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Bacillus subtilis var. *natto* promotes tobacco plant growth under normal conditions and in the presence of sodium bicarbonate

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Abstract

The coastal areas of western Taiwan feature acidic and saline soils with low fertility. Sodium bicarbonate ($NaHCO_3$), produced readily by carbon capture and storage technologies, could be suitable for the neutralization of acidic soils, but its effects on plant growth and the ability of *Bacillus subtilis* var. *natto* to confer salinity tolerance remain unclear. In this study, we examined the potential of sodium bicarbonate and *B. subtilis* var. *natto* (NTU18) to improve the growth of tobacco (*Nicotiana tabacum* L.) under salt stress conditions. We found that salt stress was the main factor affecting tobacco growth, resulting in shorter roots and shoots, a reduced leaf area and leaf number, and clustered dark green leaves. The addition of sodium bicarbonate exacerbated the symptoms of salinity stress. Inoculating the soil with *B. subtilis* did not enhance salinity tolerance, but intriguingly it increased shoot and root growth under normal conditions and in the presence of sodium bicarbonate. The mechanism of growth promotion mediated by the bacteria is unknown and should be investigated in more detail.

Keywords: acidic soil, *Bacillus subtilis* var. *natto*, *Nicotiana tabacum*, saline soil, sodium bicarbonate.

Introduction

Plants encounter many types of environmental stresses that often occur concurrently or sequentially (Coolen *et al.* 2016). For example, soil salinity (electrical conductivity of saturated extract > 4 dS m^{-1}) and soil acidity (pH < 5.5) co-occur widely and limit the productivity of major crops (von Uexküll and Mutert 1995, Abbas *et al.* 2019). Soils along the coastal areas of western Taiwan are both saline and acidic, rendering them unsuitable for agriculture (Chen *et al.* 2015). The degradation of coastal soils is mainly caused by seawater ingress, acid rain, and chemical fertilizers (Chen *et al.* 2015, Utama *et al.* 2021). Early-stage salinity causes water stress in plants, which inhibits growth, narrows the mean stomatal aperture, and causes nutrient deficiency (Chaves *et al.* 2009). Prolonged salinity induces ionic stress, triggering leaf senescence and impairing photosynthesis, thus exacerbating growth inhibition (Chaves *et al.* 2009). Soil acidity inhibits plant growth by reducing the availability of essential nutrients such as phosphorus and molybdenum, and by increasing the availability of some elements to toxic levels, particularly aluminum and manganese (Matsumoto *et al.* 2017). Lime (calcium carbonate, $CaCO_3$) has been used since the Roman civilization to neutralize acidic agricultural land (Goulding 2016).

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylate; EPS - exopolysaccharides; HKT - high-affinity potassium transporter; PGPR - plant growth-promoting rhizobacteria; SPAD - single photon avalanche diode.
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Over the last century, the burning of fossil fuels has increased the concentration of atmospheric carbon dioxide that warms the planet, contributing to climate change (Solomon *et al*. 2009). In order to mitigate the global environmental impact of greenhouse gas emissions, new technologies have emerged to capture and reuse CO_2 as a raw material for the industrial production of chemicals (Bonfim-Rocha *et al*. 2020). These include sodium bicarbonate ($NaHCO_3$), commonly known as baking soda, which is used in the chemical, food, textile, and pharmaceutical industries (Goharrizi and Abolpour 2012). Sodium bicarbonate may also be suitable as an alternative to lime to reduce the acidity of soil, but little is known about its effects in agriculture (Fageria and Baligar 2008). Gathering this information is important because the unrestricted application of sodium bicarbonate could increase soil salinity due to the accumulation of Na^+ (Ma *et al*. 2020).

Plant growth-promoting rhizobacteria (PGPR) include endophytes and bacteria in the rhizosphere that boost plant growth and confer tolerance to abiotic stresses (Akram *et al*. 2016, Shahid *et al*. 2018). For example, PGPR representing the genera *Bacillus*, *Pseudomonas*, and *Azotobacter* can improve the growth of canola, tomato, bean, lettuce, and pepper plants under salt stress (Abbas *et al*. 2019, Utama *et al*. 2021). Stress tolerance is achieved by diverse mechanisms, including the depletion of ethylene and the synthesis of growth hormones, osmolytes, and antioxidant enzymes that prevent damage caused by reactive oxygen species (ROS) and other radicals (Hmaeid *et al*. 2019). Notably, *Bacillus subtilis* var. *natto* is a probiotic bacterium isolated from natto, a traditional Japanese food made from fermented soybeans (Samanya and Yamauchi 2002). *B. subtilis* improves digestive health, reduces the accumulation of lipids, and enhances the immune response (Hitosugi *et al*. 2015). It is widely used in the manufacture of drugs and dietary supplements, and as a probiotic feed for aquaculture and livestock (Kuo *et al*. 2006, 2012; Tseng *et al*. 2009, Sun *et al*. 2010). However, its application as PGPR has not been reported.

