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Controlled Humidification of Sweet Potato Stem-Cuttings in a Self-Sustaining Humidifier: Effects on Vigour, and Implications for Climate Change

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Abstract

Predictions of extreme weather associated with global warming signify potential extremes in soil water content, and extremes of soil water tension are inimical to early plant growth. Periderm tissue [cork cells], being waterproof, restricts root and stem waterlogging whilst the promotion of early roots better prepare stem cuttings for survival in dry soils. Sweet potato stem cuttings were stored for up to six days in a chamber humidifier. Humidification was achieved by evaporation up to saturation vapour pressure [SVP] in a closed system of capillary water evaporating from a fabric which enclosed the cuttings. Treatments included vine cuttings stored (1) under grass clippings (traditional treatment) and (2) inside a high humidity (100%) chamber. Root growth occurred on 90% of the cuttings in the root length sequence: 9-, 28-, 47 mm after 3, 6 and 9 days respectively in the 100% humidity chamber at an average ambient room temperature of 25 ^{o}C. Under traditional treatments, root growth occurred on only 10% of the cuttings and was 0.5-, 2-, and 3 mm after 3, 6 and 9 days respectively. Traditional treatment did not exhibit observable periderm (cork cells) growth at any stage of the study. Periderm thicknesses of 3-, 7-, and 9mm occurred on days 3, 6, and 9 for samples held at 100% humidity. No periderm was observed for the traditionally treated samples. For field trials, the most vigorous growth in a dry soil was observed for cuttings previously subjected to chamber humidification, an important asset when early season weed competition is evident and when rapid ground cover is important. In a broader perspective, these results can be used to optimize studies of other crops grown from cuttings.

Keywords: adventitious roots, drought-resistance, humidifier, periderm, sweet potato, vine-cuttings

Introduction

1.1 Propagation Problems in Dry Soils

A lack of roots in dry soils causes wilting and desiccation of vine cuttings, but an evenly moist soil provides the best environment for root initiation and development (Meyers et al., 2014). Yet, a strong, early rooting system with its wide, deep network speeds up plant development by greatly increasing the absorptive surface area in the soil, thereby enhancing the capability to withstand prolonged water stresses (Layne & Tomlinson, 1993). Rapid root growth by transplants is therefore necessary for survival in drought-prone locations. The main methods of propagation of sweet potato are (1) shoots developed from the vascular cambium and emerging through the cortex of the root, the shoots developing adventitious roots and becoming small plants attached to the seed root, and (2) vine cuttings which also produce adventitious roots. For propagating the sweet potato, vine cuttings are advantageous in that they do not transfer soil borne diseases and nematodes as do sprouts with roots (Yamaguchi, 1983). In addition, in growing from vines, the entire tuber harvest can be saved for consumption or marketing. The use of sprouts has been discouraged as a general practice because yields are lower compared to those from vine cuttings (Ikemoto, 1971).

1.2 Mechanism of Protection

When a living plant is wounded, the plant produces waterproofing polyaromatic, polyphenolic, aliphatic domains and associated waxy material compounds (Ginzberg, 2008) known as suberin, that are deposited in the cell wall of the injured tissue. The corky layer so formed prevents microbial invasion especially as periderm tissue contains several anti-fungal constituents which block fungal invasion (Harrision et al., 2001) and water loss

(Jana, 1983) by water-proofing of the periderm (Schalk et al., 1986). Thus, cork is virtually impermeable to water (Audesirk & Audesirk, 2005; Cuthbert & Davis, 1971), providing biochemical and structural barriers against pathogen infection (Ginzberg, 2008). Morris et al. (1984) concluded that thickness of the desiccated cell layers was highly correlated with resistance to pathogens and infections. Using 19 sweet potato cultivars, Schalk et al. (1986) observed a strong positive correlation between periderm and phellem thickness and resistance to wireworm infection for several growth stages of 65 days and longer. The formation of periderm tissue therefore enhances the survival of transplants. Thus, even if roots are not initiated before transplanting, the sealing by the newly developed periderm layer increases the ease of root initiation after planting (Jana, 1983).

