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Studies on vegetative propagation in curry leaf (*Murraya koenigii* Spreng.)

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Abstract
Murraya koenigii Spreng. (Curry leaf), belonging to the family Rutaceae, is one of the important medicinal cum aromatic plant which is grown throughout the world due to its antibacterial and antifungal properties. Despite having more medicinal values and good revenue, this plant has got the least attention among scientists and researchers. Not much research work has been done on propagation of curry leaf. Hence, a study entitled, "Studies on Vegetative Propagation in Curry leaf (*Murraya koenigii* Spreng.)" was carried out to identify the better hormonal concentration for rooting of stem cuttings and to graft rootstocks of wild relatives with commercially cultivated Senkambu scions. The experiment on cuttings was laid out in FCRD (Factorial Complete Randomized Design) and the results revealed that the four nodal cuttings treated with 3000 ppm IBA (T_{14}) sprouted earlier with more number of leaves and shoots, and the highest success percentage (60.00%) whereas the three nodal cuttings treated with 3000 ppm IBA (T_9) was found to be maximum in shoot length(cm), number of roots, root length (cm), fresh root biomass (g) and dry root biomass (g) with 53.34% success percent. In grafting study, by following the wedge/cleft method of grafting, the success percent was found to be 74.28% observed after 30 days of grafting. Further studies need to be carried out to study the overall success of cuttings/graftings raised by using these hormonal concentrations.

Keywords: Studies, vegetative, propagation, leaf, *Murraya koenigii* Spreng

Introduction
Murray koenigii is known as Kari patta or Meethi neem. The word 'curry' originated from the Tamil word 'Kari' which means 'spiced sauce'. It belongs to the family Rutaceae (Citrus family) that consists of approximately 150 genera and 1500 species. *Murraya koenigii* is found to be native mainly to India and Sri Lanka (Suman Singh *et al.*, 2014) [16]. The species is named after the botanist Johann König. The genus Murraya commemorates Swedish physician and botanist Johann Andreas Murray who died in 1791. Hence the botanical name of the curry leaves is *Murraya koenigii* (Manshu Jain *et al.*, 2017) [11]. Curry leaves have been traditionally used in the Indian culinary system from ancient times as a rich source of antioxidants (Jagadheeshkanth *et al.*, 2017) [7]. The use of herbs to treat disease is almost spread among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals (Salomi *et al.*, 2016) [23]. *Murraya koenigii* were also used as a blood purifier, tonic and cure for stomachache and used as flavoring agents in curries and chutneys. Curry leaves were also used as a calcium source for those having calcium deficiency besides that they also consist of Vitamin A, Vitamin B and B2, Vitamin C and iron (Sunita *et al.*, 2018) [20]. Curry leaf is commercially propagated by seeds or suckers. Curry leaf seeds are considered to be recalcitrant seeds as they can't resist the effects of drying or exposure to temperatures less than 10ºC and thus can't be stored for long periods (Jain *et al.*, 2012) [8]. Moreover, it is a highly cross-pollinated crop and shows variability among the plant population. Attempts to propagate curry leaf through air layering have not been successful (Manshu Jain *et al.*, 2017) [11]. The freshly harvested seeds give maximum germination but they lose their viability very quickly in storage under open conditions (Thapparangana *et al.*, 2001) [21]. Raising plants through cuttings is considered to be the easiest and most reliable method to obtain true-to-type plants (Thapparangana *et al.*, 2002). The information available on vegetative propagation methods in *Murraya koenigii* is scanty. The main focus of the research work is to bring out and showcase a new propagation technology for Curry leaf. In this regard, plant propagation through cuttings with different concentrations of IBA (Indole 3-butyric Acid) for better establishment and to graft the curry leaf wild rootstock with Senkambu as scion for increased production and productivity.

