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Clonal propagation in *Pseudarthria viscida* (L.) Wight & Arn.: A threatened medicinal species

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Abstract
Pseudarthria viscida (L.) Wight & Arn., commonly known as sticky desmodium, is a perennial under shrub widely used in Ayurveda. There is no standardized clonal propagation method through stem cuttings; hence, it is necessary to fulfill the demand for *Pseudarthria viscida* in the indigenous system of medicine. To standardize clonal propagation in *Pseudarthria viscida* and to assess clones' growth characteristics, an experiment was undertaken at AICRP on Medicinal Aromatic Plants and Betelvine, Kerala Agricultural University, Thrissur, Kerala, India during 2021-2022. Stem cuttings from the stem's top, middle, and bottom portions and the primary branches from the main stem of *Pseudarthria viscida* were taken for the study. The clonal progenies were compared with seedling progenies. The experiments were laid out in a completely randomized design. The results revealed that stem cuttings from the lower portion of the main stem (T_1) had 70% sprouting at 30 DAP and registered the highest sprouting percentage among the treatments. However, it was statistically comparable to T_2 and T_4 with 65 and 55 percent, respectively, followed by T_3 (35%). The tallest plants were observed in stem cuttings from the lower portion of the main stem, measured 98.8 cm and T_4, T_3 and T_2 correspondingly recorded 80.5, 75 and 76.5 cm at 90 DAP, and were statistically on par with T_1 (40.8 cm). At 45 and 90 DAP, primary branches from the main stem (T_4) with 2.2 and 4 branches per plant recorded the highest number. At 90 DAP, stem cuttings from the upper portion of the main stem (T_3) recorded the maximum number of leaves per plant (34.8) at 90 DAP and were statistically comparable with all other treatments. From the findings of this study, we can strongly recommend stem cuttings from the basal position of the main stem for commercial cultivation of *Pseudarthria viscida*.

Keywords: *Pseudarthria viscida*, stem cuttings, sprouting percent, growth attributes

Introduction
Pseudarthria viscida (L.) Wight & Arn. is a member of the family Fabaceae, a perennial diffuse sub-shrub plant that can attain a height of one meter (Peechi, 2007) [16]. Branches are slender and covered with greyish-white hairs. Leaves are trifoliate. Seeds are brownish-black in colour (Peechi, 2007) [16]. Each pod contains 4 to 6 seeds.

Pseudarthria viscida is one of the major ingredient of dasamoolam and used to prepare various ayurvedic formulations such as Surasadi thailam, Sudarsanasava, Balajeerakadhi kashayam, Brihatyadi kashayam, Surasadi kashayam, Brahma rasayana (Warrier *et al.*, 1996) [24]. The root is the economic part, alleviating tridoshas, *viz* vata, pitta and coupha. The roots have thermogenic, astringent, emollient, digestive, febrifuge, cardiotonic and rejuvenating properties (Shaaganti *et al.*, 2019) [19]. Major chemical compounds present in the roots are gallic acid, caffeic acid, rutin, quercetin, ferulic acid and phenolic compounds (Suriyavathana and Rajan, 2011) [23] and also reported with proteins, flavonoids and leucopelargonidin (Deepa *et al.* 2004; Prasad and Nambiasan, 1976) [2]. Roots are well known for antihelmintic, anti-diuretic and anti-inflammatory properties (Kavitha *et al.*, 2018) [8]. Decoction of the root is used against heart diseases, rheumatism, asthma and piles. Root juice is used to treat headaches (Warrier *et al.*, 1996) [24]. Root has a wider claim in traditional medicines against various diseases like urinary problems, tumors, anorexia, flatulence, diarrhea, piles, vomiting and edema. Root extract of *Pseudarthria viscida* has been used in many ayurvedic preparations.

Due to over-exploitation and uprooting of the whole plant for pharmacological usage, *Pseudarthria viscida* is included under the red data list. Hence, deliberate efforts are needed to popularize this highly traded medicinal plant for commercial cultivation, ensuring continuous industry supply without depleting natural sources.

Pseudarthria viscida is commonly propagated by seeds. Low seed viability, low germination rate and high mortality of the seedlings in young stages are the limiting factors for commercial cultivation. Clonal propagation allows rapid production of large quantities of high-quality planting materials. Branch, stem, and nodal cutting were used for clonal multiplication in medicinal plants. Vegetative propagation by cuttings is easy to do but not expensive (Sumirat et *al.*, 2013) [22]. Genetic and environmental factors influence the sprouting or rooting percentage. The node position of the shoot significantly influenced the newly developing sprouts (Kraiem *et al.*, 2010) [9].

Hence, the present investigation was carried out to standardize clonal propagation in a highly traded red-listed medicinal plant and to compare it with seedling progenies.

