International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(3): 565-570 September 2014
DOI Number: 10.5958/2230-732X.2014.01361.8

Horticulture

Standardization of Propagation through cuttings in *Salacia fruticosa* Heyne ex Lawson: A Medicinal Plant Endemic to Western Ghats

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Paper No. 242 Received: June 23, 2014 Accepted: August 23, 2014 Published: September 18, 2014

Abstract

Salacia fruticosa Heyne ex Lawson, one of the red listed medicinal plants belonging to the family Celastraceae which is categorised as threatened by IUCN due to unsustainable and indiscriminate harvesting for commercial purposes. Traditional propagation techniques cannot cater to large scale planting stock production of this valuable species due to poor fruit set and seed germination hence the vegetative propagation has to be resorted to meet the planting stock requirement. Present investigation was carried out at Kerala Forest Research Institute to standardize the vegetative propagation protocol of *S. fruticosa* through stem cuttings with growth regulators in different season. Semi hard wood and hardwood cuttins were collected in three seasons (January-April, May-August and September- December) and treated with Indole Butyric Acid and Naphthalene Acetic Acid in different concentrations. Rooting response was measured after two weeks in the mist chamber. The adventitious rhizogenesis of *S. fruticosa* stem cuttings was influenced by type of cutting, season of collection, type of growth regulator and its concentration. Semi hardwood leafy cuttings, treated with 3000 mg/l of IBA during January – April was the successful method for vegetative propagation (80% rooting) to produce the planting stock. Significant increase in number of new leaves, root length also recorded for the same treatment. Hence, we recommend the same treatment combination for the large scale planting production in *S. Fruticosa*.

Highlights

The adventitious rhizogenesis of *S. fruticosa* depends on type of cutting, season of collection, type of growth regulator and its concentration

Keywords: *Salacia fruticosa,* Vegetative propagation, IBA, NAA

Medicinal plants are the local heritage of great global significance and the world is blessed with rich wealth of medicinal plants. *Salacia fruticosa* Heyne ex Lawson (Eakanayakam, Ponkarandi), one of the red listed medicinal plants belonging to the family Celastraceaes is a climbing shrub, distributed in South–West India, Ceylon, Java, Thailand and Philippines. Within India, it is mainly distributed in Karnataka, Kerala and Orissa and its is endemic to Western Ghats. In traditional system of medicine, the plants of this genus are being used as acrid, bitter, thermogenic, urinary, astringent, anodyne, anti-inflammatory, depurative, emmenagogue, vulnerary, liver

tonic and stomachic. They are useful in vitiates conditions of vata, diabetes, hemorrhoids, inflammation, leucorrhoea, leprosy, skin diseases, amenorrhoea, dysmenorrhoea, wounds, ulcers, hyperhydrosis, hepatopathy, dyspepsia, flatulence, colic, and spermatorrhoea (Padmaa *et al.,* 2008). It is one among the list of medicinal plants with proven antidiabetic and related beneficial effects and of herbal drugs used in treatment of Diabetes (Chakravarty and Kalita, 2011). It has characteristic golden coloured roots. The root and seeds has anti- diabetic properties.

S. fruticosa is facing serious threats to its survival in the wild along with habitat wipe out due to extensive extraction due to its medicinal uses. The large scale cultivation has to be initiated to meet the growing demand of industries, to conserve natural population and to facilitate availability of standard raw material to regulate market supply in the long run. Although, propagation of *S. fruticosa* through seeds is the cheapest method, due to a low rate of fruit set, and/or poor seed germination vegetative propagation methods can only cater to meet the requirements of the planting stock production on a large scale (Nalawade *et al.,* 2004). Hence, the present study was conducted at Kerala Forest Research Institute to standardize the vegetative propagation protocol of *S. fruticosa* through stem cuttings with growth regulators in different seasons.

