EFFECT OF AUXIN, LENGTH OF STEM CUTTINGS, SUBSTRATE AND SEASONAL VARIATIONS ON *JATROPHA CURCAS* L.: A BIODIESEL PLANT

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ABSTRACT

An investigation was under taken to establish the application of hormone, length of stem cuttings and influence of rooting media, on survival and rooting of *Jatropha curcas* stem cuttings. The experiments were performed in three seasons viz, monsoon, winter and spring. The first experiment evaluated the effect of different concentrations of IBA (200, 800, 1400, 2000, 4000 and 8000 mg/l.). The second experiment was set up to establish the length of stem cuttings (10, 20, 30, 40, and 50cm), while third experiment was carried out to study the effect of five media substrates viz, Sand:Soil (1:1), Sand:Soil (1:2), Sand:Soil:FYM (1:2:1), Sand:Soil:Cocopeat (1:2:1), and Sand:Soil:Vermicompost (1:2:1) on rooting and survival of stem cuttings. Cuttings treated with 2000mg/l IBA showed 78% survival during monsoon while, in spring and winter it was 62% and 54%, respectively. Sand: Soil: Vermicompost (1:2:1,) was found to be better substrate with 78% rooting and survival. Similarly, Sand: Soil: Cocopeat (1:2:1) and Sand: Soil: FYM (1:2:1) showed satisfactory survival percentage, which was 70% and 72%, respectively, in monsoon season. Stem cuttings with 20 cm length were suitable for quicker regeneration. They showed 76% of survival and rooting in monsoon season. Spring season is more suitable for quicker vegetative propagation of *Jatropha curcas*. It has been observed that 20 cm long stem cuttings, treated with 2000mg/l IBA for 5 minutes give best results in terms of rooting and survival of stem cuttings and thus raising of quality plant material.

KEYWORDS: *Jatropha*, Seasons, Vegetative propagation, IBA, Substratum, Length of stem cutting.

INTRODUCTION

Jatropha curcas L., is a multipurpose plant that belongs to the family Euphorbiaceae and is not only valued for its medicinal properties and resistance to various stresses, but also for its use as an oil seed crop (Openshaw 2000). It has drawn attention in recent years, since the demand for fuel (diesel) has increased drastically. *Jatropha* produces seeds with an oil content of 30-50% by weight. *Jatropha* plants raised from seeds reach to fruiting within three to four years after planting. However, *Jatroha* plants propagated by stem cutting, yield fruits in about one year from planting (Jones & Miller, 1992). Seeds of *Jatropha* have limited viability and can only be stored for 15 months, after which their viability is reduced to 50% (Kochar *et al.* 2008). Present study was aimed at standardizing the conditions for vegetative propagation by stem cuttings in order to raise quality planting material.

To meet the large scale demand and ensure easy supply of elite plant material, there is a need to establish mass multiplication technique. The propagation through seeds is dependent on good rainfall, moisture condition, sowing time and depth of sowing. Tissue culture technique offers rapid and continuous supply of planting material but the reports were not promising because of low multiplication rate. Stem cutting is traditional and promising method for the multiplication of this plant. Many internal factors such as auxins, rooting co-factors, carbohydrate and nitrogen levels have been shown to influence rooting of stem cuttings (Hertman and Kestar 1975). Adventitious root formation has lot of commercial interests because there are many plant species of which cuttings are difficult to root. In some plant species, adventitious root formation initiates without any treatment. Others require different growth regulators usually auxins (Soyros *et al.* 2004). Auxin induces root formation by breaking root apical dominance induced by cytokinin (Cline 2000). Indole Butyric Acid (IBA) is a synthetic rooting chemical that has been found to be reliable for root induction. IBA is widely used because it is non-toxic to most plants over a wide range and promotes root growth in large number of plant species (Hartmann *et al.* 1990).

Different planting media were used because planting medium is considered to be an important factor for the growth and development of plant. According to Larson (1980), the best planting medium must have a pH conducive to plant growth, a structure that will permit gaseous exchange to provide aeration for the rooting and permit water infiltration and movement. To standardize the methodology for propagation of this plant, the present experiments were designed with an objective to determine most suitable concentration of growth hormone, ideal length of stem cuttings and proper substrate for the propagation of *Jatropha curcas* on large scale.

