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PLANT TISSUE CULTURE

Micropropagation of sweet acacia (*Vachellia farnesiana*), an underutilized ornamental tree

JianJian Xu[1] · Sandra B. Wilson[1] · Wagner A. Vendrame[1] · David G. Beleski[1]

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Abstract

Sweet acacia (*Vachellia farnesiana* (L.) Wight and Arn) is an attractive small- to medium-sized evergreen tree native to southern North America spanning from southern California, east to southern Florida. Despite its ornamental appeal, sweet acacia has limited commercial availability. A series of experiments were conducted to determine the effect of hydrothermal scarification on *in vitro* seed germination, and an efficient level of BA (6-benzylaminopurine) for *in vitro* multiplication, auxin rooting treatments, and acclimatization of sweet acacia. Results revealed that hydrothermal scarification and visual selection of imbibed seeds are the most effective for rapid *in vitro* seed germination. The source of seed utilized for the *in vitro* germination exhibited an unusual characteristic of producing seedlings with 2, 3, or 4 cotyledons. Regardless of the cotyledon number observed, sweet acacia can be efficiently micropropagated using multiplication media supplemented with either 1.0 or 2.0 mg L^{-1} BA followed by rooting media supplemented 0.5 mg L^{-1} IBA (indole-3-butyric acid) and 0.5 mg L^{-1} NAA (1-naphthaleneacetic acid). *In vitro* derived plantlets were subsequently acclimatized with high survival ranging from 72.7 to 100%.

Keywords Fabaceae · Micropropagation · Native plants · Ornamental tree · Plant tissue culture

Introduction

The aesthetic and ecological benefits native plants can bring to our landscapes and gardens are gaining momentum. Locally adapted to the climate, soil conditions, and natural pests of a given region, native plants are naturally resilient, attract important pollinators (Kalaman *et al*. 2022a), provide necessary nectar and pollen resources (Kalaman *et al*. 2022b), and support native wildlife biodiversity (Burghardt *et al*. 2009; Pardee and Philpott 2014). Yet commercial-scale cultivation of native plants remains limited (Rupp *et al*. 2018; Tangren *et al*. 2022). This is largely attributed to consumer preference of modern selections (Wilde *et al*. 2015), lack of understanding of their benefits, and lack of species-specific propagation protocols (Dumroese *et al*. 2009; Wilde *et al*. 2015; Trigiano *et al*. 2021). White *et al*. (2018) estimate about 26% of all US native flora are under commercial production. Progress has been made in propagating and landscape trialing natives to help increase our plant palette (Thetford *et al*. 2008, 2012, 2018; Campbell-Martinez *et al*. 2021; Campbell-Martinez *et al*. 2022). Likewise, sustainable gardening is gaining interest. Landscapes with a high proportion of native plants can be appealing (Gillis and Swim 2020) and consumers are willing to pay more for well-designed yards that include native plants in place of lawns (Helfand *et al*. 2006).

Sweet acacia (*Vachellia farnesiana* (L.) Wight and Arn) [Fabaceae] is a small to medium tree characterized by even bi-pinnately compound leaves, stipular spines, prominent lenticels on stems, globose yellow flowers that are fragrant, and leguminous seed pods (Erkovan *et al*. 2016; Gilman *et al*. 2018; Gann *et al*. 2021). This species has also been widely known under the binomial of *Acacia farnesiana.* Native to the Southern United States, Central America, and the Caribbean (Parrotta 2004), sweet acacia has been historically valued for reforestation, timber, and medicinal purposes (Delgadillo-Puga *et al*. 2019; Morales-Dominguez *et al.* 2019) and can be used for phytoremediation of heavy metals in soils (Armienta *et al*. 2008; Maldonado-Magaña *et al*. 2011). In the USA, sweet acacia can be found in a wide range of environmental conditions with cold hardiness in USDA zones 8b-11 (USDA 2012). Despite its ornamental appeal, adaptive ability (UF/IFAS,

✉ JianJian Xu
jxu2s@ufl.edu

[1] Environmental Horticulture Department, University of Florida, PO Box 110670, Gainesville, FL 32611, USA