Materials and Methods

Cuttings

The study was conducted at the Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during the year 2020-21 to identify the type of cuttings for better establishment and survival from wild relatives. The cuttings of *Murraya koenigii* were collected from healthy wild relatives of Olavakkadu Village, Yercaud. Vigorously growing and disease-free plants were selected and marked after an initial survey. The 12-15 cm long pencil-sized cuttings with three different numbers of nodes (Factor 1) were collected from the marked donors. Each cutting (Table 1) was given a slanting cut at the bottom. The cuttings were then treated with different concentrations of IBA (Factor 2), which promotes rooting of the cuttings for 5-10 mins and are planted in a well-prepared media of sand, soil and well-decomposed FYM (1:1:1) and planted in mist chamber for facilitating root growth. The success rate of each treatment was noticed and recorded to determine whether there were significant differences among the different concentrations of IBA. The experiment design adopted in the study was FCRD with three replications each consisting of 75 plants.

Grafting

The standardization of grafting techniques in curry leaf wild types for vegetative propagation was carried out. Grafting was done by selecting the desired type of scion Senkambu from Karamadai, which is one of the most recognized and cultivable types by the Karamadai and Mettupalayam farmers in Coimbatore district of Tamil Nadu. The scions were procured 10 days before grafting. The grafting method used in this study was wedge or cleft grafting. The ideal time for grafting was when the scion and rootstock have the same graftable girth. Grafting was done under the shade net in the early morning and covered with polythene covers to retain moisture.

Cleft/Wedge grafting

The rootstocks were given a V-shaped cut and the scion was shaped perfectly to fit into the rootstock. After perfect interlocking of rootstock and scion, the grafted union was tied with grafting tape and covered with graft cover to create humidity for earlier sprouting. The plants are then shifted to the mist chamber for nearly 15 – 30 days at >95% RH, 25-30ºC and later transferred to the shade net house after the opening of few leaves and when the graft union is formed, the grafting tape was removed and regular watering was given. Water sprouts from the rootstock should be removed periodically for proper supply of water and nutrients to the scion. After 30 days of grafting, the success percentage was recorded and a microtome study was done to check the graft compatibility.

Results and Discussion

Cuttings

Success percentage

Sprouting percentage of cuttings was significantly affected by different hormonal concentrations (Fig. 1). The treatment T_{14} (4 nodal cuttings with 3000 ppm IBA) recorded significantly higher sprouting (60.00%), followed by T_9 (3 nodal cuttings with 3000 ppm IBA) having sprouting percentage of 53.33. The sprouting percentage observed in T_{15} (46.67%) and T10 (46.67%) were statistically at par with each other(Table 2). However, T_6 (3 nodal cuttings at Control) recorded the lowest sprouting (20%) cuttings. The highest sprouting is due to carbohydrate reserves in the cuttings. It could be due to the action of auxin, which caused hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings, resulting in accelerated cell elongation and cell division in a suitable environment, or it could be due to the action of auxin, which caused hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings, resulting in accelerated cell elongation and cell division in a suitable environment (Hartmann *et al*., 2007).

Survival percentage

The total number of plants survived and well established were noted after 60 days of planting. The total survival percentage was finally found to be 37.33%. The lower survival rate could be related to changing climatic conditions and the failure to create a vascular link between the root origin and the vascular tissue of the cuttings (Bose *et al.*, 1991).

Days taken for sprouting

The four nodal cuttings treated with 3000 ppm IBA (T_{14}) took least number of days for sprouting (16.67) followed by T_5 and T_{12}, which took 18.67 and 19.67 days respectively. Auxin induction promotes more robust cell division at the base of the cuttings and increases sugar, which promotes callus development and subsequent roots for early sprout production (Sundharai *et al.*, 2002) [9].

Number of leaves

Average number of leaves were observed at 30 and 60 days after planting. The four nodal cuttings treated with 3000 ppm IBA (T_{14}) produced more number of leaves (15.67), which is followed by T_{13} and T_9 with 14.67 leaves after 30 days of planting. After 60th day, the average number of leaves were counted and found that the plants showed gradual increase in number of leaves. The 4 nodal cuttings treated with 3000 ppm IBA (T_{14}) produced more number of leaves (23.33) followed by T_{13} (21.67). This is in concurrence with Thapparangana *et al.* (2002), on rooting of curry leaf stem cuttings where the number of leaves gradually increased with higher concentrations of IBA.