To determine whether the application of *B. subtilis* can promote plant growth and confer stress tolerance, we used tobacco (*Nicotiana tabacum* L.) as a model species. The tobacco plants were exposed to different concentrations of *1*) sodium bicarbonate, *2*) *Bacillus subtilis* var. *natto* (NTU-18), and *3*) sodium chloride (salt stress), and we investigated their effects on plant height, root length, shoot and root fresh masses, leaf number per plant, and the relative chlorophyll content.

Materials and methods

Tobacco (*Nicotiana tabacum* L.) seeds (collected from Meinong, Kaohsiung, Taiwan) were surface sterilized with 70% ethanol for 1 min, and then with 1% sodium hypochlorite for 10 min, before washing five times with sterile double distilled water (Çelik and Atak 2012). The seeds were then germinated in half-strength Murashige and Skoog (MS) medium containing 0.8% phyto-agar (*Duchefa Biochemie*, Haarlem, The Netherlands) (Yu *et al*. 2020). After 10 d, individual tobacco seedlings were transferred to 9-cm pots filled with a sterilized 1:1 mixture of *Vermiculite* and potting soil (pH 5.6), and were grown at a temperature of 25°C, a 16-h photoperiod, and irradiance of 150 µmol m^{-2} s^{-1}. Each plant was fed with 10 cm^3 of *Hyponex No. 2* fertilizer every week.

Three-week-old tobacco plants were firstly treated by inoculating the soil with 5 cm^3 of the *B. subtilis* var. *natto* suspension (2 or 1 g bacterial powder per 1 dm^3 of distilled water) alongside an untreated control. The *B. subtilis* strain NTU-18 (BCRC 80390, *Bioresource Collection and Research Center*, Taiwan) powder was used (Kuo *et al*. 2012). The plants were maintained as described above for 4 d before irrigation with 10 cm^3 of 200 or 400 mM NaCl (alongside an untreated control) with or without 10 cm^3 of 2 g dm^{-3} of sodium bicarbonate ($NaHCO_3$). The NaCl and sodium bicarbonate treatments were applied every 3 - 4 d until harvesting. The 18 treatment combinations (Table 1) were applied in four biological replicates. After 49 d, we recorded the plant height, root length, shoot/root fresh mass, leaf number per plant, and the relative chlorophyll content. Shoot and root samples were dried at 70°C for 3 d and their dry masses were also recorded.

The relative chlorophyll content was measured using a *SPAD 502 Plus* chlorophyll meter (*Spectrum Technologies*, Fort Worth, TX, USA) from four biological

Table 1. The treatments applied in this study.

Treatments	Symbol	Description
Bacillus subtilis var. *natto*	N0	control
	N0.2	5 cm^3 of 500× diluted *Bs.* (2 g dm^{-3})
	N1	5 cm^3 100× diluted *Bs.* (10 g dm^{-3})
Salt	S0	control
	S2	10 cm^3 of 200 mM NaCl
	S4	10 cm^3 of 400 mM NaCl
Sodium bicarbonate	B0	control
	B0.2	10 cm^3 of 500× diluted $NaHCO_3$ (2 g dm^{-3})

replicates. For each plant, SPAD measurements were taken from three different points on the newly expanded leaf, and the mean value was presented.

Means and standard deviations (SDs) were calculated for the measurements of plant growth. Statistical significance was determined by three-way analysis of variance (*ANOVA*) followed by *Tukey*'s *post hoc* test using *R Studio* (Fox *et al*. 2007, Lenth 2016, Hope 2022, Hothorn *et al*. 2023). Differences between treatments were considered significant at $P<0.05$.

Results

Three-week-old tobacco plants were first treated with 0, 2, or 10 g (*B. subtilis*) dm^{-3} (N0, N02, and N1), followed 4 d later by 0 or 2 g dm^{-3} of sodium bicarbonate (B0 and B0.2) and/or 0, 200, or 400 mM NaCl (S0, S2, and S4) twice per week (Table 1). There was no difference in phenotype between the various treatment groups for the first 7 d. Symptoms of phytotoxicity first appeared on day 14 in plants treated with NaCl and on day 28 in plants treated with $NaHCO_3$, and the differences between treatments were more pronounced on day 49. We therefore recorded the plant height (Fig. 1), aboveground mass (Fig. 2), leaf phenotype (Fig. 3), and root mass (Fig. 4) after 49 d.

