Somaclonal Variation in Eastern Cottonwood for Race-Specific Partial Resistance to Leaf Rust Disease

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This research was supported by a grant from the U.S. Department of Agriculture (86-CRSR-2-1900).
The investigation reported in this paper (No. 88-8-259) is a project of the Kentucky Agricultural Experiment Station and is published with approval of the director.
Accepted for publication 16 February 1989 (submitted for electronic processing).

ABSTRACT

Prakash, C. S., and Thielges, B. A. 1989. Somaclonal variation in eastern cottonwood for race-specific partial resistance to leaf rust disease. Phytopathology 79:805-808.

Forty eastern cottonwood (*Populus deltoides*) plants, derived by leaf callus regeneration in vitro (somaclones), were tested for their reaction to two races of *Melampsora medusae*, using the leaf disk assay. Most somaclones had leaf rust reactions similar to those of the parent, but some were more, or less, resistant than the parent. A significant interaction between somaclone and race was observed for two traits, latent period and uredinial number; thus the somaclonal variation for leaf rust resistance was of a race-specific nature. Somaclones as a group exhibited a longer latent period relative to the parental genotype, but complete resistance was not observed in any of the somaclones. If field studies confirm these laboratory observations, then somaclonal variation may be useful to increase the partial resistance (longer latent period and lower infection frequency) in eastern cottonwood to leaf rust and also may be used to produce congenic lines to facilitate studies on mechanisms of host-pathogen interaction.

Additional keywords: breeding, tissue culture.

Since Larkin and Scowcroft (6) suggested the term "somaclonal variation" to explain the phenotypic changes sometimes observed in plants derived from tissue culture, increasing interest has been seen in the use of this approach to generate novel traits, particularly disease resistance (2,3,17,20). However, with few exceptions (e.g., 8), somaclonal variation has not been exploited for studies of disease resistance in forest trees.

Asexual methods of generating variation circumvent the time constraints inherent in breeding trees by conventional means and thus are of interest to tree breeders. Tissue culture is increasingly employed to mass-produce planting stock in a few tree species (5) and to genetically engineer trees using *Agrobacterium*-Ti/Ri plasmid vectors (4,11).

Eastern cottonwood (*Populus deltoides* Bartr.) is an economically important hardwood tree native to North America. Production in plantations of cottonwood, its related species, and hybrids throughout the world is limited by the leaf rust disease caused by *Melampsora medusae* (Thuem.) (19). Several physiologic races of this pathogen have been identified in the United States (14,15), and host resistance to this disease is usually race-specific. Complete, hypersensitive resistance to leaf rust is inherited as a single dominant gene in *P. deltoides* (12), while partial or incomplete resistance appears to be inherited quantitatively (13). This study was designed to determine the occurrence and extent of phenotypic variation in expression of leaf rust disease in somaclones of *P. deltoides* when they were inoculated with two races of the pathogen, *M. medusae*.

MATERIALS AND METHODS

Production of somaclones. Clone K417 of *P. deltoides* was originally selected from a natural stand in Fulton County, KY (latitude 36°30′N), and was obtained for this study from T. W. Kimmerer of the Department of Forestry, University of Kentucky.

To produce somaclones, leaf segments of K417 (plants grown in the greenhouse) were surface-sterilized with sodium hypochlorite solution (0.5%) and incubated in the dark on a woody plant medium (WPM) (7) supplemented with thiamine (0.5 mg/L),

