Research Journal of Agriculture and Biological Sciences, 6(6): 691-696, 2010

Effect of IBA on Seed Germination, Sprouting and Rooting in Cuttings for Mass Propagation of *Jatropha Curcus* L Strain DARL-2

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Abstract: Recalcitrant nature and short term viability of seed, limit the mass propagation through seed in an important biofuel plant *Jatropha curcas*. Therefore, feasibility of mass propagation was assessed through hormone (IBA) induced sprouting and rooting in popular Jatropha strain DARL-2. For one year old seed with reduced viability, IBA treatment of 1.5 mg/l significantly enhanced germination percentage and shortened the time of germination by more than 350% as compared to control. In case of clonal propagation 200mg/l IBA treatment was found to be the best for sprouting and rooting in cuttings taken from basal, middle and top portion of one year old branch. Basal portion of the branch responded best (76.7% sprouting and 72% rooting) to IBA treatment (50 mg/l). Though sprouting and rooting was observed in softwood cuttings at 2.5mg/l IBA treatment but survival rate was poor hence were found unsuitable for mass multiplication. Hard wood cutting followed by semi-hard wood cutting from one year old branch gave the best response. The results suggest application of IBA for enhanced rooting for vegetative propagation of Jatropha and use of basal portion (hard wood cutting) of branch for mass propagation of Jatropha.

Key words: Jatropha, DARL-2, IBA, cuttings, vegetative propagation

INTRODUCTION

Genus Jatropha with 172 species, family Euphorbiaceae, is native to Central America and distributed in Africa and Asia[3], of which *Jatropha curcas, J. glandulifera, J. gossypifolia, J. integerrima, J. multifida, J. podagrica,* and *J. tanjorensis* are widely distributed in India. *Jatropha curcas* is found in the tropics and subtropics. *Jatropha Curcas* a semi-woody shrub, well adapted to marginal soils with low nutrient content hence posing no fear to its spread on agricultural lands used for food production. Its water requirement is extremely low and it can stand long periods of drought by shedding most of its leaves to reduce transpiration loss. *Jatropha curcas* is also suitable for preventing soil erosion and shifting of sand dunes. Besides, medicinal value, *J. curcas* has emerged as a potential biodiesel crop alternative to petro-diesel [15,13,10]. In addition to bio-diesel, it also yields by-product like glycerine and seed cakes after trans-esterification process. Glycerine can be used in soap preparation and cosmetics while seed cake can be used as bio-fertilizer, fuel briquettes, and paper making.

Jatropha curcas is usually propagated by seed. However, Jatropha seeds have very low seed germination percentage and also it looses viability upon storage. Besides, being cross-pollinated, propagation through seed leads to a lot of genetic variability in terms of growth, biomass, seed yield, oil content etc. Whereas, clonal multiplication has an advantage of developing true-to-type clones of economically and commercially important elite germplasm[8]. In most of the naturally vegetatively propagated species, adventitious root formation is there without any need for hormone treatment, while others require different growth regulators usually auxin[17]. Auxins IAA and IBA are widely used as it induces root formation by breaking root apical dominance induced by cytokinin [1]. Plant growth hormones have also been reported to affect seed germination and dormancy by affecting different parts of the seed[5,9,11]. Though there are some reports on effect of hormones for rooting in cuttings but such effects are genotype specific and before going for mass application, study needs to be conducted on the selected genotype to find out the optimum dose for hormone application. Therefore, present study was planned with the aim to improve seed germination, sprouting and rooting in cuttings through application of plant hormone for mass propagation of Jatropha strain DARL-2.

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MATERIALS AND METHODS

Germplasm Used: Jatropha strain DARL-2 (IC No. 569095, identified as promising lines by a committee constituted by Ministry of New Renewable Energy, India**)** developed through selection for high oil (36.5%) was obtained from DIBER, Field Station, Pithoragarh, India. Mother plants for continuous supply of cuttings for experimental work are being maintained at DIBER, Haldwani, India. The experiment was carried out under lab conditions before applying it under field conditions.

