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INDUCING SALT TOLERANCE IN FRENCH MARIGOLD (*Tagetes patula*) THROUGH SEED PRIMING

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ABSTRACT

The present study investigates the potential of seed priming for induction of salt tolerance in French marigold at early growth stage. Treatments were combination of priming techniques viz. hydropriming, halopriming (50 mM $CaCl_2$), hormonal priming with salicylic acid (100 ppm) and ascorbate priming (100 ppm) each for 24 h. Primed seeds were exposed to salinity levels (0; 50 and 100 mM NaCl) during germination and emergence assays. All priming treatments hastened germination and emergence attributes under saline or non-saline conditions as compared to those of non-primed (control) seeds. Seeds of French marigold primed with ascorbate followed by $CaCl_2$, salicylic acid and hydropriming enhanced final germination, speed of germination, root and shoot length and dry mass of French marigold seedlings. This was interpreted by minimized mean emergence time and time to 50% emergence. Additionally, it was also observed that all priming agents particularly calcium salt and ascorbate maintained ionic homeostasis in salt stressed seedlings. It is concluded that $CaCl_2$, ascorbate and salicylic acid were the most effective priming agents to ameliorate the adverse effects of salinity in French marigold due to lower uptake of Na^+ and higher uptake of K^+ in the seedlings.

Key words: emergence, ionic homeostasis, seedling vigor, salt tolerance, seed treatment

INTRODUCTION

One of the most alarming problems confronting the farmers in developing countries is the lack of favorable conditions in soil that reduces final germination, causes unequal emergence, heterogeneous seedling growth and competition for resources such as nutrients, light and water. On global scale land area affected by salinity is about 190 million ha [FAO 2010]. Most horticultural crops are glycophytes and range from salt-sensitive to moderately salt-tolerant [Greenway and Munns 1980]. Producers of ornamental species are therefore, reluctant to use poor quality water for irrigation because floricultural species are considered to be highly sensitive [Carter and Grieve 2008].

In recent years, marigold has come under close examination of scientists around the world for its pest control activity. The essential oil present in marigold is largely used in compounding of high grade perfumes [Afzal et al. 2009]. Salinity stress, as a result of an osmotic or specific ion effect, can stunt growth, produce foliar injury, affect nutrient balance, impair root function, and distort flower growth of marigold

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cultivars [Valdez-Aguilar et al. 2009]. Germination and emergence are sensitive stages, which are reduced by salinity in most of the crops [Ashraf and Foolad 2005]. Higher salinity level is harmful, impairs root and shoot growth and modify Na/K ratio in the tissues. Osmotic and specific ions stress reduced dry weight of plants [Al-Karaki 2000]. Salt stress impairment is primarily due to changes in water relations induced by high salt deposition in the intercellular spaces [Zhang et al. 2006]. The stand establishment of crop depends upon the germination potential of seeds while the high salt concentration decreases germination and thus upset uniform stand establishment. The researchers believe that germination speed is more affected by salinity than final germination percentage [Lovato et al. 1992].

With proper management, salinity becomes an environmentally friendly alternative to chemical growth retardants to restrict height and strengthen stems [Lee and Iersel 2008]. Many approaches have been adopted to overcome the salinity problems. The most important approach is the use of pre-sowing seed treatment to alleviate salt stress in crops. These agents may be organic and inorganic compounds [Afzal et al. 2008]. The induction of salt tolerance in crop species is one of the possible approach counteracting salt stress problems [Yamaguchi and Blumwald 2005]. Seed priming with solution of nutrients or growth substances can enhance the performance of seeds under stressful environments. This easy, low cost and low risk priming technique had positive impacts on the wider farming system and livelihoods so the technology has proved to be highly popular among farmers [Ghassemi-Golezani et al. 2008, Afzal et al. 2013]. The most common priming techniques include osmopriming, halopriming, hormonal priming and hydropriming [Ashraf and Foolad 2005, Afzal et al. 2008].

