

USE OF AFLP MOLECULAR DATA VERSUS PEDIGREE RELATIONSHIP IN BEST LINEAR UNBIASED PREDICTION OF TEST CROSS PERFORMANCE IN OIL PALM (*Elaeis guineensis*, Jacq.)

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ABSTRAK

Pemilihan hibrida terbaik yang akan direproduksi untuk keperluan komersial merupakan salah satu tujuan dari skema seleksi berulang timbal balik yang digunakan pada program pemuliaan kelapa sawit. Pada tanaman tahunan adanya metode yang memungkinkan pemulia menguji hanya hibrida yang menjanjikan akan mengurangi waktu dan lahan yang dibutuhkan untuk pengujian. Metode best linear unbiased prediction (BLUP) yang mengkombinasikan nilai hubungan antar tetua dan uji projeni mungkin adalah metode yang cocok untuk tujuan tersebut. Tujuan dari studi ini adalah membandingkan penggunaan coefficient of parentage yang diperoleh dengan teknologi molekuler amplified fragment length polymorphism (AFLP) dengan koefisien yang diperoleh dari data pedigri untuk memprediksi nilai suatu hibrida. Sebanyak 61 poin data, diperoleh dari 50 hibrida hasil persilangan 22 tetua Deli dengan 20 tetua Afrika digunakan dalam studi ini. Model aditif dari BLUP telah diaplikasikan dengan menggunakan coefficient of parentage, baik yang dihitung dari 158 marka AFLP maupun yang dihitung dari data pedigri, untuk memprediksi nilai suatu hibrida. Meskipun terdapat perbedaan nilai coefficient of parentage dari kedua metode, nilai korelasi sebesar 0,60 dan 0,84, masing-masing untuk tetua Deli dan Afrika, tampak cukup baik. Korelasi antara nilai hibrida yang diobservasi dengan yang diprediksi hampir sama untuk kedua metode dengan nilai masing-masing dari 0,39 sampai 0,79 untuk jumlah tandan, 0,31 sampai 0,68 untuk produksi tandan, 0,28 sampai 0,65 untuk produksi minyak dan 0,42 sampai 0,84 untuk pertumbuhan meninggi. Studi ini menunjukkan kegunaan metode BLUP untuk memprediksi nilai suatu hibrida yang tidak diuji. Prediksi dapat dilakukan dengan menggunakan nilai hubungan genetik yang diperoleh dari marka AFLP apabila tidak tersedia catatan pedigri ataupun bila data pedigri tersebut diragukan keakuratannya.

Kata kunci: *Elaeis guineensis* Jacq. - prediksi - AFLP - silsilah - koefisien tetua - BLUP

ABSTRACT

The choice of the best hybrids to reproduce for commercial cultivation is the ultimate aim of reciprocal recurrent selection (RRS) breeding in oil palm. For a perennial tree crop, methods that allow breeders to select the best parent and test only the promising hybrids will greatly reduce the time, effort and space needed. The best linear unbiased prediction (BLUP) method which makes use of parental relationship and progeny tests data is such a method. The objective of our study was to compare the use of the coefficient of parentage obtained by amplified fragment length

polymorphism (AFLP) molecular marker data with that obtained by pedigree data in the BLUP prediction of hybrid performance. Sixty-one data points, obtained from 50 hybrids of inter-group crossings of 22 Deli and 20 African parents were used. An additive model BLUP analysis was then applied using the coefficient of parentage, calculated from 158 AFLP marker data as well as pedigree data, to predict hybrid performances. Although there were differences in estimated genetic relationships by the two methods, their correlations of 0.60 and 0.84, respectively for Deli and African parents, were reasonably good. The correlation between observed and predicted hybrid performances were comparable for both methods of prediction with values ranging from 0.39 to 0.79, 0.31 to 0.68, 0.28 to 0.65 and 0.42 to 0.84 for bunch number, bunch production, oil production and high increment, respectively. This study demonstrated the usefulness of the BLUP method in predicting performances of untested crosses and that the prediction could be realised by using AFLP molecular marker-derived genetic relationships when the pedigree record is unavailable or its accuracy is questionable.

