Effects of Soil Media on the Growth and Survival of Micropropagated Black Cherry

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Micropropagated plantlets of black cherry (Prunus serotina Ehrh.) were transplanted in the greenhouse from an agar medium to plastic tubes containing one *of* three different soil mixtures *of* peat, perlite, vermiculite, and sand at ratios *of* 1:1:0:1, 2:1:2:0, or 3:0:2:1. Each mixture had 10 tubes, each with one plantlet, and each of three clones was represented in each soil mixture. There was no significant difference among soil mixtures for plantlet root or shoot growth, although the 1:1:0:1 mixture was preferable because it provided the greatest number *of* plantable plantlets. Tree Planters' Notes 39(3) :31-34; 1988.

Although tissue culture is commonly employed for eastern cottonwood (*Populus deltoides* Barts. ex Marsh.), to date no suitable technique exists for black cherry *(Prunus serotina* Ehrh.). Such techniques are needed, because black cherry offers landowners an alternative to planting black walnut *Juglans nigra* L.) for high-value lumber. Being able to tissue-culture black cherry would allow not only mass production of genetically improved planting stock but also production of individual plants for seed orchards.

This experiment tested the effect of soil media on the survival and growth of black cherry plantlets, originally micropropagated on agar. The objective was to improve the initial survival rate of 60% attained by Tricoli *et al.* (5), who used a soil mixture of peat, perlite, and sand in a 1:1:1 (vol) ratio.

Methods

Thirty plantlets, micropropagated on agar according to techniques developed by Tricoli *et al.* (5) and represented by three clones (B20, M018, and B9), were first obtained from Dr. Charles Maynard, forest geneticist at State University of New York at Syracuse.

Three soil mixtures of four ingredients with ten plantlets per mixture were tested in a splitplot design. The first mixture was that used for black cherry tissue culture by Tricoli et al. (5): peat, perlite, vermiculite, and sand in a 1:1:0:1 volume ratio. The second soil mixture was that used for loblolly pine (*Pinus taeda* L.) tissue culture by Amerson *et al*. (1): peat, perlite, vermiculite, and sand in a 2:1:2:0 volume ratio. The third mixture, selected to explore the effects of additional peat, was peat, perlite, vermiculite, and sand in a 3:0:2:1 volume ratio. Because the amount of peat used in the soil mixture affected soil reaction, lime was added to standardize soil reaction to pH 5.5 (about 1.5 g lime/liter of peat), according to techniques developed by Amerson *et al*. (1).

By this procedure, soil reaction was similar for all treatments.

A 3-mm screen was used to sift the soil mixtures, to concentrate the vermiculite and perlite in the top 2.5 cm of soil, thus providing a more favorable environment for developing roots. The soil mixtures were next transferred to plastic tubes, measuring 3.8 cm by 20.3 cm, and perforated on the bottom for drainage. One plantlet was then transplanted into each tube with each clone represented in each soil mixture.

Photoperiod, moisture regime, fertilizer type, and application rate were similar for all mixtures using the following procedures developed by Amerson et al. (1). Plantlets received 16 hours of light each day. Two irrigation and fertilization regimes were used for all treatments during the experiment, depending upon plantlet development. Immediately after transplanting, the plantlets were placed under a Mist-a-Matic® irrigation system.

The soil was fertilized 3 to 5 times per week until saturation using 1.2 ml of 15-30-15 fertilizer dissolved in 4.2 liters of water. Fifty cubic centimeters of captan dissolved in 4.2 liters of water was applied once a week in a like fashion. After shoot elonga-

tion began, the plantlets were removed from the irrigation system and irrigated with tap-water acidified with HCl to pH 5.5 and with a solution consisting of 39.5 ml of 20-19-8 fertilizer dissolved in 134 liters of tap-water, acidified with 35 ml of 1*N* HCl. These solutions were applied at 2-day intervals to the point of soil saturation.

Root and shoot lengths were measured to the nearest 0.5 mm when the plantlets were transplanted from the agar to the soil mixtures on May 14, 1985. Shoot lengths were measured from the shoot tip to the soil level. The measurements were repeated on August 2, 1985, after they had grown in the soil mixtures for 11 weeks. The relative value of each soil mixture was evaluated using a mean analysis based on least-square differences. In order to reduce variation, data for dead plantlets were deleted from analyses, emphasizing differences between mixtures.

