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Seed Germination of *Pongamia Pinnata (L.)* Pierre under Water Stress

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Abstract

Pongamia pinnata is one of the commercially important tree species, which provides seed oil utilized in the production of bio-diesel. Seeds of this species were subjected to different osmotic potentials induced by polyethylene glycol (PEG-6000) in order to study their response to drought conditions. Seeds were germinated in petriplates at osmotic potentials 0, -0.2, -0.5 and -0.8 MPa. Germination at high level of water stress was inhibited, but the recovery of their germinability after transferring to distilled water was high in all cases except -0.8MPa. The root length, fresh weight, dry weight, relative water and protein contents showed a significant decrease than their corresponding controls. However, carbohydrate and proline contents in the water stressed seedlings showed a significant increase.

Keywords: Seed germination, osmotic potential, PEG-6000*, Pongamia pinnata*, Proline, water stress.

Introduction

Energy is a necessary concomitant of human existence. Both transport and industry consume millions of tons of diesel every year, which is produced from crude oil. Economic development of a nation depends on self reliance in energy. India is heavily dependent on imported fossil fuels to meet its energy needs[1]. Bio-diesel, a renewable source of energy, offers a great potential to provide energy security and at the same time minimizes carbon emission. *Pongamia pinnata* is one of the plant species, which yield oil as a source of energy in the form of bio-diesel, with high oil recovery and the quality of oil[2].

Pongamia pinnata (L.) Pierre also called as *Derris indica* (Lam.) Bennet is a tree species belonging to the family Fabaceae. This species is commonly known as Pongam or karanj. The seed oil is used as a lubricant, water paint binder, pesticide, in soap making and tanning industries. The oil is used for the treatment of rheumatism and scabies. The press cake is used as poultry feed and also as organic fertilizer. It controls soil erosion and binds sand dunes[3].

The germination phase is considered critical for attaining a successful crop density and yield under arid conditions. Seed germination, one of the most important phases in the life cycle of a plant, is highly responsive to existing environment[4]. Water stress is one of the abiotic stresses, which affect seed germination, delaying its beginning or decreasing the final germinability[5].

Water is the primary abiotic factor which effects seed germination and also the subsequent metabolism of the plant. Its participation is crucial in the enzymatic reactions, solubilization and transportation of metabolites, as well as reagent in the hydrolysis of proteins, carbohydrates and lipids in the storage tissues of the seed[6].

According to Hadas[7], the critical soil water potential for seed germination, is typical for each plant species. Therefore, solutions with different osmotic potentials have been used in several studies in order to establish gradient of water stress. Osmotic agents such as mannitol and polyethylene glycol were used in germination studies to simulate low moisture conditions. PEG has been widely used as it does not penetrate the plant cell wall but can induce water stress similar to desiccation[8].

Although the impact of water stress on germination and seedling growth of different tree species was reported[9,10], Studies on the effect of water stress on seed germination are, however scanty for tropical tree species in general and pungam in particular. The study is therefore undertaken to evaluate the effect of water stress on seed germinability, seedling growth and biochemistry of seed germination of *Pongamia pinnata*, a plant taxa of contemporary relevance and diverse utility.

Material and Methods

Seeds of *Pongamia pinnata (L.)* Pierre were collected from the Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University Campus, Tirupati and stored in air tight polythene bags after sun drying. The seeds were surface sterilized using 4% hypo solution for 3min and later washed repeatedly with sterile distilled water. The seeds were then planted in germination trays filled with sterile sand. Seeds were subjected to water stress by using PEG 6000. Polyethylene glycol 6000 was used in three concentrations to maintain three levels of osmotic potentials namely -0.2MPa, -0.5MPa and -0.8MPa. Distilled water was used in place of PEG solution to maintain the control.

A completely randomized design was adopted for the experiment, with three replications of fifteen seeds each. Number of germinating seeds was counted day by day. Germination energy, speed of germination index, average time of germination and emergence energy value were then calculated using the recorded data on seed germination.

Germination percentage was calculated according to Fanti and Perez[11] using the formula G% =100 x A/N where A is the number of seeds germinated and N is the total number of seeds used in the germination test. Germination energy was calculated after Maguire[12] using the formula GE = $\frac{X_1}{Y_1}+\frac{X_2-X_1}{Y_2}+\ldots\ldots\frac{(X_n-X_{n-1})}{Y_n}$ where X_n = number of seeds germinated on the n^{th} counting date and Y_n = the number of days from sowing to the n^{th} count. Speed of germination index was calculated according to Jose et al.[13] by the formula S = $\frac{E_1}{N_1}+\frac{E_2}{N_2}+\ldots\ldots\frac{E_n}{N_n}$ where E_n = number of emerged seedlings observed in the n^{th} daily counting and N_n = number of days after the seeds were put to germinate in the n^{th} counting. Average time of germination was calculated by the formula $\frac{G_1T_1+G_2T_2+\ldots\ldots+G_nT_n}{G_1+G_2+\ldots\ldots+G_n}$ where G is the germination count on any counting period and T = time[14]. Emergence energy value is the highest value obtained when the germination percentage on a day is divided by the number of days since test when that germination percentage was reached.