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2021), and drought tolerance (Moura and Vieira 2020), sweet acacia is rarely utilized in the landscape and is difficult to find in the commercial nursery industry. Seeds are reported to possess physical dormancy (Tadros *et al*. 2011) imposed by the impermeability of the seed coat, which can be overcome through hydrothermal or mechanical scarification (Smith *et al*. 2022). When seeds are not available, asexual propagation is possible, but it is slow and at levels not satisfactory for commercial production (Smith *et al*. 2022). Noted advantages to asexual propagation include improved uniformity, maintenance of desirable traits, and reduced production time (Rupp *et al*. 2011; Dumroese *et al*. 2009; Davies *et al*. 2018). However, in situations when cuttings show recalcitrant characteristics, micropropagation may serve as an economically viable means of mass clonal propagation, maximizing the utilization of space and allowing for year-round production (Davies *et al*. 2018). Although micropropagation of native plants is less commonly employed than conventional propagation methods, protocols have been developed for a number of species, including New Jersey tea (*Ceanothus americanus*), cherry (*Prunus pumila*) and sweetballs (*Eubotrys racemosa*) (Lubell-Brand and Brank 2021), wild gingers (*Hexastylis* sp.), and many butterfly weed species (*Asclepias* sp.). Little is known regarding the applications of *in vitro* propagation of sweet acacia for ornamental purposes. A review of 17 species of *Acacia* involved the evaluation of various explant sources, media, and plant growth regulators, as well as different micropropagation techniques and cell cultures, including organogenesis and somatic embryogenesis (Vengadesan *et al*. 2002). More specifically, Morales-Domínguez *et al*. (2019) evaluated the effect of plant growth regulators on shoot multiplication and root induction of sweet acacia for reforestation purposes in Mexico. In addition, immature seed-derived zygotic embryos of *Acacia farnesiana* (syn. *Vachellia farnesiana*) were found to be capable of forming callus cultures from which somatic embryos could be formed and subsequently germinated *in vitro* (Cana Edo Ortiz *et al*. 2000). Likewise, Ho *et al*. (2022) were successful in micropropagating a closely related *Acacia confusa* using dormant buds of nodal sections taken from mature trees in the field. Despite these efforts, sweet acacia is still most commonly propagated by seed for the commercial market. Therefore, the overall goal of this study is to widen the availability of sweet acacia for use in landscapes by developing efficient methods for mass asexual propagation. Specific objectives were to determine (1) the best method for *in vitro* seed germination, (2) the effect of cytokinin concentration on multiplication, and (3) the effect of auxin concentration on *in vitro* rooting and acclimatization.

Materials and methods

Stage I: Culture establishment (*in vitro* seed germination) In late spring 2020, mature fruits were collected from a 5-yr-old specimen tree located in the Environmental Horticulture Department Teaching Garden on University of Florida's campus (Gainesville, FL). After collection, seeds were removed from seed pods and kept at room temperature in a paper bag prior to germination experiments. Seeds were washed in an aqueous solution of 1% Alconox lab detergent (Alconox Inc., White Plains, NY) for 10 min. The seeds were then surface sterilized *via* agitation in an aqueous 70% ethanol (Decon Labs, Inc., King of Prussia, PA) solution for 1 min, then rinsed in sterile deionized water, and immediately transferred to an aqueous 1% sodium hypochlorite solution for 5 min with continuous stirring. After 5 min, seeds were rinsed three times in sterile deionized water. Hydrothermal scarification was accomplished by pouring boiling (100 °C) sterile deionized water over the seeds and allowing them to soak for 24 h. As a control, seeds were soaked in water at room temperature for 24 h, as typically required for hard-coated seeds. Scarified seeds were visually sorted based on successful imbibition or not. Seeds which had imbibed were visibly swollen with the hard seed coat becoming gelatinous and rubbery (Fig. 1*A*). Seeds were then grouped according to their initial scarification treatment, classified as "scarified and imbibed," "scarified and not imbibed," or "non-scarified" (control). A total of 300 seeds, consisting of 100 seeds from each treatment group, were selected for *in vitro* germination evaluation. For each treatment, five seeds were placed in each of 20 Magenta GA7 boxes (Magenta Corp., Chicago, IL) which were previously prepared with 50 mL of sterile half-strength MS medium (Murashige and Skoog 1962) (M519, PhytoTechnology Laboratories; Shawnee Mission, KS) supplemented with 15 g L^{-1} sucrose (Thermo Fisher Scientific, Hampton, NH) without any exogenous plant growth regulators. The experiment followed a randomized complete block design with 20 replicates, each containing 5 seeds. The medium pH was adjusted to 5.65 with 0.1 N KOH (P672 PhytoTechnology Laboratories Inc.) solution prior to the addition of 7 g L^{-1} agar (A296, PhytoTechnology Laboratories Inc.) and autoclaving at 121 °C at 15 PSI for 20 min. Magenta boxes were randomly placed in incubators (Percival Scientific Inc., Perry, IA) at 23 °C without light for 1 wk, and then transferred to a growth room maintained at the same temperature with a 16-h photoperiod provided by cool-white, fluorescent lights (GE., Boston, MA) with 100 ± 5 µmol m^{-2} s^{-1} PPFD. Germination, defined as radicle protrusion (Fig. 1*B*), was recorded every 3 d for 27 d. Each seedling was also observed for the presence of 2, 3, or 4 cotyledons (Fig. 1*C*). An outline of the treatments and replicates is as follows:

