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Effect of salinity on chickpea seed germination pre-treated with salicylic acid

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is known to be highly susceptible towards soil or water salinity, whose are the primary abiotic factors that limit growth and crop of this plant in Algeria. Therefore, more efforts are needed to improve its tolerance to salinity. Salicylic acid (SA) is a key endogenous signal that mediates defense gene expression and disease resistance in many dicotyledonous species. The objective of this study was to determine the role of salicylic acid on reducing the stress sensitivity to salinity during germination of chickpea. Our observations indicate that, although SA had a positively effects exerted on salinity tolerance in seed germination in comparison with untreated seeds. It is important to notice that pre-treated seeds with salicylic acid prevent a decrease in kinetics of germination, estimated respectively to 80% in *ILC3279* and 76% in *AKIM91*, Also, a speed of radicle emergence were noted, under salt stress into both varieties. Also it provides a significant decrease in proline contents and a few variations in soluble sugar, the most important accumulation of proteins were noted with 0,5mM SA in *AKIM91* (10.25%) and *ILC3279* (8.75%). The results of polyphenol indicated an accumulation in the pretreated seeds with 0.05 mM (1.84mg/gMS in *AKIM91* and 0.45 in *ILC3279*). A relationship between this compounds and salt tolerance was observed in both varieties, under the effect of Salicylic acid concentrations during the germination period.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume crop in Algeria and is largely raise for human consumption. But many problems which are linked to environmental stress, thus moisture and increasing salinity from human disturbance and climate change is a critical problem worldwide because it has dramatic effects on plant physiology and performance and are the main causes of the low yields of their culture (Merrien and Grandin, 1990), by reducing the quality and quantity of the yield per hectare.

These weaknesses lead Algeria to import more than 355 million dollars to fill up the needs of the population (STAT. Canada, 2007). Different strategies are being employed to maximize the decrease of plant growth under saline conditions. Such as plant growth regulator; which have been used as mediators of the selections in plants because they are able of changing the expression of genes plants subjected to environmental stress (Rhodes and Orczyk, 2001). Salicylic acid (SA) is a critical signaling molecule that modulates plant responses to pathogen infection. Recent research indicated the significant role of salicylic acid (SA) in the regulation of diverse aspects of plant adaptive responses to many abiotic stresses (Senaratna *et al*. 2003; Shakirova et al. 2003).

It is essential to study the effects of the pre-treatment of seeds of this variety with different concentrations of SA during germination phase, under the influence of salinity. Our choice is focused on two varieties of chickpea (ILC3279 and AKIM90); the conducted experiment carried on the germination parameters and some biochemical compounds intervening as Osmoprotectants, until the seed impregnation and leading to the breakthrough of the radicle out coat. The aim of present work was to reveal whether seeds of chickpea plants pre-treated with different concentrations of SA could tolerant salt stress.

2. Materials and methods

2.1. Plant materiel

Tow cultivars was used in this study: ILC 3279 and AKIM 91.These cultivars were provided by ITGC (Institut Technique des Grandes Cultures) of Saïda-Algeria. Seeds are sterilized with 2 % sodium hypochlorite for 3 min and thoroughly rinsed with distilled water, then dried with filter paper.

2.2. Salt stress imposition

Seeds of both varieties of chickpea were divided into three lots: Lots 1Control (un-treated seeds exposure to 100 meq NaCl - Lots 2 (seeds treated with 0,05mM AS exposure to 100 meq NaCl) - Lots 3 (seeds treated with 0,5mM AS exposure to 100 meq NaCl).

Pre-treated seeds were imbibed in different doses of distilled water and salicylic acid for six hours. After, seeds were distributed to germinate in Petri dishes on two filter-paper discs; each dish contains 50 seeds and sprayed with 20 ml of saline solution (100 meq). The dishes are put in an incubator at 25 °C. Germination is spotted by the output of radicle from the integuments as described by (Gilles, 2009; Jaouadi *et al*. 2010). Germinated grains are counted, every 24 hours, as well as measurements of the radicle elongation. The test ends when after two successive counts no germination is registered. After 96 h, germinated grain of each dish is taken for biochemical tests (Protein, Proline, soluble sugar and Polyphenol).

