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Plant physiological stimulation by seeds salt priming in maize (*Zea mays*): Prospect for salt tolerance

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Nowadays to lessen the influence of salinity on crop performances such as germination, seedling growth and yield, several actions are undertaken; for example the use of different priming methods, antioxidants and plant growth controllers. Seed priming, best fits in this study on maize crop and is controlled by hydration process followed by re-drying that allows pre-germination which enhanced metabolic activities to proceed rapidly. The objective of this study was to investigate how seed salt priming increases maize crop performance thereby enhancing yields by stimulating the plant physiological processes. Here, sodium chloride and calcium chloride primed seeds germinated earlier than unprimed one. Primed maize seeds had better efficiency for water absorption. Likewise, energetic metabolic activities in germination process commence much earlier than radicle and plumule appearance. Priming of seeds with salt solution enables them to break their dormancy and escape from disease causing agents and competent seeds of weeds. Seed priming with NaCl and $CaCl_2$ had significant effects on germination, early growth, number branches, number of cobs and grain yield. This increase in growth traits likely helps to reduce the competition for water and nutrients with associated improvements in seed yield. Besides, it makes seed priming practice a viable option for the successful use of maize in irrigation areas. Sodium chloride (NaCl) priming increases shoot length while, calcium chloride ($CaCl_{2)}$ priming increases root length. Seeds grown in vertisol soil prefers seed priming for better stand establishment and crop yield whereas; seeds in lithosol soils prefer priming for better seed germination and increase number cob.

Key words: Seed salt priming, maize, salt tolerance, germination rate, physiology.

INTRODUCTION

Crop launching depends on the effects of seed patch of soil and seed good features (Khajeh-Hosseini et al., 2003). Salinity has been recognized as the major seed patch of soil factor, influencing crop establishment in arid and semiarid grass lands (El-kharbot et al., 2003). Particular salt effect could contribute for significant reductions in the rate and final germination percentage of plants, which in turn may lead to uneven seed emergency and initiation by means of that reduction in crop yields. Speedy, even, and complete germination is a prerequisite

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for successful plant growth, production and stand establishment in grain crops (Demir and Ermis, 2003). In area of short and low rainfall, salt accumulation is common because infiltrating moisture is insufficient to percolate salts added by irrigation. In soils comprising an extra sodium chloride salt, the available water to the plant is limited. This process results in a partial dehydration of the cytoplasm. Consequently, this results in shrinking of the protoplasm of crops from the cell wall resulting to an adverse effect on the metabolism of the cells and the functions of larger molecules which eventually outcomes in the cessation of growth (Le Rudulier, 2005).

Overdue and unpredictable germination and underprivileged preliminary establishment are the major reasons of poor maize crop production under irrigation areas and on saline fields of Ethiopia. Therefore, helping seed germination and stand establishment artificially can benefit to secure good crop yield from salt-affected areas because some findings from primed wheat under saline condition are exemplified as advantageous (Wahid et al., 2010; Jafar et al., 2012). Similarly, as a result of increased salinity problems, the prerequisite to develop crops with higher salt tolerance has increased strongly within the last decade.

To reduce the impact of salinity on crop performance, production and productivity, several treatments are carried out. For example, contrived application of different osmotic protectants (coating and priming), antioxidants, plant growth regulators, and moderate salt treatments are common one (Koyro, 2013). Moderate salt concentration treatment of the promising cash crop, *Panicum turgidum* growing at a low quality soils, enabled the crop to perform better in photosynthetic and growth responses. Its sustainable use can also help in desalinizing and reclaiming degraded land as well as sequestering CO_2 (Koyro, 2013). Furthermore, the addition of organic matter of olive mill waste compost and poultry manure are effective on the availability and uptake of plant nutrients in a highly saline soil (Walker and Bernal, 2008). Likewise, application of compost and sewage sludge increases biological activities of salt affected soils by improved soil physical-chemical properties, especially the carbon and nitrogen balance for the soil microbes (Lakhdar et al., 2010). Similarly, Jasmine rice production in salt affected soils can be improved by the application of gypsum and farmyard manure of the salt affected soils (Cha-um et al., 2011). The addition of supplemental calcium sulphate to nutrient solution containing salt significantly improves growth and physiological variables affected by salt stress (for example plant growth, fruit yield, and membrane permeability) and also increased leaf K^+, Ca^{2+}, and N contents in tomato plants (Tuna et al., 2007). Treatment of seeds with NaCl solution prior to sowing or seed priming induces good stand establishment of crops. Melon seeds primed with 18 dSm^{-1} NaCl solution for 3 days at 20°C prior to sowing increased their seedling growth evidences such that, seed priming prior to sowing improves melon physiological performance. Likewise, seed priming with NaCl induce possible physiological adjustments in pepper seeds and Dekoko seeds, especially in the early stages of development which could be used as a suitable tool for improving germination and growth physiognomies of crops under salt stress conditions (Sivritepe et al., 2003; Aloui et al., 2014; Tsegay and Gebreslassie, 2014) and potassium priming and genetic engineering, which serve as a means to improve plant resistance to salt stress (Cushman and Bohnert, 2000).

All aforementioned treatments enable plants to respond to stress physiology by overproduction of different types of responsive biological solutes (Azevedo Neto et al., 2006). Responsive biological solutes are low molecular weight, highly soluble compounds that are usually nontoxic at high cellular concentrations. Generally, they protect plants from stress through different courses which include contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins. Furthermore, since some of these solutes also protect cellular components from dehydration injury, they are commonly referred to as osmoprotectants (Saadia et al., 2012; Koyro, 2013).

Salinity tolerance of plants can be improved by soaking seeds in solutions of different salts before sowing, as plants from such treated seeds show more adaptation with enhanced physiology to saline conditions than the untreated seeds (Farooq et al., 2010a). Seed priming or osmoconditioning is one of the physiological methods which improves seed performance and provides faster and synchronized germination (Neto and Tabosa, 2000). It is an easy, low cost and low risk technique which is recently used to overcome the salinity problem in agricultural lands. It entails the partial germination of seed by soaking in either water or in a solution of salts for a specified period of time, and then re-drying them just before the radicle emerges (Neto and Tabosa, 2000). Seed priming stimulates many of the metabolic processes (physiological and chemical) involved with the early phases of germination. Moreover, it has been noted that seedlings from primed seeds emerge faster, grow more vigorously, and perform better in adverse saline conditions (Cramer, 2002). Seed priming with $CaCl_2$ and NaCl could be used as an adaptation method to improve salt tolerance of seeds. Studies conducted by Cano et al. (2001) and Cayuela et al. (2006) with tomatoes, Ahmadvand et al. (2012) with soybean, and Elouaer and Hannachi (2012) with safflower evidenced that seed priming improves seed germination, seedling emergence, growth and yield production under saline conditions. Passam and Kakouriotis (2004) also reported benefits of $CaCl_2$ and NaCl; seed priming did not persist beyond the seedling stage in cucumber, while Farooq et al. (2005) found that NaCl seed priming had positive effects on mature plants and yield of tomato.

Since $CaCl_2$ and NaCl seed priming have become an important technique to increase salt tolerance of plants, it is necessary to understand the physiological effects which mediate the responses to salinity. According to Cano et al. (2001), the higher salt tolerance of plants from primed seeds seems to be the result of a higher capacity for osmotic adjustment, since plants from primed seeds have more Na^+ and Cl^- ions in their roots and more sugars and organic acids in leaves than plants from non-primed seeds. External Ca^{2+} has been shown to ameliorate the adverse effects of salinity in plants (Kaya et al., 2006). According to Hasegawa et al. (2000), this amelioration is most probably, facilitating higher K^+/Na^+ selectivity of the plants. Calcium has often been used as a pelleting (seed coating) material. For instance, it has been known that coating rice seed with calcium peroxide increased germination and plant establishment in their various forms. Seed coatings have become an important part of modern agriculture, and some have been shown to improve emergence and seedling growth in agronomic crops like safflower (Elouaer and Hannachi, 2012).

