agronomy

MDPI

Article

Concentration-Dependent Effects of Exogenous Glycine Betaine in Alleviating Moderate Salinity Stress in Triticale (×*Triticosecale* Wittm.)

Marta Jańczak-Pieniążek [1,*], Dario Iljkić [2] and Ionel Samfira [3,4]

[1] Department of Crop Production, Faculty of Technology and Life Sciences, University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, Poland
[2] Department of Plant Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; diljkic@fazos.hr
[3] Faculty of Agriculture, University of Life Sciences "King Mihai I", Calea Aradului nr. 119, 300645 Timisoara, Romania; ionelsamfira@usvt.ro
[4] Agricultural Research and Development Station Lovrin, 307250 Lovrin, Romania
* Correspondence: mjanczak@ur.edu.pl

Abstract

Crop plants are continuously exposed to adverse environmental conditions, among which soil salinity is one of the major factors limiting plant growth and productivity. One approach to mitigating the detrimental effects of salt stress is the application of osmoprotectants such as glycine betaine (GB), which stabilise cellular structures and protect the photosynthetic apparatus. The aim of this study was to evaluate the effects of glycine betaine (10 and 20 mM) on seed germination and selected physiological parameters of triticale (×*Triticosecale* Wittm.) subjected to salt stress induced by NaCl (50, 100, and 150 mM). The study consisted of two experiments: a seed germination test conducted in Petri dishes and a pot experiment performed under controlled environmental conditions. In the pot experiment, relative chlorophyll content, chlorophyll fluorescence parameters, and gas exchange characteristics were determined. In the germination assay, salt stress impaired early seedling growth and physiological performance, whereas germination capacity remained unaffected. In the pot experiment, under non-saline stress conditions, application of 10 mM GB significantly increased F_v/F_m in Term I, F_v/Fo in Term II, and E and C_i in both terms ($p < 0.05$), whereas the increases in relative chlorophyll content and PI were not significant. In the germination test, 10 mM GB increased the number of germinal roots by 20.3% under non-saline conditions and shoot length by 62.9% under 50 mM NaCl ($p < 0.05$). No significant effect of GB application on shoot dry weight was observed ($p > 0.05$) in the pot experiment. These results indicate that exogenous application of 10 mM glycine betaine can partially alleviate the adverse effects of moderate salt stress in triticale; however, its effectiveness depends on the severity of salinity and the physiological parameter evaluated.

Keywords: ×*Triticosecale* Wittm.; salt stress; glycine betaine; germination parameters; photosynthetic apparatus; gas exchange

Check for updates

Academic Editor: Niels P. Louwaars

Received: 16 September 2026
Revised: 8 October 2026
Accepted: 13 October 2026
Published: 16 October 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Crops are constantly exposed to abiotic stresses that significantly limit their growth, development, and productivity. Among these, soil salinity is of particular importance, and its severity is increasing as a result of climate change and human activities, including improper irrigation practices and overexploitation of water resources [1,2]. It is estimated

that by 2050, up to half of the world's arable land could be affected by salinization [3], making this factor one of the key constraints on global food security. The global extent of salt-affected soils highlights the increasing importance of salinity as a constraint to agricultural production. According to the recent FAO assessment, approximately 1.38 billion hectares, corresponding to 10.7% of the world's total land area, are affected by salinity or sodicity. Moreover, approximately 10% of both irrigated and rainfed croplands are affected by salinity, although considerable uncertainty remains due to limitations in data availability. In the most severely affected countries, salinity stress can result in crop yield losses of up to 70% in crops such as rice and beans [4]. These estimates underscore the substantial impact of soil salinity on crop productivity and the need for effective strategies to improve crop tolerance to salinity, particularly through the use of approaches that can support plant performance under saline conditions.

The negative impact of salinity on plants results from the combined effects of osmotic stress, ionic toxicity, and secondary oxidative stress [5,6]. High concentrations of Na^+ and Cl^- ions lead to disturbances in water balance, reduced mineral uptake, and inhibition of photosynthetic processes. Additionally, the induced accumulation of reactive oxygen species (ROS) causes damage to cellular structures, including lipid membranes and photosynthetic apparatus, which consequently leads to a decrease in plant productivity [7].

Plants have developed complex adaptive mechanisms that enable them to function under stressful conditions, including the regulation of ionic homeostasis and the accumulation of compatible osmolytes. One of the most important osmolytic secondary metabolites is glycine betaine (GB), which plays a key role in maintaining osmotic potential, stabilizing proteins and cell membranes, and protecting the photosynthetic apparatus [8,9]. Furthermore, GB participates in regulating redox balance and mitigating oxidative stress by modulating ROS levels.

In recent years, there has been growing interest in the exogenous application of GB as a strategy to enhance plant tolerance to abiotic stresses. It has been demonstrated that GB application can improve plant physiological parameters, including photosynthetic efficiency, water status, and antioxidant enzyme activity, while reducing oxidative damage and cell membrane destabilization [10]. However, the effectiveness of this approach is highly dependent on plant species, environmental conditions, and developmental stage, and the mechanisms underlying these varied responses are not fully understood.

Despite numerous reports on the role of GB in alleviating abiotic stresses, there remains a significant research gap regarding the responses of economically important cereal crops, particularly in the context of early developmental stages and photosynthetic parameters under salt stress conditions. Particularly limited are the data on triticale (×*Triticosecale* Wittmack), a species that combines the beneficial traits of wheat (*Triticum* spp.) and rye (*Secale cereale* L.) and is characterized by relatively high tolerance to adverse environmental conditions [11]. Triticale is an important component of crop production systems, especially under conditions of limited inputs and on marginal soils, where its ability to adapt to abiotic stresses is crucial for yield stability [12,13]. However, the relative salt tolerance of triticale does not mean an absence of limitations on growth and physiological processes caused by salt stress [11,13]. Therefore, the use of exogenous biostimulants, such as GB, may be an additional strategy to support the natural tolerance mechanisms of triticale and maintain its productivity under salinity conditions. Despite this potential, the response of this species to exogenous glycine betaine application under salinity conditions remains insufficiently characterized, particularly regarding germination processes and the functioning of the photosynthetic apparatus.

Therefore, the objective of this study was to determine the effect of exogenous glycine betaine application on seed germination parameters and photosynthetic properties of

triticale grown under salt stress conditions. The hypothesis was that the application of GB may contribute to improved salinity tolerance by maintaining physiological performance, including photosynthetic activity, in triticale seedlings.

2. Materials and Methods

2.1. Experiment Design

The experiment with triticale (×*Triticosecale* Wittm.) was conducted in two stages. In the first stage, germination and early seedling growth were evaluated under laboratory conditions using Petri dishes. The second stage consisted of a pot experiment conducted under controlled conditions to assess selected physiological parameters of the plants.

The same experimental treatments were used in both stages, which allowed for a comparison of the plants' responses to the tested factor at different stages of development—from germination and early seedling growth to more advanced developmental stages.

The experiment was conducted according to a completely randomized design. Factor A (NaCl concentration): 50, 100, 150 mM NaCl. Factor B (GB concentration): 10, 20 mM. Glycine betaine (GB; Sigma-Aldrich, St. Louis, MO, USA; molecular weight 117.15 g mol^{-1}; CAS No. 107-43-7) was used at concentrations of 10 and 20 mM. The required amount of GB powder was dissolved in deionized water to obtain the desired concentrations.

For each combination of salinity × GB, n = 3 Petri dishes/pots (biological replicates).

The experimental treatments were:

1. Control (0 mM NaCl, 0 mM GB)
2. 50 mM NaCl, 0 mM GB
3. 100 mM NaCl, 0 mM GB
4. 150 mM NaCl, 0 mM GB
5. 0 mM NaCl, 10 mM GB
6. 0 mM NaCl, 20 mM GB
7. 50 mM NaCl, 10 mM GB
8. 100 mM NaCl, 10 mM GB
9. 150 mM NaCl, 10 mM GB
10. 50 mM NaCl, 20 mM GB
11. 100 mM NaCl, 20 mM GB
12. 150 mM NaCl, 20 mM GB.

2.1.1. Germination Experiment

The experiment on triticale grain germination was conducted under laboratory conditions using 90 mm diameter Petri dishes lined with a double layer of filter paper. Twenty-five grains were placed on each dish, evenly distributed, and then moistened with 10 mL of test solutions. The experiment was conducted at a temperature of 20 ± 1 °C, in complete darkness, in an incubation chamber, while maintaining constant substrate moisture. After 7 days, germination capacity was determined, expressed as the percentage of germinated seeds according to the formula:

$$\text{Germination Capacity} = (n/N) \times 100,$$

where n is the number of germinated seeds, and N is the total number of seeds sown. Seeds with visible roots were considered germinated.

In addition, morphological measurements of the seedlings were performed, including the number of seed roots, the length of the longest seed root, and the length of the above-ground part, measured using a ruler or calliper with an accuracy of 1 mm. Furthermore, the fresh weight of the seedlings was determined by weighing them immediately after the

experiment ended, after first drying them to remove excess moisture, using a laboratory balance with an accuracy of 0.01 g.

