GENETIC VARIATION, HERITABILITY AND EXPECTED GENETIC GAINS IN *MILICIA EXCELSA* (IROKO)

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OFORI. D. A., COBBINAH, J. R. & APPIAH-KWARTENG, J. 2001. Genetic variation, heritability and expected genetic gains in *Milicia excelsa* (Iroko). *Phytolyma lata* resistivity of two provenances and of 61 clones of *Milicia excelsa* was determined in our central nursery at Mesewam, approximately 15 km south-east of Kumasi, Ghana. Assessment of *Phytolyma* resistivity was carried out using the conventional breeding methods of provenance trials and mass selection followed by vegetative propagation and clonal testing. The Côte d'Ivoire provenance grew significantly faster than the Ghana provenance, suggesting that the Côte d'Ivoire provenance is more tolerant to *Phytolyma* attack than the Ghana provenance. Selection of the Côte d'Ivoire provenance resulted in 34% gain in height over the population mean. Also, substantial variabilities in number of galls and height growth were observed among individual clones. After one year of growth in the field, 24 clones were identified as being tolerant to the pest with a selection differential and a gain in height of 20.5 cm and 36.7% respectively. The results suggest that considerable variability with respect to *Phytolyma* resistivity exists within *Milicia* populations, and this can be exploited for large genetic gains.

Key words: Clonal selection - genetic resistance - provenance - *Milicia excelsa* - *Phytolyma lata*

OFORI, D. A., COBBINAH, J. R. & APPIAH-KWARTENG, J. 2001. Perubahan genetik, keterwarisan dan jangkaan pencapaian genetik dalam *Milicia excelsa* (Iroko). Daya kerintangan terhadap *Phytolyma lata* bagi dua provenans dan 61 klon *Milicia excelsa* ditentukan di tapak semaian pusat kami di Mesewam, kira-kira 15 km di tenggara Kumasi, Ghana. Penilaian daya kerintangan terhadap *Phytolyma* dijalankan menggunakan kaedah pembiakbaikan konvensional bagi ujian provenans dan pemilihan massa diikuti dengan pembiakan tampang dan ujian klon. Provenans Côte d'Ivoire tumbuh lebih cepat dengan bererti berbanding provenans Ghana. Ini mencadangkan bahawa provenans Côte d'Ivoire lebih tahan serangan *Phytolyma* berbanding provenans Ghana. Pemilihan provenans Côte d'Ivoire menghasilkan pertambahan ketinggian sebanyak 34% daripada min populasi. Kebolehubahan yang cukup dalam bilangan puru dan pertumbuhan ketinggian juga dicerap di kalangan klon individu. Selepas setahun pertumbuhan di ladang, 24 klon dikenal pasti tahan terhadap perosak dengan pembezaan pemilihan dan pertambahan ketinggian masing-masing sebanyak 20.5 cm dan 36.7%. Keputusan mengesyorkan bahawa kebolehubahan daya kerintangan terhadap *Phytolyma* wujud dalam populasi *Milicia* dan ini dapat dieksploitasi untuk mencapai faedah genetik yang besar.

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Introduction

Milicia species (*M. excelsa* and *M. regia*; trade name: Iroko) is a highly valued commercial timber species in Africa. The distribution of Iroko stretches across the entire width of humid Africa from Sierra Leone, through Central and East Africa to Mozambique (White 1964). Iroko is highly durable and has good working properties. There is considerable demand for its timber, sliced veneer, rotary veneer and profile boards for decorative and structural uses, and it is considered as the most generally useful timber species in Africa.

Iroko suffers high levels of exploitation but it is not grown in commercial plantations. Alder (1989) estimated that if the current rate of exploitation continues without regeneration, it is likely that the species will cease to be of commercial importance by the end of the 20th century. The high rate of exploitation, coupled with poor natural regeneration, threatens the future of *Milicia*.

