

Priming with Mango Peels Nanoparticles Enhances Seed Germination of Maize (*Zea mays L.*) under Salt Stress

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ABSTRACT

The main aim of this study was to evaluate the effectiveness of nano-priming application on seed germination of maize under salt stress. *Zea mays L.* seeds were primed in saline water (6.5dSm⁻¹) and mango peels nanoparticles (nMPs) at concentration of 1000mg/L. Nano-primed and non-primed seeds were germinated in petri dishes with two round filter papers moistened with 5 ml distilled water and with 10 ml solutions of 5 salinity levels (0, 2.5, 6.5, 9.5 and 12.5 dSm⁻¹). Increasing salinity levels significantly diminished radicle and plumule length, seedling fresh- dry weights, germination percentage and vigor index. Priming seeds in nMPs and saline water significantly improved germination percentage, vigor index, radicle, plumule length and total fresh weight. Such findings may serve as in vitro selection criteria for ameliorating salt stress in maize plants.

Key words: Nanoparticles, Corn, Salinity stress, Physiological parameters.

INTRODUCTION

Currently, salinity is a serious environmental problem causing great reduction in plants growth and development. More than 20% of cultivated land is currently under salinity stress and it is expected to increase in the future. Salinity stress could cause negative impact on plant growth and yield as a result of nutritional imbalance, specific ionic toxicity enhancement or combination of these factors (Wang et al. 2003). To face environmental stresses, plants develop their defense system by modulating biochemical and physiological pathways (Pottosin et al., 2014).

In arid and semi-arid environments, soil salinity and scarcity of good quality water are adversely affecting crop production through germination and stand establishment. In these areas, successful germination and seedling establishment depend on the rapid and uniform germination of the seed and the ability of the seed to germinate under water and or salinity stresses. Alleviating such stresses at the germination stage will increase the chances of getting good crop with economic yield (Kaya et al., 2006). Technologies have been

developed over the years to improve emergence and stand establishment of the crops grown in areas facing salinity stress. The technique frequently used is seed priming. It is reported that seed priming promotes seed vigor's during germination and emergence under salt stress.

Recently, nanotechnology is gaining attention of researchers because of their wide applications in resolving agricultural and environmental problems. Nano-scale particles possess high surface energy and high surface to volume ratio that enhance their biochemical activity and impact much more than their bulk counterparts (Roco, 2003). Recent results indicated significant increase in plant growth and development indices, when treated with nanoparticles (Laware 2014, Pottosin et al., 2014).

The positive role of nanoparticles on seed germination and emerging under salt stress has been reported recently. Silver and silicon nanoparticles have been reported to increase germination of tomato (*Solanum lycopersicum*) seeds under saline conditions (Siddiqui et al. 2014; Almutairi 2016). Seed priming agents including natural and synthetic compounds improve physiological processes in seeds prior to germination (Jisha et al., 2012). However, no information are available in the literature concerning the use of mango peels in seed priming. The present study was executed to study the effect of mango peel nanoparticles (1000mgL⁻¹) in presence of distilled water or saline water (6.5dSm⁻¹) on germination of corn seeds and early seedling growth under salinity stress. The objectives of this study were to produce and characterize nanoparticles of mango peels (nMPs) and to investigate the effectiveness of nano-priming application in mitigating the adverse effect of salinity on seed germination.

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MATERIALS AND METHODS

Materials

1) Maize Seeds

Corn Seeds (*Zea mays L* cv. Giza168) were obtained from Egyptian ministry of agriculture and refrigerated (4°C) until use.

2) Nanoparticles of Mango peels (nMP)

Mango peels were obtained from Juice factory at Borg Elarab city. The obtained peels were oven dried 80°C and mechanically ground to diminish the particle size to <100 nm according to the method of Elkhatib *et al.*, (2015).

Methods

1) Characterization of MPs nanoparticles

The surface morphology, composition and element contents of the MPs nanoparticles were investigated using scanning electron microscopy (SEM) with energy dispersive X-ray, EDX (INCAx-Sight model 6587, Oxford Instruments, UK), and The surface structure of nMPs was explored with Fourier transform infrared spectroscopy (FTIR) to illustrate the functional groups of the nMPs surfaces.

2) Effect of Nano mango peels priming on germination behavior of corn (*Zea mays L.*) seeds under salt stress

2.1. Seeds Sterilization and Nano-Priming

Corn seeds (*Zea mays L.*) cv. Giza 168 were first sterilized using 3% H₂O₂ solution and thoroughly washed with distilled water (DW) and then soaked in mango peels nanoparticles (nMP) suspensions (1000 mgL⁻¹) at 25°C for 24 h. The Nano-primed seeds were rinsed with distilled water and 6.5 dSm⁻¹ saline water. Seeds were rinsed in distilled water alone without nMPs, then seeds surface were dried on absorbent paper.

2.2. Germination Tests

The germination experiments were run in a completely randomized design with three replications. Nano-primed and DW-primed seeds were germinated in petri dishes with two round filter papers moistened with 5 ml distilled water and with 10 ml solutions of salinity levels (5 levels of salt equivalent including 0 (distilled water), 2.5, 6.5, 9.5 and 12.5 dSm⁻¹). Eight (8) seeds were deposited for each replicate and germinated at room temperature (25 °C) for 7 days. The papers belong to each dish were replaced every two days to maintain salinity level. At the end of the experiment, length of coleoptile, radicle roots were measured for each replicate. Only seeds with a radicle longer than 2 mm

were scored for the evaluation of percent germination and the seedlings were evaluated as described in Seedling Evaluation Handbook (AOSA. 1991).

2.3. Measurement of Physiological Parameters

a. Growth parameters

The fresh weights and volume of the shoots and roots of the seedlings were measured immediately after 7 days. The dry weights were measured after drying the shoot and root at 80°C for 48 h, to standardize the weight.

b. Germination percentage (GP):

The emergence of plumule is taken as an indication of germination process. Germination percentage was calculated using the following formula:

Germination percentage (%) = (Number of germinated seeds / Number of total seeds) *100.

C. The vigor index (VI)

Was calculated according to the formula of Elouaer and Hannachi (2012) as follows.

Vigor index (VI) = [plumule length (cm) × germination percentage%] /100.

