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Vegetative propagation of *Adhatoda vasica* a medicinal plant: effect of indole-3-butyric acid (IBA) on stem cuttings

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Abstract
Adhatoda vasica is commonly known as 'Malabar nut' and belonging to family Acanthaceae. It is important medicinal plant used in traditional system of medicine to cure various ailments. Adhatoda is well known for its efficacy in treating respiratory problems. The experiment was carried out to know the rooting ability and growth performance of *A. vasica* by treating with different concentrations of IBA (100, 200, 300, 400, 500, 1000, 1500, 2000 and 2500 ppm) on stem cuttings (softwood, semi hardwood and hardwood cuttings) and imposed the treatments in completely randomized design with three replications. The results revealed that, among the different IBA concentrations and type of cuttings, semi hardwood cuttings treated with 2500 ppm IBA showed the best results with highest shoot length (43.53± 5.31), plant fresh weight (35.66± 2.33), fresh root weight (36.10± 1.06), stem dry weight (30.93± 0.69) and root dry weight (21.33± 0.40) as compared to untreated cuttings. Hence, semi hardwood cuttings treated with IBA 2500 ppm can be used for mass multiplication and production of quality planting material for cultivation of *A. vasica*.

Keywords: *Adhatoda vasica*, IBA, softwood, semi hardwood, hardwood cuttings, multiplication

Introduction
Adhatoda vasica is an evergreen shrub belongs to the family Acanthaceae. It is used over the centuries for treating various ailments and disorders. It is commonly known as Malabar nut (English), Vasaka (Sanskrit), Arusha (Hindi), Bakas (Bengali), Alduso (Gujarati) and Adasaramu (Telugu) Adadodai (Tamil), Adusoge (Kannada), Atalotakam (Malayalam). Adhatoda is native to Indian subcontinent *i.e.* Assam, Bangladesh, India, Nepal and Sri Lanka. The plant parts of Adhatoda *i.e.* leaves, root, stem and flowers are used in treatment of asthma, cough, hepatitis and fever (Paranjpe, 2005) [1]. It is used as an herbal medicine for treating cold, cough, as a sedative expectorant. It is also well known for its activities like antiarthritis, antispasmodic, antiseptic, antimicrobial and antituberculosis properties. It is used for treating asthama and bleeding piles. *A. vasica* is highly valued for its leaves and has huge demand worldwide with potent medicinal properties due to presence of vasicine, a quinozoline alkaloid. The major groups of alkaloids present are phytosterols, polyphenolics and glycosides. The practice of collecting or harvesting the raw materials from wild or forest sources is unavoidable and about 80% of the collections are indiscriminate harvest leading to loss of biodiversity and causes threat to many medicinal plants in their natural habitat. The production of medicinal plants through cultivation can reduce the pressure on wild medicinal plants and helps in production of homogeneous raw material to the herbal industries. In additional to that this practice can prevent the environmental degradation and also loss of genetic diversity in the wild (Kala, 2005) [20]. The propagation of Adhatoda through seed is limited due to poor seed set and also poor potential of seed germination. Hence, the propagation by vegetative means is the way for mass multiplication of Adhatoda. Vegetative propagation is one of the most convenient methods for rapid multiplication with low cost of production. The rooting resulted from stem cuttings of softwood, semi hardwood and hardwood has been observed in many medicinal plants. This method helps to get the desired genetic material (Hati *et al.*, 1990) [2]. Growth regulators like auxins are generally used to induce rooting in the cuttings. The role of auxins is to hasten root initiation, increase the number and percentage of cuttings rooted as well as quality of roots produced per cutting. Larsen and Guse (1997) [3] reported that most definitive rooting hormone is indolebutyric acid (IBA) than others such as NAA and IAA. There are several reports suggested that it also be toxic to young cuttings of various species. But, IBA is still the best hormone to use for various purposes as it is non-toxic at different

concentration level (Kester *et al.*, 1990) [4]. IBA is considered an important hormone to increase the rooting percentage in the cuttings. With these facts, the present experiment was carried out to identify the suitable concentration of IBA level on vegetative cuttings of *A. vasica* for mass multiplication and production of quality planting material for large scale cultivation.

