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Standardization of Seed and Vegetative Propagation Techniques in *Saraca asoca* (Roxb.) De Wilde: An Endangered Medicinal Plant

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A B S T R A C T

Saraca asoca (Roxb.) De Wilde is one of the endangered medicinal plants of Western Ghats. An experiment was carried out to standardize seed and vegetative propagation for this very important medicinal plant. Among the different germination inducing treatments, the seed treated with GA_3 200 ppm recorded early germination (23.73), highest germination rate (2.95%), seedling vigour (6315.10), seedling height (68.40 cm), fresh weight (16.00 g) and dry weight of seedling (8.47 g). In case of vegetative propagation, hard wood cuttings and air layering was carried out. Cuttings treated with IBA2000 ppm had a significant positive effect on the percentage of rooting (33.41), sprouting (33.70) and number of days taken for sprouting (23.00). In air layering branches treated with IBA 2500 ppm had a significant influence on rooting (88.07%) number of days taken for root initiation (32.00), root diameter (3.53 mm), number of primary roots per layer (6.00), number of secondary roots per layer (40.17) and root length (13.03 cm). Results will be highly useful for large scale for large scale multiplication of the plant.

Introduction

Saraca asoca (Roxb.) De Wilde is commonly known as Sita-Ashoka or Sorrow less tree. It is an important evergreen sacred tree where bark, flowers and seeds are medicinally important. *S. asoca* is a red listed species which belongs to the family Caesalpiniaceae (Bhalerao *et al.*, 2014). It is distributed throughout India, predominantly in southern India (Western Ghats of Karnataka, Kerala and Andhra Pradesh), Odisha and Assam, and up to an altitude of 750 m above sea level in the Central and Eastern Himalaya (Patwardhan *et al.*, 2016).

It is one of the important plants of Indian system of medicine known for its variety of pharmacological activities and listed under 32 priority species of National Medicinal Plant Board (NMPB). There are several vernacular names of *S. asoca* in different languages. It is called as Kankeli in Sanskrit; Ashoka in English; Asokam in Malayalam; Vandichitrah

in Hindi; Kenkali mara, Ashokada mara in Kannada and Asogam in Tamil (Pradhan *et al.,* 2009).

S. asoca is one of the foremost plants utilized from antiquity till to date. It has high medicinal value and is used in many Ayurvedic drugs. Ayurvedic medicine manufacturers across the country use about 2,250 tons of bark and flower annually. Hindus regard this tree as highly sacred and termed as symbol of fertility. Bark is used in treatment of genitor-urinary problems, enlargement of cervical gland, thrust burning sensation, dyspepsia, piles, ulcers, menorrhagia, leucorrhoea, pimples, intestinal worms and animal poisoning (Ankur, 2015). The famous compound preparations are Ashokaristha and Ashokaghrita (Jadhav and Bhutani, 2005).

Materials and Methods

Location

The experiment was conducted during the year 2016-17 at College of Horticulture, Mudigere, Department of Plantation spices, Medicinal and Aromatic Crops and ICAR-Indian Institute of Horticultural Research, Division of Plant Genetic Resources, Hessaraghatta, Bengaluru.

Planting material

The required planting materials were collected from Indian Institute of Horticultural Research (IIHR), Bengaluru. The collected seeds were treated with mercuric chloride (0.1%) for 10 minutes, washed with water and shade dried. Seeds are subjected to 11 different pre-soaking treatments in three replications and complete randomized design was followed. In vegetative propagation stem cuttings and air-layering was done. Stem cuttings were prepared by taking the middle portion of the stem with a length of 15.00-20.00 cm without any branches and leaves with 2-3 nodes per cutting and uniform thickness. The basal portion of the cutting was given a slant cut and lower node of the cutting was planted in the polybag. Cuttings were treated with 13 different growth regulators in three replications and complete randomized design was followed. The experiment was carried out in polyhouse under partial shade condition (50% shade). This structure helps in maintaining the higher temperature and relative humidity (An average temperature of 29^0C; relative humidity of 75% and light intensity of 3500 lux was recorded inside the poly house during the period of experimentation), which in turn increases seed germination and also increases rooting in the cuttings. The data were analysed statistically as per the method suggested by Panse and Sukhatme (1985).

In air layering healthy, well matured uniform with vigorous growth mother plants were selected. On these plants previous year growth matured shoots of about pencil thickness were selected at random for air layering. Air layering was done by girdling the pencil thickness shoots of one year, about 25.00 to 30.00 cm from the top of the shoot. A complete ring with 2.50 cm width strip of bark was removed by making two cuts. The continuity of cambial layer was disrupted by gently rubbing of exposed wood portion, the required concentration of growth regulators were smeared on girdled portion of stem with soft cotton, as per the treatments then dried for few seconds.