Treatment of Seed for Germination: Seeds were washed thoroughly with tap water containing Tween-20 and then with sterilized double distilled water. The washed seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min and subsequently for 30 second with 70% ethanol followed by four washes with sterilized double distilled water. The surface sterilized seeds were soaked in different concentration of IBA (0.5 to 2.5 mg/l) and incubated overnight on incubator-shaker at 200rpm at room temperature. At the end of treatment, hormone solution was decanted and seeds were sown in pots filled with potting mix (sand: soil 1:1) for germination under glass house conditions. Control seeds without any hormone treatment was also sown keeping all other conditions identical as treated seeds.

Treatment of Cuttings for Sprouting and Rooting: Two experiments were conducted to study the effect of indole-3-butyric acid (IBA) concentrations on (1) young shoots (soft wood) and (2) semi to hard wood cuttings (Top, middle and basal portion of branch) on rooting in *Jatropha curcas* L. Soft wood cuttings from young shoots of current seasons growth with 1-2 nodes (3-4cm long, >0.5cm diameter) were surface sterilized following the same procedure as mentioned for seed sterilization and treated with different concentration of IBA (0.5, 1.0, 1.5, 2.0, and 2.5 mg/L) to study its effect on shooting and rooting. Semi to hard wood cuttings from one year old branch was treated with different concentrations of IBA (0, 50, 100, 200, 300, 400 and 500 mg/L) to assess its effect on shooting and rooting in top (dia >1cm), middle (dia >1<1.5cm) and basal portion (dia >1.5cm) with 3-4 nodes (10-15cm long). After IBA treatment by soaking for 4 hrs, the cuttings were first air dried and then sown in autoclaved soil: sand (1:1) in polythene bags. The cut surface from top was coated with paraffin wax and basal portion, about 1 cm was inserted in sand-soil mix for rooting.

Statistical Analysis: The treatments and controls of the experiments were carried out in three replications with 10 cuttings per treatment. The program CropStat for Windows (7.2.2007.2 module), developed by the Biometrics unit, IRRI, Philippines was used for analysis of variance (ANOVA) of experiments laid out in Completely Randomized Design (CRD). The percentage data was Arcsine angular transformed before analyzing statistically. The treatment means were compared by using the Duncan's Multiple Range Test (DMRT) at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Influence of Hormone on Seed Germination: Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. In the present study, there was significant influence of hormone treatment upon germination percentage and days to germination in *Jatropha curcus* DARL-2 as compared to untreated control. Germination percentage was highest in 2mg/l IBA (70%) and in 1.5mg/l IBA (65%) while days to germination were least in 1.5mg/l (7.3±1.3 days) and 1mg/l (11.0±1.3days) IBA concentration. The best treatment for germination was IBA 1.5mg/l (Table 1). The seeds were one year old and hence germination percentage was very low in case of untreated control. Jatropha seeds are oily and can not be stored viable for long. Research on viability of Jatropha seeds shows a decrease with storage duration [6,7]. Kobilke[7] investigated the viability of seeds of different ages and found that seeds older than 15 months show viability below 50 %. The result from this experiment revealed that seeds with low viability when treated with hormone, its germination percentage increases which is useful for nursery raising via seeds.

Influence of IBA on Cuttings for Mass Propagation: In case of cuttings from current season's growth (soft wood) with 1-2 nodes, 3-4 cm length, less than 5mm diameter, sprouting (63.3%) and rooting (43.3%) were the highest with 2.5mg/l IBA concentration (Table 2). Sprouting was first observed and then rooting was initiated (Fig 1). Cuttings treated with 1 mg/l IBA for 1h showed high rooting (96.7%) and short rooting period (18.2±2.0 d). Such effect has earlier been reported by Wang *et al.*,[18] in *Jatropha curcas* using the mini-stem fragment (2-3 cm) with one axillary bud but in our case the results obtained were not very encouraging**.** In the present study most of the cuttings sprouted but rooting percentage was very low and eventually the cuttings rotted before the plantlet could be established. Hence, use of soft wood cuttings is not suitable for mass multiplication.

