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Rhizome Segments Form Shoots, Whereas Leaf Cuttings Form Shoots and Rhizomes in *Eucodonia* 'Adele' Treated with Benzyladenine

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Abstract. ***Eucodonia*** **'Adele' initiates seasonal shoot growth from a scaly rhizome. Larger rhizome segments (>2.5 cm) produced shoots at a greater percentage compared with smaller rhizome segments. Shoots produced on larger segments were initiated sooner and had a longer length. However, when shoot formation efficiency was calculated as the number of potential shoots per original stock rhizome, smaller rhizome segments were more efficient, producing three to four times as many shoots. Rhizome segments (2.5 cm) soaked overnight in benzyladenine (BA) produced three to four times more shoots per rhizome (four shoots) compared with untreated or water-soaked rhizomes (0.3 and 0.7 shoot, respectively). The scaly rhizome consists of a central stem-like core surrounded by numerous leaf-like scales. Scales appear to be storage leaf tissue based on anatomy and presence of numerous amyloplasts. New shoots initiate as axillary shoots formed from the central core at the scale attachment. Isolated individual scales also have the capacity for adventitious shoot formation, but only form in about 25% of isolated scales. Leaf cuttings were capable of producing adventitious shoots, roots, and rhizomes. Untreated petiole and lamina cut leaf cuttings formed approximately three rhizomes per leaf cutting compared with less than one adventitious shoot per leaf cutting. Benzyladenine-treated leaf cuttings did not show an increase in rhizome initiation, but soaking lamina cut leaf cuttings in water or BA increased shoot formation to ~1.5 shoots per cutting. This work with isolated rhizome segments and leaf cuttings presents efficient systems for regenerating rhizomes that can be used to produce stock plants for a stem cutting system for** ***Eucodonia*** **'Adele' as a seasonal pot plant.**

Eucodonia is a small genus in the Gesneriaceae with two species that are native to Mexico. The Gesneriaceae is a diverse group with about 150 genera of mostly tropical or subtropical herbaceous terrestrial or epiphytic plants. *Eucodonia* is in the subfamily Gesnerioideae and tribe Gesnerieae with plants that form mainly from scaly rhizomes (Zimmer et al. 2002). *Eucodonia* is closely related to *Achimenes*, and species in both genera produce plants that have scaly rhizomes. When plants of *Eucodonia* or *Achimenes* are produced in temperate areas, they go dormant in winter after summer and early-fall flowering, and survive as the scaly rhizome.

Eucodonia 'Adele' is a hybrid cross with *Eucodonia verticillata* 'Frances' as one of the parents. It produces large, lavender-blue tubular flowers above dark-green pleated foliage (Fig. 1). Plants emerge from winter-dormant rhizomes and grow about 6 inches (15 cm) tall. They are summer-to-fall flowering and can be produced as a seasonal pot plant or as a partly shaded garden plant where they are winter hardy (Zone 7b US Department of Agriculture hardiness map).

Information is limited on *Eucodonia* propagation, but should follow established practices for *Achimenes* (Dole and Wilkins 2005). *Achimenes* shoots emerge from dormant rhizomes from the previous season's crop. Plants can be formed directly from rhizomes or rhizome pieces, or emerging shoots from rhizomes can be used as stock plants for cutting propagation. Several harvests of two to three node cuttings can be taken from the same stock plant from late spring into early summer.

Eucodonia 'Adele' shows potential as a commercial seasonal pot plant. To become a viable commercial crop, an efficient system to supply rhizomes would be needed from specialty growers. Therefore, the objective of this research was to investigate shoot formation efficiency related to rhizome size and cytokinin treatment in *Eucodonia*, as well as shoot or rhizome initiation in leaf cuttings. In addition, shoot formation was observed to understand the anatomic relationship between rhizome segments used for propagation and origin of new shoot formation.

Materials and Methods

Plant material. In 2020 and 2021, *Eucodonia* 'Adele' plants were grown from March to November in the greenhouse. Plants were allowed to dry seasonally. In mid-January, rhizomes were harvested from dormant plants and the substrate removed carefully by rinsing gently with water. Only healthy, intact rhizomes that ranged from 3 to 8 cm in length were selected for the study.

Growing conditions. Five rhizome pieces were pressed gently into a premoistened substrate (ProMix BX; Premier Tech Horticulture, PA, USA) in plastic inserts (7.9-cm width × 12.4-cm length × 5.9-cm height), fitting a standard 1020 open flat (T. O. Plastics, Inc., Clearwater, MN, USA). About one-half of the rhizome surface was covered with substrate. In 2021, flats were placed on greenhouse benches from mid-March to July. Greenhouse temperature set points were 21 °C days and 18 °C nights, and daily light integrals ranged from 15 to 25 $mol \cdot m^{-2} \cdot d^{-1}$. In 2022, flats were covered with a plastic dome (Humi-dome; Hummert International, Earth City, MO, USA) and placed in a growth chamber at 25 °C day/21 °C night temperatures under cool-white fluorescent lamps with a 16-h photoperiod (~80 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Rhizome size and shoot formation. Rhizomes were left intact or cut into sized segments with a razor blade before placing on the substrate. Treatments included an intact rhizome control that was between 5 and 8 cm in length and included the rhizome tip. In addition, rhizomes that had the rhizome tips removed were cut into sections that were 5.0, 2.5, 1.0, or 0.6 cm in length. The final treatment included single intact scales that had been separated gently from the rhizome core. Rhizomes were pressed gently into the substrate, whereas scales were placed directly on the surface and not covered with substrate.

