

Full Length Research Paper

Physiological potential of tamarind seeds subjected to stress conditions and storage

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Tamarind (*Tamarindus indica* L.) is a fruit species native to Equatorial Africa, India and Southeast Asia. Its seeds experience slow germination, thus, study on the influence of environmental factors, such as water and salt stresses, on seed germination, is required to assess the tolerance of these seeds to storage, and to maintain the vigor and the viability in the period between harvesting and sowing. The objective of this study was to evaluate the performance of tamarind seeds subjected to salt and water stresses and to storage. Three experiments were carried out in a completely randomized design with four replications of 25 seeds, totaling 100 seeds per treatment. For stress conditions, treatments consisted of moistening of the substrate with different concentrations of PEG 6000 (experiment 1) and NaCl (experiment 2) - 0 (control); -0.3; -0.6; -1.2. For the storage trial (experiment 3), seeds remained stored for 0 (control), 12, 24, 36 and 48 months. In the germination test, the percentage of germination, the speed of germination index, the mean speed and the mean germination time were assessed. All variables were influenced by the water and salt stress, and the seeds of tamarind sensitive to potential used in this work. As in the test with water and saline potential storage trials showed that the physiological seed quality was impaired when they were subjected to long storage period.

Key words: *Tamarindus indica* L., salinity, polyethyleneglycol, stress, storage.

INTRODUCTION

Tamarind (*Tamarindus indica* L.) is a fruit species of the Fabaceae family, native to Equatorial Africa, India and Southeast Asia. It occurs in tropical and subtropical regions, and is considered ideal to be grown in semi-arid regions, since it tolerates 5 to 6 months of drought

(Pereira et al., 2007).

The species has wide use, and can be employed in environment decoration (Donadio et al., 1988). However, the main product is the fruit, and its bittersweet flavored pulp can be used in the preparation of jam,

ice creams, liqueurs, concentrated juices and seasoning for several foods (Gurjão et al., 2006), and in folk medicine (Komutarin et al., 2004). *In natura* seeds are used as forage for domestic animals, and as gum for fabric or paper. Its trunk provides good quality wood for construction (Bourou et al., 2013), although it is difficult to work with due to its hardness (Silva et al., 2011).

Tamarind seed is orthodox, and the seedlings have epigeal germination, occurring between 15 to 45 days after sowing, with 65 to 75% germination of both freshly harvested and stored seeds (El-Siddig, 2006). Germination occurs under favorable conditions of light, temperature, water, salt and oxygen concentration (Carvalho and Nakagawa, 2012). Thus, one of the objectives of this study on seed germination is to verify the influence of these environmental factors on the germination process (Guan et al., 2009).

Environmental factors, denominated stress, or environmental disorders, which limit crop productivity (Ashraf and Harris, 2004), such as water or salt stress, may influence the germination and the development of several species in different regions (Nogueira et al., 2005). In saline soils, the salts affect the plants due to water osmotic retention and the specific ionic effect on the protoplasm. Saline solutions retain water and thereby reduce water potential, making this resource increasingly less available to plants (Nasr et al., 2011).

Water stress may adversely affect germination, vegetative growth, plant stand, yield, and in severe cases, it may cause seedlings death (Silva and Pruski, 1997) since water stress leads to the reduction of enzyme activity, and consequently to the reduction of the meristem development (Popinigis, 1985; Hadas, 1976). Very negative osmotic potential, especially at the beginning of imbibing, promotes drastic reduction of water uptake by seeds, and may derail the sequence of events of the germination process (Bansal et al., 1980).

Physiological quality of seeds has recently been one of the most studied aspects, since they are subjected to several degenerative changes that occur after maturity, which may be of biochemical, physiological and physical origin, and are associated with vigor reduction (Alizaga et al., 1990). The storage is a very important practice to maintain the physiological quality of seeds, and to ensure the maintenance of vigor and viability during the period between harvesting and sowing (Azevedo et al., 2003).

Thus, studies involving the germination behavior of seeds subjected to the condition of artificial stresses are instruments that provide a better understanding of the capacity for survival and adaptation of these species in conditions of natural stresses, such as drought and

saline soils (Pereira et al., 2012).

