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Germination Enhancement Studies in *Jatropha curcas* L. by Chemical and Physical Methods

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Abstract Physic nut (*Jatropha curcas*) *L*. is identified as the most potential tree bearing oil which can be used as biofuel crop belongs to family Euphorbiaceae. The low seed yield and low germination of seed are two important factor encountered by researchers. Therefore, enhancement of germination for raising large scale nursery assumes greater significance. The present investigation involves the use of physical (soaking of seed in water, soaking of seed in water and temperature treatment of 35°C, 40°C, 45°C) and chemicals methods (KCl, $CaCl_2$, $FeSO_4$, $ZnSO_4$, $MnSO_4$, GA_3 and Ethrel) to accelerate as well to increase the seed germination percentage. Pre-treating seed with $FeSO_4$ @ 1% and Ethrel @ 0.5% took least number of days (5 days) for initiating germination. Even pre-soaking of seed in water also substantially enhanced germination as it took 7 days to observe the first germinant. The high percentage of germinability was observed when the seed was treated with $CaCl_2$ (93.3%) followed by H_2O (86.7%). Treating seed with GA_3 @ 25 ppm recorded maximum root to shoot ratio 0.62 followed by $MnSO_4$ @ 1% 0.54, and former differed significantly with KCl @ 3% and Ethrel @ 1%. However, Soaking of *Jatropha* seeds in water for overnight found to be economical and farmer friendly practice to enhance germination in *Jatropha curcas.*

Keywords Germination, Enhancement, Treatments, *Jatropha curcas*

1. Introduction

Jatropha curcas popularly known as Physic nut or *Ratanjyo*t is a drought resistant, photo-insensitive perennial plant belonging to family Euphorbiaceae [1]. The plant grows well on poor stony soils. It has a long history of cultivation in tropical and subtropical regions of the world. It is native to Central America and occurs mainly at lower altitudes (0- 500 m) in areas with average temperatures of well above 20°C [2] and [3]. The seeds are toxic due to the presence of cursive and curative ingredients. Its first commercial applications were reported from Lisbon, where oil imported from Cape Verde was used for soap production and for lamps. It is also used as a natural dye by tribals in Uttar Pradesh, India [4]. The press cake was used as fertilizer for the cultivation of potatoes [5]. It was introduced in Asia by Portuguese [6].

In India, it is well naturalized and distributed in almost all states. It is planted as a hedge to protect the field as it in not browsed by cattle. *Jatropha curcas* is a succulent that sheds its leaves during the dry season and well adapted to arid and semi-arid condition and often used for erosion control [7]. Even today, *Jatropha curcas* is mainly cultivated for the production of oil (27 – 40 %) that has fuel substitute [8]. Extract from the plant is known for their effect on the wide range of organisms including insect pests, mouse and nematodes [9]. It is truly a multipurpose tree species fit for agroforestry and other afforestation programme [10].

The problem of great concern regarding this plant is the seed yield though the plant put forth profuse vegetative growth but a number of seed produced per plant is very low. Besides, the plant produces seeds after 2-3 years approximately, depending on environmental conditions and seeds have a limited viability (50%) within 15 months [11]. Looking to its problem of low seed setting and low seed viability, the study was planned to identify physical and chemical method suitable to enhance the seed germination of *Jatropha curcas*.

2. Materials and Methods

One-year-old mature seeds of *Jatropha curcas* L. stored in brown paper bags at ambient temperature were used in the experiment. Two separate experiments with physical and chemical methods to enhance seed germination were carried out at the National Bureau of Plant Genetic Resources (NBPGR), Regional Research station, Rajendranagar

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Firstly, The chemical method involved pre-treatment of seeds in different chemicals involving eleven treatments and three replications for each treatment. The treatments given were- T_0- Control; T_1- Soaked in water for 16 hr ; T_2- KCl @3%; T_3-$CaCl_2$ @ 1.5%; T_4- $FeSO_4$ @ 1%; T_5-$ZnSO_4$ @ 1%; T_6- $MnSO_4$ @ 1% ; T_7- GA_3 @ 25 ppm; T_8-GA_3 @ 50 ppm ; T_9- Ethrel @ 0.5% and T_{10}- Ethrel @ 1%. Treated seeds were rinsed thoroughly with distilled water and ten seeds were used for every replicate i.e., thirty seeds were used per treatment. The seeds were sown in plastic trays in sand media and trays were kept at room temperature

The Physical method involved three levels of temperature treatment of the soaked seed. Seeds were soaked overnight and subjected to temperature treatments of 35°C, 40°C, 45°C (in the oven) and a control (i.e. no temperature treatment was given). The experiment was repeated for three times and during each time 50 seeds were sown in earthen pots containing sand in glasshouse and watering was done periodically.

