able seed coat, requiring scarification and stratification. Some success with seed germination of *V. obovatum* has been reported by the nursery industry (pers. comm.).

As a general rule, based on this and the previous reports, it appears that Florida native plants should not be propagated immediately prior to or during flowering periods. Turgidity of the plants as related to available soil moisture is also a determining factor in root initiation and development. It is therefore highly recommended that, when possible, the mother plants be irrigated a few days priot to taking of cuttings. The length of time required for root initiation varies from as little as 3-4 weeks to as long as 3-4 months, depending on time of year, rainfall, flowering season, and other environmental factors. The effectiveness of IBA concentrations greater than 5,000 ppm varies with species. In some it may enhance while in others inhibit root initiation.

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VEGETATIVE PROPAGATION OF FLORIDA NATIVE PLANTS: IV. *QUERCUS* SPP. (OAKS)

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Abstract. **The difficulty in vegetative propagation of oaks and erratic seed production are known facts. Thus, successful propagation of oaks by cuttings is a desirable goal, not only to maintain some degree of uniformity in selected plants but to continue production when acorns are not available. Of the five native Florida plants studied, *Quercus laurifolia* Michx. (diamond-leaf oak) and *Q. virginiana* Mill. (live oak), produced extensive callus but did not initiate roots, irrespective of time of the year or treatment. However, *Q. geminata* Small (sand-live oak) *Q. hemisphaerica* Bartr. (Laurel oak), and *Q. nigra* L. (water oak) exhibited a definite time specificity with respect to root initiation which coincided with the first or second flush of active growth. The positive effect of IBA and/or NAA were most pronounced in *Q. hemisphaerica* *Q. nigra*, but not in *Q. geminata*. Based on our studies the best time of year for taking cuttings of *Q. geminata* and *Q. nigra* is in July and June, respectively, whereas that of *Q. hemisphaerica* is in April.**

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Although oaks can be propagated by seed easily, their vegetative propagation is notoriously difficult and has been the subject of research by many authors, often without positive results. Two factors in particular contribute to the

desirability of vegetative propagation of oaks: the erratic acorn production which is profuse in some years but non-existent in others and recurrent cross pollination of sympatric taxa which frequently results in variable fertile hybrid populations when, at least for landscaping purposes, uniformity of selected taxa is often the desired objective. In fact, lack of selected taxa (clonal cultivars) in oaks is directly attributable to the difficulty of vegetative propagation.

Attempts to propagate oaks by cuttings using traditional procedures have been only infrequently successful. Studies of Dehgan et al. (4) with *Q. phyllyraeoides* A. Gray f. *wrightii* indicated that cuttings rooted best when collected on March 31 and August 21, during first and second flushes of growth, respectively. Similar results were obtained by Deen (3) for *Q. ilex*. Rooting of cuttings from *Q. virginiana* seedlings was accomplished in 12 weeks when treated with IBA but cuttings from mature plants did not root (11). Dirr and Heuser (7) reported 73% and 81% success rate with terminal and subterminal cuttings of *Q. phellos* X, when dipped in 10,000 ppm K-IBA.

The difficulty in vegetative propagation of oaks is best reflected in employment of non-traditional methods. For example, Maynard and Bassuk (10) reported rooting of softwood cuttings of *Q. palustris*, *Q. robur*, and *Q. coccinea* by etiolation with Velcro adhesive fabric strips as the blanching material. Smith and Schwabe (12), successfully propagated *Q. robur* by growing seedlings and mature plants under high temperatures and continuous light, and further accelerated shoot growth with application of GA3 prior to taking of cuttings. Satisfactory rooting of cuttings from seedlings of *Q. robur* also was observed by Chalupa (2) when these were treated with 100 ppm IBA for 24 hr, but little improvement was observed with cuttings of mature plants. However, an unspecified high percentage of cuttings from 2 to 4 year old plants of *Q. robur* did root when treated with 80 ppm IBA for 24 hrs and maintained under 20° C and continuous light. Formation of adventitious roots on trailing branches of *Q. robur* has been reported under natural conditions but not on the upright branches (1). In a particularly interesting study Kralik, et al. (9) reported successful rooting of *Q. robur* when treated with 2-chlorethylphosphonic acid and chlorecholinechloride. They concluded that endogenous cytokinins at the basal part of the cuttings decrease with the onset of dormancy. The formation of adventitious roots is preceded by the decrease of endogenous cytokinins, emulating a dormant condition that hinders further growth of adventitious roots. This may explain the excessive formation of callus on oak cuttings and inhibition of their growth. Garbaye et al. (8) succeeded in overcoming this sudden onset of dormancy in cuttings with an unspecified period of cold treatment. Alternatively, cuttings taken from actively growing 4 to 5 year old seedlings or stump suckers were shown to have up to 90% rooting when treated with IBA.