MATERIALS AND METHODS

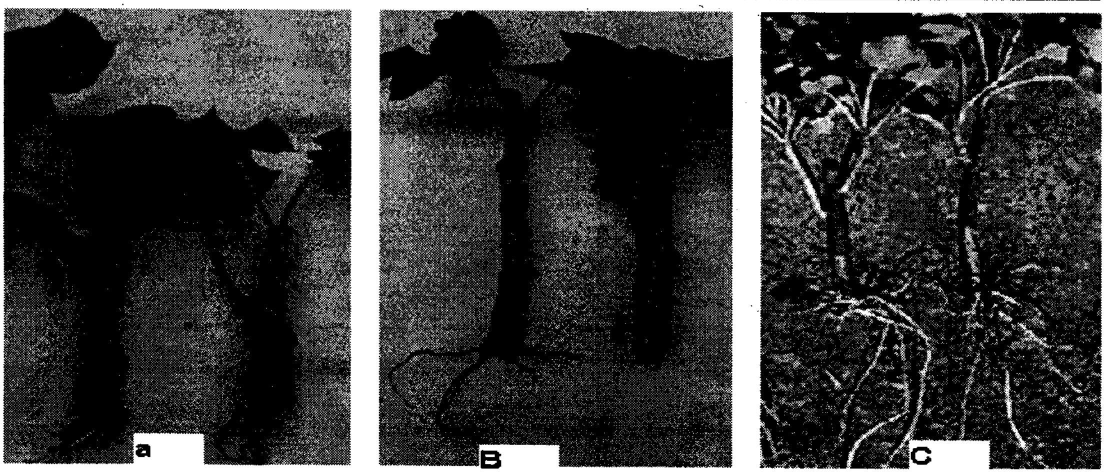
The present study was carried out at the Botanical garden of Modern College of Arts, Science and Commerce, Pune, Maharashtra. Elite plants of *Jatropha curcas* were selected from natural population around Sinhagad fort near Pune. Seasonal studies were carried out for propagation, in which the effects of growth hormone, length of stem cuttings and substratum have been evaluated. Healthy and uniform stem cuttings (20-30cm long and 2-3cm thick) of *J. curcas* were obtained from the middle portion of one year old branches of three to five year old plants during mansoon (June/July), winter (December/January) and spring (March/April) in the year 2008 - 2009. Stem cuttings were dipped in 0.1% bavistin fungicide for 2-3 minutes and subsequently washed in distilled water before giving hormonal treatment (200, 400, 800, 1400, 2000, 4000 and 8000mg/l IBA). The basal portions of cuttings (1-2 cm) were dipped in each concentration of IBA for 5min. Then the stem cuttings were planted in polythene bag filled with substrate (Sand:Soil:FYM, 1:2:1). The experiment was designed in randomized complete block design (RCBD), with factorial 3x6. Each replication consisted of three cuttings. The polybags were then kept in nursery beds and irrigated regularly depending on weather conditions. Sprouting, rooting and root character were recorded after 20, 40 and 60 days in three season viz. Monsoon, winter and spring.

To determine the optimum length of the stem cuttings for vegetative propagation, healthy cuttings of the branches were collected from selected locality. After removal of side branches and leaves stem cuttings of 10, 20, 30, 40 and 50 cm length were made, with uniform thickness i.e. 2.5- 3.0 cm, planted in polythene bags (9"x 6" size) containing loam soil and farm yard manure as substratum. This experiment was performed in triplicate for three season and observations viz, survival percentage, root length, root fresh weight and root dry weight were recorded on 20, 40 and 60 days.

To study the effect of different growing media on rooting of cuttings, the experiment was set up in RCBD with three replications and one variable factor i.e. different growing media viz, Sand:Soil(1:1), Sand:Soil (1:2), Sand:Soil:FYM(1:2:, Sand:Soil:Cocopeat(1:2:1) and Sand:Soil:Vermicompost (1:2:1). Five cuttings per treatment were inserted in black polythene bags filled with the required media and in this way a total 150 cuttings were inserted in five different media with one control. For assessing survival percentage, seedlings that survived after two months were counted and the data was recorded in percentage.

