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GENETIC AND ENZYMATIC POLYMORPHISMS OF INTERSPECIFIC *DIOSCOREA PRAENSILIS-DIOSCOREA ROTUNDATA* HYBRIDES FROM CÔTE D'IVOIRE

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ABSTRACT

Yam is a crop that is found in tropical areas. It greatly contribute to populations food in these areas, namely in Africa. In Côte d'Ivoire, yam is the first among food crops with an annual production of 5.8 million tons in 2013. In spite of the importance of this food crop, it is noticed a weak level of yield due to the lack of planting material for plantation establishment. Moreover, there is a decraese of Malgré l'importance de cette culture, il est constaté à une insuffisance de la production liée au manque de semenceaux pour l'établissement de plantations. In addition, there is a decrease of performance related to the use of unimproved varieties for which extensive culture is used to satisfy increasing demand. To face land scarcity that limits extensive culture and the constraints linked to climate change, productivity improvement appears crucial. Thus, thirty four hybrids obtained by controlled crossing between *Discorea prahensilis* (wild species) and *Dioscorea rotundata* (cultivated species) were analysed using isozymes electrophoresis technics with starch gel. Five enzymatic systems (MDH, SKDH, EST, PGD and PGI) were studied. MDH, PGD and SKDH systems were polymorphic in the tested population while EST and PGI appeared monomorphic. This study revealed a genetic diversity within yam hybrids population. The objective of the current study is to improve yams productivity. More specifically, it aims at characterizing the diversity within hybrids population for a better organization. At the end of the established organization, agronomic and morphologic traits of these hybrids need to be assessed to choose those usefull ones for productivity improvement.

INTRODUCTION

Yam (*Dioscorea spp*) is an herbaceous plant and a monocotyledonous angiosperm that multiply vegetatively by tuber. Yam, according to its global yield estimated at roughly 60.2 million tons per year (FAOSTAT, 2013) occupies worldwide the fourth position after potato, sweet potato and cassava. In terms of rentability for farmers, yam has a weak productivity with 6.95 tons/ha (FAOSTAT, 2013) while this one from potato is 30 tons/ha. In Côte d'Ivoire, yam (Dioscorea spp) occupies the first position food crops yield. Its annual yield is estimated at 5.8 millions de tons of fresh tuber in front of cassava whom annual yield reaches 2.4 million tons (FAOSTAT, 2013).Yam tubers are source of food for millions of people. They serve as basic food for 2/3 of Ivorian population. With 525 kcal/person/day, yam is ranked at the second place among the ten first products available for consuption after rice (552 kcal/person/day) and the first place as tuber cop followed by cassava (315 kcal/person/day)

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according to FAOSTAT, (2007). In spite of the first place occupied by yam among food crops its annual yield is insufficient to satisfy alimentary needs of Ivorian population characterized by a high increase rate and whom consumption is estimated to 350 kg/habitant/year (Odounfa, 1990). One of the causes of this insufficiency is the lack of planting material. Indeed, for the establishment of a yam plantation, the quantity of needed planting material if too high and is equivalent to a little bit more than 1/3 of tubers harvested for consumption. Moreover, planting material acquirement is costy for farmer setting up a first plantation and represents 33 % to 40 % of total production cost. Also the low productivity is due to the use abundant use of local varieties in comparison to improved varieties that are weakly used. Insufficiency of improved material leads to exploitation of large acreages to rise up available quantity of yam tubers. But, yam plantations could not be extended cause the lack of planting material. In addition, cultivation of yam by burn and shifting cultivation is consumer of land.

To overcome the poor performance of the cultivated varieties, one of the recommended way was the breeding to improve crops by transferring their characters of interest. Thus, crosses between Dioscorea praehensilis (wild species) and Dioscorea rotundata (cultivated variety) allowed to create hybrids that were not yet the subject of characterization. These works are therefore conducted in order to evaluate hybrids of these crosses, to estimate genetic diversity, through the enzyme variability expressed in these hybrids and highlighting the more discriminative enzymatic variables, for a better structuring of this population to meet promoting needs at research level. fait l'objet de caractérisation. Ces travaux sont donc conduits dans l'optique d'évaluer les hybrides issus de ces croisements, afin d'estimer la diversité génétique, à travers la variabilité enzymatique exprimée au sein de ces hybrides et de mettre en exergue les variables enzymatiques les plus discriminantes, en vue d'une meilleure structuration de cette population pour répondre à des besoins de valorisation au plan de la recherche.

