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Effect of auxins and associated biochemical changes during clonal propagation of the biofuel plant—*Jatropha curcas*

Sunita Kochhar, S.P. Singh, V.K. Kochhar*

National Botanical Research Institute, Lucknow 226001, India

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ABSTRACT

Rooting and sprouting behaviour of stem cuttings of biofuel plant *Jatropha curcas* and their performance under field conditions have been studied in relation to auxin application. Pretreatment with indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) increased both the rooting and sprouting. Sprouting of buds on the cuttings preceded rooting. The rooting and sprouting in *J. curcas* was more with IBA than NAA. The endogenous auxin contents were found to increase almost 15 days prior to rooting, indicating that mobilization of auxin rather than the absolute contents of auxin may be involved in root initiation. Indole acetic acid oxidase (IAA-oxidase) seems to be involved for triggering and initiating the roots/root primordia, whereas peroxidase is involved in both root initiation and the elongation processes as supported by the peroxidase and IAA-oxidase isoenzyme analysis in the cuttings.

The clonally propagated plants (cutting-raised plants) performed better in the field as compared to those raised from the seeds. The plants produced from auxin-treated cuttings produced fruits and seeds in the same year as compared to the plants raised from seeds or from untreated or control cuttings that did not produce any seeds in 1 year of this study. Jatropha plants in general produce seeds after 2–3 years.

1. Introduction

Rooting of stem cuttings is a crucial step in 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 different diseases, clonal multiplication has an advantage in developing true-to-type, disease-free varieties of economically and commercially important plants.

Jatropha curcas L. (Euphorbiaceae) is a drought-resistant, photo-insensitive [1] and perennial plant that has been attracting increasing attention as an alternate source of bio-diesel. Jatropha seeds contain viscous, non-edible oil, used as a substitute for diesel (hence called bio-diesel). The oil can also be used to manufacture candles, high-quality soaps and cosmetic products [1]. The seeds of *J. curcas* contain 48% oil. One liter oil is produced from approximately 4 kg *J. curcas* seeds and can be used for about 50 h of lighting, while 350 mL oil can last for 3 h for cooking purposes. Jatropha plants grow well on poor stony soils and are, therefore, recommended for cultivation on poor degraded soils [2–4] as a multipurpose tree with a long history of cultivation in tropical and subtropical regions of the world [2,5–7]. The seeds are toxic due to the presence of cursive ingredients, but after treatment the seeds or seed cakes can be used as an animal feed [8]. Jatropha is known to heal skin ailments such as eczema, acne, rashes and psoriasis. Jatropha is grown as a boundary fence to protect fields from grazing animals and as a hedge to prevent erosion [2,9]. Jatropha plants produce seeds after approximately 2–3 years depending on the environmental conditions

*Corresponding author. Tel.: +91 522 2205831; fax: +91 522 2205836.
E-mail address: sunitalko@hotmail.com (S. Kochhar).
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and method of propagation. In spite of all these useful properties, research on cultivation and propagation of Jatropha is extremely limited. Another problem of great concern is the rate of vegetative growth of this plant and seed yield. In spite of profuse vegetative growth, the number of seeds produced per plant is very low and the seeds show a limited viability, which is reduced by 50% within 15 months [10]. Considering these facts, it was thought to be of great interest to undertake a systematic study on the vegetative propagation of Jatropha and to undertake a comparative performance of seed-raised and cutting-raised plants under field conditions. We have recently published our results of a comparative study of two Jatropha species in relation to their rooting and growth behaviour [11]. In this paper, we report our results on the effect of auxins on clonal propagation and the accompanying biochemical changes. Despite ongoing auxin research in many plant processes, many of the original fundamental questions of auxin effects are still unanswered [12]. The data presented here form the first report of enhancement of growth, sprouting, seed set and yield in this important biofuel plant, *Jatropha curcas*, by auxins under field conditions and also strives to answer some fundamental questions in auxin physiology. The results show that mobilization and catabolism of indoles/auxin is more important for the auxin effects than the absolute indole/auxin contents. The exogenous auxin application contributes by triggering enhanced mobilization.

