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Deteriorating Harmful Effects of Drought in Cucumber by Spraying Glycinebetaine

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Abstract: In order to alleviate the shortage of irrigation water in dry regions, refining water use efficiency (WUE) is a key issue in sustainable productivity. Furthermore, glycinebetaine (GlyBet) is a vital osmoprotectant produced in crops for improving drought tolerance; however, little is known about its role in improving plant WUE under field conditions in non-accumulating plants such as cucumber. In order to elucidate the effectiveness of GlyBet concentrations (0, 2000, 4000, and 6000 mg/L) in mitigating the deleterious effects of drought (e.g., well-watered (1250 m^3/fed), moderate drought (950 m^3/fed), and severe drought (650 m^3/fed)), field experiments were conducted at Elmia village, Dakahlia, Egypt in the 2020 and 2021 seasons on vegetative growth, some physiological attributes, as well as yield and quality. Drought considerably decreased vegetative growth, yield and its components, leaf relative water content, and photosynthetic pigment concentrations compared with well-watered plants while increasing electrolyte leakage. The most harmful causes were severe drought. However, exogenous spraying with GlyBet substantially boosted the mentioned attributes, but reduced electrolyte leakage within well-watering. Commonly 6000 mg/L contributed to the maximum growth and productivity, preserving cucumber plant water status above other concentrations or untreated plants. Under extreme drought, the application of 6000 mg/L GlyBet had a beneficial effect on moderating the damage of water deficit on cucumber plant growth and productivity. Overall, using GlyBet as a cost-effective and eco-friendly biostimulant six times (10, 20, 30, 40, 50, and 60 days from sowing) has the potential to mitigate drought damage while also increasing yield; however, more research is needed to determine the optimal rate and timing of application.

Keywords: cucumber; glycinebetaine; water stress; yield

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1. Introduction

After tomato, cabbage, and onion, cucumber (*Cucumis sativus* L., Cucurbitaceae) is the fourth most popularly grown vegetable worldwide [1]. It is native to India and presumably came from the foothills of the Himalayan Mountains [2,3]. Cucumber is now widely grown around the world in both temperate and tropical climates; cucumbers require relatively high light intensity (20–30 mol/m^2/day), temperature (18–29 °C), and relative humidity (60–80%) [4]. According to FAO statistics (www.fao.org/faostat/en/ '3 February 2021'), the globally produced quantity was 87,805,086 tons of cucumber in total, with Asia producing 84.9% of that production. Cucumber has numerous uses in food, medicine, and cosmetics due to their abundance of water, nutrients, and phytochemicals [5,6]. Superior hydration and phytochemicals found in cucumbers have a variety of health benefits, including weight loss and treatment of eczema, constipation, hypertension, atherosclerosis, and cancer [6,7]. According to recent studies, cucumbers contain kaempferol, a key anti-diabetic substance [8]. In addition, cucumber is frequently used for skin treatments and natural attractiveness [6,9].

Due to abrupt climate changes and anthropogenic activities, more severe episodes of moisture unavailability, uneven rainfall distribution, and increases in average and maximum temperatures are predicted for the future, which will affect farming systems and agriculture productivity [10,11]. Drought stress (DS) has cost the global economy between USD 30 and USD 44 billion over the previous ten years [12]. In dry and semi-arid environments, DS represents the main ecological disorder that has a negative impact on plant growth and restricts sustainable production [13–15]. Owing to their direct effect on photoassimilate allocation, as well as an alteration in a sequence of biochemical processes and molecular reactions, DS undoubtedly reduced crop productivity by up to 70% [13–17]. It adjusts several metabolic processes, such as photosynthesis, water absorption, and reactive oxygen species (ROS) accumulation, leading to growth and yield deterioration [13,18,19].

Agricultural extension in Egypt requires a huge amount of water, which is now scant to fulfill the anticipated requisite, as 85% of accessible water is consumed in agriculture and utmost of the on-farm irrigation systems have little proficiency, along with poor irrigation administration. The cost-effective novel technique for enhancing water use efficiency (WUE) and boosting plant productivity has received increased attention due to the growing competition for scarce water resources. Through the use of water-saving and drought-tolerant varieties, effective agronomic practices and management, WUE can be increased [20,21]. Moreover, it is believed that the most important regulator for controlling plants' water usage within DS is chemical signaling, which involves phytohormones or osmoprotectants [22]. Plants under stress factors rapidly accumulate osmoprotectants to maintain water status and sustain typical functioning [23]. Osmoprotectants may be adjusting cellular osmotic pressure, detoxifying ROS, and preserving and stabilizing membrane integrity [24]. These findings show that the use of osmoprotectants may be a realistic strategy for increasing crop WUE under water scarcity.

Glycine betaine (N, N″, N″-trimethyl-glycine, GlyBet) is one of the most effective osmoprotectants, and it shields cellular components via maintaining an osmotic equilibrium and stabilizing the quaternary structures of complex proteins [18,25,26]. It is low-cost, reachable, eco-friendly, and provides both economic and environmental efficiency. GlyBet application increases the growth, and tolerance of a wider range of crops under stressful disorders [13,18,25,26] by regulating a number of physiological and biochemical processes [18,27], maintaining turgor pressure [27], enhancing net CO_2 assimilation rate [18], shielding the effectual proteins and enzymes, and lipids of the chloroplasts and sustaining electron stream over thylakoid membranes [28], as well as regulation of photosynthetic machinery and ion homeostasis [11]. Moreover, GlyBet may act as anti-transpirant, which permits the plant to enter extra water for a long period and facilitates photosynthesis [29]. Shemi et al. [18] mentioned that foliar-applied GlyBet increases several morphological features, yield, and accumulation of several metabolites of drought-affected maize plants. On canola, Dawood and Sadak [30] found that the application of GlyBet increased drought tolerance by enhancing shoot and root systems growth, photosynthetic pigment concentration, improving IAA, proline, soluble sugars, yield, and its components and quality (seed yield/plant, carbohydrate %, phenolic %, flavonoid level).

Different plant species have been shown to be able to tolerate drought better when GlyBet is applied exogenously. The available data infrequently differ on the plant species and water deficit severity. This study's objective was to assess how exogenously applied GlyBet affected cucumber grown under DS in terms of WUE and drought resistance. Under various irrigation regimes, the impact of GlyBet on cucumber yield and its components, photosynthetic pigments, ion percentage, relative water content, and membrane permeability was additionally tested. The findings of this study offer a new innovative water-saving technique in arid and semi-arid regions. It was assumed that GlyBet application can be used as an appropriate method for improving the growth and production of drought-affected cucumber plants.

2. Materials and Methods

2.1. Experimental Site and Soil Depiction

At a private farm in Elmia village, Dekernes district, Dakahlia governorate, Egypt (latitude 31°5′18″ N, longitude 31°35′49″ E, 8 m above sea level), two field trials were conducted in 2020 and 2021. The experiment region is classified as a semi-arid area with average precipitation and temperature of 5.39–7.52 mm and 21.3–24.7 °C, respectively, for both seasons. The physical and chemical characteristics of the experimental soil's profile, which displays clay loamy texture down to a depth of 0–60 cm, were provided in a Table 1.

Table 1. Physicochemical attributes of the experimental soil during 2020 and 2021 seasons.

Seasons	**Silt %**	**Clay %**	**Sand %**	**Soil Texture**	**F.C %**	**W.P %**	**AW %**	**pH**	**E.C (dSm^{-1})**	**O.M %**	**$CaCO_3$ %**	**N ppm**	**P ppm**	**K ppm**
2020	40.5	37.2	22.3	Clay loamy	35.7	18.9	16.8	8.22	1.51	1.8	3.39	51.9	5.7	288
2021	41.1	36.9	22.0	Clay loamy	35.2	18.4	16.8	8.13	1.78	2.o	3.45	54.1	6.2	294

F.C.—field capacity; W.P.—welting point; AW—available water; pH—potential of hydrogen; E.C—electrical conductivity; OM—organic matter; $CaCO_3$—calcium carbonate; N—nitrogen; P—phosphorus; K—potassium.

2.2. Experimental Layout

A split-plot based on a randomized complete block design and a drip irrigation system was used for the present investigation. The key plots were devoted to different three irrigation regimes: well watering (WW; 1250 m^3/fed), moderate drought (MD; 950 m^3/fed), and severe drought (SD; 650 m^3/fed), and the irrigation intervals were recognized consistent with the growth stage and local recommendation. Alternatively, the sub-plots were allocated to spraying treatments (water as control, 2000 mg/L GlyBet; 4000 mg/L GlyBet; and 6000 mg/L GlyBet). GlyBet as a pure water-soluble chemical was obtained from Sigma-Aldrich Co., LLC Company Profile | Saint Louis, MO, USA. The 12 treatments were replicated three times, making a total of 36 plots (each plot was 24 m^2 with 1.5 m wide bordered regions).

