Somatic Variation in Resistance of *Populus* to *Septoria musiva*

M. E. OSTRY and D. D. SKILLING, Research Plant Pathologists, North Central Forest Experiment Station, St. Paul, MN 55108

ABSTRACT

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Tissue culture of hybrid poplars previously susceptible to leaf spot caused by *Septoria musiva* was used to obtain poplars with putative resistance. Stem internode explants were used to obtain proliferating callus cultures on which adventitious bud formation and shoot proliferation were induced. Elongated shoots were excised and rooted in a peat-perlite medium under high humidity and transferred to the greenhouse. Variant plants were selected among the regenerants using a leaf disk bioassay that distinguished resistant plants. The incidence of somaclonal variation differed among genotypes. Tissue culture of poplars is potentially useful in detecting and recovering somaclonal variation in resistance to *Septoria*.

Additional keywords: disease, tree improvement

Tissue culture has been used primarily to clonally propagate plants. However, genetic variation has been observed among regenerated plants. Passage of plant cells through a tissue culture cycle can result in increased spontaneous phenotypic and genetic variation. Somaclonal variation is the term used to describe variation exhibited by plants obtained from aseptic culture (6). The phenomenon was recently reviewed (5). Somatic variation is now considered a general phenomenon and somaclonal variation in disease resistance has been observed in many agronomic crops.

Although the cause of somaclonal variation is not yet completely understood, the phenotypic and genetic variation found indicates that many factors are involved. Somaclonal variation can be a preexisting genetic variation that is expressed in regenerated plants, or it can be induced by the tissue culture process itself (14). Working with tomatoes (*Lycopersicon esculentum* Mill.), Evans and Sharp (2) provided classical genetic proof that tissue culture can be mutagenic. The plant species, genotype, source of explant used for culture initiation, and perhaps most importantly, the duration of the culture cycle may influence the variability observed (9). Meins (10) suggested that tissue culture induces cellular destabilization that can result in diverse heritable changes. In addition to epigenetic variation, tissue culture can cause genetic changes ranging from single base pair changes to chromosome deletions, translocations, and changes in chromosome number (3).

Tissue and cell culture techniques can be valuable tools for developing trees with improved characteristics. Additive and nonadditive traits can be captured, and aseptic culture may provide a new source of genetic diversity. Because of the long generation times of trees and the possibility of introducing desired traits not possible through traditional breeding, somaclonal variation may offer an advantage to forest tree improvement. Somatic variation in a tree species has been documented. One growing season after planting, a wide variation in height, number of branches, and leaf traits was detected in trees from five *Populus* × *euramericana* (Dode) Guinier clones regenerated from callus cultures (7).

This study was conducted to demonstrate the potential application of somaclonal variation for increasing disease resistance in a tree species. *Populus* was chosen because of its worldwide importance as a source of fiber and energy and its amenability to whole plant regeneration through a variety of cell and tissue culture systems. Additionally, biomass yields from hybrid poplar plantations are now limited by the foliar and canker diseases caused by the fungal pathogen *Septoria musiva* Peck. Somaclonal variation has the potential to enhance the productivity of hybrid poplars by increasing their resistance to *Septoria*.

MATERIALS AND METHODS

***Populus* clones.** Stock plants of five hybrid poplar clones with differing resistance to *S. musiva* were propagated in the greenhouse from hardwood cuttings (Table 1). The resistance of these clones to *Septoria* in field trials in Minnesota, Wisconsin, and Iowa has been reported (11,12).

Tissue culture. Callus cultures of the reference clones were initiated from stem internode explants taken from the greenhouse stock plants. Leaves were removed from 5-cm-long shoot tips and the stems were rinsed for 15 sec in 70% ethanol. The stems were then placed in a beaker of 2.5% NaOCl containing 10 drops of Tween 20 per liter. After 10 min, the solution was decanted and the stems received three 5-min rinses in sterile distilled water. Stem internode explants (1 cm long) were excised from the shoot tips and placed horizontally into shell vials containing 10 ml of woody plant medium (WPM) (8) plus 20 g/L of sucrose, 6 g/L of Difco Bacto agar, and 2.3 μM of 2,4-dichlorophenoxyacetic acid (2,4-D). Cultures were maintained in the dark in an incubator at 25 C.

Three to four weeks after culture initiation, the proliferating callus at the cut ends of the explants was excised, subcultured onto fresh WPM containing 0.45 μM of 2,4-D, and returned to the incubator. Proliferating callus cultures were subsequently divided and subcultured onto fresh medium every 3–4 wk. Callus cultures ranged in age from 5 to 13 mo before shoot proliferation was induced.