All seeds which were not germinated till 25 days in the previous germination tests at different PEG concentrations were placed in new poly trays moistened with distilled water and incubated under the same conditions for additional 25 days. Physiological parameters like relative water content, carbohydrates, protein and proline were determined following the procedures of Barr and Weatherly[15], Mc Cready et al[16], Lowry et al[17] and Bates et al[18], respectively.

Results and Discussion

A significant decrease was observed in germination percentage of *Pongamia pinnata* seeds subjected to all levels of water stress used in the present study (table-1). Germination percentage was found more sensitive than the average time of germination.

Table–1
Effect of water stress on seed germination percentage of *Pongamia pinnata*

Days after Sowing	Experimental group	Germination percentage Mean ± S.D
Four	Control	0.56 ± 0.08
	-0.2MPa	-
	-0.5MPa	-
Six	Control	0.76 ± 0.13
	-0.2MPa	-
	-0.5MPa	-
Eight	Control	0.97 ± 0.40
	-0.2MPa	0.48 ± 0.04
	-0.5MPa	-
Ten	Control	1.19 ± 0.01
	-0.2MPa	0.56 ± 0.42
	-0.5MPa	0.46 ± 0.01
Twelve	Control	1.39 ± 0.15
	-0.2MPa	0.58 ± 0.82
	-0.5MPa	0.48 ± 0.45
Fourteen	Control	1.39 ± 0.15
	-0.2MPa	0.58 ± 0.82
	-0.5MPa	0.48 ± 0.45
Sixteen	Control	1.39 ± 0.15
	-0.2MPa	0.58 ± 0.82
	-0.5MPa	0.48 ± 0.45

The percentage data has been transformed using $\sqrt{arc}$ sin %. Values are means of 3 replications ± standard deviation.

Reduction in the osmotic potential decreased seed germination because of low water availability for seeds. The physical process of water uptake leads to activation of metabolic process mainly due to hydration of proteins or enzymes. Elevated drought stress decreases water uptake by seeds there by inhibiting their imbibition and germination. At the low osmotic potential, PEG inhibited the process of imbibition and germination[19]. Similar decrease in seed germination of different plant taxa under moisture stress has been reported[20-22] in the recent past.

Germination percentage and speed of germination index became low as a result of reduction in the osmotic potential of the medium decreased (table-2). Germination was completely inhibited at -0.8MPa, which showed that the species resistance limit to the water stress is between -0.5MPa and -0.8MPa.

Inhibition of the primary root emergence at lower water availability is related to the reduction of enzymatic activity and subsequently, with a decrease of the seed metabolism, necessary for digestion of reserve substances and mobilization of metabolized products[23].

Table–2
Effect of water stress on Germination energy, speed of germination index, average time of germination and emergence energy value of *Pongamia pinnata*

Osmotic potential	GE	S	ATG	EEV
0MPa	2.31 ± 0.31 [a]	7.97 ± 0.54 [a]	11.33 ± 0.25 [a]	7.95 ± 0.32 [a]
-0.2MPa	0.54 ± 0.12 [b]	1.85 ± 0.38 [b]	12.23 ± 0.06 [b]	2.88 ± 0.39 [b]
-0.5MPa	0.33 ± 0.04 [c]	1.01 ± 0.13 [c, b]	13.07 ± 0.12 [c]	2.07 ± 0.12 [c, b]

Average time of germination increased as osmotic potential decreased. Speed of germination index, emergence energy value and germination energy reduced with the decrease in osmotic potential. The germination capacity of non germinated *Pongamia pinnata* seeds when subjected to PEG 6000 solutions with osmotic potentials -0.2MPa and -0.5MPa was recovered after the seeds were watered. Germination was found to be higher than 80%. But the germinability of seeds subjected to -0.8MPa osmotic potential was zero even after re-watering. Probably, this may be due to germination delay, caused by osmotic potential. Similar observations on the recovery of the seeds previously subjected to solutions with low osmotic potentials were made in *Pinus sylvestris*[24] and *Plantago*[25] after moistened with distilled water. Germination failure under water stress may be considered as secondary or induced dormancy which in many species is readily reversible when water availability is increased[26].

The relative water content of germinating seed was found decreased due to water stress (figure-2). Similar negative correlation of relative water content of germinating seeds with water stress was also found by others[27]. PEG treatment caused a reduction in the growth of root length (figure-1). Elevated water stress inhibited the root elongation[28]. Proline content increased with increasing osmotic potentials (table-3) and is found similar to other plants[29,30]. The protein content decreased in PEG treated seedlings compared to control (table-3). The lower protein content in water stressed seedling may be attributed to the damage in membranes and may result in greater degree of membrane protein proteolysis.