2.1.2. Pot Experiment

The pot experiment was conducted in pots measuring 11 × 11 × 15 cm, with 3 kg of soil per pot. The experiment was conducted using an artificial soil mixture consisting of 70% quartz sand, 25% deacidified peat, and 5% clay. The substrate was air-dried, sieved through a 2 mm mesh sieve, and thoroughly mixed to achieve a homogeneous structure. The pH of the mixture was adjusted to 6.0 (in H_2O), and soil moisture was maintained at 60% of maximum water capacity, controlled by the weighing method through regular addition of deionized water to compensate for water loss. The pots were weighed regularly, and the amount of deionized water required to restore the target weight corresponding to 60% of maximum water capacity was added. No additional NaCl or GB was applied during the experiment. Before sowing, mineral fertilizers were applied in amounts ensuring optimal plant growth conditions (N—100 mg·kg^{-1} of soil as ammonium nitrate, P—50 mg·kg^{-1} as KH_2PO_4, K—80 mg·kg^{-1} as K_2SO_4). Winter triticale seeds (×*Triticosecale* Wittmack, cv. SU Favonius, bred by SAATEN-UNION GmbH (Isernhagen, Germany)) were used in the experiment. This variety is characterized by high yield potential and tolerance to soil acidification, making it suitable for poorer sites. The experiment was conducted under controlled conditions in a growth chamber. Throughout the experiment, the temperature was maintained at 22 ± 2 °C, relative humidity at 60 ± 3%, the photoperiod at 16/8 h (day/night), and the maximum light intensity at approximately 300 µE m^{-2}·s^{-1}. The position of the pots was randomly changed every week during the experiment. The experiment was conducted according to a completely randomized design with repeated measurements over time. Factor A (NaCl concentration): 50, 100, 150 mM NaCl. Factor B (GB concentration): 10, 20 mM. For each combination of salinity and GB, n = 3 pots were used (biological replicates).

NaCl and GB were applied simultaneously as a single soil application when the plants reached the BBCH 15 stage. For each treatment, NaCl and GB were dissolved together in deionized water to prepare a single treatment solution at the respective concentrations. The treatment solution was applied directly to the soil substrate of each pot at a volume of 100 mL per pot. NaCl was applied only once at the beginning of the treatment period and was not subsequently replenished. Thereafter, plants were watered as required with deionized water to maintain adequate substrate moisture. Thus, the reported NaCl concentrations (50, 100, and 150 mM) refer to the nominal concentrations of the solutions used for the initial soil application and should not be interpreted as constant NaCl concentrations in the soil solution throughout the experimental period. Physiological parameters were measured twice: one day after the soil application (Term I) and 7 days after the application (Term II). After the measurements were taken, the aboveground parts were cut, air-dried, and weighed to determine their dry weight.

2.2. Physiological Parameter Measurements

2.2.1. Relative Chlorophyll Content Index (CCI)

The relative chlorophyll content in leaves was estimated based on the CCI using a CCM-200 chlorophyll meter (Opti-Sciences Inc., Hudson, NH, USA). Readings were taken on three fully developed leaves, obtaining a total of ten measurements for each pot.

2.2.2. Chlorophyll Fluorescence

Chlorophyll fluorescence parameters, including the maximal photochemical efficiency of photosystem II (PS II) (Fv/Fm), the efficiency of the water-splitting complex on the donor side of PSII (Fv/Fo), and the performance index (PI), were determined using a standard

measurement procedure according to Jańczak-Pieniążek et al. [14]. For each treatment, ten replicate measurements were performed per pot.

2.2.3. Gas Exchange

Plant gas exchange parameters: net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (Ci) were determined using a portable LCpro-SD photosynthesis analyzer (ADC BioScientific Ltd., Herts, UK). Measurements were conducted under controlled conditions at a photosynthetically active radiation (PAR) intensity of 1500 µmol m^{-2} s^{-1} and a temperature of 28 °C. For each treatment, three replicate measurements were performed per pot.

2.3. Statistical Analysis

Data analysis was performed using TIBCO Statistica 13.3.0. Germination data were analyzed using a two-way ANOVA with NaCl concentration and GB concentration as fixed factors. The main effects of NaCl and GB and their interaction (NaCl × GB) were tested. Post hoc comparisons were performed using Tukey's HSD test at $p \leq 0.05$.

For the parameters P_N, E, g_s, Ci, CCI, F_v/F_m, F_v/F_o, and PI, a two-factor (two-way) ANOVA with repeated measures was performed. NaCl treatment and GB application were included as between-subject factors, while measurement time (Term I and Term II) was treated as a within-subject repeated-measures factor. The pot was considered the experimental unit and was included as a random effect. Three pots were used for each treatment combination. The same experimental units were assessed at both measurement times. Multiple measurements performed within each pot were treated as subsamples of the corresponding experimental unit and were not considered independent biological replicates. The normality of the residual distribution was verified using the Shapiro–Wilk test, while the homogeneity of variances was verified using Levene's test; Post hoc comparisons were performed using Tukey's (HSD) test for marginal means at a significance level of $p \leq 0.05$.

3. Results

3.1. Germination Experiment

Table 1 presents the triticale germination parameters obtained in the first part of the experiment. Neither salinity nor the application of glycine betaine (GB) had a significant effect on germination capacity.

Salinity at 100 and 150 mM NaCl significantly reduced seedling fresh weight compared with the control, whereas GB application did not significantly affect this parameter under either control or saline conditions. Fresh weight tended to increase following GB application under salinity, although the difference was not statistically significant.

Salinity at 100 and 150 mM NaCl significantly reduced the number of seedling roots, while 50 mM NaCl had no significant effect. Under control conditions, 10 mM GB significantly increased the number of roots compared with the control. Under saline conditions, however, GB application did not significantly affect root number.

Shoot length was reduced under all applied NaCl concentrations. GB application did not significantly affect shoot length compared with the control. Under 50 mM NaCl, 10 mM GB increased shoot length, whereas 20 mM GB did not show this effect. At 100 and 150 mM NaCl, GB application did not significantly affect shoot length.

Table 1. Effects of salt stress and exogenous application of glycine betaine on selected germination parameters of triticale grains.

Variants	Germination Capacity (%)	Fresh Seedling Mass (g)	Number of Roots (pcs.)	Length of the Longest Root (cm)	Shoot Length (cm)
Control	97.3 ± 2.3 a	3.38 ± 1.03 bc	4.67 ± 1.28 c	5.79 ± 2.42 fg	2.83 ± 1.22 cd
50 mM NaCl, 0 mM GB	96.0 ± 0.0 a	2.65 ± 0.09 ab	4.42 ± 1.20 bc	4.71 ± 1.85 de	1.43 ± 1.12 b
100 mM NaCl, 0 mM GB	96.0 ± 4.0 a	2.34 ± 0.49 a	3.78 ± 1.17 a	3.74 ± 1.58 bc	1.17 ± 0.75 ab
150 mM NaCl, 0 mM GB	89.3 ± 4.6 a	2.42 ± 0.25 a	3.67 ± 0.96 a	3.31 ± 1.38 ab	0.68 ± 0.41 a
0 mM NaCl, 10 mM GB	97.3 ± 4.6 a	3.35 ± 0.32 bc	5.62 ± 0.79 d	5.95 ± 1.99 g	2.94 ± 1.18 d
0 mM NaCl, 20 mM GB	94.7 ± 2.3 a	3.52 ± 0.11 c	4.41 ± 1.13 bc	4.91 ± 1.96 ef	2.34 ± 0.89 c
50 mM NaCl, 10 mM GB	92.0 ± 8.0 a	2.72 ± 0.35 ab	4.65 ± 1.17 bc	5.34 ± 1.90 efg	2.33 ± 1.01 c
100 mM NaCl, 10 mM GB	93.3 ± 11.5 a	2.70 ± 0.36 ab	4.04 ± 1.08 ab	4.52 ± 1.85 cde	1.54 ± 0.98 b
150 mM NaCl, 10 mM GB	84.0 ± 8.0 a	2.74 ± 0.60 ab	4.14 ± 1.24 abc	3.77 ± 1.63 bcd	1.12 ± 0.87 ab
50 mM NaCl, 20 mM GB	86.7 ± 8.3 a	2.48 ± 0.06 a	4.54 ± 1.08 bc	4.38 ± 1.49 cde	1.51 ± 0.69 b
100 mM NaCl, 20 mM GB	86.7 ± 12.2 a	2.58 ± 0.37 a	4.14 ± 1.03 abc	2.68 ± 1.13 a	0.74 ± 0.43 a
150 mM NaCl, 20 mM GB	85.3 ± 9.2 a	2.45 ± 0.35 a	4.06 ± 1.13 abc	2.39 ± 1.26 a	0.82 ± 0.74 a
Mean	96.1 ± 4.93	2.78 ± 0.54	4.36 ± 1.21	4.33 ± 2.06	1.65 ± 1.18
F/*p* value	F = 1.38 ns	F = 2.51 $p < 0.05$	F = 15.13 $p < 0.0001$	F = 29.19 $p < 0.0001$	F = 54.55 $p < 0.0001$

Values are expressed as the mean ± standard deviation (SD). Means followed by different letters are significantly different according to Tukey's multiple comparison test ($p < 0.05$).

