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Comprehensive seed priming assessment of *Hibiscus sabdariffa* L. in germination and seedling growth stage under salt stress

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Abstract: This study was performed to appraise the effects of several seed pretreatment solutions and priming time on seed germination indices and growth characteristics of *Hibiscus sabdariffa* L. in various salt stress levels. Seed priming was accomplished by KCl (1 and 2 %), Na_2SO_3 (0.5 and 1 %), KNO_3 (0.5 and 1 %), and Ca_2CO_3 (1 and 2 %) as halopriming and distilled water as hydropriming at 12 and 24 h priming durations and control (non-primed), then primed seeds exposed to four levels (0, 50, 100, 200 mM) of NaCl solutions. The highest germination percentage was observed in 12 and 24 h hydropriming (63.3 and 53.3 %) and non-primed (56.6 %) under normal condition, respectively. Besides, there was no germinated seed at 24 h priming by 0.5 and 1 percentage of KNO_3. Under saline condition, 24 h 2 % Ca_2CO_3 had the highest germination percentage (43.3 %) in 50 mM, while 12 h treatment with 0.5 % Na_2SO_3 (33.3 %) had high germination percentage in 100 mM levels of saline conditions. Also, the highest germination rate index was observed in 0.5 % Na_2SO_3 with 12 h treatment time (4.05 and 3.95 respectively) in 50 and 100 mM levels of saline conditions. Overall, salt stress considerably reduced germination and growth traits of *Hibiscus sabdariffa* L. seedlings. Considering the effect of various seeds priming of *Hibiscus sabdariffa* L. on germination indices like germination percentage and mean germination time, the importance of priming duration and type of priming solutions could be concluded.

Key words: abiotic stress; medicinal plant; roselle; seed treatment

Ocena predobravnavanja semen vrste osleza *Hibiscus sabdariffa* L. v razvojnih stopnjah kalitve in kalice v razmerah solnega stresa

Izvleček: V raziskavi so bili ocenjeni učinki predobravnavanja semen z različnimi raztopinami in časi obravnavanja na kalitveni indeks in rastne lastnosti vrste *Hibiscus sabdariffa* L. v razmerah različnega solnega stresa. Predobravnavanje semen je bilo izvedeno z raztopinami KCl (1 in 2 %), Na_2SO_3 (0,5 in 1 %), KNO_3 (0,5 in 1 %), in Ca_2CO_3 (1 in 2 %) kot obravnavanje s solmi in z destilirano vodo kot vodno obravnavanje za 12 in 24 h ter kontrolo (brez predobravnavanja). Po tem so bila ta semena izpostavljena raztopinam štirih koncentracij natrijevega klorida (0, 50, 100, 200 mM NaCl). Največji odstotek kalitve je bil ugotovljen pri semenih, ki so bila predobravnavana z vodo za 12 in 24 ur (63,3 in 53,3 %) in pri netretiranih semenih (56,6 %) v normalnih razmerah. Pri predobravanavanju semen za 24 ur z 0,5 in 1 % raztopino KNO_3 ni vzklilo nobeno seme. V razmerah slanosti je imelo 24 urno obravnavanje z 2 % raztopino Ca_2CO_3 največji odstotek kalitve (43,3 %) pri 50 mM med tem, ko je imelo12 urno obravnavanje z 0,5 % raztopino Na_2SO_3 (33,3 %) še vedno velik odstotek kalitve v razmerah 100 mM slanosti. Največja vrednost indeksa kalitve je bila ugotovljena pri obravnavanju z 0,5 % raztopino Na_2SO_3, z 12 urnim časom obravnavanja (4,05 in 3,95) v razmerah 50 in 100 mM slanosti. Nasplošno je solni stres znatno zmanjšal kalitev in rastne parameter sejank osleza *Hibiscus sabdariffa* L.. Upoštevaje učinke različnih predobravnavanj semen osleza *Hibiscus sabdariffa* L. na kalitvena indeksa kot sta odstotek kalitve in poprečni čas kalitve je potrebno pri tem posebej upoštevati pomen časa obravnavanja in vrsto raztopine za obravnavanje.

Ključne besede: abiotski stres; zdravilna rastlina; *Hibiscus sabdariffa* L.; obravnava semen

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1 INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is a member of the Malvaceae family (Shruthi et al., 2018), which originally belonged to Malaysia and India (Mahadevan et al., 2009), and cultivated in tropical and subtropical climates (Da-Costa-Rocha et al., 2014). It is perennial or annual sub-shrub or woody-based herb, and widely grown in subtropical and tropical zones (Ibrahim et al., 2013). These plant species have played a key role in people's living because they provide humanity's needs that are food, clothes, shelter, and medicines (Riaz & Chopra, 2018)polysaccharides and organic acids thus having enormous prospective in modern therapeutic uses. The study aimed to review and document all the available evidence and information about the calyces of Hibiscus sabdariffa (roselle. Roselle is used in traditional medicine, due to overfill in phytochemicals like polyphenols, particularly anthocyanins, polysaccharides, and organic acids; hence, it has significant potential in modern medicinal applications (Riaz & Chopra, 2018; Sukkhaeng et al., 2018). It is traditionally cultivated owing to the usage of calyces, stems, leaves, and seeds as all organs have pharmacological and other uses (Wright et al., 2007). Calyx products are applied in indigenous medicine to treat high blood pressure, liver diseases and fever (Ali et al., 2005). Roselle extracts are increasingly developed for medications, food, and cosmetics (Farnsworth & Bunyapraphatsara, 1992).

The presence of salt in the water or soil is considerable challenge for plant production in the world. It is most prevalent in dryland and coastal areas. Due to unsuitable irrigation and drainage management, limited rain, high evaporation, and saline irrigation water, salt concentration in the soil and water is increasing inland (Ibrahim, 2016). This problem takes about 3.7 million acres of the area of food production every year (Munns & Tester, 2008). Therefore, half of the cultivation area will be lost by the Mid-21st century (Wang et al., 2003). Salt stress became a limitation factor to the production of the crops, and the majority of crops are extremely sensitive to saline soil and water (Lin et al., 2017; Ahmadizadeh et al., 2016). Seed germination and seedling growth are the susceptible stages to abiotic stress, and abiotic stress can be slowed or stopped the germination of seeds (Ahmadizadeh et al., 2011; Galal, 2017). Rouhi et al. (2011), Ahmadizadeh et al. (2011), Ansari et al. (2013), and Ebrahimi et al. (2014) stated that raising the stress had a negative impact on the germination rate.

In the past decade, several strategies have been applied to improve abiotic stress tolerance in crops. There are various methods to enhance crop growth and development in salt-affected conditions (Hussain et al., 2016; Feghhenabi et al., 2020). One of the appropriate methods is pretreatment, like prime the seeds with various materials before sowing (Ali et al., 2017; Subramanyam et al., 2019). Seed pretreatment as a practical, cost-effective, and low-risk enhancing germination of seed and seedling growth through pre-germinating metabolic processes improvement (Jime'nez-Arias et al., 2015; Migahid et al., 2019). Priming of seed is moderate stress, which activates a stress-reaction mechanism (Bhanuprakash & Yogeesha, 2016). Priming of seed is a physiological method of seed hydration and drying to ameliorate the pre-germinate metabolism under stressed conditions. The primed seeds exhibit quicker and normal seed germination (Hasanuzzaman & Fotopoulos, 2019), and seed priming adjusts the biochemical and physiological of the embryo. Priming also decreases the seeds sensitivity to unfavorable conditions (Afzal et al., 2016).