RESULTS AND DISCUSSION

Auxin treatments: Present study revealed that, rooting percentage of stem cuttings differed significantly. In case of 2000 mg/l IBA treatment, 78.4 % cuttings showed rooting in monsoon season, while 62.72% and 54.72 % in spring and winter respectively (Table-1). According to Villar (1997), better formation of roots due to auxin treatment may be due to accumulation of metabolites at the site of application, synthesis of new protein, callus formation, cell division and cell enlargement. Seasonal variation in rooting behavior of branch cuttings could be due to difference in the activities of hydrolyzing enzymes causing seasonal fluctuation in the availability of sugar, which are principal source of metabolic



energy required for cell division and differentiation during root initiation in cuttings (Jalani and Moss 1980).

The seasonal variations in the rooting response of stem cuttings have been reported by various workers (Nanda *et al.* 1984 and Handa *et al.* 2005). Such variations were due to varying levels of endogenous root forming substances influenced by climatic factors such as temperature, light and humidity (Fernquist 1966). According to Davies (1995), hormones have been shown to regulate different aspects of plant growth and development including cell division, cell elongation and cell differentiation. Auxins are substance which is produced in one tissue (Shoot) and migrates to effect the development of another tissue. It promotes cell elongation and has variety of other growth regulating effects. According to Arora (2006) the effect of auxin is specific in the development of root.

Effect of length of cuttings: The second experiment showed that, the survival percentage correlates with lengths of stem cuttings. Among the different lengths, 20 cm long stem cuttings recorded highest survival i.e. 76.0 % in monsoon season, 45.54% in winter and 64.4% in spring. In the said three seasons 10cm long stem cuttings recorded lowest survival i.e 46.8, 34.42 & 41.60 % respectively (Table-2). According to Reinhard (2003), the behavior of stem cuttings varies with age, genotypes and physiological status of mother plant, which is also one of the reasons for good performance of the medium sized stem cuttings.

Table 1. Effect of IBA on Rooting of stem cuttings of *Jatropha curcas* L.

Growth Hormone	Percentage of cuttings survived and rooted								
Treatments	Spring			Winter			Monsoon		
(mg/l)	20	40	60	20	40	60Days	20	40	60Days
S1	0	20.31	44.62	0	22.51	46.32	0	26.4	58.7
S2	0	29.37	40.28	0	21.47	49.21	Callusing	46.6	72.6
S3	0	25.57	43.39	0	26.51	53.69	0	42.8	68.2
S4	0	22.53	40.16	0	22.59	45.06	0	39.4	63.9
S5	0	30.42	58.62	0	32.72	54.72	0	44.5	78.4
S6	0	24.85	52.41	0	28.95	50.61	0	42.3	66.5
S7	0	27.33	49.44	0	30.83	49.84	0	41.5	64.2
CV (%)	0	14.01102	14.561	0	16.86506	7.07365	0	16.33988	9.516909
SE	0	1.364618	2.5861	0	1.689943	1.334694	0	2.501238	2.428011

Cv (%)= Covariance, SE= standard error

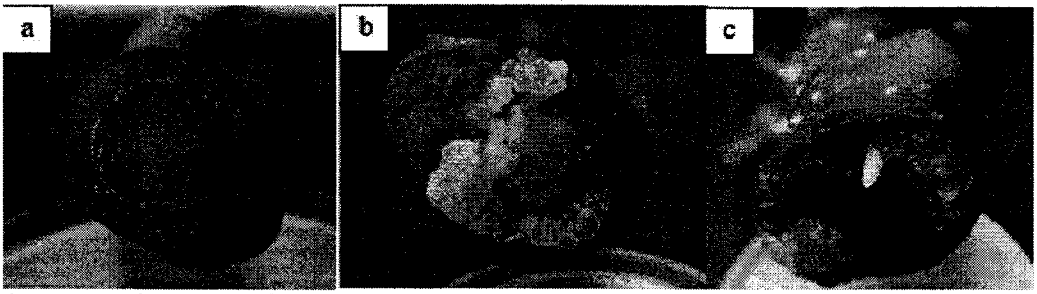


Fig. 2. Development of adventitious roots at the basal end of *Jatropha curcas* stem cutting. (a) Day 0: No changes occurred, (b) Day 10: Development of callus at the basal end of stem cutting, (c) Day 15: Root primordia emerged from the callus

