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Rooting of *Jatropha curcas* L. and *Euphorbia tirucalli* Cuttings in Response to IBA and Planting Media in North Egypt: A Potential Source for Tomorrow's Oil, Biodiesel and Biofuels

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ABSTRACT

The present study was carried out in open private commercial field in El-Behira Governorate, Egypt (30"54'34, 87"N and 30" 42' 33, 78" E) during the period from 15 February 2013 to 26 April 2013. The study deals with the effect of seven different concentrations of indole-3-butyric acid (IBA) (control zero, 500, 1000, 1500, 2000, 2500 and 3000 ppm) for 12 h and three types of planting media (sand, sand: peat moss 1:1(v/v) and peat moss) on root performance of *Jatropha curcas* L. and *Euphorbia tirucalli* cuttings. Data were collected ten weeks after planting the cuttings. Three experiments were conducted where in Experiment 1: Cuttings were taken from two years old of *Jatropha curcas* L. terminal branches 6-8 cm length treated with IBA at different concentration showed the rooting behavior in the order: 1500 ppm>1000 ppm>3000 ppm>2500 ppm >2000 ppm>500 ppm> control. In case of propagation 1500 ppm IBA treatment was found to be the best (6.8 roots/cutting, 1.32 cm root length, 71.1 rooting percentage, 3.1 g fresh weight, 1.5 leaves/cutting, 2.4 cm length of the longest leaf and 38 days to sprout). In Experiment 2: Cuttings were taken from four years old of *Jatropha curcas* L. terminal branches 15-17 cm length treated with IBA at different concentration showed the rooting behavior in the order: 2500 ppm >3000 ppm >2000 ppm>1500 ppm>500 ppm>1000 ppm>control. In case of propagation 2500 ppm IBA treatment was found to be the best for (8.3 roots/cutting, 5.6 cm root length, 68.8 rooting percentage, 14.8 g fresh weight, 3.1 leaves/cutting, 4.6 cm length of the longest leaf and 50 days to sprout). In Experiment 3: Cuttings were taken from terminal branches of *Euphorbia tirucalli* 20-22 cm length treated with IBA at different concentration showed the rooting behavior in the order: 2500 ppm>1500 ppm>2000 ppm>3000 ppm>1000 ppm>500 ppm>control. In case of propagation 2500 ppm IBA treatment was found to be the best for (8.2 roots/cutting, 11.8 cm root length, 73.3 rooting percentage, 5.2 g fresh weight, 7.7 leaves/cutting and 46 days to sprout). The types of planting media treatment showed the rooting behavior in order: Sand>sand: Peat moss 1:1 (v/v)>peat moss for both cuttings of *Jatropha curcas* and *Euphorbia tirucalli* in all three experiment.

Key words: *Jatropha curcas*, *Euphorbia tirucalli*, cutting, indole-3-butyric acid (IBA), days to sprout, No. of roots, root length, ages of stock plant, types of planting media

INTRODUCTION

The recent increase in the world oil price has prompted countries to consider biofuels option. *Jatropha curcas* L. and *Euphorbia tirucalli* are important biofuel plant that belongs to the family

Euphorbiaceae and also valued for its medicinal properties and resistance to various stresses. Increased interest in economic and scientific fields for the development of bio-energy system as one of the solutions to overcome the problems of lack of energy and climate change (Rajesh *et al.*, 2008; King *et al.*, 2009). To produce more biofuel crops for exportation to keep up with the demand for biofuels in the USA, Europe and other developed nations is partly driven by the need to replace fossil fuels and lessen dependence on high-priced imported oil. The goals being set are ambitious as the European Union (EU) has set targets of 10% biofuels in vehicle fuel by 2020. By 2017; USA aims that bio-fuels production will replace 17% of its consumption of petrol (Somma *et al.*, 2010). Developing nations are focusing on the bio-fuel markets at the same time Europe and USA are not able to produce enough amount of bio-fuel and ethanol according to its needs (Biddiger, 2007). The advantages of increased reliance on the second generation of biofuels that low in its aqueous needs and do not compete with food crops. Governments (as in Egypt) "should encourage peasant farmers to grow biofuel plants in poor and neglected land which do not compete with food crops production and planning to conduct scientific research to develop technologies that will increase productivity of agriculture per unit of land, to meet increasing global demand for both food and biofuels (Jumbe, 2007).

Biofuels are recognized for their environmental benefits, they are renewable, non-toxic, biodegradable, free of sulfur and with 60% less carbon dioxide. In fact, a 20% blend with petroleum diesel in trucks and buses would eliminate the black smoke (unburned fuel) emitted during acceleration. Biodiesels are safe to transport, to handle and to store. Biodiesels can also help reduce public health risks associated with air pollution (FARA, 2008). For every unit of fossil energy used to produce plant oil based biodiesel, 3.37 units of biodiesel energy are created. Biodiesels not only reduce the amount of carbon dioxide (CO2) released into the atmosphere but the crops used to produce biodiesel absorb also large amounts of CO2 as they grow (FARA, 2008).

At national level, poor consumers in urban areas and poorer farmers who are food consumers could be food insecure. At macroeconomic level, low income countries which are net food importing will be affected by an increase in food import bill, especially where they have low foreign currency reserves.