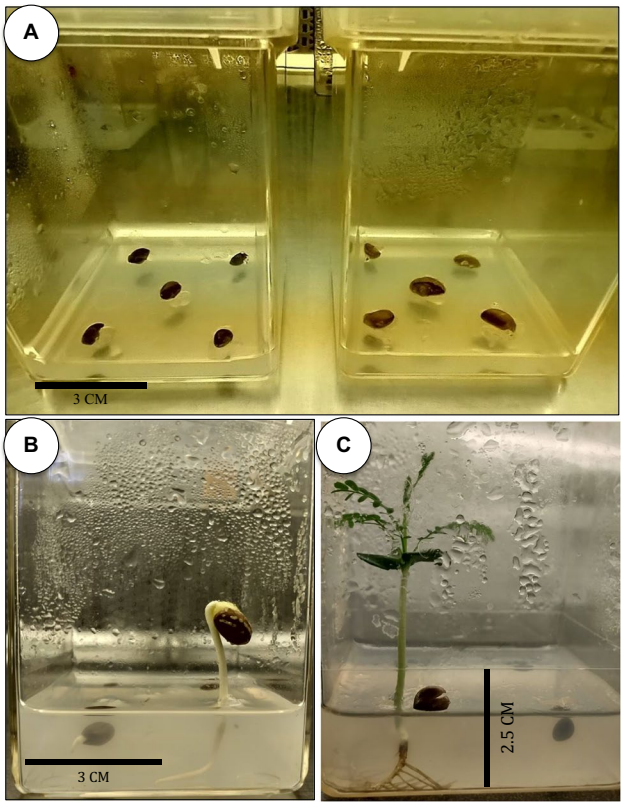

Figure 1. *A–C In vitro* seed germination of sweet acacia. Seeds were soaked in boiled water that was allowed to come to room temperature overnight. Images show (*A*) visually non-imbibed seeds (*left*) or imbibed seeds (*right*), germinated seedling after 3 d (*B*) and first true leaves after 21 d (*C*).

Treatment 1: non-scarified (control) × 20 replicates × 5 seeds
Treatment 2: scarified and imbibed × 20 replicates × 5 seeds
Treatment 3: scarified and non-imbibed × 20 replicates × 5 seeds

Stage II: Shoot multiplication From *in vitro* seedlings, 3- to 4-node shoot cuttings were used as explants, removed at about 3 mm below the cotyledons. Explants sorted by 2, 3, and 4 cotyledons were placed in 220-mL glass culture jars (PhytoTechnology Laboratories Inc.) containing 25 mL of full-strength MS medium supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} agar. Treatments consisted of cotyledon number and concentration of 6-benzylaminopurine (BA) (B800, PhytoTechnology Laboratories Inc.), a synthetic cytokinin, at 1 mg L^{-1} or 2 mg L^{-1} added to the medium prior to autoclaving as previously described. Cultures were maintained in a growth room at 23 to 24°C with a 16-h photoperiod provided by cool-white, fluorescent lights. The light intensity at shelf height was 100 ± 5 µmol·m^{-2}·s^{-1} PPFD. The experiment followed a randomized complete block design with 3 replicates, each replicate contained 3 explants. After 30 d, representative samples of shoot clusters were destructively harvested, and data was collected including shoot number and shoot length per explant. An outline of the treatments and replicates is as follows:

Treatment 1: 2 cotyledons × 1 mg L^{-1} BA × 3 replicates × 3 explants
Treatment 2: 2 cotyledons × 2 mg L^{-1} BA × 3 replicates × 3 explants
Treatment 3: 3 cotyledons × 1 mg L^{-1} BA × 3 replicates × 3 explants
Treatment 4: 3 cotyledons × 2 mg L^{-1} BA × 3 replicates × 3 explants
Treatment 5: 4 cotyledons × 2 mg L^{-1} BA × 3 replicates × 3 explants
Treatment 6: 4 cotyledons × 1 mg L^{-1} BA × 3 replicates × 3 explants

Stage III: *In vitro* rooting Excised shoots (1 to 2 cm) were rooted on full-strength MS medium supplemented with 0.5 mg L^{-1} IBA (indole-3-butyric acid) (I538, PhytoTechnology Laboratories Inc.) and either 0.05 mg L^{-1} or 0.5 mg L^{-1} NAA (1-naphthaleneacetic acid) (N600, PhytoTechnology Laboratories Inc.) as treatments. Cultures were maintained in a growth room at 23 to 24°C with a 16-h photoperiod provided by cool-white, fluorescent lights. The light intensity at shelf height was 100 ± 5 µmol·m^{-2}·s^{-1} PPFD. The experiment followed a randomized complete block design with 4 replicates, each replicate contained 3 explants. After 60 d, the percentage of explants rooting was recorded for each culture jar, and then, primary root number, root length (average of 2 longest roots), and root diameter were recorded for each explant. An outline of the treatments and replicates is as follows:

Treatment 1: 0.5 mg L^{-1} IBA and 0.05 mg L^{-1} NAA × 4 replicates × 3 explants
Treatment 2: 0.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA × 4 replicates × 3 explants

