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Effects of Gibberellic and Abscisic Acids on Germination and Seedling Growth of Okra (*Abelmoschus esculentus* L.) under Salt Stress

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ABSTRACT

This study aimed to evaluate the effect of gibberellic acid (GA_3) and abscisic acid (ABA) on the germination of okra seeds (*Abelmoschus esculentus* L.) exposed under salinity constraint. In the first part, seeds were germinated in hormonal solutions of GA_3 (5 µM and 10 µM) and ABA (5 µM and 10 µM), in the presence of NaCl (100 mM). Results showed that NaCl reduced significantly the precocity of seeds germination without influencing its final rate; contrarily, it had a negative effect on hypocotylar growth, fresh weight and seedlings water content. The application of GA_3 treatment attenuated the depressive effect of NaCl on germination by stimulating it from the first day of sowing (after 24 h) with 20% and 26.66% under the effect of 5 µM and 10 µM of GA_3 respectively. The findings showed that, this phytohormone seemed positively influencing the hypocotylar length, fresh and dry weight as well as the water content of the okra seedlings. On the contrary, ABA was not effective in inducing tolerance to salinity.

Keywords: ABA, GA_3, germination, NaCl, okra, salinity

INTRODUCTION

In their natural environment, plants are subjected to a large number of environmental constraints of biotic and abiotic stresses that will influence their growth and development (Chen et al., 2015). By 2025, global food production will need to increase by about 38% and 57% by 2050 (Wild, 2003). Most of the appropriate land has been cultivated and expansion into new areas to increase

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food production is practically possible or desirable. Additional efforts are needed to improve productivity as more and more land is degraded (Rengasamy, 2006). It is estimated that about 15% of the world's total land area has been degraded by soil erosion and physical and chemical degradation, including soil salinization (Wild, 2003).

Soil salinity is one of the major abiotic factors limiting agricultural production in arid and semi-arid regions. Nearly 400 million hectares of land is affected by salinization, 80% of which is of natural origin and 20% of anthropogenic origin (Food and Agricultural Organization [FAO], 2015) with a resultant monetary loss of 12 billion US$ in agricultural production (Shabala, 2013). In Algeria, 3.2 million hectares of land is affected by salinity (Belkhodja & Bidai, 2004). This salinity is due mainly to the presence of high amounts of minerals such as Na^+ and Cl^- in soils and water (Tavakkoli et al., 2010) which affects crop growth and development through many diverse pathways, including water stress, nutritional disorders, ion toxicity, oxidative stress, alterations to metabolic processes, cell membrane disorganization, and reduced cell expansion and division (Hanin et al., 2016; Munns, 2002; Teige et al., 2004; Zhu, 2002, 2016).

Okra (*Abelmoschus esculentus* L. Moench.) is a popular vegetable crop belonging to Malvaceae family which is widespread in the tropical, subtropical and Mediterranean regions (Abid et al., 2002). This crop has high nutritive value, and it is used as a vegetable very rich in vitamin C and calcium. The productivity of this crop in Algeria is limited because of the lack of knowledge of its pedoclimatic requirements and its adaptation especially to salty soils. Since this vegetable is often grown under irrigation, especially where dry summers are more common, principally in the Mediterranean countries, it can be subjected to salt stress (Habib et al., 2016). Salinity retarded the growth, yield and physiological growth parameters of okra (Achour, 2016; Ayub et al., 2018; Ifediora et al., 2014).

Germination is considered as a critical step in plants development cycle; indeed, it prepares the seedling's installation, its adaptation to the environment and its subsequent productivity (Zhu, 2016). In saline environments, high salt levels impair seed germination and emergence in both glycophytes such as okra (*Abelmoschus esculentus* L.) (Achour, 2016; Dkhil et al., 2014), wheat (*Triticum durum* (Desf.)) (Otu et al., 2018), broad bean (*Vicia faba* L.) (Anaya et al., 2015), rice (*Oryza sativa* L.) (Liu et al., 2018), cowpea (*Vigna unguiculata* L. Walp.) (El-Shaieny, 2015) and halophytes (Gul et al., 2013, Haraguchi & Matsuda, 2018) and perturbs the hormonal balance in plants (Atia et al., 2009). For that, the treatments that can reduce the effect of salinity during this phase will be essential.