2. Materials and Methods

2.1. Standardization of clonal propagation

A study on the standardization of clonal propagation in *P.viscida* was carried out at AICRP on Medicinal Aromatic Plants and Betelvine, Kerala Agricultural University, Thrissur, Kerala, India, during 2021-2022. For this, stem cuttings from the top, middle and bottom portions and the primary branches from the main stem were taken. The experiment followed a completely randomized (CRD) design with four treatments and five replications. Ten cuttings from each stem portion were planted in grow bags and the sprouting percentage was recorded. The treatments included T_1: stem cuttings from the lower portion of the main stem (up to 25-30 cm from the bottom); T_2: stem cuttings from the middle portion of the main stem (up to 35-40 cm from the bottom); T_3: stem cuttings from the upper portion of the main stem (up to 50-60 cm from the bottom) and T_4: Primary branches from the main stem.

2.2. Performance evaluation of clones

P. viscida can propagate normally through seeds. Hence, seedling progenies were compared with clones. For this, seeds and two-noded stem cuttings taken from the stem's top, middle and bottom portions and the primary branches from the main stem of *P. viscida* were sown/planted in the nursery. At 30 days after sowing/planting, ten seedlings and ten cuttings were transplanted into grow bags. The observations on plant height and number of branches and leaves were recorded 45 and 90 days after transplanting. The experiment followed a completely randomized design (CRD) with five treatments and five replications. The treatments include T_1: stem cuttings from the lower portion of the main stem (up to 25-30 cm from the bottom); T_2: stem cuttings from the middle portion of the main stem (up to 35-40 cm from the bottom); T_3: stem cuttings from the upper portion of the main stem (up to 50-60 cm from the bottom); T_4: primary branches from the main stem and T_5: Thirty-day old seedlings.

Results and discussion

Standardization of clonal propagation

At 15 days after planting (DAP), 80 percent of the stem cuttings taken from the lower portion of the main stem (T_1) were sprouted, and the highest value was documented. Stem cuttings from the middle portion (T_2), the upper portion (T_3) and the primary branches from the main stem (T_4), respectively, exhibited 72.5, 62.5 and 42.5% sprouting. Stem cuttings from the lower portion of the main stem (T_1) had 70 percent sprouting at 30 DAP and registered the highest sprouting percent among the treatments. However, it was statistically comparable to T_2 and T_4 with 65 and 55%, respectively, followed by T_3 (35%).

Table 1: Standardization of clonal propagation in *Pseudarthria viscida*

Treatments	Sprouting (%)	
	15 DAP	30 DAP
T1	66.52 (80.0)	56.94 (70.0)
T2	58.60 (72.5)	53.99 (65.0)
T3	40.67 (42.5)	36.22 (35.0)
T4	52.55 (62.5)	48.01 (55.0)
SEM±	4.462	4.360
CD (0.05)	14.72	11.01

Results from the present investigation stated that stem cuttings from the lower position of the main stem registered the highest sprouting percentage among the treatments. Husen and Pal (2007) [7] reported that stem cuttings taken from the middle position showed the highest rooting percentage in *Tectonia grandis*, followed by apical and basal portions, respectively. According to Benbya *et al.* (2018) [1] significant differences were noticed in the sprout lengths of rooted cuttings taken from the different positions of the branches in *Argania spinosa* and results showed that the rooting percentage was higher in cuttings obtained from the middle position than the apical meristem. Hartmann et al. (1997) [6] observed an increasing trend of rooting ability in most tree species from the apical to the basal portion of the shoots.

However, there are many variations from the general trend. This might be due to environmental conditions such as temperature, photoperiod and luminous intensity (Hansen, 1986). Factors such as leaf water potential, leaf age, stomatal distribution, stem diameter, wood structure and xylem elements may also influence the success percent of cuttings from different nodal positions (Hartmann *et al.*, 1990) [5].

Leakey and Mohammed (1985) [11] observed that the rooting percent of cuttings from the dissimilar node positions in *Triplochiton scleroxylon* was declined basipetally (Leakey and Mohammed, 1985) [11]. *Milicia excelsa* (Ofori *et al.*, 1997), *Triplochiton scleroxylon* (Leakey, 1983) [10] and *Nauclea diderrichii* (Matin, 1989) [12] showed the highest rooting percent in cuttings taken from the apical positions than the basal positions.

Therefore, these data showed that suitable stem cuttings may vary with the plant species for higher rooting percentages. Findings from the present investigation suggested that cuttings taken from the lower position of the main stem showed a higher sprouting percentage, followed by middle, upper and primary branches from the main stem, respectively. The success percentage of rooted cutting from the basal portion of the stem

might be due to the degree of secondary thickening or the changes in the extent of lignification (Girouard, 1969; Hartmann *et al.* 1990) [5]. Insufficient rooting of the upper position of the shoot might be due to either low carbohydrate concentrations in the apical portion (Palanisamy and Kumar, 1997) [15] or the inhibitory effect of auxins produced during root initiation (Smith and Wareing, 1972) [20]. Otherwise, it might be due to increased susceptibility to water stress in young cuttings (Leakey, 1983) [10].

3.2. Clonal evaluation

The tallest plants were observed in stem cuttings from the lower portion of the main stem (T_1) at 45 and 90 DAP, measuring 55.4 and 98.8 cm, respectively. T_4, T_3 and T_2 correspondingly recorded 47.25, 44 and 43.25 cm and 80.5, 75 and 76.5 cm, respectively, at 45 and 90 DAP, and were statistically on par with T_1. At 45 and 90 DAP, transplanted seedlings had lower plant heights of 21.8 and 40.8 cm, respectively, which was statistically inferior to other treatments.

