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EFFECT OF DIFFERENT PRETREATMENTS ON BREAKING SEED DORMANCY AND *IN VITRO* GERMINATION IN *JATROPHA CURCAS* L.

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Abstract

Development of rapid and efficient propagation methods for *Jatropha curcas* are highly desirable since its seed oil can be used as biofuel and hence of high economic value. Dormancy, decreased viability with age, and low germination rate are amongst the common problems associated with its conventional propagation through seeds. *In vitro* germination of seeds, specifically during the dormant periods by using some pretreatments were therefore focused to help resolve some of these limiting issues. Pretreatments included presoaking of seeds in water overnight, scarification, stratification and removal of seed coats. It was observed that the orientation of the seeds on the culture media also had an effect on its germination rate. Disinfection of naked seeds could not support germination so the seeds were disinfected before removing the seed coats. It was observed that the removal of seed coats only could break the dormancy of seeds to get 100% *In vitro* germination on full strength MS medium kept in the dark at 25 ± 2˚C in the months of December to January. Such seedlings were shifted in light conditions (16h photoperiod) after the root emergence at the same temperature to support chlorophyll development. Seedlings were successfully acclimatized by shifting to a mixture of peat, clay and silt (1:1:1 v/v) in greenhouse. It is expected that the reported method of propagation would help to develop suitable tissues for germplasm preservation, large scale rapid plant propagation and may find its application in crop improvement and hence profitability in a broader sense.

Key words: Dormancy, *In vitro* germination, *Jatropha curcas*, Pretreatments.

Introduction

Biofuels, the renewable sources of energy are gaining serious attention all over the world due to an ever-increasing energy demand and depletion of fossil fuel (non-renewable) resources. *Jatropha curcas* L. belonging to family Euphorbiaceae is a multipurpose, drought resistant, perennial and deciduous shrub. Its seed oil and press cake is considered as potential source of biofuel (Martínez-Herrera *et al*., 2006; Kamel *et al*., 2018) and its chemical specifications also match with international biodiesel standards (Azam *et al*., 2005). Due to such properties of its seed oil, Rashid *et al*., (2010) also recommended its cultivation in Pakistan on large scale for biodiesel production. It can grow on a wide variety of soils and is resistant to various environmental stresses (Francis *et al*., 2005; Maes *et al*., 2009; Silva *et al*., 2010; Hishida *et al*., 2014; Mudalkar *et al*., 2017). Therefore, propagation of *Jatropha curcas* is of high value around the world. It is propagated normally through seeds or vegetative cuttings. Plants propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than plants propagated by seeds (Heller, 1996). Seed set has also been reported to be low in vegetatively-propagated plants later (Sujatha *et al.*, 2006). Problems associated with *Jatropha curcas* propagation through seeds are also many such as poor seed viability, low germination, scanty and delayed rooting of seedlings (Heller, 1996 and Openshaw, 2000). *Jatropha curcas* seeds are usually unreliable in terms of germination which varies from 10-95 per cent (Niranjan *et al.*, 2010). Freshly harvested seeds, on the other hand show dormancy and after-ripening and pre-treatment is therefore necessary before the seeds can germinate. Poor germination of seeds is due to water impermeable testa that exerts physical exogenous dormancy (Holmes *et al*., 1987). Seeds germinate best only in the month of October or in March-April and during rest of the year are usually dormant. Being an oil crop, it cannot be stored for long as it loses up to 50 percent of its viability upon 15 months storage (Kobilke, 1989). In the present research work, different pretreatments and *In vitro* techniques were applied to break seed dormancy and to enhance rate of germination. Kureel (2006) reported that the seeds of *J. curcas* when soaked in water and GA_3 solutions (0, 10 and 20 mg l^{-1}) for 24 h, germinated within 12, 8 and 5 days, respectively. Soaking *J. curcas* seeds in di-sodium hydrogen orthophosphate (Na_2HPO_4) for 6 h enhanced emergence by 25% and improved the quality of seedlings (Srimathi & Paramathma, 2006). Effect of plant growth hormones (Idu *et al.*, 2007 and Koorneef *et al.*, 2007) and seed soaking time individually or in combination (Pascual *et al.*, 2009) on seed germination have also been reported.