Table 1—*Average root and shoot length of surviving plantlets by clone and soil mixture*

			May 14, 1985		August 2, 1985	
	Clone no.	No. of plantlets	Root length (mm)	Shoot length (mm)	Root length (mm)	Shoot length (mm)
Peat/perlite/vermiculite/sand (1:1:0:1)						
	B20	4	1.5	14.0	171.0	17.0
	M018	2	32.5	27.5	174.0	27.5
	B9	2	1.0	24.5	135.0	24.5
Peat/perlite/vermiculite/sand (2:1:2:0)						
	B20	3	0.0	19.0	146.7	18.0
	M018	2	17.0	30.0	104.0	32.0
	B9	4	9.2	15.7	154.2	15.7
Peat/perlite/vermiculite/sand (3:0:2:1)						
	B20	2	0.0	10.0	100.0	9.5
	M018	2	20.0	26.0	171.5	19.0
	B9	2	10.5	24.5	209.0	17.0

Results

Prior to experimentation, the plantlets showed considerable variation in root length among the three clones. In addition, many roots were broken during shipment from Syracuse. Clone B20 had the shortest roots and clone M018 the longest roots (table 1). Less variation occurred among clones in shoot length.

At the end of the experiment, root growth was greater than shoot growth. Even plantlets that initially lacked visible roots developed fibrous root systems during the experiment. Root growth of the 1:1:0:1 mixture averaged 148.3 mm, growth of the 2:1:2:0 mixture averaged 126.2 mm, and growth of the 3:0:2:1 mixture averaged 150.0 mm, but these differences were not statistically different.

Shoot growth was very slow, with an overall average increase of 2.3 mm; shoot length of some plantlets actually decreased. Growth of the 1:1:0:1 and 2:1:2:0 mixtures both averaged 1.0 mm; growth of the 3:0:2:1 mixture averaged - 4.7 mm, but none of these differences were statistically significant either. All plantlets developed some reddening of the new leaves between the leaf margin and midrib as early as 1 week after transplanting. Reddening continued throughout the experiment. Red mottling and tissue necrosis of leaf tips and margins developed on older leaves.

Forty-three percent of the plantlets did not develop adequate root and shoot systems by the end of the experiment to allow field planting. Plantlets were judged to be field-plantable when the roots were at least 10 cm long. The variation in root and shoot length between plantlets of the same clone after 11 weeks was dramatic (fig. 1). Although the survival rate in all three treatments equaled or exceeded that obtained by Tricoli et al. (S), the proportion of plantlets suitable for field planting was much lower: 40% for 1:1:0:1, 30% for 2:1 :2:0, and

