Improvment Of Phaseolus Vulgaris Seed Germination With GA_3 Under Salt Stress

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Abstract
Salinity is one of the main abiotic stresses that causes a serious damage to agricultural production. This study aimed to investigate and evaluate the impact of increasing salt stress levels on seeds germination of bean species (Phaseolus vulgaris L.) This research is based on biochemical and physiological measurements. To select salt-tolerant genotypes and propose a biological alternative, the effect of different concentrations of NaCl (50, 100, 150 and 200 mM) and an exogenous application of GA_3 on the seeds were evaluated. Generally, GA_3 is a hormone that improves plant growth. The negative effect of salt stress induced by NaCl was observed on the percentage of imbibition, the radicles length. under this stress, an increase in soluble sugar amount was recorded compared to the control (0 mM). Indeed, the high value of soluble sugars was recorded by Cocorose variety. In this study, the α-amylases activity after 72 hours seems sensitive to salinity. Consequently, 0.759 mg of maltose are obtained by Coco rose genotype, While, Djadida genotype registred 0.516 mg. At 72 hours, this activity seems lower, Coco rose genotype showed 0.136mg in 0mM. The application of GA_3 (10^{-5} mM) exerted a notable effect on the increase in the activity of α-amylases.

The results proposed that GA_3 can be used as an alternative to improve the physiological and biochemical response of plants under abiotic stress.

Keywords: GA_3. Phaseolus vulgaris L.. Salt stress. Starch. α-amylases. Germination. Soluble sugars.

1. INTRODUCTION

Salinity is one of the most important abiotic stresses that negatively affect plant growth and development around the world. It affects about 20% of the world's irrigated land and results in the loss of 1.5 million hectares of agricultural land each year (FAO, 2015 ; Hiz et al. 2014; Afridi et al, 2019). Saline areas continue to increase in size because of mishandled irrigation.

In addition, in arid and semi-arid regions the salinization process occurs because of high evaporation and inadequate amounts of precipitation for considerable leaching (Liang et al. 2018). According to George, (2012), the soil is considered saline when the conductivity of the soil saturation extract (ECe) exceeds 4 dS m^{-1} (> 40 mM NaCl). Physiological dehydration and ionic imbalance present the salinity effect form (Bohnert et al. 1995; Nagy and Galiba, 1995). Na+ and Cl- ions are osmolytes that are biologically aggressive. Due to their small atomic diameter and high charge, these two ions lower the soil's water potential and accumulate in plant tissues, leading to water stress, soil toxicity and finally an antagonism in the mineral nutrition of the plant (Zhu et al. 2001; Nagy and Galiba, 1995; Bohnert et al., 1995, Munns; James ; L¨auchli, 2006).

The effects of salinity on plant rely upon on its intensity, the duration of its persistence and the stage of plant development during which it occurs. Therefore, the study of salt tolerance during different growth phases is essential to detect salt limits (Zapata et al. 2004).

Salinity inhibits crop growth and development, through complex traits that include osmotic stress, ion toxicity, mineral deficits, and physiological and biochemical defects. Higher salinity negatively affects nutrient balance, carbon metabolism, plant development, photosynthesis (Khan et al. 2015; Singh and Thakar 2018; Amjad et al. 2020). Indeed, it causes damage to cell membranes and reduces antioxidant activity and osmotic regulation (Hahm et al. 2017; Liang et al. 2014; Farouk and Al-Amri, 2019).

The seed germination phase represents a crucial stage of development that determines the successful completion of the subsequent phases of the plant's morphogenesis (Wolny et al. 2018). Although most plant species are salt-sensitive at all stages of their lifecycle, their sensitivity differs among growth stages, with seed germination being viewed as the most critical stage when salt stress impairs water absorption during seed imbibition and turgescence. The productivity of the species is greatly impacted by any modification of seed germination potential caused by negative environmental actions. During germination, the resumption of cellular activity is conditioned by a strong enzymatic activity especially the amylases, involved in the remobilization of carbohydrate reserves which are the main sources of energy synthesis in the amylaceous seeds (Nandi; Das; Sen-Mandi, 1995; Sulpice et al. 2009; Yue et al. 2019). α-amylase is regarded as an important enzyme in seed germination (Lovegrove et al. 2000).

