Effects of Priming on Seed Germination of Wheat (Triticum aestivum L.) Under Salinity Stress

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Abstract

The current study was aimed at evaluating the potential of hydro and aspirin (ASP) priming technique to improve germination of soft wheat seeds (Triticum aestivum L.) under salinity stress. Seeds of two wheat genotypes were first primed and then germinated and grown in saline and non-saline condition (0 and 120mM NaCl) for 8 days. The results showed that priming of seeds in the presence or absence of salinity stress enhanced germination traits. Under saline condition ASP primed seeds were highest in germination percentage (79%) as compared

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to hydro primed seeds (71%) and non- primed seeds (70%). Also shoot and root length were the highest in ASP primed seeds compared to hydro primed and non-primed seeds. The result revealed that priming with ASP resulted in highest seedling dry weight (0.75g) compared to hydro priming (0.67g) and no-priming (0.27g). In addition, seedling vigor index was higher in ASP priming (10) compared to hydro priming (8) and no-priming (7). Although both priming treatments improved germination and seedling performance of wheat under saline and non-saline condition, ASP priming was more effective for germination and seedling performance. These results suggested that priming of wheat seeds with ASP can be used as an effective method to improve seeds germination and seedling performance.

***Keywords**: Seed priming; Aspirin; Triticum aestivum L; Germination; Salinity stress.*

1. Introduction

To meet the need for food security worldwide, wheat production is required to be increased. At present wheat is the most produced food crop globally with almost 30% of the world grain production and 50% of the world grain trade (Akter and Islam, 2017). Amongst the most important factors affecting wheat production in arid and semi-arid regions is salt stress (Munns and Tester, 2008). The toxic effects of specific ions principal in the saline environment disrupt the structure of enzymes, disrupt different metabolic activities, induce ions deficiency, creates nutrient imbalance and causes physiological drought (Bensidhoum and Nabti, 2019). High salt concentration affects plant life from the stage of seed germination to the stage of maturity of plants (Akbarimoghaddam et al., 2011). Salinity stress significantly influences germination of wheat seeds (Ehtaiwesh and Rashed, 2019). Shoot and root growth also reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point.

The problem of salinity has been approached through better management practices and lately different approaches are being employed to enhance salinity tolerance One of these approaches is seed priming to overcome negative effects induced by salinity. Seed priming treatments have previously found to enhance seed germination. The increasing of seed germination, root and shoot length and fresh and dry weights were reported in previous studies (Afzal et al, 2005). A study found that halo-priming and hydro-priming were effective in improving germination and seedling growth of wheat varieties (Afzal et al, 2006). Recent study reported that Moringa leaves extract can be effectively used as priming agents for hard wheat germination and (Ehtaiwesh and Yarboa, 2020). Also recent study on wheat germination under saline condition reported that wheat seeds priming with aspirin successfully improved the negative effects of salinity on germination and seedling growth (Hussain et al., 2018; Djebar et al., 2020). The objective of this study was to evaluate the potential of aspirin (ASP) priming technique to improve germination of Libyan soft wheat cultivars under salinity stress.

Materials and methods

Libyan soft wheat cultivars Sabha and Germa were used in this study, seeds were obtained from Libyan National GenBank in Tajoura. Aspirin obtained from the local pharmacy. Seeds of wheat were sterilized with 3% sodium hypochlorite for 3 minutes, washed twice in tape water and air dried. Then seeds were subjected to three treatments: A non-primed control (NP), hydro (H_2O) and ASP solution (ASP) priming. For priming with H_2O and ASP solution, the seeds were soaked in H_2O and 120 ppm ASP solution for 24 h (Hussain et al., 2018). After that, seeds were washed with tap water and let to dry. A set of 20 wheat seeds were placed in a Petri dishes with Whatman no. 1 filter paper discs; and 10ml of designed saline solution (0 and 120mM NaCl) was added to the correspondent Petri dishes. The filter paper was moisturized on daily basis till the end of experiment and filter papers were changed once in every two days to prevent salt accumulation. The unprimed seeds which moisturized with tape water instead of NaCl solution saved as absolute control for the experiment. Seeds were considered germinated when both shoot, and root extended more than 2 mm from the seed (Islam et al., 2012).

