



Adventitious root formation in leaf petiole cuttings of lemon (*Citrus limon* L. Burm.)

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ABSTRACT

Lemon (*Citrus limon* Burm.) is a valuable citrus fruit for its adaptability, market value, and uses in food, cosmetics, and pharmaceutical industries. The demand for quality planting materials of lemon is rising; however, propagation by hardwood cuttings requires a relatively large number of mother plants. Therefore, a study on the rooting behaviour of leaf petiole cuttings in lemons and identification of suitable rooting substrates was conducted to potentially improve multiplication efficiency while reducing the maintenance cost of mother plants. A leaf petiole cutting of lemon cv. "Assam Lemon" was planted in five different substrates, viz., river sand, cocopeat, vermicompost, river sand + cocopeat (1:1 v/v) and river sand + vermicompost (1:1 v/v) under polyhouse conditions. Results indicated that leaf petiole cuttings have potential for rooting, though vary significantly with substrates. The river sand recorded the lowest rooting attributes, except for the number of primary roots (3.67 ± 0.4). Cocopeat produced the maximum number of secondary roots (14.07 ± 0.33) and root diameter (0.49 ± 0.011 mm). Furthermore, the highest rooting percentage (44.67 ± 3.4) and root traits (length, surface area, volume, tips, forks, and crosses) were recorded in cocopeat + river sand, followed by vermicompost + river sand. Therefore, the leaf petiole cuttings of lemons showed positive rooting; however, there was no shoot initiation or formation. The most suitable substrates for root initiation and growth were found in cocopeat + river sand. The study demonstrated the preliminary rooting capability of lemon leaf petiole cuttings in suitable substrates, indicating potential for the future development of an alternative propagation system.

Key words: Lemon, propagation, leaf petiole cuttings, leaf cutting, rooting media.

INTRODUCTION

Lemon (*Citrus limon* Burm.) is an important citrus fruit of the world. It occupies the third largest in area (1.56 million hectares) and production (25.56 million metric tonnes) in the world after orange and mandarin. India is leading the world in lemon production (3.84 million metric tonnes) followed by Mexico and China (FAOSTAT, 6). The fruits of lemons are primarily used for dessert and fruit juice preparation; hence, the trade of lemons includes both fresh fruits and value-added products. Lemon and its derivatives play a multifaceted role across the food, pharmaceutical, and cosmetic industries owing to their rich biochemical composition and functional properties (Long and Mohan, 11). Beyond its sensory attributes, lemon peel is a potent source of bioactive compounds, including phenolics, essential oils, and mucilage, which exhibit strong antioxidant, anti-inflammatory, and antimicrobial activities (Jiang *et al.*, 8). There is continuous expansion of lemon cultivation area due to its suitability for diverse agro-climatic conditions, high market value, and potential uses in the processing and pharmaceutical industries.

Therefore, the demand for quality planting materials is increasing.

Efficient propagation techniques are essential to the success of the citriculture industry, as they substantially influence orchard establishment, crop productivity, and the maintenance of varietal characteristics. The propagation of lemons by seed is generally used for raising rootstocks and/or seedlings and for crop improvement programmes. However, commercial orchard development is primarily dependent on vegetative methods to ensure genetic uniformity and reduce juvenility. The conventional commercial propagation techniques for lemon are hardwood cuttings (Kumar and Kumar, 9), semi-hardwood cuttings (Satpal *et al.*, 17), and single-leaf bud cuttings (Kumar and Kumar, 9; Maurya *et al.*, 12). The propagation ability of cuttings is influenced by several internal and external factors, including age, vigour, growth regulators, environmental conditions, nutrient status, and genetic makeup of the genotype. Among these, the genetic potency and the hormonal responses of tissues to form new organs such as lateral roots, shoots, and flowers, including meristems, are significant. The meristems coordinate the activation of newly formed organs, continuously

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supply new cells for plants, and are all regulated by a dynamic system. Thus, this enables plant growth and development in these organs (Dello Iorio *et al.*, 4).

