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ALLEVIATION OF SALT STRESS IN TURFGRASS BY NANO-COMPOST WITH INOCULATION OF MICROBIAL ISOLATES FROM COMPOST

LIAN DUO, XUJIAO HOU, CHUNXIANG LIU AND SHULAN ZHAO*

Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University, Tianjin 300387, China
**Corresponding author's email: zhaosl_tjnu@126.com*

Abstract

High soil salinity is an important factor to limit turfgrass growth due to insufficient precipitation, inappropriate water management and salts from road deicers or fertilizers. A greenhouse experiment was performed to examine the roles of nano-compost, either alone or in combination with salinity-tolerant microbial isolates, in alleviating the detrimental effects of salt stress on turfgrass. Municipal solid waste (MSW) compost was processed into nano-sized particles, and microorganisms in the compost were screened for salinity stress tolerance using increasing concentrations of NaCl. Tall fescue plants were treated with nano-compost and microbial isolates under three salt levels (0.3, 0.6 and 0.9% of NaCl). The results showed that the turfgrass quality decreased, malonyldialdehyde (MDA) content and superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities in the plants increased as salt level increased. However, the application of nano-compost, either alone or in combination with a microbial inoculum, improved the turfgrass growth, increased the chlorophyll (Chl) contents of the plants, decreased the plant MDA contents and further enhanced antioxidant enzyme activities in the salinity-stressed plants. Our results suggest that nano-compost combined with a microbial inoculation enhances the salinity tolerance of turfgrass by alleviating the oxidative stress.

Key words: Tall fescue; Salinity stress; Nano-compost; Microbial isolates; Oxidative stress.

Introduction

As an important and common stress factor, high salinity significantly limits plant growth, development and productivity in agriculture (Husain & Ismail, 1994; Saddiqe *et al.*, 2016). Approximately 20% of total cultivated land in the world are salinity-stressed (Shrivastava & Kumar, 2015). High salinity levels affect photosynthesis and plant growth by the negative effects of osmotic potential, ionic toxicity, and nutritional disorders (Munns &Tester, 2008; Bizhani & Salehi, 2014). In addition, salinity stress can cause oxidative stress with the excessive production of ROS, leading to damage to membrane lipids, biomacromolecules, ultimately to the cell structure (Wu *et al.*, 2010). However, plants possess a variety of antioxidant enzymes to mitigate the damages induced by ROS. As a key enzyme, superoxide dismutase (SOD) can scavenge O_2^- in ROS metabolism to form O_2 and H_2O_2, and then H_2O_2 can be scavenged by POD, CAT or ascorbate peroxidase (APX) (Bowler *et al.*, 1992).

To improve the salinity resistance of plants, municipal solid waste (MSW) compost has been applied in salt-affected soils as an organic amendment (Lakhdar *et al.*, 2009). MSW compost improved some enzymatic activities and microbial biomass of salt-affected soil due to the substantial increase in the organic matter (Ounia *et al.*, 2013). With the development of nanotechnology, nano-fertilizers have been proposed to promote crop growth, increase crop yields and enhance the utilization of fertilizer nutrients in agricultural production (Zhang *et al.*, 2002). They have large specific surface and small size, which enable them to increase their nutrients dissolved in water and their availability for plant uptake (Nair *et al.*, 2010). Numerous studies have showed various beneficial effects of nano-fertilizers, for instance, increased growth and yield, enhanced enzyme activities and improved resistance to various abiotic stresses (Huang *et al.*, 2015).

Microorganisms such as AM fungi and rhizobacteria are very effective in improving nutrient uptake, plant growth and controlling diseases (Abeer *et al.*, 2016). The beneficial microbes colonize plant rhizosphere and accelerate the growth of plants via various mechanisms (Ramadoss *et al.*, 2013; Nia *et al.*, 2012). Certain bacteria could, under stress conditions, produce exopolysaccharides that are beneficial to the root growth of plants (Sandhya *et al.*, 2009). In the last few years, inoculation with microbes has been proposed to mitigate high salinity stress on plants. Upadhyay *et al*., (2011) found that *Arthrobacter* sp. and *B. subtilis* co-inoculation could mitigate the adverse influences of salt stress in wheat.