In the last two decades, physiological treatments such as hormone inputs have been intensively studied, assuming that the depressive effect of salinity on germination could be related to either a decrease or an increase in endogenous hormone levels

(Bahrani, 2015). Reduction in amounts of phytohormons especially of cytokinins and gibberellic acid and increase in the abscissic acid (Wang et al., 2001), in several species of plants, under salt and drought stresses, have been reported (Cao et al., 2014; Mizrahi et al. 1971; Zhang et al. 2006). Under saline stress, ABA can improve water permeability (Glinka & Reinhold, 1971) and induces late-embryogenesis abundant proteins (LEA proteins), and the osmolyte biosynthesis (Xiong et al., 2001).

Gibberellic acid plays an important role at different stages of plant development from germination to flowering; it participates in the lifting of seed dormancy, growth of stems and leaves, and flower development (Gupta & Chakrabarty, 2013; Yamaguchi, 2008).

Baskin and Baskin (2014) reported that gibberellins were often used to overcome seed dormancy, and could significantly improve seed germination in many species, mainly through the activation of embryo growth, mobilization of reserves, and weakening of the endosperm layer.

The GA_3 is the most active to attenuate the inhibitory effect of salinity on germination, by increasing nutrient uptake, dry weight, seedlings growth under saline conditions (Bahrani & Pourreza, 2012; Tsegay & Andargie, 2018; Waleed et al., 2019). It has also been proven that the growth of wheat, rice, cotton and some halophytes has been significantly improved in the presence of GA_3 under salt stress (Colebrook et al., 2014; Javid et al. 2011; Iqbal & Ashraf, 2013). Such improvement can be attributed to increased level of some endogenous GA_3 with a concurrent decrease in level of ABA and improvement of ions uptake under salinity-stressed conditions.

For this purpose the exogenous application of GA_3 on germination and seedling growth in saline conditions offers an attractive approach to alleviate the harmful effects of salt stress.

Therefore, the objectives of this experiment were to study the effects of gibberellic acid (GA_3) and abscisic acid (ABA) on the germination and seedlings growth of okra seeds under salinity constraint.

MATERIALS AND METHODS

Vegetal Material

Seeds were harvested in July 2012 from the fruits of okra (*Abelmoschus esculentus* L.) that was grown on a parcel of agricultural land.

Seed Preparation for Germination Tests

Seeds were disinfected with 2% of sodium hypochlorite during 3 minutes, rinsed thoroughly with distilled water and then dried on filter paper and placed in Petri dishes lined with two layers of sterile filter paper. Each test was carried out on 30 seeds which made 3 repetitions of 10 seeds per Petri dish. Control seeds received 10 ml of distilled water and the same volume of the different saline and hormonal solutions: NaCl (100 mM), ABA (5 and 10 μM), GA_3 (5 and 10 μM), ABA / NaCl, GA_3 / NaCl was added to the treated seeds.

The Petri dishes were put in an incubator that was set at 28°C. Daily, a count of germinated seeds was carried out for a week. The germination was marked by the exit of the radicle out of the seed's coat.

During this test the following parameters were studied:

The Precocity of Germination. Represented by the rate of the first germinated seeds, so be after 24 hours of sowing (Belkhodja & Soltani, 1992).

Final Rate of Germination (Tg). This parameter is the best way to identify the germination ability of the seeds under the different treatments used. It was calculated after 7 days from sowing date and expressed as percentage according to the equation as described by (Kandil et al., 2012). It is expressed as the ratio of the percentage of germinating seeds (Ni) to the total number of seeds recorded at the end of the test (Nt).