pyridoxine (0.5 mg/L), nicotinic acid (1 mg/L), glycine (2.0 mg/L), myoinositol (100 mg/L), casein hydrolysate (100 mg/L), calcium gluconate (6 mM), glucose (20 g/l), α-napthalane acetic acid (NAA) (1 μM), and 6-benzylaminopurine (BAP) (1 μM). After 2–3 wk, light green, compact callus developed around the edge of leaf explants (primarily near the cut end of veins) and was transferred to a medium as described above, supplemented with 0.1 μM thidiazuron (gift of Nor-Am Company, Wilmington, DE) but without NAA or BAP. Transfer callus cultures were incubated in light (50 μmole photons $m^{-2}sec^{-1}$) to attain shoot regeneration. After 2 wk, the resultant adventitious shoot clumps were cultured successively on a supplemented WPM devoid of thidiazuron but with reduced concentration of BAP (0.5 μM) to achieve internode elongation. Excised microshoots were grown in Magenta vessels (Magenta Corp, Chicago, IL) containing WPM with BAP (0.1 μM) for 4 wk and were subsequently rooted in Techniculture peat plugs (Castle and Cooke, Salinas, CA) under intermittent mist in the greenhouse. To produce control parent plants, axillary shoot tips (3–4 cm) from greenhouse-grown K417 plants were directly rooted in the Techniculture plugs. All plants (somaclones and parents) were grown in a soilless medium (vermiculite and perlite, 1:1) in the greenhouse (25 C; 16 hr light) under standard cultural conditions. To minimize ontogenetic variation in disease expression, all plants were cut back three times, and leaves (3 mo old) were selected for testing only from shoots of comparable maturity. Somaclones and parents of uniform age (12 mo) were used for this test.

Pathogen races. Two monourediospore-derived isolates, each representing two races of *M. medusae* collected at Iowa (IO2-SS1) and Mississippi (MS-SS2), were employed in this study (characteristics of the two pathogen races are described elsewhere [15]). Cultures were increased on detached leaves of susceptible *P.* × *euramericana* Dode (Guinier) 'I-488' as described earlier (14,15). Parental genotype K417 was initially determined to be susceptible to the leaf rust race IO2-SS1 and moderately resistant to race MS-SS2 (15).

Inoculation. Six replicate leaf disks from each somaclone ($n = 40$), and four ramets ($n = 4$) of the parent K417 (controls) were separately inoculated with fresh, dry urediniospores (6 mg) of the two leaf rust races, using a spore settling tower (18). Deposition and germination of urediniospores was uniform, as assessed on the five coverglasses included as inoculation standards. Inoculated leaf disks were placed on plastic foam soaked with gibberellic acid solution (10 mg/L), located in sealed petri plates, and incubated in a growth chambers (16 C; 100 μmole photons $m^{-2}sec^{-1}$ and a 16-hr photoperiod).

Disease observation. After 18 days, leaf disks were qualitatively assayed macroscopically for disease severity on a scale of 0–7 for infection types (ITs) as follows (15): 0, no macroscopic symptoms of disease and highly resistant; 1, necrotic flecks only; 2, necrotic flecks, some with very small uredinia and very restricted sporulation; 3, necrotic flecks with a few, medium-sized uredinia; 4, moderate number of small uredinia; 5, moderate number of large uredinia; 6, numerous small uredinia; 7, numerous large uredinia and highly susceptible. Infection types 0–2 represent incompatible interactions, and 4–7 represent compatible interactions; IT = 3 indicates intermediate interaction of mesothetic type.

Two quantitative parameters of disease expression were also recorded: 1) latent period (LP1)—time, in days, from inoculation to the appearance of the first uredinium, which indicates timing of disease expression and 2) uredinia produced per leaf disk (ULD)—recorded at the end of the monocycle, when no new uredinia erupt (usually between 14 and 20 days), which indicates infection frequency.

Statistical analysis. Analyses of variance (ANOVAs) for the traits LP1 and ULD were performed using the SAS statistical package. Values for ULD were square roots transformed to improve the homoscedasticity and to achieve normality of the error variance. Least significant difference at $P = 0.05$ was used to test for significances of difference in mean values of LP1 and ULD between plants.

RESULTS

All parental ramets exhibited IT = 7 (highly compatible) to race IO2-SS1 and IT = 3 (incompatible intermediate) to race MS-SS2 of *M. medusae* (Fig. 1). No significant differences were found among the four parental ramets in disease expression traits LP1 and ULD ($P > 0.01$).