In particular, salt stress is interlinked with lipid peroxidation in cellular membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown and impairment of enzymatic activity, as well as metabolic adaptations, mainly involving the accumulation of osmolytes. GA are endogenous plant hormones that are essential and potent regulators of plant growth and development, including promotion of seed germination, stem elongation, regulation of bolting, flowering and fruiting (Shan et al. 2014; Yan et al. 2017). Furthermore, pre-treatment with phytohormones such as GAs could improve seed performance under various environmental conditions (Rady et al. 2019; Ren et al. 2020). Previous researchs have shown that AG and ethylene weaken the seed coat, break seed dormancy and stimulate seed germination (Xiao et al. 2018; Zhang et al. 2014).

This study aimed to estimate of the enzymatic activity involved in the remobilization of carbohydrate reserves and to evaluate the exogenous effect of gibberellic acid (GA_3) on this activity in two varieties of beans under saline conditions.

2. MATERIEL AND METHODS

The used plant material is composed of two bean genotypes seeds (Phaseolus vulgaris L.), Coco rose and Djadida. The experiments was carried out in the plant improvement laboratory of Ibn khaldoun University in Tiaret (Algeria) and the IUT of biological engineering of the Paul Sabatier University of Toulouse (France).

The saline solutions of the germination medium are prepared from distilled water and NaCl with four increased concentrations 0mM, 50mM, 100mM and 200mM. Firstly, the seeds are disinfected with sodium hypochlorite (4%) for 5 min, then rinsed thoroughly with distilled water. Each germination test is conducted in four replicates of 10 seeds each at 25°C (LAMBERT et al., 2014). In fact, a seed is considered germinated when the radicle pierces the seed coat.

Imbibition rate and radicle length

Seed weight is performed every four hour until 72 hours, when seed radicle length was measured after 7 days of germination with a sliding caliper.

Soluble sucres

The simple sugars (glucose, fructose, and sucrose) are extracted by a solvent responsible for their solubilisation and capable of ihibiting the enzymatic activities that can degrade them (Gomez, Rubio, Lescourret, 2003). The reaction principle is based on the condensation of neutral monosaccharide degradation products with sulfuric acid.

100mg of seed cotyledons is maintaining at 5.25 ml of 80% ethanol for 20 hours. Then, 2 ml of the reaction mixture are diluted 10 times with 80% ethanol (reagent A). 4 ml of reagent, prepared 4 hours in advance, composed of 2 g of pure anthrone added to 1L of sulfuric acid (reagent B) are added to reagent A. The medium is delicately mixed and held in the melting ice. After shaking, the tubes are placed in a water bath at 92°C for 8 min and then are cooled for 30 min in the dark. The prepared medium was measured spectrophotometrically at 585 nm ($mg.g^{-1}$ of DM).

3. Estimation of the enzyme complex

The extraction of the enzymatic complex is carried out after 72 hours of seed germination. α-amylase activity was measured at 570 nm according to the method of LI et al. (2019). The same principle of assaying the enzymatic activity is carried out by adding gibberellic acid to the germination medium at concentrations of 10^{-5} mM.

Determination of osmotic potential

The osmotic potential of the germination media solutions (10µl), as well as the germinated seeds, is determined at different times of the experiment. The operation is carried out using a VAPOR **WESCOR type micro-osmometer.**

Statistical analysis

The obtained results were statistically processed by analysis of variance at a safety threshold of 5% using SPSS software.

