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WATER PRE-HYDRATION AS PRIMING FOR *Moringa oleifera* Lam. SEEDS UNDER SALT STRESS

[PRE-HIDRATACIÓN EN SEMILLAS DE *Moringa oleifera* Lam. BAJO ESTRÉS SALINO]

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SUMMARY

The *Moringa oleifera* belongs to the Moringaceae family and presents different uses meanly for family farming. Aiming to establish plantations in areas of family agriculture of the Sergipe State (Brasil), the objective of this study was to evaluate the effect of pre-soaking on *M. oleifera* seeds as a way to overcome salinity stress at first stages of development aiming the planting in marginal areas subjected to salinity. For the test we used two batches of seeds, with 0 and 3 months of storage. Both seed batches were treated for two times of pre-soaking in water (0 and 24 hours). After the treatment the seeds were placed on germination paper soaked 2.5 times with saline solutions (0, 25, 50, 100, 200 and 250 mol.m^{-3}) and kept into germination chamber at 25°C and continuous light. The variables analyzed were percentage, speed of germination index, length and dry matter of seedlings. The treatment with submersion in water for 24 hours was effective to promote higher average values for vigor on Moringa seeds.

Keywords: priming; salinity; vigor.

RESUMEN

La *Moringa oleifera* pertenece a la familia Moringaceae y presenta diferentes usos en la agricultura familiar. Con el objetivo de establecer plantaciones en áreas con problemas de salinidad en el Estado de Sergipe (Brasil), el objetivo del presente estudio fue evaluar el efecto del remojo de las semillas de *M. oleifera* en etapa de germinación como una forma de superar el estrés salino. Para el estudio, se emplearon dos lotes de semillas, con 0 y 3 meses de almacenamiento. Ambos lotes de semillas fueron sometidas a dos tiempos de remojo en agua (0 y 24 horas). Después del tratamiento las semillas fueron colocadas sobre papel de germinación y regadas con solución salina (0, 25, 50, 100, 200 y 250 mol.m^{-3}). Los tratamientos fueron mantenidos en cámara de germinación a 25°C y luz continua. Las variables analizadas fueron: porcentaje, el índice de velocidad de germinación, la longitud y la materia seca de las plántulas. El tratamiento con inmersión en agua durante 24 horas fue eficaz para promover los promedios más altos para el vigor de las semillas de moringa

Palabras clave: priming; salinidad; vigor.

INTRODUCTION

The physiological quality of seeds is affected in all stages of production, ranging from fertilization trough sowing. The main factors that affect quality include genetics, environmental conditions during seed development, seed position on the mother plant, season and harvesting techniques, storage conditions and pre-germination treatments (Basu, 1995). Thus, the assessment of physiological seed quality is essential for the various segments that take part of a production system, since the discovery of factors that may affect the quality of seeds is directly dependent on the efficiency of methods used to determine it (Marcos Filho *et al.*, 1987).

In particular, the salinity influences the response to seed germination. The excess of soluble salts causes a reduction in soil-water potential, leading to less water evaluable to be absorbed by seeds. This water potential reduction and the toxic effects of salt interfere on water absorption process influencing on germination (Cavalcante and Perez, 1995).

The salt promotes changes of functions of the cell membranes and the cell walls and may affect the water potential of the cytosol and cellular extensibility, and thus, may affect seed germination and seedling growth. According to Jamil *et al.* (2006) the seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease. These salts interfere with seed germination and crop establishment. Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Salinity stress can affect seed germination through osmotic effects. Studies to examine salinity effects on the initial growth of plants (Tobe *et al*., 2004). Plants respond to salt stress, germination, and consequently the percentage and germination rate, and vary with the level of salinity evaluable, there is a limit of resistance (tolerance), and critical levels can inhibit germination (Fanti and Perez, 1996).

The adaptability of the plants to saline soils depends on several factors as a highlighting the physiological constitution and its stage of development (Brady, 1979). Some species, such as sorghum (*Shorgum* spp.), maize (*Zea mays* L.), beans (*Phaseolus* spp.) and wheat (*Tricum* spp.), are less affected during the initial phase of their cycle (François *et al.*, 1984, Maas *et al.*, 1986). However, in rice (*Oryza sativa* L.), the sensitivity to salinity increases during the flowering and fruiting phases (Guerra, 1976). According to Torres *et al.* (2000), in arid and semi-arid areas, the excess of salts in the soil has limited agricultural production which can affect adversely the germination, the crop growth and the crop yield (Silva and Pruski, 1997).