Germination traits

Germination percentage (*G %*) was expressed following (Nasri et al., 2011).

$$G\% = (NSG/TNSS)X100$$

Where *NSG* is the number of seeds germinated at the end of the experiment. *TNSS* is the total number of seeds sown.

The germination speed (GS) was calculated as following (Rubio-Casal et al., 2003).

$$GS = n1/d1 + n2/d2 + n3/d3 + \cdots$$

Where n_1 is the number of seeds germinated in day one of sowing, t_1 is the number of days taken for germination from day of sowing.

Mean daily germination (MDG) was calculated as following (Gairola et all., 2011).

$$MDG = TNGS/TNDG$$

Where *TNGS* is total number of germinated seeds and *TNDG* is total number of days taken for final germination.

Seedling growth

Morphological traits viz., shoot and root length and fresh and dry weight were subsequently measured from 4 uniform seedlings from each treatment. The length from the seed to the tip of the root and leaf blade was calculated to measure the root length and shoot length, respectively. The fresh weight of shoot and root was recorded using a weighing balance, and then dried in an oven at 50 °C till it attains stable weight. After that, shoot and root dry weights were recorded. Using the morphological traits, the salinity tolerance index (STI) and Seedling vigor index (SVI) were calculated. The following formula was used to calculate STI (Tsegay, and Gebreslassie, 2014).

STI = (SdwTrt/ SdwCon) x 100

Where *SdwTrt* is dry weight of seedling from priming treatment, *SdwCon* is dry weight of seedling from control treatment. Also the following formula was used to calculate SVI (Majid et al., 2013).

SVI = (SL x G%) x 100

Where *SL* is seedling length and *G%* is germination per cent.

Statistical analysis:

The experimental design was a randomized complete design with four replications. Data were analyzed using GLM procedure in statistical software SAS 9.4 for mean and standard error estimation. Separation of means was carried out using the LSD test ($P < 0.05$).

Results

The P-values for germination and seedling growth traits are presented in table 1. The independent effects of priming, salinity and genotype were highly significant (P < 0.0001) for almost all germination and seedling growth traits included in this study, but mean daily germination was significant (P< 0.0007) (Table 1). Interaction effects of priming x salinity was highly significant (P < 0.0001) for all germinate traits and for most of seedling growth trait except for shoot and root length, which were significant (P< 0.01). The interaction effect of priming x genotype was highly significant (P < 0.0001) for root length and seedling vigor index, and significant (P< 0.05) for all other germination and seedling growth traits. The interaction of salinity x genotype was highly significant (P < 0.0001) for seedling dry weight, seedling vigor index and salinity tolerance index traits, and was significant (P< 0.05) for all other germination and seedling growth traits. The interaction effect of priming x salinity x genotype was significant (P < 0.05) for all seed germination and growth related traits excluding seedling fresh weight, which was non-significant (Table1).

Table 1. Probability values of effects of priming (P), salinity (S), P x S interaction, genotype (G), P x G interaction, S x G interaction and P x G x S interaction on various germination and seedling growth traits

	Variables						
Traits	Priming (p)	Salinity (S)	P x S	Genotype (G)	P x G	S x G	P x G x S
Germination percentage (%)	<.0001	<.0001	<.0001	<.0001	0.0498	0.0053	0.0498
Mean daily germination	<.0001	<.0001	<.0001	0.0007	0.0340	0.0069	0.0464
Germination Speed	<.0001	<.0001	0.0001	<.0001	0.0440	0.0216	0.0440
Shoot length (cm)	<.0001	<.0001	0.0065	<.0001	0.0302	0.010	0.0346