High salinity is becoming a more prevalent issue in turfgrass establishment because of insufficient precipitation, inappropriate water management and salts from road deicers or fertilizers (Zhang *et al.*, 2013). *Festuca arundinacea*, one of the most widely used turfgrasses in North China, experiences salinity stress in varying degrees. To our knowledge, very little has been done to explore the effects of nano-compost and salinity-tolerant microbes on turfgrass under saline conditions.

MSW compost contains high salt content and consists of a rich variety of microbial communities, with some adapted to the osmotic environment. Here, MSW compost was processed into nanoparticles and screened for potential microbial strains, and turfgrass plants were treated with the nano-compost and microbial inoculum under three levels of NaCl. The objective of the research was to examine the effects of nano-compost combined with microbial inoculation on plant growth and physiological parameters under salt stress. The mechanism of salinity tolerance was observed by determining the antioxidant enzyme activities and MDA accumulation.

Materials and Methods

Soil and compost samples: The loamy soil was obtained from the 0–20 cm layer of an experimental site in the campus of Tianjin Normal University, China, and the saline soil was from the Huanghua Seashore, Hebei Province. Some physical and chemical properties of the loamy and saline soils are listed in Table 1. Desired soils with three saline levels (0.3%, 0.6% and 0.9%) were created by mixing different proportions of the loamy soil with the saline soil. MSW compost was collected from the Tianjin Xiaodian Compost Plant and processed into nano-particles with an average size of 30 nm.

Bacterial screening for salinity stress tolerance and identification: Ten grams of homogenized compost was suspended in sterile water and shaken for 15 min. Then, 10 ml of this suspension was grown in beef extract-peptone medium and continuously shaken at 220 rpm under 28°C for 3 days. The microbes were cultured in nutrient broth supplement with increasing NaCl concentration (0.5%, 1%, 1.5%, 2%, 2.5% and 3%). For isolation, 1 ml of the suspension was diluted to obtain a dilution of 10^{-1}–10^{-6}. The dilutions were plated on beef extract-peptone medium and Rose Bengal medium for growing bacteria and fungi, respectively. The most abundant microbial strains were selected and purified for the later experiment.

The purified bacteria and fungi were identified by sequencing their 16S rDNA and 18S rDNA genes, respectively. Microbial cells were extracted, diluted, lysed and directly used as templates in the PCR reactions. Database searches for 16S rRNA or 18S rRNA sequence similarities unambiguously identified two strains of bacteria as *Bacillus subtilis* and *Lysinibacillus* sp. and one strain of fungi as *Penicillium chrysogenum*. The three identified strains were inoculated in liquid culture medium, incubated and mixed at a volume ratio of 1:1:1 to prepare the microbial inoculum. The microbial inoculum was then diluted 100 times.

Plant growth conditions and treatments: The experiment was randomly designed with three soil salinity levels (0.3%, 0.6% and 0.9% NaCl) and three treatments in each salinity level: (1) no nano-compost and no inoculation (control), (2) nano-compost alone (M1), and (3) nano-compost combined with the microbial inoculum(M2). Each treatment was replicated four times.

The surface sterilized tall fescue seeds were sown at the rate of 160 $g \cdot m^{-2}$ in plastic pots filled with sterile saline soils containing 1.5% (w/w) nano-compost, except for the controls. The soil was irrigated to 70% field water capacity daily. After 10 days, the plants in the M2 treatments were treated with 15 ml of microbial inoculum, and the plants in the M1 and control treatments were treated with 15 ml of sterilized microbial broth. During the experiment, the temperature was 23–28°C and relative humidity ranging from 30–60%.

Parameter determination

Plant biomass: Fifty days later, the seedlings were gently uprooted and rinsed thoroughly. Next, roots and shoots were separated and dried at 80°C for dry weights determination.

Chlorophyll contents: The chlorophyll contents were assayed according to Zhang (1992).

