Effect of Two Blue- green Algae and Tragacanth Coated Seed in Maize under Salinity Stress

Mahsa Soltani[1], Niloofar Mostafavi[1], Mohamad Reza Ghalamboran[1], Seyedeh Batool Hassani[1*], Françoise Bernard[1]

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Abstract

Soil salinity gradually become a major problem in arid and semi-arid regions of the world. Seed coating through bio-materials is a new method that maintains humidity around the seed. The aim of this study is using bio-compounds absorbent to increase the percentage of germination and improving growth of *Zea mays* L. under salt stress. The experiment was conducted with maize seed, which coated with tragacanth, tragacanth +*Anabaena vaginicola* and tragacanth+ *Pseudoanabaena* sp. when exposed to 0, 120 and 160 mM NaCl per kg of coco-peat. All seed treatments effectively promoted seed germination percentage in 160 mM NaCl. Seed coating with tragacanth led to a significant increase of growth indices compared to the non-coated seeds under salt stress. While, the combination of tragacanth with *A. vaginicola* or *Peusodoanabaena* sp. reduced the growth parameters. Coating treatment of maize seeds resulted in decreasing of Na content in the plants compared to non-coated seeds. The results suggested that maize *seed coating* with tragacanth could improve Na tolerance in the plants associated with a positive regulation of the seed germination and plant growth.

Keywords: *Trifolium alexandrinum* L., (Tragacanth gum), *Pseudoanabaena* sp., *Anabaena vaginicola* L.

Introduction

Salinity is one of the most important environmental stresses that affecting growth and yield of plants. In all areas where irrigation is essential for crop production, soil salinization is also unavoidable. This phenomenon has gradually become a major problem in arid and semi-arid regions of the world (Flowers and Flowers, 2005). In fact, salinity stress is due to the excessive accumulation of cations and anions in the soil solution. These minerals are primarily sodium and chlorine and then bicarbonates, sulfates, calcium, magnesium, boron and rarely nitrates (Shannon, 1997). Sodium ions (Na^+) that play key role in soil salinization, are toxic for most the plants except halophytes. On the other hand, high salt levels decrease osmotic potential, less water or osmotic stress (Zhu, 2001). Salt stress frequently inhib-

1- Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
*e-mail: b_hassani@sbu.ac.ir

its growth and influences key developmental stages, triggering premature senescence and death during prolonged exposure (Larcher, 1995). Salinity reduces growth for two reasons: (i) the effect of water scarcity or osmotic effect; (ii) the special effect of salt or ionic enhancement causing toxicity in plants. One of the best strategies for the plants is adapting to salinity by seed pre-treatment and seed coating with organic compounds, which can improve germination and seedling growth at the beginning of the plant lifecycle and cause better establishment of the plant. Seed coating technique uses for different purposes such as changing the appearance and size of the seed, possibility of better and easier seed placement, preventing environmental contamination, possibility of delayed planting of plants where the climate is not suitable and preventing animals from eating the seeds (Scott, 1989; Harris et al., 2002; Rasool et al., 2019).

Cyanobacteria or green-blue algae is one of the biological compounds recommended for seed coating (Seyfabadi et al., 2011). Seed coating with tragacanth gum due to its moisture absorber properties can protected seedlings at early growth (Balaghi et al., 2010).

The purpose of this project was to develop suitable seed coating materials to improve seed germination, seedling establishment and growth indices in maize plant under salinity stress. The results of this study indicated the positive effects of seed coating with tragacanth gum on reducing salinity effects and improving the evaluated factors in maize.

Material and Methods

Biological material

Zea mays L. seeds were prepared from Seed and Plant Reproduction Institute in Karaj, Iran. *Anabaena vaginicola* L. and *Pseudoanabaena* sp. were provided from Shahid Beheshti University. *A. vaginicola* and *Pseudoanabaena* sp. were cultured in liquid BG-11 medium (Waterbury and Stanier, 1981) without Nitrate and BG11 culture medium with Nitrate, respectively.

Seed coating

3 gram of tragacanth gum was dissolved in 100 ml distilled water. The resulting gel solution was coated over the seed; so that the seeds in the solution were covered with a thick layer of the tragacanth gum. The seeds were dried at room temperature for one day. For tragacanth + *A. vaginicola* or *Pseudoanabaena* sp. treatment, 2-3 g of each algae samples were homogenized with distilled water and was separately added to tragacanth gel solution. The seeds were rolled up in the algae plus tragacanth solution.