Variables							
Traits	Priming (p)	Salinity (S)	P x S	Genotype (G)	P x G	S x G	P x G x S
Root length (cm)	<.0001	<.0001	0.0012	<.0001	<.0001	0.0004	0.0207
Seedling fresh weight (g)	<.0001	<.0001	<.0001	<.0001	0.1227	0.7381	0.0587
Seedling dry weight (g)	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	0.0147
Seedling vigor index	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0453
Salinity tolerance index	<.0001	<.0001	<.0001	<.0001	0.0047	<.0001	0.0325

Results of the independent effect of priming strategy on germination and seedling growth traits (Tables 2). All germination and seedling traits were increased with both priming strategies. Plus, ASP priming was more effective in improving germination and seedling growth under saline condition as compared to H_2O priming.

Table 2. The main effect of different priming strategy on seed germination and seedling traits of two wheat genotypes. Data are averaged across two genotypes, four replications of each genotype and two salinity levels. Means was estimated using the GLM procedure in SAS.

Traits	No-priming	Hydro-priming	ASP priming
Germination percent (%)	80^{c*}	84^{b}	87^{a}
Mean daily germination	4.6^{b}	6.8^{a}	7^{a}
Germination Speed	7.3^{c}	7.8^{b}	8^{a}
Shoot length (cm)	8.4^{c}	9^{b}	9.6^{a}
Root length (cm)	5.7^{b}	6.1^{a}	6.2^{a}
Seedling fresh weight (g)	1^{c}	1.2^{b}	1.6^{a}
Seedling dry weight (g)	0.5^{c}	0.6^{b}	0.8^{a}
Seedling vigor index	12^{c}	13.2^{b}	14.2^{a}
Salinity tolerance index	63^{c}	85^{b}	106^{a}

[*]Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Results of the independent effect of salinity on germination and seedling growth traits are presented in (Tables 3). All germination and seedling parameters decreased under saline condition (120 mM NaCl). The data on table 3 clearly showed the decreasing of all parameters.

Table 3. The main effect of salinity on seed germination and seedling traits of two wheat genotypes. Data are averaged across two genotypes, four replications of each genotype and three priming strategy. Means was estimated using the GLM procedure in SAS.

Traits	0 mM NaCl	120 mM NaCl
Germination percent (%)	97[a*]	71[b]
Mean daily germination	8.6[a]	3.6[b]
Germination Speed	9.6[a]	5.9[b]
Shoot length (cm)	10.6[a]	7.4[b]
Root length (cm)	8.8[a]	3.3[b]
Seedling fresh weight (g)	1.9[a]	0.7[b]
Seedling dry weight (g)	0.8[a]	0.4[b]
Seedling vigor index	18.7[a]	7.6[b]
Salinity tolerance index	112[a]	57[b]

[*]Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

The results of the independent effect of genotype on germination and growth related traits are presented in (Tables 4). Under non-saline conditions (0 mM NaCl) both genotypes showed no considerable differences in all germination and seedling traits. Nevertheless; both genotypes responded differently to salinity stress and priming strategy.

the wheat genotype Sabha showed to be more tolerance to salinity than Germa genotype as indicated by reduced germination and seedling growth traits.

Table 4. The main effect of genotype on seed germination and seedling traits of two wheat genotypes. Data are averaged across two salinity levels, three priming strategy and four replications. Means was estimated using the GLM procedure in SAS.

Traits	Sabha	Germa
Germination percent (%)	85[a*]	82[b]
Mean daily germination	6.4[a]	5.9[b]
Germination Speed	7.9[a]	7.6[b]
Shoot length (cm)	9.4[a]	8.6[b]
Root length (cm)	6.3[a]	5.7[b]
Seedling fresh weight (g)	1.4[a]	1.1[b]
Seedling dry weight (g)	0.7[a]	0.5[b]
Seedling vigor index	14[a]	12.5[b]
Salinity tolerance index	94[a]	75[b]

[*] Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

The result showed that salinity stress reduced germination traits of wheat seeds under saline condition (120 mM NaCl) as compared non-saline condition (0 mM NaCl). However, both seed priming strategies improved germination traits of both wheat genotypes as compared with no-priming treatment. Butt ASP seed priming strategy was more effective and increased the attributes of germination traits compared with control and hydro-priming (Figure 1).

