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RESEARCH ARTICLE

Studies in the seedling phenology and role of seedling in clonally propagated species *Hellenia speciosa*

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Abstract

Hellenia speciosa, a clonally propagated plant, widely known as crepe ginger, is an important medicinal plant of tropical Asia. It is a rich source of diosgenin - a phytosteroid sapogenin used in the commercial synthesis of progesterone. Despite clonal propagation, the plant produces a substantial number of viable seeds, which later become short-lived seedlings. Therefore, this research aims to comprehend the phenological behaviour of the seedling and its significance in clonal development. The study will also help understand the role of ramets in establishment vis-à-vis rapid colonization of the plant. Studies on seed germination and phenology of seedling genet have revealed significant phenological and morphological features. Seed germination is of epigeal and phanerocotylar type producing seedling genet with orbicular obovate cotyledon, glabrous hypocotyl and spiromonostichous phyllotaxy. Leaf shape varies from narrowly ovate to lanceolate. Horizontal rhizome develops from the base of the seedling epicotyl and produces 4–5 ramets in the same growing season. Morphometry of leaf length and breadth of seedling is positively correlated with that of reproductive ramet of the previous growing season (leaf length: $r=0.99$, $p<0.001$; leaf breadth: $r=0.79$, $p<0.05$). The plant shows rhizomatous dependency for maturity as seedling genets are short-lived. So it could be concluded that the primary role of the short-lived seedling genet is to provide a foundation for rhizome development. Additionally, the seedling appears as a miniature of the adult reproductive ramet. Here, during the first growing season, the species allocate more resources for the vegetative growth of its ramet rather than reproductive development. The reproductive development starts from the next growing season.

Keywords

Clonal growth; genet; ramet; rhizome

Introduction

The study of seedling phenology is crucial to understanding the reproductive ecology of sexually reproducing species (1). Studies on seedling features may help to elucidate the taxonomic and phylogenetic relationship between taxa (2). Seedling is the final stage of plants regeneration from seed (3) and also represents any plant's juvenile stage (1). Some monocotyledonous families can produce genetically identical offspring through rhizome mediated clonal propagation. Various authors have been previously studied different aspects of clonally propagated plants such as nutrient sharing between sister ramets, dynamics of genets, fitness, evolution and benefits of clonal propagation (4–7). However, no attempts have been made to study seedling phenology and the significance in establishing clonal plants.

Hellenia speciosa (J.Koenig) S.R.Dutta is a perennial herbs that belongs to the family Costaceae, a potent source of bioactive compounds such as diosgenin, dioscin, costusosides and eremanthin (8). It is an erect, unbranched herb with spiromonostichous phyllotaxy (9). The terminal cone like inflorescence bears a crepe paper-like white zygomorphic flower with a large labellum. The plant is propagated through clonal ramets, stem cutting as well as seeds. It flowers during July to September and fruits mature into a dehiscent capsule in October. Considering the entire flowering season in different months, a fruit of *H. speciosa* requires about 80–120 days for complete maturation. Though it is vegetatively propagated through ramets, it produces large showy flower with ample amounts of nectar. The flower is pollinated by different kinds of bees and produces a considerable number of viable seeds. Hence the primary objective of this study is to understand the importance of seedlings in clonal growth of this plant.

Materials and Methods

Study site and study plant

Phenological events of *Hellenia speciosa* studied in research plot of Vidyasagar University campus, Midnapore, (latitude 22°25´, longitude 87°17´) as well as wild habitat of Kharagpur (latitude 22°20´, longitude 87°17´) West Bengal, India.

Seed structure, germination and phenological events

The study was conducted in March 2019 and 2020 under *in-vivo* conditions with an average maximum and minimum temperature of 18.73 °C and 16.89 °C respectively. The relative humidity of 66.57% (max) and 44.21% (min) was recorded during the experimental period. Seeds were collected from mature and dehisced capsules in November 2018. After collection, seeds were thoroughly washed with a running tap and sun-dried for two to three days. Seeds were then stored in a desiccator. In March 2019, the seeds were sown in suitable potting soil for germination. The phenological data of ten seedlings were recorded for two consecutive years (2019–2020). Different terminologies were used to describe the seed and seedling as described by Bose and Paria (2). Phenological events were documented with drawings and photographs. Photographs were taken using a Canon 800D Digital SRL camera and Zeiss Stemi 508 Stereo microscope.

