Chromatographic Analysis of Pinus rigida × taeda hybrids and their parents[1])

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(Received April / June 1980)

Summary

Needle phenolics of pitch and loblolly pine hybrids and parents were analysed using two-dimensional paper chromatography. The results indicate that considerable variation exists in hybrid plant phenolics. Many phenolics are present in F_1 progeny which are not useful hybrid plant markers; phenolics in parents may not necessarily appear in F_1 progeny. A considerable amount of genetic analysis is needed before chromatography of phenolics can be used at the applied level to identify hybrids of pitch and loblolly pine.

Key words: plant phenolics, hybrids, *Pinus rigida* × *P. taeda*, paper chromatography.

Zusammenfassung

Phenole aus Nadeln von *Pinus rigida* × *P. taeda*-Hybriden sowie deren Eltern wurden mittels zweidimensionaler Papierchromatographie untersucht. Die Ergebnisse weisen auf eine beträchtliche Variation der Phenole in den Hybriden hin. Es gibt zahlreiche Phenole in der F_1, die nicht zur Identifizierung von Hybriden geeignet sind; Phenole der Eltern sind nicht unbedingt in der F_1 anzutreffen. Bevor die Chromatographie von Phenolen zur Identifizierung von *P. rigida* × *P. taeda*-Hybriden anwendungsreif ist, müssen noch genetische Untersuchungen erfolgen.

Introduction

Hybrid progeny of the pitch pine (*Pinus rigida* Mill.) loblolly pine (*Pinus taeda* L.) cross exhibit survival and vigor superiority over either parental type on some sites (Little and Trew, 1977). Because of the potential commercial value of the hybrid, methods of obtaining large amounts of hybrid seed are being investigated (Personal Communication, S. Little). The mist blowing of pollen may be one way to ensure a relatively high percentage of hybrid seed from unisolated female strobili (Franklin, 1971). However, identification of hybrid seedlings to measure success of mass pollination techniques is difficult since no simple salient morphological feature is available. To circumvent this problem, a biochemical approach was taken using paper chromatography (Alston and Turner, 1959, 1962).

The objective of this study was to determine if foliar phenolics could be used to determine parental origins of pitch × loblolly hybrids. A secondary objective was to study the inheritance of chromatographically isolated biochemicals from foliar tissue. Previous work with the genus *Pinus* (Mirov, 1958; Erdtman, 1963; and Thielges, 1968) has shown the approach to be of mixed value in hybrid and species identification.

Material and Methods

Needles were collected from five loblolly pine parents and five pitch pine parents and their hybrid progeny (eight families). Foliage from pitch pine parental trees was collected in August from trees growing in New Jersey. Loblolly pine foliage was collected both from trees growing in a grafted orchard in New Jersey and from trees in a seed orchard in South Carolina. Hybrid progeny foliage was sampled from five year-old trees growing in Virginia. Needles were air dried and then ground in a Wiley mill until passing a 2 mm mesh screen. Ground needles (150 grams from each sample tree, replicated twice) were extracted in 1000 ml of 80 % methanol for 24 hours on a mechanical shaker. The extracts from each sample tree were combined and evaporated to dryness. The residue was then separated into chloroform-soluble, water-soluble and water-chloroform insoluble (tannin) fractions. This was done by resuspending the methanol residue in 100 ml water, washing it three times with 20 ml chloroform, and twice with 10 ml chloroform. Insoluble tannins were separated by decanting and resuspended in 3-5 ml 80 % methanol.

Two-dimensional paper chromatography was performed using Whatman 3 mm paper in a descending paper chromatography apparatus. Chromatograms were run for 24 hours in one direction using TBA, acetic acid and water in a 3:1:1 v/v solvent. Chromatograms were dried, rotated 90° and rerun for six hours in the second dimension using 15 % acetic acid. After drying U. V. visible spots were recorded and identified by color and Rf values. Three replicate chromatograms were run for each sample. Spots appearing in at least two of three chromatograms were considered repeatable.

Results and Discussion

Marker substances were found in chromatograms of the eight individual crosses investigated *(Table 1)*. Pitch pine parents contributed more markers than loblolly parents in three crosses. The pitch pine parent contributed three markers in hybrid 64 × 22 and five markers in hybrid 59 × 7-56. To these hybrids, loblolly pine parents contributed one marker. The pitch pine parent of hybrid 78 × 7-56 contributed two markers whereas the loblolly parent produced none. In two other crosses, loblolly parents provided more markers than pitch parents. Loblolly parents contributed four and pitch parents contributed one marker in hybrids 64 × 16 and 78 × 11-9. It is evident, based on the above results, that some parents exhibited dominance. However, neither parent exhibited dominance in three other crosses. Loblolly pine and pitch pine parents each furnished four marker substances in hybrid 59 × 11-9. Pitch pine parents 54 and 51 and loblolly pine parents 23 provided three and five markers respectively to the hybrids among the three trees. Each pitch parent provided four markers to these two hybrid families *(Table 1)*. In general, neither species was clearly dominant with respect to the number of markers associated with progeny.