2.3. Crop Husbandry

The experimental field was deeply tilled, and the soil smoothed before seeding. Prior to sowing, 10 m^3/fed (4200 m^2) of farmyard manure had been added and properly mixed with the top 0–30 cm of soil. As fertigation at 2-day intervals starting one week after planting, 70, 45, and 65 kg fed^{-1} of ammonium nitrate (33.5% N), phosphoric acid (50% P_2O_5), and potassium sulfate (50% K_2O), were delivered correspondingly according to the recommendations of Ministry of Agriculture and Land Reclamation, Egypt. On the first and third of August in both seasons, respectively, cucumber seeds (*C. sativus* L., cv. *JABBAR, F1,* were secured from Fine Seeds Company, Giza, Egypt) were manually sown at two sides of the dripper.

Irrigation was started once sowing for identical seedling emergence 14 days before irrigation regime treatment. The GlyBet concentrations with 0.01% (*v*/*v*) Tween-20 (a witting agent) were sprayed six times at 10, 20, 30, 40, 50, and 60 days from sowing. The spraying (15 L/plot) was performed to a run-off in the early morning via a back-sprayer. For each treatment, irrigation was used to replenish the water loss accumulated every 2 days.

2.4. Data Recording and Measurements

At 35 days from sowing, five randomly chosen plants from each plot were selected to evaluate vegetative growth trials as well as some physiological trials within the shoot.

2.4.1. Vegetative Growth Characteristics

Vine length (cm), number of branches and leaves per plant, leaf area (cm^2), leaf dry matter percentage, foliage fresh weight (g/plant).

2.4.2. Photosynthetic Pigment Concentration

Photosynthetic pigments were extracted for 48 h with ice-cold methanol at lab temperature, and subsequently the optical density of extraction was read, and then its concentration was calculated (mg g^{-1} FW) following the equation of Lichtenthaler [31].

2.4.3. Leaf Chemical Composition

The N, P, and K percent were analyzed according to AOAC [32].

2.4.4. Leaf Relative Water Content (LRWC)

The Farouk et al. [33] protocol was used to assess LRWC. Leaves from the middle of each plant were independently collected and the fresh weight was assessed. Following this, leaves were kept for 24 h in closed Petri dishes containing distilled water, and their turgid weight was recorded. To determine the dry weight, the completely turgid leaves were dried in an oven at 80 °C until a consistent weight was reached. Finally, the following equation was used to calculate LRWC (%):

$$\text{LRWC}\ (\%) = \frac{\text{fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

2.4.5. Electrolyte Leakage (EL)

The EL was deliberate next to the scheme designated by Lutts et al. [34]. Leaves were cut into 1 cm segments and were carefully washed with deionized water (DW) thrice to eradicate any surface contamination. Then, leaf segments were incubated for 24 h at 25 °C on a rotary shaker by keeping the samples in closed and precleared vials containing 20 mL of DW. The electrical conductivity (EC1) of each solution was recorded using a conductivity meter. Then the samples were autoclaved for 20 min at 120 °C, and the electrical conductivity of each solution was measured (EC2) once incubated solutions were cooled down at 25 °C. Electrolyte leakage (%) was deliberated by the subsequent equation:

$$\text{EL}\ (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

2.4.6. Water Use Efficiency (WUE)

The WUE was considered following the equation of Howell [35]:

$$\text{WUE} = \frac{\text{Fruit yield}\ (\text{kg}/\text{fed})}{\text{Crop water consumption}\ (\text{m}^3/\text{fed}/\text{season})}$$

2.4.7. Sex Expression, Fruit Yield, and Their Components

Five plants from each plot were selected for counting the number of male and female flowers for each treatment at two day intervals up to the end of the experiment. Sex ratio was considered as male flowers/female flowers. Additionally, these selected plants were used for estimating fruits' weight and numbers per plant, and total yield (ton/fed).

2.4.8. Fruit Chemical Quality

Fruit quality parameters, such as dry matter percentage, ascorbic acid concentration, and total soluble solids (TSS), were assessed [32]. Fruit dry matter % was estimated by taking a known weight of the fruit and drying at 105 °C; then, the fruit dry matter % was calculating via dividing the dry weight by the fresh weight and indicated as a percent. TSS (°Brix) of each fruit was assessed with a digital refractometer (Model HI96801, Hanna Instruments, Woonsocket, RI, USA). Meanwhile, ascorbic acid (vitamin C) concentration (mg/100 g fruit fresh weight) was estimated using 2,6-dichlorophenol indophenol reagent.

2.5. Statistical Analysis

Utilizing Costat software, a two-way ANOVA was carried out for statistical analysis (CoHortSoftware, 2006; Birmingham, UK). All data were examined using a split-plot methodology, with the replications treated as a random variable in the model and the interactions between GlyBet and irrigation water treatment treated as permanent effects. Data were tested for outstanding normality previous to analysis, and when an ANOVA showed that there were significant treatment effects, means were separated at $p \leq 0.05$ using the LSD pair-wise comparison test.

3. Results

3.1. Vegetative Growth Characters

The application GlyBet concentration had a significant impact on plant growth ($p \leq 0.05$) (Table 2). In both seasons, compared to well-watered cucumber plants, severe drought significantly reduced vine length (21.46 and 21.12%), foliage fresh weight (46.48 and 46.25%), branches number per plant (29.05 and 28.7%), leaves number per plant (26.2 and 25.9%), and leaves area per plants (27.9 and 27.6%) (Table 2).

The plant growth trials were greatly improved by exogenous GlyBet administration. In comparison to untreated plants, 6000 mg/L GlyBet recorded the maximum vine length (42.12 and 42.05%), foliage fresh weight (25.06 and 24.89%), branches per plant (36.05 and 35.95%), leaves per plant (20.16 and 20.22%) and leaves area per plants (12.11 and 14.55%) in both seasons (Table 2).

In comparison to untreated, well-watered plants, the spraying of GlyBet levels in particular 6000 mg/L under mild drought lessened the harmful effects of drought. The application of 6000 mg/L GlyBet resulted in the greatest vine length (38.78 and 38.91%), foliage fresh weight (49.33 and 49.23%), branches per plant (61.66 and 61.47%), leaves per plant (19.96 and 19.86%), and leaves area per plants (14.84 and 14.84%) in the 1st and 2nd years, in comparison to untreated, severely drought-affected plants (Table 2).

3.2. Photosynthetic Pigments

The data in Table 3 demonstrate that within drought stress, the concentration of photosynthetic pigments in cucumber leaves drastically decreased. Well-watered plants had the maximum concentration, which was followed in both seasons by moderate and then severe drought stress.

Additionally, Table 3 also proves that cucumber plants elicited with GlyBet displayed an encouraging impact on photosynthetic pigment accumulation related to control plants. The prime efficient was 6000 mg/L, which boosted Chl a (11.25 and 11.24%), Chl b (11.23 and 11.24%), and carotenoids (11.25 and 11.26%) compared with untreated plants.

The current study found that, in comparison to untreated plants at such drought levels, the application of GlyBet concentrations increased the concentrations of photosynthetic pigment. By boosting Chl a (11.79 and 11.77%), Chl b (11.75 and 11.82%), and carotenoid (11.83 and 11.80%) levels over untreated plants growing in extreme drought, foliar application of 6000 mg/L GlyBet reduces the negative impacts of drought.

3.3. Ion Content

Drought stress significantly reduced the percentages of N, P, and K, with the maximum reductions of N (28.01 and 25.99%), P (27.96 and 17.92%), and K (28.12 and 22.83%) recorded under severe drought linked to well-watered in both years, respectively (Table 4). Spraying GlyBet at all concentrations significantly raises the shoot's N, P, and K% compared to untreated plants (Table 4). In both growing seasons, spraying with 6000 mg/L GlyBet produced the maximum N, P, and K%. Data in Table 4 show spraying with GlyBet in special 6000 mg/L under moderate or severe drought nullified the depression impacts of drought above non-treated plants under such drought levels.

Table 2. Cucumber plant growth as affected by GlyBet concentration, irrigation regimes, and their interactions at 35 days from sowing in both seasons 2020 and 2021.