Statistical analysis

The Pearson's correlation between morphometric data was performed with R statistical tools. The plot of comparisons was obtained using Python statistical tools.

Results

Seed structure and germination

The ovary of *Hellenia speciosa* matures into three locular capsule with 19.59 seeds per locule and an average number of seeds per flower is 57.93. Seeds are dark black with hard four-sided seed coats. The upper operculum region is convex and the flat hilum region remains covered by white and wet aril during dehiscence (Fig. 1-A). Seed size was 3–5 × 2–3 mm. It takes 15–24 days for cotyledon to emergence. The germination starts with the emergence of hypocotyl from the embryo. The spherical tip of the hypocotyl, i.e. the collet, develops into primary root of 8–9 hyaline rootlets (Fig. 1-C). At this stage, a minute flattened area with 1 mm diameter becomes visible on the hypocotyl near the hilum. This area gradually develops into a cotyledonary leaf bearing the seed at its apex, exhibiting epigeal and phanerocotylar types of germination (Fig. 1-E). The cotyledonary leaf pushes the soil by its lower surface to emerge.

Phenological events

The phenological events of *H. speciosa* could be divided into two phases: 1) The seedling genet phase and 2) The phases of ramets (the phase of rhizome-generated shoots). Rhizome starts to develop along with the seedling genet phase and emerges its ramets. After vegetative growth, the rhizome produces 4-5 ramets horizontally.

Seedling genet phase

On the 1st day after cotyledon emergence (DACE), the seedling genet is represented by an erect hypocotyl (5 mm), cotyledonary blade (9 × 6 mm) and a cotyledonary sheath. The cotyledonary blade is sessile, glabrous with an entire margin, ex-stipulate, having parallel venation of 10–12 primary veins (Fig. 1-E). The upper surface cotyledonary blade is dark green and the lower surface is pale green in appearance and it performs the assimilatory function.

The first internodal growth starts from the axils of the cotyledon. First eophyll becomes visible on the 3rd DACE when hypocotyl attains a height of 10 mm (Fig. 1-F). The glabrous hypocotyl is erect and pale green. First eophyll and blade-like cotyledon remain arranged in alternate phyllotaxy. The blade of the cotyledon gradually swollen and became orbicular.

The first eophyll completes its development and attains a size of 35 × 23 mm at 10–15 DACE. A prominent leaf sheath surrounds the slender internode, which remains covered by minute white to hyaline hair. The growth of hypocotyls comes to an end after attaining a length of 22 mm after the appearance of the 2nd leaf (50 × 25 mm). The first and second internodes also attain a length of 8 mm and 5 mm respectively. Rhizomes start to develop from the epicotyl region by rupturing the leaf sheath of the first foliage leaf at 15 DACE.

The 3rd leaf (60 × 30 mm) and 4th (71 × 33 mm) leaf completes their development by 20–25 DACE. The completion of leaf development is marked by yellowing and subsequent drying up of the leaf tip. The primary root attains a