The observation regarding time to initiate germination, time to maximum germination was recorded at regular interval. The data was analysed and transformed to percentage values and following parameters were determined.

$$Germinability = \frac{Number\ of\ germinating\ seeds}{Number\ of\ seeds\ initiated} \times 100$$

$$Relative\ Germinability = \frac{Number\ of\ germinating\ seeds}{Number\ of\ viable\ seeds\ initiated} \times 100$$

$$Dormancy = \frac{Number\ of\ ungerminated\ but\ viable\ seeds}{Number\ of\ viable\ seeds\ initiated} \times 100$$

$$Mortality = \frac{Number\ of\ viable\ seeds}{Number\ of\ seeds\ initiated} \times 100$$

Table 1. Effect of chemical treatments on seed germination and different parameters in *Jatropha curcas*

Treatment	Time to first observed germination (days)	Time to maximum germination (days)	Germinability (%)	Mortality (%)
T0-(Control)	11.0	18	43.3	16.7
T1-H2O (16Hr)	7.0	10	86.7	3.3
T2-KCl 3%(16Hr)	8.0	13	80.0	6.7
T3-$CaCl_2$ 1.5%	8.0	12	93.3	3.3
T4-$FeSO_4$ 1%	5.0	13	70.0	13.3
T5-$ZnSO_4$ 1%	8.0	14	73.3	13.3
T6-$MnSO_4$ 1%	6.0	12	70.0	6.7
T7-GA3 25ppm	12.0	16	23.3	20.0
T8-GA3 50ppm	5.0	11	83.3	6.7
T9-Ethrel 0.5%	5.0	10	73.3	6.7
T10-Ethrel 1%	6.0	12	76.7	6.7
S.E(±)	0.77	0.73	6.07	1.67

Table 2. Effect of chemical treatments on different parameters in *Jatropha curcas*

Treatment	Root length (cm)	Shoot length (cm)	Root to shoot ratio (cm)	Seedling dry weight (g)
T0-(Control)	4.02	13.23	0.30	1.87
T1-H2O (16Hr)	6.40	15.78	0.38	3.88
T2-KCl 3%(16Hr)	4.43	15.80	0.27	3.98
T3-$CaCl_2$ 1.5%	5.52	16.74	0.32	5.92
T4-$FeSO_4$ 1%	5.78	16.30	0.34	4.78
T5-$ZnSO_4$ 1%	4.52	15.04	0.30	4.06
T6-$MnSO_4$ 1%	8.06	15.86	0.54	4.67
T7-GA3 25ppm	5.76	09.91	0.62	2.52
T8-GA3 50ppm	7.38	15.06	0.48	3.63
T9-Ethrel 0.5%	4.86	15.56	0.30	5.45
T10-Ethrel 1%	4.23	17.34	0.24	4.74
C V (%)	25.37	11.53	30.05	8.58
CD (%)	2.39	2.97	0.19	1.32

3. Results and Discussion

The effect of the chemical treatments on the Jatropha seed germinability (%) and mortality (%) are presented in table 1. Pre-treating seed with $FeSO_4$ @ 1% and Ethrel @ 0.5% took least number of days (5 days) for initiating germination followed by imbibition of seeds in $MnSO_4$ @ 1% and Ethrel @ 1% which took 6 days for initiating germination. Ethrel is known to reduce the activity of total phenol content in pericarp and embryo of sunflower seeds which makes the seed coat permeable to water results in better germination as per earlier report [12]. The earlier report also suggested the effectiveness of Ethrel in enhancing the germination of dormant sunflower seeds [13]. Even pre-soaking of seed in water also substantially enhanced germination as it took 7 days to observe the first germinant. Maximum of 12 days to complete germination were recorded with GA_3 at 25 ppm. The time to reach maximum germination was recorded when seeds pre-soaked in water for 16 hours (10 days). Water may be the best, cheapest and farmer friendly method to enhance germination in *Jatropha curcas*. The untreated (control) seed took almost 18 days to reach maximum germination. This highlights that water can be used as pre-soaking media for enhancing germination as it would facilitate imbibition of embryo resulting in quick germination and better growth of the seedling.

