Inducing Adventitious Rooting in Cycad Leaves

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Cycads are exotic, ancient and rare, hence their popularity as collectibles. They also tend to reproduce very slowly. The time from the pollination of the female cone to the emergence of the first leaf in a seedling can be measured in years. Faced with a steady decline in habitat and pressure from both poachers and collectors, many species are at risk of extinction. To quote Chamberlain, the living cycads "are the last of their race, restricted in geographic distribution, and struggling for their very existence." If cycads are to continue, discovering faster, more efficient means of reproduction is paramount. These should be simple techniques that can be used effectively by almost anyone having basic horticultural skills. Fortunately, the "primitive" heritage of the cycad has bestowed upon it a unique survival advantage. Almost every part of a cycad, even individual pinnae (those that have a midrib), are capable of regenerating roots. Tom Broom, in a recent article in the Cycad Newsletter, described numerous techniques for deriving new plants from pieces of trunk, stem, and roots.

Leaf cuttings can also an excellent source of new plants. Cycad leaves are remarkably resistant to desiccation, staying fresh for days after being cut. Cycads also show a tendency to develop roots from old leaf bases on the sides of the trunk (Pant, 1990). In South Africa, observations by Roy Osborne (1988) that "whole leaves removed from the parent plant and placed in a warm, porous medium . . . (produce) callogenesis and occasional rhizogenesis . . . ." Supporting this, a happy accident planting dislodged leaves of *Encephalartos lehmannii*, one of which rooted and produced a new plant after several years, prompted Nat Grobbelaar to experiment with excised leaves from ten different species of *Encephalartos*. He was able to show that *Encephalartos* leaf bases rooted easily (even without being treated with rooting hormones) and that these rooted leaves were capable of surviving for long periods (7.5 years). Of these survivors a small percentage regenerated the terminal bud (apical meristem) that gives rise to leaves, cataphylls, sporophylls, and internal stem and root systems, and produced new leaves (Grobbelaar, 1993). In 1994, Osborne and Dalzell performed a similar experiment with excised *Encephalartos woodii* leaves treated with "Seradix" rooting powder and placed in pots of clean sharp river sand. These were maintained with bottom heat and intermittent spray mist for six years and of these, several of these explants also produced new leaves.

Figures 1 and 2. Callus and roots on leaf tip cutting of *Z. furfuracea* (note nitrogen fixing nodules).

In 1972, I excised the pinnae from a *Cycas circinallis* leaf, sliced the rachis into 3 inch segments, dipped the segment ends into "Rootone" rooting powder and placed the cuttings in a mist tank under fluorescent GRO-lights. After 3 months, a few of the pinnae had produced small callus buds, and of these, two developed roots. Several rachis cuttings also swelled and split at the leaf base and rooted. However, the roots ceased growing when the pinnae withered. Cuttings of this size possessed the ability, but lacked the starch reserves necessary for long term regeneration.

I began to experiment with larger leaf cuttings, using orchid bark as the support medium. I found that some leaves would root readily (see Figures 2, 7 & 8), but that once the leaf itself died, the new roots and callus ceased growing. The photosynthic support of the leaf seemed to be the driving impetus for regeneration. It appeared that for leaf cuttings to live long enough to regenerate into a new plant the candidate had to be a young, newly hardened leaf supplied with proper humidity, light, and abundant nutrients.

In the Fall of 1997, I began to work with leaves of *Microcycas calocoma* applying what I had learned

Figure 3. The donor *Microcycas* plant remains in good health.

Figure 4. A rooted *Microcycas* leaf cutting potted in a mixture of pumice and sand

from the failures and near successes of past years. A mist tank was used to keep the leaf hydrated long enough to generate callus tissue and roots. I also switched from using orchid bark as the potting medium to sterilized sharp sand and pumice. Once the leaf cuttings had formed roots, I moved them into an Aerojet hydrophonic unit that sprayed the suspended cuttings with nutrient solution every 5 minutes for 40 seconds. This caused more rapid root formation than would soil culture. This is important because the cutting has to develop a large callus mass and an extensive root system *before* the leaf reaches it natural senescence and began to die.

Over a period of two and a half years eight excised Microcycas leaves were cultured using this process, a small sample, admittedly, but all my sole specimen of *Microcycas calocoma* could support without sustaining harm.

Of these eight leaves, three have died: one from accidental dehydration while in the mist tank, one never produced callus or root tissue and died after six months in the mist tank, and a healthy, fully rooted leaf died due to being "pushed" to produce a new foliage leaf before it was ready. Three long term survivors have now developed large root systems, and two are in an early callus stage (not all leaves were started at the same time).