Ethiopia has been reported to possess over 11 million hectares of unproductive naturally salt which affected wasteland (PGRC, 2000). These areas are normally found in the arid and semi-arid lowlands and in rift valley and other areas that are characterized by higher evapotranspiration rates in relation to low precipitation (PGRC, 2000). According to Tamirie (2004), salt-affected flats have increased from 6 to 16% of the total land area of Ethiopia in recent years where 9% of the population lives in these areas. About 44 million ha (36% of the country's total land areas) are potentially susceptible to salinity problems.

Claiming back these salt affected areas and the use for agricultural production is very costly and time consuming. The practice of maize production in Ethiopia ranges from large-scale commercial farms to smallholders and subsistence farmers; and consumed as main food, with its crop residues and by-products commonly fed to livestock. Hence, Priming of maize seeds (one of the most important crop in Ethiopia) with $CaCl_2$ and NaCl could be important in improving the growth performance and yield enhancement of maize in areas that are potentially susceptible to salinity problems and totally unproductive naturally salt affected wastelands. Such study was not done on physiological tolerance of maize seeds, by seed priming in Ethiopian agricultural landscapes and particular in the study sites. Therefore, this study investigated the evaluation on physiological stimulation of priming on improving seed germination, seedling growth and finally yield enhancement of maize under different salinity levels at open field condition.

MATERIALS AND METHODS

Geographical location of the assay

The study was conducted in Sirinka Agricultural Research Center (SARC). Sirinka Agriculture research center is one of the regional research centers under ARARI. It is located in the dryland areas of north eastern Ethiopia (north Wollo Administrative Zone) 508 km from Addis Ababa on the main road to Mekelle and 12 km south eastern of Woldia. SARC is found in Habru district at Sirinka kebele. The center has 2 sub-centers (Kobo at 1450 m.a.s.l and Hayik at 1680 m.a.s.l). With regard to climatic condition, it has an altitude of 1850 m.a.s.l, 11°45'00'' north latitude 39°36'36''east longitude (Figure 1) with major soil type of Eutric vertisol. The average annual rain fall of the study area is 945 mm and average maximum and minimum temperature is 26 and 13°C, respectively (Administrator, 2014).

Seed collection and soil sampling

The improved variety of *Zea mays*, Melkasa-2 multiplied in Sirinka Agricultural Research Center (SARC) which was released from this research center was used for this study. Melkasa 2 seeds were obtained from the certified seed supplier of north Wollo zone found in SARC located in south eastern of Woldia. A total of 800 seeds were sown in 80 green plastic pots each with four replications. Soil samples were taken from the Kobo and SARC for nutrient analysis. This was done purposely for the determination of the amount of the salt treatments containing the respective Na^+ and Ca^{2+} ionic nutrients.

Representative soil samples were collected from the fields to estimate their soil fertility through soil analysis using multiple sampling methods. Samples were collected from soil surface layer down to the depth of 15 to 25 cm which is the standard depth in cultivated fields. The samples were taken from five soil cores both in Kobo and SARC to show variations in fertility from one part of the field to another and the value of mean were taken, after mixing and sieving the soil samples thoroughly.

Seed priming methods

Seed priming is applied preferably in this study. It is a controlled hydration process followed by re-drying that allows pre-germination metabolic activities to proceed rapidly (Farooq et al., 2005a). The addition of $CaCl_2$ and NaCl approaches was applied for this study prior to sowing of the maize seeds. Melkasa 2 seeds were sterilized superficially with sodium hypochloride (Na_2HCl) solution on smooth layered flow for 3 min, followed by thoroughly washing for 5 min with distilled water.

Subsequently, the seeds were primed by soaking 5g/l NaCl and $CaCl_2$ solutions for 12 h, under shade and the ratio of seed weight to solution volume were set at 1:5 (g/ml). After priming, seeds were removed and washed with tap water and then rinsed three times in distilled water. Finally seeds were left in air between two filter papers to re-dry to their original moisture level as a method proposed by Afzal et al. (2009b).

Design of the experiment assay

The experimental design was three factors factorial, arranged in a randomized block design, with four replications. The first factor was salinity types and stresses due to salt solutions (0, 5, 7, 9 and 11 dS/m NaCl and $CaCl_2$), the second factor was the Melkasa-2 seed types (primed and unprimed seeds) and the third factor was soil types of black and red soils. This experiment was conducted in 80 green plastic pots (40 for NaCl treatment and 40 for $CaCl_2$ treatments and each divided into 20 pots per the two soil types arranged randomly in a mixed manner).

Ten seeds were sown in each 50 L contain pot (40 cm height and 35 cm in diameter) at 2 cm depth and replicated four times. The

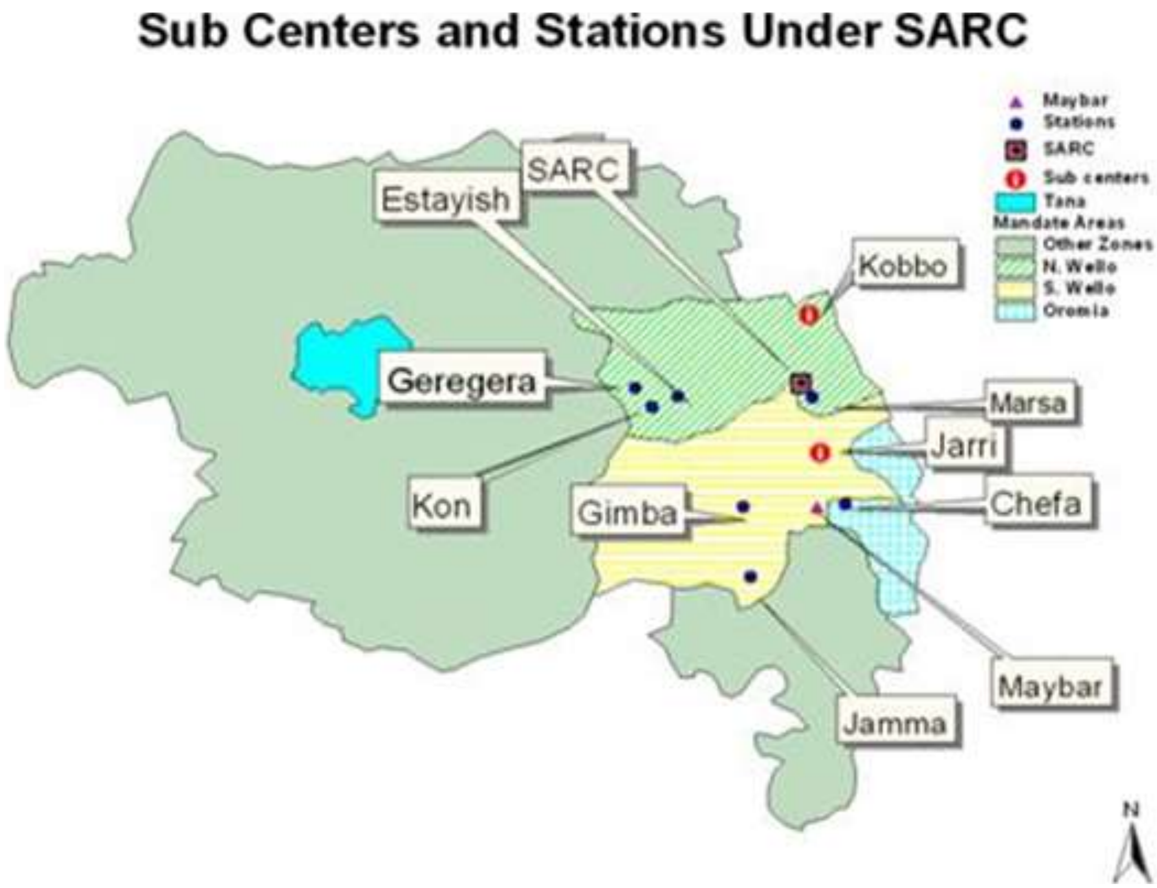

Figure 1. Map of the study area. Sirinka Agricultural Research Center is found in the Northern part of Ethiopia. Source: (Administrator, 2014).

pots were internally covered with black plastics to maintain constant environmental temperature. The bottom of each pot was lined with drainage sand to keep the soil well-drained and was filled with 2:6:2 sand to local soil (the growth medium) and organic manure fertilizer, respectively, instead of drilling to have bottom water drainage and side aeration holes (Ahmadvand et al., 2012).

