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GERMINATION AND GROWTH IN CONTROL AND PRIMED SEEDS OF PEPPER AS AFFECTED BY SALT STRESS

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ABSTRACT. Salinity is an important abiotic stress which can affect crop production in the world. One of the simplest methods for improving salinity tolerance of plants is seeds priming. This experiment was conducted to evaluate the effects of seeds priming with three solutions (KCl , NaCl and $CaCl_2$) in germination and later growth of three pepper (*Capsicum annuum* L.) cultivars: Beldi, Baklouti and Anaheim Chili. Seeds germination was conducted in a completely randomized design under seven salinity levels (0, 2, 4, 6, 8, 10 and 12 g L^{-1}) at room temperature for primed and control seeds. Plants derived from these germinated seeds (control and primed) were transplanted and cultivated in a greenhouse for 4 months and were irrigated permanently with seven salinity levels (0, 2, 4, 6, 8, 10 and 12 g L^{-1}). The results showed that salinity affected all parameters under study like total germination percentage and chlorophyll level (a and b). As well, proline content increased as response to increasing salinity. The plants derived and grown from primed seeds showed a considerable tolerance to salt stress and gave better results. In fact, priming improved the salt resistance of pepper owing to more chlorophyll and proline accumulation. These results suggest that seed priming induced possible physiological adjustments in pepper seeds, especially in the early stages of development, and could be used as a suitable tool for improving germination and growth characteristics under salt stress conditions.

Key words: Seed priming; Pepper; Proline; Chlorophyll level.

INTRODUCTION

The necessity of developing crops with higher salt tolerance become an evidence. In fact, salinity affects 20% of the irrigated land in the

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world (Yeo, 1999). It causes harmful damages to seeds germination and seedling growth either by preventing water uptake or by the toxicity of sodium and chloride ions (Hopper *et al.,*1979). This loss of plant productivity conflicts with the needs of the world population, which is projected to increase by 1.5 billion over the next 20 years (Yamaguchi and Blumwald, 2005). So, to avoid a famine and insufficient food production, researchers are trying to improve the tolerance of plants cultivated under salt stress by various methods such as genetic selection, biotechnology and others methods . Germination is considered as a critical step in the development cycle of the plant and several environmental factors such as salinity can affect it especially in arid and semi-arid areas. In this context, seeds priming is a physiological method based in a controlled hydration treatment in which seeds are allowed to imbibe water before radical protrusion (Bradford, 1986). Different substances are used as osmotica such as Polyethylene glycol (PEG), Sodium chloride (NaCl), Potassium nitrate (KNO_3), Zinc sulfate ($ZnSO_4$), Potassium chloride (KCl) and Calcium chloride ($CaCl_2$). It has been shown that priming, especially NaCl priming, improves salt tolerance of seeds in many species such as vegetables: tomatoes (Cano *et al.,* 1991), asparagus (Pill *et al.,* 1991), cucumber (Passam and Kakouriotis, 1994) and condiment crops: fennel (Neamatollahi *et al.,* 2009) and fenugreek (Souguir *et al.,* 2013). Many authors have concluded that seed priming ameliorates the uniformity of germination and the final germination percentage of seeds compared with control (Basra *et al.,* 2002; Farooq *et al.,* 2004; Souguir *et al.,* 2013). Also, priming improves seed performance by activating the synthesis of many proteins and enzymes involved in cell metabolism such as carbohydrates (α and β amylases) and lipids mobilization (isocitrate lyase), which are implicated in the mobilization of storage reserves (Varier *et al.,* 2010). So, priming enhances seed performance under normal as well as under saline conditions and this technique is considered as feasible and very cheap (Souguir *et al.,* 2013).

Therefore, this study evaluated the effects of priming with three different solutions (KCl, NaCl and $CaCl_2$) in germination (laboratory) and later growth (unheated greenhouse) of three pepper cultivars (*Capsicum annuum* L.).