In fact, one of biodiesels greatest benefit is that it requires no change to the immediate market infrastructure namely, public transport (buses) and commercial transport (freight trucks). Biodiesel can be used either as blend with petroleum diesel or as a pure fuel. However, due to the limited supply of biodiesel today, as well as the non-linear emission benefits of burning biodiesel, it is actually more environmentally beneficial to fuel 5 vehicles with a blended biodiesel (20% biodiesel/80% petroleum diesel) than one vehicle with 100% biodiesel (FARA, 2008). The first two end users of *Jatropha* seeds in Egypt forms a relatively small market but the world markets are big and can absorb any quantity of *Jatropha* seed. The demand for seeds is driven by the demand for biofuel which increase day by day and year by year. The recent records of increasing oil prices and the trend of processing biofuels from plant material are of great interest worldwide. Global biofuel production has tripled from 4.8 billion gallons in 2000 to about 12.6 billion gallons in 2007. However, it still accounts for less than 3 percent of the global transportation fuel supply. About 90 percent of production is concentrated in the United States, Brazil and the European Union (EU) (El-Gamassy, 2008). The data showed that the price of *Jatropha* seed jumped in 2008. That reached to 215% of its average in 2007, reflecting a great shift on the demand side. At the same time it reflects a great concern about *Jatropha* seed for other uses than biofuel. The domestic Egyptian market is absorbing the remaining branches taken after pruning. Three tons of prunings residual per feddan could be collected on average per year. These could be used in compost production or the aromatic industry. The price is about L.E.1,000/ton which is equal to the price of citrus tree pruning (El-Gamassy, 2008).

Euphorbiaceae is a good starting point for a search for phytomedicines of human, veterinary or pesticidal nature Fig. 1a. The genus *Jatropha* is a morphological diverse genus comprising 170 species of shrubs subshrubs and herbs belonging to family Euphorbiaceae. *Jatropha curcas* L. is a multipurpose plant 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 forfuel (diesel) has increased drastically. *Jatropha* produces seeds with an oil content of 30-50% by weight.

Jatropha curcas L. is a biofuel crop, widely cultivated in Africa, Central and South America, India and Southeast Asia (Katembo and Gray, 2007; Maes *et al*., 2009; Trabucco *et al*., 2010), mainly because of the high quality oil it produces and its ability to reclaim dry, marginal and degraded areas (Achten *et al*., 2008; Achten *et al*., 2010). It is a tropical, drought resistant, stem succulent tree. *Jatropha curcas* L. is an ideal plant for afforest ration of wasteland under both irrigated as well as rain fed conditions. The cultivation of *jatropha* species is also reported to prevent and control erosion (Gubitz *et al*., 1999). Besides, medicinal value, *J. curcas* has emerged as a potential biodiesel crop alternative to petro-diesel (Openshaw, 2000; Mandpe *et al*., 2005; Kou and Chun, 2007). In addition to bio-diesel, it also yields byproduct like glycerine and seed cakes after transesterification process. Glycerine can be used in soap preparation and cosmetics while seed cake can be used as bio-fertilizer, fuel briquettes and paper making (Kumari *et al*., 2010).

The plant of *Euphorbia tirucalli* Fig. 1b belongs to family-Euphorbiaceae is commonly known as Barki-thohar. This plant is native to America but has become acclimatized and grows freely in all parts of India. This is a common medicinal plant of India; the plant parts used milky juice and stem bark. Milky juice in small doses is a purgative but in large doses it is acrid, counter-irritant and emetic. *E. tirucalli* latex seems to reduce the specific cellular immunity associated with the virus Epstein-Barr injection by activating the virus lytic cycle (Wal *et al*., 2013). The bark/latex of *E. tirucalli* presents pharmacological activities as an antibacterial, molluscicide, antiherpetic and anti-mutagenic. It also shows co-carcinogenic and anticarcinogenic activities. In the northeast of region in Brazil, the latex of *E. tirucalli* is used as a folk medicine against syphilis. As an antimicrobial; a laxative agent to control intestinal parasites to treat asthma, cough, earache, rheumatism, verrucae, cancer, epithelioma, sarcoma and skin tumours. *E. tirucalli* contains a large quantity of terpenes and sterols among its constituent and the following substances which have been isolated; alcohol, eufol, alfaeuforbol and taraxasterole tirucallol (Imai *et al*., 1994). *E. tirucalli* used as a source of hydrocarbon has been investigated for long. The sap called latex is similar to that of the rubber tree. Originally *E. tirucalli* was known as petroleum plant and Nobel Prize winner Melvin Calvin included the plant in his research on hydrocarbons produced from vegetative origin. Calvin notes that cuttings with a longitude of 5 cm increased in one growing season attaining more than 50 cm height in the first growing season (Calvin, 1980). It was reported that latex of *E. tirucalli* is composed of petroleum like hydrocarbons largely C_{30} triterpenoids which on cracking yield high-octane gasoline. It was estimated that a crude gasoline yield between 4 and 8 barrels per hectare from an *E. tirucalli* planted field per year and calculated at about three dollars per barrel, it is three times cheaper than normal crude oil (Calvin, 1978; Prusty *et al*., 2008). *E. tirucalli* is still looked at as a potential source of biodiesel as it can produce a high biomass and grow in marginal areas unfit for production of other crops. Of late, there has been increasing attention on biodiesel production in order to reduce over dependence on fossil fuel (Rajasekaran *et al*., 1989). Associated with biodiesel production is methane and biogas generation; many scientists, considering its reported high biomass production and ease with which it ferments, note that it is a potential source of methane and biogas (Van Damme and Gebruik, 1990; Sow *et al*., 1989). It was experimentally demonstrated that *E. tirucalli* produces suitable biomass for biogas generation especially through chopped material under thermophilic (Mahiri, 2002). For the same reason, it has been recommended for commercial fuel wood production projects for purposes of woodlot restocking in semi-arid parts of Kenya (Mahiri, 1998). *E. tirucalli* is preferred for this purpose due to its fast growth rate, high productivity, quick acclimatizion to an area and ease with which it dries.

