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Metabolic response of tomato (*Lycopersicon esculentum* Mill.) under salt stress combined with hormones

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Abstract

Effect of the combined the phytohormones-salinity (ABA, salicylic acid and GA_3) on the total soluble protein and soluble sugars content contained in germinated seeds of two tomato varieties (*Lycopersicon esculentum* Mill.) is realized to characterize the soluble metabolite involvement in the processes of adaptation to salinity. The research of plant species tolerant to salinity requires an analysis of the behavior of the plant during its cycle especially during the phase of seed germination. At this stage of the seed, salinity is not the only constraining factor, the hormonal action is to be included. Thus, we propose an analysis of the seeds of two varieties of Rio Grande and Imperial tomatoes during their germination under salt stress at 100 mM $NaCl.l^{-1}$ of distilled water, with or without added ABA at 0.005 mM, GA_3 0.005 mM and SA at 0.5 mM. The observations are carried out in two stages at 48 and 96 hours of sowing show that the total soluble protein content and the soluble sugars are influenced by the different treatments; on the other hand, salicylic acid such as GA_3 improves protein contents, salt stress and SA increase the soluble sugar content in germinated seeds. In contrast, the proteins decrease in seaweed associated with the ABA's seeds of two varieties of processing tomato. On the other hand, a variability of the two quantified parameters of the germinated seed is expressed according to the treatment and between the two stages of germination.

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Introduction

Since a long time, salt is considered a key factor that affects seed germination (Darwin, 1856). In a soil environment, salinity has a strong impact on seed germination and impairs the establishment of seedlings in many species (Zapata *et al.*, 2004). However, the mechanism of salt inhibition of seed germination remains largely unknown. According Huang and Redmann (1995), this salt-induced inhibition in seed germination could be attributed to osmotic stress or specific ion toxicity. Effectively, a decrease in the movement of water in the seeds during imbibition causes the inhibition of germination (Jamil *et al.*, 2007). By delaying germination, soil salinity decreases enzymatic activity, thus, the mobilization rate of the metabolites is affected (Ashraf *et al.*, 2002).Seed germination is inherently related to seed metabolism, which changes throughout its maturation, desiccation and germination processes (Fait *et al.*, 2006). During its maturation, the seed accumulates transcripts and metabolites necessary for its germination (Rosental *et al.*, 2014). However, during germination, glucose at high levels can support abscisic acid (ABA) signalling, delaying germination and starch degradation in tomato (Bradford *et al.*, 2003).To resist or tolerate the salt stress, the seed triggers hormonal, physiological and biochemical mechanisms (Eraslan *et al.*, 2015). Also, this leads to major alternations in carbohydrate metabolism (Kaur *et al.*, 2002). However, sugar signalling pathways interact with stress pathways to modulate metabolism. Indirectly, sugars play an important role during the growth and development of plants under abiotic stresses by regulating carbohydrate metabolism. A large number of stress-sensitive genes are indicated by glucose, highlighting the role of sugars in environmental responses (Price *et al.*, 2004).Amino acids are also used as sources of energy production during the early stages of germination by various pathways (Rajjou *et al.*, 2012). Cell wall metabolism is essential for the relaxation of the endosperm heading in the tomato and for the prolongation of the radicle leading to germination (Martinez-Andujar *et al.*, 2012). Despite these studies, understanding the relationship between seed primary metabolism and germination is still poor (Nonogaki *et al.*, 2010).

The need to understand the hormonal action on the germination process, since it represents a major key to improve this important physiological step in sensitive species and their establishment in saline-soils.

The present work focuses on the role of ABA, GA_3 and salicylic acid on the germination of two processing tomato varieties in the presence of NaCl, in order to obtain more information on the mechanisms of inhibition due to salinity. This research is important because it can reveal the role of hormones that give stress resistance and give insight into the potential of tomato plants to adapt to salt conditions.

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 ormixed.

