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Asexual propagation of *Spondias pinnata* Linn. as influenced by cutting length and sucrose concentration

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ABSTRACT

An experiment was conducted to study the effect of different cutting length (10, 15 and 20 cm) in combination with sucrose treatment (15, 30, 45 and 60 g sucrose l^{-1} of water, and control) for better rooting and performance of Spondias pinnata. The cuttings were treated with various concentrations of sucrose separately each for 1 hour. The crop performance was better in terms of the earliest bud (8.88 DAP) and leaf (12.11 DAP) initiation, highest cuttings success (83%), shoot length (27.80 cm), root length (15.08 cm), collar diameter (22.22 mm), root number (23.53), root diameter (3.06 mm), fresh shoot weight (40.28 g), fresh leaf weight (15.69 g), dry shoot weight (20.68 g), dry root weight (4.65 g) and total biomass (31.31 g $plant^{-1}$) with the use of cuttings (15 cm) treated with sucrose solution (15 g l^{-1}), compared with control.

***Keywords*:** Cutting length, spondias pinata, sucrose, vegetative propagation

Spondias pinnata Linn. (Family: Anacardiaceae), locally known as 'Amra', is a potential minor fruit crop and sparsely distributed in all rural villages and forests of India. It is an aromatic and fast growing deciduous tree, living in 550-1,500 m mean sea level. The fruit of this species is greatly appreciated in the tropics because of its taste and nutritional value. Also it possesses high medicinal value. The raw fruits are mostly consumed and sold in local markets, serving a great food diet to the people. Different processed products like jellies, sauces and chatni *etc*. can be prepared from both the fresh and dried fruits. It is a rich source of different minerals and vitamin C (Campbell and Sauls, 1994). Its fruit contains food energy 189-203 kcal g^{-1}, crude fat 12.23-12.54%, total carbohydrate 16.30-23.54%, sodium 0.96-1.38%, calcium 0.15-0.93% and iron 1.3-1.5% (Andola and Purohit, 2010). Traditionally, this species plays a pivotal role in meeting the nutritional requirements of the tribal populations for providing different nutrients for human body. However, among different species, amra has not been fully domesticated as evidenced from great morphological diversity. It is conventionally propagated through seeds. Deer, monkeys and squirrels eat the fruit, leading to a low germination percentage (Purohit, 2006). It is also damaged by birds and insects during germination. Its propagation is not sustaining the fast reproduction in the wild that can keep up with its increased demand (Tripathi and Kumari, 2010). Considering the increasing trend towards genetic improvement and mass multiplication of underutilized fruit crops, it is very much essential to develop economical methods of producing large scale planting materials and their large scale cultivation particularly on unproductive and barren lands. In last few years, different experiments have been done to develop suitable techniques to propagate *Spondias* sp. through cutting (Souza and Lima, 2005; Lima *et al*., 2002 and Paula *et al*., 2007) and by grafting (Espindola *et al*., 2004; Gomes *et al*., 2010). Though vegetative propagation is very useful for propagation of elite plants, but a little success has been reported by Sundriyal and Sundriyal (2001) in *Spondias axillaris*. During propagation through cuttings, auxin has been commercially used for quick rooting. But it is an expensive process, whereas the selection of better quality cuttings and their treatment with carbohydrates may be a cost-effective way and easily available. Therefore, the present study was conducted to standardize sucrose treatments along with standard cutting length for obtaining better rooting in the cuttings of *S. pinnata* for ensuring their survival and establishment.