Treatments		**Vine Length (cm)**		**Foliage FW g/Plant**		**Branches No/Plant**		**Leaves No /Plant**		**Leaves Area (cm^2)/Plant**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Irrigation regimes (m^3/fed)											
WW		125.3 ± 42.6 ^{a}	108.4 ± 36.7 ^{a}	540 ± 83 ^{a}	467 ± 71 ^{a}	8.33 ± 1.60 ^{a}	7.20 ± 1.40 ^{a}	78.0 ± 15.9 ^{a}	67.5 ± 14.4 ^{a}	5062 ± 623 ^{a}	4379 ± 533 ^{a}
MD		108.9 ± 30.0 ^{b}	94.3 ± 25.0 ^{b}	433 ± 62 ^{b}	375 ± 51 ^{b}	6.91 ± 1.37 ^{b}	5.99 ± 1.16 ^{b}	65.3 ± 8.7 ^{b}	56.5 ± 7.7 ^{b}	4457 ± 530 ^{b}	3861 ± 449 ^{b}
SD		98.4 ± 25.9 ^{c}	85.5 ± 22.2 ^{c}	289 ± 92 ^{c}	251 ± 79 ^{c}	5.91 ± 2.18 ^{c}	5.13 ± 1.89 ^{c}	57.5 ± 9.3 ^{c}	50.0 ± 8.1 ^{c}	3646 ± 499 ^{c}	3167 ± 401 ^{c}
Glycinebetaine (mg/L)											
GlyBet 0		90.9 ± 17.8 ^{d}	78.7 ± 13.6 ^{d}	367 ± 231 ^{c}	318 ± 195 ^{c}	5.88 ± 2.61 ^{d}	5.09 ± 2.19 ^{d}	60.5 ± 17.2 ^{d}	52.4 ± 14.4 ^{c}	4093 ± 1175 ^{c}	3544 ± 968 ^{b}
GlyBet 2000		103.4 ± 18.5 ^{c}	89.6 ± 15.4 ^{c}	415 ± 225 ^{b}	360 ± 196 ^{b}	6.77 ± 2.05 ^{c}	5.87 ± 1.79 ^{c}	65.1 ± 9.8 ^{c}	56.4 ± 8.8 ^{b}	4283 ± 1229 ^{c}	3713 ± 1085 ^{b}
GlyBet 4000		120.2 ± 34.6 ^{b}	104.2 ± 30.6 ^{b}	441 ± 210 ^{a}	383 ± 185 ^{a}	7.55 ± 1.79 ^{b}	6.55 ± 1.64 ^{b}	69.5 ± 22.5 ^{b}	60.3 ± 20.5 ^{a}	4488 ± 1288 ^{b}	3891 ± 1156 ^{a}
GlyBet 6000		129.1 ± 32.5 ^{a}	111.8 ± 25.8 ^{a}	459 ± 215 ^{a}	397 ± 180 ^{a}	8.00 ± 2.11 ^{a}	6.92 ± 1.73 ^{a}	72.7 ± 23.9 ^{a}	63.0 ± 20.1 ^{a}	4689 ± 1346 ^{a}	4060 ± 1109 ^{a}
Interaction											
WW	GlyBet 0	100.0 ± 11.1 $^{d–g}$	86.0 ± 7.5 ^{de}	487 ± 42 ^{bc}	419 ± 29 ^{c}	7.33 ± 0.39 ^{de}	6.30 ± 0.32 ^{cd}	70.3 ± 3.7 ^{b}	60.4 ± 4.7 ^{bc}	4716 ± 330 ^{cd}	4056 ± 288 ^{bc}
	GlyBet 2000	113.0 ± 12.6 $^{c–e}$	97.7 ± 8.4 $^{b–d}$	532 ± 46 ^{ab}	460 ± 32 ^{b}	8.00 ± 0.42 ^{c}	6.92 ± 0.32 ^{b}	71.3 ± 3.7 ^{b}	61.7 ± 4.8 ^{b}	4939 ± 345 ^{bc}	4272 ± 303 ^{ab}
	GlyBet 4000	140.3 ± 15.6 ^{ab}	122.8 ± 10.6 ^{a}	559 ± 48 ^{a}	489 ± 34 ^{ab}	8.66 ± 0.45 ^{b}	7.58 ± 0.39 ^{a}	83.0 ± 4.3 ^{a}	72.6 ± 5.6 ^{a}	5178 ± 362 ^{ab}	4531 ± 322 ^{a}
	GlyBet 6000	148.0 ± 16.5 ^{a}	127.2 ± 11.1 ^{a}	582 ± 50 ^{a}	501 ± 35 ^{a}	9.33 ± 0.49 ^{a}	8.02 ± 0.41 ^{a}	87.6 ± 4.6 ^{a}	75.3 ± 5.8 ^{a}	5415 ± 379 ^{a}	4656 ± 331 ^{a}
MD	GlyBet 0	90.6 ± 10.1 ^{fg}	78.4 ± 6.7 ^{ef}	390 ± 34 ^{de}	337 ± 23 ^{d}	6.00 ± 0.31 ^{g}	5.19 ± 0.27 ^{f}	60.7 ± 3.2 ^{c}	52.4 ± 4.1 ^{de}	4166 ± 291 $^{e–g}$	3604 ± 256 $^{d–f}$
	GlyBet 2000	102.3 ± 11.3 $^{d–f}$	89.5 ± 7.8 $^{c–e}$	436 ± 38 ^{cd}	381 ± 26 ^{c}	6.66 ± 0.34 ^{f}	5.83 ± 0.30 ^{de}	62.3 ± 3.2 ^{c}	54.5 ± 4.2 $^{c–e}$	4354 ± 304 $^{d–f}$	3809 ± 270 $^{c–e}$
	GlyBet 4000	117.2 ± 13.1 ^{cd}	100.8 ± 8.8 ^{bc}	447 ± 38 ^{c}	384 ± 26 ^{c}	7.33 ± 0.39 ^{de}	6.30 ± 0.32 ^{cd}	68.3 ± 3.6 ^{b}	58.7 ± 4.5 $^{b–d}$	4555 ± 318 $^{c–e}$	3917 ± 278 $^{b–d}$
	GlyBet 6000	125.7 ± 14.0 ^{bc}	108.7 ± 9.4 ^{b}	458 ± 39 ^{c}	396 ± 27 ^{c}	7.66 ± 0.40 ^{cd}	6.63 ± 0.34 ^{bc}	70.0 ± 3.7 ^{b}	60.5 ± 4.7 ^{bc}	4754 ± 332 $^{b–d}$	4112 ± 292 ^{bc}
SD	GlyBet 0	82.0 ± 9.1 ^{g}	71.7 ± 6.2 ^{f}	225 ± 19 ^{h}	197 ± 13 ^{g}	4.33 ± 0.23 ^{h}	3.79 ± 0.19 ^{g}	50.6 ± 2.6 ^{d}	44.3 ± 3.4 ^{f}	3396 ± 237 ^{i}	2971 ± 211 ^{h}
	GlyBet 2000	95.0 ± 10.6 $^{e–g}$	81.7 ± 7.1 ^{ef}	277 ± 24 ^{gh}	238 ± 16 ^{f}	5.67 ± 0.29 ^{g}	4.87 ± 0.25 ^{f}	61.6 ± 3.2 ^{c}	53.0 ± 4.1 ^{de}	3557 ± 249 ^{hi}	3059 ± 217 ^{gh}
	GlyBet 4000	103.0 ± 11.5 $^{d–f}$	89.1 ± 7.8 $^{c–e}$	319 ± 27 ^{fg}	276 ± 19 ^{ef}	6.66 ± 0.34 ^{f}	5.76 ± 0.30 ^{e}	57.3 ± 3.0 ^{c}	49.5 ± 3.8 ^{ef}	3730 ± 261 $^{g–i}$	3226 ± 229 $^{f–h}$
	GlyBet 6000	113.8 ± 12.6 ^{cd}	99.6 ± 8.7 ^{bc}	336 ± 29 ^{ef}	294 ± 20 ^{e}	7.00 ± 0.37 ^{ef}	6.12 ± 0.31 ^{de}	60.7 ± 3.2 ^{c}	53.1 ± 4.1 ^{de}	3900 ± 273 $^{f–h}$	3412 ± 242 $^{e–g}$

WW—well-watered (1250 m^3/fed); MD—moderate drought (950 m^3/fed); SD—severe drought (650 m^3/fed); S1—first season; S2—second season; GlyBet—glycinebetaine, FW—fresh weight; fed—feddan. Means values ± standard error within each column for every trial with a similar lower-case letter are not significantly different following Tukey's HSD at $p \leq 0.05$.

Table 3. Photosyntheic pigment concentration of cucumber plant as affected by GlyBet concentration, irrigation regimes and their interactions at 35 days from sowing in both season 2020 and 2021.