The propagation through leaf petioles is recognised as an essential vegetative multiplication technique enabling clonal regeneration of plants. This method has been extensively adopted for micropropagation of ornamental, medicinal, and endangered taxa. Such regenerative responses are driven by cellular totipotency, and their success is influenced by factors such as explant age, genotype, and hormonal conditions (Rachmawati *et al.*, 14). Given the increasing demand for ex situ conservation of threatened species and the commercial-scale propagation of elite cultivars, leaf-based propagation protocols have become a focal area of plant biotechnology (Arif *et al.*, 1). Standardizing the rooting substrates is extremely important for efficient propagation in plants. The quality of seedlings, including rooting, shoot growth, and establishment, as well as the development and maintenance of a highly functional rooting system, is strongly influenced by the rooting substrates (Rymbai *et al.*, 17). High-quality substrates provide the plant with sufficient support or anchorage. It also facilitates gas exchange between the roots and the atmosphere, acts as a reservoir for water and nutrients, and allows oxygen to diffuse to the roots (Rymbai *et al.*, 16).

Considering the increasing demand for lemon planting materials, although vegetative propagation of lemon through hardwood, semi-hardwood, and single-node cuttings has been extensively investigated, these methods require a large number of mother plants, are relatively labour-intensive, time-consuming, expensive and often exhibit variable rooting success depending on genotype and environmental conditions. To date, there is little or no documented information on the rooting potential of detached leaf petiole cuttings in lemon under ex vitro conditions. Leaf-derived explants in ornamentals can produce adventitious roots and regenerate complete plants with proper physiological and hormonal conditions. Whether a similar regenerative response exists in citrus remains largely unexplored. Demonstrating the ability of lemon leaf petiole cuttings to initiate adventitious roots would provide new insights into the regenerative competence of citrus tissues and could form the basis for developing a low-cost, space-efficient propagation system requiring only minimal donor material. Such an approach would be particularly valuable for rapid multiplication of elite genotypes, conservation of valuable germplasm, and future integration with tissue culture and regeneration protocols. Given the remarkable plasticity and adaptability of plants in forming new organs, there may be a possibility of

rooting in leaf petioles. Therefore, the present study aimed to investigate, for the first time, the adventitious rooting potential of lemon (*Citrus limon* L. Burm.) leaf petiole cuttings under ex vitro conditions and to identify suitable rooting substrates for root initiation and development. This work serves as a preliminary step toward developing an alternative propagation strategy for lemon using leaf petiole cuttings.

MATERIALS AND METHODS

The present experiment was carried out during June-August of 2024-25 in the Horticulture Section, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India, located at 25°41'91" North (latitude) and 91°55'15" East (longitude) with an altitude of 960 m above mean sea level. A mother stock plant of Lemon (*Citrus limon* Burm.) var. "Assam Lemon", maintained in the field gene bank of the Institute, was used as a source of planting material for the experiment. Healthy plants with high-yielding and quality fruit characteristics, free from pests and diseases, were selected as mother plants for the collection of leaf petioles. Fully expanded, mature, dark-green, and healthy leaves, along with intact petioles, were harvested. The bases of the petioles were sliced very thinly (0.5-1 mm) with a sterilised, sharp blade knife. The different substrates were sterilised in an autoclave at 121 °C and 15 psi for one hour. Five different growing substrates and their mixtures were used in the experiment, viz., river sand (T1), cocopeat (T2), vermicompost (T3), river sand + cocopeat (1:1 v/v, T4) and river sand + vermicompost (1:1 v/v, T5). The basal end of the leaf petiole was treated with a rooting hormone powder (Rotoon 2.0) through dipping for 10 minutes and then planted in a pot (10 cm x 7 cm) containing growing substrates. A planting hole was made in the rooting substrate, and the treated leaf petioles were inserted at a 45-degree angle to the substrate. The medium was gently pressed around the leaf petioles, and the leaf petioles were irrigated immediately. The leaf petiole cuttings were placed under a polyhouse with an atmospheric temperature (25.8/20.7°C, day/night), relative humidity (87.9/80.6%, maximum/minimum), and light (780.6 lux). To maintain high humidity and reduce transpiration stress, each pot was covered with a perforated polyethylene dome, creating a micro-environment favorable for rooting.

