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Germination of the tomato (*Lycopersicon esculentum* Mill.) in response to salt stress combined with hormones

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Abstract

Abstract

Salt stress is a major environmental constraint most limiting plant productivity. Seeking salt-tolerant crops requires an examination of the behavior of the plant development including seed germination stage. The effects of NaCl stress (100 mM) combined or not with different solutions of phytohormones ABA (0.005 mM), GA_3 (0.005 mM) and SA (0.5 mM) on germination of two processing tomato cultivars named Rio Grande (Rg) and Imperial (Ip) were investigated. Seeds were subjected to salt stress, combined or not with hormones, at two stages of development (48 and 96 hours). The results shows that the germination rate and germinate value of the two processing cultivars of tomato were influenced by the different treatments. On the other hand, salicylic acid as GA_3 attenuate the effect of NaCl. Instead, the middle enriched with ABA inhibits seed germination. Moreover, the tested stress conditions had shown a significant variability in the germination and handling between the two germination stages. Nevertheless, the effect of GA_3 and SA on the germination of two processing tomato varieties to improve the inhibitory effect of ABA and salinity was discussed.

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Introduction

Salinity and drought are the main constraints that limit agricultural production in the world and in particular in arid and semi-arid regions. In many crops, the seed germination and early seedling growth are the most sensitive stages to environmental stresses, such as salinity (Foolad, 2004). However, plant species differ in their sensitivity or tolerance to salt stress (Cony and Trione, 1998). NaCl in the seed middle cause the inhibitory effects on germination (Kaveh *et al.*, 2011), significantly reduces the rate and the final percentage of germination (Liopa-Tsakalidia *et al.*, 2012), also, it retards the development of the plant and reduces the yield of crops (Iqbal and Ashraf, 2007). However, these negative effects on the seeds germination can be mitigate by phytohormones (Nasri *et al.*, 2012).

According Wang *et al.* (2001), in response to salinity, abscisic acid (ABA) and jasmonic acid (JA) increase, while the indole-3-acetic acid (IAA) and salicylic acid (SA) decrease. Egamberdieva, (2009) reports that the exogenous application of plant growth regulators (PGRs), produced some benefit in alleviating the adverse effects of salt stress and they improve germination, as gibberellins (Afzal *et al.*, 2006), auxins (Khan *et al.*, 2004), cytokinins (Gul *et al.*, 2000).

The phytohormones abscisic acid (ABA) controls various aspects of plant growth and development (Hirayama and Shinozaki, 2007). During vegetative growth, one of the major roles of ABA is to mediate adaptive responses to various environmental stresses, such as drought, high salinity (Javid *et al.*, 2011). However, an exogenous supply of ABA inhibits the germination and accumulates in plants in response to salt stress (Jakab *et al.*, 2005). This inhibition is, in part, due to a sharp drop of gibberellic acid (GA) during imbibition (Atia *et al.*, 2009).

Gibberellins (GAs) are endogenous plant growth regulators, stimulates the seed germination (Gupta and Chakrabarty, 2013). Liopa-Tsakalidia *et al.* (2012) confirmed that an exogenous supply of GA_3 in the seed middle increases the germination percentage and the seedling growth, and provided an interesting approach to counter the salt effects. In a saline environment for seeds with germination were inhibited can be grown with applied GA_3 (Iqbal and Ashraf, 2013).

Salicylic acid (SA) is a plant phenolic compound, it is used as a growth regulator (Agamy *et al.*, 2013), it promotes various physiological processes, such as germination, growth, photosynthesis, transport and uptake of solutes, and finally, it induces anatomical leaf and chloroplast structural changes (Sakhabutdinova *et al.*, 2003). Horvath *et al.* (2007), showed that pretreatment of seeds with low concentrations of salicylic acid (SA) improves the tolerance to abiotic stresses from several plant species, through the strengthening of antioxidant capacity.