2.4. Statistical Analysis

Experimental data were analyzed by FAOSTAT (2005). Treatments means were compared using least significant test (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

3. Characterization of Mango Peels Nanoparticles (nMPs)

3.1. Main components of nMPs

Mango (*Mangifera indica L. Anacardiaceae*) is ranked 5th in total world production among fruit crops (FAO, 2004) and Mango Peels represent 15-20% of the fruit production. Mango Peels are not utilized for commercial purposes and discarded as a waste. Therefore, the innovative use of the peels might introduce various benefits to human. The proximate composition of mango peel was 72% moisture, 28% carbohydrate, 2% protein, 2.22% fat, 5.8% fiber and 1.16 Ash as reported by Ajila *et al.* (2007).

3.2. X-ray diffraction

XRD pattern of the nMPs is shown in Fig. (1). The produced curve has a broad hump with no sharp diffraction peaks. The absence of sharp diffraction peaks is an indication of amorphous materials. The presence of a significant amount of amorphous materials in nMPs samples could be due to lignin and tannin in the sample.

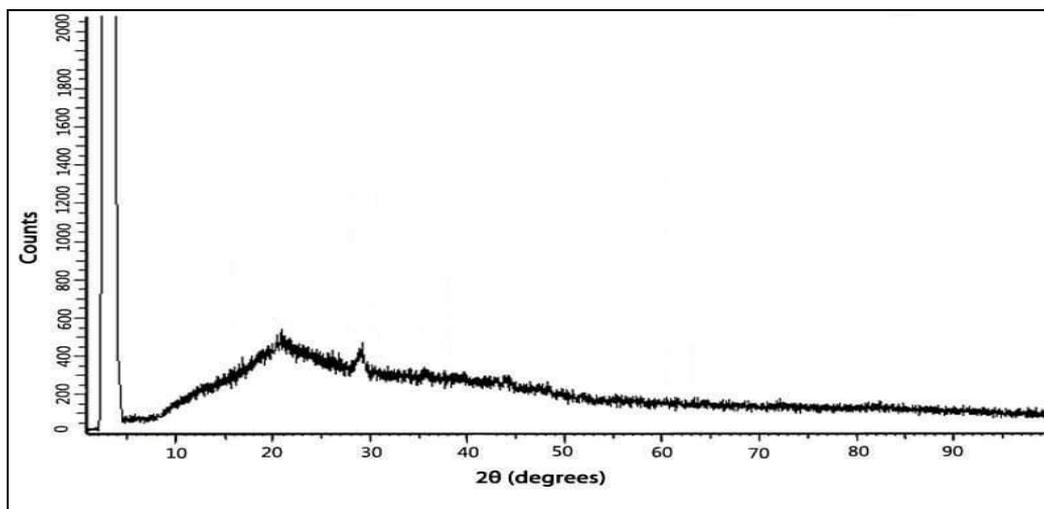


Fig. 1. The X-ray diffraction (XRD) analysis of nMPs.

3.3. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analyses

The structure of surface morphology and elemental analyses of mango peels nanoparticles were examined using SEM equipped with EDX. SEM and EDX analyses of mango peels nanoparticles are shown in Fig. (2). The SEM image clearly showed different shapes and different sizes of MPs nanoparticles, all of which lie in the nanostructure range (1-100) nm. The EDX analysis revealed that potassium, calcium, silicon, iron, sulphur and Phosphorus elements represent 84.5% of the total elements of nMPs illustrated in Fig.(2).

3.4. Fourier transform infrared (FTIR) spectroscopy for surface characterization of nMPs

Surface characterization of nMPs using FTIR spectroscopy contributes to identify the functional groups present in the Mango Peels Nanoparticles structure (Ngah & Hanafiah, 2008). Figure (3) shows FTIR spectra of nMPs. Spectral bands of nMPs showed the appearance of intense bands at 3412 cm^{-1} attributed to the vibrational stretching of the O–H bond present in carbohydrates, fatty acids, proteins, alcohols, Phenols and lignin (Stuart 2004; Han et al., 2010; Gonçalves et al., 2011, Feng et al., 2011). Spectral peak at 2922 cm^{-1} referred to vibrational stretching of C–H bond of alkane groups (Barbosa, 2007).

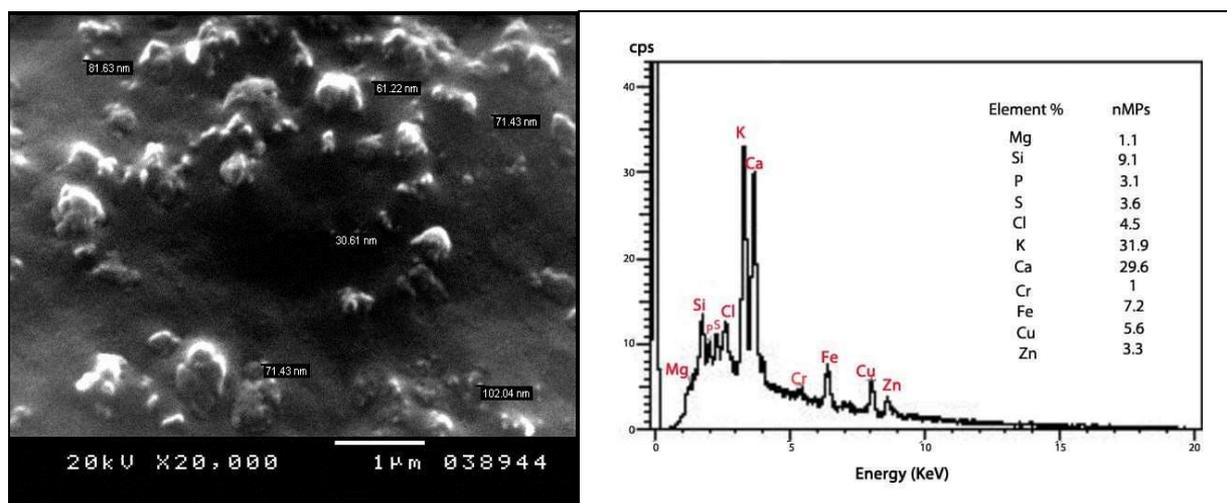


Fig. 2. Scanning electron microscopy (SEM) image (a) and energy dispersive X-ray (EDX) elemental distribution (b) of nMPs.