Treatments		**Chl. a (mg/100 FW)**		**Chl. b (mg/100 FW)**		**Carotenoids (mg/100g FW)**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**
Irrigation regimes (m^3/fed).							
WW		76.09 ± 9.12^{h}	65.82 ± 7.28^{h}	33.04 ± 4.56^{h}	32.91 ± 3.51^{h}	21.36 ± 2.22^{h}	18.47 ± 2.03^{h}
MD		67.17 ± 6.55^{b}	58.19 ± 4.91^{b}	33.59 ± 3.28^{b}	29.09 ± 2.30^{b}	18.85 ± 1.46^{b}	16.33 ± 1.36^{b}
SD		53.12 ± 6.14^{c}	46.15 ± 4.76^{c}	26.56 ± 3.07^{c}	23.07 ± 2.28^{c}	14.91 ± 1.48^{c}	12.95 ± 1.33^{c}
Glycinebetaine (mg/L)							
GlyBet 0		61.40 ± 19.19^{b}	53.16 ± 15.64^{b}	30.70 ± 9.60^{b}	26.58 ± 7.78^{c}	17.23 ± 5.28^{c}	14.92 ± 4.38^{b}
GlyBet 2000		65.61 ± 21.41^{h}	56.89 ± 18.64^{h}	32.81 ± 10.70^{h}	28.45 ± 9.28^{b}	18.41 ± 5.90^{b}	15.97 ± 5.22^{h}
GlyBet 4000		66.52 ± 20.03^{h}	57.68 ± 17.65^{h}	33.25 ± 10.01^{h}	28.84 ± 8.78^{ab}	18.67 ± 5.51^{ab}	16.19 ± 4.95^{h}
GlyBet 6000		68.31 ± 22.14^{h}	59.14 ± 18.07^{h}	34.15 ± 11.07^{h}	29.57 ± 8.99^{h}	19.17 ± 6.10^{h}	16.60 ± 5.07^{h}
Interaction							
WW	GlyBet 0	$70.79 \pm 6.17^{b–d}$	60.88 ± 3.79^{bc}	$35.40 \pm 3.08^{b–d}$	30.44 ± 1.58^{b}	19.87 ± 1.05^{b}	17.09 ± 1.05^{bc}
	GlyBet 2000	$76.31 \pm 6.64^{a–c}$	66.01 ± 4.12^{ab}	$38.15 \pm 3.33^{a–c}$	33.01 ± 1.71^{h}	21.42 ± 1.13^{h}	18.53 ± 1.13^{ab}
	GlyBet 4000	76.91 ± 6.70^{ab}	67.29 ± 4.20^{h}	38.45 ± 3.35^{ab}	33.64 ± 1.75^{h}	21.59 ± 1.14^{h}	18.89 ± 1.16^{h}
	GlyBet 6000	80.36 ± 7.00^{h}	69.11 ± 4.31^{h}	40.18 ± 3.50^{h}	34.55 ± 1.79^{h}	22.55 ± 1.18^{h}	19.39 ± 1.19^{h}
MD	GlyBet 0	63.74 ± 5.56^{de}	55.13 ± 3.44^{d}	31.87 ± 2.77^{de}	27.56 ± 1.43^{c}	17.89 ± 0.95^{c}	15.47 ± 0.95^{d}
	GlyBet 2000	67.93 ± 5.91^{cd}	59.44 ± 3.71^{cd}	33.96 ± 2.96^{cd}	29.72 ± 1.54^{bc}	19.07 ± 1.00^{bc}	16.68 ± 1.02^{cd}
	GlyBet 4000	67.98 ± 5.91^{cd}	58.46 ± 3.65^{cd}	33.99 ± 2.96^{cd}	29.23 ± 1.51^{bc}	19.08 ± 1.01^{bc}	16.41 ± 1.00^{cd}
	GlyBet 6000	$69.05 \pm 6.01^{b–d}$	59.72 ± 3.72^{cd}	$34.53 \pm 3.00^{b–d}$	29.86 ± 1.54^{b}	19.38 ± 1.02^{b}	16.76 ± 1.02^{cd}
SD	GlyBet 0	49.68 ± 4.33^{f}	43.47 ± 2.71^{e}	24.84 ± 2.16^{f}	21.73 ± 1.13^{e}	13.94 ± 0.72^{e}	12.20 ± 0.741^{e}
	GlyBet 2000	52.61 ± 4.58^{f}	45.28 ± 2.83^{e}	26.30 ± 2.28^{f}	22.62 ± 1.17^{de}	14.76 ± 0.78^{de}	12.70 ± 0.77^{e}
	GlyBet 4000	54.66 ± 4.76^{f}	47.28 ± 2.95^{e}	27.33 ± 2.38^{f}	23.64 ± 1.23^{de}	15.34 ± 0.80^{de}	13.27 ± 0.81^{e}
	GlyBet 6000	55.54 ± 4.84^{ef}	48.59 ± 3.02^{e}	27.76 ± 2.42^{ef}	24.30 ± 1.26^{d}	15.59 ± 0.82^{d}	13.64 ± 0.84^{e}

WW—well-watered (1250 m^3/fed); MD—moderate drought (950 m^3/fed); SD—severe drought (650 m^3/fed); S1—first season; S2—second season; GlyBet—glycinebetaine; Chl. A—chlorophyll a; Chl. B—chlorophyll b; FW—fresh weight; fed—feddan. Means values ± standard error within each column for every trial with a similar lower-case letter are not significantly different following Tukey's HSD at $p \leq 0.05$.

3.4. Leaf Relative Water Content and Electrolyte Leakage

LRWC decreased under drought, and the lowest value (19.16 and 18.79%) was observed once severe drought was present compared to control (Table 4). Application of GlyBet had a favorable impact on the plant's LRWC %. In comparison to untreated control plants in both seasons, more than 12.15 and 12.17% were produced by the application of 6000 mg/L GlyBet (Table 4). The damages of DS on LRWC % were lightened by GlyBet spraying, resulting in an enhancement in LRWC % under moderate or severe drought, as compared with untreated plants grown under drought single (Table 4).

The data in the same table verified that cucumber plant EL % was dramatically enhanced by intensified drought, with the maximum EL % reported under severe drought, which increased by 11.98 and 12.43% in comparison to well-watered plants in the first and second seasons. Additionally, the use of GlyBet resulted in a non-significant reduction in EL %. The data additionally noted that, in general, the GlyBet spraying under irrigation levels increased EL % compared to untreated plants under such irrigation regimes when considering the interaction between irrigation treatment and GlyBet.

3.5. Water Use Efficiency

Data shown in Figure 1 demonstrated that, in comparison to well-watered plants, WUE increased dramatically as DS increased. When there was a severe drought, the WUE was at its highest. The maximum WUE was obtained by applying GlyBet, which enhanced it by 38.52% in the 1st season and by 39.18% in the 2nd season. Moreover, Figure 1 displays that GlyBet usage under moderate or severe drought enhanced WUE compared with untreated well-watered plants. The highest WUE in both seasons was acquired by application of 6000 mg/L GlyBet under moderate or severe drought stress, respectively.

Table 4. Ion percentage, relative water content and electrolyte leakage of cucumber plant as affected by GlyBet concentration, irrigation regimes and their interactions at 35 days from sowing in both 2020 and 2021 seasons.