Several researchers have indicated that seed priming enhances the well establishment and growth of plants (Farooq et al., 2010; Kerchev et al., 2020; Feghhenabi et al., 2020). The beneficial impacts of seed priming in saline conditions have been shown in several crops for instance, pepper (Khan et al., 2009), okra (Dkhil et al., 2014), tomato (Ebrahimi et al., 2014), rosella (Galal, 2017), and *Silybum marianum* (L.) Gaertn. (Migahid et al., 2019). Latef et al. (2020) studied the impact of priming with Al_2O_3 nanoparticles on the growth of roselle, and the results showed that Al_2O_3 nanoparticles influenced growth traits, like dry mass, fresh mass, root, and shoot length. Shruthi et al. (2018) concluded pretreatment with GA_3, KNO_3, and hot water to study the influence of seed priming on germination of Roselle (*Hibiscus sabdariffa* L.), they indicated the positive impact of seed pretreatment on the properties of germination speed and germination percentage. Nassar (2010) reported the positive results of seed priming and organic fertilizer on the yield and quality of roselle. Sheyhakinia et al. (2020) showed ameliorate of salt stress tolerance by jasmonic acid in roselle. Their results showed that jasmonic acid protected roselle seedlings against salinity damage. Germination indices and seedling traits of two tomato cultivars are influenced by the great potential value of seed treatment with $CaCl_2$ and KNO_3 solution under salinity conditions. In contrast, enhanced salinity concentrations led to a significant reduction in germination indices and seedlings growth (Ebrahimi et al., 2014).

Soil salinity is one of the principal widespread abiotic stresses, which has adverse effects on crop production (Ismail et al., 2007; Ahmadizadeh et al., 2021). Appropriate seed germination is a prerequisite for the successful stand establishment of plants in unfavorable

environments such as low moisture, saline water, and soil, which are limiting germination factors (Ahmadizadeh, 2013). The fast and uniform germination and seedling establishment are influential factors for plant performance. There are several priming techniques, which are helpful for successful stand establishment of plants in abiotic stress conditions. Therefore, the study aimed to investigate the effects of various priming including KCl, Na_2SO_3, KNO_3, and Ca_2CO_3 and hydropriming of seeds on germination at different priming durations under normal and various levels of salinity conditions.

2 MATERIAL AND METHODS

In order to assess the effects of various seed priming compounds and priming durations on seed germination indices and growth characteristics of *Hibiscus sabdariffa* L. seedlings in various levels of salt stress conditions were studied at the agricultural laboratory of Minab higher education center, university of Hormozgan. An experiment was conducted in a factorial experiment based on a completely randomized design with three replications. Priming treatments consisted of halopriming with KNO_3 (0.5 and 1 %), Na_2SO_3 (0.5 and 1 %), KCl (1 and 2 %) and Ca_2CO_3 (1 and 2 %), and hydropriming with distilled water and control (non-primed), priming durations was 12 and 24 h, then seeds exposed to four levels (0, 50, 100, 200 mM) of NaCl solution. For any treatments, disinfected seeds were immersed in 50 ml of the prepared solution for 12 and 24 h in covered glass containers to preserve evaporation loss. The seeds were then rinsed with distilled water several times, afterward dried back at room temperature (25 °C) for 24 h to be dried (Ebrahimi et al., 2014; Ibrahim, 2016; Aghdaei et al., 2019).

Fifteen healthy primed seeds of roselle were placed in petri dishes on two layers of filter paper, then 8 ml of the salinity solutions (0, 50, 100, and 200 mM NaCl). To germinate the seeds, the petri dishes were put in an incubator at 26 ± 1°C. Germination of *Hibiscus sabdariffa* L. the seeds were counted as a germinated seed once they displayed extension of radicle almost 2 mm. The germination count was recorded every 24 h up to 7 days. At the end of the first week, and germination percentage (*GP*), germination rate index (*GRI*), mean germination time (*MGT*), germination index (*GI*), and vigor index (*VI*) were calculated based on the following equations (Al-Mudaris, 1998):

$$GP = (N/M) * 100 \quad (1)$$

where *GP* is germination percentage, *N* is the total number of germinated seeds at the end of seven days, and *M* is the total number of cultivated seeds.

$$GRI = (G1/1) + (G2/2) + \ldots + (Gx/x) \quad (2)$$

G1 = germination percent in first day. *G2* = germination percent in second day to final experiment,

$$MGT = (fx) / f \quad (3)$$

where *f* is the number of newly germinated seeds on each day and *x* is the day of counting,

$$GI = (7 * N_1) + (6 * N_2) + (5 * N_3) + \ldots \quad (4)$$

N1, N2, . . . = the number of germinated seeds in first day, second day and . . .,

$$VI = GP \times \text{Seedling length } (SL) \quad (5)$$

(El-ouaer & Hannachi, 2012),

Also, fresh shoot mass, fresh root mass, shoot length, root length, dry shoot mass and dry root mass were measured. Mass of root and shoot were measured from the sample mass before and after drying at 70 °C for 12 h. the data were analyzed using SAS software, and means comparisons were done by the least significant difference test (LSD) at $p < 0.05$ level of confidence. The Excel software was used to draw figures.

3 RESULT AND DISCUSSION

Seed germination and early establishment of seedling are the crucial stages for the crops, and these two stages are the delicate growth stages in unfavorable environments (Begcy et al., 2018). Seed germination is sometimes prevented or delayed under different abiotic stresses (Fazlali et al., 2013; Muhie et al., 2020a,b). Roselle is sensitive to germination and early seedling development in saline conditions (Bahaeldeen et al., 2012; Al-Tohafi et al., 2015; Kadamanda, 2019). Considering to the value and privilege of the *Hibiscus sabdariffa* products, particularly medicinal value, this study was conducted to evaluate the influence of various priming approaches in different NaCl concentrations on germination indices and seedling traits of roselle plants in petri dish at a controlled experimental environment. The analysis of variance showed a highly significant ($p < 0.01$) difference between various priming treatments in terms of germination indices and seedling growth traits (Table 1). Also, the result revealed a significant difference among the salinity levels. The priming × salinity interaction effect was significant for all studied traits (Table 1). High concentrations of salinity prevent and reduce the performance of most plants, but seed emergence is the utmost momentous process for well seed germination in medicinal plants (Nadjafi et al., 2006; Reed et al., 2022). The highest germination

Table 1: Analysis of variance for salinity and priming effects on some germination characteristics of *Hibiscus sabdariffa*

S.O.V	DF	Mean Square										
		GP	*GRI*	*MGT*	*GI*	*VI*	*ShL*	*RL*	*FShM*	*FRM*	*DShM*	*DRM*	*Sh/R*
Prim	18	861.39**	15.25**	4.78**	907.27**	49425.72**	14.93**	5.62**	0.02**	0.00044**	0.00033**	0.0000035**	202.13**
Salinity	3	12156.73**	235.69**	33.82**	884.45**	1001023**	213.34**	57.56**	0.34**	0.0048**	0.0034**	0.0000372**	1394.11**
P*S	54	197.69**	4.51**	1.19**	13323.17**	21367.41**	2.73**	1.33**	0.004**	0.00014**	0.000068**	0.00000077**	88.44**
Error	152	35.41	0.98	0.69	43.14	3152.86	0.50	0.18	0.001	0.00001	0.0000089	0.00000013	25.12

**: is significant at 1 %,
GP: Germination Percentage, GRI: Germination Rate Index, MGT: Mean Germination Time, GI: Germination Index, VI: Vigor Index, *ShL:* Shoot length, *RL:* Root Length, *FShM:* Fresh Shoot Mass, *FRM:* Fresh Root Mass, *DShM:* Dry Shoot Mass, *DRM:* Dry Root Mass, *Sh/R:* Shoot/Root ratio

rate and percentage of T. polium seeds were obtained at concentrations of 500-2500 ppm GA3. Washing and chilling (5°C. The highest germination percentage (*GP*) (36.93 %) was observed in normal condition, while under the salinity conditions the highest *GP* (23.24 %) was observed under 50 mM salinity condition (Table 2).