Seed priming improved final germination, stand establishment, seedling vigor and reduced time to 50% germination in horticultural crops [Ashraf and Foolad 2005, Ghassemi-Golezani et al. 2008] under saline or non-saline conditions. Many researchers have studied salt tolerance induction in field crops through seed priming [Afzal et al. 2008]. Seed priming with osmoticum increased the final emergence, uniformity of emergence, shortened the time to emergence and improved the flower quality of African marigold under wide range of environmental conditions [Bosma et al. 2013]. Mukhtar et al. [2013] found that priming marigold seeds with $CaCl_2$ improved the emergence attributes, increased α-amylase activity, total soluble and reducing sugars. Therefore, this study was carried out to look into the impact of various priming strategies on germination capacity and stand establishment of French marigold under salinity stress.

MATERIALS AND METHODS

French marigold (*Tagetes patula* L.) seeds were obtained from Chanan Din Nursery, Lahore, Pakistan and were primed in solutions of distilled water (hydropriming), $CaCl_2$ (50 mM), salicylic acid (100 ppm) and ascorbate (100 ppm). Relative performance of primed and untreated seeds was assessed, based upon germination scores in germination and emergence assays.

Priming procedure

Five gram seed was soaked in each treatment solution by keeping seed to solution ratio 1 : 5 for 24 h in a glass beaker at 25°C. Continuous aeration was provided to the soaked seeds separately in each beaker. Seeds were removed from priming solutions after specified time and washed under running distilled water and then re-dried around 10% seed moisture contents (near to original weight) on filter paper sheets for 48 h [Afzal et al. 2013].

Germination assay

Twenty five treated and non-treated seeds were allowed to germinate separately by placing them in 9 cm diameter petri dishes, furnished with Whatman No. 1 filter paper. Filter papers were kept wet by 2 ml saline (50 and 100 mM NaCl solution) or non-saline water as salinity treatments supply. Petri dishes were then shifted in seed incubator (SANYO MIR-254) adjusted at 20°C provided with continuous fluorescent light for 14 days [ISTA 2015]. Three petri dishes were used for each treatment. Seeds were considered as germinated when 2 mm radicle emerged out of seed.

Germination was recorded on the daily basis. Time to 50% germination (T_{50}) was calculated according to following formula given by Coolbear et al. [1984]:

$$T_{50} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i}$$

Where N is the number of final seeds germinated and n_i and n_j are cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_i$. Mean germination time (MGT) was counted according to the equation of Ellis and Roberts [1981].

$$\text{MGT} = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

Final germination percentage (FGP) was calculated by dividing total number of germinated seeds to the total seeds sown and multiplying this with 100. Seedlings were harvested after two weeks and washed under running deionized water. Five randomly selected seedlings from each replicate were assessed for root and shoot lengths. Seedlings were dried in an oven at 60°C for 48 h and dry weight was recorded with help of electric weighing balance.

Emergence assay

Treated and untreated seeds were raised in plastic trays (25 in each) containing acid washed dry sand. 50 ml of saline solution was applied in each tray on daily basis. Time to 50% emergence (E_{50}), mean emergence time (MET), final emergence percentage (FEP), root length, shoot length and seedling dry weights were calculated according to the methods mentioned in germination assay.

Seed biochemical analysis

The activity of α-amylase enzyme was determined in primed and non-primed seeds of marigold. Seed (0.1 g) samples were extracted by using potassium phosphate buffer (pH 7.0). After preparation of sample, α-amylase activity was determined by following the modified DNS method [Varavinit et al. 2002]. Total soluble sugars were quantified in (0.1 g) seed sample after grinding with the help of mortal pistol followed by hydrolysis with 2.5 N HCl and then neutralized by sodium carbonate. Distilled water was used to make final volume of 10 ml, centrifuged at 10,000 x g and the supernatant was used for measurement of total soluble sugars following the phenol sulphuric acid method [Thimmaiah 2004]. Total reducing sugars were measured by DNS method from the marigold seed sample (0.1 g) extracted in 80% ethanol twice using 5 ml volume each time.

Determination of ions

For the determination of sodium and potassium in shoots, the dried samples of two week old seedling were digested with nitric acid (HCl) and perchloric acid ($HClO_4$) in 2 : 1 ratio on volume basis [Zarcinas et al. 1987] and ionic contents were determined with the help of flame photometer (Sherwood, Model 360, UK).