MATERIALS AND METHODS

Matériel végétal

Plant material used consists of thirty-four (34) hybrids accessions from crosses between *Dioscorea praehensilis* (wild: IB 120 female parent) and *Dioscorea rotundata* (cultivated: krengle OA 20 male parent) in conservation at the in vitro culture laboratory of the Central Laboratory of biotechnology (LCB) at the Centre National de Recherche Agronomique (CNRA).

Experimental method

Method used is the technique of isozyme electrophoresis on starch gel described by Zoundjihekpon (1993), Dansi *et al.* (1999) and (Koffi *et al.* 2015).

Yam isozymes electrophoresis

Technique described by Second and Trouslot (1980) in rice was used in this study. Four polymorphic enzymatic systems with a good resolution (Zoundjihékpon, 1986) were used for testing all planting material studied. These systems are (Jarret and Litz, 1986): phosphoglucose isomerase (PGI), esterase (EST), phosphatase acide (PAC) and malate dehydrogenase (MDH).

Electrophoresis implementation

Buffers and solutions

Three types of buffer were used: gel preparation buffer, migration or electrode buffer and enzymes extraction buffer. Before migration reaches the end frontline, revelation solutions containing specific substrates from different enzymes and their cofactors were mixed in a 50 ml beaker. Revelation solutions composition and those of different enzyme buffers (PGI, EST, PGD, SKDH and MDH) were made according to Lidah (2005) and Lidah *et al.* (2006).

Gel preparation

14 % Starch was prepared as gel one day before use. 50 g of hydrolised starch potato (SIGMA STARCH S-4501 USA) for electrophoresis was dissolved in 400 ml buffer and heated to 300°C in 1 L Erlenmeyer flask. Gas in the gel was pulled out with a vacuum pomp and poured in a 20 cm x 17 cm mould. Gels were cooled at room temperature overnight and covered with a plastic film.

Enzyme extraction

Young leaves (600 mg) from yam apexes were harvested on individuals from accessions and ground in mortar with extraction buffer (Zoundjihépkon and Touré, 1983). Crude extract contains enzymes and Polyvinylpolypyrolidone (PVP) in extraction buffer enables chelating phenolic compounds. Dealing with 2-mercaptoéthanol, it avoids enzymes oxidation. Whatman paper number 3 was cut in small square pieces and put on top of toilet paper (Kleenex) laid on crude extract in each mortar. An average of 20 samples was ground at each extraction for the two gels, leading to a total of 40 samples per manipulation.

Migration

Whatman paper number 3 pieces embedded with proteic crude extract were inserted into wells made 4 to 5 cm under the top of the gel. Drops of Bromophenol Blue (BB) were laid down on top of wells as migration front indicator. 20 samples were put along the 19 cm gel and run. Mould containing gel was set on a horizontal support constituted of two tanks filled with migration buffer and connected to power supply. Two designs were established at each manipulation: one containing Histidin pH 6 buffer for MDH, PGI and EST systems and the other contained Histidin pH 8 buffer for SDH and PGD systems. Gel at pH 6 was run under a constant current at 25 mA for 1 hour, from 35 mA for 3 hours and 40 mA for 2 hours. Gel at pH 8 was submitted to a constant current at 30 mA for 1 hour, 40 mA for 3 hours and 45 mA for 2 hours. All these steps were undergone at low temperature to avoid any warming. For that, a tank filled with ice was placed on top of the gel. Protein migrated from cathod (-) to anod (+) under influence of the

electric field that was proportional to its charge and spacial volume. Migration took end when Bromophenol Blue migrated to an equivalent distance of 9 cm.

Enzymes staining

Staining was made after migration. Gel was cut in many thin slides of 1.5 mm thickness. Each slide was used to stain one enzymatic system. So, it was possible to stain more enzymatic systems. Staining consists in evidencing presence of a given enzymatic system through its specific coloring that is a result of its reaction with its substrate (Harris et Hopkinson, 1976; Pasteur *et al.*, 1987). Each slide was added a reacting mixture including the specific substrate of the given enzyme. The reacting mixture added to the slide of gel was incubated in an oven at 45°C until appearance of enzymatic bands. When coloring intensity of bands was thought sufficient, reaction was stopped by adding a 10 % acetic acid solution.