Materials and Methods

The present investigation on "effect of indolebutyric acid on vegetative propagation of *Adhatoda vasica*" was carried out at Lambhvel Farm, ICAR-Directorate of Medicinal and Aromatic Plant Research, Boriavi, Anand, Gujarat during the year 2018(fig. 1). The experiment was executed using completely randomized design having ten treatments with the application of indolebutyric acid at different concentration *viz.*, 100 (T_1), 200 (T_2), 300 (T_3), 400 (T_4), 500 (T_5), 1000 (T_6), 1500 (T_7), 2000 (T_8), 2500 ppm (T_9) and a control (T_{10} without any treatment). Three types of stem cuttings were used for rooting *i.e.* softwood, semi hardwood and hardwood cuttings. Fifteen stem cuttings were used for each treatment and replicated thrice. A softwood cutting with a average diameter of 6.66 mm having 2 pairs of leaves on its apical portion of stem, whereas the cuttings approximately with 7.45 mm and 9.09 mm diameter with 2-3 internodes were selected as semi hardwood and hardwood cuttings, respectively. The cuttings were collected from the healthy mother plant maintained at nursery ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand. The lower end of each cutting was dipped for 30 minutes in different concentration of indole butyric acid and excluded control cuttings. Further, the set of treated each forty five softwood, semi hardwood and hardwood cuttings were prepared for each treatment and replicated thrice. During the preparation of the cuttings, at distal end the straight cut was made for semi hardwood and hardwood cuttings and at lower end of stem the slant cut was made just below the node. The cuttings were planted in the well prepared beds consists of sand, soil and farmyard manure in the ratio of 1:2:1 under polyhouse condition. Watering was carried out regularly considering the moisture requirement of the media and environment. The observations were recorded at 90 days for shoot length, fresh weight of plant, number of sprouts, number of roots, root length, root weight and foliage weight of softwood, semi hardwood and hardwood cuttings respectively by randomly selected five rooted cuttings in each treatment and replication and mean values was calculated. The prominent roots were only taken for counting the number of roots and rests (smaller) root hairs are not considered. The root length was measured in centimeter of the longest root and the mean value was calculated. The significance of difference of among mean values was carried out using Duncan's multiple range test (DMRT) at $P<0.05$ and results are expressed as mean ± SE of the experiment. The experimental data analyzed using statistical package SPSS version 16.

Results and Discussions

Effect of IBA on softwood cuttings

The different concentrations of IBA treatment on softwood cuttings of *A. vasica* showed significant effects for various root and shoot characters such as fresh weight of plant, number of sprouts, number of roots, root length, foliage weight, dry weight of stem and dry weight of root as compared to control and the data presented in the table 1. Among the different concentrations of IBA treatment highest shoot length (46.86±4.43) was observed for cuttings treated with 2500 ppm of IBA but it was statistically non significant. The results were in agreement with the findings of Yeshiwas *et al*. (2015)[5] and they reported that shoot length was maximum in rose softwood cuttings treated with 2500 ppm IBA followed by 1000 ppm IBA. The fresh weight of plant was observed significantly maximum in cuttings treated with 1500 ppm IBA (26.00 ± 1.52) as compared to untreated cuttings (18.00±1.52). It was statistically at par with the treatment 300 ppm IBA (20.00 ± 1.15) while cuttings treated with 400 ppm IBA significantly less effective on fresh weight of plant. It was observed that the numbers of sprouts were maximum (1.20± 0.11) in cuttings treated with 100 ppm IBA and it was found on par with treatment of 300 ppm and 400 ppm IBA. Among all the IBA treatments, 500 ppm and 2000 ppm showed least effective on number of sprouts per rooted cutting. Significantly maximum root length was recorded in cuttings treated with 200 ppm IBA (20.79± 1.33) as compared to untreated cuttings. It was statistically at par with 2500 ppm (15.04± 1.52). The minimum root length (11.94± 2.17) was recorded in the treatment received with 500 ppm IBA. These results are in accordance with Shepherd and Winston (2000) in Bougainvillea. The results are also in acceptance with the findings of Ramtin *et al.* (2011) [6] in poinsettia. The IBA controls cell division, multiplication and specialization which helps in the production of roots (Davis and Hassig, 1990) [7]. Non significant results were observed for root weight among different levels of IBA treatment received for softwood cuttings.