Biochemical analysis was conducted on germinated seeds of chickpea divided into three lots:

For the protein content; 1g of dried powder of germinated seeds of chickpea for any treatment was estimated by multiplying the total nitrogen content by the factor 6.25 (Kdjeldhal, 1983). The Proline content: 100 mg of dried powder of germinated seeds of chickpea for any treatment was homogenized in 1.25 ml ethanol 70%, and then determined with ninhydrine by spectrophotometer at 505 nm by the method of (Bergman and Loxley, 1970). The soluble sugar was determined by adding 50 mg of dried powder of germinated seeds of chickpea for any treatment to 50 ml distilled water for 24 h and carbohydrates were measured by phenol-sulfuric acid method as described by [Dubois et al. 1956]. For Polyphenol concentration; 100 mg of dried sample of germinated seeds of chickpea for any treatment were homogenized with 1ml of acetone, then extract with Ultrasons 45mn at 4°C. The polyphenol are commonly determined using Folin-Ciocalteu reagent (Gallet and Lebreton, 1995).

2.3. Statistical analysis

Analysis of variance with student test was used to test for differences in effects of the different doses of treatment, and response of both varieties (kinetic of germination, radicle elongation, biochemical compounds) to salt stress.

3. Results

Obtained results indicated that the germination of control of ILC 3279 and AKIM 91 grains starts at 24 h (Fig 1) with a percentage which reached respectively (45% et 38%) in two cultivars and increases at (70, 9% and 94%) at 96 h and a longer of radicle noted of 24 h in a linear way, during the following hours to attain respectively (4.25-18.5-24.8-25 mm) in the grain of ILC3279 and (16-22-30-32 mm) in the grain of AKIM91. On the other hand salt stress has eventually reduced the percentage of germinated seeds; the speed of germination generally shows a phase of latency, where there is a total absence of germination since 24 h in the two varieties (Fig 1). The kinetics of germination of un-treated seeds showed inhibition over time, starting in 48 h (Fig 1), it's not exceeding the: 4.3%, 29% and 30% in ILC3279 and 21%, 25% and 27% in 48 h, 72 h and 96 h AKIM91. It's characterized by a level indicating a stop of germination at 72 h. Also, the emergency of radicle is very low in untreated seeds (Fig 2), it begins at 48 h to reach only respectively (3-17.1 – 17.3 mm) in the grain of ILC 3279 and (3.7 – 6.3 -12.5 mm) in the AKIM 91 seed's in 48 h, 72 h and 96 h. While an variant acceleration of germination in seeds treated with increasing doses of salicylic acid (0.05 - 0, 5 mM) exposed to 100 meq of salinity in both varieties since 48 h and lasts up to 96 h, before reaching the stationary phase, where maximum of sprouted grains reaching respectively (70-80%) in ILC3279 and (76 and 79%) in AKIM91 under 0.05 and 0.5 mM SA. The concentration 0.05 mM was more reliable to the grain of AKIM91, since 48 h (Fig. 1). Also, the seeds treated with SA show a linear elongation of radicle since 48 h and hard until 72 h, 96 h to reach respectively (17- 29 -31 mm) with 0.05 mM SA and (17 – 30 – 33 mm) with 0.5 Mm SA in ILC3279 and (11 -23 -25 mm) with 0.05 mM SA and (12 -27- 30 mm) with 0.5 mM SA for AKIM91 (Fig. 2). Student statistical test shows no significant differences between mean values (P\0.05) of control and treated seeds in comparison with no treated seeds, in both varieties, also the effect of concentration of AS; 0.5 mM is significant than 0.05 mM on the kinetic and the rate of germination, thus the radicle length. To assess the interaction of salicylic acid and physiological tolerance during germination phase under salt stress in both varieties, some biochemical substances having a relation with osmoprotection of cells and whose play a protective role in stress were performed (Tab 01). Obtained results illustrated a few augment in the percentage of the proteins in untreated seeds of the two varieties (tab 01), under the effect of the saline stress compared to the control (4.5%) and with (3.37%) in ILC 3279 and (5.27%) and with (4.2%) in AKIM 91. However, a gradual accumulation is noted in the pre-treated seeds of both varieties, with variations according to the concentrations of SA; With 0,05 mM SA an increase is recorded in ILC 3279 (5.25%) and AKIM 91 seeds (7.15%), while with 0,5mM SA, an accumulation is most noticed at variety AKIM 91 seeds (10.25%) and ILC 3279 (8.75%). Statistical analysis indicated significant differences between mean values (P\0.05) of proteins which are reduced under the effect of the saline stress in untreated seeds and significantly higher in the seeds treated in both varieties of chickpea, especially with 0,5mM SA. The proline analyses indicate an increase in its rate in seeds untreated and subjected to salt compared to the control (tab 01); (19.8 µg) against (15.7 µg) in the grains of the ILC 3279 variety, and (17.6 against 12.7 µg) in the grains of the AKIM 91 variety. In contrast, there is a gradual decrease in the levels of proline as a function of concentration of SA (0- 0.05 - 0.5 mM) in both varieties, respectively (19.8-16.8-14.8 µg) in ILC 3279, (17.7 -12.2-11.7 µg) in AKIM91. Student's t statistical test showed a highly significant effect of SA on the reduction of proline content, with variations according to variety and depending on the concentration of this compound. The results representing sugars show an increase in this compound levels in seeds under the influence of salinity compared to controls for both varieties (tab 01); (1.23 against 0.94 mg) in grain of ILC 3279 and (1.07 against 0.86 mg) for AKIM 91. But in case, there is a variation in the accumulation of this compound according to the concentrations of SA, a decline in level of sugar is showed in the seeds of two varieties pretreated in the presence of 0.05 mM (1.04 and 1.05 mg). However, in the presence of 0.5 mM a decrease was reported in ILC 3279 (0.97 mg) against an increase in AKIM 91 (1.33 mg). Statistical analysis shows that the level of sugar in seeds pretreated of ILC 3279 has significantly diminished regardless of the dose of SA in comparison to the non-treated seeds. But significant changes are clearly noted in the variety AKIM 91, depending on the concentration of SA. Polyphenol content in un-treated seeds varied from (1.95 against 0.31 mg) in grain of ILC 3279 and (1.55 against 0.22 mg) in grain of AKIM 91, under the influence of salinity compared to controls (tab 01). However the level decreased in response to salinity in the treated seeds with 0,05mM SA; respectively (0.45 and 1.84 mg) and increased with 0.5mM SA (0.35 and 0.53 mg) in both varieties. Student's test showed a highly significant effect of SA and salinity on the change of content of polyphenol, with variations in both varieties according to the concentration of SA.

