View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Universiti Putra Malaysia Institutional Repository

Research Journal of Agriculture and Biological Sciences, 5(4): 338-343, 2009
© 2009, INSInet Publication

Improvement on Rooting Quality of *Jatropha curcas* Using Indole Butyric Acid (IBA)

Noor Camellia, N.A., Thohirah, L.A., N.A.P Abdullah, and Mohd Khidir, O.

Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia,
43400 Serdang, Selangor Darul Ehsan.

Abstract: The effects of indole-3-butyric acid (IBA) concentrations and three types of cutting (softwood, semi hardwood and hardwood) on root performance of *Jatropha curcas* L. cuttings were investigated. Two experiments were conducted where in experiment 1, conducted on July 2007, the hardwood cutting gave the highest mean value for root dry weight (0.2g), number of roots (22) with percentage of rooted cuttings (56%) compared to the one using softwood cutting. 10, 000 mg/L IBA gave the highest mean value for root length (13.6cm), number of roots (28), percentage of rooted cuttings (74%) with root dry weight (0.5g). In experiment 2, conducted on January 2008, the hardwood cutting once again gave the highest mean value for root length (12.1cm) and root dry weight (0.4g). The greatest root number (59) was obtained at 20, 000 mg/L. There was no significant effect on percentage of rooted cuttings, root dry weight, root length and rootball diameter. The histological studies showed that adventitious roots were originated directly from the endodermis. The root primordial started to develop on day 8 and emerged through the epidermis after day 11.

Key words: *Jatropha curcas*, Vegetative propagation, Indole butyric acid, Root initiation

INTRODUCTION

Jatropha curcas L., is a multipurpose plant that belongs to the family Euphorbiaceae and is valued not only for its medicinal properties and resistance to various stresses but also for its use as an oilseed crop [4,10]. It has drawn attention in recent years since demand for fuel (diesel) has increased drastically. Jatropha produce seeds with an oil content of 30-50% by weight. Seed production of Jatropha plant propagated by seed will come into production within three to four years after planting. However propagation through cuttings can probably yield earlier which is about 1 year from planting [6]. Furthermore, seeds of Jatropha have a limited viability and can only be stored for 15 months after which its viability is reduced by 50% [7]. Studying the vegetative propagation of Jatropha through stem cutting and the use of rooting hormone is one approach in order to get more planting material and fast yielding tree.

Adventitious root formation has a lot of commercial interests because there are many plant species cutting that are difficult to root. In some plant species, adventitious root formation initiate without any treatment, while others required different growth regulators usually auxin [12]. Auxin induces root formation by breaking root apical dominance induced by cytokinin [2]. Indole Butyric Acid (IBA) is a synthetic rooting chemical that have been found to be reliable in the promotion of rooting in cuttings more proficiently than Indole Acetic Acid (IAA) which is a native auxin. IBA is widely used because it is non-toxic to most plants over a wide range and promotes root growth in a large number of plant species [3].

MATERIALS AND METHODS

This study was conducted under rainshelter in Field 2, Universiti Putra Malaysia, Serdang, Selangor. The duration of the study was from July 2007 to September 2007 for experiment 1 and January 2008 to March 2008 for experiment 2. To achieve the objectives, two experiments were conducted to study the effects of indole-3-butyric acid (IBA) concentrations and three types of cuttings (softwood, semi hardwood and hardwood) on root performance of *Jatropha curcas* L. For adventitious root initiation and development studies, the histological analysis was carried out using protocol by Johansen[5] to determine the rooting time line to confirm when and where adventitious roots are formed. Cuttings that have been treated with 0 mg/L and 2000 mg/L of IBA were harvested 7 to 11 days after planted for histological analysis.