MATERIALS AND METHODS

Seed material

Seed material is composed of three pepper cultivars (*Capsicum annuum* L.): "Beldi", which is a local medium-early cultivar with a slightly spicy aftertaste, "Baklouti", which is a local late cultivar with a very spicy aftertaste and "Anaheim Chili", which is an Americain mild cultivar. These three cultivars are commonly cultivated in Tunisia.

Priming protocol

A quantity of 30 grams of seeds from each cultivar were superficially sterilized with sodium hypochlorite solution (1%) for 3 minutes and then thoroughly washed for 5 minutes with distilled water. After, seeds of Beldi cultivar were primed with 10 millimoles (mM) of KCl solution for 36 h, seeds of Baklouti cultivar were primed with 10 mM of $CaCl_2$ solution for 36 h and seeds of Anaheim Chili cultivar were primed with 50 mM of NaCl solution for 24 h. For control, seeds were soaked in distilled water for the same duration. Then, seeds were removed from priming media, given three surface washings with distilled water, redried under shade to their original weight and finally stored in refrigerator at 5°C until future use (Farooq *et al.*, 2006). The optimum priming conditions (duration and concentration) were determined based on a preliminary experiment (data not shown).

Germination protocol

Later, dry seeds from each treatment (priming and control) were placed in 90 mm diameter Petri dishes between two layers of Watman filter paper and then moistened with 5 ml of seven NaCl concentration (0, 2, 4, 6, 8, 10, 12 g L^{-1}) for 14 days. Seeds were kept for germination at room temperature (25°C ± 1°C) under normal light in a completely randomized design. Each treatment includes five Petri dishes, which contains each of them 20 homogenous seeds. Germinated seeds were counted daily and the appearance of 2 mm or more of radicle was considered as germination.

Growth in greenhouse

Primed and control seedlings were replicated in plastic pots filled with black fertilized peat and placed in an unheated greenhouse (18-30°C) in order to complete their vegetative cycle (4 months from January to April). Plants derived from primed and control seeds were irrigated permanently with seven salinity levels (0, 2, 4, 6, 8, 10, 12 g L^{-1}). The experiment was arranged in a completely randomized design with four replicates .

Parameters measured

Parameters measured in this experiment are following: germination percentage, chlorophyll a and b content and proline content.

Germination percentage (GP), which was calculated based on the equation described by Ashraf and Foolad (2005): GP = (Total germinated seed) / (Total number of seed). Radicle and stem length (cm) were measured with a graduated ruler after 14 days of germination. Before flowering, chlorophyll a and b content in milligrams (mg) were calculated after estimating the absorbance of the chlorophyll solution, which was measured with a spectrophotometer (T60 Uv /vis) at 645 and 663 nm using the formula of Arnon (1949). Proline content (µ mol /g FW) was estimated based on proline's reaction with ninhydrin and the absorbance of the solution was read at 520 nm using toluene as blank by UV-visible spectrophotometer (Bates *et al.,* 1973).

RESULTS

Total germination percentage

Effect of NaCl concentration on the percentage of germination in the three pepper cultivars (Beldi, Baklouti and Anaheim Chili) during 14 days is shown in *Figs. 1 and 2*. Total germination from both primed and control seeds decreased with increasing NaCl concentration. But, germination was higher in primed seeds compared to control. In fact, for control seeds, we have recorded a reduction of 76%, 92% and 37% (respectively for "Beldi", "Baklouti" and "Anaheim Chili") on total germination due to an increase in salinity from 0 g L^{-1} to 12 g L^{-1} .

For primed seeds, the reduction on total germination due to an increase in salinity from 0 g L^{-1} to 12 g L^{-1} was 22%, 87% and 37%, respectively for "Beldi", "Baklouti" and "Anaheim Chili".

