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Micropropagation and regeneration of *Ulmus parvifolia* 'Pathfinder', the Chinese elm tree

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Abstract

Micropropagation and regeneration systems were established for the U.S. National Arboretum accession, *Ulmus parvifolia* 'Pathfinder'. Experiments were done using two lines of *U. parvifolia*, P-6 and P-10, each derived from a different, open-pollinated seed. Because micropropagation of *U. parvifolia* required the addition of a cytokinin to the Driver and Kuniyuki Walnut (DKW) medium, BA, mT, and TDZ were compared. Stem cuttings cultured on DKW containing 2.4 mg L^{-1}mT for line P-10 or 4.8 mg L^{-1} mT for both lines P-6 and P-10 produced a significantly higher number of axillary shoots than cuttings on DKW lacking phytohormones. Stem cuttings on TDZ (0.25, 0.50, or 1.0 mg L^{-1}) produced a significantly lower number of axillary shoots. Axillary shoots of line P-6 formed roots at a low frequency (6 %) as compared to those of P-10 shoots (58 %) when cultured on DKW with either NAA (1.0 mg L^{-1}) or IBA (1.0 mg L^{-1}). Most of the shoots, 96 % of P-6 and 99 % of P-10, survived in soil in the greenhouse. Shoots were regenerated from leaves taken from *in vitro*-grown plants, and most of the regenerated shoots came from leaves of line P-10 and very few from line P-6. P-10 leaves cultured on DKW containing 7.2 mg L^{-1}mT regenerated significantly more plants than leaves cultured on DKW lacking hormones. The frequency of regenerated shoots from leaves cultured on DKW with mT(2.4, 4.8, or 7.2 mg L^{-1}) that formed roots *in vitro* was 47, and 98 % of the shoots survived in soil in the greenhouse. This study indicates the benefit of using mT for micro propagation and regeneration from leaves of *U. parvifolia*.

Key words: Ornamental trees, aromatic cytokinins, tissue culture

Introduction

The American elm, *Ulmus americana*, was the predominant tree lining the streets in the early 20th century until Dutch elm disease killed most of them. Currently there are four major groups of elms of horticultural importance in the U.S.: *U. parvifolia* (Chinese elm), Asian elms other *than U. parvifolia*, European and European-Asian hybrids, and *U. americana* (American elm) (Warren and Schmidt, 2000). *Ulmus* is once again becoming an important nursery tree resulting from the introduction of select *U. americana* trees that were found to be resistant to Dutch elm disease and the development of elm hybrids created by hybridizing *U. americana* with Asian species resistant to Dutch elm disease.The role that the Chinese elm has played during the past 85 years in both Europe and North America to develop an elm with resistance to Dutch elm disease are described in a review (Mittempergher and Santini, 2004). Only four elm species, one of them being *U. parvifolia*, out of 13 species listed are resistant to Dutch elm disease. *U. parvifolia* has high resistance to the elm leaf beetle as well, and the shape of the Chinese elm tree resembles a vase similar to that of the American elm (Mittempergher and Santini, 2004). The J. Frank Schmidt & Son Company, a leader in the U.S. nursery industry, now has 19 cultivars of elm in production and is evaluating over 12 more (Warren and J. Frank Schmidt & Son Co., 2000). One of the main problems that has slowed the progress of these introductions is the difficulty in propagating elms.

Ulmus parvifolia, is native to China, Japan, North Korea, and Vietnam. It is sometimes called the "lacebark elm" because of its bark which appears as a beautiful mosaic of tan, orange, green, and gray colors. Currently there is interest in *U. parvifolia* as a tree for the urban environment because of its tolerance to heat, cold, Dutch elm disease, and air pollution, and its relatively short stature makes it an excellent choice for planting under power lines (Gilman and Watson, 1994). These traits also permit Chinese elm to be grown in parking lots and planters along streets or on a patio (Moore and King, 2006).

