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Effect of priming on germinability and salt tolerance in seeds and seedlings of *Physalis peruviana* L.

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In some species, pre-germination treatments such as priming can increase germinability and the speed of the process, besides conferring tolerance to abiotic stress. The central effect of priming is the slow and controlled absorption of water in seed tissues, allowing the membranes to reorganize and synthesize protective substances against stress. This study was performed to assess the effects of priming on the invigoration of seeds and seedlings of *Physalis peruviana* subjected to salt stress. Seeds of *P. peruviana* were primed in polyethylene glycol 6000 to -0.8 MPa and were germinated in solutions with different salt concentrations (0, 4, 8 and 12 dS m^{-1}). In addition to the rate of radical emergence, post-seminal development was also evaluated until the emergence of the cotyledons. Germinability decreased with increasing salt solution concentrations in both types of seeds. Priming appeared to alleviate the effects of salt stress in the early stages of development of *P. peruviana*. Total dry mass of seedlings increased under saline conditions, suggesting possible physiological adjustments induced by priming.

Key words: Germination, priming, salt stress, Solanaceae.

INTRODUCTION

For many plant species, seeds are the main means of dispersal. Germination and seedling establishment are the most sensitive stages of development with regards to environmental conditions, and affect the expansion of a species in a new environment (Natale et al., 2010). However, abiotic factors such as water availability and salinity of soil water drastically affect this process. Plants need minerals to grow and develop; however, salt in excess can be extremely dangerous to plants (Xiong and Zhu, 2002). The successful development of some species subjected to salt stress depends on their ability species to tolerate such conditions. Seeds with better germinality and salt tolerance can survive more effectively. Priming has been recommended as a pre-germination treatment for the production of cultivars in order to increase the rate of germination and seedling

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Abbreviations: NP, Non-primed; **P,** primed; **PEG,** polyethylene glycol.

establishment under adverse conditions (Heydecker and Coolbear, 1977; Bradford, 1986; Nakaune et al., 2012). This technique involves the absorption of water by the seeds followed by drying and rehydration when the early stages of germination begin, at radicle protrusion (Basu, 1994; McDonald, 2000). Besides increasing germination rate, the benefits of this technique include an increase in uniformity and germinative process. According to De Castro et al. (2000), the effects of priming are prolonged growth and vigour of the seedling. This technique has been successfully used in crops such as wheat (Iqbal and Ashraf, 2007), chickpeas (Kaur et al., 2002), sunflowers (Kaya et al., 2006) and cotton (Casenave and Toselli, 2007).

Physalis peruviana L. (family Solanaceae) is a native plant of the Andes and it is produced on a large scale in Colombia and South Africa. The species is known as a medicinal herb in Peru (Fischer et al., 2007; Mazorra et al., 2006). Its fruit is considered a good source of natural anti-oxidants and other components, e.g. vitamins A, B, C, E and K1, phytosterols and essential minerals (Puente et al., 2011).

In Brazil, the fruit of *P. peruviana* L. is considered exotic and has a high market value (Lima et al., 2009). The fruit ripens and acquires an orange color, and its persistent calyx becomes papery and light brown. This adds aesthetic value to the fruit which is sold primarily for decoration of fine pastries, pies and jams or as a fresh fruit. In Brazil other species of the genus, such as *Physalis angulata* L., have been widely studied in the Northeast region, primarily for their medicinal potential (Souza et al., 2013).

There is potential for the cultivation of this species in arid regions where excessive salt in the soil has been a limiting factor in agriculture. Studies on the effect of salinity on crop growth are gaining importance, and our understanding of how the genus *Physalis* responds to such conditions can add to this. Thus, this study assesses the effect of seed priming on salinity tolerance during germination and early development of *P. peruviana*.

MATERIALS AND METHODS

The seeds of *P. peruviana* used in the study were collected in June 2011, at the Horto Florestal Station at the Universidade Estadual de Feira de Santana, Bahia, Brazil. They were removed from the fruit under running water until complete separation of the pulp. The seeds were dried in a desiccator containing super-saturated calcium chloride solution at 20°C. Water content was determined on a fresh weight basis by placing samples of 200 seeds for 17 h in an oven set at 103°C (ISTA, 2007). Remaining seeds were placed in 5 ml tubes and stored at 4°C until further use.