3.2. Physiological Parameters

3.2.1. Relative Chlorophyll Content

Salinity reduced relative chlorophyll content compared with the control, with significant decreases observed at 100 mM NaCl in Term I and at 150 mM NaCl in both terms (Figure 1). Under non-saline conditions, both 10 and 20 mM GB significantly increased relative chlorophyll content compared with the control at both measurement times. Under saline conditions, GB application resulted in an increase in relative chlorophyll content compared with the corresponding NaCl treatments without GB; however, these differences were not statistically significant. An overall increasing trend in relative chlorophyll content was observed between the two measurement times, although a significant increase was detected only in plants treated with 50 mM NaCl without GB.

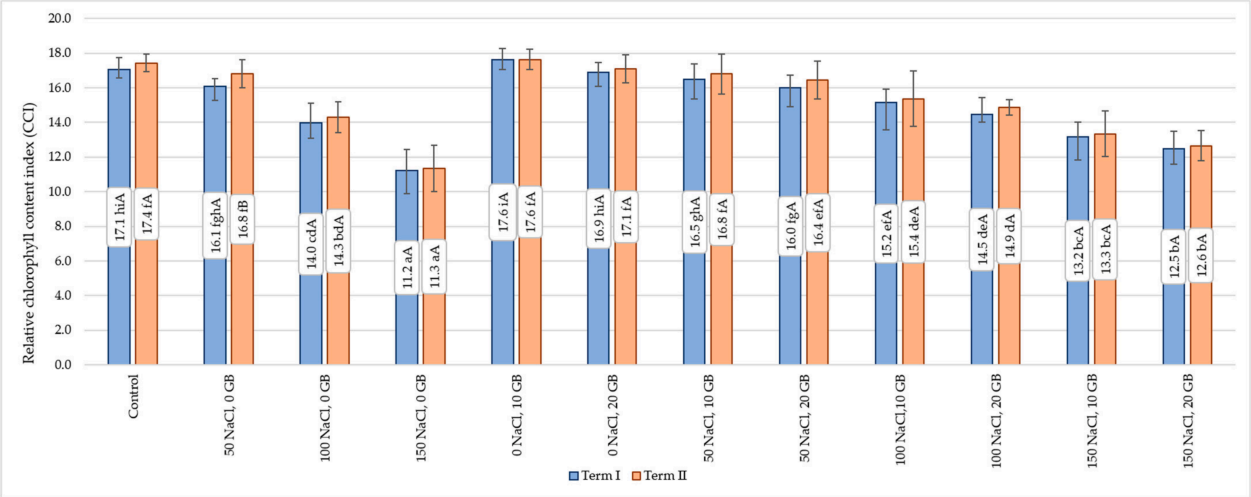


Figure 1. Relative chlorophyll content in triticale seedlings under salt stress and glycine betaine (GB) treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were assessed using Tukey's multiple comparison test ($p < 0.05$).

3.2.2. Chlorophyll Fluorescence

(a) Maximal photochemical efficiency of PS II (F_v/F_m)

Soil salinity significantly decreased the F_v/F_m ratio compared with the control, with the lowest values recorded under 150 mM NaCl (Figure 2a). The decrease relative to the control reached 12.0% in Term I and 12.3% in Term II. Under non-saline conditions, 10 mM GB significantly increased the F_v/F_m ratio in Term I, whereas 20 mM GB had no significant effect. Under saline conditions, GB significantly increased the F_v/F_m ratio in several treatment combinations, namely at 50 mM NaCl with 10 mM GB in both terms and with 20 mM GB in Term II, at 100 mM NaCl with 10 mM GB in both terms, and at 150 mM NaCl with 10 mM GB in Term I. A significant increase in F_v/F_m between Term I and Term II was also observed in all variants except 100 mM NaCl without GB.

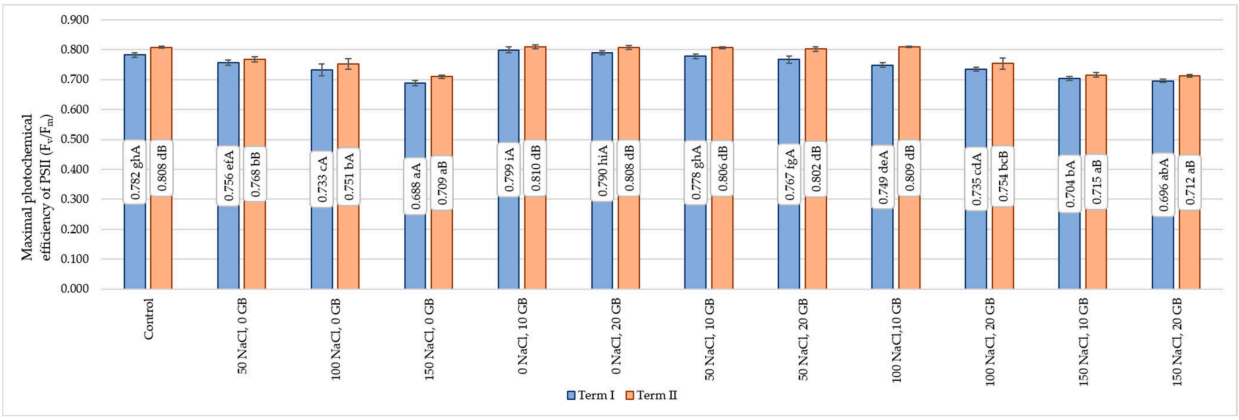
(b) Efficiency of the water-splitting complex on the donor side of PSII (F_v/F_o)

Salt treatment significantly decreased the F_v/F_o ratio at 50 mM NaCl in Term I and at 100 and 150 mM NaCl in both measurement terms (Figure 2b). The greatest decrease was observed at 150 mM NaCl, reaching 21.8% in Term I and 14.6% in Term II compared with the control. GB application under saline conditions generally resulted in higher F_v/F_o values than the corresponding NaCl treatments without GB; however, significant increases were observed only at 100 mM NaCl with 10 mM GB in Term I and at 150 mM NaCl with 10 mM GB in Term I. Fv/F_o increased between Term I and Term II in most treatments, with no significant increase observed in the control and in the 100 mM NaCl + 10 mM GB treatment.

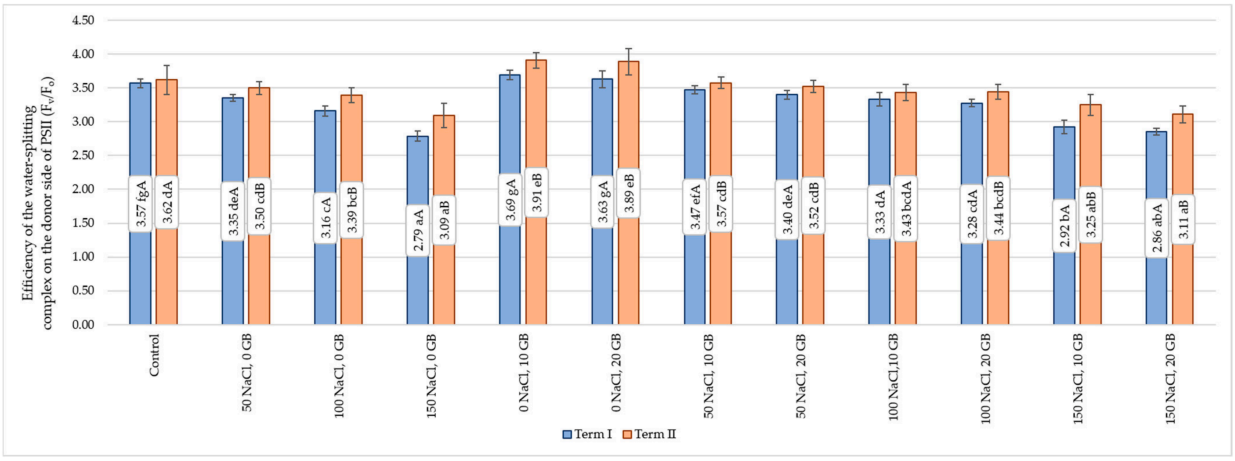
(c) Performance Index (PI)

Salt treatment significantly decreased the F_v/F_o ratio at 50 mM NaCl in Term I and at 100 and 150 mM NaCl in both measurement terms (Figure 2b). The greatest decrease was observed at 150 mM NaCl, reaching 21.8% in Term I and 14.6% in Term II compared with the control. GB application under saline conditions generally resulted in higher F_v/F_o values than the corresponding NaCl treatments without GB; however, significant increases were observed only at 100 mM NaCl with 10 mM GB in Term I and at 150 mM NaCl with 10 mM GB in Term I. Fv/F_o increased between Term I and Term II in most treatments,

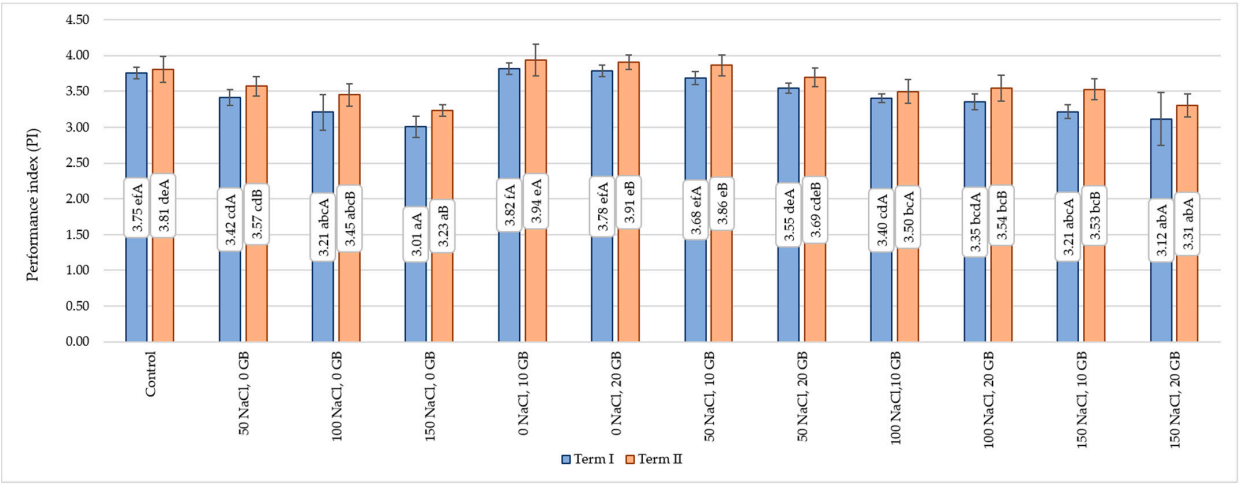
with no significant increase observed in the control and in the 100 mM NaCl + 10 mM GB treatment.