Thus, given the lack of information on the germination of tamarind seeds under stress conditions, as well as the influence of storage on seeds vigor, the objective of this study was to evaluate the behavior of tamarind seed subjected to stress conditions and storage.

MATERIALS AND METHODS

Three experiments were carried out in the Plant Physiology Laboratory of the Applied Biology Department of FCAV/UNESP, Campos de Jaboticabal-SP, with *T. indica* seeds. Before the establishment of the three trials, seeds were mechanically scarified with sandpaper (220 grit), on both surfaces of the seed coat. In all the trials, the experimental design was completely randomized with four replications of 25 seeds, totaling 100 seeds per treatment.

Experiment 1 (water stress)

To verify the effect of different water potentials in the germination process, polyethylene glycol (PEG 6000) was used as osmotic agent. Seeds were sown on a sheet of paper moistened with distilled water (control treatment) and PEG solution (polyethylene glycol) of 2.5 times the weight of the paper, at the potential of -0.3; -0.6; -1.2 Mpa, placed in gerbox-type boxes with four replications of 25 seeds. The boxes remained in biochemical oxygen demand (B.O.D) chambers, equipped with fluorescent lamps, under constant temperature of 30°C.

The concentrations of the different potentials of PEG solutions used for 30°C were obtained based on the equation of Michel and Kayfmann (1973).

Experiment 2 (salt stress)

To simulate salt stress, seeds were sown on a sheet of paper moistened with distilled (control treatment) and NaCl solution (sodium chloride) of 2.5 times the paper weight, at potentials of -0.3; -0.6; -1.2 Mpa, placed in gerbox-type boxes with four replications of 25 seeds. The boxes remained in B.O.D chambers, equipped with fluorescent lamps, under constant temperature of 30°C.

NaCl solutions used to simulate salt stress and determine the tolerance limit of tamarind seeds to NaCl were prepared using the Van't Hoff equation (Salisbury and Ross, 1992).

Experiment 3 (storage)

To verify the influence of storage on tamarind seed germination, seeds were stored in cold chamber at a temperature of 10°C and 50% relative humidity (RH) for different times: 0 (freshly harvested), 12, 24, 36 and 48 months.

Germination tests in the three trials were carried out according to the recommendations of the Rules for Seeds Analysis – RAS” (Brasil, 2009), using B.O.D chambers, equipped with fluorescent lamps, under constant temperature of 30°C.

Germination percentage was performed at 30 days after sowing,

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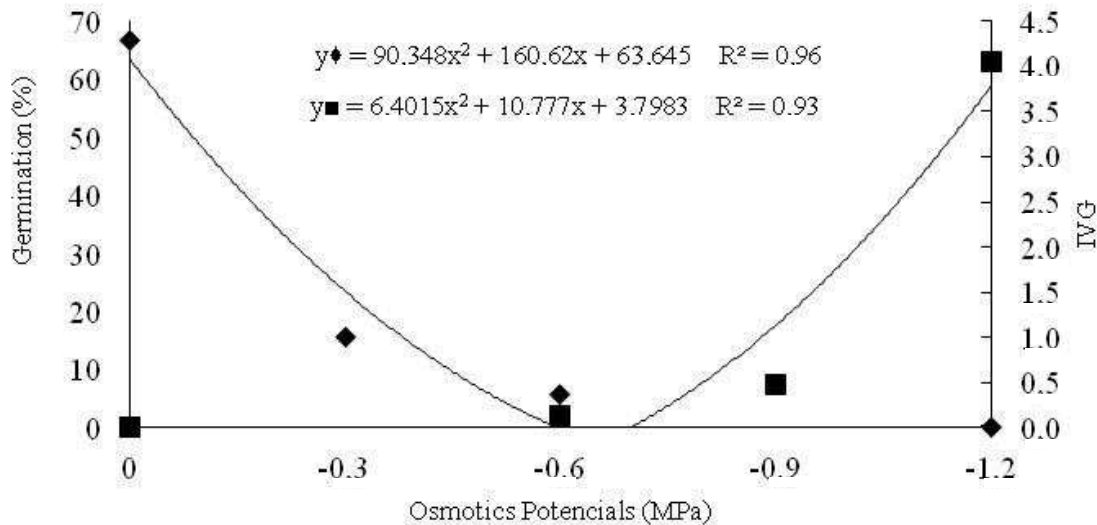