There were no branches in transplanted seedlings at 45 DAP, but 1.6 per plant at 90 DAP. At 45 and 90 DAP, primary branches from the main stem (T_4) with 2.2 and 4 branches per plant recorded the highest number. However, it was statistically comparable to T_1, T_3 and T_2 at 45 and 90 DAP with 1.6, 1.6 and 1.4 and 3.6, 3 and 2 branches per plant, respectively.

Primary branches from the main stem (T_4) with 17.8 leaves per plant, recorded the highest number of leaves per plant at 45 DAP, and were on par with T_3, T_1 and T_2. However, at 90 DAP, stem cuttings from the upper portion of the main stem (T_3) recorded the maximum number of leaves per plant (34.8) and were statistically comparable with all other treatments.

Table 2: Performance evaluation of clonal progenies

Treatments	Plant height (cm)		No. of branches plant^{-1}		No. of leaves plant^{-1}	
	45 DAP	90 DAP	45 DAP	90 DAP	45 DAP	90 DAP
T1	55.40	98.80	1.60	3.60	15.40	30.40
T2	43.25	76.50	1.40	2.00	11.00	22.60
T3	44.00	75.00	1.60	3.00	16.20	34.80
T4	47.25	80.50	2.20	4.00	17.80	33.80
T5	21.80	40.80	0	1.60	4.80	15.00
SEM±	2.78	5.06	0.23	0.26	1.15	2.42
CD (0.05)	11.64	23.90	1.264	1.334	4.71	13.63

Results of the present study displayed that the tallest plants were observed in stem cuttings from the lower portion of the main stem (T_1) at 45 and 90 DAP. Similar findings were observed by Severino *et al.* (2011) [18]. Stem cuttings taken from the lower portion of the branches produced more buds, leaves, stems and higher shoot and dry weight, whereas rooted cuttings from the upper portion of the shoot showed minimal growth in *Jatropha curcas* (Severino *et al.*, 2011) [18]. This might be because shoot cuttings from the basal portion of the stem have more carbohydrate reserve, providing more nutrients for the better growth of the stem cuttings than the apical stems (Palanisamy and Kumar, 1997) [15].

According to Solikin (2018) [21], cuttings from the top of the stem reported the highest shoot growth and branchlet number. In contrast, cuttings from the middle and bottom positions of the stem showed the lowest growth in *Andrographis paniculata* (Solikin, 2018) [21]. Higher growth of the upper portion of the stem might be due to more auxin content and axillary buds present in the top position (Solikin, 2018) [21]. A higher number of nodes in the upper position of the stem produces a higher number of branches. Findings from the present study suggested that primary branches from the main stem reported higher number of branches at 45 and 90 DAP. This might be due to more nodes in the laterals or the lateral branches also tend to produce more side shoots than the main stem. According to Paembonan *et al.* (2019) [14], branch position does not significantly affect the number of shoots in *Gigantochloa atter*. However, branch cuttings from the lower position of the stem showed the highest number of shoots, ranging from 3 to 4 shoots, with an average value of 3.56. The apical position showed the lowest number of shoots, ranging from 2.5 to 4.5, with an average of 3.31 (Paembonan *et al.*, 2019) [14].

Results of the present investigation suggested that stem cuttings from the upper portion of the main stem recorded the maximum number of leaves at 90 DAP. This might be because the apical portion of the stem contains more auxin content, which stimulates nodes to produce more leaves. Benbya *et al.* (2018) [1] found that during the entire investigation period, cuttings obtained from the lower position showed the highest number of leaves, followed by the middle position in *Argania spinosa*. Paembonan *et al.* (2019) [14] reported that the effect of the branch on the number of leaves was found to be non-significant in *Gigantochloa atter*. However, branch cuttings from the base of the stem showed the best average number of leaves (Paembonan *et al.*, 2019) [14]. Solikin, (2018) [21] reported that the middle stem cuttings of *Andrographis paniculata* showed the highest leaf number but a non-significant difference from the upper cuttings of the stem.

Based on the results obtained from this study, the position of stem cuttings significantly affected plant height, number of branches and number of leaves. Shoot cuttings have a limited reserve of organic matter, requiring stimulating hormones for better growth results. However, branch cuttings from the different nodal positions tend to provide the best growth.

The present investigation focuses on the importance of stem positions in clonal propagation through stem cuttings, standardizes the best stem propagation method for *Pseudarthia viscida*, and analyzes the growth attributes of clonal progenies. Results of this study revealed that sprouting percent and growth attributes of *Pseudarthria viscida* were significantly influenced by the cutting position of the stem. Higher sprouting percent was shown by cuttings from the main stem's basal portion followed by the main stem's middle, upper and primary branches, respectively. From the findings of this study, we can strongly recommend stem cuttings from the basal position of the main stem for commercial cultivation of *Pseudarthria viscida*.

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