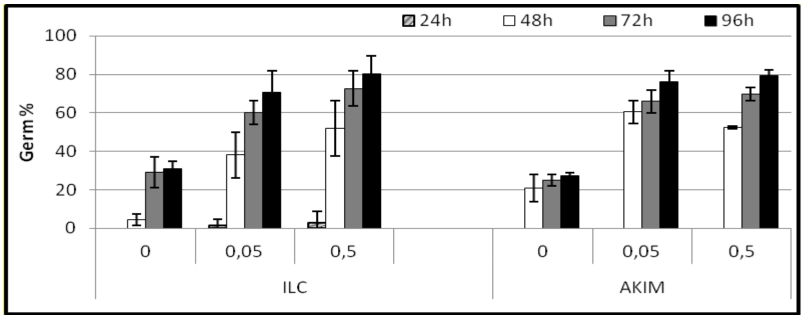

Fig. 1. Kinetic of Chickpea seed's germination (ILC 3279 and AKIM 91) treated with salicylic acid and subjected to saline stress.

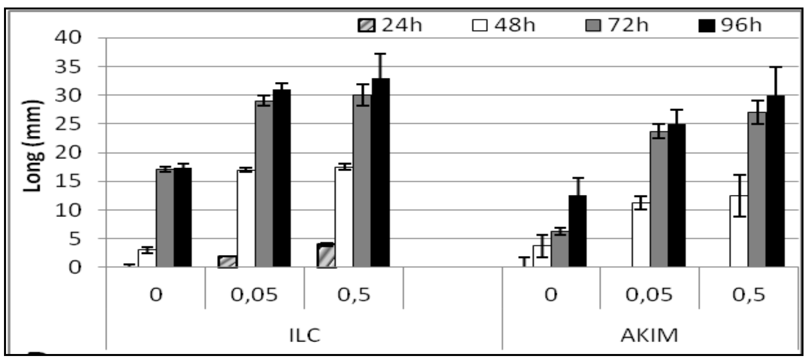

Fig. 2. Radicle length of Chickpea seed's germination (ILC 3279 and AKIM 91) treated with salicylic acid and subjected to saline stress.