Attempts to establish plantations of Iroko have been largely unsuccessful because of the damage caused to *Milicia* seedlings by *Phytolyma lata* (White 1964, Wagner *et al.* 1991).

Masses of galls are formed on young tissues (stems and leaves) of seedlings, engulfing *Phytolyma* nymphs after infestation. The nymphs develop in the galls and the galls erupt to release the adults. This is followed by a decay of the galls. The shoots and leaves containing the galls then become a putrefying mass and the stem dies-back to the lignified tissues. This disrupts physiological processes and after continuous attack, growth is retarded, the plants remain in a deformed stunted state and most seedlings are killed (White 1964, Cobbinah 1990, Wagner *et al.* 1991). Although older leaves and tissues often have galls, they are small individual galls which usually do not open to release adult pests.

Insecticidal control has proved uneconomical and inefficient because of the cryptic habit of the insect (White 1964, Cobbinah 1993). Parasitism has so far been ineffective in controlling *P. lata* (White 1964, Bosu, pers. comm.). The problem has therefore attracted a great deal of attention from foresters, ecologists, entomologists and tree breeders. Partial genetic resistance to *P. lata* has, however, been observed within the natural populations of *Milicia* (Cobbinah 1990, Cobbinah & Wagner 1995) that can be cloned for rapid genetic gains (Leakey & Ladipo 1987).

In this study, two provenances were screened to identify resistant genotypes with respect to *Phytolyma* attack, followed by verification and quantification of the observed resistance through vegetative multiplication and clonal selection.

Materials and methods

Study site

This study was conducted at the Mesewam research nursery of the Forestry Research Institute of the Council for Scientific and Industrial Research (CSIR), near Kumasi, Ghana. This area is located at latitude 6.38° N and longitude 1.17° W. It falls within the moist semi-deciduous forest zone, where mean annual precipitation ranges between 1200 and 1800 mm with a pH of 5 to 6 (Hall & Swaine 1981). There is a long dry season between December and March with a short dry season between August and September.

Provenance trials

Seeds from eight half-sib families (Tain II, EAK 1, Fosu 4, EA 7, EK 4, AA 24, EAS 1 and EAS 4) collected in Ghana and four half-sib families (Kani 30, Kani 34, Kani 35 and Kani 36) from Côte d'Ivoire were used. The seeds from each country were bulked together to form a provenance. They were germinated on nursery beds and transferred into poly-pots (12 × 19 cm) at two leaf stage. The seedlings received daily watering to field capacity except whenever it rained.

When seedlings were three months old, 112 seedlings from each provenance were re-potted into large polythene bags (24 × 30 cm) and placed in a walk-in screen house made of mosquito mesh. The bags were arranged in groups of 28 seedlings (7 × 4 rows) with four replications in completely randomised block design. The door of the screen house was kept closed to prevent the seedlings from *P. lata* attack. After seven months of isolation from the pest, the plants attained a uniform growth and the door of the screen house was opened to allow infestation by *Phytolyma*. After one year of infestation, data on height growth, number of galls and die-back scars were taken from 10 middle plants per provenance per replicate.

Vegetative propagation

Individual trees that showed superior phenotypic characteristics (namely, low incidence of gall formation, high recuperative ability and apical growth) were selected from the two provenances and multiplied vegetatively. These candidate trees were first pruned to induce coppicing and the coppiced shoots were collected at semi-hardwood stage for propagation by cuttings (Ofori 1994, Ofori *et al.* 1996a, b, Ofori *et al.* 1997), in a low technology non-mist propagation system (Leakey *et al.* 1990). The rooted cuttings were potted into polythene bags containing sandy loam soil. The ramets were kept in a walk-in screen house to exclude them from the pest, *Phytolyma lata*, until they were ready for field planting (three months after rooting). This rendered all the ramets free from *Phytolyma* galls at the time of planting.