The foliage weight was found highest (44.13± 7.71) for 100 ppm of IBA and found statistically at par with 300 ppm IBA (31.13± 5.81). Minimum foliage weight (11.33± 1.39) recorded in 500 ppm IBA. The dry weight of stem was maximum (16.66± 0.17) in cuttings treated with 100 ppm IBA and the minimum effect was observed for treatment given at 1000 ppm IBA (7.33± 0.51) and on par results was recorded for treatment given at the rate of 300 ppm IBA (12.23± 0.95). The dry weight of root was observed maximum in 2500 ppm IBA (15.56± 0.43) as compared to control (5.30± 0.26) and found statistically on par with 1000 ppm IBA (13.86± 0.80). Significantly minimum effect on dry weight of root was observed in cuttings treated with 200 ppm IBA (5.80± 0.05) in comparison to all the treatments.

Effect of IBA on semi hardwood cuttings

The results on effect of different concentration of IBA levels on semi hardwood cuttings are presented in table 2. It was observed that significantly highest shoot length (43.53± 5.31) and fresh weight of plant (35.66± 2.33) recorded in semi hardwood cuttings treated with 2500 ppm of IBA as compared to the untreated cuttings (9.75± 9.75, 35.52± 0.55 respectively). The number of sprouts per rooted cutting was found significant among all the treatments and found application of 100 ppm IBA had significantly highest number of sprouts (3.33± 0.26) and foliage weight (30.43± 0.87) of the cuttings. This treatment remained statistically on par with treatment of 200 ppm IBA (2.73± 0.13) and 2000 ppm IBA (2.40± 0.11). The lowest number of sprouts per rooted cutting was observed in cuttings received 1500 ppm IBA (1.73± 0.13). These findings are partially agreement with Tiwari and Das (2010) [18]. Significantly maximum numbers of roots (6.73 ± 1.43) were found in cuttings treated with 200 ppm IBA in comparison with control (1.46 ± 0.68). The maximum number of roots was in 200 ppm IBA whereas minimum number found in cuttings treated with 1500 ppm IBA. The cuttings treated with 400 ppm IBA had significantly maximum root length (16.26 ± 0.93) as compared to control (2.33 ± 2.33) and also other concentrations of IBA. In a similar study Deepika *et al*. (2015) reported that at higher concentration of IBA produced maximum number of roots, root length and rooting percentage in Karonda. The root weight has been increased in higher concentration of IBA level and significantly maximum root weight (36.10± 1.06) recorded in cuttings treated with 2500 ppm IBA as compared to controlled cuttings and less root weight (15.13± 3.24) recorded for the cuttings treated with 100 ppm IBA. The traits dry weight of stem and roots was significantly maximum in semi hardwood cuttings treated with 2500 ppm IBA (30.93± 0.69 and 21.33± 0.40 respectively) as compared to the control (24.93± 0.20 and 5.56± 0.07 respectively). The results showed that semi hardwood cuttings with 2500 ppm IBA showed effective on growth of root and shoot shown in fig.3. Similar results were reported by Das and Jha (2014) [17] where the semi hardwood cuttings treated with IBA had higher percentage of rooting than hardwood cuttings in *Taxus wallachiana*. Tiwari *et al*. (2016) [19] also reported that best rooting response from semi hardwood cuttings compared to long and hardwood cuttings of *Dillenia pentagyna* where they concluded 500 ppm IBA was optimum for rooting. The process of formation of roots, the fresh weight and dry weight of stem, the fresh weight and dry weight of root is affected by external and internal factors. The enhanced hydrolysis of carbohydrates, accumulation of metabolites where auxin is applied, protein synthesis, cell division and cell enlargement causes to increase the root length when it is treated with different concentrations of IBA (Strydem and Hartman, 1960) [8]. Hamooh (2014) [9] and Porghorban *et al.* (2014) [10] also reported that increased the root length in olive cuttings treated with IBA. The improvement in the rooting quality increased with the treatment of IBA and it helped to increase the total fresh and dry weight of root in the cuttings of pine (Jones and Van Staden, (1997) [11] and (Thorsen *et al.* 2010) [12].