Samples of 500 seeds of *P. peruviana* were primed by immersion in polyethylene glycol solution ("PEG 6000"), with an osmotic potential of -0.8 MPa, (Villela et al., 1991). This priming potential was established according to the method developed for *P. angulata* by Souza et al. (2011). The immersion was performed in 20 ml test-tubes coupled with an artificial aeration system for 10 days at 25°C in a germination chamber, adjusted for a 12 h photoperiod. The osmotic solution was replaced every two days until day 10 when, after the pre-germination period, the seeds were taken from the solution and dried in an incubator saturated with calcium chloride solution at 20°C for 4 h until the initial weight was reached.

For the germination experiments, 400 primed (P) and 400 non-primed (NP) seeds were placed in Petri dishes made of glass containing two sheets of germitest paper (sterilized in a drying oven at 105°C for 4 h), moistened with solutions of different NaCl concentrations (0, 4, 8 and 12 dS m^{-1}). The concentration of each solution was measured by electrical conductivity. The seeds were kept in the germination chamber at 25°C, adjusted to a 12 h photoperiod for 30 days. Daily observations were performed, with seeds those that issued a radicle of at least 1 mm. recorded as "germinated." Each treatment consisted of four batches of 25 seeds.

The parameters assessed for germination were germinability (%), relative frequency (%), average speed of germination (day^{-1}) germination speed index of seeds (GSI) ($seed.day^{-1}$) and coefficient of uniformity of germination (CUG). Daily the germinated seeds were transferred to another Petri dish moistened with solutions of different NaCl concentrations (0, 4, 8 and 12 dS m^{-1}) for 10 days for analysis of post-seminal development. Seedlings considered normal according to internationally standardized rules (ISTA, 2007), had the length of the radicle and shoots (from the insertion of the cotyledons) measured with the aid of a digital caliper and placed in a forced-air circulation oven at 40°C for 10 days to dry. After this period, the samples were stored in a desiccator with silica and subsequently weighed on a precision balance. The samples ranged according to the germinability and normal seedlings per treatment. The mass per replicate was determined in mg/seedling. To analyze the post-seminal development, normal seedlings (%), total dry mass of normal seedlings (mg/seedling) and ratio of the length radicle/shoots were assessed. Data analysis of germination and post-seminal development were performed using the SISVAR computer program (Ferreira, 2011).

RESULTS

Germinability (%) of primed and non-primed seeds of *P. peruviana* showed a negative quadratic trend, with a decrease in the percentage as the concentration of the salt solutions increased (Figure 1). In the solution with electrical conductivity (EC) of 8 dS m^{-1} seeds showed an 89% rate of germination when compared to non-primed seeds with a rate of 58%. At the highest salt concentration used in this assay (12 dS m^{-1}) 41% of the primed seeds of *P. peruviana* germinated *versus* 31% of the non-primed seeds.

The germination speed index (GSI) increased significantly in primed seeds (4.12) grown in a control solution (0 dS m^{-1}) compared to non-primed (3.4 $seed.day^{-1}$) and to salt concentration with EC of 8 dS m^{-1} (2.4 and 1.3 $seed.day^{-1}$, respectively) (Table 1).

The relative frequency polygon was polimodal in solutions with electrical conductivity of 12 dS m^{-1} and unimodal in other concentrations (Figure 2). In unimodal polygons, the peaks were higher in primed seeds versus non-primed, reflecting a greater uniformity of germination, supported by the uniformity coefficients presented in control treatment (1.520 and 0.664, respectively).

