Effects of Seedling Age and Dehydration on the Hypocotyl Cracks of Green Braided Seedling in Pachira macrocarpa (Cham. & Schl.) Schl.

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Effects of Seedling Age and Dehydration on the Hypocotyl Cracks of Green Braided Seedling in *Pachira macrocarpa* (Cham. & Schl.) Schl.

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The objective of this study was to examine the effects of seedling age and dehydration on the hypocotyl cracks of green braided seedling in *Pachira macrocarpa*. When we braided the 12 cm tall dug up seedlings 8 or 10 days after sowing, the hypocotyl crack indices of these seedlings were smaller than 1.5 (little cracking). Crack indices increased to greater than 3.5 (severe cracking) when we braided the dug–up seedlings aged 12, 14, or 16 days. In addition, dug–up seedlings were placed in a indoor environment to dehydrate and soften the hypocotyl. The mechanical stress in hypocotyls rapidly decreased to 29.7 kpa after undergoing dehydration for 36 hours. The crack index of green braided seedling, which decreased with increased duration of dehydration, was lower than 1.5 after being dehydrated for 36 or 48 hours. However, for those that were dehydrated for 72 hours, the crack index increased to 3.2. These results imply that the cracking on the hypocotyls can be minimized by braiding the very young seedling that are 10 days old and have been dehydrated for 36 or 48 hours. Consequently, the resulting hypocotyls of the braided seedlings develop the emerald color and intact epidermis that is preferred by the consumers.

Key words: malabar chestnut, mechanical stress, ornamental quality

INTRODUCTION

Malabar chestnut (*Pachira macrocarpa* (Cham. & Schl.) Schl.) is a tropical evergreen tree native to Central and South America, in the family of Bombacaceae (Liou, 1960; Liou and Liau, 1981). *P. macrocarpa* is highly appreciated by consumers and has gradually become a popular indoor foliage plant internationally because of its growth vigor and versatility to be shaped into various styles (Zhang, 1995). In Taiwan, 95% of locally grown *P. macrocarpa*, which is a top five potted plant regarding export values, are exported to the China, Japan, Netherlands, South Korea and the United States (Wang, 2005). In 2011, the total export value of *P. macrocarpa* seedlings reached US $7.2 million (Chung, 2008).

The commercially available *P. macrocarpa* is categorized into single–trunk and braided types. The braided type consists of a "5–stem braided seedling" that is 60–120 cm tall and a "small 5–stem braided seedling" that is 25 to 30 cm tall (Taiwan Pot Plants Asso., 2008). The new *P. macrocarpa* product, which is an 18 cm tall "green braided seedling", is immensely popular in the American, European and Japanese markets. Green braid seedling is one of the small 5–stem braiding products, which is commonly appreciated for the slender green hypocotyls being elongated by shading the seeds after sowing (the hypocotyl length is longer than 12 cm) and the beautiful plant type formed by braiding and the dense lobulars. It is suitable as a desktop potted plant and indoor ornamental skit (Wang, 2011).

However, the production of green braided seedling of *P. macrocarpa* has not yet been standardized. The braiding can result in serious cracks on the surface of the *P. macrocarpa* hypocotyl, which affects the product quality. Therefore, this study examined the effects of seedling age and dehydration duration on the hypocotyl cracks of green braided *P. macrocarpa* seedling. We endeavored to identify a balance between the post–sowing digging period and the post–digging dehydration duration. This may ensure that the hypocotyl remains intact and retain emerald color after braiding and hence facilitate the export industry of *P. macrocarpa* seedlings in Taiwan.

MATERIALS AND METHODS

Plant materials

Mature *P. macrocarpa* fruits were harvested and transported to the laboratory within 1 day, and then placed in shade until they split naturally. After splitting, seeds were extracted for testing. The experiment was conducted in a seedling greenhouse at the internship farm owned by the Department of Horticultural Science of National Chiayi University. Seeds were sown on a mix of equal volumes of river sand and rick husks (particle size is about 2–4 mm) in a 3×3 cm density and covered by a supporting tunnel similar to that used for growing muskmelons in Taiwan. The shed was covered by a shade net (Hun–Kun Enterprise Co., Ltd, Changhua, Taiwan) with an 80% shading rate. The photosynthetic photon flux density (*PPFD*) inside the shed was kept at 14.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a temperature of 33±2°C. When

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the hypocotyls reached 12 cm long, which was commercially sellable standard, the seedlings were dug up for experiment.