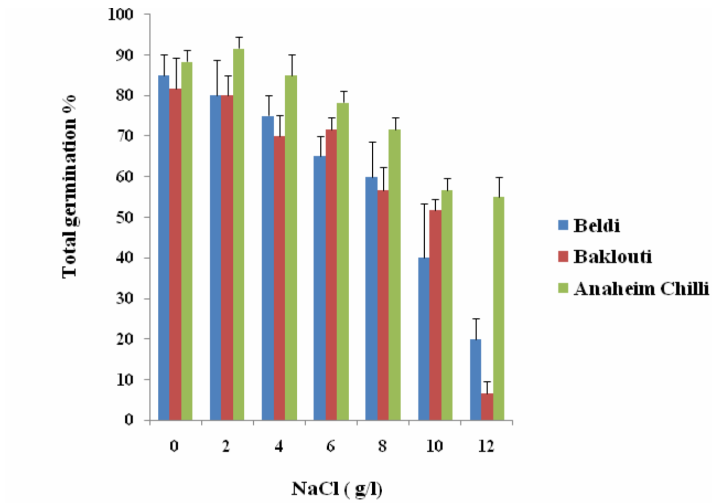


Figure 1 - Effect of salinity (NaCl) on germination of control pepper seeds (cultivars Beldi, Baklouti and Anaheim Chili)

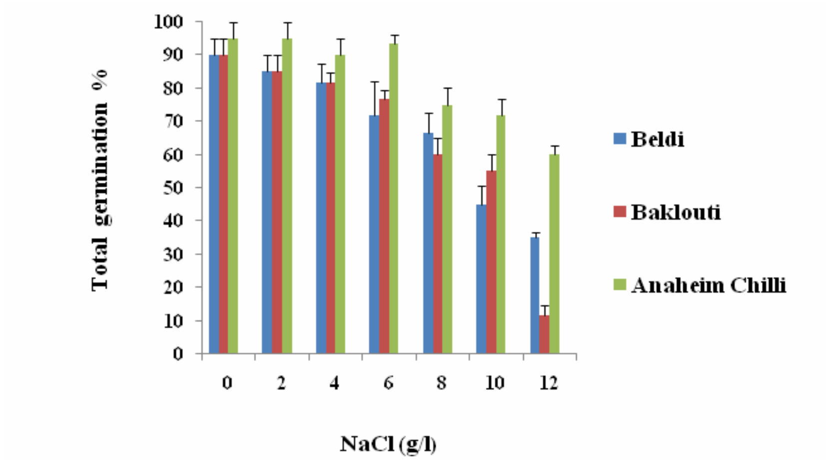


Figure 2 - Effect of salinity (NaCl) on germination of primed pepper seeds (cultivars Beldi, Baklouti and Anaheim Chili)

Radicle and stem length

The effect of salinity stress on radicle and stem length is shown in *Figs. 3 and 4*. Results showed that increased salt stress level reduces the length of the radicle in all cultivars . The radicle length of Anaheim Chili cultivar was longer than other cultivars at 12 g L^{-1}. Similar to length of the radicle, length of the stem also reduced by increase salinity levels in all cultivars. Indeed, NaCl concentration of 12 g L^{-1} sharply reduced the length of the stem. On the other side, plants which derived from primed seeds have developed longer radicle and stem for all salinity levels and for all cultivars.