Recently, many results shows the importance of the SA to determine the plants sensitivity to salinity, especially during the seeds germination; examples *Hordeum vulgare* (El-Tayeb, 2005), *Zea mays* (Günes *et al.*, 2007), *Phaseolus vulgaris* (Palma *et al.*, 2009). Experiments conducted to determine the concentration of salicylic acid to bring the germination meddle in order to improve salt tolerance of plants. El-Tayeb (2005) had confirmed that tolerance to salinity increases for barley grains after being soaked in a solution of 1 mM SA before planting. Shakirova *et al.* (2003) applying a concentration of 0.05 mM SA, shows that growth of was improved in saline. These reports shows that the SA had an effect on the induction of tolerance to stress that depends on the planted plant and the SA concentration. El-Mergawi and Abdel Wahed (2004) confirmed that SA effect on the physiological process is variable; it stimulates certain processes or inhibits others, depending on its concentration, the cultivated species and environmental conditions. The present work was to investigate the effect of GA_3 and SA on the germination of two processing tomato varieties to improve the inhibitory effect of ABA and salinity.

Materials and methods

Plant materials

Two processing tomato varieties (*Lycopersicon esculentum* Mill. *cvs* "Rio Grande (Rg) and Imperial (Ip)") were used as plant material for this experience.

Germination experiment

Seeds were treated for 10 min in 50 % of sodium hypochlorite solution, then washed and rinsed with distilled water. For each variety, 30 seeds were sown in a Petri dish 9 cm in diameter, containing a double layer of sterile filter paper. Then, dishes were moistened with equal amounts of 5 ml consecutively water distilled and various hormonal solutions only or mixed.

NaCl and hormones treatments

The treatments were designed from the following solutions: (a) H_2O (control), (b) 0.005 mM ABA, (c) 0.005 mM GA_3, (d) 0.5 mM SA, (e) 100 mM NaCl. The Petri dishes were placed in oven at 25°C. Each treatment was repeated three times. Finally, counting seeds was carried out at 24 h intervals, to determine the germination rate and germination value, with or without NaCl. Then, observations were carried on two stages of seed germination, 48 h and 96 h of the seedlings.

Measured parameters

Germination rate

The germination rate (G %) is expressed as the ratio of the number of germinated seeds on the total number of seeds. G = 100% (XT / N) where XT is the total number of germinated seeds and N is the total number of seeds germinated.

Germination value

The germination value (VG) as integrated quality seed extent, had used by several specialists seed (Djavanshir and Pourbeik, 1976). Its formula is as follows:

$$GV = \left(\Sigma \frac{SDG}{N}\right)\left(\frac{GP}{10}\right)$$

Were GV: Germination Value; SDG: GP: Germination Percentage; Speed Daily Germination; obtained by dividing the percentage of germination accumulated by the number of days since sowing.

∑SDG: total obtained by aggregating the various SDG determined from daily counts.

Statistical analysis

The data collected was transformed to normalize before analyzed using analysis of ANOVA and the mean comparison using the Newman-Keuls test ($P< 0.05$). Statistical analysis were conducted using Statistical Analysis System STATSTICA 10.1 version (Stat Soft, Inc. France).

Results

Combined effects of ABA, GA_3, SA and NaCl on the germination rate

After 48 hours of sowing

48 hours after the sowing, the germination of *Lycopersicon esculentum* Mill. was influenced by the presence of phytohormones in the middle of the seed (Fig. 1).

Both varieties of Rg and Ip tomato unregistered respectively under the effect of GA_3/AS (73.7 and 70.03%), salicylic acid (70 and 66.7%) and GA_3 (63.33 and 60%) compared to the control which posted (70.03 and 70%). However, germination inhibited by ABA.

Except in meddle supplemented with GA_3 or salicylic acid unregistered successively (9.96 for Rg and 10% for Ip) and (10 for the two cultivars).

Moreover, salt stress (100 mM NaCl) stimulated germination of tomato (Fig. 1).

Since a maximum of germination obtained by treating SA/NaCl (73.33 and 70%). However, these rates have fallen under the influence of GA_3/NaCl and only NaCl, which marked germination with a similar (60 and 56.7%) respectively Rg and Ip, finally the ABA/NaCl treatment inhibited germination (Fig.1).