Since the soft wood cuttings from new flush with <5mm diameter did not respond better in terms of shooting and rooting percentage, semi-hard wood and hard wood cuttings from one year growth and >1cm in

Table 1: Effect of hormone on seed germination in Jatropha strain DARL-2.

IBA Conc. (mg/l)	Germination (%)	Days to germination
0.0	18.3c	19.0a
0.5	28.3c	16.0a
1.0	55.0b,c	11.0b
1.5	65.0a,b	7.3b
2.0	70.0a	17.3a
2.5	51.7c	13.7a,b
LSD	12.3	4.2

Means with different alphabets in each column are significantly different at P ≤ 0.05, according to DMRT.

Table 2: Effect of hormone on sprouting and rooting in cuttings from current seasons flush (young shoots) in Jatropha strain DARL-2.

IBA Conc. (mg/l)	Sprouting (%)	Rooting (%)	Days to sprout	Days to root
0.0	13.3e	0.0d	32.0a	41.7[a]
0.5	26.7d	3.3d	25.0b	38.0a,b
1.0	36.7c	6.7c,d	22.0c	34.7b
1.5	43.3c	13.3c	19.0d	25.7c
2.0	53.3b	23.3b	14.0e	22.0c
2.5	63.3a	43.3a	8.7f	16.0d
LSD	9.2	8.1	2.3	3.7

Means with different alphabets in each column are significantly different at P ≤ 0.05, according to DMRT.

Fig. 1: Effect of IBA treatment on sprouting in A: soft wood cuttings from new flush; B: semi-hard wood to hard wood cutting from one year old branch; C: Sprouting in cuttings

diameter with more than 2 nodes (10-15cm long) were treated with higher concentrations of IBA to study the sprouting and rooting response. Hormonal treatment significantly increased sprouting and rooting percentage in all types of cuttings tried (Table 3). Sprouting in cuttings was initiated earlier to roots (Fig 2). There was also significant difference in response to hormonal treatment for sprouting and rooting in different types of cuttings. Sprouting % was best in cutting from basal portion followed by middle and top position in 50mg/L IBA (76.7, 73.3 and 60% respectively) and (66.7, 63.3 and 53.3% respectively) in 200mg/l from one year old branch (Table 3). Days to sprouting were the least in 50mg/l IBA whereas for rooting it was the least in 50 and 200mg/l IBA concentrations (Table 3). For rooting all the sprouted cuttings were grouped in to three clusters (top, middle and basal). Rooting percentage was the highest in basal>middle>top in 50mg/l IBA and 200mg/l IBA was the second best (Fig 3). Our result reveals that cuttings treated with IBA induced rooting hence can be used to enhance the vegetative propagation in Jatropha. Some basal callusing was observed in almost all the cuttings prior to root development but no histological study was carried out in the present study. This phenomenon has also been reported by Noor Camelia *et al.*,[14].

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation and also promote the production of other hormones and in conjunction with cytokinins[16]. Some previous reports on the effect of hormone on shooting and rooting in cuttings from Jatropha revealed its positive effect on rooting and sprouting behaviour of stem cuttings in *Jatropha curcas*. Kochhar *et al.*,[8] found that pretreatment with 100mg/l IBA and 100mg/l NAA increased both rooting and sprouting. Sprouting of buds on the cuttings preceded rooting. The rooting and sprouting in *J. curcas* was more with IBA than NAA. Kumar and Swarnkar[12] observed that IBA concentrations of 50 and 75 mg/l for the 24-h dip and IBA concentrations of 1000 and 1500 mg/l in the 3 to 4 min dip promoted higher rooting, survival and vigour of *Jatropha gossypiifolia* stem cuttings. Dhillon *et al.*, [2] studied the effect of auxins (IAA, IBA and NAA) and vitamin B1 (thiamine) on rooting response of cuttings of *Jatropha curcas* during spring and monsoon seasons. Spring season was found best for clonal multiplication of genetically superior material in Jatropha. Cuttings treated with 600 and 800 mg/l thiamine showed 100% sprouting during both seasons in comparison to auxins during both seasons. In the present study, also there was significant effect of auxin