Cuttings were obtained from Sungai Besi, Selangor for the first experiment and from Merang, Terengganu for the second experiment. For the first experiment, cuttings were treated with six concentrations of IBA (0 mg/L, 2000 mg/L, 4000 mg/L, 6000 mg/L, 8000 mg/L,

Corresponding Author: Thohirah, L.A. Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.

and 10, 000 mg/L) and for second experiment, the concentrations of IBA were increased (0 mg/L (control), 10, 000 mg/L, 15, 000 mg/L, 20, 000 mg/L and 25, 000 mg/L). To prepare 2000 mg/L solution, 25 g of the pure material was dissolved in about one liter of 50 % alcohol (ethanol). Method is the same for other concentrations of the solution.

Healthy and uniform stem cuttings of 25cm long were prepared. Then, they were dipped in fungicide solution (Benlate) which contains 50 % w/w Benomyl for 30 seconds. Then, the basal of the cuttings (1-2 cm length) were dipped in each concentration of IBA for 5 – 10 seconds. After that, the cuttings were allowed to air dry for 30 second before inserted in sand 1/3 proportion of the cutting inside propagating tray (Figure 1). The experimental design used for both experiments were RCBD with factorial 3 x 6. Each treatment consisted five replications and each replication consisted of three cuttings. Data from both experiments were collected eight weeks after planting to measure parameters such as root length, number of roots, percentage of rooted cuttings, root dry weight and root ball diameter.

Fig. 1: Experimental site showing propagation area where cuttings of *Jatropha curcas* were propagated in plastic trays and irrigated under mist system.

RESULTS AND DISCUSSION

Experiment 1: All softwood cutting were decayed 40 days after planting (Figure 2). There were significant differences between type of cuttings (hardwood and semi-hardwood) on number of roots (P<0.01), root percentage (P<0.01) and root dry weight (P<0.05). The hardwood cutting gave the highest mean value for root dry weight (0.2g), root number (22) with percentage of rooted cuttings (56%) (Table 1, Figure 3). All six IBA concentrations (0 mg/L, 2000 mg/L, 4000mg/L, 6000 mg/L, 8000 mg/L, 10000 mg/L) showed highly significant differences on all parameters accept for root ball diameter. 10, 000 mg/L IBA gave the highest mean value for root length (13.6cm), number of roots (28), percentage of rooted cuttings (74%) with root dry weight (0.5g) (Table 1). There were no significant interaction between types of cuttings and IBA concentrations. Hardwood treated with 10, 000 mg/L IBA showed the highest percentage of rooted cuttings, root number, and root dry weight.

Experiment 2: There were significant differences between types of cutting (hardwood, semi hardwood and softwood) on root length (P<0.05) and root dry weight (P<0.05). The hardwood cutting once again gave the highest mean value for root length (12.1cm) and dry weight (0.4g) (Table 2, Figure 4).Highly significant effect was obtained between IBA concentrations (0 mg/L (control), 10, 000 mg/L, 15, 000 mg/L, 20, 000 mg/L and 25, 000 mg/L) for root number (59) but no effect on the other parameters. The greatest root number (59) was achieved at 20, 000 mg/L. There was no interaction between types of cutting and IBA concentrations for all parameters.

Adventitious Root Initiation and Development of *Jatropha Curcas:* *Jatropha curcas* cuttings that were treated with 2000 mg/L IBA rooted faster than control (0 mg/L). On day 7, some callus developed at the basal end of cutting (figure 5b). The basal end was swollen and the colour was changed from green to whitish green. The formation of callus still continued to the following days until the 11th day, root primordial emerged from the callus (figure 5c). Although the root primordial seems to be emerged from the callus, closer examination from histological analysis showed that the root was emerging from an area near the vascular bundle and not from the callus itself.

Cross section of stem on day 0 showed no changes occurred. On day 7, the cells dedifferentiation occurred near the vascular bundle. On day 8, cells started to dedifferentiate and develop to be root initials. On day 9, subsequent development of these root initials into organized root primordial was completely done as well as the root cap. Root primordial elongated towards the epidermis on day 10 (Figure 6). The root primordial grew and emerged through epidermis on day 11 and the vascular connections between the root primordial and the conducting tissues of the cutting itself was formed.