Maximum number of sprouts and shoot length in *A. vasica* cuttings treated with higher concentration of IBA in the present study might be due to better root growth which augmented absorption and translocation of nutrients from soil which take active part in various plant metabolic processes. Rooting in *A.vasica* with high concentrations of IBA might lead to advanced bud break and maximum rooting and sprouting resulted in better shoot proliferation. Moreover, the root and shoot growth are linked to endogenous levels of hormones and food materials (Husen and Pal, 2006) [13].

Effect of IBA on hardwood cuttings

The results on effect of different concentration of IBA levels on hardwood cuttings are presented in the table 3. The hardwood cuttings exhibited significant results for the number of sprouts, number of roots, root length, foliage weight, dry weight of stem and root for different concentration of IBA treatment. In hardwood cuttings, significantly highest numbers of sprouts (2.93± 0.40) were found with 100 ppm of IBA and on par with 500 ppm and 300 ppm IBA. The lowest numbers of sprouts were observed in cuttings treated with 2500 ppm IBA (1.60± 0.0). Significantly highest number of roots (4.33± 0.74) and foliage weight (28.30± 3.08) recorded with 500 ppm IBA as compared to control. Significantly maximum root length was gained by cuttings treated with 300 ppm IBA (14.00± 1.73) and the minimum root length was obtained in 2000 ppm IBA (10.33± 0.66). Similar trend has been observed for dry weight of stem where the cuttings received with 300 ppm IBA and found significantly maximum dry weight of stem (40.76± 0.28). The cuttings treated with 2000 ppm IBA had exhibited significantly maximum dry weight of root (26.53± 0.26) as compared to untreated cuttings while it was minimum in the cuttings treated with 1500 ppm of IBA (16.00± 0.83). The results were in accordance with the findings of Bojja *et al.* (2018) [14] in *Terminalia arjuna*. In *A. vasica*, Gnanamani and Panneerselvam (2015) [16] reported that IBA treated cuttings showed highest number of roots, root length, root hairs, fresh weight and dry weight of shoots as compared to triademefon and hexaconazole treatment.

Auxin promotes the starch hydrolysis and the mobilization of sugars and nutrients at the base of the cuttings during the regeneration of adventitious roots (Husen and Pal, 2006) [13]

Auxin promotes the starch hydrolysis and the mobilization of sugars and nutrients at the base of the cuttings during the regeneration of adventitious roots (Husen and Pal, 2006) [13]

Conclusion

Among different concentration of IBA levels and three types of cuttings i.e. soft wood, semi hardwood and hard woodcuttings; semi hardwood cuttings exhibited significant response with treatment of 2500 ppm of IBA for shoot length (43.53± 5.31), fresh weight of plant (35.66± 2.33), fresh root weight (36.10± 1.06), dry weight of stem (30.93± 0.69) and dry weight of root (21.33± 0.40). Further, it's suggested that semi hardwood cuttings with application of 2500 ppm IBA can be used for mass multiplication of *A. vasica*.