The plants exposed to NaCl exhibited stunting, which was more severe at the highest NaCl concentration (Fig. 1*A*) whether or not the plants were also treated with *B. subtilis* and/or sodium bicarbonate (Fig. 1*B*). In the presence of NaCl, the leaves were also relatively flat to the soil surface (Fig. 1 Suppl.). Inoculation with *B. subtilis* does not offset the damage caused by salt. There was no significant difference in plant height between the N0, N0.2, and N1 treatments, but progressive stunting was observed when comparing the S0, S2, and S4 treatments.

The sodium bicarbonate treatment appeared to exacerbate the effect of salt on plant height, even in the presence of *B. subtilis* (Fig. 1*B*). The average plant heights following treatments N0+B0.2+S2 (7.4 cm) and N0+B0.2+S4 (4.1 cm) were lower than the average heights following treatments N0+B0+S2 (7.9 cm) and N0+B0+S4 (5.8 cm), respectively. Similarly, the average plant heights following treatments N1+B0.2+S2 (6.4 cm) and N1+B0.2+S4 (3.9 cm) were lower than the average heights following treatments N1+B0+S2 (7.9 cm) and N1+B0+S4 (4.6 cm), respectively.

A comparison of the aboveground tobacco tissues (Fig. 2*A*, Fig. 2 Suppl.) revealed that higher NaCl concentrations resulted in more darkening and wrinkling of the leaves. Treatment with NaCl and $NaHCO_3$ (S2+B0.2 and S4+B0.2) caused production of new leaves that were small and clustered, and unable to expand. We also measured the fresh and dry masses of the aboveground tissues (stems and leaves). The fresh mass fell progressively with higher NaCl concentrations regardless of the presence of *B. subtilis* and/or sodium bicarbonate, and there was a significant difference between the S4, S2, and control (S0) treatments (Fig. 2*B*). A similar trend was observed for

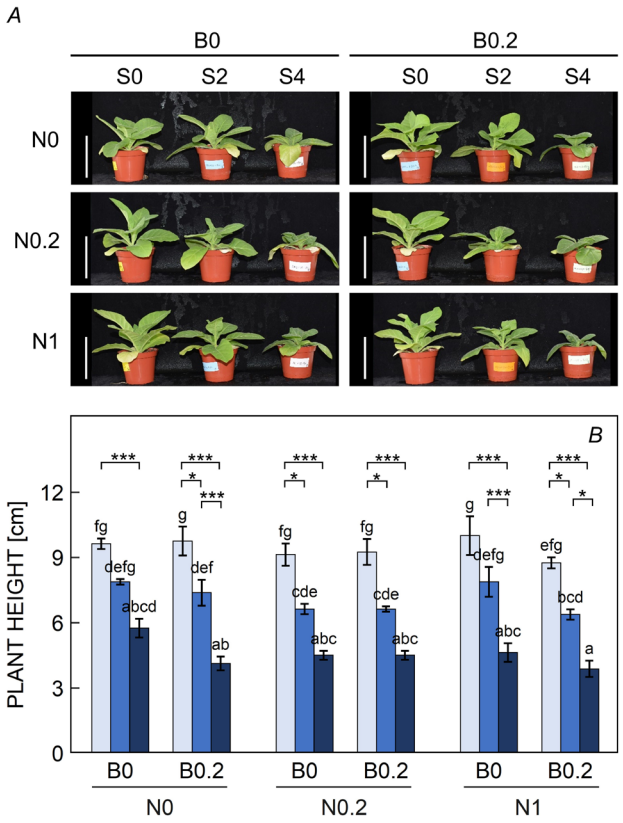

Fig. 1. Height of tobacco plants following treatment with NaCl, $NaHCO_3$, and/or *Bacillus subtilis* for 49 d. *A* - Pictures of representative plants. *B* - Plant height. B0.2 = 2 g dm^{-3} of sodium bicarbonate (B0 is the control). S2 and S4 refer to 200 and 400 mM NaCl, respectively (S0 is the control). N0.2 and N1 refer to 2 and 10 g dm^{-3} of *B. subtilis*, respectively (N0 is the control). The *scale bar* is 10 cm. The *blue bars* represent the salt treatments (S0, S2, and S4 from light to dark). Means ± SDs ($n = 4$). Data were analyzed by three-way *ANOVA* followed by *Tukey*'s *post hoc* test. Different letters represent significant differences ($P<0.05$). Brackets indicate the relevant comparisons (*- $P<0.05$, **- $P<0.01$, ***- $P<0.001$).

the dry mass of aboveground tissues (Fig. 2*C*). However, in this case the low dry mass caused by NaCl stress was exacerbated by the presence of sodium bicarbonate. For example, the dry masses under treatments N1+S2+B0.2 (784.9 mg) and N1+S4+B0.2 (347.2 mg) were lower than the corresponding treatments N1+S2+B0 (1 042.3 mg) and N1+S4+B0 (588 mg), respectively. The pattern was similar for the N0 and N0.2 treatments and the high NaCl stress (S4).

