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Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes

Jatropha, a drought-resistant, photo-insensitive¹ perennial plant belonging to the family Euphorbiaceae is attracting increasing attention as an important source of bio-diesel. The seeds of *Jatropha* plant contain a viscous, non-edible oil, which besides being a source of bio-diesel can also be used for manufacturing other useful products such as candles, high quality soaps and cosmetics as well as for healing several skin disorders. Because of its above-mentioned industrial and medicinal uses, Central and State Governments have drawn ambitious programmes for its large-scale cultivation.

Two species of *Jatropha* that are grown include *J. curcas* and *J. glandulifera*. Although only *J. curcas* is being promoted for bio-diesel, *J. glandulifera* is known for its beautiful flowers. The seeds of *J. curcas* contain 48% oil, while that of *J. glandulifera* contain 27% oil. Both the species need to be studied in detail for their fat and oil content. *Jatropha* plants grow well on poor stony soils²⁻⁴. *Jatropha* is a multipurpose tree with a long history of cultivation in tropical and subtropical regions of the world^{2,5-7}. It is a native of Central America and occurs mainly at lower altitudes (0–500 m) in areas with annual temperatures of well above 20°C. The seeds are toxic due to the presence of curative and curative ingredients, but after treatment the seeds or seed cakes can be used as animal feed⁸. *Jatropha* is grown as a boundary fence to protect fields from grazing animals and as a hedge to prevent erosion^{2,9}. The problem of great concern regarding this plant is the rate of vegetative

growth of plants and seed yield. The plants have profuse vegetative growth, but the number of seeds produced per plant is very low. Besides, the plants produce seeds after approximately 2–3 years depending on environmental conditions and seeds have a limited viability, they lose almost 50% viability within 15 months¹⁰. In spite of all these properties, research on cultivation and propagation of *Jatropha* is limited. Thus it was considered useful to undertake a systematic study on the vegetative propagation of *Jatropha* through stem cuttings as rooting is a crucial step in the propagation of woody plants and there is a great variability in the rooting ability of different species. While propagation through seeds leads to genetic variability and makes the crops prone to diseases, propagation through vegetative means offers an advantage in developing true-to-type, disease-free varieties of economically and commercially important plants for clonal multiplication¹¹.

We report here the results of our trials on the vegetative propagation of two species of *Jatropha*, namely *J. curcas* and *J. glandulifera* through stem-cuttings and the accompanying biochemical changes. This is a report of the effect of auxins in rooting and sprouting behaviour of the two *Jatropha* species. It is observed that during the rooting process many changes take place both at the physiological and biochemical levels and activities of many enzymes are up- and down-regulated. The initial levels of endogenous auxin and its oxidation enzymes – IAA-oxidase and peroxidase play a significant part in the process, these being more in *J. glandulifera* than in *J. curcas*.

IAA-oxidase activity is involved in triggering and initiating the roots/root primordia, whereas peroxidase is involved in both root initiation and elongation of roots. Position of the cuttings on the mother branch also plays a significant role in rooting and sprouting. Cuttings made from the middle portions of the mother branches exhibit better rooting as compared to the most apical or most basal cuttings. These results are supported by the peroxidase isoenzyme analysis in the cuttings.

Healthy and uniform stem-cuttings (8–10 inches in length) of *J. curcas* and *J. glandulifera* were obtained from both the apical, middle and basal portions of branches of 2–3 year-old *Jatropha* plants. They were pre-treated with 10 and 100 mg/l indole-3 butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) for 24 h, with one group of untreated cuttings serving as control. After 24 h pre-treatment with IBA or NAA, the cuttings were transferred to the field. Observations on the number of cuttings rooted, number of roots and shoots/sprouts produced on each cutting and their length were recorded in each treatment at biweekly intervals up to 45 days. Samples from sprouted portions and from rooted portions were also collected till 45 days for biochemical analysis. Endogenous auxin content and changes in the activities of IAA-oxidase and peroxidase were determined¹²⁻¹⁴. Records of growth were maintained even after 45 days. The isoenzyme pattern of peroxidase was also studied by electrophoresis¹⁵. The protein was determined by the method of Lowry *et al.*¹⁶.