All the time, watering was made continually by the respected salt concentrations for various treatments. Each pot was irrigated with 250 ml of saline solution, produced by sodium chloride and calcium chloride of desired treatment during early morning and evening every day. After the 10th day seedling emergence, some seedlings were removed per two weeks interval to maintain nutrient nourishment level, aeration, water usage and other environmental factors optimum for those that long last for the continual observation and assessment with good spacing. Three seedlings per pot were left for last measurements. The salinity of water in the experiment was similar to that used by farmers to irrigate their corn fields, even at the end.

Shoot and root length were measured starting from the 2nd week of planting and were done for four successive months. After five months from the start of the experiment, the total number of leaves of every plant in each pot was counted. Subsequently, root length, shoot and root fresh weights were determined immediately in each treatment. Seedlings were dried out in drying oven for 48 h at 70°C, shoot and root dry weights were measured (Elouaer and Hannachi, 2012). Then plants were harvested carefully to prevent falling of the seeds. Finally, biomass difference, length variation, yield enhancement and overall performance of the treatments were, analyzed.

Parameters assayed during the salinity experiment

Some among the measured growth features of the crop were:

Total germination percentage (TG %) which was measured in 147 day using the formula

$$(\mathrm{TG\%}) = \frac{\mathrm{n}}{\mathrm{N}} \times 100 \quad (1)$$

Where n, number of seeds germinated per day and N total number of seeds sown.

Mean germination is the number of seeds germinated in the intervals of time, established for data collection (The number of days required for seeds to complete the germination process). It was calculated according the formula of Kader and Jutzi (2004).

$$MGT\ (d) = \frac{\sum(\mathrm{TiNi})}{\sum \mathrm{Ni}} \quad (2)$$

Where Ni = number of germinated seeds on the ith days and Ti= rank order of day i (number of days counted from the beginning of germination).

Germination index (GI) was calculated using the equation used

Table 1. Analyses of variance of different parameters.

Source of variance	TG (%)	MGT	GI	CV	SL	RL	CY	SB	VI
unprimed	80*	9.11*	238.96*	4.87*	299.50*	361.86*	7.40*	8.00*	76.91*
Primed	69.47*	15.24*	183.11*	7.14*	212.00*	344.70*	36.40*	9.00*	70.30*
Unprimed x primed	79.75*	6.53*	116.498*	3.14*	0.14*	0.13*	5.467*	0.18*	0.01*
Error	0.03*	0.07*	6.12*	0.92*	11.72*	2.18*	0.98*	0.56*	0.05*

TG = Total germination of seeds in percentage; MGT = mean germination time; GI = germination index; CV = coefficient of variation; SL = shoot length; RL = root length; CY =crop yield; SB = shoot branch; VI = vigor index. *Significant at 0.05 according to Duncan test.

Table 2. Means comparison of the measured assays under different salt type treatments.

Parameters	TG (%)	MGT	GI	CV%	SL	RL	CY	SB	VI
Control	70.50[c]	8.15 [c]	118.98[a]	3.97[c]	133.25[b]	289.51[a]	4.03[c]	2.5[b]	42.98[c]
NaCl priming	77.51[b]	12.78 [a]	70.69[c]	5.17[b]	167.25 [a]	220.27[b]	19.55[a]	2.3[c]	50.06[b]
$CaCl_2$ priming	82.5[a]	9.33 [b]	83.22[b]	6.11[a]	124.25[c]	207.72[c]	9.65[b]	3.5[a]	50.94[a]

*Means with the same letters in each column are not significantly different at 0.05 according to Duncan test. TG = Total germination of seeds in percentage; MGT = mean germination time; GI = germination index; CV = coefficient of variation; SL = shoot length; RL = root length; CY =crop yield; SB = shoot branch; VI = vigor index.

by Kader and Jutzi (2004).

$$GI = \frac{\sum TiNi}{Ti} \quad (3)$$

Where, Ti is the number of days after sowing and Ni is number of germinated seeds in the observation day.

Coefficient of velocity of germination (CVG), gives an indication of the rapidity of germination, which was calculated following the method used by Kader and Jutzi (2004).

$$CVG = \frac{\sum Ni}{\sum (TiNi)} \times 100 \quad (4)$$

Where, Ti is number of days after sowing and Ni is number of germinated seeds in an observation day.

The vigour index (VI) was used to assess the power of surviving, growing and thriving of the germinated seeds and was measured using the formula of Elouaer and Hannachi (2012) calculated as follows:

$$VI = \frac{[TGP(\%) \times Seedling\ \ Length(cm)]}{100}$$

RESULTS

Salt stress response evaluation of the assayed parameters

Analysis of variance showed that all salinity levels, salt types and soil types have significant effects on the studied parameters of *Z. mays* (Melkasa 2) seeds ($p < 0.05$). Moreover, the interaction of salinity level and soil type with different salt solutions, had significant effect on all parameters tested at 5% significant level except the early emerged roots measured in the first and second weeks after the emergence of plumules and radicals (Tables 1 and 2).

Priming with NaCl and $CaCl_2$ increases the germination (total germination of seeds in percentage, mean germination time, germination index and coefficient velocity of germination) and growth parameters (grain yield, root to shoot ratio and vigor index) of maize (*Z. mays*), as compared to unprimed seeds (control) under different levels of saline conditions. For example NaCl and $CaCl_2$ priming is an energetic supplement for uniform start of germination for maize seedling under saline conditions (Figure 2).

At the lower salinity level (7 dS/m) total germination of seeds decreased as compared with other salinity levels. However, the number of days for the completion of seed germination increased at 7 dS/m salinity level like the other higher salinity levels in black soil type at control (Figure 3).

Conversely, in red soil type the total germinated seeds were almost similar apart from the highest concentration level (11 dS/m) that showed lower germinated seeds of *Z. mays*. When primed seeds of maize are sown in black soil, their root length at the first instances of radical and plumule emergence was less susceptible to salt stress. In all the treatments the root length at the first two weeks was approximately similar, 35 cm except for 7 dS/m showed longer root length. Here unprimed seeds have longer root length than the primed once (Figure 3).

As it can be seen from this figure with four replication and five different salt treatments for Melkasa 2 seeds, total germinated seeds were highly pronounced at 7

Figure 2. Uniform stand establishment of salt (NaCl and $CaCl_2$) primed maize seeds.

