Research Article

Film coating with sodium alginate improves seed germination of sweet corn (Zea mays var. Saccharata) under osmotic stress conditions

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Abstract

Drought stress influences seed germination and seedling growth of many plants. Seed coating is a technique of covering seeds with adhesive agents to improve seed performance and plant establishment and could be used to alleviate the negative effects of osmotic stress. In order to solve the problem of low germination and poor vigor of sweet corn under osmotic stress conditions (0, -0.3, -0.6 and -0.9 MPa), film coating with different concentrations of sodium alginate (NaAlg) (0, 1 and 2%) was performed. Results showed that osmotic stress reduced percentage and the rate of germination, seedling length, seedling vigor index and soluble protein content whereas increased the content of proline and soluble sugars. In all levels of osmotic stress, coating with NaAlg 1% improved germination and biochemical indices of sweet corn seeds compared to NaAlg 0 and 2%- and non-coated seeds. The highest percentage of germination (67 %) was related to osmotic potential of 0 MPa and the lowest (6.68 mg g⁻¹ FW) amount of soluble protein were obtained from NaAlg 1% at 0 MPa and NaAlg 0% at -0.9 MPa, respectively. The results suggested that seed coating with NaAlg 1% could serve as an appropriate treatment to increase the germination and early seedling growth of sweet corn under osmotic stress conditions.

Keywords: Film coating, Germination, Osmotic stress, Seedling vigor index, Soluble protein content

Introduction

Corn as a fomous plant from Poaceae family is cultivated in a large part of the world and is exploiting due to its extraordinary diversity in form, quality and habit of growth. Among the various subspecies of maize, sweet corn (*Zea mays* var. *Saccharata*) is one of the most important subspecies. Sweet corn is produced by introducing a gene mutation in the locus of Su from chromosome 4 of common maize. This mutation makes the endosperm to save sugar about twice the amount of common corn (Schultheis, 2010). These mutations lead to less seed vigour in sweet corn grains compared to the conventional corn. In addition, low germination rate of sweet corn has been attributed to seed sensitivity to soilborn diseases (Khalid *et al.*, 2012).

In the life cycle of plants, germination time and germination rate are important ecological concepts and they have significant effect on seedling survival and subsequent growth of plants until plant maturity (Xiao *et al.*, 2010). Therefore, disruption of the germination process and desirable establishment causes a lack of uniformity in maturity and thus reduces yield (David, 2015).

Biotic and abiotic stresses are the most important factors that severely limit plant growth and metabolism (Makbul *et al.*, 2011). Abiotic stress is the primary cause of crop loss worldwide, reducing average yields

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for most of the major crop plants by more than 50% (Bray *et al.*, 2000). Moreover, when the usable areas on the earth are classified in view of stress factors, drought stress is one of the most widespread environmental stresses (SaruhanGuler *et al.*, 2012). Although each stress factor produces its own specific effect on plants, in general, all stress types can cause an increase in reactive oxygen species (ROS). Reactive oxygen species, are known as detrimental to biological systems because they cause oxidation of lipids, proteins, deoxyribonucleic acid, and carbohydrates (Baysal Furtana and Tıpırdamaz, 2010). Therefore, it is necessary to alleviate the adverse effects of drought stress for achieving good crop yields (Ashraf and Rauf, 2001).

Among various strategies adopted to improve seed performance under drought stress, seed priming and seed coating are thought to be two effective approaches. Seed coating can be broadly classified into three categories namely seed pelleting, film coating and seed encrusting. Film coating is the technique of encapsulating seeds with a thin layer of synthetic slurry form consisting of polymers, plasticizers, pigments, and solvents, using rotating drum machines. The thin coating layer does not greatly change the size and shape of seeds, however, it enhances handling characteristics

of seeds (Taylor et al., 2001). Recently, film coating has gained wide acceptance in the seed industry as a reliable approach to improve the productivity of many important crop species, such as Brassica napus, Glycine max, Gossypium sp., Helianthus annuus, Medicago sativa, Triticum aestivum and Zea mays (Accinelli et al., 2016; Oliveira et al., 2016; Zhou et al., 2017).

The results of Saadat and Ehteshami (2016) showed that film coating with absorbant materials and micronutrients has improved the germination indices of maize including germination percentage, root and shoot length, root and shoot fresh weight and alpha amylaseenzyme activity. In another study on Onobrychis sativa seeds coated with different coating materials (organic coating, hydro gel coating and mineral coating), coating significantly enhanced germination indices in both normal and drought stressed conditions (Mehrabi and Chaichi, 2012).