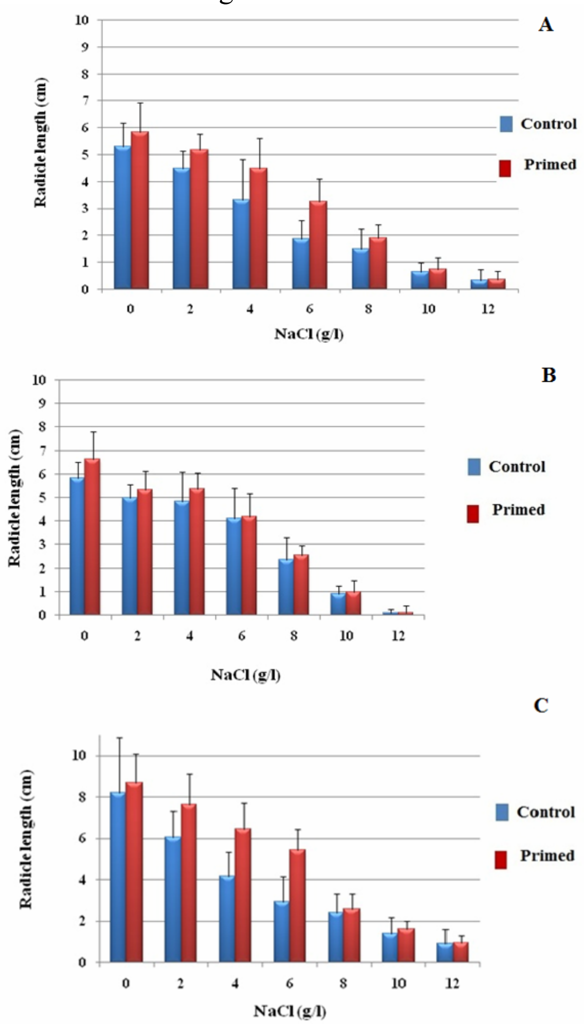


Figure 3 - Effect of different salinity levels on radicle length of three pepper cultivars derived from control and primed seeds.
A= "Beldi", B= "Baklouti" and C= "Anaheim Chili"

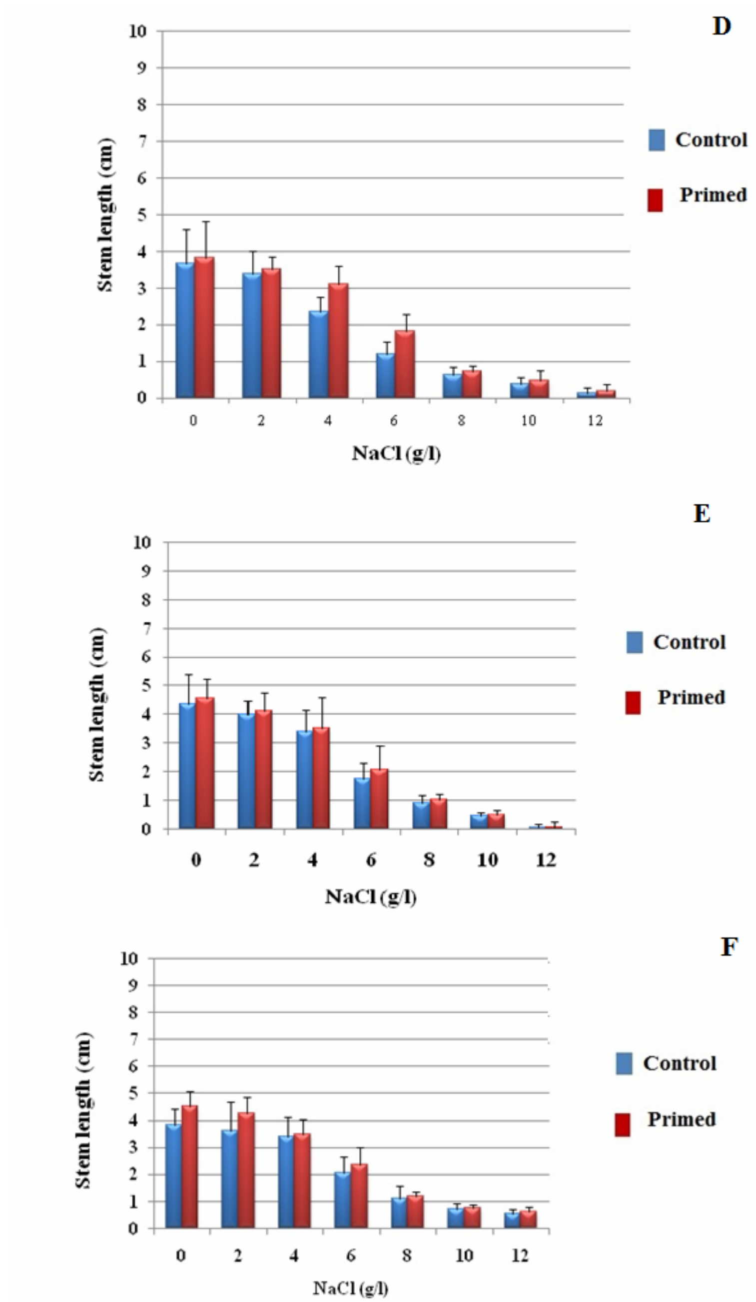


Figure 4 - Effect of different salinity levels on stem length of three pepper cultivars derived from control and primed seeds.
D= "Beldi", E= "Baklouti" and F= "Anaheim Chili"