Table 1. *Populus* reference clones and their resistance to *Septoria musiva* in field trials

Clone	Parentage	Disease resistance[a]
NE 41	*P. maximowiczii* × *P. trichocarpa* 'Androscoggin'	M
NE 314	*P. nigra* var. *charkowiensis* × *P. nigra* var. *caudina*	H
NE 299	*P. nigra* var. *betulifolia* × *P. trichocarpa*	L
NE 319	*P. nigra* var. *charkowiensis* × *P. deltoides*	M
NE 293	*P. nigra* var. *betulifolia* × *P. nigra* 'Volga'	H

[a] H = slight leaf spot, no defoliation; M = moderate leaf spot, premature defoliation in lower and mid crown; L = severe leaf spot, premature defoliation throughout crown.

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Adventitious bud formation and shoot proliferation were induced by transferring callus cultures to WPM containing 1.1 μM of 6-benzylaminopurine (BA) and 0.27 μM of 1-naphthaleneacetic acid (NAA). Cultures were incubated under light (3,000 lx) with a 16-hr photoperiod at 25 C.

Elongated shoots were excised and rooted in a 2:1 peat-perlite medium under 100% humidity in a continuously lighted growth room (20–25 C, 2,000 lx). After acclimation to lower humidity, plants were transferred to the greenhouse (18–30 C, 18-hr photoperiod).

Screening for somaclonal variants. Cultures of *S. musiva* were isolated from surface-sterilized diseased hybrid poplar leaves by placing 5 mm^2 pieces of infected leaves onto a potato-dextrose agar medium and incubating them at 20 C under continuous light (3,000 lx). To induce sporulation, cultures were transferred to an agar medium containing 180 ml/L of V-8 juice and 2 g/L of $CaCO_3$ and incubated under continuous light at 20 C.

Conidia of *S. musiva* were removed from 7- to 10-day-old cultures by flooding them with distilled deionized water and gently agitating the plates. A conidial suspension was adjusted to a concentration of 1×10^6 conidia per ml of water. An in vitro bioassay (13), developed to identify poplars with resistance to *S. musiva*, was used to screen for variant plants. Recently expanded leaves were collected from tissue culture-derived plants and from the parent stock plants (source plants) of each clone tested. All tissue culture-derived plants had been growing in the greenhouse for at least 1 mo. Leaves were rinsed with distilled water, and 18-mm-diameter disks were extracted using a cork borer. Corresponding wells were made in 2% water agar in petri plates, and the leaf disks were placed into them abaxial surface up.

Twelve leaf disks from each plant were inoculated with 0.1 ml of the spore suspension. Two leaf disks received distilled deionized water only and served as uninoculated controls. Leaf disks were incubated in a continuously lighted (2,000 lx) growth room maintained at 20–25 C.

Disease progression was monitored by measuring the necrotic area on each disk every 2 days by using a dot grid (25 dots/1.8 cm^2). Measurements continued until the control leaf disks started to become necrotic.

Data analysis. A regression analysis of the pooled leaf disk measurements for all tissue culture regenerants from a single clone was performed with percent of green leaf area and elapsed time as the dependent and independent variables, respectively. From this, we estimated the time elapsed to 50% necrosis and the rate at which 50% necrosis occurred. Based on these estimates, we compared the *Septoria* resistance of the tissue culture-derived plants to that of the parent plants.

RESULTS

Adventitious shoot proliferation from callus cultures differed by clone. Some *Populus* genotypes were more recalcitrant than others under identical culture conditions. Clone NE 299 yielded the greatest number of rootable shoots.

We found no obvious mutant phenotypes among the regenerated plants from any of the clones except for two plants with mutant leaves that did not root. Phenotypically, all plants that rooted resembled their parent source plants.