A Chinese elm given to the U.S. by the King of Prussia has been growing in the Central Park of New York City for many years indicating the ability of this particular Chinese elm cultivar, *U. parvifolia* 'A/Ross Central Park', to withstand cold temperatures. A propagation and regeneration procedure using newly-flushed, nodal segments was devised for this particular tree because of the interest in maintaining it for the future (Thakur and Karnosky, 2007). More than 1, 000 offspring of the original tree, now patented as Central Park Splendor®, have been planted in New York City parks. Axillary shoot development was optimal when nodal segments of *U. parvifolia* 'A/Ross Central Park' were cultured in Woody Plant Medium (WPM) (Lloyd and McCown, 1980) supplemented with 6-benzylaminopurine (BA).

Topolins are naturally occurring hydoxylated analogues of BA that were first isolated from poplar (Horgan *et al.*, 1975). In several plant micropropagation systems, the replacement of BA with a topolin resulted in higher shoot multiplication rates and higher rates of root formation from the shoots (Aremu *et al.*, 2012). Various topolins have been applied to several woody plant species with varying results.

The purpose of this project was to develop an efficient system of both micropropagation and regeneration for *U. parvifolia* by comparing the effect of three cytokinins on axillary shoot development, regeneration of shoots, and their effect on subsequent root formation as well as characterizing the role of genotype (Lines P-6 and P-10). Micropropagated shoots can be used to multiply *U. parvifolia* accessions at the U.S. National Arboretum, and they provide a source of explants for future experiments in genetic engineering.

Materials and methods

Stock Plants: Seeds were collected in October 2012 from trees of *Ulmus parvifolia* 'Pathfinder' trees grown at the USDA's South Farm in Beltsville, MD. 'Pathfinder' was officially registered at the Arnold Arboretum (Spongberg, 1991). In March, 2013, the papery coating was removed from the seeds prior to their sterilization in 15 % Clorox with 15 drops of Tween 20 $L^{-1}$for 15 min followed by three 5 min rinses in sterile water. There was 100 % germination, and none of the seeds were contaminated. Two plants (P-6 and P-10) that originated from two different seeds out of 25 sterilized seeds were chosen to compare a vigorously growing line, P-10, with a not so vigorous grower, P-6. Plantlets were maintained on DKW medium (Driver and Kuniyuki, 1984) (Phytotechnology, Overland Park, Kansas, USA) supplemented with 3 % sucrose, 1.0 mg L^{-1} thiamine, 0.5 mg L^{-1} pyridoxine, 0.5 mg L^{-1} nicotinic acid, 100 mg L^{-1} m-inositol, 0.1 mg L^{-1} BA (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), adjusted to pH 5.8 and solidified with 0.2 % Phytagel (Sigma-Aldrich). Plants were transferred each month to fresh medium and grown at 25 °C under a 12-h light photoperiod provided by cool white fluorescent light bulbs (75 $\mu Em^{-2}s^{-1}$).

Micropropagation: Cuttings from the stock plants were cultured on DKW medium supplemented with either BA (0.1, 0.5, 1.0 mg^{-1}), thidiazuron (TDZ) (0.25, 0.5, 1.0 mg L^{-1}), or meta-topolin (mT) (2.4, 4.8, 7.2 mg L^{-1}), and the pH was adjusted to 5.8. BA, mT (Phytotechnology), and TDZ (Phytotechnology) were added to DKW medium prior to autoclaving at 121°C for 18 min at 20 psi. DKW medium lacking a cytokinin was the control. Each Magenta GA-7 vessel (77 x 77 x 97 mm vessel containing 60 mL medium)contained a single shoot cutting that had three nodes and lacked an apical meristem. Shoot cuttings were subcultured each month to fresh medium. Cultures were grown under the same photoperiod and light conditions as the stock plants.

After approximately one year *in vitro*, the stock plants of 10 open-pollinated elms were no longer vigorously proliferating. Ten Magenta vessels of cuttings containing DKW with 0.1 mg L^{-1} BA (one cutting/Magenta vessel and one vessel/line) were placed in the dark at 4°C and the same lines and number of Magenta vessels remained in the light conditions as used for their maintenance. After 40 days at 4 °C, the cuttings were removed from the cold and placed at 25 °C following a transfer to fresh medium.