The spectral strong bands observed at 1626cm^{-1} and 1741cm^{-1} may be attributed to the vibrational stretching of C–O bond due to non-carboxyl groups ($-\text{COOH}$, $-\text{COOCH}_3$) or from Carboxylic acids or esters. In the region of $1441\text{--}1237\text{ cm}^{-1}$ could be referred to the vibrational stretching of C–O bond of amide and carboxylic groups and C–O stretching with phenols (Han et al., 2010). The band at 1062 cm^{-1} attributed to corresponds to C=O bonds of ether, ester or phenol, which have compounds such as carboxyl groups (Guo et al., 2008). The peaks observed at 878 cm^{-1} correspond to stretching vibration of NH_2 group of proteins (Sekhar et al. 2009).

4. Effectiveness of Nano-priming application in mitigating the adverse effect of salinity on seed germination

4.1. Germination percentage (GP):

The germination percentage of *zea mays L.* seeds under salt stress as affected by different priming treatments are presented in Table 1. Germination percentage decreased gradually with increasing salinity stress level. Seeds germination was greatly inhibited (50%) at the salinity level of 12.5 dSm^{-1} even though seeds were primed with DW (Table 1). Meanwhile, the germination percentage at 12.5dSm^{-1} salinity level was significantly improved when seeds were primed with nMPs in SW. It is also worth noticing that the germination of corn seeds primed with mango peels nanoparticles biostimulant (nMPs) in DW at 12.5dSm^{-1} improved to a lesser extent than that of seeds primed with nMPs in SW. It is clear that priming seeds with

nMPs and SW have significant beneficial effects on germination performance of *Zea mays L.* seeds under salt stress. The use of biostimulants such as karrikinolide (KAR1) (Kulkarni et al., 2011) and Kelpak® (seaweed extract) in order to mitigate salinity effect is well-documented (Bulgari et al., 2014; Sharma et al., 2014). Khodakovskaya et al. (2009) reported that carbon nanotubes increased seed germination and growth of tomato plants due to its ability to penetrate the seed coat and enhance the crucial water uptake.

4. 2. Growth Parameter

The effects of seed priming on maize growth parameters (root length, plumule length, seedling fresh weight) under salt stress are shown in (Figs. 4b, 5b, and 6b). In general, seedlings grown without salt stress were more healthy and vigorous than those grown under salt stress, regardless of priming treatments in (Figs. 4a, 5a, and 6a). In all priming treatments, increasing salinity stress significantly reduced root length, plumule length, seedling fresh weight, seedling volumes, roots and shoots dry weights. However, the deleterious effect of salt stress was less pronounced in nMPs-SW primed plants than DW and nMPs-DW primed plants. The reduction in growth parameters studied of maize seedlings under salt stress is attributed to leaf elongation inhibition in maize seedlings grown in saline environment (Szalai and Janda, 2009). Farooq et al (2015) postulated that the deleterious effect of salt stress on plant growth could be due to toxic effects of sodium and chloride ions and the disturbance of biosynthesis and plasma membrane integrity.

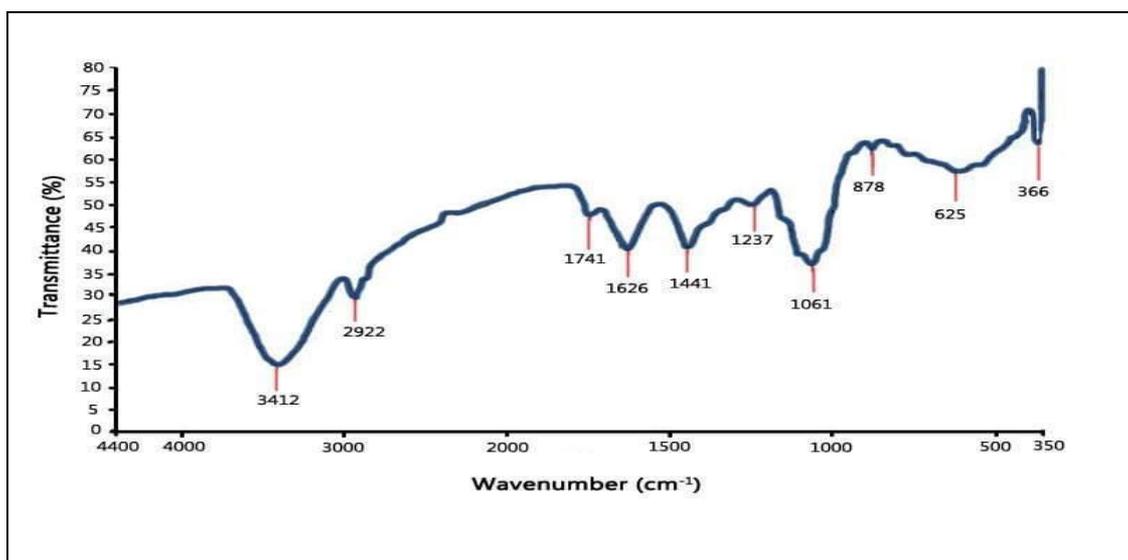


Fig. 3. Fourier transmission infrared (FTIR) spectra of nMPs

Root and plumule lengths of nMP-SW primed seedlings were ~2-3 times longer than those of DW and nMP-DW primed seedlings under 12.5 dSm⁻¹ salt stress (Figure 4b & 5b). Similarly, vigor index and seedling volume of nMP-SW primed seedlings significantly improved by ~ 3.5 and 1.4 times compared to hydroprimed and 2.23 and 1.75 times compared to nMP-SW primed seedlings respectively (Table 2, Fig. 4b, 7b).

Results of fresh weight, dry weight of shoots, and dry weight of roots (Fig. 6b, 8b, 9b) showed that nMPs-SW treatment significantly increased seedling growth under salinity stress as compared to DW and nMP-DW treatments. Several researchers have reported that seed priming with salts, especially NaCl, clearly improved germination and growth of many crops under stressed conditions (Esmailpour *et al.*, 2006; Sivritepe *et al.*, 2007). In the current study seed treatments with nMP in

saline water improved the germination rate and enhanced the seedling vigor as indicated by increased and seedling fresh and dry weights. Farooq *et al.* (2007) indicated that improved seedling fresh and dry weights might be due to increased cell division within the apical meristem of seedling roots. Different seed priming agents are capable of regulating pathways in the early development stages that leads to a faster defense response by plants (Jisha *et al.*, 2012). Thus, application of bio-stimulants such as nMPs may induce biochemical changes (hydrolysis, activation of enzymes, breaking seed dormancy) to help seeds to grow faster under stress (Jisha *et al.*, 2012; Paparella *et al.*, 2015). Thus, these biostimulants alleviated the detrimental effects of salinity stress during seed germination and seedling growth in maize.