Typically, a callus will form at base of the petiole after roughly three months. The size of callus will vary. Roots appear simultaneously, or soon after callus formation. Root growth accelerates in the hydrophonic solution. Roots that develop in the hydrophonic unit are long and straight, but once potted in fast draining soil, the roots begin to resemble normal cycad roots. Apical meristem regeneration and new leaf formation, based on documented development in *Encephalartos* leaf cuttings, requires another 3 to 4 years. It remains to be seen if hydrophonic culture will speed up this process.

As long as proper light, warmth and humidity are provided, cycad leaves readily produce both callus

Preparation and Culture of Excised Cycad Leaves:

1. Remove healthy leaf from donor plant (leaf should be at least 4 months old, well hardened). This can be the tip of a large frond, or the base. 2' long cuttings work best. Soak entire cutting in 10% sodium hypochlorite for 10 minutes to discourage bacteria and fungal spores.

2. Rinse cutting in tap water for several minutes and shake dry. Soak cut base in B1 rooting solution for 10 minutes, then dip base in a commercial rooting & fungicide powder such as Rootone, or equivalent.

3. Boil enough sharp sand to fill a small, clean plastic pot (about 20 minutes). Drain and allow to cool.

4. Fill small plastic pot with sand and place on a petri dish. Push a hole into the sand with a sterile spoon. Place the base of the cutting in the sand and position so that it leans at a low angle. Tamp down the sand to hold the cutting in place.

5. Place pot in mist tank. For new cuttings, spray briefly with distilled water once a day for two months. Keep temperature at 90˚F during day, no lower than 75˚F at night. Maintain humidity between 80 to 90%. Use a "fogger" set to come on 4 times a day. Put mist tank lighting on a timer (16 hours ON, 8 hours OFF).

6. Once a callus and roots have formed, transfer cutting to Aerojet net pot with its bottom cut out. Place the leaf stem through the hole in the neoprene plug and replace pot in Aerojet unit under plant lights. Mix per 1 gallon H2O: (2.8 grams Base 5-11-26, 3.5 grams 15.5-0-0 Cal. Nitrate, 1.12 grams Magnesium Sulfate, pH= 6.0/6.5)

7. Monitor root growth. When roots become too large for the Aerojet spray tray, remove the cutting from the net pot and transfer to a deep pot filled with pumice, sand, and loam. Place in bright shade, keep moist and fertilize.

Figure 5. A rooted leaf cutting is placed through a hole in the pot's neoprene plug. This cutting is now ready for the Aerojet unit

Figure 6. An Aerojet hydrophonic unit available from seaofgreen.com, Phoenix, Arizona, U.S.A.

material and roots. I have observed callus and root growth in the genus *Cycas, Encephalartos, Zamia, Dioon*, and *Microcycas*. Not all cuttings respond as well as those pictured.

Leaf cuttings of *Stangeria, Bowenia, Lepidozamia, Ceratozamia*, and *Macrozamia* have so far shown negative results, but this may have been due to the cuttings not being good candidates; root cuttings of *Stangeria* produce new plants readily - *Stangeria* was one of the first cycads to be successfully tissue cultured (Osborne & van Staden, 1987).

In the early stages of callus development, it's important to monitor hydration (turgor) of the cutting. In *Microcycas*, the rachis will show a distinct ridge between leaflets when it is beginning to dry out. Soon after, leaflets will begin to turn yellow and drop off unless water is added to the potting sand and the plant is misted more frequently. A rounded rachis without indentations is the sign of a fully hydrated cutting.

Experimental cuttings of leaf tip material are also being tested to determine their growth potential. When severed at mid-leaf and cultured with several rows of pinnae removed, callus material has been observed swelling the rachis and splitting the area where the pinnae were formerly attached.

Apical Meristem Regeneration - This is crucial for the creation of a new plant from a leaf cutting. The cells that form new leaves (leaf primordium) surround and overlap this area. If an apical meristem

Figure 7. This root of this cutting has broken away from base of the leaf. Callus tissue was unusually small in this cutting. This 2 year old leaf of *D. spinulosum* was cultured for 7 months.

does not regenerate within the callus, the rooted leaf will never be more than a clonal "monster". An extensive study was made of the structure and growth of the apical meristem (shoot apex) in *Cycas* (Foster, 1939). Sixty 2-year old *Cycas revoluta* seedlings were sectioned and examined microscopically to determine if there was a single "permanent" specific apical cell responsible for shoot growth. (A rooted