A nonreplicated study was conducted to observe the capacity of scales and rhizome fractions to produce shoots. Surgical treatments included single and paired twin scales along with 2-cm rhizomes cut longitudinally in half or quarters. These were placed on germination paper wetted with 5 mL deionized (DI) water in petri dishes held under growth chamber conditions.

Fig. 1. *Eucodonia* 'Adele' plants indicating flower color and shape.

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Table 1. Shoot formation in *Eucodonia* 'Adele' rhizomes sections or scales.

Rhizome size (cm)	2021			2022		
	Rhizomes with shoots (%)	Shoots per rhizome section	Shoot formation efficiency[i]	Rhizomes with shoots (%)	Shoots per rhizome section	Shoot formation efficiency[i]
5.0	94.5	1.3 a[ii]	1.2	96.7	1.4 a	1.4
2.5	94.5	1.1 ab	2.1	96.7	1.2 ab	2.3
1.0	—	—	—	86.7	0.86 b	4.3
0.6	53.0	0.8 b	3.5	86.7	0.93 ab	6.7
Single scale	23.9	0.3	3.5	—	—	—

[i] Calculated as Percentage × Shoot number × Sections per original rhizome. A conservative estimate of scales per rhizome piece was set at 50 scales per rhizome.
[ii] Means followed by the same letter within a column were not different at 0.05 by Tukey's test.

Cytokinin-treated rhizomes. In 2022, 2.5-cm rhizome pieces without rhizome tips were either untreated (placed directly into substrate) or soaked for 24 h in DI water or benzyladenine (BA) (PhytoTech Laboratories, Lenexa KS, USA) at 25, 50, or 100 mg·L^{-1} (111, 222, or 444 μM). Solutions were three times the rhizome volume in 500-mL containers. Treated rhizomes were rinsed briefly in DI water before being placed on substrate and then moved to the growth chamber as described previously.

Experimental design. Each insert tray contained five rhizomes or 20 scales. In the rhizome size experiments, there were nine replicate trays per treatment in 2021 and six replicate trays in 2022. For the cytokinin experiment, there were five replicate trays per treatment. The percentage of rhizome pieces or scales producing shoots and the number of shoots per rhizome piece or scale were evaluated 4 months after placing them on the substrate. Shoot production efficiency was calculated as Percentage of rhizomes with shoots (expressed as a decimal) × Shoots per rhizome section × Number of sections excised per intact rhizome. For example, for 1-cm rhizome sections from Table 1, the percentage of rhizome sections with shoots (0.867) × shoots per segment (0.86) × number of sections per rhizome (5) = 4.3 shoots per original rhizome. Shoot height (measured in centimeters) was recorded after 5 months. Insert trays were assigned randomly into the flats, and mean separation was by Tukey's test at the 5% level using SigmaPlot version 12.3 (Systat Software, Richmond, CA, USA).

Table 2. Stem height in *Eucodonia* 'Adele' plants derived from rhizome pieces of various sizes.

Rhizome size (cm)	Nodes per stem	Stem ht (cm)
Intact (∼5 cm)	3.3 a[i]	4.3 ab
2.5	3.4 a	4.7 a
0.6	3.3 a	3.2 bc
Single scale	2.8 a	2.4 c

[i] Means followed by the same letter within a column were not different at 0.05 by Tukey's test.

Shoot and rhizome initiation in leaf cuttings. Leaf cuttings were prepared by removing fully expanded leaves from stock plants and either using a razor blade to cut ∼2 mm from the petiole end or to cut the lower base of the lamina just above the petiole attachment. Leaf cuttings were left untreated or soaked for 24 h in DI water or BA at 50 or 100 mg·L^{-1} (222 or 444 μM). Leaf cuttings were placed upright in 500-mL beakers with only the lower 1.0 cm submerged in water or the BA solution, and were left uncovered on the benchtop. Leaf cuttings were placed in six-pack containers (one leaf cutting per cell) filled with vermiculite. Flats were covered with a plastic dome and placed in a growth chamber at 25 °C day/21 °C night temperatures under light-emitting diode lamps with a 16-h photoperiod (∼80 μmol·m^{-2}·s^{-1}). There were four replicate six packs (24 cuttings in total) per treatment. Cuttings were taken Oct 2022 and evaluated for rhizome or shoot formation after 60 d.

Rhizome and shoot initiation anatomy. Rhizome sections or scales at various stages of shoot formation development were fixed in formalin aceto-alcohol and vacuum-infiltrated for 24 h. Tissue was rinsed twice with 50% ethyl alcohol and dehydrated using a tertiary butyl alcohol dehydration series. Tissue was embedded in paraffin blocks, attached to a rotary microtome (model 820; American Optical, Buffalo, NY, USA), and sectioned at 12 to 15 μm. Tissue sections were affixed to glass slides (8 × 3 cm) and stained with safranin-fast green. In addition, hand sections of rhizome tissue were stained with 5.0% iodine-potassium iodide to visualize cellular starch. Sections were observed and photographed under a light microscope (Olympus BX40; Evident Corp., Tokyo, Japan) equipped with a digital camera (Olympus DP25, Evident Corp.).