Table 1. Effect of IBA and NAA on sprouting of stem-cuttings of *J. curcas* and *J. glandulifera*. Observations on per cent cuttings that sprouted and number of sprouts per cutting were taken 15, 30 and 45 days after planting

Treatment	<i>J. curcas</i>						<i>J. glandulifera</i>					
	Per cent cuttings sprouted			No. of sprouts per cutting			Per cent cuttings sprouted			No. of sprouts per cutting		
	15 D	30 D	45 D	15 D	30 D	45 D	15 D	30 D	45 D	15 D	30 D	45 D
Control	60 ± 4.1	68 ± 3.7	73 ± 4.3	1.25 ± 0.002	2.25 ± 0.01	1.5 ± 0.01	93 ± 4.7	100 ± 5.1	100 ± 6.7	1.83 ± 0.02	2.25 ± 0.01	1.5 ± 0.02
IBA 10 mg/l	70 ± 3.7	95 ± 2.6	100 ± 5.7	2.75 ± 0.003	2.75 ± 0.01	2.75 ± 0.01	60 ± 3.5	75 ± 4.5	85 ± 5.2	1.13 ± 0.02	2.75 ± 0.03	2.75 ± 0.01
IBA 100 mg/l	85 ± 3.9	91 ± 2.8	100 ± 4.9	2.5 ± 0.001	2.5 ± 0.02	2.5 ± 0.03	83 ± 5.1	80 ± 5.1	76 ± 4.1	1.39 ± 0.01	2.5 ± 0.01	2.5 ± 0.02
NAA 10 mg/l	60 ± 5.1	81 ± 4.1	88 ± 3.1	1.5 ± 0.01	2.0 ± 0.01	2.2 ± 0.01	89 ± 3.3	100 ± 4.2	100 ± 3.2	2.17 ± 0.01	2.0 ± 0.008	2.2 ± 0.03
NAA 100 mg/l	80 ± 6.2	85 ± 3.8	85 ± 3.8	2.0 ± 0.002	2.5 ± 0.01	2.5 ± 0.01	93 ± 4.8	98 ± 3.8	100 ± 2.8	1.33 ± 0.03	2.5 ± 0.005	2.5 ± 0.02

D, Days after planting the cuttings.



Figure 1. Rooting and sprouting of cuttings of *Jatropha curcas* (a–c) and *Jatropha glandulifera* (d, e). a, d, Sprouting of buds but no rooting; other figures show the status of stem cuttings after 45 days of planting. b, c, Rooting of *J. curcas* with auxin application; e, Rooting of stem cuttings of *J. glandulifera* after 45 days. Both roots and shoots can be seen with IBA and NAA treatments.

Our results have shown that sprouting of buds took place much earlier than rooting in both the species. While 100% of the cuttings of both species was fresh up to 15 days, only 85–90% survived after 30 days. Application of IBA and NAA increased survival percentage, IBA being more effective in case of *J. curcas* and NAA in the case of *J. glandulifera* (data not given). The cuttings looked healthy with a number of shoots, but without roots (Figure 1 a, d; Table 1). Sprouting on stem cuttings of *J. glandulifera* occurred earlier than *J. curcas* (Table 1). Data on the per cent cuttings sprouted and number of sprouts per cutting and their length indicate that 100 mg/l of both IBA and NAA application increased the percentage of cuttings sprouted after 15 days in *J. curcas*, and this continued even after 45 days (Table 1). In *J. glandulifera*, more than 90% control cuttings sprouted within 15 days and all 100% sprouted within 30 days. IBA application

reduced the percentage of cuttings sprouted, but with NAA 89 and 93% cuttings sprouted with 10 and 100 mg/l NAA respectively after 30 days and all the 100% sprouted after 45 days. Both IBA and NAA increased the number of sprouts per cutting in *J. curcas* and *J. glandulifera*, and the effect was pronounced throughout the experiment up to 45 days. IBA was again more effective in *J. curcas* and NAA was more effective in *J. glandulifera* in the number of sprouted buds and their length (data not given).

