Variation among slash pine families in chlorophyll fluorescence traits

Anita C. Koehn, James H. Roberds, and Robert L. Doudrick

Abstract: Photochemical quenching, nonphotochemical quenching, and yield of photosystem II were measured on seedlings of full-sibling, open-, and self-pollinated slash pine (*Pinus elliottii* Engelm. var. *elliottii*) families. Our results reveal that genetic variation in photochemical quenching and yield of photosystem II exists within this species. The pattern of variation found in these traits is consistent with the variance profile expected to occur as a result of segregation among nuclear genes. Variation among families accounted for 17% of the total variation observed in photochemical quenching, whereas the component for trees within families made up slightly more than 25% of the total. Less variation, both among families as well as among trees within families, was found for yield of photosystem II. A strikingly different pattern was observed for nonphotochemical quenching. Other than the error term, only pretreatment effects contributed significantly to the variation observed. This suggests that nonphotochemical quenching is largely influenced by environmental factors. With regard to associations between fluorescence and growth traits, both height and diameter growth were found to be positively correlated with photochemical quenching (0.36 and 0.33, respectively) when selfed and open-pollinated families were analyzed along with control-pollinated families.

Résumé : Les auteurs ont mesuré la saturation photochimique, la saturation non photochimique et le rendement du photosystème II chez des semis issus d'autofécondation, de pollinisation libre et de descendances biparentales chez le pin de Floride (*Pinus elliottii* Englm. var. *elliottii*). Les résultats obtenus démontrent qu'une variation de nature génétique existe chez cette espèce pour la saturation photochimique et le rendement du photosystème II. Le patron de variation observé pour ces caractères est congruent avec le profil de variance qui devrait normalement découler de la ségrégation parmi des gènes nucléaires. La variation interfamiliale compte pour 17 % de la variation totale observée pour la saturation photochimique alors que la composante intrafamiliale est responsable d'un peu plus de 25 % de la variation totale. Une variation moindre a été observée pour le rendement du photosystème II, tant pour les composantes interfamiliale qu'intrafamiliale. Un patron de variation très différent a été observé pour la saturation non photochimique. Mis à part le terme d'erreur, seuls les effets de prétraitement contribuent significativement à la variation observée. Cette observation suggère que la saturation non photochimique est largement affectée par les facteurs environnementaux. Quant aux relations entre la fluorescence et les caractères de croissance, tant la croissance en hauteur qu'en diamètre sont corrélées positivement avec la saturation photochimique (respectivement 0,36 et 0,33) lorsque les familles issues d'autofécondation ou de pollinisation libre sont analysées avec les familles issues de croisements dirigés.

[Traduit par la Rédaction]

Introduction

Plants have the capacity to optimize light utilization during photosynthesis and to minimize damage to electron transport protein complexes caused by excessive light energy during periods of environmental stress (Demmig-Adams and Adams 1992; Long et al. 1994). Factors that influence the efficiency of light energy captured by photosystem II (PS II) reaction centers of the electron transport system can be studied using chlorophyll fluorescence methods. The ratio of variable chlorophyll fluorescence to maximum fluorescence (F_v/F_m) is a relative measure of the maximum efficiency of excitation energy captured by open PS II centers (measured on dark-adapted leaves). Photochemical quenching (q_P) is the fraction of open PS II centers under steady-state light conditions and represents the degree by which primary acceptors of electrons in the reaction centers of PS II are reduced. The quantum yield of noncyclic electron transport of PS II (yield) is directly proportional to the product of q_P and F_v/F_m. Changes in nonphotochemical processes that involve the deactivation of excitation energy (e.g., photoinhibition) within the PS II centers also influence yield of PS II. These nonphotochemical processes measured by chlorophyll fluorescence are defined as nonphotochemical quenching (q_N) (Adams et al. 1989; Genty et al. 1989; Havaux et al. 1991).

Received 8 October 2002. Accepted 10 January 2003. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 15 May 2003.

A.C. Koehn.[1,2] Department of Plant and Soil Science, Alabama A&M University, P.O. Box 1208 Normal, AL 35762, U.S.A.
J.H. Roberds. USDA Forest Service, Southern Research Station, 23332 HWY 67, Saucier, MS 39574, U.S.A.
R.L. Doudrick. USDA Forest Service, Sidney R. Yates Federal Building, 201 4th Street at Independence Avenue, SW, Washington, DC 20250, U.S.A.

[1]Corresponding author (e-mail: akoehn@kimberly.uidaho.edu).
[2]Present address: University of Idaho, Kimberly Research and Extension Center, Kimberly, ID 83341-5076, U.S.A.

 doi: 10.1139/X03-035 © 2003 NRC Canada

For the most part, q_N occurs when energy is released as heat during the de-excitation of light-harvesting chlorophyll–protein complexes associated with PS II. Photons are converted to heat when light intensities exceed those required for photosynthetic electron transport. In fully functional leaves, over one-half of the light absorbed by PS II can be redirected and released as heat. In leaves acclimated to their light environment, the processes of photosynthetic electron transport and nonradiative thermal dissipation of absorbed light energy indicated by q_N interact dynamically to optimize photosynthetic efficiency and photoprotection of PS II (Demmig-Adams and Adams 1992; Ruban and Horton 1995; Ort 2001).

