African Journal of Biotechnology Vol. 7 (20), pp. 3550-3553, 20 October, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Molecular variability from two selection of BRT10 population in an inbreeding program of oil palm (*Elaeis guineensis* Jacq.) in Côte d'Ivoire

Désiré Allou[1]*, Benjamin Adon[2] and Abdourahamane Sangare[1, 2]

[1]Laboratoire de génétique, Université de Cocody, UFR des Biosciences, 22 B P 723 Abidjan 22, Tel (225), Cote d'Ivoire.
[2]Centre National de Recherche Agronomique, 01 B P 1740 Abidjan 01, Tel (225) 23 47 24 24 Fax (225) 23 47 24 11, Cote d'Ivoire.

Accepted 15 February, 2008

Genetic variability of two groups of palms composed with four progenies selected in BRT10 improved populations resulting from successive self-fertilizations of two parents LM2T and LM10T was studied using four polymorphic microsatellites DNA markers of *Elaeis guineensis* Jacq. The molecular variability of those progenies was analyzed and genetic diversity of each group of palms was deduced. The results of analysis of molecular variance revealed that 59.68% of variance can be explained by palms within each progeny, while 31.24% of total variance was partitioned between the two groups of palms. The program selection of self-fertilizations has created a distinct molecular structure within BRT10 improved populations resulting from LM2T and LM10T. Molecular analysis showed that the partitioning of variance in this breeding program was higher among palms within each progeny than among the two groups of palms. Only the exploitation of molecular data revealed the truly diversified progeny. The use of molecular data analysis in support with agro-morphological assessment should improve the cross performance while maintaining the genetic variation for further progress in subsequent selection cycles.

Key words: Selection, oil palm, molecular variability, BRT10 population.

INTRODUCTION

Reciprocal recurrent selection (RRS) was proposed by Comstock et al. (1949) as a cyclical method for simultaneous improvement of two groups of populations. Following this proposal, Meunier and Gascon (1972) adapted in Côte d'Ivoire a RRS program for oil palm breeding. In this program, completed by a program of self-fertilization, two complementary sets of populations A and B were used as a basis for oil palm breeding. The A group characterized by production of small number of big bunch is composed of DELI populations coming from various geographical origins. The B group characterized by production of big number of small bunch is composed of population from several geographical origins in which BRT10 population was the most used. The primary goal of RRS is to improve the cross performance between the two complementary populations while maintaining the genetic variation for further progress in subsequent selection cycles. The program of self-fertilization accompanying the RRS process has for objective a better knowledge of the material and possibly a classic selection beginning by self-fertilization. The program of self-fertilization permits the elimination of some parents chosen after test of progenies but holders of flaws. The main population of BRT10 was initially composed of 79 oil palm trees and was demonstrated to possess a limited genetic variability (Meunier, 1969; Adon, 1995). After two program of selection, the BRT10 improved population was more restricted (Cao, 1995). It was finally composed of progenies resulting from successive self-fertilization of two parents (LM2T and LM10T) among 79 oil palm trees which composed the initial parents' population.

Genetic variability assessment based on agro-morphological information which has been widely used in

*Corresponding author. E-mail: desire_allou@yahoo.fr. Tel: (225) 07 57 12 31.

Table 1. List of microsatellites used for PCR amplification of some oil palm ADN samples.

Microsatellite primers of oil palm		Specification	
Primer	**Code**	**Hybridization temperature (°C)**	**Allele average size (pb)**
mEgCIR3362	Eg3362	52	151
mEgCIR3785	Eg3785	52	284
mEgCIR3546	Eg3546	52	286
mEgCIR3543	Eg3543	52	232

the oil palm evaluation is no longer sufficient. Therefore, assessment of genetic variability within BRT10 improved parent populations was limited. Microsatellites (i.e., SSRs) are becoming the markers of choice for genetic diversity studies in a wide range of living organisms (Gupta and Varshney, 2000). The availability and abundance of Simple Sequence Repeats (SSR) markers throughout the oil palm genome, their polymorphic nature, codominance, and polymerase chain reaction (PCR)-based assay make SSRs useful in detecting real genetic variability (Billotte et al., 2001).

The aim of this study was to estimate genetic variability based on molecular information within BRT10 improved population after two cycles of selection. Analysis of molecular variance (AMOVA) was performed to detect molecular variability.