Table 2. Effect of different lengths of stem cuttings on the rooting and survival of *Jatropha curcas* L.,

	WINTER		SPRING		MONSOON	
Cuttings (cm)	Rooting	No. of roots	Rooting	No. of roots	Rooting	No. of roots
	(%)	per cutting	(%)	per cutting	(%)	per cutting
10	34.42	[redacted]	41.6	2.6	46.8	8.2
20	45.54	2.5	64.64	2.8	76	12.3
30	47.41	2.7	67.61	3.0	64.2	10.2
40	36.38	3.3	44.28	4.9	62.7	14.3
50	32.42	3.2	57.92	4.3	40.4	12.9
CV (%)		18.04333		8.7938857		86.85834
SE		0.222711		0.4554119		1.072101

Cv (%) = Covariance SE = standard error

Table 3. Effect of Substrate on the rooting of stem cuttings of *Jatropha curcas* L.

	Percentage of cuttings survived and rooted								
Substrate	**Spring**			**Winter**			**Monsoon**		
Treatment	20	40	60Days	20	40	60Days	20	40	60Days
Control (Fine Sand)	0	8.45	26.27	0	9.54	26.11	0	41.62	64
Sand: Soil (1:1)	0	12.56	29.47	0	10.4	22.21	0	46.31	69.8
Sand:Soil (1:2)	0	11.94	36.81	0	10.73	32.41	0	44.41	70.8
Sand:Soil:FYM (1:2:1)	**0**	**19.43**	**58.94**	**0**	**16.45**	**37.34**	**0**	**56.34**	**74.4**
Sand:Soil:Cocopeat (1:2:1)	0	24.31	42.54	0	15.94	36.4	0	54.31	72.4
Sand:Soil: Vermicompost (1:2:1)	0	36.47	56.64	0	22.61	45.63	0	74.53	78.2
CV(%)		54.9022	32.6908		35.29405	25.20959		22.74	6.6595
SE		3.913654	5.162114		1.904715	3.177698		04.54	1.8022

Cv (%)= Covariance, SE= standard error

Fig. 3 Sprouting of stem cuttings in spring season on the substrate (Sand:Soil:Vermicompost,1:2:1

Reductions in number of roots were observed with reduction in size of cuttings (Table 2.). According to Good and Tukey (1966), poor performance of shorter stem cuttings is due to inadequate supply of nutrients and leaching of nutrients in shorter cuttings and According to Hegde (1988), the poor performance of small size cutting was due to the reason that cuttings were still under maturity and may be devoid of sufficient food material for induction of roots and shoots. The under performance of large sized cuttings may be attributed to reason that these cuttings are more woody and might have converted most of food material for lignification, which resulted in over lignified stem caused lower rooting and shooting percentage.(Kochhar *et al.* 2005).

Role of substratum: This experiment has revealed that the substratum and seasons play important role in the survival and establishment of stem cuttings. The stem cuttings planted in Sand: Soil: Vermicompost (1:2:1) showed 78.2, 56.64, and 45.63 % rooting and survival in monsoon, spring and winter seasons, respectively (Table-3). It has been concluded that in Sand: Soil: Vermicompost (1:2:1) is most efficient substrate. Similarly, 20cm long stem cuttings showed best performance, treated with an IBA planted. It showed that 74.4% of rooting with the Fine Sand:Soil:F.Y.M (1:2:1) in spring while, 58.9 % and 37.34% in monsoon and winter, respectively (Table-3). This could be due to the availability of good amount of nutrients in vermicompost and due to seasonal factors.

It is concluded from the present study that a trend of relatively better survival and rooting of stem cuttings were obtained with Sand: Soil: Vermicompost (1:2:1) as compared to other substrate and 20cm length of cuttings. The observed results could be due to better aeration and high water holding capacity of substrate. Furthermore, the study has revealed that vegetative propagation with treatment of hormone 2000mg/l (IBA) found better. The further study should evaluate the field performance of such plant produce by stem cuttings.

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