Here, we describe genetic variation patterns observed in slash pine (*Pinus elliottii* Engelm. var. *elliottii*) for traits associated with these utilization and dissipation systems. Because these traits (q_P, q_N, and yield) are known indicators of plant stress caused by high and low temperatures, high light intensity, and drought (Schreiber et al. 1994), determination of whether genetic effects influence the expression of chlorophyll fluorescence characteristics is of considerable importance. Moreover, because conifers make up the only known collection of species in higher plants to have evolved strictly paternal transmission for chloroplast DNA (Neale and Sederoff 1988; Strauss et al. 1989), it is of interest to ascertain whether these processes vary primarily according to paternal lineage. Such a pattern would suggest that the control of the chlorophyll fluorescence processes is influenced by genes from the chloroplast genome. On the other hand, variation among families, along with genetic variation among trees within families, indicate that genes segregating in the nuclear genome are actively influencing trait expression.

Few studies have investigated genetic variation in chlorophyll fluorescence traits. Pettigrew and Turley (1998) demonstrated that genetic variation existed in CO_2 exchange rates and the ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) in *Gossypium hirsutum* L. (cotton) genotypes that were bred for increased crop yield. Open-pollinated families representing tall and short height classes in *Pinus ponderosa* Dougl. ex P. & C. Laws. (ponderosa pine) differed in F_v/F_m during spring and fall seasons, but no variation was detected among height classes in populations of *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco (Douglas-fir) and *Pinus monticola* Dougl. ex D. Don (western white pine) (Marshall et al. 2001). Several studies on *Yucca* s*pp.* (Huxman et al. 1998), deciduous tree species (Kitao et al. 2000), *Hordeum vulgare* genotypes (Planchon et al. 1989), and *Glycine* spp. (Kao and Tsai 1998) have used chlorophyll fluorescence methods to investigate species response to environmental changes.

Genetic differences among both families and populations, however, have been studied for other photosynthetic traits in conifers. Johnsen et al. (1999) reported high heritability in *Picea mariana* (Mill.) BSP (black spruce) for carbon isotope discrimination, an integrated measure of internal physiological processes influenced by environmental factors. Populations of Douglas-fir have been found to differ in carbon isotope discrimination and gas exchange, but no differences were detected among ponderosa pine populations (Zhang and Marshall 1995). Boltz et al. (1986) also found significant differences in net photosynthesis for provenances of *Pinus taeda* L. (loblolly pine).

Other inquiries have focused on regulation of specific gene expression by source–sink relationships and feedback regulation in photosynthesis (Van Oosten et al. 1994; Krapp and Stitt 1995; Nie et al. 1995; Van Oosten and Besford 1996). Both molecular and classical genetic approaches can effectively contribute to unraveling the interactions that take place between genes and environmental factors involved in regulating photosynthesis (Koornneef and Stam 2001). To obtain information about how genetic effects influence these processes in slash pine, we studied variation patterns among and within families for q_P, q_N, and yield of PS II (yield). To our knowledge, this is the first investigation of genetic variation in these traits for conifers. Our goal is to determine whether there is sufficient evidence for genetic variability to warrant further detailed investigation; therefore, we included only a limited number of families in this exploratory study.

Materials and methods

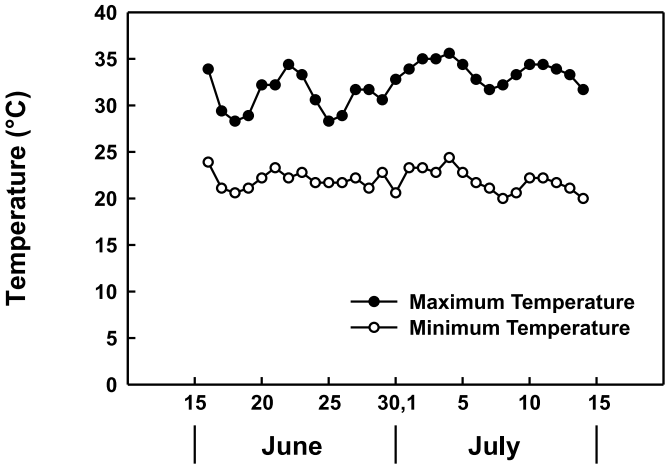
Plant material

Chlorophyll fluorescence, height, and diameter were measured on five seedlings from six slash pine families (30 seedlings total). This family collection consisted of full-sib progenies obtained by mating a single maternal parent (18-26) to itself and to four other individuals (8-7, 9-2, 18-27, and 18-62) plus open-pollinated progeny of the maternal parent. In subsequent discussion, these families are identified, respectively, as 18-26 × 18-26, 18-26 × 8-7, 18-26 × 9-2, 18-26 × 18-27, 18-26 × 18-62, and 18-26 OP. Parent trees originated from natural stands on the Harrison Experimental Forest (30°37′N, 89°03′W), located near Saucier, Miss., and the De Soto National Forest in Harrison County, Miss.