The plant grows well at pH 6 to 8.5 and is highly tolerant to high salt content and is sometimes grown in gardens near the sea beach in gardens (Christman, 2000). The plant can withstand to just under 5.000 p.p.m. arsenic35 (Barnes, 2009).

Under optimal conditions, *Euphorbia tirucalli* produces between 200 and 500 MT “Metric ton, an alternative term for tonne, a measurement of mass equal to one thousand kilograms” of fresh biomass per hectare per year (22-55 MT of dry matter) (Van Damme, 2001; Kumar, 2000). The gross energy content of dry *E. tirucalli* is 17,600 Kj kg^{-1} (Orwa *et al*., 2009). *E. tirucalli* can be of great importance as a bioenergy source in developing countries in the tropics. As a crop it is easy to establish and manage and the whole plant can be harvested all year round as a feedstock for bioenergy without having to wait for flowers or fruits. *E. tirucalli* can withstand severe drought without problems.

Especially in areas that are not connected to an electricity grid and rely on expensive electricity generated from fossil fuels *E. tirucalli* can be of importance. It is possible to convert the plant into biogas and the effluent that is produced can be used to fertilize the plots that have just been harvested. This creates a virtually closed nutrient balance reducing the need for external fertilization. Yield of *E. tirucalli* stems for biofuel production varies greatly with density of planting, number of cuttings per year, annual rainfall and soil type. Measurements by Eco-energia showed dry matter contents of larger stems 26.2%, leaves (16.4%), flowers (9.75%) and roots (41.2%). FACT measured the dry matter content of small young stem segments and found a dry matter content of around 11% (John *et al*., 2011).

Jatropha plants raised from seeds reach to fruiting within three to four years from planting Fig. 4. However, *Jatropha* plants propagated by stem cutting, yield fruits in about one year from planting (Jones and Millers, 1992). Seeds of *Jatropha* have limited viability and can only be stored

Fig. 1(a-b): *Jatropha curcas* L. (a) and (b) *Euphorbia tirucalli*

Fig. 2: Three types of planting media as sand (S), Sand: Peat moss 1: 1(v/v) (M) and peat moss (P)

for 15 months. Their viability is reduced to 50% (Kochhar *et al*., 2008). 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. Advantages are that only vegetative material is needed and there is no need to wait for flowering and fruit production; when cut back, the plant rapidly grows back by itself and plantation can easily be established by vegetative propagation (Orwa *et al*., 2009).

Many internal factors such as auxins, rooting co-factors, carbohydrate and nitrogen levels have been shown to influence rooting of stem cuttings (Hartmann 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 (Syros *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).

Auxin play multifarious roles related to the division and elongation of meristematic cell, differentiation of cambial initials into root primordial and in the mobilization of reserve food material, thereby enhancing the activities of the hydrolyzing enzymes (Nanda, 1970; Nanda and Kochar, 1985).

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 plan.

Aim of study: Present study was aimed to standardize the conditions for vegetative propagation using terminal stem cuttings in order to raise quality of planting material. The present experiments were designed with an objective to determine the most suitable IBA concentration, ideal types of planting media and proper substrate for the propagation of *Jatropha curcas* and *Euphorbia tirucalli* on large scale.

MATERIALS AND METHODS

The present study was carried out in open private commercial nursery conditions in El-Behira Governorate, Egypt falls in (30"54'34, 87"N and 30"42' 33, 78"E). During Spring of 2013 started 15 February and ended in 26 April 2013.

Three experiments were conducted to study the effect of seven different concentrations of Indole-3-butyric Acid (IBA) and three different planting media on rooting of stem cuttings Experiment 1: Two years old *Jatropha curcas* mature thick terminal branches 6 8 cm length, 3-4 cm (circumference) thickness with 4-6 nodes Fig. 3a Experiment 2: Four years old

Fig. 3(a-c): Stem cuttings taken from terminal branches of (a) Two years old of *Jatropha curcas*, (b) Four years old of *Jatropha curcas* and (c) *Euphorbia tirucalli*

Jatropha curcas mature thick terminal branches 15-17 cm length, 4-5 cm (circumference) thickness with 8-10 nodes Fig. 3b were used and Experiment 3: Terminal branches of *Euphorbia tirucalli* 20-22 cm length, 1.5-2 cm (circumference) thickness with 2 top branches during Spring 2013 Fig. 3c.