MATERIALS AND METHODS

Healthy and uniform plant of *S. pinnata* was selected, and stem cuttings (18 mm diameter) were taken from one year old branches during rainy season in 2014. After collection and complete defoliation, they were

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treated in fungicide solution for 2-3 minutes to check any fungal attack and then washed in distilled water prior to the treatment of sucrose. After treatment these cuttings were planted in polybags filled with media mixture (sand : soil : FYM at 1 : 2 : 1). The polybags were then placed in the mist chamber with maintaining average relative humidity of 93% and temperature of 27.5^0C, and watered at two hours interval. The experiment was laid out in a two-factor factorial randomized block design with 15 treatment combinations and 3 replications having ten cuttings per replication. The treatments included three levels of cutting length *viz.*, 10 cm (C_1), 15 cm (C_2) and 20 cm (C_3), and five levels of sucrose treatments *viz*. control-no sucrose and sucrose of 15, 30, 45 and 60 g l^{-1} of water solution (represented as S_1, S_2, S_3, S_4 and S_5, respectively). Fifteen treatment combinations were T_1- C_1+S_1, T_2- C_2+S_1, T_3-C_3+S_1, T_4-C_1+S_2, T_5-C_2+S_2, T_6-C_3+S_2, T_7-C_1+S_3, T_8-C_2+S_3, T_9-C_3+S_3, T_{10}-C_1+S_4, T_{11}-C_2+S_4, T_{12}-C_3+S_4, T_{13}-C_1+S_5, T_{14}-C_2+S_5, and T_{15}-C_3+S_5.

The basal part of cuttings was dipped in different concentrations of sucrose separately, each for 1 hour (Pinto *et al*., 2011) and observed daily up to 45 days after planting (DAP). The observation on the percentage of cutting success, days taken for initiation of first bud and leaf, and number of buds were recorded. Five random seedlings from each replication were chosen at 45 DAP. Afterwards the seedlings were placed in bucket of water to clean the adhered soil particles from root and then used blotting paper to soak extra water. Length of root and shoot, collar diameter, root diameter, fresh and dry weight of shoot leaf and root, and total biomass per plant were also recorded.

Statistical Analysis System (SAS) software (version 9.3) was used for Analysis of Variance (ANOVA) for each parameter. Mean values for different parameters under different treatments were performed using Least Significant Difference (L.S.D.) test at 5% level. Shapiro-Wilk test was used to check the normality of residuals under the assumption of ANOVA. Prior to performing ANOVA, angular transformation on percentage of cutting success was carried out (Gomez and Gomez, 1983).

RESULTS AND DISCUSSION

Cutting success

It was observed that the success percentage of cutting, days taken for bud and leaf initiation, number of buds, collar diameter, shoot length, root length, root diameter and number of roots were influenced by both cutting length and sucrose concentrations, and showed significant difference among the all the treatments *i.e*. in sole and combination (Table 1). The percentage of cutting success ranged from 36.67 to 83% among the treatments. Maximum cutting success (83.00%) was observed in T_5: C_2S_2, followed by 76.67 % in T_6: C_3S_2, whereas it was minimum (36.67%) in T_1: C_1S_1. Cutting success was not satisfactory in shorter and larger size of cuttings in comparison to the medium sized cutting which exhibited maximum cutting success and sucrose of 15 g l^{-1} was sufficient for enhancing rooting and shooting. This finding was well in line with the results of Gautam *et al*. (2010), Gopale and Zunjarrao (2011) and Pinto *et al*. (2011) in *Spondias cytherea*. For rooting, certain energy was required, and for this the nutritional status of mother plants was very important. The age, genotypes and physiological status of mother plant were the factors influencing good success in the medium sized stem cuttings (Reinhard, 2003). Poor rooting success of shorter stem cuttings was reported by Good and Tukey (1956) due to improper distribution of nutrients and leaching loss of nutrients whereas the large sized cuttings contained more woody portion which might be converted into most of food materials for lignification, causing lower rooting and shooting percentage (Kochhar *et al*.,2005). The important effect of sucrose on cutting success by increasing vascular regeneration was also reported by Wilson *et al*. (1994).