In new agriculture, water-absorbent polymershave been used as seed coating materials in order to reduce the harmful effects of abiotic stresses and enhances crop yield and quality. These polymers are biodegradable and compatible with the environment. In this relation, Alginate polymer has attracted the attention of many researchers. Alginate is a polysaccharide extracted from brown seaweed (Phaeophyceae), including kelp. Sodium alginate is one of the alginate salts that presents high water uptake, does not repel water and allowing proper imbibition of the seed, which favors the germination process (McHugh, 1987). Also, in agriculture, alginate is used to cover seeds, fruits and tip of the stem (Cisneros-Zevallos and Krochta, 2006).

Currently, the most researches have been done on the use of sodium alginate for the production of synthetic seeds, and there is no research on the effect of sodium alginate on germination indices and seedlings of different seeds. Research findings showed that NaAlg 2% and 3% in Ferulago angulate L. were used to produce synthetic seed with maximum germination (Sorbi and Moradi, 2018). The results of an experiment on persian oak (Quercus brantii L.) synthetic seeds showed that NaAlg 3.5% has improved the germination percentage of this plant (Fayzi, 2016).

However, since no research has done on film coating on sweet corn seed, this study was done to investigate the effect NaAlg on the improvement of germination and biochemical indices of sweet corn under osmotic stress in laboratory conditions.

Material and Methods

Seeds of sweet corn (Zea mays var. Saccharata) hybrid Challenger were film coated in three levels of sodium alginate (Sigma Aldrich) 0 (hydrated in distilled water as coating control), 1 and 2%, The treated seeds were dried in air. Also, non-treated (dry) seeds were used as main control. Coated and non-coated (control) seeds were germinated in petri dishes (12 cm) with one layer of filter papers moistened with 10 ml appropriate solutions. Four levels of osmotic potentials including 0 (distilled water), -0.3, -0.6 and -0.9 MPa were induced by PEG 6000 using Michel and Kaufmanm (1973) method. Four replications of 25 seeds were used for each treatment. Petri dishes were placed in a germinator at $25 \pm 1^{\circ}$ C under dark conditions for 7 days. Seeds germination recorded daily and the seeds were considered germinated when the radicle visibly protruded through the seed coat. At the end of experiment, 5 seedlings were randomly selected from each petri dish, and seedling length measured (Akramian et al., 2007). Germination percentage (GP), germination rate (GR) and seedling vigor index (SVI) were calculated using the following equations: G

$$SP = \times 100$$

n is the total germinated seeds and N is the total number of seeds (Ikic et al., 2012).

$$GR = \sum \frac{NI}{Ti}$$

 N_i is the number of germinated seeds per day, and T_i is the day from the start of testing (Pagter et al., 2009). $SVI = seedling lenght (mm) \times GP (ISTA, 2010).$

To determine the seed moisture content, the oven method was used. In this method, 10 gr of sweet corn seeds were weighed and incubated in oven at 130 °C for 4 hours. After drying, the seeds were weighed and seed moisture was calculated according to the following formula (ISTA, 2010).

Seed moisture content= $\frac{M_2 - M_B}{M_2 - M_1} \times 100$

 M_1 = Container weight

 M_2 = Container weight+ Seed sample before drying

 M_3 = Container weight+ Seed sample after drying

The content of free proline was determined according to the method described by Pquin and Lechasseur (1979) using L-proline as a standard (0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.5 and 0.6 µmol ml⁻ ¹). UV-Spectrophotometer was used for determination of the absorbance at 515 nm. Free proline content was expressed as μ mol g⁻¹ FW of seed.

Soluble sugars content was measured as the method described by Irigoyen et al. (1992). To measure the content of soluble sugars, 100 µL alcoholic extract (ethanol 80%, v/v) was mixed with 3 mL Anthrone reagent. After centrifugion, at 3500 rpm for 15 min at 4 °C, the supernatant obtained was placed in a boiling water bath for 10 minutes and then cooled in an ice bath. The content of soluble sugars was determined using a UV-Spectrophotometer by reading the absorbance at 625 nm. Before performing the above steps, standards of glucose with concentrations of zero, 25, 50, 100, 200, 500, 1000, 1500 and 2000 mg. 1⁻¹ were prepared and all the mentioned steps were done on prepared Standard samples. Then the calibration curve was plotted using the glucose standard and the amount of soluble sugars in the samples was calculated based on mg per gram of fresh seed weight.

