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Improvement of Salinity Tolerance in Rice Seedlings by Exogenous Magnesium Sulfate Application

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Abstract: This study was conducted to develop the salt tolerance of rice by exogenous application of magnesium sulfate supplement ($MgSO_4$). The salinization was carried out on 7-day-old rice seedlings including BC15 (salinity tolerant) and DT84DB (salinity susceptible) varieties with 0.5 mM $MgSO_4$. The exogenous application of $MgSO_4$ significantly improves the growth of seedlings of both varieties. In addition, antioxidant activities increase in line with the raise of total phenolic and total flavonoid contents. Remarkably, the contents of momilactone B (MB) and phenolic compounds including tricin, *ρ*-coumaric, salicylic, cinnamic, benzoic, and ferulic acids simultaneously rise in both varieties treated by salinity and 0.5 mM $MgSO_4$. Interestingly, MB was not found in the salt-treated samples but presents with considerable contents in the salt and $MgSO_4$-treated cultivars. The findings imply that $MgSO_4$ may significantly improve the salt tolerance of rice seedlings through the enhancement of secondary metabolic synthesis pathways, of which phenolic acids and momilactone B may play a crucial role in the response of rice to salt stress. In contrast, momilactone A (MA) did not show any contribution in salinity tolerance of examined rice cultivars at the early seedling stage. Further investigations on the effect of $MgSO_4$ exogenous application in improving salinity tolerance of various rice varieties at other growing stages should be carried out.

Keywords: salinity; rice; tolerance; magnesium sulfate

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1. Introduction

Salt intrusion has seriously affected 20% of farmland area in the world and caused worrisome negative effects on agricultural crops by resulting in 70% yield loss of wheat, maize, rice, and barley [1]. Salt concentrations affect plant development by osmotic homeostasis, ionic homeostasis, and oxidative stress [2]. At the level of low to moderate salinities, salt stress leads to stomatal closure and reducing leaf size, while at high-moderate concentrations, it may cause leaf burn and growth inhibitions [3]. Salt stress causes an accumulation of toxic sodium (Na^+) that competes with K^+ for binding sites of essential enzymes for metabolic pathways [3]. Moreover, the osmotic stress reduces the leaf size and limits the photosynthetic area of salt-affected plants [4]. Salt stress also increases the formation of reactive oxygen species (ROS), which disrupt the antioxidant defense system and consequently causes oxidative stress. The influenced physiological processes affect all growth parameters; therefore, salt stress reduces crop productivity.

Rice (*Oryza sativa* L.) is one of the most essential crops that is the major food source for more than half of the world's population. Among monocot cereals, rice is highly sensitive to salt stress which can reduce productivity by 30–50% [5]. Naturally, rice adaptively responds to salt stress via major mechanisms consisting of ion exclusion, osmotic tolerance,

and tissue tolerance [6] which are regulated by specific genes. The gene expression and quantity of secondary metabolites are the most popular indices which properly determine the rice response to environmental stresses. Hitherto, together with a good understanding of rice physiology, modern breeding and genetic technologies have been creating more salt-tolerant rice varieties, of which novel breeding-integrated QTLs, transgene, genetic modification, and mutation are the most prevalent approaches. However, most tolerant varieties have only a resistance to environmental stresses but do not involve high yield and good quality yet [7]. In addition, salt tolerance is a quantitative trait that is regulated by various genes [7]; therefore, direct engineering effects on the genome probably carry the risk of undesired mutations which may take a long time and laborious efforts to correct. Hence, other potential solutions such as water management, irrigation control, fertilization, and exogenous application of supplements are proposed to alleviate the effect of salt stress on rice production.

In fact, the exogenous application of minerals or substances to improve salt tolerance in rice is not a novelty. For example, the use of aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) as ethylene inhibitors help maintain and increase grain yield of rice under salt stress [8]. Additionally, the application of proline and glycinebetaine positively improves salt tolerance in rice and rice seedlings [9]. Recently, plant growth regulators as phytohormones auxin (IAA), gibberellin (GA), and abscisic acid (ABA) have been externally applied to enhance salt tolerance in rice [10]. Nevertheless, previous studies mainly focus on the application of organic compounds, there are few investigations on the utilization of inorganic nutrients in recovering rice from salinity condition. Although the primary macronutrients nitrogen (N), phosphorus (P), and potassium (K) have been proven in terms of their function in response to salt stress in rice via both rooting medium and foliar application [10], the role of secondary macronutrients such as magnesium (Mg) is not well known.

Developing ionic and osmotic balances as well as detoxifying ROS are some salt-stress tolerances of plants [11,12]. Plants have an antioxidant defense system to detoxify the overproduction of ROS. Moreover, plants can produce endogenous compounds to sustain osmotic balance by maintaining the water status and stabilizing protein and enzyme complexes [13]. Phenolics are secondary metabolites that are involved in the stress protection of plants. Phenolic acids are considered as powerful antioxidants that can scavenge the damage from overproduced ROS in plants under abiotic stress [14]. Particularly, the role of endogenous phenolic acids in plant-tolerant mechanisms against abiotic stress has been recorded in many plant species [15–17]. Our previous study indicated that water deficit after anthesis improved phytochemicals in rice [18].