Periderm growth ideally requires cool, shaded (but not hot and sunny) conditions (Jana, 1983). But when stored on the ground (turf or soil) for several days prior to transplanting, cuttings are vulnerable to soil insect infestation. Rapid acquisition of periderm tissue at the wounded sites while cuttings are stored off the ground may therefore enhance vine growth and survival, while escaping the threat of soil borne pathogens. But off-the-ground storage increases desiccation of vine cuttings because relative humidity varies inversely with height above ground level. Turgor pressure can be maintained with increased humidity because transpiration is reduced under such conditions (Tibbits & Gottenberg, 1976). In addition, Briske and Wilson (1978) showed that very high levels of atmospheric humidity dramatically accelerates the growth rate of adventitious roots.

1.3 Significance of Sweet Potato

Ipoema batatas (sweet potato) is ranked among the top five crops (yield tonnage) in the tropics (Harrison & Peterson, 2001). It is highly nutritious, high in vitamins A and D (Chada & Dakshinamurthy, 1965), and compared with other local root crops such as *Dioscorea* (yam), the root, and leaves in particular, contain relatively high levels of protein (Adewolu, 2008; Hognan et al., 2014; Winarno, 1983). Though sweet potato plants are hardy, yields are substantially reduced by low rainfall (Jones, 1961; McKeown, 2000), especially in the early growth stages (McKeown, 2000) and drier soils (and wetter soils) are included in predicted climatic fluctuations associated with global warming.

1.4 Hypothesis

To increase potential survival rates of transplanted sweet potato in dry soils, a study investigating the effects of two levels of humidity on periderm and/or root growth on cut vines was done. This was because Morris et al. (2004) found that optimum conditions were 25 ºC, and 98% relative humidity (RH), while citing temperature as the most important factor affecting deposition of lipid, lignin, and periderm formation. Based on the above information, it was hypothesized that the treatment of artificially maintaining a high relative humidity around vine cuttings of *I. batatas* away from direct contact with soil would produce periderm and/or root initiation more rapidly than would the traditional method. Therefore the effects of maintaining a high relative humidity in a closed system would be compared to those of ambient humidity in the traditional manner of ground storage. Furthermore, avoiding contact with the soil removes the risk of contamination with soil pathogens.

2. Materials

2.1 Area & Treatments

This study was begun in November 2006 in Portland, northeastern Jamaica and completed in field plots at the campus of Northern Caribbean University, Mandeville Jamaica, in August 2013. The well-drained kaolinite-dominated red bauxite soils (Harris & Omoregie, 2007) of south-central Jamaica (Mandeville) provided the dry soil conditions required for field testing the vigour of stem cuttings after transplanting. There were fifty sweet potato stem cuttings per treatment of 30 cm in length. Each cutting contained approximately ten nodes. These were freshly cut from field plots and stored under the following conditions: 100% relative humidity (H1), or < 95% relative humidity (H2). For H1, the cuttings were incubated in a humidity chamber (section 2.2.1) which was stored indoors in the shade at mean ambient temperatures of average 25 ºC (± 5 ºC) for 9 days. The humidity was known to be 100% because a thick layer of condensation droplets appeared on the inside surface of the polythene covering within 12 hours. Despite temporary disturbances for observations, the condensation droplets persisted for the duration (9 days) of the study. The H2 samples were placed on the ground (soil) under a 10-cm-thick cover of cut grass mulch, and were perpetually shaded from direct sunlight. Shading was accomplished by locating the samples under a large tree, the lower branches of which were no more than 2 m above ground level.

2.1.1 Techniques

The objective was to produce a system of self-sustained relative humidity of 100% around stem cuttings continuously for several days. A pore radius of 0.2 mm in a glass tube would raise water only 70 mm whereas

that of 10 μm would cause a 1.4 m rise, and 1 μm would give a maximum rise of about 15 m (Rank, 2010). However, due to the effects of evaporation, in practice the rise would be considerably lower (Hall & Hoff, 2007). Yet in a humidified chamber, evaporation is required initially for an increase in humidity, after which it must be reduced to achieve 100% relative humidity (RH). Hence there was a need to control the evaporation. Such a closed system of controlled humidity would operate according to the relationship:

$$E = eh \quad (1)$$

where the total evaporation E depends on the wetted height h and the evaporation rate e (per unit area) is established by the microenvironment (Hall & Hoff, 2007). The evaporation rate e must match the rate of capillary rise which decreases over time. Therefore, to achieve a steady-state in the system, a plastic polythene shroud of thickness .5 mm was used to cover and tightly seal the fabric (Figure 1) thereby reducing evaporation and water loss from the system. A permeable medium of suitable capillarity could rapidly attain and maintain saturation vapour pressure (SVP) while inside the chamber. To determine the most suitable material for such capillary transmission of water from the basin, equidimensional lengths of various fabrics and textures: nylon, closed-weave cotton cloth (1 mm-thick denim), open weave thick cotton cloth (1 mm-thick "terry cloth"), or 1 mm- thick jute hemp were placed each in a water-filled narrow-diameter graduated cylinder and observed until the stabilization point for capillary rise was indicated and recorded.

Figure 1. Humidifier showing condensed water vapour maintained for six days on the internal surface of the polyethylene covering. The water vapour was produced and maintained by capillary forces in a fabric soaked in a bucket of water at the base of the system and suspended on a hollow wire frame

The fabric with the highest capillary rise and water content, and hence the most effective potential supplier of water vapour molecules was found to be the tightly woven "denim" cotton cloth (weighing approximately 650 g m^2). This was due to its narrow pore columns and, possibly, its tortuosity, which reduces gravitational flow downwards, negative charge (polarity), and sufficient thickness.

2.1.2 Humidity Measurements

Humidity for the traditional treatments was determined using a hair tension dial hygrometer.

2.1.3 Frame of Humidifier

A closed chamber facilitated sustained humidity at SVP. This required a sufficiently large evaporative surface which enhanced capillarity. An enlarged evaporative surface area and a steady state height of water column were

maintained by a source of sufficient liquid water in a basin at the base of the gas-tight enclosure.

A 120 cm long, 35 cm diameter cylindrical cage was made from 8 mm (1/4-inch) squared aperture wire netting (Figure 2). On each of five plastic-coated wire shelves were placed 10 stem cuttings of 30 cm length. Pre-wet denim cloth (above-mentioned) was wrapped around the wire frame and the whole system enclosed in a 0.5 mm thick polythene covering with a closed end at the top. The open end was placed over the wet cloth (Figure 1) supported by the netting and the whole assembly placed in a plastic bucket containing water. The cloth was submerged in the water up to a height of 15 cm. In terms of the transfer of matter, this was a closed system because the external polythene shield was to prevent the loss of water vapour. Heat loss was also reduced due to polyethylene's opacity to far infra-red rays. This retained heat was to increase the rate of evaporation.

2.3 Statistics

The objective of the study was to compare the effects of one treatment against that of the other (control group). To accomplish this, a *t*-test was used to determine the probability that the difference between the means was authentic and significant.

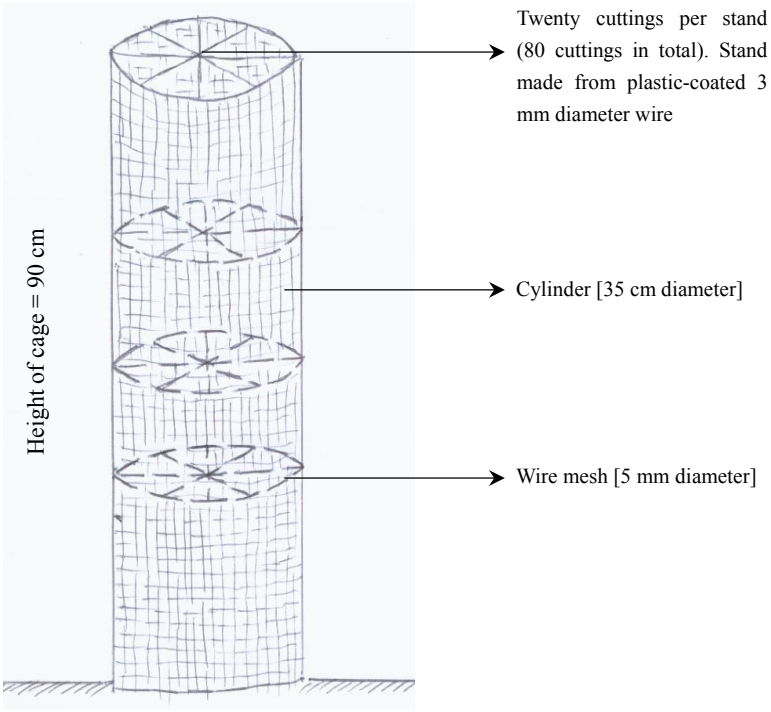

Figure 2. Frame of humidity chamber [relative humidity = 100%]