These observations showed a highly significant effect between treatments (Table 1).

Table 1. ANOVA of normalized rate of germination after 48 hours.

Source of variance	df	F	P
Variety	1	2.80	ns
Treatment	10	298.35	***
Variety*Treatment	10	0.244	ns
Error	50		

df: degree of freedom, F: coefficient of Fisher-Snedecor (test at level 5 %), ns: non-significant, * P< 0.05, **: P< 0.01, *** : P< 0.001.

The study of the comparison of means using the Newman-Keuls test at 5%, mean of four groups have emerged. The group "a" (control, SA/NaCl, SA/GA_3 and SA) is significantly higher than "b" (GA_3, GA_3/NaCl and NaCl), it is significantly higher than "c" (ABA/GA_3 and ABA/SA), finally, the group "d" (ABA alone or with NaCl) gave no germinated seed, this group is significantly lower than the three groups mentioned above (Fig. 1).

After 96 hours of sowing

The observation of germination after 96 hours in both varieties of *Lycopersicon esculentum* Mill. had rebounded significantly (Fig. 2). However, control seeds registered a germination rate respectively for Rg and Ip (97.8 and 94.4%). Similarly, germination gave similar numbers of relatively under the effect of SA/GA_3 (98.9 and 95.6%) and SA (96.7 and 93.3%) and GA_3 (95.6 and 92.2%) of these four treatments form the group "a" (Fig. 2).

In addition to that, the data showed that the saline constraints 100mM NaCl-induced led to a gradual reduction in the percentage of germination with the prolongation of germination time compared to the control (Fig. 2). This last registered rate above 94% which is significantly higher than the group "d" of stressed seeds with NaCl (100 mM) significantly match the lower (70 and 66.7%) respectively for Rg and Ip. Supplemented NaCl phytohormones in the middle, germination is progressing under the GA_3/NaCl effect (80.03 and 73.04%) respectively for Rg and Ip, these rates were changing more workplace SA/NaCl, where respectively Rg and Ip express (89.9 and 86.6%).

Finally, the two varieties had their germination inhibit under effect ABA alone or with NaCl. However, the ABA associated with GA_3 or SA showed a weakness response seeds, which manifested itself with germination rates varying between (10 for both varieties) or (16.7 and 14.4%) respectively for Rg and Ip. At this time of germination, a highly significant difference was observed between the different treatments (Table 2).

Table 2. ANOVA of normalized rate of germination after 96 hours.

Source of variance	df	F	P
Variety	1	8,74	**
Treatment	10	781,82	***
Variety*Treatment	10	0,44	ns
Error	50		

df: degree of freedom, F: coefficient of Fisher-Snedecor (test at level 5 %), ns: non-significant, * P< 0.05, **: P< 0.01, *** : P< 0.001.

Combined effects of ABA, GA_3, AS and NaCl on the germinate value

After 48 hours of sowing

Concerning the effects of combined ABA, GA_3, AS and NaCl on the germinate value after 48 hours of the sowing, the results shown clearly that the germinate value of the tomato expressed no value under the ABA and ABA/NaCl effect (Fig. 3). In contrast, the addition of salicylic acid and gibberellic acid to the ABA, helped raise seed dormancy, however, in both ABA/SA and ABA/GA_3, Rg and Ip respectively have expressed a germinate value (2.5 and 2.65) and (1.65 and 1.6).

The hormonal effect was felt in seeds exposed to GA_3 presenting data (60.2 and 53.9) and SA (73.8 and 66.9) respectively for Rg and Ip. These values increase as a result of the GA_3/SA association which displays (91.01) for Rg and (73.83) for Ip. These data presented in table 3 shows a highly significant between treatments.

Table 3. ANOVA of germinate value after 48 hours.