Stage IV: Acclimatization After data collection, shoots from both stage III auxin treatments were dipped in 3000 mg L^{-1} IBA (Hormex rooting powder #3, Westlake Village, CA) and transferred into 6-cell packs (width 3.8 cm × length 3.8 cm × depth 5.8 cm) (T.O. Plastics, Inc., Clearwater, MN) containing Metro-Mix 852 (6:3:1 vol% bark:Canadian peat:perlite) (Sun Gro Horticulture, Agawam, MA). Plants were misted with water from a spray bottle to keep them from wilting prior to transfer to the mist house. Three plants from each treatment were spaced in 6-cell packs and placed into one of four flat trays (width 27.3 cm × length 54.5 cm × depth 6.2 cm) (T.O plastics Inc.). To minimize excessive

water on foliage, plant trays were covered by plastic domes for the first 3 wk and placed on a greenhouse bench set to mist frequency every 5 min for 5 s. The experiment followed a randomized complete block design with 4 replicates, each replicate contained 3 explants. Plants were checked weekly for moisture control, root initiation, pest interference, and foliage senescence. The average temperature in the mist house was 25.1 °C with a maximum temperature of 36.4 °C and a low temperature of 13.7 °C (HOBO Pendant® MX Water Temperature Data Logger; Onset Computer Corp., Bourne, MA). After 4 wk in the mist house, plants were moved to an environmentally controlled, open-aired, sawtooth greenhouse set to a minimum of 20.0 °C and maximum of 23.9 °C for an additional 4 wk prior to evaluation. Stage IV rooted transplants were assessed for root quality using a scale from 1 to 4 that indicated the following: 1 = no roots (plants are alive), 2 = roots developed but do not hold media, 3 = roots hold some media, 4 = roots are fully developed and completely hold media. Plants were then soaked in water to remove soil from roots to determine total number of primary roots, mean root length (from two longest roots), plant height, and primary leaf number.

Statistical analysis Data was collected, transformed if appropriate, and submitted to analysis of variance (ANOVA) using the OriginPro® 2021b software (OriginLab, Northampton, MA). When significant, Tukey's *post hoc* multiple comparison adjustment ($\alpha = 0.05$) was used for all pairwise comparisons of means. A two-way ANOVA was used to determine the main effects of scarification treatment and time on *in vitro* seed germination. For the shoot multiplication experiment, a two-way ANOVA was used to determine the effects of cytokinin concentration and cotyledon number and their interaction. For the rooting experiment, a one-way ANOVA was applied to determine the effects of auxin concentration on stage III rooting performance. A one-way ANOVA was also used to determine the effects of auxin concentration on stage IV survival, and root and shoot traits.

Results

Results presented herein describe *in vitro* seed germination of *Vachellia farnesiana* (duration: 27 d, treatments: non-scarified, scarified + imbibed, and scarified/non-imbibed), stage II shoot multiplication (duration: 30 d, treatments: 3 types of cotyledon number and 2 levels of BA), stage III rooting (duration: 60 d, treatments: 2 levels of NAA/IBA combinations), and stage IV acclimatization (duration, 60 d).

***In vitro* seed germination** Scarification of seeds had a positive effect on seed germination ($P < 0.0001$). Germination was not significantly influenced by time ($P < 0.8966$), nor was there a significant scarification × time interaction