The decreasing in the percentage of germination may be associated with the increase of external osmotic pressure that has an impact on the water absorption of the seed, as well as, owing to the accumulation of some ions in the embryo, which may result in stimulation of the metabolic processes of germination and ultimately leading to cells death in the embryo (Maher et al., 2013; Feghhenabi et al., 2020). Afkari Bajehbaj (2010) and Shereiwy et al. (2021) demonstrated that enhancing salinity levels decreased final germination in seeds, but, the adverse impact of salinity on primed seeds was less than unprimed seeds. The highest germination rate index (*GRI*) (5.06) was observed in normal condition, while under the salinity conditions the highest *GRI* was observed under 50 mM salinity condition that had significantly different from normal condition (Table 2).

The highest *GI* was observed in normal condition, under the low level of salinity conditions the *GI* reduction was 46 percentage, and the highest *GI* in stress condition was observed under 50 mM salinity condition (Table 2). The highest *VI* was observed in normal condition, and there was significantly difference between the various levels of salinity stress (Table 2). The means comparison under different levels of salinity stress revealed the highest *MGT* under 50, and 100 mM salinity conditions (Table 2). In this respect, Kaveh et al. (2011), Thiam et al. (2013), and Ibrahim (2016) indicated that enhancing the concentration of salinity improved germination time and reduced the germination percentage. In general, low levels of salinity cause a dormancy and low impact on the germination rate, but ascending concentration of salt prevents the seed germination and reduces the percentage of germination (Khan & Weber, 2006; Shannon & Grieve, 1998).

There was a significant difference between control and salinity conditions in terms of shoot and root mass. The highest of shoot fresh mass (0.19 g), root fresh mass (0.023 g) and root dry mass (0.002 g) were observed at non-salinity condition (control) but the highest shoot dry mass (0.019) was achieved at salinity condition (Table 2), suggesting that root growth is more sensitive to salinity than shoot growth. Amiri et al. (2010) with the study of germination characteristics of *Cynara scolymus* L. and *Echinacea purpurea* (L.) Moench under salinity stress demonstrated that shoot dry mass was reduced by enhancing salt concentration in studied medicinal plants. The similar finding was reported by research-

Table 2: Mean comparison of salinity levels on germination indices and seedling growth traits in *Hibiscus sabdariffa*

Salinity *mM*	*GP* (%)	*GRI*	*MGT* (day)	*GI*	*VI*	*ShL* (cm)	*RL* (cm)	*FShM* (g)	*FRM* (g)	*DShM* (g)	*DRM* (g)	*Sh/R*
0	36.93 a	5.067 a	1.666 b	38.63 a	314.5 a	4.859 a	2.628 a	0.1900 a	0.0236 a	0.0167 b	0.00202a	8.350 c
50	23.24 b	2.297 b	2.367 a	20.84 b	136.7 b	3.363 b	1.601 b	0.1673 b	0.0161 b	0.0181 a	0.00157b	11.711 b
100	19.38 c	1.869 c	2.274 a	17.49 c	80.3 c	2.394 c	1.217 c	0.1273 c	0.0114 c	0.0190 a	0.00137c	14.611 a
200	1.49 d	0.161 d	0.693 c	1.35 d	2.84 d	0.243 d	0.196 d	0.0156 d	0.0015 d	0.0026 c	0.00013d	3.092 d
LSD	2.2024	0.3665	0.3092	2.4309	20.78	0.2642	0.161	0.0122	0.0012	0.0011	0.0001	1.8549

GP: Germination Percentage, *GRI*: Germination Rate Index, *MGT*: Mean Germination Time, *GI*: Germination Index, *VI*: Vigor Index, *ShL*: Shoot length, *RL*: Root Length, *FShMW*: Fresh Shoot Mass, *FRM*: Fresh Root Mass, *DShM*: Dry Shoot Mass, *DRM*: Dry Root Mass, *Sh/R*: Shoot/Root ratio

ers in other species consisting of roselle (Galal, 2017), safflower (Kaya et al., 2003; Khodadad, 2011), triticale (Atak et al., 2006), and tomato (Ebrahimi et al., 2014).

There are multiple pretreatment approaches applied and classification based on the priming compounds. These comprise halo-priming, hydro-priming, hormone priming, osmo-priming, solid matrix, hardening, stratification, and thermal shock and humidification. The hydropriming, osmo-priming, halopriming, and hormone priming techniques commonly had been used for seed treatment (Ashraf & Foolad, 2005; Eskandari, 2013; Paparella et al., 2015). Water potential, temperature, seed vigor, priming duration, seed primed storage condition, and plant species are the factors that influence the response of the seed to priming. Therefore, the optimization and fine-tuning of the priming approach is substantial to obtain the best outcome (Ratikanta & Kalipada, 2013).

Hydropriming affect some of the required metabolic processes for germination to happen without germination be accomplished, faster imbibition, further, softening of seed coat led to lesser mechanical prevention as a result of priming (Askari-Nejad & Farahmand, 2012). Seed pretreatment by inorganic salts enhances the enzymes activity engaged in the germination of seed and changes the mobilization of organic substances' to various embryo parts (Aghdaei et al., 2019). Priming for 12 h with 0.5 % Na_2SO_3, 12 and 24 h with 2 % Ca_2CO_3, 12 h with 1 % KCl, 12 h with 1 % Na_2SO_3 and various hydro-prim showed the highest *GP* (Table 3). The highest *GRI* was in 12 h priming with 0.5 % Na_2SO_3, 1 % Na_2So_3 and hydro-prim (Table 3). The *GRI* illustrate the percentage of germination on every day of the germination period. Higher *GRI* values display prompt and high germination (Fuller et al., 2012). Priming with 0.5 % Na_2SO_3 and 1 % Ca_2CO_3 in 12 h revealed the lowest median germination time (*MGT*) (Table 3). The lower *MGT* showed the faster germination of a seeds population (Fuller et al., 2012). The highest *GI* was in 12 h priming with 0.5 % Na_2SO_3, hydro-prim, and 24 h with 2 % Ca_2CO_3 (Table 3). Priming with 12 h 0.5 % Na_2SO_3, hydro-prim, and 24 h with 2 % Ca_2CO_3 showed the highest *VI* (Table 3). In contrast, 12 and 24 h priming with two percentage KCl had the lowest *GRI, GI, VI*, and *GP* (Table 3).

The highest shoot length was in 24 h priming with 2 % Ca_2CO_3 (4.19 cm), 1 % Ca_2CO_3 (3.91 cm), 1 % Na_2SO_3 (3.89 cm), and 12 h priming with 1 % KCl (3.74 cm), there were no statistically significant difference among these treatments. Priming with 24 h with 2 % Ca_2CO_3 showed the highest root length (Table 3). In terms of shoot mass at 24 h priming with 2 % Ca_2CO_3 (0.19 g), 1 % Ca_2CO_3 (0.18 g), and 1 % Na_2So_3 (0.17

g) had the highest shoot mass that there were no statistically significant difference among these treatments. Also, 24 h priming with 2 % Ca_2CO_3 root mass (Table 3). The highest dry shoot mass was in 24 h priming with 1 % Ca_2CO_3, and 2 % Ca_2CO_3, priming with 24 h 2 % Ca_2CO_3, hydro_prim, and 12 h with 2 % Ca_2CO_3 showed the highest dry root mass (Table 3). The positive effects of various priming approaches like priming the tomato seed by potassium nitrate on germination (Lara et al., 2014), hydropriming on sorghum and rice germination percentage (Farooq et al., 2006; Moradi & Younesi, 2009), and salicylic acid on *Solanum melongena* L. seed germination percentage (Mahesh et al., 2017), as well as, enhance of the shoot and root length of cotton (*Gossypium hirsutum* L.) in hydropriming (Shaheen et al., 2015) have been reported.