Source of variance	df	F	P
Variety	1	25,88	***
Treatments	10	245,62	***
Variety*Treatment	10	8,136	***
Error	50		

df: degree of freedom, F: coefficient of Fisher-Snedecor (test at level 5 %), ns: non-significant, * P< 0.05, **: P< 0.01, *** : P< 0.001.

Also, the treatment of 100 mM of NaCl affected the germinate value of the tomato, both Rg and Ip varieties have presented respectively (54.1 and 48.22), these values are displayed under GA_3/NaCl effect. However, the SA/NaCl treatment gave a maximum value of 80.72 by Rg and 73.59 by Ip. However, these data are still lower than the control (88.08 and 54.25) successively by Rg and Ip.

Table 4. ANOVA of germinate value after 96 hours.

Source of variance	df	F	P
Variety	1	2,42	ns
Treatment	10	185,92	
Variety*Treatment	10	0,57	***
Error	50		ns

df: degree of freedom, F: coefficient of Fisher-Snedecor (test at level 5 %), ns: non-significant, * P< 0.05, **: P< 0.01, *** : P< 0.001.

Analysis of variance revealed a significant effect between treatments (Table. 3), in addition, the comparison of means, using the Newman-Keuls test at the 5%, threshold had emerged more mean groups (a , b, c, d, e, f and g) (Fig. 3).

After 96 hours of sowing

Regarding the germinate value after 96 hours of the sowing (Fig. 4), the results indicated that the addition of salicylic acid in the medium of the seed gave germinate values of tomato significantly higher compared to other treatments. Thus, treatment SA/GA_3 unregistered germinate values (73.36 and 68.5) higher than the control (71.72 and 66.9), respectively by Rg and Ip. However, salicylic acid alone presented by 70.14 by Rg and 65.56 by Ip, these values remain lower than the control (65.5 and 70.2) respectively for Rg and Ip. To a lesser degree, the seeds treated with GA_3 expressed 52.5 by Rg and 60.8 by Ip. However, the ABA alone or in combination with NaCl canceled the germination value. However, the ABA associated with GA_3 or salicylic acid gave low germination value of 0.91 the two varieties for the first treatment and 2.25 by 1.8 by Rg and Ip for the second treatment.

Moreover, salt stress (100 mM NaCl) decreased the germinate value of the tomato over time compared to seeds irrigated with distilled water (control) (Fig. 4). However, the two varieties have registered under the influence of 100 mM NaCl worth 37 by Rg and 33.58 by Ip, these figures are much lower than that of irrigated seeds with H_2O (71.72 per Rg and 66.9 per Ip).

Phytohormones environments combined with NaCl gave in response to GA_3/NaCl germinate values of 48.11 per Rg and 40.49 by Ip, at the same time, the combination SA/NaCl allowed to display values greater with 60,89 per Rg and 56,4 per Ip. Finally, the ABA/NaCl treatment expressed no germination value for both tomato varieties.