Treatments		**Nitrogen %**		**Phosphorus %**		**Potassium %**		**Relative Water Content %**		**Electrolyte Leakage %**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Irrigation regimes (m^3/fed).											
WW		3.07 ± 0.35 ^{a}	2.77 ± 0.38 ^{a}	0.372 ± 0.046 ^{a}	0.318 ± 0.037 ^{a}	3.84 ± 0.44 ^{a}	3.46 ± 0.41 ^{a}	87.21 ± 9.30 ^{a}	75.43 ± 6.99 ^{a}	68.03 ± 7.77 ^{b}	58.85 ± 7.48 ^{b}
MD		2.70 ± 0.29 ^{b}	2.60 ± 0.28 ^{b}	0.327 ± 0.039 ^{b}	0.298 ± 0.023 ^{b}	3.38 ± 0.38 ^{b}	3.25 ± 0.27 ^{b}	79.09 ± 6.36 ^{b}	68.51 ± 3.92 ^{b}	75.00 ± 6.59 ^{a}	64.98 ± 6.71 ^{a}
SD		2.21 ± 0.24 ^{c}	2.05 ± 0.24 ^{c}	0.268 ± 0.033 ^{c}	0.261 ± 0.023 ^{c}	2.76 ± 0.32 ^{c}	2.67 ± 0.32c	70.50 ± 13.16 ^{c}	61.25 ± 11.13 ^{c}	76.18 ± 7.28 ^{a}	66.17 ± 6.29 ^{a}
Glycinebetaine (mg/L)											
GlyBet 0		2.48 ± 0.70 ^{d}	2.31 ± 0.62 ^{b}	0.301 ± 0.086 ^{b}	0.274 ± 0.044 ^{c}	3.10 ± 0.88 ^{d}	2.99 ± 0.68 ^{b}	74.48 ± 17.89 ^{c}	64.50 ± 14.18 ^{c}	74.88 ± 6.55 ^{a}	64.90 ± 6.51 ^{a}
GlyBet 2000		2.60 ± 0.73 ^{c}	2.51 ± 0.69 ^{a}	0.315 ± 0.090 ^{b}	0.297 ± 0.051 ^{ab}	3.25 ± 0.92 ^{c}	3.14 ± 0.84 ^{a}	77.04 ± 19.05 ^{bc}	66.79 ± 16.44 ^{c}	74.57 ± 15.17 ^{a}	64.63 ± 13.32 ^{a}
GlyBet 4000		2.72 ± 0.76 ^{b}	2.58 ± 0.76 ^{a}	0.330 ± 0.094 ^{a}	0.305 ± 0.060 ^{a}	3.40 ± 0.96 ^{b}	3.26 ± 0.83 ^{a}	80.68 ± 11.96 ^{ab}	69.95 ± 10.46 ^{b}	72.02 ± 3.25 ^{b}	62.42 ± 3.82 ^{ab}
GlyBet 6000		2.84 ± 0.80 ^{a}	2.47 ± 0.69 ^{ab}	0.345 ± 0.099 ^{a}	0.293 ± 0.052 ^{b}	3.56 ± 1.01 ^{a}	3.19 ± 0.57 ^{a}	83.53 ± 14.50 ^{a}	72.35 ± 10.96 ^{a}	70.81 ± 10.58 ^{b}	61.39 ± 10.23 ^{b}
Interaction											
WW	GlyBet 0	2.86 ± 0.12 ^{cd}	2.56 ± 0.26 ^{b}	0.3470 ± 0.024 $^{b–d}$	0.294 ± 0.015 ^{cd}	3.58 ± 0.19 ^{cd}	3.20 ± 0.20 ^{cd}	82.53 ± 6.75 $^{a–c}$	70.97 ± 3.70 ^{bc}	71.53 ± 3.11 ^{d}	61.51 ± 3.86 ^{cd}
	GlyBet 2000	2.99 ± 0.12 ^{bc}	2.79 ± 0.29 ^{ab}	0.3633 ± 0.025 ^{bc}	0.321 ± 0.016 ^{ab}	3.75 ± 0.20 ^{bc}	3.50 ± 0.21 ^{ab}	87.08 ± 7.12 ^{ab}	75.33 ± 3.92 ^{ab}	64.97 ± 2.83 ^{e}	56.20 ± 3.53 ^{de}
	GlyBet 4000	3.14 ± 0.14 ^{ab}	2.96 ± 0.30 ^{a}	0.3807 ± 0.026 ^{ab}	0.340 ± 0.017 ^{a}	3.93 ± 0.21 ^{ab}	3.70 ± 0.22 ^{a}	87.18 ± 7.13 ^{ab}	76.29 ± 3.97 ^{a}	71.55 ± 3.11 ^{d}	62.60 ± 3.93 ^{bc}
	GlyBet 6000	3.28 ± 0.14 ^{a}	2.76 ± 0.28 ^{ab}	0.3983 ± 0.028 ^{a}	0.317 ± 0.016 ^{b}	4.11 ± 0.21 ^{a}	3.45 ± 0.21 $^{a–c}$	92.04 ± 7.53 ^{a}	79.15 ± 4.12 ^{a}	64.08 ± 2.79 ^{e}	55.10 ± 3.46 ^{e}
MD	GlyBet 0	2.53 ± 0.11 ^{fg}	2.46 ± 0.25 ^{bc}	0.3063 ± 0.022 $^{e–g}$	0.283 ± 0.014 $^{c–e}$	3.16 ± 0.17 ^{fg}	3.08 ± 0.19 ^{de}	77.44 ± 6.33 ^{c}	66.99 ± 3.49 ^{cd}	78.35 ± 3.41 ^{ab}	67.77 ± 4.26 ^{ab}
	GlyBet 2000	2.64 ± 0.12 ^{ef}	2.65 ± 0.27 ^{ab}	0.3203 ± 0.023 $^{d–f}$	0.305 ± 0.016 ^{bc}	3.30 ± 0.18 ^{ef}	3.33 ± 0.20 $^{b–d}$	77.97 ± 6.38 ^{bc}	68.22 ± 3.54 ^{cd}	77.15 ± 3.36 $^{a–c}$	67.51 ± 4.23 ^{ab}
	GlyBet 4000	2.76 ± 0.11 ^{de}	2.65 ± 0.28 ^{ab}	0.3350 ± 0.023 $^{c–e}$	0.305 ± 0.015 ^{bc}	3.45 ± 0.18 ^{de}	3.32 ± 0.21 $^{b–d}$	79.68 ± 6.52 ^{bc}	68.52 ± 3.57 ^{cd}	71.31 ± 3.10 ^{d}	61.32 ± 3.85 ^{cd}
	GlyBet 6000	2.88 ± 0.13 ^{cd}	2.62 ± 0.27 ^{ab}	0.3497 ± 0.024 $^{b–d}$	0.302 ± 0.015 ^{bc}	3.61 ± 0.19 ^{cd}	3.28 ± 0.20 $^{b–d}$	81.29 ± 6.65 ^{bc}	70.31 ± 3.65 ^{bc}	73.19 ± 3.19 ^{cd}	63.31 ± 3.97 ^{bc}
SD	GlyBet 0	2.05 ± 0.08 ^{j}	1.93 ± 0.20 ^{d}	0.2500 ± 0.017 ^{i}	0.246 ± 0.013 ^{g}	2.58 ± 0.14 ^{i}	2.48 ± 0.16 ^{g}	63.47 ± 5.19 ^{e}	55.54 ± 2.89 ^{e}	74.77 ± 3.25 $^{b–d}$	65.42 ± 4.10 $^{a–c}$
	GlyBet 2000	2.15 ± 0.08 ^{ij}	2.08 ± 0.22 ^{cd}	0.2617 ± 0.018 ^{hi}	0.266 ± 0.014 $^{e–g}$	2.70 ± 0.14 ^{hi}	2.60 ± 0.16 ^{fg}	66.08 ± 5.40 ^{de}	56.83 ± 2.95 ^{e}	81.60 ± 3.55 ^{a}	70.17 ± 4.41 ^{a}
	GlyBet 4000	2.26 ± 0.09 ^{hi}	2.13 ± 0.22 ^{cd}	0.2743 ± 0.020 $^{g–i}$	0.272 ± 0.014 $^{d–f}$	2.83 ± 0.15 ^{hi}	2.76 ± 0.17 ^{fg}	75.18 ± 6.15 ^{cd}	65.03 ± 3.39 ^{d}	73.21 ± 3.19 ^{cd}	63.33 ± 3.98 ^{bc}
	GlyBet 6000	2.36 ± 0.09 ^{gh}	2.04 ± 0.21 ^{d}	0.2867 ± 0.020 $^{f–h}$	0.260 ± 0.013 ^{fg}	2.96 ± 0.15 ^{gh}	2.84 ± 0.17 ^{ef}	77.25 ± 6.32 ^{c}	67.60 ± 3.52 ^{cd}	75.15 ± 3.27 $^{b–d}$	65.76 ± 4.12 $^{a–c}$

WW—well-watered (1250 m^3/fed); MD—moderate drought (950 m^3/fed); SD—severe drought (650 m^3/fed); S1—first season; S2—second season; GlyBet—glycinebetaine; fed—feddan. Means values ± standard error within each column for every trial with a similar lower-case letter are not significantly different following Tukey's HSD at $p \leq 0.05$.