On the other consideration, magnesium (Mg) is the second most abundant element that is involved in all metabolic pathways of plants [19]. As a central atom of chlorophyll molecule, Mg plays an essential role in plant photosynthesis. Moreover, Mg is important for energy synthesis, enzyme activators, ion transporters, and maintaining osmotic balance [20]. In addition, the foliar application of Mg at growth and reproductive stages improves the productivity of rice under submergence stress [21]. Previous studies reported that an adequate amount of Mg is imperative for the salt tolerance of plants [22]. For instance, the application of Mg increases chlorophyll content of sunflower [23] and shoot and leaf growth of castor oil plants [24] under salt stress. Furthermore, the transportation of Mg by OsMGT1 in the root might play a crucial role in the salt tolerance of rice [25]. However, the application of Mg and Mg-including supplements and/or fertilizers in reducing salt-induced damage in rice has not been comprehensively scrutinized. On the other hand, the seedling is an important stage during rice growth; the response of rice seedlings to abiotic effects can be regarded as the initial signal that helps accurately evaluate rice growth at the later stages. Therefore, this study, for the first time, investigates the potential effect of supplemental magnesium sulfate ($MgSO_4$) on rice responsive mechanisms to salt stress by improving growth parameters, antioxidant activities, and secondary metabolites including phenolics and momilactones at the seedling stage.

2. Results

2.1. Phenotypic Performances

Salinity treatment decreased plant growth performances (root length except for T1, plant height, fresh weight, and dry weight) of all rice samples, in which the changes in phenotypic responses of tolerant cultivars were smaller compared to those of susceptible rice (Figure 1).

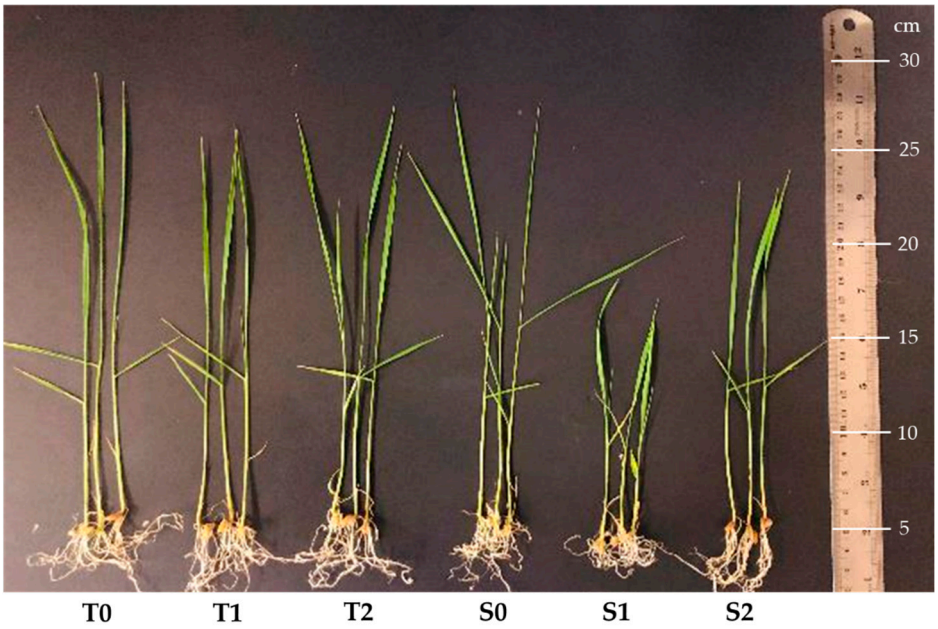


Figure 1. Phenotypic responses of rice under salt stress. T0, tolerant variety (BC15) control; T1, salted BC15; T2, salted BC15 + $MgSO_4$; S0, susceptible variety (DT84DB) control; S1, salted DT84DB; S2, salted DT84DB + $MgSO_4$.

Under salt stress, the injury score of T2 was the lowest (3.00) while the highest score (5.23) was found in S1 (Table 1). T2 and S2 had better growth performances compared to T1 and S1, respectively (Table 1). The results showed that samples performed lower injury scores and better growth parameters with $MgSO_4$ application.

Table 1. Phenotypic responses of rice under salt stress.