Key words: *Elaeis guineensis* Jacq. - prediction - AFLP - pedigree - coefficient of parentage - BLUP

INTRODUCTION

The RRS scheme developed by the Institut de Recherche pour les Huile et Oléagineux (IRHO) (23) has been adopted in the Indonesian Oil Palm Research Institute (IOPRI) oil palm breeding programme. In this scheme, the two heterotic groups are characterised by the palm with few big bunches (A group) and the palm with many smaller bunches (B group). The aim of this selection procedure is to improve, at the same time, the parental combining ability of both groups. The parents that have a good combining ability in the progeny-test are then reproduced to produce commercial hybrids.

A method of making a prediction of hybrid performance that is close to the field progeny-test results is of utmost interest to oil palm breeders. For a perennial tree crop with a long breeding cycle (12-15 years), a typical oil palm

breeding programme requires a large commitment in terms of land, effort and time. In this context, the progeny-testing phase of the reciprocal recurrent selection (RRS) programme, in which the best hybrids for reproduction as commercial hybrid seeds are identified, is important. Consequently, much time, land and expense can be saved by reducing the choice of the best parents and testing only their most promising hybrids.

To predict the performance of a cross, use is usually made of different types of parental information, such as pedigree, combining ability and progeny-test results and in recent years molecular marker data (10). General combining ability (GCA) combined with genetic distance calculated from molecular marker data was effective in maize and rapeseed (*Brassica napus* L.) in predicting cross performances (10, 12). Nevertheless, the GCA value is sometimes difficult to assess with acceptable

precision. In the case of the oil palm breeding programme in Indonesia, improperly designed trials and limited resources led to imprecise parental evaluations due to highly unbalanced data sets (24).

The incorporation of all pedigree data into the model, even with unbalanced data sets, should improve genetic parameter estimation (13). However, due to incomplete pedigree information, the calculation of parentage coefficient is always a problem in some cross-pollinated species, particularly when the genotypes are genetically unrelated (22). To predict the coefficient of parentage as a measure of genetic relationship, genetic distance estimations, based on molecular markers, were found to be highly correlated with pedigree information (8, 19, 20). Estimated differences were based on the assumption that the parents make equal allelic contribution to their progeny. This assumption, taken into account in the calculations of coefficient of parentage by the pedigree method, may not be completely true due to selection and/or drift in the developmental process (8).

Molecular data have frequently included in several methods of hybrid performance prediction. Molecular data have been integrated into yield prediction models by using them (i) to calculate genetic distances (general and specific) and then correlating them with yield (1, 9, 18), (ii) to generate covariates for specific combining ability (SCA) in the distance and factorial regression interaction models (10), and (iii) in the calculation of the coefficients of parentage (3) in order to use those

coefficients as covariances between single-crosses in the best linear unbiased prediction (BLUP) method (4, 5, 10).

The BLUP method has been successfully used in predicting maize single-cross performances (6, 10). In oil palm, Purba *et al* (24) used this method to predict the untested hybrid performances by using the agronomic performances of tested hybrids and their relationships with the former. The correlation between predicted and observed performances, ranging from 0.42 to 0.71 for the five characters studied, were high enough to justify the use of BLUP in predicting oil palm hybrid performances. One of the major advantages of the BLUP approach is its flexibility in combining with other data sources (30).

Molecular marker techniques such as random amplified polymorphic DNA (RAPD), restriction fragments length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) were developed for studying genetic polymorphism in oil palm germplasm (27), genotyping (16), linkage mapping (17, 21), and genetic diversity studies of Indonesian oil palm breeding materials (25). In the near future, these technologies may be routinely used to identify the best materials to be integrated in breeding programmes.

We adopted Bernardo's work (4, 5) to integrate AFLP molecular data into the prediction of oil palm hybrid performance with the BLUP method. Our objective was to compare the use of coefficient of parentage, obtained from molecular marker data generated by AFLP to that obtained by using the

pedigree data, in the prediction of hybrid performance using the BLUP method.

MATERIALS AND METHODS

Plant materials and trial conditions

Forty-two genotypes were used as parents representative of the selection materials used in the Indonesian Oil Palm Research Institute (IOPRI) breeding programme. They comprised 22 parents of the Deli group and 20 parents of the African group. These parents had been analysed using 158 AFLP markers (25).