During post-seminal post evaluation normal seedling development of *P. peruviana* took place under low salinity conditions (Figure 3A). The highest number of normal seedlings came from primed seeds, regardless of electrical conductivity (66.7 and 61.5% at EC of 0 dS m^{-1}), and this difference was significant for those kept in saline

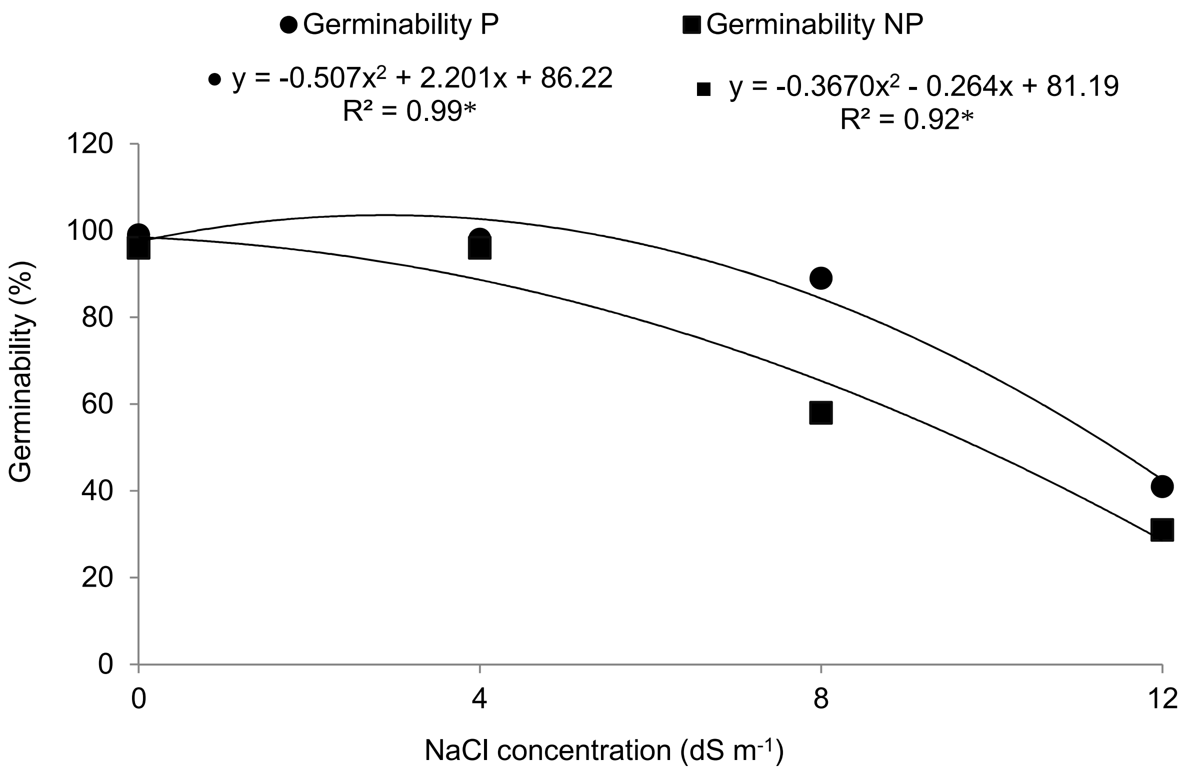

Figure 1. Germinability of seeds of *P. peruviana* L. originated from primed (P) and non-primed (NP) seeds. *Significant at 5% probability.

Table 1. Average speed (day^{-1}), germination speed index (GSI, seeds.day^{-1}) and coefficient of uniformity of germination (CUG) of primed (P) and non-primed (NP) seeds of *Physalis peruviana* L. under different salt concentrations.

Seed	NaCl concentration (dS m^{-1})			
	0	4	8	12
Average speed (day^{-1})				
P	0.163^{a}	0.120^{b}	0.100^{b}	0.081^{c}
NP	0.137^{a}	0.119^{a}	0.084^{b}	0.051^{c}
GSI (seeds. day^{-1})				
P	4.116^{a}*	3.090^{b}	2.365^{b}*	0.846^{c}
NP	3.429^{a}	2.963^{a}	1.300^{b}	0.586^{c}
CUG				
P	1.520^{a}	0.388^{a}	0.199^{a}	0.306^{a}
NP	0.664^{a}	0.540^{a}	0.138^{a}	0.086^{a}

Means followed by the same letter in the line are not significantly different according Tukey test, at 5% probability. *Significant by Student's t-test at 5% probability.