3. Results

Vine cuttings were examined on days 1, 2, 3, 6, 9, of treatment. After one day, only the H1 treatment produced roots (Table 1). At 100% humidity (H1), roots were observed on 90% of the cuttings in the root length sequence: 9-, 28-, and 49 mm after 3, 6, and 9 days respectively. Root vigour appeared very high compared to that of the traditionally-treated samples, based on eye observation. Of the fifty cuttings in the H2 treatment, only 10% had developed new roots (Table 1). Further, roots appeared less vigorous than the H1 samples even after 9 days, by which stage they had withered and dried. Thus even the traditional method of curing in the field did not produce roots and probably depends on the maintenance of specific humidity and/or temperature levels (see section 4). Losses of cuttings can be high under such conditions without careful monitoring, even within hours of storage.

Table 1. Roots on fifty sweet potato vine cuttings: number and length at nodes [50 samples treatment $^{-1}$]

Treatments* [4 replicates]	Samples with roots [no.]	Day 1	Day 3	Day 6	Day 9
		--------[mean root length mm]-------			
H1	45	0^{a}	9^{d}	28^{e}	47^{f}
H2	5	0^{a}	0.5^{b}	2^{c}	3^{c}

Note. *H1 = 100% humidity, H2 =<95% humidity & exposed to weather. Column values followed by different letters are significantly different ($P < .01$). S.D.: H1 d3 = 2, d6 = 17, d9 = 27; H2 d3 = .1, d6 = 1, d9 = 1.3.

3.1 Periderm Growth

After just two days, the H1 treatment produced visually observed periderm tissue in the form of toughened cells at cut points on stems on every vine cutting. After 6 days, the H1 periderm was more than twice the thickness of that observed on day 3 (Table 2).

Table 2. Mean visually observed periderm growth [mm] on vine cuttings [50 samples treatment $^{-1}$]

Treatments* [4 replicates]	Number	Day 1	Day 3	Day 6	Day 9
H1	50	0	3^{b}	7^{c}	9^{d}
H2	0	0	0	0	0

Note. *H1 = 100% humidity, H2 =< 95% humidity & exposed to weather. Column values followed by different letters are significantly different ($P < .01$). S.D. for H1: d1 = .2, d3 = .7, d6 = 1.7, d9 = 3.

3.2 Temperature and Humidity Changes

Temperature readings were recorded at 10 a.m. on the mornings of days 1, 3, 6 and 9. The temperature in the H1 was substantially warmer than determined for all other treatments, while differences among all controls were insignificant (Table 2). It had been anticipated that the energy required for evaporation from the chambered treatments would have decreased temperatures inside all chambers. However, average temperatures were 35 ºC, 34 ºC, and 31 ºC for the H1 chamber, thereby constantly remaining at >30 ºC after three days. This was a mean of 7 ºC higher than the ambient temperatures of the traditional treatment (Table 3).

Table 3. Mean Temperature (˚C) During Storage of Potato Vine Cuttings at 10 a.m. [average values]

Treatments* [4 replicates]	Day 1	Day 3	Day 6	Day 9	Mean
H1	23^{a}	35^{c}	34^{c}	33^{c}	31
H2	22^{a}	24^{a}	24^{a}	26^{a}	24

Note. *H1 = 100% humidity, H2 =< 95% humidity, exposed to weather. Column values followed by different letters are significantly different ($P < .05$). S.D.: H1 d1 = 2, d3 = 1, d6 = 1.6, d9 = 1.5; H1 d1 = 2, d3 = 1, d3 = 3, d9 = 1.

3.3 Field Growth

The results of vine cuttings planted in a dry, porous soil are depicted in Figure 3. This shows that after three days in the ground, the treated cuttings with root and periderm appeared more robust and had not wilted, in contrast to the controls.