Reaction of the 40 somaclones to the two races of leaf rust was clearly variable. Whereas the infection types of most somaclones were identical to those of the parental ramets, there were somaclonal variants located toward resistance to race IO2-SS1 and variants located toward resistance and toward susceptibility to race MS-SS2 (Fig. 1). The majority of the somaclones, however, exhibited a longer latent period to both races and was characterized by substantial variation (Fig. 2). In terms of uredinial production, the distribution of the somaclones was near normal in their interaction with race IO2-SS1; most approximated the parental mean, but several somaclones were either more susceptible or more resistant than the parental reaction range (Fig. 3). Against race MS-SS2, most somaclones exhibited disease levels similar to those of the parent, but an appreciable number of somaclones exhibited a highly susceptible reaction (Fig. 3).

The disease interaction trend of the somaclones with both races of *M. medusae* was tested by ANOVA (Table 1). The "race" term was the largest contributor to the variation in both latent period and uredinial number, followed by the term for "somaclones" ($P < 0.0001$). However, the "race × somaclone" term was also a significant ($P < 0.0001$) but smaller source of variation in both traits, indicating the race-specific nature of the disease reaction in somaclones. Two somaclones exhibiting extreme disease reactions, and one somaclone exhibiting a similar reaction to that of the parent were retested with both pathogen races. The reactions and ranking of these three somaclones were the same as in the original test.

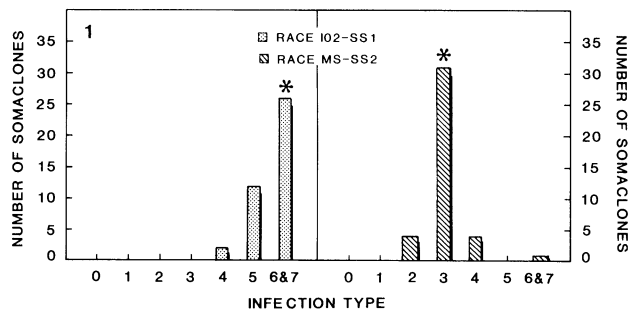

Fig. 1. Frequency distribution of the somaclones of *Populus deltoides* clone K417 to two races of leaf rust for infection type. Mean reaction of the parental replicates to two races of the pathogen is indicated by asterisks; there was no variation in this reaction.

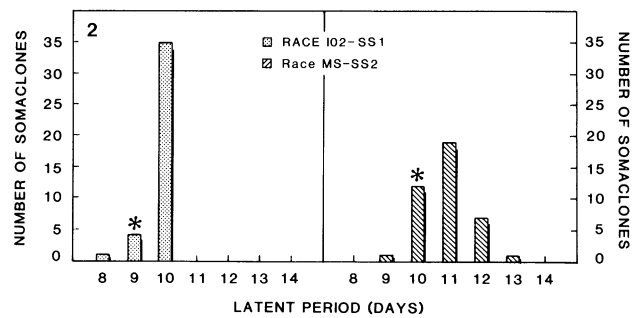

Fig. 2. Frequency distribution of the somaclones of *Populus deltoides* clone K417 to two races of leaf rust for latent period. Mean reaction of the parental replicates to two races of the pathogen is indicated by asterisks; there was no variation in this reaction. Least significant difference for latent period = 0.33 days.

DISCUSSION

Eastern cottonwood plants derived adventitiously in vitro exhibit phenotypic variation to leaf rust races. The disease reactions of these somaclones were significantly dissimilar with the two pathogen races tested (Table 1, Figs. 1–3). That is, the direction of change in disease expression of any specific somaclone was not necessarily similar in reaction against both pathogen races; some somaclonal plants were more susceptible than the parental ramets to one race but less susceptible to the other race. The race-specific nature of such variation can perhaps be explained by assuming a polygenic gene-for-gene interaction model as proposed by Parlevliet and Zadoks (10).

The somaclones, as a group, developed leaf rust disease more slowly than the parent K417. Although it is highly unlikely that all of them may have undergone a genetic change for this trait, it is tempting to speculate whether the process of dedifferentiation of the cells during callus regeneration and redifferentitation during adventitious shoot regeneration may have conferred a degree of partial resistance, as observed in some somaclones. Further conditions of plant culture are unlikely to have caused this change, as parental ramets were subjected to the same conditions for rooting and growth as the somaclones, before sampling and testing. Techniques employed for culturing the plants, selecting leaves, and inoculating and incubating the leaf disks ensured the uniformity of testing conditions and thus minimized unknown sources of error.

