SEED PRIMING IMPROVES GERMINATION AND ENHANCES THE INITIAL GROWTH OF PEPPER SEEDLINGS UNDER SALINE CONDITION

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ABSTRACT

Three equal samples of graded seeds of a commercial pepper variety were used in the experiment. The first was used as control (ctr), and the next were subject of priming with respectively KNO_3 (1%), $CaCl_2$ (1%) and PEG osmotic solution (-1.5 bar). Primed variants were incubated for 48 hours at a germination room (24 °C, 80% RH), then removed from the respective solutions, dried and kept for next 48 hours at same conditions (24 °C, 80% RH). The control variant was also kept under the same conditions for the last 48 hours. Following that procedure, all seeds were sown in polysterol trays, filled with peat moss compost (100 cm^3 per each module) and transferred in growth chambers with equal microclimate regimes; 24°C, 80% RH and artificial lightening (12 hrs, PPFD 180µmol $m^{-2}s^{-1}$). The total number of seeds for each variant was split in half, and regularly irrigated with normal tap water, or saline (100mM NaCl) water. KNO_3 and $CaCl_2$ priming has significantly improved germination parameters of pepper seedlings under saline irrigation water conditions. In addition, it increased the dry matter, length, surface area and volume of the root system compared with control plants, and enhanced the initial growth and vigor of pepper seedlings.

Keywords: seed priming, KNO_3, $CaCl_2$, PEG, root length, root surface area, root volume, RGR

1. INTRODUCTION

High salinity in soil/substrate or irrigation water is one of most serious abiotic stressors in agriculture. It affects almost every aspect of the physiology and biochemistry of plants (Evelin *et al*., 2009), and significantly reduces growth rate (Balliu *et al*., 2012; 2015), nutrient's uptake (Meça *et al*., 2016; Sallaku *et al*., 2019), and the yield of cultivated plants (Balliu *et al*., 2008b; Veselaj *et al*., 2018a). The direct effects of salt on plant growth involves reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant, toxicity due to excessive Na^+ and Cl^- ions into

plant cells, and nutrient imbalances (Evelin *et al*., 2009). In addition, a secondary aspect of salinity stress in plants is the stress-induced production of reactive oxygen species (ROS). The enhanced production of ROS during salinity stress led to the progressive oxidative damage and ultimately cell death and growth suppression (Fatemi 2014).

The increasing deleterious effects of salinity over the plant was explained by a two-phase growth response to salinity; at the beginning growth reduction is essentially a water stress or osmotic phase, and the growth reduction is presumably regulated by hormonal signals coming from the roots. Then there is a second phase of growth reduction, which is due to salts accumulation in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuole (Munns 2002). Biochemical and molecular mechanisms have evolved to reduce the negative effects of salinity plants. These mechanisms may act in a concerted manner and constitute the integrated physiological response to soil salinity. The most important plant strategies are synthesis and accumulation of compatible solutes, control of ion uptake by roots, compartmentation, and transport into plant tissues, fine regulation of water uptake and distribution to plant tissues by the action of aquaporins, and reduction of oxidative damage through improved antioxidant capacity (Ruiz-Lozano *et al*., 2012).

However, the power of these internal plant' mechanisms is limited. Therefore, several agronomic interventions to alleviate the negative effects of salinity stress are developed. In addition to the development of salt-tolerant cultivars (Krishnamurthy *et al*., 2022), the use of arbuscular mycorrhizal fungi (AMF) (Babaj *et al*., 2014; Meça *et al*., 2016) or combined applications of AMF and plant growth promoting bacteria (Veselaj *et al*., 2018a; 2018b) have been reported to improve crop plants' tolerance under salinity stress, whereas grafting (Balliu *et al*., 2008b; Sallaku *et al*., 2019) and the use of higher nitrogen doses (Balliu *et al*., 2007; Balliu et al., 2008a) were also proved to be effective measures to enhance plant growth under saline conditions.