Gel analysis after staining

Reading of gels and codification of different profiles were made from the positioning of alleles or colored migration bands and according to the direction of migration.

Statistical analysis

Method used is the Principal Component Analysis (PCA) using the XLSAST software. The PCA is a method of analysis that allows to evaluate the similarity between individuals, in order to evidence homogeneous groups of individuals using relationships among variables.

RESULTS

Enzymatic profiles description

Enzymatic systems EST and PGI were monomorphic for all individuals, while the three others MDH, PGD and SKDH were polymorphic with one or two areas of activities. In monomorphic systems (EST and PGI), revealed individuals expressed identical profile consisting of a single band. They are homozygous with genotype ESTa/ESTa and PGIa/PGIa (Figures 1 and 2).

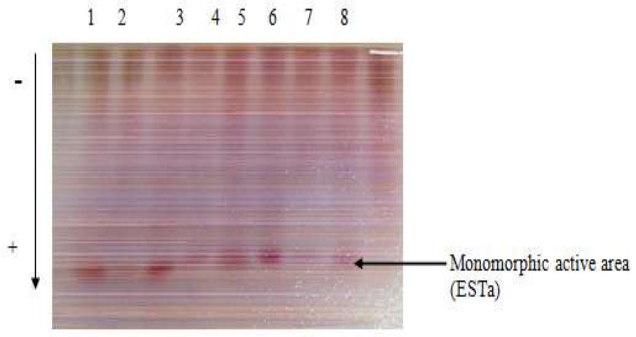

Figure 1. Enzymatic pattern of Esterase system (EST)
1 to 8: yam hybrides samples analysed
1 à 8: ESTa

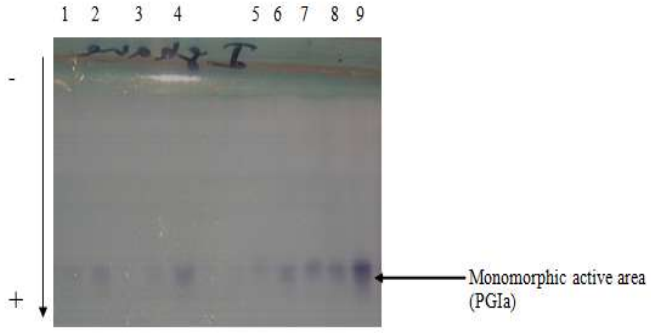

Figure 2. Enzymatic pattern of Phosphoglucose Isomerase system (PGI)
1 to 9 : yam hybrides samples analysed
1 to 9 : PGIa

Dealing with the other systems, observed enzymatic profiles were different, showing variability in the hybrids analyzed. This variability focused on one locus for each of MDH, PGD, and SKDH systems. In the case of the MDH system, at the level of this locus, two bands were evidenced corresponding each to phenotype MDH-Ia (band 1) and MDH-Ib (band 2). The observed individuals were all homozygous (Figure 3). PGD and SKDH systems presented different profiles. Indeed, they showed, in addition to the variability on the two bands corresponding to the homozygous genotypes, individuals with both the two bands indicating that they are heterozygous (Figure 4 and 5).

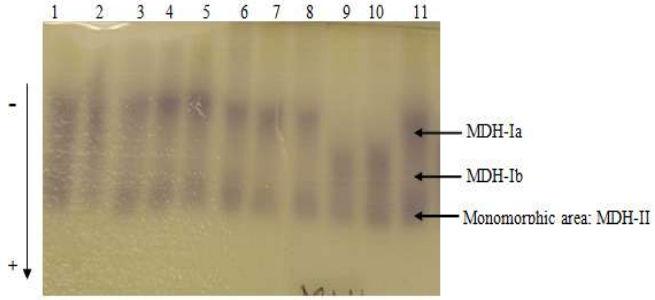

Figure 3. Enzymatic pattern of Malate deshydrogenase system (MDH)
1 to 11 : yam hybrides samples analysed
1, 2, 3, 4, 5, 6, 7, 8, 11: MDH-Ia and 9,10 : MDH-Ib