Seed from family collections were sown in April 1996 and the seedlings grown in a greenhouse in Normal, Ala. The seedlings in the greenhouse were grown at ambient photoperiod, light intensity, and relative humidity (no supplemental light was used) and watered uniformly as needed. Minimum greenhouse temperatures were maintained between 16 and 22°C, and the maximum temperatures ranged from 21 to 27°C. In August 1996, seedlings were transported to the Harrison Experimental Forest near Saucier, Miss., and transplanted to 4-L pots containing a medium composed of 75% bark, 12.5% peat moss, and 12.5% vermiculite amended with 6 g/L Osmocote fertilizer (14:14:14, $N–P_2O_5–K_2O$). Transplanted seedlings were subsequently grown outdoors at the Harrison Experimental Forest in a 10 × 15 m growing area in full sunlight and were uniformly watered as needed. In April 1997, seedlings were transplanted a second time to 12-L pots using the same soil medium and fertilizer amendment as previously described. Minimum and maximum ambient temperatures at the Harrison Experimental Forest for 16 June 1997 through 14 July 1997 are shown in Fig. 1. Height and root collar diameter measurements were taken during January 1997 when seedlings were dormant. Heights were measured from the top of the pot to the tip of the terminal bud using a metre stick. Stem diameter was measured at the top of the pot point using a caliper. Chlorophyll fluorescence measurements were

© 2003 NRC Canada

Fig. 1. Minimum and maximum daily temperatures for June and July 1997 at the Harrison Experimental Forest, Saucier, Miss. (Data source: http://www.ncdc.noaa.gov/).



taken between 16 June 1997 and 14 July 1997 from intact, mature needles on the first growth flush produced in 1997.

Chlorophyll fluorescence

Chlorophyll fluorescence readings for F_m, F_o, F'_m, F'_o, and F_t were obtained using a PAM-2000 chlorophyll fluorometer (Walz, Effeltrich, Germany) with a quartz-halogen lamp (Walz 2050-HB, Walz). F_o and F_m represent minimal and maximal fluorescence yields of dark-adapted needles, respectively. F'_o and F'_m are the minimal and maximal fluorescence yields of illuminated needles, and F_t is the measured fluorescence yield under steady-state light conditions (Schreiber et al. 1994). Intact needles of slash pine seedlings were dark-adapted for 15 min before taking readings of F_v/F_m (maximal yield of open PS II centers: $F_v = F_m - F_o$). The F_v/F_m was about 0.84 for all seedlings of all families in all treatments, indicating healthy unstressed plants. Because there was no variation in F_v/F_m and it has been found to vary little among species in plants growing with no environmental stress, we did not include it in the statistical analysis (Björkman and Demmig 1987). Following the measurement of F_v/F_m, readings for F'_m, F'_o, and F_t were taken at six light intensities (10, 50, 100, 200, 500, and 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$), beginning with the lowest light intensity and sequencing through to the highest intensity. Needles were illuminated for 5 min, and readings were taken at temperatures ranging from 23 to 26°C. Temperature was measured at the needle surface using a NiCr–Ni thermocouple attached to the leaf clip holder (Walz 2030-B, Walz). q_P was calculated as $(F'_m - F_t)/(F'_m - F'_o)$, q_N as $(F_m - F'_m)/(F_m - F'_o)$, and yield as $(F'_m - F_t)/F'_m$ (Schreiber et al. 1994).

Fluorescence was measured from each seedling following three different pretreatments. The pretreatments represent prospective methods for reducing the decline in photosynthesis in the afternoon during periods of high temperature and high light intensity (data not shown). The first pretreatment consisted of moving seedlings into the laboratory 1 h before assessment, the second involved moving seedlings into the laboratory 2–3 h before assessment, whereas the third pretreatment involved moving seedlings into the laboratory the evening prior to measurement. Temperature in the laboratory was maintained between 22 and 25°C, and light intensity was kept at approximately 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During the third pretreatment, seedlings were subjected to the normal daily dark period. Following each pretreatment and set of chlorophyll fluorescence measurements, seedlings were returned to the outdoor growing area. An interval of 6–7 days separated seedling exposure to different pretreatments.

Experimental design and statistical analysis

Pots containing single seedlings were randomly assigned positions in the experimental growing area irrespective of family identity. The seedlings were selected for pretreatment implementation and subsequent fluorescence measurement by order of placement in the growing area. Since families were represented by equal numbers of seedlings, our experimental arrangement conforms to the layout for a balanced, completely random experimental design. Data were analyzed using analysis of variance procedures (procedure ANOVA, SAS Release 8.0, SAS Institute Inc., Cary, N.C.) for a mixed model in which pretreatment and family effects are treated as main effects and tree effects are treated as nested within families. The appropriate linear model for our experiment then may be written as

$$[1] \qquad Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_{jk} + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} represents value of a trait for tree k in family j following pretreatment i; μ indicates the population mean; α_i is the effect for pretreatment i; β_j is the effect for family j; τ_{jk} is the effect for tree k in family j; $(\alpha\beta)_{ij}$ is the interaction effect associated with pretreatment i and family j; and ε_{ijk} denotes random error effects. In our analyses, pretreatment effects were assumed to be fixed, whereas family and tree within family effects were considered to be random. Variance attributed to each effect in the model was estimated from the mean squares in the analysis of variance using standard estimation procedures derived from expected mean squares. We tested uniformity of error variances associated with pretreatments for q_P, q_N, and yield and found no significant differences between pretreatments. Duncan's multiple range test was used to test multiple comparisons between pretreatment and family means for each trait. To study associations between fluorescence traits and seedling growth, Pearson's correlation coefficients were computed (procedure CORR, SAS Release 8.0, SAS Institute Inc.) on an individual tree basis using mean fluorescence values obtained across pretreatments and individual seedling growth measurements. Because F tests for pretreatment × family effects were not significant, pooled error mean squares were formed by pooling error and pretreatment × family sum of squares and used as a basis for F tests involving the trees within families and pretreatment sources of variation. Mean squares for trees within families were used to calculate F tests for families effects. The arcsin transformation was used to transform the quenching and yield data prior to conducting statistical tests.