The Bradford (1976) method was used to measure the quantity of soluble protein content. The amount of 0.2 g seed sample was homogenized with 2 ml of 0.1 M

mg ml⁻¹). For extracting hydrogen peroxide, 0.2 g of seed samples were homogenized with 2 ml trichloroacetic acid 1% (w/v) in a mortar and centrifuged at 15,000 rpm for 15 min at 4 °C and the resulting supernatant was used to measure hydrogen peroxide. The light absorption of each sample was estimated at 390 nm using a UV-Spectrophotometer. Contents of hydrogen peroxide were determined using H_2O_2 standard at concentrations (100-1000 µmol ml⁻¹) and expressed as µg g⁻¹ FW of seed (Loreto and Velikova, 2001).

concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.156

Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) reaction (Heath and Packer, 1968). The extraction was carried out by mixing 0.2 g of seed sample with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA). Then, the mixture was homogenized on ice and centrifuged at 13,000 rpm for 30 min at 4 °C. The supernatant and 0.5% (w/v) TBA in 20% (w/v) TCA were mixed and put in water bath at 95 °C for 30 min. After that, the mixture was cooled on ice for 10 min and centrifuged at 10,000 rpm for 10 min. Absorbance was read at 532 and 600 nm. The Lipid peroxidation was expressed as MDA content in nM per gram fresh weight, by using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

A completely randomized design was used in the experiments. Statistical analysis was performed using SAS statistical software (version 9.4). Arc SinX was used for converting germination percentage data. Analysis of variance (ANOVA) was used to analyses treatments effects, and significant differences of means were separated using Duncan's multiple range test ($P \le 0.05$).

Results

Seed moisture content: In Figure 1, seed moisture content is shown in various concentrations of sodium alginate and non-coated seeds. Seeds coated with NaAlg 1% had the highest moisture content (56 %) and caused 33% increase in moisture content compared to the lowest ratio of this index (42 %) at non-coated seeds. However, the amount of this trait decreased with decreasing osmotic potential.

Proline content: With increasing osmotic level, proline content increased in both coated and non-coated seeds. In seed coated with NaAlg 1%, proline content was higher than those coated with NaAlg 0%. The highest proline content (8.16 μ mol g⁻¹ fresh seed weight) was observed in osmotic potential of -0.9 MPa and NaAlg 1%, while the lowest amount (3.12 μ molg⁻¹ fresh seed weight) was detected at 0 MPa and NaAlg

0%, which had 62% difference (Figure 2).

Soluble sugars content: The results showed that with increasing osmotic stress, soluble sugars content increased. At all levels of osmotic potential, coating with NaAlg 1% caused the highest amount of soluble sugars. Among the different levels of osmotic potential, the highest content of soluble sugars (34.75 mg g⁻¹ fresh seed weight) was obtained in osmotic potential of -0.9 MPa and NaAlg 1%. Also, it caused 37% increase in soluble sugars content compared to the lowest ratio of this index (25.36 mg g⁻¹ fresh seed weight) at 0 MPa and NaAlg 0% treatment (Figure 3).

Soluble protein content: Unlike what was observed in soluble sugars content trait, figure 4 shows that the soluble protein content was decreased with increasing osmotic potential levels. So that the highest soluble protein content (19.17 mg g⁻¹ fresh seed weight) was observed in osmotic potential of 0 MPa and NaAlg 1% which was about 2-fold of this amount in -0.9 MPa and NaAlg 0% (6.68 mg g⁻¹ fresh seed weight).

Hydrogen peroxide: Contrast to soluble protein content, hydrogen peroxide content increased with increasing PEG concentration. Non-coated seeds showed higher hydrogen peroxide content compared to the coated seeds. According to the results, the highest hydrogen peroxide content (5.69 Mm g⁻¹ fresh seed weight) was obtained at -0.9 MPa. However, at all levels of osmotic potential, the use of sodium alginate reduced the level of this compound; This reduction was between 29 to 44% (Figure 5).

Malondialdehyde content: Similar to what was observed in the H_2O_2 content, the malondialdehyde (MDA) content increased with increasing osmotic potential (Figure 6a). The highest amount of MDA (0.0026 Mm g⁻¹ fresh seed weight) belonged to the osmotic potential of -0.9 MPa with no significant difference with osmotic potentials of -0.3 and -0.6 MPa. The increase in this amount was about 40% by increasing osmotic potential from 0 to -0.9 Mpa. However, the application of NaAlg was able to mitigate this effect to a large extent, so that NaAlg 1% treatment showed about 35% less MDA content than the non-NaAlg treatment (Figure 6b).