Materials and Methods

Branch cuttings of mother plants were collected from the medicinal plant garden, KFRI campus, Peechi. The collections were made during January-April (Season I), May-August (Season II) and September- December (Season III). Hardwood and semi hardwood leafy shoot cuttings having a length of 15 cm with 2 pairs of leaves intact were prepared. In order to minimize the transpiration rate, the area of leaflets of compound leaves was trimmed to two-third, retaining the apical bud intact. To prevent fungal attack, cuttings were treated with 0.05% aqueous solution of Bavistin for 45 minutes. Both hard wood and semi-hard wood cuttings were treated with various concentrations (500 to 8000 mg/l) NAA (Naphthole Acetic Acid, make SRL) and IBA (Indole Butyric Acid, make Merk) prepared in talc by quick dip method (Table 1). The treated cuttings were inserted immediately to the vermiculite (rooting medium) in root trainers and kept inside the mist chamber. Temperature inside the mist chamber was maintained at 28 ± 2 °C and relative humidity at 92%. Regular misting for 10 seconds was carried out at half an hour intervals. Cuttings started to sprout and root within a period of two weeks and the observations on number of rooted cuttings in each tray and number of roots per rooted cuttings were recorded at this stage. The observations were also made on the number of new leaves and root, and root length. There were three replications per treatment combination and each replication contained 12 cuttings.

Table 1. Different concentrations of IBA and NAA tried in the rooting of cuttings of *Salacia fruticosa*

Treatment	IBA (mg/l)	Treatment	NAA (mg/l)
1	500	14	500
2	600	15	600
3	700	16	700
4	800	17	800
5	900	18	900
6	1000	19	1000
7	2000	20	2000
8	3000	21	3000
9	4000	22	4000
10	5000	23	5000
11	6000	24	6000
12	7000	25	7000
13	8000	26	8000

Statistical analysis

Uni-variate analysis of variance was carried out in SPSS 16 for Windows taking type of cutting, collection time, growth regulators and their concentration as independent variable and rooting attributes as dependent variable.

Results and Discussion

In general, *S. fruticosa* is a difficult to root species as the root induction was poor in most of the treatment combinations. However, the best treatment combination could produce up to 80% rooting. Although lower concentrations of IBA and NAA were tried initially (500-1000 mg/l) the rooting was observed only between 2000 to 5000 mg/l. The higher concentrations (6000-8000 mg/l) of IBA and NAA also were unable to induce

rooting. Hence, data for 2000-5000 mg/l were taken for analysis and the results as follows. Analysis of variance revealed significant difference in rooting parentage, number of new leaves and roots and length of roots due to interaction effect of type of cutting x season x growth regulator x concentration at one per cent level.

With regards to individual effects of cutting, season, growth regulator and its concentration the rooting response was significant. Of the two type of cutting, semi hardwood cuttings recorded a higher rooting percentage (based on observed means) and among three collection times, season I and III recorded the highest rooting percentage. Of the two growth regulators IBA was superior to NAA. With regard to concentration, the order of rooting was 3000 mg/l > 4000 mg/l > 5000 mg/l >2000 mg/l.

The highest rooting (80%) was achieved in semi hardwood leafy cuttings treated with 3000 mg/l IBA in season I, followed by 4000 mg/l IBA treated cuttings in season III (60%). The semi hardwood cuttings treated with IBA 5000 mg/l in season I also recorded 50 percent rooting. Although rooting was poor in hardwood cuttings, a maximum of 35% occurred during season 1 on treating with IBA 3000 mg/l followed

Table 2. The rooting attributes of *Salacia fruticosa* as affected by type of cutting, collection time, growth regulator and its concentration.