Sixty-one data points, obtained from 50 hybrids of the inter-group crossings (Deli x African) of the parents considered, were tested in 18 progeny trials, planted between the years 1974 and 1980. Some of these hybrids had been tested in different trials. Trial conditions, statistical designs and analytical procedures used for several observed characters have been described by Purba *et al.* (24). The characters analysed were mean bunch production, mean oil yield of the second period of observation (age period from 7 to 9 years) and mean height increment measured at 6 and 8 years. All the agronomic data used in this study were the average performances of a hybrid in each trial.

Calculation of the coefficient of parentage

The coefficients of parentage or co-ancestry between parents were calculated by (i) Malécot's method using the

pedigree data, and (ii) the molecular method using 158 AFLP marker data following Bernardo (3):

$$f_{AB}^M [S_{AB} - 1/2(\bar{A} + \bar{B})] / [1 - 1/2(\bar{A} + \bar{B})]$$

where f_{AB}^M was the coefficient of parentage estimated by using AFLP marker data, S_{AB} was the similarity indices (proportions of AFLP loci with shared variants) calculated by the Simple Matching method (28), and \bar{A} and \bar{B} were the average proportions of common bands from non-related parents of the Deli and Africa groups, respectively.

Data analysis

Based on the results obtained from an analysis using the same data sources (24), the additive genetic model was adopted. Suppose that a and a' are two parents of populations A (Deli group in the oil palm RRS scheme) and b and b' are two parents of population B (African group in the oil palm RRS scheme). Assuming no epistasis and Hardy-Weinberg equilibrium of both populations studied, the genetic covariance between an individual of an $a \times b$ cross and an individual of an $a' \times b'$ cross can be expressed as follows (29):

$$\text{Cov}(ab, a'b') = f_{aa} V_{A(A)} + f_{bb} V_{A(B)} \quad [1]$$

where,

f_{aa} and f_{bb} are the coefficients of parentage between a and a' and b and b' , respectively.

$V_{A(A)}$ and $V_{A(B)}$ are the additive variances of alleles of the A group and of the B group, respectively.

Variances estimation

The variances in equation [1] were estimated with the following mixed linear model applied to the n experimental observations resulting from p crosses derived from p_A parents of A and p_B parents of B which have been tested in t progeny tests:

$$y_{AB} = X + Z_1 a_{(A)} + Z_2 a_{(B)} + e \quad \dots\dots\dots [2]$$

where,

- y_{AB} = $n \times 1$ vector of the observed
- β = $t \times 1$ vector of the trial effects (fixed effect),
- $a_{(A)}$ = $p_A \times 1$ vector of the additive genetic effects of the parents from A (random effect), which are supposed to have a normal distribution with a zero mean and a variance of $V_{A(A)} \times A_1$, where A_1 is the $(p_A \times p_A)$ matrix of the coefficient of parentage between the parents in A group,
- $a_{(B)}$ = $p_B \times 1$ vector of the additive genetic effects of the parents from B (random effect), which are supposed to have a normal distribution with a zero mean and a variance of $V_{A(B)} \times A_2$, where A_2 is the $(p_B \times p_B)$ matrix of the coefficient of parentage between the parents in B group,
- e = $n \times 1$ vector of the residual effects, with a zero mean and the variance of $V_R I_n$,

$X(n \times t)$, $Z_1(n \times p_A)$ and $Z_2(n \times p_B)$ are the incidence matrices that connect the above effects with y_{AB}

The fixed effect β and the genetic and non-genetic variances were estimated by using the restricted maximum likelihood (REML) method. The calculations were done by the MIXED procedure of SAS software (26).

Hybrid performance prediction

The phenotypic value of a hybrid was computed as the average of its observed value corrected for the trial effect. If Z is the $(n \times p)$ design incidence matrix that relate each observed value to its cross, then the vector y_P of phenotypic value is $(ZZ')^{-1} Z (y_{AB} - X)$. Any random vector y_M of untested hybrids performances correlated with y_P can then be estimated by:

$$y_M = \text{Cov}(y_M, y_P) V(y_P)^{-1} y_P \quad \dots\dots\dots [3]$$

which is its best linear unbiased predictor. The elements of $\text{Cov}(y_M, y_P)$ and $V(y_P)$ were estimated with equation [1] where $V_{A(A)}$ and $V_{A(B)}$ were replaced by their estimations. The variance due to the environmental effect e , i.e. the quantity V_R /number of observations of the i^{th} hybrid, was added to the i^{th} diagonal element of $V(y_P)$.