(**a**)



(**b**)



(**c**)

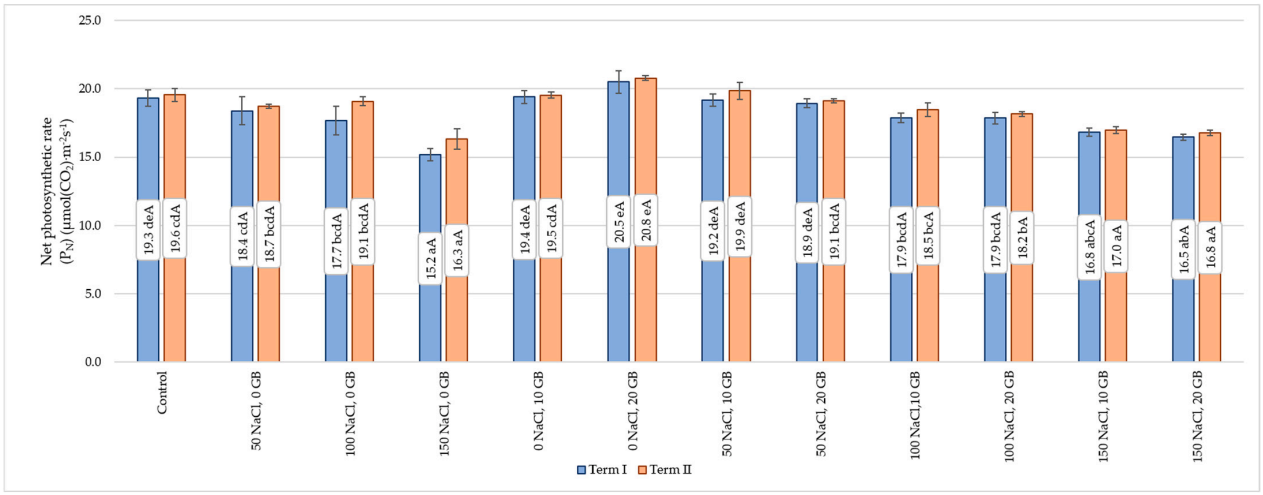
Figure 2. (**a**) Maximal photochemical efficiency of PSII (F_v/F_m) in triticale seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$). (**b**) Efficiency of the water-splitting complex on the donor side of PSII (Fv/Fo) in triticale

seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$). (**c**) Performance Index (PI) in triticale seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$).

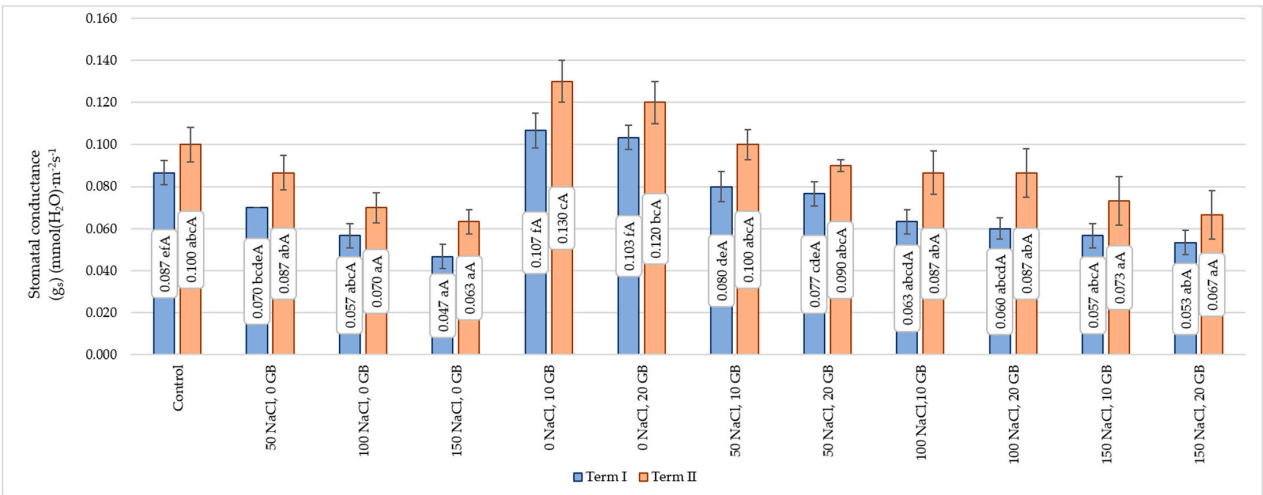
3.2.3. Gas Exchange

(a) Net photosynthetic rate (P_N)

Salt stress significantly reduced P_N only at 150 mM NaCl, with decreases of 21.2% in Term I and 16.8% in Term II compared with the control (Figure 3a). Under non-saline conditions, 20 mM GB significantly increased P_N by 6.2% and 6.1% in Term I and Term II, respectively. Under saline conditions, GB application resulted in numerically higher P_N values than the corresponding NaCl treatments without GB; however, these differences were not statistically significant. No significant differences in P_N were observed between Term I and Term II.

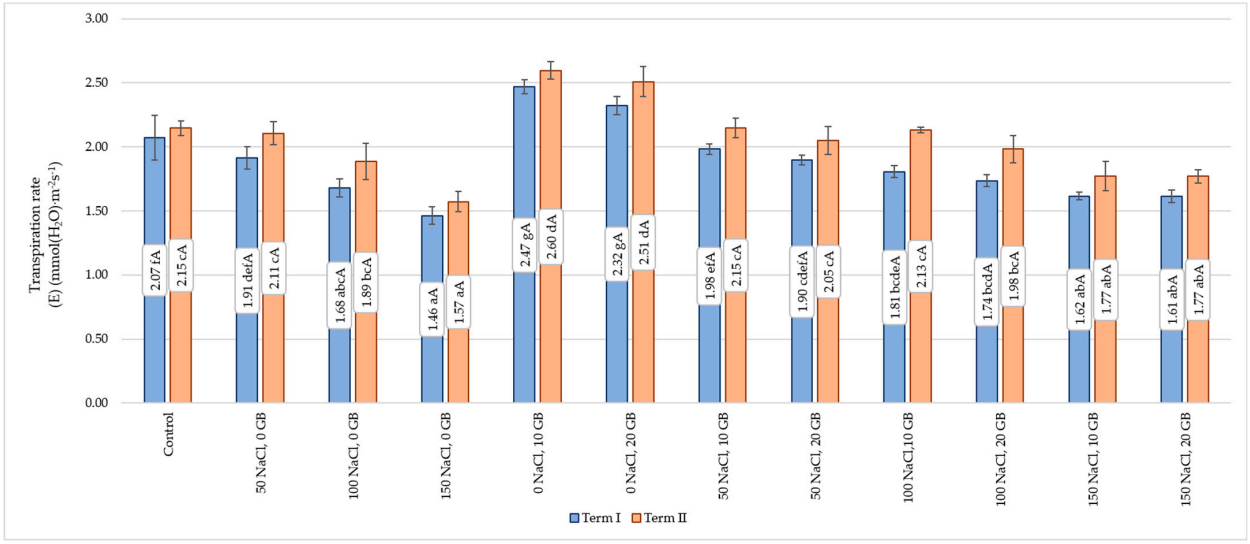


(**a**)