The girdled portion was covered with sterilized moist sphagnum moss and coco peat and secured firmly with a polythene sheet and both the ends were tied firmly using thread in field. Air layered branches were treated with 13 different growth regulators in three replications and randomized complete block

design was followed. The data were analysed statistically as per the method suggested by Panse and Sukhatme (1985).

Results and Discussion

The data pertaining to various germination and growth parameters as influenced by different growth regulators for seed propagation of *S. asoca* are presented in Table 1.

There was significant influence of treatments on germination and growth parameters such as days to initiate germination, rate of germination, germination percentage, seedling vigour, seedling height (cm), fresh weight (g) and dry weight of seedling (g). The growth regulator treatment increased the overall germination and growth parameter values as compared to control. Significantly minimum days to initiate germination (23.73), rate of germination (2.95) and germination percentage (92.33) was registered in $GA_3$200ppm which was followed by $GA_3$100 ppm (Table 1).

This may be due to instigative action of GA_3 for germination of seeds. GA_3 induces the *de-novo* synthesis of proteolytic enzymes like a-amylase and ribonuclease. Amylases in turn hydrolyse starch in the endosperm, providing the essential sugars for the initiation of growth processes (Copeland and Mc-Donald, 1995). GA_3 treatment is also known to overrule the photo dormancy, thermo-dormancy, dormancy imposed by incomplete embryo development, mechanical barriers and presence of germination inhibitors (Diaz and Martin, 1971).

Among different growth regulator treatments maximum seedling vigour (6315.10), seedling height (68.40 cm), fresh weight (16.00 g) and dry weight (8.47 g) of seedling was also recorded in seeds treated with GA_3 200ppm when compared to control (Table 1). Similar results were also obtained by Masoodi and Masoodi (2000) for *Ulmus wallichiana*, an endangered tree species. This is also due to the effect of gibberellic acid in inducing the formation of hydrolytic enzymes which in turn might have increased carbohydrates accumulation thereby increasing the fresh weight and dry weight of plant (Bhattacharjee *et al.,* 1994).

Lalithkumar (2008) obtained higher germination per cent of 77. 9, 74. 9, 82. 0 and 71. 0 per cent against control (51. 0, 43. 0, 38. 0 and 31. 9 per cent), in tulsi, ashwagandha, periwinkle and kalmegh, respectively, when the seeds were treated with GA_3 at 250 ppm. This may be due to GA3 role in cell division and cell enlargement and are largely controlled by endogenous level of gibberellic acid which has been proved in number of crops. The increased cell division and cell elongation reflected in increased plant height was observed in hybrid lilies (Gorden *et al.*, 1980).

The data pertaining to various growth parameters as influenced by different growth regulators for cuttings propagation of *S. asoca* are presented in Table 2.

There was significant influence of different treatments on parameters such as days taken for sprouting of stem cuttings, number of sprouts per cutting, sprouting and rooting percentage.

Cuttings treated with IBA showed good results for all the parameters as compared to control. It is well known that auxins are associated with the division and elongation of meristematic cells and has been attributed to enhanced transport of carbohydrates to the base of the cuttings and also increase the activity of hydrolyzing enzymes and there by consequently stimulate rooting (Ali *et al.*, 2008).

Fig.1 Best treatment for germination of *Saraca asoca* seeds (a. Early germination in $GA_3$200 ppm b. GA_3 200 ppm treated seedlings at 120 DAS)

Fig.2a Root initiation in *Saraca asoca* cuttings

Fig.2b Effect of IBA 2000 ppm on sprouting and rooting of *Saraca*

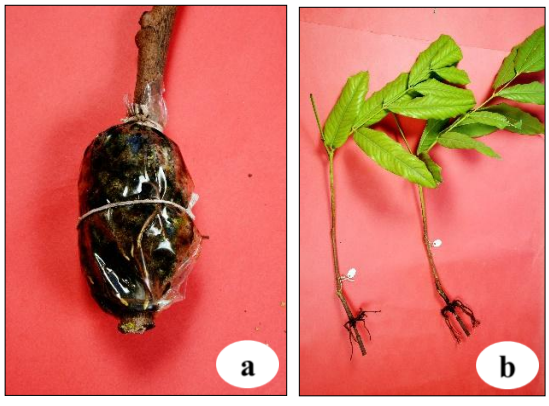

Fig.3a a. Root initiation in air layers b. Effect of IBA 2500 ppm on rooting of *Saraca asoca* air layers at 45 days