Table 1. Germination (%) of *Zea mays L.* seeds primed with 3 different treatments under salinity stress conditions

Priming Treatments	¥Germination Percentage (%)				
	Salinity level(dSm ⁻¹)				
	0	2.5	6.5	9.5	12.5
DW	100 ^a	80 ^b	70 ^c	60 ^d	50 ^e
nMPs-DW	100 ^a	90 ^b	85 ^c	80 ^d	70 ^e
nMPs-SW(6.5dSm ⁻¹)	100 ^a	100 ^a	95 ^b	90 ^c	80 ^d

#DW: Distilled Water; SW: Saline Water ¥ values for each column and treatment with different letter are significantly different (P ≤ 0.05) based on Duncan's Multiple Range Test.

Table 2. Effect of Different Priming Treatments on Vigor Index of *Zea Mays* Seedlings at Different Salinity Levels.

Treatment	Saline Water (dSm ⁻¹)	Vigor Index
nMPs-DW	0	10.86±0.3a
	2.5	4.98±0.2b
	6.5	4.17±0.1c
	9.5	3.22±0.3d
	12.5	2.21±0.5e
Mean		5.09 ^b
nMPs-SW	0	12.86±0.8a
	2.5	11.66±0.15b
	6.5	10.77±0.2c
	9.5	7.16±0.4d
	12.5	4.93±0.50e
Mean		9.48 ^a

Mean values ±standard error (n = 3) for each column and treatment with different letter(s) are significantly different (P ≤ 0.05) based on Duncan's Multiple Range Test.

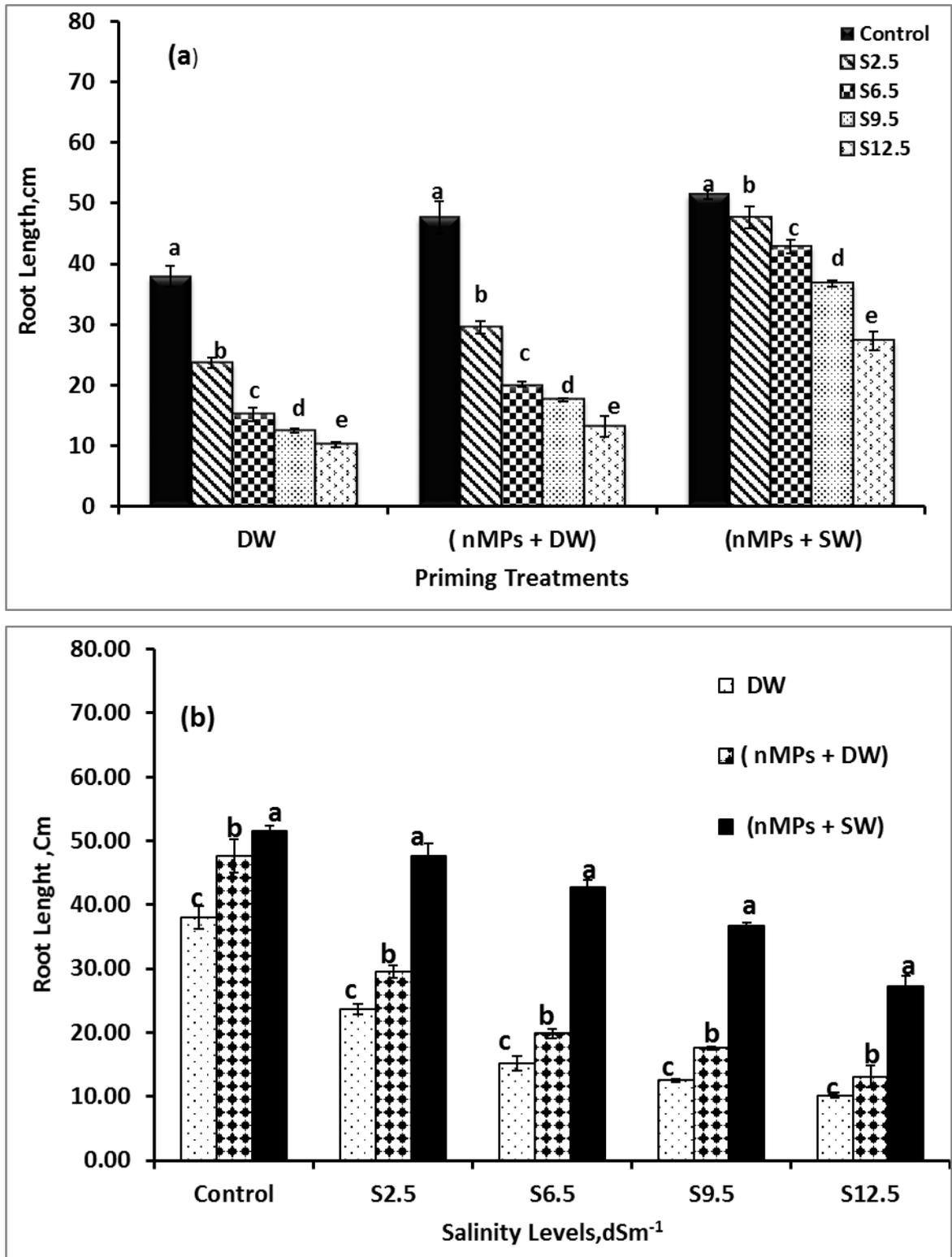


Fig. 4. Effect of seed priming with three different priming solutions on root length (cm) of *zea mays* seedlings under different salinity levels. Letters represent statistically significant differences between the means at (a) the same priming treatment and (b) the same salinity level ($p \leq 0.05$).