Although *B. subtilis* did not confer salt stress tolerance on the tobacco plants, we observed a surprising ability to promote growth in the absence of NaCl (or in the presence of small amounts of NaCl) whether or not sodium bicarbonate was also present (Fig. 2*C*). Accordingly, the dry mass of aboveground tissues in all treatments with the bacteria – N1+S0+B0 (1 298.1 mg), N1+S0+B0.2 (1 237.8 mg) and N1+S2+B0 (1 042.3 mg) – were higher than the corresponding treatments without

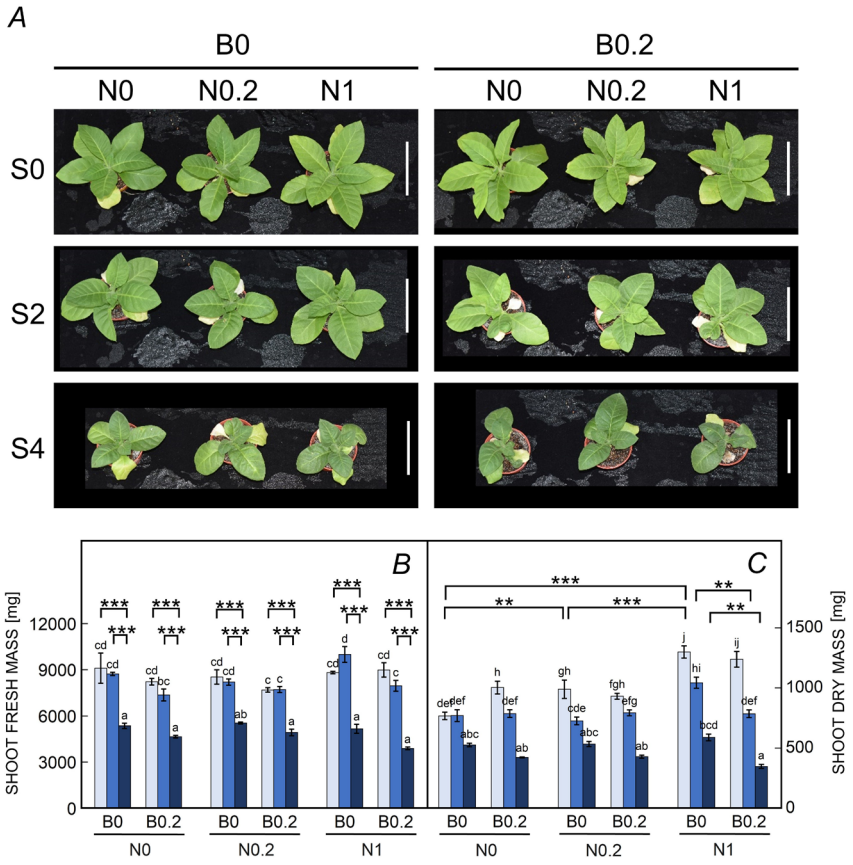

Fig. 2. Top view showing the tobacco plants following treatment with NaCl, $NaHCO_3$, and/or *Bacillus subtilis* for 49 d. *A* - Pictures of representative plants. *B* - Fresh mass, and *C* - dry mass of tobacco shoots. B0.2 = 2 g dm^{-3} of sodium bicarbonate (B0 is the control). S2 and S4 refer to 200 and 400 mM NaCl, respectively (S0 is the control). N0.2 and N1 refer to 2 and 10 g dm^{-3} of *B. subtilis*, respectively (N0 is the control). The *scale bar* is 10 cm. The *blue bars* represent the salt treatments (S0, S2, and S4 from light to dark). Data are means ± SDs (n = 4) analyzed by three-way *ANOVA* followed by *Tukey*'s *post hoc* test. Different letters represent significant differences (P<0.05). Brackets indicate the relevant comparisons (*- P<0.05, ** - P<0.01, *** - P<0.001).

bacteria: N0+S0+B0 (766.6 mg), N0+S0+B0.2 (1 002.5 mg) and N0+S2+B0 (769 mg), respectively.