NaCl treatment and culture conditions

For salinity stress, 120 and 160 mM NaCl was added per kg cocopeat as culture medium. The treated cocopeat was sown in a culture tray to cultivate the desired plants. Seeds were transplanted into the trays with dimensions of 51×32 cm and 5 cm depth. To achieve the effect of coating materials on germination, coated seeds incubated at photoperiodic lighting16:8 h, temperature 23±2°C. During the period of seed germination, daily irrigation was carried out at a rate of 5 ml per seed.

Seed germination measurements

Coated seeds cultured in the trays and the number of germinated seeds in each tray was counted and recorded at intervals of 3 days. Germination was recorded when radicals removed the seed coats. The seed germination percentage was calculated by the following relation where N was the total number of seeds and N* was the number of germinated seeds.

$GP= (N^*/N) \times 100$.

Measurement of growth indices

After 21 days of cultivation, the roots and organs were washed with water and dried with a filter paper. Plant fresh weight were determined immediately after harvesting. root and seedling length were measured by the ruler. The number of leaves was also counted.

Measurement of Na^+ content in shoot

To measure the sodium (Na^+) content of the leaves, the shoots were first harvested and then dried in an oven at 70°C for 12 hours. Preparation of the samples for Na^+ content measurement were done according to Hamada and EL-enany (1994) method. The amount of Na^+ in samples was measured by a flame photometer.

Statistical analysis

To analyze the results of the experiments conducted in this study, SPSS software (version 22) was used. The data were presented as the mean ± standard error.

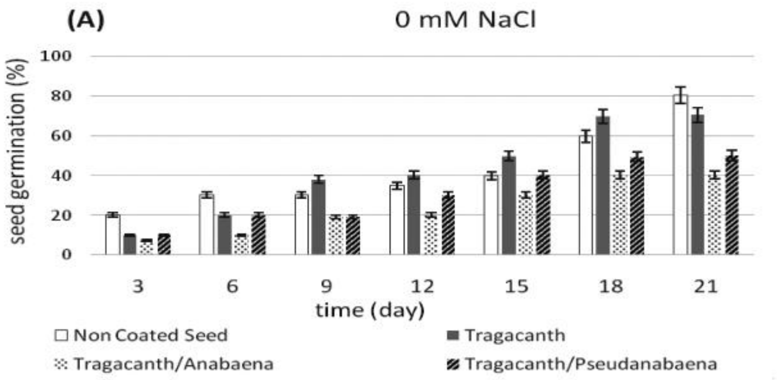
Results

Seed coating accelerate germinationin maize under salt stress

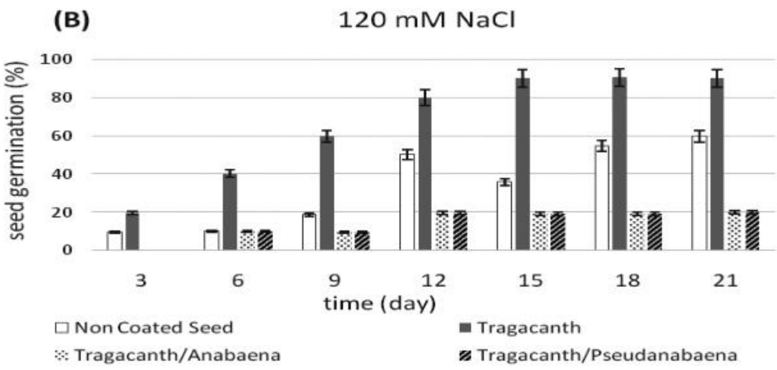
The results showed that in control culture (without NaCl), non-coated seeds represented the maximum germination percentage during 21 days (Fig. 1a). In culture with 120 mM NaCl, coated seeds with tragacanth showed the maximum germination during 21 days (Fig. 1b). The positive effect of seed coating on germination percentage was observed in culture with 160 mM NaCl. Non-coated seeds represented minimum germination percentage in 160 mM NaCl (Fig. 1c). Maximum germination (100%) was obtained in seeds treated with tragacanth in culture with 160 mM NaCl, however germination percentage in *Pseudo-anabaena* sp. or *A. vaginicola* treated seeds remarkably increased compared with the non-coated seeds (Fig. 1c).