Figure 1. Germination (♦) and germination speed index (■) of *T. indica* seeds subjected to water stress.

considering as normal the seedlings which had all the essential structures well-developed (Brasil, 2009). In the germination test, the following were evaluated:

1. Germination speed index (GSI), calculated according to the formula: $GSI = G1 / N1 + G2 / N2 + \dots + Gn / Nn$, in which GSI= germination speed index, G1, G2 and Gn = number of normal seedlings, computed in the first, second ... and last count, respectively; N1, N2, Nn = number of days from the sowing to the first, second ... and last count, respectively (Maguire, 1962);
 2. Mean germination time (MGT), calculated according to the formula cited by Labourial and Valadares (1976): $T = (\sum ni) / \sum ni$, in which T = germination time; ni = number of germinated seeds per day; ti = incubation time;
 3. Mean germination speed (MGS), calculated according to the formula cited by Labourial and Valadares (1976): $V = 1/t$, in which V = mean germination speed; T = mean germination time.
- For all the trials, the data obtained from the variables measured in the experiment were subjected to analysis of variance using the F test, and the means were compared by the Tukey test at 5% probability. Quantitative data were subjected to polynomial regression analysis, by testing the linear and quadratic models, choosing the highest R^2 . The analyses were carried out using the software System for Analysis of Variance - SISVAR (Ferreira, 2000).

RESULTS AND DISCUSSION

Experiment 1

Influence of PEG solutions on the variables analyzed was observed. Figure 1 shows the results for germination and germination speed index of tamarind seeds subjected to water stress. It was found that PEG solutions reduced the percentage and the speed germination index with the increase of water stress, and there was no germination from potential of -0.6 MPa.

When seeds are subjected to contact with a given

concentration of aqueous solutions containing solutes, water soaking occurs naturally. However, the process is interrupted when there is equilibrium with the osmotic potential of the external solution, without radicle protrusion. Low enough potential inhibits radicle growth, even if the seed is metabolically active for germination and cell elongation (Fonseca and Perez, 2003).

In a study with genipap seeds (*Genipa americana* L.), Santos et al. (2011) obtained similar results, when germination percentage and germination speed index reduced with the increase of water stress, with no radicle emergency at potential of -0.3 and -0.4 MPa. For mangabeira seeds (*H. speciosa* Gomes), the increase of PEG osmotic solutions caused drastic reductions when the seeds were submitted to osmotic potential of -0.6 (Maseto and Scalón, 2012). The germination of mimosa-de-calf (*Piptadenia moniliformis* Benth) was committed from potential water below -0.6 MPa PEG 6000 (Azerêdo et. al., 2016). In *Jatropha* seeds (*Jatropha curcas*) there was marked decrease in germination rate in the potential of 0.2 MPa, reaching zero at the potential of 0.8 MPa (Pereira and Lopes, 2011).

The Figure 2 shows the values of mean germination time and mean germination speed of *T. indica*. The increase in water stress induced by the increase in PEG 6000 concentration in the substrate solution was responsible for the reduction of mean germination speed and for significant increase in the mean germination time. The reduction of the osmotic potential of the substrate solution influenced the slow and uneven germination over time.

This fact can be explained by the decrease in the metabolism of the seeds in function of the low water

