POTENTIAL OF IN VITRO SCREENING OF LOBLOLLY PINE FOR FUSIFORM RUST RESISTANCE

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Abstract.-- Twelve loblolly pine (Pinus taeda L.) embryos from each of 24 full-sib families (arranged in a 3 x 8 factorial mating design) were inoculated in vitro with Cronartium quercuum (Berk.) Miyabe ex Shirae f. sp. fusiforme basidiospores. Assessments of two types of responses, incompatible rapid necrosis and the appearance of a dark staining substance, typically at the inoculation point were made. These traits showed high family correlation with field resistance and high family heritabilities and so should be useful in the development of a rapid in vitro resistance screening technique.

Additional keywords: Indirect selection, early selection, Pinus taeda, Cronartium quercuum f. sp. fusiforme.

Presently, use of resistant planting stock is the primary and most economically feasible method of managing fusiform rust in plantations of loblolly (Pinus taeda L.) and slash (Pinus elliottii var. elliottii Englem.) pines. In order to reduce the time and effort involved in obtaining resistant ratings from field progeny tests, a greenhouse method of assessing resistance with artificial inoculations is being utilized by the U.S. Forest Service Resistance Screening Center. Select trees are rated based on their progeny performance for six symptom types assessed six months after inoculation (USDA For. Pest Mgt. Rep. #82-1-18, 1982). This method of screening is very useful and may account for 40 to 60 percent of the variation in field resistance (Walkinshaw et al. 1980, Carson 1983). However, development of an in vitro method of resistance screening could offer further benefits such as shortening assessment time and effort, better environmental control and the opportunity to better investigate host-pathogen relationships.

Recently, Gray and Amerson (in press, 1983) observed several in vitro responses of loblolly pine embryos to infection by Cronartium quercuum f. sp. fusiforme. Two responses, rapid incompatible necrosis (RN) and the formation of appositions which effectively barricaded the fungus from entering the cell, were regarded as effective resistance responses. The former response, RN, was characterized by the rapid death of host tissue and often resulted in haustorial necrosis. This response showed significant family differences and family rankings corresponding to field resistance rankings for a susceptible, an intermediate and a resistant full-sib family of loblolly pine. However, a family x assessment time and family x inoculum density interaction were present, implying that cultural conditions are crucial when assessing this in vitro response.

The purpose of this study is to further investigate the potential of in vitro screening of loblolly pine for fusiform rust resistance.

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METHODS

Plant material.--Fifteen embryos from 24 full-sib families arranged in a 3x 8 factorial mating design were used in this study. These families were part of a 22 parent half-diallel of resistant trees that were established in a series of plantings in high rust hazard areas by the North Carolina State University-Industry Cooperative Tree Improvement Program. Seeds were nicked at the micropylar end and incubated 4 to 5 days at 30°C in 1 percent aqueous hydrogen peroxide, changed daily. Seed coats were then removed and the gametophytes were surface sterilized with 0.8 percent (w/v) sodium hypochlorite solution and rinsed three times with sterile distilled water. Embryos were aseptically excised and their radicles inserted into half-strength Greshoff and Doy medium (Mott and Amerson 1981). Embryos were maintained at 22±2 C in mixed incandescent fluorescent light, 3000-4000 lux, with a 16 hour photoperiod for 13 days.

Inoculum preparation and inoculation.--Several Northern red oak (Quercus rubra L.) trees with telia bearing leaves were obtained from the U.S. Forest Service Resistance Screening Center. Leaves of these trees were suspended over pH 2.2 sterile distilled water for 36 hours. Basidiospores cast from telia into the water were then concentrated and rinsed on a Millipore filter (5pm pore size) and rediluted in pH 5.5 sterile distilled water. A Coulter counter was used to adjust the concentration to 1,375,000 spores/ml. One pl drop of Inoculum was placed on the upper hypocotyl region of 12 embryos from each family with a Hamilton syringe and repeating dispenser. Three uninoculated embryos/family served as controls. Inoculum was continuously agitated in an ice bath during preparation and inoculation to reduce premature germination and clumping. The embryos were incubated at 23±2^{8}C in darkness for two days and subsequently returned to the previously described light environment for five days