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Fig 1: Propagation of *Adhatoda vasica* under polyhouse

Fig 2: Rooting of semihard wood cuttings of *Adhatoda vasica*

Fig 3: Rooting of semi hardwood cuttings treated with 2500 ppm IBA

Table 1: Effect of different concentrations of IBA on soft wood cuttings of *Adhatoda vasica*

Treatment	Shoot length	Fresh weight of plant	No. of sprouts	No. of roots	Root length	Root weight	Foliage weight	Dry weight of stem	Dry weight of root
100 ppm	45.33± 1.60	23.66± 0.66	1.20± 0.11	6.93± 1.56	15.54± 2.80	16.13± 1.94	44.13± 7.71	16.66± 0.17	7.76± 0.65
200 ppm	43.13± 3.06	22.33± 2.02	1.06± 0.17	9.46± 0.74	20.79± 1.33	15.03± 3.72	36.66± 0.83	9.73± 1.47	7.43± 0.59
300 ppm	42.73± 1.90	20.00± 1.15	0.80± 0.30	12.66± 0.63	18.94± 2.04	20.20± 4.55	31.13± 5.81	12.23± 0.95	11.30± 0.29
400 ppm	34.53± 0.99	15.33± 1.85	0.60± 0.20	16.53± 1.39	13.60± 1.02	14.53± 1.80	14.70± 2.02	11.53± 0.72	11.80± 0.75
500 ppm	36.80± 4.10	17.66± 4.70	0.13± 0.06	13.00± 0.57	11.94± 2.17	11.03± 1.08	11.33± 1.39	8.70± 0.20	5.80± 0.05
1000 ppm	39.60± 3.47	20.33± 1.45	0.20± 0.11	9.60± 1.74	16.58± 3.52	24.33± 3.96	19± 6.97	7.33± 0.51	13.86± 0.80
1500 ppm	42.13± 1.65	26.00± 1.52	0.33± 0.17	6.33± 1.18	13.64± 3.01	22.03± 2.52	21.43± 2.80	9.63± 0.55	11.53± 1.25
2000 ppm	37.73± 1.04	24.66± 1.20	0.13± 0.06	7.93± 0.35	12.88± 1.36	20.03± 4.77	25.26± 5.00	10.16± 0.48	11.26± 0.29
2500 ppm	46.86± 4.43	23.33± 2.18	0.20± 0.11	8.33± 1.31	15.04± 1.52	20.93± 2.43	15.56± 5.89	8.56± 0.61	15.56± 0.43
Control	40.66± 3.33	18.00± 1.52	0.00± 0.00	0.86± 0.86	2.02± 2.02	8.13± 8.13	14.90± 7.93	7.40± 0.83	5.30± 0.26

Values are means (n = 5) ± SE according to Duncan's multiple range test ($P \leq 0.05$).

Table 2: Effect of different concentrations of IBA on semi hardwood cuttings of *Adhatoda vasica*