Cross validation test

To assess the effectiveness of BLUP estimations, the observed performances of some tested hybrids were compared to their predictions when using the other

hybrids as predictors. A total of $p = 5, 10, 15, 20, 25$ and 30 hybrids were used as predictors. For a given number of predictor hybrids, p hybrids were randomly chosen and the performances of the $(50 - p)$ missing hybrids were predicted according to equation [3]. This process was repeated 100 times and for each sampling the correlation between the predicted and observed values was computed. The pooled correlation coefficients were then calculated across 100 repetitions for each number of p .

RESULTS AND DISCUSSION

Coefficient of parentage

We calculated and compared the coefficient of parentage of 42 parents (22 parents of the Deli group and 20 parents of the African group) used in the BLUP analysis. Within the Deli group, these coefficients varied from zero to 0.665 with AFLP assays and from 0.125 to 0.563 using Malécot's method (Table 1). The corresponding values ranged from zero to 0.712 and from zero to 0.313 for the African group, based on 158 AFLP markers and Malecot's pedigree-based method, respectively (Table 2). The correlation between the coefficients of parentage obtained from the two methods was 0.65 and 0.85, respectively, for Deli and African groups.

Differences found between the methods for predicting genetic relationships may be due to different estimates of parental contribution. This was probably caused by selection and/or

drift during development of the parents (8, 22) as selection pressure for oil palm tended to be very high.

Malécot's coefficient of parentage, calculated from genealogical information, is based on the assumptions underlying the available pedigree data set. Moreover, in Malécot's method the coefficients of parentage of a genotype i with itself, Φ_{ii} , depend on the level of inbreeding of its parents. The corresponding value was systematically equated to 1 when calculated by AFLP markers using Simple-Matching indices. Consequently, a comparison of the coefficients of parentage resulting from pedigree and molecular data should be viewed with caution due to the different underlying assumptions of the methods.

Correlation between predicted and observed performances

The objective of this study was to compare between predicted and observed crosses performances obtained using either the AFLP-derived or the pedigree-derived genetic relationships in the BLUP analyses. Despite the small number of crosses used, the correlation between the predicted and observed performances was similar for the two methods, although Malécot's method tended to be slightly better. The values ranged from 0.39 to 0.79, 0.31 to 0.68, 0.28 to 0.65 and 0.42 to 0.84 for the performances of bunch number, bunch production, oil production and high increment, respectively. As expected, the correlation increased with the number of crosses used as predictors (Figure 1).

AFLP-BLUP in oil palm breeding

The use of AFLP marker-derived genetic relationships in the BLUP prediction of untested hybrid performance has no advantage over the usual BLUP with known pedigree information. When pedigree information is unavailable or unreliable, molecular marker data could be used in the prediction.

In conclusion, this study demonstrated the usefulness of the AFLP-based coefficient of parentage BLUP method in the prediction of untested cross performance. Considering the biological constraints of a perennial tree crop and the increasing availability of molecular markers, BLUP combined with molecular markers should be more widely used in oil palm breeding programmes.

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Table 1. Coefficients of parentage of the Deli group calculated by AFLP (above diagonal) and by Malécot's method (below diagonal)