There is insufficient information on enhancement of seed germination of *J. curcas* around the world. Moreover information on seed germination using *In vitro* means during the dormant period is also extremely scanty. In this paper, we present various methods to break seed dormancy and subsequent germination both under *In vitro* as well as glasshouse conditions in *J. curcas* with an objective of improving mass propagation of this valuable plant species.

Materials and Methods

Seeds used in the present investigation were supplied by 'Jatropha Pakistan Four Friends Group, Multan' in the month of December. Experiments were performed in the months of December 2010 and January 2011 when seeds and whole plants were dormant. Seeds were pretreated by seven different methods. Scarification of seeds (S1) was done with emery sand paper to the extent that black coating of seed coats was removed. For stratification (S2), cold shock was given by keeping the seeds at 4-5˚C for 24 hours. S3 seeds were first scarified and then stratified. S4 seeds were soaked in water overnight for 24 hours at

room temperature. Seed coats were removed after sterilization in Laminar Air-flow cabinet (S5) or seeds were sterilized after removing the whole seed coats (S6). Some seeds were flamed for 2-3 seconds on Bunsen burner after dipping in ethyl alcohol (S7). S0 were considered as non-treated control. All treatments were given to 60 seeds in 3 replicates of 20 seeds each. Out of which half were sown on full strength and the rest on half strength MS medium. In another lot, each treatment was given to 10 seeds for soil germination.

Seeds from treatments S0 to S7 after thorough washing with tap water were dipped in a mixture of Tween-20 and household detergent and kept on magnetic stirrer at 50˚C for 10-15 minutes. Seeds were then rinsed several times with water and dipped in 0.1% $HgCl_2$ solution for 10 minutes, rinsed three to four times with distilled autoclaved water and then dipped in 20 % bleach solution (NaClO) for 15-20 minutes and rinsed 4-5 times again with distilled autoclaved water. The same method was used for S5 seeds except that Tween-20 was not used with detergent and also kept on magnetic stirrer at room temperature for 5-10 minutes. Instead of 20%, seeds were dipped in 10% NaClO solution for 10 minutes.

Pre-treated seeds were sown on full or ½ strength MS media (Murashige & Skoog, 1962) in 200×30 mm culture tubes. The medium was adjusted to pH 5.8±0.2 and solidified with 0.8% Agar. Sterilization of media was achieved by autoclaving at 15lb in^{-2} pressure at 120˚C for 10-15 minutes. Seeds were either oriented dorsally or ventrally in the culture tubes. All the cultures were kept initially in dark and then after root emergence shifted to 16 h photoperiod for chlorophyll development in shoots.

Developed seedling after 10-15 days were removed from agar medium and roots were washed with tab water and shifted to the soil mix (peat, clay and silt 1:1:1 v/v). After watering, plantlets were covered with glass jars for 24 hours at 25±2˚C and 16 h photoperiod. Seedlings were then shifted in glasshouse conditions after 3-4 days.

Results and Discussion

There was no seed germination in the soil by using any pretreatment method. Even the removal of seed coats in December to January did not help in this study. Our results therefore were indirectly in agreement with a previous study from Thailand in which the author suggested October to be the most suitable month for *J. curcas* plantation (Ratree, 2004). Rahman *et al*., (2009), on the other hand have suggested March to early April as best time for *J. curcas* plantation in Bangladesh. There was, on the other hand, 100% *In vitro* germination using coatless seeds oriented dorsally in full strength MS medium (Table 1). This result is in agreement with Abdelgadir *et al*., (2012) who reported that seed coat removal accelerated water imbibition and germination occurred within 48 h. Different pretreatments to enhance germination of seeds were reported by several workers (Idu *et al.*, 2007, Koorneef *et al.*, 2007 and Pascual *et al.*, 2009) but most pretreatments in the present research work did not help support even *In vitro* seed germination in *J. curcas*. This may be due to the prevailing dormancy in the months of December and January. Poor germination of seeds is perhaps also due to seed water impermeable testa (seed coats) which exerts physical exogenous dormancy (Holmes *et al*., 1987). Low germination rate of *J. curcas* is also possibly due to seed coats that form mucilage surrounding the seeds which prevents diffusion of oxygen to the embryo and hence inhibits germination (Kumari *et al*, 2011). *J. curcas* seeds are usually dark brown to blakish-brown in colour. Dark colour of seeds is also considered to be a factor for enhancing dormancy (Duran & Retamal, 1989). Therefore removal of seed coats in the present investigation might have been helpful in breaking dormancy and enhancing seed germination. Rodrigues & Rodrigues (2014) in their studies on *Macaranga peltata* seed germination confirmed that seed coat dormancy is responsible for inhibited germination. Mwang`Ingo *et al.*, (2004) in their investigation also suggested the complete removal of seed coats and soaking in hot water for enhanced germination and better seedling growth. Islam *et al*., (2009) also reported that pre-sowing treatments significantly enhanced seed germination parameters of *J. curcas*. Some workers used pre-soaking of seeds in GA_3 (100 ppm) for that purpose and attained 67.38% germination (Kumari *et al*., 2011).