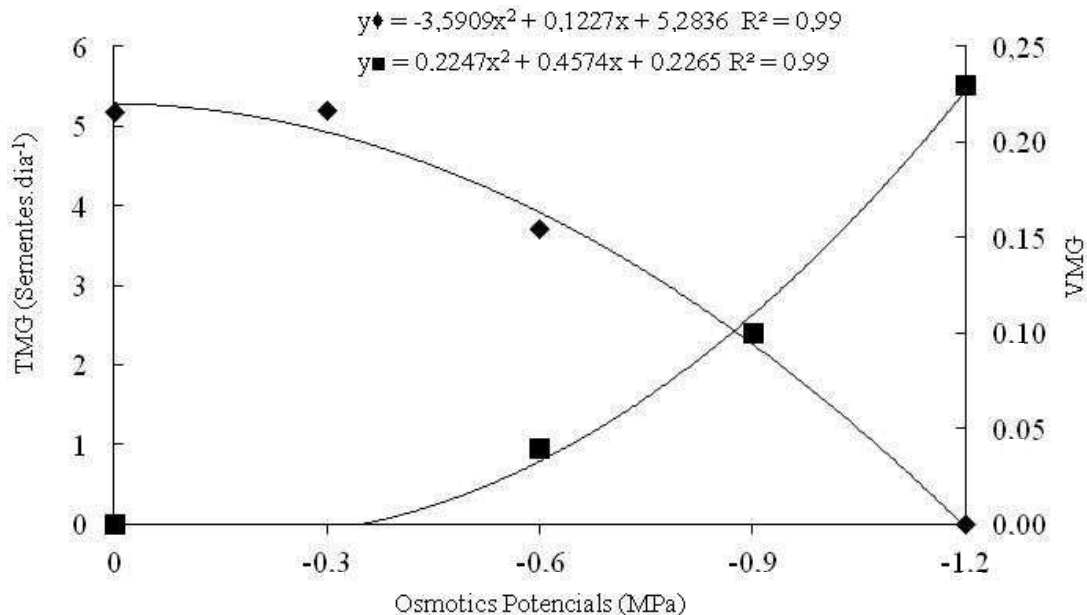


Figure 2. Mean germination time (♦) and mean speed germination (■) of *T. indica* seeds subjected to water stress.

availability for the digestion of reserves and translocation of metabolized products. Water stress can reduce both the percentage and the germination speed, with a wide range of responses between the most sensitive and the resistant species (Bewley and Black, 1994). Therefore, it is necessary to provide adequate level of hydration during seeds imbibition stage, in order to allow the reactivation of the metabolic processes, culminating in the growth of the embryonic axis (Marcos, 2005).

Similar results were found by Teixeira et al. (2011) in a study with crambe seeds (*Crambe abyssinica* Hochst), in which the increase in PEG 6000 concentrations in the substrate solution caused significant increase in the mean germination time, and reduction in germination speed. Avila et al. (2007) concluded that with the reduction of the osmotic potential, there was an increase in the number of days to the initial germination of canola seeds (*Brassica napus* L.). Rabbani et al. (2012) found a decrease in the mean germination speed and mean germination time of moringa seeds (*Moringa oleifera*), with the increase in water stress.

Experiment 2

Figure 3 shows the results of germination (A), germination speed index (B) and mean germination speed (C) of tamarind seeds subjected to salt stress with NaCl solutions. The germination behavior of seeds was similar to those that were subjected to PEG 6000

solutions, with reduction in the germination potential. There was reduction in germination and in the mean germination speed when the seeds were submitted to NaCl solutions, from the potential of -0.3 MPa. However, unlike water stress in saline, there was no germination for any of the treatments, showing that the tamarind seed are more sensitive to drought stress to saline.

The high concentration of salts is a stress factor in plants, as water is osmotically retained in saline, thus increasing the salt concentration becomes less and less available to plants (Munns, 2002). The species are sensitive to salinity and when sown in saline, initially a decrease in water absorption was observed, which acts by reducing the speed of the physiological and biochemical processes (Flowers, 2004), so the presence of higher levels of ions in plants less tolerant to water stress, can have adverse effects on the permeability of cell membranes (Greenway and Munns, 1980), also causing a reduction in the germination process in terms of higher levels of salt stress, pointing out that these levels vary with the species (Pereira et al., 2012). This information become important for purposes of recommendations for the planting of species able to withstand different conditions of osmotic potential in various ecological situations, especially when considering the saline soils and areas with low water availability (Rego et al., 2011).