The basal portion of the cuttings were used dipped in the seven different concentrations of Indole-3-butyric Acid (IBA) as (control treated with distilled water only) Zero, 500, 1000, 1500, 2000, 2500 and 3000 ppm for 12 h by dilute solution soaking method described by (Hartmann and Kester, 2007), The treated cuttings were planted in polyethylene bags with size of (12.5×22.5 cm) (Heller, 1992). Polyethylene bags were filled with three different types of planting media sand, sand: peat moss 1: 1(v/v) and peat moss (Fig. 2). The drainage holes were provided at the bottom of the polyethylene bags. All cuttings were planted on a depth of 4-5 cm. The cuttings were watered twice a week using 1liter of water per bag per week.

Statistical analysis: The experimental design used for the three experiments were randomized complete block design RCBD. Each treatment contains three replications and each replication consisted of five cuttings. Data were subjected to analysis of variance (ANOVA) using the SAS program (SAS, 2002) and the mean values were compared using Tukey's test at $LSD_{0.05}$ level (Snedecor and Cochran, 1974). Data from the three experiments were collected ten weeks from planting to measure parameters collected were No. of roots per cutting, root length, percentage of rooted cuttings, cutting fresh weight, No. of leaves per cuttings, length of the longest leaf and days to sprout.

Fig. 4(a-h): *Jatropha curcas* L. Plant and different stages (a) Seeds, (b) Seed nursery, El-Behira, Egypt, (c) Seedling 1.5 years old, (d) Tree 2 years old, (e) Tree 4 years old, (f) Leaf and flowers, (g) Fruiting branch, (h) and fruits at different stages of maturity

RESULTS AND DISCUSSION

Experiment 1: The analysis of variance showed that, the F-values of Types of planting media, IBA concentration and interactions between them were significant. Generally, data on means of number of roots per cutting, root length (cm), % rooting, fresh weight (g), number of leaves per cutting, length of the longest leaf (cm) and days to sprout in Table 1 showed that, using Sand as a type of

Table 1: Effect of IBA concentration (ppm) and types of planting media on rooting of stem cuttings taken from terminal branches of two years old of *Jatropha curcas* L. for Experiment 1

Types of planting media	No. of roots/cutting	Root length (cm)	Rooted cuttings (%)	Fresh weight (g)	No.of leaves/cuttings	Length of the longest leaf (cm)	Days to sprout
Main effect of types of planting media (M)							
Planting media							
Sand	8.00^{a}	1.90^{a}	87.6^{a}	3.60^{a}	1.42^{a}	2.93^{a}	44.70^{c}
Sand: Peat moss (1:1)	3.60^{b}	0.50^{b}	46.6^{b}	2.50^{b}	0.85^{b}	1.78^{b}	47.10^{b}
Peat moss	2.00^{c}	0.19^{c}	31.4^{c}	1.50^{c}	0.80^{b}	1.18^{c}	48.60^{a}
Main effect of IBA concentration (ppm)							
IBA concentration (ppm)							
Zero	2.30^{e}	0.61c	35.5^{b}	1.86^{c}	0.44^{b}	1.55^{a}	55.44^{a}
500	3.20^{de}	0.63c	53.3^{ab}	2.11^{bc}	0.66^{ab}	1.72^{a}	51.66^{b}
1000	6.00^{ab}	1.33^{a}	57.7^{a}	3.00^{ab}	1.22^{ab}	2.22^{a}	41.10^{d}
1500	6.80^{a}	1.32^{ab}	71.1^{a}	3.04^{a}	1.55^{a}	2.48^{a}	38.80^{e}
2000	4.20^{cd}	0.81^{c}	53.3^{ab}	2.36^{abc}	1.00^{ab}	1.83^{a}	50.30^{b}
2500	4.30^{cd}	0.86^{bc}	57.7^{a}	2.66^{abc}	1.11^{ab}	1.91^{a}	45.80^{c}
3000	5.00^{bc}	0.87^{ab}	57.7^{a}	2.93^{ab}	1.22^{ab}	2.05^{a}	44.60^{c}

Main effect of interaction between (M×IBA) types of planting media and IBA concentration (ppm)

Types of planting media	IBA concentration (ppm)	No. of roots/cutting	Root length (cm)	Rrooted cuttings (%)	Fresh weight (g)	No.of leaves/cuttings	Length of the longest leaf (cm)	Days to sprout
Sand	Zero	4.00	1.50	40.00	3.50	0.66	2.66	54.3
	500	6.30	1.60	100.00	3.00	1.33	3.16	47.0
	1000	8.60	1.90	100.00	3.30	2.66	2.83	38.6
	1500	12.30	2.50	100.00	3.50	1.66	2.83	38.0
	2000	5.60	2.00	100.00	3.70	2.00	2.50	47.0
	2500	10.00	2.20	73.00	3.60	1.33	2.73	44.6
	3000	9.00	2.00	100.00	4.70	0.33	3.83	43.6
Sand: Peat moss (1:1)	Zero	1.60	0.13	33.30	1.00	0.33	1.00	55.3
	500	1.60	0.16	33.30	2.30	0.33	1.00	53.0
	1000	6.60	1.70	46.60	3.00	0.33	2.83	41.3
	1500	5.60	1.20	73.30	2.80	1.33	2.66	38.6
	2000	3.60	0.23	33.30	2.30	0.66	2.00	51.0
	2500	1.60	0.20	60.00	3.40	1.33	1.66	45.6
	3000	4.60	0.30	46.60	2.90	1.66	1.33	45.0
Peat moss	Zero	1.30	0.13	33.30	1.00	0.33	1.00	56.6
	500	1.60	1.60	26.60	1.00	0.33	1.00	55.0
	1000	2.60	0.30	26.60	2.60	0.66	1.00	43.3
	1500	2.60	0.26	40.00	2.70	1.66	1.96	40.0
	2000	3.30	0.20	26.60	1.00	0.33	1.00	53.0
	2500	1.30	0.13	40.00	1.00	0.66	1.33	47.3
	3000	1.30	0.20	26.60	1.10	1.66	1.00	45.3
$LSD_{0.05}$ for (M×IBA)	0.77	0.25	10.00	0.49	0.58	0.55	0.77	