$Tg = Ni \times 100 / Nt$ (Tg: germination rate)

Hypocotyl Length. This parameter is a good step to evaluate the salt stress effect and the hormonal treatments effect on the hypocotyl's growth, this length is estimated using a caliper at the end of the germination test (after 7 days of sowing) (International Seed Testing Association [ISTA], 1996).

Water Content (WC %). The water content of the seedlings is determined by the calculation of seedlings fresh weight (FW) of the before drying them in the oven at 80°C during 48 hours. The dry weight is then determined (DW) and the water content is calculated by the following equation: WC (%) = (FW-DW/FW) * 100 (Chen et al., 2009).

Each treatment was replicated three times and results were statistically analyzed by calculating variance and the standard error (Sx) of the mean. Statistical analysis was performed based on SPSS (version 17.0) program. In order to detect the significance of differences ($p<0.05$) of variables, a multiple comparison (LSD) test was performed.

RESULTS

Precocity of Germination

In Different Hormones Concentration. The LSD test indicated significant effects of GA_3 on the germination precocity ($p<0.05$); the first germinations occurred from the first day of sowing for the control seeds and those treated with GA_3. It should be noted that the germination rate increased considerably for the seeds receiving the hormonal solution at 5 and 10 µM of GA_3 with respective levels 76.66% and 83.33%. On the other hand, significant effect of ABA on germination was noted ($p<0.05$), Indeed, any germination was observed on seeds under the ABA treatment during the first 48 hours; beyond that, the seeds germination appeared in a slow way compared to those treated with GA_3 (Figure 1).

In Different Hormone and NaCl Combination. Analysis of variance results indicate significant effect of NaCl and NaCl/hormone on okra seeds germination ($p<0.05$) compared to control seeds. Under 100 mM, the seeds germinate only from the second day of sowing. When the seeds receive the GA_3 at 5 and 10 µM associated with the saline solution, the germination starts the first day of sowing with respective rates of 20% and 26.66% (Figure 1). On the other hand, a very pronounced germination delay is recorded in the seeds treated with ABA.

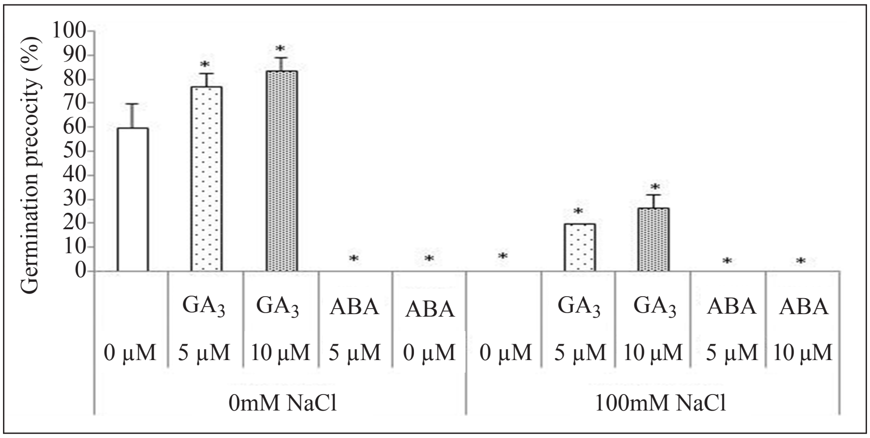

Figure 1. Effect of hormone/salinity interaction on germination precocity (%)

Table 1
Values are mean and standard errors of measurement made on three replicates. Asterisks within the means of each column (*) indicate significant difference among the means (LSD test, p <0.05) compared to control