Recently, seed priming is getting an increased attention of both scientist and commercial producers as a tool to enhance plant resistance to abiotic stresses. Priming is based on seed imbibition which allows seeds to go through the first reversible stage of germination and then dehydrated again and stored until final sowing. During subsequent germination, primed seeds exhibit a faster and more synchronized germination and young seedlings are often more vigorous and resistant to abiotic stresses than seedlings obtained from unprimed seeds (Lutts *et al*., 2016). Seed priming improves germination because same major metabolic events which includes repair of DNA, enhanced synthesis of RNA and protein and increases in the respiration activity of seeds take place before radicle emergence (Ruttanaruangboworn *et al*., 2017). Mostly, the benefits of priming are linked with the enhanced

capabilities of plant seeds (Elkoca *et al*., 2007; Pill and Kilian 2018). However, recent studies show that seed priming could also alter the root morphology (Sallaku *et al*., 2021) and improve plant growth under adverse environmental conditions (Sallaku *et al*., 2020).

Despite numerous recently published data, still there are a lot of non-elaborated issues regarding the specific effects of different priming methods, or different priming compounds on specific crops under adverse environment conditions. The effect of different priming compounds on initial growth of just emerged plants remains an open question. Therefore, the present investigation aims to estimate the effects of three different priming compounds; calcium chlorate ($CaCl_2$), potassium nitrate (KNO_3) and polyethylene glycol (PEG) on seed germination, root morphology and post-germination growth of pepper plants under high sodium (Na) content in the irrigation water.

2. MATERIALS AND METHODS

The experiment was carried out in the spring of 2021 under growth-controlled conditions, in the experimental premises of Agricultural University of Tirana, Albania. Graded seeds of a commercial pepper variety (*Capsicum annum* L. cv. Belorozec F1, SEMO a.s., Smirzice, Czech Republic) were used in the experiment. Three different priming compounds, $CaCl_2$ (10 gr L^{-1}), KNO_3 (10 gr L^{-1}), and PEG (10 gr L^{-1}) were tested alongside with a control (ctr) variant.

The seeds were incubated for 48 hours in the respective priming solutions, in dark conditions, at a germination room (24 °C, 80% RH) for priming purposes. The ratio of seed weight to solution volume (w/v) was 1:5. After 48 h, the primed seeds were washed with distilled water for 2 min, surface-dried using blotting paper, and transferred again for next 48 hours at the same conditions (24 °C, 80% RH). The control variant was also kept at the same conditions for the last 48 hours. Following that procedure, all seeds were sown in polysterol trays, filled with peat (100 cm^3 per each module). Immediately after sowing the substrate was brought to full water capacity thorough the irrigation with tap water. After that, all (control included) variants were split into two equal parts and uniformly irrigated with tap water (one part) and saline (100 mM) tap water (the next part). The plants were kept in two different growth chambers with identical environment conditions; air temperature 24°C, relative humidity 80% and artificial lightening for 12 hrs., at a PPFD 180µmol $m^{-2}s^{-1}$.

Seed germination was closely monitored, and germinated seeds were daily counted, until no new seeds were germinated (day 12 after sowing). Based on counted germinated seeds final germination (FG, %), germination speed (GS, seeds day^{-1}), mean germination time (MGT, day) and the synchronization

index (SI / E, unitless) were determined according to (Ranal0 and De Santana 200) and (Damalas *et al*., 201).

At DAS (day after sowing) 17 and 25, ten plants of each variant were randomly selected and harvested. The root system was gently washed free of adhering peat particles, and scanned with an Epson Expression/STD 4800 Scanner. Subsequently, the acquired root images were analysed with the WinRHIZO Arabidopsis software (Regent Instruments Inc., Quebec, Canada), and the growth parameters of root system; root length (RL, cm), root surface area (RSA, cm^2), average root diameter (AvgD, mm) and root volume (RV, cm^3) were measured and recorded. The plant organs were subsequently dried (65°C, 48 h) and weighted separately to an accuracy of 0.001 g (TP 303; Denver Instruments GmbH, Göttingen, Germany). Based on the determined dry matter, the relative growth rate of roots (RGR_{root}) and the entire plant (RGR_{plant}) between DAS 17 and 25 were calculated for each treatment, according to methods described in (Hoffmann and Poorter 2002; Hunt 2003).

A randomized block design with a total of 8 variants (4 priming methods x 2 levels of salinity) was established. Four replications, thirty seeds for each one, were used for each variant. Residuals of all variables were tested for homogeneity of variances and normality using the tests after Brown-Forsythe and Shapiro-Wilk, respectively. Differences regarding germination (FG, GS, MGT, SI), root morphology parameters (RL, RSA, AvgD, RV) and plant growth parameters (DM, RGR), were tested by two-way ANOVA, using the PC program SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA). Each significant ANOVA result ($p< 0.05$) was followed by a Tukey-Kramer tests at $p<0.05$ as post-hoc test. Values given throughout the text are means ± SE.