Most markers were in the tannin (17) and water-soluble (15) fractions while fewer (11) were in the chloroform fraction *(Table 2)*. Generally, less than 50 % of the reproducible substance found in the chromatograms could serve as markers *(Table 3)*. In some cases, progeny produced

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Table 1. — Reproducible marker substances found in parents and hybrids by solvent fraction. Not listed are many compounds which appeared in parents but not progeny, or appeared in progeny but not parents.

Solvent Fraction	Pitch pine	Loblolly pine	Hybrid Progeny
	54	23	54 x 23
Tannin		B.68/.74[1]	B.68/.80
Water	P.58/.50		P.54/.46
	P.58/.40		P.57/.38
	B.18/.24	B.20/.27	B.21/.25
Chloroform	B.84/.86		B.76/.89
		B.58/.81	B.64/.84
	51	23	51 x 23
Tannin	P.27/.77		P.22/.79
		B.64/.36	B.68/.39
Water	Y.66/.20		Y.65/.26
	P.52/.32		P.54/.32
		B.73/.46	B.73/.51
Chloroform	Y.04/.52	Y.06/.48	Y.04/.49
		B.58/.81	B.65/.79
		B.73/.82	B.76/.84
	64	22	64 x 22
Tannin	B.76/.76		B.71/.68
Water	P.55/.18		P.58/.17
		P.49/.32	P.49/.17
Chloroform	B.74/.71		B.70/.78
	78	7-56	78 x 7-56
Water	Y.68/.20		Y.67/.14
Chloroform	P.06/.87		P.12/.80
	59	11-9	59 x 11-9
Tannin	B.78/.28		B.79/.25
	P.49/.67		P.50/.70
	Y.03/.58		Y.03/.63
		P.42/.69	P.45/.53
		P.11/.83	P.16/.86
Water	P.54/.35	P.56/.33	P.53/.34
Chloroform		B.82/.74	B.83/.87
	59	7-56	59 x 7-56
Tannin	B.78/.28		B.85/.21
	P.49/.67		P.54/.68
	P.22/.73		P.20/.81
	Y.03/.58		Y.03/.62
		P.49/.55.	P.49/.53
Water	P.54/.35		P.53/.38
	64	16	64 x 16
Water		B.64/.41	B.69/.44
		P.43/.36	P.47/.41
		P.41/.50	P.46/.53
Chloroform	B.74/.71		B.79/.83
		Y.04/.57	Y.02/.61
	78	11-9	78 x 11-9
Tannin		B.84/.15	B.85/.18
		P.42/.69	P.44/.68
		P.11/.83	P.11/.78
Water		B.75/.25	B.68/.27
Chloroform	P.06/.87		P.08/.82

[1]) The first letter designates color of the compound; B = blue, Y = yellow, P = purple. The first number designates Rf value associated with the TBA, acetic acid solvent and the second number designates the 15% acetic acid solvent Rf value.

Table 2. — Distribution of hybrid markers between fractions.

Hybrid Family	Number of Tracers: Tannin Fraction	Water Fraction	Chloroform Fraction
54 x 23	1	3	2
51 x 23	2	3	3
64 x 22	1	2	1
78 x 7-56	0	1	1
59 x 11-9	5	1	1
59 x 7-56	5	1	0
64 x 16	0	3	2
78 x 11-9	3	1	1
TOTAL =	17	15	11

(It should read markers)

Table 3. — Fraction[1]) of total compounds found which could serve as hybrid markers.

Parent	Fraction	Parent	Fraction	Hybrid	Fraction
78	1/6	11-9	4/13	78 x 11-9	5/23
54	4/23	23	3/19	54 x 23	6/7
51	4/10	23	5/19	51 x 23	8/11
64	3/14	22	1/7	64 x 22	4/11
78	2/6	7-56	0/2	78 x 7-56	2/14
59	4/16	1109	4/13	59 x 11-9	7/13
59	5/16	7-56	1/2	59 x 7-56	6/12
64	1/14	16	4/12	64 x 16	5/22

[1]) Numerator = number of markers. Denominator = total number of reproducible compounds (spots) isolated.

substances that were not found in either parent. New substances may therefore have been synthesized by hybrid gene combinations. The appearance of new compounds may also have been due to accumulation of substances in hybrids normally present in smaller undetectable amounts in either parent (Levy and Levin, 1971). In contrast, other substances produced in parents were not found in progeny. The ability to synthesize some substances may have been suppressed or lost in hybrids or their appearance may be a function of plant age or the growth environment.

When crossed with different individuals, a particular parent did not have the same number or type of markers in progeny *(Table 1)*. For example, only substance B .58/.81 of the chloroform fraction was common to the progenies of both crosses when loblolly parent 23 was crossed with different pitch parents. Dominance or epistatic effects may have influenced the ability of hybrids to synthesize chromatographically identifiable substances.