MATERIALS AND METHODS

BRT10 improved material

Four progenies (LM9344, LM10129, LM12011 and LM12030) resulting from successive self-fertilization of two parents (LM2T and LM10T) were identified to be the better population representing BRT10 improved material. A first group of palms (LM2TSFSF) resulting from successive self-fertilization of LM2T was composed of progenies LM9344 and LM10129. A second group of palms (LM10TSFSF) resulting from successive self-fertilization of LM10T was composed of progenies LM12011 and LM12030. Molecular analysis was performed on a total of 18 oil palm trees selected among the better bunch producer: namely 9 palms identified within LM2TSFSF group of palms and 9 palms identified within LM10TSFSF groups of palms.

DNA extraction and SSR procedure

Total genomic DNA was extracted from young leaves of 1-week-old by using modified cetyl trimethyl ammonium bromide (CTAB) method (Murry and Thompson, 1980) according to Rusterucci et al. (2000). DNA preparations were quantified by comparing fluorescence intensities of ethidium bromide stained samples to standards DNA (1, 0.50, 0.25 and 0.10 µg) on 0.8% agarose gels. DNA amplifications were performed according to Billotte et al. (2001) in a Biometra UNO II thermal cycler (Whatman Company) with 25 µL reaction volume. SSR reaction contained 5 µL (5 ng) genomic DNA, 2 µL forward primer (1 pmol μL^{-1}), 2 µL reverse primer (1 pmol μL^{-1}), 0.25 µL MgCl (25 mM), 2.5 µL reaction buffer (10 x), 2 µL dNTPs (50 mM), 1 µL Taq DNA polymerase (10U μL^{-1}) and 10.25 µL ddH_2O. PCR reaction conditions consisted of 5 min at 94°C for initial denaturation, followed by 35 cycles of polymerization reaction. Each reaction consist in a denaturation step of 30 s at 94°C, an annealing step of 1 min at 52°C, a polymerization step of 1 min at 72°C, followed by a final polymerization step of 8 min at 72°C. A total of 4 primer pairs published by Billotte et al. (2005) (Table 1) were used for PCR amplifications. An equal volume of formamide loading buffer was added and samples were denatured at 94°C for 3 min. 1 µL of each sample was loaded on to a 25 cm, 6%w/v denaturing polyacrylamide gel according to Creste et al. (2001). The fragment sizes were compared to a 50 to 350 bp standard ladder (MWG Biotech AG, Ebersberg, Germany).

Molecular data scoring

DNA fragments generated by SSR procedure were scored manually as binary data with presence "1" and absence "0," based on the SSR pattern amplified by each primer combination. Each column of the resulting binary (0/1) matrix represented one allele of the corresponding SSR locus were achieved by computer using Microsoft Office Excel (2003).

Statistical analysis

Genetic variability assessment based on molecular information was calculated by Genetix v4.05 as number of polymorphic loci, average number of alleles per locus, and expected mean heterozygosity. Analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed by Arlequin (Schneider et al., 2000) to detect differences in the distribution of multilocus genotypes with respect to the following patterns of variation: between group of palms, among progeny within group, and among palms within progeny. The sources of variation include two groups of palms (i.e., LM2TSFSF and LM10TSFSF), 4 progenies (i.e., LM9344, LM10129 from LM2T self-fertilization, and LM12011, LM12030 from LM10T self-fertilization) and 18 selected palms that represent total sample of BRT10 improved population. AMOVA was performed to evaluate variation in multilocus genotypes between and within the two groups of palms. The statistics tests of this analysis are composites of the expected mean squares associated with each source of variation. The AMOVA statistical model used was based on the formula:

$$Y_{lki(j)} = Y + r_l + a_{l(k)} + b_{lk(i)} + w_{lki(j)}$$

Where $Y_{lki(j)}$ = included value between 0 and 1 for j allele from i palm from k progeny from l group, r_l = effect of l group which variance is r^2, $a_{l(k)}$ = effect of k progeny from l group which variance is a^2, $b_{lk(i)}$ = effect of i palm from k progeny from l group which variance is b^2, $w_{lki(j)}$= effect of j locus from i palm from k progeny from l group witch variance is c^2.

RESULTS AND DISCUSSION

In BRT10 improved population, the 4 loci revealed a total of 10 alleles, varying from 1 to 4 alleles per locus with an average of alleles varying from 1.75 to 3 alleles per locus

Table 2. Numbers of allele and expected heterozygoty detected by four microsatellite marker in BRT10 improved population.