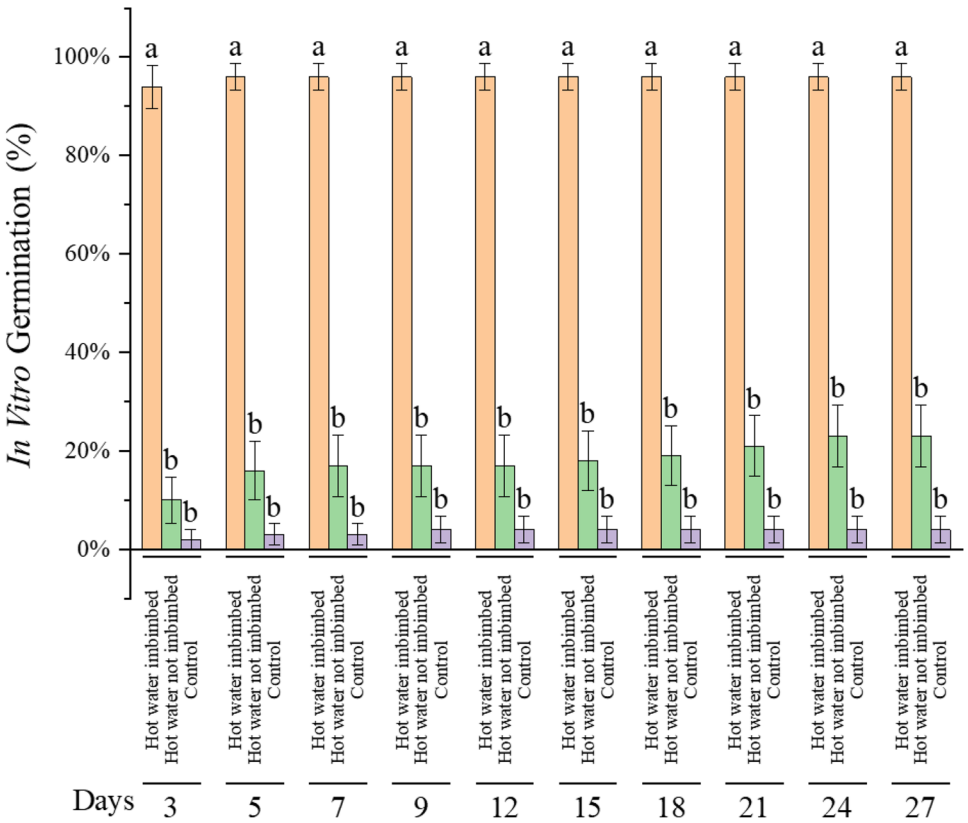

Figure 2. Effect of scarification on seed germination. *In vitro* germination of *Vachellia farnesiana* seeds placed on agar based half-strength MS medium without hormones for 27 d. Seeds were scarified with a hot water treatment, allowed to soak overnight, and then sorted by the visual presence or absence of imbibition. Control seeds were not scarified and only soaked overnight at room temperature. Means within each time interval (days) with different *letters* are significantly different (Tukey, $P \leq 0.05$).

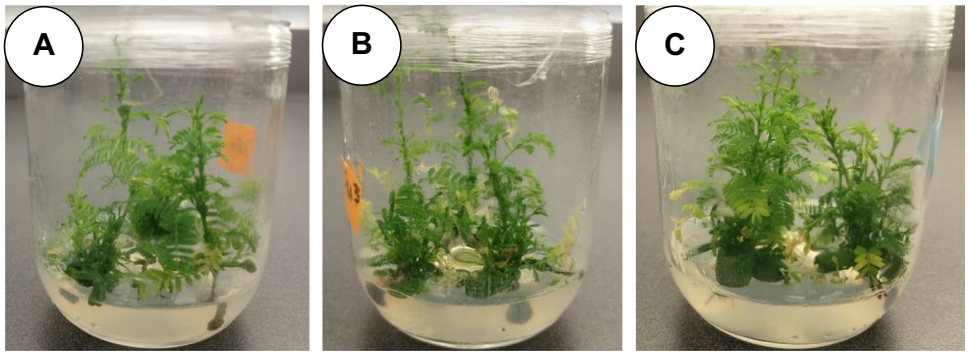

Figure 3. Multiplication of shoot cultures taken from *in vitro* seedlings with 2 (*A*), 3 (*B*), or 4 (*C*) cotyledons after 30 d in MS medium supplemented with BA.

($P < 0.9998$). Within 3 d after sowing, seeds that were hot water imbibed achieved 94.0% germination compared to hot water-treated seeds that did not imbibe at only 10.0% germination, while seeds from the control group only showed 2.0% germination (Fig. 2). From days 5 to 27, maximum germination was 96.0% for hot water imbibed, 23.0% for hot water non-imbibed, and 4.0% for control, and was found to be statistically similar to germination on day 3.

***In vitro* shoot multiplication (stage II)** Regardless of the tested BA concentration or observed cotyledon number, stage II explants cultured for 30 d on multiplication medium produced 5.3 to 7.1 shoots ranging from 7.4 to 8.8 mm in length (Fig. 4*A–D*). The multiplication rate, expressed as number of shoots per explant, was similar for explants cultured on medium supplemented with 1.0 or 2.0 mg L^{-1} BA ($P < 0.4914$) (Fig. 4*C*), as was the length of shoots ($P < 0.2344$) (Fig. 4*D*). There was no interaction between BA concentration and cotyledon number, either ($P < 0.6010$). Cotyledon number (2, 3, or 4) had a significant effect on shoot multiplication ($P < 0.0236$) and shoot length ($P < 0.0370$) (Fig. 3, Fig. 4*A*, *B*). Explants with 4 cotyledons produced 1.3 times more shoots than explants with 2 or 3 cotyledons (Fig. 4*A*) but were shorter in length compared to explants with 3 cotyledons (Fig. 3, Fig. 4*B*).