Germination percentage: Results showed that germination percentage decreased significantly with increasing osmotic potential. Germination percentage decreased from 67 % in 0 MPa to 37 % at -0.9 MPa (Figure 7a). Film coating with NaAlg 1% had significant effect on germination percentage as compared with non-coated seeds, so that the highest germination percentage (56 %) was observed in NaAlg 1%, while NaAlg 0% and 2% had negative effect (Figure 7a).

Germination rate: Results showed that germination rate decreased significantly with increasing osmotic potential (Figure 8). In all osmotic levels, NaAlg coating treatment improved germination rate compared to non-coated treatment. Among the different concentration of NaAlg, NaAlg 1% had significant

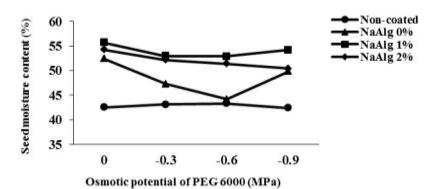


Figure 1. Changes in the moisture content of seeds coated with different concentrations of sodium alginate (NaAlg) under osmotic stress induced with PEG 6000.

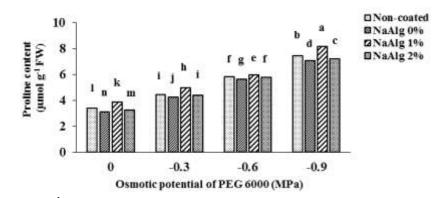


Figure 2. Proline content (Mm g^{-1} FW) of sweet corn affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential of PEG 6000. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

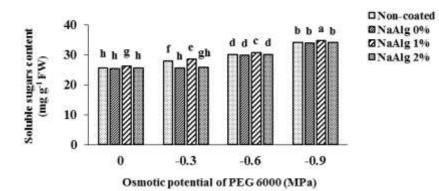


Figure 3. Soluble sugars content (mg g^{-1} FW) of sweet corn affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential of PEG 6000. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

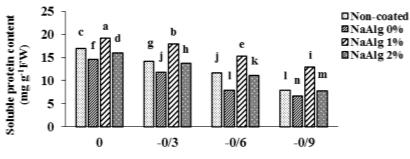
effect. The highest germination rate (24.60 seed d^{-1}) was observed in 0 MPa and NaAlg 1% that had a difference of 92% with the lowest amount (1.75 seed d^{-1}) in -0.9 MPa and NaAlg 0%.

Seedling length: According to the results of means comparision, seedling length decrearsed significantly with increasing concentration of PEG. The highest seedling length was obtained at 0 MPa osmotic potential, while with increasing osmotic potential to -0.9 MPa, this trait decreased to its lowest rate. In all stress levels, film coating with NaAlg 1% improved seedling length. In osmotic potential of 0 MPa and NaAlg 1%,

seedlings had the highest length (131.95 mm), which was about 3-fold of -0.9 MPa and NaAlg 0% (30.55 mm) (Figure 9).

Seedling vigor index: Seedling vigor index decreased with increasing osmotic stress. Film coating with NaAlg 1% had a positive effect on seedling vigor index compared to the NaAlg 0, 2% and non-coated seeds. The highest seedling vigor index (120.09) was observed in osmotic potential of 0 MPa and NaAlg 1%, that had a difference of 91% with the lowest seedling vigor index (9.77) at osmotic potential of -0.9

MPa and NaAlg 0%. However, this treatment had no



Osmotic potential of PEG 6000 (MPa)

Figure 4. Soluble protein content (mg g^{-1} FW) in sweet corn affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential of PEG 6000. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

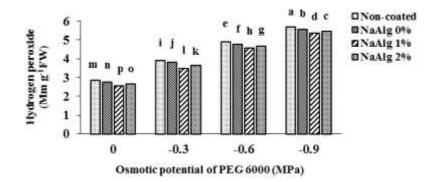


Figure 5. Hydrogen peroxide (Mm g^{-1} FW) content in sweet corn affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

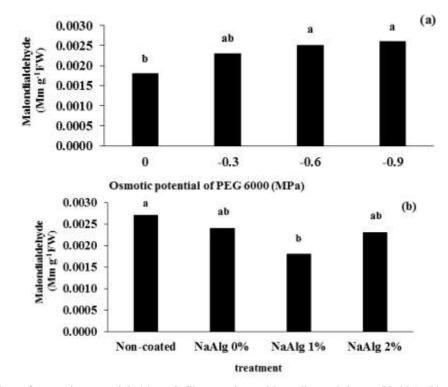


Figure 6. The effect of osmotic potential (a) and film coating with sodium alginate (NaAlg) (b) on the content of malondialdehyde (Mm g^{-1} FW) in sweet corn. Means with the same letter are not significantly different according to Duncan test at P< 0.05.