Priming is one of the physiological methods, which improves seed performance and provides faster and synchronized germination (Sivritepe and Dourado, 1995). These primed seeds in general give earlier, more uniform and sometimes greater germination and seedling emergence (Bradford, 1986). Cayuela *et al*. (1996) reported that the higher salt tolerance of plants from primed seeds is the result of a higher capacity for osmotic adjustment since plants from primed seeds have more Na^+ and Cl^-, in roots and more sugars and organic acids in leaves than plants from non-primed seeds. Idris and Aslam (1975) and Afzal *et al.* (2007) found that osmopriming of wheat seeds hastened germination under varying sodium chloride (NaCI) regimes. Similarly, soaking in distilled water enhanced germination under saline conditions as much as any seed pretreatment used on cotton (Shannon and Francois, 1977).

Pre-germinative treatments have to be viable for some species, as they can assist in germination, since they may promote the rapid and uniform seedling emergence in adverse environments (Sguarezi *et al*., 2001), exposure to soaking the seed has been one of the technologies tested in several species to facilitate the germination of seeds depending on the situation and even promote plant tolerance in the event of stress (Henkel, 1961; Salim and Todd, 1968; Idris and Aslam, 1975).

Moringa (*Moringa oleifera* Lam.) is a species that grows rapidly and can survive in poor soil, requiring little care for long periods of drought (McConnachie *et al.*, 1999). According to Joly (1979) moringa is a perennial tree, which origin is from northwest region of India, widely distributed around the globe and with multiple uses (Ramachandran *et al.*, 1980; Pio-Corrêa, 1984). The specie is recommended for regions with low agricultural development aiming to purify water for human use (Morales Avelino *et al.*, 2009).

Furthermore, despite the importance, studies involving salinity and pre-germination treatments lack for this species, and there is no technology for field production. This essay can contribute to generate of information for cultivation in areas not adequate for other crop cultivation. Thus the mean goal of this work is to evaluate the effect of salt stress on germination and vigor of moringa seeds by the pre-soaking.

MATERIAL AND METHODS

The study was carried out in the Laboratory of Seed Technology at Departament of Agronomy, at the Federal University of Sergipe. Fruits of Moringa were harvested from Aracaju provenance. Initially were determinate the volume by weight of 100 seed, using methods recommended by Rules for Seed Analysis (Brasil, 2009) with adaptations.

The obtained seeds composed two batches, the first stored using plastic bags in cold chamber for three months and second without storage. In order to obtain seeds with uniformity were selected seeds for color and size. The seeds of both batches were submitted to pre-soaking in distilled water for 24 hours. The seeds without pre-soaking and both batches were taken as control.

Both groups were tested under different saline concentrations (0, 25, 50, 100, 200 and 250 $mol.m^{-3}$) in order to evaluate the behavior of seeds under salt stress conditions. For each treatment were used four replications composed by 25 seeds per treatment. The constancy of temperature in germination tests was reached keeping seeds in germination chamber at 25°C ± 2 under continuous light. The evaluation occurred during 14 days with periodically evaluation every two days. The normal seedlings were counted as germinated seeds.

It was estimated the percentage of germination (% G) (Labouriau and Valadares, 1976), the Index of Speed Germination (Nakagawa, 1994), and the length and dry matter of seedlings (Ayala-Cordero *et al.*, 2006). The data obtained were analyzed with the software SISVAR (Ferreira, 2000).

RESULTS

The weight of 100 seeds was 30 grams and the volume by weight of 23,75 kg hl^{-1}. The summary table of the F-values obtained of analysis of variance (ANOVA) (Table 1), was only possible to observe significant differences on the viability and vigor (P<0.001).

Table 1. F values for the variables quantified in three batches of *Moringa oleifera* Lam subjected to six levels of salinity.

Source	Salinity	Lots	Sal x Lost
% Germination	42,08***	23,77***	3,56***
Speed Germination Index	47,32***	6,516**	3,98***
Seedling Size	32,84***	$0,170^{ns}$	$1,42^{ns}$
Dry Matter	$0,61^{ns}$	$3,046^{ns}$	$0,49^{ns}$

*** - P <0.001, ** - P <0.01, ns - not significant.

In accord to the regression curve, the patterns of seeds' behavior under salt stress conditions show a decreasing on germination with the increasing of saline concentration (Figure 1).

It was also observed that for both parameters (percentage of germination and SGI) the pre-hydrated seeds presented higher values, while the lowest averages values were observed for seeds without soaking. For 100 $mol.m^{-3}$ concentration it was observed a decreasing of values, and the level of 250 $mol.m^{-3}$ presented strongly negative responses.