Number of shoots

The highest number of shoots were found at 5.67 (30 DAP) and 7.67 (60 DAP) in 4 nodal cuttings treated with 3000 ppm IBA (T_{14}), which is followed by T_8 with 4.67 and 6.33 at 30 and 60 DAP respectively. As the number of roots increased under IBA treatment, leading to more number of apical roots also increased which are responsible for synthesis of cytokinin thus lead to the formation of more number of shoots per cutting and total number of leaves. This result was in conformity with the findings of Abdul *et al* (2002).

Shoot length (cm)

Different hormonal concentrations exhibited variation in shoot length (per cutting).The 4 nodal cuttings with 2000 ppm IBA produced shoots with maximum length (6.80 cm) followed by T_9 which produced 6.15 cm long shoots. Rest of the treatments were statistically at par with each other.

Number of roots

The total number of lateral roots were counted and found that cuttings with 3 nodal cuttings at 2000 ppm IBA (T_8) produced highest number of roots (7.00). The average number of roots were on par with each other, as the number roots were found between 4, 5 and 6. For instance, in the cuttings in which roots are not induced often developed callus like growth at the basal end of cutting in response to auxin treatments. The basal end was swollen and the colour was changed from green to whitish green. Auxin plays an important role in the metabolic activities and cell division which results in an increase in the growth of roots (Sulaiman *et al.,* 2014).

Root length (cm)

The average root length differences were statistically significant by different hormonal concentrations. Maximum root length (1.36 cm) was conspicuous in T_9 (3 nodal cuttings with 3000 ppm IBA)) and the differences were statistically at par with T_{13} (4 nodal cuttings with 2000 ppm IBA) and T_{14} (4 nodal cuttings with 3000 ppm IBA) bearing respective values of 1.20 cm and 1.10 cm. The above results might be due to the reason that the sprouted cuttings were having high foliage and leaves are the main site of food production which is translocated to the roots for development. On the other hand, IBA helped the roots to grow deeper in to the soil for locating more nutrients and thus resulted in increased root length. These results are in partial agreement with the findings of Krishan Kumar *et al.*, (2018) [9], who reported that IBA produced significantly longer roots and maximum root percentage in citrus species than control.

Fresh root biomass (g)

The data on fresh root biomass showed maximum of 0.040 g in T_9 (3 nodal cuttings with 3000 ppm IBA) followed by 0.038 g in T_{14} (4 nodal cuttings with 3000 ppm IBA). The differences in fresh root biomass in rest of the treatments were statistically at par with each other.

Dry root biomass (g)

The 3 nodal cuttings with 3000 ppm IBA (T_9) recorded the highest dry root biomass of 0.020 g, which is followed by 0.019 g in T_{14} (4 nodal cuttings with 3000 ppm IBA). Increase in dry weight of roots might be due to the fact that the increase in the number of roots and length of roots resulted in higher accumulation of dry matter (Thapparangana *et al.,* 2002).

Grafting study

In the present grafting study, the wild rootstocks collected from Olavakkadu village in Yercaud (Shevaroy hills) in Salem district of Tamil Nadu were grafted (Fig. 2) with Senkambu cultivar and the resulted seedlings were assessed by analyzing the following growth parameters for better establishment and increased productivity. The results were furnished in Table 3.

Graft success percentage

It is observed that the success percentage of Senkambu scions grafted with wild rootstocks was 74.28%. Compatibility between scion and rootstock leads to successful grafting, which was due to cell division in the scion and rootstock at the graft union called dedifferentiation and redifferentiation of the callus tissue, rapid connection between the vascular bundles of the scion and rootstock (Shehata *et al.,* 2000) [15]. Concurrent to this finding, Sandhiya *et al*.,I (2019) observed 66 percent success when grafting Senkambu variety onto wild types collected from Shevaroy hills. The highest success percentage was due to the compatible graft union for the formation of callus tissue in the area of both rootstock and scion (Hartmann and Kester, 2002), and also the combined effect of rootstocks and precuring (defoliation) levels. This is in accordance with the findings of Sundaramahalingam *et al*. (2016) which implies the highest survival percentage (57.63) recorded in *A. muricata* grafted on *A. muricata.*

Number of days taken for graft bud break

The Senkambu grafted on to wild rootstocks took about 11.1 days for the first bud break after grafting. Early sprouting was due to consequent higher level of photosynthates and/or dry matter production (Amrita *et al*., 2019) [2].