3. RESULTS

Priming the pepper seeds with the tested osmo regulatory compounds ($CaCl_2$, KNO_3, PEG) was proved to have a significant impact on their germination parameters. Indeed, final germination (FG), germination speed (GS), mean germination time (MGT) and germination uniformity (SI / E) were strongly influenced by the priming agent and the salinity of the irrigation water. Under normal germination conditions, the control variant shows similar values of FG (nearly 80%) with $CaCl_2$, KNO_3 and PEG primed seeds (Figure 1). No difference was found between control variant and $CaCl_2$ and KNO_3 primed seeds regarding GS. As an exception, different from other priming compounds, PEG primed seeds have shown significantly smaller GS values compared with the control variant (Fig 1). The non-germinated seeds have demonstrated smaller values of MGT and E (SI) than the primed variants (Figure 1). Among the primed seeds, PEG recorded the highest values of MGT and E under non-saline germination conditions (Fig. 1).

Obviously, seed priming was more helpful under saline germination conditions. In this case, the final germination (FG) of non-primed seeds was significantly lower (37.5%) than $CaCl_2$, KNO_3 and PEG primed seeds (Fig. 1). The highest rate of germination under saline conditions was achieved by KNO_3 priming (nearly 78%), followed by $CaCl_2$ (62%) and PEG (55%). The germination speed of control variant was drastically reduced under saline conditions from 43.9 germinated seeds per day to 19.4 germinated seeds per day. Similarly, GS was significantly reduced under saline conditions in $CaCl_2$ and PEG primed seeds, although remain significantly higher than the control variant. Very interestingly, KNO_3 primed seeds maintained their germination speed under saline conditions (Fig. 1), proving to be the best priming compound of pepper seeds under saline conditions. Not many differences were found regarding MGT between non-saline and saline conditions. Except of PEG which significantly reduced mean germination time compared to non-saline conditions, increased salinity in the irrigation water had no significant influence on mean germination time on either non-primed, or $CaCl_2$ and KNO_3 primed seeds (Fig. 1). In general, the presence of high salinity in the irrigation water increases the synchronization index (E) values, which means that the uniformity of germination was deteriorated. Interestingly again, KNO_3 primed seeds were the only variant which slightly improved the germination uniformity (reduced E value) under saline conditions (Fig. 1).

The differences in germination parameters between the non-primed and primed variants were followed by significant differences regarding growth parameters of respective variants. Even immediately after germination (DAS 17) the dry matter weight (DM_{plant}) of non-primed variant was significantly smaller than the dry matter of $CaCl_2$, KNO_3 and PEG primed variants. However, so far, the differences in total plant dry matter under non-saline conditions were only due to differences in the dry matter weight of the aboveground part of the plants (DM_{shoot}). All primed variants have significantly higher DM_{shoot} than the non-primed seeds. No difference was found among non-primed and primed variants regarding DM_{root} (Table 1).

The dry matter of all variants was significantly reduced under saline conditions, but the primed variants maintain their advantage over the non-primed variant (Table 1). Both, DM_{root} and DM_{shoot} were reduced, but a significantly higher DM_{root} than the control was recorded in $CaCl_2$ primed seeds, and a higher DM_{shoot} than the control was recorded for both, $CaCl_2$ and KNO_3 variants (Table 1). Despite different priming compounds and salinity level, no difference was found regarding the ratio of root dry matter weight to the whole plant dry matter weight (Table 1).

The effects of priming compounds and salinity were even more distinguished 25 day after sowing (DAS 25), especially under saline conditions. The dry matter weight of plants DM_{plant} in $CaCl_2$ and KNO_3

primed variants was significantly higher than the non-primed variant (Table 1). Different from them, PEG primed variant did not show any advantage over the control plant regarding DM_{plant}. Generally speaking, the same trend was also seen regarding DM_{root} and DM_{shoot}. Similar to DAS 17, neither priming compound nor the salinity level in the irrigation water had influenced the ratio of root dry matter weight to the whole plant dry matter weight (Table 1).

