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Response of *in vivo* propagation techniques for *Viola pilosa* on growth and flowering behaviour

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Abstract
In vivo propagation of *Viola pilosa*, an ornamental plant renowned for its medicinal properties, is essential for understanding the impact of various vegetative propagation techniques (from cuttings, runners, etc.) or seed germination, on its growth and flowering potential. This study evaluates the growth and flowering responses of *Viola pilosa* propagated through different methods: mother plant, cuttings, runners, and seeds. The study focuses on various growth parameters such as petiole length, lamina size, flower size, rosette diameter, tillers per plant, leaves per plant, and flowering characteristics, including flower count, flower weight, and flower yield per plant. Results suggest that different propagation methods significantly affect the growth and flowering behavior of *Viola pilosa*, with runners and mother plants showing superior performance in several traits.These propagation methods are more cost-effective as they require less specialized equipment and facilities andare less time-consuming than *in vitro* propagation for large-scale multiplication. These methods also ensure the new plants are genetically identical to the parent plant for maintaining desired traits like yield or any specific characteristics, making them more accessible for farmers and researchers with limited resources.

Keywords: Lamina size, rosette diameter, runners, vegetative propagation

Introduction
Viola pilosa is a perennial flowering plant native to various regions and is highly regarded for its ornamental value and potential medicinal uses. This herb is glabrous, stoloniferous, and has short, tufted rootstocks. The fruit is a globose capsule with very few seeds. The stem is short and covered in withered scales, which makes it distinct. The leaves are crenate-serrate and ovate-cordate, and the flowers are blue or occasionally white, 6-12 mm in diameter, on the main stem. The herb is used to make roghani-banafsha, a therapeutic oil that is sold alongside commercial banafsha. This plant serves as the primary element in Joshanda, a Unani remedy that is mostly used as a decoction for coughs and colds. The violin, an acrid and bitter emetic component found in all sections of the plant, is present in the blossoms. In addition, this plant has glucoside (methyl salicylate), an alkaloid (violine), and a glycoside (viola quercitrin) in its roots and leaves. It also contains the volatile oils saponin, cyanin, and rutin. Due to its many therapeutic applications, including the preparation of a medicinal oil called Rohani-banafsha, the treatment of blood diseases and lung problems. The plant is highly on demand in both Ayurveda and other medical systems (Raizada and Saxena, 1978; Bhattacharjee, 2001) [14, 2] as a laxative in bilious diseases, an expectorant, a diaphoretic, an antipyretic, a diuretic, and a home remedy for cough, cold, sore and throat for infants (Kheterpal *et al.*, 1987) [7]. Additionally, it helps with constipation, fever, headaches, bleeding piles, asthma, and skin conditions. One of the main factors contributing to the extinction of many species has been determined to be the unsystematic exploitation of significant drug-producing plants by the pharmaceutical industry (Lewin, 1986; Wilson, 1988) [9, 18]. The species has many kinds of bioactive ingredients, including β-sitosterol, violine, viola quercitrin, and methyl salicylate (Prajapati *et al.* 2006; Kumar *et al.* 2011). It also has antipyretic, bilious, and diaphoretic properties (Man and Samant 2011) [11]. Habitat It is found in the hills of Meghalaya, Nagaland, and Manipur to the east, as well as in the temperate Himalayas up to an elevation of roughly 2000 meters.

At an elevation of 1500-2000 meters, it can also be found in the Ganjam hills of Orissa and the Nilgiri and Palani hills of Tamil Nadu.

Preparations: Banafshadikwath, Gulkandbanafsha, Banafsha syrup, and Banafsha oil

Propagation of this plant can be achieved through several methods, including vegetative propagation (via cuttings and runners) and sexual propagation (via seeds). Understanding how these different propagation methods affect growth, flowering behaviour, and yield can provide insights into optimizing cultivation practices for commercial or medicinal purposes. *in vivo* propagation, involving methods that maintain the plant in its natural environment, is a widely used technique for producing new plants from an existing stock. However, the effectiveness of different propagation methods in terms of growth and flowering behaviour is not well-established for *Viola pilosa*. This study aims to evaluate the responses of *Viola pilosa* propagated through various methods and assess their impact on both vegetative growth and flowering behaviour.