Discussion: From the results obtained, the hardwood cutting produced the highest average dry weight and root number in both experiments because hardwood cutting contained higher stored carbohydrate than semi-hardwood and softwood cutting which enable better root production [3]. The root percentage increased as the

Fig. 2: All softwood cutting of *Jatropha curcas* were decayed after 40 days (Arrow).

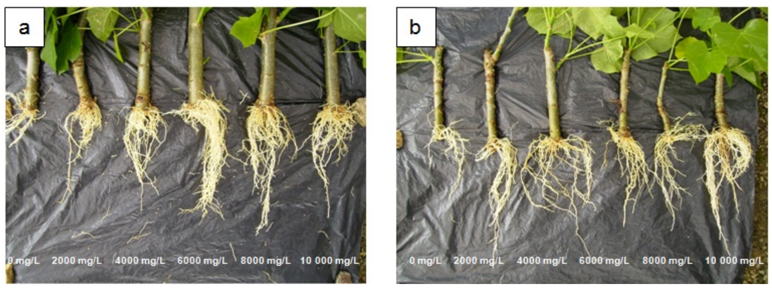

Fig. 3: Effect of IBA concentrations on rooting performance for hardwood (a) and semi hardwood (b) cuttings of *Jatropha curcas* eight weeks after planting.

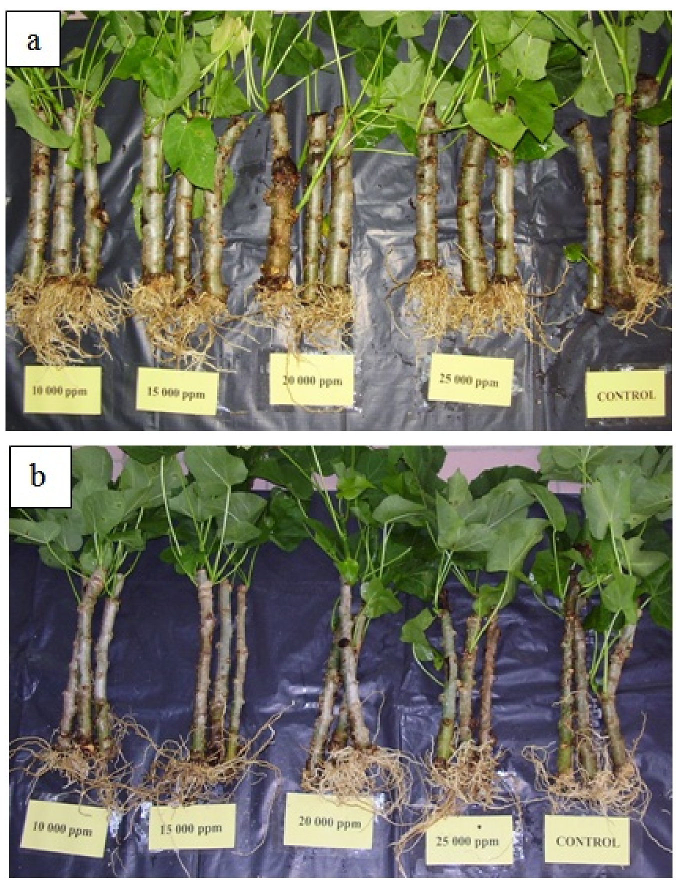

Fig. 4: Effect of IBA concentrations on rooting performance for hardwood (a), semi hardwood (b) and softwood (c) cuttings of *Jatropha curcas* eight weeks after planting.

Table 1: Effects of IBA concentrations and types of cutting of *Jatropha curcas* for Experiment 1.