Treatments	Germination precocity (%)	Final rate of germination (%)	Hypocotyl length (cm)	Seedlings fresh weight (g)	Seedlings dry weight (g)	Seedlings water content (%)
Control	60±10	100	8±1.11	0.46±0.04	0.03±0.003	93.26
5µM GA_3	76.66±5.77*	100(ns)	8.1±0.95(ns)	0.48±0.03(ns)	0.032±0.002(ns)	93.33(ns)
10µM GA_3	83.33±5.77*	100(ns)	12±0.98*	0.58±0.06*	0.035±0.002(ns)	94.03(ns)
5µM ABA	0*	93.33±5.77(ns)	0.28±0.03*	0.07±0.005*	0.03±0.004(ns)	55*
10µM ABA	0*	76.66±5.77*	0*	0.07±0.01*	0.03±0.003(ns)	55.68*
100 mM NaCl	0*	96.66±5.77(ns)	1.25±0.5*	0.14±0.04*	0.03±0.006(ns)	77.93*
5µM GA_3/NaCl	20*	100(ns)	8.47±0.73(ns)	0.45±0.06(ns)	0.035±0.005(ns)	92.22(ns)
10µMGA_3/NaCl	26.6±5.77*	100(ns)	10.4±1.39*	0.58±0.06(ns)	0.036±0.003(ns)	92.96(ns)
5µM ABA/NaCl	0*	23.33±11.54*	0 *	0.06±0.01*	0.03±0.002(ns)	52.34 *
10µMABA/NaCl	0*	6.66±11.54*	0*	0.06±0.005*	0.03±0.002(ns)	44.92*

Note: (*) signifiant at 0.05 level; (ns) non-signifiant

Final Rate of Germination

In Different Hormones Concentration. Any significant effect of GA_3 treatment was noted on final rate of germination (Table 1). Indeed, a final rate of 100% was recorded in the control seeds and those irrigated with GA_3. This rate decreased to 93.33% in the presence of ABA at 5 µM while the lowest rate was recorded in seeds receiving 10 µM ABA with 76.66% sprouted seeds.

In Different Hormone and NaCl Combination. The final rate of germinated seeds reached 93.33% then it increased until all of the seeds were germinated under the combination of GA_3 with NaCl solution (Figure 2), on the other hand, the combined ABA/ NaCl treatments (5 and 10 µM of ABA) decreased significantly final rate of germination with respective rates of 23.33% and 6.66%.

Hypocotyl Length

In Different Hormones Concentration. The hypocotylar length was positively influenced by the presence of GA_3 in the imbibing medium; in fact GA3 increased significantly ($p<0.05$). The highest length was recorded in seeds receiving 10 µM of GA_3 (12 cm) followed by control seeds and those receiving 5 µM of GA_3. In addition, it should be noted that treatment with ABA acted by slowing the hypocotylar growth (Figure 3).

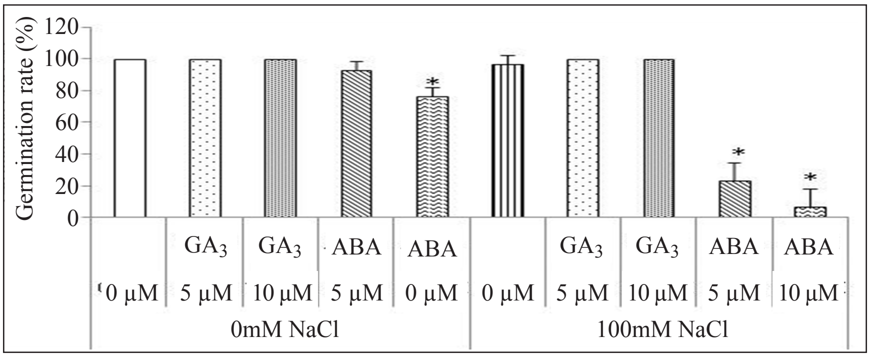

Figure 2. Effect of hormone/salinity interaction on the final rate of germination (%)