Samples	Injury Score	Root Length (mm)	Plant Height (mm)	Fresh Weight (mg)	Dry Weight (mg)
T0	1.00 ± 0.00^{e}	64.93 ± 0.55^{ab}	190.67 ± 1.63^{a}	97.57 ± 0.64^{a}	41.43 ± 0.67^{a}
T1	3.43 ± 0.10^{c}	65.37 ± 1.44^{a}	170.83 ± 2.26^{b}	79.67 ± 0.58^{c}	36.00 ± 1.00^{bc}
T2	3.00 ± 0.00^{d}	65.30 ± 0.38^{a}	175.10 ± 2.70^{ab}	90.93 ± 0.41^{b}	38.97 ± 2.37^{b}
S0	1.00 ± 0.00^{e}	59.80 ± 2.17^{bc}	187.43 ± 3.60^{a}	96.56 ± 0.58^{a}	39.67 ± 0.41^{a}
S1	5.23 ± 0.07^{a}	58.03 ± 0.70^{c}	145.63 ± 0.20^{c}	70.47 ± 0.58^{d}	26.67 ± 0.88^{d}
S2	4.73 ± 0.03^{b}	60.00 ± 0.56^{abc}	166.97 ± 2.32^{bc}	80.60 ± 0.90^{c}	28.57 ± 1.33^{c}

Values represent mean ± standard errors (SE); superscripted different letters in a column indicate a significant difference ($p < 0.05$) by Tukey's test. T0, tolerant variety (BC15) control; T1, salted BC15; T2, salted BC15 + $MgSO_4$; S0, susceptible variety (DT84DB) control; S1, salted DT84DB; S2, salted DT84DB + $MgSO_4$.

2.2. Chemical Performance

Total phenolic (TPC) and flavonoid (TFC) contents and antioxidant activities of salted rice with application of $MgSO_4$ are presented in Figure 2.