4. RESULTS

The process of imbibing germinated seeds

The obtained results (Tab. 04) indicated that the variations in the quantity of absorbed water are strongly influenced by the nature of the tested genotypes. The exception is noted after 4h and 20h of germination. This parameter is affected by the effect of saline solutions after 24h of germination. Indeed, after 72 hours, it is shown the maximum water absorption (0.621 g) by the seeds in the control (0 mM) compared to the other saline treatments (Fig. 1). In contrast, in 200 mM, the lowest level of hydration is registred by the seeds with an amount of absorbed water evaluated at 0.488g (Djadida) and 0.491g (Coco rose). The tested genotypes showed distinct behaviors with regard to the imposed salt stress. Thus, the obtained average results (Fig.) showed a predominance of water absorption by Coco rose compared to Djadida.

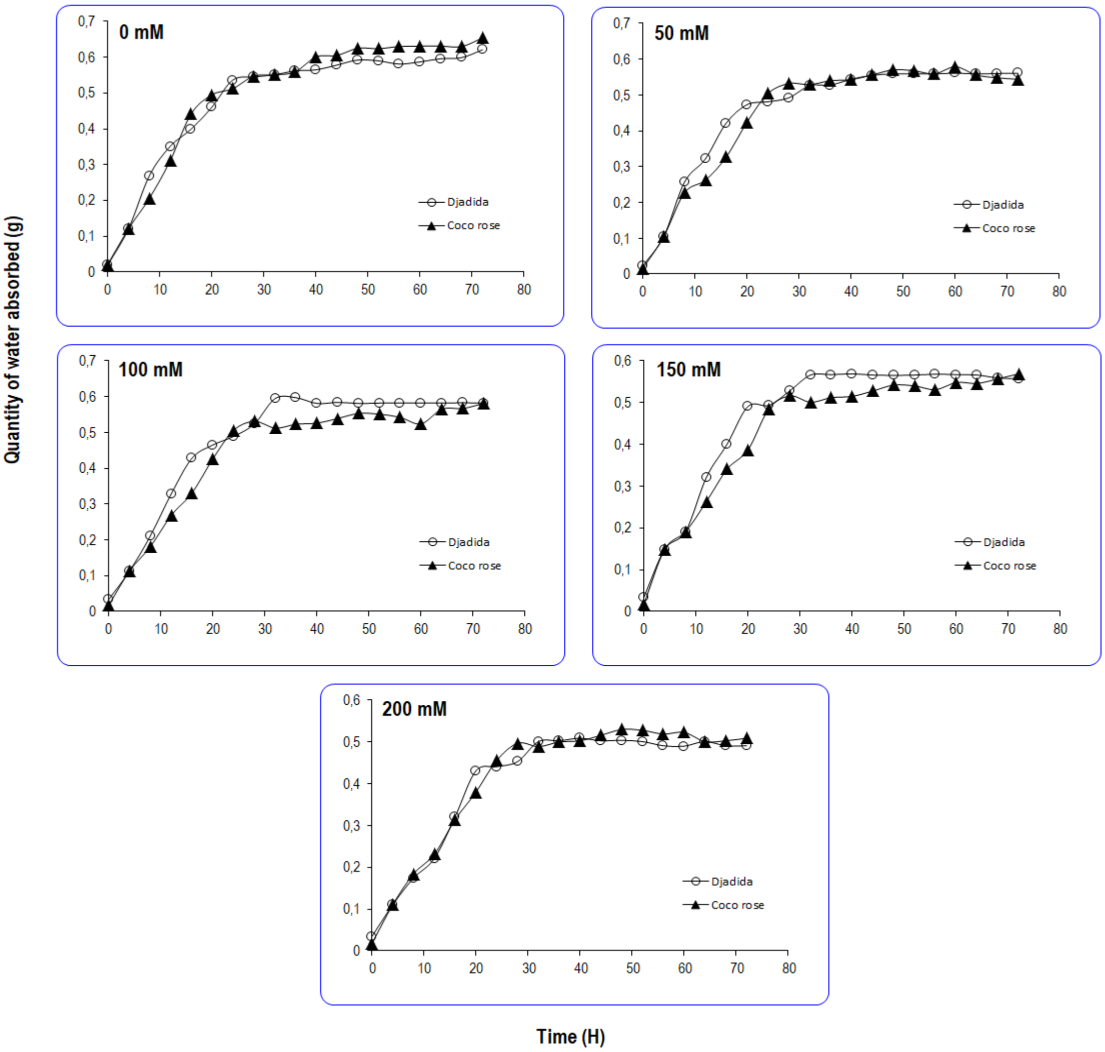

Figure 1: Quantities of water absorbed by the seeds of the two bean genotypes Djadida and Coco rose subjected to different salinity concentrations.

Radicle length

The average results (Fig2, A), showed a significant variations in the length across the different saline treatments. However, it is in the control that the length values are the highest. Indeed, in this treatement (0mM), Coco rose genotype is characterised by the highest value (19.6080 mm), while Djadida genotype recorded a value of 16.2560 mm.

According to the obtained results, it is shown that the intensification of the NaCl content in the germination medium is accompanied proportionally by a reduction in the elongation of the length of the root. Therefore, the relative reductions in 50 and 100meq, are of the order of 3.67 and 33.85% for Coco rose genotype and 19.57 and 28.13% for Djadida genotype respectively. However, under severe conditions (150 and 200 mM), no growth of the radicle was observed. Consequently, the recorded values for the two studied genotypes are zero.

Soluble sugars content