To evaluate the post-germination phase, the comportment of treatments was similar, which seedling height decreased with an increasing of saline concentration (Figure 2).

For the seedlings-size, it was noted that the highest (108.87mm) and the lowest (4.47 mm) values was observed in control treatment (108.87) mm.

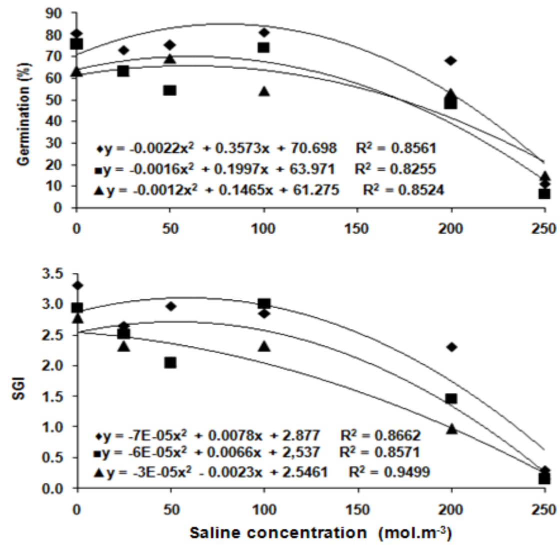

Figure 1. Percentage and Speed Germination Index (SGI) for Moringa seeds (*Moringa oleifera* Lam.) submitted to pre-soaking and salt stress. Not- stored-seeds (♦), stored seeds (■), and control (▲).

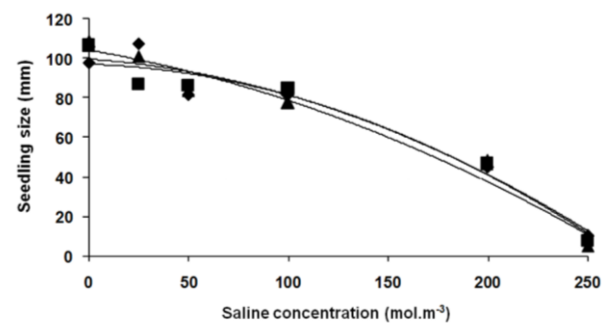

Figure 2. Seedlings-size of Moringa seeds (*Moringa oleifera* Lam) submitted pre-soaking and after to salt stress. Not-stored-seeds (♦), stored seeds (■) and control (▲).

Different results were observed for dry matter. Not-stored-seeds behavior shows uniformity for weight under different saline concentrations that initiate at 50 $Mol.m^{-3}$. For stored-seeds and control there was a variation according to salinity level (Figure 3).

The highest values were found for 25 $mol.m^{-3}$, while the lowest were observed at 250 $mol.m^{-3}$.

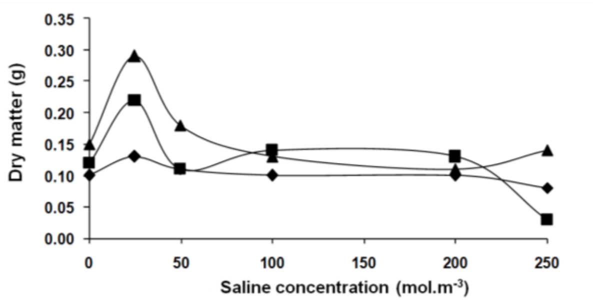

Figure 3. Dry matter of moringa seedlings (*Moringa oleifera* Lam) submitted to pre-soaking and salt stress. Not-stored-seeds (♦), stored-seeds (■) and control (▲).

DISCUSSION

The viability and vigor are the two characteristics to be considered when analyzing seed lot (Popinigis, 1985). The viability, estimates the maximum germination (e.g. percentage of germination), while the vigor comprises a set of characteristics that determine seed vigor and is influenced by environmental conditions and handling during the stages of pre-and post-harvest (e.g. SGI) (Vieira and Carvalho, 1994).

The effect of seed germination under salt stress was reported by many authors for many halophyte and non halophyte species (Belaqziz *et al.*, 2009). The increasing of averages for SGI and percentage of germination from concentrations at 100 $mol.m^{-3}$ in Moringa, can be explained by pre-hydration, which works as conditioning and physiological accumulation of solutes (sugars, organic acids and ions) at the beginning of the seed metabolism, resulting in increased turgor and protrusion of the primary root in a short time (Bradford, 1986). The results obtained in this study are in line with Dianati *et al.* (2008), Ramazani *et al*. (2009), Khan and Gulzar (2003), Kader and Jutzi (2004) and Mauromicale and Licandro (2002). They found that salinity increase results in decrease in germinability and delayed rate of germination.