Malondialdehyde (MDA) content: The MDA content was measured according to Zhang & Qu (2003).

Antioxidant enzyme activities: At 4°C, fresh leaves were homogenized in phosphate buffer (pH 7.8), and then centrifuged at 12,000 g for 20 min. The supernatant is used for the determination of POD, SOD and CAT activities.

SOD was assayed following the method described by Giannopolitis & Ries (1977). POD was determined using the guaiacol method (Kochba *et al.,* 1977). CAT was determined according to Aebi (1984).

Statistical analyses: The obtained data were analyzed by one-way ANOVA using SPSS software (17.0). The means were compared by the Duncan's multiple range test at the significance level of p=0.05.

Results and Discussion

As shown in Fig. 1, the dry weights of plant roots and shoots decreased as salt level increased. This reduction could be ascribed to the Na^+ toxicity to the plants and reduced water availability (Farhangi-Abriz &Torabian, 2017). High NaCl levels inhibit plant growth by disturbing ionic balance in plant cells and causing nutritional imbalances (Wang *et al.,* 2012). However, root and shoot biomass increased markedly in the plants treated with nano-compost alone or nano-compost in combination with the microbial inoculum under saline conditions. When compared with their corresponding controls, the combined applications increased shoot dry weights by 40%, 87%, 186% under 0.3%, 0.6%, 0.9% salt conditions, respectively. With small sizes within the nanometric scale and high specific surface areas, nano-fertilizers have been found to be beneficial to plant yield (Sabir *et al.,* 2014). The application of nano-fertilizers improves the yield and quality of plants by increasing the dissolution of their nutrients in water, which increases their availability to plants (Nair *et al.,* 2010) and thus improves the uptake of the nutrients. The assayed *Bacillus subtilis*, *Lysinibacillus* and *Penicillium chrysogenum* showed osmotic stress tolerance (growing at 3% NaCl). Kumar *et al.*, (2017) reported that *Lysinibacillus* sp. And *Pseudomonas aeruginosa* effectively promoted paddy growth under saline conditions. Higher dry weights were found in nano-compost treated and inoculated plants than the control and the nano-compost treated plants (Fig. 1), implying that nano-compost combined with microbes can reduce the deleterious influences of high salinity.

Chlorophyll contents decreased with increasing salinity level (Fig. 2). This was in agreement with the report of Liu *et al.* (2018), who observed decreased Chl a, Chl b and total Chl contents with increased NaCl concentrations in *Punica granatum* L. Under saline conditions, the decrease in the content of chlorophyll is due to reduced chlorophyll synthesis, the instability of pigment proteins, and the destruction of chlorophyll pigments (Levitt, 1980). Higher chlorophyll contents were observed in nano-compost treated plants under salinity stress than in control plants, and especially higher in nano-compost treated and inoculated plants. Higher chlorophyll contents suggest higher rates of photosynthesis resulting in a better growth status of treated plants under salt stress, because nano-compost and microbial inoculation provided the growing plants with more energy and carbon sources. Therefore, nano-compost treatment and microbial inoculation can help plants to maintain high photosynthetic abilities under stress, thus maintaining plant productivity.

As a stress marker, the content of MDA is often used to evaluate the extent of oxidative damage under abotic stresses (Elkahoui *et al.*, 2005). In this study, growth inhibition was positively correlated with enhanced MDA contents under saline conditions, which was also found by other studies (Koca *et al.*, 2007; Yazici *et al.*, 2007). The reason may be membrane leakage after excess ROS production and membrane lipid peroxidation (Koyro *et al.*, 2013). The present work observed that MDA levels increased as salinity stress increased, but they were significantly lower in the nano-compost-treated and inoculated seedlings under salinity stress (Fig. 2) than in the untreated control seedlings, which implies that lower amounts of ROS accumulate in treated and inoculated seedlings, and less associated membrane damage occurs. This result agrees with previous results obtained from AM-inoculated citrus exposed to salt stress (Wu *et al.*, 2010). Compared to the control plants, microbial inoculation decreased MDA contents in the nano-compost-treated plants by 51.0%, 56.7% and 51.5% under 0.3%, 0.6% and 0.9% saline conditions, respectively. The findings suggest that nano-compost combined with microbial inoculum can mitigate cellular oxidative damages under salt stress.