Cutting	Season	Growth regulator	Concentration (mg/l)	Rooting%	No. of leaves	No. of roots/ plant	Root length (cm)
Semi hardwood	1	IBA	2000	40.00±2.00	0.16±0.03	0.25±0.03	0.73±0.03
			3000	80.00±1.00	2.41±0.28	3.25±0.26	13.08±0.16
			4000	50.00±3.00	0.25±0.03	0.83±0.02	2.82±0.03
			5000	25.00±1.00	0±0.	0.16±0.02	0.30±0.02
		NAA	2000	8.00±1.00	0±0	0.16±0.01	0.35±0.01
			3000	25.00±1.00	0.08±0.02	0.66±0.03	1.71±0.11
			4000	20.00±2.64	0.16±0.02	0.58±0.01	1.43±0.12
			5000	12.00±2.64	0.16±0.03	0.41±0.03	1.20±0.10
	2	IBA	2000	10.00±1.00	0.41±0.04	0.66±0.02	2.08±0.04
			3000	20.30±3.20	1.00±0.50	2.00±1.73	7.20±0.26
			4000	15.00±1.00	0.58±0.20	1.10±0.10	3.75±0.25
			5000	5.00±1.00	0.25±0.02	0.16±0.01	0.15±0.01
		NAA	2000	2.33±1.15	0.16±0.02	0.25±0.04	0.51±0.02
			3000	10.00±1.00	0.50±0.05	1.00±0.50	3.75±0.50
			4000	12.00±2.00	0.58±0.02	1.33±0.10	4.25±0.50
			5000	5.66±0.57	0.25±0.03	0.33±0.07	0.88±0.07
	3	IBA	2000	30.00±2.64	0.08±0.01	0.33±0.01	1.09±0.02
			3000	60.00±3.00	1.25±0.02	0.75±0.05	2.82±0.11
			4000	40.00±1.00	0.33±0.01	0.66±0.02	2.05±0.11
			5000	20.00±2.00	0.08±0.02	0.25±0.02	0.50±0.10
		NAA	2000	5.00±1.00	0.08±0.01	0.33±0.02	0.65±0.03
			3000	20.00±2.64	0.41±0.01	1.25±0.13	2.77±0.06
			4000	15.30±2.10	0.25±0.02	0.41±0.01	2.24±0.04
			5000	15.00±2.00	0.25±0.01	0.58±0.05	1.80±0.10

Hardwood	1	IBA	2000	5.00±2.00	0±0	0.16±0.02	0.40±0.20
			3000	35.00±1.00	0.41±0.02	0.83±0.02	1.83±0.11
			4000	25.00±2.00	0.33±0.03	0.66±0.04	1.41±0.14
			5000	20.00±3.60	0.08±0.02	0.33±0.03	0.82±0.06
		NAA	2000	5.00±1.00	0±0	0.08±0.01	0.20±0.10
			3000	18.00±1.00	0±0	0.16±0.02	0.47±0.02
			4000	13.00±1.00	0±0	0.16±0.01	0.31±0.05
			5000	5.00±0.57	0±0	0.16±0.02	0.25±0.05
	2	IBA	2000	5.00±1.00	0±0	0.08±0.01	0.12±0.02
			3000	10.00±1.00	0.08±0.01	0.66±0.02	1.19±0.07
			4000	10.00±1.00	0±0	0.41±0.026	0.72±0.06
			5000	5.00±1.00	0±0	0.33±0.02	0.65±0.05
		NAA	2000	5.00±0.06	0±0	0.16±0.02	0.36±0. 02
			3000	5.00±1.00	0±0	0.25±0.03	0.41±0.04
			4000	5.00±1.00	0±0	0.16±0.03	0.24±0.03
			5000	5.00±2.00	0±0	0.16±0.04	0.20±0.10
	3	IBA	2000	5.00±1.00	0±0	0.08±0.01	0.24±0.01
			3000	33.00±3.00	0±0	0.25±0.04	0.41±0.03
			4000	18.00±1.00	0±0	0.16±0.04	0.36±0.04
			5000	10.00±1.00	0±0	0.16±0.02	0.24±0.02
		NAA	2000	5.00±1.00	0±0	0.08±0.01	0.21±0.04
			3000	15.00±1.00	0±0	0.41±0.01	1.17±0.05
			4000	7.00±1.00	0±0	0.16±0.01	0.30±0.10
			5000	8.00±1.00	0±0	0.16±0.02	0.33±0.04

by those treated with IBA 3000 mg/l in season III. Most of the treatment combinations produced new leaves on both semi-hardwood and hardwood cuttings. Among which the highest number of leaves was recorded for those treated with IBA 3000 mg/l (2.41) in season I followed by 3000 mg/l (1.25) in season III. With regards to number of roots per cutting, higher number of roots was observed in semihard wood cuttings treated with IBA 3000 mg/l in season I followed by season II and III. Highest root length was observed in semi hardwood cuttings treated with IBA 3000 mg/l in all seasons of which the first season gave maximum root length. In nutshell, the best rooting attributes were observed in the semi hardwood cuttings collected during season I treated with 3000 mg/l IBA.