Clonal testing and selection

Ramets of 61 clones selected from individual superior plants identified from the provenance trial were used for this test. The trial was established at the Mesewam central research nursery of Forestry Research Institute of Ghana (see study site). The design consisted of 2-tree plots per clone per block, planted at 1 × 1 m with four replications. Data on the number of galls and height growth at four months intervals were collected for 12 months. This enabled the plants to grow through the two main seasons in Ghana; rainy season (March–July and October–November) and dry season (December–March). *Phytolyma* populations and galls are seasonally controlled. They increase during rainy season and decrease during dry season.

Statistical analysis

For provenance analysis, data were analysed using *t*-test and the means separated by LSD. For clonal trials, data on the two plants per clone per block were averaged and the data analysed using analysis of variance using Systat statistical package. Broad sense heritability and genetic gains were estimated using the method adopted by Zobel and Talbert (1991) and Lepisto (1993).

For all the statistical analyses performed, the probability to reject the null hypothesis was set at 0.05.

Estimation of broad sense heritability, selection differential and genetic gain

Heritability (H^2), the proportion of variation in the population that is attributable to genetic differences among the individual clones, was derived from the analysis of variance table as shown below.

SV	df	MS	F
Among clones	(f-1)	$MS_1 = V_E + rV_G$	MS_1/MS_3
Block	(r-1)	MS_2	
Within clones	f(r-1)	$MS_3 = V_E$	

$H^2 = (V_A + V_{NA})/(V_A + V_{NA} + V_E) = V_G/(V_G + V_E)$
but $V_G = (MS_1 - MS_3)/r$

where

V_G = variation due to true differences among clones
V_A = additive genetic variance
V_{NA} = non-additive genetic variance
V_E = within clone variance (environment)
r = number of replicates
f = number of clones
MS_1 = mean square for clones
MS_2 = mean square for blocks
MS_3 = mean square error

Selection differential (S), the difference in the average phenotypic value before and after selection, was estimated as:

$S = X_s - X_\mu$

where

X_s = phenotypic value after selection
X_μ = phenotypic value before selection

Genetic gain (G), a shift in the clone mean relative to the mean of the original population brought about by directional selection, was estimated as: $G = H^2S$

Per cent phenotypic gain, selection differential (response to selection) expressed as a percentage of population mean, was estimated as: $R = (S/X_\mu) \times 100\%$

Results and discussion

Provenance trials

Highly significant difference in height growth was observed between the two provenances (Table 1). The mean height of the Ghana provenance (38.4 cm) was significantly lower than the Côte d'Ivoire provenance (78.2 cm). The Côte d'Ivoire provenance developed two types of galls. The first were small hard galls, usually less than 3 mm in diameter, which appeared as isolated nodules on the upper surface of the leaves, while the second are large galls, usually more than 3 mm in diameter. The Ghana provenance, on the other hand, developed only large masses of galls. The small hard galls, predominant in the Côte d'Ivoire provenance, often did not erupt to release adult psyllid for re-infestation. In contrast, the large galls erupted and released adult psyllids. The ensuing die-back after gall eruption was largely responsible for growth disruption and mortality of seedling. Selection of the Côte d'Ivoire provenance resulted in a selection differential of 19.8 cm representing 34% gain in height over the population mean (Table 2).

Clonal trials at four months

Results of the clonal testing at four months showed wide variability in height growth, ranging from -13 cm to 30.5 cm and the number of galls ranging from 0 to 104 per plant. Three categories of plants were identified with respect to type and incidence of *P. lata* attack and die-back development. The first group had predominantly small hard galls and devoid of die-back. These were categorised as resistant strains. The second group had mainly large galls formed on them. Although die-back was prevalent in this group, positive growth was recorded because of their recuperative potential.