Although a few of the somaclones exhibited a higher degree of partial resistance ("slow-rusting") compared to the parents (Fig. 2), we did not observe complete resistance to either of the pathogen races (Figs. 1 and 3). This may have been partly due to the small population of somaclones screened. Complete, hypersensitive-type resistance to certain races of *M. medusae* in *P. deltoides* is known to be inherited as a single dominant gene (12). Mutations from recessive to dominance are less likely to occur than the reverse (16), and thus it may be difficult to obtain mutants with complete resistance from a susceptible wild type.

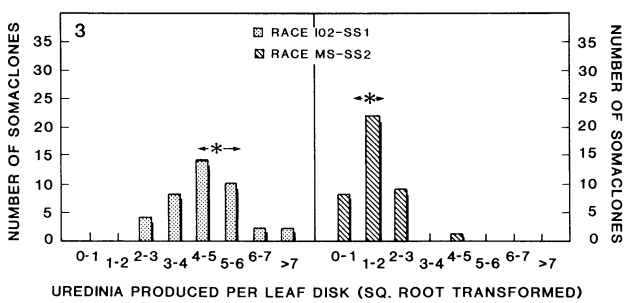

Fig. 3. Frequency distribution of the somaclones of *Populus deltoides* clone K417 to two races of leaf rust for uredinial number. Mean reaction of the parental replicates to two races of the pathogen is indicated by asterisks, and the parental range for the uredinial number is indicated by the two-directional arrow. Least significant difference for uredinia produced per leaf disk = 1.04.

TABLE 1. Analysis of variance for latent period and uredial number of *Populus deltoides* somaclones inoculated with two races of leaf rust, *Melampsora medusae*

Source of variation	df	Latent period		Uredial number[c]	
		MSS[a]	*F* value[b]	MSS[a]	*F* value[b]
Race	1	124.49	406.91	1,170.35	1,362.52
Somaclone	39	2.69	8.80	10.79	12.56
Race × somaclone	39	1.23	4.04	3.46	4.03
Error	400	0.30	···	0.85	···

[a]Mean sum of squares.
[b]All *F* values are highly significant ($P < 0.0001$).
[c]Using transformed square-root values.

Nevertheless, this study shows that it is possible to increase the level of partial resistance through induction of somaclonal variation. It is useful to test whether higher levels of partial resistance can be achieved by subjecting the select somaclones to additional cycles of tissue culture followed by selection. Some workers (e.g., 9) believe that partial resistance to disease is relatively more durable than the complete resistance, as it permits some level of disease expression in the plant population and thus does not exert strong selection pressure on the pathogen. Additional studies are necessary to determine whether partial resistance components measured in leaf disks are reflected in mature poplar trees in the field under polycyclic disease situations.

Because the somaclonal variants possess a common genetic background, they are likely to be congenic or near-isogenic lines. Such lines are almost impossible to produce in perennial woody plants by conventional means and are potentially useful for comparative biochemical and molecular studies of resistance. A few somaclones exhibited a high level of susceptibility (about three times more uredinial pustules than the parent). Although such plants are not of much interest for breeding, they are useful in studying the fundamental aspects of host-pathogen interaction aimed at understanding the mechanisms of resistance (3). A casual observation of somaclonal plants in the greenhouse did not reveal any apparent morphological or growth-related variation. The somaclones also had little variation in six enzyme systems when tested by starch gel electrophoresis (Dong Jinsheng and C. S. Prakash, *unpublished*).

The significant variation in leaf rust disease expression observed in the somaclones of *P. deltoides* suggests that a tissue culture process relying on adventitious regeneration of the plant may not be a useful method to mass-produce trees for commercial plantation where uniformity is desired. This is especially significant considering the rather large number of somaclonal variants generated in this study that were more susceptible to leaf rust than the parental line. The significant isolate × somaclone interaction, however, suggests the occurrence of noncorrelated changes in somaclones in their reactions with two races of the pathogen. Such diversity in the somaclonal population may confer a degree of stability by buffering the disease pressure through host heterogeneity (1).