Table 1

Effect of Salicylic acid applications through Chickpea seeds, during germination,on biochemical parameters: proteins, proline, sugar, polyphenol, subjected to saline stress.

Variety	Treatments AS (mM)	PROT (%)	PROL (µg/ 100-1g dry wt)	SUCRE (mg/ 100-1g dry wt)	Polyphenol (mg/100^{-1g} dry wt)
ILC3279,	0	4.37 ± 1.1	19.8 ± 0.25	1.23 ± 0.06	0.31 ± 0.03
	0.05	5.25 ± 2.6	16.8 ± 0.2	1.04 ± 0.14	0.45 ± 0.04
	0.5	8.75 ± 4.1	14.8 ± 0.3	0.97 ± 0.085	0.35 ± 0.065
AKIM91	0	5.27 ± 2.1	17.6 ± 0.85	1.07 ±0.14	1.55 ± 0.06
	0.05	7.15 ± 2.9	12.2 ± 0.65	1.05 ± 0.02	1.84 ± 0.053
	0.5	10.25 ± 2.3	11.7 ± 0.4	1.33 ± 1.14	0.53 ± 0.085

4. Discussion

The results obtained show a delay in the germination of un-treated seeds submit to salt stress of both varieties, either on the decrease in the percentage of germinated grains or on the elongation of radicle (Chandrashekhar et al. 1986), in comparison with control or with pre-treated seeds SA. We note that in the witness, the emergence of the radicle it follows the kinetics of germination; it seems more intense in the AKIM91 varieties. Several studies have pointed out that the seeds of glycophytes respond to salt stress by reducing the total number of germinated seeds and accusing a delay in the initiation of the process of germination and emergence of radicle (Mauromicale and Licandro, 2002; Wojciechet *et al*. 2008). This toxic effect may lead to alteration of the metabolic processes of germination and in extreme case the death of the embryo by excess ions (Kaya *et al*., 2008). Either by an increase in external osmotic pressure, which affects the absorption of water by the grains and / or well has an accumulation of Na and Cl ions in the embryo (Groome, 1991).

The contrasting effects of SA on seed germination found in previous studies, SA is considered as a signal molecule that is involved in several physiological processes of controlling the germination (Krasavina, 2007; Lucas and Lee, 2004). The positive effect of both doses of SA under salt stress was shown, not only in percentage and speed of germination but also on the elongation of the radicle. These results accord those of (Senaratna *et al*. 2003; Shakirova 2003). Szalai *et al*. (2005) suggested that the interaction between salinity and SA possibly induced genes encoding of resistance, this molecule seems to act on germination by increasing the physiological activity, marked by a mobilization of the reserves necessary to growth (Bois, 2005). It also been shown that SA increases the degree of growth of radicle cells both by division and expansion of meristem cells (Janda *et al*. 2007)**.**

Differences in the accumulation patterns of proline, total soluble sugars, protein and polyphenol were found under salinity stress.

Seed germination is an important agronomic trait that profoundly affects plant growth and productivity; A diminishes in percentage and speed in seed germination is showed, with an alteration of the length of the radicle. However, the rate of protein is low in un-treated seeds with an increase in proline content. These results correspond with the work of (Gomes, 1983). This can be explained by the high availability of NH_3 issues from the degradation of proteins in the stressed plants.

In contrast, we showed that physiological concentrations of SA greatly reduced the inhibitory effects of salt on germination by reducing osmotic compounds. Also, a linear accumulation of proteins and a linear decline of proline are observed. Also, a decrease of sugars is reported in ILC 3279 seeds against an increase in AKIM 91 seeds according to high concentrations of SA.

Janda et *al*. (2007) reported that there is an antagonism between the effect of salinity and SA on some genes and metabolites. The effect of NaCl is exercised on the enzyme activity and on the transport of reserves to the embryo (Gomes *et al*. 1983). Also, Kim *et al*. (2007) have revealed that SA inhibits the genes expression salinity (SALT gene).