To determine whether leaf growth and development were affected by treatments with NaCl, $NaHCO_3$, and *B. subtilis*, we compared the sixth leaf phenotype, relative leaf chlorophyll content, and leaf number in the different treatment groups (Fig. 3). The tobacco leaves became smaller and darker green when exposed to NaCl (S2 and S4) compared to the control treatment (S0) (Fig. 3*A*). The NaCl treatments also resulted in leaf wrinkling, but the sodium bicarbonate and bacterial treatments had no effect on the leaf phenotype. The relative leaf chlorophyll content (SPAD values) of plants under high-NaCl (S4) treatment (45.6 - 50.4) were higher than those of plants exposed to the intermediate NaCl (S2) and control (S0) treatments (25.6 - 38.6) (Fig. 3*B*). After 49 d, the S4 plants also had fewer leaves (5 - 8 leaves) than those in the S2 (7 - 10 leaves), and S0 (10 - 12 leaves) groups (Fig. 3*C*). The combination of $NaHCO_3$ and NaCl reduced the number of leaves even more, as shown by comparing the S2+B0.2 (8 - 10 leaves) and S4+B0.2 (5 - 7 leaves) groups with the corresponding S2+B0 (9 - 10 leaves) and S4+B0 (7 - 8 leaves) groups. In summary, our results showed that salinity delays the growth and development of tobacco leaves, and that sodium bicarbonate has a small additional effect.

Finally, we compared the root phenotype, root length, and root dry mass in the different treatment groups (Fig. 4). The root length was highly sensitive to salinity (Fig. 4*A*), with the S4 group developing much shorter roots (12.5 - 15.2 cm) than the S2 (19.0 - 26.2 cm) and S0 (17.8 - 22.4 cm) groups (Fig. 4*B*). The treatments with sodium bicarbonate and *B. subtilis* had no significant effect on root length. The root dry mass was reduced progressively by increasing the NaCl concentration (Fig. 4*C*). Accordingly, the dry mass was lowest in S4 (47.4 - 61.6 mg), followed by S2 (110.1 - 149.3 mg) and the control (199.6 - 303.7 mg). Interestingly, the root dry mass increased in the N1 treatment compared to N0.2 and N0 but only in the absence of NaCl. For example, the root dry masses in the treatment combinations N1+S0+B0 (248.3 mg) and N1+S0+B0.2 (303.7 mg) were higher compared to the N0/N0.2+B0/B0.2 treatments, which ranged from 199.6 to 220.7 mg. These data suggest