The obtained results (Fig.2, B) showed that an evolution of the soluble sucre values is highlighed by all the genotypes according to the increase in the salinity of the germination solutions. Djadida genotype recorded 6.55 mg g-1 DM (0mM) and 9.75 mg g-1 DM (200mM) of soluble sugars content. Also, Treatment with 50 mM, 100 mM and 150 mM saline solutions exteriorizes respective sugar contents of 7.32 mg g-1 DM, 7.93 mg g-1 MS and 8.67 mg g-1 DM. However, for Coco rose genotype, the values are higher under high salinity. They are 8.065, 8.82, 9.065 and 9.555mg g-1 DM in 50 mM, 100mM, 150mM and 200mM respectively.

α-amylases activity of germinated seeds

During seed germination, α-amylase mainly hydrolyzes starch into soluble sugar. Figure 2 (C) showed that the saline solutions of 50, 100, 150 and 200 mM NaCl led to a significant decrease in α-amylase activity. Nevertheless, this decrease was higher for Coco rose than Djadida genotype. For that, in Djadida genotype, starch degradation decreased significantly by 15.38%, 16.66%, 25.64% and 35.89% in 0 mM compared to 50, 100, 150 and 200 mM of NaCl respectively.

α-amylase activity after exogenous application of GA$_3$

The obtained results from the activity of α-amylase with exogenous supply of gibberellins at a concentration of 10^{-5} mM showed that GA3 attenuated the decrease in α-amylase activity in both studied genotypes (Fig 2, D). These results suggest that the decrease in α-amylase activity plays a role in suppressing seed germination. For Djadida genotype, the quantities of remobilized starch are of values of 0.078 mg (0 mM), 0.066 mg (50mM), 0.058 mg (100 mM), 0.054mg (150 mM) and 0.050 mg (200mM). While, They are of the order of 0.145mg (0 mM), 0.084mg (50mM), 0.071mg (100 mM), 0.063mg (150 mM) and 0.057mg (200 mM) for Coco rose genotype.

The exogenous application of GA$_3$ caused significant increases in α-amylase activities compared to the control in the Coco rose genotype and which are of the order of 40.43%, 41.38, 41.14 and 62.20% recorded in 50, 100, 150 and 200 mM NaCl respectively. While, the variation oscillates between 15% (50Mm) and 35% (200mM) for Djadida genotype.