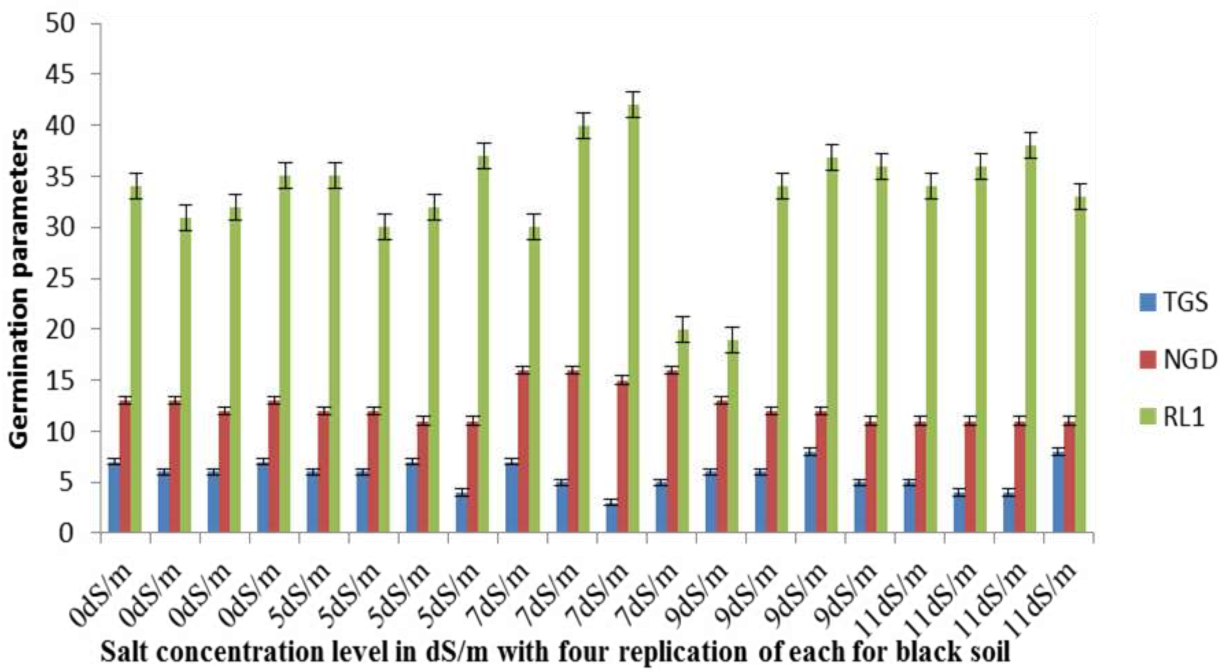

Figure 3. The effect of salinity on total germination of seeds (TGS), number of days for germination compellation (NGD), and root length (RL) of Melkasa 2 seeds.

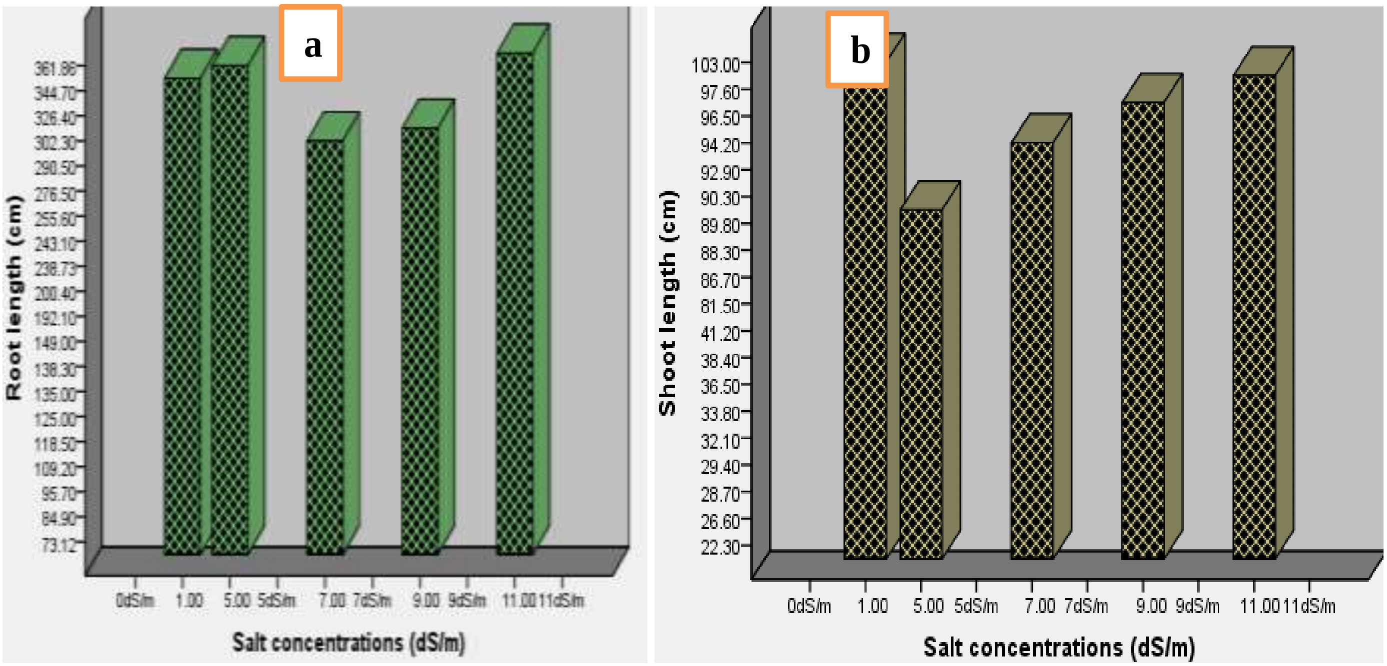

Figure 4. The effect of different salinity levels of (a) NaCl on root length and (b) $CaCl_2$ on shoot length of maize seeds.

dS/m in the third replication. Moreover, the highest averaged root length was found as 34 cm at 7 dS/m similar to the control (0 dS/m) followed by 33.5 cm for 11 dS/m, 33.25 cm for 5 dS/m, and 30.5 cm for 9 dS/m, respectively. Similarly, the number of days for germination compellation was higher at 7 dS/m.

Root length and shoot length susceptibility to salt stress

Priming seeds with NaCl and $CaCl_2$ prior to sowing, decreased root length at the medium salinity levels (7 and 9 dS/m) as compared with the control. However, the lowest and highest salinity levels (5 and 11 dS/m), respectively indicated longer average root length compared with the control (Figure 4a). There was a 17.16 cm root length difference between the medium salt concentrations and the lowest and highest salt concentrations.

Moreover, *Z. Mays* seeds showed a reduction in shoot length compared to the control as the salinity level of $CaCl_2$ increases, except the highest salinity level (11 dS/m) that possess similar shoot length with the control. But with salt treatments as salinity level increases, shoot length of primed seeds also increases. At the highest salinity level, primed seeds have similar average shoot length with an unprimed maize seeds (Figure 4b). This may be because external Ca^{2+} ameliorates the adverse effects of salinity by facilitating higher K^+/Na^+ selectivity of the plant.

Germination percentage, mean germination time, germination index and shoot branch

Sodium chloride and calcium chloride priming decreased earlier in the population of seeds to be germinated compared with the control though there was a satisfactory increase later. Total germination percentage of primed maize seeds increased significantly ($P < 0.05$) with an increasing NaCl and $CaCl_2$ salinity level except the lower salt treatment (5 dS/m) (Figure 5a).

Moreover, the rise in total germination percentage was significantly higher for seeds primed with higher salinity levels (9 dS/m and 11 dS/m) as compared to the control and lower salinity level primed seeds (Figure 4a), indicating that priming increases root physiology such as water absorption and nutrient uptake. The higher salt tolerance of the roots from primed seeds seems to be the result of a higher capacity for osmotic adjustment in the root system, since plants from primed seeds have more Na^+ and Cl^- ions in their roots and more sugars and organic acids in leaves than plants from non-primed seeds. The rise with 20% on total germination percentage due to an increase in salinity level from 0 to 11 dS/m showed that, seed priming increase germination percentage when compared to unprimed seeds except the lowest salt treatment with 10% reduction (Figure 5a).