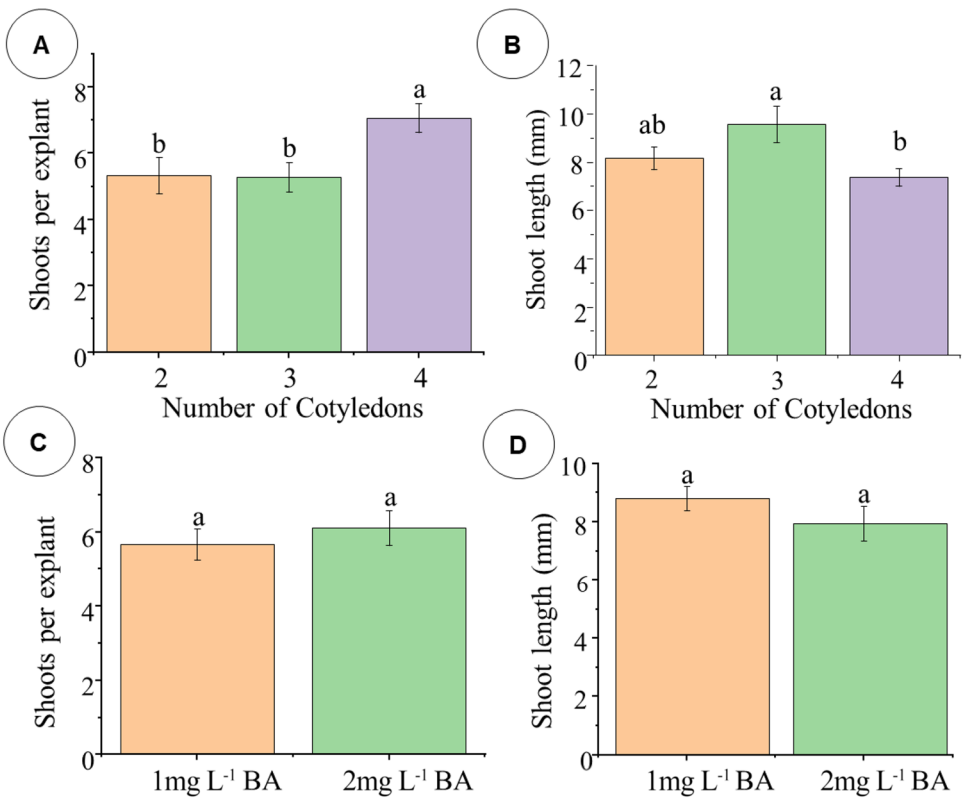

Figure 4. Multiplication rate and mean length of shoot explants grown on full-strength MS medium for 30 d and having 2, 3, or 4 cotyledons (*A*, *B*) and supplemented with 1.0 or 2.0 mg L^{-1} BA (*C*, *D*). Explants were derived from *in vitro* seedlings.

Table 1. Rooting percentage, root width, primary root number, and average root length of shoot explants grown on full-strength MS medium supplemented with 0.5 mg L^{-1} IBA and either 0.05 or 0.5 mg L^{-1} NAA for 30 d. Only shoots with roots were included in performance analysis ($n = 21$)

Treatments	Rooting percentage (%)[z]	Root width (mm)	Root number	Root length (mm)[y]
0.05 NAA	62.5 ± 14.2^{b}	0.50 ± 0.04^{b}	8.9 ± 1.8^{a}	37.0 ± 5.1^{a}
0.50 NAA	100.0 ± 0.0^{a}	0.64 ± 0.04^{a}	5.8 ± 0.6^{a}	30.0 ± 1.9^{a}

[z]Values represent mean ± SE (standard error). Means with different *letters* are significantly different (Tukey, $P \leq 0.05$)

[y]Root length was determined by averaged the two longest roots of each plantlet

***In vitro* rooting (stage III)** Stage III shoots grown for 60 d on rooting media with 0.50 mg L^{-1} NAA had a greater positive effect on rooting percentage ($P < 0.0388$) and width ($P < 0.0152$) compared to those rooted in media containing 0.05 mg L^{-1} NAA. When 0.50 mg L^{-1} NAA was used in the media, 100% of the shoots rooted, whereas only 62.5% of the shoots rooted with 0.05 mg L^{-1} NAA concentration (Table 1). While the root number and root length were similar among rooting treatments, shoots grown on 0.50 mg L^{-1} NAA produced roots that were 1.3 times the diameter in comparison to roots under 0.05 mg L^{-1} NAA (Table 1; Fig. 5*A*, *B*).

Acclimatization (stage IV) After 60 d of greenhouse acclimatization (Fig. 6*A*, *B*), 72.7% and 100% of shoots rooted from stage III shoots under 0.05 and 0.50 mg L^{-1} NAA, respectively (Table 2). There was a positive effect of NAA concentration on stage IV root quality ($P < 0.045$) and plantlet height ($P < 0.047$), but this difference was not observed for root length ($P < 0.149$), root number ($P < 0.747$), or primary leaf number ($P < 0.733$). Shoots grown on 0.50 mg L^{-1} NAA rooting media prior to acclimatization had 1.12 times greater root quality (3.9 on a scale of 1 to 4) and 1.73 times greater plantlet height than shoots grown on 0.05 mg L^{-1} NAA (Table 2; Fig. 6*C*, *D*).

Discussion

Results presented herein reveal that *Vachellia farnesiana* can be successfully micropropagated using 3 to 4-node shoot explants derived from *in vitro* seedlings. When seeds were treated with hot water and had visual signs of imbibition (swelling), maximum germination (96%) was achieved within 5 d, revealing that physical dormancy of seeds can easily be overcome by hydrothermal scarification. This is consistent with 97% emergence reported for *V. farnesiana* seed that were also hydrothermally scarified but instead germinated in cell trays filled with soilless media and placed under a greenhouse mist system (Smith *et al.* 2022). Interestingly, in this *in vitro* study, hydrothermally scarified, non-imbibed seeds showed only 23.0% germination, while in a prior greenhouse study (Smith *et al.* 2022), non-imbibed seeds germinated to a maximum of 86% over the course of 4

Figure 5. Stage III shoot and root performance of explants grown on full-strength MS medium supplemented with 0.5 mg L^{-1} IBA and either 0.05 mg L^{-1} NAA (*A*) or 0.5 mg L^{-1} NAA (*B*) for 30 d.