Observations were recorded at 3 months after planting (MAP) for various rooting attributes. The rooting percentage was calculated by dividing the number of rooted leaf petiole cuttings by the total number of planted leaf petiole cuttings and expressed as a percentage. The number of primary and secondary roots was counted. Primary roots are

the adventitious roots that emerge from the basal portion of the cutting, while secondary roots are the first-order lateral roots branching from the primary. The rooting attributes, including root diameter (mm), root surface area (cm²), root volume (cm³), number of root tips, number of forks and number of crosses, were taken using a root scanner (Epson Perfection V850 Pro). The experiment was laid out in a completely randomised block design (CRD) with three replications; each replication comprised five pots, and each pot contained ten leaf petiole cuttings. The statistical analysis was carried out using IBM SPSS (version 26.0), and the graphical presentation was prepared using RStudio (4.4.2).

RESULTS AND DISCUSSION

The results showed that the rooting responses of leaf petiole cuttings of lemon varied significantly ($p \leq 0.05$) among the different growing media (Fig. 1-2). The rooting percentage was recorded as the highest in cocopeat + river sand ($44.67 \pm 3.4\%$), followed by vermicompost + river sand ($34 \pm 4.3\%$), while the lowest was observed in river sand ($8.67 \pm 3.4\%$). The highest number of primary roots (3.67 ± 0.4 per leaf petiole cutting) was obtained in river sand (T1), followed by cocopeat + river sand (2.67 ± 0.45 per leaf petiole cutting), while the minimum was recorded in cocopeat (0.98 ± 0.02). The number of secondary roots was recorded as the highest in cocopeat (14.07 ± 0.33 per leaf petiole cutting), followed by cocopeat + river sand (11.73 ± 0.38), and the lowest in river sand (8.67 ± 0.47). Similarly, cocopeat + river sand had the maximum root length (57.83 ± 3.04 cm), root surface area (5.67 ± 0.3 cm²), volume of roots (0.08 ± 0.005 cm³), number of root tips (382.5 ± 8.57), number of root forks (210.83 ± 11.43), and number of root crosses (25.17 ± 0.62), which was followed by vermicompost + river sand. However, the root diameter was recorded as the maximum in cocopeat (0.49 ± 0.011 mm), followed by river sand (0.41 ± 0.012 mm). The variation in responses of the leaf petioles indicates the critical influence of substrate composition on adventitious root induction and development. River sand alone recorded the lowest values for all rooting attributes, except for the number of primary roots. This suggests that substrate combinations with balanced aeration and moisture retention are more conducive to root initiation than inert media alone (Rymbai *et al.*, 17). Our findings have shown that lemon leaf petiole cuttings exhibited a high capacity for root initiation and root attributes. This was due to the potency of leaf tissues for forming adventitious roots, as earlier reported in *Helianthus verticillatus* (Nowakowska *et al.*, 13) and *Jasminum sambac* (Baskaran *et al.*, 2).

The present study found that the number of primary roots was 38% greater in river sand than in cocopeat + river sand. The present study is corroborated by the earlier reports that the maximum number of roots in cuttings of *Cratoxylum arborescens* was obtained in river sand (Wahyuningtyas *et al.*, 21). This could possibly be because the river sand provides a better texture with sufficient aeration for higher initiation and formation of adventitious roots from the food reserve of the cutting (Hartmann *et al.*, 7); however, the limited nutrients in the sand could not sustain further growth and development of roots. Therefore, the rooting growth attributes, such as root length, surface area of roots, root volume, number of root tips, and number of forks, were higher in cocopeat + river sand by 152%, 91%, 168%, 498%, and 652%, respectively, as compared to river sand alone. The higher rooting attributes observed in the substrate mixture of cocopeat and river sand can be attributed to the favourable physico-chemical properties of this combination, which meets the optimum pH range (5-6.5) required for rooting and plant growth (Rymbai *et al.*, 17). Furthermore, it indicates that while river sand favours root initiation, cocopeat, as an organic substrate, promotes subsequent root proliferation and branching, resulting in superior root traits.

Similarly, differential responses between the root initiation and development phases have been reported in vegetative propagation studies, in which substrate aeration primarily influences root initiation, whereas nutrient availability governs root growth and differentiation (Hartmann *et al.*, 7). Previous studies have established the positive effects of cocopeat-based mediums on rooting (Rajkumar *et al.*, 15), as cocopeat is known to retain and release nutrients over time, enhancing nutrient uptake. Additionally, superior oxygenation, phenolic composition, moisture retention, and air content in cocopeat create ideal biological conditions for root development (Treder, 20). Varieties grown in cocopeat-amended media also show increased production of total proteins and amino acids in the stems of ericaceous plants (Scagel, 18) and improved potassium uptake in rough lemon seedlings (Bhagat *et al.*, 3), which likely contribute to enhanced root growth characteristics.