Days taken for bud and leaf initiation

The data pertaining to the days taken for initiation of first bud and leaf (Table 1) showed that the bud initiation varied from 8.88 to 19.11 DAP whereas leaf initiation varied from 12.11 to 23.35 DAP. The treatment T_5: C_2S_2 (15 cm length of cutting with sucrose of 15 g l^{-1} water) took the lowest days (8.88 and 12.11 DAP) for first bud and leaf initiation, followed by 9.20 and 13.50 DAP inT_6: C_3S_2 (20 cm length with sucrose 15 g l^{-1} water) whereas the bud and leaf initiation (19.11 and 23.35 DAP) was delayed in T_1: C_1S_1 (10 cm length with no sucrose). It was inferred that the number of buds in cutting ranged from 2.1 to 7.27. The highest (7.27) and lowest (2.1) number of buds was obtained in T_5: C_2S_2 and T_1: C_1S_1, respectively. It might be due to accumulation of food reserve through addition of sucrose and dry mass which was required for bud and leaf differentiation, resulting in earlier bud and leaf formation along with number of buds.

Shoot length and collar diameter

The highest shoot length and collar diameter (27.8 cm and 22.22 mm) and the lowest shoot length (11.52 cm) and collar diameter (9.85 mm) were observed in T_5: C_2S_2 and T_1: C_1S_1, respectively, whereas the root length varied from 6.54 to 15.08 cm among the treatments. Maximum root length (15.08 cm) was exhibited in T_5: C_2S_2, followed by T_6: C_3S_2 (12.74 cm),

Table 1: Effect of cutting length and sucrose concentrations on cutting success and different growth parameters of *Spondias pinnata*

Treatments	Cutting success (%)	First bud initiation (DAP)	Number of buds	First leaf initiation (DAP)	Shoot length (cm)	Collar diameter (mm)	Root length (cm)	Number of roots	Root diameter (mm)
C_1	37.29(37.29)	19.11	2.10	23.35	11.52	9.85	6.54	3.13	1.10
C_2	46.67(43.11)	14.02	2.33	22.11	15.73	13.14	7.45	4.93	1.38
C_3	40.00 (39.23)	15.17	2.27	22.96	14.39	12.06	6.71	3.60	1.30
S. Em (±)	0.38	0.30	0.13	0.32	0.42	0.36	0.40	0.37	0.12
L.S.D. (0.05)	1.11	0.87	0.37	0.92	1.23	1.03	1.15	1.07	0.34
S_1	36.67(37.29)	19.11	2.10	23.35	11.52	9.85	6.54	3.13	1.10
S_2	71.00(57.42)	9.78	5.73	13.86	23.63	19.92	11.42	14.27	2.34
S_3	60.00(50.77)	10.86	4.70	15.62	22.85	16.42	9.78	10.67	1.96
S_4	53.33(46.89)	11.79	3.50	17.52	20.89	14.91	9.01	8.33	1.90
S_5	46.67(43.11)	13.72	2.37	20.39	16.49	13.43	7.86	5.53	1.55
S. Em (±)	0.49	0.39	0.17	0.41	0.55	0.46	0.51	0.48	0.15
L.S.D. (0.05)	1.43	1.12	0.48	1.18	1.58	1.33	1.49	1.38	0.44
T_1 (C_1S_1)	36.67(37.29)	19.11	2.10	23.35	11.52	9.85	6.54	3.13	1.10
T_2 (C_2S_1)	46.67(43.11)	14.02	2.33	22.11	15.73	13.14	7.45	4.93	1.38
T_3(C_3S_1)	40.00 (39.23)	15.17	2.27	22.96	14.39	12.06	6.71	3.60	1.30
T_4(C_1S_2)	71.00 (57.42)	9.78	5.73	13.86	23.63	19.92	11.42	14.27	2.34
T_5 (C_2S_2)	83.00 (65.65)	8.88	7.27	12.11	27.80	22.22	15.08	23.53	3.06
T_6(C_3S_2)	76.67(61.14)	9.20	5.83	13.50	25.69	22.01	12.74	17.67	2.53
T_7(C_1S_3)	60.00 (50.77)	10.86	4.70	15.62	22.85	16.42	9.78	10.67	1.96
T_8(C_2S_3)	70.00 (56.79)	10.50	5.40	14.43	23.38	19.21	10.91	14.20	2.23
T_9(C_3S_3)	63.33(52.71)	10.53	5.37	14.58	23.09	16.67	10.04	13.80	2.14
T_{10}(C_1S_4)	53.33(46.89)	11.79	3.50	17.52	20.89	14.91	9.01	8.33	1.90
T_{11}(C_2S_4)	60.00 (50.77)	11.54	4.17	17.27	21.80	16.34	9.74	9.13	1.94
T_{12}(C_3S_4)	56.67(48.85)	11.73	4.17	17.50	21.78	16.03	9.69	8.73	1.92
T_{13}(C_1S_5)	46.67(43.11)	13.72	2.37	20.39	16.49	13.43	7.86	5.53	1.55
T_{14}(C_2S_5)	53.33(46.89)	12.40	3.20	17.76	20.40	14.43	8.54	7.20	1.80
T_{15}(C_3S_5)	53.33(46.89)	12.64	2.67	18.01	18.29	14.32	8.41	7.07	1.74
S. Em (±)	0.86	0.67	0.29	0.71	0.95	0.80	0.89	0.83	0.26
L.S.D. (0.05)	2.48	1.94	0.84	2.05	2.74	2.31	2.58	2.40	0.77