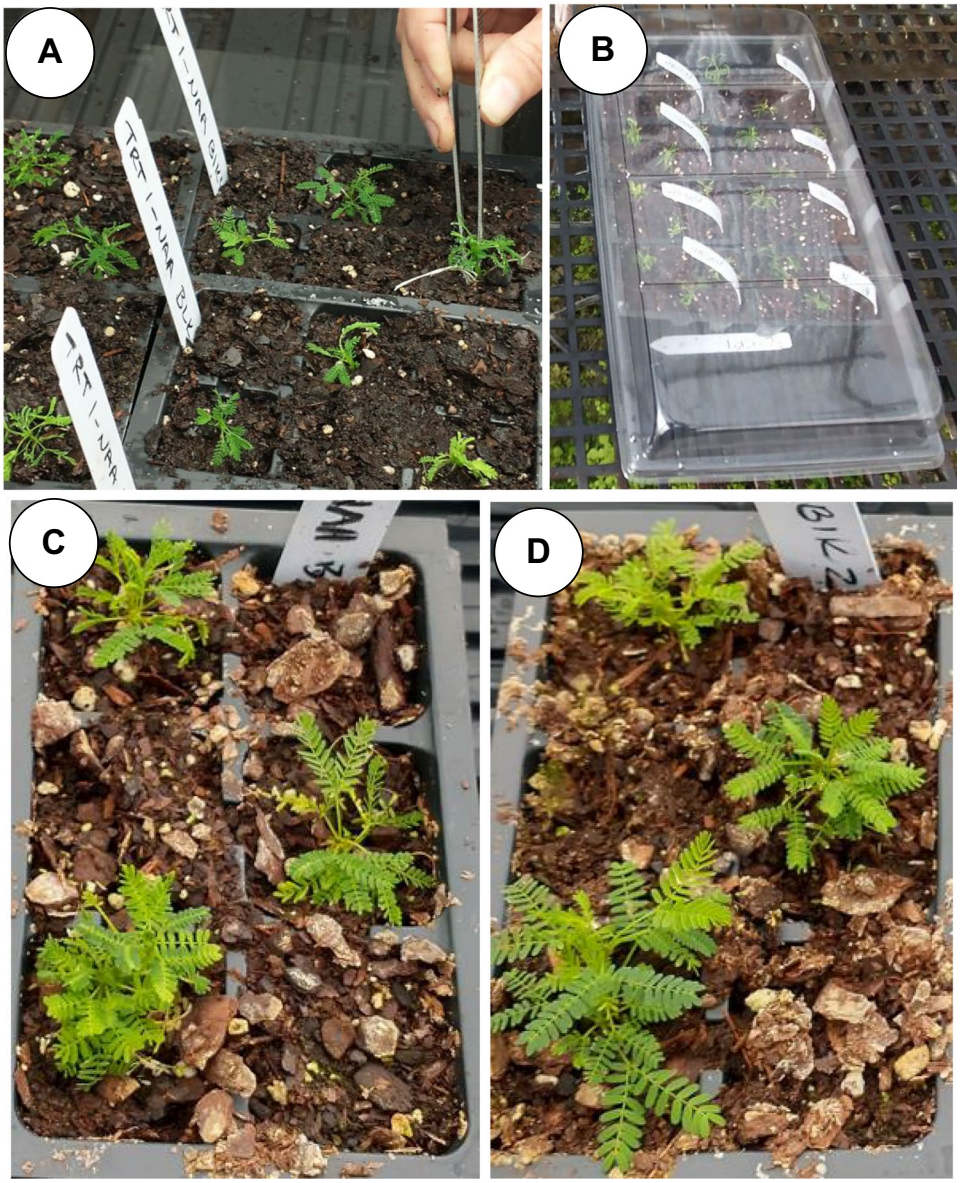

Figure 6. Acclimatization of sweet acacia (*Vachellia farnesiana*) from stage III *in vitro* to stage IV *ex vitro* conditions. (*A*) Rooted shoots were dipped with 3000 mg L^{-1} IBA, transferred into trays filled with soilless media, placed in a mist house, and covered by plastic domes (*B*). Representative images after 60 d of acclimatization of stage IV shoots cultured on MS medium supplemented with 0.5 mg L^{-1} IBA and either (*C*) 0.05 mg L^{-1} NAA or (*D*) 0.5 mg L^{-1} NAA.