The lower mean germination time is 10 days for control group, indicating the lower average length of time for maximum germination of seed lots (Figure 5b). Germination index however was lower in salt treated seeds compared with the control. Seed priming prior to

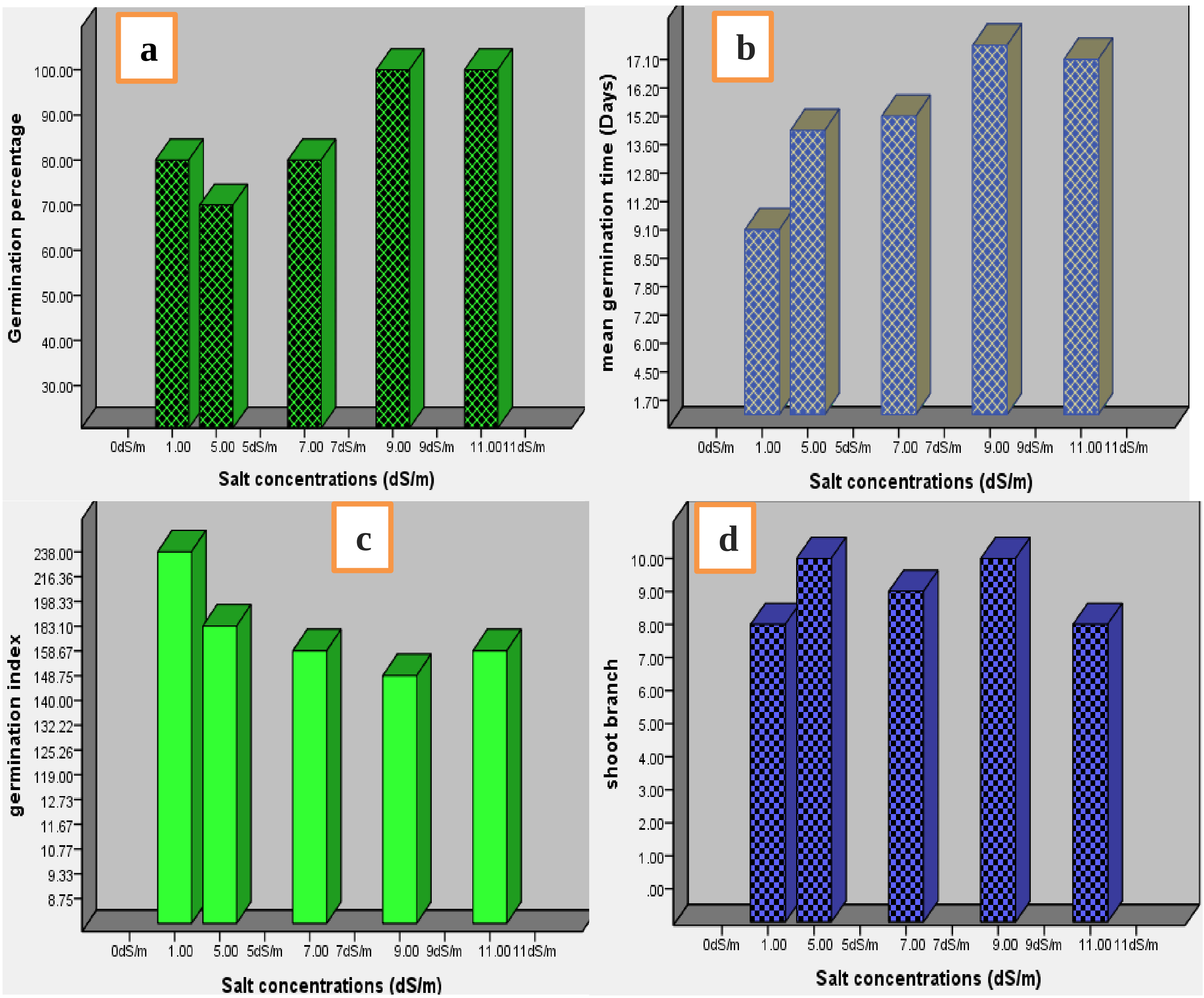

Figure 5. The effects of different salt concentrations on (**a**) germination percentage, (**b**) means germination, (**c**) germination index time and (**d**) shoot branch of *Zea mays.*

sowing with NaCl and $CaCl_2$ also enhances shoot branch in *Z. mays* crops particularly of Melkasa-2, compared with seeds sown without any salt treatment. There is two more branch increment in 5 and 9 dS/m salt solutions compared with the control (Figure 5d). The addition of NaCl and $CaCl_2$ might increase leaf size by increasing the membrane permeability which increases the leaf N^+ and Ca^{2+} thereby the number of leaf branches.

These is a statistically significant (P <0.05) difference in all the growth features of Melkasa 2 maize, where primed with different salt types (NaCl and $CaCl_2$ (Table 2). Compare with the unprimed seeds, $CaCl_2$ and NaCl priming respectively in descending order showed better total germination percentage and vigor index. However, mean germination time, shoot length and crop yield were better in NaCl primed seeds as compared with $CaCl_2$ primed seeds (Table 2). This is because $CaCl_2$ is preferable for seed coating and germination and did not persist beyond the early seedling growth whereas NaCl seed priming had positive effects on mature plants and yield.

Coefficient of velocity, vigor index, number of cobs and grain yield per pot

Salinity causes an increase in the coefficient of velocity which ranges from 5 germinated seeds per day at 0 dS/m