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 and (e) 100 mM NaCl. The Petri dishes were placed in oven at 25°C. Eachtreatment was repeated three times. Finally, countingseeds 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

Soluble sugar content

Total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are determined by the method Dubois *et al.* (1956). It consists in taking 100 mg of plant material, in test

tubes, is added 3 ml of 80% ethanol in order to extract the sugars and then left at room temperature for 48 hours. At the time of dosing, the tubes are placed in the oven at 80°C. To evaporate the alcohol. In each tube 20 ml of distilled water is added to the extract, 2 ml of the test solution are placed in clean glass tubes, with one ml of 5% phenol added; And 5 ml of concentrated sulfuric acid are then rapidly added while avoiding the pouring of acid against the walls of the tube. After obtaining an orange-yellow solution on the surface, the vortex is passed to homogenize the solution. The tubes are then left for 10 minutes and placed in a water bath for 10 to 20 minutes at a temperature of 30°C. The optical density is read at a wavelength of 585 nm.

Soluble proteins content

2.5 g of fresh material of each sample are extracted with 15 ml of each medium, previously cooled: distilled water pH 7.0, tris-HCl buffer 0.05 M at pH 9.0, sodium borate buffer 0.05 M. at pH 9.0; the samples are first crushed with a mortar in the presence of silica sand washed with acid and then taken up in a potter to complete the extraction. The extracts are vigorously stirred and placed in the cold (4°C), where extraction is continued for 16 h. They are then filtered through a cloth "city of MOP" centrifuged at 10500 g for 10 minutes at 0°C, and then stored at -20°C. These samples can be used directly without freezing. Determination of Bradford's technique (1976).

Statistical analysis

Data of soluble sugar content and soluble protein content were analyzed by the Student's t-test. Means were compared between treatments by LSD (least significant difference) at the 0.05 confidence level.

Results and discussion

Conjugated effect of ABA, GA_3, SA and NaCl on soluble sugar content

After 48 hours of sowing

After 48 hours of sowing, the tomato seeds react differently in presence of phytohormones and/or NaCl (Fig. 1). The ABA and NaCl influence the soluble sugar content; since the sugars rate recorded in Rg and Ip vary between 7.81 10^{-4} and 7.87 10^{-4} µg.ml^{-1} are lower than those recorded by the control varieties, which express 7.89 10^{-4} and 7.92 10^{-4} µg.ml^{-1}; the GA_3 registers higher rates than the other two treatments (ABA and NaCl), but its values remain lower than the control. A very significant fact appeared with the exogenous contribution of SA alone or associated with other hormones or NaCl medium. The soluble sugar levels recorded under SA are higher than the control.

Table 1. ANOVA of soluble sugars content after 48 hours of germination.

Source of variance	df	F	P
Variety	1	0.19	ns
Treatment	10	9.1189	***
Interaction	10	0.28	ns
Within the group	44		

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

Thus, the germinated seeds of the two varieties of tomato (Rg and Ip) treated with SA and the two combinations ABA/SA and GA_3/SA express respectively (8.01 10^{-4} and 8.05 10^{-4} µg.ml^{-1}), (8.05 10^{-4} and 8.09 10^{-4} µg.ml^{-1}) and (7.93 10^{-4} and 7.96 10^{-4} µg.ml^{-1}). However, the SA/NaCl solution showed significantly higher levels of sugars than those recorded by the control (Rg at 8.05 10^{-4} µg.ml^{-1} and Ip at 8.02 10-4 10^{-4} µg.ml^{-1}). Analysis of variance of the two-factor shows a highly significant treatment effect (Table 1), however, no significant effect of the variety or treatment varieties.

After 96 hours of sowing

After 96 hours of germination, the soluble sugars content increase in the seeds of both varieties of tomato (Rg and Ip) under the hormonal effect compared to the control.

Indeed, the two treatments ABA and GA_3 show identical values with 8.11 10^{-4} and 8.14 10^{-4} µg.ml^{-1} respectively by Rg and Ip. However, the salicylic acid alone or combined gives values much higher than the control. Thus, the treatments GA_3/SA, SA, and ABA/SA combination expressed respectively (8.48 10^{-4} and 8.45 10^{-4} µg.ml^{-1}), (8.26 10^{-4} and 8.24 10^{-4} µg.ml^{-1}) and (8.23 10^{-4} and 8.2 10^{-4} µg.ml^{-1}) successively by Rg and Ip; these data remain higher than those displayed by the control (Rg at 8.05 $10^{-4}$and Ip at 8.02 10^{-4}µg.ml^{-1} In addition, the two tomato varieties (Rg and Ip) stressed with 100 mM NaCl respectively recorded soluble sugars ranging from 8.08 10^{-4} to 8.03 10^{-4} µg.ml^{-1}, these contents remaining higher than those displayed by the control.

