. Binns, J. Farm to Fork Strategy. Available online: https://ec.europa.eu/food/farm2fork_en (accessed on 26 March 2021).
15. du Jardin, P. Plant Biostimulants: Definition, Concept, Main Categories and Regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
16. Sible, C.N.; Seebauer, J.R.; Below, F.E. Plant Biostimulants: A Categorical Review, Their Implications for Row Crop Production, and Relation to Soil Health Indicators. *Agronomy* **2021**, *11*, 1297. [CrossRef]
17. Madende, M.; Hayes, M. Fish By-Product Use as Biostimulants: An Overview of the Current State of the Art, Including Relevant Legislation and Regulations within the EU and USA. *Molecules* **2020**, *25*, 1122. [CrossRef]
18. Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The Role of Biostimulants and Bioeffectors as Alleviators of Abiotic Stress in Crop Plants. *Chem. Biol. Technol. Agric.* **2017**, *4*, 5. [CrossRef]
19. Sofy, M.R.; Elhawat, N. Tarek Alshaal Glycine Betaine Counters Salinity Stress by Maintaining High K+/Na+ Ratio and Antioxidant Defense via Limiting Na+ Uptake in Common Bean (*Phaseolus vulgaris* L.). *Ecotoxicol. Environ. Saf.* **2020**, *200*, 110732. [CrossRef]
20. Rasheed, R.; Iqbal, M.; Ashraf, M.A.; Hussain, I.; Shafiq, F.; Yousaf, A.; Zaheer, A. Glycine Betaine Counteracts the Inhibitory Effects of Waterlogging on Growth, Photosynthetic Pigments, Oxidative Defence System, Nutrient Composition, and Fruit Quality in Tomato. *J. Hortic. Sci. Biotechnol.* **2018**, *93*, 385–391. [CrossRef]
21. Hamani, A.K.M.; Wang, G.; Soothar, M.K.; Shen, X.; Gao, Y.; Qiu, R.; Mehmood, F. Responses of Leaf Gas Exchange Attributes, Photosynthetic Pigments and Antioxidant Enzymes in NaCl-Stressed Cotton (*Gossypium hirsutum* L.) Seedlings to Exogenous Glycine Betaine and Salicylic Acid. *BMC Plant Biol.* **2020**, *20*, 434. [CrossRef]
22. de Oliveira Maia Júnior, S.; de Andrade, J.R.; dos Santos, C.M.; Santos, J.V.; dos Santos Silva, L.K.; Aprígio Clemente, P.R.; Ferreira, V.M.; Silva, J.V.; Endres, L. Foliar-Applied Glycine Betaine Minimizes Drought Stress-Related Impact to Gas Exchange and the Photochemical Efficiency of PSII in Sugarcane. *Theor. Exp. Plant Physiol.* **2020**, *32*, 315–329. [CrossRef]
23. Shemi, R.; Wang, R.; Gheith, E.-S.M.S.; Hussain, H.A.; Hussain, S.; Irfan, M.; Cholidah, L.; Zhang, K.; Zhang, S.; Wang, L. Effects of Salicylic Acid, Zinc and Glycine Betaine on Morpho-Physiological Growth and Yield of Maize under Drought Stress. *Sci. Rep.* **2021**, *11*, 3195. [CrossRef]
24. Jander, G.; Kolukisaoglu, U.; Stahl, M.; Yoon, G.M. Editorial: Physiological Aspects of Non-Proteinogenic Amino Acids in Plants. *Front. Plant Sci.* **2020**, *11*, 2057. [CrossRef]
25. Zhao, X.; Tong, C.; Pang, X.M.; Wang, Z.; Guo, Y.; Du, F.; Wu, R. Functional Mapping of Ontogeny in Flowering Plants. *Brief. Bioinform.* **2011**, *13*, 317–328. [CrossRef] [PubMed]
26. Jiménez-Arias, D.; García-Machado, F.J.; Morales-Sierra, S.; Suárez, E.; Pérez, J.A.; Luis, J.C.; Garrido-Orduña, C.; Herrera, A.J.; Valdés, F.; Sandalio, L.M.; et al. Menadione Sodium Bisulphite (MSB): Beyond Seed-Soaking. Root Pretreatment with MSB Primes Salt Stress Tolerance in Tomato Plants. *Environ. Exp. Bot.* **2019**, *157*, 161–170. [CrossRef]
27. HOFFMANN, W.A.; POORTER, H. Avoiding Bias in Calculations of Relative Growth Rate. *Ann. Bot.* **2002**, *90*, 37–42. [CrossRef] [PubMed]
28. Hoagland, D.R.; Arnon, D.I. The Water-Culture Method for Growing Plants without Soil. *Circ. Calif. Agric. Exp. Stn.* **1950**, *347*, 32.