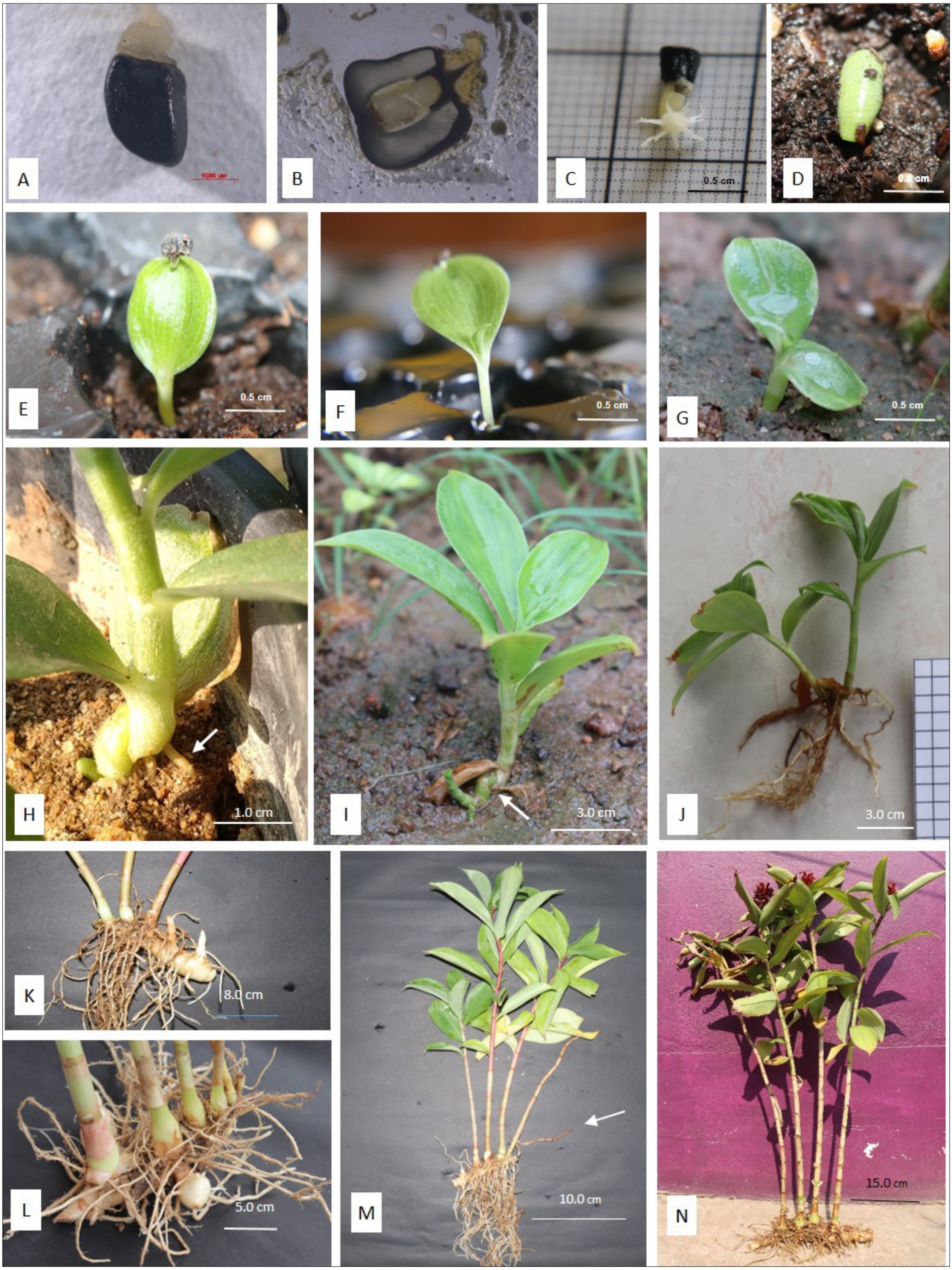

Figure 1. Photographs of different stages of seedling phenology of *H. speciosa*. (**A**) seed with aril. (**B**) L.S through seed. (**C**) Germinated seed with collet and rootlets. (**D**) Emergence of cotyledon from the soil. (**E**) cotyledon bearing seed at its tip. (**F-G**) Seedling showing cotyledon with first eophyll. (**H**) Stilt like adventitious roots developing from hypocotyl base. (**I**) Rhizome initial developing from the leaf sheath of first eophyll. (**J**) Seedling genet with first ramet. (**K-L**) Ramets on horizontal rhizome arranged alternately. (**M**) Mature and vegetative ramets (arrow indicates the remnant of dead seedling). (**N**) Mature and reproductive ramets of the previous growing season.

length of 70 mm with many branches. In addition, adventitious branches also arise. These adventitious branches bear no additional branches or hair. The adventitious branches were 67 mm, 52 mm and 25 mm in length (Fig. 2-D). The hypocotyl begins to inflate and the stilt-like 4–5 adventitious root arises from the base of the hypocotyl (Fig. 1-H). The length of the first and second internode increases very slowly. The first internode completes its growth after attaining a length of 10 mm by 25 DACE. From the developing rhizome, a lateral, slender, erect and green ramet of 4 mm starts to emerge from the soil (Fig. 1-I). The cotyledonary leaf blade completely dries off.

When the seedling attains a height of 81 mm (at 30–35 DACE), the 5th (80 × 37 mm) to 8th (110 × 40 mm) leaf completes its development. The first foliage leaf i.e. the first eophyll dries off. The growth of the primary root continues and reaches up to 85 mm.