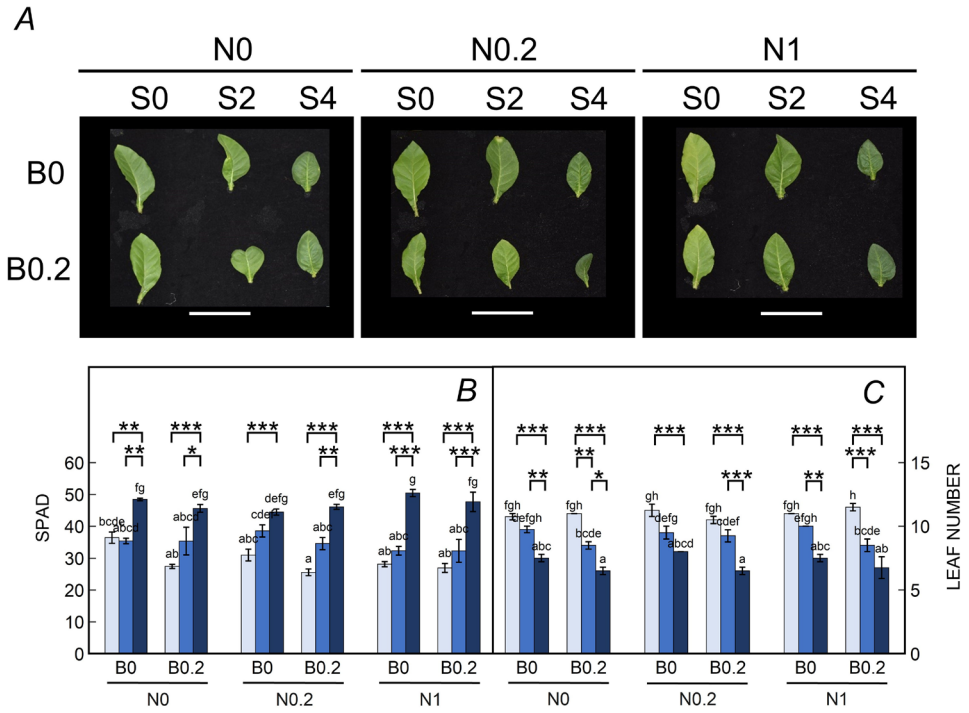

Fig. 3. Leaf phenotypes of tobacco plants following treatment with NaCl, $NaHCO_3$, and/or *Bacillus subtilis* for 49 d. *A* - The phenotype of the sixth leaf from representative plants. *B* - The relative chlorophyll content (SPAD) of tobacco leaves. *C* - Mean leaf number per plant. B0.2 = 2 g dm^{-3} of sodium bicarbonate (B0 is the control). S2 and S4 refer to 200 and 400 mM NaCl, respectively (S0 is the control). N0.2 and N1 refer to 2 and 10 g dm^{-3} of *B. subtilis*, respectively (N0 is the control). The *scale bar* is 10 cm. The *blue bars* represent the salt treatments (S0, S2, and S4 from light to dark). Data are means ± SD (n = 4) and analyzed by three-way *ANOVA* followed by *Tukey*'s *post hoc* test. Different letters represent significant differences (P<0.05). Brackets indicate the relevant comparisons (*- P<0.05, ** - P<0.01, *** - P<0.001).

that *B. subtilis* enhances the growth of tobacco roots in the absence of salt stress and in the presence of sodium bicarbonate.

Discussion

By definition, slightly saline water contains 17 - 51 mM NaCl, moderately saline water contains 51 - 170 mM NaCl, and highly saline water contains 170 - 595 mM NaCl (Krieger 1963). Both of our salt treatments therefore fall under the "highly saline" category. High salinity disrupts water potential and ion distribution in plants (Zhang *et al.* 2010). Sodium and/or chloride toxicity inhibit germination, development, photosynthesis, protein synthesis, and lipid metabolism (Yang *et al.* 2020). Similar to our results, previous studies have shown that salinity reduced the shoot and root length, biomass, leaf area, and leaf number of tobacco plants (Çelik and Atak 2012, Gautam *et al.* 2020, Yu *et al.* 2020). Nevertheless, salt stress can have more serious effects in a susceptible cultivar than in tolerant one (Çelik and Atak 2012). Instead of leaf chlorosis as reported previously (Çelik and Atak 2012, Yu *et al.* 2020), we found that salinity caused leaf darkening (Fig. 3*A*), which was consistent with the higher SPAD values of the plants in the S4 treatment groups (Fig. 3*B*). The discrepancy between these studies may reflect the use of different tobacco cultivars. For example, leaf chlorosis was observed in two oriental tobacco cultivars (İzmir Özbaş and Akhisar 97) following exposure to NaCl concentrations ≥ 150 mM (Çelik and Atak 2012), whereas darker leaves were observed in the cv. Samsun following exposure to 200 mM NaCl (Yu *et al.* 2020). Despite the darker leaf color, the total chlorophyll content and the rate of photosynthesis were significantly lower in the cv. Samsun subjected to salinity stress (Yu *et al.* 2020). Therefore, the darker leaf color and higher SPAD values that we observed in tobacco may not necessarily reflect a higher total chlorophyll content, but may be a consequence of attenuated mitosis or cell elongation as suggested by the reduced leaf area and wrinkled leaves (Fig. 3*A*).

The general response of many plants to salinity stress is to maintain low content of Na^+ and Cl^- in the cytosol by sequestering these ions into vacuoles and thus protecting the cytosolic water potential (Yildiz *et al.* 2020). Plants can also increase their salt tolerance by other mechanisms, including the regulation of ion translocation from roots to leaves, the sequestration of ions into cellular compartments, the production of osmoprotectants, and the synthesis of hormones and antioxidant enzymes (Chaves *et al.* 2009). We found that the combination of salt and sodium bicarbonate reduced plant height, shoot biomass, and leaf number to a greater extent than the NaCl alone (Figs. 1*B*, 2*B*,*C*; 3*C*). This can be explained by the additional Na^+