IBA (mg/L)	No of roots		Rooted cuttings %		Rootball diameter (cm)		Root length (cm)		Root dry weight (g)	
	Hardwood	Semi Hardwood	Hardwood	Semi Hardwood	Hardwood	Semi Hardwood	Hardwood	Semi Hardwood	Hardwood	Semi Hardwood
Control (0 mg/L)	12^{c}	8^{d}	30^{d}	23^{d}	9.3^{a}	9.1^{a}	8.5^{c}	6.6^{b}	0.11^{d}	0.05^{c}
2 000	13^{c}	13^{bcd}	40^{c}	34^{d}	9.4^{a}	9.3^{a}	9.9^{bc}	9.0^{b}	0.13^{d}	0.09^{bc}
4 000	20^{b}	11^{cd}	52^{b}	36^{cd}	9.7^{a}	9.3^{a}	10.7^{abc}	9.2^{b}	0.17^{c}	0.12^{b}
6 000	23^{b}	16^{bc}	59^{b}	52^{bc}	10.1^{a}	9.6^{a}	11.8^{ab}	9.5^{b}	0.20^{bc}	0.21^{a}
8 000	29^{a}	19^{ab}	74^{b}	55^{ab}	10.3^{a}	10.2^{a}	12.0^{ab}	12.9^{a}	0.23^{ab}	0.23^{a}
10 000	32^{a}	23^{a}	79^{a}	69^{a}	11.0^{a}	10.5^{a}	13.0^{a}	14.2^{a}	0.25^{a}	0.27^{a}
Average	22^{a}	15^{b}	56^{a}	45^{b}	10.0^{a}	9.7^{a}	13.0^{a}	14.2^{a}	0.18^{a}	0.16^{b}

Means with the same letter are not significantly different ($P \leq 0.05$) according to LSD

Table 2: Effects of IBA concentrations and types of cutting of *Jatropha curcas* for Experiment 2.

IBA (mg/L)	No of roots			Rooted cuttings %			Rootball diameter (cm)			Root length (cm)			Root dry weight (g)		
	Hard	Semi	Soft	Hard	Semi	Soft	Hard	Semi	Soft	Hard	Semi	Soft	Hard	Semi	Soft
Control (0 mg/L)	22^{b}	18^{c}	17^{b}	80^{a}	100^{a}	93^{a}	10.9^{a}	14.7^{a}	13.4^{a}	10.1^{a}	8.9^{a}	9.6^{a}	0.25^{a}	0.28^{a}	0.28^{a}
10 000	41^{ab}	36^{bc}	30^{ab}	93^{a}	93^{a}	87^{a}	12.3^{a}	13.2^{a}	10.5^{a}	12.9^{a}	11.1^{a}	7.8^{a}	0.45^{a}	0.39^{a}	0.20^{a}
15 000	41^{ab}	48^{ab}	50^{a}	87^{a}	93^{a}	100^{a}	14.0^{a}	14.4^{a}	14.0^{a}	13.8^{a}	9.7^{a}	11.2^{a}	0.33^{a}	0.44^{a}	0.35^{a}
20 000	64^{a}	67^{a}	46^{a}	87^{a}	100^{a}	73^{a}	13.8^{a}	14.0^{a}	9.2^{a}	11.9^{a}	11.1^{a}	5.4^{a}	0.47^{a}	0.51^{a}	0.20^{a}
25 000	68^{a}	47^{ab}	42^{ab}	93^{a}	73^{a}	87^{a}	13.8^{a}	9.8^{a}	8.6^{a}	11.6^{a}	9.0^{a}	8.6^{a}	0.41^{a}	0.22^{a}	0.27^{a}
Average	47^{a}	43^{a}	37^{a}	88^{a}	92^{a}	88^{a}	13.0^{a}	13.2^{a}	11.1^{a}	12.1^{a}	10.0^{ab}	8.5^{b}	0.38^{a}	0.37^{ab}	0.26^{b}

Means with the same letter are not significantly different ($P \leq 0.05$) according to Tukey

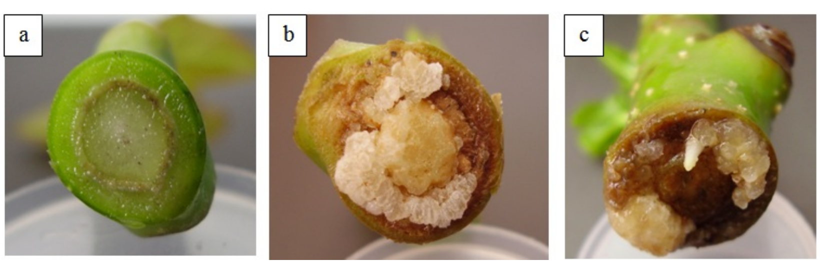

Fig. 5: Development of adventitious root at the basal end of *Jatropha curcas* cutting . (a) day 0 no changes occurred, (b) day 7 some callus developed at the basal end of cutting, (c) day 11 root primordial emerged from the callus