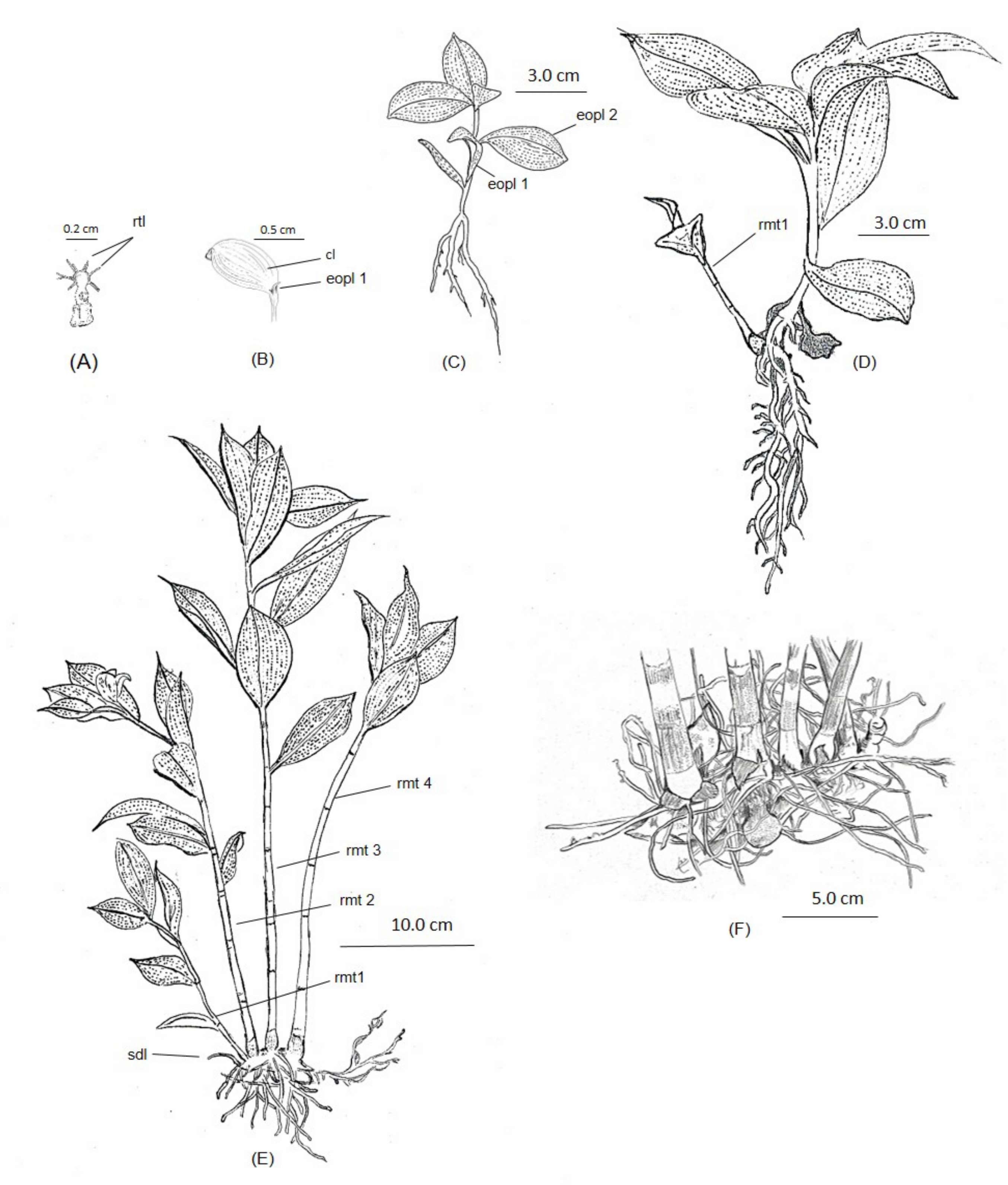

Figure 2. Line drawing of different stages of seedling phenology of *H. speciosa*. (**A**) Germinated seed. (**B**) Cotyledon. (**C**) Seedling with cotyledon and eophyll. (**D**) Seedling with first ramet. (**E**) Mature and vegetative ramets. (**F**) Ramets on horizontal rhizome with adventitious roots. (rlt, rootlet; cl, cotyledon; eopl, eophyll; rmt, ramet; sdl, seedling).