Fig.3b Effect of IBA 2500 ppm on rooting of *Saraca asoca*air layers at 60 days

Table.1 Effect of different pre sowing treatments on growth parameters as influenced in *Saraca asoca* (Roxb.) De Wilde seeds

Treatment	Days to initiate germination	Rate of germination (%)	Germination (%)	Seedling height (cm)	Seedling vigour	Fresh weight of seedling (g)	Dry weight of seedling (g)
T_1 – Control	56.60	0.60	43.44 (41.23)	38.20	1659.43	4.13	1.86
T_2 - GA_3 100 ppm	25.13	2.63	90.11 (71.66)	63.26	5700.13	14.20	7.10
T_3 - GA_3 200 ppm	23.73	2.95	92.33 (73.92)	68.40	6315.10	16.00	8.47
T_4 - KNO_3 0.5%	52.47	1.05	58.00 (49.60)	42.86	2485.57	5.67	2.83
T_5 - KNO_3 1.0%	48.93	1.26	63.22 (52.66)	46.48	2938.20	6.80	3.26
T_6 – H_2SO_4 1.0%	38.40	2.06	83.00 (65.66)	54.23	4501.17	11.00	5.50
T_7 - H_2SO_4 2.0%	36.20	2.27	85.22 (67.38)	57.37	4888.60	12.13	6.35
T_8 –HCl 1.0%	39.40	1.51	76.44 (60.96)	49.87	3811.67	8.70	4.36
T_9 - HCl 2.0%	37.13	1.88	80.56 (63.84)	52.30	3997.70	10.27	5.10
T_{10} – Ascorbic acid 500 ppm	42.00	1.47	72.74 (58.52)	49.20	3578.57	8.27	3.94
T_{11} – Ascorbic acid 1000 ppm	40.40	1.71	78.00 (62.04)	52.36	4083.53	9.90	4.93
Mean	40.04	1.76	74.82 (59.66)	52.23	3996.33	9.73	4.88
S. Em ±	0.22	0.04	0.45	0.42	17.37	0.21	0.13
C D @ 5%	0.63	0.11	1.32	1.22	50.95	0.62	0.39

Table.2 Effect of different growth regulator treatments on growth parameters as influenced in *Saraca asoca* (Roxb.) De Wilde cuttings

Treatment	Days taken for sprouting of stem cuttings	Number of sprouts per cutting	Sprouting percentage	Rooting percentage
T_1 –Control	0.00	0.00	0.00 (0.46)	0.00 (0.46)
T_2 –IBA 250 ppm	28.33	0.83	20.43 (26.90)	17.30 (24.60)
T_3 – IBA 500 ppm	26.67	1.00	23.30 (28.90)	20.27 (26.80)
T_4 – IBA 1000 ppm	26.00	1.16	26.10 (30.70)	24.29 (29.50)
T_5 _ IBA 1500 ppm	25.33	1.16	30.06 (33.20)	29.04 (32.60)
T_6 – IBA 2000 ppm	23.00	1.33	33.70 (35.50)	33.41(35.30)
T_7 _ NAA 50 ppm	0.00	0.00	0.00 (0.46)	0.00 (0.46)
T_8 _ NAA 100 ppm	0.00	0.00	0.00 (0.46)	0.00 (0.46)
T_9 _ NAA 150 ppm	0.00	0.00	0.00 (0.46)	0.00 (0.46)
T_{10} _ NAA 200 ppm	0.00	0.00	0.00 (0.46)	0.00 (0.46)
T_{11} _ NAA 50 ppm + IBA 250 ppm	31.00	0.66	18.73 (25.60)	14.20 (22.10)
T_{12} _ NAA 100 ppm + IBA 500 ppm	28.00	0.83	21.70 (27.80)	16.24 (23.80)
T_{13} _ NAA 150 ppm + IBA 1000 ppm	26.33	1.00	24.33 (29.60)	22.25 (28.10)
Mean	16.51	0.61	15.26 (23.20)	13.62 (21.80)
S. Em ±	0.29	0.12	0.49	0.55
C D @ 5%	0.85	0.36	1.43	1.59

Table.3 Effect of different growth regulator treatments on growth parameters as influenced in *Saraca asoca* (Roxb.) De Wilde air layering