The high percentage of germinability was observed when the seed treated with $CaCl_2$ (93.3%) followed by H_2O (86.7%). This might be due to enhanced viability of Calcium chloride treated seeds as per previous reports in Groundnut where it increased the activity of dehydrogenase which prolonged seed viability [14]. The least germinability (23.3%) and highest mortality (20%) was recorded with GA_3 @ 25 ppm which indicates GA_3 at lower concentration may not effective in enhancing germination and may have preventive effect on *Jatropha* seed by inhibiting enzyme activity because the same growth regulator at higher concentration of 50 ppm has increased the germinability of *Jatropha* seed by 40% over control and even shown less mortality rate of 6.7%, this may be due to GA_3 at higher concentration not only effective in increasing rate of germination but also increases the activity of lipase which helps in the hydrolysis of stored lipids and provides the respiratory fuel for the seedling growth in Brassica spp as reported by [15] and [16] respectively. Most pronounced effect of GA_3 was more rapid germination of *Tripsacum dactyloides* L. seeds as per earlier reports [17]. The rose root (*Rhodiola rosea*) seed treated with 100 mg gibberellic acid/ litre and exposed to 20-24°C recorded highest germination (87%) as confirmed by previous results [18]. Gibberellic acid at 50 ppm gave the highest germination percentage (83.33%) in Aswagandtha (*Withania somnifera*) as per earlier report [19].

The significant difference existed among treatments with respect to root length of the germinated seedlings (Table 2). The seeds treated with $MnSO_4$ @1% and GA_3 50 ppm recorded highest root length of 8.06 cm and 7.38 cm respectively and differed significantly with other treatments *viz.,* Ethrel @ 0.5%, $ZnSO_4$ 1%, KCl 3%, Ethrel 1% and control. The $MnSO_4$ not only enhances germination it also increases seed yield of sunflower as per previous reports [20]. The seeds treated with Ethrel 1%, differed significantly and recorded highest shoot length of 17.34 cm and lowest was 9.91 cm with GA_3 at 25 ppm. In Apricot GA_3 was effective in increasing the total length of seedlings as reported by previous workers [16] and this finding corroborates present investigation as GA_3 @ 25 ppm recorded maximum root to shoot ratio 0.62 cm followed by $MnSO_4$ @ 1% 0.54, and former differed significantly with KCl @ 3% and Ethrel @ 1%. The control recorded the lowest root to shoot ratio of 0.27. The maximum dry weight of 5.92 g was recorded with $CaCl_2$ @ 1.5% and differed significantly with rest of the treatments except for $FeSO_4$ @ 1%, Ethrel @ 1% and $MnSO_4$ @1% and minimum dry weight was recorded in control 1.874 g. This indicates lanky growth of the seedlings which may not be vigorous and withstand extreme conditions in the field.

Even in the physical methods, there was a significant difference among the treatments (Table 3). Observing the result, the best temperature for *Jatropha* seed germination is 40°C, where 44% of the seeds germinated. This corroborates previous results [21] who suggested pre-heating increased the speed of germination and in this study also maximum germination percentage was achieved within 15 to 30 days after incubation. This indicates warm temperature is pre-requisite for better germinations and good crop stand under adverse conditions in the field. This highlights neither moderate temperature nor high-temperature favours germination. However, seed germination behaviour is good at room temperature as well as at 40°C.

Table 3. Effect of Physical method (temperature treatments) on germination of *Jatropha* seeds

Treatment	Germination (%)
T1- Control	39.33
T2 - 35˚C	16.66
T 3 - 40˚C	44.00
T 4 - 45˚C	16.66
C V (%)	11.13
C D (%)	6.49

Fig.1 indicates that treating seeds with $CaCl_2$ at 1.5% increased the relative germinability by 96.6% followed by soaking seed in H_2O for 16 hours (89.7%) in comparison to control (52%). This may be attributed better role in enhancing cell division and differentiation as per earlier reports [21]. In groundnut also, $CaCl_2$ enhanced germination as per previous results [22]. However, GA_3 has recorded 29.2% of germination which is lower than the control. The rate of germination varied significantly among various treatments. The high rate of germination was observed when seeds were soaked in H_2O followed by $CaCl_2$ at 1.5%. (Fig 2). It was reported that water plays an important role in determining the time of germination.