Plant cell turgor reduction and decrease of shoot and root length caused by salinity stress (Werner & Finkelstein, 1995). Also, it was suggested that salinity stress acts firstly on water uptake. Moreover, Na^+ and Cl^- accumulation prevent the metabolism of cells dividing and expanding (Neumann, 1997), less germination, and even resulting in seed or embryo death. In addition, salt stress leads prevent and decrease the enzymes activities that may be significantly associated with seed germination (Katembe et al., 1998). Priming approaches have been utilized for better germination of seeds in both normal and unfavorable environments (Jisha et al., 2012). The positive and affirmative effects of priming were observed in adverse condition than optimal conditions (Ashraf & Foolad, 2005; Chen & Arora, 2011; Ibrahim, 2016). Suggested priming mechanisms were consisting of the incidence of epigenetic alterations, also the transcription factors accumulation and inactive and inhibition of signaling proteins. These mechanisms are induced against the stress, hence improved resulting in a well and effective defense mechanism (Tanou et al., 2012). Some treatments and techniques were able to develop well establishment of crops in stressful conditions (Soeda et al., 2005; Patade et al., 2009).

The highest *GP* was in 0, 12, 24 hours hydropriming under normal condition. Also, 2 % Ca_2CO_3, 0.5 % Na_2So_3, and 1 % KCl in 12 hours treatment showed high GP under normal condition (Figure 1). enhancing the growth characteristics resulting from seed priming by water soaking could be owing to the impact of seed hydropriming on the fast and sound establishment of plants (Ashraf & Foolad, 2005). The lowest *GP* was observed in 0.5 % KNO_3 in 24 hours pretreatment in all studied conditions, 0.5 % KNO_3 in 12 hours pretreatment under 200 mM salinity condition, as well as, the same results were observed by 24 pretreatments of 1 %

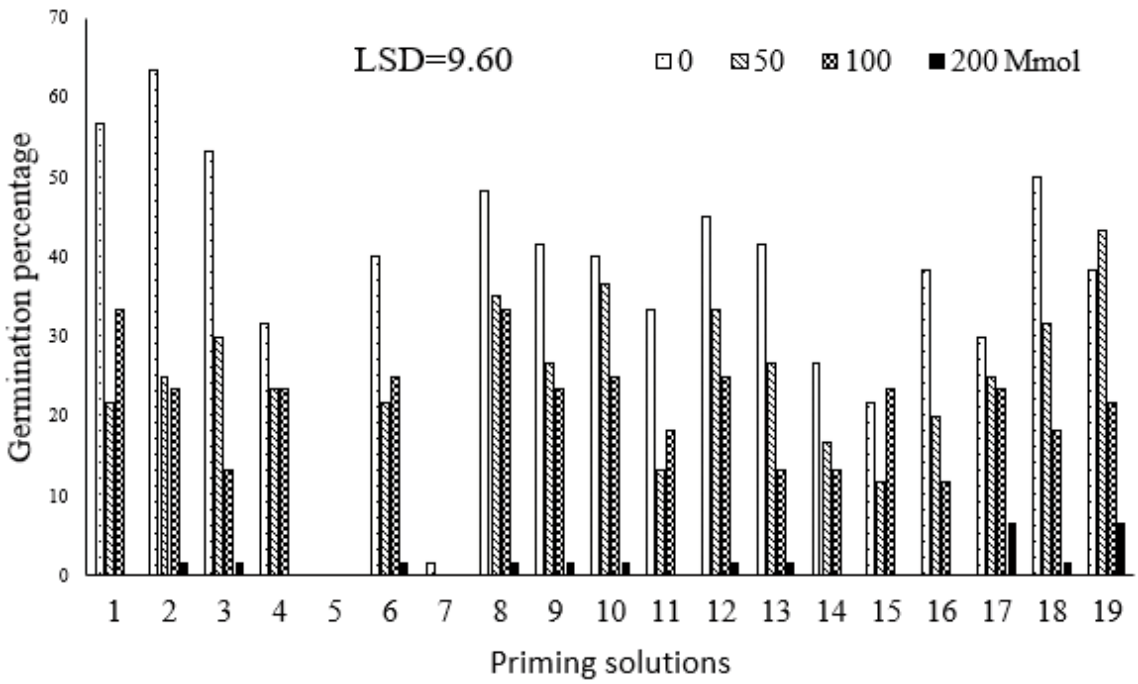

Figure 1: Effect of various priming under four level of salt stress on germination percentage of *Hibiscus sabdariffa* L.
1: non-primed, 2: Hydro 12h , 3: Hydro 24 h, 4: KNO_3_0.5 % 12 h , 5: KNO_3_0.5 % 24 h , 6: KNO_3_1 % 12 h, 7: KNO_3_1 % 24 h, 8: Na_2SO_3_0.5 % 12 h, 9: Na_2SO_3_0.5 % 24 h , 10: Na_2SO_3_1 % 12 h, 11: Na_2SO_3_1 % 24 h, 12: KCl_1 % 12 h, 13: KCl_1 % 24 h, 14: KCl_2 % 12 h , 15: KCl_2 % 24 h, 16: Ca_2CO_3_1 % 12 h, 17: Ca_2CO_3_1 % 24 h, 18: Ca_2CO_3_2 % 12 h , 19: Ca_2CO_3_2 % 24 h