Plants possess antioxidant enzymes, such as POD, CAT and SOD to prevent their cells from the oxidative stress induced by ROS. SOD is involved in the scavenging of O_2^- into O_2 and H_2O_2. Then, H_2O_2 is further scavenged by CAT, POD into H_2O and O_2 (Farooq *et al.*, 2009). The enhancement of antioxidant defenses can thus increase plant resistance to different stress factors and prevent stress damage. In our results, the salinity stressed plants that were treated with the nano-compost and microbial inoculation kept higher antioxidant enzyme activities than their untreated counterparts (Table 2). Compared with the control plants, microbial inoculation increased POD, SOD and CAT activities in nano-compost-treated plants by 2.1-, 6.1- and 2.0-fold (0.3% salt level), 1.8-, 6.4- and 2.4-fold (0.6% salt level), or 2.1-, 7.6- and 3.2-fold (0.9% salt level), respectively. Higher enzyme activities help plants to mediate the quick removal of ROS imposed by salinity stress to maintain metabolism stability. The increased antioxidant enzyme activities in the AMF-inoculated and salinity stressed plants support previous findings for soybeans (Ghorbanli *et al.*, 2004) and tomatoes (Latef & He, 2011).

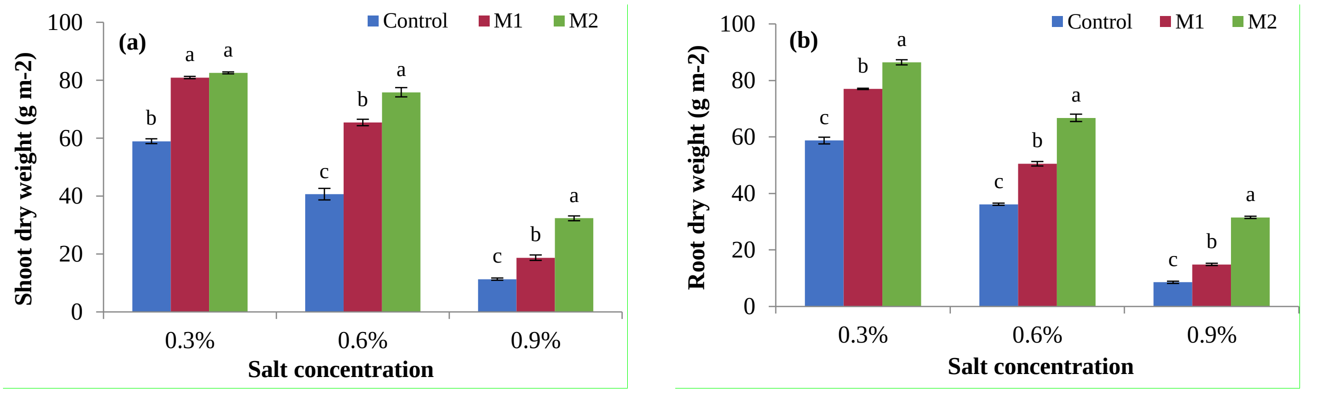

Fig.1. Shoot (a) and root (b) dry weights of *F.arundinacea* treated with nano-compost and microbial inoculum under salinity stress. Control, M1, and M2 correspond to non-treated, nano-compost-treated, and nano-compost-treated and inoculated plants, respectively. Different letters indicate significant differences among treatments at *p*<0.05.