Test methods

The tests were divided into two parts to test the effects of seedling age and dehydration on the hypocotyl cracks of green braid seedlings. To test of the effects of age, seedlings with over 12 cm long hypocotyls were dug–up at 8, 10, 12, 14, or 16 days after sowing. The bare–root seedlings were subjected to dehydration for 24 h in a indoor environment, which was modified from production environment of producer, at temperature of 25±1°C (air–condition, RAS–56BD, Hitachi, Ltd., Taiwan) and relative humidity of 60% (dehumidizer, RD–200J, Hitachi, Ltd., Taiwan). In the experiment 2 regarding the effect of dehydration on the hypocotyl cracks, seedlings were braided after they had been subjected to dehydration under the same conditions as experiment 1 for 0, 6, 12, 24, 36, 48, or 72 h. Then every 5 seedlings were braided together as a replication, and each treatment was repeated 10 times. The test period was from Aug. 15, 2011 to Aug. 31, 2011.

Investigated items

After the seedlings underwent dehydration treatments, we examined the mechanical stress of the hypocotyls, crack indices after braiding, and number of fractured seedling per braid. The method for measuring mechanical stress of hypocotyls was a modification of the method used by Szot *et al.* (2009) to measure stress for strelitzia (*Strelitzia reginae* Banks). Mechanical stress was represented the degree of elasticity of hypocotyl. A customized stress device was employed to measure the mechanical stress in the hypocotyls of the seedlings (Fig. 1A). The measurement was conducted first by selecting seedlings with 12 cm long hypocotyls. The seedlings were placed flat on the stress device to measure the weight for bending the hypocotyl at length of 10 cm downward to 0.5 cm (Fig. 1B). The results were converted into mechanical stress units (Pan, 2003) using the conversion equation Pa=0.101 kg·m^2 (Pa is a mechanical stress unit. The numerical value is larger means the hypocotyl is harder; kg and m are the pressing weight and hypocotyl diameter, respectively). Crack index (CI) is a method for classifying visually estimated levels of cracks in the hypocotyls after the dug–up seedlings were braided. After braiding, every seedlings of green braided seedling were investigated the crack index. Crack index was defined to 0–4 level. Level 0 meant that no cracks were observed (Fig. 2A). Level 1 referred to hypocotyl cracks having areas with a length less than 1.5 cm (Fig. 2B). Level 2 referred to cracks having areas that ranged from 1.5 to 3.0 cm in length with no blocks formed (Fig. 2C). Level 3 referred to hypocotyl cracks having areas that were greater than 3.0 cm in length, and each crack area was large enough to form a connected surface with other cracks (Fig. 2D). Level 4 meant that the hypocotyl fractured during the braiding (Fig. 2E).

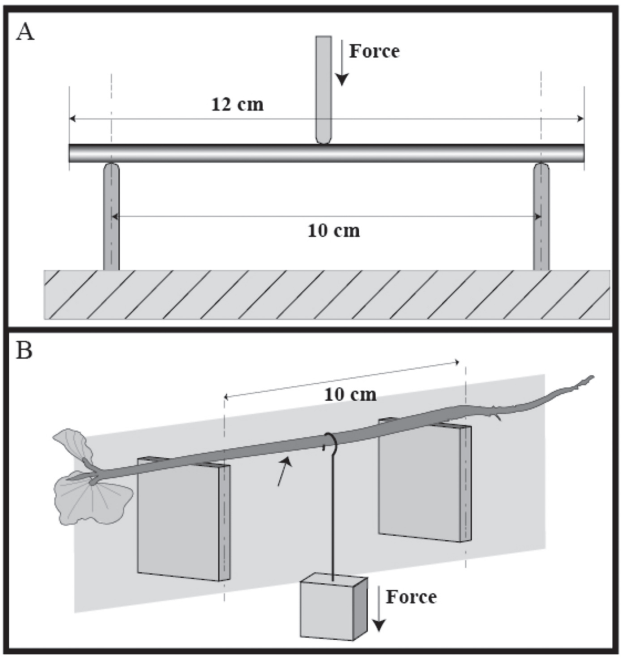

Fig. 1. Schematic diagrams of (A) mechanical stress testing and (B) mechanical stress testing for *Pachira macrocarpa* hypocotyls.
The hypocotyl was placed on the self–made stress device for measuring the mechanical stress (arrow–heads, B).