Levels of inbreeding depression were estimated from outcrossed and selfed family means computed over pretreatments as (1) absolute units (mean of outcrossed families minus the selfed family), (2) percentage of the mean of outcrossed families (outcrossed mean minus selfed mean divided by outcrossed mean multiplied by 100), and (3) per-

© 2003 NRC Canada

centage of the absolute units divided by the standard deviation of the outcrossed families (Falconer and Mackay 1996). Open-pollinated family data were excluded from inbreeding depression determinations. Depression values are expressed as inbreeding depression per 10% increase in inbreeding. Since the inbreeding coefficient for selfed progeny is 0.5, total depression values were divided by 5 (10% × 0.5) (Falconer and Mackay 1996).

Results

Of the light intensities studied, the greatest genetic variation was observed at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the intensity that approaches light saturation; therefore, our focus here will be on the measurements taken at this light intensity. Results from the analysis of variance for q_P indicated that families contributed 17% to the total variation observed (Table 1). Family 18-26 × 18-27 had the highest mean value for q_P, whereas mean q_P for 18-26 × 18-62 and the selfed family were significantly lower (0.70 vs. 0.58 and 0.57, respectively; Table 2). In contrast, genetic sources of variation did not contribute to the variation in q_N, and no significant differences were detected among families (Tables 1, 2, and 3). Families contributed less to the total variation observed for yield in comparison with q_P (10–11% vs. 17%, respectively; Table 1). Family 18-26 × 18-27 showed significantly higher yield of PS II than 18-26 × 18-62 and the selfed family (0.47 vs. 0.37; Table 2).

Trees within families contributed more than families to total variation observed in q_P (27–32% vs. 17%, respectively; Table 1). The trees within families component contributed 11–14% to the total variation in yield, a smaller contribution than was obtained for q_P (Table 1). In contrast, the trees within families component contributed little or not at all to the variation in q_N (Table 1).

Pretreatments contributed more to the variation in q_N than any other source (22–26%, Table 1). The second pretreatment (2–3 h in the laboratory, q_N = 0.57) resulted in a q_N value 20% lower than that which resulted from the first pretreatment (1 h in the laboratory, q_N = 0.71), while the third pretreatment (overnight in the laboratory, q_N = 0.65) had an intermediate effect. Using Duncan's multiple range test, these pretreatment means were shown to differ significantly from each other at the 5% level.

A somewhat different pattern was observed for yield of PS II. For this measure, the second pretreatment produced the highest average value (0.45, significant at the 5% level), whereas the first and third pretreatments resulted in equivalent lower readings (0.38 and 0.39, respectively). Pretreatments had no significant effect on q_P (0.61–0.65) and contributed little to the total variation observed in this trait (0.04–0.09, Table 1).

Because our results indicate that the selfed family yielded lower values than the other families for all fluorescence traits except q_N (Table 2), we computed levels of inbreeding depression for both growth and chlorophyll fluorescence. Substantial inbreeding depression was found for growth traits and two of the fluorescence traits (q_P and yield, Table 4). In contrast with other fluorescence traits, q_N showed little reduction in value for the single selfed family studied as compared with outcrossed families. At the other extreme,

Table 1. Estimates of variance components in *Pinus elliottii* Engelm. var. *elliottii* full-sibling families with open-pollinated (OP) and selfed (Self) families included (+) or excluded (–).

	Photochemical quenching (q_P)				Nonphotochemical quenching (q_N)				Yield			
	–Self and –OP		+Self and +OP		–Self and –OP		+Self and +OP		–Self and –OP		+Self and +OP	
Variance component	Variance ($\times 10^{-3}$)	% Total variance	Variance ($\times 10^{-3}$)	% Total variance	Variance ($\times 10^{-3}$)	% Total variance	Variance ($\times 10^{-3}$)	% Total variance	Variance ($\times 10^{-3}$)	% Total variance	Variance ($\times 10^{-3}$)	% Total variance
θ_m *	0.04	0	0.09	1	6.05	26	4.93	22	2.04	21	1.22	14
$\sigma_c^{2\dagger}$	1.77	17	1.68	17	0.0	0	0.0	0	1.09	11	0.89	10
$\sigma_{ct}^{2\ddagger}$	3.45	32	2.62	27	0.0	0	1.59	7	1.35	14	0.95	11
$\sigma_{mc}^{2\S}$	0.44	4	0.27	3	0.0	0	0.0	0	0.01	0	0.17	2
$\sigma_e^{2\|}$	4.98	47	5.12	52	17.09	74	16.13	71	5.39	55	5.56	63
$\sigma_T^{2\P}$	10.67	—	9.77	—	23.14	—	22.65	—	9.87	—	8.80	—

Note: Measurements were taken at a light intensity of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The % values are based on the total of the positive estimates; when variance values were negative, they were set to zero. *m*, pretreatments; *c*, families; *t*, trees; *e*, error; *T*, total; MS, mean square.

*Pretreatments = [MS(Pretreatments) – MS(Pretreatments × Families)]/*tc*.

†Families = [MS(Families) – MS(Trees in Families)]/*mt*.