(70.5 and 48.1 at EC of 4 dS m^{-1}).

Data from the root:shoot ratio demonstrated that seedlings from primed seeds showed a radicle length 3.15-fold greater than the shoots kept in the control solution (0 dS m^{-1}). However, in seedlings from non-primed seeds this value was only 1.58, and this difference is significant (Figure 4). Seedlings subjected to salt stress had shoots of a greater length for both seedlings, whether derived from primed or non-primed seeds (0.55 and 0.57). Under saline conditions, although the total dry mass increased, the allocation of this mass was not influenced by priming.

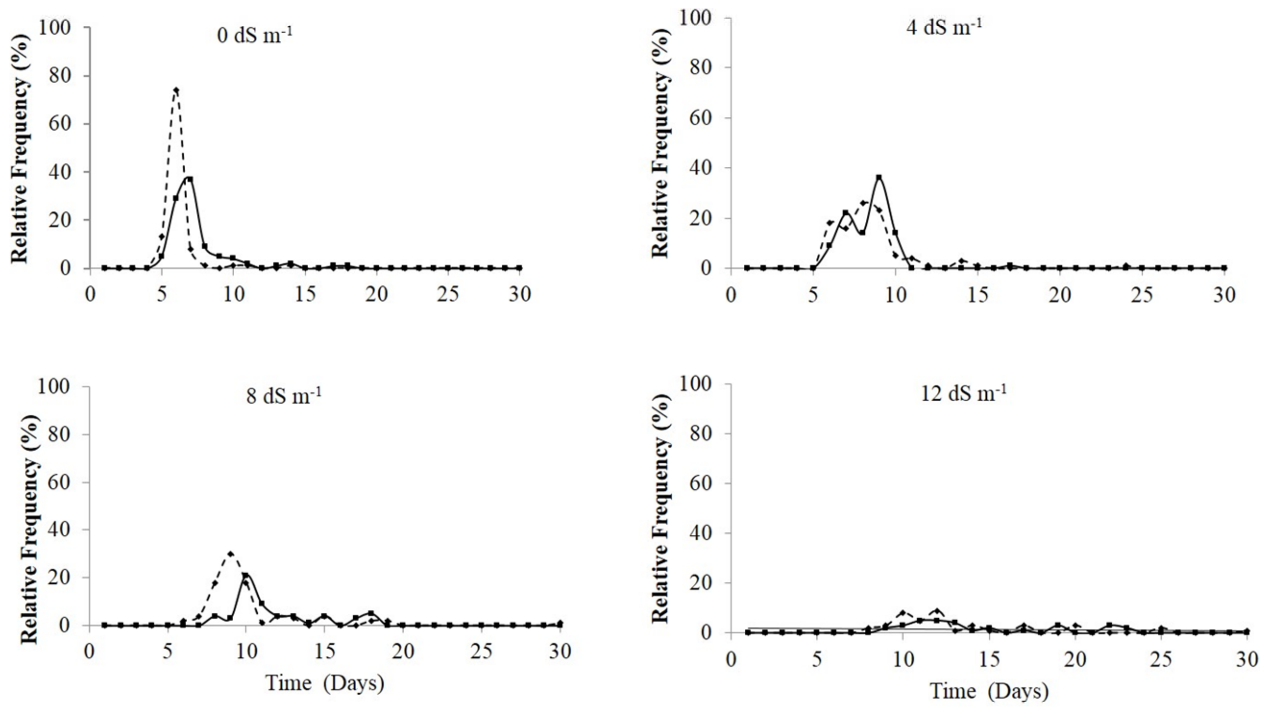

Figure 2. Relative frequency polygons (FR%) of germination of *Physalis peruviana* L. for different salt concentrations. P (- -♦- -) e NP (—■—) .