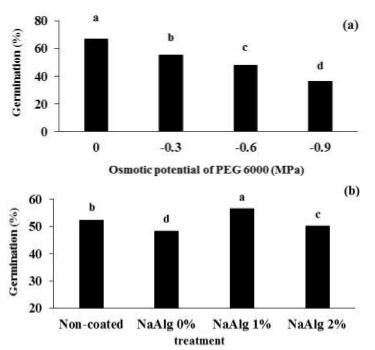


Figure 7. The effect of osmotic potential (a) and film coating with sodium alginate (NaAlg) (b) on germination percentage of sweet corn. Means with the same letter are not significantly different according to Duncan test at P< 0.05.

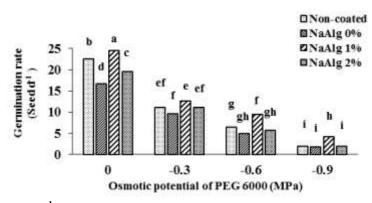


Figure 8. Germination rate (Seed d⁻¹) in sweet corn as affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential of PEG 6000. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

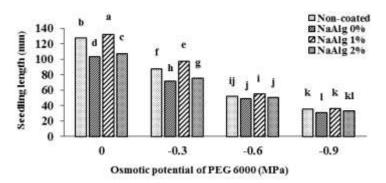


Figure 9. Seedling length of sweet corn affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential of PEG 6000. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

significant difference with -0.9 MPa and NaAlg 2% treatment (Figure 10).

Discussion

Drought stress is a major abiotic agent that seriously

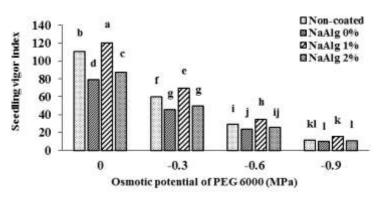


Figure 10. Seedling vigour index of sweet corn affected by film coating with sodium alginate (NaAlg) at different levels of osmotic potential of PEG 6000. Means with the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

decreases crop productivity in the arid and semi-arid regions of the world (Lipiec et al., 2013). The findings of the present study have indicated that seed germination of sweet corn was inhibited by drought stress, whereas film coating with sodium alginate reversed this effect. The negative effects of drought stress on maize (Zea mays L.) seed germination have been reported in previous researches. In a research on maiz Queiroz et al. (2019) reported that increasing osmotic potential, decreased seed germinated, germination rate, root and shoot length and seedling vigor index (SVI), while mean germination time (MGT) and root: shoot ratio (RSR) increased. In a study on cumin (Cuminum cyminum L.), with increasing the levels of drought stress, the germination indices (germination percentage and rate, seedling vigor index and seedling length) decreased (Sohrabiani et al., 2016). Also, in a study on fennel (Foeniculum vulgare L.), it was found that with increasing the severity of drought germination indices decreased (Hoseini stress, Moghaddam et al., 2017).

Germination involves many metabolic steps of the enzymatic hydrolysis of stored materials and making new tissues using hydrolyzed materials. Drought stress limits water uptake by seeds that delay or inhibit germination. Water uptake directly effects cell division and elongation within growing seedlings and then shoot and root system development (Abd Allah et al., 2010). Hydrophilic or water absorbent compounds that increase the amount of water available for germination and seedling growth, enzyme activity, and improvement of biochemical processes are recommended as a promising solution for improving plant establishment under abiotic stresses condition (Berdahl and Barker, 1980). Qiu et al. (2005) have conducted an experiment on three varieties of rape (Brassica napus L.) and observed that seed coating enhanced root vigour, increased root length, root volume and root dry weight.