$LSD_{0.05}$ = least significant differences at 0.05 probability. Means with the same letter are not significantly different ($p \leq 0.05$) according to Tukey planting media, IBA at 1500 ppm followed by 1000 ppm and the interactions between them led to an increase the number of roots per cutting (12.3-8.6), root length (2.5-1.9 cm), % rooting (100-100%), fresh weight (3.5-3.3 g), number of leaves per cutting (1.66 -2.66), respectively of terminal cuttings compared to Sand: Peat moss (1:1) or Peat moss with IBA at control. The highest two significant increase in the number of roots per cutting, root length, % rooting, fresh weight, number of leaves per cutting were obtained by using Sand as a type of planting media combined with IBA at 1500 ppm followed by 1000 ppm. The earliest days to sprout in the same two treatments (38-38.6 days), respectively.

The IBA treated cuttings were taken from two years old of *Jatropha curcas* L. terminal branches 6-8 cm length. Showed the rooting behavior in the order: 1500 ppm>1000 ppm>3000 ppm >2500 ppm>2000 ppm>500 ppm>control and the types of planting media showed the rooting behavior in the order: Sand>Sand: Peat moss (1:1)>Peat moss. These results may be due to the changes in the rooting process on both physiological and biochemical levels and the enzymatic activities that may become more regulated by IBA. Further it has been evident that IBA is more effective in case of *J. curcas* which increased the number of roots per cutting, root length (cm) and % rooting (Bijalwan and Thakur, 2010; Kochhar *et al.*, 2005) also IBA treated juvenile cuttings have significant effect at 1000 ppm and 1500 ppm which promoted higher rooting and sprouting in *J. curcas*. It has been observed that number of leaves per cutting, length of the longest leaf declined beyond the range of optimum concentration. These results are in conformity with the results reported by Tewary *et al.* (2004) for *Vitex negundo*.

Nanda and Kochar (1985) showed the effectiveness of exogenously applied auxins (IBA) changes with morphophysiological conditions related to bud dormancy and maturity, applying IBA at the base of the cuttings appears to stimulate rooting in *J. curcas*. The enrichment of rooting may be due to the transformation of auxin after absorption. Auxin plays multifarious roles related to the division and elongation of meristematic cells, differentiation of cambial initial into root primordial and in the mobilization of reserve food material, thereby enhancing the activities of the hydrolyzing enzymes. Most of the IBA treated cuttings produced greater result in root elongation and days to sprout in *J. curcas* as compared to control which is in agreement with (Teklehaimanot *et al.*, 1996; Chauhan *et al.*, 1994).

Experiment 2: The analysis of variance showed that, the F-values of Types of planting media, IBA concentration and interactions between them were significant. Generally, data on means of number of roots per cutting, root length (cm), % rooting, fresh weight (g), number of leaves per cutting, length of the longest leaf (cm) and days to sprout in Table 2 showed that, using Sand as a type of planting media, IBA at 2500 ppm followed by 3000 ppm and interactions between them led to an increase in the number of roots per cutting (13-7.66), root length (12.3-7.16 cm), % rooting (73.3-53.3%), fresh weight (20-18.33 g), number of leaves per cutting (6.33-4.66), respectively terminal cuttings compared to Sand: Peat moss (1:1) or Peat moss with IBA at control. The most two effective treatments significant increase in the number of roots per cutting, root length, % rooting, fresh weight, number of leaves per cutting were the application of Sand as a type of planting media combined with IBA at 2500 ppm followed by 3000 ppm. The earliest days to sprout (48.66-51.33 days), respectively in the same two treatments.

The IBA treated cuttings were taken from four years old *Jatropha curcas* L. terminal branches 15-17cm length. Showed the rooting behavior in the order: 2500 ppm>3000 ppm>2000 ppm> 1500 ppm>500 ppm>1000 ppm>control and the types of planting media showed the rooting behavior in the order: Sand>Sand: Peat moss (1:1)>Peat moss. These results may be attributed to the auxins that positively influence cell enlargement, bud formation and root initiation and also

Table 2: Effect of IBA concentration (ppm) and types of planting media on rooting of stem cuttings taken from terminal branches of four years old of *Jatropha curcas* L. for Experiment 2