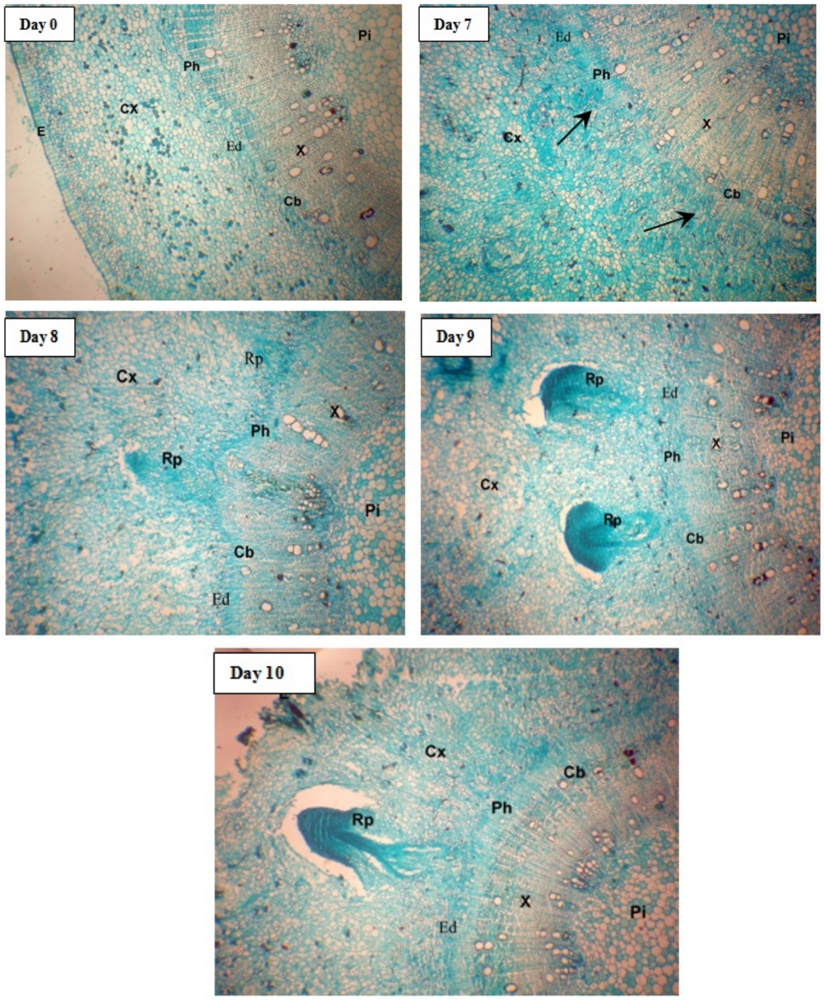

Fig. 6: Cross section of *Jatropha curcas* stem (X 3.2) showed the adventitious root development of on day 0, 7, 8, 9 and 10 [epidermis (E), cortex (Cx), Root primordial (Rp), endodermis (Ed), phloem (Ph), vascular cambium (Cb), xylem (X), and pith (Pi)].

IBA concentrations increased because application of IBA on cutting increased the root percentage, hasten root initiation and increase uniformity of rooting. The root number increased as the IBA concentration increased in both experiments in all types of cuttings. According to Krishnankutty[9], the application of IBA can increase the root number. In both experiments, there were no significant differences on root ball diameter either between types of cuttings or different concentration of IBA. This will only differed if different types of rooting media are used.