The intent of the present research was to determine the best time of year and possible treatments for propagation of Florida native oaks by cuttings. Except for the paper on the Texas ecotypes of *Q. virginiana* by Morgan (11), to our knowledge, no other reports have been published for the indigenous oaks of Florida.

Table 1. Effect of various concentrations of IBA, NAA, and time of year on rooting of cuttings of *Quercus geminata* Small, taken from the White Springs site.

Date stuck	Date rated	Treatment (ppm)	Mean % rooting	Root condition*
7/31/86	10/31/86	Control	53.33 ± 17.16	2.5
7/31/86	10/31/86	2,500 IBA	36.67 ± 17.16	2.0
7/31/86	10/31/86	5,000 IBA	48.33 ± 36.29	2.0
7/31/86	10/31/86	1,000 NAA	55.00 ± 18.71	2.5
7/31/86	10/31/86	2,000 NAA	40.00 ± 13.33	<2.0
7/31/86	10/31/86	2,500 IBA + 1,000 NAA	38.33 ± 22.73	2.0
7/31/86	10/31/86	2,500 IBA + 2,000 NAA	43.33 ± 26.56	2.0
7/31/86	10/31/86	5,000 IBA + 1,000 NAA	45.00 ± 25.06	2.0
7/31/86	10/31/86	5,000 IBA + 2,000 NAA	16.67 ± 0.00	<2.0
9/26/86	12/29/86	Control	1.67 ± 3.33	<2.0
9/26/86	12/29/86	2,500 IBA	1.67 ± 3.33	<2.0
9/26/86	12/29/86	5,000 IBA	1.67 ± 3.33	<2.0
9/26/86	12/29/86	1,000 NAA	3.33 ± 4.08	<2.0
9/26/86	12/29/86	2,000 NAA	1.67 ± 3.33	<2.0
9/26/86	12/29/86	2,500 IBA + 1,000 NAA	5.00 ± 6.67	<2.0
9/26/86	12/29/86	2,500 IBA + 2,000 NAA	0.00 ± 0.00	<2.0
9/26/86	12/29/86	5,000 IBA + 1,000 NAA	3.33 ± 6.67	<2.0
9/26/86	12/29/86	5,000 IBA + 2,000 NAA	1.67 ± 3.33	<2.0

*<2: Unacceptable
2: Inferior
3: Good-Acceptable
4: Very Good
5: Excellent

Materials and Methods

Terminal softwood-semihardwood cuttings of *Q. geminata* Small (sand-live oak), *Q. hemisphaerica* Bartr. (laurel oak), *Q. laurifolia* Michx. (diamond-leaf oak), *Q. nigra* L. (water oak), and *Q. virginiana* Mill. (live oak), were collected from undisturbed areas of Occidental and W. R. Grace mines in White Springs and Bartow, at approximately six week intervals for two consecutive years. Cuttings were dipped in water (control) and various concentrations and combinations of IBA and NAA (see Tables 1-3 for details). Rooting media, greenhouse environmental conditions, and data collection were the same as previously described by Dehgan et al. (5, 6, and in this volume).

Results and Discussion

Cuttings of *Q. laurifolia* and *Q. virginiana* did not root regardless of treatment or time of year (data not shown). Although Morgan (11), has reported successful rooting of cuttings taken from seedling and juvenile plants of *Q. virginiana*, we are not aware of other reports for rooting of cuttings of these or any other Florida native species. Successful propagation of *Q. virginiana* by root cuttings has been reported by some Florida nurserymen (pers. comm.) and growth of adventitious shoots from root pieces has been observed by the authors in phosphate mines where top soil is transferred from one site to another for reclamation purposes. The results of experiments for the other three species are presented in Tables 1-3, for the only times when rooting occurred.