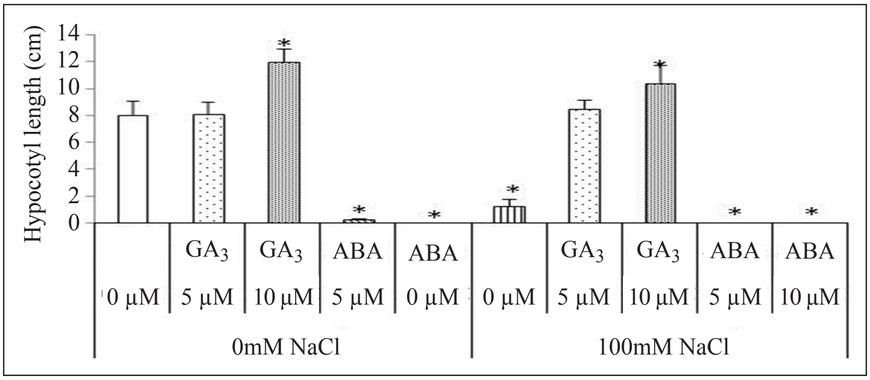

Figure 3. Effect of hormone/salinity interaction on the hypocotyl length (cm)

In Different Hormone and NaCl Combination. The hypocotyl's growth was slowed down, whereas the treatment with GA_3 (10 and 5 μM) associated with NaCl had positively affected this parameter so that the respective lengths varied between 10.4 cm and 8.47 cm. On the other hand, it should be noted that the growth of the hypocotyl was stopped in the seeds subjected to the treatment ABA/NaCl which was confirmed by the statistical study showing a significant effect of combined treatment ABA/salt on the hypocotyl growth of okra seedlings ($p<0.05$).

Seedling Fresh and Dry Weight

In Different Hormones Concentration. The FW seems positively influenced by the GA_3 effect. Thus, the highest FW was noted in treated seeds at 10 μM GA_3 (0.58 g). In contrast, the seeds exposed to ABA record a relatively low FW (0.07 g).

On the other hand, it should be noted that the DW did not seem to be influenced by the different treatments. Indeed, statistical analysis using the LSD test showed no significant effect of the different treatments compared to the control on the dry weight of seedlings ($p>0.05$) except for GA_3 at 10 μM. The DW of seedlings oscillated between 0.03 g and 0.035 g (Figure 4).

In Different Hormone and NaCl Combination. The fresh weight of the seedlings decreased significantly (0.14 g). On the other hand, it amounted to 0.45 g in seeds receiving GA_3 at 5 μM combined with NaCl and reached up to 0.58 g in the presence of 10 μM. The treatment with ABA at 5 μM and 10 μM combined with NaCl induced a significant reduction (($p<0.05$) of FW (0.064 g and 0.061 g) (Figure 5).

It should be noted that the DW did not seem to be influenced by the different treatments. In fact, the dry weight of seedlings varied between 0.03 g and 0.036 g.

Seedlings Water Content

In Different Hormones Concentration. The highest water contents were recorded in control seedlings and those treated with gibberellic acid with a maximum value of

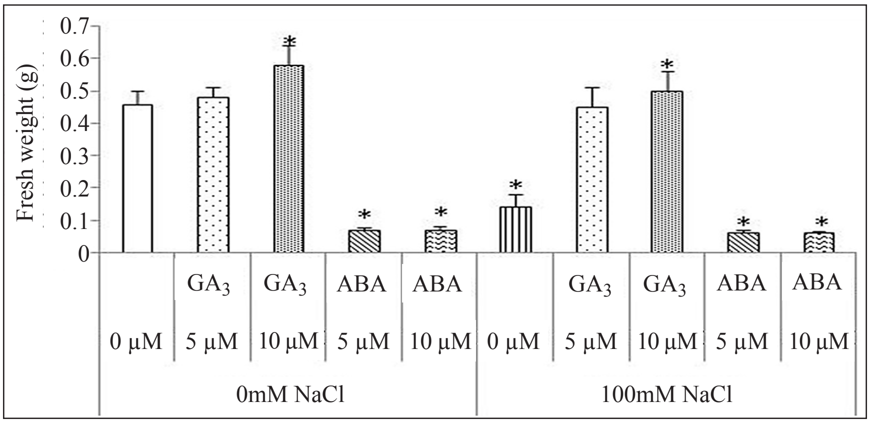

Figure 4. Effect of hormone/salinity interaction on seedlings dry weight (g)