Values in parentheses are angular transformed values. DAP: Days after planting

and the lowest length (6.54 cm) was in T_1: C_1S_1. Maximum number of roots (23.53) was obtained in T_5: C_2S_2 followed by T_6: C_3S_2 (17.67) whereas it was minimum (3.13) in T_1: C_1S_1. Number of root was less in shorter and larger size of cuttings as compared to the medium sized cutting where it showed the highest. Root diameter varied from 1.10 to 3.06 mm among the treatments. The treatment T_5: C_2S_2 exhibited the highest (3.06 mm) root diameter whereas the lowest diameter (1.10 mm) was in T_1: C_1S_1. The present findings were similar to the results of Okunlola (2013) and Gopale and Zunjarrao (2011). This might be due to the cause of the cell elongation by increasing the food reserve and dry mass when 15 cm length of cutting was treated with sucrose of 15 g l^{-1} water. The increment in root length might be attributed to the fact that sucrose as growth regulator enhanced the hydrolysis of carbohydrates, accumulation of metabolites at the site of application of synthesis of new proteins, cell enlargement and cell division (Strydem and Hartmen, 1960). Janick (1986) reported that the nutritional status of the plant served as an essential factor of the ability for the formation of root. The early and vigorous rooting helped the cuttings to absorb more nutrients which ultimately produced more leaves and could elongate the shoot length. For greater rooting process, the physiological status of stock plant during cutting duration was of higher importance, and sucrose was considered as an essential carbohydrate for the development of roots in stem cuttings.

Fresh and dry weight of shoot, leaves, root and total biomass

The data pertaining to fresh and dry weight of leaves along with root, shoot and total biomass (Table 2) showed significant differences (p = 0.05) among all treatments. The fresh weight of leaves, root and shoot

Table 2: Effect of cutting length and sucrose concentration on fresh, dry and total biomass of shoot, leaf and root of *Spondias pinnata*