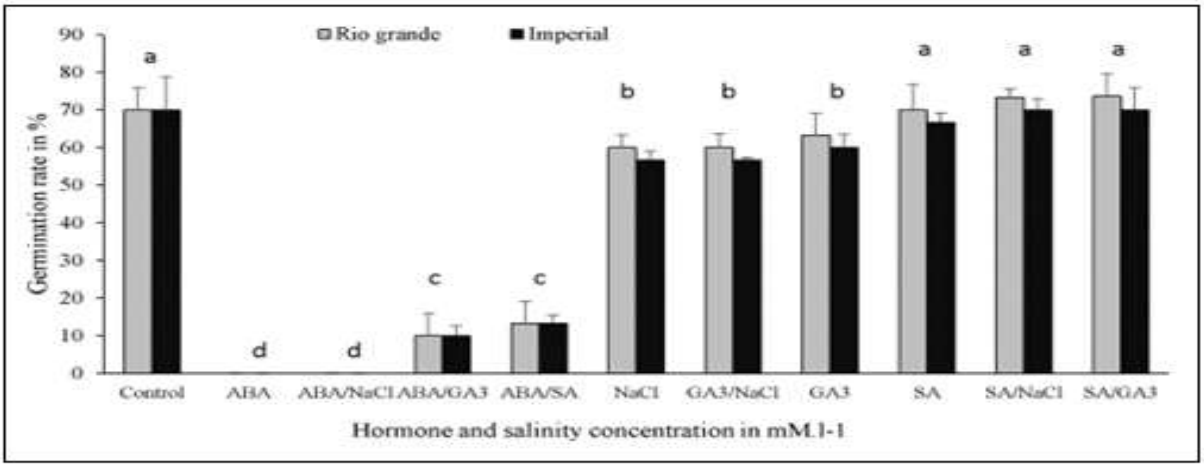

Fig. 1. Germination rate after 48 hours of sowing of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at $p<0.05$. The same letters show no significantly different values.

Statistical analysis using the Newman-Keuls test showed a highly significant effect between treatments (Table. 4). In addition, the comparison of means identified six groups (a, b, c, d, e and f) (Fig. 4).

Discussion

Strategies were used to improve seed growth in saline conditions. One of them was to screening salinity tolerant genotypes. Attempts to improve salt tolerance in plant breeding methods are time consuming and laborious, and rely on the existing genetic variability. In addition, many other attempts were made to overcome this problem, including the proper management and exogenous application of phytohormones. In this context, gibberellic acid (GA_3), Abscisic acid (ABA) and salicylic acid (SA) under salt stress were tested during this experiment carried out on seeds of two processing tomato varieties with or without NaCl in two germination stages (48 and 96 hours) concluded the following:

After 48 hours of sowing, ABA reveals his inhibiting effect on seeds germination of Rg and Ip, either with or without NaCl. This inhibition is thrown under ABA/SA effect. The ABA inhibitory effect on the seeds germination occurs again, after 96 hours of sowing and the ABA middle supplemented with the SA allows the end of seeds dormancy of two tomato varieties. ABA in the seed middle controls the maturation of the seed; in addition, it is responsible for the induction and maintenance of the primary state of dormancy. According to Gimeno-Gilles, (2009), ABA does not improve germination; on the contrary, the ABA inhibits germination. However, ABA-deficient plants are not primary dormancy, whereas overexpression of genes of this hormone biosynthesis pathway leads to a state of deep dormancy (Finch-Savage and Leubner-Metzger, 2006).

The exogenous application of GA_3 on seed increases the germination rate. After that, Gimeno-Gilles

(2009), deficient plant species in GA_3 are unable to complete this phase of development. This shows that the seed's germination is mainly conducted by the opposite action of the ABA and gibberellins, this phenomenon is reported by other researchers as Weitbrecht *et al.* (2011). Furthermore, the addition of SA to the irrigation solution of seeds increases the germination rate of Rg and Ip. Szepesi *et al.* (2006), confirm our results, using 0.5 mM SA concentration, they notice an increase tomato germination. Others evoke the beneficial effect of SA on seeds germination (Linkies and Leubner-Metzger, 2012). Our test shows the effect of the combination SA/GA_3, manifesting itself with very high germination rates.