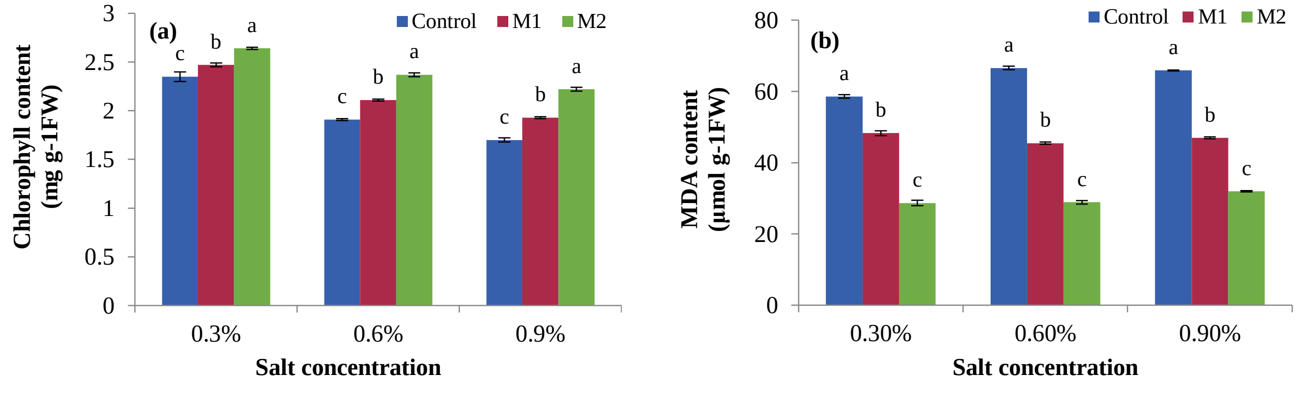

Fig. 2. Chlorophyll (a) and MDA (b) contents of *F.arundinacea* treated with nano-compost and microbial inoculum under salinity stress. Control, M1, and M2 correspond to non-treated, nano-compost-treated, and nano-compost-treated and inoculated plants, respectively. Different letters indicate significant differences among treatments at *p*<0.05.

Table 1. The physicochemical properties of the soils.

Soils	pH	Organic matter (%)	Total N (%)	Total K (g kg^{-1})	Available P (mg kg^{-1})	Salt content (%)
The loamy soil	7.44	4.68	0.21	45.61	22.03	0.29
The saline soil	7.85	2.21	0.26	28.96	15.26	1.58

Table 2. The antioxidant enzyme activities of *F. arundinacea* treated with the nano-compost and microbial inoculum under salinity stress.

Salt concentration	Treatments	POD ($U \cdot g^{-1}$)	SOD ($U \cdot g^{-1}$)	CAT ($U \cdot g^{-1}$ fresh weight$\cdot min^{-1}$)
	Control	2042.7 ± 83.4c	92.27 ± 0.71c	37.33 ± 1.39c
0.3%	M1	3608.9 ± 39.6b	494.67 ± 1.41b	65.33 ± 1.73b
	M2	4275.6 ± 94.1a	559.73 ± 3.73a	75.89 ± 3.04a
	Control	1448.9 ± 108.1c	74.13 ± 1.48c	25.67 ± 2.03c
0.6%	M1	2179.6 ± 58.9b	408.00 ± 2.57b	50.11 ± 0.91b
	M2	2608.0 ± 37.5a	470.13 ± 2.08a	60.89 ± 0.81a
	Control	1095.1 ± 55.5c	55.47 ± 2.54c	18.67 ± 1.73c
0.9%	M1	1777.8 ± 46.1b	352.00 ± 5.68b	42.56 ± 2.63b
	M2	2257.8 ± 24.7a	422.13 ± 2.54a	60.00 ± 1.07a

Control, M1, and M2 correspond to non-treated, single nano-compost-treated, and nano-compost-treated and inoculated plants, respectively. Different letters indicate significant differences among treatments at $p<0.05$

Conclusion

Our research provides a new approach to mitigate salinity stress on turfgrass through the combined application of nano-compost with salinity-tolerant strains from compost. Compared with control plants, the nano-compost treated and inoculated plants responded to salinity stress with increased biomasses, decreased MDA contents and higher levels of antioxidant enzymes. Overall, nano-compost with microbial inoculation made great contribution to the promotion of turfgrass growth, biomass and quality under saline soil conditions. Therefore, this approach is proposed to mitigate salinity stress for the sustainable turfgrass establishment.

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