Types of planting media	No. of roots/cutting	Root length(cm)	Rooted cuttings (%)	Fresh weight(g)	No.of leaves/cuttings	Length of the longest leaf (cm)	Days to sprout
Main effect of types of planting media (M)							
Planting media							
Sand	5.57^{a}	5.44^{a}	54.2^{a}	15.3^{a}	3.33^{a}	4.69^{a}	54.95^{c}
Sand: Peat moss (1:1)	4.19^{b}	1.81^{b}	45.7^{a}	12.9^{b}	2.00^{b}	2.80^{b}	56.00^{b}
Peat moss	2.00^{c}	0.82^{c}	34.2^{b}	10.9^{c}	0.66^{c}	1.23^{c}	56.80^{a}
Main effect of IBA concentration (ppm)							
Zero	1.77^{d}	1.12^{d}	28.8^{c}	11.5^{a}	0.44^{c}	1.11^{c}	62.55^{a}
500	3.00^{bcd}	2.02^{cd}	37.7^{bc}	12.3^{a}	2.33^{ab}	1.94^{bc}	57.33^{c}
1000	2.55^{cd}	1.86^{cd}	37.7^{bc}	11.6^{a}	0.66^{bc}	1.72^{c}	59.66^{b}
1500	3.22^{bcd}	2.13^{bcd}	40.0^{bc}	12.5^{a}	2.33^{ab}	3.27]	55.30^{d}
2000	3.88^{bc}	2.87^{bc}	48.8^{abc}	14.1^{a}	2.33^{ab}	3.44^{a}	54.33^{e}
2500	8.33^{a}	5.62^{a}	68.88^{a}	14.8^{a}	3.11^{a}	4.61^{a}	50.00^{g}
3000	4.66^{b}	3.24^{b}	51.10^{ab}	14.4^{a}	2.77^{a}	4.27^{a}	52.22^{f}

Main effect of interaction between (M×IBA) types of planting media and IBA concentration (ppm)

Types of planting media	IBA concentration (ppm)	No. of roots/cutting	Root length (cm)	Rooted cuttings (%)	Fresh weight (g)	No.of leaves/cuttings	Length of the longest leaf (cm)	Days to sprout
Sand	Zero	2.66	1.53	40.00	14.00	0.66	1.33	61.66
	500	4.66	3.66	46.60	13.00	3.66	2.50	56.33
	1000	3.66	2.66	53.30	14.66	1.33	3.16	58.66
	1500	3.33	3.26	40.00	13.00	3.00	4.33	54.66
	2000	4.00	7.50	73.30	14.66	3.66	5.50	53.33
	2500	13.00	12.30	73.30	20.00	6.33	7.66	48.66
	3000	7.66	7.16	53.30	18.33	4.66	8.33	51.33
Sand: Peat moss	Zero	1.66	1.53	26.60	11.60	0.33	1.00	62.66
(1:1)	500	2.66	1.40	40.00	13.00	3.00	2.30	57.33
	1000	3.00	2.66	40.00	10.66	0.33	1.00	59.66
	1500	3.33	2.20	40.00	12.66	2.00	3.16	55.33
	2000	4.00	0.86	46.60	14.00	3.00	3.80	54.33
	2500	9.30	2.66	73.30	15.33	2.33	5.16	50.33
	3000	5.33	1.40	53.30	13.00	3.00	3.16	52.33
Peat moss	Zero	1.00	0.30	20.00	9.00	0.33	1.00	63.33
	500	1.66	1.00	26.60	11.00	0.30	1.00	58.33
	1000	1.00	0.26	20.00	9.66	0.33	1.00	60.66
	1500	3.00	0.93	40.00	12.00	2.00	2.33	56.00
	2000	3.66	0.26	26.60	13.66	0.33	1.00	55.33
	2500	2.66	1.86	60.00	9.33	0.66	1.00	51.00
	3000	1.00	1.16	46.60	12.00	0.66	1.33	53.00
$LSD_{0.05}$ for (M ×IBA)	0.98	0.66	11.80	02.03	0.97	0.75	0.43	

$LSD_{0.05}$ = least significant differences at 0.05 probability. Means with the same letter are not significantly different ($p \leq 0.05$) according to Tukey

promote the production of other hormones (Osborne and McManus, 2005). From the results obtained the IBA treated at 2500 ppm gave best response that is in agreement with (Bijalwan and Thakur, 2010) they found that the IBA treated mature cuttings at 2000 ppm promoted higher rooting and sprouting results in *J. curcas*.

Experiment 1 and Experiment 2: Cuttings from 4 years old *J. curcas* tree gave better response of rooting and sprouting than cutting from 2 years old *J. curcas* tree, this may be because cuttings from mature tree (4 years old *J. curcas*) contain higher stored carbohydrate than cuttings from (2 years old *J. curcas*) which enable better root production, these results are in harmony with those obtained by Hartmann *et al.* (1990). Cuttings age play an important role in rooting, moreover, cutting size (length, thickness and number of buds per cutting) that response of shoot cuttings (Bijalwan and Thakur, 2010).

The cuttings taken and propagated in Sand performed excellent rooting results compared to Sand: Peat moss (1:1) or Peat moss, this might be due to sufficient sand moisture content, better aeration and favorable growth condition to the cuttings; while using peat moss only as planting media was the least significant effect in all results, that may be due peat moss proprieties high water holding capacity and bad aeration caused rotted cuttings (Fig. 5 and 6).