Seed coating with tragacanth enhanced the seedling growth under salt stress

The results of seedling growth represented that in both NaCl free/exposed conditions, seed coating with tragacanth significantly increased the seedling length compared with the non-coated and algae treated seeds (Fig. 2). The length of the seedling decreased in NaCl concentration dependent manner, which was more observable in non-treated seeds (Fig. 2). The negative effect of algae treatment on seedling length was observed in both NaCl free/exposed conditions. Investigating of the root length showed a decrease by elevated NaCl concentration (Fig. 3). In both NaCl free/exposed conditions, there was no significant difference between tragacanth treated seeds and non-coated seeds. Seed coating with algae resulted in remarkable decrease of root length in both NaCl free/exposed conditions (Fig. 3). At 120 and 160 mg NaCl level, seed coating with tragacnth caused increase in leaf number when compared to the non- and algae- treated plants (Fig. 4). In both NaCl free/exposed



(a)



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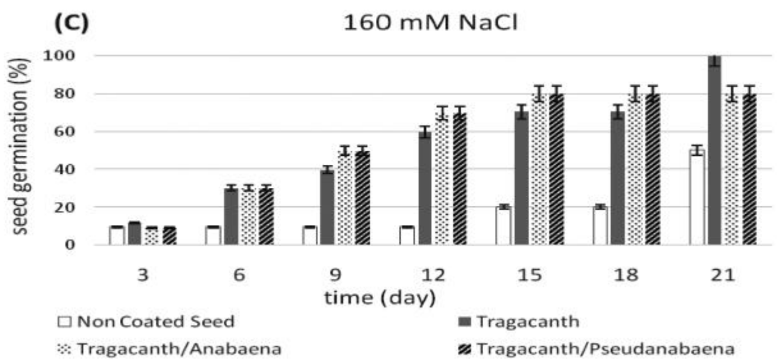

(c)

Fig. 1. Effects of seed coating (water (control), Tragacanth, Tragacanth + *A. vaginicola* and Tragacanth + *Pseudanabaena* sp.) on germination percentage under control (a), 120mM NaCl /kg cocopeat (b) and 160 mM NaCl /kg cocopeat (c) in maize plants. The bars are the means of three repetitions ± standard error.

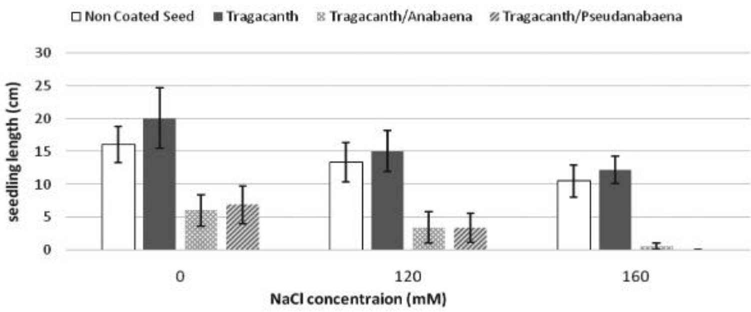

Fig. 2. Effects of seed coating (water (control), Tragacanth, Tragacanth + *A. vaginicola* and Tragacanth + *Pseudanabaena* sp.) on seedling length under different concentrations (0, 120 and 160 mM) of NaCl /kg cocopeat in maize plants. The bars are the means of three repetitions ± standard error.

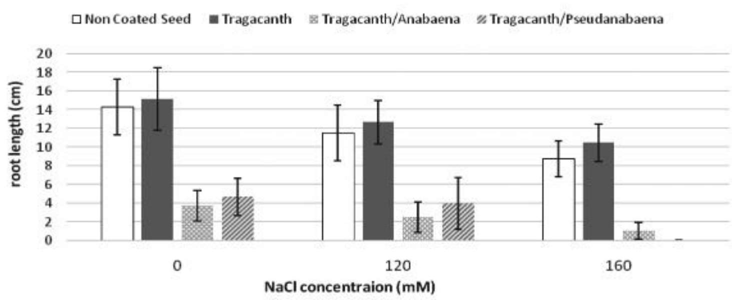

Fig. 3. Effects of seed coating (water (control), Tragacanth, Tragacanth + *A. vaginicola* and Tragacanth + *Pseudanabaena* sp.) on root length under different concentrations (0, 120 and 160 mM) of NaCl/kg cocopeat in maize plants. The bars are the means of three repetitions ± standard error.