Martins et al. (2011) also observed an adverse effect on speed and percentage of seed germination melaleuca (*Melaleuca quinquenervia*) according to the increase of the amount of NaCl in the solution. In cedar seeds

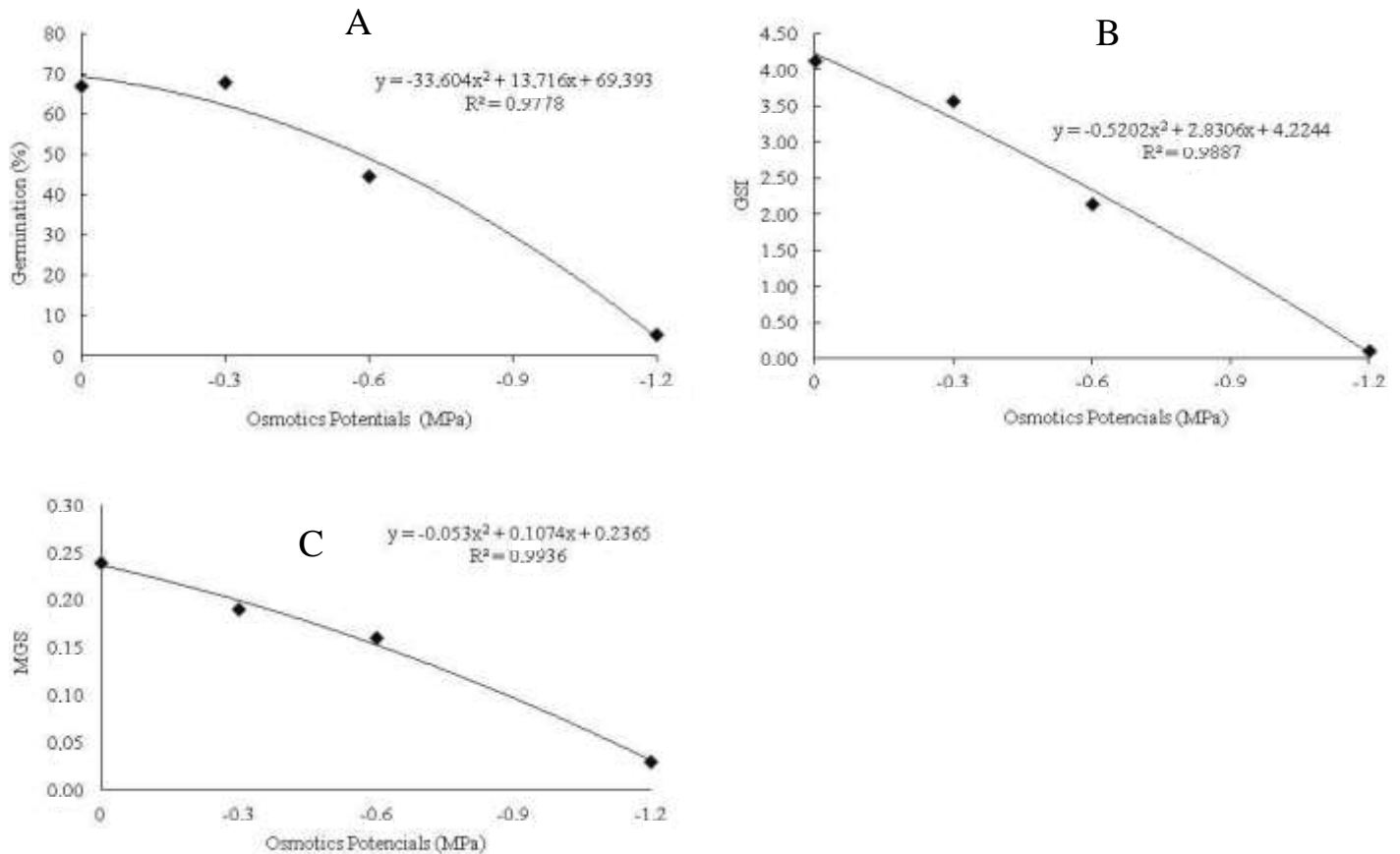


Figure 3. Germination (A), germination speed index (B) and mean germination speed (C) of *T. indica* seeds subjected to salt stress.