Under drought stress condition, the H_2O_2 content increased, causing oxidative stress and damaging the cell membrane structure. One of the mechanisms that plants are used to withstand drought stress is regulating osmotic potential of the cell, especially if drought stress increases gradually from mild stress to severe one (Lipiec et al., 2013). Plant usually accumulates organic materials, such as proline and soluble sugars to counter the osmotic stress (Farhad et al., 2011; Liu et al., 2011). In the present study, the increase in the content of proline and soluble sugars can also be interpreted in this regard (Figures 2 and 3). These results are in accordance with previous results that drought stress increased sugar and free proline content, which enhanced germination and seedling growth of plant under drought stress (Irigoyen et al., 1992; Krasensky and Jonak, 2012). In a research on Malaysian rice (Oryza sativa cv. MR219) seedling, proline and total soluble sugar in increased with increasing PEG concentrations, (Kalhori et al., 2018). As a compatible osmolyte, proline exerts a protective role by regulating osmotic potential and scavenging free radicals (Hasegawa et al., 2000). Sugar accumulation in drought stress conditions helps to maintain the stability of the membrane and protein (Lipiec et al., 2013). Protein synthesis in growing plants is the basic mechanisms to regulate plant metabolism, which increases the plant's ability to response drought stress (Patil, 2010). Under drought stress, concentration of soluble proteins reduced due to increased activity of protein degrading enzymes, decreased protein synthesis, and the accumulation of free amino acids, including proline (John et al., 2009). Piri (2017) showed that the amount of soluble protein in green cumin (Cuminum cyminum L.) decreased with increasing drought stress.

Under normal growth conditions, many metabolic processes cause the production of reactive oxygen species, but plants have antioxidant mechanisms that are effective in eliminating these molecules. Under stress conditions, this balance is mixed up and the amount of active oxygen species is increased. The presence of these active species is harmful to the plant and damages cell structures such as membranes, proteins and nucleic acids (Laspina *et al.*, 2005). The findings of the present study indicated that hydrogen peroxide content increased with increasing osmotic potential (Figure 5). An increase in this reactive oxygen species has also been reported in maize (Ragab Moussa and Abdel-Aziz, 2008) and sunflower (*Helianthus annuus* L.) (Baloglu *et*

al., 2012). Increased reactive oxygen species can lead to lipid peroxidation. The increase in malondialdehyde content by increasing the osmotic potential (Figure 6) can be interpreted in this regard. Sodium alginate is a good oxygen barrier that protects the seeds against oxygen entry and therefore decrease lipid peroxidation (Varela and Fiszman, 2011). In addition, sodium alginate is a highly water-absorbent seed coat that by increasing seed water content (Figure 1) improves the metabolism of stored materials, enhances the proline and soluble sugars content and other osmotic regulating compounds ultimately led to reduced lipid peroxidation of cell membranes under environmental stresses (Figures 5 and 6).

Seed coating technology have been used as an effective approach for improving seed germination and seedling growth of many important crop species, such as Brassica napus, Glycine max, Gossypium sp., Helianthus annuus, Medicago sativa, Triticum aestivum and Zea mays (Zhou et al., 2017). In rice, seed coating significantly increased the activities of the antioxidant enzymes such as POD, CAT and SOD, and enhanced soluble sugars content and improved the performance and physical properties of seeds, especially under adverse environmental conditions (Manjunatha et al., 2008; Zeng et al., 2009). In the present study, seed coating with sodium alginate enhanced seedling vigour, germination rate and germination percentage of sweet corn (Figures 7, 8 and 9). Also, film coating of sweet corn seed significantly increased soluble protein and proline contents, and decreased MDA and H₂O₂ contents (Figures 2, 4, 5 and 6). Seed coating with sodium alginate reduces the harmful effects of drought stress. Sodium alginate as a polysaccharide and hydrophilic compound can play a significant role in increasing resistance to drought stress and salinity in germinating seeds (Dehyadegari *et al.*, 2016). Film coating with NaAlg 1%, improved germination of seeds due to improving water uptake efficiency (Figure 1), wherea, higher concentration (NaAlg 2%) had negative effect. Since sodium alginate have high viscosity especially in higher concentration, negative effects of this treatment on germination traits might be due to its limiting effect on oxygen entry or resistance to water entry from the germination medium to seed.

Conclusion

The seed coating process involves application of pesticides, fertilizers, absorbent materials, oxygen agents or growth regulators to seeds in order to resist diseases and pests, tolerance to adverse environmental conditions and to promote seed germination and seedling growth. The results of this experiment revealed that drought stress significantly effected sweet corn germination. Using film coating with sodium alginate (NaAlg) improved seed germination and helps to improve biochemical and germination indices of sweet corn. Film coating with NaAlg 1% increased sweet corn seedlings tolerance under drought stress by improving germination and seedling growth indices and direct effect on non-enzymatic antioxidant defense system of plant. However higher level of sodium alginate (NaAlg 2%) had negative effect on sweet corn germination.

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