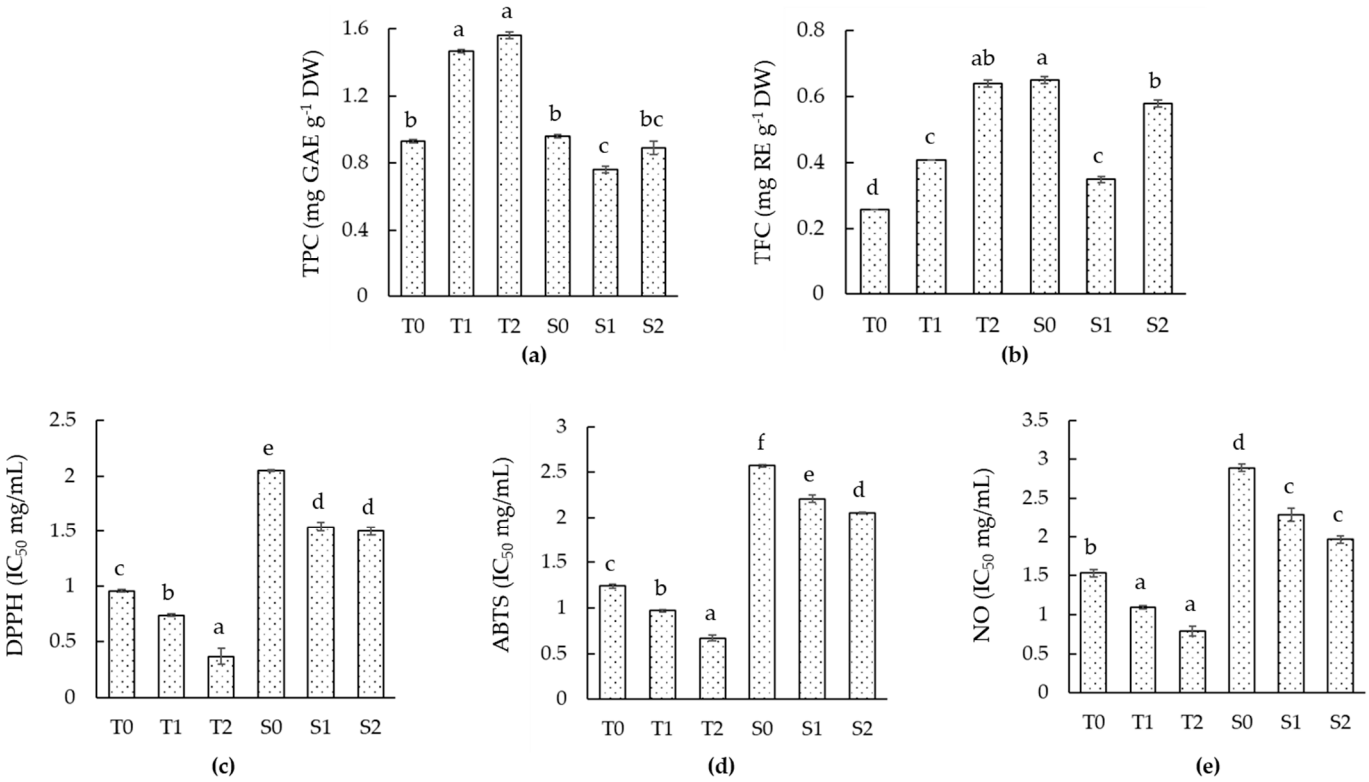
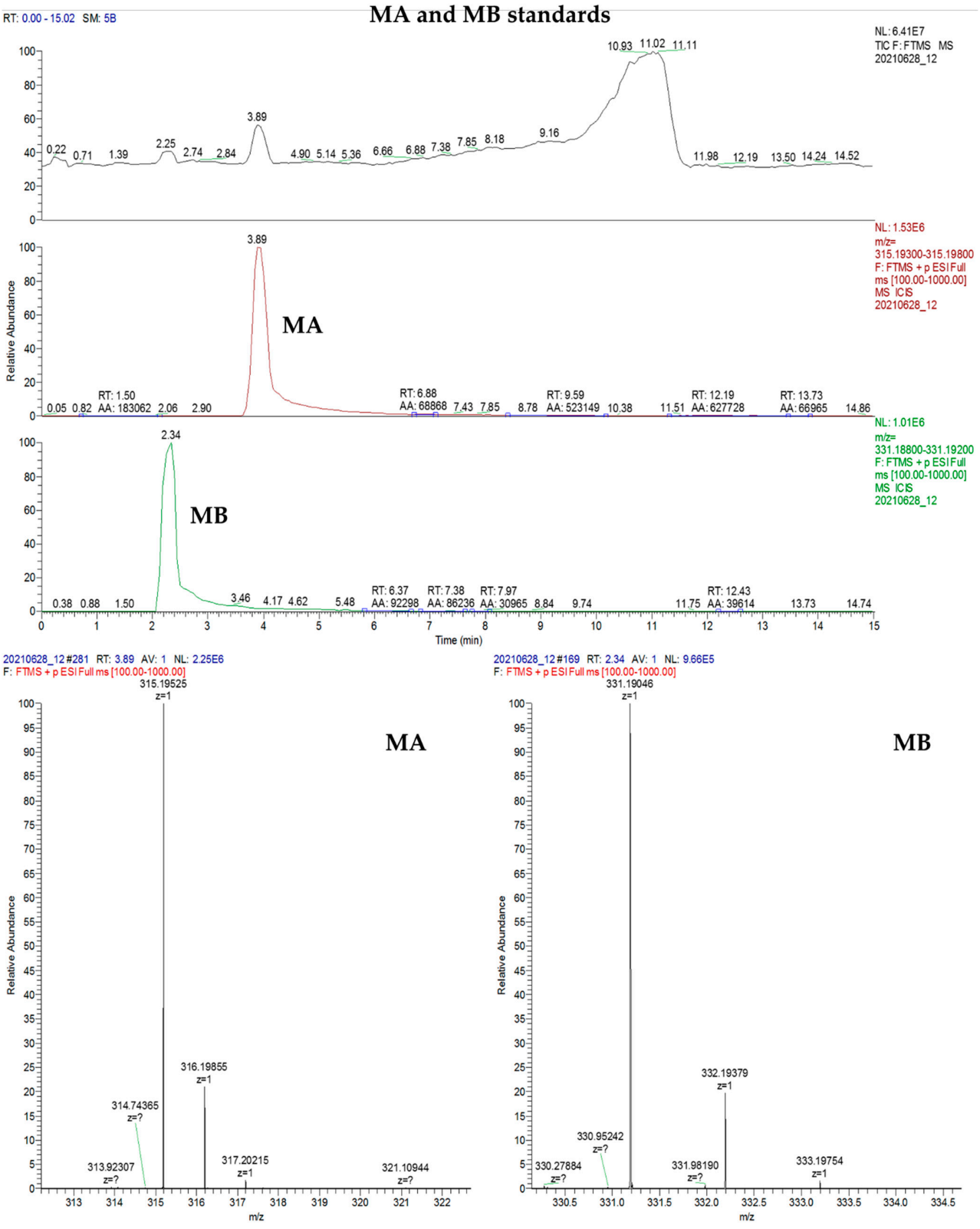


Figure 2. Total phenolic content (**a**), total flavonoid content (**b**), and antioxidant activities (**c**–**e**) of rice under salinity stress. Different letters in a bar chart indicate significant difference ($p < 0.05$) by Tukey's test. TPC, total phenolic content; GAE, gallic acid equivalent; TFC, total flavonoid content; RE, rutin equivalent; DW, dry weight; DPPH, 2,2−diphenyl−1−picrylhydrazyl assay; ABTS, (2,2−azinobis−(3−ethylbenzthiazoline−6−sulfonic acid)) assay; NO, nitric oxide assay; T0, tolerant variety (BC15) control; T1, salted BC15; T2, salted BC15 + $MgSO_4$; S0, susceptible variety (DT84DB) control; S1, salted DT84DB; S2, salted DT84DB + $MgSO_4$.