‡Trees in Families = [MS(Trees in Families) – MS(Error)]/*m*.

§Pretreatments × Families = MS(Pretreatments × Families) – MS(Error)/[*tm*/(*m* – 1)].

‖Error = MS(Error).

¶Estimated total variance = $\sigma_e^2 + \sigma_{mc}^2 + \sigma_{ct}^2 + \sigma_c^2 + \theta_m$.

© 2003 NRC Canada

Table 2. Mean values for chlorophyll fluorescence (photochemical quenching (q_P), nonphotochemical quenching (q_N), and yield), height, and diameter for *Pinus elliottii* Engelm. var. *elliottii* families.

Cross	q_P	q_N	Yield	Height (cm)	Diameter (mm)
18-26 × 18-27	0.70a	0.63a	0.47a	29.8a	8.1a
18-26 × 9-2	0.67ab	0.68a	0.42ab	25.8b	6.6b
18-26 OP	0.64abc	0.61a	0.43ab	20.1c	7.5ab
18-26 × 8-7	0.62abc	0.64a	0.40ab	23.2bc	7.3ab
18-26 × 18-62	0.58bc	0.66a	0.37b	23.7b	7.1ab
18-26 × 18-26	0.57c	0.63a	0.37b	15.4d	6.6b

Note: Letters indicate Duncan's multiple range test groupings at $\alpha = 0.05$ ($n = 5$). OP, open-pollinated.

Table 3. *F* values from analyses of variance for chlorophyll fluorescence (q_P, q_N, and yield) calculated from full-sibling *Pinus elliottii* Engelm. var. *elliottii* families with open-pollinated (OP) and selfed (Self) families, excluded (–) or included (+).

Source of variation	df	–Self and –OP (1000 μmol·m^{-2}·s^{-1})	df	+Self and +OP (1000 μmol·m^{-2}·s^{-1})
Photochemical quenching (q_P)				
Pretreatment	2,38	1.33	2,58	1.51
Families	3,16	2.74†	5,24	2.95*
Trees (families)	16,38	2.59**	24,58	2.30**
Nonphotochemical quenching (q_N)				
Pretreatment	2,38	8.34**	2,58	10.22**
Families	3,16	0.39	5,24	0.40
Trees (families)	16,38	1.05	24,58	1.35
Yield				
Pretreatment	2,38	8.56**	2,58	7.40**
Families	3,16	2.63†	5,24	2.51†
Trees (families)	16,38	1.82†	24,58	1.52†

Note: *F* tests were calculated using the error terms described in the text. †, significance at 10% level; *, significance at 5% level; **, significance at 1% level.

height growth had the greatest level of inbreeding depression of the traits examined.

Discussion

Our results clearly demonstrate that substantial differences exist among slash pine families in the photochemical-quenching capacity of first year seedlings. The highest ranking families (18-26 × 18-27 and 18-26 × 9-2) had a mean q_P that was 16% greater than that of the lowest ranking families (18-26 × 18-62 and 18-26 × 18-26). The theoretical state of complete utilization of energy without the complete closure of PS II centers occurs when q_P is near 1.0 or all reaction centers are open while receiving light energy. Therefore, the higher q_P observed in the high-ranking families indicates that their PS II reaction centers are more "open" at a light intensity of 1000 μmol·m^{-2}·s^{-1}, which means they have a higher oxidation–reduction capacity at this light intensity. When q_P is below 0.6, as was the case for the lowest ranking families, susceptibility to photoinhibition is increased (Ruban and Horton 1995). The ability to acclimate to environmental conditions, as reflected by q_P, may be a trait of breeding interest in the future. The two highest ranking families also had a 17% higher yield than the two lowest ranking families, indicating a higher excitation capture efficiency of light energy (Schreiber et al. 1994).

Table 4. Inbreeding depression of chlorophyll fluorescence (photochemical quenching (q_P), nonphotochemical quenching (q_N), and yield), height, and diameter of *Pinus elliottii* Engelm. var. *elliottii* families expressed (1) in absolute units, (2) as percentage of the noninbred mean, and (3) as the percentage of the total standard deviation of the noninbred mean (σ_T).