Axillary shoots were excised and cultured nine weeks in DKW medium lacking cytokinins but containing either 1.0 mg L^{-1}1-naphthalene acetic acid (NAA) or 1.0 mg L^{-1} indole butyric acid (IBA) to stimulate rooting. Shoots were acclimated *ex vitro* in trays containing super coarse perlite, and shoots that had not formed roots were first dipped in Hormodin 1 (0.1 % IBA) (OHP, Inc., Mainland, PA, USA). After nine weeks on a mist bench that cycled 7 min for 30 s from 6:00 AM to 8:00 PM, the shoots were transplanted into plastic pots (3 shoots/15 cm diameter) containing BX Mycorrhizae Pro-Mix (Griffin Greenhouse Supplies, Inc., USA), and survival was recorded 12 weeks later. The greenhouse was maintained at 24-26 °C during the day and 21-23 °C during the night.

Regeneration from leaves: Leaves taken from elms cultured *in vitro* were cultured on DKW medium supplemented with either BA (0.1, 0.5, 1.0 mg L^{-1}), TDZ (0.25, 0.5, 1.0 mg $L^{-1,}$) or mT (2.4, 4.8, 7.2 mg L^{-1}). Each Petri plate contained one leaf placed abaxial side down and scored perpendicular to the midvein using a scalpel. After four weeks, leaves were subcultured to the same cytokinin-containing medium. After an additional six weeks, regenerated shoots were cultured on DKW with either 1.0 mg L^{-1} NAA or 1.0 mg L^{-1} IBA to induce roots and then plantlets were established in the greenhouse in the same way as the micropropagated shoots.

Statistical analysis for micropropagation experiments: The number of shoot cuttings used for experiments was based on availability as plantlets had been put into culture in March, 2013. In February 2014, four shoot cuttings and in August, 2014, 12 shoot cuttings, each in its own Magenta vessel, were cultured for each cytokinin concentration. The number of axillary shoots from each treatment within each line (P-6 or P-10) were compared using a One Way Anova followed by the Tukey test as selected by Systat (www.systat.com) to determine treatments that resulted in significantly more or less axillary shoots than the control at the 0.05 significance level.

Statistical analysis for regeneration experiments: For the regeneration from leaves experiments, nine leaves were cultured in February, 2014 nine leaves and 12 in August, 2014 on DKW medium containing each cytokinin concentration based upon availability of leaves. Each leaf was cultured in a separate Petriplate. The number of shoots that regenerated from leaves following each treatment within each line (P-6 or P-10) were compared to the control using a One Way Anova followed by the Tukey test as recommended by Systat to determine treatments that were significantly better than the control at the 0.05 significance level.

Results and discussion

Micropropagation: DKW basal salts was selected for the micropropagation of *U. parvifolia* in this study based upon its use for micropropagation of other *Ulmus* species. Shoot tips of *Ulmus procera* (English elm) were successfully cultured on DKW but did not grow on WPM or MS media (Fenning *et al.*, 1993). Micropropagation rates of *U. americana* shoots (American elm) were higher on DKW, followed by WPM and lowest on Murashige and Skoog's (MS) (Murashige and Skoog, 1962) medium (Shukla *et al.*, 2012). DKW contains a higher calcium and magnesium content than WPM or MS media. Shoot development from nodal segments of *U. parvifolia* 'A/Ross Central Park' was reported to be better on WP M than MS medium, but DKW was not tested (Thakur and Karnosky, 2007).

Axillary shoots initially developed from stem cuttings of *U. parvifolia* 'Pathfinder' cultured on DKW medium without