Table 1. Phenotypic differences in height growth (cm), number of galls and die-back scars between *Milicia excelsa* from Ghana and Côte d'Ivoire provenances (values are means ± SE)

Provenances	Height ± SE (cm)	Number of galls ± SE		Die-back ± SE
		Large	Small	
Ghana	38.4b ± 4.7	4.1a ± 0.7	0.0b ± 0.0	3.4a ± 0.3
Côte d'Ivoire	78.2a ± 5.2	1.8b ± 0.3	15.0a ± 3.9	2.3b ± 0.1

Means in a column followed by the same letter are not significantly different (p = 0.05 probability level)

Table 2. Selection differential and genetic gain observed in *Milicia excelsa*, after selecting the Côte d'Ivoire provenance

Selection differential (S)	Per cent gain in height
$S = X_s - X_\mu$	$G = [(X_s - X_\mu) / X_\mu] \times 100\%$
$= 78.2 - 58.3$	$= (S / X_\mu) \times 100\%$
$= 19.8$ cm	$= (19.8/58.3) \times 100\%$
where X_s – mean of Côte d'Ivoire provenance	= 34%
X_μ – population mean	

These were categorised as tolerant strains. The third group referred to as susceptible strain was associated with large masses of galls with serious incidence of die-back and, as a consequence, had heights lower than heights at planting (Table 3).

Clonal trials at twelve months

One year after the establishment of the clonal trials, however, all clones including those devoid of large galls at the early stages, had some large galls developing on them. Infested parts of these ramets died-back and these necessitated a reviewing of the earlier categorisation.

Two strains of plants were finally identified with respect to *Phytolyma* attack and subsequent die-back. These were referred to as tolerant and susceptible strains, consisting of 39 and 61% of the population respectively (Table 4). All the clones selected (at four months) as resistant became tolerant and those classified as tolerant joined the susceptible group.

The heritability for height growth increased substantially after one year, but that of gall formation remained almost unchanged (Table 5). The change in the degree of resistance or tolerance of the clones subsequently may be due to progressive adaptation of the local *P. lata* biotype to these clones. Similar observation in the variation of heritability and resistivity of *Phytolyma* over time has also been reported by Roulund *et al.* (1986). This makes it important to consider the age at which the final selection for *Phytolyma* resistance is done. Considering the heritabilities for height at four months (H^2 = 0.01) and at twelve months (H^2 = 0.2), it seems that some of the resistant genes turned on as the trees grew resulting in morphological and

Table 3. Broad sense heritability, selection differential and genetic gains observed in *Milicia excelsa* clones at four months after establishment

Group	% of population	Selection differential (S cm)	Genetic gain (G cm)	Per cent gain in height = $(S/X_{\mu})100\%$
Resistant	39.3	4.6	0.4	54.2
Tolerant	36.1	0.5	0.002	5.9
Susceptible	24.6	- 0.4	- 0.6	- 4.71

Table 4. Broad sense heritability, selection differential and genetic gains observed in *Milicia excelsa* clones at twelve months after establishment

Group	% of population	Selection differential	Genetic gain (G cm)	Per cent gain in height
Tolerant	39	20.5	4.1	36.7
Susceptible	61	−14.9	-3	−26.7

Table 5. Changes in broad sense heritability with age among *Milicia excelsa* clones

Time	H^2 for height	H^2 for gall formation
4 months	0.01	0.14
12 month	0.2	0.13

physiological changes that may make the trees more resistant. This suggests that selection for resistance solely on the basis of early infestation may not be appropriate and may confirm inefficiency in very early selection until juvenile-mature correlation is established.

This study has revealed evidence of variability in *Milicia* half-sib families to *P. lata* induced damage. To broaden the genetic base and to forestall or reduce the rate at which more virulent strains could develop, selection of materials for planting should not be restricted to only the highly resistant genotypes but also include some genotypes with low levels of resistance so as to increase the durability of resistance (Gaud 1986).

This study clearly showed that genotypes of *Milicia* species which are tolerant or resistant to the attack of *P. lata* exist in the natural populations, which can be selected and cloned for quicker genetic gains.

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