Materials and Methods

The experiment was conducted at Banthra Research Station, CSIR-National Botanical Research Institute, Lucknow, in a controlled environment under standard greenhouse conditions, with consistent temperature. The plant material was obtained from healthy *Viola pilosa* mother plants, which were then subjected to four different propagation methods (table 1), Taken as four treatments in randomized block design with four replications,

Table 1: Details of treatments.

Treatment (Propagation means)	Treatment details
T_1 (Mother plant)	Plan was propagated directly from the original mother plant.
T_2 (Cuttings)	Stem cuttings of uniform size were taken from mature plants, rooted, and grown.
T_3 (Runners)	Lateral shoots or runners were used to propagate new plants.
T_4 (Seeds)	Fresh seeds were sown to propagate the plants.

The saplings were planted in the pots as well as in the field, and before the plants were planted, well-decomposed compost was added to the field. The field was irrigated as soon as the transplanting was done. Five plants were selected for each plot, while in pots, a single plant was planted. Observations were taken on several growth and flowering parameters to evaluate the response of each propagation method. Growth parameters included: Petiole Length (cm)-the length of the petiole/leaf stalk, Lamina Size (cm)-the length and width of the lamina (leaf blade), Flower Size (cm)-the size of individual flowers from the tip to the base, Rosette Diameter (cm)-the diameter of the plant rosette (the cluster of leaves around the stem base), Tillers/Plant-the number of tillers (side shoots) produced by each plant, Leaves/Plant-the number of leaves produced by each plant was counted. Flowering characteristics measured included, Flowers/Plant-the number of flowers produced by each plant, Fresh Flower Weight/Plant (g)-the fresh weight of flowers harvested from each plant, Dry Flower Weight/Plant (g)-the dry weight of flowers after dehydration, Flower Yield (g/day/plant)-the rate of flower production per plant per day.

Biometrical analysis

Experimental data were subjected to biometrical analysis as per the standard as procedure given by (Gomez and Gomez 1984) [3]. Significant difference between treatment means was tested through 'F' test and critical difference (CD) was worked out wherever 'F' value was found to be significant for treatment effect.

Results and Discussion

Response of propagation means on growth and production potential of *Viola pilosa*

Table 2: Response of propogation means on growth and production potential of *Viola pilosa*

Treatment details (Propagation means)	Petiole length (mm)	Size of lamina (cm)		Rosett Diameter (mm)	Tillers/plant	Leaves/plant
		Length (mm)	Width (mm)			
T_1: Mother plant	14.0	7.8	5.8	39.3	20.0	120.0
T_2: Cuttings	12.3	5.8	4.8	38.3	11.3	50.0
T_3: Runners	14.3	8.0	6.7	45.0	24.3	92.3
T_4: seeds	11.3	6.3	5.2	23.7	8.3	25.0
SEm (±)	0.93	0.24	0.27	4.2	2.59	9.05
CD (P = 0.05)	NA	0.85	0.97	NA	9.21	31.93

The growth parameters across the different propagation methods showed significant variation (Table 2). Petiole length was longest in plants propagated from the runners (14.3 cm), followed closely by mother plants (14.0 cm). Plants propagated from seeds had the shortest petioles (11.3 cm), indicating that vegetative propagation methods may promote more vigorous vegetative growth. Similarly, significantly higher values obtained for lamina size (both length and width) for the plantlets produced from runners (8.0 cm × 56.7 cm), followed by mother plants (7.8 cm × 5.8 cm). The higher rosette diameter (45.0 cm) was also obtained in those propagules produced from runners, followed by mother plants (39.3 cm). The values for tillers per plant were also significantly higher for runners (24.3), followed by the mother plant (20.0) however, the significantly higher number of leaves per plant was reported from mother plants (120.0), followed by runners (92.3). These results demonstrate that saplings produced from the runners support more robust growth than other propagation methods. This can be attributed to the established growth potential of the runners, which likely provided a more vigorous source for the new plants. The lower values for the same growth parameters suggest that sexual propagation via seeds may result in less vigorous vegetative growth compared to vegetative methods like runners or mother plant propagation.