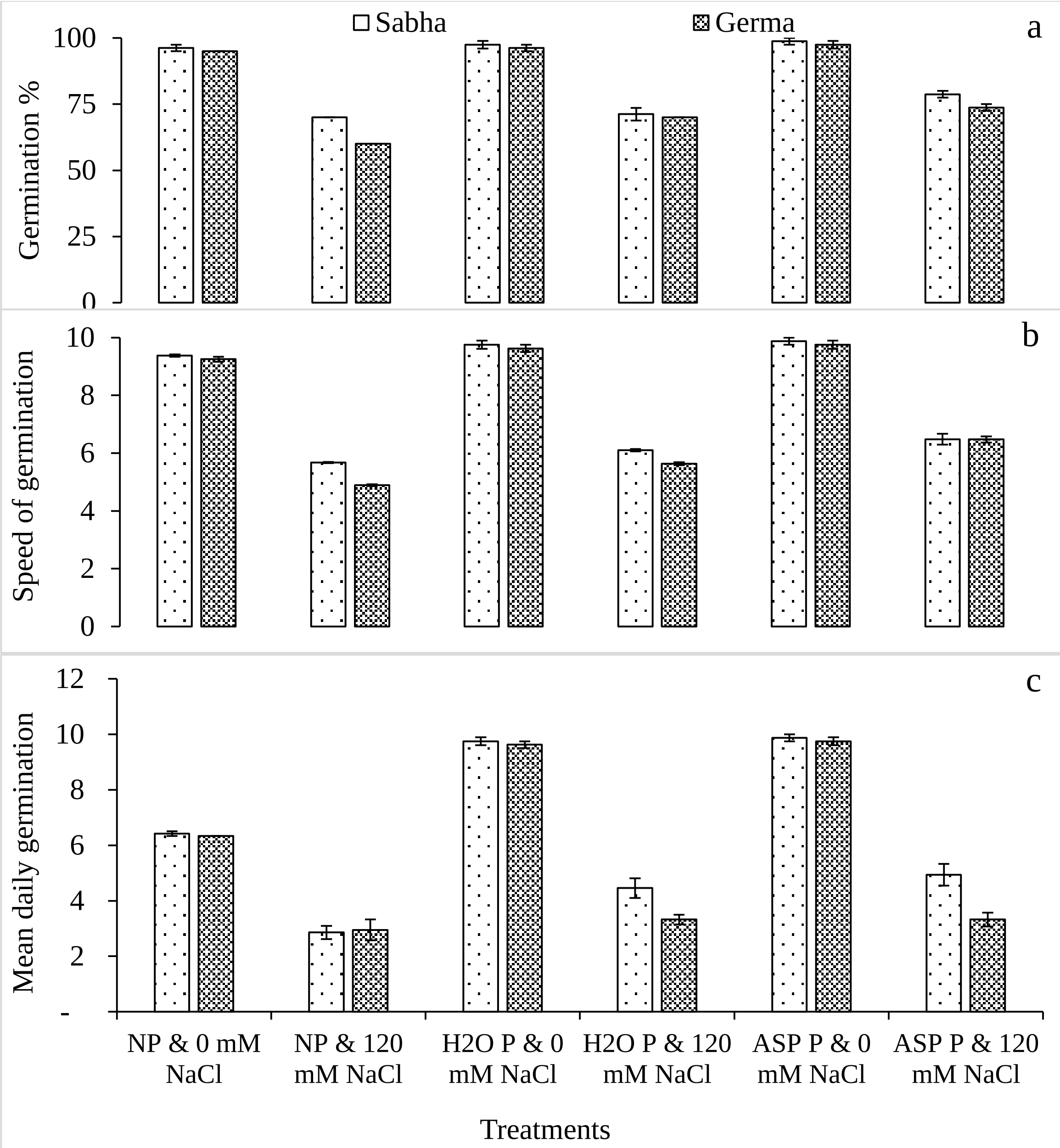

Figure 1. The effects of different priming strategies on (a) germination percentage, (b) speed of germination and (c) mean daily germination of two wheat genotypes. Vertical lines on top of bars indicate standard error of means (n = 4).