Group	Progeny	Number of alleles					Expected heterozygosity (%)
		Eg3543	Eg3362	Eg3785	Eg3546	Mean	
LM2TSFSF	LM9344	3	1	1	1	1.5	14.50
	LM10129	2	2	1	1	1.5	23.44
LM10TSFSF	LM12011	4	2	-	3	3	55.56
	LM12030	2	2	-	2	2	38.89

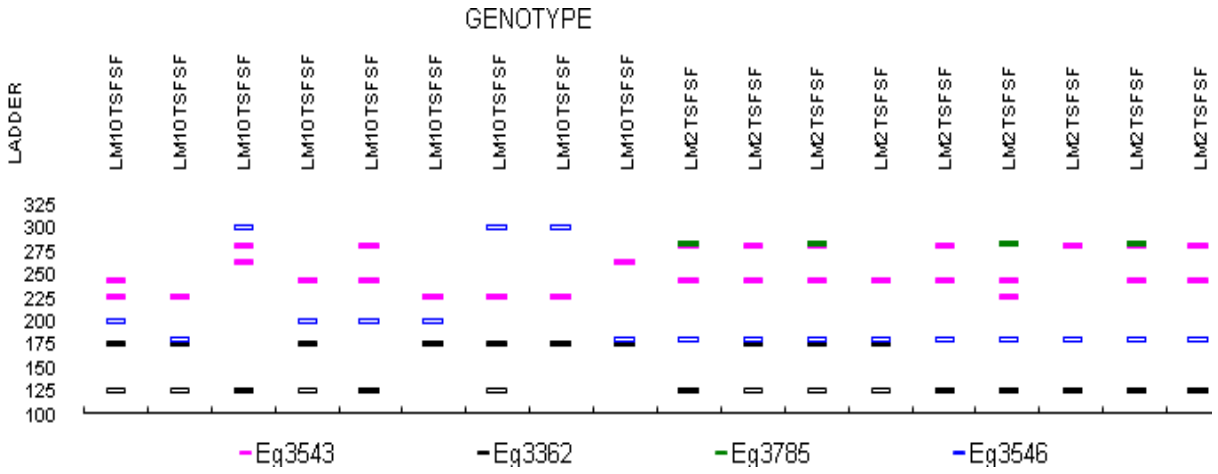

Figure 1. A model DNA fingerprint of the 18 samples used for evaluation of molecular variability after two 2 cycles of RRS based on microsatellite data.

Table 3. Analysis of molecular variance for the BRT10 improved population sampled from two groups of palms (LM2TSFSF and LM10TSFSF) after two cycle of selection program over 4 SSR loci.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	9.111	0.40159 Va	31.24
Among progenies within group	2	3.506	0.11671 Vb	9.08
Among palms within progenies	32	24.550	0.76719 Vc	59.68
Total	35	37.167	1.28549	

Fixation indices. FSC: 0.13204, FST: 0.40320, FCT: 0.31240.

(Table 2). Respectively, 7 and 9 different alleles were detected in LM2TSFSF and LM10TSFSF group of palms (Figure 1). One allele (Eg3785_283 pb) was unique in LM2TSFSF group and 3 alleles (Eg3543_263 pb, Eg3546_200 and Eg3546_300) were specific in LM10TSFSF group. There was a large difference for expected heterozygosity between the two groups of palms. Expected mean heterozygosity was 20.68% for LM2TSFSF group and 59.88% for LM10TSFSF group. Analysis of molecular variance revealed significant differentiation of alleles among BRT10 improved populations (FST = 0.40320, Pr = 0.0001). When molecular structure among palms was tested, the greatest percentage of variation was observed among pairs of palms within progeny (Table 3). Under AMOVA, 59.68% of the variance can be explained by palms within progeny, while 31.24% of total variance was partitioned between LM2TSFSF and LM10TSFSF groups. The amount of variation found among progeny within group of palms was the smallest (9.08%) of the three sources of variation. More genetic variation was observed for individual oil palm tree within progeny than for progeny within group of palms when determining molecular structure in BRT10 improved population germplasm.

After 2 cycle of selection, phenotypical variability observed in BRT10 improved population was practically

remained low for the character related to bunch number (Bakoume et al., 2001). Those authors have proposed the introduction of Yocoboue material, in the selection program, to improve the genetic variability of B group. However, low phenotypical variability was also observed in Yocoboue wild population (Bakoume, 1999) and even in certain families of clones (Soh et al., 2003). The use of molecular data showed strong genetic variability in BRT10 selected population. Allelic variability was stronger in LM10TSFSF group than in LM2TSFSF group. Using molecular data in support of oil palm genetic selection remain an effective means to evaluate the real genetic variability among the improved material. Indeed, the oil palm selector can imperatively control the impact of self-fertilization effect due to agro-morphological assessment of genetic variability in order to reduce, in a very significant way, all risks related to the weakness of genetic variability in the selected populations. The genetic originalities of certain progenies resulting from self-fertilization of LM2T and LM10T were exploited for the 2nd cycle of RRS by Gascon et al. (1988) and this originality can nowadays be confirmed by the molecular data analysis. Even if strong phenotypical variability was not observed in improved population of BRT10, it is not exclusive due to genotypic variability. In fact, effects of environment can bring error to genetic diversity judgments. The exploitation of molecular data in support of the agro-morphological data can reveal that the diversification of BRT10 improved population is due to a distinct molecular structure within and between progenies and in particular between certain plants within progenies.