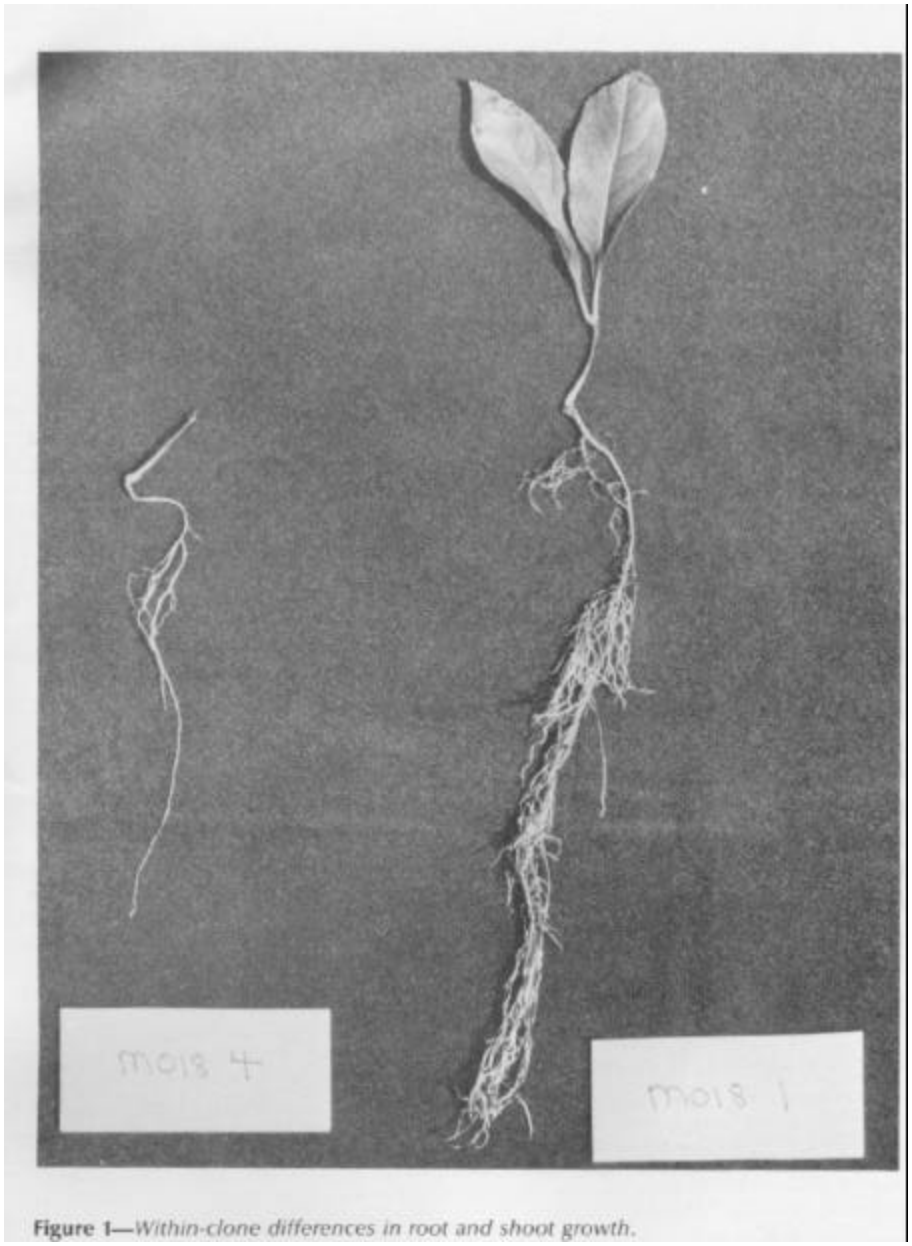

Figure 1—*Within-clone differences in root and shoot growth.*

30% for 3:0:2:1 (table 2). The soil mixture containing equal parts of peat, perlite, and sand (1:1:0:1) gave the largest number of field-plantable plantlets.

Table 2—*Survival rate and plantability of black cherry plantlets grown for 3 weeks in three different soil mixtures after micropropagation*

Peat/perlite/vermiculite/sand (vol)	% Survival	% Plantable
1:1:0:1	80	40
2:1:2:0	90	30
3:0:2:1	60	30

Discussion

The large differences in plantlet condition prior to experimentation may be partly due to the rough treatment during shipment from Syracuse. The plantlets exhibited different amounts of root development and elongation, possibly because some of the plantlets may have not received enough root-inducing hormones during the micropropagation stages conducted at Syracuse. Better methods that promote uniformity in size of shoots and roots need to be developed for the micropropagation stages.

There were no significant differences in root growth among soil mixtures. Nevertheless, the 1:1:0:1 mixture provided the largest number of field-plantable plantlets, and thus it is the preferred mixture. However, we consider these results to be preliminary because of the experimental limitations provided by the initial plantlet condition and

by the soil nutrition discussed below. The decrease in shoot length in some clones may be due to slight variations in the site of measurements. Because the soil level varied slightly within a container due to splashing during irrigation, a more accurate method would be to measure from the shoot tip to the lowest node.

The reddening and necrosis observed on leaves may be due to soil nutrient deficiencies or toxicities caused by soil reaction. At low soil reaction, many nutrients are fixed and not available for plant uptake; in addition, low soil reaction may result in aluminum and manganese toxicities (3). The symptoms observed resemble those associated with phosphorus or possibly nitrogen deficiency (2). The specific nutrient requirements for black cherry and interactions between soil reaction, nutrient absorption, and aluminum and manganese toxicity need to be determined in future research.

Since shoot growth was slow, a technique to stimulate shoot growth is needed. Spraying the bud and foliage with gibberellic acid, which promotes cell elongation and division (4), at time of transplanting, might increase shoot growth.

Conclusions

Two preliminary conclusions may be drawn from this experiment: a) of the three soil mixtures tested, the mixture containing equal parts of peat, perlite, and sand gave the most field-plantable plantlets, and thus is preferable; b) more research on the interactions of shoot elongation, soil reaction, and nutrient availability is needed to successfully grow black cherry tissue culture plantlets in the greenhouse.

Acknowledgments

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