There was no rooting till 15 days, except for some callusing with 10 mg/l IBA in both species. The root formation took much longer and even after one month, the cuttings did not have any visible roots and showed only some callusing (Figure 1 a, d; Table 2). Compared to no rooting in control after 30 days, pre-treatment with IBA prior to planting of cuttings enhanced rooting in *J. curcas*, where 48 and 75% cuttings

rooted with 10 and 100 mg/l IBA respectively, and all of the cuttings rooted in 45 days (Table 2). In comparison, only 31 and 33% cuttings of *J. curcas* rooted with NAA after 30 days and 69 and 79% after 45 days, showing thereby that IBA was more effective than NAA in *J. curcas* (Table 2). The number of roots per cutting also increased with both IBA and NAA, the effect of IBA being again more pronounced (Table 2). In contrast, in *J. glandulifera*, 33% control cuttings rooted after 30 days and 87% cuttings rooted after 45 days. IBA application had almost no effect, except with 100 mg/l IBA which only slightly increased the number of roots (Table 2). However, NAA application increased both the percentage of cuttings rooted and number of roots per cutting. The effect was being more after 45 days, indicating that NAA treatment was more effective over IBA treatment in *J. glandulifera* (Table 2). This shows a differential response of the two *Jatropha* species to auxin application. In addition, it was seen that cuttings taken from the middle portion of the branches showed more rooting and better response to auxin application compared to the extreme apical or basal cuttings (data not given). After 45 days the cuttings were transferred to the field and they started producing flowers and seeds after six months in *J. glandulifera* and after 8 months in *J. curcas* (Figure 2 a and b).

The levels of endogenous auxin, IAA-oxidase and peroxidase activities were initially higher in *J. glandulifera* than in *J. curcas*. The level of endogenous auxin declined considerably up to 30 days and then levelled-off in *J. glandulifera*. In contrast, in *J. curcas*, the auxin level increased up to 15 days and declined thereafter. IAA oxidase and peroxidase activities which were higher in *J. glandulifera* than in *J. curcas* initially, increased further in *J.*

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Table 2. Effect of IBA and NAA on rooting of stem cuttings of *J. curcas* and *J. glandulifera*. Observations on per cent cuttings that rooted and number of roots per cutting were taken 15, 30 and 45 days after planting

Treatment	<i>J. curcas</i>						<i>J. glandulifera</i>					
	Per cent cuttings rooted			No. of roots per cutting			Per cent cuttings rooted			No. of roots per cutting		
	15 D	30 D	45 D	15 D	30 D	45 D	15 D	30 D	45 D	15 D	30 D	45 D
Control	0	0	61 ± 2.6	0	0	1.5 ± 0.10	0	33 ± 1.5	87 ± 5.2	0	1.0 ± 0.02	2.4 ± 0.03
IBA 10 mg/l	Callusing	48 ± 1.80	100 ± 3.2	0	0.75 ± 0.014	3.75 ± 0.13	Callusing	30 ± 1.3	73 ± 6.1	0	0.8 ± 0.002	2.24 ± 0.02
IBA 100 mg/l	0	75 ± 2.30	100 ± 3.7	0	1.0 ± 0.001	5.25 ± 0.17	0	40 ± 2.1	76 ± 5.8	0	1.3 ± 0.01	2.75 ± 0.02
NAA 10 mg/l	0	31 ± 1.05	69 ± 4.1	0	0.75 ± 0.001	1.5 ± 0.11	0	43 ± 1.9	90 ± 4.3	0	1.0 ± 0.03	3.25 ± 0.01
NAA 100 mg/l	0	33 ± 1.65	79 ± 3.8	0	0.98 ± 0.02	4.25 ± 0.16	0	50 ± 2.4	100 ± 5.1	0	1.9 ± 0.03	4.25 ± 0.03

D, Days after planting the cuttings.