Table 2. ANOVA of soluble sugars content after 96 hours of germination.

Source of variance	df	F	P
Variety	1	25.77	***
Treatment	10	133.69	***
Interaction	10	0.72	ns
Within the group	44		

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

Table 3. ANOVA of soluble protein content after 48 hours of germination.

Source of variance	df	F	P
Variety	1	0.536	ns
Treatment	10	1.34	ns
Interaction	10	0.144	ns
Within the group	44		

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

On the other hand, the NaCl associated with the various hormones improves the sugar content, thus, under the respective effect of ABA/NaCl, GA_3/NaCl and SA/NaCl, the two varieties Rg and Ip show varying contents Between 8.09 $10^{-4}$and 8.06 10^{-4} µg.ml^{-1}, 8.27 $10^{-4}$and 8.24 10^{-4} µg.ml^{-1} and 8.25 $10^{-4}$and 8.23 10^{-4} µg.ml^{-1}.

Table 4. ANOVA of soluble proteins content after 96 hours of germination.

Source of variance	df	F	P
Variety	1	0.005	ns
Treatment	10	0.72	ns
Interaction	10	0.055	ns
Within the group	44		

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

These values express an advantage over the control. The statistic study gives a highly significant varietal effect to the treatment effect; however, the interaction between treatments shows no significant effect (Table 2).

Conjugated effect of ABA, GA_3, SA and NaCl on soluble protein content

After 48 hours of sowing

The soluble protein content recorded fluctuations under the combined effect of phytohormones-NaCl (Fig. 3).

After 48 hours of sowing, the seeds of Rg and Ip under the effect of ABA/GA_3 show respective total soluble protein rates varying between (0.0187 and 0.0185 µg.g^{-1} of FM).

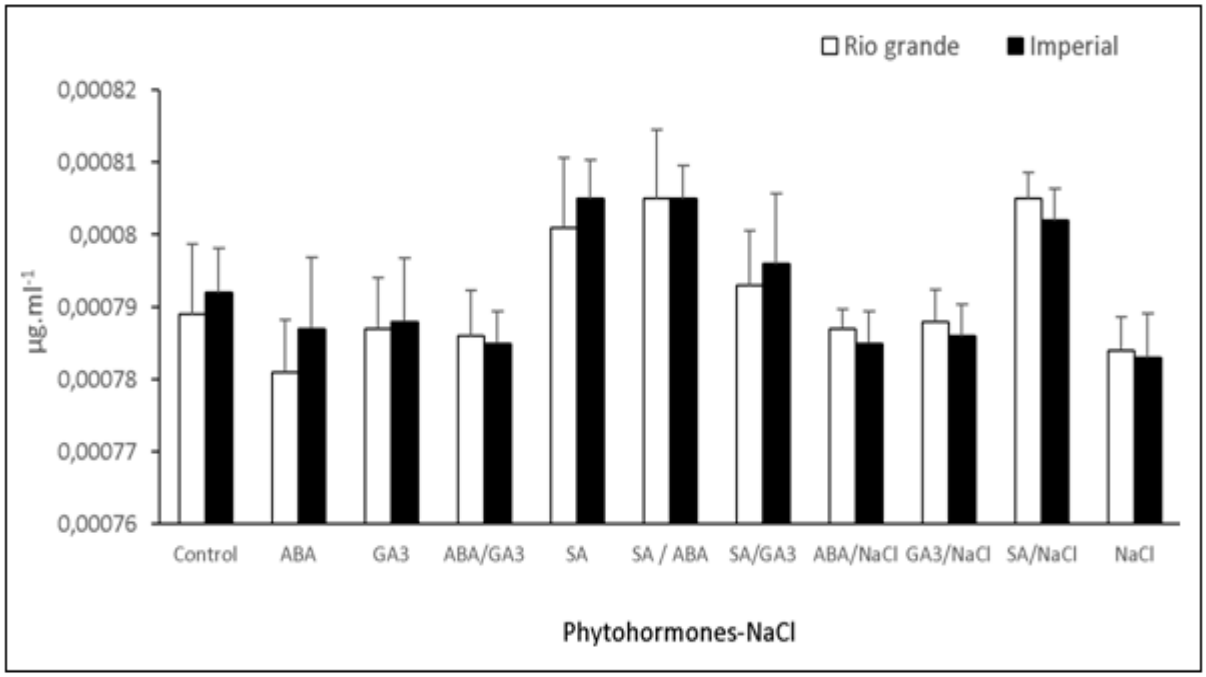

Fig. 1. Soluble sugar concentrate after 48 hours of sowing of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD.