An increase in proteins accumulation in pre-treated seeds with SA is reported, these results are consistent with those of (Nemet, 2002). Alem and Amri (2005) have shown that the use of exogenous SA activates the synthesis of stress's proteins under abiotic conditions, such as LEA proteins, that ensure the protection of all vital cellular proteins. Such, the growth of the embryo depends on the accumulation of proteins in the seeds and available nitrogen (Gilles, 2009). SA has antagonistic effects on proline in comparison with salt; a low accumulation is noted with low dose and a decline with high dose (Hanan, 2007). Fabro et *al*. (2003) also, indicated an accumulation of proline due to the deficiency of SA in *Arabidopsis thaliana*, during biotic stress. Yoshiba (1997) confirms that the highest rates of proline are considered as an indicator of protein degradation. It is therefore possible that a correlation exist between the accumulation of proteins and the reduction of proline under the effect of SA.

The rates of sugars were higher in un-treated grains compared with the control ones, in parallel with a reduction in the rate of seeds germinated, especially in AKIM 91 variety. Price et al. (2003) have recently shown that sugars such as sucrose and some hexose inhibit the growth of young seedlings, regardless of their osmotic effect. Conversely, a decrease in soluble sugar content in tissues of the pre-treated seeds was noted in ILC 3279 variety against an increase in AKIM 91 variety. The sugar levels depend on the concentrations of SA used (Janda et al. 2007). Bhuja et al. (2004) indicate that the levels of sugars depend on the physiological state of grain during germination. These results agree with those of (Krasavina, 2007), which demonstrated that the accumulation of sugar in the grain of Mays pretreated with SA is not stable all the time. While, Korkmaz *et al*. (2007); Szepesi *et al*. *(*2005) ensure that SA increases the soluble sugars in melon seedlings. Gille, (2009) says that the event of germination is correlated with callose's synthesis, formed during cytokinesis. So, Bhuja, (2004)] noticed that SA active the callose synthesis during the abiotic stress. However, an increase of osmolites is not always related to a reliable treatment or an increased tolerance (Maggio et al. 2002).

Differences of polyphenol content are observed through un-treated seeds or pre-treated seeds in both varieties. Which were accumulated more in AKim91 towards a decline in ILC in un-treated seeds in comparison with the control, which were higher in ILC and reduce in AKIM (Agastian *et al*. 2000; Mane *et al*. 2011). Consequently, an increase in the content of polyphenol in pre-treated seeds with low concentration of SA, and a progressive decline with high doses of SA was noted.

Polyphenol played a key role in the plants towards stress. Mane *et al*. (2011) reported that plants vary widely in their phenolic composition and content, with both genetics, physiological and environment effects. Also, the excess accumulations of many polyphenol inhibit the germination by reduce the O_2 (Vadez *et al*. 2007). So, the content of total phenol diminishes in pre-treated seeds with high concentration of SA and a level of germination. This could be due to the effect of SA, which may play a role in activation of the polyphenoloxidase and cause the degradation of polyphenol (Khandelwal *et al*. 2010) or binding the polyphenol with other organic substances such as carbohydrate or protein (Saharan *et al*. 2002).

Furthermore, examination of the germination would clarify the possible link between SA and biochemical compounds in seed germination, under salt stress. However, Salicylic acid treatment had a pronounced ameliorative role as well as, germination, emergence of radicle under saline conditions. The ameliorative effect of SA might be linked to the observable increase in protein, necessary for cell division of the embryo. Consequently the changes in content of proline, soluble sugar and polyphenol were due to SA treatments.

This may indicate that SA might alleviate the imposed salt stress, either via osmotic adjustment or by conferring desiccation resistance to plant cells as reported by other investigators (Gunes *et al*., 2007). But its exact role on the biochemical compounds is not fully illustrated.

5. Conclusion

In conclusion, we found that SA has inhibitory effects of salt on germination, depending on the concentration applied and the genotypes of chickpea, which have little different mechanism to adapt to salt stress during germination. But we can't establish a good correlation between its roles in the metabolism of biochemical compounds that facilitate retention of water and synthesis of osmotic active metabolites.

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