		Dell parents																				TI 221 D
		BJ 3 D	BJ 7 D	BJ 9 D	BJ 13 D	BJ 42 D	BJ 88 D	BJ 126 D	BJ 129 D	BJ 331 D	BJ 342 D	BJ 367 D	DA 8 D	DA 115 D	DA 128 D	DS 29 D	DS 139 D	LM 27 D	MA284 D	MA370 D	MA401 D	PA 118 D
	BJ 3 D		0.559	0.310	0.598	0.442	0.174	0.605	0.455	0.383	0.296	0.542	0.459	0.395	0.421	0.447	0.222	0.366	0.204	0.307	0.517	0.317
	BJ 7 D	0.399		0.189	0.662	0.506	0.112	0.425	0.458	0.324	0.482	0.483	0.401	0.271	0.296	0.633	0.164	0.369	0.085	0.376	0.455	0.382
	BJ 9 D	0.399	0.399		0.296	0.201	0.247	0.368	0.160	0.083	0.302	0.425	0.106	0.338	0.363	0.209	0.353	0.247	0.332	0.248	0.330	0.077
	BJ 13 D	0.399	0.399	0.399		0.546	0.226	0.584	0.438	0.427	0.401	0.463	0.441	0.502	0.466	0.610	0.331	0.412	0.192	0.420	0.623	0.363
	BJ 42 D	0.234	0.234	0.234	0.234		0.126	0.553	0.525	0.395	0.550	0.431	0.351	0.220	0.307	0.578	0.238	0.379	0.159	0.386	0.401	0.330
	BJ 88 D	0.125	0.125	0.125	0.125	0.125		0.303	0.022	0.130	0.042	0.172	0.091	0.268	0.563	0.071	0.156	0.238	0.074	0.447	0.259	0.254
	BJ 126 D	0.234	0.234	0.234	0.234	0.234	0.125		0.622	0.436	0.470	0.530	0.508	0.388	0.599	0.439	0.522	0.665	0.264	0.367	0.567	0.134
	BJ 129 D	0.234	0.234	0.234	0.234	0.234	0.125	0.399		0.350	0.560	0.328	0.539	0.178	0.263	0.529	0.256	0.273	0	0.339	0.354	0.168
	BJ 331 D	0.289	0.289	0.289	0.289	0.234	0.125	0.234	0.234		0.552	0.374	0.413	0.286	0.248	0.520	0.423	0.320	0.043	0.260	0.404	0.273
	BJ 342 D	0.234	0.234	0.234	0.234	0.399	0.125	0.234	0.234	0.234		0.349	0.271	0.137	0.347	0.613	0.458	0.295	0.199	0.234	0.377	0.248
	BJ 367 D	0.234	0.234	0.234	0.234	0.399	0.125	0.234	0.234	0.234	0.399		0.331	0.324	0.411	0.258	0.219	0.358	0.083	0.364	0.503	0.190
	DA 8 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125		0.183	0.206	0.415	0.320	0.217	0.125	0.343	0.297	0.232
	DA 115 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125		0.385	0.289	0.311	0.330	0.106	0.203	0.287	0.156
	DA 128 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125		0.251	0.337	0.420	0.129	0.564	0.378	0.180
	DS 29 D	0.453	0.453	0.453	0.453	0.344	0.125	0.344	0.344	0.453	0.344	0.344	0.125	0.125	0.125		0.305	0.262	0.048	0.264	0.469	0.336
	DS 139 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125		0.346	0.307	0.222	0.430	0.114
	LM 270 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125		0.284	0.372	0.452	0.191
	MA 284 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125		0.075	0.159	0.088
	MA 370 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.328	0.256
	MA 401 D	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.365
	PA 118 D	0.234	0.234	0.234	0.234	0.234	0.125	0.399	0.399	0.234	0.234	0.234	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.273
	TI 221 D	0.344	0.344	0.344	0.344	0.344	0.125	0.344	0.344	0.344	0.344	0.344	0.125	0.125	0.125	0.563	0.125	0.125	0.125	0.125	0.125	0.357

Table 2. Coefficients of parentage of the African group calculated by AFLP (above diagonal) and by Malécot's method (below diagonal)