Auxin is a substance which is produced in one tissue (shoots) and migrated to effect the development of another tissue which in this case the lower portion of stem cuttings. It promotes cell elongation and has variety of other growth-regulating effects. The effect of the auxin is specific [1]. The results support the earlier findings in *Jatropha curcas* and other plant species, revealed that cuttings treated with IBA could induced rooting and very effective to enhance the vegetative propagation and growth [8].

Jatropha curcas cuttings treated with 2000 mg/L IBA started to form root primordial 8 days after planting (Figure 6) and emerged through epidermis after day 11(Figure 5c). The histological studies revealed that adventitious root originated directly from endodermis (near the vascular bundle just outside the cambium) but not from the callus. Callus is an irregular mass of meristematic parenchyma cells formed in response to wound and reproduces from young cells at the base of cutting in the region of the vascular cambium. Its main function is to seal off the wound and produced suberin [3]. Suberin is a complex mixture of oxidation and condensation products of fatty acids which are present in the walls of cork-cells, thereby rendering them impervious to water and preventing water loss from the tissues below [1]. Usually, the first roots appear through the callus [3]. The use of hormones during adventitious rooting enhances the formation of callus in addition to inducing the formation of roots [11].

Conclusion: The application of IBA increased number of roots. Cuttings with high number of roots have the advantage by enhancing good anchorage when it is planted in the field. The hardwood cutting is the best type of cutting because it gave the best performance compared to the one using semi-hardwood and softwood cuttings of *Jatropha curcas*. IBA application at 10, 000 mg/L gave the best rooting performance in experiment 1 and 20, 000 mg/L IBA was found to elicit the best rooting response in experiment 2. *Jatropha curcas* can be considered as easy-to-root plant cutting because it started to form adventitious roots primordial on day 8 and emerged through epidermis after day 11.

ACKNOWLEDGEMENT

The authors would like to thank the Head and personnel in Ladang 2, UPM and The Department of Crop Science, Faculty of Agriculture, UPM for the technical cooperation.

REFERENCES

1. Arora, D., 2006. Dictionary of Botany. A.I.T.B.S.
2. Cline, M.G., 2000. Execution of the auxin replacement apical dominance experiment in temperate woody species. American Journal of Botany, 87(2): 182–190.
3. Hartmann, H.T., D.E. Kester and F.T. Davies, Jr. 1990. Plant Propagation: Principles and Practices 5th edition. Prentice-Hall, Inc.
4. Heller, J., 1996. Physic nut. Jatropha curcas L. Promoting the conservation and use of underutilized and neglected crops. Unpublished report. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute (IPGRI), Rome.
5. Johansen, D.A., 1940., Plant Micro Technique. Mc Grow Hill, New York.
6. Jones, N. and J.H. Miller, 1992. *Jatropha curcas* : A multipurpose species for problematic sites. The World Bank Asia Technical Department Agriculture Division.
7. Kochhar, S., V.K. Kochhar, S.P. Singh, R.S. Katiyar and P. Pushpangadan, 2005. Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes. Current Science, 89(6): 936 -939.
8. Kochhar, S., S.P. Singh and V.K. Kochhar, 2008. Effect of auxins and associated biochemical changes during clonal propagation of the biofuel plant- *Jatropha curcas.* Biomass and Bioenergy, 32 (12): 1136-1143.
9. Krisnankutty, N., 2005. Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes. Current Science, 89(6): 936-939.
10. Openshaw, K., 2000. A review of Jatropha curcas: an oil plant of unfulfilled promise. Biomass and Bioenergy, 19(1): 1-15.
11. Sutter, E.G., 1989. Relationship Between Indole-3-Acetic Acid Levels in Apple (*Malus pumila* Mill) Rootstocks Cultured *in Vitro* and Adventitious Root Formation in the Presence of Indole-3-Butyric Acid. University of California Davis. California.
12. Syros, T., T. Yupsanis, H. Zafiriadis and A. Economou, 2004. Activity and isoforms of peroxidases, lignin and anatomy, during adventitious rooting in cuttings of Ebenus cretica L. Journal of Plant Physiology, 161: 69–77.