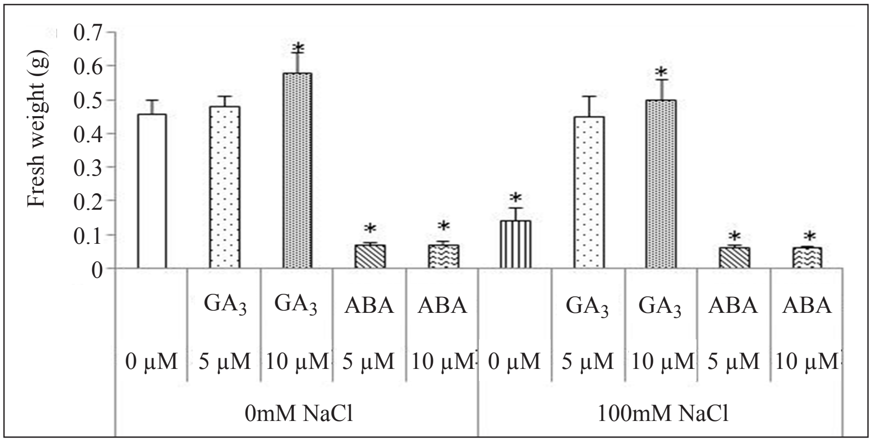

Figure 5. Effect of hormone/salinity interaction on seedlings fresh weight (g)

94.03% in the presence 10µM GA_3. The application of the ABA at 5 µM and 10 µM decreased significantly water content at 55% and 55.68% respectively.

In Different Hormone and NaCl Combination. The water content of seedlings reached only 77.93%, and increased to 92.22% and 92.96% in the presence of GA_3 in saline solution. However, the application of NaCl in combination with ABA greatly reduced the seedlings' water content (Figure 6), which was confirmed by the LSD test which showed significant effect of combined treatment ABA / salt on the water content of okra seedlings ($p<0.05$).

DISCUSSION

The results of this study showed that the application of salt stress at 100 mM significantly reduced the precocity of okra seeds germination without affecting its final rate. This delay of germination caused by this level of salinity is due to a difficulty of seeds' hydration because of a high osmotic potential and can be

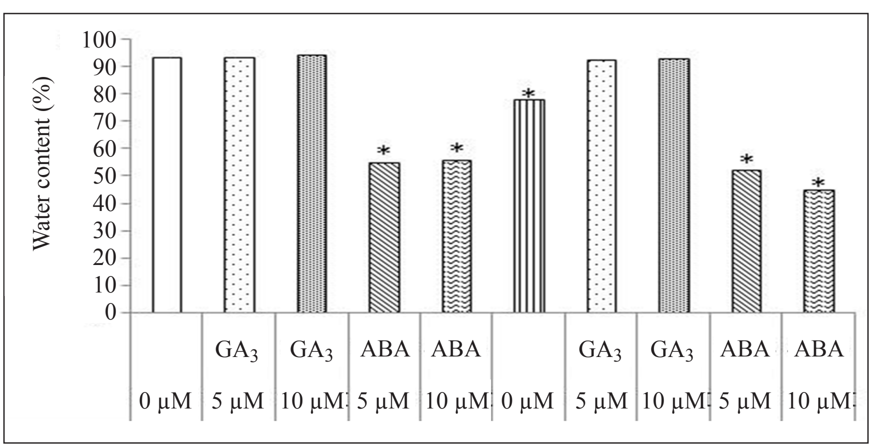

Figure 6. Effect of hormone/salinity interaction on seedlings water content (%)