Treatments	Fresh shoot wt. (g plant^{-1})	Fresh leaf wt. (g plant^{-1})	Fresh root wt. (g plant^{-1})	Dry shoot wt. (g plant^{-1})	Dry leaf wt. (g plant^{-1})	Dry root wt. (g plant^{-1})	Total biomass (g plant^{-1})
C_1	12.79	4.78	2.91	7.38	3.01	2.56	12.95
C_2	15.11	7.49	3.59	9.34	3.21	2.86	15.41
C_3	14.84	7.35	3.50	8.07	3.16	2.68	13.91
S. Em (±)	0.56	0.43	0.21	0.53	0.20	0.10	0.57
L.S.D. (0.05)	1.61	1.23	0.61	1.53	0.58	NS	1.66
S_1	12.79	4.78	2.91	7.38	3.01	2.56	12.95
S_2	34.61	12.12	5.65	19.54	5.37	3.77	28.68
S_3	25.57	10.16	4.68	13.34	4.22	3.27	20.83
S_4	24.11	9.41	4.35	12.05	3.99	3.19	19.23
S_5	21.21	8.15	4.00	10.81	3.69	2.99	17.49
S. Em (±)	0.72	0.55	0.27	0.68	0.26	0.13	0.74
L.S.D. (0.05)	2.08	1.59	0.78	1.97	0.75	0.38	2.14
T_1 (C_1S_1)	12.79	4.78	2.91	7.38	3.01	2.56	12.95
T_2 (C_2S_1)	15.11	7.49	3.59	9.34	3.21	2.86	15.41
T_3(C_3S_1)	14.84	7.35	3.50	8.07	3.16	2.68	13.91
T_4(C_1S_2)	34.61	12.12	5.65	19.54	5.37	3.77	28.68
T_5 (C_2S_2)	40.20	15.69	7.15	20.68	5.98	4.65	31.31
T_6(C_3S_2)	37.50	12.53	6.28	19.86	5.51	4.27	29.64
T_7(C_1S_3)	25.57	10.16	4.68	13.34	4.22	3.27	20.83
T_8(C_2S_3)	31.93	11.98	5.44	16.76	4.93	3.40	25.09
T_9(C_3S_3)	27.38	10.54	5.07	14.50	4.45	3.30	22.25
T_{10}(C_1S_4)	24.11	9.41	4.35	12.05	3.99	3.19	19.23
T_{11}(C_2S_4)	25.47	9.62	4.67	13.23	4.19	3.22	20.64
T_{12}(C_3S_4)	25.14	9.58	4.44	12.82	4.12	3.21	20.15
T_{13}(C_1S_5)	21.21	8.15	4.00	10.81	3.69	2.99	17.49
T_{14}(C_2S_5)	23.36	9.04	4.30	11.65	3.81	3.07	18.53
T_{15}(C_3S_5)	21.35	8.80	4.26	11.07	3.76	3.03	17.86
S. Em (±)	1.25	0.95	0.47	1.18	0.45	0.23	1.28
L.S.D. (0.05)	3.61	2.76	1.36	3.41	1.30	0.65	3.71

were 4.78 15.69, 2.91-7.15 and 12.79-40.20 g plant^{-1}, respectively. The treatment T_5: C_2S_2 recorded the highest (15.69, 7.15 and 40.20 g plant^{-1}) fresh weight of leaves, root and shoot, followed by T_6: C_3S_2 (12.53, 6.28 and 37.5 g plant^{-1}) while these were the lowest (4.78, 2.91 and 12.79 g plant^{-1}) in T_1: C_1S_1. The same trend was followed in case of dry weight of leaves, root and shoot and total biomass, respectively. The highest (31.31 plant^{-1}) total biomass was noted in T_5: C_2S_2 (15 cm length with 15 g sucrose l^{-1} water), followed by 29.64 and 25.09 g plant^{-1} in T_6: C_3S_2 (20 cm length with 15 g sucrose l^{-1} water) and T_8: C_2S_3 (15 cm length with 15 g sucrose l^{-1} water), respectively, whereas T_1: C_1S_1 (10 cm length of cutting with no sucrose) showed the lowest (12.95 g plant^{-1}). These findings were in agreement with the results of Pinto *et al*. (2011) in *Spondias cythera* and *S. purpurea* cutting. It might be due to the reserve food in the cutting that influenced higher leaf, root and shoot fresh weight corresponding to the dry weight of respective parameters and total biomass, respectively.

The success of root and shoot regeneration through stem cutting was proved to be an effective method for multiplication by showing a strong synergistic interaction between the optimum length of cutting along with sucrose concentration. It might be concluded that the cuttings (15 cm length) treated with sucrose (15 g l^{-1} water) would be most promising tool for rooting of *Spondias pinnata* in nursery industry.

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