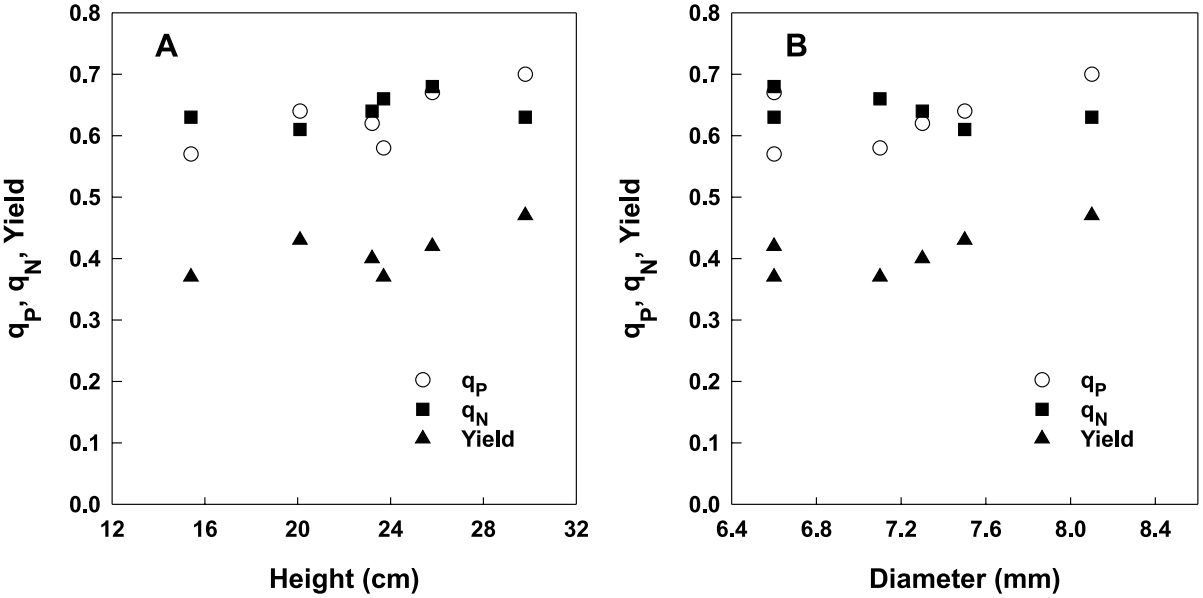
	Units	% of mean	% of σ_T
q_P	0.015	2.3	14.2
q_N	0.004	0.6	2.8
Yield	0.009	2.1	8.7
Height (cm)	2.04	8.0	52.6
Diameter (mm)	0.14	1.9	14.5

Note: The values are expressed as an increase in inbreeding depression per 10% increase in inbreeding; the inbreeding coefficient for the selfed progeny is 0.5.

In addition to a higher q_P and yield, the two high-ranking families have 30% greater height growth than the two lowest ranking families. The Pearson's correlation coefficient between height and q_P was estimated to be 0.36 (significant at the 5% level), while that between diameter and q_P was estimated to be 0.33 (significant at the 10% level). However, evidence for a positive association was less compelling when the selfed and open-pollinated families were excluded. The upward trend of increasing family means for height and diameter with increasing q_P and yield (Fig. 2) suggests that

© 2003 NRC Canada

Fig. 2. Relationships between (A) height and photochemical quenching (q_P), nonphotochemical quenching (q_N), and yield and (B) diameter and q_P, q_N, and yield. The values represent mean values of the families listed in Table 2.



genetic factors might be involved in producing a positive association among growth and these fluorescence traits. Relationships among growth and various photosynthetic characteristics result from many factors, and a single physiological parameter may be unreliable for early selection in conifers (Greenwood and Volkaert 1992; Johnsen and Major 1995). The advantage of the chlorophyll fluorescence measurements is that they are fast, nondestructive measurements, and repeated measurements can be taken on the same plant material over a long period of time. We realize that the results in this study must be treated with caution and should not be interpreted as applicable to slash pine populations in general. Johnsen and Major (1995) concluded from their study of black spruce families that relationships between photosynthesis and growth generally can result from many factors and that associations are likely to be specific for particular populations. Ledig (1976) also found genetic variation in photosynthetic processes in conifers and stated that although it can be a major component of growth variation, it is often obscured by the influence of season and the rate at which photosynthate is allocated to leaf growth.

Our findings are not unique with respect to evidence for a positive relationship between growth and photosynthetic traits in conifers. While attempts to establish presence of such associations have been met with mixed results (Greenwood and Volkaert 1992; Ledig 1976; Zhang and Marshall 1995), evidence for their existence has been reported for several photosynthetic traits. Boltz et al. (1986) found strong positive correlations between seedling dry mass and net photosynthesis in loblolly pine seedlings when measurements were averaged over an entire growing season. In an investigation of seasonal measurements for photosynthetic traits in outcrossed families of *Pinus banksiana* Lamb. (jack pine), Blake and Yeatman (1989) found a positive correlation between September measurements of photosynthesis and diameter; also, stomatal conductance and transpiration were positively correlated with height and diameter growth in August and September. Marshall et al. (2001) also report evidence from a field test that implies a positive association between a photosynthetic trait and growth in open-pollinated families of ponderosa pine. In spring and fall measurements, these investigators discovered that tall families possessed higher variable fluorescence (F_v/F_m) than short families of the same age. Based on these results and other considerations, they concluded that for the northern latitude of their experiment, genotypic differences in photosynthetic response to seasonal temperature changes are a significant component of the physiological basis for height growth differences in ponderosa pine.

Our observation that variation exists among families as well as among trees within families in q_P plus evidence that inbreeding depression occurs in q_P implies that some processes involved in chlorophyll fluorescence are primarily affected by nuclear genes segregating in slash pine populations. The level of inbreeding depression that we detected for height growth falls in the upper range of values reported by Snyder (1968) for first year height in slash pine. This suggests that the values we observed for inbreeding depression are probably not excessive for this species. Because of the patterns of variation detected and inbreeding depression observed, we suspect that nuclear genes having nonadditive effects are involved to some degree in influencing expression of all but one of the traits we investigated, the lone exception being q_N. If we had observed little variation among trees within families, no inbreeding depression, and strong evidence for variation among families, we would have surmised that q_P is strongly paternally influenced and that its expression is probably regulated by genes residing in the chloroplast genome.

In contrast with our results for q_P and yield, Johnsen et al. (1999) found that inbreeding had no impact on carbon isotope discrimination values, but it did adversely affect height and diameter growth. This led them to hypothesize that although selfing does not disrupt the photosynthetic potential

© 2003 NRC Canada

of black spruce trees, it disrupts physiological processes contributing to growth.