Results

Rhizome size and shoot formation. Intact and fragmented rhizomes showed a high capacity for initiating adventitious shoots (Table 1). In general, intact and larger rhizome sections produced at a greater percentage (∼95%) compared with shorter sections (50%–87%). Single scales showed the lowest capacity for shoot formation at 24%. Shoots-per-rhizome section did not vary greatly in relation to section size,

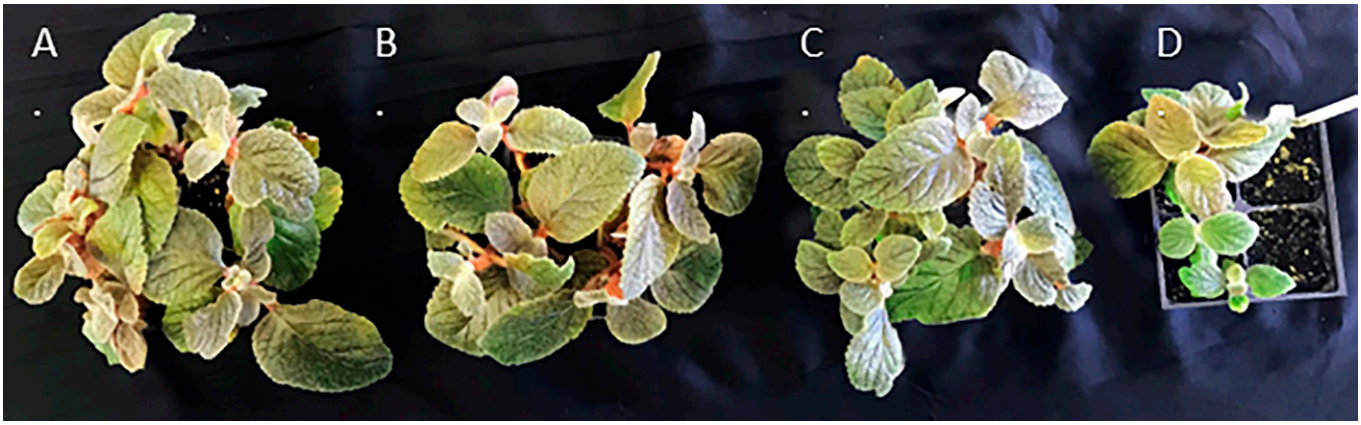

Fig. 2. Shoot formation in *Eucodonia* 'Adele' rhizomes four months after rhizomes placed on substate. (**A**) Intactrhizome. (**B**) Rhizome (2.5 cm) without rhizome tip. (**C**) Rhizome (1.0 cm) without rhizome tip. (**D**) Individual scales.

ranging from 0.8 to 1.4 shoots per section. However, shoot formation efficiency expressed as the number of shoots that could be formed per intact rhizome after sectioning demonstrated that more total shoots were initiated using smaller sections. For example, in the 2022 experiment, using 0.6-cm rhizome sections produced approximately five more shoots compared with intact 5-cm rhizomes. Interestingly, even though using single scales showed a low percentage conversion for shoot formation, by using a conservative estimate of 50 scales per rhizome section, there was an equivalent number of shoots per rhizome to the 0.6-cm section, and the efficiency would improve dramatically if the percentage of scales converting to shoot production could be increased.

Shoot development, as indicated by nodes per stem after transfer to a greenhouse environment, was not significantly different (Table 2). However, stem height was greater in shoots from larger rhizome segments, with 5- and 2.5-cm segments averaging ~2 cm more growth (Fig. 2). Plants grown under greenhouse conditions used resources from the original rhizome for shoot development and began new rhizome development from stolons coincident with flower formation (Fig. 3).

Cytokinin-treated rhizomes. There was a substantial decrease in time to shoot formation and a significant increase in shoot number in 2.5-cm rhizome sections treated with a 24-h soak with BA before planting (Table 3, Fig. 4). All rhizome segments formed shoots when treated with BA after 30 d, whereas those receiving no treatment or only a water soak formed shoots at 60% and 24%, respectively. Benzyladenine concentration did not impact shoot formation in BA-treated rhizomes, but shoot numbers were increased significantly over untreated and water-treated rhizomes (4.1 shoots vs. 0.5 shoot per rhizome section).

Rhizome and shoot initiation anatomy. Eucodonia produces a scaly rhizome typical to members of the Gesneriaceae tribe in the Gesneriaceae (Fig. 5). The scales appear to be modified leaves attached to a central stem-like core of the polar rhizome (Figs. 5 and 6).

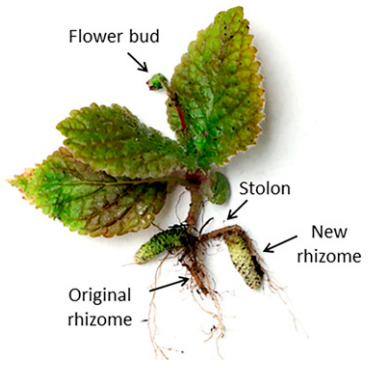

Fig. 3. *Eucodonia* 'Adele' rhizome formation from stolons on a plant derived from the previous season's rhizome section cutting.

Table 3. Shoot formation in 2.5-cm *Eucodonia* 'Adele' rhizomes after a 24-h soak in water of benzyladenine (BA).