	African parents																			
	BJ 210 P	BJ 216 P	BJ 220 P	BJ 248 P	DS 70 P	LM 2 T	LM 7 T	LM 9 T	LM 239 T	LM 312 P	LM 451 T	LM 718 T	MA 300 P	MA 313 P	MA 315 P	MA 840 T	MA 845 T	MA 851 T	RS 3 T	YO 4 T
BJ 210 P	0.524	0.620	0.448	0.042	0	0	0.061	0	0	0.104	0	0	0	0	0	0	0.133	0	0.375	0.103
BJ 216 P	0.313	0.463	0.441	0.330	0	0	0.124	0.034	0	0	0.163	0.071	0	0	0	0	0.123	0	0.237	0.234
BJ 220 P	0.313	0.313	0.467	0	0	0	0.089	0.067	0	0	0.129	0	0	0	0	0	0.085	0	0.329	0.129
BJ 248 P	0.313	0.313	0.313	0.009	0	0	0.030	0	0	0	0.008	0	0	0	0	0	0.019	0	0.088	0.003
DS 70 P	0.250	0.250	0.250	0.250	0	0	0	0	0	0	0	0.134	0.038	0.017	0	0	0	0	0	0.022
LM 2 T	0	0	0	0	0	0.329	0.194	0.1095	0.252	0.712	0.110	0.212	0.116	0.168	0.166	0	0.086	0	0	0.282
LM 7 T	0	0	0	0	0	0.125	0.175	0	0.279	0.373	0	0.192	0.002	0	0	0	0	0	0	0.259
LM 9 T	0	0	0	0	0	0.125	0.125	0	0.162	0	0.249	0.104	0.014	0	0	0.102	0.058	0.076	0	0.424
LM 239 T	0	0	0	0	0	0	0	0	0.088	0.161	0.460	0	0	0	0	0.136	0	0	0.196	0.192
LM 312 P	0	0	0	0	0	0.250	0.063	0.063	0	0.340	0.039	0.069	0.094	0	0	0	0	0	0	0.185
LM 451 T	0	0	0	0	0	0.250	0.063	0.063	0	0.125	0.056	0.151	0	0.051	0	0.165	0	0.087	0	0.444
LM 718 T	0	0	0	0	0	0	0	0.172	0	0	0	0	0	0.069	0.323	0	0	0	0.259	0.198
MA 300 P	0	0	0	0	0	0	0	0	0	0	0	0	0.250	0.089	0.371	0.087	0	0.313	0	0.121
MA 313 P	0	0	0	0	0	0	0	0	0	0	0	0	0.250	0.250	0.250	0.202	0.028	0.187	0	0
MA 315 P	0	0	0	0	0	0	0	0	0	0	0	0	0.250	0.250	0.250	0.250	0.169	0.320	0.027	0.077
MA 840 T	0	0	0	0	0	0	0	0	0	0	0	0	0.250	0.125	0.125	0.125	0.125	0	0	0.031
MA 845 T	0	0	0	0	0	0	0	0	0	0	0	0	0.250	0.250	0.250	0.250	0.125	0	0	0
MA 851 T	0	0	0	0	0	0	0	0	0	0	0	0.125	0	0	0	0	0	0	0	0.028
RS 3 T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
YO 4 T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

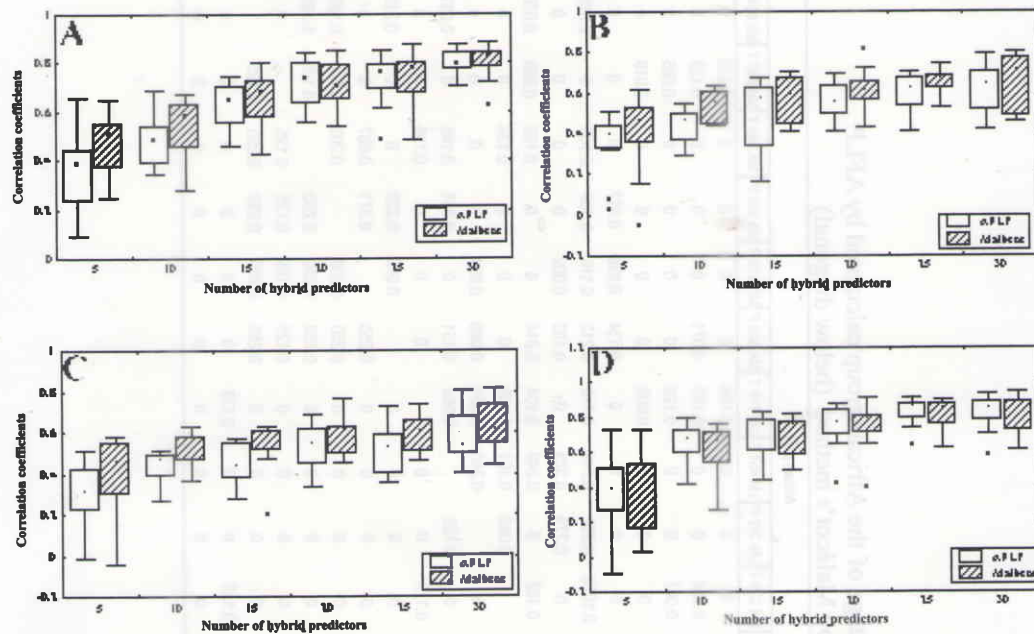


Figure 1. Correlation between observed and predicted oil palm performances according to the nature of coefficients of parentage calculated using AFLP data and Malécot's method (A = bunch number; B = bunch production; C = oil production; D = height increment rate).