These values remained significantly higher than the seeds that received H_2O (control), which expressed levels ranging from (Rg to 0.0184 µg.g^{-1} of FM and Ip to 0.0182 µg.g^{-1} of FM). On the other hand, these protein levels decrease with the effect of other hormones and their association.

However, NaCl associated with GA_3 recorded higher protein levels than the control with (Rg at 0.0186 µg.g^{-1} of FM and Ip at 0.0184 µg.g^{-1} of FM), so NaCl alone allows the variety Imperial to accumulate 0.0184 µg.g^{-1} of FM, this rate is significantly higher than that of the control of the same variety (Fig. 3).

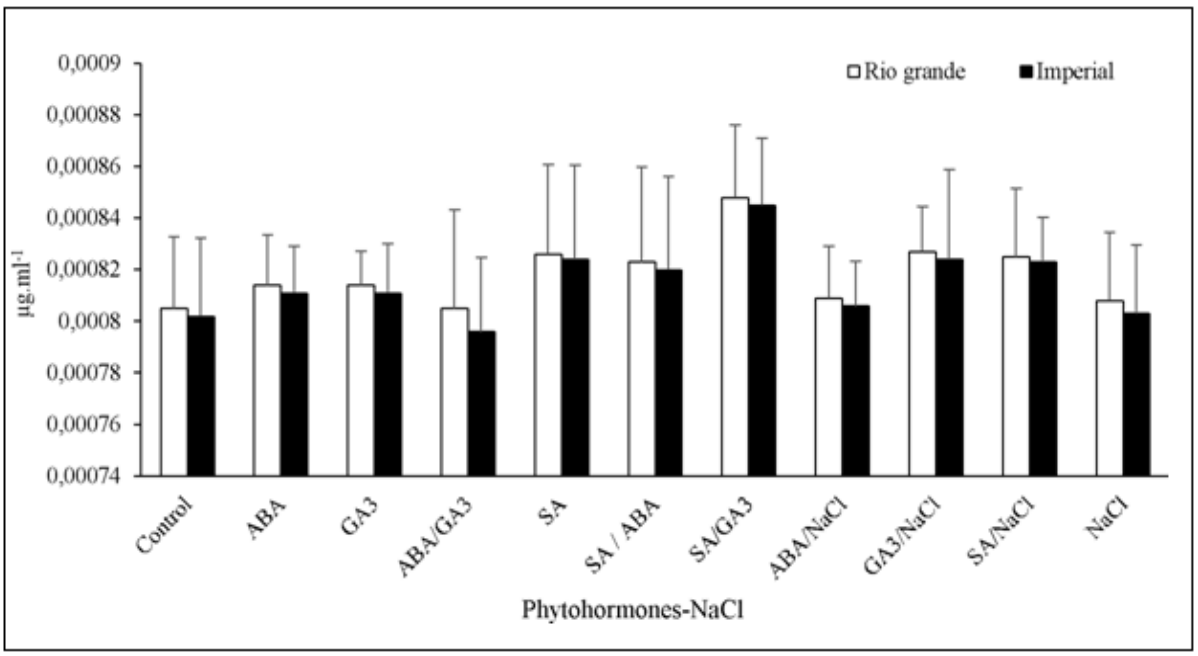

Fig. 2. Soluble sugars content after 96 hours of germination of two processing tomato varieties

The phytohormones SA and ABA associated with NaCl do not allow the seed to express large quantities of proteins because under both these effects the two tomato varieties have lower levels than the control (Fig 3). Analysis of the two-factor variance gives no significant effect (Table 3).

After 96 hours of sowing

After 96 hours of germination (Fig. 4), the results indicate that the addition of SA to the seed solution resulted in significantly higher levels of soluble total protein than other treatment conditions.

Thus, the combinations SA/NaCl, SA/ABA and SA/GA_3 respectively cause accumulation of protein levels ranging from (0.01869 and 0.01864 µg.g^{-1} of FM), (0.0183 and 0.0182 µg.g^{-1} of FM) to (0.0181 and 0.018 µg.g^{-1} of FM) successively for Rg and Ip.