Statistical analysis

The experiment was conducted in Completely Randomized Design (CRD) with factorial arrangements and was replicated thrice. Data collected was analyzed statistically by using analytical software, Statistix (version 8.1) and treatment means were compared by using Least Significant Difference (LSD) test at 5% probability level.

RESULTS

Elevated salt concentrations in the medium significantly reduced final germination and seedling growth of French marigold. Under both saline and non-saline conditions seeds soaked in priming solutions improved germination traits (i.e. final germination, mean germination time and time to 50% germination) in comparison with non-primed seeds (tab. 1). Maximum final germination was found in seeds primed with ascorbate (AsA). At higher salinity level, seeds primed with ascorbate took minimum time to

germinate as compared with differently treated or non-treated seeds. Increase in salinity stress enhanced time to 50% germination of French marigold. At 100 mM NaCl, priming with AsA accelerated the seed germination to the greatest degree, followed by priming with SA, $CaCl_2$ and hydropriming. Results depict that ascorbate priming and halopriming ($CaCl_2$) maximally increased root length in non-saline conditions while at 50 and mM NaCl maximum root length was produced by the AsA priming followed by SA priming. Shoot lengths were maximally increased as a result of AsA priming followed by $CaCl_2$ priming compared to control treatment under both saline and non-saline conditions (tab. 2). Likewise, seeds sown after ascorbate treatment minimized salinity effects and enhanced dry weight of French marigold seedlings as compared to other priming agents under saline conditions.

Less emergence potential i.e. lower FEP, higher MET and E_{50} was recorded with the rise of salinity level for especially for untreated seeds (tab. 3), whereas most of the priming treatments significantly improved final emergence of marigold seeds. Ascorbate priming followed by $CaCl_2$ priming maximally contributed to salt resistance and improved final emergence. Salt stress significantly affected root length, shoot length and dry weight of seedlings while most of the priming treatments improved all these attributes (tab. 4). Ascorbate priming along with halopriming also contributed to enhanced root and shoot lengths and dry weight of seedlings as compared to other treatments under salinity stress.

Table 1. Final germination, mean germination time and time to 50% germination of French marigold, influenced by seed priming, under saline (50 and 100 mM NaCl) and non-saline (control) regimes

	Priming treatments	0 mM NaCl	50 mM NaCl	100 mM NaCl
Final germination (%)	Untreated seeds	74.00 h	72.00 i	70.00 j
	Hydropriming	86.66 e	84.00 f	77.33 g
	Priming with $CaCl_2$	92.00 bc	90.66 cd	90.00 d
	Priming with SA	94.66 a	86.66 e	84.00 f
	Priming with AsA	94.66 a	93.33 a	92.00 bc
		LSD at 0.05 = 1.5311		
Mean germination time (days)	Untreated seeds	4.65 c	4.73 b	4.80 a
	Hydropriming	4.56 ef	4.49 i	4.57 de
	Priming with $CaCl_2$	4.43 k	4.45 j	4.53 g
	Priming with SA	4.51 h	4.56 ef	4.58 d
	Priming with AsA	4.32 l	4.55 f	4.43 k
		LSD at 0.05 = 0.0189		
Time to 50% germination (days)	Untreated seeds	2.17 c	2.86 a	2.73 b
	Hydropriming	1.56 h	1.43 k	1.79 d
	Priming with $CaCl_2$	1.41 l	1.50 j	1.74 e
	Priming with SA	1.52 i	1.60 g	1.65 f
	Priming with AsA	1.32 m	1.60 g	1.44 k
		LSD at 0.05 = 0.0131		

SA – salicylic acid, AsA – ascorbate. Means with the same letters don't differ significantly at $P < 0.05$

Table 2. Root length, shoot length and seedling dry weight of French marigold, influenced by seed priming, under saline (50 and 100 mM NaCl) and non-saline (control) regimes in germination assay