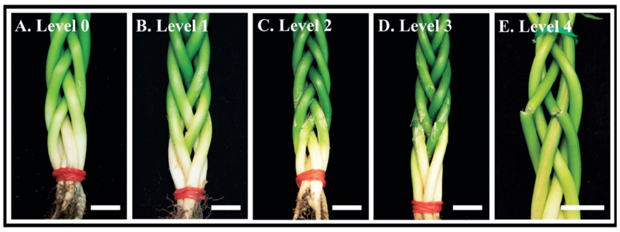

Fig. 2. Photograph of crack index from level 0–4 (A–E) after breading of *Pachira macrocarpa*.
The crack index (CI) is the level of cracks: Level–0 indicates no crack, Level–1 indicates the formation of hypocotyl cracks with an area length <1.5 cm; Level–2 indicates crack area length in the range of 1.5 to 3.0 cm without blocks; Level–3 indicates the hypocotyl crack area length >3 cm and a single crack area has formed on the surface; Level–4 indicates the fracture of hypocotyls in braiding. Bars=2 cm

Statistical analysis

All data were adopted a time–series design and analyzed by ANOVA, using the CoStat statistical software package (CoHort Software, USA). Differences between treatments were separated by least significant differences (LSD) testing at a probability level of 0.05. SigmaPlot® 10.0 statistical software package (Systat software Inc. USA) was used for plotting and comparing the experiment results.

RESULTS

Experiment 1. Effects of seedling age on hypocotyl cracks

The effects of seedling age on the hypocotyls cracks of green braided seedling could be represented by a sigmoidal curve (Fig. 3), which showed a highly positive correlation (R^2=0.94) between seedling age and the severeness of hypocotyl cracks. Variations in the cracks could be categorized into three stages. If the seedling was less than 10 days, its crack index after braiding was smaller than 1.5 and no significant cracks were observed (Fig. 3 and 4A). In addition, hypocotyls of seedlings that were grown in the greenhouse for 2 months remained emerald (Fig. 4C). If the seedlings were 12 days or older, the crack index of the hypocotyl increased linearly with increase in age. If the seedling was 14 or 16 days old, the crack index reached a peak of greater than 3.5 (Fig. 3) and significant cracks were observed (Fig. 4B). Cracks of this braided seedling type could not be repaired after growing in the greenhouse for 2 months, and the epidermal tissue appeared brown and mottled (Fig. 4D).

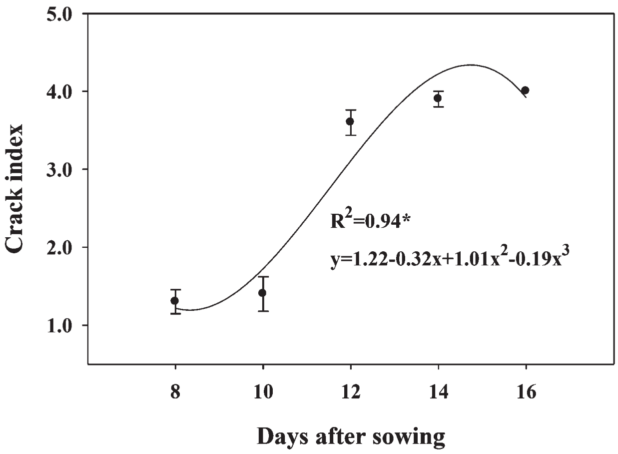

Fig. 3. Effects of seedling age on the crack index of the hypocotyls of green braided seedling in *Pachira macrocarpa*.
The crack index (CI) is the level of cracks: Level–0 indicates no crack, Level–1 indicates the formation of hypocotyl cracks with an area length <1.5 cm; Level–2 indicates crack area length in the range of 1.5 to 3.0 cm without blocks; Level–3 indicates the hypocotyl crack area length >3 cm and a single crack area has formed on the surface; Level–4 indicates the fracture of hypocotyls in braiding.