Chlorophyll content

Chlorophyll content a and b (mg /g FW) decreased by salinity regardless of cultivars (*Figs 5, 6 and 7*). The lowest leaf chlorophyll content was observed at the concentration of 12 g L^{-1}. For exemple, at this concentration, chlorophyll a decreased for Beldi cultivar with 71% (plants derived from primed seeds) and 73% (plants derived from control seeds) compared to 0 g L^{-1}. In the same way, production of chlorophyll b decreased in all cultivars but this decrease was higher in control plants than in plants derived from primed seeds.

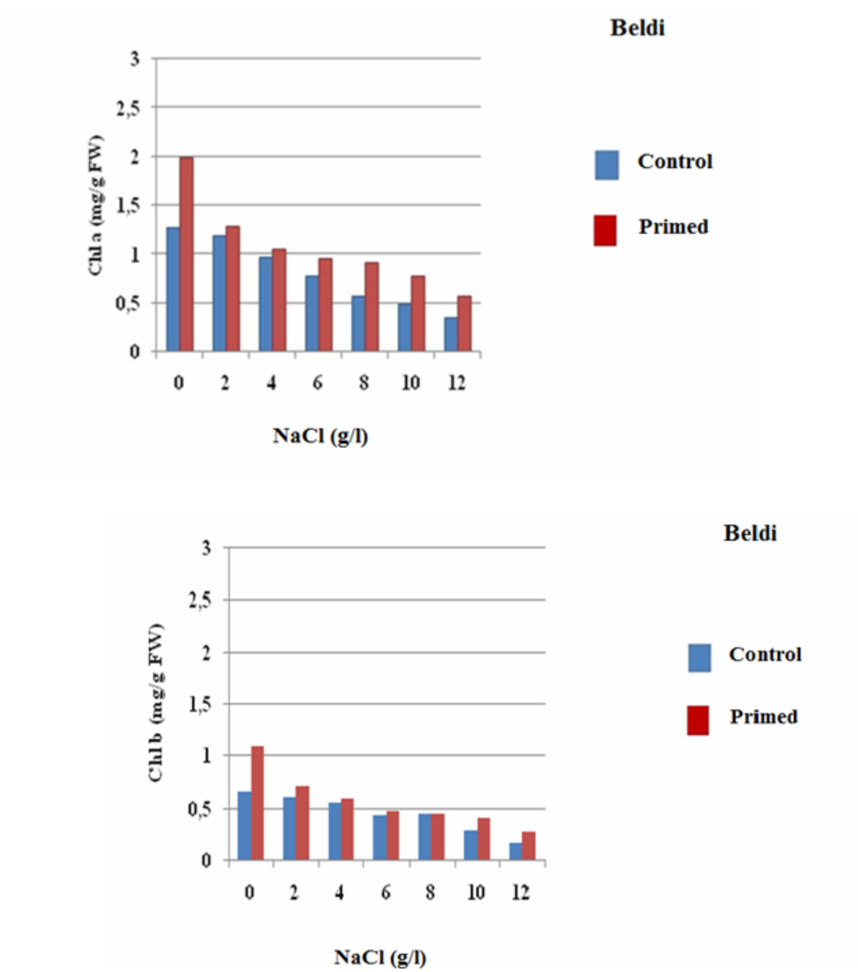
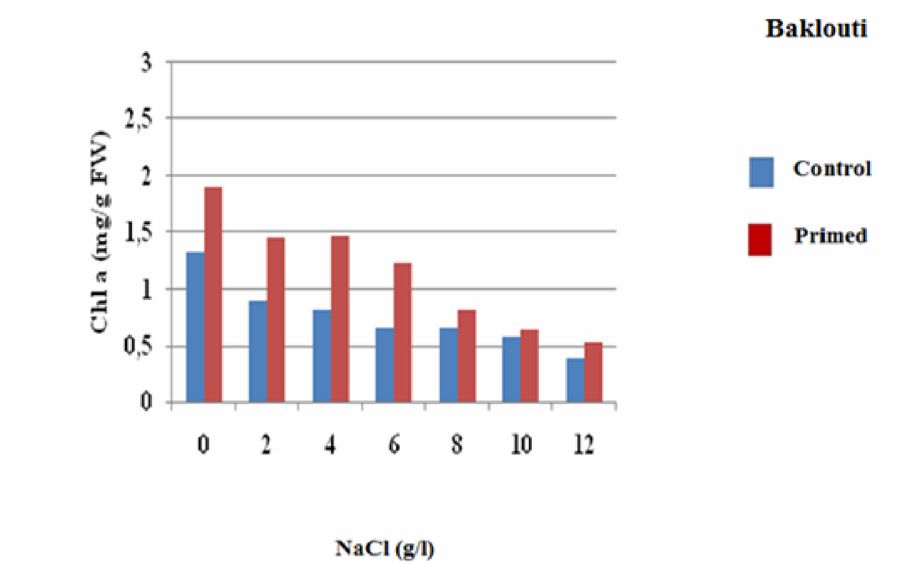