As shown in Figure 2, the TPC and antioxidant activities of tolerant variety are better than those of sensitive rice. T2 had the highest TPC (1.56 mg GAE g^{-1} DW) while the greatest TFC was found in S0 (0.65 mg RE g^{-1} DW). S0 performed the strongest antioxidant capacities (in DPPH, ABTS, and NO assays). It was also found that rice plants obtained higher TPC, TFC, and stronger antioxidant activities with $MgSO_4$ supplement (Figure 2). Especially, antioxidant properties increased in tolerance but decreased in susceptible rice under salt stress.

Figure 3 shows UPLC and extracted ion chromatograms of standard momilactones A (MA) and B (MB) and detected MA and MB in sample T0. Accordingly, MB was detected at 2.3 min and identified with a principal ion of 331.19 *m/z*; meanwhile, MA was detected at 3.9 min with a major ion of 315.19 *m/z*.



(a)

Figure 3. *Cont.*

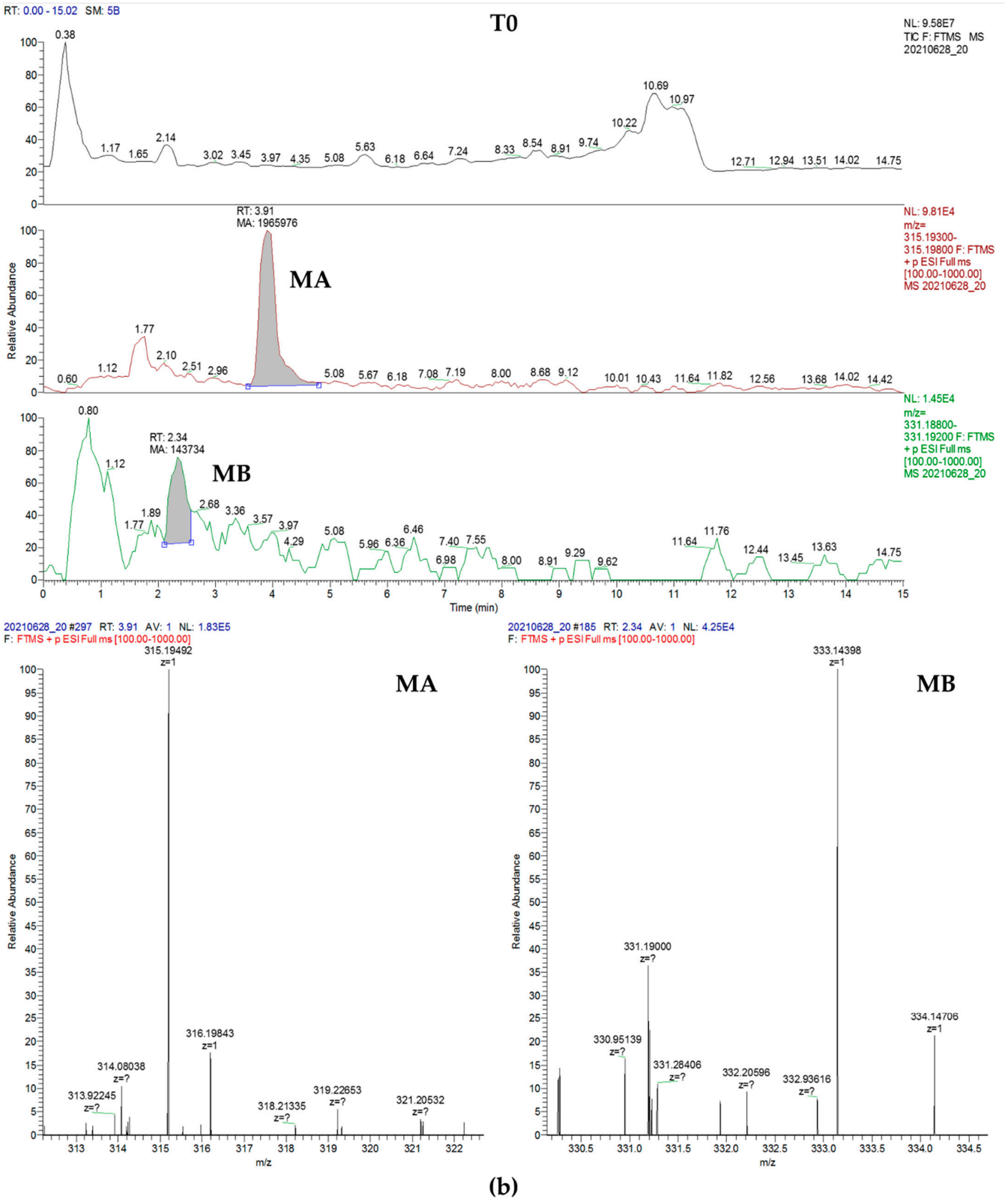


Figure 3. Ultra-performance liquid chromatography chromatograms and mass spectra of momilactones A (MA) and B (MB) standards (**a**); and MA and MB in T0 sample (BC15-Control) (**b**).