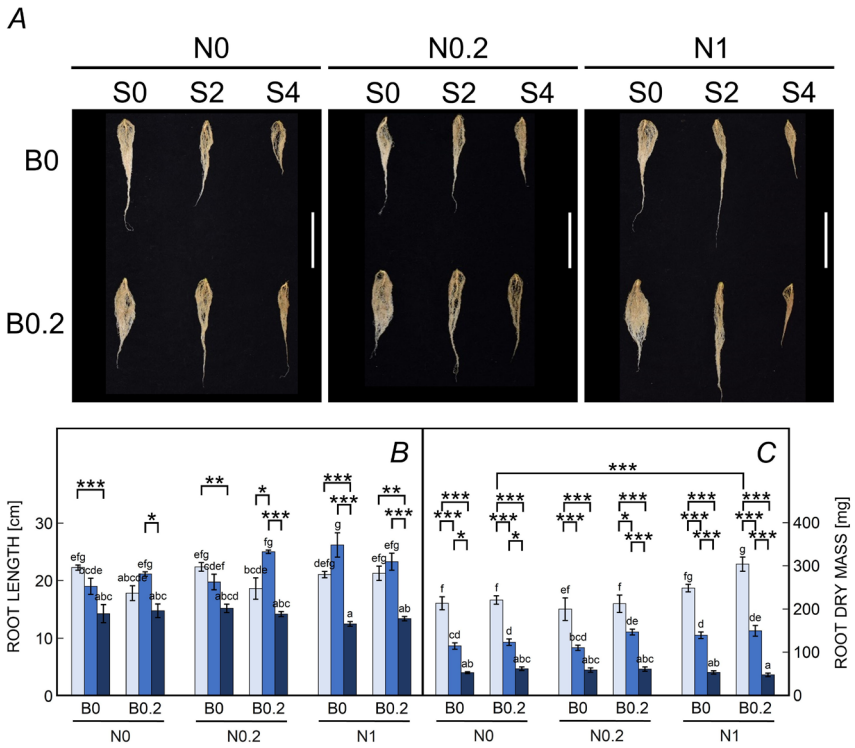

Fig. 4. Root phenotypes of tobacco plants following treatment with NaCl, $NaHCO_3$, and/or *Bacillus subtilis* for 49 d. *A* - Roots of representative plants. *B* - Root length. *C* - Root dry mass. B0.2 = 2 g dm^{-3} of sodium bicarbonate (B0 is the control). S2 and S4 refer to 200 and 400 mM NaCl, respectively (S0 is the control). N0.2 and N1 refer to 2 and 10 g dm^{-3} of *B. subtilis*, respectively (N0 is the control). The *scale bar* is 10 cm. The *blue bars* represent the salt treatments (S0, S2, and S4 from light to dark). Data are means ± SD ($n = 4$) and analyzed by three-way *ANOVA* followed by *Tukey*'s *post hoc* test. Different letters represent significant differences ($P<0.05$). Brackets indicate the relevant comparisons (*- $P<0.05$, ** - $P<0.01$, *** - $P<0.001$).

presented in the sodium bicarbonate treatment, which is likely to exacerbate the sodium toxicity caused by NaCl.

Bacillus is one of the largest genera of Gram-positive PGPR, and the species naturally inhabit soil, water, and plants (Bulgari *et al*. 2022). The ability of *B. subtilis* to promote plant growth has not been reported before. However, we found that the inoculation of soil with *B. subtilis* var. *natto* (N1) significantly increased tobacco shoot dry mass in the absence of NaCl (S0), in the presence of low concentrations of NaCl (S2), and in the presence of $NaHCO_3$ (B0.2) without NaCl (Fig. 2*C*). *B. subtilis* treatment also significantly increased the root dry mass in the presence of sodium bicarbonate and the absence of NaCl (N1+B0.2+S0) (Fig. 4*C*). However, the growth promoting effects of *B. subtilis* disappeared under high salt stress (S4) (Figs. 2*C*, 4*C*). This is likely to reflect the severity of the high salt stress treatment (S4), because the bacteria were still able to increase tobacco shoot dry mass when exposed to intermediate NaCl concentration (S2) in the absence of sodium bicarbonate (Fig. 2*C*). Nevertheless, previous studies have shown that other strains of *Bacillus* can enhance the salinity tolerance of crops such as maize (Li and Jiang 2017), wheat (Pourbabaee *et al*. 2016, Khan *et al*. 2017), rice (Nautiyal *et al*. 2013), tomato (Damodaran *et al*. 2013), soybean (Kumari *et al*. 2015), mungbean (Patel *et al*. 2015), and cucumber (Nadeem *et al*. 2016).

To understand why *B. subtilis* does not confer salinity tolerance in tobacco plants, we have to consider how other *Bacillus* strains are able to exert such an effect. Previous studies have shown that *Bacillus* spp. can enhance nutrient availability (*e.g.*, nitrogen, phosphorus, and iron), increase 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and boost the synthesis of growth hormones (*e.g.*, auxins, cytokinins, and gibberrellins), osmoprotectants (*e.g.*, proline and glycine betaine), antioxidant enzymes and exopolysaccharides (EPS) (Khan *et al*. 2017, Abbas *et al*. 2019). For example, *B. mojavensis* produces ACC deaminase and alleviates salinity stress in wheat by inhibiting stress-induced ethylene biosynthesis (Pourbabaee *et al*. 2016). Certain EPS-producing rhizobacteria ameliorate Na^+ toxicity by forming rhizosheaths around the roots, trapping Na^+ and limiting its uptake by plants (Rossi and De Philippis *et al*. 2015). The inoculation of *Arabidopsis thaliana* and *Puccinellia tenuiflora* (halophyte grass) with *B. amyloliquefaciens* GB03 reduces the influx of Na^+ by suppressing the *AtHKT1/K*$^+$ and *PtHKT2;1* transporter genes (Zhang *et al*. 2008, Niu *et al*. 2016). The inability of *B. subtilis* to confer salinity tolerance in tobacco plants may reflect its lack of access to these aforementioned PGPR strategies.