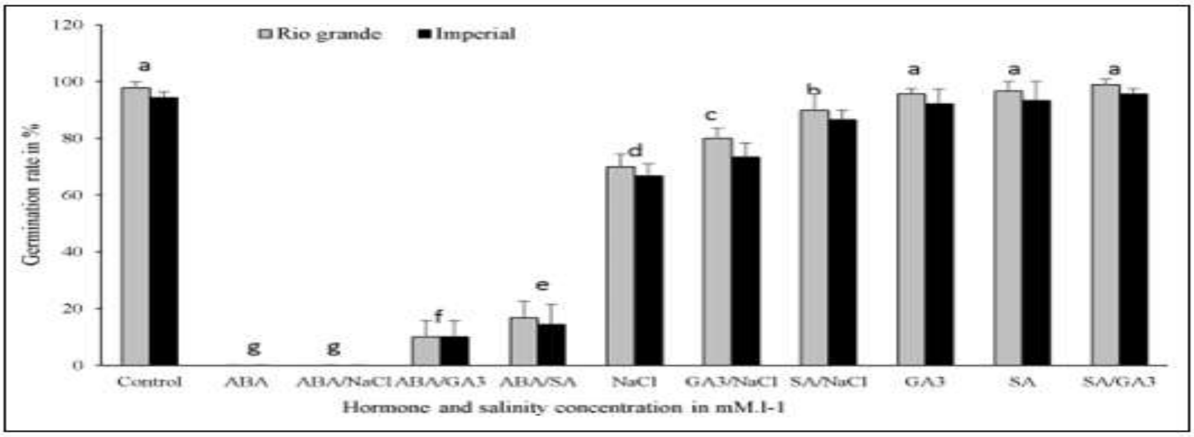

Fig. 2. Germination rate after 96 hours of germination of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at $p<0.05$. The same letters show no significantly different values.

The concentration of 100 mM NaCl applied on tomato seeds (Rg and Ip), after 48 hours of sowing, stimulates and increases germination. On the other hand, the germination is delayed after 96 hours of sowing. These results indicate that the reaction of tomato at the salinity differs with the variety and time of germination. The duration time in the saline conditions weakened the faculty and retards the process of germination for both varieties, although, the seeds of the Rg variety show some tolerance than the Ip variety.

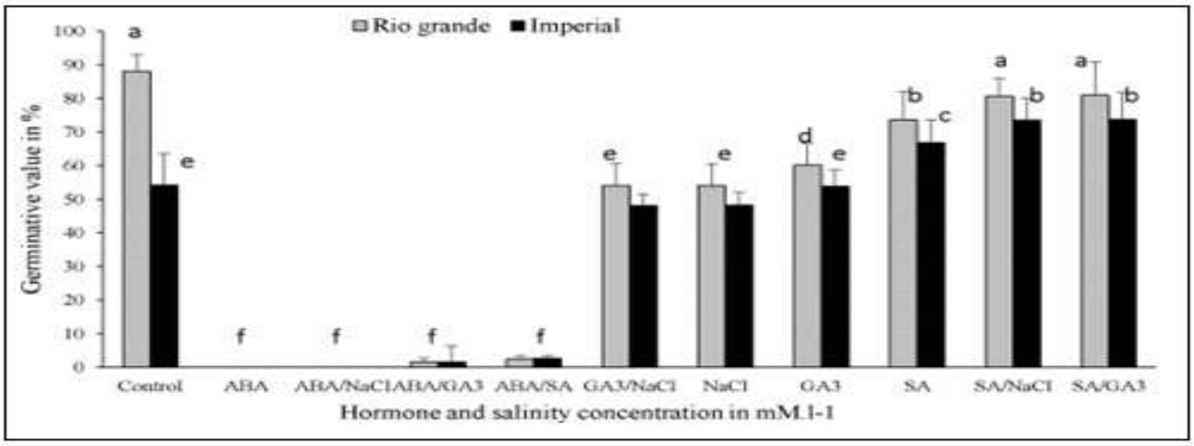

Fig. 3. Germinate value after 48 hours of sowing of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at $p<0.05$. The same letters show no significantly different values.

These results are in agreement with those obtained by Ashraf and O'Leary, (1997) and Afzal *et al.* (2006), show that the germination of tomato seeds is significantly delayed by the salt. In addition, Cuartero and Fernandez-Munoz (1999) report that with 80 mM of NaCl-treated tomato seeds are germination is delayed by 50% of germination time, this delay is multiplied by two for the seeds irrigated with 190 mM NaCl. Others like Shahba *et al.* (2010) indicate that there is a delay and a decrease in seeds germination in several plants under the influence of salt stress.