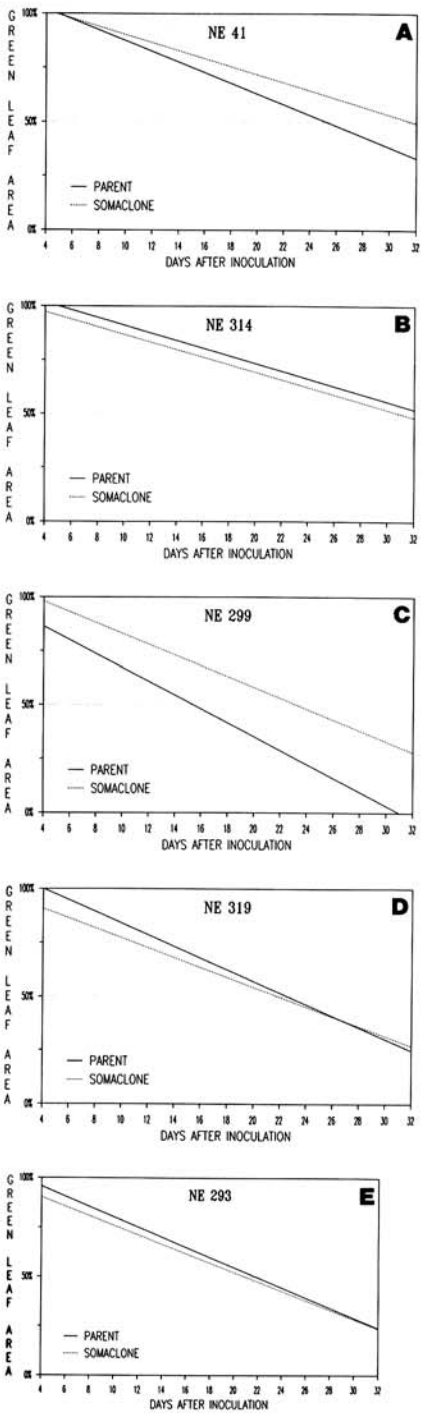

Fig. 1. Estimated green leaf area-time regression lines from leaf disk bioassay for *Populus* source clones NE41, NE314, NE299, NE319, and NE293 (solid lines) and tissue culture regenerants (dashed lines) after inoculation with *Septoria musiva*.

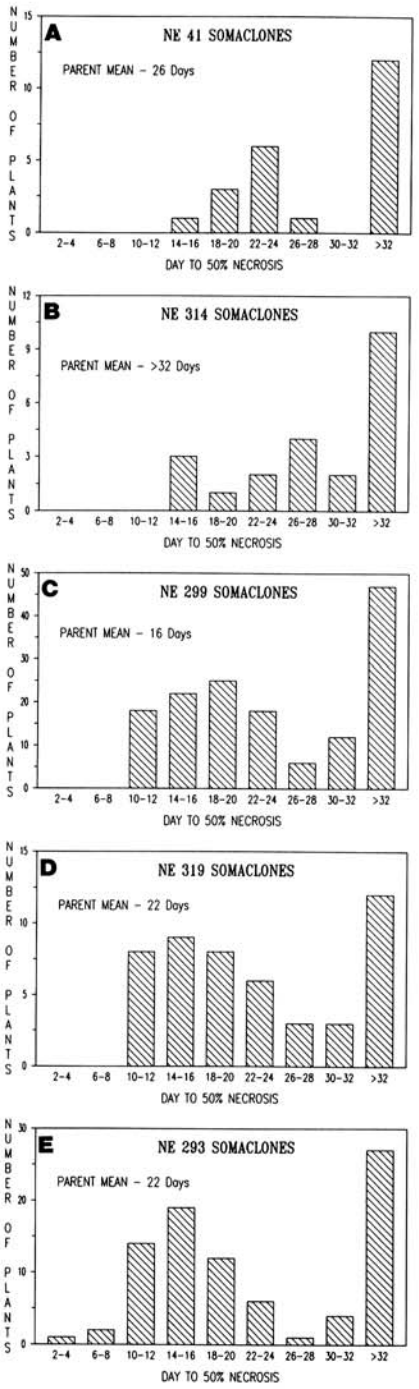

Fig. 2. Range in disease severity exhibited by somaclones in the leaf disk bioassay of *Populus* clones NE41, NE314, NE299, NE319, and NE293. Only those somaclones that clearly were less diseased than their source clones in the leaf disk bioassay were considered somatic variants.