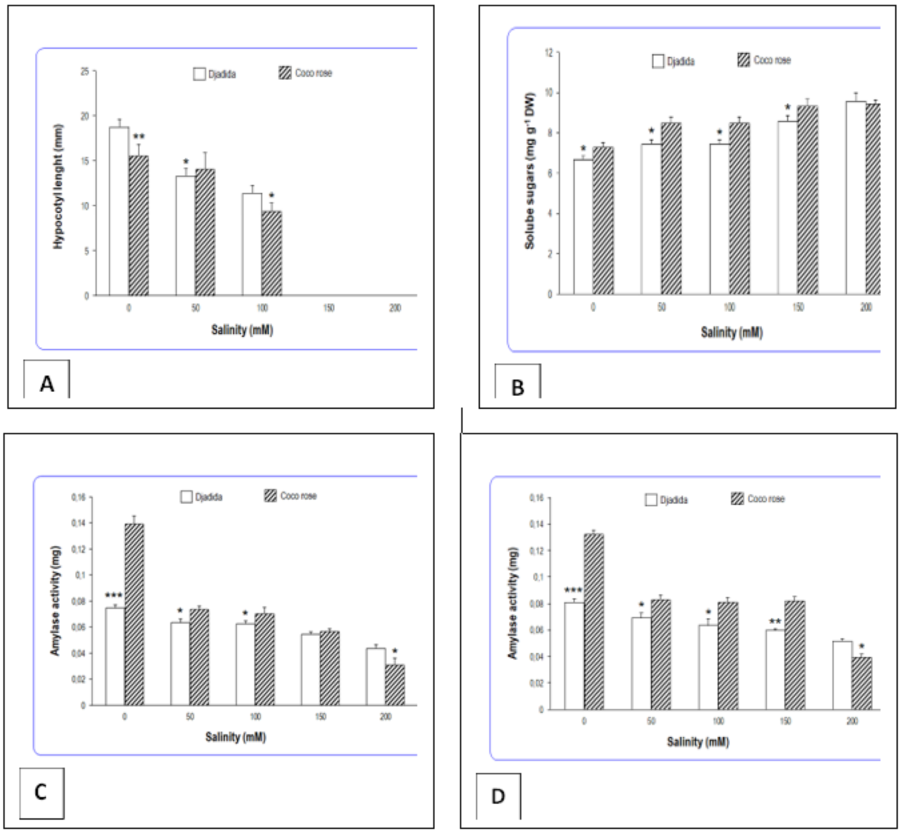

Figure 2: Effect of salt stress on hypocotyl length (A), soluble sugars (B), amylase activity without exogenous application of GA$_3$ (C) and amylase activity with exogenous application of GA$_3$ (D).

5. DISCUSSION

Seed germination is one of the most fundamental and vital phases of the plant growth cycle, as it determines plant establishment and productivity. It is also the most sensitive stage to environmental factors during the plant growth process (Weitbrech et al. 2011). Salt stress is one of the main abiotic stress that reduces the germination potential including the germinal index and biochemical and enzymatic processes (Gao et al. 2019; Yu et al. 2016; Castanares et al. 2018; Zeng et al. 2018). The seed imbibition is a physiological step, essential for the seeds germination of plant (Johansson et al. 2000). The rehydration constitutes the mechanism where the cellular water potential becomes favorable to a metabolic reactivation of the cells of the seed and essentially those of the primary meristems of the growing points of the embryo. Imbibition inevitably depends on the water quantity and quality. The obtained results showed that the osmotic potential of the seeds germinated is maintained at a lower level throughout the imbibition period. The osmotic potentials of the adopted solutions in this study are of the order of -514 kpa, -652 kpa, -802 kpa, -964 kpa and -1115 kpa for 0, 50, 100, 150 and 200 mM of NaCl respectively. The results of the present study corroborate with the previous reports indicating that salt stress decreases water uptake during seed germination (Shine et al. 2012; Thomas et al. 2013). Jeanette et al. (2002) indicated that the Phaseolus species presents a large variability of tolerance to salinity during the germination. Indeed, at concentrations of 0, 60, 120 and 180 mM of NaCl, the seed imbibition process unfolded in a optimal way. These results indicated that under saline conditions, a collection of 28 bean genotypes exhibited sufficient rehydration to allow cell reactivation and germination initiation.