Figure 5 - Effect of salinity on chlorophyll a and b content in Beldi cultivar



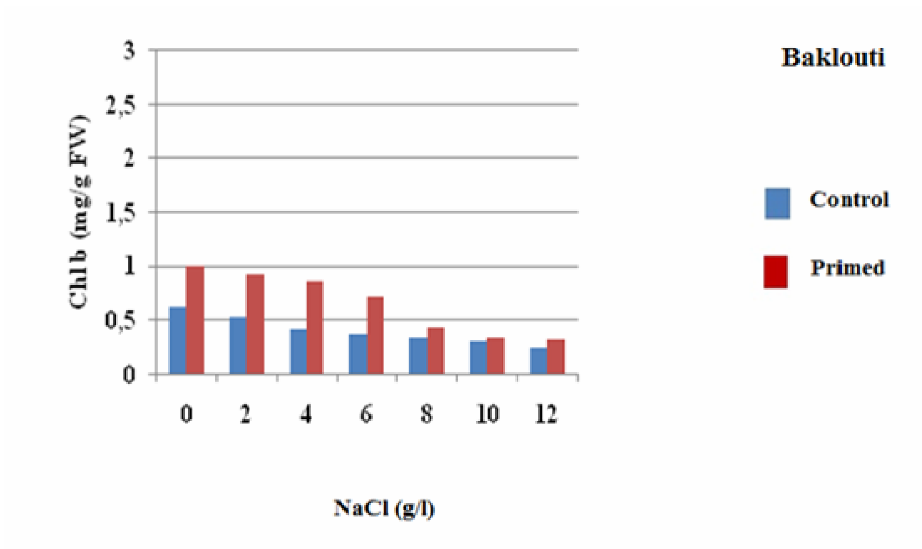
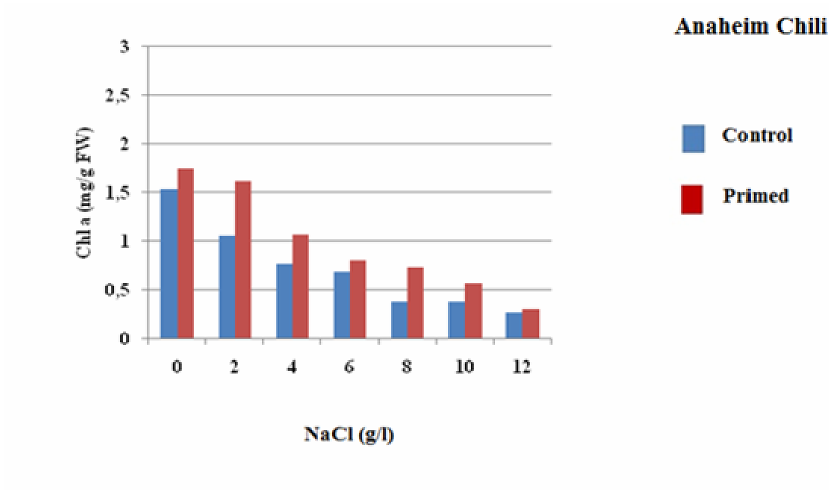