	Priming treatments	0 mM NaCl	50 mM NaCl	100 mM NaCl
Root length (cm)	Untreated seeds	4.53 h	4.12 hi	3.70 m
	Hydropriming	5.09 c	4.52 h	3.91 l
	Priming with $CaCl_2$	5.22 b	4.64 g	4.23 j
	Priming with SA	5.04 d	4.86 f	4.25 i
	Priming with AsA	5.55 a	4.91 e	4.87 f
		LSD at 0.05 = 0.0153		
Shoot length (cm)	Untreated seeds	4.33 j	4.06 m	3.93 n
	Hydropriming	4.77 e	4.30 k	4.25 l
	Priming with $CaCl_2$	5.03 b	4.72 f	4.54 h
	Priming with SA	4.87 c	4.55 h	4.51 i
	Priming with AsA	5.34 a	4.84 d	4.64 g
		LSD at 0.05 = 0.0168		
Seedling dry weight (mg)	Untreated seeds	40.67 j	35.00 l	34.00 l
	Hydropriming	52.67 f	42.67 i	36.67 k
	Priming with $CaCl_2$	63.67 b	54.67 e	53.00 f
	Priming with SA	57.67 d	50.67 g	48.00 h
	Priming with AsA	72.67 a	61.67 c	62.00 c
		LSD at 0.05 = 1.0494		

SA – salicylic acid, AsA – ascorbate. Means with the same letters don't differ significantly at $P < 0.05$

Table 3. Final emergence, mean emergence time and time to 50% emergence of French marigold, influenced by seed priming, under saline (50 and 100 mM NaCl) and non-saline (control) regimes

	Priming treatments	0 mM NaCl	50 mM NaCl	100 mM NaCl
Final emergence (%)	Untreated seeds	70.67 fg	62.67 h	68.00 gh
	Hydropriming	76.00 d-f	69.33 g	77.33 de
	Priming with $CaCl_2$	81.33 cd	89.33 ab	85.33 bc
	Priming with SA	73.33 e-g	73.33 e-g	80.00 cd
	Priming with AsA	80.00 cd	93.33 a	89.33 ab
		LSD at 0.05 = 6.20		
Mean emergence time (days)	Untreated seeds	6.46 i	6.74 b	6.99 a
	Hydropriming	6.32 l	6.34 k	6.55 g
	Priming with $CaCl_2$	6.34 k	6.58 e	6.61 d
	Priming with SA	6.52 h	6.69 c	6.70 c
	Priming with AsA	6.28 m	6.44 j	6.57 f
		LSD at 0.05 = 1.56		
Time to 50% emergence (days)	Untreated seeds	2.37 c-e	3.43 a	2.92 a-c
	Hydropriming	2.08 d-f	1.56 f	2.54 b-e
	Priming with $CaCl_2$	2.21c-f	3.12 ab	2.70 b-d
	Priming with SA	2.39 c-e	2.77 a-d	2.80 a-c
	Priming with AsA	1.93 ef	3.20 ab	2.77 a-d
		LSD at 0.05 = 0.72		

SA – salicylic acid, AsA – ascorbate. Means with the same letters don't differ significantly at $P < 0.05$

Table 4. Root length, shoot length and seedling dry weight of French marigold, influenced by seed priming, under saline (50 and 100 mM NaCl) and non-saline (control) regimes in emergence assay

	Priming treatments	0 mM NaCl	50 mM NaCl	100 mM NaCl
Root length (cm)	Untreated seeds	3.52 k	3.44 l	2.99 m
	Hydropriming	3.44 l	3.68 h	2.94 n
	Priming with $CaCl_2$	4.32 c	4.19 d	3.76 g
	Priming with SA	3.95 e	3.59 i	3.55 j
	Priming with AsA	4.48 a	4.38 b	3.86 f
		LSD at 0.05 = 0.03		
Shoot length (cm)	Untreated seeds	4.34 f	4.23 i	4.04 j
	Hydropriming	4.34 f	4.24 h	3.99 l
	Priming with $CaCl_2$	4.66 b	4.41 e	4.28 g
	Priming with SA	4.62 c	4.34 f	4.03 k
	Priming with AsA	4.87 a	4.52 d	4.41 e
		LSD at 0.05 = 0.01		
Seedling dry weight (mg)	Untreated seeds	71.00 l	66.00 n	62.00 o
	Hydropriming	81.00 i	77.00 j	67.00 m
	Priming with $CaCl_2$	89.33 d	87.33 e	82.00 h
	Priming with SA	90.00 c	86.00 f	75.00 k
	Priming with AsA	94.00 a	91.00 b	84.00 g
		LSD at 0.05 = 0.59		