Phase of ramets

The phase of ramets starts at 25 DACE with the emergence of ramet from developing rhizome. The rhizomes start to produce adventitious roots by this stage. The phenological changes of the ramets were very quick compared to the growth of seedling genet, hence we took phenological measurements in two-day intervals for ramets. The first ramet reaches up to 30 mm at 30 DACE with a single leaf (5 × 5 mm). After two days i.e. at 32 DACE, the ramet increases by 10 mm and the leaf increases up to 15 × 10 mm. The 2nd leaf (40 × 10 mm) on the ramet arises at 34 DACE. The third leaf (40 × 6 mm) on the ramet becomes visible at 36 DACE. At 38 DACE, the second and third leaf attains 80 × 40 mm and 90 × 40 mm respectively. The 4th leaf (60 × 32 mm) and unfurled immature fifth leaf (32 mm in length) were also visible during that time. The stem at this point attains 80 mm height.

The 2nd leaf completes its development at 40 DACE. The 3rd, 4th and 5th leaf grows up to 90×45 mm, 95×40 mm and 60×10 mm without any further increase of stem height.

Growth of 3rd leaf ceases after 41 DACE. The 4th and 5th leaf continues to grow and attains 105 × 45 mm and 100 × 42 mm respectively. The 6th leaf attains a length of 75 mm and starts to unfurl. The length of five internodes starting from the rhizome to the first foliage leaf cumulatively contributes to the increase in the height of the shoot up to 87 mm, which increases by 13 mm more in the next two days. The stem attains a height of 100 mm with a mean internode length (cumulative) of 91.66 mm at 43 DACE. Here, the 4th and 5th leaf completes their growth. 6th leaf completes unfurling and attains 90 ×45 mm and the next leaf i.e. 7th leaf starts to open (55 × 2 mm). The 7th leaf matures (110 × 40 mm) and the 8th leaf starts to unfurl (60 × 02 mm) by 40–45 DACE. The 4th to 7th leaves complete their growth at 45 DACE with a leaf measurement of 115 × 45 mm to 110 × 40 mm. Such leaf measurement indicated gradual changes in leaf shape from narrowly ovate to lanceolate. The seedling attains 95mm height and completes its 9th (110 × 40 mm) to 12th (80 × 30 mm) leaf development at this stage. From this stage, the seedling genet gradually dries off. It never produces any inflorescence or flower. Here the 2nd lateral ramet begins to sprout. Likewise, the 3rd, 4th and 5th ramet arises from the horizontally developing rhizome. The 5th ramet completes its growth at 120–125 DACE. Ramets rarely produce inflorescence in the current growing season. A plot (Fig. 3) showing comparisons of leaf length and leaf breadth in seedling genet, vegetative ramets and reproductive ramet from previous season (control). Pearson's correlation study found a strong positive correlation between genet and control (mature and reproductive ramets from previous growing seasons) concerning leaf length and breadth (r=0.99, p<0.001) and (r=0.79, p<0.05) respectively (Fig. 4 and 5). Such strong correlations were not observed for ramets.