Figure 3. Sweet potato vine cuttings two days after transplanting in dry soil. Prior to transplanting, the cuttings at left were subjected to ambient relative humidity of 100%. Cuttings at right were exposed to < 95% relative humidity

Due to the low proportion of lignified tissue in sweet potato shoots (Noggle & Fritz, 1983), this water stress would have readily reduced cell turgidity not only past its critical value, but to "permanent" wilting, causing destruction of cells and leaf losses. After 5 days, the traditional samples had recovered, such that no difference between treatments was observed at that stage. However, it is reasonable to suggest that had the soil been drier, more losses could have occurred for the humidified samples compared to those traditionally treated.

4. Discussion

Maintaining a high relative humidity (100%) around vine cuttings of *I. batatas* away from direct contact with soil produced periderm more rapidly than did the traditional method. Further, the treatment produced adventitious roots far more successfully than the traditional treatment of ground storage in the shade. As shown above (3.3), pre-rooted humidified cuttings appeared more vigorous than those without roots after transplanting in a dry soil. Yet, Holwerda and Ekanayake (1991) found that for two sweet potato cultivars they used, pre-rooting the cuttings were of no benefit in terms of survival after planting. As this study involved samples from a single cultivar, replicate experiments should determine the extent that these results can be applied among strains within the *I. batatas* species. Moreover, the roots of samples exposed to 100% humidity deteriorated, failed to retain turgidity, shrivelled and died within five minutes of subsequent exposure to < 90% humidity (Table 4). In other words, such treated roots failed to withstand what are normal soil humidity levels even for a few minutes.

Table 4. Effect of root length on root longevity after decreasing ambient humidity from 100% to < 90% [average values, four replicates]

Root length [mm]	10	5	2	1
Longevity [seconds]*	180[a]	225[b]	340[c]	630[d]

Note. Column values followed by different letters are significantly different ($P < .05$). *S.D. [longevity]: 180 = 7, 225 = 4, 340 = 11, 630 = 4.

Therefore, with such a high susceptibility to lowered humidity, it is reasonable to suggest that the pre-formed H1 roots rapidly deteriorated in the dry soil of the field trial. Yet, in the dry soil, the same vine cuttings were more vigorous than traditionally treated samples (Figure 4). The explanation could lie in the age of the roots: not only had the H1 cuttings borne more abundant roots, but at several points on the stems, potential adventitious roots were almost breaking through the stem epidermis (Table 5).

Table 5. Effect of ambient humidity on the total number of potential adventitious roots observed on 50 sweet potato vine cuttings [for each treatment of four replicates] after three days

Ambient Humidity	<90%	100%
Number of Potential Roots	4[a]	208[b]
Mean number of roots on a cutting	0.8	4.2

Note. Column values followed by different letters are significantly different ($P < .05$).

Table 5 reveals that the mean number of potential adventitious roots on each H1 sample exceeded that of each H2 sample in a ratio exceeding 52:1. Thus on the vast majority of H2 samples no potential adventitious roots were observed. As shown in Table 4, the survival rate on exposure to lowered ambient humidity varied inversely with root length (and hence with root age). Tensiometer readings for the kaolinite-dominated red soil revealed a soil water potential of < -33 kPa, thereby indicating a soil water content below soil field capacity. Therefore it is reasonable to suggest that the pre-emergent potential roots observed during humidification (on the H1 samples), and which emerged after transplanting, resisted the lowered humidity of the dry soil, just as younger roots did in the laboratory study. It is likely that the great proportion of emerging short roots supported by the stem would have remained viable for a longer time in the dry soil than the older roots. In a study of the effect of root cell size and transplant age on yield of transplanted eggplant Harmon et al. (1991) significantly increased early yields with larger root cell sizes. These can be equated with the younger, robust root cells of H1 treatment in this study, as compared with the withering older ones subjected to < 95% humidity. The youthful vigour extended the life of the root, thereby providing nutrients for the shoot. Similarly, Harris (2015) found that prolonging the metabolism of inflorescences on *Mangifera indica* until fruit-set, produced fruit on previously non-fruiting trees. The relatively high vigour of the H1 samples could thereby be probably explained.

4.1 Periderm and Temperature

The periderm thickness on the H1 samples after just 3 days was found to have exceeded 0.5 mm. Walter and Schadel (1983) observed that wounds were healed when the wound-periderm layer attained a thickness of just 3-7 cells in diameter. Further, Schalk et al. (1986) found that resistance to soil insects was most effective early in root development, varied directly with phellem (one of three periderm layers) and periderm thickness, and that the number of cells in phellem tissue increased with thickness of the periderm. Such high early rates of periderm increase are therefore advantageous because, as stated earlier, the physical and chemical barrier of a thick periderm provide a degree of resistance to pathogen incursion (Ginzberg, 2008) during the early stages of establishment in the soil. It is therefore predicted that the rapid thickening of the H1 periderm observed in this study should improve the vigour and longevity of particular vine cuttings after transplanting.