(*Cedrelela odorata* L.) the adverse effects of salt stress on seed germination was evident at concentrations of 25 mM NaCl, KCl and CaCl₂ (Ferreira et al., 2013). In a study on okra seeds (*Abelmoschus esculentus* L.) Dkhil and Denden (2010) found that germination was impaired when the seeds were subjected to solutions of 80 and 100 mM NaCl.

Experiment 3

Figure 4 shows the influence of storage times on the germination (A) and on the germination speed index (B) of tamarind seeds stored for different times. It was found that storage caused reduction in percentage and in germination speed in all storage times, and tamarind seeds are sensitive to prolonged storage.

Probably this reduction in germination is related to the fluctuations in water content, which was enough to promote higher respiratory rates, resulting in increased consumption of seeds reserves during respiration, and thus accelerating the deterioration speed (Guedes et al., 2010). Decreases in viability and vigor during the storage time can be attributed to degenerative changes,

which are typical of deterioration, reflecting the reduction of physiological quality (Corvello et al., 1999).

Similar results were observed by Scalon et al. (2012) in a study on the storage of uvaia seeds (*Eugenia pyriformis* Cambess), in which the percentage of freshly harvested seed germination was higher when compared with those subjected to storage. Contrary results were reported by H6ring et al. (2011) in jatropha seeds, in which stored seeds presented higher GSI. Souza et al. (2016) found that in quinoa seeds (*Chenopodium quinoa* Willdenow), the physiological quality was not affected by prolonged storage for over 300 days.

Understanding the dynamics of seed germination is essential to add information that is necessary to promote improvements in working conditions and germination. Overall, the results presented in the 'experiment 3' provided a better understanding of the survivability of the tamarind seeds in conditions of natural stress.

Conclusion

The tamarind seeds are sensitive to water and salt

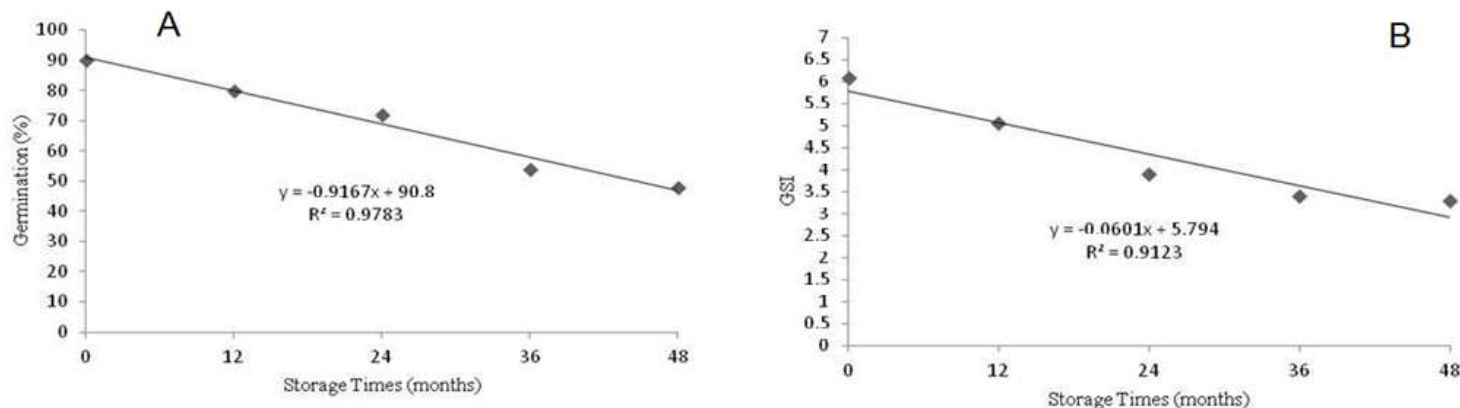


Figure 4. Germination (A) and germination speed index (B) of *T. indica* seeds subjected to storage.

stress, but water stress caused major damage to the seeds with the increase of osmotic solutions of PEG, being sensitive from the potential of -0.6 MPa. The seeds were sensitive to prolonged storage, this is not recommended for species.

Conflict of Interests

The authors have not declared any conflict of interests.

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