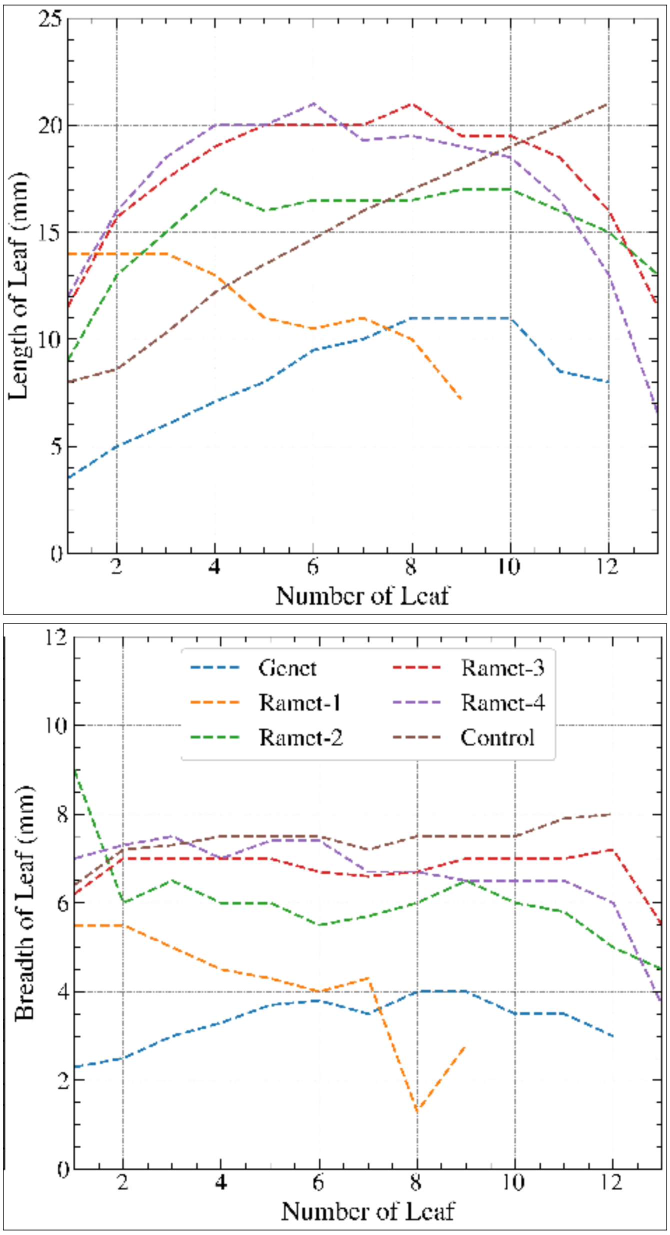

Figure 3. Plot showing a comparison between leaf length and leaf breadth in seedling genet and its ramets.

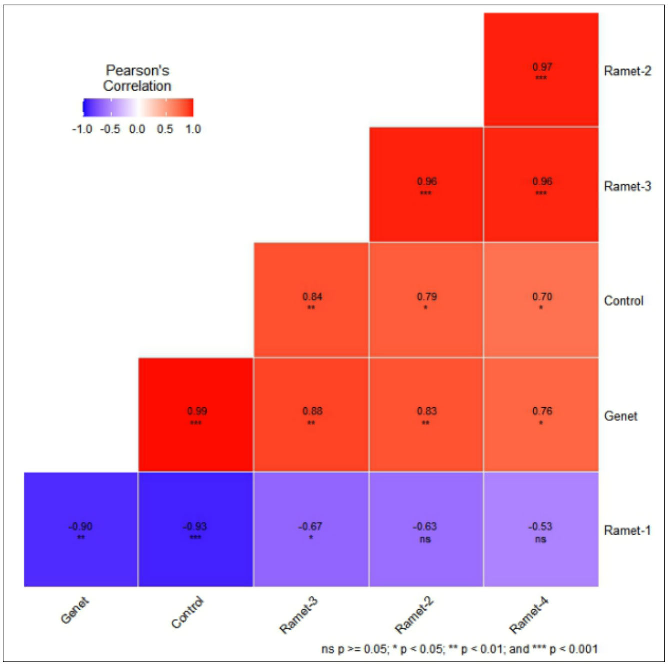

Figure 4. Correlation matrix plot among leaf length of genet, ramets and control (Control-mature reproductive ramet from previous growing season).

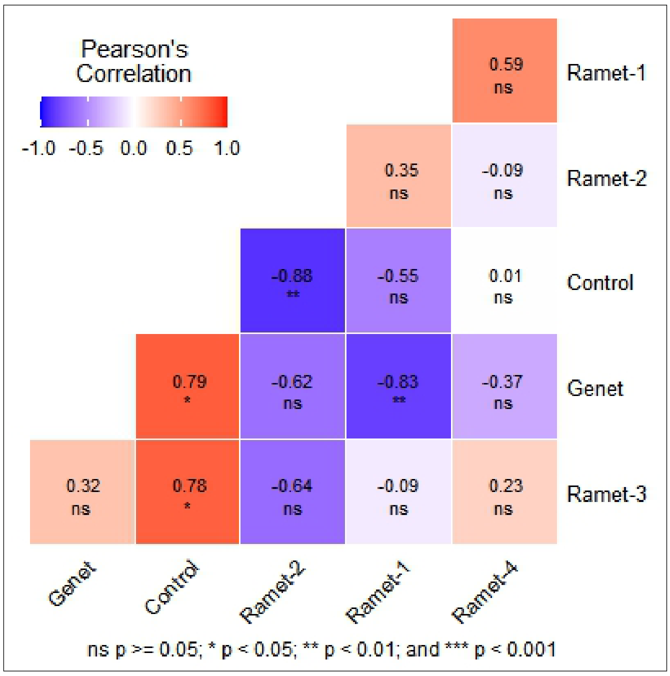

Figure 5. Correlation matrix plot among leaf breadth of genet, ramets and Control (Control=mature reproductive ramet from previous growing season).