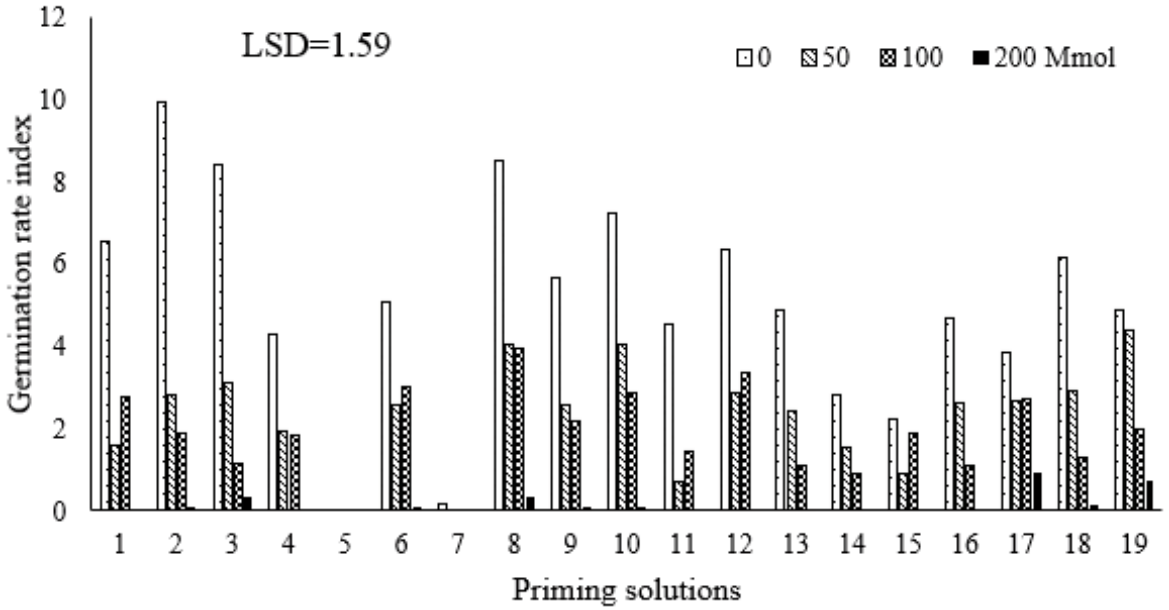

Figure 2: Effect of various priming under four level of salt stress on germination rate index of *Hibiscus sabdariffa* L.
1: non-primed, 2: Hydro 12h , 3: Hydro 24 h, 4: KNO_3_0.5 % 12 h , 5: KNO_3_0.5 % 24 h , 6: KNO_3_1 % 12 h, 7: KNO_3_1 % 24 h , 8: Na_2SO_3_0.5 % 12 h, 9: Na_2SO_3_0.5 % 24 h , 10: Na_2SO_3_1 % 12 h, 11: Na_2SO_3_1 % 24 h, 12: KCl_1 % 12 h, 13: KCl_1 % 24 h , 14: KCl_2 % 12 h , 15: KCl_2 % 24 h, 16: Ca_2CO_3_1 % 12 h, 17: Ca_2CO_3_1 % 24 h, 18: Ca_2CO_3_2 % 12 h , 19: Ca_2CO_3_2 % 24 h

Na_2SO_3, and 2 % KCl under 200 mM salinity condition, and 1 % Ca_2CO_3 in 12 hours pretreatment under 200 mM salinity condition (Figure 1). In saline conditions, 2 % Ca_2CO_3, 0.1 % Na_2SO_3 under 50 mM salinity, and 0.1 % Na_2SO_3 in 100 mM salinity showed the high *GP*. However, there was significantly different with the control condition (Figure 1). The result implied that the best treatments in terms of *GP* were 12 h Hydro, 12 h Na_2SO_3_0.5 %, 24 h Ca_2CO_3_1 %, 24 h Ca_2CO_3_2 % in control, 100 mM salinity, 200 mM salinity, and 50 mM salinity conditions, respectively (Figure 1).

Hydropriming (12 and 24 h), 0.5 % Na_2SO_3, and 1 % Na_2SO_3 in 12 h had the highest *GRI* in the normal condition. In saline conditions, 12 h seed priming by 0.5 % Na_2SO_3 under 50 mM salt stress condition and 12 h priming with 1 % KCl in 200 mM salt stress condition, while there were zero *GRI* in 24 h pretreatment of seeds with 0.5 % KNO_3 and 1 % KNO_3 under salinity conditions, as well as, 1 % Na_2SO_3, 1 % Ca_2CO_3 and 2 % KCl under 200 mM salinity condition (Figure 2). The highest *MGT* was in 1 % Ca_2CO_3 and 2 % KCl with 12 and 24 priming hours under 200 and 100 mM salinity conditions, respectively. Also, there were no significant differences in 24 h priming with 2 % Ca_2CO_3 under 50 mM salinity conditions, 0.5 % Na_2SO_3, hydro-prim and 1 % Na_2SO_3 under 100 mM salinity conditions, and 12 h priming with 1 % Na_2SO_3 and 1 % Ca_2CO_3 in 100 mM salinity condition (Table 4). Similar results were reported by (Farooq et al., 2006) and (Qadir et al., 2011), who reported reducing *MGT* using $CaCl_2$ primed seeds.

Hydropriming (12 and 24 h) had the highest *GI* in normal conditions, and there were no significant differences with hydropriming (control), 0.5 % Na_2So_3, and 1 % Na_2SO_3 in 12 h in normal condition (Table 4). The seeds with twelve-hour priming of 1 % KCl in 100 mM salinity, 0.5 % Na_2So_3, and 0.1 % Na_2SO_3 under 50 mM salinity showed high *GI*, but there were significant differences with the control condition (Table 4). Enhancing the germination rate in treatment seeds can be illustrated through the enhanced synthesis of protein, the less term of metabolism in the germination stage, the influence on cell membrane phospholipids (Ansari et al., 2013), enhance of cell division rate (Taylor & Harman, 1990), and faster absorption of water, better development in these seeds, which all eventually lead to enhancing of seed germination duration. Shahverdi et al. (2017), with priming the stevia seeds, indicated a considerable correlation between the germination percentage enhancement and seed germination improvement factors. It seems that the efficiency of pretreatment of seed is affiliated with the elements like type and concentration of priming compound, duration of seed treatment by compounds (duration of priming).

The 12 h treatment with hydro-prim, 1 % Na_2So_3,

Table 3: Mean comparison of various priming on germination indices and seedling growth traits in *Hibiscus sabdariffa* L.

Prim	%	Time	*GP* (%)	*GRI*	*MGT* (day)	*GI*	*VI*	*ShL* (cm)	*RL* (cm)	*FShM* (g)	*FRM*(g)	*DShM* (g)	*DRW* (g)	*Sh/R*
Hydro	-	Control	27.917	2.7319	1.9104	25.75	143.33	1.9417	1.2058	0.13333	0.015333	0.016375	0.001633	8.833
		12	28.333	3.6903	1.9492	28.167	200.46	2.6833	1.8083	0.13583	0.015417	0.016367	0.001642	7.608
		24	24.583	3.2569	1.7405	25.083	155.67	2.3917	1.625	0.1375	0.017833	0.011717	0.001883	4.772
KNO_3	0.5	12	19.58	2.0139	1.825	18.333	88.29	2.4667	0.775	0.13167	0.013333	0.015667	0.001458	8.277
		24	0	0	0	0	0	0	0	0	0	0	0	0
	1	12	22.08	2.6875	1.7681	22.083	126.83	2.825	1.075	0.125	0.014992	0.015125	0.001333	12.993
		24	0	0	0	0	0	0	0	0	0	0	0	0
Na_2SO_3	0.5	12	29.583	4.2111	1.3682	31.583	184.92	3.3083	0.1	0.135	0.015158	0.018292	0.001367	11.886
		24	23.333	2.6319	2.0141	22.417	160.33	3.5083	1.7417	0.1425	0.012792	0.016933	0.001367	13.181
	1	12	25.833	3.5514	2.0231	24.75	158.67	2.925	1.475	0.12167	0.011342	0.015167	0.001217	10.36
		24	16.25	1.6764	2.3915	13.917	155.67	3.8917	1.9583	0.17417	0.01435	0.013575	0.00105	11.225
KCl	1	12	26.25	3.1625	1.967	25.917	195.5	3.7417	1.525	0.14167	0.013375	0.014875	0.001175	10.414
		24	20.833	2.1208	2.2943	19.667	139	2.725	1.425	0.13167	0.01155	0.01655	0.001325	11.548
	2	12	14.167	1.3125	1.9494	12.75	63.88	2.2833	0.9083	0.10583	0.007083	0.015258	0.000898	13.817
		24	14.167	1.25	2.0431	12.25	67.17	2.2917	1.1083	0.10833	0.008408	0.012975	0.000733	14.143
Ca_2CO_3	1	12	17.5	2.1042	1.6296	17.333	133.24	3.175	1.5575	0.12917	0.016833	0.012475	0.001492	7.633
		24	21.25	2.5486	1.9756	21.167	145.25	3.9167	2.1917	0.18083	0.017833	0.019433	0.001733	12.976
	2	12	25.417	2.6375	2.0192	23.833	189.04	3.1417	1.875	0.1375	0.019417	0.015967	0.0018	6.902
		24	27.5	2.9986	2.2199	26.583	239.38	4.1917	2.9417	0.19	0.02525	0.020867	0.002075	12.033
LSD	5%		4.8001	0.7987	0.6738	5.298	45.289	0.5758	0.3508	0.0265	0.0027	0.0024	0.0003	4.0427