Microscopic examination.--Embryos were harvested seven days after inoculation, fixed in FAA (formalin-acetic-acid-EtOH), dehydrated in a graded EtOH-TBA (tertiary butyl alcohol) series, embedded in paraffin, sectioned at lOμm Johansen 1940) and stained with orseillen BB and analine blue (Jewell et al. 1962). RN was rated via light microscopy using the following three scales (Gray and Amerson 1983):

1 - percentage of epidermal necrosis (RN epidermis) 0 - 100
2 - tissue necrosis (RN time)
    0 none
    1 epidermal necrosis
    2 cortical necrosis
    3 vascular necrosis
    4 pith necrosis
3 - severity of cellular necrosis (RN severity)
    0 none
    1 necrotic cytoplasm
    2 necrotic collapsed cell walls.

Gray and Amerson (1983) observed a compatible necrosis which was not associated with resistance. This type of necrosis typically developed slowly and is difficult to observe via light microscopy. Therefore, all necrosis

evaluated in this study probably corresponds to the incompatible rapid type noted by Gray and Amerson (1983).

Another response characterized by the presence of a dark-red staining substance (RSS) at the inoculation point was observed and recorded. This substance was observed primarily on the epidermis but occasionally extended into the cortical region. The percentage of epidermis covered by this substance and the tissue affected (RSS epidermis and RSS tissue, respectively) were recorded using scales identical to those for the RN responses.

Data Analysis.--Phenotypic correlations among the traits measured were calculated. Field resistance at age four based on a one to five scale which was then standardized to a zero to 100 scale over several tests (Hatcher et al. 1981) were available for the eight paternal half-sib families and for 10 of the 24 full-sib families. Individual and multiple regressions of these family performance levels on the in vitro traits were performed. An analysis of variance appropriate for a factorial mating design was conducted and genetic parameters were estimated from the Type IV mean squares and their expected values using standard quantitative techniques (e.g., Becker 1967).

RESULTS

Phenotypic correlations (Table 1) were high ($0.41 > r > 0.72$) among the RN traits and between the two RSS traits ($r = 0.65$) as expected. RN epidermis showed negligible phenotypic correlation with the two RSS traits ($r = -0.0062$ and 0.046) while the other two RN traits showed weak correlations ($0.19 > r > 0.27$) with the RSS traits.

Table 1.-- Phenotypic correlations among in vitro traits

Trait	Trait			
	RN Epidermis	RN Tissue	RN Severity	RSS Epidermis
RN Tissue	0.44 0.0001[a/]			
RN Severity	0.41 0.0001	0.72 0.0001		
RSS Epidermis	-0.062 NS	0.20 0.0007	0.19 0.002	
RSS Tissue	0.046 NS	0.27 0.0001	0.20 0.0006	0.65 0.0001

[a/] Prob > |r| under Ho:rho = 0

Regression of rust field performance levels on the half-sib family means of individual in vitro traits produced low coefficients of regression for these traits (Table 2); however, RN epidermis and RSS epidermis, both yielded moderate regression coefficients (r - square = 0.43 and 0.55, respectively). Inclusion of two and three variables simultaneously in the regression increased the regression coefficients to 0.86 and 0.94, respectively. All the in vitro traits produced low regression coefficients (r - square > 0.12) when their individual full-sib means were used to predict full-sib field performance. The best three-variable-regression in this case yielded

Table 2.-- Single and best multiple regression of rust field performance levels on half-sib and full-sib means of in vitro traits

	Half-Sib		Full-Sib	
	r-square	Prob > F	r-square	Prob > F
RN Epidermis	0.43	0.08	0.22	NS
RN Tissue	0.093	NS	0.0052	NS
RN Severity	0.0045	NS	0.00096	NS
RSS Epidermis	0.55	0.04	0.12	NS
RSS Tissue	0.078	NS	0.044	NS
RSS Epidermis and RSS Tissue	0.86	0.006	--	--
RN Epidermis and RN Severity	--	--	0.29	NS
RN Epidermis, RSS Epidermis and RSS Tissue	0.94	0.006	0.44	NS

a moderate r-square value (0.44); however, this was not significant due to the small sample size. RN epidermis, RSS epidermis and RSS tissue compromised the best three variable regression for both half- and full-sib field performance.