The last stage of germination is marked by the proliferation and growth of the cells engendering the development of the radicle of the embryo (Schiefelbein et al. 1997). Changes in growth and biomass are important indicators of salt toxicity in plants (Manaa et al. 2014; Mimouni et al. 2016). The results of our study showed a dramatic decline in radicle length under NaCl stress. The salinity effect on germination is significant during multiplication and growth of cells of the meristems, cauline and roots of the embryo. Thus, the remobilization of carbohydrate reserves is insufficient to allow vegetative growth through the development of the radicle. It has been revealed by this study that 150 and 200 mM of NaCl concentration inhibit the radicle growth. This indicates that the effect of salinity, by toxicity and lowering of the osmotic potential is expressed at later stages of germination. According to the obtained results, these effects are mainly explained by a physiological water deficit, caused by the low of the osmotic potential of the germination medium. Growth inhibition induced by salinity was positively correlated with high accumulation of Na+, accompanied with high Na+/K+ ratio at the root. salt stress decreases root plasma membrane permeability which induces accumulation of intracellular Na+ content by substituting K+ and other plant tissue nutrients (Assaha et al., 2017). Ion toxicity, in turn, limits water and mineral nutrient uptake, thereby limiting seed growth and development, which is supported by previous studies (Hamayun et al. 2015; Nedjimi and Daoud , 2009; Tiwari et al. 2010). Multiplication and cell growth processus inevitably require sufficient water availability in quantity and quality (Pessarakli, 1991; Taiz et al. 2003). The remobilization of carbohydrate reserves is an essential physiological step in the germination process of starchy seeds (Heldt, 2005; Soriano et al. 2014; Zhao et al. 2018). Soluble sugars hydrolyzed from starch in the endosperm are transported into the embryo to provide energy for its development and facilitate seed germination (Kaneko et al. 2002; Kaur et al. 1998). The biogenesis of these sugars results from a catalytic activity of the polysaccharides accumulated during the seed maturation. The improvement in the activity of antioxidant defense systems is produced under the salinity effect on the seed amylases activity responsible for the accumulation of soluble sugars (Boyer, 1982; Bray et al. 2000; Yildirim et al. 2009; Elham et al. 2014; Ren et al. 2020). Increase in consumption of energy presents one of the strategies adopted to tolerate salt stress (Liu et al. 2018; Acosta-Motos et al. 2020).

In this study, the obtained results indicated that the mobilization of carbohydrate reserves in the form of soluble sugars is stimulated by the increase in NaCl levels in the germination medium. Thus, the increase in the soluble sugars could be explained by the early installation of osmotic adjustment mechanisms (Pattangul and Thitisaksakul, 2008; Khalil et al. 2017). These preliminary results indicated that the manifestation of salinity tolerance mechanisms by osmotic adjustment can be expressed at different stages of plant development (Greenway and Munns 1980; Maury et al. 2000; Nounjan et al. 2018).

The species Phaseolus vulgaris presents an intra-specific genetic variability of tolerance to salinity during the first phases of plant development. Thus the two tested genotypes during showed different behaviors against salt constraint. Generally, abiotic stress and plant hormones affect α-amylase activity (Appleford and Lenton, 1997; Kaneko et al. 2002; Li et al. 2019; Li et al. 2019; Liu et al. 2018). In fact, the osmotic potential of the seeds cells and that of the germination medium have a significant influence on this activity. The synthesis of α-amylases is initiated in the aleurone cell layer where the inducible environment is conditioned by endogenous gibberellins in germinating seeds (Zou et al. 2008). This process is variable depending on different plant species, different tissues and environmental conditions (Laura et al. 1997; Kommineni et al. 1995; Masao Ishimoto et al. 1996).