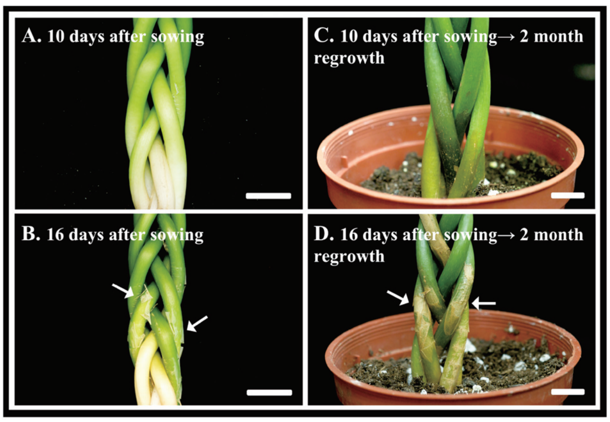

Fig. 4. The seedling ages affect the hypocotyl crack in green braid seedling of *Pachira macrocarpa*.
Photograph of 10–day–old (A) and 16–day–old (B) seedlings were braided that were dug from the sowing bed for 24 h curing; After transplanting into the pot for 2–month regrowth, the braided seedlings showing green and complete appearance (C), and brown–massive spot (arrowheads, D). Bars=1 cm

Experiment 2. Effects of dehydration on the hypocotyl cracks

Results showed that the relationship between dehydration duration and hypocotyls mechanical stress can be represented by a reverse sigmoidal curve (Fig. 5A). The coefficient of determination (R^2) of a regression analysis was 0.97. In the first phase of mechanical stress variation, the mechanical stress of hypocotyls remained greater than 42 kpa after the dug–up seedlings were dehydrated for less than 24 h. In the second phase, the mechanical stress showed a linear decrease with 24 to 48 h of dehydration. After 48 h of dehydration, the mechanical stress decreased to 29.7 kpa (Fig. 5A) in the

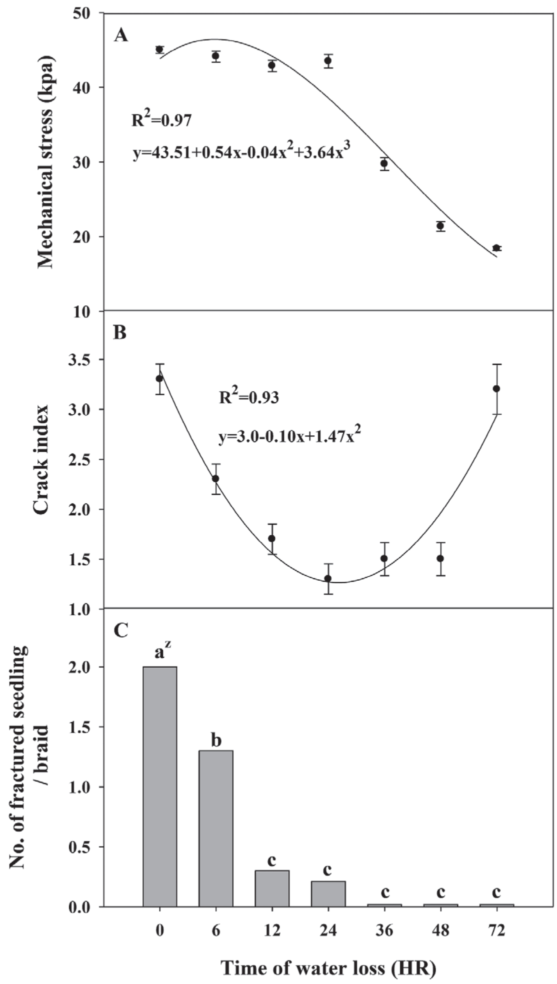

Fig. 5. Effects dehydration duration on the mechanical stress (A), crack index (B) and fractured seedling number(C) of hypocotyls in green braid seedling of *Pachira macrocarpa*.
The crack index (CI) is the level of cracks: Level–0 indicates no crack, Level–1 indicates the formation of hypocotyl cracks with an area length <1.5 cm; Level–2 indicates crack area length in the range of 1.5 to 3.0 cm without blocks; Level–3 indicates the hypocotyl crack area length >3 cm and a single crack area has formed on the surface; Level–4 indicates the fracture of hypocotyls in braiding.
[z] Means followed by the same letter(s) are not significantly different at 5% level by LSD test.