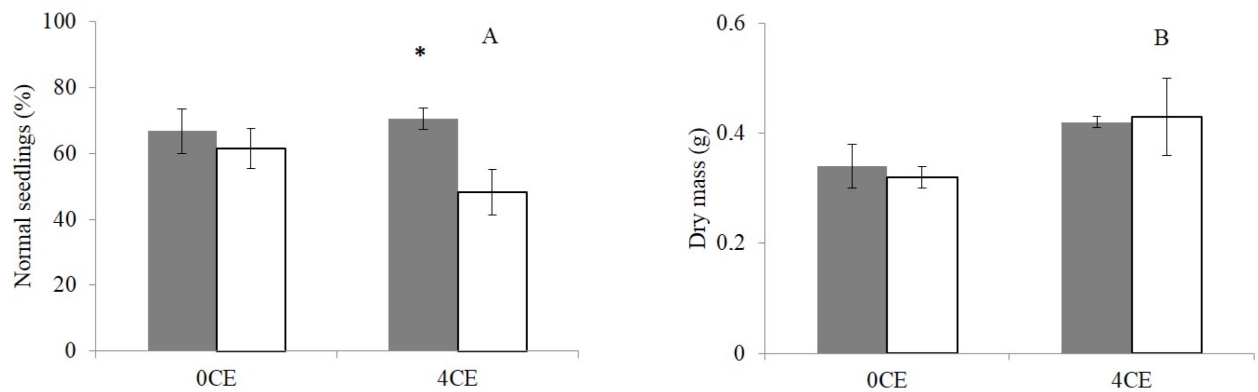

Figure 3. Percentage (A) and dry mass (B) of normal seedlings of *Physalis peruviana* L. from P (■) and NP (□) seeds and post-germination in water and saline (4 EC). *Significant at 5% probability.

DISCUSSION

The 31% difference in germinability between primed and non-primed seeds in saline with EC of 8 dS m^{-1} demonstrates that priming induced higher salinity tolerance. During priming the metabolic energy in primed seeds is greater than in non-primed seeds because increases in adenosine triphosphate (ATP), energy charge (EC) and ATP/ADP (adenosine diphosphate) ratio (Corbineau et al., 2000; Varier et al., 2010).

Investigations using *P. angulata* have also demonstrated that priming provides salt tolerance, even at high concentrations (Souza et al., 2011). However, at the highest salt concentration (12 dS m^{-1}) used in the present assay, seeds of *P. peruviana* demonstrated around half the germination success of those of *P. angulata* (41% compared with 83%, respectively), when kept in saline with the same electrical conductivity.

Despite belonging to the same genus, *P. angulata* and *P. peruviana* responded differently to the priming technique, with a more significant effect on salt tolerance in *P. angulata*. However, to a lesser extent, the benefits