Length of the new scion shoots (cm)

The grafted plants producesnew shoots of about 0.98 cm in 30 days. Probably this may be due to better growth of grafts and weather condition like temperature and humidity, which played important role in growth of grafts (Gohil *et al*., 2019) [5].

Increased scion diameter (cm)

The increased scion diameter was found to be approximately 0.014 cm, which is comparatively on par with all the plants. The growth is very slightly higher (0.01cm) than before, is associated with auxin destruction, ascorbic acid changes, physiological and biochemical interference (Singh *et al*.,2000)

Number of leaves

The number of leaves of new growth per graft is found to be 8.5 and 12.6 at 15th and 30th days after grafting. Production of higher number of leaflet was due to relative increase in anticlinal division (Suja *et al*. 2016) [17]

Length of the largest leaf (cm)

The length of the largest leaf was found to be 0.66 cm at 30 days after grafting.This could be due to a longer period of active growth in meristamatic cells, as well as improved physiological processes such as photosynthesis and lower respiration, and higher levels of nutrients in scion shoots, as well as favourable temperature and humidity during the monsoon season, which aided faster growth (linear and radial growth) that benefits both the rootstock and the scion shoot. Earlier reported by Dhakar *et al*. (2017) [4] in litchi, recorded highest length of leaflet (9.37 cm) by wedge grafting method for standardization of grafting technique.

Breadth of the largest leaf (cm)

The breadth of the largest leaf produced was 0.34cm. The maximum leaf area in early grafting dates may be due to early healing of graft union, which in turn produced maximum leaflet area (Rehman *et al*., 2000)

Number of laterals per graft

Senkambu scion grafted onto wild rootstocks produced 4.0 and 5.7 laterals at 15 and 30 days after grafting. Number of leaves got increased in jamun might be due to the active growth of both rootstock and scion followed by favourable climatic conditions for the cambial activity and in turn favouring growth of grafts (Uchoi *et al.,* 2012) [22]. This can also be correlated to higher cell activity and early sprouting which are responsible for higher number of leaves and shoot length.

Conclusion

In Curry leaf stem cuttings, among the various hormonal concentrations with a varying number of nodes, T_{14} (4 nodal cuttings with 3000 ppm IBA) showed better production and higher success percentage (60%) followed by T_9 (3 nodal cuttings with 3000 ppm IBA). The grafting of the wild rootstock with Senkambu scion, by wedge/cleft grafting, showed better graft compatibility and the highest success percentage of 74.28. Based on the results of the current study, it is advised that the study has to be continued and reconfirmed to see the survival percentage. The success of cuttings/graftings may be improved by altering their favorable climatic conditions and by using scions at different maturity level.

Table 1: Treatment details

Treatment	Factor 1	Factor 2
T_1	2 nodal cuttings	Control
T_2		1000 ppm IBA
T_3		2000 ppmIBA
T_4		3000 ppmIBA
T_5		4000 ppm IBA
T_6	3 nodal cuttings	Control
T_7		1000 ppm IBA
T_8		2000 ppm IBA
T_9		3000 ppm IBA
T_{10}		4000 ppm IBA
T_{11}	4 nodal cuttings	Control
T_{12}		1000 ppmIBA
T_{13}		2000 ppm IBA
T_{14}		3000 ppm IBA
T_{15}		4000 ppm IBA