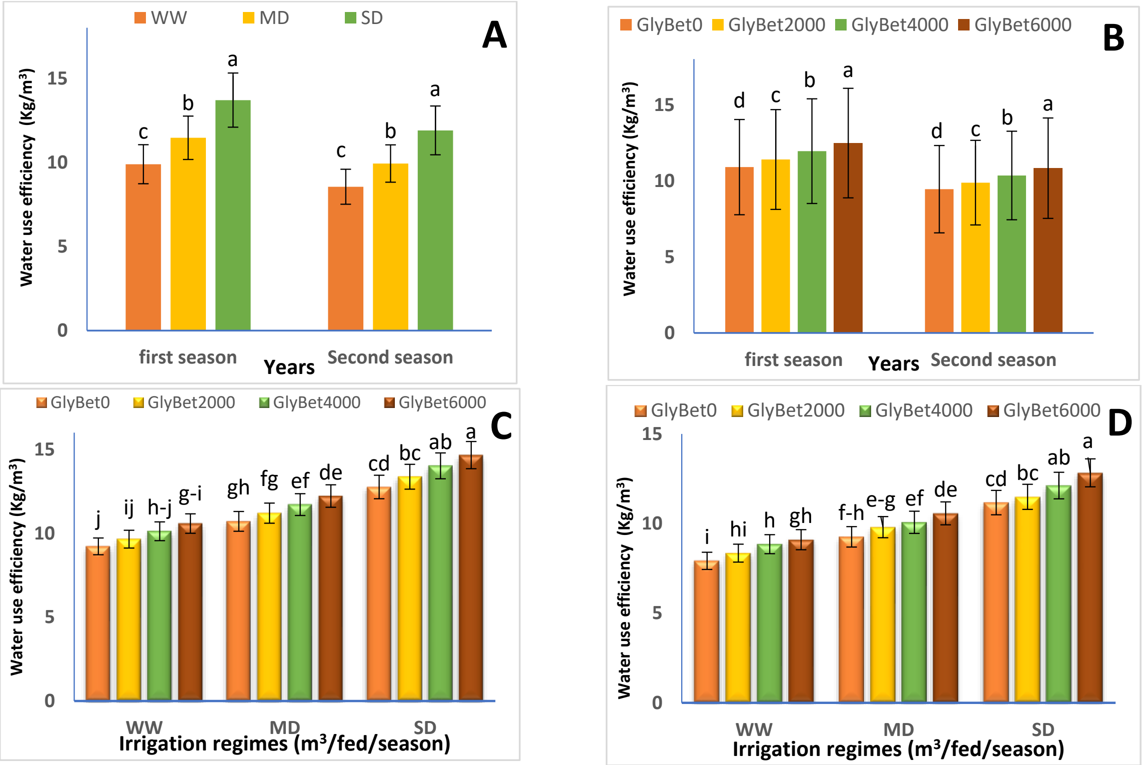

Figure 1. Water use efficiency of cucumber plant as affected by irrigation regimes (**A**), glycinebetaine concentration (**B**), and their interactions (**C**,**D**) in both seasons—2020 and 2021. WW—well-watered (1250 m^3/fed); MD—moderate drought (950 m^3/fed); SD—severe drought (650 m^3/fed); fed—feddan. Values followed by the same lower-case letter are not significantly different following Tukey's HSD at $p \leq 0.05$.

3.6. Sex Expression, Fruit Yield, and Their Components and Quality Parameters

The data in Table 5 clearly show that DS caused a dramatic reduction in yield and its component. In comparison to well-watered plants, there was a substantial decrease in fruit number per plant (55.87 and 55.31%), fruit weight/plant (28.01 and 27.70%), and total yield (27.99 and 27.68%) in the 1st and 2nd seasons, correspondingly. GlyBet spraying significantly improved all yield and its components over untreated plants. The utmost effective treatment was 6000 mg/L GlyBet, which boosted fruit number/plant (53.69 and 47.84%), fruit weight/plant (14.49 and 14.56%), and total yield (14.50 and 14.68%) in both seasons, correspondingly, compared with untreated plants (Table 5). GlyBet spraying moderates the drastic injuries of DS on cucumber crop yield. Since the supplementation of 6000 mg/L GlyBet during an extreme drought, all yield parameters in the first and second seasons have been decreased in relation to untreated drought-affected plants.

The data on the sex ratio indicates that it rose with drought and fell with the use of GlyBet application. The findings also showed that the use of GlyBet under moderate or severe drought lessens the negative effects of drought on sex ratio (Table 5).

Data in Table 6 show that drought levels increased fruit dry matter % and decreased both ascorbic acid and TSS of fruits relative to control plants. The maximum fruit dry matter was noted within severe drought. On the other hand, the greatest concentration of vitamin C and TSS was obtained under normal conditions in both seasons. Data indicated in Table 6 show that applying GlyBet considerably affected the previous parameters. The highest values were obtained while adding GlyBet at a rate of 6000 mg/L in both seasons, relative to other concentrations or untreated plants. Data presented in Table 6 indicate the interaction effects between irrigation regimes and GlyBet rates. Results show that application of GlyBet concentration under irrigation regimes significantly increased fruit dry matter %, vitamin

C, and TSS as compared with untreated plants under each irrigation regime. The highest percentages of fruit dry matter were evidently achieved with severe drought in combination with 6000 mg/L GlyBet. meanwhile, the greatest concentration of vitamin C and TSS was recorded under normal conditions and sprayed with 6000 mg/L GlyBet.

Table 5. Sex expression and yield components of cucumber plant as affected by GlyBet concentration, irrigation regimes, and their interactions in both seasons 2020 and 2021.

Treatments		**Sex Ratio**		**Fruit Weight (g)/Plant**		**Fruit No/Plant**		**Total Yield (ton/fed)**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Irrigation regimes (m^3/fed).									
WW		5.42 ± 4.48 ^{c}	5.50 ± 4.50 ^{c}	589 ± 69 ^{h}	509 ± 62 ^{h}	12.08 ± 0.94 ^{h}	10.25 ± 0.84 ^{h}	12.36 ± 1.47 ^{h}	10.69 ± 1.27 ^{h}
MD		10.85 ± 5.72 ^{b}	11.02 ± 5.75 ^{b}	518 ± 58 ^{b}	449 ± 50 ^{b}	7.08 ± 0.62 ^{b}	6.00 ± 0.25 ^{b}	10.89 ± 1.25 ^{b}	9.43 ± 1.02 ^{b}
SD		16.97 ± 4.51 ^{h}	17.29 ± 4.56 ^{h}	424 ± 49 ^{c}	368 ± 45 ^{c}	5.33 ± 0.60 ^{c}	4.58 ± 0.33 ^{c}	8.90 ± 1.06 ^{c}	7.73 ± 0.92 ^{c}
Glycinebetaine (mg/L)									
GlyBet 0		14.58 ± 9.60 ^{h}	14.85 ± 9.91 ^{h}	476 ± 135 ^{d}	412 ± 111 ^{d}	6.22 ± 0.66 ^{c}	5.33 ± 0.24 ^{c}	10.00 ± 2.85 ^{d}	8.65 ± 2.33 ^{d}
GlyBet 2000		10.94 ± 11.27 ^{b}	11.10 ± 11.32 ^{b}	498 ± 141 ^{c}	432 ± 125 ^{c}	8.11 ± 0.52 ^{b}	7.00 ± 0.26 ^{b}	10.46 ± 2.98 ^{c}	9.07 ± 2.62 ^{c}
GlyBet 4000		9.81 ± 9.44 ^{c}	9.95 ± 9.52 ^{c}	522 ± 148 ^{b}	452 ± 133 ^{b}	8.78 ± 0.48 ^{ab}	7.55 ± 0.31 ^{ab}	10.96 ± 3.12 ^{b}	9.50 ± 2.79 ^{b}
GlyBet 6000		8.99 ± 10.49 ^{d}	9.17 ± 10.79 ^{d}	545 ± 155 ^{h}	472 ± 128 ^{h}	9.56 ± 0.81 ^{h}	7.88 ± 0.33 ^{a}	11.45 ± 3.27 ^{h}	9.92 ± 2.67 ^{h}
Interaction									
WW	GlyBet 0	8.77 ± 0.66 ^{e}	8.860 ± 0.46 ^{e}	548 ± 29 ^{cd}	472 ± 29 ^{cd}	8.00 ± 0.88 ^{d}	6.660 ± 0.70 ^{de}	11.52 ± 0.70 ^{cd}	9.910 ± 0.51 $^{c–e}$
	GlyBet 2000	5.77 ± 0.44 ^{f}	5.860 ± 0.31 ^{f}	574 ± 30 ^{bc}	497 ± 31 ^{bc}	10.33 ± 0.75 ^{c}	8.670 ± 0.67 ^{c}	12.06 ± 0.73 ^{bc}	10.43 ± 0.54 ^{bc}
	GlyBet 4000	3.89 ± 0.29 ^{g}	3.990 ± 0.21 ^{g}	602 ± 32 ^{ab}	527 ± 33 ^{ab}	14.00 ± 0.83 ^{b}	12.00 ± 0.58 ^{b}	12.65 ± 0.76 ^{ab}	11.07 ± 0.57 ^{ab}
	GlyBet 6000	3.27 ± 0.25 ^{g}	3.300 ± 0.16 ^{g}	630 ± 33 ^{h}	541 ± 34 ^{h}	16.00 ± 1.02 ^{h}	13.67 ± 0.49 ^{h}	13.23 ± 0.80 ^{h}	11.37 ± 0.59 ^{h}
MD	GlyBet 0	15.25 ± 1.15 ^{c}	15.48 ± 0.80 ^{c}	485 ± 25 ^{fg}	419 ± 26 ^{ef}	5.660 ± 0.24 ^{fg}	5.000 ± 0.28 ^{fg}	10.18 ± 0.61 ^{ef}	8.800 ± 0.45 ^{fg}
	GlyBet 2000	8.820 ± 0.67 ^{e}	9.030 ± 0.46 ^{e}	506 ± 27 ^{ef}	443 ± 28 $^{d\ e}$	8.660 ± 0.33 ^{cd}	7.660 ± 0.61 ^{d}	10.63 ± 0.64 ^{de}	9.300 ± 0.48 ^{ef}
	GlyBet 4000	10.93 ± 0.82 ^{d}	11.04 ± 0.57 ^{d}	530 ± 28 ^{de}	455 ± 29 $^{c–e}$	6.330 ± 0.41 ^{f}	5.330 ± 0.50 ^{f}	11.13 ± 0.67 $^{c–e}$	9.570 ± 0.49 ^{de}
	GlyBet 6000	8.390 ± 0.63 ^{e}	8.520 ± 0.44 ^{e}	553 ± 28 ^{cd}	478 ± 30 ^{cd}	7.670 ± 0.66 ^{e}	6.000 ± 0.37 ^{e}	11.61 ± 0.70 ^{c}	10.04 ± 0.52 ^{cd}
SD	GlyBet 0	19.73 ± 1.49 ^{h}	20.23 ± 1.06 ^{h}	395 ± 21 ^{i}	346 ± 22 ^{h}	5.000 ± 0.15 ^{i}	4.330 ± 0.29 ^{h}	8.290 ± 0.50 ^{h}	7.260 ± 0.37 ^{i}
	GlyBet 2000	18.23 ± 1.37 ^{b}	18.41 ± 0.96 ^{b}	413 ± 22 ^{hi}	356 ± 22 ^{gh}	5.330 ± 0.25 ^{h}	4.660 ± 0.31 ^{g}	8.690 ± 0.52 ^{gh}	7.470 ± 0.38 ^{i}
	GlyBet 4000	14.60 ± 1.09 ^{c}	14.82 ± 0.76 ^{c}	433 ± 23 ^{hi}	375 ± 23 ^{gh}	6.000 ± 0.22 ^{g}	5.330 ± 0.62 ^{f}	9.110 ± 0.55 ^{gh}	7.880 ± 0.41 ^{hi}
	GlyBet 6000	15.32 ± 1.15 ^{c}	15.70 ± 0.82 ^{c}	454 ± 24 ^{gh}	397 ± 25 ^{fg}	5.000 ± 0.40 ^{i}	4.000 ± 0.33 ^{i}	9.530 ± 0.57 ^{fg}	8.330 ± 43 ^{gh}