This study highlighted that the salinity exerts a very depressive effect on the germination of bean seeds. Results confirmed by Zeid (2004) and Kaymakanova (2009). This inhibition is mainly due to an imbalance of water uptake, destruction of the cell membrane and reduction of enzymatic activity, which limits the hydrolysis of seeds (Guo et al. 2004; Ke et al. 2002; Qi et al. 2007; Deivanai et al. 2011). Also, this abiotic stress could inhibit seed germination through inhibition of key enzymes, α- and β-amylase, which hydrolyze stored starch during germination (Hua-Long et al. 2014; Arnao and Hernandez-Ruiz, 2018).

Salinity has a notable effect on the availability and functioning of gibberellins during germination. This is confirmed by the variations in the α-amylases activity by exogenous application of this phytohormone. Biosynthesis and catabolism of phytohormones play essentiel roles in seed germination, seed dormancy and seedling growth (Hamayun et al. 2015; Ahanger et al. 2018). Salt stress increases the concentration of ABA and reduces GA in germinating seeds, decreasing consequently the GA/ABA ratio which is a key factor for seed germination (Shuai et al., 2017). However, exogenous application of GA3 has significantly affected the endogenous GA3, ABA and IAA content (Zhang et al. 2016). Under the effect of abiotic stresses, the inhibition of seed germination is mainly due to the reduction of the concentrations of bioactive GA (Chen et al. 2018; He et al. 2019; Liu et al. 2019; Wang et al. (2019). Kaneko et al. (2002) and Miransari et al. (2014) demonstrated that during seed germination, bioactive GA increase the biosynthesis of hydrolases in the aleurone layer. These Hydrolases are responsible for converting starches into monosaccharides and disaccharides, which provide substrates and energy for seed germination.

According to our results, the exogenous application of GA was effective for mitigating the effect of NaCl salinity on the germination of several other halophytes, such as Zygophyllum simplex., Arthrocnemum indicum L., and Prosopis juliflora (Khan et al. 2002, EL-KEBLAWY, Al-Ansari et al. 2005). Herein, it was strongly correlated with the increase of antioxidant enzyme activities (Maggio et al. 2010). This could be explained by the fact that GA can reduce the level of ABA in seeds by activating their catabolism enzymes or by blocking the biosynthetic pathway (Toyomasu et al. 1994). Any increase in the activity of these enzymes can result in early and vigorous germination and optimal crop establishment (Berhanu and Melkamu, 2018). Li (2015) suggested that under salt stress conditions, bioactive GA is a limiting factor during Suaeda salsa seed germination.

Salt stress can concomitantly decrease the content of compounds that stimulate seed germination such as enzymes and growth hormones (e.g., gibberellins), increase ABA levels, and induce changes in membrane permeability. Exogenous supply of GA_3 could be a useful approach to improve the response of Phaseolus vulgaris L. at early stages of to high salinity.

6. CONCLUSIONS

The results of our study showed that salt stress greatly inhibits the germination of bean seeds mainly due to ion toxicity, oxidative damage and hormonal imbalance. Growth hormones are commonly used in agriculture to improve productivity. Gibberellic acid (GA) is one of the plant hormones involved in the mechanism of growth and development of vegetative organs. Indeed, it has effects on seed germination, leaf expansion, stem elongation and flowering. Additionally, gibberellins interact with other hormones to regulate different metabolic processes in plants. This study provided valuable information on seed pretreatment with growth hormone (GA_3). Therefore, GA_3 application reduced the negative effects of salinity produced by NaCl. It leads to higher activity of α-amylases, which promoted seed germination. Treated bean seeds with GA_3 showed greater starch degradation under salt stress than those untreated.

Finally, treatment with regulating hormones can be used as an effective strategy for seed germination and seedling establishment under abiotic stress.

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