	Rhizomes with shoots (%)		Shoots per rhizome section	
Treatment	After 30 d	After 50 d	After 30 d	After 50 d
No treatment	24 c[1]	80 b	0.3 b	0.8 a
Water	60 b	84 b	0.7 b	1.0 a
BA 25 mg·L^{-1}	100 a	100 a	3.7 a	—
BA 50 mg·L^{-1}	100 a	100 a	4.1 a	—
BA 100 mg·L^{-1}	100 a	100 a	4.1 a	—

[1] Means followed by the same letter within a column were not different at 0.05 by Tukey's test.

Each scale is connected to the central core with a narrow petiole-like attachment (Figs. 5C and 6B). The central core has an epidermis, cortex, and vascular ring around a central pith (Fig. 6B). The rounded pith cells include mostly collenchyma, with some cells showing sclereid formation (Fig. 6C and D).

Eucodonia scales are leaf-like, as evidenced by the preponderance of parenchyma cells, vein-like vascular system, and the "jigsaw"-shaped epidermal cells (Fig. 7A–D). Scales function as storage organs for the dormant rhizome with numerous starch amyloplasts (Fig. 7E and F).

Shoot formation in rhizome sections occurs in the axil between the stem-like core and the leaf-like scale (Fig. 8). In longitudinal rhizome sections, it is evident that shoot origin is from stem tissue (Fig. 9A and B). In scales, shoots were initiated from the proximal portion of the leaf-like scale, and adventitious roots form after shoot initiation on the proximal portion of the new shoot (Fig. 9C and D).

Shoot and rhizome initiation in leaf cuttings. Eucodonia leaf cuttings were able to initiate adventitious shoots, roots, and rhizomes (Table 4, Fig. 10). There was no difference in the percentage of untreated leaves forming rhizomes for leaves with a petiole or cut lamina. However, the ability to form adventitious shoots was greater for untreated leaves with the cut lamina surface compared with petiole leaf cuttings (Table 4). Overall, there were fewer adventitious shoots initiated compared with rhizomes and very few leaf cuttings with a petiole-formed shoot. Soaking leaf cuttings with or without BA had no effect or a negative effect on the number of shoots or rhizomes per cutting (Table 4). Benzyladenine had a negative impact on rhizome initiation in petiole leaf cuttings, reducing the percentage of leaves forming rhizomes to ~56% compared with 81% for untreated leaves or leaves soaked in water. Benzyladenine had no impact on rhizome initiation in leaf cuttings with a cut lamina, but there was a reduction in the number of leaf cuttings forming shoots after BA treatment.

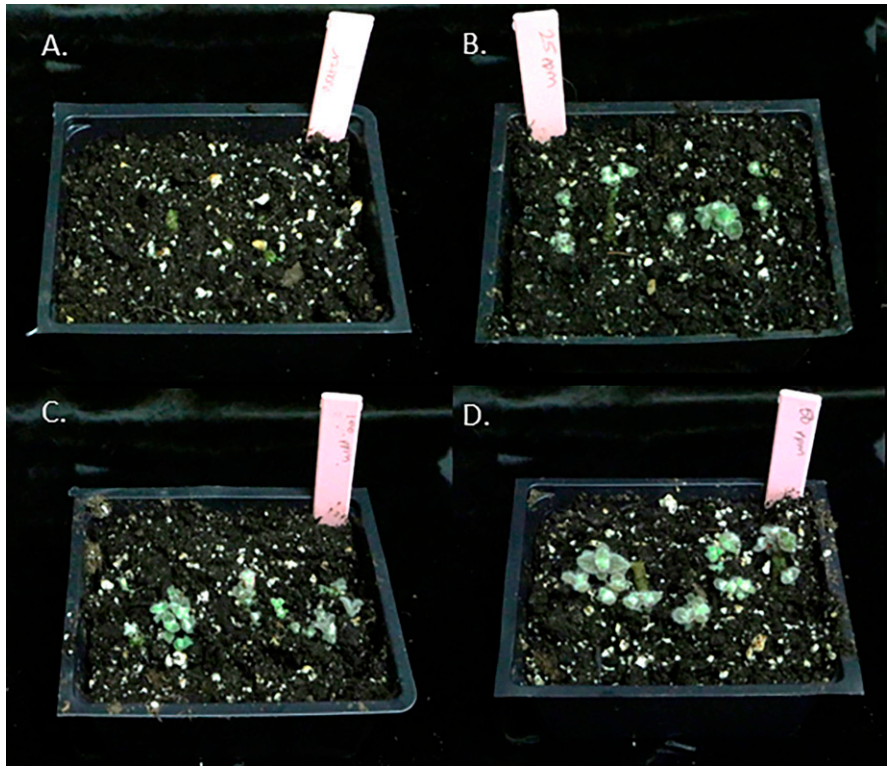

Fig. 4. Shoot formation in *Eucodonia* rhizomes treated with benzyladenine. (**A**) Water. (**B**) Benzyladenine at 25 mg·L^{-1}. (**C**) Benzyladenine at 50 mg·L^{-1}. (**D**) Benzyladenine at 100 mg·L^{-1}.