Figure 6 - Effect of salinity on chlorophyll a and b content in Baklouti cultivar



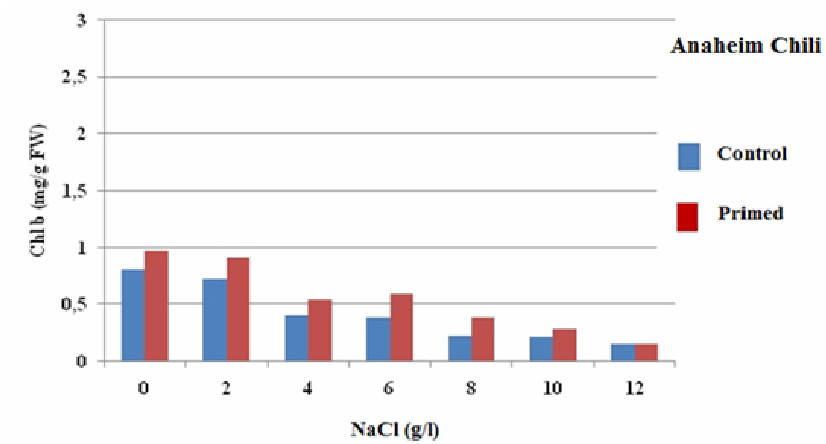


Figure 7 - Effect of salinity on chlorophyll a and b content in Anaheim Chili cultivar

Proline content

Proline content (µ mol/ g FW) increased in control, as well as in plants which derived from primed seeds for all cultivars (*Figs. 8 and 9*). However, proline content was higher in primed groups. This increase was maximum at 12 g L^{-1}. Anaheim Chili cultivar maintained higher content of proline, compared to Beldi and Baklouti cultivars especially in primed group (concentrations above g L^{-1}). For exemple, we observed a higher proline production *(>six* times*)* for Anaheim Chili cultivar at 12 g L^{-1}, compared to 0 g/l in primed group.

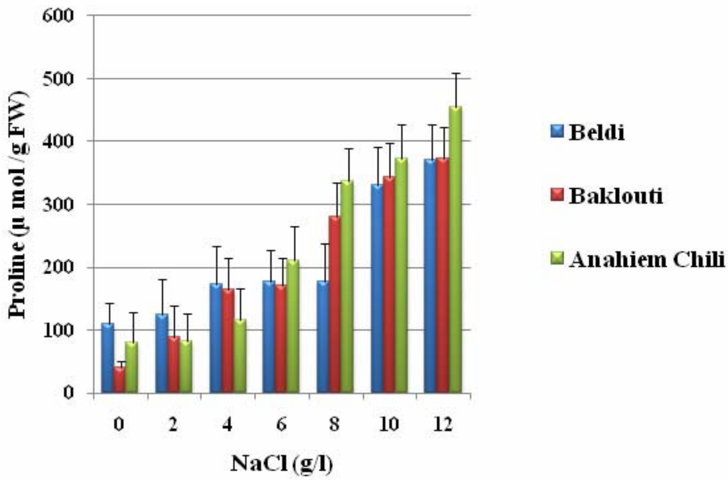


Figure 8 - Effect of salinity (NaCl) on proline content in plants which derived from primed seeds (cultivars Beldi, Baklouti and Anaheim Chili)