phytohormones, but phytohormones were required for continous micropropagation beyond one year. This study compared the phytohormones BA, mT, and TDZ for micropropagation of *U. parvifolia*. The number of axillary shoots was significantly enhanced when stem cuttings of both lines, P-6 and P-10, were cultured on DKW containing 4.8 mg L^{-1} mT and P-10 stem cuttings on 2.4 mg L^{-1} mT. Axillary shoots appeared phenotypically normal (Fig. 2A, B, C). The highest percentage of axillary shoots came from nodal explants of the *U. parvifolia* cultivar 'A/Ross Central Park'when explants were cultured on WPM containing 0.5 mg L^{-1} BA instead of zeatin, kinetin, 4-CPPU, or TDZ, although mT was not tested (Thakur and Karnosky, 2007).Our study included mT because it has been beneficial for inhibiting senescence during micropropagation and for stimulating root formation in other woody plant species. The micropropagation rate was highest for *Sorbus torminalis* using BA, but shoots grown on a topolin, 6-(3-methoxybenzylamino) purine-9-β-D-ribofuranoside (MemTR) showed a higherrate of root formation than shoots from medium containing BA (Malá *et al.*, 2009). The topolins meta-fluorotopolin (FmT) and 6-(3-fluorobenzylamino) purine-9-riboside (FmTR) resulted in the highest multiplication rate of rose shoots *in vitro*, and MemTR was beneficial because it inhibited the senescence that occurs rapidly when rose shoots were cultured in BA (Bogaert *et al.*, 2006).

Our stem cuttings of *U. parvifolia* 'Pathfinder' cultured on DKW with TDZ produced large amounts of callus and a few phenotypically abnormal shoots with thick stems and leaves (Fig. 2D). Thakur and Karnosky (2007) cultured nodal segments of *U. parvifolia* 'A/Ross Central Park' on WPM containing 2 mg L^{-1} 4-CPPU and 0.5 mg L^{-1} TDZ to induce meristematic nodules from the callus growing from the base of the explant, but meristematic nodules were never observed on the callus of *U. parvifolia* 'Pathfinder'. Nodules of 'A/Ross Central Park' transferred to WPM containing 0.25 mg L^{-1} zeatin resulted in elongation of the shoots. At times yellow callus that appeared to be non-embryogenic developed along the stems of *U. parvifolia* 'Pathfinder' (Fig. 2E). This callus was attached loosely to the stem and would sometimes fall off. Stems with callus were not propagated further.

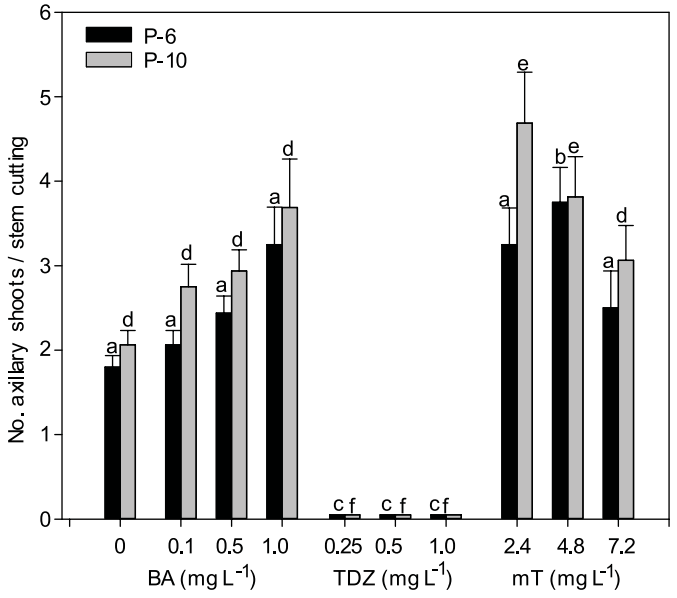

Fig. 1. Axillary shoots of *U. parvifolia* that grew from stem cuttings of lines P-6 and P-10 were cultured on DKW containing either no hormones, BA, TDZ, or mT at the concentrations indicated. Thirteen stem cuttings from two replicates were cultured for each hormone level. Standard error bars are shown (n=16). The number of axillary shoots for each treatment were compared within each line (P-6 or P-10) using a One Way Anova followed by a Tukey test. Means with different letters (a, b, c for P-6 and d, e, f for P-10) are significantly different at $P \leq 0.05$.