GP: Germination Percentage, *GRI*: Germination Rate Index, *MGT*: Mean Germination Time, *GI*: Germination Index, *VI*:Vigor Index, *ShL*: Shoot length, *RL*: Root Length, *FShM*: Fresh Shoot Mass, *FRM*: Fresh Root Mass, *DShM*: Dry Shoot Mass, *DRM*: Dry Root Mass, *Sh/R*: Shoot/Root ratio

and 1 % KCl had the highest *VI* in normal condition, and there were no significant differences with 0.5 % Na_2SO_3, 1 % Ca_2CO_3, 2 % Ca_2CO_3, and 1 % KNO_3 in 12 h treatments under normal condition, and 0.5 % Na_2SO_3 (12 h priming) in 50 mM salinity condition, 1 % Na_2SO_3, and hydro-prim in 24 h treatment at normal condition (Figure 3). The 12 h treatment with 0.5 % Na_2SO_3 in 50 mM salinity condition, 1 % KCl, 1 % Ca_2CO_3, 1 % Na_2SO_3, and the 24 h treatment with 1 % Na_2SO_3 had the highest shoot length in normal condition. Also, there were no significant differences with 12 h treatment by 1 % KNO_3 under 100 mM salinity, 1 % KNO_3,

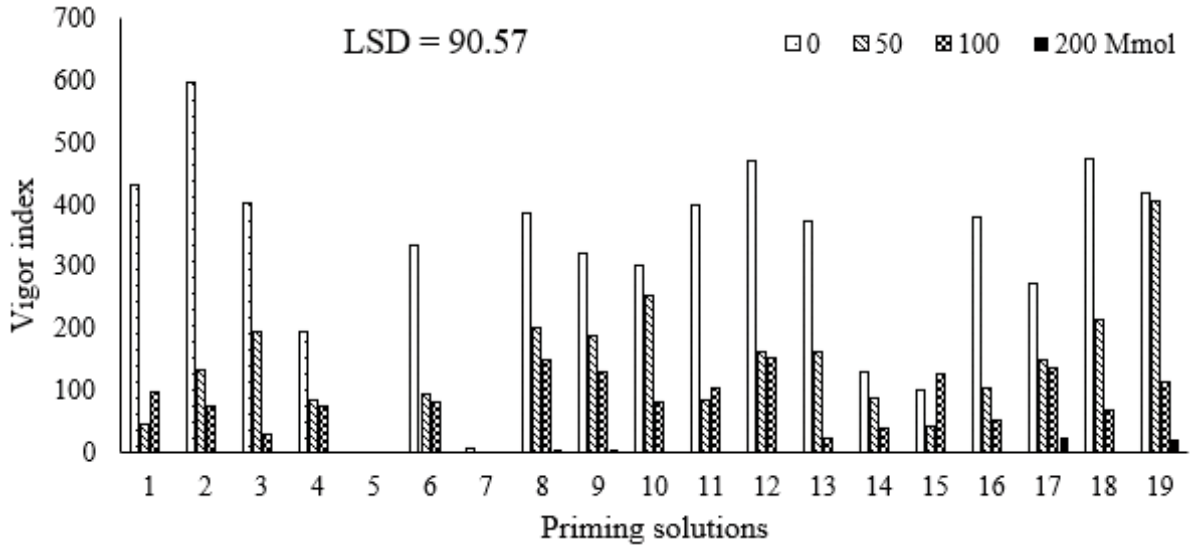

Figure 3: Effect of various priming under four level of salt stress on vigor index of *Hibiscus sabdariffa* L.
1: non-primed, 2: Hydro 12h , 3: Hydro 24 h, 4: KNO_3_0.5 % 12 h , 5: KNO_3_0.5 % 24 h , 6: KNO_3_1 % 12 h, 7: KNO_3_1 % 24 h , 8: Na_2SO_3_0.5 % 12 h, 9: Na_2SO_3_0.5 % 24 h , 10: Na_2SO_3_1 % 12 h, 11: Na_2SO_3_1 % 24 h, 12: KCl_1 % 12 h, 13: KCl_1 % 24 h , 14: KCl_2 % 12 h , 15: KCl_2 % 24 h, 16: Ca_2CO_3_1 % 12 h, 17: Ca_2CO_3_1 % 24 h, 18: Ca_2CO_3_2 % 12 h , 19: Ca_2CO_3_2 % 24 h

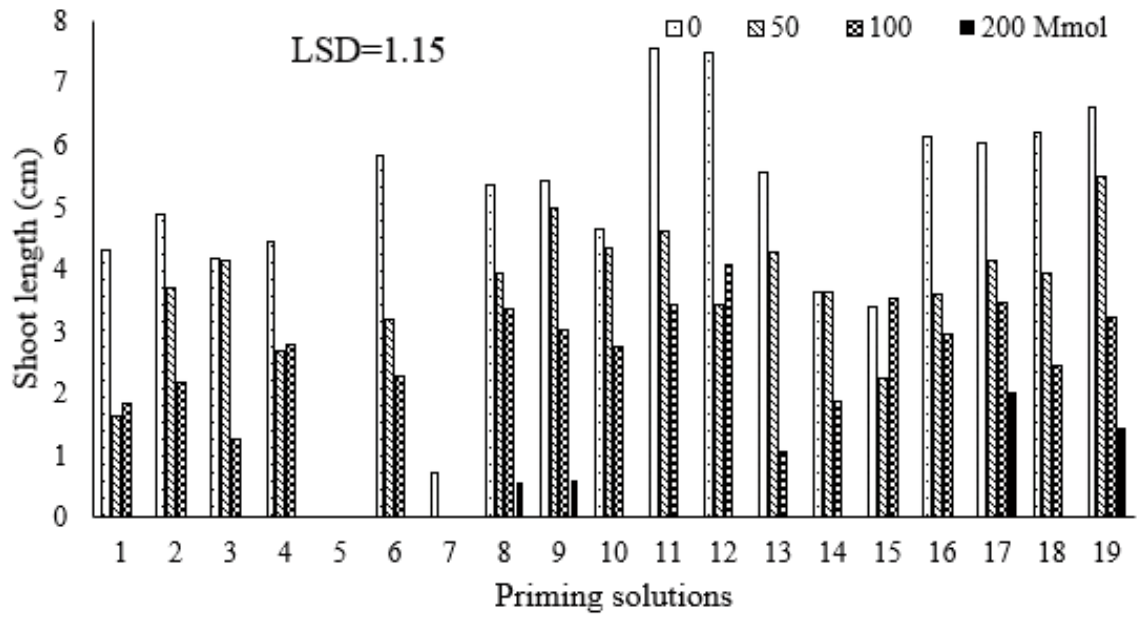

Figure 4: Effect of various priming under four level of salt stress on shoot length of *Hibiscus sabdariffa* L.
1: non-primed, 2: Hydro 12h , 3: Hydro 24 h, 4: KNO_3_0.5 % 12 h , 5: KNO_3_0.5 % 24 h , 6: KNO_3_1 % 12 h, 7: KNO_3_1 % 24 h , 8: Na_2SO_3_0.5 % 12 h, 9: Na_2SO_3_0.5 % 24 h , 10: Na_2SO_3_1 % 12 h, 11: Na_2SO_3_1 % 24 h, 12: KCl_1 % 12 h, 13: KCl_1 % 24 h , 14: KCl_2 % 12 h , 15: KCl_2 % 24 h, 16: Ca_2CO_3_1 % 12 h, 17: Ca_2CO_3_1 % 24 h, 18: Ca_2CO_3_2 % 12 h , 19: Ca_2CO_3_2 % 24 h

2 % Ca_2CO_3 in normal condition, 24 h treatment by 1 % KCl, and 0.5 % Na_2SO_3 in normal condition (Figure 4).