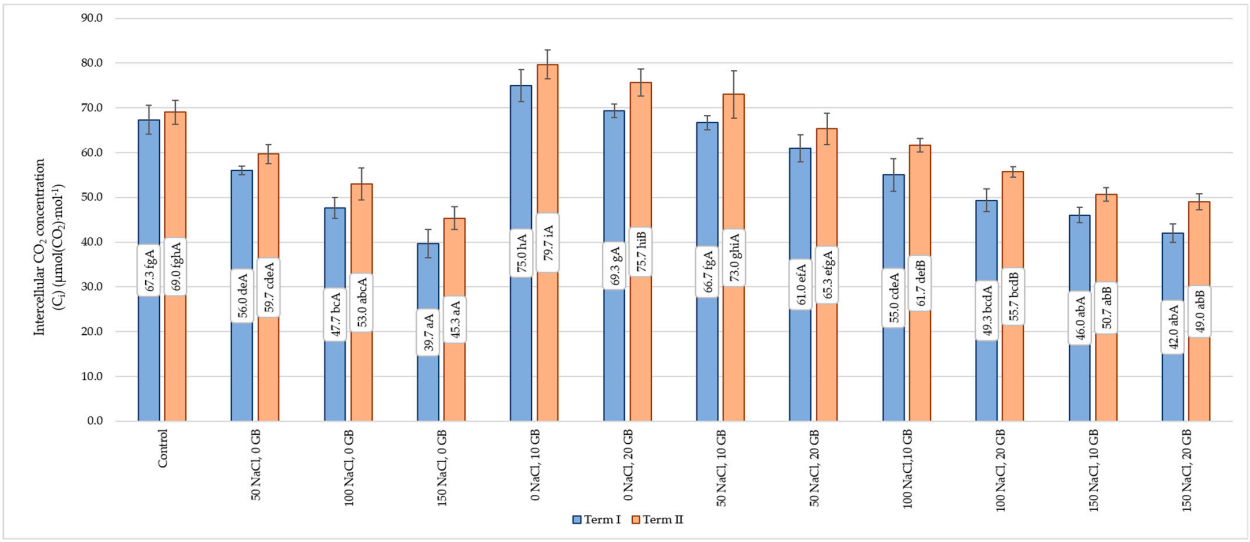


(**b**)

Figure 3. *Cont.*



(**c**)



(**d**)

Figure 3. (**a**) Net photosynthetic rate (P_N) in triticale seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$). (**b**) Stomatal conductance (g_s) in triticale seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$). (**c**) Transpiration rate (E) in triticale seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$). (**d**) Intercellular CO_2 concentration (Ci) in triticale seedlings under salt stress and glycine betaine treatment at different measurement times. Values are presented as mean ± standard deviation (SD). Lowercase letters indicate significant differences among treatments within the same measurement time, whereas uppercase letters indicate significant differences between measurement times. Differences were determined using Tukey's multiple comparison test ($p < 0.05$).

(b) Stomatal conductance (g_s)

Soil salinity significantly reduced g_s at 100 and 150 mM NaCl in Term I compared with the control (Figure 3b). GB application resulted in a numerical increase in g_s under both control and saline conditions; however, these differences were not statistically significant. No significant differences in g_s were observed between Term I and Term II.

(c) Transpiration rate (E)

Soil salinity reduced E, with significant decreases observed at 100 mM NaCl in Term I and at 150 mM NaCl in both measurement terms (Figure 3c). The lowest E values were recorded at 150 mM NaCl, corresponding to decreases of 29.5% in Term I and 27.0% in Term II compared with the control. Under non-saline conditions, GB significantly increased E compared with the control, with significant increases observed for both 10 and 20 mM GB in both measurement terms. In contrast, GB application did not significantly affect E under saline conditions compared with the corresponding NaCl treatments without GB. No significant differences in E were observed between Term I and Term II.

(d) Intercellular CO_2 concentration (Ci)

Soil salinity significantly decreased C_i at all analyzed NaCl concentrations (Figure 3d). Under non-saline conditions, 10 mM GB significantly increased C_i compared with the control by 11.4% in Term I and 15.5% in Term II. Under saline conditions, GB significantly increased C_i compared with the corresponding NaCl treatments without GB at 50 mM NaCl with 10 mM GB in both terms and at 100 mM NaCl with 10 mM GB in Term II. Significant increases in C_i between Term I and Term II were observed in the 0 mM NaCl + 20 mM GB, 100 mM NaCl + 10 mM GB, 100 mM NaCl + 20 mM GB, 150 mM NaCl + 10 mM GB, and 150 mM NaCl + 20 mM GB treatments.

3.3. Aboveground Dry Weight

Soil salinity resulted in a numerical decrease in the dry mass of the aboveground part of triticale plants; however, the values did not differ significantly from the control (Figure 4). Under non-saline conditions, 10 mM GB resulted in a dry mass of 2.18 g, which did not differ significantly from the control (2.04 g) but was significantly higher than that observed in the corresponding salt-stressed variant without GB. However, under saline conditions, GB application did not significantly affect aboveground dry mass compared with the respective NaCl treatments without GB.

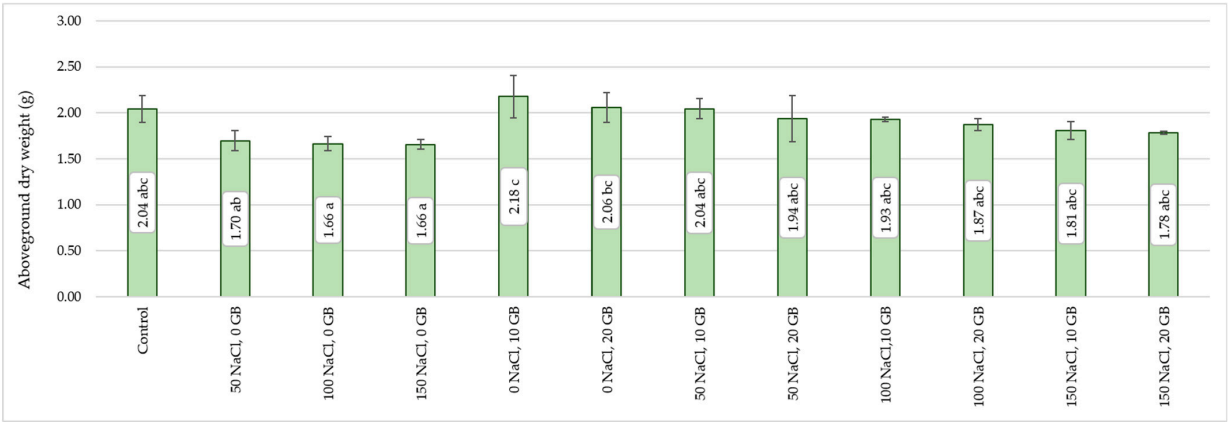


Figure 4. Aboveground dry weight in triticale seedlings under salt stress and glycine betaine treatment. Values are presented as mean ± standard deviation (SD). Different lowercase letters indicate significant differences among treatments according to Tukey's multiple comparison test ($p < 0.05$).

4. Discussion

Salinity is a significant abiotic stress factor affecting plant function. Its impact is primarily linked to osmotic stress and the toxic effects of ions. Long-term exposure of plants to elevated salinity leads to disturbances in water and mineral homeostasis, reduced photosynthesis and a decline in productivity [15,16]. Among cereals, triticale is considered a species that is relatively tolerant to adverse environmental conditions; however, its growth and development may also be restricted by increasing soil salinity. Therefore, assessing this species' response to salt stress is significant, both from a scientific and practical perspective [17].

The first experiment examined whether GB application could alleviate the adverse effects of salinity on germination and morphological parameters of triticale, whereas the second evaluated plant responses to GB under salinity in pot conditions. Thus, the two experiments provide complementary information on GB-mediated responses at different stages of plant development, and differences in the magnitude of responses between experiments should not be interpreted as direct quantitative comparisons. It was shown that the application of salt solutions resulted in reduced seedling growth, as evidenced by a lower number of roots, as well as shorter root and shoot lengths compared to the control. However, a significant reduction in the parameters under investigation occurred with the application of solutions with higher concentrations, i.e., 100 and 150 mM. The results obtained indicate a dose-dependent response of triticale seedlings to salinity. As the NaCl concentration increased, there was a gradual reduction in seedling growth and root development, confirming the negative impact of salt stress on the early stages of cereal plant development.

The reduction in the growth parameters of triticale seedlings under salinity stress may be primarily due to the occurrence of osmotic stress. High salt concentrations in the growing medium lower the water potential, limiting water uptake by germinating seeds and young seedlings. Limited water availability leads to a reduction in the rate of cell division and elongation, resulting in shorter roots and shoots. Furthermore, the accumulation of sodium (Na^+) and chloride (Cl^-) ions can induce ionic stress, disrupting the uptake of essential nutrients, particularly potassium (K) and calcium (Ca). Consequently, root system growth is inhibited, the number of primary roots is reduced, and the development of the plants' above-ground parts is impaired [16,18,19]. However, ion accumulation and nutrient uptake were not directly assessed in the present study. The observed reduction in seedling growth is therefore discussed in the context of these mechanisms described in the literature. These findings are consistent with the research by Karim et al. [20] and Ramadan et al. [21], who demonstrated that increasing NaCl concentrations lead to reduced growth in triticale seedlings.

In the experiment conducted, a slight decreasing trend in triticale grain germination was observed following the application of salt solutions. Ionic toxicity caused by various types of salinity disrupts cellular processes, causing damage to plant tissues and hindering the normal growth of seedlings. Ramadan et al. [21] demonstrated that high accumulation of Na^+ ions inhibited triticale germination. This was evident in the germination parameters, which declined as the concentration of NaCl increased. This can be explained by a disruption in the uptake of nutrients and water, leading to growth inhibition, leaf wilting and other symptoms of physiological stress. In the experiment conducted, the decline in germination capacity was not statistically significant. The lack of a significant effect of salinity on triticale germination capacity may result from the seeds' greater tolerance to osmotic stress compared with seedlings. This is confirmed by studies indicating that the germination phase is less sensitive to NaCl than the growth phase [22,23].