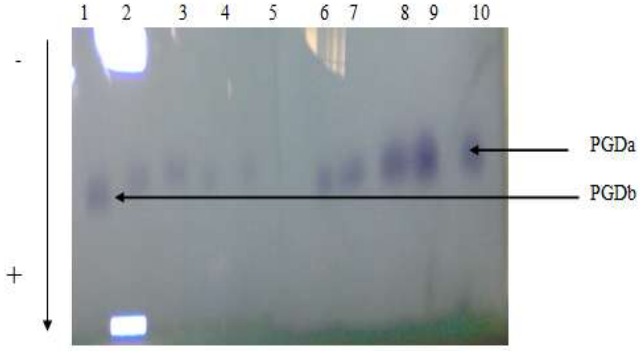

Figure 4. Enzymatic pattern of Phosphoglucose Dehydrogenase system (PGD)
1 to 10 : yam hybrides samples analysed
1 : PGDa and 2, 3, 4, 5, 6, 7, 8, 9,10 : PGDb

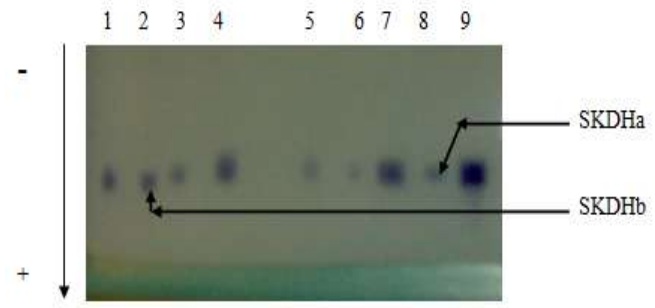

Figure 5. Enzymatic pattern of Shikimate Dehydrogenase system (SKDH)
1 to 9 : yam hybrides analysed
1,2 : SKDHb ; 3, 5, 6,8 : SKDHa and 4,7,9 : KDHa/SKDHb

Variability assessment within all individuals

Codified representation of different enzymatic systems showed existence of several profiles in polymorphic systems. Homozygous profiles were in majority in all systems: 100% (MDH), 94.1% (PGD) and 76.5% (SKDH) at the opposite of heterozygous profiles that were in minority (0%: MDH; 5.9%: PGD and 23.5%: SKDH). SKDH system recorded the highest percentage of heterozygous in comparison to other systems (Table 1).

Table 1. Variability evidenced in three polymorphic enzymatic systems (MDH, PGD and SKDH) after reading of gels

	Enzymatic systems					
N° accession	PGDa	PGDb	SKDHa	SKDHb	MDH-Ia	MDH-Ib
kp01	0	1	0	1	1	0
kp05	0	1	0	1	0	1
kp01hi04	0	1	1	0	0	1
kp01hi71	0	1	0	1	0	1
kp06hi698	0	1	0	1	1	0
kp01hi284	0	1	0	1	1	0
kp06hi563	0	1	0	1	1	0
kp06hi582	0	1	0	1	0	1
kp06hi524	1	0	0	1	1	0
kp06hi578	0	1	0	1	1	0
kp06hi407	0	1	0	1	1	0
kp06hi577	0	1	0	1	1	0
kp08	1	1	0	1	0	1
kp01hi282	0	1	1	1	1	0
kp06hi603	0	1	0	1	0	1
IB120	1	0	0	1	1	0
OA20	0	1	1	0	0	1
kp06hi572	1	0	1	0	0	1
kp06hi589	0	1	1	1	1	0
kp06	0	1	0	1	0	1
kp01hi29	0	1	0	1	1	0
kp06hi559	1	0	1	1	0	1
kp01hi112	0	1	0	1	0	1
kp06hi558	1	0	1	0	0	1
kp03	0	1	1	1	0	1
kp06hi605	0	1	0	1	1	0
kp06hi376	0	1	0	1	1	0
kp06hi699	1	0	1	0	0	1
kp06hi695	0	1	0	1	1	0
kp01hi27	0	1	0	1	1	0
kp06hi416	1	0	1	1	1	0
kp01hi91	0	1	0	1	1	0
Kp06hi418	1	0	1	0	1	0
Kp01hi190	0	1	1	1	1	0
Kp01hi523	0	1	1	1	1	0
Kp06hi619	1	1	1	1	0	1

Légende :
0 : lack of band ; 1 : presence of band ; IB120 : female parent ; OA20 : male parent