WW—well-watered (1250 m^3/fed); MD—moderate drought (950 m^3/fed); SD—severe drought (650 m^3/fed); S1—first season; S2—second season; GlyBet—glycinebetaine; fed—feddan. Means values ± standard error within each column for every trial with a similar lower-case letter are not significantly different following Tukey's HSD at $p \leq 0.05$.

Table 6. Some fruit quality trials of cucumber as affected by GlyBet concentration, irrigation regimes, and their interactions in both seasons—2020 and 2021.

Treatments		**Fruit Dry Matter (%)**		**Vit. C (mg/100 g Fresh Weight)**		**TSS (°Brix)**	
		S1	**S2**	**S1**	**S2**	**S1**	**S2**
Irrigation regimes (m^3/fed).							
WW		3.07 ± 0.46 ^{b}	2.56 ± 0.40 ^{c}	29.31 ± 7.36 ^{h}	25.35 ± 6.32 ^{h}	3.03 ± 0.33 ^{h}	2.66 ± 0.31 ^{h}
MD		3.27 ± 0.55 ^{h}	2.83 ± 0.47 ^{b}	27.67 ± 6.38 ^{b}	23.96 ± 5.35 ^{b}	2.85 ± 0.24 ^{b}	2.47 ± 0.21 ^{b}
SD		3.38 ± 0.54 ^{h}	2.93 ± 0.47 ^{h}	24.51 ± 6.80 ^{c}	21.30 ± 5.90 ^{c}	2.49 ± 0.26 ^{c}	2.16 ± 0.21 ^{c}
Glycinebetaine (mg/L)							
GlyBet 0		2.91 ± 0.23 ^{d}	2.52 ± 0.23 ^{d}	22.76 ± 4.17 ^{d}	19.71 ± 3.24 ^{d}	2.62 ± 0.47 ^{b}	2.27 ± 0.36 ^{b}
GlyBet 2000		3.16 ± 0.38 ^{c}	2.74 ± 0.33 ^{c}	25.91 ± 4.80 ^{c}	22.46 ± 4.23 ^{c}	2.80 ± 0.54 ^{h}	2.43 ± 0.52 ^{h}
GlyBet 4000		3.35 ± 0.32 ^{b}	2.90 ± 0.25 ^{b}	28.66 ± 4.27 ^{b}	24.85 ± 3.77 ^{b}	2.84 ± 0.48 ^{h}	2.45 ± 0.45 ^{h}
GlyBet 6000		3.54 ± 0.36 ^{h}	3.06 ± 0.35 ^{h}	31.31 ± 4.97 ^{h}	27.12 ± 3.77 ^{h}	2.91 ± 0.55 ^{h}	2.53 ± 0.45 ^{h}
Interaction							
WW	GlyBet 0	2.81 ± 0.17 ^{g}	2.42 ± 0.15 ^{f}	24.57 ± 1.73 $^{e–g}$	21.13 ± 1.09 ^{ef}	2.83 ± 0.19 $^{b–e}$	2.43 ± 0.23 $^{b–e}$
	GlyBet 2000	2.94 ± 0.18 $^{e–g}$	2.54 ± 0.15 ^{ef}	28.08 ± 1.98 ^{cd}	24.29 ± 1.25 ^{cd}	3.03 ± 0.21 ^{ab}	2.63 ± 0.23 $^{a–c}$
	GlyBet 4000	3.19 ± 0.19 $^{d–h}$	2.79 ± 0.17 $^{c–e}$	30.69 ± 2.16 ^{bc}	26.85 ± 1.39 ^{b}	3.07 ± 0.21 ^{ab}	2.70 ± 0.20 ^{ab}
	GlyBet 6000	3.34 ± 0.20 $^{b–d}$	2.87 ± 0.18 $^{b–d}$	33.89 ± 2.39 ^{h}	29.14 ± 1.51 ^{h}	3.20 ± 0.22 ^{h}	2.76 ± 0.11 ^{h}
MD	GlyBet 0	2.90 ± 0.18 ^{fg}	2.51 ± 0.15 ^{f}	23.50 ± 1.66 ^{fg}	20.33 ± 1.06 ^{f}	2.70 ± 0.18 $^{c–f}$	2.36 ± 0.11 $^{c–f}$
	GlyBet 2000	3.22 ± 0.20 $^{c–e}$	2.82 ± 0.17 ^{cd}	26.65 ± 1.88 $^{d\ e}$	23.32 ± 1.21 ^{cd}	2.86 ± 0.20 $^{b–d}$	2.53 ± 0.23 $^{a–d}$
	GlyBet 4000	3.38 ± 0.21 $^{b–d}$	2.91 ± 0.18 ^{bc}	29.07 ± 2.05 $^{b–d}$	25.00 ± 1.30 ^{c}	2.90 ± 0.20 $^{b–d}$	2.46 ± 0.11 $^{b–e}$
	GlyBet 6000	3.59 ± 0.22 ^{ab}	3.10 ± 0.19 ^{ab}	31.46 ± 2.22 ^{ab}	27.21 ± 1.41 ^{b}	2.93 ± 0.20 $^{a–c}$	2.53 ± 0.23 $^{a–d}$
SD	GlyBet 0	3.01 ± 0.18 $^{e–g}$	2.64 ± 0.16 $^{d–f}$	20.21 ± 1.42 ^{h}	17.69 ± 0.91 ^{g}	2.33 ± 0.16 ^{g}	2.06 ± 0.11 ^{g}
	GlyBet 2000	3.31 ± 0.20 $^{b–d}$	2.85 ± 0.17 $^{b–d}$	23.00 ± 1.62 ^{gh}	19.78 ± 1.02 ^{f}	2.46 ± 0.17 ^{fg}	2.13 ± 0.20 ^{fg}
	GlyBet 4000	3.49 ± 0.21 $^{a–c}$	3.02 ± 0.18 $^{a–c}$	26.24 ± 1.85 $^{d–f}$	22.70 ± 1.17 $^{d\ e}$	2.56 ± 0.17 $^{e–g}$	2.20 ± 0.20 $^{e–g}$
	GlyBet 6000	3.68 ± 0.23 ^{h}	3.22 ± 0.20 ^{h}	28.60 ± 2.02 ^{cd}	25.02 ± 1.30 ^{c}	2.60 ± 0.18 $^{d–g}$	2.30 ± 0.11 $^{c–f}$

WW—well-watered (1250 m^3/fed); MD—moderate drought (950 m^3/fed); SD—severe drought (650 m^3/fed); S1—first season; S2—second season; GlyBet—glycinebetaine; Vit. C—vitamin C or ascorbic acid; TSS—total soluble solid; fed—feddan. Means values ± standard error within each column for every trial with a similar lower-case letter are not significantly different following Tukey's HSD at $p \leq 0.05$.

4. Discussion

Over 30–70% of crop yields are lost due to drought, one of the major obstacles that climate change has posed to crop production [10,11,15]. Therefore, it is essential to increase plants' resistance to drought in order to protect them from such yield losses and sustain productivity for food security [16,17]. Applying exogenous materials, especially those that are already compatible with plants, i.e., GlyBet, is one of the low-cost and eco-friendly, innovative water-saving techniques for improving plants' drought tolerance.