In many cases, moderate concentrations of NaCl do not completely inhibit the germination process but only delay its progression or restrict the rate of seedling development. This is because, in the initial phase of germination, seeds utilise their stored reserves, and their metabolism is only to a limited extent dependent on external conditions. It is only during the seedling growth phase that the negative effects of osmotic and ionic stress become more pronounced, leading to reduced cell elongation and organ development. Consequently, a reduction in seedling length and impaired root system development are observed, which is associated with disturbances in water balance and a slowing down of cell division and elongation processes under the influence of excess Na^+ and Cl^- ions [24,25].

Salt stress also led to a decline in the physiological parameters of triticale plants in the pot experiment. Rapid and non-destructive phenotyping methods are becoming increasingly important in assessing plant responses to salinity, as they enable the early detection of stress even before morphological changes become apparent. The most used parameters include chlorophyll fluorescence (including F_v/F_m), the relative chlorophyll content index (CCI) and photosystem II (PSII) activity indices, which allow the functioning of the photosynthetic apparatus to be assessed under stressful conditions. Previous studies have shown that chlorophyll fluorescence enables the detection of disturbances in PSII function as early as the initial stage of NaCl exposure [26]. Furthermore, these methods are widely used in high-throughput phenotyping, enabling rapid comparison of genotypes in terms of salt stress tolerance without the need to destroy plant material [27]. Non-destructive physiological measurements are therefore a valuable tool in the selection of tolerant plants and in the assessment of the intensity of abiotic stress, as demonstrated in studies by Jańczak-Pieniążek et al. [14], Singh et al. [28], Angmo et al. [29], and Hniličkа et al. [30].

The experiment conducted demonstrated that salt stress, following the application of a salt solution—particularly at higher concentrations of 100 and 150 mM—led to a reduction in the values of the physiological parameters examined in the plants.

The results obtained indicate that salinity significantly altered the physiological functioning of triticale seedlings, a finding consistent with the literature describing the response of triticale plants to salt stress [31,32]. Salt stress adversely affects various processes, including photosynthetic metabolism, chlorophyll concentration in leaves, photosynthetic capacity and stomatal conductance [33]. In the later stages of stress development, the ionic component plays a significant role, linked to the accumulation of Na^+ and Cl^- in plant tissues. A disruption in ionic balance leads to reduced availability of key elements, such as K^+ and Ca^{2+}, resulting in the destabilisation of cell membranes and impaired functioning of chloroplast structures. Consequently, a reduction in the efficiency of photosystem II (PSII) is observed, which may manifest as a decrease in chlorophyll fluorescence parameters and an overall decline in photosynthetic efficiency [34]. The observed reduction in relative chlorophyll content under salinity stress indicates disturbances in the metabolism of photosynthetic pigments, resulting from both a reduction in chlorophyll biosynthesis and its accelerated degradation under conditions of oxidative stress. This process is commonly described as one of the early responses of plants to salt stress, associated with the destabilisation of chloroplast membranes and increased ROS production [22,35].

At the same time, changes in chlorophyll fluorescence parameters, particularly a decrease in PSII efficiency, indicate disturbances in the functioning of the electron transport chain. A reduction in fluorescence parameters, such as F_v/F_m, is interpreted as a sign of photoinhibition and a reduced ability of the photosynthetic apparatus to utilise light energy efficiently. This phenomenon is strongly linked to the accumulation of Na^+ and Cl^- ions in chloroplasts and the induction of oxidative stress [34,35].

Another important aspect of plants' response to salinity is the reduction in gas exchange resulting from a decrease in stomatal conductance. Stomatal closure is a protective mechanism that limits transpiration and water loss; however, it also leads to a reduction in CO_2 availability in the leaf mesophyll, which results in a reduction in the rate of photosynthesis. Consequently, there is a decrease in carbon assimilation and a reduction in biomass growth [35,36].

The negative effects of abiotic stress, including salinity stress, can be mitigated using biostimulants that support plants' natural defence mechanisms. One of the most well-known compounds with such an effect is glycine betaine (GB), which is classified as a compatible osmolite. Although some plant species synthesise GB in response to adverse environmental conditions, this process is energetically costly; therefore, the exogenous application of this compound is an effective method of increasing plants' tolerance to abiotic stress, particularly salinity [37–39].

Studies on seedling germination showed that the addition of GB did not significantly affect the germination of triticale seeds, either in treatments without salt stress or in those where salinity was applied. The application of GB at a concentration of 10 mM resulted in a significant increase in the number of roots compared with the control, while it did not significantly affect the fresh weight of the seedling, the length of the longest root or the length of the shoot. For most of the parameters analysed, particularly following the addition of 10 mM GB, an improvement in the morphological parameters of the seedlings was observed compared to the salt-stressed treatments, especially in the case of salt concentrations of 100 and 150 mM. The beneficial effect of GB is already evident at the seed germination stage. Salama et al. [40] demonstrated that priming wheat grains (*Triticum aestivum* L.) with a GB solution before sowing effectively mitigated the negative effects of salt stress on seedling growth. The application of GB improved the relative water content in tissues, reduced damage to cell membranes by decreasing lipid peroxidation, increased glutathione levels and helped maintain ionic homeostasis. As a result, higher fresh and dry weights of the above-ground parts and roots were recorded compared with plants that had not undergone priming. The authors Salama et al. [40] and Zhu et al. [7] indicate that the improvement in salt tolerance stems primarily from the protection of cell membranes, the reduction in oxidative stress and the maintenance of osmotic balance.

The studies also demonstrated a positive effect of GB application on the values of the physiological parameters examined. In treatments where salt stress was not applied following the addition of GB, particularly at a concentration of 10 mM, an increase in physiological indicators was observed; however, this was statistically significant only for F_v/F_m (Term I), F_v/F_o (Term II), E and Ci. Although GB applications increased E and C_i in some treatments, these changes were not accompanied by a significant increase in P_N under saline conditions. Thus, the observed changes in gas exchange should be interpreted cautiously and cannot be taken as direct evidence of improved carbon assimilation or water use efficiency.

Previous studies indicate that the protective effect of GB stems from its multi-directional impact on plant physiology and metabolism. The primary mechanism is osmotic regulation of cells, enabling the maintenance of water potential and the reduction of water loss during stress. At the same time, GB stabilises cell membranes and protein structures, protecting them from denaturation and aggregation, and reduces lipid peroxidation and membrane damage caused by reactive oxygen species (ROS) [37,38]. The accumulation of compatible osmolytes, including GB, depends on salinity levels, water availability and plant species [41]. These mechanisms have been proposed based on previous studies; however, they were not directly assessed in the present experiment and therefore should be considered as possible explanations for the observed responses.

A key aspect of GB's action is the reduction of oxidative stress. Previous studies by other researchers have shown that exogenous application of GB increases the activity of antioxidant enzymes and supports the functioning of the ascorbate-glutathione cycle. This leads to more effective removal of ROS and a reduction in damage to proteins, lipids and nucleic acids [28,42,43]. Furthermore, GB may increase the expression of genes encoding enzymes responsible for ROS elimination, which further strengthens the plants' antioxidant system. However, oxidative stress and antioxidant responses were not directly assessed in the present study; therefore, these mechanisms are discussed based on the previous literature and cannot be considered directly demonstrated by our results.

Many studies also highlight the protective effect of GB on the photosynthetic apparatus. This compound stabilises the structure of PSII, protects thylakoid membranes and maintains the activity of Rubisco and the Calvin cycle enzymes, thereby limiting the decline in photosynthetic efficiency under stressful conditions [39,44,45]. Furthermore, GB contributes to the repair of stress-induced damage to PSII and protects photosynthetic complexes from excessive ROS production [43]. This is confirmed by the results of experiments in which, following the application of GB, an increase in chlorophyll content, higher F_v/F_m values and improvements in gas exchange parameters and photosynthetic rate were observed [9,46].

Another important mechanism enhancing plants' tolerance to salinity is the regulation of ion homeostasis. Studies have shown that GB limits the uptake of toxic Na^+ and Cl^- ions, reduces stress-induced K^+ efflux from cells, and increases the activity of the plasma membrane H^+-ATPase, which helps maintain ionic homeostasis and normal cell function [7,47]. However, ion concentrations and ion transport were not directly assessed in the present study; therefore, this mechanism cannot be considered directly demonstrated by our results.

Numerous studies also indicate that GB acts as a regulator of the molecular processes occurring during the stress response. In addition to its direct osmoregulatory effect, it influences the expression of genes associated with the stress response, the activity of antioxidant enzymes, the synthesis of heat shock proteins, and genes that respond to auxin and regulate cell division [28,39]. It has also been shown that GB interacts with phytohormones such as abscisic acid, salicylic acid, jasmonic acid, ethylene and polyamines, participating in the regulation of signalling pathways responsible for plant adaptation to adverse environmental conditions [28].

The effectiveness of exogenous GB application has been confirmed in many crop species. In maize, foliar application of GB reduced the negative impact of salinity on growth, increased chlorophyll content, and improved photosynthetic parameters and the activity of antioxidant enzymes [7,48]. Similar effects were observed in wheat, where leaf water content and PSII efficiency were improved [44,46]. In tomatoes, by contrast, exogenous application of GB improved photosynthetic activity under salinity conditions and alleviated the inhibition of seed germination caused by low-temperature stress [49,50]. The beneficial effect of GB has also been demonstrated in oilseed rape [10], stevia [51], *Glycyrrhiza uralensis* [42] and Indian mustard, where biomass, gas exchange parameters and osmoregulatory capacity were improved [9]. Furthermore, Zuzunaga-Rosas et al. [52] demonstrated that a biostimulant containing GB stimulated root system development, increased the content of photosynthetic pigments and reduced the uptake of Na^+ and Cl^- ions by the roots. These findings are consistent with the latest reports by Saidur Rhaman et al. [1], who demonstrated that exogenous application of GB effectively helps plants to overcome the negative effects of salinity by improving water management, reducing oxidative stress and maintaining the efficiency of the photosynthetic apparatus.