The highest root length was in hydro-prim, 1 % Ca_2CO_3 at 12 h in normal condition, and 0.5 % Na_2SO_3 at 12 h in 50 mM salinity condition, and there were no significant differences with 2 % Ca_2CO_3 at 12 h, 1 % Na_2SO_3, and hydroprim at 12 h in normal condition (Figure 5). Neumann (1995) also reported that salinity could quickly prevent root growth and thus the ability to uptake water and essential mineral nutrition. The 12 h treatment with 1 % Na_2SO_3, in 50 mM salinity condition, 1 % Ca_2CO_3 and 1 % KCl had the highest shoot mass in normal condition. Also, there were no significant differences with 1 % Na_2SO_3 at 24 h, 2 % Ca_2CO_3 in 12 h treatment in normal condition and 1 % KNO_3 in 12 treatment under 100 mM salinity (Table 4).

The 24 h treatment with 1 % Na_2SO_3, 1 % KCl, and the 12 h treatment with 2 % Ca_2CO_3 had the highest root mass in normal condition. Also, there were no significant different with 12 treatment of 1 % Na_2SO_3, 0.5% Na_2SO_3 in normal condition and 1 % Ca_2CO_3 under 50 mM salinity (Table 4). The results showed several priming had high dry shoot mass under various salinity conditions, for instance 2 % Ca_2CO_3, 1 % Ca_2CO_3, 1 % Na_2SO_3, 0.5 % Na_2SO_3, 0.5 % KNO_3, and 1 % KCl illustrated the highest dry shoot mass under 100 mM salinity condition. Besides, 0.5 % Na_2SO_3, 1 % Ca_2CO_3, 0.5 % KNO_3, 2 % Ca_2CO_3, 1 % KCl, 1 % KNO_3 and 2 % KCl had the highest dry shoot mass under 50 mM salinity condition (Table 4). Seed priming by $CaCl_2$, KCl, and NaCl were figured out to be effective in diminishing the negative impact of salinity on wheat via their effects on changing the levels of various plant phytohormones (Iqbal et al., 2006).

The highest dry root length was in hydro-prim, 1 % Na_2SO_3, and 1 % KCl at 24 h, and 1 % Ca_2CO_3 at 12 h in normal condition, also, there were no significant differences with hydro-prim (12 h), 2 % Ca_2CO_3 (12 h), 0.5 % Na_2SO_3 (12 and 24 h), and 1 % KCl (24 h) in 50 mM salinity condition, 0.5 % Na_2SO_3 (24 h), 1 % Na_2SO_3 (24 h), and 1 % KNO_3 (12 h) in 100 mM salinity condition (Table 4). Abdollahi & Jafari (2012) demonstrated that KNO_3 3 % treatment enhanced root length to primary shoot ratio more than NaCl 1 % under saline condition. This enhances the water uptake by the plant that may help the growth development of seedlings in saline conditions. In addition, application of the four potassium nitrate concentrations (0, 0.5, 1, and 2 %) on time to 50 percentage germination, and germination percentage of amaranth seeds revealed that using 0.5 percent of potassium nitrate decreased time to 50 % seed germination (Musa & Lawal, 2015).

The 24 h treatment with 1 % KCl, 1 % Ca_2CO_3, and 2 % Ca_2CO_3 had the highest shoot and root ratio in 100 mM salinity condition. Also, there were no significant differences with 12 treatments of 1 % Ca_2CO_3, 2 % KCl,

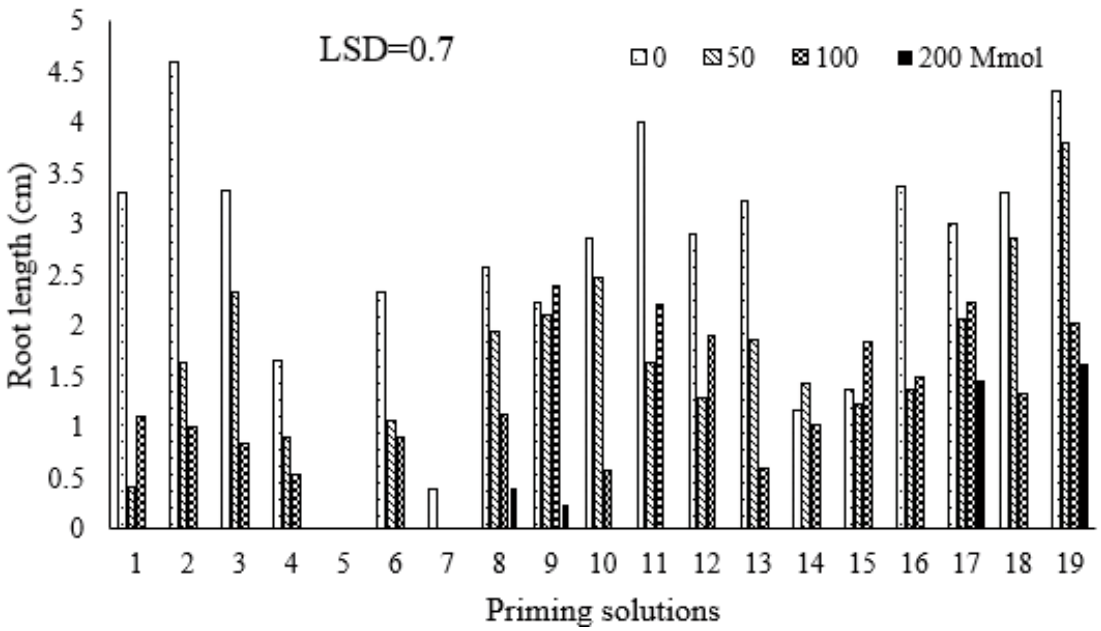

Figure 5: Effect of various priming under four level of salt stress on root length of *Hibiscus sabdariffa* L.
1: non-primed, 2: Hydro 12h , 3: Hydro 24 h, 4: KNO_3–0.5 % 12 h , 5: KNO_3–0.5 % 24 h , 6: KNO_3–1 % 12 h, 7: KNO_3–1 % 24 h , 8: Na_2SO_3–0.5 % 12 h, 9: Na_2SO_3–0.5 % 24 h , 10: Na_2SO_3–1 % 12 h, 11: Na_2SO_3–1 % 24 h, 12: KCl_1 % 12 h, 13: KCl_1 % 24 h , 14: KCl_2 % 12 h , 15: KCl_2 % 24 h, 16: Ca_2CO_3–1 % 12 h, 17: Ca_2CO_3–1 % 24 h, 18: Ca_2CO_3–2 % 12 h , 19: Ca_2CO_3–2 % 24 h.

Table 4: Mean comparison of priming and salinity interaction effect on germination indices and seedling growth traits in *Hibiscus sabdariffa* L.