Long term micropropagation: A benefit of growing micropropagated plants in mT is that generally the shoots will form roots more readily than shoots grown in BA presumably because mT is degraded more rapidly than BA (Werbrouck *et al.*, 1996). In this study the mean frequency of rooting P-6 shoots *in vitro* that had been grown on DKW (no hormones) was 7 %, on BA was 2.5 %, and on mT was 8.5 % (Table 1). P-10 shoots had higher frequencies of rooting *in vitro* than P-6 shoots, and P-10 shoots grown on DKW, BA, and mT showed rooting frequencies of 62, 66, and 52 %, respectively. Although P-6 axillary shoots on mT formed roots at a higher frequency (8.5 %) than shoots on BA (2.5 %), this was not the case for P-10 shoots indicating that axillary shoots of *U. parvifolia* grown on mT did not result in shoots that formed roots more frequently than BA. Both 1.0 mg L^{-1} NAA or 1.0 mg L^{-1} IBA were used to induce root formation on the axillary shoots excised from the shoot cuttings, and there was no difference in the frequency of rooting (data not shown). This result was in agreement with the result by Conde *et al.* (2008)

Table 1. Effect of BA, mT, and TDZ on the ability of axillary shoots of *U. parvifolia* to survive, form roots, and acclimatize to soil in the greenhouse

Hormone (mg L^{-1})	Number of shoots			
	After excision from explant	With roots (% total)	Planted in soil	Survived in soil (% total planted)
Line P-6				
0 Control	32	20 (62 %)	31	31 (100 %)
0.1 BA	43	30 (70 %)	40	40 (100 %)
0.5 BA	43	29 (67 %)	42	42 (100 %)
1.0 BA	56	34(61 %)	52	52 (100 %)
2.4 mT	72	39 (54 %)	60	59 (98 %)
4.8 mT	61	34 (56 %)	51	51(100 %)
7.2 mT	43	18(42 %)	41	40 (98 %)
0.25 TDZ	2	0	0	0
0.50 TDZ	0	0	0	0
1.0 TDZ	0	0	0	0
Line P-10				
0 Control	29	2 (7 %)	8	8 (100 %)
0.1 BA	32	2 (6 %)	11	11 (100 %)
0.5 BA	38	0 (0 %)	7	6 (86 %)
1.0 BA	50	1 (2 %)	15	14 (93 %)
2.4 mT	42	4(10 %)	10	10 (100 %)
4.8 mT	49	3 (6 %)	12	12 (100 %)
7.2 mT	27	3 (11 %)	11	10 (91 %)
0.25 TDZ	0			
0.50 TDZ	0			
1.0 TDZ	0			

Axillary shoots that developed from 16 stem cuttings/cytokinin concentration were cultured nine weeks on DKW containing either 1.0 mg L^{-1} NAA or 1.0 mg L^{-1} IBA, respectively, to induce root formation. Plants lacking roots were dipped in Hormodin 1, and plants with roots were then transferred to a soilless mix in the greenhouse. The number of shoots that survived was determined 12 weeks later.