Quercus geminata (Table 1. Figs. A-B), Cuttings rooted only when taken on 31 July, 1986. None of the cuttings

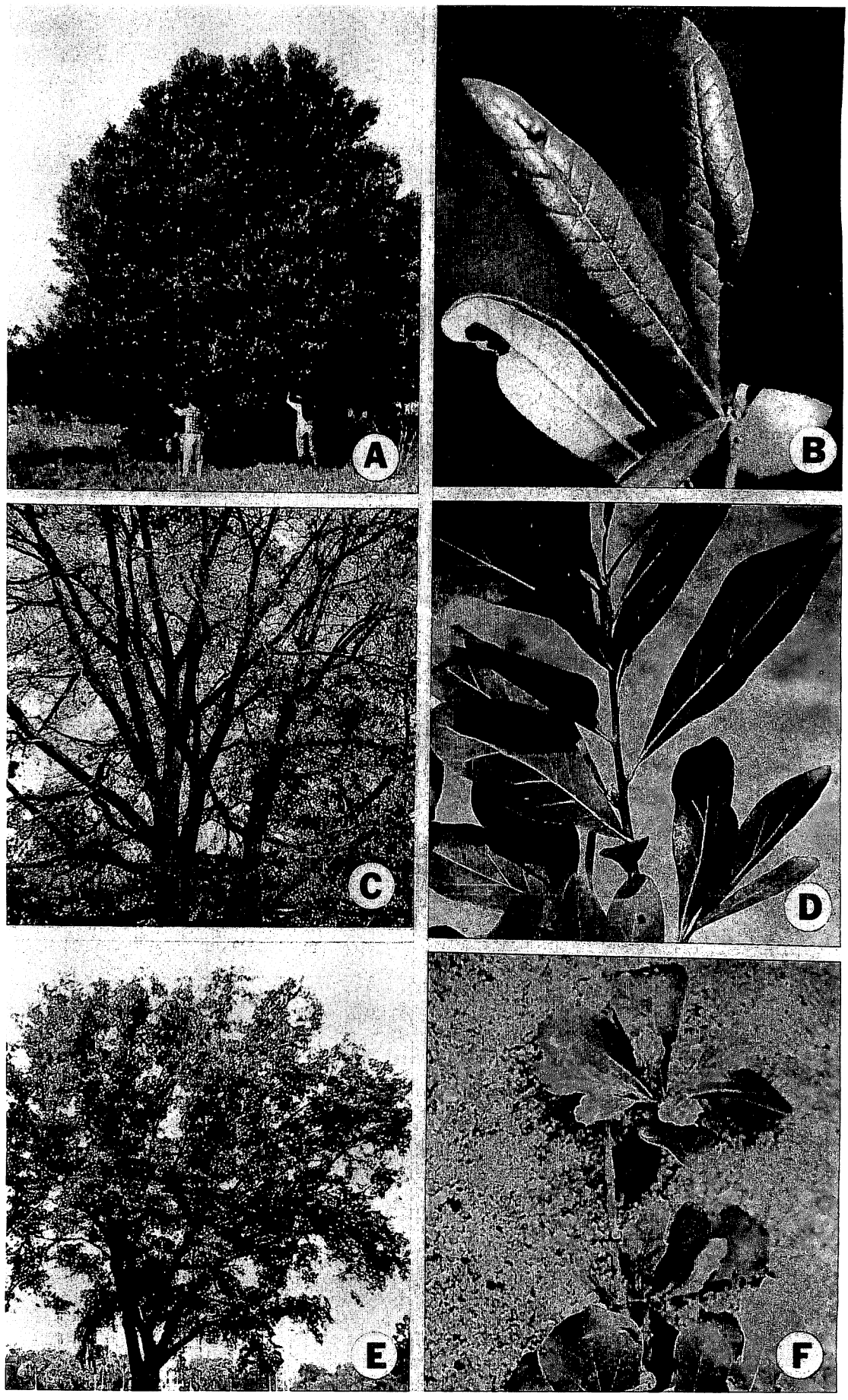

Fig. A-F. Growth habit and leaf characteristics of *Quercus* spp.: Figs. A-B, *Q. geminata*; Figs. C-D, *Quercus hemmisphaerica*; Figs. E-F, Q. nigra.

Table 2. Effect of IBA concentrations on rooting of *Quercus hemisphaerica* Bartr. cuttings taken from Four Corners Mine site.

Date stuck	Date rated	Treatment (ppm)	Mean % rooting	Root condition*
4/24/87	6/22/87	Control	2.50 ± 4.33	<2.0
4/24/87	6/22/87	2,500 ppm IBA	7.50 ± 8.29	<2.0
4/24/87	6/22/87	5,000 ppm IBA	32.50 ± 4.33	<2.0
4/24/87	6/22/87	10,000 ppm IBA	32.50 ± 10.90	<2.0
4/24/87	6/22/87	250 ppm NAA	37.50 ± 8.29	<2.0
4/24/87	6/22/87	500 ppm NAA	55.00 ± 15.00	<2.0
4/24/87	6/22/87	250 ppm NAA + 2,500 ppm IBA	37.50 ± 4.33	<2.0
4/24/87	6/22/87	250 ppm NAA + 5,000 ppm IBA	42.50 ± 21.65	<2.0
4/24/87	6/22/87	250 ppm NAA + 10,000 ppm IBA	45.00 ± 15.00	<2.0
4/24/87	6/22/87	500 ppm NAA + 2,500 ppm IBA	50.00 ± 12.25	<2.0
4/24/87	6/22/87	500 ppm NAA + 5,000 ppm IBA	35.00 ± 18.03	<2.0
4/24/87	6/22/87	500 ppm NAA + 10,000 ppm IBA	25.00 ± 15.00	<2.0