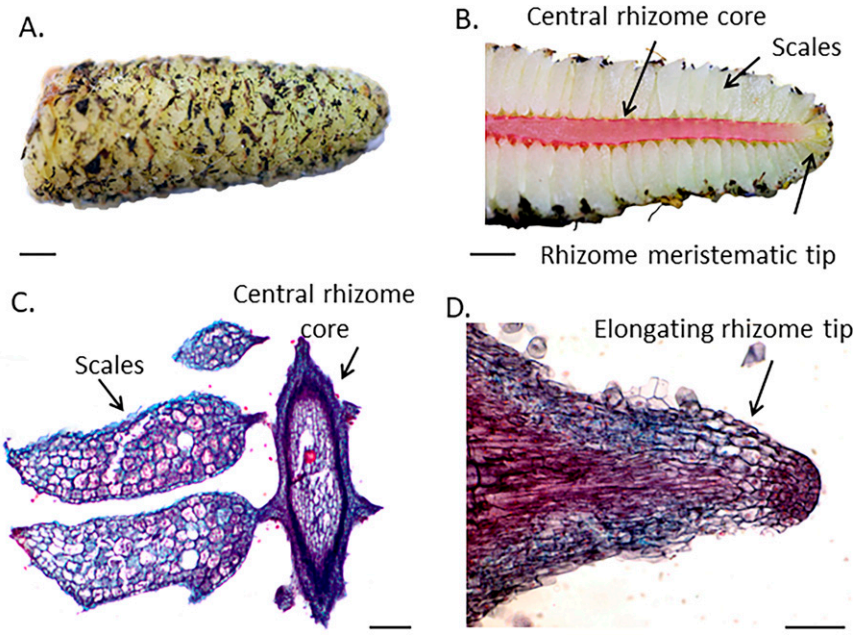

Fig. 5. *Eucodonia* 'Adele' rhizome anatomy. (**A**) Intact rhizome (scale bar = 2 mm). (**B**) Longitudinal rhizome hand section (scale bar = 2 mm). (**C**) Transection of rhizome core and scales (scale bar = 200 μm). (**D**) Elongating rhizome tip (scale bar = 200 μm).

Leaf cuttings initiated both shoots and rhizomes from the same leaf at 16.7% for leaf cuttings with a cut lamina and 3.1% for petiole leaf cuttings, and there were only a few instances when shoots were initiated on cuttings that did not also form rhizomes (Fig. 10). Rhizome initiation was usually at the cut surface of either the petiole or the lamina, but occasionally rhizomes were seen to initiate along the uncut leaf margin (Fig. 10E) or the lamina midvein (Fig. 10F).

Discussion

Rhizome size and shoot formation. The percentage of rhizome pieces forming shoots was greater in larger segments compared with smaller segments (Table 1), but when the number of shoots produced per original stock rhizome was calculated, the efficiency was greater in smaller segments. The increase in shoot production efficiency came with a delay in shoot formation and reduced shoot growth (Table 2). This is most likely related to the annual growth cycle pattern in which the original rhizome is used completely as shoots form, and a new group of rhizomes is produced on a short stolon that eventually becomes winter dormant (Fig. 3). It does not appear that includinicg the rhizome apical tip with intact rhizomes affected subsequent shoot growth significantly compared with the 2.5-cm rhizome segment without a tip. In a study using *Achimenes* rhizomes split into two halves, the rhizome segment that included the apical tip produced shoots that

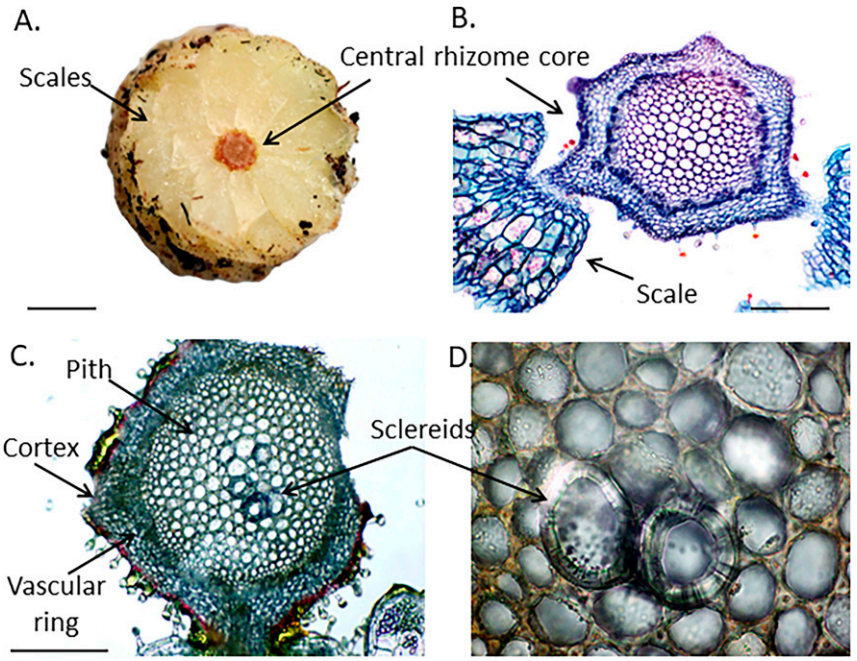

Fig. 6. *Eucodonia* 'Adele' rhizome in cross section. (**A**) Rhizome hand cross section (scale bar = 2 mm). (**B**) Micrograph of the central rhizome core and scale attachment (scale bar 200 = μm). (**C**, **D**) Micrograph of central rhizome core including sclereids in the pith (scale bar = 200 μm).