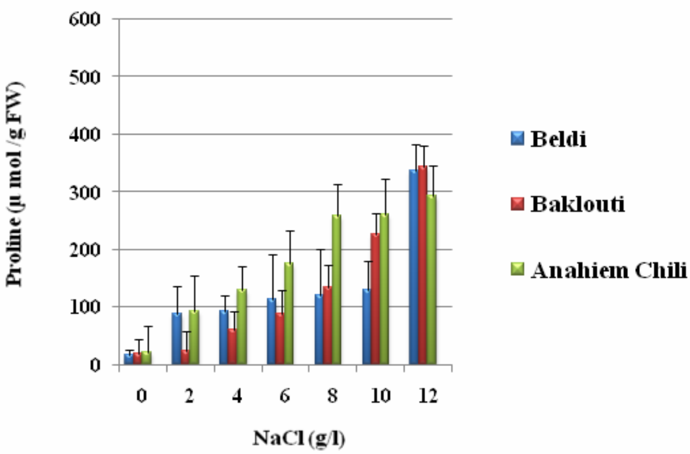


Figure 9 - Effect of salinity (NaCl) on proline content in plants which derived from control seeds (cultivars Beldi, Baklouti and Anaheim Chili)

DISCUSSION

For most plants, the increase of salt (NaCl) causes a decrease in germination percentage; this can be the results of toxic effects of Na^+ and Cl^-. This toxicity changes the activity of some enzymes included in germination process, changes protein metabolism and interrupts hormonal balance (Gomes-Filho *et al.,* 2002). Also, Demir and Van De Venter

(1999) reported that salinity may influence germination by decreasing the water uptake. However, seed priming with NaCl improved germination and growth of many crops under salinity conditions (Sivritepe *et al.,* 2003; Souguir *et al.,* 2013).

In this study, stem and radical length for three pepper cultivars (Beldi, Baklouti and Anaheim Chili) have been improved due to earlier germination induced by priming treatment. These results are in accordance with Mavi *et al.* (2006), who reported that priming treatments increased seedling length. Also, Liu *et al.* (1996) demonstrated that osmo priming improves radicle and plumule length in treated tomato seeds. Primed seeds of pepper might have better water absorption from the growing media that enabled faster metabolic activities in seeds and leads to better germination and earlier radicle and plumule appearance. So, priming improved germination by accelerating imbibition, which facilitated the multiplication of radicle cells and led to an earlier emergence. Indeed, the effects of seed priming are the results of suitable and efficient osmosis regulation and a better efficiency for water absorption for primed derived plants compared with control derived plants. These results are in accordance with McDonald (1999).

The decrease in the rate of photosynthesis is the result of the toxic effect of salt (NaCl) at high salt levels, which damage the roots and decrease their ability to absorb water and nutrient which cause marked effects in leaves number and area. The accumulation of toxic ions like Na^+ and Cl^- in leaves inhibits metabolic processes for photosynthesis, therefore plants become unable to develop new leaves and chlorophyll deficit in leaves accelerate leaf senescence. Results also showed that plants derived from primed seeds produced more chlorophyll (a and b), which indicate that these plants were more salt tolerant. Khan *et al.* (2010) reported that, under salinity conditions, the chlorophyll a and b contents decreased significantly. Priming resulted in enhancing the relative chlorophyll content of the leaves. The increase in the chlorophyll content for plants derived from primed seeds could be due to an increase in the number of chloroplasts in leaves or to a moderate levels of toxic ions in leaves which cannot cause early leaf senescence or chlorophyll degradation.

Many plants accumulate proline as a protective osmolyte under stress conditions. In fact, a positive correlation between proline accumulation and salt tolerance can be used like an index for salt tolerance between plants (Misra and Gupta, 2005). But some others authors reported that proline accumulation cannot be used as the only criterion for salt tolerance (Moradi and Ismail, 2007). In this present study, the proline content increased in all plants (derived from primed seeds or not), but this increase was higher in plants derived from priming. It can be due to metabolic changes induced by high salinity. Sivritepe *et al.* (2003) found that priming enhanced proline and leads to salt tolerance in melon seedling. Our results showed that seeds priming can especially be useful in economically disadvantaged, arid crop growing areas to reduce the effects of salinity.

CONCLUSION

Seeds priming is a simple, cheap and environmentally friendly technique that does not need expensive chemical products. But biochimical analysis of changes in plants associated with seed priming may be very useful in future for advancing the understanding of plant salt tolerance. So, additional work can be recommended to know all priming mechanisms, especially in pepper cultivars.

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