The response to GB was not consistently concentration-dependent. In several cases, 10 mM GB produced a more pronounced beneficial response than 20 mM GB, while the

higher concentration did not provide additional improvement and, for some parameters, resulted in less favorable responses. This may indicate that the beneficial effects of exogenous GB occur within an optimal concentration range and that increasing its concentration beyond this range does not necessarily enhance plant responses to salinity. However, the mechanisms underlying the different responses to 10 and 20 mM GB were not directly investigated in the present study and therefore remain to be elucidated. The optimal concentration of exogenous GB may depend on plant species, developmental stage, salinity level, and experimental conditions, which may explain differences in the response to 10 and 20 mM GB.

In the present study, improvements in the values of the physiological indicators under investigation were observed in some of the analysed cases in the pot experiment in Term II. A similar relationship had previously been demonstrated in studies on wheat seedlings conducted by Jańczak-Pieniążek et al. [14]. Higher values of the physiological indicators under investigation were obtained at subsequent measurement dates. This can be explained by the activation of defence mechanisms that play a role in detoxifying excess ROS produced by cells under stress conditions [53,54].

A limitation of the study is that the experiments were conducted under controlled conditions and at an early stage of plant development, which restricts the ability to directly extrapolate the results to field conditions and later stages of triticale growth. Furthermore, the efficacy of the applied GB may depend on its concentration, the method of application, and the species and cultivar of the plant under study. The use of NaCl to induce salt stress does not fully reflect the complex ionic composition and conditions found in naturally saline soils. In addition, NaCl concentration or electrical conductivity of the soil solution was not monitored throughout the experimental period. Therefore, subsequent watering may have caused some dilution or redistribution of NaCl in the substrate. The effects observed in the present study should consequently be interpreted in relation to the initial NaCl treatment rather than as responses to a continuously maintained soil salinity level. Therefore, further field studies are needed to determine whether the observed short-term physiological responses persist under prolonged salinity and translate into effects on grain yield and yield components. Such studies should also evaluate different GB application methods, concentrations, and application timings, including foliar application at key developmental stages such as anthesis and grain filling, under field conditions before practical agronomic recommendations can be made.

5. Conclusions

Salinity negatively affected triticale growth and physiological performance, whereas germination capacity remained unaffected. Exogenous application of GB, particularly at 10 mM, improved selected physiological responses under moderate salinity (50 mM NaCl), whereas its protective effects were limited under severe salinity (100–150 mM NaCl). These findings indicate that 10 mM GB may have potential to support triticale performance under short-term moderate salinity stress; however, the observed effects were obtained under controlled conditions and do not provide sufficient evidence for direct agronomic recommendations. Further field studies under diverse environmental and salinity conditions are needed to determine whether these responses persist under prolonged stress and translate into improvements in grain yield and yield components, as well as to assess the practical feasibility of GB application.

Author Contributions: Conceptualization, M.J.-P., D.I. and I.S.; methodology, M.J.-P. and D.I.; validation, M.J.-P., D.I. and I.S.; formal analysis, M.J.-P.; investigation, M.J.-P. and D.I.; resources, M.J.-P., D.I. and I.S.; writing—original draft preparation, M.J.-P. writing—review and editing, M.J.-P., D.I. and I.S.;

visualization, M.J.-P. and D.I.; supervision, M.J.-P., D.I. and I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

F_v/F_m	Maximal photochemical efficiency of photosystem II (PSII)
F_v/F_o	Efficiency of the water-splitting complex on the donor side of PSII
PI	Performance Index
P_N	Net photosynthetic rate
g_s	Stomatal conductance
E	Transpiration rate
Ci	Intercellular CO_2 concentration

References

1. Rhaman, M.S.; Tahjib-Ul-Arif, M.; Kibria, M.; Hoque, A. Climate change and its adverse impacts on plant growth in South Asia: Current status and upcoming challenges. *Phyton* **2022**, *91*, 695–711. [CrossRef]
2. Shahid, M.A.; Sarkhosh, A.; Khan, N.; Balal, R.M.; Ali, S.; Rossi, L.; Gómez, C.; Mattson, N.; Nasim, W.; Garcia-Sanchez, F. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy* **2020**, *10*, 938. [CrossRef]
3. Butcher, K.; Wick, A.F.; DeSutter, T.M.; Chatterjee, A.; Harmon, J. Soil salinity: A threat to global food security. *Agron. J.* **2016**, *108*, 2189–2200. [CrossRef]
4. Srarfi, F.; Majar, A. *Global Status of Salt-Affected Soils*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2024. [CrossRef]
5. Flowers, T.J. Improving crop salt tolerance. *J. Exp. Bot.* **2004**, *55*, 307–319. [CrossRef] [PubMed]
6. Yang, Y.; Guo, Y. Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytol.* **2018**, *217*, 523–539. [CrossRef] [PubMed]
7. Zhu, M.; Li, Q.; Zhang, Y.; Zhang, M.; Li, Z. Glycine betaine increases salt tolerance in maize (*Zea mays* L.) by regulating Na+ homeostasis. *Front. Plant Sci.* **2022**, *13*, 978304. [CrossRef] [PubMed]
8. Annunziata, M.G.; Ciarmiello, L.F.; Woodrow, P.; Dell'Aversana, E.; Carillo, P. Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. *Front. Plant Sci.* **2019**, *10*, 230. [CrossRef] [PubMed]
9. Islam, S.; Mohammad, F.; Shakeel, A.; Corpas, F.J. Glycine betaine: A multifaceted protectant against salt stress in Indian mustard through ionic homeostasis, ROS scavenging and osmotic regulation. *Physiol. Plant.* **2024**, *176*, e14530. [CrossRef] [PubMed]
10. Khalid, A.; Athar, H.U.R.; Zafar, Z.U.; Akram, A.; Hussain, K.; Manzoor, H.; Ashraf, M. Photosynthetic capacity of canola (*Brassica napus* L.) plants as affected by glycinebetaine under salt stress. *J. Appl. Bot. Food Qual.* **2015**, *88*, 78–86. [CrossRef]
11. Alagoz, S.M.; Hadi, H.; Toorchi, M.; Pawłowski, T.A.; Lajayer, B.A.; Price, G.W.; Farooq, M.; Astatkie, T. Morpho-physiological responses and growth indices of triticale to drought and salt stresses. *Sci. Rep.* **2023**, *13*, 8896. [CrossRef] [PubMed]
12. Grieve, C.M.; Grattan, S.R.; Maas, E.V. Plant salt tolerance. In *Agricultural Salinity Assessment and Management*, 2nd ed.; Wallender, W.W., Tanji, K.K., Eds.; ASCE: Reston, VA, USA, 2012; pp. 405–459, Chapter 13. [CrossRef]
13. Salehi, M.; Arzani, A. Evaluation of triticale genotypes for salt tolerance using physiological traits. *Emir. J. Food Agric.* **2014**, *26*, 277–283. [CrossRef]
14. Jańczak-Pieniążek, M.; Migut, D.; Piechowiak, T.; Balawejder, M. Assessment of the impact of the application of a quercetin–Copper complex on the course of physiological and biochemical processes in wheat plants (*Triticum aestivum* L.) growing under saline conditions. *Cells* **2022**, *11*, 1141. [CrossRef] [PubMed]
15. Arif, Y.; Singh, P.; Siddiqui, H.; Bajguz, A.; Hayat, S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiol. Biochem.* **2020**, *156*, 64–77. [CrossRef] [PubMed]
16. Lindberg, S.; Premkumar, A. Ion changes and signaling under salt stress in wheat and other important crops. *Plants* **2024**, *13*, 46. [CrossRef] [PubMed]
17. Blum, A. The abiotic stress response and adaptation of triticale—A review. *Cereal Res. Commun.* **2014**, *42*, 359–375. [CrossRef]