4.2 Explanation for Root Growth

The above findings are in agreement with Briske and Wilson (1978) who showed that elongation rates for the longest root per seedling in a 100% humidity soil environment at 15 ºC, 20 ºC, 25 ºC, and 30 ºC, were 0.40, 0.74, 1.04, and 1.22 cm/day respectively. Hence root growth increased up to 30 ºC. Briske and Wilson (1978) also observed an approximately 50% reduction in root elongation with just a 4% lowering of ambient humidity from 100% to 96%. Therefore at 96% humidity, the corresponding root lengths for the same temperatures were 0.28, 0.36, 0.38, 0.44. Similar results (at least qualitatively) were observed in the present study, which is in agreement with Morris and others (2004) who found 98% relative humidity ideal for root growth.

Interestingly, the aim of traditional treatment is to "cure cuttings in the shade." Yet, despite treatments in this study having all been conducted in the shade, the main effective factor was not shade (contrary to expectations), but a high level of ambient humidity. Thus in this study, shade *per se* did not trigger root initiation. But being of high porosity, grass mulch failed to maintain water vapour levels as efficiently as polythene. Grass mulches trap moisture and reduce desiccation more effectively in the shade than at higher temperatures; this probably accounts for the traditional view of shade being an imperative for curing of transplant cuttings. However, this study shows water vapour (despite heat), and not shade, as the single most critical factor studied, for root initiation on potato vine cuttings.

4.3 Source and Role of Heat

Three factors in the system affected the temperature in the H1 chamber: evaporation/condensation, metabolic processes, and the design of the chamber. As the samples were stored in the shade and surrounded by a long-wave infra-red barrier, the major heat source would have been from the rapid cell division producing roots

and periderm tissue. The perpetual wetness of the fabric surrounding the samples would have transported this heat, because increasing moisture content of a material increases its thermal conductivity (Bouguerra et al., 1998). However the thick external polythene barrier to infra-red rays prevented the loss of that heat to the external atmosphere. The role of the polythene was thus to (1) retain water and (2) increase the evaporation rate by retaining heat in the humidifying system. The heat released in the system by condensation was only that recycled from evaporation on the inside. This increased heat energy would have helped to maintain high humidity levels. Nevertheless, as root and periderm tissues appeared before the occurrence of extra heat, it is clear that rapid growth of those (periderm) tissues occurred despite, and not because of, the higher temperatures.

4.4 Applications

Prior to transplanting, large farms require extensive areas for storing (curing) vine cuttings. A major problem with the humidifier of this study is the small volume of the chamber, which accommodates only 80 vine cuttings. A possible cost-effective alternative which mimics the chamber humidifier may produce similar results. It was therefore postulated that spreading vine cuttings on a table and covering them with wet material under a polyethylene covering may be just as effective. For large farms, such an intervention may be sufficient to accommodate thousands of vine cuttings. This postulation was tested at the end of this study, and shown to be correct. Thus vine cuttings stored under wet newspaper sheets sealed with polythene sheets on large tables produced similar results to those for the humidified chamber.

Forced humidity (being 100% efficient) requires no addition of water in contrast to grass-covered vine cuttings at intervals during hot, dry days to prevent desiccation of vine cuttings. Cut vines left lying on the ground in the shade are subject to infection by soil-borne pathogens, and vagaries of the weather, such as removal by wind or flooding. In contrast, during forced humidity, all cuttings are suspended in the air high above ground level in protected chambers. Chamber humidification promises significant protection as well from soil-pathogen attacks in transplanted sweet potato vine cuttings. Moreover, if these results are transferable to other plant species, benefits in dry soils could accrue. Improved ability of fresh stem cuttings to withstand extremes of soil water tension inimical to early plant growth improves the potential to survive increased climate changing conditions. However, it is stressed that the vigorous growth, longevity and high level of turgidity at 100% R.H. achieved in this study were not achieved for plants having woody stems.

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