Discussion

The epigeal and phanerocotylar seed germination in *H. speciosa* is favourable since they grow more quickly with increased light (10). Although the seedling genet has a brief lifespan, it is nonetheless crucial for the development of ramets since it starts the rhizome's emergence from its epicotyl. Previous research on *Asparagus* showed how the base epicotyl develops into the rhizome (11). In contrast, rhizome growth from the hypocotyl base was observed in *Dioscoria glabra* (12). Adventitious roots that resemble stilts have grown from the hypocotyl base. Such adventitious roots play a significant role under stress conditions such as flooding, nutrient deficiency and wounding (13) and considered as unique feature in clonal growth (14). The plant exhibits considerable clonal proliferation during the growing season, producing 4–5 clonal ramets horizontally, and a seedling genet. Vertical growth favours to horizontal clonal development. This type of proliferation is regarded as advantageous. (14). Such clonal growth through ramets increases floral display size and the potential for outcrossing, contributing to reproductive fitness (15). Clonal growth and production of ramets also increase the survivability of the plant. The clonally propagated genet shares the risk of mortality with its ramets (16). Thus, in the premature death of any ramet, the genetic stock remains alive representing the original genotype. Such a survival benefit is noticed in *H. speciosa*. Additionally, clonal and sexual reproduction with repeated seedling requirements is a strategy for establishing new genets that helps to deal with changing environment (17). Ramets are potentially independent (5) and immortal (6), representing seedling genotype, although somatic mutations may bring about variations in genotypes within ramets. The gradual transformation of leaf shape from ovate to lanceolate is a notable feature. The architecture of clonal growth of *H. speciosa* shows "phalanx strategy," i.e. close aggregation of ramets on growing rhizome (15) with linear "zig zag" pattern (Fig. 1-L). Later, the rhizome escapes from this zig zag pattern by producing additional ramet initials from the inflated internode of the base of the previous ramets. This indicates strategic accumulation of storage material to support the developing ramets. This distance from the rhizome to the first assimilatory leaf increases steadily in each ramet i.e. 20, 25, 40, 54 cm for ramet, 2nd, 3rd, 4th, and 5th respectively. That indicates rhizomatous dependency for nourishment in early growing phases. Positive correlation in morphometric measurements of leaves of seedling genet with that of mature ramets indicates the end of seedling phase as it appears as a miniature of an adult plant (1). The increasing number of ramets with rare occurrence of reproductive structure indicates an increased allocation of resources towards vegetative functions than reproductive functions. Such allocation trade-offs between vegetative and reproductive structures in clonal species have been studied previously (18).

Conclusion

The seedling phenology and growth behaviour of *H. speciosa* revealed specific adaptive strategies that support reproductive fitness. Firstly, the plant grows through clonal propagation, which aids the plant's survivability. Additionally, the genetic diversity of the clonal population is increased through repeated seedling recruitment. Furthermore, the "phalanx strategy" of clonal ramets benefits successful reproduction by expanding flower display size and enabling biotic pollinators to outcross. Despite having a very brief lifespan, seedling has played a crucial role in clonal proliferation by establishing the rhizome. The strategic allocation of resources to the vegetative growth of ramets produces more clones and significantly more rhizome growth. Rapid colonization and accumulation of sufficient photosynthates are made possible by this growth pattern, which ultimately aids the reproductive system's maturity in succeeding growing seasons. The study suggests that the clonal propagation from seed and seedling imparts genetic diversity in the clonal population that can be used to collect rhizomes which is a rich source clinically important secondary metabolites.

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Authors contributions

AS carried out the collection of necessary data and statistical analysis. BB participated photography and hand drawing of plant's developmental phases. PK participated in the design of the study and preparation of manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: **:** Authors declare that they don't have any conflict of interest.

Ethical issues: None

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