conditions, *seed coating* with algae had negative effect on leaf number (Fig. 4). As shown in Figure 5 by increasing of the NaCl concentration, a decrease in plant fresh weight was observed in all plants. In both NaCl free/exposed conditions, a considerable increase in plant fresh weight caused by *seed coating* with tragacnth. Fresh weight of plants from algae treated seeds decreased when compared to the non- and tragacanth- treated plants (Fig. 5). In free NaCl condition, low amount of Nawas obtained in shoots from all non- and treated-seeds. Increasing of the NaCl concentration in culture medium resulted in enhancement

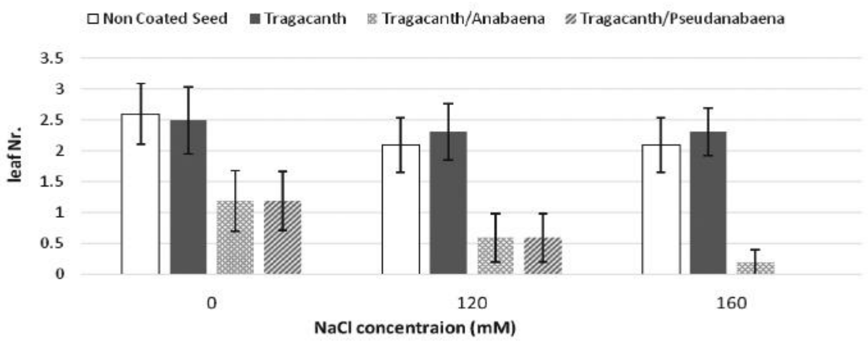

Fig. 4. Effects of seed coating (control, Tragacanth, Tragacanth +*A. vaginicola* and Tragacanth+ *Pseudanabaena* sp.) on leaf number under different concentrations (0, 120 and 160 mM) of NaCl/kg cocopeat in maize plants. The bars are the means of three repetitions ± standard error.

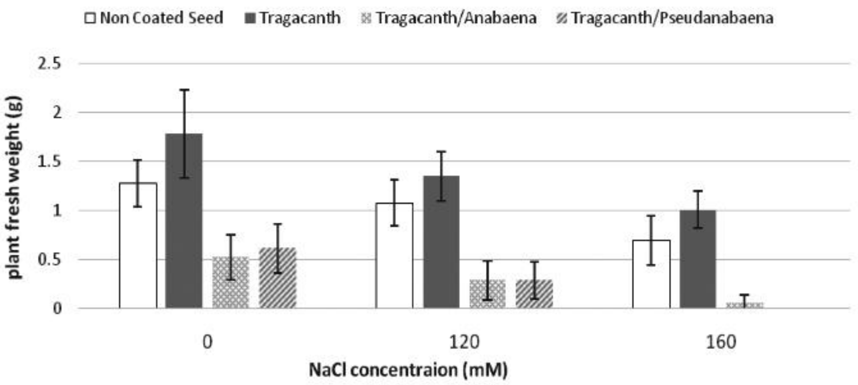

Fig. 5. Effects of seed coating (water (control), Tragacanth, Tragacanth + *A. vaginicola* and Tragacanth + *Pseudanabaena* sp.) on plant fresh weight under different concentrations (0, 120 and 160 mM) of NaCl/kg cocopeat in maize plants. The bars are the means of three repetitions ± standard error.

of Na content in plant shoots (Fig. 6). At 120 and 160 mg NaCl level, *seed coating* with tragacnth or algae caused decrease in shoot Na content when compared with the non-treated plants (Fig. 6). This reduction in Na content in plants from tragacanth treated seedwas more than algae treatment (Fig. 6).

Discussion

Salinity is an environmental factor that more or less affects all stages of plant growth from germination to biomass, seed and fruit production. Salinity in environments are characterized by low osmotic potential and high solute concentration. Plants are generally

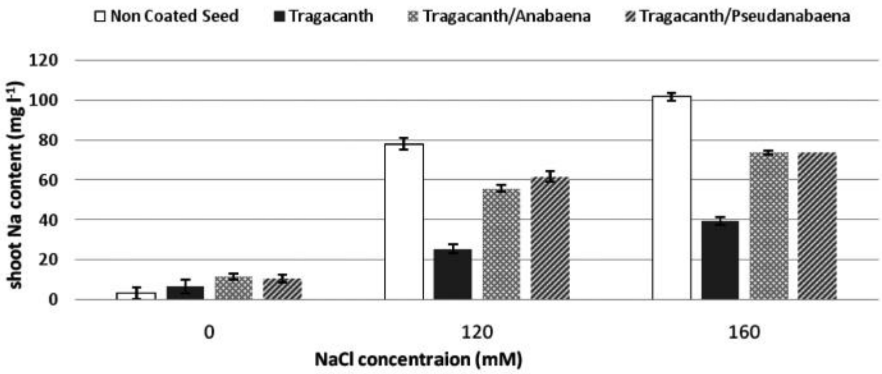

Fig. 6. Effects of seed coating (water (control), Tragacanth, Tragacanth +*A. vaginicola* and Tragacanth +*Pseudanabaena* sp.) on shoot NaCl content under different concentrations (0, 120 and 160 mM) of NaCl/kg cocopeat in maize plants. The bars are the means of three repetitions ± standard error.