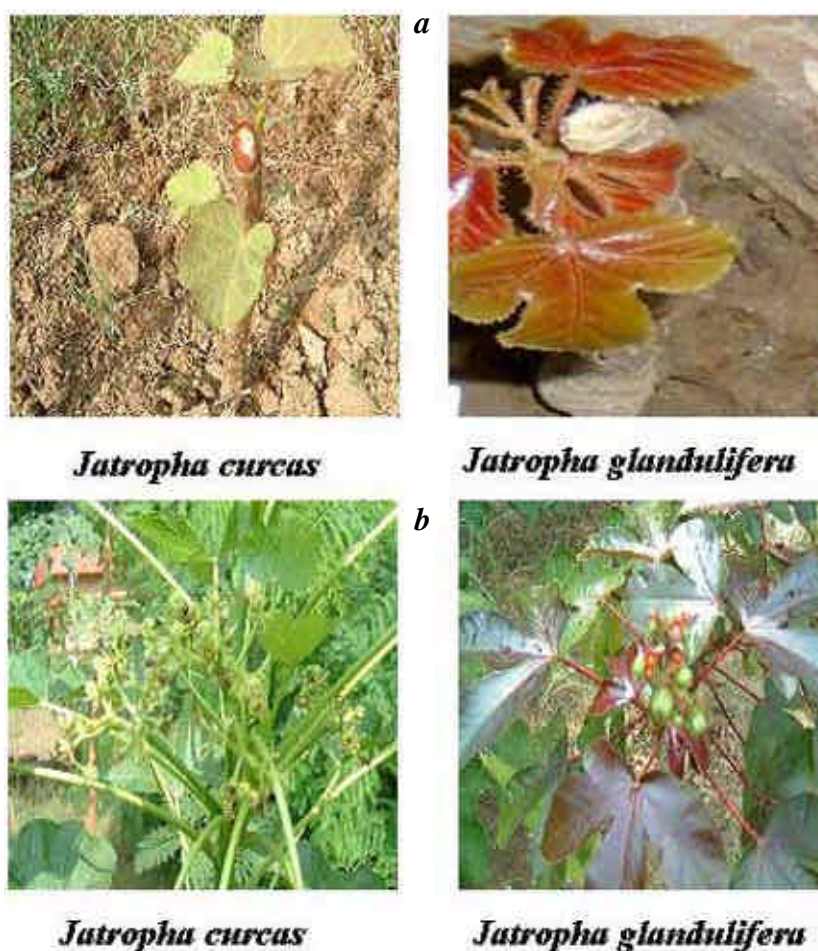


Figure 2. Forty-five-day-old cuttings of *J. curcas* and *J. glandulifera* (a) transferred to field and the same after 8 months bearing flowers and fruits (b).

glandulifera up to 15 days and decreased after that. In the case of *J. curcas*, IAA oxidase activity started increasing after 15 days, peaking after 30 days. The peroxidase activity declined initially up to 15 days, but increased later in *J. glandulifera*. In *J. curcas*, peroxidase activity did not change till 15 days, but increased considerably after that (data not presented).

Only one major peroxidase band was detected in both *J. curcas* and *J. glandulifera*. The density of this band was much higher in *J. glandulifera* than in *J. curcas*, which supports the observed high activity in *J. glandulifera*. Peroxidase isoenzyme analysis from apical, middle and basal parts of the mother branches (from which the cuttings were made)

showed that cuttings from the middle portion of the branch exhibited most dense band compared to the extreme apical or basal portions in both species. Better rooting of middle portion of the branches could be ascribed to this observation (data not given).

Auxins have been shown to regulate different aspects of plant growth and development by affecting numerous processes, including cell division, cell elongation and differentiation¹⁷. The induction of rooting by auxins application has also been reported by many workers in other plant species¹⁸⁻²⁰. The induction of rooting with IBA pretreatment in *J. curcas* supports the earlier findings in poplar²⁰. However, our results suggest that early metabolization of auxin rather than total auxin content matters in the process of root initiation. These findings also suggest that auxin pre-treatment could be effective in enhancing the vegetative propagation of this biofuel plant. It is interesting that the two *Jatropha* species exhibit differential response to external auxin application, which is dependent on the endogenous auxin status of the cuttings. While IBA is more effective in the rooting of *J. curcas*, NAA is more effective in *J. glandulifera*, indicating the selective response of the two *Jatropha* species to auxin application.