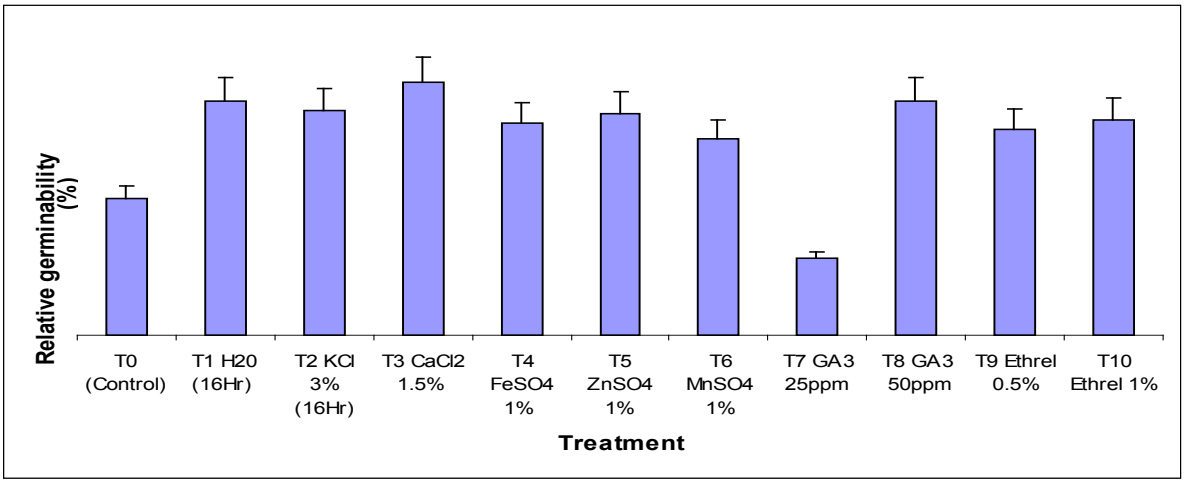

Figure 1. Comparison of various chemical treatments to relative germinability of *Jatropha curcas*

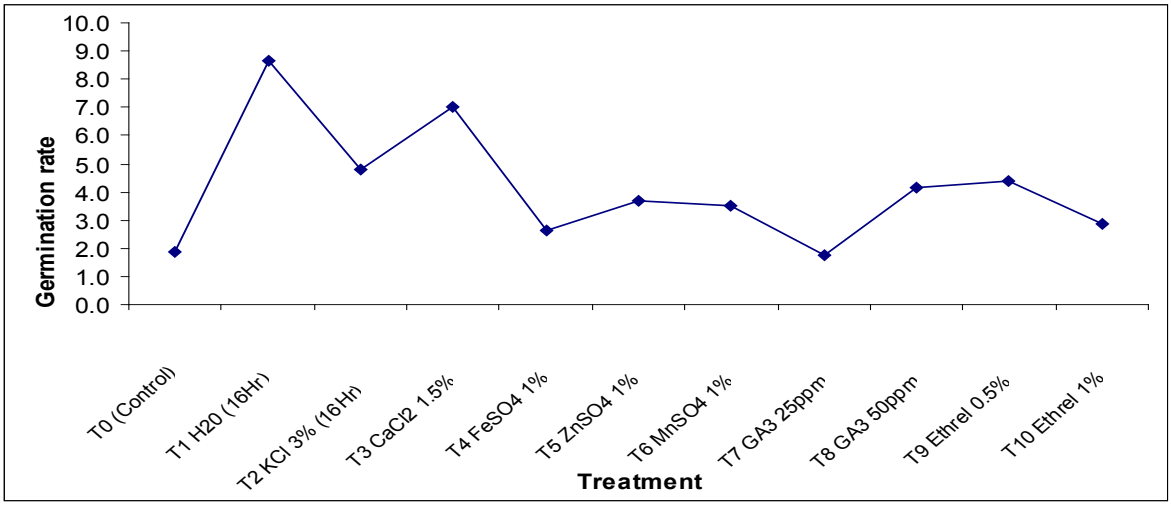

Figure 2. Comparison of various chemical treatments on germination rate of *Jatropha*

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