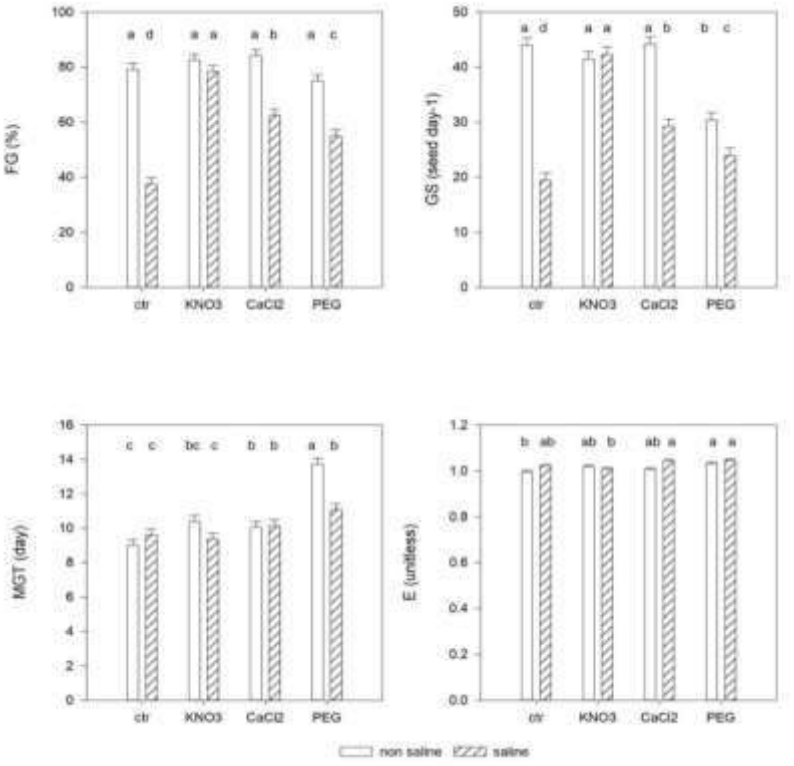

Fig. 1: Final germination (FG, %), germination speed (GS, seed day^{-1}), mean daily germination (MDG, day) and germination uniformity (SI, unitless) under two different levels of irrigation water salinity (0 and 40 mM). Mean values over non primed (ctr) and primed ($CaCl_2$, KNO3, PEG) variants. Different letters indicate significant differences within following parameters (Holm-Sidak method, $p < 0.05$; mean±SE).

There were no differences among non-primed and $CaCl_2$ and KNO_3 primed variants regarding the growth intensity of root system (RGR_{root}) and of the entire plant (RGR_{plant}) under normal growing conditions. On the contrary, PEG primed variant has shown significantly lower relative growth rates of roots and the entire plant (Fig. 2). The raise of salinity in the irrigation water from 0 to 100 mM, was generally reflected to a significant reduction on RGR_{root} and RGR_{plant}, in the non-primed and $CaCl_2$ and KNO_3 primed variants, but not in

PEG primed variant. Although with the lowest RGR_{root} values, PEG was able to maintain the relative growth rate of plants roots stable, depend less of salinity level in the irrigation water (Fig. 2).

The raise of salinity has also drastically reduced the relative growth rate of plant (RGR_{plant}) in the non-primed and $CaCl_2$ primed variants, but not in KNO_3 and PEG variants (Figure 2). However, there was a significant difference between these two variants. KNO_3 priming was able to maintain RGR_{plant} under salinity condition at the level of non-saline environment. Hence, RGR_{plant} of KNO_3 primed plants was significantly higher compared to all other variants. As for PEG primed variant, although it maintained the RGR_{plant} similar with non-saline conditions, still was not able to make any difference with the control plants (Fig. 2).

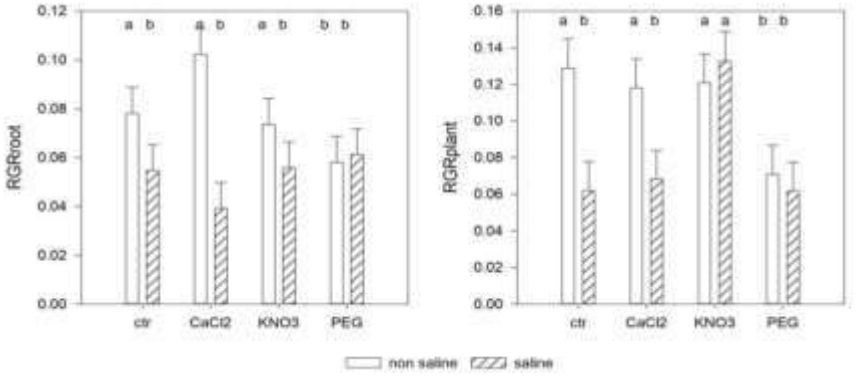

Fig. 2: The relative growth rate of root system (RGR_{root}, g $g^{-1}day^{-1}$) and the relative growth rate of entire plant (RGR_{plant}, g $g^{-1}day^{-1}$) under two different levels of irrigation water salinity (0 and 100 mM). Mean values over non primed (ctr) and primed ($CaCl_2$, KNO_3, PEG) variants. Different letters indicate significant differences within following parameters (Holm-Sidak method, $p < 0.05$; mean±SE).