Treatment	Days taken for root initiation	Rooting percentage	Root diameter (mm)	Number of primary roots	Number of secondary roots	Root length
T_1 –Control	40.33	64.40 (53.36)	2.17	1.64	13.57	5.17
T_2 –IBA 500 ppm	36.67	81.33 (64.43)	2.73	3.30	24.10	9.23
T_3 – IBA 1000 ppm	34.40	84.67 (66.96)	3.07	4.50	26.40	11.42
T_4 – IBA 1500 ppm	34.77	85.27 (67.45)	3.09	4.83	27.60	11.60
T_5 _ IBA 2000 ppm	33.33	86.03 (68.02)	3.17	5.43	32.07	12.10
T_6 – IBA 2500 ppm	32.00	88.07 (69.81)	3.53	6.00	40.17	13.03
T_7 _ IBA 3000 ppm	33.83	85.57 (67.68)	3.10	4.90	28.27	11.73
T_8 _ NAA 100 ppm	38.50	73.03 (58.72)	2.48	2.53	19.20	7.67
T_9 _ NAA 200 ppm	38.27	76.20 (60.81)	2.62	2.72	22.13	8.53
T_{10} _ NAA 300 ppm	39.07	71.33 (57.63)	2.43	2.23	17.50	7.37
T_{11} _ NAA 100 ppm + IBA 2000 ppm	35.30	82.20 (65.04)	2.94	3.92	26.27	10.17
T_{12} _ NAA 200 ppm + IBA 2500 ppm	34.93	84.33 (66.70)	3.06	4.33	27.10	11.20
T_{13} _ NAA 300 ppm + IBA 3000 ppm	36.30	81.60 (64.61)	2.82	3.60	25.00	9.47
Mean	35.98	80.31 (63.55)	2.86	3.84	25.34	9.90
S. Em ±	0.23	0.72	0.10	0.19	0.70	0.36
C D @ 5%	0.68	2.10	0.30	0.55	2.05	1.06

Parameters like number of days taken for sprouting (23.00), number of sprouts per cutting (1.33), sprouting (33.70%) and rooting (33.41%) was also found to be maximum in cuttings treated with IBA 2000 ppm. Similar results were obtained in *Embelia tsjeram-cottam* cuttings treated with IBA 2000 ppm and there was significant increase in rooting percentage (42.00%) and sprouting percentage (35.80%) against the control (Sharma *et al.,* 2010). Yashaswini *et al.,* (2011) reported that a rare medicinal plant species *Premna integrifolia* propagated by hard wood cuttings showed higher rooting percentage (86.00%) when cuttings treated with 2000 ppm IBA against control (57.66%).Husen (2003) reported that *Rauvolfia serpentine* cuttings treated with IBA 2000 ppm showed significant increase in rooting and sprouting percentage against the control. Nanda and Kochar (1985) found that some group of plants including most fruit and nut species and many woody plant rich in tannins and phenolic compounds are difficult to propagate by cuttings because of poor rooting and the same was true even in case of *S. asoca.*

The data pertaining to various rooting parameters as influenced by different growth regulators for air layering propagation of *S. asoca* are presented in Table 3.

Parameters like rooting (88.07%) number of days taken for root initiation (32.00), root diameter (3.53 mm), number of primary roots per layer (6.00), number of secondary roots per layer (40.17), root length (13.03 cm) was found to be maximum in air layers treated with IBA 2500 ppm. Similar results were obtained in litchi air layers treated with 2500 ppm of IBA showed maximum number of roots (9.94), root length (10.94 cm), number of leaves (10.55) when compared to control (Rahman *et al.,* 2000). Good success on rooting and root characteristics of air layering in *Chebulic mycobalum* has been recorded by Misra and Jaiswal (1994) after treatments with IBA. IBA has been found to stimulate root initiation in air layers of many plant species like *Carissa carandas* and *Dalbergia sissoo* (Puri and Nagpal, 1988). Kunal and Syamal (2005) reported that shoots treated with IBA 3000 ppm influenced maximum number of primary roots (14.80), number of secondary roots (10.72), length of the primary root (11.30 cm), diameter of the roots (2.20 mm), rooting (93.34%) and survival percentage of air layers in guava (75.90) followed by NAA at 2000 ppm and lest was observed in control.

The results presented in the paper constitute the study on seed and vegetative propagation of *Saraca asoca* by cuttings and air layering. The success of germination in seed (90%) and among vegetative propagation, rooting success of cuttings was very meagre, however, air layering was best over cuttings by producing (88.07%) rooting.

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