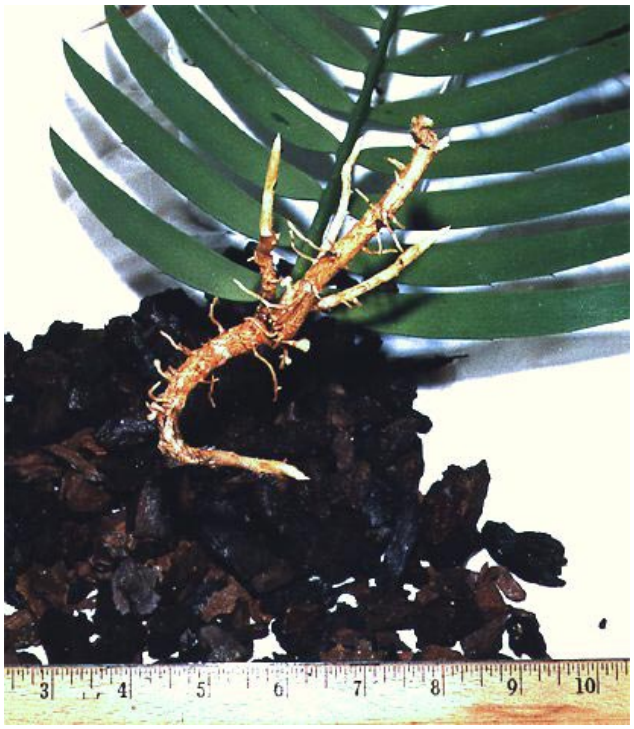

Figure 8. Two views of root development on an *E. lebomboensis* cutting after eight months. This cutting came from the tip of the leaf, rather than its base.

leaf would be incapable of producing new foliage *unless some portion of this meristem tissue had been captured along with the leaf base as it was cut from the stem*. For this reason, Osborne recommends that at least 1 mm of stem tissue be included in the leaf cutting.) However, Foster's, and other studies have shown that there is no single apical cell, but rather several initial cells (an initiation zone) that lie above a mound of cells known as the central mother zone and these give rise to all other types of zones in the apex by cyclic episodes of division.

So here we have an interesting mystery. Is it possible for a new initiation zone to spontaneously appear in the mass of undifferentiated regenerative tissue that forms in a cultured leaf base? This is currently unclear. Two early rooted cuttings were from rooted leaf tips, not leaf bases. There is also the incidence of *Cycas* pinnae rooting. Additionally, *Stangeria* is capable of regenerating an initiation zone (and new leaves) from cuttings of root tissue alone (of course, being subterranean, *Stangeria* lacks a true stem). Time will show if leaf base cuttings and leaf tip cuttings both have the ability to give rise to new plants. Further experimentation in cloning offsets from leaves may reveal that the basic structure of the apical meristem in cycads is more generic and adaptable than we currently believe possible.

Despite having limited culture material available for *Microcycas*, the cuttings produced seem promising for the artificial propagation of this rare genus. Hopefully, experiments similar to this one and those performed by Grobbelaar and Osborne will inspire others to experiment on their own and help ensure that the "Living Cycads" remain just that.

Figure 9. Root development (left) in a *Microcycas* cutting after 4 months in mist tank and (right) a cutting after 4 months in a mist tank and 3 months in the Aeroject unit (shown below).

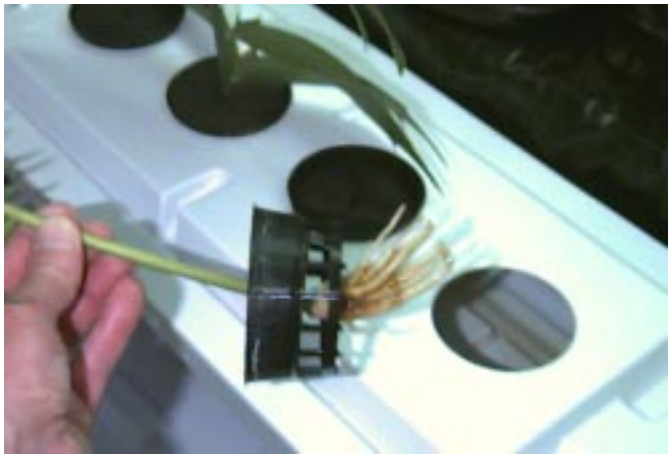

Figure 10. A *Microcycas* cutting being moved into the Aerojet unit.

Figure 11. Two views of the roots of the *Microcycas* cutting shown in Figure 4. Photo at left shows the still expanding callus area (note cracks). This cutting is 18 months old.

Acknowledgements:

Roy Osbornewas kind enough to review the first draft of this article and provide helpful suggestions for improvement. Thanks also to Roy Osborne, Libby Besse and Severn Doughty for providing copies of hard to find reference material, which proved to be invaluable.

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