In addition, salinity stress effected plant growth and seedling performance, which indicated by reduced shoot and root length. the result evidently showed a reduction in shoot and root length. However, seed priming treatments improved shoot and root length under salinity stress as compared with non-primed seeds (Figure 2a and b).

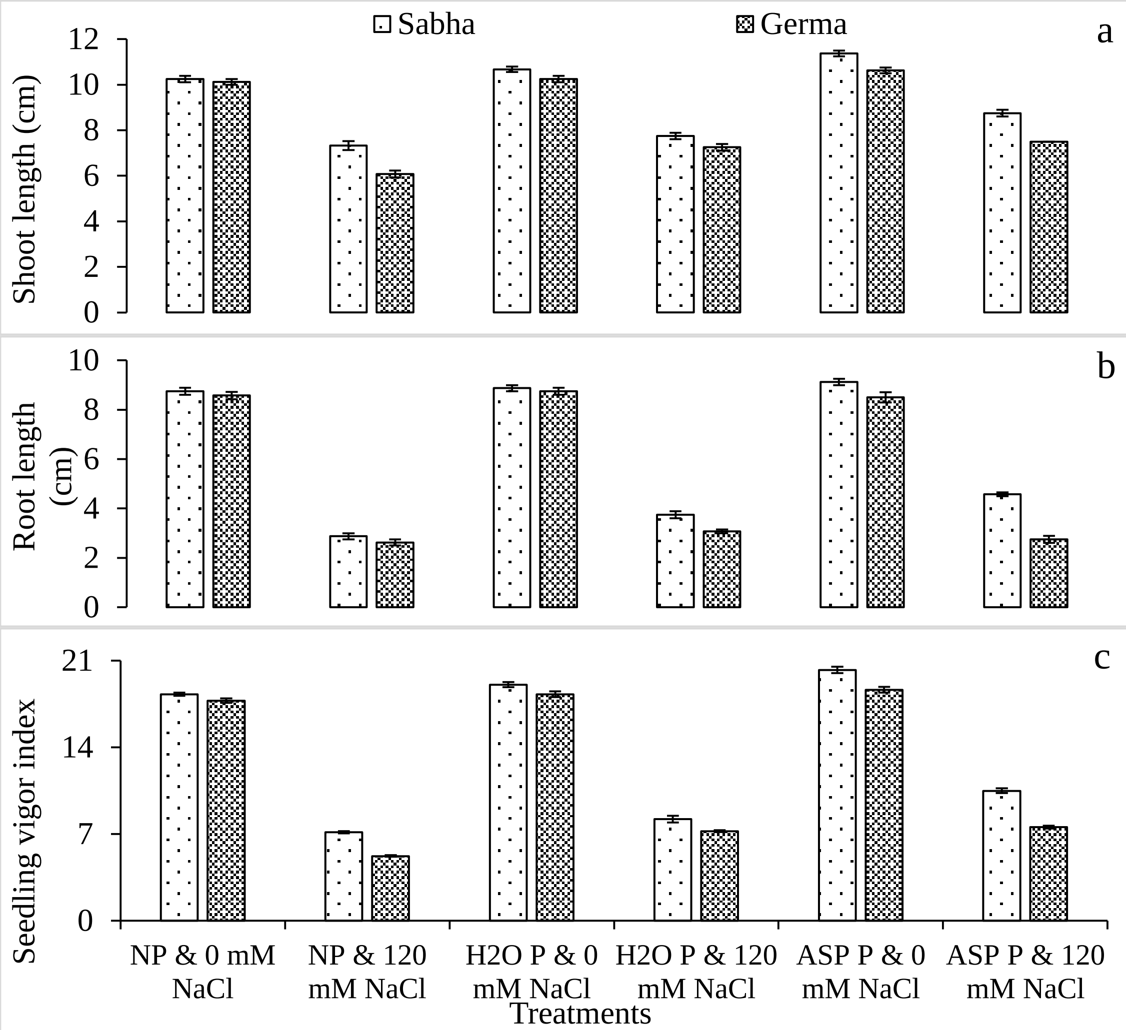

Figure 2. The effects of different priming strategies on (s) shoot length, (b) root length and (c) seedling vigor index of two wheat genotypes. Vertical lines on top of bars indicate standard error of means (n = 4).