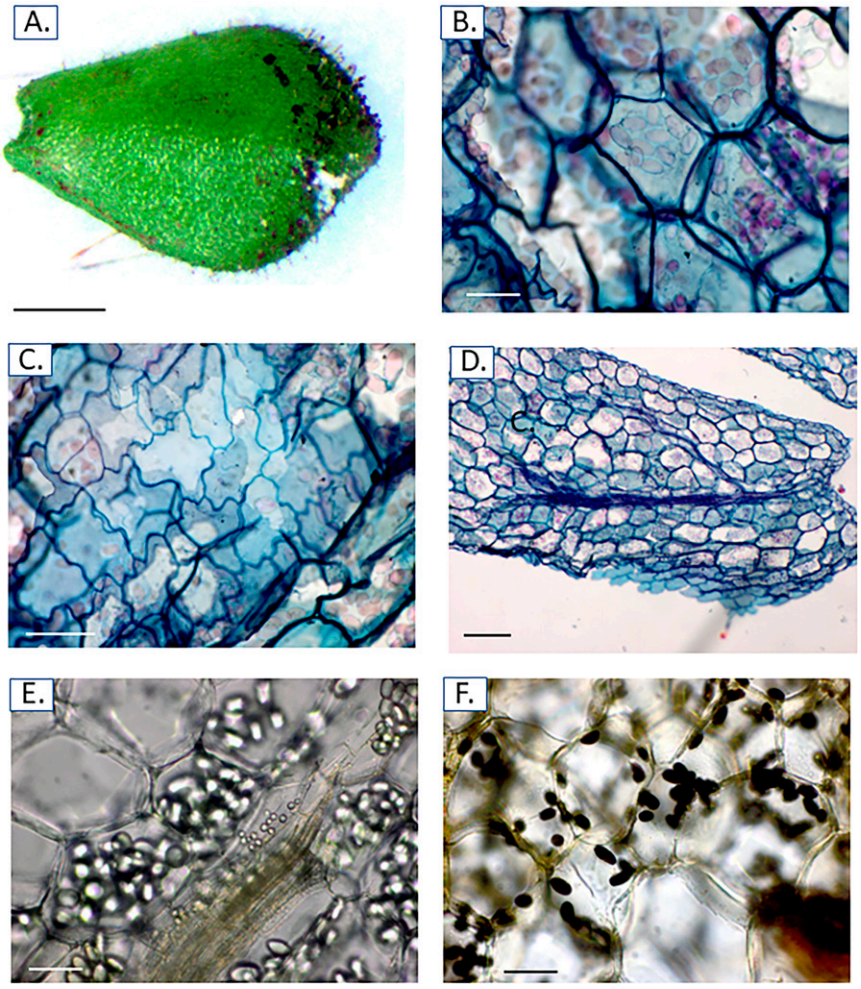

Fig. 7. *Eucodonia* 'Adele' scales. (**A**) Single scale (scale bar = 1 mm). (**B**) Scale parenchyma cells. (**C**) Adaxial surface scale cells. (**D**) Scale micrograph with parenchyma cells and vascular system (scale bar = 200 μm). (**E**) Parenchyma cells with starch amyloplasts next to a vascular strand. (**F**) Iodine-stained starch amyloplasts. (**B, C, E, F**) Scale bar = 40 μm.

were three times greater in length compared with the half without a tip (Vlahos 1985).

Choice of *Eucodonia* rhizome segment size could depend on the production scheme. When rhizomes were used to produce a final pot plant crop or for stock plant production of stem cuttings, larger rhizome segments provided greater quality shoot growth. However, for a specialty grower attempting to produce increased quantities of rhizomes for next season's crop, smaller rhizome segments are more efficient.

Cytokinin-treated rhizomes. Treating rhizomes with BA before planting induced a greater number of shoots to form per rhizome and reduced the time to shoot formation (Table 3). The action of cytokinins on in vitro axillary and adventitious shoot formation are well documented (Davies et al. 2018). Alternatively, cytokinins play a role in dormancy release in a number of geophytic structures by accelerating axillary budbreak (Zhao et al. 2022). Benzyladenine treatment of rhizomes in *Achimenes* was shown to increase the percentage of rhizomes with shoots that appeared earlier compared with untreated rhizomes (Vlahos 1985). It was suggested by Vlahos (1985) that the impact of BA was to reduce dormancy or apical dominance in the rhizomes promoting earlier shoot growth.

Rhizome and shoot initiation anatomy. Eucodonia shoots form from a polar scaly rhizome with an apical-growing tip at the distal end (Fig. 5). It forms tight concentric rings of scales around a central stem-like core (Fig. 6). Our study provides strong evidence that *Eucodonia* rhizome scales are modified storage leaves (Fig. 7). Shoots that form on rhizome segments occur in the axil of these modified leaves, suggesting they should be considered axillary shoots rather than adventitious shoots (Fig. 8). However, the shoots that form from isolated leaf scales appear to be formed from the proximal end of the scale and should be considered adventitious (Fig. 9C and D). Isolated scales could be considered analogous to a leaf cutting.