As can be seen in Table 2, the amounts of phenolics in S2 (salinity susceptible) were significantly higher than those of T2 (salinity tolerant). However, salinity induced a dramatic reduction in phenolic amounts of sensitive rice samples. Particularly, the quantity of *ρ*-coumaric, salicylic, benzoic, and ferulic acids increased in both tolerant and susceptible rice varieties under salinity stress together with $MgSO_4$ treatment. Quantities of caffeic acid, cinnamic acid, benzoic acid, and tricin remarkably declined in all salted-rice seedlings. On the other hand, MA was only detected in T0 (salinity tolerant cultivar-control). MB was found with minor content in salted samples in both tolerant and susceptible varieties. Remarkably, MB was found to be higher in T0 and T2 (tolerant cultivar) than in S0 and S2 (susceptible rice), respectively. The results indicated that under salt stress, the amounts of phenolics (except caffeic acid) and MB in rice seedlings were improved after treatment with 0.5 mM $MgSO_4$ (Table 2).

Table 2. Phenolic compound (μg/g DW) and momilactone (ng/g DW) contents of rice under salinity stress.

Samples	Caffeic Acid	*ρ*-Coumaric Acid	Salicylic Acid	Cinnamic Acid	Benzoic Acid	Ferulic Acid	Tricin	MA	MB
T0	0.05 ± 0.00^{b}	0.12 ± 0.01^{d}	0.18 ± 0.01^{d}	0.04 ± 0.00^{b}	0.65 ± 0.03^{d}	0.11 ± 0.00^{d}	0.03 ± 0.00^{cd}	3.72 ± 0.07^{a}	1.45 ± 0.11^{a}
T1	0.01 ± 0.00^{c}	0.16 ± 0.00^{c}	0.29 ± 0.00^{d}	0.01 ± 0.00^{d}	0.62 ± 0.02^{de}	0.15 ± 0.01^{c}	0.02 ± 0.00^{e}	-	-
T2	0.04 ± 0.00^{b}	0.17 ± 0.00^{bc}	0.49 ± 0.03^{c}	0.02 ± 0.00^{cd}	1.00 ± 0.04^{c}	0.24 ± 0.01^{b}	0.09 ± 0.00^{a}	-	0.61 ± 0.01^{b}
S0	0.23 ± 0.01^{a}	0.47 ± 0.01^{a}	2.45 ± 0.05^{a}	0.07 ± 0.01^{a}	2.74 ± 0.10^{a}	0.34 ± 0.01^{a}	0.04 ± 0.00^{bc}	-	0.68 ± 0.04^{b}
S1	0.02 ± 0.00^{c}	0.09 ± 0.00^{e}	0.45 ± 0.02^{c}	0.02 ± 0.00^{cd}	0.42 ± 0.02^{e}	0.10 ± 0.00^{d}	0.03 ± 0.00^{de}	-	-
S2	0.01 ± 0.00^{c}	0.18 ± 0.01^{b}	0.76 ± 0.01^{b}	0.03 ± 0.00^{bc}	1.35 ± 0.02^{b}	0.16 ± 0.00^{c}	0.05 ± 0.00^{de}	-	0.53 ± 0.01^{b}

Values represent mean ± standard errors (SE); '-': nonquantifiable (a very low concentration that could not be measured, henceforth will be interpreted as undetectable); superscripted different letters in a column indicate a significant difference ($p < 0.05$) by Tukey's test. MA, momilactone A; MB, momilactone B; T0, tolerant variety (BC15) control; T1, salted BC15; T2, salted BC15 + $MgSO_4$; S0, susceptible variety (DT84DB) control; S1, salted DT84DB; S2, salted DT84DB + $MgSO_4$.

3. Discussion

In recent decades, researchers have used phytoprotectants to enhance the salinity tolerance of rice. Exogenous osmoprotectants (glycine betaine, trehalose, sorbitol, and ectoine); plant hormones (abscisic acid, auxin, and cytokinin); signal molecules (nitric oxide and hydrogen peroxide); and trace elements (Si, Zn, and Mn) were found to contribute to rice salt-tolerance [26]. However, the success of these micronutrient applications was mostly dose-dependent [26]. On the other hand, magnesium (Mg) is a macronutrient for plants. It is the central component of chlorophyll molecules and plays an important role in plant photosynthesis. In addition, it is an abundant element that is involved in a vast physiological process of plants. However, studies about the application of Mg in developing salinity-tolerant rice have still been limited. In this study, $MgSO_4$ was used to improve the salinity tolerance of rice. The results showed a promising result that $MgSO_4$ recovers inhibited growth and improve the antioxidant, phenolic profiles, and momilactone B of the rice seedlings under salt stress.