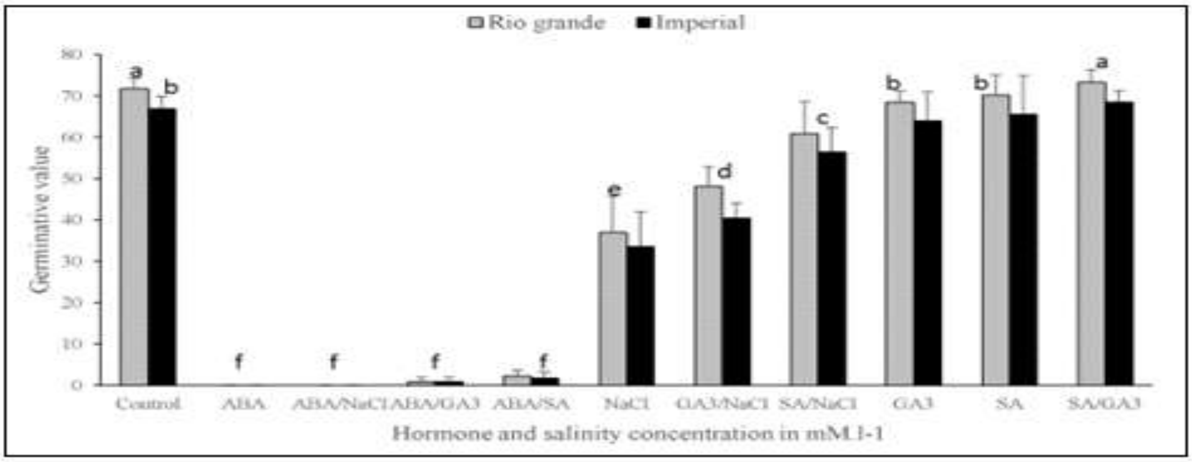

Fig. 4. Germinate value after 96 hours of sowing of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at $p<0.05$. The same letters show no significantly different values.

Addition of GA_3 in middle saline (100mM NaCl) is beneficial to the tomato, these observations confirm that GA_3 improves germination under saline conditions. Thus, Liopa-Tsakalidia *et al.* (2012) attest that the GA_3 is involved in the governance of the plants response at salt stress and the harmful effects of stress. Bejaoui (1985) notes that NaCl associated with GA_3 improves seeds germination, a metabolic response of the seed, through the activity of a specific enzyme, which is involved in the synthesis of RNA and protein. This can be also explained by the fact that GA_3 reduced the ABA level in seed through the activation of their enzymes for catabolism or blocking the way of its biosynthesis (Atia *et al.*, 2009). It should be noted, the involvement of ABA in response to stress, in particular, salt stress, but is also involved in response to biotic stresses and in the development process (Nitsch *et al.*, 2012).

In addition, the SA exhibits significant responses of seeds germination for both tomato varieties, and its association with NaCl mitigates the salt effect. This effect was also observed under the GA_3 treatment with NaCl. However, the association SA/GA_3 occurs very high germination rate. These results are related to previous work, Afzal *et al.* (2006) which noted an improvement in seeds pretreated with a solution SA compared to untreated seeds. These data are consistent with those El-Tayeb (2005) and Motamedi *et al.* (2013). According Gunes *et al.* (2007), the SA could be used as potential growth regulator to improve plants tolerance to salinity.

Finally, the important role of phytohormones for plants under saline stress is essential in the variation of physiological responses that eventually lead to adaptation to an unfavorable environment. However, the functional level of phytohormones, and their relative concentration in the tissues, can have different impacts on the germination of seeds and salt stress tolerance. Thus, understanding of hormone action on the germination process is a major key to improve species germination sensitive to salinity, and subsequently their development in saline conditions.

Conclusion

This study was carried out to test the seed tolerance of tomato to salinity, using regulators growth as ABA, GA_3 and SA conjugated to NaCl. At this germination stage, the results show that the SA and GA_3 minimize the negative salt effects. Salicylic acid (SA) at 0.5mM concentrations the seeds middle, significantly improved the tomato germination performance under salt stress (100mM NaCl) and could be used to mitigate the adverse effect of salt stress. Imperial variety was very promising with 100 mM NaCl.

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