Drought commonly causes significant injury to plants, as the current study and earlier findings have shown [13–15]. In general, the occurrence of DS induces numerous physio–biochemical, morphological, and molecular alterations [13,14], such as the contraction of vascular tissues, decreased water absorption [36], and photoassimilate translocation [37]. Additionally, DS inhibited ion absorption, induced the accumulation of ROS [13,38]; and impaired ATP biosynthesis, which accelerated oxidative injury and subsequently reduced plant development [39]. Additionally, according to González-Villagra et al. [40], DS disrupts the production of endogenous phytohormones by increasing ABA concentration, lowering IAA and GAs, and rapidly decreasing zeatin concentration. The hormonal imbalance slowed down the growth of plant cells by reducing their turgor, elongation, and volume, leading to a decline in growth attributes [36]. In comparison to well-watered or unsprayed drought-affected plants, the current study has shown that GlyBet supplementation exhibits exceptional impacts on plant growth under normal or stress settings [13,18,25]. Tisarum et al. [41] also noted that by enhancing growth vigor, exogenous GlyBet treatment could mitigate the negative impacts of drought. Several potential strategies coupled with stress moderation by GlyBet have been accepted: (1) preserving water status, as demonstrated by an increase in LRWC in the recent study [18,25]; (2) an acceleration of growth promoters (IAA, GAs, salicylic acid, and cytokinin), and a reduction in ABA [42,43]; (3) increasing cell division and enlargement due to activation of water absorption and rising P concentration [44]. As for the interactions, there are a few studies confirming the current outcomes [13,18] that indicate application of GlyBet under drought mitigates the harmful effects of drought on plant growth.

Our findings demonstrated that the DS caused a significant decrease in photosynthetic pigments, which was reversed by the addition of GlyBet (Table 3). Drought-induced chlorophyll loss in several crops is a frequently seen occurrence [13,14,18]. The reduction in photosynthetic pigments within water deficit may be attributable to: (1) inhibition of the assimilation of the chlorophyll pigment complexes encoded by the cab gene family [28]; (2) destruction of chiral macro-aggregates of the light-harvesting pigment–protein complexes that offer defense to chloroplasts [45] as well as the formation of chlorophyllase [46]; and/or (3) the defeat of chloroplast membranes, exciting enlargement, modification of the lamellae vasculation and the presence of plastoglobules [47,48]. Previous studies have supported GlyBet's dramatic increase in photosynthetic pigment [13,18,25,41]. These rises could be attributable to well-organized ROS illumination mechanisms, as antioxidant enzymes and solutes would otherwise have destroyed the chlorophyll [13,18,25]. Additionally, carotenoids have the capacity to accumulate as a light receptor and photosystem shield against ROS [49]. As a result, the application of GlyBet [13,25] hastens the over-abundance of carotene in photosynthetic tissues. Finally, GlyBet protects the chloroplasts, with RUBISCO stabilizing membrane structure under drought [27]. Although the physiological effect of GlyBet is not clear, recent research showed that GlyBet acts a crucial function similar to cytokinin in enhancing the chlorophyll accumulation [50], increasing the number of chloroplasts [27].

Numerous crops have previously been shown to exhibit a decrease in ion percentage within DS [51,52]. At the soil-root interface, factors such as root form and growth rate, ion absorption kinetics, and soil nutrient supply dominate ion absorption [53]. This loss in N % is able to be connected to a decline in nitrate reductase activity that is interrelated with photosynthetic activity and decreased availability of carbon skeletons within DS [54]. The decline in K % under DS may be elucidated via the statement that a lack of water disturbs stomatal control, which reduces photosynthetic capability, and likewise the uptake of K for sustaining and regulating turgidity and stomatal control as recorded by Sarani et al. [55]. The role of GlyBet in increasing N, P, and K % is not totally implicit and there are plentiful comparable investigators who have recognized the present research. Estaji et al. [56] and Khoshkharam et al. [57] postulated that GlyBet supplementation increases ion concentrations in plant tissue. This encouraging impact might be ascribed to enhanced ion uptake, preserving membrane permeability (Table 4), and/or possibly providing a better-developed root system [27].

Drought cessations affect plant—water balance firstly, hence disturbing the plant's typical physio-biochemical occupations. LRWC % was first presented as a useful criterion for plant water status under water deficiency in the middle of the 1980s. The injuries of DS on LRWC % were alleviated by exogenous application of GlyBet, leading to an improvement in LRWC % under DS, as compared with untreated plants grown under drought only (Table 4). Previously, it was discovered that LRWC % decreased in this area during a drought [14,18,19,58]. Currently, the application of GlyBet mitigated the reducing trend of LRWC % of cucumber under DS, which was approved by Dustgeer et al. [25], Shemi et al. [18], and Yang et al. [26]. Genard et al. [59] stated that GlyBet not only preserves plant water in an arid site that may be owing to its solid hydrophilicity and solubility, but also plays a role in osmotic defense of plant tissues. Alasvandyari et al. [60] recorded that GlyBet can support plants' ability to maintain their leaves' water content by encouraging sodium elimination and K^+ accretion under drought conditions. Moreover, GlyBet spraying motivated the development of the root system and reinforces the capability of water absorption in addition to upregulation of aquaporin genes, so as to boost water preservation and enhanced WUE [26,27].

Drought causes an overabundance of ROS, which speeds up membrane lipid peroxidation and hence raises membrane permeability [14,18,19]. In this research, DS distinctly boosted EL % in cucumber leaves, which was in line with the findings of Nawaz and Wang [61] and Nazar et al. [62]. Such mutilation can be caused by oxidation and cross-linkage of protein thiols, and inhibition of key membrane proteins such as H^+-ATPase [63]. However, GlyBet application lightened the adverse effects of DS by decreasing EL % under stress or under well-watered conditions [18,25]. Current outcomes approved that GlyBet spraying could decreasing EL % by adjusting ROS homeostasis and lowering lipid peroxidation to defend cell micro-organelles from the negative injuries of drought [26]. This suggests that the use of GlyBet could maintain the stabilities of membranes in wheat plants under DS.

Typically, plant biomass significantly decreases in response to DS [48]. As a result, the total water losses from transpiration were cut in half, which significantly increased WUE [64]. The goal is to increase plant WUE in drought conditions, which can be accomplished in two ways: by improving the plant's ability to adapt and by increasing the crop's ability to produce biomass per unit of water. However, the impact of a drought on a plant's WUE often depends on the plant's cultivar and drought severity [65]. A foliar spray of GlyBet, on the other hand, increases cucumber WUE by influencing photosynthesis, enhancing root development, which leads to better water absorption, and increasing the plant's resistance to water scarcity. In some plants, such as wheat [66], increased WUE brought on by GlyBet treatment under well-watered or water-deficient conditions has already been documented.

When compared to well-watered plants, crop yield is consistently reduced by up to 70% under DS [13–15,18,41]. This decrease could be instigated by decreasing branch number and leaf size, which would decline biomass production, hinder the movement of photoassimilate to the developing fruits, and/or cause flower and fruit abortion [14]. Additionally, Song et al. [67] provided that DS caused pollen to swell, filament growth to reduce filament fertility, and grain production to decrease. According to Anjum et al. [68], drought stress decreased agricultural output by reducing photosynthetic pigments and Calvin cycle enzyme activity [18,69]. The drought-induced decline in the yield might have resulted from a diminished photosynthetic rate [14,18] and disturbed assimilate partitioning [70]. GlyBet's effects on stimulating metabolic processes and morphological modification and anatomical changes may be the cause of the increase in cucumber yield [18,27,41]. These findings agreed with the values provided by other authors [13,18,57]. The preservation of a greater net photosynthetic rate and an improvement in the source–sink relationship were both correlated with the yield enhancement by GlyBet [18,27]. GlyBet stimulates plant growth and yield owing to its osmoprotective influence on photosynthetic machinery and control of ion homeostasis [11] along with enhancing drought-affected plant CO_2 assimilation [18,57], and because of its role in biosynthesis and transport of hormones such as cytokinins that may have a role in the transport of photoassimilates [49]. Adak [71] found that TSS and vitamin C decreased by drought meanwhile increasing with GlyBet application. As for the interaction effects, several research [13,18,29,57] confirmed our results, which proved that application of GlyBet alleviated the drastic effect of drought on crop yield

5. Conclusions

Our results unequivocally show that applying GlyBet is a successful strategy for reducing drought injury and enhancing plant performance within water scarcity. Overall, spraying drought-affected cucumber plants six times at 10, 20, 30, 40, 50, 60 days after planting with 6000 mg/L GlyBet may be a potential method for reducing the effects of water deficit and therefore improving water use efficiency as well as crop yield and quality. This has a significant impact on both regional and national economic development, as well as water conservation in dry and semi-dry regions in the context of climate change adaptation efforts.

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