The conventional propagation technique through stem cuttings has reported relatively high rooting (70–90%) and survival percentages, particularly with appropriate concentrations of IBA, accompanied by vigorous shoot development, making them suitable for commercial nursery production (Kumar and Kumar, 9; Maurya *et al.*, 12; Satpal *et al.*, 17). In contrast, the present study recorded a maximum rooting percentage of 44.67% in leaf petiole cuttings grown in a cocopeat-river sand (1:1) substrate,

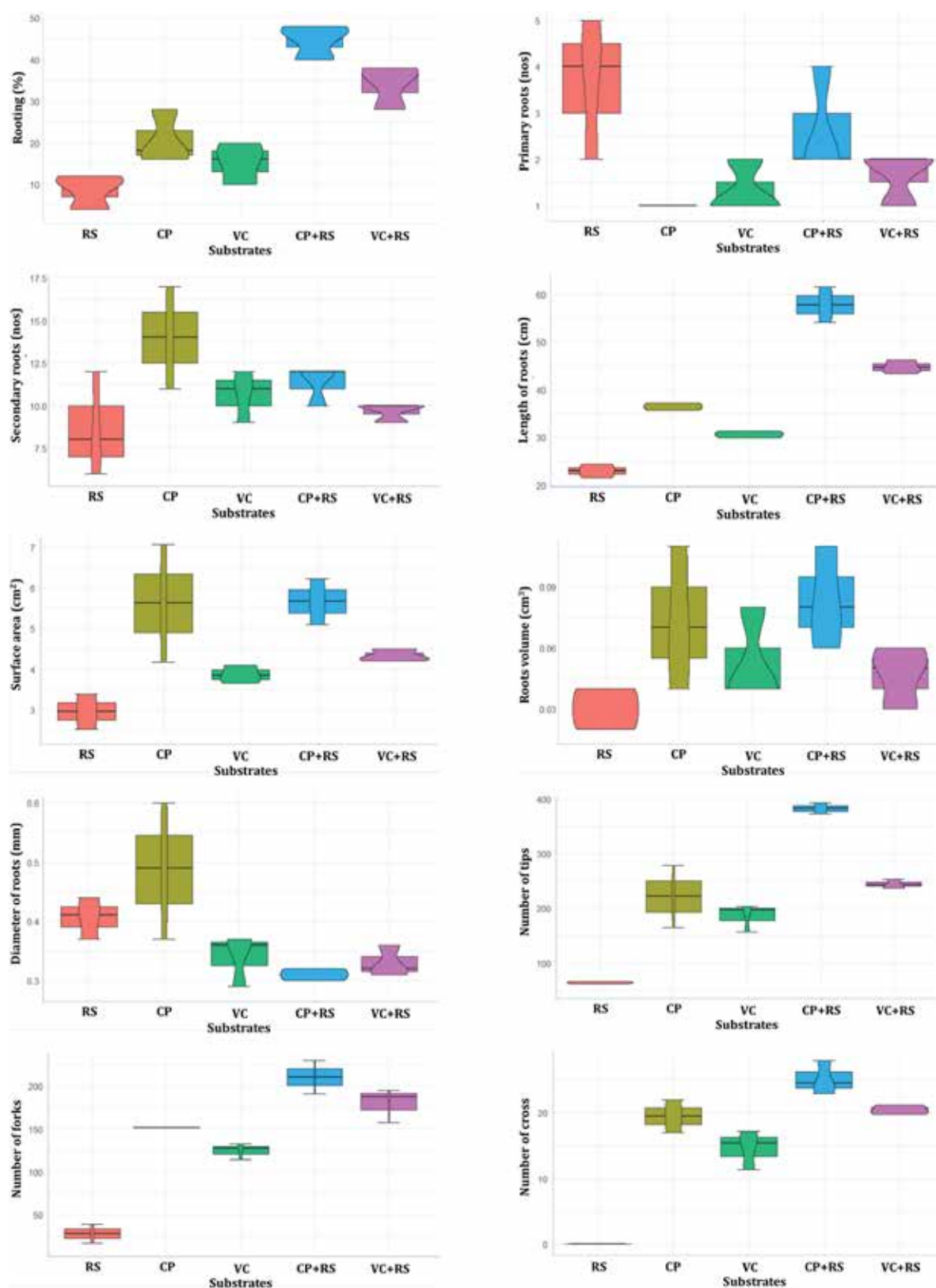


Fig. 1. Root initiation and their growth attributes in lemon cv. Assam Lemon under different rooting substrates. RS – river sand, CP – cocopeat, VC – vermicompost.