Table-3
Effect of water stress on carbohydrate, Protein and Proline contents during seed germination and early seedling growth of *Pongamia pinnata*

Treatments	Biochemical Parameters		
	Carbohydrates (mg/g.d.w)	Proteins (mg/g.d.w)	Proline (µmol/g.f.w)
Control	1.028 ± 0.001[d]	19.26 ± 0.06[a]	6.92 ± 0.09[d]
-0.2 MPa	1.047 ± 0.003[c]	11.08 ± 0.21[b]	89.0 ± 1.21[c]
-0.5MPa	1.087 ± 0.001[b]	9.94 ± 0.12[c]	190.47 ±0.99[b]
-0.8MPa	1.280 ± 0.00[a]	8.03 ± 0.25[d]	258.47 ± 1.44[a]

Values are means of 3 replications ± standard deviation

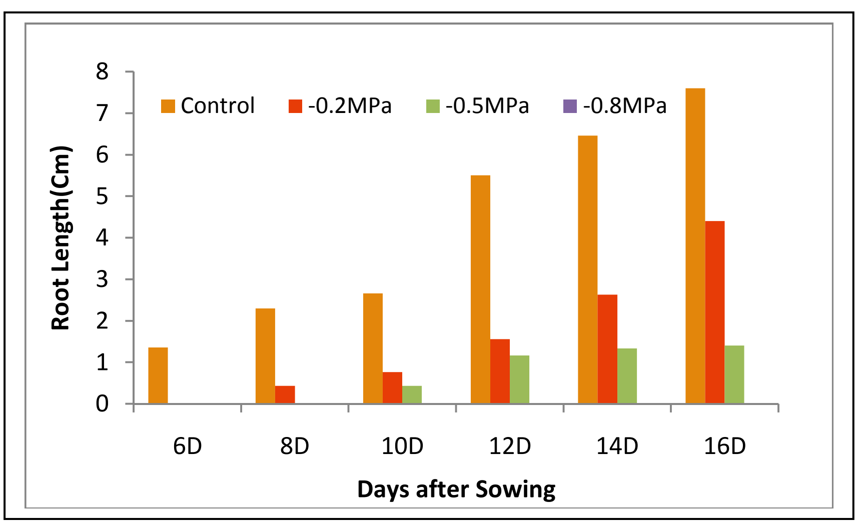

Figure-1
Effect of water stress on root length of *Pongamia pinnata* (L.) Pierre

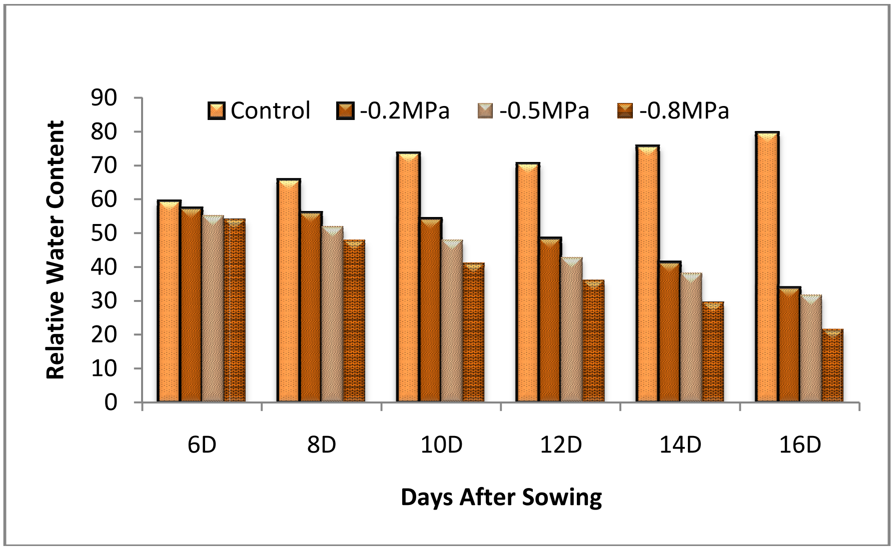

Figure-2
Effect of water stress on relative water content of *Pongamia pinnata* (L.) Pierre

The carbohydrate content increased in the seedlings under water stress compared to control. Similar increase in total sugar content under water stress in forage sorghum has also been reported[20].

Conclusion

The basic results reported in the present investigation are highly significant and useful in increasing the growth and productivity of field grown *Pongamia pinnata*, a potential resource for the production of bio-diesel. Severe water stress caused the reduction in seed germination percentage as well as early seedling growth. But the seeds under mild and moderate water stress recovered when adequate water is supplied.

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