*<2: Unacceptable
2: Inferior
3: Good-Acceptable
4: Very Good
5: Excellent

taken within a few days of this date in 1987 rooted. The best results (55%) were obtained when plants were still growing actively but some secondary wood had formed in the basal portion of the cuttings. No meaningful differences were observed between the control and all other treatments, except for the reduction with the highest concentrations of IBA + NAA (16.67%). Cuttings taken in September (during second flush of growth) of the same year rooted very poorly, none exceeding 5%. Periodic examination of the cuttings indicated that root development took place only in the last 3-4 weeks prior to collection of the data, although extensive callus was formed during the first two months. Quality and quantity of the roots were not affected by IBA or NAA treatments.

Quercus hemisphaerica (Table 2, Figs. C-D). In contrast to *Q. geminata* which rooted only in the summer, cuttings of this taxon rooted only when taken on 24 April, 1987. None of the other attempts were successful. This time period coincided with the first flush of growth. The highest percentage rooting occurred when cuttings were treated with 500 ppm NAA alone (55%) and in combination with 2,500 ppm IBA (50%). The untreated cuttings had the lowest rooting percentage (2.5%) and 2,500 ppm IBA resulted in only a slight improvement (7.5%). It is noteworthy that although cuttings of this species were collected from both sites at approximately the same date, only those from Four Corners Mine (Bartow) rooted. This area had received considerable rainfall just prior to collection of cuttings.

Table 3. Effect of IBA concentration on rooting of *Quercus nigra* L. cuttings, taken from the White Springs site.

Date stuck	Date rated	Treatment (ppm)	Mean % rooting	Root condition*
6/11/86	9/3/86	Control	17.33 ± 19.14	<2.0
6/11/86	9/3/86	2,500 IBA	42.67 ± 12.36	2.5
6/11/86	9/3/86	5,000 IBA	37.33 ± 18.67	2.5
6/11/86	9/3/86	10,000 IBA	38.67 ± 16.55	2.5

*<2: Unacceptable
2: Inferior
3: Good-Acceptable
4: Very Good
5: Excellent

Quercus nigra (Table 3, Figs. E-F). The cuttings of this species rooted best when taken on 11 June, 1986. The only difference was between the control (17.33%) and all IBA treated cuttings (37.33-42.67%), among which no notable difference was observed. Only a few of the cuttings taken on 12 May, 1986 rooted (data not shown). Once again, the shoots were still growing actively but had become somewhat woody.

Clearly, species of *Quercus* are exceptionally exact as to the time of year when they are capable of root initiation. There is a direct correlation between the growth stage of the mother plants and rooting of cuttings. This is in agreement with most reports that oak cuttings root only when taken from juvenile (actual or forced) plant or during active growth (1, 2, 3, 4, 8, 9, 10, 11, 12). Cytokinins and probably all phytohormones exhibit peaks of activity which are dependent on environmental stimuli (13). Whether adventitious root formation is the result of high or low concentrations of certain hormones such as cytokinins is a matter of some controversy (see a detailed review and discussion in 13). Such time specificity in rooting of oaks is undoubtedly related to the activity of one or more endogenous hormones. Thus, future studies should determine the presence or absence of hormones in roots as well as shoots, which in turn should facilitate propagation of oaks by providing clues for appropriate treatments. It is also interesting to note that in none of the previous studies NAA has been used as a treatment, while at least in *Q. hemisphaerica* it promoted root initiation. Additional studies are needed to determine the effect of NAA on rooting of other oak species.

The root quality data in Tables 1-3 are somewhat deceiving, as all cuttings produced only a few but rapidly elongating healthy roots. Growth subsequent to transplanting was initially slow but survival was satisfactory.

Acorns of all five species germinated while cold stored (about 40° F) in plastic bags. There does not appear to be any seed dormancy in the Florida native taxa.

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IN VITRO PROPAGATION OF FLORIDA NATIVE PLANTS: *STYRAX AMERICANA* AND *PERSEA PALUSTRIS*

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Additional index words. acclimatization, micropropagation, tissue culture, woody plants.