explained by the time necessary for the seeds to trigger the mechanisms allowing them to adjust their osmotic pressure (Diallo et al., 2013; Ouis et al., 2015), which was also reported by Zemani (2009). Results showed that the NaCl acted negatively on the hypocotylar length and seedlings water content (Table 1), indicating that NaCl affected not only germination rate but also the seedlings' growth. Similar results have been highlighted by Bahrani and Pourreza (2012) on wheat. According to Werner and Finkelstein (1995), high salinity can inhibit the lengthening of roots and hypocotyls by slowing water absorption by the plant.

During seeds germination and seedling development stages, salinity negatively regulates GA biosynthesis (Horvath et al., 2015; Zhang et al., 2016). Shu et al. (2017) reported that NaCl delayed soybean seed germination by negatively regulating gibberellin (GA) while positively mediating abscisic acid (ABA) biogenesis, inducing a decrease in the GA/ABA ratio.

This study showed that the application of GA_3 at 5 µM and 10 µM in the 100 mM saline solution modified the responses of the seeds in particular the precocity and the final rate of germination which corroborates with the work of Chouhim (2011) on the same species and Samad and Karmoker (2012) on triticale seeds. Similarly, this phytohormone has a positive effect on hypocotylar growth, fresh and dry weights and water content of seedlings. The same results were reported by Turkyilmaz (2012) on soft wheat, Abdel-Hamid and Mohamed (2014) on barley, Achour (2016) on okra and Waleed et al. (2019) on wheat.

Under salt stress, gibberellins induce the lifting of physiological seeds' dormancy, increase water absorption and stimulate the synthesis and the activation of hydrolytic enzymes mainly α-amylase, thus releasing the reducing sugars and amino acids that are essential for embryo development (Ajmal Khan et al., 2004). Liu et al. (2018) demonstrated that NaCl treatment significantly reduced the activity of α-amylase and the germination rate of rice seeds. These effects can be mitigated by exogenous GA_3 during germination of rice. In addition, they found a positive relationship between bioactive GA content and α-amylase activity and between α-amylase activity and germination rate of rice seeds. Il the other side, gibberellins improve seeds germination by inhibiting ABA activity by either activating the enzymes involved in its catabolism or by blocking the pathway of its biosynthesis (Miransari & Smith, 2014).

In parallel, the application of ABA associated with NaCl on okra seeds does not seem to attenuate the depressive effect of salinity on the studied parameters. This observation expresses the inhibitory effect of ABA on germination was already confirmed by the work of Thakur and Sharma (2005). However, this ABA acts by limiting water absorption and inhibiting the synthesis of germination-specific enzymes such as α-amylase by counteracting the stimulatory effect of GA_3 on them (Kondhare et al., 2014). The decrease in germination rate observed under ABA treatment can be attributed to the induction of secondary dormancy and the inhibition of seeds germination by limiting the availability of energy and metabolism (Leymarie et al., 2008). Besides GA, ABA also plays an important role in regulating seed germination. It was recognized that GA and ABA antagonistically regulate seed germination (Li et al., 2016; Shu et al., 2016), and NaCl inhibited soybean seed germination by decreasing the ratio of GA/ABA via decreased bioactive GA and increased ABA contents (Shu et al., 2017).

CONCLUSION

During seeds germination stage, the presence of NaCl at 100 mM negatively affected the precocity, but did not appear to influence the final rate of sprouted seeds. However, at the seedlings growth phase, this saline concentration significantly reduced seedlings growth and their development. The hormonal supply of GA_3 increased the seeds germinal capacity in the presence and in the absence of NaCl, moreover this phytohormone positively affected the fresh weight, the hypocotylar length and the water content of okra seedlings under the two tested concentrations. On the other hand, the presence of ABA in the imbibing medium did not improve the response of the seeds under salt stress, in particular under the concentration of 10 µM of ABA.

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