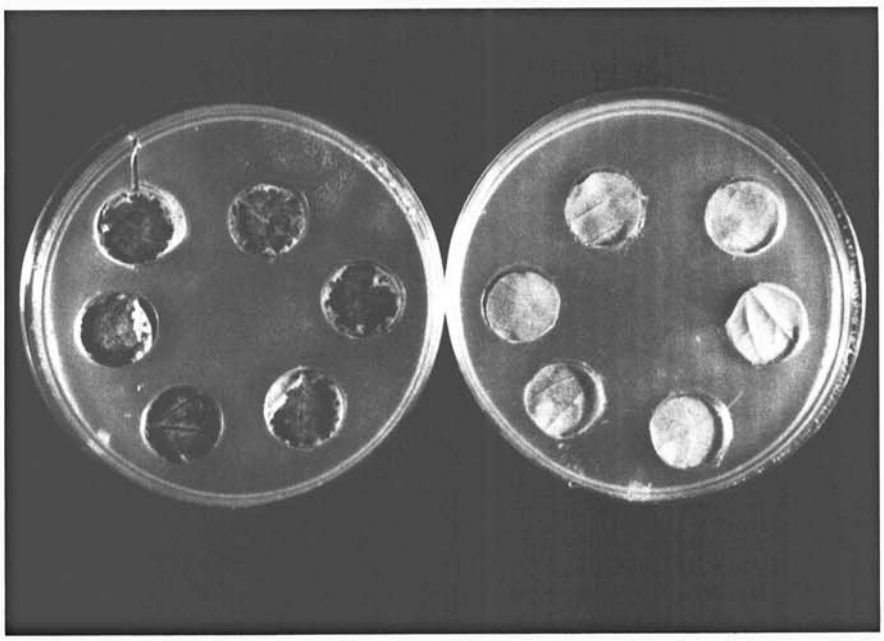

Fig. 3. Disease severity on leaf disks from susceptible *Populus* clone (left) and its resistant tissue culture-derived somaclone (right). Several tissue culture-derived plants were more resistant to *Septoria musiva* than the source plant based on lack of leaf necrosis in the leaf disk bioassay.

Table 2. Somaclones of *Populus* hybrids not reaching 50% necrosis in the leaf disk bioassay after inoculation with *Septoria musiva*

Source clone	Regenerated plants		
	Tested (no.)	Not reaching 50% necrosis[a]	
		Number	Percent
NE 41	23	12	52
NE 314	25	11	44
NE 299	157	48	31
NE 319	84	11	14
NE 293	90	27	30

[a] Necrosis assessed for 32 days after inoculation.

Plants with increased resistance were recovered from two of the three *Septoria*-susceptible clones used in this study (NE 41, NE 299, and NE 319). Many regenerants from clones NE 41 and NE 299 were significantly more resistant to *Septoria* than their source plants.

Regression analysis showed the regenerants from clone NE 319 were as susceptible to *Septoria* as the parent plant (Fig. 1). Most regenerants from the two resistant clones (NE 293 and NE 314) were as resistant as their parent plants. However, a range in disease resistance was exhibited by the regenerants from all the clones (Fig. 2). The variation in disease severity exhibited among the regenerants depended on clone. Some of the regenerated plants were as susceptible or more susceptible than the parent clone and some were clearly more resistant. Some of the leaf disks never reached 50% necrosis, and several did not exhibit any disease symptoms (Fig. 3, Table 2). Sporulation of *S. musiva* was common on infected leaf disks of all susceptible clones.

We could not detect a clear trend in the rate of somaclonal variation in regenerants from callus cultures of different ages. Variants with increased levels of resistance were recovered from callus of all ages.

DISCUSSION

Based on our results, tissue culture can be used to increase disease resistance in poplar clones. The putative resistant clones regenerated from tissue culture reacted differently in the bioassay than their parent source plants. Because the cause for this variation is not known, these plants cannot be considered true mutants until this observed phenotypic change is shown to result from a permanent heritable genetic change. However, even a stable epigenetic variant of an elite poplar clone would be valuable because poplars are usually propagated vegetatively.

We do not know if traits other than disease resistance have been altered in these plants. The desirable qualities of the clones must be retained, and the new trait must be expressed for somaclonal variation to be of any value. In addition, a change in the resistance to one pathogen may either increase, decrease, or have no influence on the resistance of a clone to other pathogens or insect pests. This underscores the importance and need for field testing plant genotypes regenerated from tissue culture. The plants from our study have been planted and are being monitored for growth and for insect and disease resistance in the field.

Somaclonal variation may be simpler to exploit than gene transfer. Scowcroft and Larkin (14) proposed that somaclonal variation generates mutant genotypes similar to those resulting from spontaneous or induced mutation, but at a much higher frequency. They believe that most of the genetic changes responsible for somatic variation are induced by the tissue culture cycle itself. Somatic variation may be a better means for tree improvement than either direct gene insertion or *Agrobacterium*-mediated transformation because of the many technical and legal difficulties that need to be overcome with the latter methods.

In the work reported here, we screened regenerated plants. A far more powerful technique would be selection at the cellular level, favoring growth of only the variant cells resistant to the pathogen. We are currently evaluating techniques to use partially purified *S. musiva* culture filtrates to select resistant *Populus* cells. The advantages and limitations of in vitro screening and selection for disease resistance have recently been reviewed (1,4). Of primary importance is that the desired trait, in this case resistance to *Septoria*, be expressed or selectable in culture.

Somaclonal variation and cellular selection for disease resistance, coupled with early, rapid screening of regenerated plants, is a new and efficient technology for tree improvement. This technology may provide a valuable option for the tree breeder and plant pathologist and should be incorporated into conventional breeding programs to meet both short- and long-term goals. Moreover, the ability to introduce new traits, such as disease resistance, into elite lines via somaclonal variation eliminates the segregation of other desirable characteristics that occur in hybridization. In addition, we may have the potential to use somatic variation to respond more quickly to adaptive changes in pathogen populations.

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