Fig. 5(a-c): Effect of types of planting media, (a) Sand, (b) Sand: Peat moss 1:1 and (c) Peat moss on rooting performance at 1500 ppm IBA after 10 weeks for Experiment 1

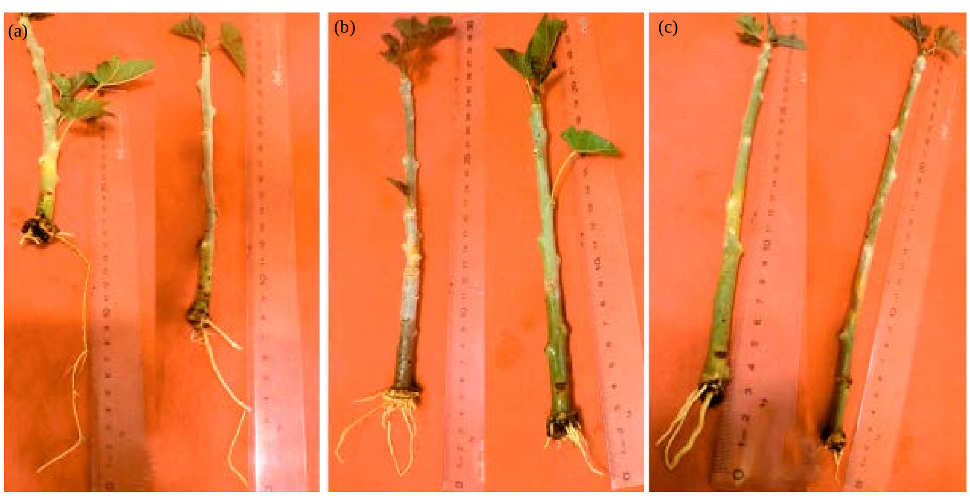

Fig. 6(a-c): Effect of types of planting media (a) Sand, (b) Sand: Peat moss 1:1 and (c) Peat moss on rooting performance at 2500 ppm IBA after 10 weeks for Experiment 2

Experiment 3: While using peat moss only as planting media was the least significant effect in all results, that may be due peat moss proprieties high water holding capacity and bad aeration caused rotted cuttings. In general, all means of No. of roots per cutting, root length (cm), % rooting, fresh weight (g), number of leaves per cutting and days to sprout in Table 3 showed that, using Sand as a Type of planting media, IBA at 2500 ppm after 1500 ppm and the interactions between them led to an increase in the number of roots per cutting (12-7.33), root length (14-15 cm),

Table 3: Effect of IBA concentration (ppm) and types of planting media on rooting of stem cuttings taken from terminal branches of *Euphorbia tirucalli* for Experiment 3

Types of planting media	No. of roots/cutting	Root length (cm)	Rooted cuttings (%)	Fresh weight (g)	No.of leaves/cuttings	Days to sprout
Main effect of types of planting media (M)						
Planting media (M)						
Sand	7.85^{a}	13.00^{a}	75.238^{a}	5.50^{a}	7.47^{a}	53.57^{b}
Sand: peat moss(1:1)	5.47^{b}	9.90^{b}	51.42^{b}	4.51^{b}	4.90^{b}	55.33^{a}
Peat moss	3.76^{c}	7.25^{c}	41.90^{b}	4.25^{b}	3.85^{c}	56.23^{a}
Main effect of IBA concentration (ppm)						
IBM concentrtion (ppm)						
Zero	4.33^{d}	7.10^{e}	46.66^{b}	4.07^{b}	3.00^{d}	61.55^{a}
500	4.66^{d}	8.88^{d}	46.66^{b}	4.33^{b}	3.44^{d}	59.77^{ab}
1000	5.00^{cd}	10.11^{c}	48.88^{b}	4.45^{b}	4.88^{c}	57.77^{b}
1500	6.00^{b}	11.55^{ab}	66.66^{ab}	5.28^{a}	7.33^{a}	53.00^{cd}
2000	6.00^{b}	10.72^{bc}	53.33^{ab}	5.155^{a}	5.77^{b}	52.00^{d}
2500	8.22^{a}	11.88^{a}	73.33^{a}	5.24^{a}	7.77^{a}	46.22^{e}
3000	5.66^{bc}	10.11^{c}	57.77^{ab}	4.76^{ab}	5.66^{b}	55.00^{c}

Main effect of interaction between (M×IBA) types of planting media and IBA concentration (ppm)

Types of planting media	IBA concentration (ppm)	No. of roots/cutting	Root length (cm)	Rooted cuttings (%)	Fresh weight (g)	No.of leaves/cuttings	Days to sprout
Sand	Zero	6.33	9.66	73.33	4.33	3.00	61.00
	500	6.33	11.33	73.33	5.70	3.66	59.00
	1000	7.33	14.00	60.00	4.83	9.00	57.00
	1500	7.33	15.00	73.33	6.76	9.66	47.66
	2000	8.33	15.00	73.33	5.70	11.00	50.66
	2500	12.00	14.00	86.66	6.00	9.33	45.33
	3000	7.33	12.00	86.66	5.23	6.66	54.33
Sand: Peat moss (1:1)	Zero	3.33	9.66	33.33	3.96	3.00	61.66
	500	4.33	7.66	33.33	3.70	3.66	59.66
	1000	5.33	8.66	46.60	4.56	3.00	57.66
	1500	5.33	12.00	73.33	4.56	7.66	55.33
	2000	6.33	9.66	46.66	5.00	3.66	52.00
	2500	7.33	12.00	73.33	5.20	7.66	46.00
	3000	6.33	9.66	53.33	4.63	5.66	55.00
Peat moss	Zero	3.33	1.96	33.33	3.93	3.00	62.00
	500	3.33	7.66	33.33	3.60	3.00	60.66
	1000	2.33	7.66	40.00	3.96	2.66	58.66
	1500	5.33	7.66	53.33	4.53	4.66	56.00
	2000	3.33	7.50	40.00	4.76	2.66	53.33
	2500	5.33	9.66	60.00	4.53	6.33	47.33
	3000	3.33	8.66	33.33	4.43	4.66	55.66
$LSD_{0.05}$ for (M×IBA)	0.49	0.59	13.10	0.38	0.42	1.51	