As the plant-specialized metabolites, phenolics have attracted attention from researchers due to their crucial functions in physiological processes throughout the plant life cycle, including responses to stress [27]. Plant phenolic acids are powerful antioxidants that can scavenge the overproduction of ROS in plants under different abiotic stresses [14]. The stimulation of the biosynthetic pathways that induce the synthesis of phenolic acids is one cause of the activated plant's antioxidant system [14]. The accumulation of endogenous phenolic acids is determined as a mechanism of plant tolerance against abiotic stresses in many plant species including rice [16–18]. Based on the upregulation of key genes phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), phenolic biosynthesis is increased, resulting in the improvement of rice tolerance to abiotic stress, including salinity [16,17]. In the present study, TPC and TFC of rice samples treated with 0.5 mM MgSO4 under 100 mM NaCl were significantly higher than those of salted samples in both tolerant and susceptible varieties. Remarkably, the results implied that MgSO4 might contribute a role in increasing the synthesis of salicylic, benzoic, ferulic, and *ρ*-coumaric

acids which were proven to involve salt tolerance of plants in general and rice in particular [28–30]. These findings coincided with previous studies that recognized salicylic acid as a phytohormone signaling disease and salt tolerance [31].

In the current study, momilactone A was only detected in BC15 (tolerant cultivar) under control conditions at the seedling stage. However, momilactone B was identified in both tolerant and susceptible samples, in which, the amount of momilactone B in BC15 was higher than that of DT84DB (susceptible genotype). This result is in line with our previous hypothesis that momilactones A and B are involved in the salt tolerance of rice [32], and momilactone B has a greater correlation with rice salinity tolerance compared to momilactone A [32]. Significantly, momilactone B could not be detected in salted samples but appeared with smaller amounts in $MgSO_4$-treated samples compared to the control. This indicated that $MgSO_4$ might help rice recover MB synthesis to cope with salinity stress. Moreover, with $MgSO_4$ application, the quantity of both momilactone B and important phenolic compounds was enhanced in rice seedlings under salt stress. These results suggest that the simultaneous synthesis of salicylic, benzoic, ferulic, ρ-coumaric acids, and momilactone B may be involved in the rice response to salt stress. Further studies should be implemented to authenticate these preparatory findings.

Previous studies showed the potential of supplemented elements (Mn, Si, Zn) in the improvement of plant growth, photosynthesis, and antioxidant activities [13,26,33]. However, different plants have different mineral demands; therefore, exceeding application of those elements can damage plant growth and development. Different from the above elements, Mg is a macronutrient of a plant. Plant requires a huge amount of Mg for its essential structure components as well as metabolisms. Mg is the central component of chlorophyll molecules and particularly essential to plants, with 75% of leaf Mg involved in protein synthesis and 15–20% of total Mg associated with chlorophyll pigments [34]. Furthermore, this element is involved in many enzyme activities and the structural stabilization of tissues such as nucleic acids, proteins, cell membranes, and walls [35–37].

The crucial role of Mg in the regulation of ROS, cation–anion balance, and cell turgor was determined [37]. Researchers stated that the special roles of Mg in plants that cannot be found in other elements may be caused by its unique chemical properties such as great hydrated radius and preference for acting with oxygen [38]. However, studies on Mg have mostly focused on the detoxification of heavy metals such as Al and Cd [39]. Especially, the application of Mg in reducing salt damages on rice is still limited. On the other hand, Chen et al. [25] stated that magnesium uptake via the OsMGT1 transporter in the roots might be a promoting signal for upgrading OsHKT1;5 activity, subsequently, eliminating sodium accumulation in the shoots [25]. Results of this research showed that exogenous $MgSO_4$ enhances the inhibited growth, water status, antioxidant activities, phenolic profiles, and momilactone B of salted rice seedlings. It is a potential strategy for breeding crops with Mg enrichment. The present study suggested that $MgSO_4$ may be useful to develop fertilizer for rice growing in saline soil. However, the application of exogenous $MgSO_4$ needs to be further investigated in field scale.

4. Materials and Methods

4.1. Materials and Treatments

Rice cultivars consisting of BC15 (salinity tolerant cultivar) and DT84DB (salinity susceptible cultivar) [6] were used in this study. Initially, rice seeds were soaked in 0.1% NaOCl for 30 min. After washing several times with distilled water, the seeds were immersed in water at 30 °C for 5 days for germination. The germinated seeds were then grown in a plant growth chamber (28 °C day; 25 °C night; 12 h light; 12 h dark). Rice seedlings were hydroponically cultivated by supplying with Yoshida nutrient solution [40,41]. The solution was salinized at the seedling stage (14 days after sowing) by NaCl powder at a concentration of 100 mM (moderate salinity, United State Department of Agriculture). A preliminary screening of magnesium sulphate ($MgSO_4$) concentrations (0.1–1.0 mM) on rice seedling growth was carried out to select the optimal dose of $MgSO_4$ (data not

shown). According to the best phenotypic performance of corresponding rice samples, the concentration of 0.5 mM $MgSO_4$ was chosen and supplemented for rice seedlings in this study. Trays without NaCl were considered as control. The list of samples and treatments is presented in Table 3. The pH and EC (electronic productivity) were adjusted at 2-day intervals for the control (except T0) and treatments to be 5.0 and 10 dS m^{-1}, respectively.