The results of this study indicate that it is possible to generate eastern cottonwood plants with either higher or lower levels of partial resistance to races of Melampsora leaf rust than those of the parental line, through tissue culture-induced (somaclonal) variation. Clearly, additional work involving the generation and screening of a larger population, field testing of the somaclonal variants, and inheritance studies is needed to critically examine the usefulness of this approach in producing host cultivars and populations with durable resistance to this disease.

LITERATURE CITED

1. Browning, J. A. 1974. Relevance of knowledge about natural ecosystems to development of pest management programs for agro-ecosystems. Proc. Am. Phytopathol. Soc. 1:190-199.
2. Daub, M. E. 1986. Tissue culture and the selection of resistance to pathogens. Annu. Rev. Phytopathol. 24:159-186.
3. Evans, D. A., and Sharp, W. R. 1986. Applications of somaclonal variation. Bio/Technology 4:528-532.
4. Fillatti, J. A., Sellmer, J., McCown, B., Haissig, B., and Comai, L. 1987. *Agrobacterium* mediated transformation and regeneration of *Populus*. Mol. Gen. Genet. 206:192-199.
5. Haissig, B. E., Nelson, N. D., and Kidd, G. H. 1987. Trends in the use of tissue culture in forest improvement. Bio/Technology 5:52-56.
6. Larkin, P. J., and Scowcroft, W. R. 1981. Somaclonal variation A novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.
7. Lloyd, G., and McCown, B. 1980. A commercially feasible micropropagation for mountain laurel, *Kalmia latifolia*, by using shoot-tip culture. Int. Plant Propag. Soc. Proc. 30:421-427.
8. Ostry, M. E., and Skilling, D. D. 1988. Somatic variation in resistance of *Populus* to *Septoria musiva*. Plant Dis. 72:724-726.
9. Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.
10. Parlevliet, J. E., and Zadoks, J. C. 1977. The integrated concept

of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26:5-21.
11. Parsons, T. J., Sinkar, V. P., Stettler, R. F., Nester, R. W., and Gordon, M. P. 1986. Transformation of poplar by *Agrobacterium tumefaciens*. Bio/Technology 4:533-536.
12. Prakash, C. S., and Heather, W. A. 1986. Inheritance of resistance to races of *Melampsora medusae* in *Populus deltoides*. Silv. Genet. 35:74-78.
13. Prakash, C. S., and Heather, W. A. 1989. Inheritance of partial resistance to two races of leaf rust *Melampsora medusae* in *Populus deltoides*. Silv. Genet. 38:(In press).
14. Prakash, C. S., and Thielges, B. A. 1987. Pathogenic variation in *Melampsora medusae* leaf rust of poplars. Euphytica 36:563-570.
15. Prakash, C. S., and Thielges, B. A. 1989. Interaction of geographic isolates of *Melampsora medusae* and *Populus*: Effect of temperature. Can. J. Bot. 67:486-490.
16. Pryor, A. J. 1977. Plant disease resistance and the acquisition of pathogen virulence. Proc. 3rd Int. Conf. SABRAO, Canberra 4:15-28.
17. Scowcroft, W. R. 1985. Somaclonal variation: The myth of clonal uniformity. Pages 218-245 in: Genetic Flux in Plants. B. Hohn and E. S. Dennis, eds. Springer-Verlag, New York.
18. Sharma, J. K., Heather, W. A., and Winer, P. 1980. Effect of leaf maturity and shoot age of clones of *Populus* species on susceptibility to *Melampsora larici-populina*. Phytopathology 70:548-554.
19. Thielges, B. A. 1985. Breeding poplars for disease resistance. Forestry Paper No. 66. FAO, Rome.
20. Wenzel, G., Bolik, M., Deimling, S., Debnath, S. C., Foroughi-Wehr, B., and Schuchmann, R. 1987. Breeding for disease resistant crop plants by cell culture techniques. Pages 343-358 in: Plant Tissue and Cell Culture. C. E. Green, D. A. Somers, W. P. Hackett, and D. D. Biesboer, eds. Alan R. Liss, New York.