Treatment	Shoot length	Fresh weight of plant	No. of sprouts	No. of roots	Root length	Root weight	Foliage weight	Dry weight of stem	Dry weight of root
100 ppm	41.06± 3.16	28.53± 2.93	3.33± 0.26	5.40± 0.40	14.07± 1.45	15.13± 3.24	30.43± 0.87	19.06± 0.12	7.63± 0.56
200 ppm	34.53± 1.24	23.86± 1.27	2.73± 0.13	6.73± 1.43	11.40± 1.44	18.66± 2.25	16.23± 3.33	25.30± 0.97	10.43± 0.27
300 ppm	38.33± 2.21	31.66± 1.59	2.20± 0.20	5.20± 1.17	13.65± 2.19	24.93± 1.31	28.06± 4.18	24.76± 0.92	13.90± 0.45
400 ppm	40.93± 1.35	30.13± 2.99	2.13± 0.06	6.00± 0.46	16.26± 0.93	25.66± 0.71	25.50± 3.25	22.36± 0.60	12.80± 0.25
500 ppm	33.4± 3.91	23.46± 1.04	2.20± 0.30	4.26± 0.52	11.14± 1.03	18.63± 4.83	16.80± 7.34	20.20± 2.42	9.36± 0.27
1000 ppm	32.46± 2.26	26.86± 0.29	2.13± 0.26	4.93± 0.37	13.38± 1.15	32.76± 3.55	12.26± 1.38	21.13± 1.44	19.63± 0.79
1500 ppm	34.26± 1.04	22.66± 1.90	1.73± 0.13	3.60± 0.91	12.83± 1.16	23.06± 1.31	12.66± 2.20	21.60± 0.26	12.10± 1.96
2000 ppm	35.73± 1.62	26.13± 2.19	2.40± 0.11	5.66± 0.52	15.14± 0.45	22.00± 3.04	17.50± 2.78	22.40± 0.56	11.03± 0.56
2500 ppm	43.53± 5.31	35.66± 2.33	2.33± 0.06	6.00± 0.30	13.94± 1.57	36.10± 1.06	25.23± 1.60	30.93± 0.69	21.33± 0.40
Control	9.75± 9.75	22.52± 0.65	1.33± 0.68	1.46± 1.46	2.33± 2.33	9.20± 9.20	10.76±10.76	24.93± 0.20	5.56± 0.07

Values are means (n = 5) ± SE according to Duncan's multiple range test ($P \leq 0.05$).

Table 3: Effect of different concentrations of IBA on hardwood cuttings of *Adhatoda vasica*

Treatment	Shoot length	Fresh weight of plant	No. of sprouts	No. of fibrous root	Root length	Root weight	Foliage weight	Dry weight of stem	Dry weight of root
100 ppm	43.80± 2.42	29.13± 2.25	2.93± 0.40	3.00± 0.20	11.00± 1.00	27.60± 0.76	24.00± 3.93	27.93± 0.52	13.86± 0.86
200 ppm	41.20± 1.60	35.66± 4.25	2.60± 0.11	3.33± 0.24	11.33± 0.33	35.00± 5.81	22.33± 4.37	36.20± 0.50	17.53± 1.20
300 ppm	41.00± 1.79	36.06± 4.06	2.53± 0.17	4.00± 0.52	14.00± 1.73	38.03± 8.59	17.46± 4.79	40.76± 0.28	20.86± 0.51
400 ppm	44.26± 3.30	36.73± 3.23	2.20± 0.11	4.06± 0.43	12.66± 1.45	44.60± 2.33	14.00± 1.76	38.06± 0.27	19.46± 0.34
500 ppm	35.33± 5.02	30.20± 4.40	2.26± 0.17	4.33± 0.74	11.66± 1.85	32.06± 3.38	28.30± 3.08	24.30± 0.55	16.60± 0.47
1000 ppm	40.73± 1.79	30.13± 3.03	1.86± 0.17	3.33± 0.17	10.66± 0.88	39.23± 2.72	23.20± 3.68	33.96± 0.96	19.16± 0.73
1500 ppm	36.46± 1.62	31.53± 3.74	1.93± 0.29	4.00± 0.20	12.00± 1.00	31.83± 2.11	15.33± 0.31	29.33± 0.50	16.00± 0.83
2000 ppm	36.00± 1.50	37.46± 5.44	2.20± 0.23	2.33± 0.46	10.33± 0.66	50.03± 5.23	15.70± 2.55	37.10± 0.95	26.53± 0.26
2500 ppm	34.13± 2.36	33.46± 4.50	1.60± 0.0	3.53± 1.07	13.00± 1.00	39.13±13.38	16.93± 3.31	27.63± 0.89	22.76± 0.86
Control	37.25± 1.42	39.66± 1.64	2.08± 0.08	1.00± 1.00	1.66± 1.66	18.16±18.16	6.80± 6.80	25.36± 0.03	3.96± 0.29

Values are means (n = 5) ± SE according to Duncan's multiple range test ($P \leq 0.05$).

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