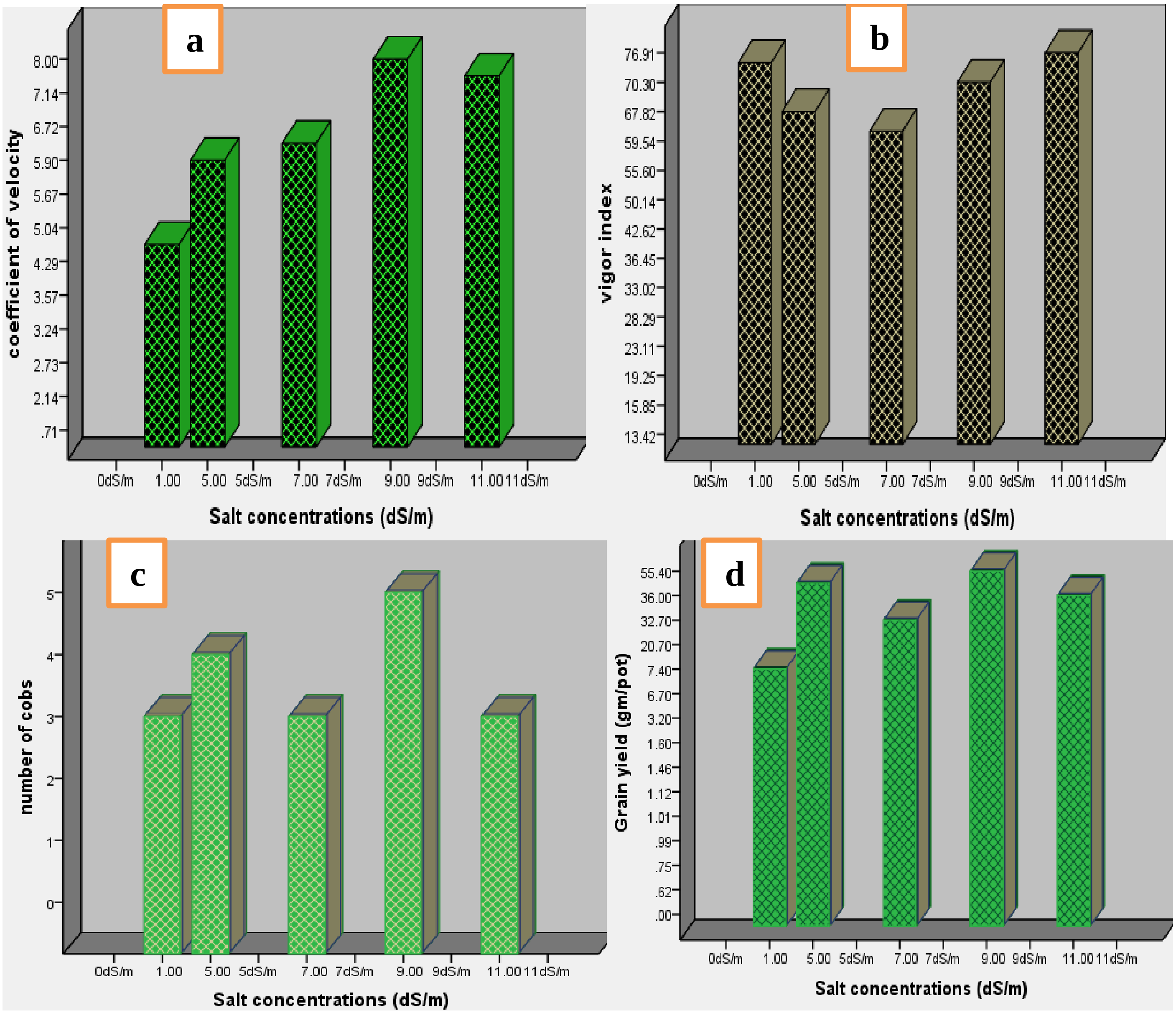

Figure 6. Effect of different salinity levels on (**a**) coefficient of velocity, (**b**) vigor index, (**c**) number of cobs and (**d**) grain yield *Zea mays.*

to reach 10 germinated seeds per day at 11dS/m. Coefficient of velocity was higher in $CaCl_2$ seed priming (10%) compared to those with NaCl seed priming (8%) and control seeds (5%) (Table 2 and Figure 6a). In fact, $CaCl_2$ seed priming had significantly ($P<0.05$) increased coefficient of velocity of about 2.96 in comparison with control seeds. Likewise, coefficient of velocity of NaCl primed seed also significantly ($P<0.05$) increased compared with control. Generally coefficient of velocity of Melkasa-2 indicated a faster germination when exposed to saline environmental condition than in non-saline areas. A similar results are observed from the means where the CV values are $CaCl_2$ > NaCl > control (Table 2). From the present findings seeds primed prior to sowing germinated two times more rapidly than the control.

Vigor index is not greatly significant with regard to salt treatment compared with the other growth parameters. There is no significant difference between the control and the highest salt treatment (Figure 6b). Seeds treated with lower salinity have lower chance for growth and survival. Increasing salinity causes a significant increase ($P < 0.05$) in maize vigor index for primed seeds. This value increases form 67.82 at 7dS/m to gain 78.20 at 11 dS/m (Figure 6b). Besides, NaCl and $CaCl_2$ seed priming increases significantly ($P < 0.05$) vigor index of maize (77.01 and 80.06, respectively) with $CaCl_2$ having better role compared with seedlings from control seeds (76.91)

(Figure 6b). Numbers of cobs per plant were different in salt treatments 5 dS/m and 9 dS/m as compared to unprimed seeds with 4 and 5 cobs plant values, respectively (Figure 6c).

As salt concentration increases from 5 to 11dS/m, grain yield per pot showed a significant increment as compared with the control. Grain yield of the maize crop were found higher in primed seeds which might be as a result of good stand establishment, speedy and uniform germination of primed seeds (Figure 6d). The salinity treatments 9 dS/m followed by 7 dS/m is better for study crop in terms of cobs per plant and grain yield. This is an indication that this released variety of maize is moderate to salt tolerant crop which can improve growth and physiological variables that can be affected by salt stress. This may be that, crop which can overproduce different types of responsive biological solutes have low molecular weight, highly soluble and usually nontoxic at a high cellular concentrations, contributing to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins.

Soil type based salt treatment responses of maize crop assayed parameters

All growth parameters except the early emerging roots, coefficient of velocity and salt type showed a significant effect with respect to the soil types treated with different salinity levels and salts types ($P < 0.05$). Number of days to complete germination, total germinated seeds from the sown one, root length, shoot length, mean germination time, germination index, germination percentage, vigor index, coefficient of velocity, number of cobs per plant and grain yield per pot respond differently in the two types of soils (Figure 7b). Seeds primed with NaCl have wider leaf area and longer shoot compared to the seeds primed with $CaCl_2$ (Figure 7a to d).

Moreover, the kernel that contains the seed and husk of the maize is larger in plants primed with NaCl. Salt primed maize seedlings grow in black soil types are more effective than the maize seeds grown on red soils from both salt treatments (Figure 7a and b). The reason may be due to the water holding capacity and moisture content of black soils that help to adjust the osmotic potential of the crop. The results of this study also indicted salt priming of maize seeds grown on red soils, affect negatively at seedling stage though, the lower treatments are indicators of medium salt tolerance of the crop on red soils (Figure 7c and d).

Germination percentage, root length, shoots length, and vigor index

A decrease in germination percentage of the black soil and increase in germination percentage of the red soil is observed initially. While on seedlings from vertisol soils, there is a higher germination percentage as compared with lithosol soils (Figure 8a). The results also suggested a reduction by 20% in germination percentage in lithosol soil sown maize seeds germination percentage compared with maize seeds sown in vertisol soil type (Figure 8a). Germination percentage increased in both soil types with increase in salt concentration. The increment was higher at 9 and 11 dS/m salt concentrations for *Z. mays* seeds in black soil and at 7 dS/m for *Z. mays* seeds in red soil which show that, all salinity levels showed a significant ($P <0.05$) increase in germination percentage of primed *Z. mays* seeds compared with the control (Table not presented here).

Compared with lithosol soil, vertisol soils showed better performance in root length when seeds are sown by priming with different salt solutions. During the early seedling, growth stage seeds on red soils perform well in root length, but after adapting the salt stress condition seeedlings from black soil grow well (Figure 8b). Unlike root length, shoot length has a different response at the early seedling growth stage. Lithosol soils have a longer shoot length as compared with the vertisol soils. There was an 86.15 cm root length difference between black soil and red soil and 87 cm difference in shoot length between red soil and black soil, respectively. As salinity level increases shoot length in black soil also increases. The increase in salt stress in the growth medium causes a final increase in root length of black soil and shoots length of red soil.

Contrarily, vigor index of *Z. mays* seeds response better for red soils as compared with the one grown in black soil (Figure 8d). Increasing salinity level causes significant ($P < 0.05$) increases in *Z. mays* vigor index in both the primed and unprimed seeds with both NaCl and $CaCl_2$ treated *Z. mays* seeds. The survival and growth power of germinated seeds was highly pronounced in black soil at the higher salinity levels (Figure 7). However, primed maize seeds sown in red soil were highly thriving in medium salinity levels particularly of 7 dS/m NaCl and $CaCl_2$ treatments.