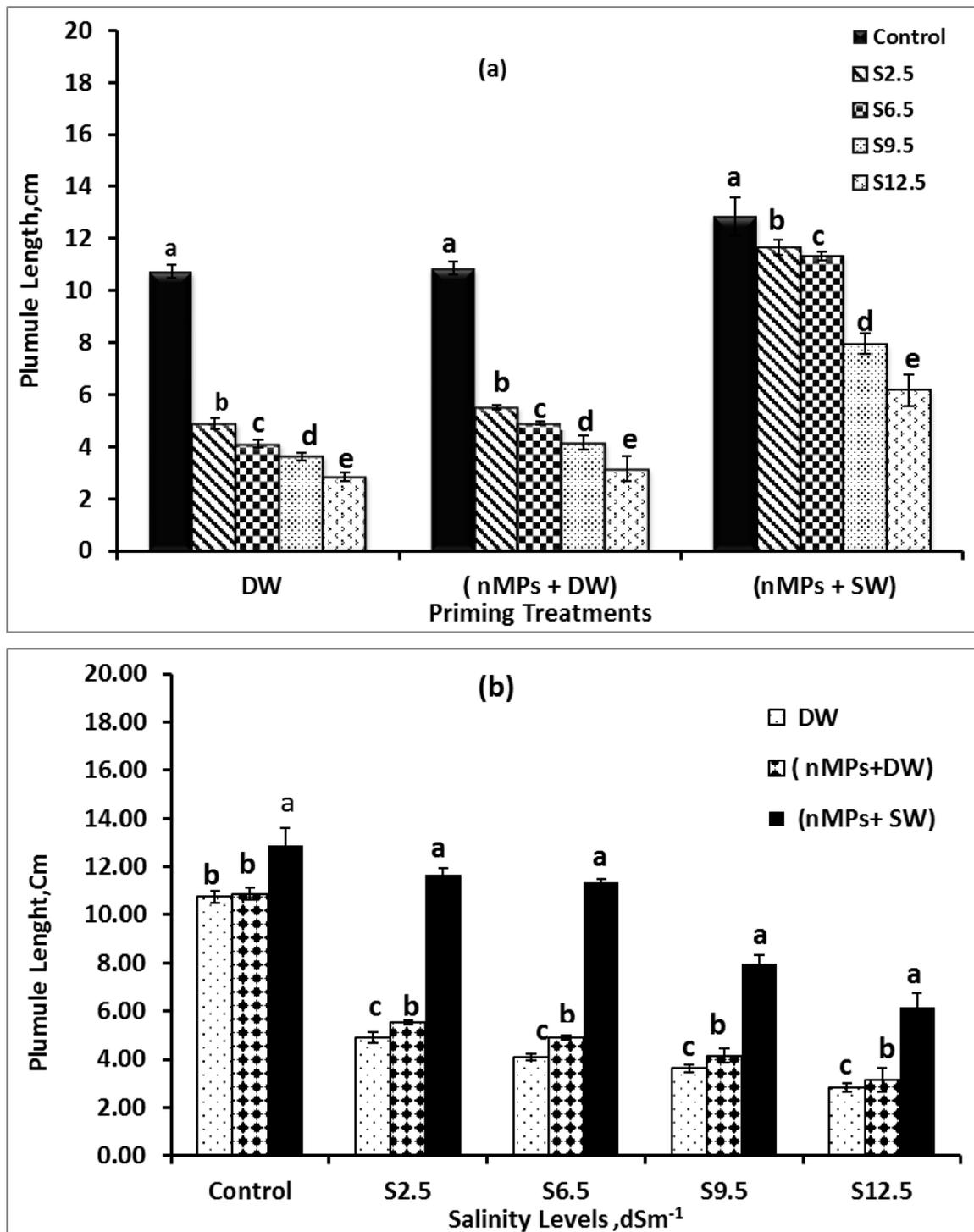


Fig. 5. Effect of seed priming with three different priming solutions on plumule length (cm) of *zea mays* seedlings under salinity levels. Letters represent statistically significant differences between the means at (a) the same priming treatment and (b) the same salinity level ($p \leq 0.05$).