SA – salicylic acid, AsA – ascorbate. Means with the same letters don't differ significantly at $P < 0.05$

Seed treatments had significant effect in reducing the uptake of sodium ions. Under elevated salinity levels, Na^+ uptake in the shoots was high but $CaCl_2$ followed by AsA and SA reduced uptake of Na^+ from medium (fig. 1). Another important finding of this study is that $CaCl_2$, AsA and SA also enhanced K^+ uptake in French marigold plants as compared to hydropriming and untreated seeds. Significantly higher α-amylase activity was measured in seeds primed with AsA followed by those primed with SA. Control seeds showed less α-amylase activity compared with all other treatments. Similarly seed priming with AsA increased total soluble sugars and reducing sugars in marigold seeds (fig. 2).

DISCUSSION

Salt stress severely hampered the germination and seedling establishment in glycophytes. Seed priming is helpful in decreasing the risk of poor and irregular stand establishment under salt stress in agricultural [Afzal et al. 2013] and horticultural crops [Bradford 1986]. Seed priming can enhance the performance of marigold under normal environmental conditions [Afzal et al. 2009, Afzal et al. 2013]. Our results strongly demonstrate that an increased germination potential through seed priming can improve the performance of marigold seedlings under salt stress (tabs 1 and 2). Better germination potential i.e. higher FGP and lower MGT and T_{50} in primed seeds is the base of heightening salt tolerance in marigold. It may due to early reserve breakdown and mobilization, synchronization of germinated seeds or may be ascribed to promoting effect of Ca^{+2} or ascorbate during priming process as reported by other researchers [Demir and Oztokat 2003, Afzal et al. 2008].