Coefficient of velocity, number of cobs per plant and grain yield per pot

Results from figure 8 revealed that primed seeds grown in black soil have higher coefficient of velocity (Figure 9a), which have more number of cobs per plant and more grain yield than primed *Zea mays* seeds grown in red soil. Compared with the control, NaCl and $CaCl_2$ primed seeds showed higher coefficient of velocity, more cobs and better yield than the unprimed seeds. Primed seeds of maize from red soil have two more less number of cobs per plant than primed seeds from black soil (Figure 9b). Similarly, *Z. mays* seeds treated with NaCl and

Figure 7. Differences in growth traits of *Zea mays* seedling treated with similar salts ($CaCl_2$ and NaCl) and effect of the salt priming on cob number and size of red and black soil types. (**a**) Maize seedlings grown on black soil primed with $CaCl_2$ (**b**) maize seedlings grown black soil primed with NaCl (**c**) maize seedlings grown on red soil primed with $CaCl_2$ (**d**) maize seedlings grown on red soil primed with NaCl.

$CaCl_2$ grown in black soil have 52.2 g more grain yield per pot than the similarly treated seeds from red soil (Figure 9c).

DISCUSSION

Salt priming significantly affect maize physiology starting from germination up to yield. Germinations from primed seeds were better than un-primed seeds when exposed to different salinity levels and soil types. Similar findings were reported by Kaya et al. (2006), where seed treatment of sunflower overcomes salt and drought stress during germination. Priming with NaCl and $CaCl_2$ increases the germination parameters (total germinated seeds in percentage, mean germination time, germination index and coefficient velocity of germination) and growth parameters (grain yield, root to shoot ratio and vigor index) of maize (*Z. mays*), as compared to unprimed seeds (control). The study revealed that seed priming treatments not only improved the stand establishment but also provided physiological improvements.

A significant decrease in emergence time and increase in final emergence count of the plant may be because of

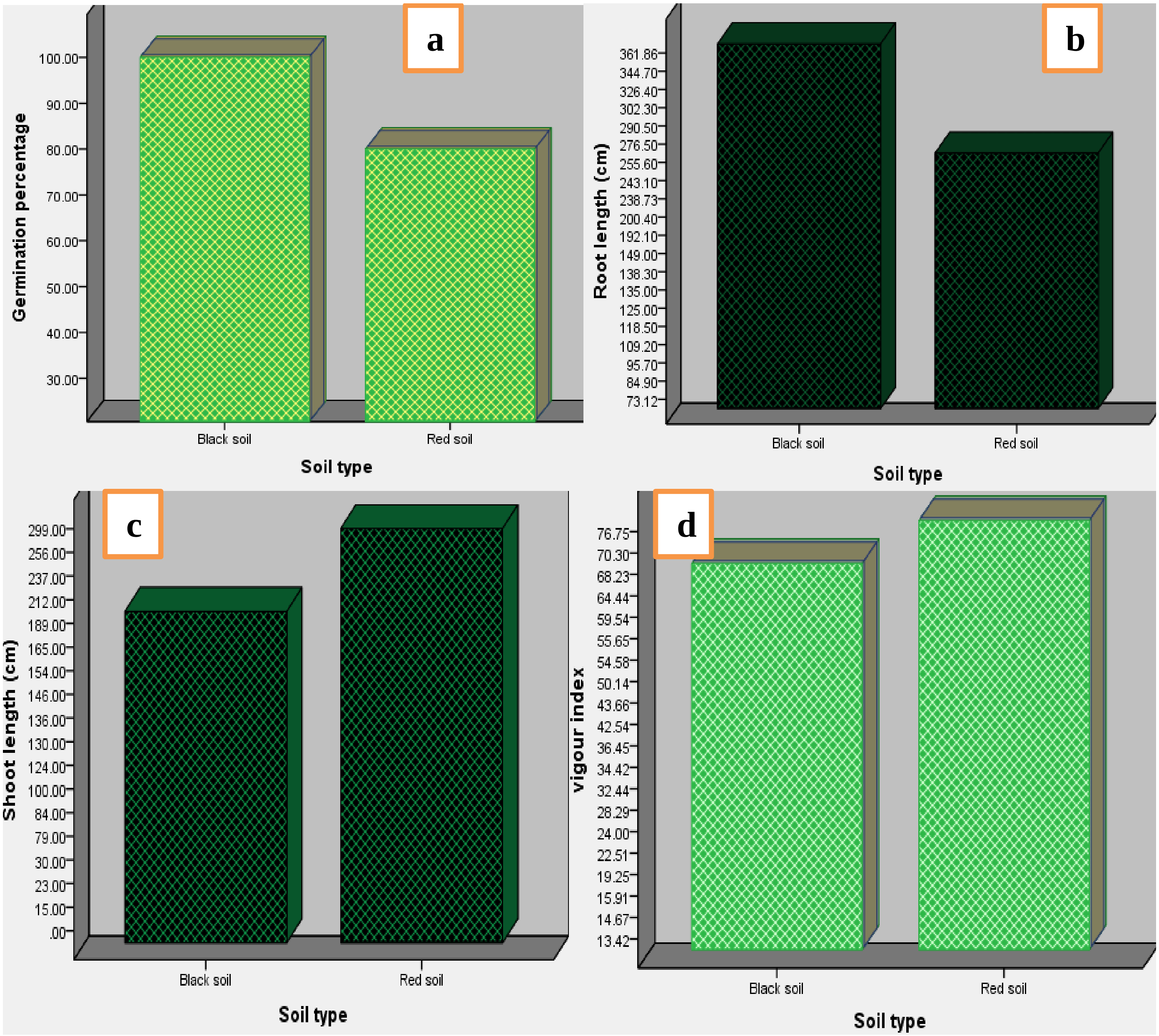

Figure 8. Effect of NaCl and $CaCl_2$ priming on (**a**) germination percentage, (**b**) root length, (**c**) shoot length, and (**d**) vigor index of *Zea mays* seeds grown on black and red soils.

the fact that seed priming induces a range of biochemical changes such as hydrolysis, activation of enzymes and dormancy breaking (Aziza et al., 2004; Farooq et al., 2010b), which are required to start the germination process, this edge of primed seeds over nonprime (control) resulted in improvement of maize grain yield. Higher salinity decreases maize germination and let seedling growth deteriorate; this might be due to the toxic effects of Na^+, Ca^{2+} and Cl^- in the process of germination which terminates and starts of seedling growth even with soil types. Also, salinity stress affects seed germination through the restriction of seed water absorption (Tsegazeabe and Berhane, 2012), imbalanced use of nutrient pool and creation of disorders in protein synthesis (Bordi, 2010).

At the lower salinity level, 7dS/m total germinated seeds decreased compared with the other salinity levels even though the number of days for the completion of seed germination is increased at 7dS/m salinity level like the other higher salinity levels in black soil type at control. Conversely, in red soil type the total germinated seeds are almost similar apart from the highest concentration level (11dS/m) that showed lower germinated seeds of *Zea mays* (Melkasa-2). When primed seeds of maize are