Response of propagation means on the flowering behaviour of *Viola pilosa*

Table 3: Response of Propogation means on growth and flowering behaviour of *Viola pilosa*

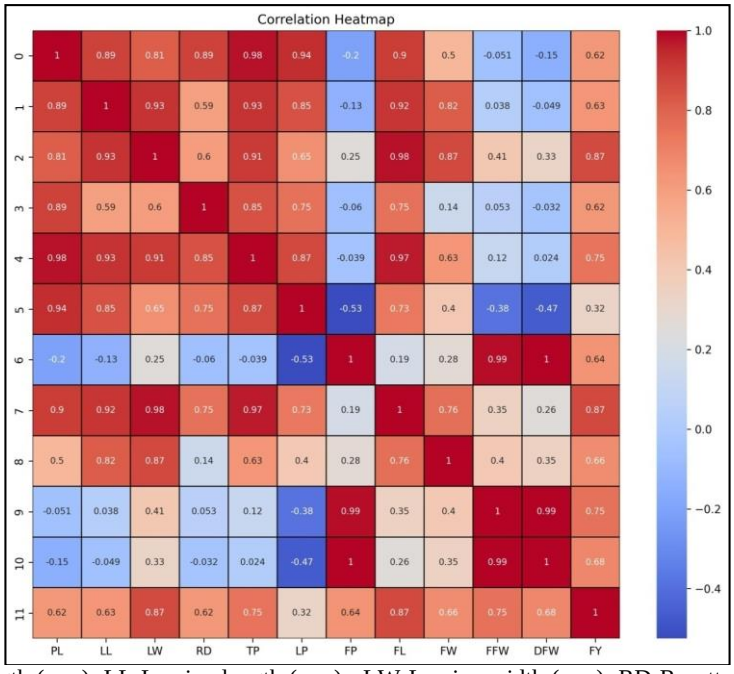
Treatment details (Propagation means)	Flower/plant	Flower size (cm)		Fresh Flower wt/plant (g)	Dry Flower wt/plant (g)	Flower yield (g)/plant
		Length (cm)	Width (cm)			
T_1:Mother plant	1.5	1.9	1.7	0.17	0.05	0.2
T_2: Cuttings	2.3	1.8	1.5	0.21	0.07	0.2
T_3: Runners	2.9	2.0	1.8	0.26	0.09	0.3
T_4: seeds	2.6	1.8	1.7	0.23	0.08	0.2
SEm (+)	0.09	0.11	0.06	0.006	0.002	0.05
CD (P = 0.05)	0.3	NA	NA	0.02	NA	NA

Table 3 highlights the flowering behavior of *Viola pilosa* propagated by different propagation methods. The runner propagated plants produced a significantly higher number of flowers per plant (2.9), with higher flower size (2.0 cm x 1.8 cm), followed by the propagules obtained from mother plants, suggesting that plants propagated through runners and mother plants tend to produce more number of flowers with higher size, compared to those propagated through other vegetative methods like cuttings and seeds.

The higher values for flower weight (for fresh and dry) were obtained from runner propagated plants, followed by mother plants, which reflects the higher flowering potential of runner propagated plants. The highest flower yield per plant was also reported from the plants propagated through runners. These higher values for flowering parameters support that plants propagated from runners tend to produce higher flower yield with larger and heavier flowers, which could be important for ornamental purposes. This could be beneficial in the commercial cultivation of Viola pilosa, where both vegetative growth and flowering are important.

Discussion

The present work examined the effects of several *in vivo* propagation techniques on the growth and flowering behavior of *Viola pilosa*, a medicinally and ornamentally important plant. The results show that propagation techniques greatly affect both vegetative growth and reproductive performance, with notable advantages seen in plants propagated via runners and mother plants. Vegetative growth parameters such as petiole length, lamina size, rosette diameter, tiller number, and leaf count were all markedly higher in plants propagated through runners and mother plants. These results suggest that vegetative propagation methods, particularly runners, support more vigorous growth compared to sexual propagation via seeds (Kaloo *et al.* 2013) [5]. The study revealed that petiole length had a positive and significant relationship with tillers per plant (0.981*) and flower length (0.98*) showed a positive and significant association with lamina width, lamina length (0.926) and tillers per plant (0.967 *) fig 1. This superiority can be attributed to the fact that vegetative propagation ensures the transfer of established physiological and genetic advantages from the parent plant to the offspring, bypassing the juvenile growth phase that seed-propagated plants must undergo (Sharma *et al.* 2004) [17]. Runners, being naturally generated lateral shoots with pre-developed roots, probably provide an instantaneous and strong establishment in the growing media, which enables more development rates and bigger plant structures. This finding was corroborated by the quantity of tillers and leaves per plant (Martin *et al.* 2006; Bhatia *et al.* 2008) [12, 1].