Marker compounds consistently present within a species and hence suitable for differentiating between pitch and loblolly pines were not found. Substances which appeared most frequently in pitch pine were also commonly found in loblolly pine *(Table 4)*. For example, P .49/.33 was found in three of five loblolly pine individuals and four of five pitch pines. Similarly Y .05/.49 and B .71/.70 appeared in both species. Those compounds unique to a hybrid were not found in enough parental individuals to be diagnostic at the species level, but in many cases could serve to identify hybrid trees when analyses were limited to F_1 progeny and their parents.

Table 4. — Marker compounds common to two or more individuals within a species by fraction.

	Loblolly Pine				
Fraction	22	23	16	11-9	7-56
Tannin			B.65/.84	B.64/.82	
Water	P.49/.32[1]	P.49/.33	P.43/.36		
	B.73/.50	B.73/.46			
			P.54/.34	P.56/.33	
Chloroform		Y.06/.48	Y.04/.57		
				B.71/.70	B.74/.72
		P.19/.63			P.12/.69
	Pitch Pine				
Fraction	59	64	78	54	51
Tannin	P.49/.61		P.46/.64		P.53/.63
Water	P.54/.35		P.50./36	P.49/.32	P.52/.32
	P.55/.48			P.58/.50	P.58/.46
Chloroform	B.72/.67	B.74/.71	B.75./79		
	Y.05/.46	Y.05/.48		Y.07/.53	Y.04/.52

[1]) Numerator = number of markers. Denominator = total number of reproducible compounds (spots) isolated.

Conclusion

Two-dimensional paper chromatography shows promise for identifying pitch × loblolly pine hybrids. The large amount of variation in chromatographically identifiable substances in both pitch pine and loblolly pine requires more complete understanding before these methods can be practiced at the operational level. The genetic inheritance of the compounds that appear to be repeatable hybrid plant markers needs considerable study before variation in chromatographic profiles of hybrids can be fully understood. Identification of the chemical structure of markers would be advised.

Acknowledgment

We wish to thank Dr. S. Little, Retired, U. S. Forest Service, Pennington, N. J., and Ms. J. Bivens, WESTVACO, Summerville, S. C., for assistance in collecting foliage of parental trees used in this study.

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Altitudinal Variation in juvenile Characteristics of southern Appalachian black Cherry (Prunus serotina Ehrh.)*

(Received May / June 1980)

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Summary

Open-pollinated families of *Prunus serotina* Ehrh. from high- and low-elevation provenances in the southern Appalachian mountains were grown for five years in replicated reciprocal plantings. In the low-elevation planting, height superiority of high-elevation seedlings, established during first-year growth in the nursery, was eliminated after five years, resulting in no significant source differences. In the high-elevation test, material from high-elevation parents was superior in height and survival to low-elevation material. Low-elevation families exhibited earlier spring budbreak and began flowering earlier in ontogeny than high-elevation trees most of which had not flowered at five years.

Key words: shoot growth, flowering, budbreak

Zusammenfassung

Mit frei abgeblühten Familien von *Prunus serotina* Ehrh. aus dem Hoch- und Tiefland der Südappalachen wurde ein wiederholter, reziproker Versuch angelegt und fünf Jahre lang beobachtet. In der Tieflandpflanzung wurde an Hochland-Sämlingen schon im ersten Jahr im Gewächshaus Überlegenheit im Höhenwachstum festgestellt, die aber nach fünf Jahren verschwunden war, so daß keine signifikanten Unterschiede mehr bestanden. Im Hochland-Test war das aus dem Hochland stammende Pflanzenmaterial dem aus dem Tiefland in Wuchsleistung und Überlebensfähigkeit überlegen. Familien aus dem Tiefland trieben im Frühjahr eher aus und begannen auch während der Ontogenese früher zu blühen, als die aus dem Hochland, von denen auch mit fünf Jahren noch keine zu blühen begonnen hatte.

Introduction

In eastern Tennessee the phenotypic quality of black cherry (*Prunus serotina* Ehrh.) from high elevations (800+ m) is apparently better, in terms of growth, form, and pest resistance, than that of cherry from low elevations. As a consequence, most selections from natural populations made in genetic improvement programs have been from high elevations. If high elevation material retains superior qualities on low-elevation sites, its use on such sites could expand opportunities for planting this high-value species. Earlier experiments (Farmer and Barnett, 1972a, b) have demonstrated altitudinal variation in seed and flowering characteristics. In this study we have examined variation in 5-year heights and other characteristics in a reciprocal planting at high- and low-elevation sites, with special emphasis on the performance of high-elevation material at the low- elevation planting site.

Methods

Seed collections were made in the summer of 1969 from 87 trees at altitudes of 275 to 1370 m in Monroe and Anderson Counties, Tennessee. Six replications of 87 families were planted in the TVA Norris Nursery, where seedling growth characteristics were evaluated in 1970 (Farmer and Barnett, 1972a). In the spring of 1971, a low-elevation (275 m) planting was established on Jones Island in the Clinch River below Melton Hill Dam in Anderson County, Tennessee, where the soil varies from silt loam to sandy loam. A fescue sod cover was reduced by discing and

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