Significant differences due to priming compound and salinity level were also found regarding the morphology of root system. By DAS 17, $CaCl_2$ and KNO_3 primed variants recorded significantly higher total length of roots (RL) than the non-primed variant, either under normal or saline conditions (Table 2). No differences were found between PEG primed and control plants. There was no difference between $CaCl_2$ and PEG primed variants with the non-primed plants regarding root surface area (RSA) and root volume (RV). On the contrary, KNO_3 primed variant significantly overpassed the control and $CaCl_2$ and PEG primed variants regarding RSA and RV (Table 2). The non-primed plants were however characterized by a significantly larger root diameter (AvgD), either under non-saline or saline conditions, compared to all primed variants (Table 2).

The situation remains largely the same by DAS 25. Although there were no significant differences under non-saline conditions, KNO_3 and $CaCl_2$ primed seeds demonstrated significantly longer roots, a significantly larger root surface area and a significantly higher root volume than the control and PEG primed seeds under saline conditions. No differences were found regarding RL, RSA and RV between control and PEG primed plants (Table 2).

4. DISCUSSION

Although all stages of plant growth are affected by salt stress, the seed germination stage is the most sensitive (Cuartero *et al*., 2006). Indeed, many studies have already proven that salt stress can significantly reduce seed vigour and inhibit germination in many species (Hussain 2013; Oliveira *et al*., 2019; Mohammed and Nulit 2020). Similarly, our results shown a significant reduction in final germination (FG) and germination speed (GS), whereas the mean daily germination (MGT) was extended and the uniformity of germination deteriorated under 100 mM NaCl in the irrigation water. The inhibition of seed germination under high salinity is explained with the high concentrations of sodium and chloride ions which reduce the osmotic potential of the surrounding environment and suppress seed imbibition and embryo growth (Chen *et al*., 2021). In addition, the high concentrations of sodium and chloride ions negatively effects the energy utilization and metabolism during seed germination (Chen *et al*., 2021).

Table 1. Dry matter weight of roots (DM_{root}, g), shoots (DM_{shoot}, g), plant (DM_{plant}, g) and the ratio of root dry weight to plant dry weight (R:P ratio) under two different levels of water salinity (0 and 100 mM), 17 days after sowing (DAS 17) and 25 days after sowing (DAS 25). Mean values, different letters indicate significant differences within following parameters (Holm-Sidak method, $p < 0.05$; mean±SE).

Priming agent	Salinity	DAS 17				DAS 25			
		DM_{root}	DM_{shoot}	DM_{plant}	R:P ratio	DM_{root}	DM_{shoot}	DM_{plant}	R:P ratio
Control	0	0.0018±0.00a	0.005±0.001b	0.007±0.002b	0.31±0.12	0.0036±0.000a	0.016±0.002a	0.020±0.002a	0.17±0.01
$CaCl_2$	0	0.0016±0.00a	0.010±0.00a	0.012±0.00a	0.22±0.06	0.0041±0.000a	0.024±0.003a	0.028±0.003a	0.14±0.01
KNO3	0	0.0020±0.00a	0.009±0.00a	0.011±0.00a	0.21±0.03	0.0031±0.000a	0.019±0.003a	0.022±0.003a	0.14±0.02
PEG	0	0.0016±0.00a	0.010±0.00a	0.011±0.00a	0.18±0.04	0.0020±0.000b	0.014±0.002a	0.016±0.002a	0.12±0.02
Control	50	0.0012±0.00ab	0.005±0.00b	0.006±0.00b	0.26±0.03	0.0022±0.000b	0.008±0.001b	0.011±0.001b	0.20±0.02
$CaCl_2$	50	0.0018±0.00a	0.009±0.00a	0.011±0.00a	0.21±0.04	0.0020±0.000b	0.015±0.001a	0.017±0.001a	0.11±0.02
KNO3	50	0.0008±0.00b	0.008±0.00a	0.009±0.00ab	0.12±0.08	0.0031±0.000a	0.020±0.003a	0.023±0.003a	0.14±0.02
PEG	50	0.0010±0.00b	0.004±0.00b	0.005±0.00b	0.23±0.08	0.0011±0.000c	0.006±0.001b	0.007±0.001b	0.22±0.02
Significance									
Priming method (Pm)		0.610	0.023	0.027	0.082	0.043	0.002	0.004	0.059