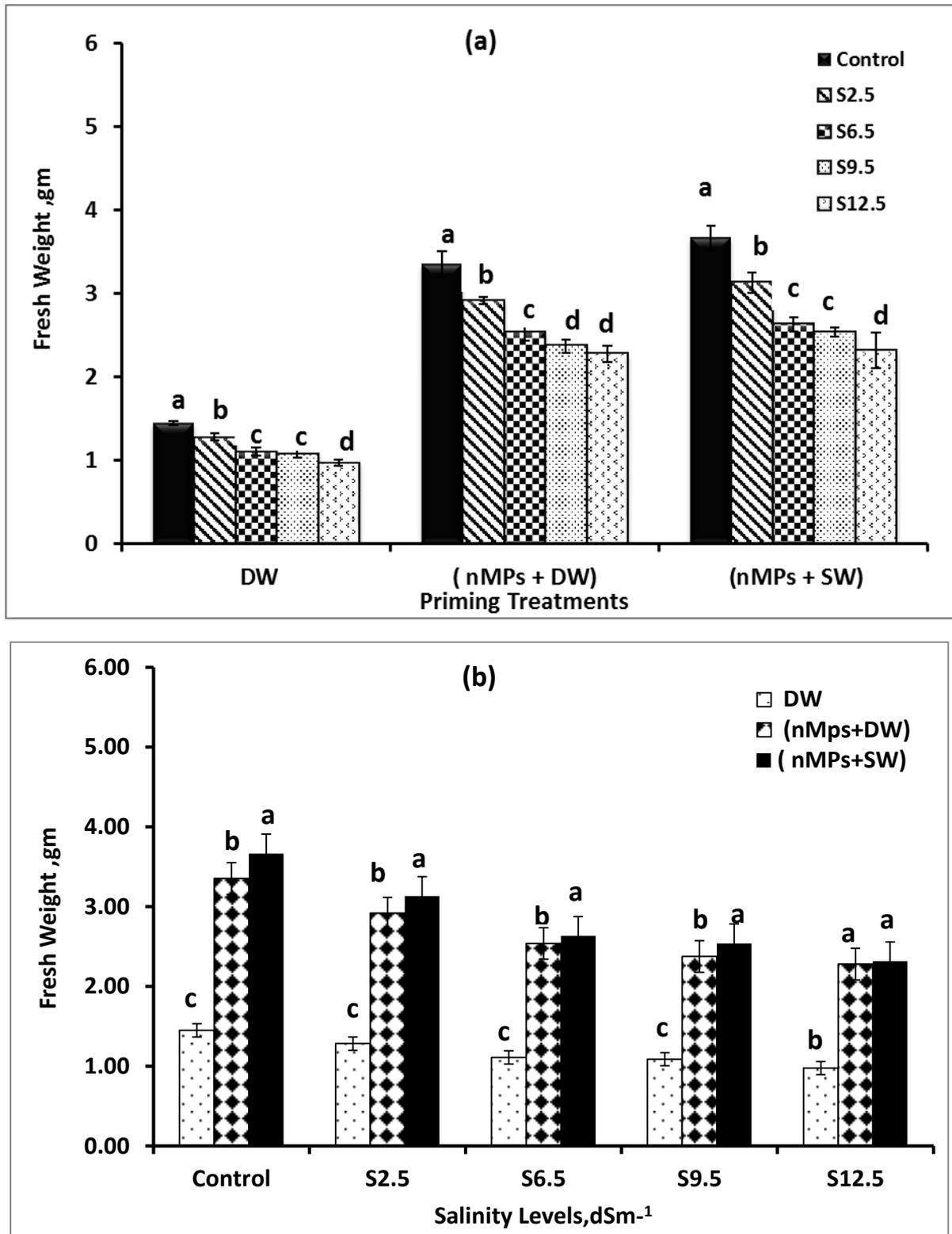


Fig. 6. Effect of seed priming with three different priming solutions on fresh weight (gm) of *zea mays* seedlings under salinity levels. Letters represent statistically significant differences between the means at (a) the same priming treatment and (b) the same salinity level ($p \leq 0.05$).

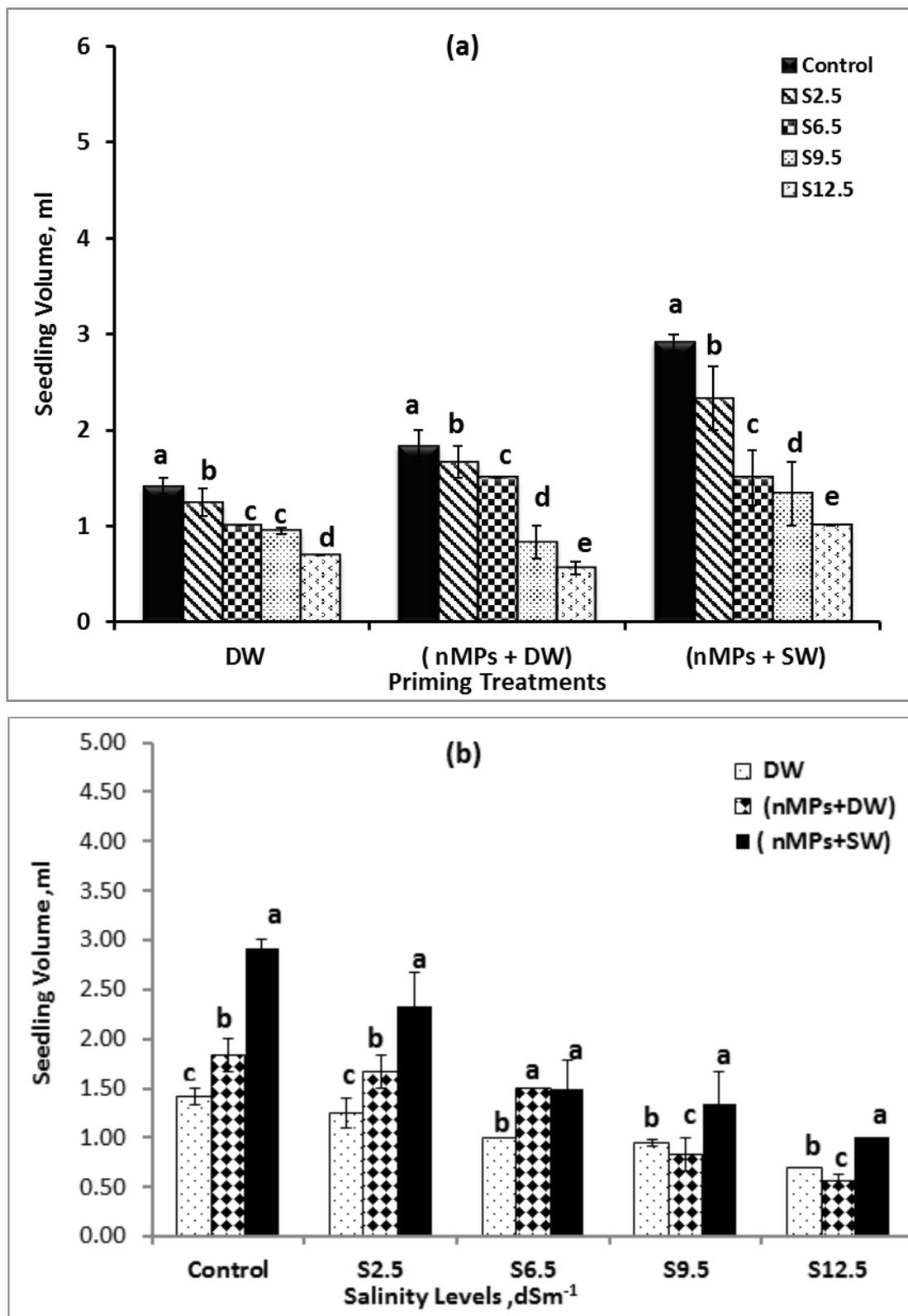


Fig. 7. Effect of seed priming with three different priming Solutions on seedling volume of *zeamays* seedlings under salinity levels. Letters represent statistically significant differences between the means at (a) the same priming treatment and (b) the same salinity level ($p \leq 0.05$).