Shoot and rhizome initiation in leaf cuttings. Leaf cuttings require adventitious organ formation to be a viable propagation method. Numerous gesneriads have demonstrated the capacity to form adventitious shoots from leaf cuttings (Davies et al. 2018; Parliman and Stushnoff 1979). In *Eucodonia*, only shoot formation was observed in isolated scales (Fig. 9C and D), whereas shoots, roots, and rhizomes were initiated from leaf cuttings, but no stolon formation was observed (Fig. 10). In *Achimenes*, shoots, stolons, or rhizomes were observed to be the adventive organs formed on petiole leaf cuttings (Miller and Bridgen 2005). Miller and Bridgen (2005) also demonstrated that petiole leaf cuttings from "old" *Achimenes* stock plants (27 weeks after planting) formed a greater percentage of rhizomes compared with "young" stock plants (13 weeks after planting). In contrast, leaf cuttings from young *Achimenes* stock plants formed a greater percentage of shoots compared with old stock plants. In our study with *Eucodonia*, the stock plants were at ~30 weeks after planting, which would be at a similar stage to the old *Achimenes* stock plants described previously, and in petiole leaf cuttings they also showed a greater percentage of rhizome initiation compared with shoot initiation (83% and 2.5 rhizomes per cutting compared with 4% and 0.1 shoot per cutting). The same pattern was observed in leaf cuttings with cut lamina (87% and 3.5 rhizomes per cutting compared with 20% and 0.3 shoot per cutting). At the time leaf

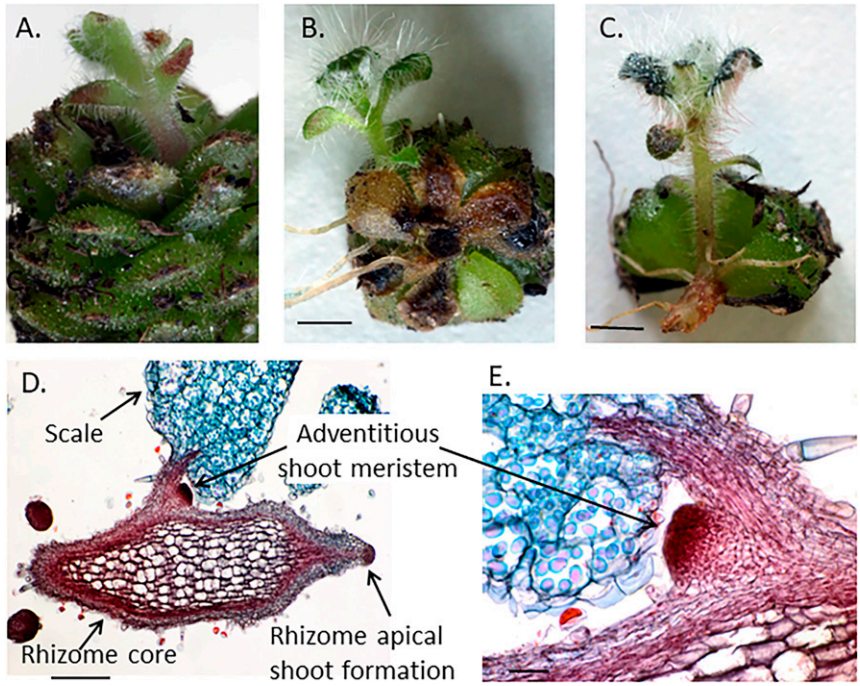

Fig. 8. *Eucodonia* 'Adele' shoot formation in rhizome pieces (**A**–**C**). Shoots emerging between rhizome scales (**D**, **E**). Longitudinal micrograph rhizome section with adventitious shoot initials emerging between the rhizome central core and scale axial junction. Scale bars in **B** and **C** = 2 mm; **D** scale bar = 200 μm; **E** scale bar = 20 μm.

cuttings were made, *Eucodonia* stock plants were in the process of new-rhizome formation from short stolons (Fig. 3), and it could be possible that internal signals associated with natural rhizome initiation were active in leaf cuttings, leading to rhizome formation rather than shoot formation.

In contrast to shoot formation in rhizome segments, *Eucodonia* leaf cuttings did not respond to BA to initiate shoots or rhizomes. The promotive impact of exogenous cytokinins on shoot initiation on in vitro leaf segments is well documented (Davies et al. 2018; Kumar and Reddy 2011). Benzyladenine has also been reported to increase adventitious shoot formation in selected leaf cuttings such as begonia and kalanchoe (Davies and Moser 1980; Heide 1965a, 1965b), or have no effect on leaf cuttings as observed in the succulent *Echeveria* (Cabahug et al. 2016). It could be that BA had no impact in *Eucodonia* leaf cuttings based on the stage of stock plant development or the application method. In Rieger begonia (*Begonia* ×*hiemalis*) leaf cuttings, BA applied in talc or foliar spray was more effective than a 12-h basal soak (Davies and Moser 1980).