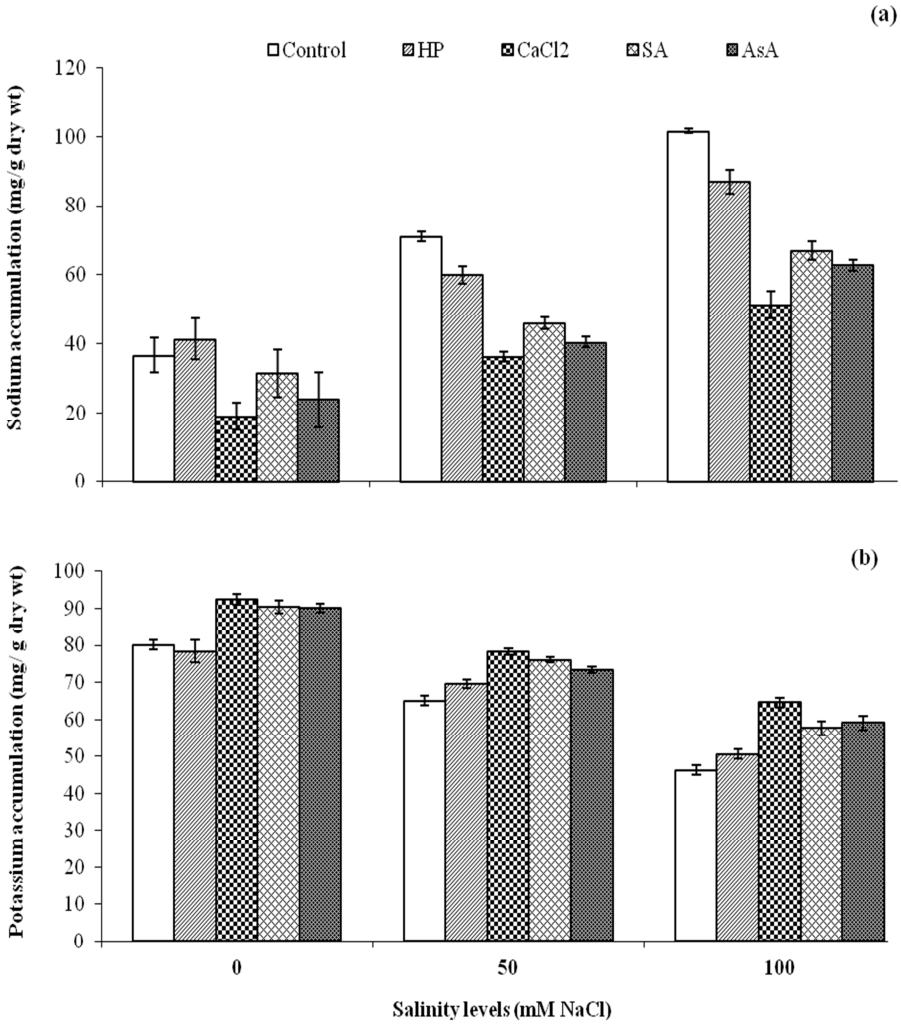

Fig. 1. Effect of seed priming on ionic contents of marigold seedlings grown under various salinity levels. HP – hydropriming, SA – salicylic acid, AsA – ascorbate

Efficient germination potential is essential for successful stand establishment, which in turn is of great importance for plant survival under salt stress. Presently our results indicate that higher salinity level decreased root and shoot lengths and dry weight of marigold seedlings. But seed priming with ascorbate enhanced root and shoot lengths and dry weight of seedlings under saline and non-saline conditions and the similar results have been reported by Mukhtar et al. [2013]. Salicylic acid and ascorbate might play an important role as antioxidant compounds and protect plants from the oxidative damage by scavenging ROS that are generated during salt stress conditions [Athar et al. 2008].

The response of priming treatments was also sustained during emergence experiment in terms of higher emergence potential evident from higher FEP and lower MET and E_{50} with the application of ascorbate and $CaCl_2$ (tab. 3). This enhancement due to priming could be attributed to higher reducing and total sugars as well as higher α-amylase activity in primed seeds [Afzal et al. 2013]. Moreover, maxi-

mum root, shoot lengths and dry weight of seedlings could be due to earlier start of emergence by lowering MET and T_{50} under salinity stress in seeds primed with ascorbate and $CaCl_2$ (tab. 4). These findings are supported by Kathiresan et al. [1984] who reported maximum seedlings vigor and emergence in sunflower seeds as a result of priming with ascorbate and $CaCl_2$. The observed ability of seeds primed with calcium salt, to sustain germination under salt stress may be due to the influence of calcium ions on membranes [Shannon and Grieve 1999, Afzal et al. 2008]. Thus, calcium protects marigold seedlings from side effects of salt stress and triggers the seedling growth under saline environment.