Prim (%)	Time	salt	*MGT* (day)	GI	*FShM* (g)	*FRM* (g)	*DShM* (g)	*DRM* (g)	Sh/R
Hydro	Control	0	2.05	56.33	0.19	0.020	0.0192	0.0025	7.79
		50	2.85	18	0.19	0.021	0.0209	0.0017	16.36
		100	2.73	28.66	0.14	0.02	0.0253	0.0023	11.17
		200	0	0	0	0	0	0	0
	12 h	0	1.58	68.66	0.15	0.025	0.0227	0.0023	10.07
		50	2.30	23.33	0.21	0.023	0.0213	0.0020	10.52
		100	2.9	19.33	0.17	0.013	0.0213	0.0022	9.82
		200	1	1.33	0	0	0	0	0
	24 h	0	1.49	59	0.19	0.03	0.0196	0.0033	5.90
		50	2.19	28.66	0.21	0.021	0.0155	0.0023	6.71
		100	2.94	10.66	0.14	0.016	0.0116	0.0018	6.46
		200	0.33	2	0	0	0	0	0
KNO_3_0.5 %	12 h	0	1.72	33.66	0.18	0.016	0.0201	0.00196	10.35
		50	2.72	20.33	0.18	0.023	0.0221	0.0022	10.28
		100	2.85	19.33	0.16	0.013	0.0203	0.0016	12.47
		200	0	0	0	0	0	0	0
	24 h	0	0	0	0	0	0	0	0
		50	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0
		200	0	0	0	0	0	0	0
KNO_3_1 %	12 h	0	2.02	40.33	0.21	0.043	0.0189	0.003	6.34
		50	1.94	22	0.19	0.011	0.0216	0.0013	17.68
		100	2.10	24.66	0.096	0.005	0.0198	0.0010	27.94
		200	1	1.33	0	0	0	0	0
	24 h	0	0.66	1.66	0.06	0.001	0.005	0.00053	3.12
		50	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0
		200	0	0	0	0	0	0	0
Na_2SO_3_0.5 %	12 h	0	1.26	55.66	0.20	0.023	0.0189	0.00163	11.57
		50	1.97	35	0.17	0.018	0.0239	0.00206	11.61
		100	1.89	33.66	0.11	0.015	0.0228	0.00126	19.35
		200	0.33	2	0.04	0.003	0.0075	0.0005	5
	24 h	0	1.64	44.66	0.18	0.017	0.0202	0.0018	12.81
		50	2.85	22.66	0.2	0.016	0.0211	0.00203	10.45
		100	2.55	21	0.15	0.015	0.0236	0.00156	15.62
		200	1	1.33	0.03	0.002	0.0027	0.0002	13.83

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Na_2SO_3_1 %	12 h	0	1.36	45.33	0.14	0.016	0.0136	0.002	6.78
		50	3.09	30	0.19	0.018	0.0217	0.00163	13.32
		100	2.63	22.33	0.15	0.009	0.0252	0.00123	21.32
		200	1	1.33	0	0	0	0	0
	24 h	0	2.12	33.33	0.31	0.034	0.0188	0.0021	9.12
		50	4.77	6.66	0.20	0.009	0.0116	0.00073	16.92
		100	2.66	15.66	0.17	0.013	0.0238	0.0013	18.85
		200	0	0	0	0	0	0	0
KCl_1 %	12 h	0	1.66	48	0.27	0.031	0.016	0.0018	8.91
		50	2.70	28.66	0.14	0.010	0.0207	0.00173	12.33
		100	1.83	26	0.15	0.011	0.0228	0.00117	20.40
		200	1.66	1	0	0	0	0	0
	24 h	0	1.89	42.66	0.21	0.025	0.0202	0.00243	8.64
		50	2.66	23.66	0.19	0.015	0.0251	0.00203	12.32
		100	2.61	12	0.12	0.005	0.0207	0.00083	25.22
		200	2	0.333	0	0	0	0	0
KCl_2 %	12 h	0	2.21	25.66	0.15	0.007	0.0162	0.001267	13.56
		50	2.58	15	0.18	0.009	0.0223	0.001467	15.48
		100	3	10.33	0.09	0.0116	0.0224	0.00086	26.21
		200	0	0	0	0	0	0	0
	24 h	0	2.36	19.33	0.15	0.0103	0.0172	0.0012	15.12
		50	3.22	9	0.12	0.0143	0.0173	0.00073	24.05
		100	2.58	20.66	0.16	0.009	0.0174	0.001	17.4
		200	0	0	0	0	0	0	0
Ca_2CO_3_1 %	12 h	0	1.96	38.66	0.23	0.041	0.0172	0.0032	5.33
		50	2.05	20.33	0.14	0.0143	0.0137	0.001	13.67
		100	2.5	10.33	0.13	0.012	0.0189	0.001767	11.51
		200	0	0	0	0	0	0	0
	24 h	0	1.81	31	0.24	0.032	0.0171	0.00213	8.66
		50	2.11	24	0.20	0.009	0.0198	0.0015	13.54
		100	2.13	22.66	0.14	0.019	0.0206	0.00233	8.88
		200	1.83	7	0.13	0.010	0.0201	0.000967	20.81
Ca_2CO_3_2%	12 h	0	1.91	50.66	0.21	0.034	0.0169	0.0026	6.62
		50	2.46	28.66	0.21	0.030	0.0253	0.00246	10.54
		100	3.03	14.33	0.12	0.012	0.0215	0.00213	10.44
		200	0.66	1.66	0	0	0	0	0
	24 h	0	1.879	39.33	0.28	0.034	0.0199	0.0025	7.88
		50	2.45	40	0.22	0.039	0.0196	0.003	6.65
		100	2.21	20.66	0.16	0.014	0.0239	0.0017	14.47
		200	2.33	6.33	0.09	0.013	0.0203	0.0010	19.11
LSD 5 %	-	-	1.34	10.59	0.05	0.005	0.0048	0.0006	8.0855

MGT: Mean Germination Time, *GI*: Germination Index, *FShM*: Fresh Shoot Mass, *FRM*: Fresh Root Mass, *DShMW*: Dry Shoot Mass, *DRM*: Dry Root Mass, *Sh/R*: Shoot/Root ratio

and hydro-prime in 50 mM salinity condition (Table 4). Seed priming is one of the simple, low risk and cost approaches used to cope with the adverse effect of salinity in agricultural lands. The privilege of seed priming or pretreatment in unfavorable conditions have been studied in several crops, instance hot pepper (Khan et al., 2009), tomato (Ebrahimi et al., 2014), pepper (Aloui et al., 2014), lettuce (Nasri et al., 2011), pea (*Pisum sativum* L.) (Naz et al., 2014), and maize (Abraha & Yohannes, 2013). Soil salinity has adverse effects on agriculture productivity. Therefore, agronomic and genetic solutions to enhancing salt tolerance are urgently required. We concluded that applying easy and low-cost techniques such as priming can remarkably increase the germination seed in salinity condition.

4 CONCLUSIONS

Priming is a technique that is capable to improve the performance of seeds in salinity stress conditions. Under saline condition, 24 h 2 % $Ca2CO_3$ had the highest germination percentage (43.3 %) in 50 mM, while 12 h treatment with 0.5 % $Na2SO_3$ (33.3 %) had high germination percentage in 100 mM levels of saline conditions. Also, the highest germination rate index was observed in 0.5 % Na_2SO_3 with 12 h treatment time (4.05 and 3.95 respectively) in 50 and 100 mM levels of saline conditions. There was no geminated seed at 24 h priming by 0.5 and 1 percentage of KNO_3, while priming with 1 % of KNO_3 at 12 h showed good performance in terms of shoot mass trait under saline condition. The result of various priming on studied traits revealed the importance of the type of priming compound and priming duration. The result implied that the best treatments in terms of *GP* were 12 h hydropriming, 24 h Ca_2CO_3_2 %, 12 h Na_2SO_3_0.5 %, 24 h Ca_2CO_3_1 %, in control, 50 mM salinity, 100 mM salinity, and 200 mM salinity conditions, respectively. We suggested performing the same studies with the suitable material at the precise concentration on similar species to determine and understand the reliability and efficiency of the approaches. Also, supplementary research should concentrate on molecular, metabolic, and physiological stimulate with priming agents in salt stress. Moreover, future studies need to assess germination and early seedling growth at the field condition.

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