third stationary phase. In addition, the relationship between dehydration duration and hypocotyl cracks could be plotted by a regular U–curve (Fig. 5B) with the R^2 of 0.93. The seedlings that were dehydrated for 0, 6, and 12 h experienced the first variation phase of hypocotyl cracks, where the crack index decreased with the increase in dehydration duration. The lowest crack indices were observed in seedlings that were braided after 36 or 48 h of dehydration. These seedlings were in the second variation phase (i.e., the U–curve stationary phase) and exhibited crack indices lower than 1.5. Seedlings that were braided after 36 h of dehydration were grown in the greenhouse for 2 months and displayed an appearance that was more emerald in their hypocotyls (Fig. 6C and G). However, the crack index for seedlings that were braided after 72 h of dehydration significantly increased to a maximum of 3.2 (Fig. 5B and 6D). The cracks in these seedlings could not be recover after growing in the greenhouse for 2 months and the epidermal tissue appeared brown and mottled (Fig. 6H). Additionally, we examined seedlings that were braided after 0 and 6 h of dehydration and found an average of 2 and 1.3 fractured seedlings in each braid respectively (Fig. 5C, 6A–B). These dehydration durations severely impacted the quality of the braided seedling. The number of fractures in each braid decreased with the increase of dehydration duration. Thus, no cracks were observed in the seedlings that dehydrated loss for 36 h (Fig. 5C).

DISCUSSION

The 18 cm green braided seedling of *P. macrocarpa* is an emerging export foliage product for Taiwan. This plant resembles braided *P. macrocarpa* in appearance, but is smaller in size, corresponding to the trend of ornamental plant minimization. Different from 25 to 30 cm tall *P. macrocarpa* that possess corpulent hypocotyls, the 18 cm tall green braid seedling is appreciated for its slender emerald hypocotyls. This small plant can be exquisitely shaped by artificially braiding its seedlings. In this study, we modified the production environment of producer, and further on established the standardization of seedling age and dehydration duration. The results indicate that seedlings that are younger than 10 days are less likely to crack after braiding because their immature epidermal tissues contribute to their tenderness (Fig. 4A). However, when the seedlings are older than 12 days of age, the forces exerted by braiding cause the epidermal tissue to crack. Sánchez–Bravo *et al.* (1992) found that the thickness of hypocotyl epidermal tissues of lupin younger than 12 days increased with seedling age. Seedlings older than 12 days of age obtained a maximum thickness and the epidermal tissue near the base of their hypocotyls was thicker than the middle and the top of the hypocotyls. In this study, the part close to the base of the hypocotyls in green braid seedling cracked more severely (Fig. 4B), suggesting that the epidermal layer thickness should be related to the cracks after braiding of the hypocotyls. Kutschera and Niklas (2007) contended that the outer layer of epidermal tissue and the inner cortical tissue of a plant jointly control the growth rate of hypocotyls. In addition, they argued that the upward–growing cortical tissue provides the elongating strength of hypocotyls, whereas the epidermal tissue exerts an opposite tension that restricts the elongation of hypocotyls. Based on this theory, we inferred that the hypocotyl cracks that occurs during braiding is caused by the force of twisting the hypocotyl during braiding that exceeds the tension limit of the epidermal tissue. Conversely, because hypocotyls younger than 10 days are thinner and incompletely developed, they possess lower tension that better tolerates the forces being applied during braiding. Thus, these hypocotyls are less likely to crack.

The results of the experiment on the effects of dehydration duration on hypocotyl cracks showed that the decrease in mechanical stress in hypocotyls measured after various durations of dehydration can be categorized into three phases. Dehydration between 0 and 24 h maintained the mechanical stress of hypocotyls at a high peak. This suggests that despite dehydration, the remaining water sustained the turgor pressure in the hypocotyl tissue, maintaining the mechanical stress. Because of higher mechanical stress and turgor pressure in the hypocotyl, braiding seedlings that were dehydrated for