Germination of *J. curcas* seeds was attained in just 2-3 (Fig. 1a, b) days as compared to previous studies where seeds germinated normally within 8-9 days (Kumari *et al.*, 2011). Germination in *J. curcas* is epigean (Fig. 1a-g) in nature (cotyledons emerge above ground). Cotyledonary leaves soon wither away or fall-off after new leaves are developed (Fig. 1g). This fact was also supported by Becker & Francis, (2003). Sterilization of seeds after removing the seed coats did not favor germination process. This may be attributed to the effect of strong sterilizing agents on delicate embryo tissues.

Table 1. Effect of various pretreatments and MS medium strength on *In vitro* seed germination of *Jatropha curcas* L.

Pretreatments*	Full strength MS medium			Half strength MS medium		
	Root induction	Shoot induction	%age germination	Root induction	Shoot induction	%age germination
S 0	0	0	0	0	0	0
S1	0	0	0	0	0	0
S2	0	0	0	0	0	0
S3	0	0	0	0	0	0
S4	0	0	0	0	0	0
S5	30	24	80	14	4	13.3
S6	0	0	0	0	0	0
S7	0	0	0	0	0	0

*S0 non treated, S1 scarified, S2 stratified, S3 scarified+ stratified, S4 seeds soaked in water, S5 seed coats removed after sterilization, S6 seed coats removed before sterilization, S7 seeds flamed on Bunsen burner. Each treatment given to 30 seeds in 3 replicates