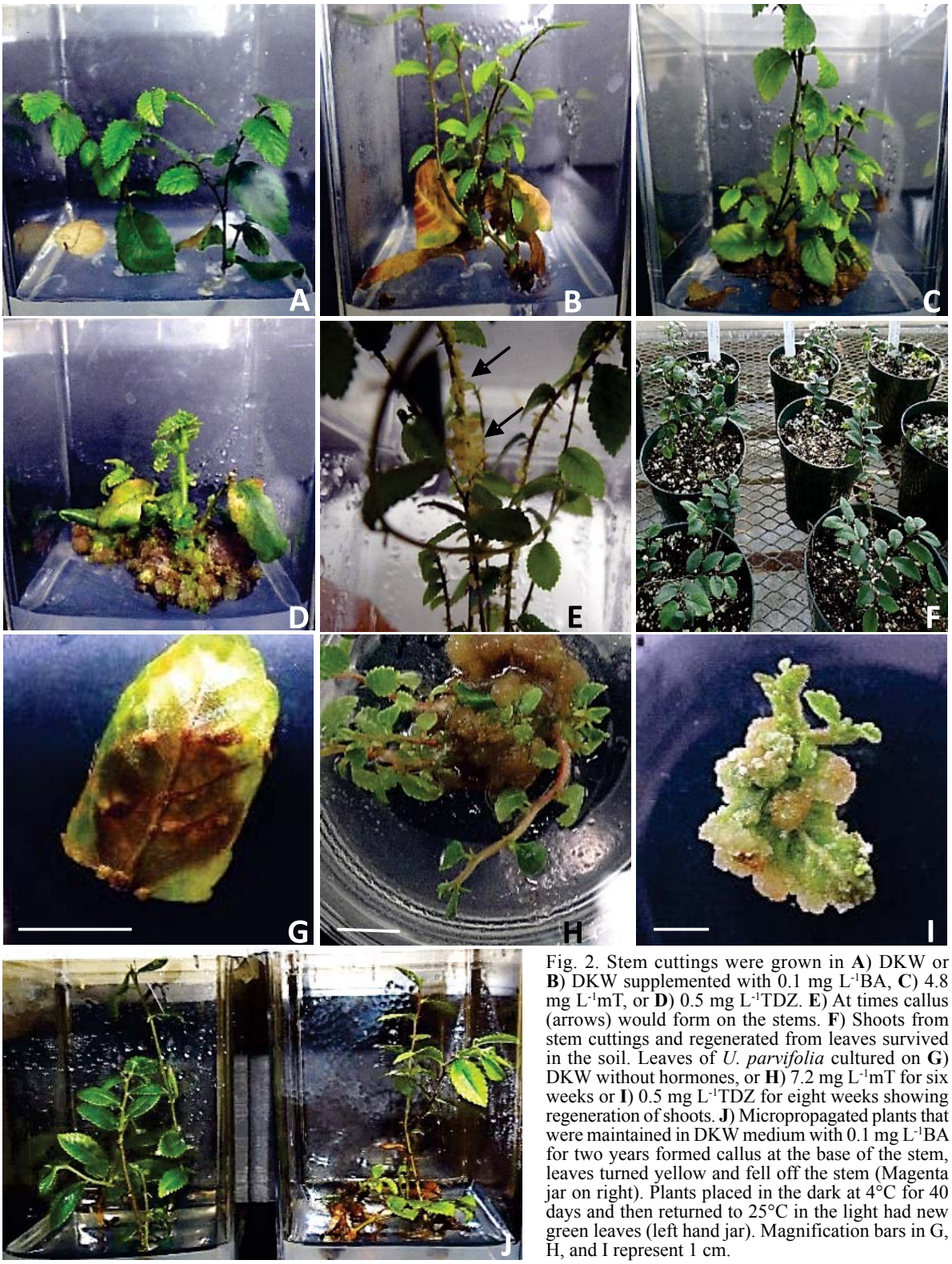

Fig. 2. Stem cuttings were grown in **A**) DKW or **B**) DKW supplemented with 0.1 mg L^{-1}BA, **C**) 4.8 mg L^{-1}mT, or **D**) 0.5 mg L^{-1}TDZ. **E**) At times callus (arrows) would form on the stems. **F**) Shoots from stem cuttings and regenerated from leaves survived in the soil. Leaves of *U. parvifolia* cultured on **G**) DKW without hormones, or **H**) 7.2 mg L^{-1}mT for six weeks or **I**) 0.5 mg L^{-1}TDZ for eight weeks showing regeneration of shoots. **J**) Micropropagated plants that were maintained in DKW medium with 0.1 mg L^{-1}BA for two years formed callus at the base of the stem, leaves turned yellow and fell off the stem (Magenta jar on right). Plants placed in the dark at 4°C for 40 days and then returned to 25°C in the light had new green leaves (left hand jar). Magnification bars in G, H, and I represent 1 cm.

who found similar frequencies of rooting micropropagated shoots of *U. minor* using NAA or IBA.

Survival of the axillary shoots of *U. parvifolia* 'Pathfinder' in the soil was high for both P-6 and P-10 (96 and 99 %, respectively), and the plants appeared phenotypically normal (Fig. 2F). Acclimatization from *in vitro* to growth in soil occurred at a high frequency for *U. parvifolia* 'A/Ross Central Park', *U. minor*, and *U. americana* indicating that *Ulmus* species readily adapt from an *in vitro* to *ex vitro* environment (Thakur and Karnosky, 2007; Conde *et al.*, 2008; Shukla *et al.*, 2012).