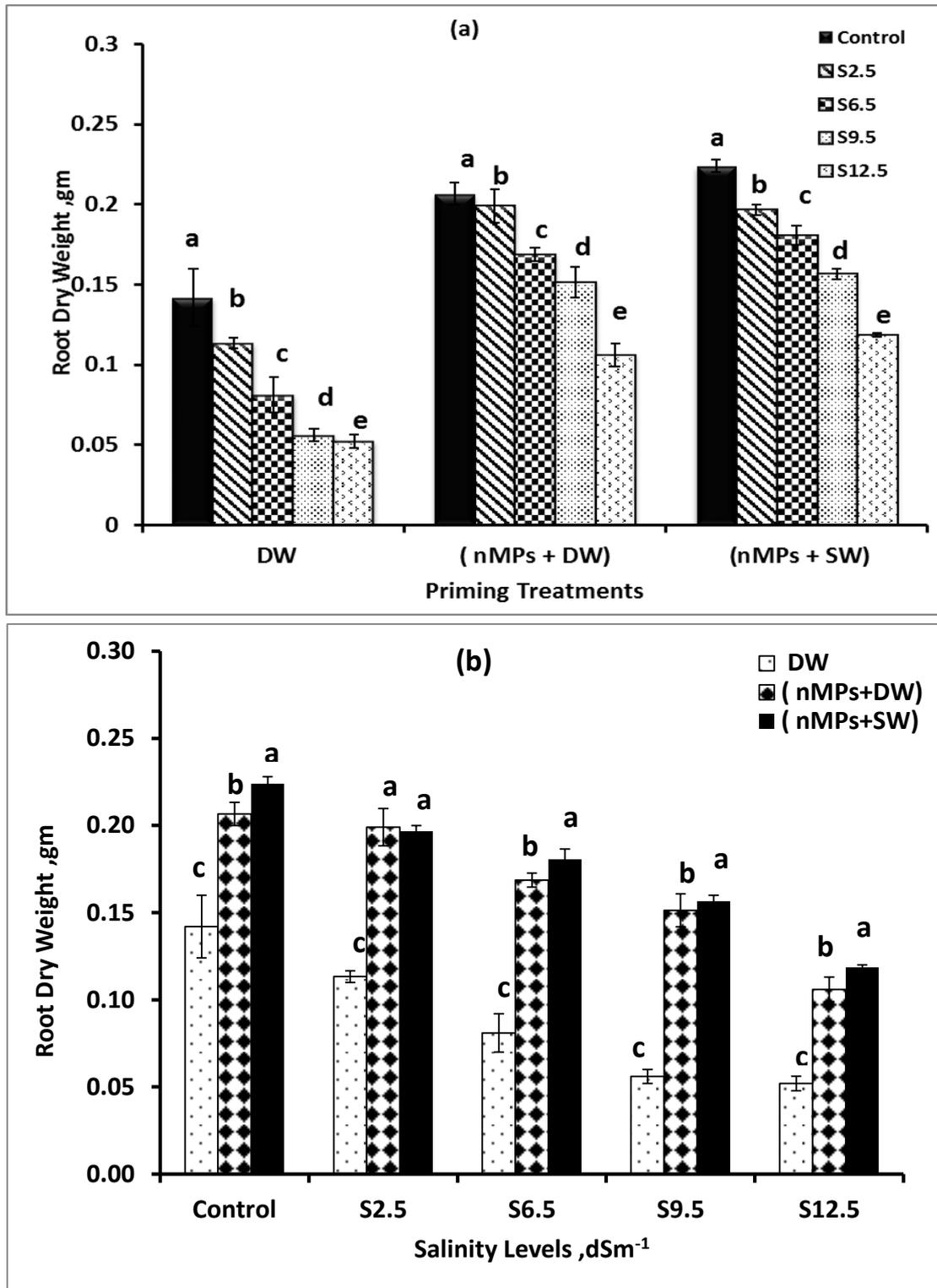


Fig. 8. Effect of seed priming with three different priming solutions on root dry weight of *Zea mays* seedlings under salinity levels. Letters represent statistically significant differences between the means at (a) the same priming treatment and (b) the same salinity level ($p \leq 0.05$).

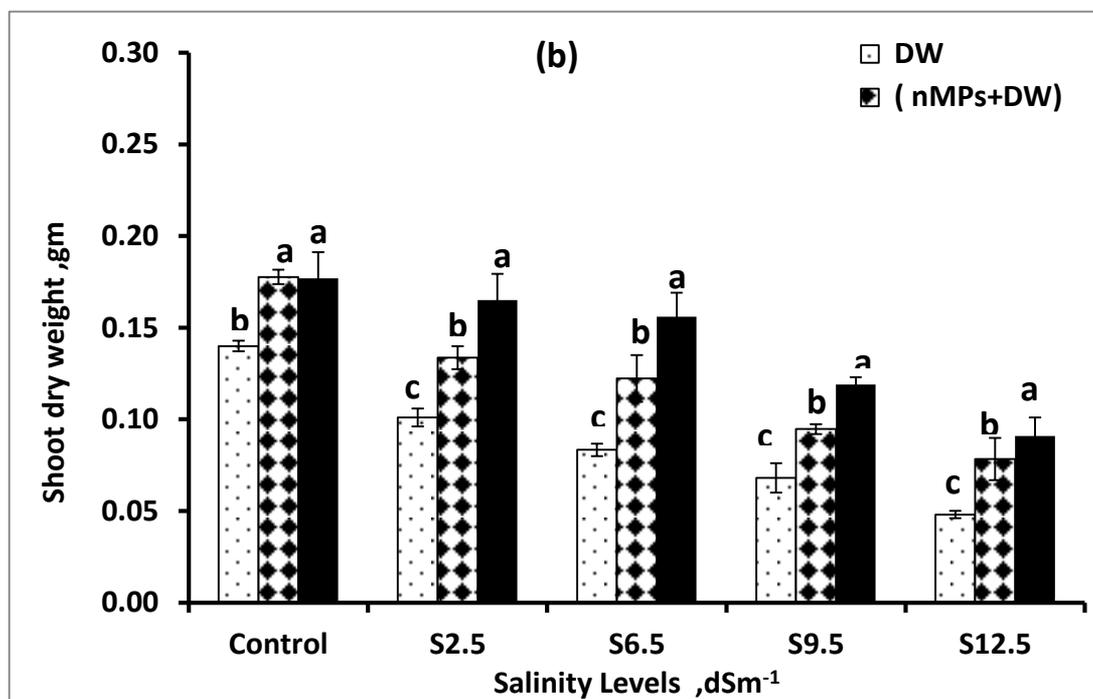
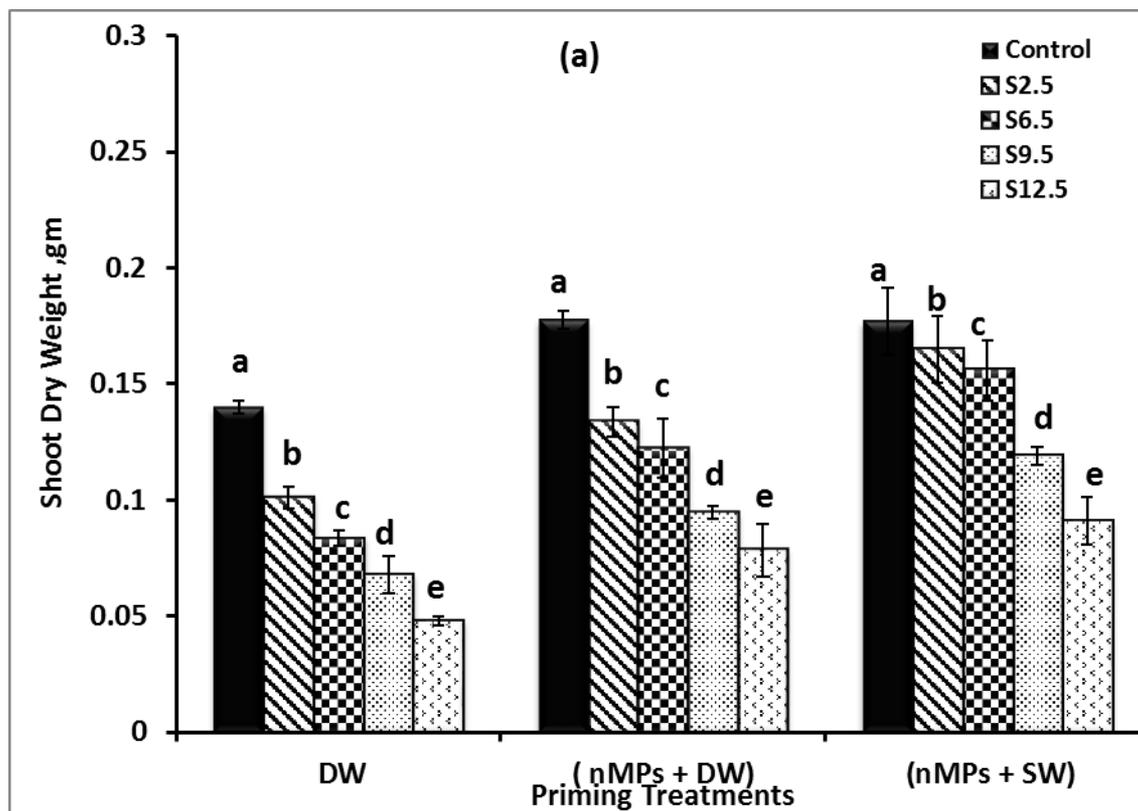