Conclusion

RRS program has created a distinct molecular structure within and between LM2TSFSF and LM10TSFSF genotypes of BRT10 improved populations. Molecular analyses of oil palm tree in Côte d'Ivoire show that the partitioning of variance in the breeding program of BRT10 material is higher among plants within progenies than among progenies within group of palms. Certain progenitor lines were highly variable. Some of that variation can be explained by the allele variability among plants within progenies. This repartitioning of the variance can be exploited by molecular studies even if phenotypic studies show a decrease in variance of BRT10 population over time.

REFERENCES

Adon B (1995). Evaluation des introductions de palmier à huile (*Elaeis guineensis* Jacq.): utilisation dans le schéma de sélection récurrente réciproque. Thèse de Doctorat 3è cycle. Génétique et amélioration des productions végétales. Faculté des Sciences et Techniques. Abidjan, Côte d'Ivoire, p. 117.

Bakoume C (1999). Evaluation des palmiers à huile (*Elaeis guineensis* Jacq.) issus du peuplement naturel de la région de Yocoboué émoire de D.E.A. Université de Cocody. UFR Biosciences. Abidjan, Côte d'Ivoire, p 27.

Bakoume C, Adon B, Cochard B, Potier F, Durand-Gasselin T, Amblard P (2001). Assessment of yocoboue wild oil palm (*Elaeis guineensis* Jacq.) from Côte d'Ivoire, Euphytica, 121: 59-64.

Billotte N, Rusturicci AM, Barcelos E, Noyer JP, Amblard P, Baurens FC (2001). Development, characterization, and across-taxa utility of oil palm (*Elaeis guineensis* Jacq) Microsatellite markers. Genome. 44: 413-425

Billotte N, Marseillac N, Risterucci AM, Adon B, Brottier P, Baurens FC, Singh R, Herran A, Asmady H, Billot C, Amblard P, Durand-Gasselin T, Courtois B, Asmono D, Cheah SC, Rohde W, Ritter E, Charrier A (2005). Microsatellite-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.) TAG Theor. Appl. Genet. 110: 754-765.

Cao TV (1995). Organisation de la variabilité génétique chez le palmier à huile (*Elaeis guineensis* Jacq.). Conséquences pour l'amélioration des populations et la création variétale. Thèse de Doctorat, Institut National Agronomique, Paris-Grignon, p. 200.

Comstock RE, Robinson HF, ET Harvey PH (1949). A breeding procedure designed to make maximum use of both general and specific combining ability. J. Am. Soc. Agron., 41: 360-367.

Creste S, Tulmann Neto A, Figueira A (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. Plant Mol. Biol. Rep. 19: 299-306.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplo types: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Gascon JP, Le Guen V, Nouy B, Asmady, Kamga Fondjo F (1988). Résultats d'essais de second cycle de sélection récurrente réciproque chez le palmier à huile Elaeis guineensis JACQ. Oléagineux, 43: 1-7.

Gupta PK, Varshney RK (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with the emphasis on bread wheat. Euphytica 113: 163-185.

Meunier JET, Gascon JP (1972). Le schéma de sélection du palmier à huile à l'I.R.H.O. Oléagineux. 27: 1-12.

Meunier J (1969). Etude des populations naturelles d'*Elaeis guineensis* Jacq. en Côte d'Ivoire. Oléagineux 24: 195-201.

Microsoft Office Excel (2003). Partie de Microsoft Office Professional Edition 2003. Microsoft Corporation.

Murry HG, Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321-4325.

Schneider S, Roessli D, Excoffier L (2000). *Arlequin* ver. 2. A software for population genetics data analysis. Genetics and Biometry Laboratory, Univ. of Geneva, witzerland. (Available on-line from http://lgb.unige.ch/arlequin; verified 14 September 2007).

Soh AC, Gan Hh, Wong G, Hor TY, Tan CC (2003). Estimates of within family genetic variability for clonal selection in oil palm. Euphytica 133: 147-163.