$LSD_{0.05}$ = least significant differences at 0.05 probability. Means with the same letter are not significantly different ($p \le 0.05$) according to Tukey

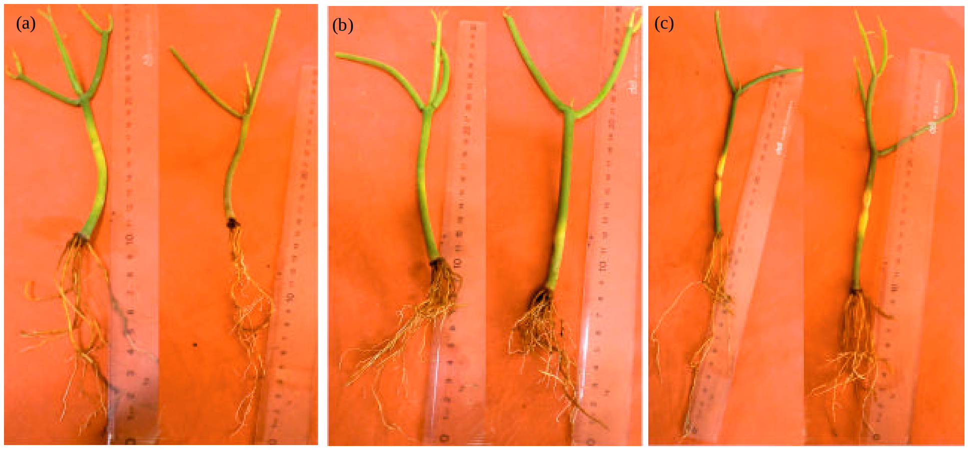

Fig. 7(a-c): Effect of types of planting media, (a) Sand, (b) Sand: Peat moss 1:1 and (c) Peat moss and on rooting performance at 2500 ppm IBA after 10 weeks for Experiment 3

% rooting (86.66-73.33%), fresh weight (6-6.76 g), No. of leaves per cutting (9.33-9.66), respectively of *Euphorbia tirucalli* terminal cuttings compared to Sand: Peat moss (1:1) or Peat moss with IBA at control. Furthermore, the best two significant increases resulted in the number of roots per cutting, root length, % rooting, fresh weight, number of leaves per cutting were obtained by using and as a type of planting media combined with IBA at 2500 ppm after 1500 ppm. The earliest days to sprout (45.33-47.66 days), respectively in the same two treatments.

The IBA treated cuttings were taken from of *Euphorbia tirucalli* 20-22 cm length. Showed the rooting behavior in the order: 2500 ppm>1500 ppm>2000 ppm>3000 ppm>1000 ppm>500 ppm> control and the types of planting media showed the rooting behavior in the order: Sand>Sand: Peat moss (1:1)>Peat moss. These results were probably due to the effect of auxin on adventitious root induction and elongation is highly dependent on the plant type (Nandagopal and Kumari, 2007). IBA is derived from Indole Acetic Acid (IAA) via a chain elongation reaction similar to that found in fatty acid biosynthesis. Besides that, IBA can be converted to IAA after being broken down by peroxisomes through the process of β-oxidation (the same process used to metabolize fatty acids) (Roberts, 2007). Thus, IBA may also be part of the machinery that maintains IAA homeostasis (Srivastava, 2002). Consequently, additional energy would be used up and might eventually lead to insufficient energy needed for cell growth and development (Zolman *et al*., 2008). The cuttings taken and propagated in sand performed excellent rooting results compared with using Sand: Peat moss (1:1) or Peat moss, this might be due to sufficient sand moisture content, better aeration and favorable growth condition to the cuttings; while using Peat moss only as planting media was the lowest significant effect in all results, that may be due peat moss proprieties such as high water holding capacity and bad aeration caused rotted cuttings (Fig.7).

CONCLUSION

The application of IBA increased number of roots, root length and percentage of rooting in *Jatropha curcas* and *Euphorbia tirucalli* compared to control. Cutting with high number of roots have the advantage by enhancing good anchorage when planted in the field. IBA application at 1500 ppm followed by 1000 ppm gave the best rooting performance in Experiment 1, 2500 ppm IBA followed by 3000 ppm were found to elicit the best rooting response in Experiment 2 and 2500 ppm

IBA followed by 1500 ppm were the best concentrations for rooting response in Experiment 3. The sand was the best planting media because it gave the best performance followed by the sand: Peat moss (1:1) than peat moss for both cuttings of *Jatropha curcas* and *Euphorbia tirucalli*. *Euphorbia tirucalli* could be considered to be easier in rooting than *Jatropha curcas* because it formed higher number of adventitious roots, root length at the same concentration of IBA and faster than *Jatropha curcas*. Cuttings from 4 years old *Jatropha curcas* were the best choice followed by cuttings from 2 years old *Jatropha curcas* for vegetative propagation.

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