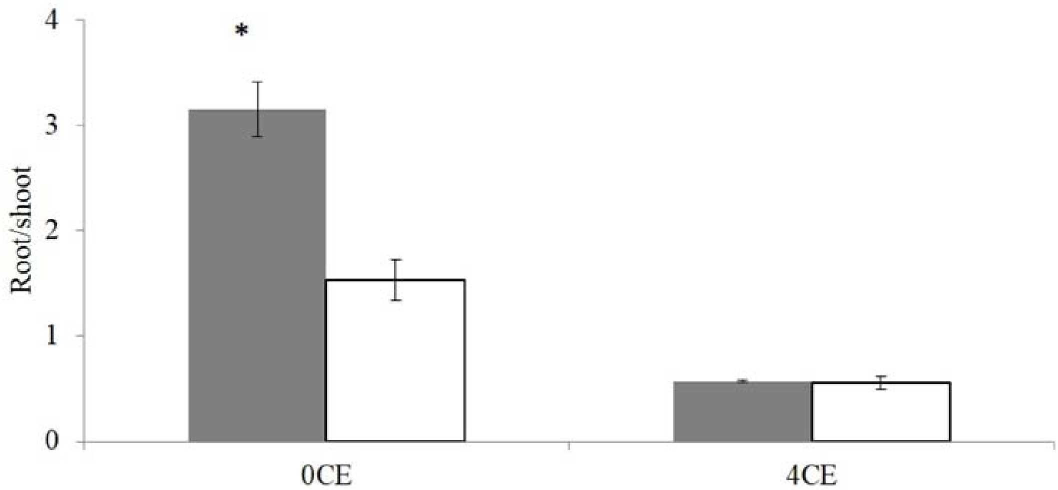

Figure 4. Ratio of length between root and shoot of *Physalis peruviana* L. seedlings from P (■) and NP (□) seeds and posterior germination in water and saline (4 EC). *Significant at 5% probability.

of priming were also observed in *P. peruviana*, which confirms that this technique can be used as pre-germination treatment.

Yildirim et al. (2011) observed that in seeds of *P. peruviana* and *Physalis ixocarpa* the germinability (%) decreased, and the average time increased, as the concentration of saline the seeds were treated with increased. According to the authors, *P. peruviana* is tolerant to salinity during germination but it becomes sensitive during seedling development.

Germinability (%), germination speed index (seeds.day^{-1}) and average speed (day^{-1}) results observed in the present study are corroborated by Bradford (1986) and Nakaune et al. (2012). They claim that, under saline conditions, priming has a protective effect on oxidative damage caused by the accumulation of sodium in the cell cytoplasm.

Data from seedling dry mass did not agree with those presented by Yildirim et al. (2011), which indicated that the salt stress affected the seedlings of *P. peruviana* and *P. ixocarpa* adversely, with a decrease in dry mass as salt concentration increased. However, according to Miranda et al. (2010), plants of *P. peruviana* showed a positive effect when subjected to saline at a concentration of 30 mM (around 4 dS m^{-1}). According to these authors, *P. peruviana* is moderately salt tolerant, since the relative growth rate was stimulated by moderate salt stress (30 mM, around 4 dS m^{-1}). This suggests possible physiological and osmotic adjustments to maintain the water potential in the tissues of the plant (Miranda et al., 2010).

As priming is a technique that promotes greater speed and uniformity of germination, the positive effects were also observed in the increase in the root:shoot ratio. Primed seeds produced a radicle in a shorter time than non-primed seeds. We suggest that cell elongation was also faster, reflected in the fact that the radicles from primed seedlings had a 3.15-fold greater length than the shoots. According to De Castro et al. (2000), the activation of genes that occurs during priming is maintained beyond the seed and seedling stages, lasting into the adult stage. As for the decrease in root length in seedlings kept in saline (4 dSm^{-1}), this could relate to a strategy developed by plants under such conditions to limit the absorption of toxic ions such as Na^+ and Cl^- (Munns, 2002; Alarcón et al., 2006).

Priming was effective in the invigoration of seeds and seedlings of *Physalis peruviana* under different saline conditions; however, this response was less effective when compared to other species of the genus in the same conditions, especially during post-seminal development.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Alarcón JJ, Morales MA, Ferrández T, Sánchez-Blanco MJ (2006). Effects of water and salt stresses on growth, water relations and gas exchange in *Rosmarinus officinalis*. J. Hortic. Sci. Biotechnol. 81: 845-853.

Basu RN (1994). An appraisal of research on wet and dry physiological seed treatments and their applicability with special reference to tropical and subtropical countries. Seed Sci. Technol. 22: 107–126.

Bradford KJ (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience. 21: 1105-1112.

Casenave EC, Toselli ME (2007). Hydropriming as a pre-treatment for cotton germination under thermal and water stress conditions. Seed Sci. Technol. 35: 88-98.

Corbineau F, Ozbingol N, Vineland D, Come D (2000). Improvement of tomato seed germination by osmopriming as related to energy metabolism. In Black M, Bradford KJ, Vasquez-Ramos J (Eds). Seed Biology Advances and Applications: Proceedings of the Sixth International Workshop on Seeds, Mérida, Mexico, 1999. New York, NY : CABI. 467-474.