Furthermore, due to priming treatments seedling vigor index was also increased under saline condition as compared with untreated seedlings (Figure 2c). Nevertheless; ASP seed priming was more effective and increased the attributes of seedling vigor index with respect to control and with hydro-priming. Remarkably; the result indicated that under salt stress, the seedling fresh and dry weight were reduced (Figure 3a, b) and seed priming significantly improved those seedling traits. Yet, priming with ASP was the most effectiveness with regard to hydro and non-priming treatments (Fig 3 a & b).

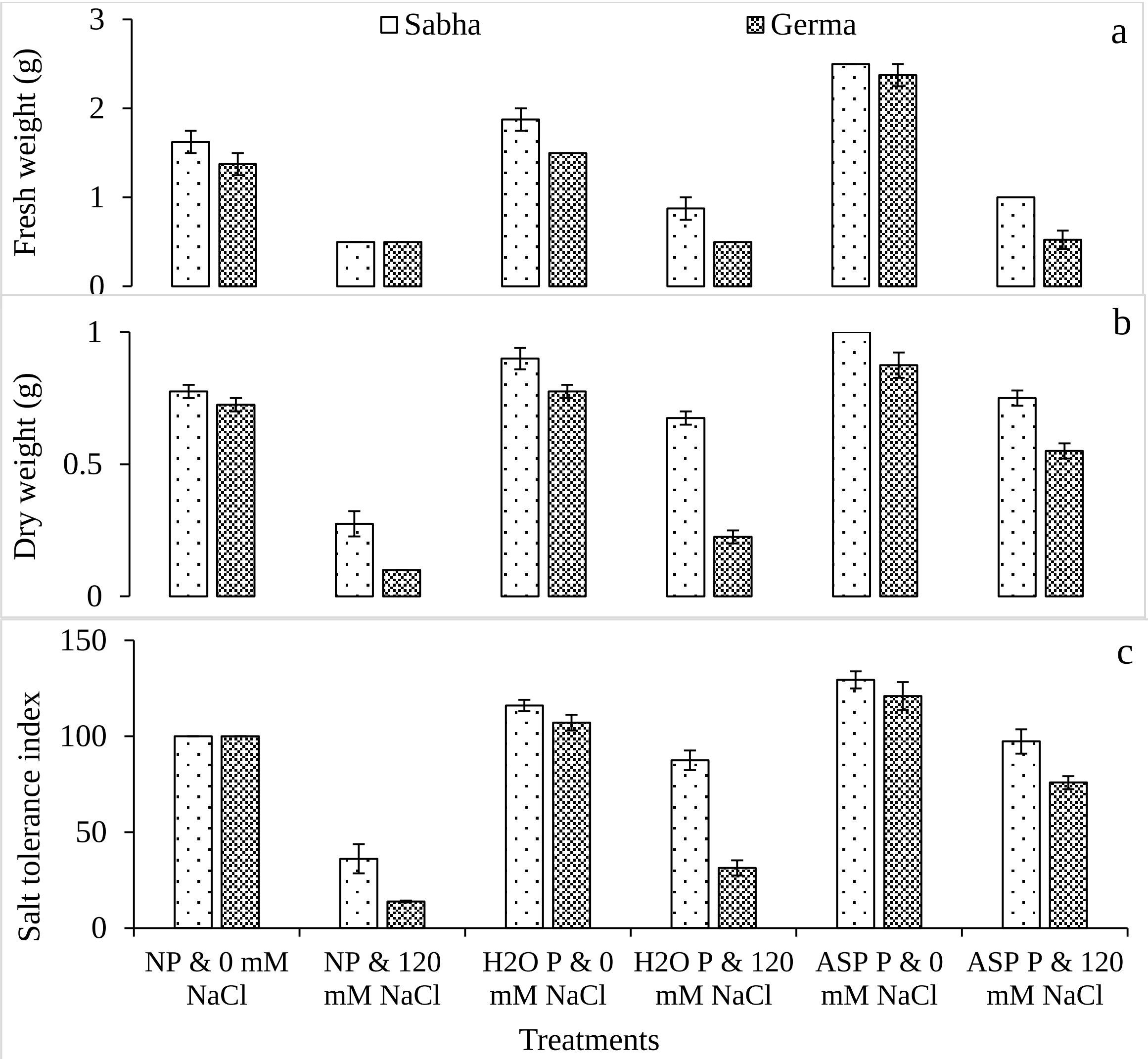

Figure 3. The effects of different priming strategies on (a) seedling fresh weight, (b) seedling dry weight and (c) salinity tolerance index of two wheat genotypes. Vertical lines on top of bars indicate standard error of means (n = 4).

Also, the result showed that salinity tolerance index was significantly decreased by salinity stress (Fig 3c). Nonetheless, priming improved salinity tolerance index as compared with non-primed seeds.

The results regarding salinity tolerance index (STI) of different wheat genotypes showed that genotypes Sabha was more tolerant to salinity stress than Germa genotype.

Discussion

The study found that the germination traits of wheat under saline condition (120 mM NaCl) is strongly affected. However, priming treatment increased germination traits, which to some extent exceeded the rate observed in the non-primed seeds. Under abiotic stresses, improved crop performance through pre-sowing treatments depends on compounds used for priming but all of them are involved in initiating pre-germination metabolism of seeds (Lutts et al., 2016; Ginzburg and Klein, 2019). In seeds that germinated under salinity stress conditions (120mM NaCl) pretreatment with ASP avoided the inhibition of germination observed following salt stress. This finding was corresponding to previous study (Gebreegziabher and Qufa, 2017; Tabassum et al., 2017). Figure 2 shows that seedling length were reduced due