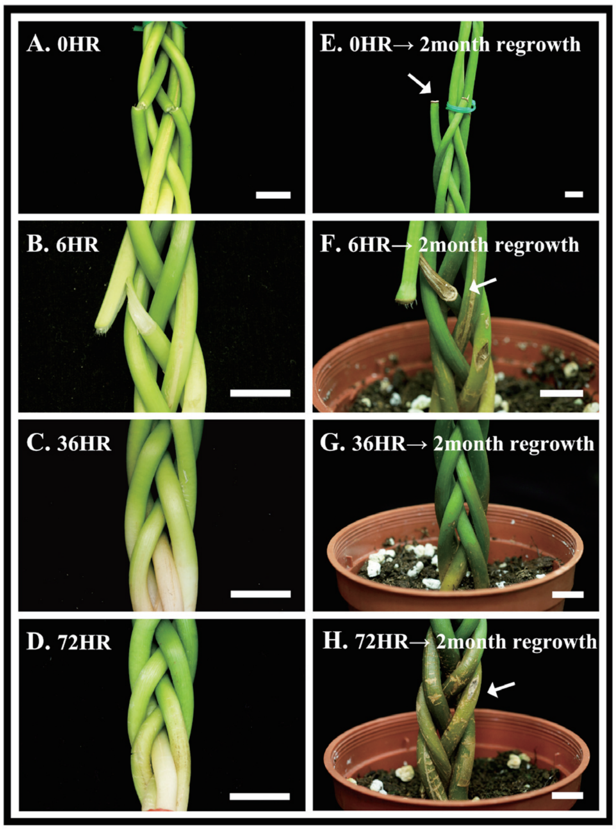

Fig. 6. Photograph of dehydration affect the hypocotyl crack in green braid seedling of *Pachira macrocarpa*.
The seedlings that were dug and dehydration for 0 (A), 6 (B), 36 (C) and 72 h (D) before braided operation; After transplanting into the pot for 2–month regrowth, the braided seedlings showing a few breaking seedlings (arrow–heads, E and F), better appearance performance(G) and brown–massive spot (arrow–head, H). Bars=1 cm

less than 24 h resulted in the highest number of fractures (Fig. 5C). Nevertheless, 36 or 48 h of dehydration caused a sharp decline in the mechanical stress of hypocotyls. During this period, rapid dehydration resulted in loss of turgor pressure in the hypocotyl tissues, thereby substantially softening the hypocotyls. These hypocotyls exhibited the lowest crack index and were in the optimal condition for braiding.

The respiration and transpiration of asparagus (*Aspanrague officinalis* L.) were high after harvesting that resulted in rapid weight loss reaching 70% loss at 7 days after harvesting that severely reduced the product quality (Zhao *et al.*, 2004). Such situation is similar to the softening of *P. macrocarpa* hypocotyl by dehydration. However, unlike the asparagus weight loss after harvesting which reduced quality of the spears, dehydration softened the hypocotyls of the *P. macrocarpa* seedlings and facilitated braiding with minimal or no injury. Furthermore, an optimal amount of dehydration reduced the hypocotyl cracks after braiding (Fig. 6C). Dehydration decreased the water potential in the hypocotyls, lowering the turgor pressure in the cortical tissue and reducing the tension of the epidermal tissue (Kutschera and Niklas, 2007). The tension of the epidermal tissue counteracted the forces exerted during braiding. Consequently, because the epidermal tissue of the *P. macrocarpa* hypocotyls that did not undergo dehydration possessed high tension, braiding forces could easily surpass the limit that could be tolerated by the epidermal tissue, leading to cracks or fractures (Fig. 6A–B). Dehydration for 36 h decreased the turgor pressure in the hypocotyls and further reduced their tension. Thus, because the forces exerted by the braiding process were less likely to exceed the tension limit in the epidermal tissue, cracks caused by braiding were prevented and optimal appearance was obtained following regrowth (Fig. 7). In this experiment, the green braid seedling was soft after 72 h of dehydration and the hypocotyl mechanical stress gradually diminished. During this period of mechanical stress variation, although braiding was facilitated by the soft hypocotyl, hypocotyl cracks increased after braiding. We infer that these cracks were caused by over dehydration of the seedlings. Due to the overly softening of the hypocotyl, the forces exerted by braiding contrarily induced friction and pulling between hypocotyls, which intensified the cracking.

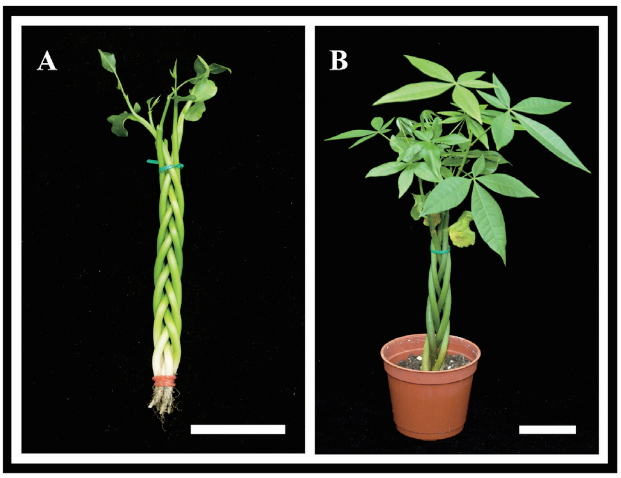

Fig. 7. Photograph of 10–day–old and 36 h dehydration braided seedling (A) and 2–month regrowth of transplanting into the pot (B) showing optimal ornamental quality in green braid seedling of *Pachira macrocarpa*. Bars=5 cm

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