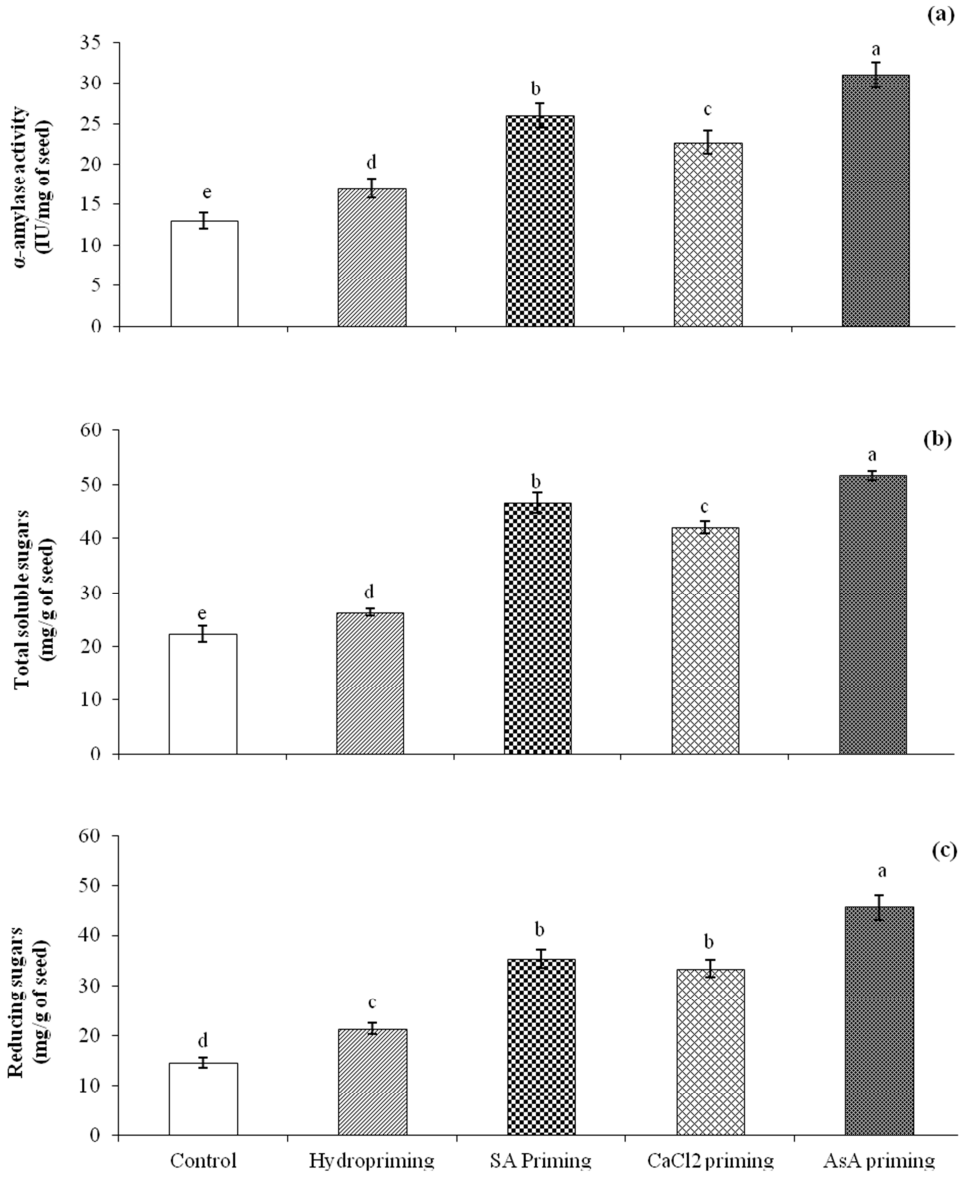

Fig. 2. Influence of seed priming on seed α-amylase activity (a), total soluble sugars (b) and reducing sugars (c). SA – salicylic acid, AsA – ascorbate

Negative effects of salinity are due to toxic ions which can compromise the mobilization of reserves, the emergence and early growth of seedlings [Ashraf and Foolad 2005]. Priming agents (AsA and $CaCl_2$) counteract negative consequences of salt treatments reducing Na^+ uptake and increasing K^+ uptake as compared to other primed and non-primed seeds (fig. 1). Overall, all priming treatments reduced harmful effects of salinity and maintained low Na^+/K^+ ratios in seedlings of marigold but seeds treated with $CaCl_2$, AsA and SA reduced the uptake of Na^+ uptake and enhanced K^+ uptake. Seed soaking in $CaCl_2$ solution reduced the Na^+ uptake as Ca^{2+} signaling elicited plasma membrane Na^+/H^+ antiporter which enhanced Na^+ extrusion under salt stress [Sun et al. 2009, Kong et al. 2012, Bose et al. 2015]. Increased α-amylase activity along with total soluble and reducing sugars in primed seeds was observed in the present study (fig. 2). This highlights the possible role of seed priming in either inducing the *de novo* synthesis or increasing the activities of hydrolytic enzymes hence producing germination metabolites in requisite amounts [Sung and Chang 1993, Lee and Kim 2000].

CONCLUSION

On the basis of findings, it is concluded that priming ameliorated the inhibitory effects of salinity on the seedling growth of French marigold. Seed priming with ascorbate, calcium salt and salicylic acid proved effective for the induction of salt tolerance through increased seedling vigor, improved stand establishment and balanced ionic homeostasis in marigold seedlings.

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