18. Wang, C.F.; Han, G.L.; Yang, Z.R.; Li, Y.X.; Wang, B.S. Plant salinity sensors: Current understanding and future directions. *Front. Plant Sci.* **2022**, *13*, 859224. [CrossRef] [PubMed]
19. Zou, Y.; Zhang, Y.; Testerink, C. Root dynamic growth strategies in response to salinity. *Plant Cell Environ.* **2022**, *45*, 695–704. [CrossRef] [PubMed]
20. Karim, M.A.; Utsunomiya, N.; Shigenaga, S. Effect of sodium chloride on germination and growth of hexaploid triticale at early seedling stage. *Jpn. J. Crop Sci.* **1992**, *61*, 279–284. [CrossRef]
21. Ramadan, E.; Freeg, H.A.; Shalaby, N.; Rizk, M.S.; Ma, J.; Du, W.; Ibrahim, O.M.; Alwutayd, K.M.; AbdElgawad, H.; Jo, I.H.; et al. Response of nine triticale genotypes to different salt concentrations at the germination and early seedling stages. *PeerJ* **2023**, *11*, e16256. [CrossRef] [PubMed]
22. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]
23. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J.I. Plant salt-tolerance mechanisms. *Trends Plant Sci.* **2014**, *19*, 371–379. [CrossRef] [PubMed]
24. Atak, M.; Kaya, M.D.; Kaya, G.; Çikili, Y. Effects of NaCl on the Germination, Seedling Growth and Water Uptake of Triticale. *Turk. J. Agric. For.* **2006**, *30*, 39–47. Available online: https://journals.tubitak.gov.tr/agriculture/vol30/iss1/5/ (accessed on 12 June 2026).
25. Manono, B.O. Effects of salinity on seed germination: Mechanisms, impacts, and mitigation strategies. *Seeds* **2026**, *5*, 1. [CrossRef]
26. Mehta, P.; Jajoo, A.; Mathur, S.; Bharti, S. Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. *Plant Physiol. Biochem.* **2010**, *48*, 16–20. [CrossRef] [PubMed]
27. Shah, S.H.; Houborg, R.; McCabe, M.F. Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy* **2017**, *7*, 61. [CrossRef]
28. Singh, G.M.; Goldberg, S.; Schaefer, D.; Zhang, F.; Sharma, S.; Mishra, V.K.; Xu, J. Biochemical, gas exchange, and chlorophyll fluorescence analysis of maize genotypes under drought stress reveals important insights into their interaction and homeostasis. *Photosynthetica* **2022**, *60*, 376–388. [CrossRef] [PubMed]
29. Angmo, D.; Sharma, S.P.; Kalia, A.; Brar, N.S.; Bhardwaj, V. Effect of cold stress on field performance, chlorophyll fluorescence, electrolyte leakage and leaf gas exchange parameters of potato (*Solanum tuberosum* L.) genotypes. *Potato Res.* **2023**, *66*, 641–661. [CrossRef]
30. Hnilička, F.; Hniličková, H.; Rýgl, T. Gas exchange and chlorophyll fluorescence of four sorghum genotypes under drought stress and rehydration. *Plant Soil Environ.* **2024**, *70*, 543–551. [CrossRef]
31. Gołębiowska-Paluch, G.; Stawoska, I.; Jelonek-Kozioł, M.; Wesełucha-Birczyńska, A.; Kornaś, A. Soil salinity differentiates winter triticale genotypes in physiological and biochemical characteristics of seedlings and consequently their yield. *Int. J. Mol. Sci.* **2024**, *25*, 12971. [CrossRef] [PubMed]
32. Aghaei, F.; Sharifi, R.S.; Farzaneh, S. Effects of nano iron–silicon oxide on yield and some biochemical and physiological characteristics of triticale under salinity stress. *Silicon* **2024**, *16*, 3267–3279. [CrossRef]
33. Akter, M.; Mahmud, A.; Akter, M.; Siddiqui, M.N.; Khan, M.A.R. Dissecting the salt tolerance potential of cowpea genotypes based on morphophysiology and yield-related attributes. *Ann. Appl. Biol.* **2021**, *180*, 428–437. [CrossRef]
34. Kalaji, H.M.; Jajoo, A.; Oukarroum, A.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Łukasik, I.; Goltsev, V.; Ladle, R.J. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiol. Plant.* **2016**, *38*, 102. [CrossRef]
35. Negrão, S.; Schmöckel, S.M.; Tester, M. Evaluating physiological responses of plants to salinity stress. *Ann. Bot.* **2017**, *119*, 1–11. [CrossRef] [PubMed]
36. Yildirim, E.; Ekinci, M.; Turan, M.; Ağar, G.; Ors, S.; Dursun, A.; Kul, R.; Akgül, G. Physiological and biochemical changes of pepper cultivars under combined salt and drought stress. *Gesunde Pflanz.* **2022**, *74*, 675–683. [CrossRef]
37. Gupta, B.; Huang, B. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Int. J. Genom.* **2014**, *2014*, 701596. [CrossRef] [PubMed]
38. Bai, M.; Zeng, W.; Chen, F.; Ji, X.; Zhuang, Z.; Jin, B.; Wang, J.; Jia, L.; Peng, Y. Transcriptome expression profiles reveal response mechanisms to drought and drought- Stress mitigation mechanisms by exogenous glycine betaine in maize. *Biotechnol. Lett.* **2022**, *44*, 367–386. [CrossRef] [PubMed]
39. Hernandez-Leon, S.G.; Valenzuela-Soto, E.M. Glycine betaine is a phytohormone-like plant growth and development regulator under stress conditions. *J. Plant Growth Regul.* **2023**, *42*, 5029–5040. [CrossRef]
40. Salama, K.H.A.; Mansour, M.M.F.; Al-Malawi, H.A. Glycinebetaine priming improves salt tolerance of wheat. *Biologia* **2015**, *70*, 1334–1339. [CrossRef]
41. Desoky, E.S.M.; Ibrahim, S.A.; Merwad, A.R.M. Mitigation of salinity stress effects on growth, physio-chemical parameters and yield of snapbean (*Phaseolus vulgaris* L.) by exogenous application of glycine betaine. *Int. Lett. Nat. Sci.* **2019**, *76*, 60–71. [CrossRef]
42. Dong, X.; Ma, X.; Zhao, Z.; Ma, M. Exogenous betaine enhances salt tolerance of *Glycyrrhiza uralensis* through multiple pathways. *BMC Plant Biol.* **2024**, *24*, 165. [CrossRef] [PubMed]

43. Jarin, A.; Ghosh, U.K.; Hossain, M.S.; Mahmud, A.; Khan, M.A.R. Glycine betaine in plant responses and tolerance to abiotic stresses. *Discov. Agric.* **2024**, *2*, 127. [CrossRef]
44. Ahmed, N.; Zhang, Y.; Yu, H.; Gabar, A.; Zhou, Y.; Li, Z.; Zhang, M. Seed priming with glycine betaine improves seed germination characteristics and antioxidant capacity of wheat (*Triticum aestivum* L.) seedlings under water-stress conditions. *Appl. Ecol. Environ. Res.* **2019**, *17*, 8333–8350. [CrossRef]
45. Huang, S.; Zuo, T.; Ni, W. Important roles of glycinebetaine in stabilizing the structure and function of the photosystem II complex under abiotic stresses. *Planta* **2020**, *251*, 36. [CrossRef] [PubMed]
46. Khedr, R.A.; Sorour, S.G.; Aboukhadrah, S.H.; Shafey, N.M.; Elsalam, H.E.; El-Sharnouby, M.E.; El-Tahan, A.M. Alleviation of salinity stress effects on agro-physiological traits of wheat by auxin, glycine betaine, and soil additives. *Saudi J. Biol. Sci.* **2022**, *29*, 534–540. [CrossRef] [PubMed]
47. Li, D.; Zhang, T.; Wang, M.; Liu, Y.; Brestic, M.; Chen, T.H.; Yang, X. Genetic engineering of the biosynthesis of glycine betaine modulates phosphate homeostasis by regulating phosphate acquisition in tomato. *Front. Plant Sci.* **2019**, *9*, 1995. [CrossRef] [PubMed]
48. Irshad, I.; Anwar-ul-Haq, M.; Akhtar, J.; Maqsood, M. Enhancing maize growth and mitigating salinity stress through foliar application of proline and glycine betaine. *Pak. J. Bot.* **2024**, *56*, 9–17. [CrossRef] [PubMed]
49. Niu, T.; Zhang, J.; Li, J.; Gao, X.; Ma, H.; Gao, Y.; Chang, Y.; Xie, J. Effects of exogenous glycine betaine and cycloleucine on photosynthetic capacity, amino acid composition, and hormone metabolism in *Solanum melongena* L. *Sci. Rep.* **2023**, *13*, 7626. [CrossRef] [PubMed]
50. Zhang, Y.; Dai, T.; Liu, Y.; Wang, J.; Wang, Q.; Zhu, W. Effect of exogenous glycine betaine on the germination of tomato seeds under cold stress. *Int. J. Mol. Sci.* **2022**, *23*, 10474. [CrossRef] [PubMed]
51. Ghorbani, A.; Ghasemi-Omran, V.O.; Chen, M. The effect of glycine betaine on nitrogen and polyamine metabolisms, expression of glycoside-related biosynthetic enzymes, and K/Na balance of stevia under salt stress. *Plants* **2023**, *12*, 1628. [CrossRef] [PubMed]
52. Zuzunaga-Rosas, J.; González-Orenga, S.; Tofei, A.M.; Boscaiu, M.; Moreno-Ramón, H.; Ibáñez-Asensio, S.; Vicente, O. Effect of a biostimulant based on polyphenols and glycine betaine on tomato plants' responses to salt stress. *Agronomy* **2022**, *12*, 2142. [CrossRef]
53. Huchzermeyer, B.; Menghani, E.; Khardia, P.; Shilu, A. Metabolic pathway of natural antioxidants, antioxidant enzymes and ROS providence. *Antioxidants* **2022**, *11*, 761. [CrossRef] [PubMed]
54. Dos Santos, T.B.; Ribas, A.F.; de Souza, S.G.H.; Budzinski, I.G.F.; Domingues, D.S. Physiological responses to drought, salinity, and heat stress in plants: A review. *Stresses* **2022**, *2*, 113–135. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.