Table 2: Growth parameters of Curry leaf cuttings on various treatments

Treatment	Days taken for Sprouting	No. of leaves		No of Shoots		Shoot length	No. of roots	Root length	Root biomass		Success %
		30 DAP	60DAP	30 DAP	60 DAP				Fresh	Dry	
T1	22.67	6.67	6.33	1.67	4.67	2.83	4.00	0.37	0.013	0.007	26.67
T2	21.00	4.00	7.00	2.33	3.33	3.03	3.33	0.47	0.016	0.008	33.33
T3	23.00	6.67	9.00	2.67	4.00	3.07	4.00	0.80	0.017	0.009	26.67
T4	24.33	5.00	11.67	2.67	4.33	4.40	3.33	0.93	0.012	0.006	40.00
T5	18.67	7.33	11.67	3.33	4.67	3.57	2.67	0.87	0.030	0.015	40.00
T6	24.00	6.00	9.33	2.99	3.99	3.85	2.99	0.63	0.017	0.009	20.00
T7	22.33	11.00	15.00	2.00	4.00	5.67	6.00	0.70	0.014	0.007	33.33
T8	20.33	13.33	19.00	4.67	6.33	5.97	7.00	0.70	0.032	0.016	26.67
T9	20.00	14.67	16.67	2.99	5.98	6.15	6.64	1.36	0.040	0.020	53.33
T10	25.33	6.00	9.00	4.67	4.67	5.11	6.00	0.73	0.021	0.011	46.67
T11	21.67	7.67	10.67	3.01	3.68	4.45	5.35	0.77	0.017	0.008	33.33
T12	19.67	6.67	16.33	2.33	3.33	5.90	5.67	1.00	0.015	0.008	26.67
T13	18.33	14.67	21.67	4.67	5.33	6.80	5.33	1.20	0.031	0.016	40.00
T14	16.67	15.67	23.33	5.67	7.67	5.43	5.33	1.10	0.038	0.019	60.00
T15	23.33	8.00	10.00	3.67	4.67	6.00	5.33	0.50	0.034	0.017	46.67
S.Ed	1.06	0.46	0.67	0.17	0.23	0.24	0.24	0.04	0.001	0.97	
CD (0.05)	2.16**	0.94**	1.38**	0.35**	0.48**	0.49**	0.49**	0.08**	0.002**	1.98**	

Table 3: Growth parameters of grafted curry leaf plants

Number of days taken for graft bud break	Length of the new scion shoots (cm)	Increased scion diameter (cm)	Number of leaves of new growth per graft		Length of the largest leaf (cm)	Breadth of the largest leaf (cm)	Number of laterals per graft	Number of sprouts per graft		Success %
			15 DAP	30 DAP			15 DAP	30 DAP		
11.1	0.98	0.014	8.5	12.6	0.66	0.34	4	5.7	1.7	74.28%

Fig 1: Cuttings in Curry leaf

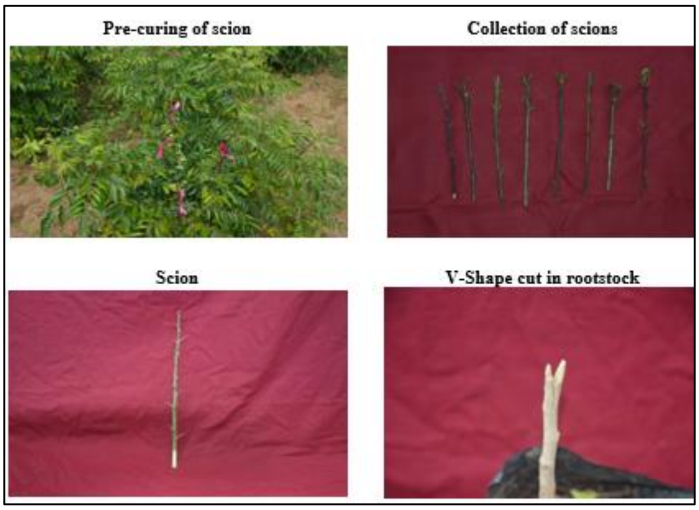

Fig 2: Grafting in Curry leaf

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