Long-term propagation of woody plants *in vitro* is generally a problem which is thought to be partially due to their natural cycle of dormancy. The micropropagated *U. parvifolia* shoots that had been maintained *in vitro* on DKW with 0.1 mg L^{-1} BA for two years had large (1 cm diameter) balls of callus that formed at the base of the stem rather than roots, and their leaves turned yellow and began to drop. Plants placed in the dark at 4 °C for 40 days lost all of their leaves by 40 days. Following the 4 °C cold

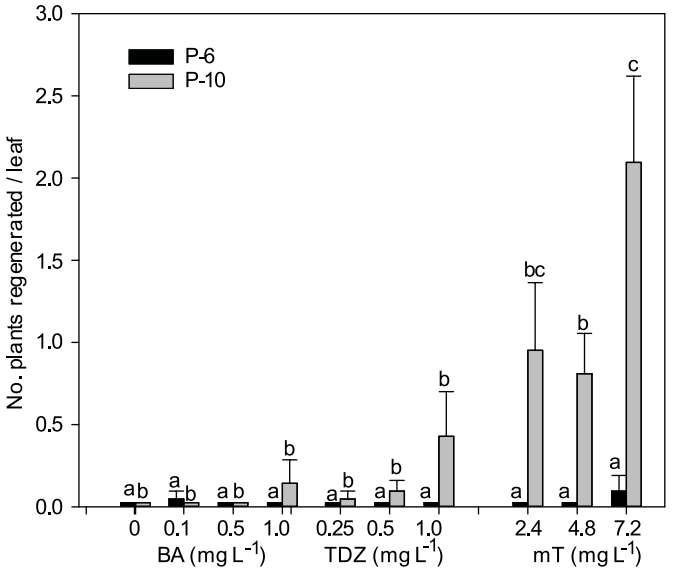

Fig. 3. Shoots of *U. parvifolia* were regenerated after culturing leaves on DKW containing either no hormones, BA, TDZ, or mT at the concentrations indicated. Twenty one leaves from two replicates were cultured for each hormone level. Standard error bars are shown (n=21). The number of regenerated shoots was compared for each treatment within each line (P-6 or P-10) using a One Way Anova followed by Tukey test. Means with different letters (a for P-6 and b, c for P-10) are significantly different at $P \leq 0.05$.

treatment, stem cuttings were transferred to fresh DKW medium containing 0.1 mg L^{-1} BA and grown in a 12 h-light photoperiod. The buds broke dormancy and formed new leaves (Fig. 2J). Cold treatment has routinely been used in the micropropagation of other woody plant species (Gayle Suttle, Microplant Nurseries, Inc., conference talk). An antiauxin, p-chlorophenoxyisobutyric acid, has been found to prolong proliferation of *U. americana* shoots *in vitro*, but the cold treatment is preferable because it avoids potential effects of a chemical on the phenotype of the plant (Shukla *et al.*, 2012).

Regeneration: In a preliminary experiment to determine which organ of the *U. parvifolia* seedlings might be capable of regeneration in response to select hormone concentrations, it was found that seedling roots, hypocotyls, and cotyledons produced callus when cultured on DKW supplemented with 2, 4-D (0.25, 0.50, or 1.0 mg L^{-1}). Seedling roots, hypocotyls, and cotyledons produced shoots when cultured on BA (0.25, 0.50, or 1.0 mg L^{-1}) and TDZ (0.25, 0.50, or 1.0 mg L^{-1}) indicating the responsiveness of several tissues to regenerate shoots. Leaves from micropropagated plants growing *in vitro* were chosen for further experiments because it was possible to obtain many leaves derived from a single seed rather than using open-pollinated seeds as a source of seedling roots, hypocotyls, and cotyledons.