Fig. 9. Effect of seed priming with three different priming solutions on shoot dry weight of *zea mays* seedlings under salt stress. Letters represent statistically significant differences between the means at (a) the same priming treatment and (b) the same salinity level ($p \leq 0.05$).

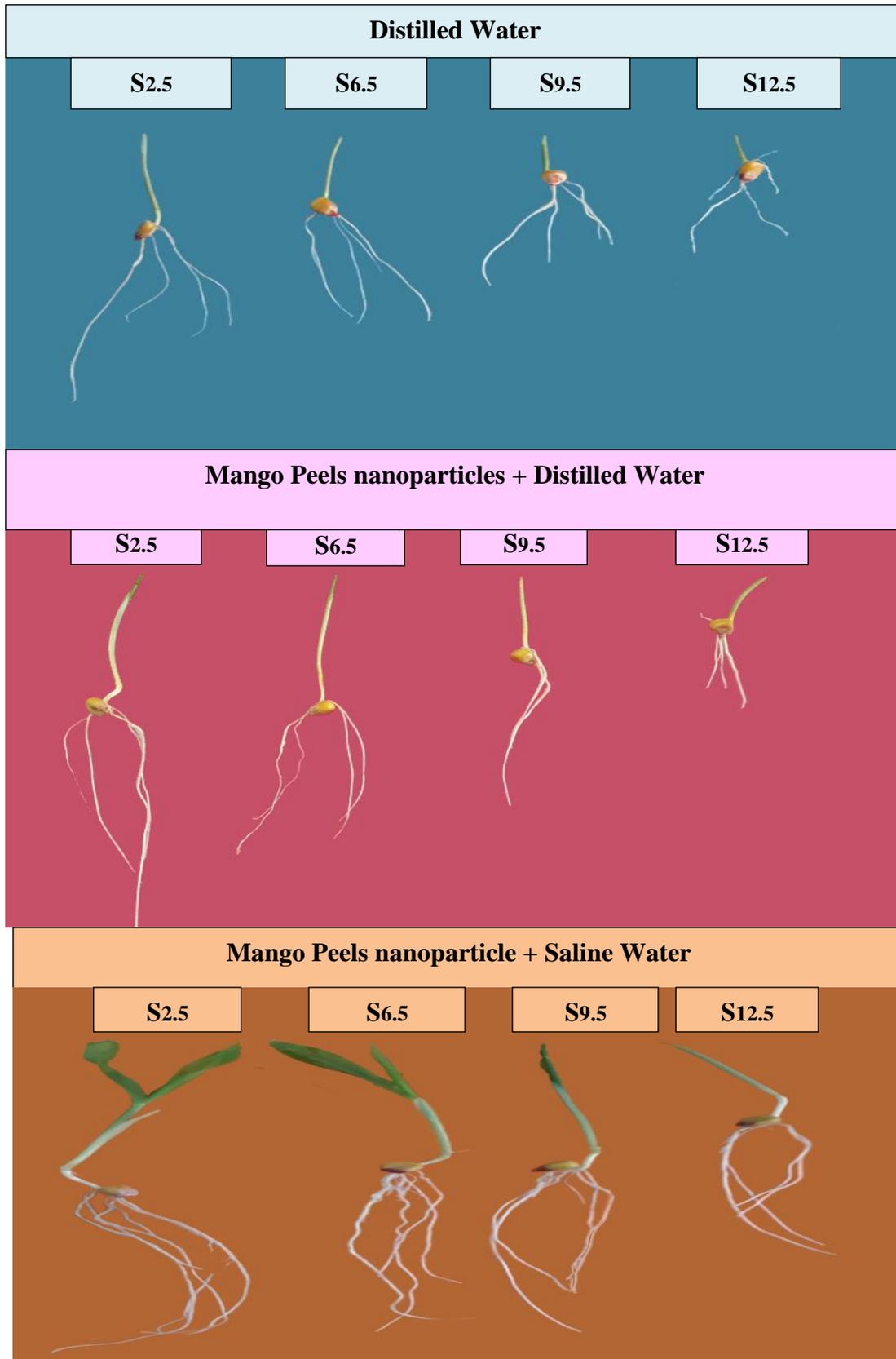


Fig.10. Effect of three priming treatments at different Salinity levels (2.5, 6.5, 9.5 and 12.5 dSm⁻¹) .

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