According to Castro and Hilhorst (2004), the water exerts great influence on the germination process observed in pre-hydrated seeds in solution. The germination occurs more rapidly and uniformly, promoting the recovery of vigor. In this sense, the water plays a fundamental role in seed understanding biology, particularly on germination and plant development (Villela, 1998). The treatment at 200 $mol.m^{-3}$ reduces the germination. This reduction was probably promoted at the beginning of the treatment by progressive reduction of the evaluable water, and consequently interferes on hydrolytic enzymes activity affecting the supplement and velocity of water assimilation (Lobato *et al.*, 2009).

The water potential promotes enzyme activation responsible by carrying out starch hydrolysis, as well as enzymes responsible to liberate ATP used for root protrusion and consequently seed germination (Bewley and Black, 1999; Uriyo, 2000; Lobato *et al.*, 2009).

Powell and Burgasser (1984) reported the seed pre-conditioning may be associated with a better performance of seeds through hydration trigger mechanisms to repair membranes, which could act over deteriorated seeds. Kaur *et al*. (2000) explain that pre-soaking seeds can overcome stress conditions, since these conditions growth regulators, like gibberellic acid and kinetin, can partially reverse the adverse effects of stress during germination by inducing changes in activities enzymes of carbohydrate metabolism.

The progressive reduction of seedlings-size for Moringa showed the severity of the salt stress. Lobato *et al.* (2009) explains this occurrence due to lower rate of cell division and ethylene production in seedlings subjected to this kind of stress. This hormone presents as consequence the decreasing of seedling-size. Besides of this, it promotes the reduction of length and increasing of the diameter of root as demonstrated by several authors in different species (Rodrigues *et al.*, 2002; Dantas *et al.*, 2007)

Another possibility is this seedlings-size reduction is a survival strategy in according to reports of Ungar (1987), and Jefferis Rudmik (1991) and Houle *et al.* (2001) with reduction on transpiration rate with a needy for water absorption by roots, which leads to lower salt intake (Long and Mason 1983).

The progressive increasing of dry matter at 25 $Mol.m^{-3}$ is due to salt accumulation on cell wall and, consequently, an increasing of weight. During the seeds imbibitions process occur the absorption of solution which contents NaCl and water. The salt enters through the cell and acts directly on biochemistry pathways and consequently on germination process. This process depends on the stress intensity, which can lead to enzymes activation/denaturation (Smith and Comb, 1991; Marcos, 2005; Lobato *et al.*, 2009).

According to Jeannette *et al*. (2002) that faster rate of germination allowed the emerging seedlings to accumulate more biomass relative to the control but conversely, being significantly reduced with increased salt stress. These results are also similar in line with Shannon and Grieve (2000). Hasegawa *et al.* (2000) explains that the plants need to keep alive and grow in saline environments, and one of the alternatives is storage salt inside organelles and tissues, in compartmentalized way, preventing the vital functions of vegetables to be affected.

In our results, from 50 $Mol.m^{-3}$, the dry matter of moringa seedling's decreasing, which can probably be associated with metabolic changes and decontrol of the cells process under stress condition.

Izzo *et al.* (1991) studied the salt stress over maize seedlings. The exposition leads to a decreasing ratio of shoot/root. The seedlings-decreasing (miniaturization phenomenon) might be associated to reduction on biomass in response to increased salinity. This event is commonly observed in halophytes (Waisel 1972) as well glycophytes plants (Houle *et al.* 2001).

Our results demonstrated that pre-hydration influences the germination process and, moreover, also influences the behavior of post-germination which affects probably the establishment on field. The better development promotion can be reached, even with development under stress conditions, since the water pre-hydration for 24 hours showed a better seed performance under salt stress. In wheat (Afzal et al., 2007) a similar situation as Moringa was ocurred, where the hydropriming by 24 hours was affective in inducing salt tolerance during the germination and emergence.

Pre-hydration is a simple method of priming that requires no special equipment and technique, is probably the most simple and inexpensive priming (Fujikura *et al.*, 1993), this technique can be adapted easily to small farmers, as found in semi-arid of Brazil, to promote a better response of seed germination and in short time under salt stress.

CONCLUSIONS

The *Moringa oleifera* is a sensitive species to salt stress levels equal or higher than 100 $Mol.m^{-3}$.
The water pre-hydration of Moringa seeds promotes better behavior during the germination process until 50 $Mol.m^{-3}$ level for salt stress.

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