Salacia fruticosa is an important medicinal plant species which has to be conserved with higher priority. As the propagation through seed is not adequate to meet the large scale planting stock requirement, vegetative propagation methods such rooting of hardwood, semi hardwood cuttings can be resorted for mass multiplication of this species. Perusal of literature indicated that the rooting response of cuttings depends on species, type of cutting, season of collection, growth regulator and its concentration etc (Hartman *et al*., 1993). Hence a species-specific protocol need to be standardised after preliminary experiments considering all these parameters before going to large scale of planting stock production. The present investigation conducted with a view to standardise a protocol for large scale production of planting stock using cuttings revealed that rooting response can be as high as 80% in this species but it was obtained only on specific treatment combinations.

The maturity of cutting played a vital role in inducing better rooting *S. fruticosa* with semi hard wood cuttings giving a significantly higher rooting as compared to hardwood cuttings. Similar results were obtained in leafy cuttings of *Saraca asoka* (Surendran, 1998) and *Oroxylum indicum* (Saumya *et al.*, 2013a). The highest of rooting (100%) was achieved in semi hardwood cuttings of *O. indicum* treated with 1000 mg/l IBA in summer season. Hence, comparison of rooting success between material taken from juvenile and mature *S. fruticosa* stem cuttings revealed that physiological age affects rooting of stem cuttings. The environmental conditions during collection like light, temperature, humidity, rainfall plays a significant role in root induction of cuttings (Hoffman, 1979; Bunce, 1984; Karaguzel, 1997). Which may be related to endogenous plant growth regulator levels or carbohydrates (Day and Loveys, 1998) collection season of cuttings played significant role on successful rooting of cuttings and the cuttings collected during the season I (during dry periods) recorded higher rooting. Similar seasonal influence was observed during root induction of *Saraca asoka*, *Oroxylum indicum, Embelia ribes* and bamboos (Surendran, 1998, Saumya *et al.,* 2013 a-b, Raveendran *et al.,* 2010 a-b). Adventitious rooting in shoot cuttings of neem (*Azadirachta indica*) and karanj (*Pongamia pinnata*) also indicated that the maximum rhizogenesis coincided with the emergence of new sprouts in February (neem) and March (karanj)

The growth regulators and their concentration significantly affected the rooting of *S. fruticosa*. The positive response of growth regulating substances such as NAA, IBA, and chemicals such as boric acid, coumarin etc on rooting has been reported in earlier works (Surendran and Seethalakshmi 1985; Sharma and Aier 1989; Zeng *et al.*, 2005). However, the root promoting effect varied with auxin concentrations and types of auxin. In the present study, cuttings treated with both IBA and NAA above 5000 mg/l failed to initiate rooting. The high auxin application is reported to produce toxicity and NAA is more toxic than IBA (Zeng and Lu 1988). The superiority of IBA in rooting of cuttings might be because IBA being an auxin, generally has distinct advantage over NAA as it is slowly destroyed by the auxin destroying enzyme linked system (Pearse 1948). Likewise Weaver (1972) suggested that, since IBA translocates poorly, it is retained near the site of application and is therefore very effective. Farooqi *et al.,* (1994) conducted an experiment on *Rosa damascena* Mill and studied the effect of IBA. They found the increasing trend of rooting percentage, number of roots per cutting, length of the longest root (cm), thickness of root (cm), fresh weight of root and dry weight of root with increasing concentration of IBA from 100 mg/l to 300 mg/l.

Conclusion

Based on the results of the present investigation we recommend that semi hardwood cuttings collected summer period treated with 3000 mg/l IBA can be used for mass propagation of this species. Further studies are to be carried out to understand the biochemical and anatomical changes during rooting and to reduce the cost of production.

Acknowledgements

We express our sincere gratitude to Dr.K.V. Sankaran, Former Director, KFRI for his valuable suggestions and encouragement. We also extend our gratitude to Dr. C.K. Somen, HOD, and Dr. K.K. Seethalakshmi, Programme Coordinator (Retd) for their whole hearted support. The valuable help rendered by Mr. T.P. John, during the course of investigation is also duly acknowledged.

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