Table 3. List of samples and treatments.

Treatment Code	Description
T0	BC15-Control
T1	BC15-Stress (100 mM NaCl)
T2	BC15-Stress & Treatment (100 mM NaCl + 0.5 mM $MgSO_4$)
S0	DT84DB-Control
S1	DT84DB-Stress (100 mM NaCl)
S2	DT84DB-Stress snd Treatment (100 mM NaCl + 0.5 mM $MgSO_4$)

BC15, salt tolerant cultivar; DT84DB, salt susceptible cultivar.

4.2. Phenotypic and Chemical Measurements

At 7 days after treatment, the salinity tolerance of rice materials was scored by standard evaluation score (SES) [41]. Their growth parameters including injury score, root length, plant height, fresh weight, and dry weight also were measured, in which injury score was determined as individual plant or group of genotypes scored usually on a 1 to 9 scale where lower score toward 1 states tolerant and higher score denotes sensitive genotypes [41], following the observed injuries described in Table 4. Root length and plant height of samples were measured with the longest root and leaf. Dried biomass of samples was extracted with methanol in 3 days using a magnetic stirrer. The extract was then separated by hexane and finally dried by an evaporator at 50 °C. The obtained powder was kept in methanol at 4 °C in the dark for further measurements. Chemical analyses including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), and NO (nitric oxide) assays of samples were conducted. Protocols were followed for the chemical assessments described previously [32,42,43].

Table 4. Standard evaluation score (SES) of visual injury.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or a few leaves whitish and rolled	Tolerant
5	Growth severely retarded most leaves rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dried, some plants are dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

4.3. Identification and Quantification of Phenolics and Momilactones A and B

Eleven phenolic and relative compounds including gallic acid, catechol, protocatechuic acid, caffeic acid, chlorogenic acid, ρ-coumaric acid, salicylic acid, cinnamic acid, benzoic acid, ferulic acid, and tricin were used as standards. Phenolic profiles of samples were identified and quantified by high performance liquid chromatography (HPLC) method [44] with the following conditions: pump: PU-4180 RHPLC; controller: LC-Net II/ADC controller; detector: UV-4075 UV/Vis (Jasco, Tokyo, Japan); stationary phase: XBridge BEH Shield RP18 (USA); mobile phase: solution A (0.1% aqueous formic acid) and solution B (100% acetonitrile); program: 5% B during 0–2 min, 5–70% B during 2–12 min, 100% B from 12–16 min and maintained for 6 min, 100% B to 5% during 22–24 min, equilibration: 10 min; injection volume: 5 µL; and flow rate: 400 µL/min. The operation was carried out in 35 min under room temperature (24–26 °C). Identification and quantification of phenolics

compounds were carried out based on the corresponding peak and its area compared to the curve built from the standard chemicals.

Momilactones A (MA) and B (MB) were identified and quantified by the authenticated protocol using ultra-performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS) [37–39]. Briefly, 3 µL of the sample was injected into the system including the Acquity UPLC® BEH C18 (1.7 µm, 50 × 2.1 mm i.d.) column (Waters Cooperation, Milford, MA, USA) as the stationary phase and the mobile phases of 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B). The gradient program of the mobile phase was set as follows: 0–5 min, solvent A and solvent B (1:1, *v*/*v*); 5–10 min, 100% solvent B. The electrospray ionization (ESI) and mass spectroscopic conditions and quantification of MA and MB in samples were reported in the previous studies [44–46].

4.4. Data Analysis

Physiological and chemical measurements were conducted with 3 replications. Data were expressed as means ± standard errors. Significant variations among samples were analyzed by analysis of variance (ANOVA) via the Minitab software version 16.0 (Minitab Inc., State College, PA, USA).

5. Conclusions

The results of this study conclude that exogenous application of $MgSO_4$ reduced salt-induced damages by improving growth parameters, antioxidant activities, phenolic acids, and momilactone B of the rice seedlings. Moreover, *ρ*-coumaric acid, salicylic acid, ferulic acid, and momilactone B may be the major contributors to rice tolerance ability against salt stress at the seedling stage. The involvements of these compounds in recovery mechanisms of rice under salt stress should be further elaborated at different growth stages and for other rice varieties. This study also indicates that momilactone A does not play any role in the salinity tolerance of the examined rice cultivars. Additional studies on the mechanism of $MgSO_4$ transport from soil to rice and determining suitable $MgSO_4$ dosages to different saline soil conditions should be implemented in the future.

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