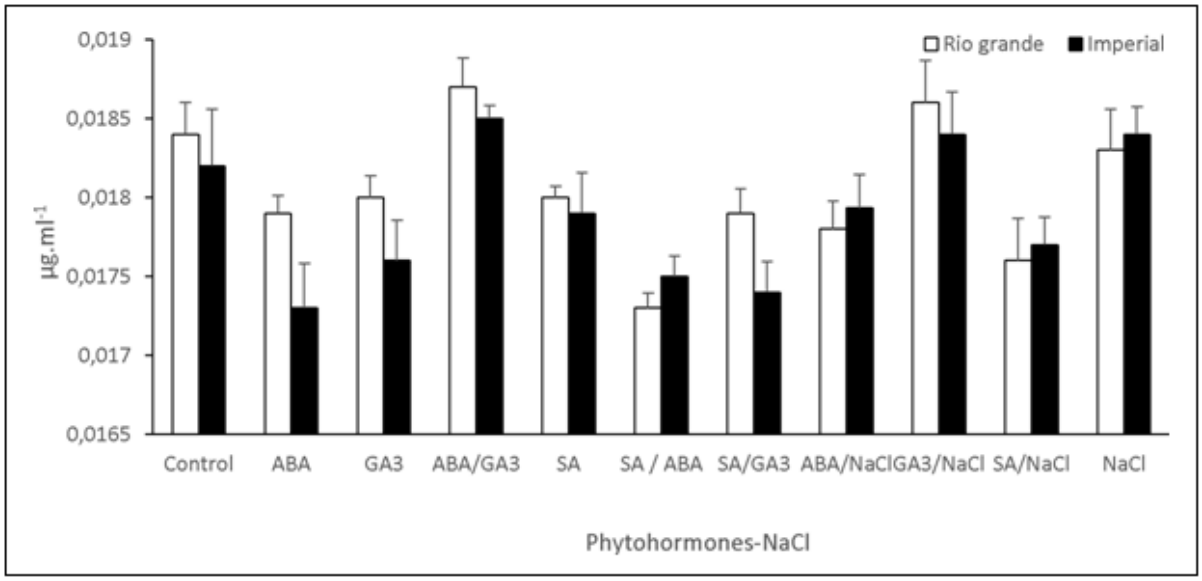

Fig. 3. Soluble protein content after 48 hours of germination of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD.

This effect is also felt under the association GA_3/NaCl which records (0.01803 µg.g^{-1} of FM per Rg and 0.0185 µg.g^{-1} of FM per Ip), these data are superior to those recorded by the control (Rg at 0.0179 µg.g^{-1} of FM and Ip at µg.g^{-1} of FM).The seeds germinated under different treatments (GA_3, SA, ABA and ABA/GA_3) express data below the control. Saline stress (100 mM NaCl) affects the accumulation of total soluble proteins, the amounts recorded are also low for the two varieties Rg and Ip which show respectively 0.0175 and Ip 0.0177 µg.g^{-1} of FM. On the other hand, the addition of ABA to NaCl improves this accumulation of proteins relative to NaCl alone, since Rg and Ip respectively recorded 0.01782 and 0.01779 µg.g^{-1} of FM (Fig. 4). The analysis of the two-factor variance shows no varietal effect, treatment, as well, for combination varieties-treatments (Table 4).

Discussion

Understanding the hormonal action on the germination process is a key to improving the germination of saline-sensitive species and their development under saline conditions. Furthermore, the hormonal action is a response of the seed to salt stress through osmoticum-like metabolites responsible for tolerance to environmental constraints. To verify the extent of salinity tolerance of our seeds, we assessed the soluble protein and soluble sugar content. After 48 h of germination, the protein content increased under the two treatments GA_3/NaCl and NaCl; on the contrary, the protein decreased under the effect of ABA/NaCl and SA/NaCl. On the 5thday of germination, under the effect of the various treatments associated with NaCl, the protein content in the seeds increased, except under the effect of NaCl alone. These results confirm those of Shahba *et al.* (2010).

Other studies indicate a decrease in protein content under saline conditions in several plant species, *Oriza sativa* L. (Amirjani, 2010), *Capsicum annum* L. (Argyropoulou, 2011) and *Triticum durum* (Hameed *et al.*, 2010). This reduction may be attributed to the inhibitory effect of accumulated ions such as sodium and chloride. However, the exogenous contribution of GA_3 and SA to the saline solution appears to improve the protein content of germinated seeds. These data are confirmed by Agamy *et al.* (2013) in tomato and by Iqbal and Ashraf (2013) in wheat. However, Shi *et al.* (2014) explains that SA is involved in the synthesis of a particular protein, kinase, and this protein plays an important role in the regulation and differentiation of cell division.