vulnerable to environmental pressures during seed germination. Under stress conditions, early stage of plant development and growth is regarded as the most sensitive stage of development (Radosevich, 1997). Salinity tolerance during this stage is important for plant establishment due to less germination and reduced seedling growth (El-Keblawy and Al-Rawai, 2005). Salinity stress by limiting water absorption, reducing material degradation of seed reserves, and disturbing storage protein reduces germination (Voigt et al., 2009). In this study, NaCl increasing affected negatively germination percentage. In investigating the effect of salinity on the germination process, some researchers call osmotic effects as an effective factor, but others regard ion toxicity as an inhibitor of germination (Katerji et al., 1994). Seeds are well protected against environmental stresses, but as soon as they absorb water and germination process started, they expose to stress (Li et al., 2005). Our results indicate *seed coatings* (tragacanth, tragacanth +*A. vaginicola* and tragacanth + *Pseudoanabaena* sp.) increased germination percentage in maize under 160 mM NaCl condition in compare with control seeds. The treated seeds did not initially (after 3 days) show any increase in germination percentage compare with the control, however germination percentage elevated during several days post cultivation. Among treated seeds, tragacanth treated seeds showed maximum germination in both NaCl free/exposed conditions. Maintenance of requisite water status is a paramount importance during seed germination which is facilitated by *seed coating* with tragacanth as water retaining resources (Souza and Marcos-Filho, 2001). Moreover, *seed coating* with cyanobacteria such as *A. vaginicola* and *Pseudanabaena* sp. may act as growth regulator and modulates plant metabolism and supply metabolites involved in stress tolerance (Essa et al., 2015; Chittapun et al., 2018). Mehrabi (2009) examined the effect of *seed coating* on germination of *Sanguisorba minor* and showed *seed coating* had a significant effect on germination of the species, with a 97% increase in germination.

The application of tragacanth alone due to its absorbent properties and water requirement of plant under salt stress indicates application of the mentioned coatings increased the germination percentage and growth parameters at 120 and 160 mg NaCl. However using a combination of *A. vaginicola* and *Pseudanabaena* sp. with tragacanth gum failed to increase plant growth under salinity stress. It suggested that mismatch of required growth pH with the pH of tragacanth gum reduces the yield of the cover, ultimately leading to decrease plant growth under salinity stress.

Salinity affects all aspects of plant metabolism and causes changes in plant anatomy and morphology. Some of these changes lead to adaptations help plant tolerance stress, however, most of the changes are a sign of salinity damage. The reduction in leaf expansion after the salinity expose is one of these changes. Salt stress reduces shoot, root and leaf dry weight, leaf number, leaf area and stem length in wheat, maize, barley, rice, sorghum, and sesame (Zeng et al, 2001).

Generally, younger roots are more at risk. They are present only in the upper layers of soil containing higher concentrations of salt, with severe salt stress leading to short root, in which case canopy growth is stopped. The symptoms of necrosis can be seen on the roots, buds, leaf margins, and stems (Zeng et al., 2001). It has been reported using of coated seeds increase growth in plants under different stresses. The positive effect of inorganic, organic and hydrogel based coatings on dry weight gain was reported for several plant species under drought stress (Gorim and Asch, 2012). Furthermore, increasing root length and plant height has been reported in coated seeds by polymers (Suma et al., 2014). Colla et al. (2015) showed that application of *Arbuscular mycorrhiza seed coating* in rice plant increased root, shoot and leaf dry weight compared to non-coated seeds. The result showed that tragacanth coating increased growth in maize by increasing relative water content, nutrient absorption, regulating osmotic pressure, maintaining moisture around the seed. However, application of co-treatment of *A. vaginicola* and *Pseudanabaena* sp. with tragacanth gum had negative effect on maize growth. Using these algae alone or in combination with other useful materials can be suggested as a suitable coating to increase plant resistance under salinity stress.

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