PL-Petiole length (mm); LL-Lamina length (mm) ; LW-Lamina width (mm); RD-Rosett diameter (cm); TP-Tillers/plant; LP-Leaves/plant; FP-Flower/plant; FL-Flower length (cm); FW-Flower width (cm); FFW-Fresh flower weight (g)/plant; DFW-Dry flower weight (g)/plant; FY-Flower yield (g)/plant

Fig 1: Heat map of correlation studies of plant attributes contributing flower yields

Variables	PL	LL	LW	RD	TP	LP	FP	FL	FW	FFW	DFW
LL	0.89										
LW	0.82	0.93									
RD	0.89	0.59	0.60								
TP	0.98 *	0.93	0.91	0.85							
LP	0.94	0.85	0.65	0.75	0.87						
FP	-0.21	-0.17	0.25	-0.06	-0.04	-0.52					
FL	0.9	0.92	0.98 *	0.75	0.97 *	0.73	0.19				
FW	0.50	0.82	0.87	0.15	0.63	0.4	0.26	0.76			
FFW	-0.05	0.04	0.41	0.05	0.12	-0.38	0.99 *	0.35	0.40		
DFW	-0.15	-0.05	0.33	-0.03	0.02	-0.47	1.0 **	0.26	0.35	1.0 **	
FY	0.62	0.63	0.87	0.62	0.77	0.32	0.64	0.87	0.66	0.75	0.68

Compared to plants propagated using other techniques, runners produced a significantly higher number of tillers (24.3), indicating an increased capacity for vegetative multiplication. Mother plants, on the other hand, produced more leaves per plant (120), indicating that they might be able to maintain denser foliage, which is essential for photosynthesis and the build up of biomass overall (Kaur *et al.* 2010) [6]. These variations might result from the saplings' superior resource mobilization and innate physiological maturity. All flowering parameters, such as flower number, flower size, and fresh and dry flower weights, were superior in runner-propagated plants in terms of reproductive traits. Runner-propagated plants had the most flowers per plant (2.9), followed by mother plants. The study revealed that fresh flower weight per plant had a positive and significant relationship with dry flower weight per plant (0.995**) and flowers per plant (0.986*). The general horticultural understanding that vegetative propagation preserves genetic uniformity and retains desirable traits of the parent plant, such as higher flowering capacity and larger, more aesthetically pleasing blooms, is consistent with these superior flowering traits in vegetatively propagated plants, especially runners (Sharma and Mishra 2009) [16]. This can be explained by the introduction of genetic variability during sexual reproduction, which could lead to less homogeneous and possibly weaker offspring (Joshi and Dhawan 2007) [4]. Figure 2 Furthermore, because plants go through a lengthy juvenile phase before reaching reproductive maturity, seedling establishment usually takes longer.

Fig 2: Healthy vegetative growth of plants.
A- Healthy Seed.; B-One Month Plants; C-Two Month Plants; D-Three Months plants with runners; E- Successful Pots demonstration F- First successful flowering; G- Multiplication Demonstration; H-Overall life span: seed, flower, seed coat, healthy plant

Conclusion

The study validates that *in vivo* vegetative propagation techniques, especially runners, are more successful for improving *Viola pilosa*'s growth and flowering behaviour. These techniques guarantee the genetic integrity of desired features in produced plants, in addition to being reasonably priced and easily available for researchers and farmers. This makes vegetative propagation, particularly from runners, a better approach for the commercial growing of *Viola pilosa*, so complementing the plant's dual use as a medicinal and ornamental resource.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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