Our findings suggest that is not the case for slash pine seedlings, i.e., inbreeding does affect some photosynthetic processes, at least those that influence q_P and yield. Blake and Yeatman (1989) also found that inbreeding negatively affected both net photosynthesis and growth traits in *Pinus banksiana*.

Although our results indicate that nuclear genes strongly influence regulation of processes involved in q_P, we realize that chloroplast genes may also play a role. Allen and Raven (1996) discuss the evolutionary advantage of having both nuclear and organelle (i.e., chloroplast) genetic regulation in plants. If genes coding for key regulatory proteins in photosynthetic pathways are present in the chloroplast, control of gene expression can occur by oxidation–reduction reactions. However, genes in the chloroplast and other organelles are known to suffer high mutation rates, so it is advantageous to have regulatory genes located in the nucleus (Allen and Raven 1996). Both nuclear and chloroplast genes have been discovered that code for proteins involved in electron transport processes (Mackerness et al. 1999).

We failed to detect evidence for genetic variation in q_N. Because pretreatment effects were highly significant, q_N appears to be under strong environmental control. Furthermore, as q_N increases, yield of PS II is reduced, indicating a tradeoff between efficiency and photoprotective processes associated with q_N (Horton et al. 1996; Ort 2001). Such a pattern is clearly evident in our results for pretreatment means. For the second pretreatment, average yield is 0.45 and q_N is 0.57, but for the first pretreatment, yield and q_N are 0.38 and 0.71, respectively. We suspect the second pretreatment may have been more effective than the first and third pretreatments in preventing the reduction in photosynthetic activity that occurs with high temperature, high light intensity, and induction influences. Our conclusion that q_N processes in slash pine are primarily environmentally regulated is consistent with results obtained in molecular genetics research by Li et al. (2000). Their analysis linked the gene that codes for PsbS, a chlorophyll-binding protein, to nonphotochemical quenching in *Arabidopsis thaliana*. Furthermore, they concluded that expression of several genes, all which encode for chlorophyll-binding proteins, is induced by high light stress, an environmental cue. Other investigators also have found environmental regulation of photosynthesis to be dominated by the thermal dissipation processes associated with q_N (Demmig-Adams and Adams 1992; Horton et al. 1996).

Because conifers are long-lived perennials, they must survive fluctuations in temperature, vapor deficits, and light conditions for extended periods, while at the same time maintaining growth (Larcher 1980). Regulation of electron transport processes by both genetic and environmentally mediated mechanisms would thus seem to be advantageous for these species. Genetic regulation of photosynthesis in crop plants is thought to be a coarse mechanism of control, while environmental regulation appears to operate as a fine control (Nelson 1988). Our results suggest that electron transport processes active in q_P are influenced by genetic effects in slash pine, while q_N processes, which are important for photoprotection in plants, provide a finer control that is more strongly influenced by environmental conditions.

Investigation of a large number of families over a wide range of environmental conditions in field tests is required before further progress can be made in our understanding of the segregation of genes that regulate chlorophyll fluorescence and their effect on growth in slash pine. Determination of modes by which components of photosynthesis in conifers are genetically and environmentally regulated will contribute fundamental information that may be used in genetic improvement programs and will provide the groundwork for research directed at determining how photosynthetic processes evolved in coniferous species.

Acknowledgements

This study was funded by the U.S. Forest Service Southern Research Station Cooperative Agreement 19-95-078 with Alabama A&M University, Normal, Ala. The research was conducted while A.C.K. was in a postdoctoral position at Alabama A&M University. We express our appreciation to Lynn Lott for technical support, Dr. Tom Sharkey and Dr. Gerry Edwards for initial reviews, and two anonymous reviewers.

References

Adams, W.W., Díaz, M., and Winter, K. 1989. Diurnal changes in photochemical efficiency, the reduction state of Q, radiationless energy dissipation and non-photochemical fluorescence quenching in cacti exposed to natural sunlight in northern Venezuela. Oecologia, **80**: 553–561.

Allen, J., and Raven, J.A. 1996. Free-radical-induced mutation vs. redox regulation: costs and benefits of genes in organelles. J. Mol. Evol. **42**: 482–492.

Björkman, O., and Demmig, B. 1987. Photon yield and O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta (Berlin), **170**: 489–504.

Blake, T.J., and Yeatman, C.W. 1989. Water relations, gas exchange, and early growth rates of outcrossed and selfed *Pinus banksiana* families. Can. J. Bot. **67**: 1618–1623.

Boltz, B.A., Bongarten, B.C., and Teskey, R.O. 1986. Seasonal patterns of net photosynthesis of loblolly pine from diverse origins. Can. J. For. Res. **16**: 1063–1068.

Demmig-Adams, B., and Adams, W.W. 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43**: 599–626.

Falconer, D.S., and Mackay, T.F.C. 1996. Inbreeding and crossbreeding 1. Changes in mean value. *In* Introduction to quantitative genetics. Longman Group Ltd, Essex, U.K. pp. 247–262.

Genty, B., Briantiais, J., and Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta, **990**: 87–92.

Greenwood, M.S., and Volkaert, H.A. 1992. Morphophysiological traits as markers for the early selection of conifer genetic families. Can. J. For. Res. **22**: 1001–1008.