29. Medrano, H.; Tomás, M.; Martorell, S.; Flexas, J.; Hernández, E.; Rosselló, J.; Pou, A.; Escalona, J.-M.; Bota, J. From Leaf to Whole-Plant Water Use Efficiency (WUE) in Complex Canopies: Limitations of Leaf WUE as a Selection Target. *Crop J.* **2015**, *3*, 220–228. [CrossRef]

30. Allen, R.G.; Food and Agriculture Organization of the United Nations (Eds.) *Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements*; FAO Irrigation and Drainage Paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 1998; ISBN 978-92-5-104219-9.
31. Leakey, A.D.B.; Ferguson, J.N.; Pignon, C.P.; Wu, A.; Jin, Z.; Hammer, G.L.; Lobell, D.B. Water Use Efficiency as a Constraint and Target for Improving the Resilience and Productivity of C3 and C4 Crops. *Annu. Rev. Plant Biol.* **2019**, *70*, 781–808. [CrossRef]
32. Barrs, H.D.; Weatherley, P.E. A Re-Examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. *Aust. J. Biol. Sci.* **1962**, *15*, 413–428. [CrossRef]
33. Kirk, P.L. Kjeldahl Method for Total Nitrogen. Available online: https://pubs.acs.org/doi/pdf/10.1021/ac60038a038 (accessed on 24 November 2021).
34. Spinoni, J.; Vogt, J.V.; Naumann, G.; Barbosa, P.; Dosio, A. Will Drought Events Become More Frequent and Severe in Europe? *Int. J. Climatol.* **2018**, *38*, 1718–1736. [CrossRef]
35. Veldkamp, T.I.E.; Wada, Y.; Aerts, J.C.J.H.; Döll, P.; Gosling, S.N.; Liu, J.; Masaki, Y.; Oki, T.; Ostberg, S.; Pokhrel, Y.; et al. Water Scarcity Hotspots Travel Downstream Due to Human Interventions in the 20th and 21st Century. *Nat. Commun.* **2017**, *8*, 15697. [CrossRef]
36. Boretti, A.; Rosa, L. Reassessing the Projections of the World Water Development Report. *NPJ Clean Water* **2019**, *2*, 15. [CrossRef]
37. Martínez-Alvarez, V.; Maestre-Valero, J.F.; González-Ortega, M.J.; Gallego-Elvira, B.; Martin-Gorriz, B. Characterization of the Agricultural Supply of Desalinated Seawater in Southeastern Spain. *Water* **2019**, *11*, 1233. [CrossRef]
38. Christ, K.L.; Burritt, R.L. Water Management Accounting: A Framework for Corporate Practice. *J. Clean. Prod.* **2017**, *152*, 379–386. [CrossRef]
39. Rabêlo, V.M.; Magalhães, P.C.; Bressanin, L.A.; Carvalho, D.T.; dos Reis, C.O.; Karam, D.; Doriguetto, A.C.; dos Santos, M.H.; Santos Filho, P.R.d.S.; de Souza, T.C. The Foliar Application of a Mixture of Semisynthetic Chitosan Derivatives Induces Tolerance to Water Deficit in Maize, Improving the Antioxidant System and Increasing Photosynthesis and Grain Yield. *Sci. Rep.* **2019**, *9*, 8164. [CrossRef] [PubMed]
40. Blum, A. Drought Resistance, Water-Use Efficiency, and Yield Potential—Are They Compatible, Dissonant, or Mutually Exclusive? *Aust. J. Agric. Res.* **2005**, *56*, 1159–1168. [CrossRef]
41. Passioura, J. Increasing Crop Productivity When Water Is Scarce—From Breeding to Field Management. *Agric. Water Manag.* **2006**, *80*, 176–196. [CrossRef]