Table 2. *Ex vitro* performance of stage IV plantlets acclimated for 60 d in a greenhouse. Prior to acclimatization, shoot explants were cultured on medium supplemented with 0.5 mg L^{-1} IBA and either 0.05 or 0.5 mg L^{-1} NAA

Treatments	Rooting percentage (%)[z]	Root quality (0–4)[y]	Root length (mm)[x]	Root number	Shoot length (mm)	Leaf (no.)
0.05 NAA	72.7 ± 12.9^{b}	3.5 ± 0.17^{b}	7.1 ± 0.2^{a}	4.1 ± 0.4^{a}	2.0 ± 0.2^{b}	11.6 ± 0.6^{a}
0.50 NAA	100 ± 0^{a}	3.9 ± 0.08^{a}	7.5 ± 0.2^{a}	4.3 ± 0.3^{a}	3.5 ± 0.7^{a}	11.2 ± 1.2^{a}

[z]Values represent mean ± SE (standard error). Means with different *letters* are significantly different (Tukey, $P \leq 0.05$)

[y]Root quality was determined using a scale from 1 to 4 that indicated the following: 1 = no roots (plants are alive), 2 = roots developed but do not hold media, 3 = roots hold some media, 4 = roots are fully developed completely hold media

[x]Root length was determined by averaged the two longest roots of each plantlet

wk. It is likely that the *in vitro* conditions were not sufficient to completely overcome physical dormancy of non-imbibed seeds compared to seeds germinated in a greenhouse mist system in warmer summer months. For this species and possibly other species with hard seed coats, this emphasizes the benefit of sorting visually imbibed hydrothermally scarified seeds prior to germinating *in vitro*. Alternatively, chemical seed scarification (sulfuric acid) has been found effective for *in vitro* germination of *V. farnesiana* (Morales-Domínguez *et al*. 2019); however, associated safety hygiene and hazardous waste removal can be cost prohibitive.

When using shoot explants of sweet acacia, BA was found to be an effective cytokinin for stage II multiplication, and the lower concentration (1 mg L^{-1} BA) was as effective as the higher concentration (2 mg L^{-1} BA) in the medium. In addition, for stage III micropropagation, IBA and NAA were found to be effective auxins for *in vitro* rooting with the higher NAA concentration (0.50 mg L^{-1}) resulting in the greatest rooting response. These results are consistent with those reported by Morales-Dominguez *et al.* (2019) who evaluated the effect of plant growth regulators on shoot multiplication and root induction of sweet acacia. They found MS multiplication medium supplemented with 1 mg L^{-1} BA achieved the greatest number of shoots per explant (5.42), and MS rooting medium supplemented with 3 mg L^{-1} IAA (indole-3-acetic acid) achieved the greatest rooting percentage (95%). This indicates that another type of auxin, IAA, can also be used for *in vitro* rooting of sweet acacia. The combination of NAA and IBA in the rooting medium has been found to produce greater *in vitro* rooting response than either auxin used independently (Khalisi and Al-Joboury 2012).

Interestingly, about two-thirds of the germinated seedlings of *V. farnesiana* had 3 or 4 cotyledons rather than the expected 2 cotyledons, polycotyledonary development has been noted in some related species (Reddy *et al*. 2000). Regardless, all explants having 3 to 4 nodes had a 100% multiplication response. In a similar study with presumably 2-cotyledon sweet acacia explants, Morales-Dominguez *et al.* (2019) also reported a 100% multiplication response, greater than that reported by Khalisi and Al-Joboury (2012) who used 1.5-cm nodal segments with only a 75% multiplication response. This research found the presence of 4 cotyledons to be advantageous in shoot multiplication producing 34% more shoots than explants with 2 or 3 cotyledons. While the origin of polycotyledons remains unclear, this serves as a first report of its occurrence for sweet acacia in tissue culture.

Stage III plantlets from both NAA treatments (0.05 and 0.50 mg/L) were successfully acclimated to *ex vitro* conditions with a 100% survival rate. However, a higher auxin concentration in the medium resulted in a greater rooting percentage (100%) and root quality (3.9 of a 1 to 4 scale) compared to the lower concentration that yielded 72.7% rooting and 3.5 root quality. The primary root length, root number, and leaf number were similar between treatments, but plantlets grown in the higher NAA concentration grew taller (had longer shoot lengths) than plantlets grown in the lower NAA concentration, presumably due to their better root quality. The use of 3000 mg L^{-1} IBA talc prior to sticking the shoots was not sufficient to improve the rooting percentage of the shoots which were under the lower *in vitro* auxin treatment. The finding of this study would suggest using a combination of 0.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA in stage III culture of sweet acacia. Compared to a mist greenhouse system often used for stage IV micropropagation (Davies *et al*. 2018), in this study, the use of humidity domes in a shaded greenhouse worked well to prevent water from collecting on the delicate compound leaves and subsequent nutrient leaching.

Conclusion

Along with sweet acacia's many desirable traits for Florida landscapes, the results from this study show that this species is relatively easy to micropropagate. Using the *in vitro* system described herein, commercial nurseries can expect to have finished sweet acacia liners in a relatively fast 150-d production cycle (30-d stage II, 60-d stage III, and 60-d stage IV). It is hopeful that continued advances in propagation knowledge for this species as well as for other related species will help expand the nursery industry's plant palette and facilitate the widespread use of sweet acacia in landscapes and gardens.

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