to salinity stress, but priming treatments caused stimulation of seedling growth whose values increased especially in seeds primed with ASP. This could be due to the increased level of cell division of shoot and roots. The same conclusion was found in earlier studies (Ali et al., 2017; Hussain et al., 2018; Khan et al., 2019). Also the results showed that salinity effected fresh and dry weight and priming techniques were effective in elevating the injury caused by salt stress. Similar result was obtained in other studies (Feghhenabi et al., 2020; Ghafoor et al., 2020). The increased fresh and dry weight of wheat seedling could be due to the effects of pretreatment with ASP and H_2O. The results indicated that germination percentages, vigor index increased by presoaking of seeds in ASP solutions. This finding agreed with early study (Dolatabadian et al., 2009). This is maybe due to the ability of seed priming treatment to activate the metabolic processes within the seeds to get early emergence even under saline conditions, which was in agreement with earlier studies (Ibrahim. 2016; Saddiq et al., 2019; An et al., 2020). Moreover, results suggest that ASP pretreatment could induce some mechanisms involved in resistance or adaptive responses of seeds which is agreed with (Shatpathy et al., 2018; Soliman et al., 2018).

Conclusion

The purpose of the study was to study the character of changes in germination and seedling growth induced by ASP and H_2o in wheat germination and growth under salt stress conditions. The result conclude that the injuries caused by salinity stress to wheat seeds during germination and early seedling stages were alleviated and the salt-tolerance of wheat were raised by aspirin and hydro priming. In fact, ASP priming treatment was better than hydro priming as it reduced the damaging action of salinity stress on embryo growth and enhanced growth processes; subsequently it may be effective technique for the enhancement of seed germination in saline effected soil.

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