Conclusion

An efficient system was developed for producing shoots from *Eucodonia* rhizome segments that could be used for pot plant production or to multiply rhizomes. Shoot formation efficiency was improved by using smaller

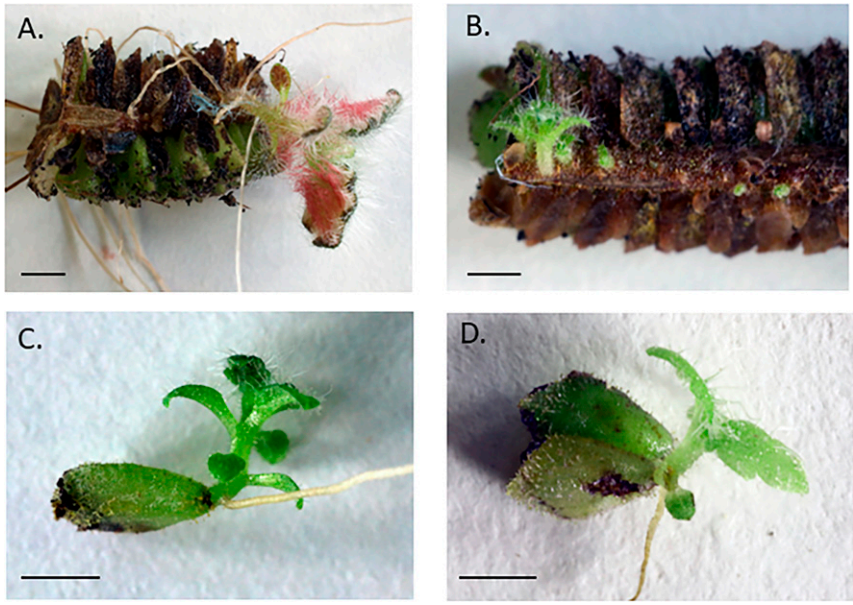

Fig. 9. *Eucodonia* 'Adele' shoot formation in longitudinally sectioned rhizome pieces and intact single scales. (**A**) A 2-cm rhizome piece without a rhizome tip cut in half longitudinally. (**B**) A 2-cm rhizome piece without a rhizome tip cut into quarters longitudinally. (**C**, **D**) Single (**C**) and twin (**D**) scales forming a shoot at the proximal end of the scale. Scale bar = 2 mm.

Table 4. Adventitious shoot and rhizome production in *Eucodonia* leaf cuttings from the cut petiole or lamina after a 24-h soak in 0, 50, or 100 mg·L^{-1} benzyladenine (BA).

Leaf cutting	BA (mg·L^{-1})	Cuttings with shoots (%)	Shoots per cutting	Cuttings with rhizomes (%)	Rhizomes per cutting
Leaf with petiole	None	4.2 b[1]	0.1	83.3 a	2.5 ab
	0	0	0	79.2 a	1.7 ab
	50	8.3 b	0.1	50.0 b	1.3 b
	100	0	0	62.5 b	1.4 b
Leaf with cut lamina	None	20.1 a	0.3	87.5 a	3.5 a
	0	37.5 a	0.5	91.7 a	3.1 a
	50	12.5 b	0.3	87.5 a	3.3 a
	100	8.3 b	0.1	87.5 a	3.3 a

[1] Means followed by the same letter within a column were not different at 0.05 by Tukey's test.

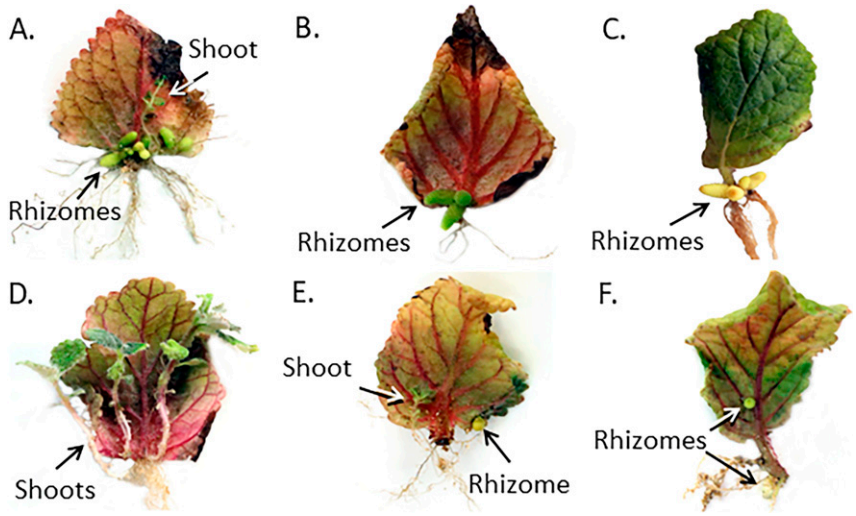

Fig. 10. *Eucodonia* 'Adele' rhizome and shoot formation from leaf cuttings. (**A**) Leaf cutting forming rhizomes and a shoot along the cut lamina. (**B**) Rhizomes forming near major veins on the cut lamina. (**C**) Rhizomes forming on the cut petiole. (**D**) Multiple shoots forming from the cut lamina. (**E**) Shoot forming from the petiole and a rhizome forming from the lamina margin. (**F**) Rhizomes forming from the cut petiole and along the midvein on the lamina.

rhizome segments, with 2.5-cm segments producing a greater percentage of shoots that showed good shoot length growth. Shoot formation efficiency improved further by treating 2.5-cm rhizome segments with a BA solution overnight. In addition to increasing the number of shoots per rhizome, BA-treated rhizomes also showed reduced time to visible shoot formation. Leaf cuttings proved to be a good system for rhizome multiplication. On average, untreated petiole and lamina cut leaf cuttings formed three rhizomes per leaf cutting. Single stock plants could have five or six leaves suitable as leaf cuttings (Fig. 2), and if 85% of leaf cuttings initiate rhizomes, they could yield 13 to 15 rhizomes per stock plant compared with the three or four natural offset rhizomes produced on an actively growing stock plant (Fig. 3).

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