Salinity (S)	0.022	0.070	0.028	0.346	0.014	0.005	0.005	0.125
Pm x S interaction	0.257	0.339	0.356	0.482	0.353	0.306	0.312	0.025

Table 2. Total root length (RL, cm), root surface area (RSA, cm^2), average root diameter (AvgD, mm) and root volume (RV, cm^3) under two different levels of water salinity (0 and 100 mM), 17 days after sowing (DAS 17) and 25 days after sowing (DAS 25). Mean values, different letters indicate significant differences within following parameters (Holm-Sidak method, $p < 0.05$; mean±SE).

Priming agent	Salinity	DAS 17				DAS 25			
		RL	RSA	AvgD	RV	RL	RSA	AvgD	RV
Control	0	3.38±0.49c	0.48±0.07b	0.47±0.01a	0.005±0.001b	11.2±1.41b	1.65±0.24a	0.46±0.02a	0.015±0.001a
$CaCl_2$	0	6.81±0.44b	0.72±0.04b	0.34±0.03b	0.006±0.01b	16.2±2.17a	1.97±0.25a	0.39±0.01ab	0.019±0.002a
KNO_3	0	10.8±0.150a	1.28±0.19a	0.37±0.11b	0.012±0.002a	16.4±1.53a	1.73±0.14a	0.33±0.01b	0.015±0.001a
PEG	0	4.30±0.34c	0.51±0.04b	0.38±0.01b	0.005±0.000b	13.3±0.98b	1.53±0.11a	0.36±0.01b	0.014±0.001a
Control	50	4.27±0.19c	0.63±0.01b	0.48±0.03a	0.007±0.001b	3.85±0.29c	0.54±0.04c	0.38±0.04ab	0.006±0.000c
$CaCl_2$	50	6.06±0.53b	0.68±0.04b	0.36±0.02b	0.006±0.000b	5.87±0.60bc	0.83±0.07bc	0.47±0.07a	0.010±0.002b
KNO_3	50	7.98±0.20b	0.98±0.05a	0.39±0.02b	0.009±0.001ab	9.61±0.93b	1.18±0.14b	0.43±0.14a	0.011±0.002b
PEG	50	5.12±0.84c	0.67±0.11b	0.41±0.01ab	0.007±0.001b	4.63±0.53c	0.55±0.06c	0.41±0.06ab	0.005±0.001c
Significance									
Priming method (Pm)		<0.001	<0.001	<0.001	<0.001	<0.001	0.017	0.154	0.005
Salinity (S)		0.351	0.819	0.251	0.461	<0.001	<0.001	0.058	<0.001
Pm x S interaction		0.036	0.033	0.954	0.097	0.482	0.196	0.006	0.105

The germination of pepper seeds under saline conditions can be improved by priming. According to our results, KNO_3 priming provided the best results. The KNO_3 primed seeds-maintained FG, GS and MGT at the level of non-saline conditions. The uniformity of germination (SI) was slightly deteriorated, but still was better than the rest of variants. Similar results were previously reported in tomato (Ali *et al*., 2020) and cucumber (Oliveira and Steiner 2017). K^+ represents the most abundant inorganic ion in plants which accumulates at cytoplasmic concentrations of ~100 mM (Manishankar *et al*., 2018). When challenged with salt stress, plants attempt to maintain a high K^+ to Na^+ ratio in the cytosol. They do this by regulating the expression and activity of K^+ and Na^+ transporters and H^+ pumps that generate the driving force for transport (Manishankar *et al*., 2018). It is already proved that externally supplied K may also increase endogenous K^+ content of seeds, suggesting this is a possible mechanism of K primed seeds to tolerate salinity at germination, and ameliorate abiotic-stress effects (Umar *et al*., 2011). In addition to that, The beneficial effect of the KNO_3 application on seed germination are explained with the synthesis of substances that release nitric oxide (NO) (Oliveira and Steiner 2017). These substances act in membrane

permeability, preventing or reversing the damage caused by environmental stresses (Oliveira *et al*., 2020).