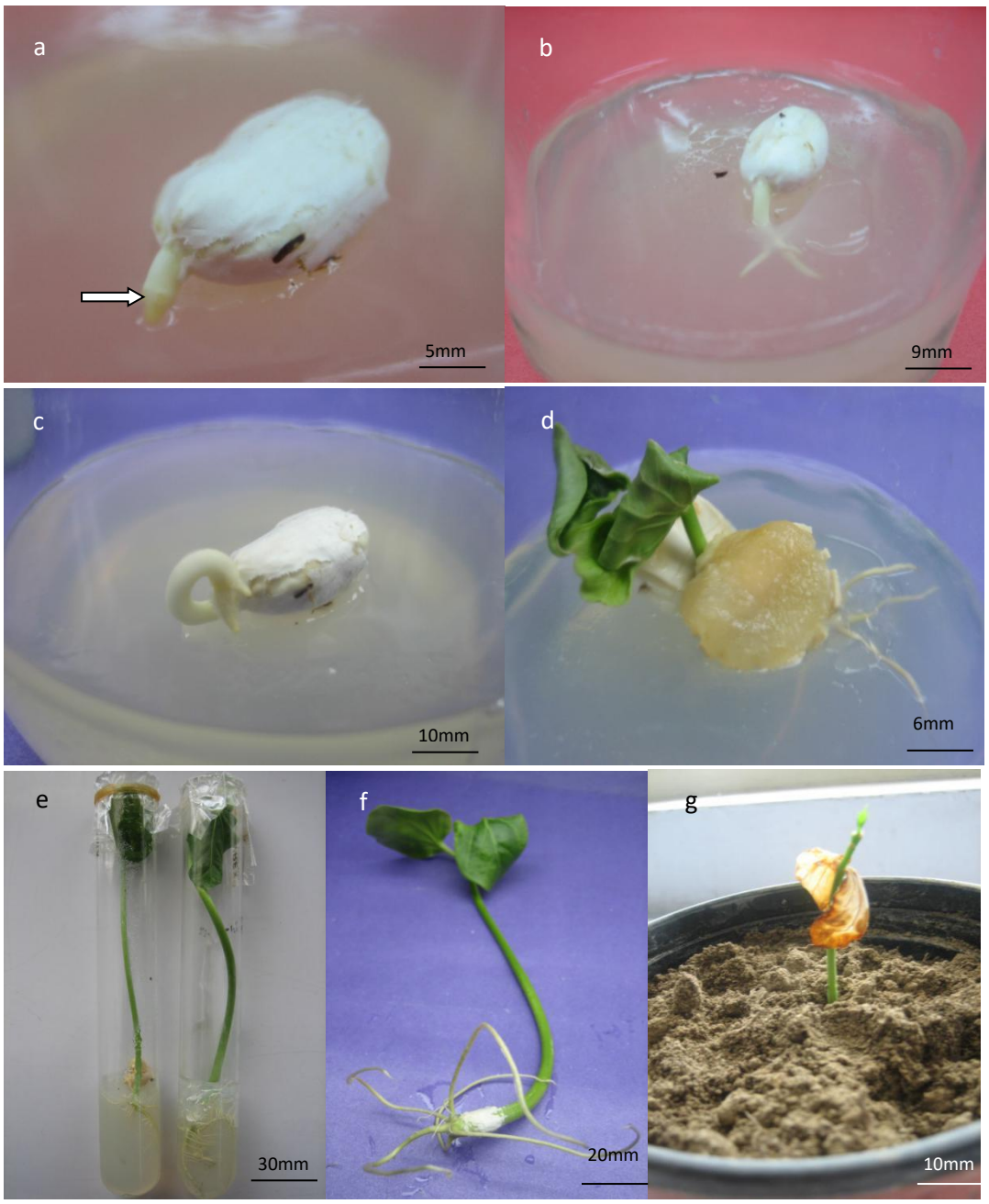

Fig. 1. Root induction (arrow) after 2 days from a *J. carcus* seed sown dorsally on full strength MS medium (a), after 3 days of sowing (b), root emergence in ventrally oriented seed (c), Developed shoot after 7-8 days of sowing (d), further shoot elongation after 15 days of sowing (e), seedling taken out of the culture tube for acclimatization in glasshouse after 15 days of sowing (f), withdrawal of cotyledonary leaves and emergence of a new leaf 7-8 days after acclimatization (g).

Table 2. Effect of orientation of S5 seeds on germination behavior on full strength MS medium.

Orientation	Root induction	Shoot induction
Dorsal	100	100
Ventral	15	60

Out of total 30 S5 seed, half were oriented dorsally while the other half ventrally

Seeds soaked in water overnight could not germinate even after 15-20 days of sowing. These results are not in agreement with those of Feike *et al*., (2008) who reported that seeds soaked in water showed highest survival and germination rate. This contradiction was perhaps due to dormant season in which the present investigation was carried out. Mechanical scarification and stratification both increase germination but the germination rate was highest and fastest when both treatments were applied together (Kaye & Kuykendall 2001) but presently both the treatments either separately or in combination had no effect on germination. Our results also disagree with a recent work by Geisler *et al.*, (2017) who reported that wet shocks of 40 to 50ºC proved helpful in breaking physical dormancy of seeds. Seeds oriented dorsally in the culture medium resulted in better germination and seedling development as compared to ventral orientation (Table 2, Fig. 1c). This may be due to an easy approach of radical to the growth medium in dorsally-placed seeds. Zewdie & Welka (2015) also suggested that orientation played important role in germination of seeds. They further reported that sowing of seeds in such a way that micropyle

was directed downwards showed best results in terms of germination. Same was the case in our experiments where micropyle was directed downwards in dorsally-placed seeds. Developed seedlings (30%) from both the treatments were successfully acclimatized in the soil.

Thus the study highlighted that removal of seed coat could help germinate seeds of *J. curcas* even in dormant season (December, January) on simple full strength MS medium. This method takes least time for germination (2-3 days) as compared to previous studies and will be of help for future propagation of this plant at mass scale level. This study also opens new horizon to explore more suitable methods in the near future for acclimatization and hardening of these *In vitro*-developed plantlets.

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