The higher initial level of endogenous auxin in *J. glandulifera* cuttings compared to *J. curcas* cuttings explains better rooting of *J. glandulifera* without auxin application. Because of higher initial endogenous auxins present in *J. glandulifera*, cuttings of this species sprouted buds earlier than *J. curcas*. These studies also suggest that both IAA-oxidase and peroxidase help in auxin catabolism and in triggering the root initiation process. While IAA-oxidase seems to be involved only in triggering and initiating the roots/root primordia, peroxidase is involved in both root initiation

and elongation processes and oxidation products of auxin catabolism may be involved in the initiation of roots. A more interesting observation is that shoots are formed much earlier in *Jatropha* species than roots. Shoots thus formed earlier due to reserve carbohydrates, start producing auxins which moves downward, thereby accumulating in the lower portion of the cuttings. When the concentration reaches a threshold value, endogenous auxins at the extreme basal end start getting metabolized and signal the process of root initiation. Nanda and Kochhar¹¹, while studying the rooting behaviour of 100 Indian forest tree species, had shown that a balance of auxin and carbohydrates determines the ability of cuttings to root.

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The first dolphin fossil from the Miocene of Korea

Cetacea (whales, dolphins, and porpoises) are one of the most diverse and aquatically adapted mammals in the world. The cetacean fossil record extends back to the Middle Eocene, based on the relatively highly evolved nature of the earliest known whales¹.

Extant dolphins consisting of 17 genera, including 32 species are distributed worldwide, except in polar waters. The extinct family Kentriodontidae, fossil long-beaked dolphins of the Platanistoidea, have been discovered from rocks of the Late Oligocene to Late Miocene. Kentriodontids are small dolphins, approximately 2 m in length, with a nearly symmetrical vertex of the skull in contrast with the asymmetrical vertex of most living dolphins^{2,3}. The skulls of the Kentriodontidae are relatively small and delicate. The subfamily Kentriodontinae had a wide distribution, including North America, Italy, Germany, Switzerland and Japan^{4–7}. Kentriodontids have been divided into four groups. Kentriodontines include the genera *Kentriodon*, *Delphinodon*, *Macrokentriodon*, *Kampholophus* and *Rudicetus*^{5,7}.

Kentriodon, one of the most successful and widespread kentriodontids, is a small Miocene dolphin and known from several localities including Peru, Patagonia, New Zealand, Japan and North America. Only three species of *Kentriodon*, i.e. *K. pernix*, *K. obscurus* and *K. hobetsu* are described. *K. pernix*, the type species of *Kentriodon*, was named on the basis of two skulls from the Middle Miocene Calvert Formation in Maryland, USA⁴. *K. obscurus*, a second species, is known from the Sharktooth Hill Bonebed in California, USA and is named on the basis of a partial skull and some periotics⁵. *K. hobetsu*, a third species, was named based on a partial skull from the Middle Miocene Takinoue Formation in Hokkaido, Japan⁵. The partial skull of *K. hobetsu* lacks the distal part of its rostrum. The Takinoue Formation was deposited under warm-water environments⁵, at approximately 15.5 Ma.

An incomplete upper jaw of a fossil dolphin was discovered from the Miocene Duho Formation (the Yeongil Group in the Pohang Basin) in South Korea. The middle portion of the premaxilla and maxilla

are preserved. The preserved premaxilla is convex laterally, as viewed anteroposteriorly. It also becomes broader posteriorly. Only eight teeth (four on the each side) are preserved in the maxilla (DWF 10001; DongHae City Whale Fossil Museum). The eight preserved teeth of DWF 10001 indicate a homodont dentition, and are recurved laterally as in *Kentriodon pernix* (Figure 1). The alveoli are more broadened laterally rather than elongated anteroposteriorly. The overall morphology of the incomplete rostrum (DWF 10001) is similar to that of *Kentriodon*. As in Figure 1 (bottom), the tooth row is not symmetrical toward the margin of the maxilla. The tooth row also curves laterally as in *Kentriodon*. The teeth of the left maxilla are oriented at a wider angle than do those in the right maxilla (Figure 2, bottom). The surface of the preserved premaxilla is smoother than the adjacent maxilla, as in *K. obscurus*.

K. obscurus is different in having significantly smaller teeth compared to DWF 10001 and *K. pernix*. The alveoli of *K. pernix* have a uniform diameter (3 mm), while those of *K. obscurus* have only one-