$CaCl_2$ provided second best results regarding germination parameters under saline condition. FG and GS in $CaCl_2$ primed seeds were significantly higher than non-primed and PEG primed seeds. Previous publication has also identified $CaCl_2$ as one of the most promising priming agents for sorghum (Chen *et al*., 2021) and rice (Afzal *et al*., 2012). Calcium ions play an important role in the regulation of plant metabolism, and free calcium ions inhibit the influx of extracellular sodium ions and maintain the intracellular potassium and sodium ion balance reducing sodium ion toxicity and improving salt tolerance during germination (Chen *et al*., 2021). Early studies have already revealed strong indications that Ca^{2+} signaling is an early and prominent feature of the plant responses to environmental salinity and that the plants exhibit a rapid increase in cytosolic calcium concentration ($[Ca^{2+}]cyt$) within seconds of being exposed to NaCl (Manishankar *et al*., 2018). Seeds primed with $CaCl_2$ have the advantage in maintaining germination under saline conditions perhaps due to the facilitation of a higher K^+/Na^+ selectivity, enhanced antioxidant proteins, and enhanced oxygen uptake and mobilization of nutrients from cotyledon to embryonic axis (Afzal *et al*., 2012).

The raise of salinity in the irrigation water has also significantly reduced DM_{plant}, which was a consequence of the reduction in both dry matter weight of roots (DM_{root}) and dry matter weight of shoots (DM_{shoot}). The reductions in DM_{root} and DM_{plant} were consequences of significant reductions in the respective relative growth rates of roots (RGR_{root}) and the entire plant (RGR_{plant}). Whereas seedlings invariably develop roots first, their relative investment into leaves versus roots as carbon-and nutrient-acquiring organs, respectively, can also change over time (Mcconnaughay and Coleman, 1999). Clearly, as plants grew on, shoots were gaining more weight versus the root systems, but different from what was reported on priming effect on cold stress alleviation (Sallaku *et al*., 2021) we found no significant effect of priming effects on the ratio of DM_{root} to DM_{plant}, either at non-saline or saline conditions. Similar with dry matter weight, root length (RL), root surface area (RSA) and root volume (RV), but not average root diameter (AvgD) was significantly reduced by raised salinity.

Priming with KNO_3, $CaCl_2$ and PEG did not provide any advantage over the non-primed seeds regarding DM_{root}, DM_{shoot} and DM_{plant} under non-saline condition. However, the effects were highly significant regarding most of root parameters. KNO_3 and $CaCl_2$ priming, but not PEG priming, provided higher RL, RSA and RV values. The only parameter that the non-primed seeds had an advantage was the average root diameter. On the contrary, priming effects were distinguished and statistically significant under saline conditions. It was KNO_3 and then $CaCl_2$ which maintained DM_{plant} under saline condition at the

level of non-saline conditions. Similar results of $CaCl_2$ priming were reported in rice (Afzal *et al*., 2012), but on the contrary priming of seeds with KNO_3 had an insignificant effect on the initial growth and vigor of cucumber seedling (Oliveira *et al*., 2020). We found that both, DM_{root} and RGR_{root} were significantly reduced under saline condition. As such, this is another indication of the fact that the high salinity is primarily affecting the root system, in direct contact with raised salinity, rather than above ground plant organs.

Obviously, apart from improved germination, the overall growth of plants was enhanced due to the seed-priming treatments. Although, the physiological reasons behind the improved germination and seedling establishment after seed priming are rather unclear (Zhang *et al*., 2015), several hypotheses are proposed. The drop in H_2O_2 accumulation, restoration of catalase activity, increased protein content in various plant tissues via improved performance of protein synthesis system and increased production of specific enzymes that plays an important role in repairing plant tissue proteins, and enhanced activity of protease and amylase that hydrolyze protein and starch into simple forms to make them available for the embryo are some of them (Waqas *et al*., 2019). More specifically, the higher activities of amylase, invertase, sucrose synthase and sucrose phosphate synthase in shoots of primed seedlings in comparison to non-primed seedlings suggests a rapid hydrolysis of transitory starch formed in the shoots of primed seedlings leading to more availability of glucose for seedling growth (Kaur et al., 2002; Zhang et al., 2015).

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