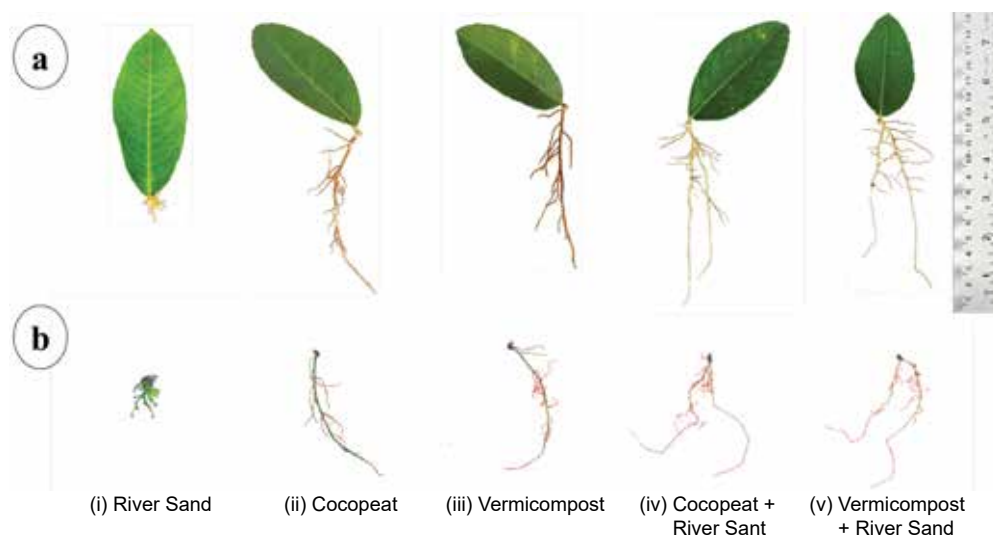


Fig. 2. Pictorial presentation of rooting characteristics in lemon cv. Assam Lemon under different rooting substrates. (a) – rooted leaf petiole and (b) root characteristics taken from a Root Scanner (Epson Perfection V850 Pro).

with successful adventitious root formation but no shoot initiation. Although lemon petiole cuttings successfully formed adventitious roots but failed to produce shoots, primarily because petioles lack pre-existing axillary buds or meristematic tissues required for shoot regeneration (De Klerk *et al.*, 5). The application of auxin promotes root induction but, in the absence of sufficient cytokinin activity, suppresses shoot organogenesis, resulting in rooted but non-regenerating cuttings (Li *et al.*, 10). Furthermore, depletion of carbohydrate reserves and the inherently low regenerative capacity of mature citrus petiole tissues under *ex vitro* conditions likely contributed to the eventual senescence of the rooted cuttings (Steffens and Rasmussen, 19). Although the rooting efficiency was lower than that reported for conventional stem cuttings, the ability of detached leaf petioles to produce adventitious roots demonstrates the inherent regenerative competence of citrus leaf tissues, which has not been previously documented in lemon under *ex vitro* conditions.

The present study concluded that the lemon leaf petiole exhibited higher root initiation and growth. Leaf petiole cuttings from lemon produced the highest rooting percentage and root growth attributes in the substrate mixture (cocopeat + river sand). However, no adventitious shoots were initiated or formed in the present study. This study will lead to a better understanding of rootability and potential uses of leaf petioles as propagules in lemons. Further research is essential on hormonal treatments for shoot generation and development in lemon to establish an efficient multiplication technique for quality planting materials in the future.

AUTHOR'S CONTRIBUTION

Conceptualisation, data curation and original drafting of the manuscript (HR); Revision of the manuscript, validation (HT); Interpretation and revision of manuscript (NAD); Performed the computation, editing of the manuscript (MBD); Critical revision and statistical analysis (VKV).

DECLARATION

No potential conflict of interest was reported by the authors.

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