42. El-Hendawy, S.E.; Kotab, M.A.; Al-Suhaibani, N.A.; Schmidhalter, U. Optimal Coupling Combinations between the Irrigation Rate and Glycinebetaine Levels for Improving Yield and Water Use Efficiency of Drip-Irrigated Maize Grown under Arid Conditions. *Agric. Water Manag.* **2014**, *140*, 69–78. [CrossRef]
43. Goodarzian Ghahfarokhi, M.; Mansurifar, S.; Taghizadeh-Mehrjardi, R.; Saeidi, M.; Jamshidi, A.M.; Ghasemi, E. Effects of Drought Stress and Rewatering on Antioxidant Systems and Relative Water Content in Different Growth Stages of Maize (*Zea mays* L.) Hybrids. *Arch. Agron. Soil Sci.* **2015**, *61*, 493–506. [CrossRef]
44. Blum, A. Effective Use of Water (EUW) and Not Water-Use Efficiency (WUE) Is the Target of Crop Yield Improvement under Drought Stress. *Field Crops Res.* **2009**, *112*, 119–123. [CrossRef]
45. Da Ge, T.; Sui, F.G.; Nie, S.; Sun, N.B.; Xiao, H.; Tong, C.L. Differential Responses of Yield and Selected Nutritional Compositions to Drought Stress in Summer Maize Grains. *J. Plant Nutr.* **2010**, *33*, 1811–1818. [CrossRef]
46. Rosanoff, A.; Weaver, C.M.; Rude, R.K. Suboptimal Magnesium Status in the United States: Are the Health Consequences Underestimated? *Nutr. Rev.* **2012**, *70*, 153–164. [CrossRef]
47. Hermans, C.; Conn, S.J.; Chen, J.; Xiao, Q.; Verbruggen, N. An Update on Magnesium Homeostasis Mechanisms in Plants. *Metallomics* **2013**, *5*, 1170–1183. [CrossRef] [PubMed]
48. Guo, W.; Nazim, H.; Liang, Z.; Yang, D. Magnesium Deficiency in Plants: An Urgent Problem. *Crop J.* **2016**, *4*, 83–91. [CrossRef]
49. Ciríaco da Silva, E.; Nogueira, R.J.; Silva, M.; Albuquerque, M. Drought Stress and Plant Nutrition. *Plant Stress* **2010**, *5*, 32–41.
50. Rakszegi, M.; Darkó, É.; Lovegrove, A.; Molnár, I.; Láng, L.; Bedő, Z.; Molnár-Láng, M.; Shewry, P. Drought Stress Affects the Protein and Dietary Fiber Content of Wholemeal Wheat Flour in Wheat/Aegilops Addition Lines. *PLoS ONE* **2019**, *14*, e0211892. [CrossRef] [PubMed]
51. Lu, D.; Cai, X.; Zhao, J.; Shen, X.; Lu, W. Effects of Drought after Pollination on Grain Yield and Quality of Fresh Waxy Maize. *J. Sci. Food Agric.* **2015**, *95*, 210–215. [CrossRef]
52. Ignjatovic-Micic, D.; Vancetovic, J.; Trbovic, D.; Dumanovic, Z.; Kostadinovic, M.; Bozinovic, S. Grain Nutrient Composition of Maize (*Zea mays* L.) Drought-Tolerant Populations. *J. Agric. Food Chem.* **2015**, *63*, 1251–1260. [CrossRef] [PubMed]
53. Chen, Q.; Qu, Z.; Ma, G.; Wang, W.; Dai, J.; Zhang, M.; Wei, Z.; Liu, Z. Humic Acid Modulates Growth, Photosynthesis, Hormone and Osmolytes System of Maize under Drought Conditions. *Agric. Water Manag.* **2022**, *263*, 107447. [CrossRef]
54. Sandhu, H.; Scialabba, N.E.-H.; Warner, C.; Behzadnejad, F.; Keohane, K.; Houston, R.; Fujiwara, D. Evaluating the Holistic Costs and Benefits of Corn Production Systems in Minnesota, US. *Sci. Rep.* **2020**, *10*, 3922. [CrossRef] [PubMed]