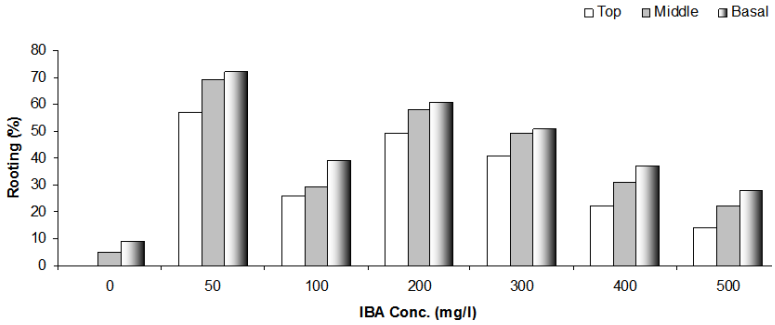

Fig. 2: Effect of hormone on rooting percentage in cuttings from top, middle and basal portion of the branch in Jatropha strain DARL-2.

Table 3: Effect of hormone on sprouting and rooting in cuttings from top, middle and basal portion of the branch in Jatropha strain DARL-2.

IBA Conc. (mg/l)	Sprouting Top (%)	Sprouting Middle (%)	Sprouting Basal (%)	Days to sprout	Days to root
0.0	0.0	6.7e	13.3d	26.0a	43.3a
50	60.0a	73.3a	76.7[a]	11.7d	30.7d
100	30.0c	33.3c,d	43.3c	20.7b	41.0b
200	53.3[a],b	63.3[a],b	66.7[a],b	16.0c	31.7d
300	46.7b	53.3b	56.7b	18.0b,c	37.0c
400	26.7c,d	36.7c	43.3c	19.0b	36.0c
500	16.7d	26.7d	33.3c	20.0b	38.0c
LSD	12.6	10.3	10.6	2.7	2.1

Means with different alphabets in each column are significantly different at $P \leq 0.05$, according to DMRT.

Fig. 3A: Nursery raising from cutting at Jatropha farm plantation, Secunderabad; B & C: Rooted cuttings ready for transplantation

IBA on sprouting and rooting of cuttings wherein 50 and 200mg/l IBA concentration was found to be the best. Basal cuttings responded better than middle and top portion of the branch. From the results obtained, the hardwood cutting (Basal) gave best response. This may be because hardwood cutting contain higher stored carbohydrate than semi-hardwood and softwood cutting which enable better root production [4]. Noor Camellia *et al.*,[14] investigated the effects of IBA concentrations in three types of cutting (softwood, semi hardwood and hardwood) and on root performance of *Jatropha curcas*. 10, 000 mg/L IBA gave the highest mean value for root length and 74% rooting while greatest root number was obtained at 20, 000 mg/L IBA. Results obtained from the present study were applied on mass scale multiplication of DARL-2 strain (Fig. 3) under field condition at our Jatropha farm plantation in Secunderabad, AP. The hormone concentration found best for sprouting and rooting (200 mg/L) was used for mass propagation of *Jatropha curcus* accession DARL-2, by raising nursery (Fig. 3A-C) from cuttings taken from basal portion of one year old branch with 3-4 nodes. The plants are still under vegetative phase and are expected to flower and fruit in next season. Yield and yield attributing data will be compiled in next season. Thus, the present study established the practical application of the results in clonal mass propagation of Jatropha, an important biofuel plant.

Conclusion: IBA treatment on cuttings increased rooting in Jatropha strain DARL-2 compared to non treated control. IBA application at 200 mg/L gave the best rooting percentage. Soft wood cuttings from new flush though sprouted early but failed to establish and eventually got rotted. Hard wood cuttings are the best choice followed by semi-hard wood cuttings from one year old branch for vegetative propagation. Thus, selection of stem type (soft wood, semi-hard or hard) is important from establishment point of view. IBA treatment of one year old seeds at lower concentrations also enhanced germination percentage in seeds with low viability as Jatropha seeds loose viability upon storage.

ACKNOWLEDGMENT

The authors express their thankfulness to the technical staff members for their support in carrying out the field work.

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