Abstract. **Micropropagation protocols for *Styrax americana* L. (American snowbell) and *Persea palustris* (Raf.) Sarg. (Swamp bay persea) were developed and the post-transplant performances of rooted microcuttings were evaluated. In *Styrax*, maximum shoot regeneration (13-fold increase per 7 week culture cycle) from nodal explants occurred on agar-solidified Woody Plant Medium (WPM) supplemented with 1.0 mg/liter N^6-benzyladenine (BA). In *Persea*, maximal shoot regeneration (4-fold increase per 6 week culture cycle) occurred when nodal explants were cultured in liquid Murashige & Skoog medium (MS) supplemented with 0.25 mg/liter BA and 0.5 mg/liter gibberellic acid (GA_3). *In vitro* rooting of 1.5-cm-long *Styrax* microcuttings increased 74% on agar-solidified MS medium supplemented with 1.0 mg/liter indolebutyric acid (IBA). Transplant survival of rooted *Styrax* microcuttings was 83%, but acclimatized plantlets grew slowly, increasing only 52% in length 28 days post-transplant. One hundred percent *ex vitro* rooting of 3.0 cm long *Persea* microcuttings was attained by pre-dipping microcuttings in 5)) mg/liter IBA for 15 minutes prior to direct sticking in Vergro Klay Mix A, a soilless growing medium. Although rooted *Persea* microcuttings readily acclimatized to greenhouse conditions (88% survival), post-transplant growth was slow.**

In Florida, native woody plants have emerged as desirable alternatives to exotics for use in the urban landscape and are being utilized in revegetation of disturbed sites. The market for these "new plants" is reflected by the presence of more than 50 Florida native plant nurseries. Limited information is available on the traditional propagation techniques required for successful production of many native woody species (4, 5, 6). Although *in vitro* propagation (microprogation) systems have been developed for the efficient and large-scale clonal propagation of many fruit and forest tree species (1, 2, 6, 7, 9, 10, 14), procedures are not available for the micropropagation of most native trees and shrubs possessing potential commercial value (3, 8, 10). The application of *in vitro* culture techniques to woody native plant production could be useful for production of difficult to propagate or rare and endangered species and selection of elite clonal lines exhibiting superior growth, form, enhanced disease resistance, stress tolerance, and other commercially valuable characteristics (10). The objectives of this study were to determine the feasibility of developing protocols for the *in vitro* propagation of *Styrax americana* (American snowbell) and *Persea palustris* (Swamp bay persea) and to evaluate the *ex vitro* post-transplant performance of both species.

Materials and Methods

Culture Protocol for Styrax americana. Shoot-tip cuttings of *Styrax americana* were collected from flowering plants growing on undisturbed sites at W. R. Grace and Company, Four Corners Mine, Hillsborough County, Florida. Single node stem explants were surface sterilized by successive immersion in 50% (v/v) ethanol for 30 seconds and 1.0% (v/v) aqueous sodium hypochlorite for 15 min, followed by three 5-min rinses in sterile deionized water. Surface sterilized nodal explants were transferred individually into 150 X 25 mm culture tubes containing 15 ml sterile establishment (Stage I) medium consisting of Murashige and Skoog [MS] inorganic salts (13), 100 mg/liter myo-inositol, 0.4 mg/liter thiamine-HCL and 3) g/liter sucrose supplemented with 0.5 mg/liter N^6-benzyladenine (BA). The medium was solidified with 8 g/liter TC Agar (Hazelton Research Product, Inc., Lenexa, KS). All media were adjusted to pH 5.7 with 0.1 N KOH before autoclaving at 1.6 Kg cm^{-2} for 20 min at 121°C. Unless stated otherwise, all cultures were maintained at 15±2°C under a 16-hr photoperiod provided by cool-white fluorescent lights at 90 μmol $m^{-2}s^{-1}$ as measured at culture level.

Nodal explants (each 5 mm long bearing two axillary buds with attached subtending leaves) were excised from Stage I cultures and transferred into 150 x 25 mm culture tubes containing either 15 ml sterile MS or Woody Plant Medium [WPM] inorganic salts and vitamins (11) supplemented with 30 g/liter sucrose and solidified with 8 g/liter TC agar. Media were supplemented with BA at five concentrations (0, 0.1, 0.5, 1.0, and 5.0 mg/liter) alone or with 0.1 mg/liter 1-naphthaleneacetic acid [NAA]. Nodal explants were oriented horizontally onto the medium. A

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