De Castro RD, Van Lammeren AAM, Groot SPC, Bino RJ, Hilhorst

HWM (2000). Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. Plant Physiol. 122: 327-335.

Fischer G, Ebert G, Lüdders P (2007). Production, seeds and carbohydrate contents of cape gooseberry (*Physalis peruviana* L.) fruits grown at two contrasting Colombian altitudes. J. Appl. Bot. Food Qual. 81: 29-35.

Ferreira DF (2011). Sisvar: a computer statistic alanalysis system. Cienc. Agrotecnol. 35: 1039-1042.

Heydecker W, Coolbear P (1977). Seed treatments for improved performance - survey and attempted prognosis. Seed Sci. Technol. 5: 353-425.

Iqbal M, Ashraf M (2007). Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. J. Integr. Plant Biol. 49: 1003-1015.

ISTA (2007) International Rules for Seed Testing Association, Bassersdorf, Switzerland.

Kaya MD, Okçu G, Atak M, Çikili Y, Kolsarici O (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.) Eur. J. Agron. 24: 291-295.

Kaur S, Gupta AK, Kaur N (2002). Effect of osmo and hydropriming of chickpea seeds on seedling growth and carbohydrate metabolism under water deficit stress. J. Plant Growth Regul. 37: 17-22.

Lima CSM, Manica-Berto R, Silva SJP, Betemps DL, Rufato AR (2009). Establishment and operating costs of a cape gooseberry orchard in the south region of Rio Grande do Sul. Rev. Ceres. 56: 555-561.

Mazorra MF, Quintana AP, Miranda D, Fischer G, De Valencia MC (2006). Anatomic Aspects of Formation and Growth of the Cape Gooseberry Fruit *Physalis peruviana* (Solanaceae). Acta Biol.Colomb.11: 69-81.

McDonald MB (2000). Seed priming. In: Black M, Bewley JD (Eds). Seed Technology and Its Biological Basis. Sheffield: Sheffield Academic Press. UK. 287-325.

Miranda D, Fischer G, Ulrichs C (2010). Growth of cape gooseberry (*Physalis peruviana* L.) plants affected by salinity. J. Appl. Bot. Food Qual. 83: 175-181.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell Environ. 25: 239-250.

Nakaune M, Hanada A, Yin YG, Matsukura C, Yamaguchi S, Ezura H (2012). Molecular and physiological dissection of enhanced seed germination using short-term low-concentration salt seed priming in tomato. Plant Physiol. Biochem. 52: 28-37.

Natale E, Zalba SM, Oggero A, Reinoso H (2010). Establishment of *Tamarix ramosissima* under different conditions of salinity and water availability: Implications for its management as an invasive species. J. Arid Environ. 7: 1399 -1407.

Puente LA, Pinto-Muñoz CA, Castro ES, Cortés M (2011). *Physalis peruviana* Linnaeus, the multiple properties of a highliy functional fruit: a review. Food Res. Int. 44: 1733-1740.

Souza MO, Souza CLM, Pelacani CR (2011). Germination of osmoprimed and non-osmoprimed seeds and initial growth of *Physalis angulata* (Solanaceae) in saline environments. Acta Bot. Bras. 25: 105-112.

Souza MO, Souza CLM, Pelacani CR, Soares M, Mazzei JL, Ribeiro IM, Rodrigues CP, Tomassini TCB (2013). Osmotic priming effects on emergence of *Physalis angulata* and the influence of abiotic stresses on physalin content. S. Afr. J. Bot. 88: 191-197.

Varier A, Vari AK, Dadlani M (2010). The subcellular basis of seed priming. Curr. Sci. 99: 450-456.

Villela FA, Doni Filho LD, Sequeira EL (1991). Table of osmotic potential as a function of polyethileneglycol 6000 concentretion and temperature. Pesqui. Agropecu. Bras. 26: 1957-1968.

Xiong L, Zhu JK (2002). Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ. 25: 131-139.

Yildirim E, Karlidag H, Dursun A (2011). Salt tolerance of *Physalis* during germination and seedling growth. Pak. J. Bot. 43: 2673-2676.