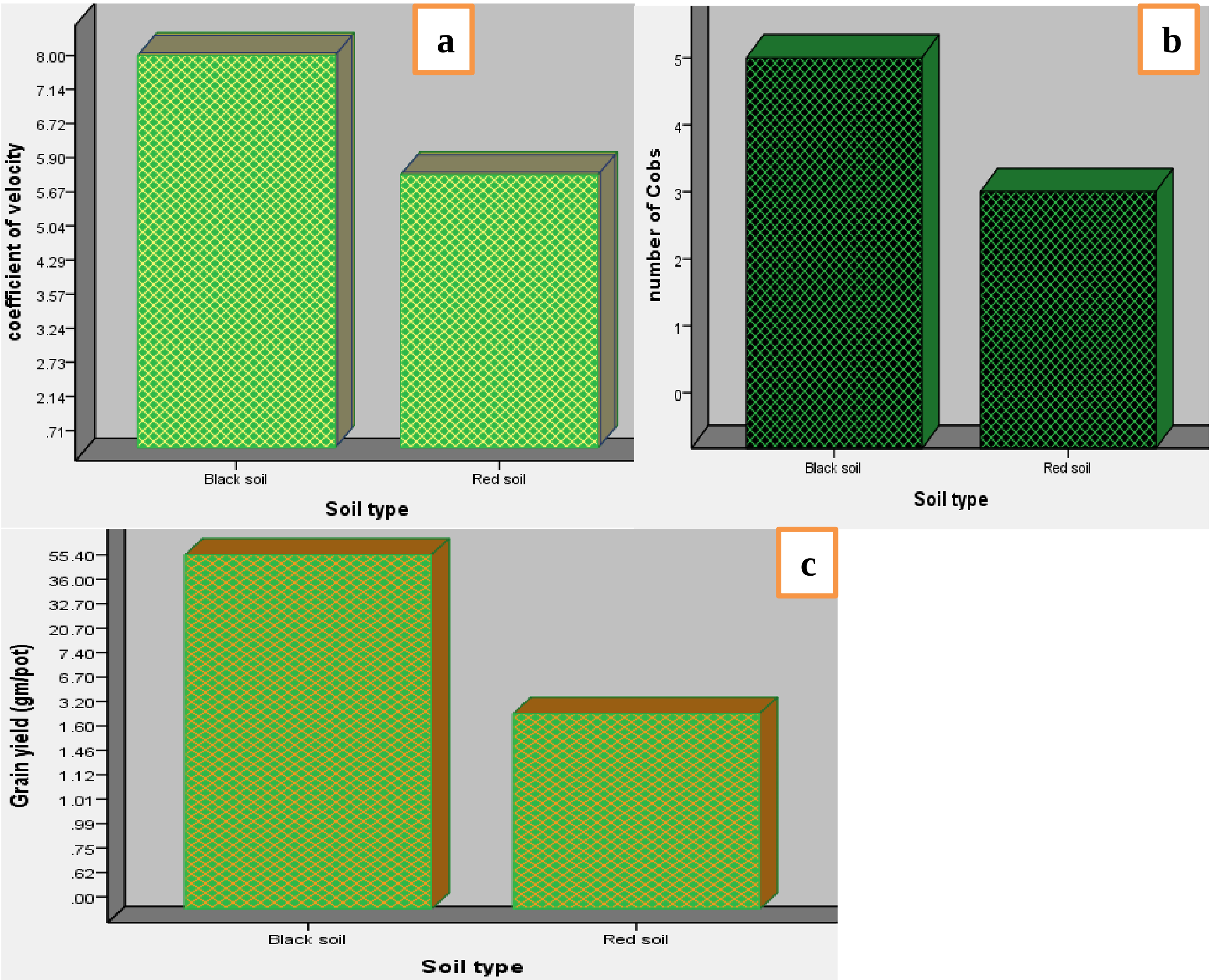

Figure 9. Effect of NaCl and $CaCl_2$ priming on (a) coefficient of velocity, (b) number of cobs per plant and (c) grain yield per pot of *Zea mays* seeds grown on black and red soils.

sow in black soil, their root length at the first instances of radical and plumule emergence is less susceptible to salt stress. In all the treatments, the root length at the first two weeks is approximately similar 35 cm except for 7dS/m, which showed longer root length. Here unprimed seeds have longer root length than the primed once.

Priming with $CaCl_2$ and NaCl, improves the performance of maize germination, seedling growth and production. This is similar with the idea osmopriming with $CaCl_2$ and NaCl priming which improves maize performance on salt treated soils (Ashraf et al., 2003; Nawaz et al., 2013). Cellular membrane integrity can be maintained by Ca^{2+} which reduces Na^+ and favours the K^+ absorption (Ashraf et al., 2003). The effectiveness of osmopriming with $CaCl_2$ has already been reported for yield improvement in several crops including wheat (Farooq et al., 2008), maize (Ashraf and Rauf, 2001) and rice (Farooq et al., 2006). Primed seeds of maize might had better competency for water absorption from the growing media that enable metabolic activities in seeds during the start of germination process, much earlier than when radicle and plumule appearances (Elouaer and Hannachi, 2012). The earlier and superior germination of a primed maize seeds is correlated with breakdown of dormancy due to priming (Butler et al., 2009). Farooq et al. (2005) found faster emergence of germination, emergence of radicle and plumule in primed seeds. Similarly Afzal et al. (2009) reported that increased solubilization of seed storage proteins like the beta subunit of the globulin and reduction in lipid per-oxidation and enhanced anti-oxidative activity in primed seeds facilitated germination. This faster germination is due to the synthesis of DNA, RNA and protein during priming. Osmopriming with $CaCl_2$ gives higher benefit-to-cost ratio in the soils. The easy availability and relatively non-toxic nature of $CaCl_2$, may lead to wider on-farm adaptation of this osmoticum and has been suggested to farmers for usage.

Although, salt priming decrease mean germination time for primed maize seeds on both soil types, the primed seeds have significantly lower mean germination time compared to unprimed seeds. Similar results were reported by Ashraf and Rauf (2001) working with other priming treatments, such as polyethylene glycol (PEG), inorganic salts or even ABA. According to Kaya et al. (2006), seed priming leads to the initiation of primary metabolic processes and the time required for germination decreases. This positive effect is probably due to the stimulatory effect of priming on later stages of the germination process through the mediation of cell division in germinated seeds and rapid synthesis of DNA, RNA and protein during priming (Sivritepe et al., 2003). Farooq et al. (2005) reported the swelling of the embryo inside primed tomato seed speeding up germination by facilitating water absorption. This is due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient which allow water uptake during seed germination (Kaya et al., 2006). Seed priming, upgrades early stand establishment and vigorous seedling growth, earlier flowering, and increased seed yield and harvest index in maize. Priming improved seedling vigor which can be attributed to stimulated starch metabolism triggering earlier emergence and vigorous seedling growth (Farooq et al., 2010b; Berhane and Berhanu, 2016).

Improved performance of $CaCl_2$ is related to involvement of Ca^{2+} in membrane repairs and activation of enzymes, when starch reserve mobilization and radicle protrusion are in progress (Farooq et al., 2010a). Earlier and vigorous stand might reduce crop weed competition and increased absorption of water and nutrients by vigorous root system producing increased number of branches, tillers/plant, cobs/plant and yields/pot (Berhane and Berhanu, 2016). Increased emergence, tillers number and number of branches by seed osmopriming with $CaCl_2$ reported in rice and wheat are consistent (Farooq et al., 2008; Singh et al., 2015) with present study. Increased seed yield was also associated with an increase in yield contributing traits. Increase in biological yield was due to increased plant height from healthy and vigorous seedlings which might be due to, improved crop growth rate and net assimilation rate (Farooq et al., 2006; Singh et al., 2015). Similarly, increase in harvest index from primed seeds might be due to an increase in dry matter, and partitioning towards the growing pods thereby increasing grain yield. Increased growth and yield in sunflower by seed treatment with $CaCl_2$ reported by Kaya et al. (2006), confirms present study findings.

Conclusions

Seed priming prior to sowing, stimulates the physiological activities of maize seeds which resulted in rapid and uniform seed germination, as a result of the permeability of the roots changed by the treatments. The priming methods improved growth and enhances yield of crops through stimulation and the seeding up of the physiological processes of the plant although the seeds of the plant showed variation in responses to different salt types/concentrations and soil types they grew.

Priming with sodium chloride improves the crop maturity and yield whereas calcium chloride priming serves as seed coat and increases the Na^+ and Ca^{2+} concentration of roots thus speeds up germination process. Black soils require moderate salt priming supplements for better crop production than red soils.

Conflict of Interests

The authors have not declared any conflict of interests.

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