2. Material and methods

2.1. Source of cuttings

Healthy and uniform stem cuttings (8–10 in long) of *J. curcas* were obtained from the branches of 2–3 years old Banthra Selection-1 plants growing at the Banthra Research Station of National Botanical Research Institute, Lucknow, in the month of February and were divided into five groups. One group was kept in water to serve as control and the other four groups were treated with 10 and 100 mg L^{-1} of indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA), respectively. After 24 h pretreatment with IBA or NAA, the cuttings were transferred to pots till 45 days and subsequently were planted in the National Botanical Research Institute's research field in Genetics Block. The cuttings were irrigated daily. The 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 collected after every 15, 30 and 45 days for biochemical analysis. Endogenous total indole (auxin) contents and changes in the activities of IAA-oxidase and peroxidase were determined. The isoenzyme pattern of IAA-oxidase and peroxidase were also studied. The records of growth were also maintained for 1 year.

2.2. Extraction and estimation of the IAA content

The auxin content was measured according to the method of Donate-Correa et al. [13]. One gram material from the stem cuttings was weighed in duplicate. Sample A was extracted with 5.0 mL of 35% perchloric acid (CAS 7601-90-3) and sample B with 5.0 mL of modified Salkowski Reagent and kept in the dark for 1 h as described [13]. The solutions were centrifuged at 10,000 rpm for 15 min and the supernatant was collected and the optical density of the solution was read at 530 nm. The relative auxin content was determined by subtracting the extinction of adjusted A from extinction of adjusted B, using the IAA standard curve and is expressed as $\mu g\, g^{-1}$ fresh wt. Each value presented is the mean of three replicates.

2.3. Extraction for enzyme activities

Two gram tissue from the cuttings was extracted in 100 mM phosphate buffer (pH 7.0) containing 2 mM ethylenediaminetetraacetic acid, 1% polyvinylpyrrolidone and 1 mM β mercaptoethanol in the ratio of 1:2 (w/v) using liquid nitrogen. The homogenate was centrifuged at 10,000 rpm and the supernatant was collected and stored for subsequent enzyme analysis. All operations were carried out at 4 °C. The total protein content was estimated by Lowry et al. [14]. Enzyme assays were performed at room temperature.

2.4. IAA-oxidase activity

The activity was determined by the method of Liu et al. [15]. Reaction mixture contained 0.2 mL of enzyme extract, 0.78 mL of 50 mM potassium phosphate buffer (pH 6.0), 0.01 mL of 5 mM $MnCl_2$, 0.01 mL of 5 mM 2,4-dichlorophenol (DCP), 50 μg of IAA for 30 min at 37 °C in dark. The reaction was terminated with 2.0 mL of Salkowski reagent. The destruction of IAA was determined by measuring O.D of the reaction mixture at 535 nm after 30 min, and the amount of IAA-oxidase activity was expressed as the amount of IAA oxidized $\mu g\, h^{-1}\, g^{-1}$ tissue. Each value was the mean of three replicates.

2.5. Peroxidase activity

Peroxidase was measured using guaiacol as substrate following the method of Kochhar et al. [16]. The assay mixture contained 100 mM phosphate buffer (pH 6.8), 2.7 mM guaiacol and 4 mM H_2O_2 and 50 μg of enzyme protein. Each value was the mean of three replicates.

2.6. Isoenzyme analysis

Isoenzymes were separated using 8% separating and 4% stacking polyacrylamide native gels. Electrophoresis was carried out at 4 °C under non-denaturing conditions as described by Laemmli [17]. Following electrophoretic separation, the gels were stained for peroxidase and IAA-oxidase isoenzymes. Peroxidase bands were visualized by incubating the gels in 50 mM phosphate buffer (pH 6.5), 4 mM H_2O_2 and 2.7 mM guaiacol as described by Kochhar et al. [16]. Gels were scanned using the Bio-Rad Gel Documentation System.

For IAA-oxidase isoenzymes, gels were incubated overnight in a reaction mixture containing 2 mM IAA, 0.1 mM DCP and 0.1 mM $MnCl_2$ in a 0.1 M phosphate buffer, pH 6.0. The isozymes were visualized by transferring the gels, at the end of the incubation period, to a modified reaction mixture