Salt stress may also affect the viability of *B. subtilis*, thus compromising its beneficial traits. Researchers have therefore sought to isolate halotolerant PGPR strains

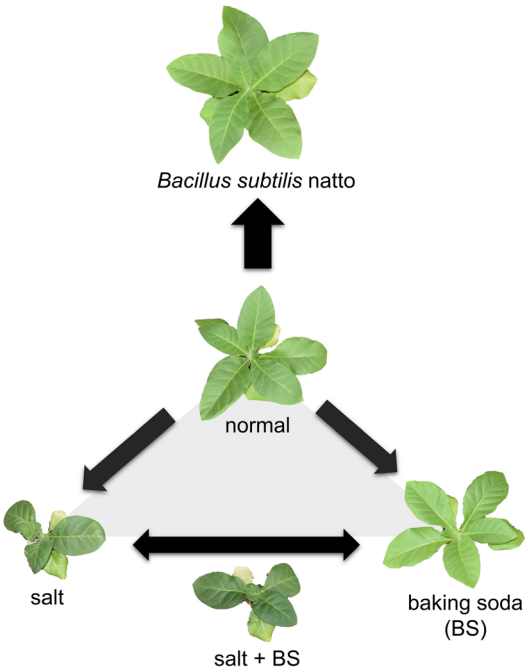

Fig. 5. Model of the tobacco plant response to NaCl, sodium bicarbonate, and *Bacillus subtilis* treatments. The application of *B. subtilis* enhanced the growth of tobacco plants resulting in a higher shoot and root dry masses. In contrast, salt stress inhibited the growth of tobacco roots and shoots (shorter roots and shoots, reduced shoot fresh and dry masses, and root dry mass), increased SPAD (darker and smaller leaves), and reduced the number of leaves. Although sodium bicarbonate had no significant change on the phenotype of tobacco plants, it aggravated the salt stress response by introducing additional Na^+.

to improve the performance of crops (Ramadoss *et al.* 2013, Patel *et al.* 2015). For example, by isolating 84 halotolerant bacterial strains from saline habitats, two strains (*Hallobacillus* sp. SL3 and *B. halodenitrificans* PU62) were found to mitigate severe salinity stress (320 mM NaCl) efficiently in wheat seedlings (Ramadoss *et al.* 2013). In another study, 50 halotolerant bacterial strains were isolated from alkaline-saline soil, and two (*B. subtilis* BN7 and *B. megaterium*) were shown to alleviate salt stress in mungbean plants (Patel *et al.* 2015). Although *B. subtilis* var. *natto* is not effective under salt stress, it promotes the growth of tobacco plants under normal conditions. Further studies should therefore be carried out to identify the underlying mechanisms. It would also be useful to examine its plant growth promotion activity in soils differing in pH, given that this bacterial strain thrives in the digestive tract where the pH varies greatly (Evans *et al.* 1988).

Conclusion

In the coastal region of Taiwan, soil salinity and acidity limit the use of the soil for agriculture, because these conditions inhibit the growth and productivity of crops (Chen *et al.* 2015). Sodium bicarbonate, a major product from the fixation of CO_2, has a pH of 8.1 and could be applied to soil in order to neutralize its acidity (Bonfim-Rocha *et al.* 2020). However, the effect of sodium bicarbonate on plants has not been investigated in detail (Fig. 5). We used tobacco (*Nicotiana tabacum* L.) as a model plant to explore the response to NaCl and $NaHCO_3$ treatments, and to understand whether *B. subtilis* var. *natto* (NTU-18) can work as a PGPR by promoting plant growth and conferring tolerance to salt stress. We found that the tobacco plants subjected to salt stress were stunted, with lower shoot fresh and dry masses, fewer leaves, shorter roots, a lower root dry mass, and higher SPAD values (corresponding to smaller, darker leaves). Sodium bicarbonate had no significant effect on the tobacco phenotype, but it aggravated the response to salt stress, probably by increasing the already toxic concentration of Na^+. Finally, the application of *B. subtilis* enhanced tobacco growth under normal conditions and in the presence of sodium bicarbonate, resulting in higher shoot and root dry masses but it did not mitigate the effects of salt stress. Further research is required to evaluate the use of *B. subtilis* as a PGPR to promote the growth of crops.

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