Leaves cultured on DKW lacking phytohormones formed a small amount of callus at the wounded regions, then the leaves turned brown and died (Fig. 2G). A few plants regenerated from leaves cultured on BA (0.1, 0.5, or 1.0 mg L^{-1}) (Fig. 3). The highest number of regenerated plants were from leaves of line P-10 cultured on DKW containing 7.2 mg L^{-1} mT, and this was statistically significant (Fig. 3). Plantlets regenerated on mT appeared phenotypically normal (Fig. 2H) whereas those regenerated on TDZ had abnormally thick stems and leaves (Fig. 2I). Most of the regenerated plants came from leaves of P-10 and only a few plants from P-6 (Fig. 3). The P-10 plantlets showed a

Table 2. Effect of BA, mT, and TDZ on shoots of *U. parvifolia* regenerated from leaves of line P-10 and their ability to form roots and survive transplanting to soil in the greenhouse

Hormone (mg L^{-1})	After excision from explant with roots (% total)	Number of shoots		
		Planted in soil Survived in soil (% total planted)	After excision from explant With roots (% total)	Planted in soil Survived in soil (% total planted)
Line P-10				
0 (Control)	0	0	0	0
0.1 BA	0	0	0	0
0.5 BA	0	0	0	0
1.0 BA	3	2 (66 %)	2	2 (100 %)
2.4 mT	13	5 (38 %)	6	6 (100 %)
4.8 mT	14	6 (43 %)	13	13 (100 %)
7.2 mT	28	15 (54 %)	24	23 (96 %)
0.25 TDZ	2	1 (50 %)	2	2 (100 %)
0.50 TDZ	2	2 (100 %)	1	1 (100 %)
1.0 TDZ	6	6 (100 %)	6	6 (100 %)

47 % mean frequency of root formation *in vitro*, and survival of the regenerated plants was high (98 %) when grown in soil in the greenhouse (Table 2). These results indicate the superiority of mT for regenerating plants from leaves of *U. parvifolia*. Regeneration has been achieved from leaves of several *Ulmus* sp. Shoots were regenerated from leaves of the American elm cultured with either TDZ or BA, and several shoots formed roots on medium with indole butyric acid (IBA) (Bolyard, 1991; George and Tripepi, 1994). Leaves of American elm were also used for *Agrobacterium*-mediated transformation, and regenerated plants were transformed (Newhouse *et al.*, 2007). Leaves of the Dutch elm hybrid 'Commelin' formed shoots when cultured in Murashige and Skoog (MS) medium with TDZ combined with indoleacetic acid (IAA) (Ben Jouria *et al.*, 2000). Shoots were regenerated from leaves of Chinese elm cultured on TDZ and callus induced on MS medium containing TDZ and NAA, but no attempt was made to root the shoots (Bolyard *et al.*, 1991; Aziz *et al.*, 2003).

There were obvious differences in both micropropagation and regeneration of shoots between lines P-6 and P-10 indicating differences in open-pollinated seeds of the *Ulmus parvifolia* 'Pathfinder'. Axillary shoot development was significantly stimulated when stem cuttings were cultured on mT (2.4 and 4.8 mg L^{-1}) added to the DKW medium. The optimum 4.7 shoots/stem occurred when P-10 stems were cultured on 2.4 mg L^{-1} mT as compared to the P-10 control stems on DKW without a hormone that produced 2.12 shoots/stem. TDZ significantly inhibited axillary shoot formation. Root formation was 6 % and 58 % for P-6 and P-10 micropropagated shoots, respectively, and 98 % survived in soil in the greenhouse. Shoot regeneration was higher from leaves of line P-10 than P-6. Maximum regeneration occurred when line P-10 leaves were cultured on DKW with 7.2 mg L^{-1} mT, and 47 % of these regenerated shoots formed roots *in vitro* with 98 % survival in the greenhouse. This study indicates the benefit of using mT for both micropropagation and regeneration of Chinese elm.

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Mention of a trademark, proprietary product or vendor does not imply its approval to the exclusion of other products or vendors that may also be suitable. Sue Bentz (U.S. National Arboretum) is thanked for collecting the elm seeds used in this study and for critical review of the manuscript.

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