Havaux, M., Strasser, R.J., and Greppin, H. 1991. A theoretical and experimental analysis of the q_P and q_N coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. Photosynth. Res. **27**: 41–55.

Horton, P., Ruban, A.V., and Walters, R.G. 1996. Regulation of light harvesting in green plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47**: 655–684.

© 2003 NRC Canada

Huxman, T.E., Hamerlynck, E.P., Loik, M.E., and Smith, S.D. 1998. Gas exchange and chlorophyll fluorescence responses of three south-western *Yucca* species to elevated CO_2 and high temperature. Plant Cell Environ. **21**: 1275–1283.

Johnsen, K.H., and Major, J.E. 1995. Gas exchange of 20-year-old black spruce families displaying a genotype × environment interaction in growth rate. Can. J. For. Res. **25**: 430–439.

Johnsen, K.H., Flanagan, L.B., Huber, S.A., and Major, J.E. 1999. Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in *Picea mariana*: analyses from a half-diallel mating design using field-grown trees. Can. J. For. Res. **29**: 1727–1735.

Kao, W.-Y., and Tsai, T.-T. 1998. Tropic leaf movements, photosynthetic gas exchange, leaf $\delta^{13}C$ and chlorophyll *a* fluorescence of three soybean species in response to water availability. Plant Cell Environ. **21**: 1055–1062.

Kitao, M., Lei, T.T., Koike, T., Tobita, H., and Maruyama, Y. 2000. Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant Cell Environ. **23**: 81–89.

Koornneff, M., and Stam, P. 2001. Changing paradigms in plant breeding. Plant Physiol. **125**: 156–159.

Krapp, A., and Stitt, M. 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta (Berlin), **195**: 313–323.

Larcher, W. 1980. Carbon utilization and dry matter production. *In* Physiological plant ecology. Springer-Verlag, Berlin, Heidelberg, New York. pp. 73–157.

Ledig, F.T. 1976. Physiological genetics, photosynthesis and growth models. *In* Tree physiology and yield improvement. *Edited by* M.G.R. Cannell and F.T. Last. Academic Press Inc., New York. pp. 21–54.

Li, X.-P., Björkman, O., Shih, C., Grossman, A.R., Rosenquist, M., Jansson, S., and Niyogi, K.K. 2000. A pigment-binding protein essential for regulation of photosynthetic light harvesting. Nature (London), **403**: 391–395.

Long, S.P., Humphries, S., and Falkowski, P.G. 1994. Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45**: 633–662.

Mackerness., S.A.H., Surplus, S.L., Blake, P., John, C.F., Buchanan-Wollaston, V., Jordan, B.R., and Thomas, B. 1999. Ultraviolet-B-induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signaling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. Plant Cell Environ. **22**: 1413–1423.

Marshall, J.D., Rehfeldt, G.E., and Monserud, R.A. 2001. Family differences in height growth and photosynthetic traits in three conifers. Tree Physiol. **21**: 727–734.

Neale, D.B., and Sederoff, R.R. 1988. Inheritance and evolution of conifer organelle genomes. *In* Genetic manipulation of woody plants. *Edited by* J.W. Hanover and D.E. Keathley. Plenum Publishing Corporation, New York. pp. 251–264.

Nelson, C.J. 1988. Genetic associations between photosynthetic characteristics and yield: review of the evidence. Plant Physiol. Biochem. **26**: 543–554.

Nie, G., Hendrix, D.L., Webber, A.N., Kimball, B.A., and Long, S.P. 1995. Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO_2 concentration in the field. Plant Physiol. **108**: 975–983.

Ort, D. 2001. When there is too much light. Plant Physiol. **125**: 29–32.

Pettigrew, W.T., and Turley, R.B. 1998. Variation in photosynthetic components among photosynthetically diverse cotton genotypes. Photosynth. Res. **56**(1): 15–25.

Planchon, C., Sarrafi, A., and Ecochard, R. 1989. Chlorophyll fluorescence transient as a genetic marker of productivity in barley. Euphytica, **42**: 269–273.

Ruban, A.V., and Horton, P. 1995 Regulation of non-photochemical quenching of chlorophyll fluorescence in plants. Aust. J. Plant Physiol. **22**: 221–230.

Schreiber, U., Bilger, W., and Neubauer, C. 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. *In* Ecophysiology of photosynthesis. *Edited by* E.D. Schulze and M.M. Caldwell. Springer-Verlag GmbH & Co. KG, Berlin, Heidelberg. pp. 49–70.

Snyder, E.B. 1968. Seed yield and nursery performance of self-pollinated slash pines. For. Sci. **14**: 68–74.

Strauss, S.H., Neale, D.B., and Wagner, D.B. 1989. Genetics of the chloroplast in conifers. J. For. **87**: 11–17.

Van Oosten, J.-J., and Besford, R.T. 1996. Acclimation of photosynthesis to elevated CO_2 through feedback regulation of gene expression: climate of opinion. Photosynth. Res. **48**: 353–365.

Van Oosten, J.-J., Wilkins, D., and Besford, R.T. 1994. Regulation of the expression of photosynthetic nuclear genes by CO_2 is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO_2? Plant Cell Environ. **17**: 913–923.

Zhang, J.W., and Marshall, J.D. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in different environments. Funct. Ecol. **9**: 402–412.

© 2003 NRC Canada