Partially due to the small degrees of freedom of the "Female-Male" interaction (df = 14), the "Male" and "Female" effects in the analyses of variance were nonsignificant (Prob F 0.10 for all the in vitro traits with the exception of the "Male" effect for RSS time. Conversely, the "Female-Male" interaction effect was significant (Prob > F < 0.10) for all the in vitro traits with the exception of RSS tissue.

Several estimates of genetic variance components were negative (Table 3). Estimates of the additive genetic variances were small for the RN traits relative to their respective dominance variance estimates. The reverse phenomenon was true for the RSS traits. RSS tissue had the largest individual heritability estimate ($h_I^2 = 0.16$) due to a relatively large amount of additive variance. With a few exceptions, family heritability estimates were moderate to large ($0.35 < h^2 < 1.04$) for all traits.

Table 3.-- Estimates of genetic parameters for in vitro traits

Trait	Additive Variance	Dominance Variance	Individual Heritability	Half-Sib Family Heritability	Full-Sib Family Heritability
RN Epidermis	0.00843[a/]	0.102	-0.049	-0.29	0.66
RN Tissue	0.0482	0.115	0.068	0.35	0.70
RN Severity	-0.00198	0.150	-0.0039	-0.026	0.54
RSS Epidermis	0.000314	-0.000376	0.045	0.37	0.12
RSS Tissue	0.0771	-0.0483	0.16	0.73	1.04

[a/] Negative estimate due to sampling error. The estimate is assumed to be zero.

DISCUSSION

Gray and Amerson (in press, 1983) discussed the histology and ultrastructure of the RN response and related this response to histological reactions observed by others (Jewell and Spiers 1975, Miller et al. 1976 and Walkinshaw 1978). The composition and mode of action of the RSS response is currently unknown. RN appears to be a common response among resistant families of loblolly pine and occurred to some degree in 94 percent of the embryos observed in this study. RSS, however, was present in only 30 percent of the embryos observed. Although these two types of responses commonly occurred simultaneously, the phenotypic correlations among the traits measured indicate that the degree of development of these responses are somewhat independent. Furthermore, a relatively large portion of the genetic variance of RN appears to be due to dominance while the genetic control of the RSS response appears to be predominantly additive. Therefore, these two types of response appear to be distinct from one another.

The genetic parameters estimated from this data are not necessary representative of the loblolly pine population at large since only families with some degree of field resistance were sampled. (Half-sib field performance levels (Hatcher et al. 1981) ranged from 48 to 75). However, for the population

sampled, this study has demonstrated that the RN and RSS responses possess the necessary genetic characteristics for developing an in vitro resistance screening program. The correlated response in field resistance due to selection on an in vitro trait is proportional to the genetic correlation between field performance and the in vitro trait as well as the square root of the heritability of the in vitro trait appropriate for the type of selection applied (Falconer 1960). In light of the high values for these parameters, development of a selection index based on several of these and perhaps other in vitro traits appears promising. Such an index should be based on a large number of families that are more representative of the entire loblolly pine population.

Development and implementation of an in vitro screening index could be enhanced by further reduction of the time and effort involved in assessment. For instance, it may be possible to develop a biochemical assay to detect the presence and degree of RN which is associated with compounds most likely of phenolic origin (Gray and Amerson 1983). Thus, the tedium and time involved in histological preparation and assessment could be bypassed. The RSS response is also a candidate for such an assay. Another potential enhancement would be the development of techniques to successfully infect and assess callus of loblolly pine. Select trees could be directly screened rather than their progeny. Unfortunately, to date attempts to inoculate loblolly pine callus in vitro have proven unsuccessful (Jacobi 1982).

In conclusion, the future of developing an in vitro method of screening loblolly pine for resistance to fusiform rust is promising. Coupled with the developing tissue culture technology such a method may provide the tree breeder with an effective and rapid mode of assessing substantial genetic gains in fusiform rust resistance.

NOTE: The use of trade names in this manuscript does not imply endorsement of the product named nor criticism of similar products not named.

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