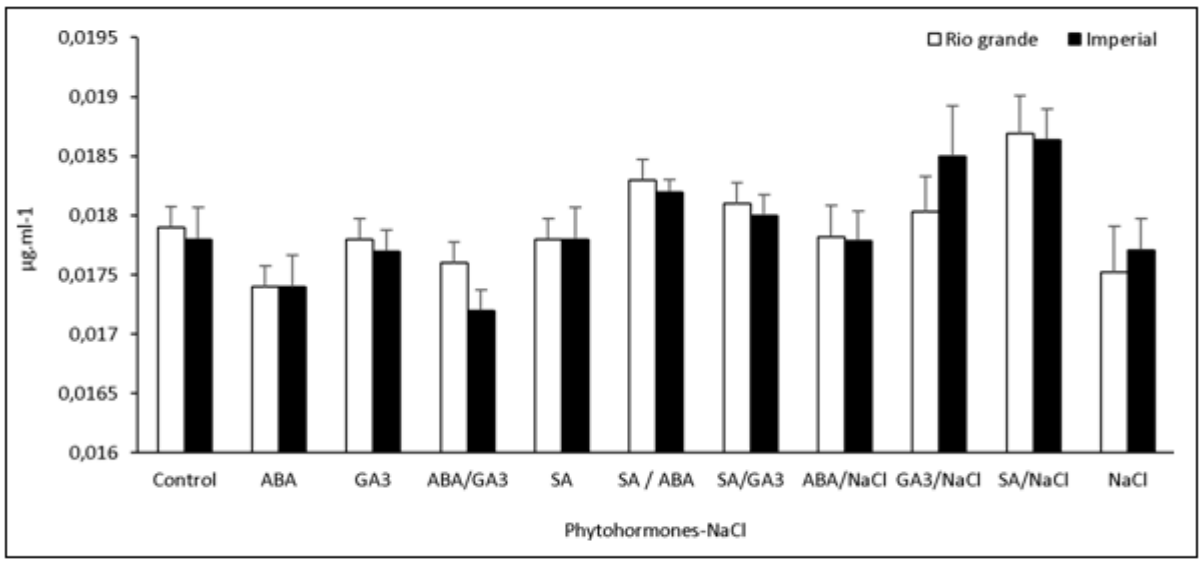

Fig. 4. Soluble protein content after 96 hours of germination of two processing tomato varieties (Rg and Ip) under combined phytohormones-NaCl effect. Data represent the mean of tree replication and error bars indicate SD.

The salt stress (100 mM NaCl) increases the soluble sugar content of the germinated seeds of the two industrial tomato varieties (Rg and Ip). These observations are in agreement with those of Nawaz *et al.* (2013) and Hameed *et al.* (2013). Some investigations by Parvaneh *et al.* (2012) conclude that sugar concentrations (reserve polysaccharides) always rise after exposure of the plant to salinity. Others, Wu *et al.* (2013), discuss the importance of these accumulations of organic solutes (soluble and insoluble carbohydrates) to play an important role in increasing the internal osmotic pressure.

This reaction of the plant has already been considered as a response to salt stress (Afzal *et al.*, 2006). Also, Jat and Sharma (2006) note an increase in sugars on wheat seeds pre-treated with GA_3 in saline medium. Also, the exogenous contribution of SA alone or associated with salinity increases the level of sugars in the sprouting seed of the tomato.

These results are confirmed by Dong *et al.* (2011), on cucumber seeds, who have noticed that the exogenous contribution of SA to saline increases the sugar content. According to the same authors, this accumulation of soluble sugars, after an addition of SA to the germination of the seed in saline medium, can restore the osmotic balance. It has been shown that SA plays a role in plant adaptive responses to osmotic stress and intervenes in the defence mechanism as a regulator against abiotic stresses (Shahba *et al.*, 2014). Similarly, Lee *et al.*, (2010) discuss the importance of SA by contributing to the regularization of the various aspects of plant responses to abiotic stresses by extensive signalling with other growth hormones.

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