Distribution frequencies data from observed phenotypes within all the individuals tested (Table 2) showed that MDH systems presented two different phenotypes A and B whom distribution frequency was as followed: 58.82% for phenotype A and 41.19% for phenotype B. Concerning PGD and SKD systems they expressed three distinctive phenotypes (A, B and C). Distribution frequencies observed were 20.59 (PGD A) and 61.76% (SKD A), 73.53% (PGD B) and 14.71% (SKD B) and 5.88 (PGD C) and 25.53% (SKD C). Contrary to previous enzymatic systems, EST and PGI systeme presented one phenotype (A) with 100% of distribution frequency for each.

Table 2. Observed phenotypes frequencies after revelation of five enzymatic systems tested with all individuals studied

Enzymatic system	Phenotype	Genotype	Frequency (%)
MDH	A	Homozygous	58.82
	B	Homozygous	41.18
PGD	A	Homozygous	20.59
	B	Homozygous	73.53
	C	Heterozygous	5.88
SKDH	A	Homozygous	61.76
	B	Homozygous	14.71
	C	Heterozygous	23.53
EST	A	Homozygous	100.00
PGI	A	Homozygous	100.00

Variability assessment between parents and hybrides

SKD enzymatic profiles compared between parents and hybrids (Figure 6) showed that some hybrids have phenotypes and genotypes similar to parents, while others have different genotypes and phenotypes. Indeed, genotype of the latter is the combination of the genotypes of both parents. Proportion of heterozygous hybrids is ¼ (8) against ¾ (26) for homozygous hybrids. Hybrids phenotypically and genotypically similar to female parent presented a relatively high frequency (61.76%) in comparison to those resembling the male parent (14.71).

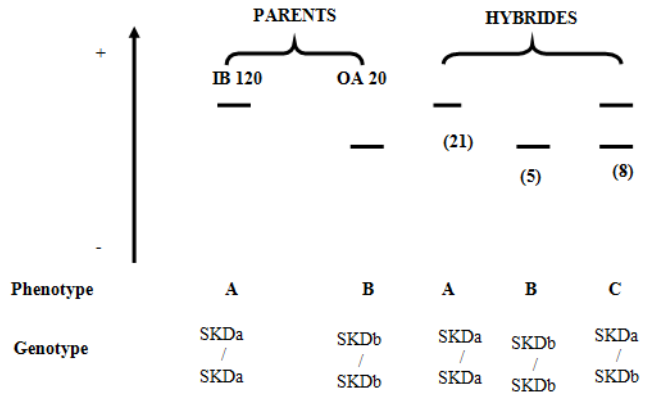

Figure 6. Enzymatic pattern of Shikimate dehydrogenase system (SKDH) compared between parents and hybrides
IB120 : female parent
OA20 : male parent
NB : Numbers in parenthesis are individuals recorded per phenotype

In regards to the PGD (Figure 7) system, hybrids exhibited phenotypes and genotypes similar to SKDH system, but proportions were different. Hybrids exhibiting female parent phenotype and genotype registered a frequency of 73.53% against 20.59% for those expressing phenotype and the

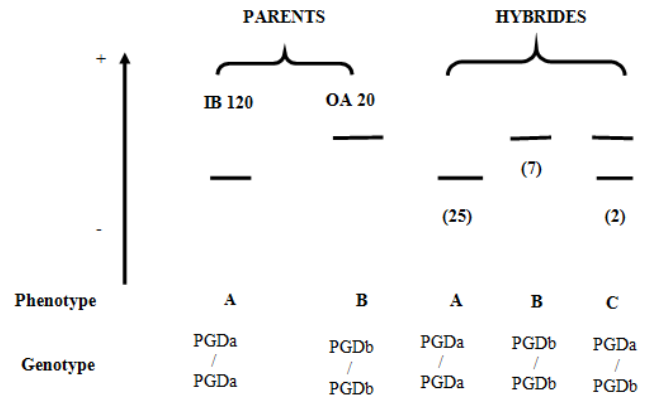

Figure 7. Enzymatic pattern of Phosphogluconate dehydrogenase system (PGD) compared between parents and hybrides (PGD)

IB120 : female parent

OA20 : male parent

NB : : Numbers in parenthesis are individuals recorded per phenotype

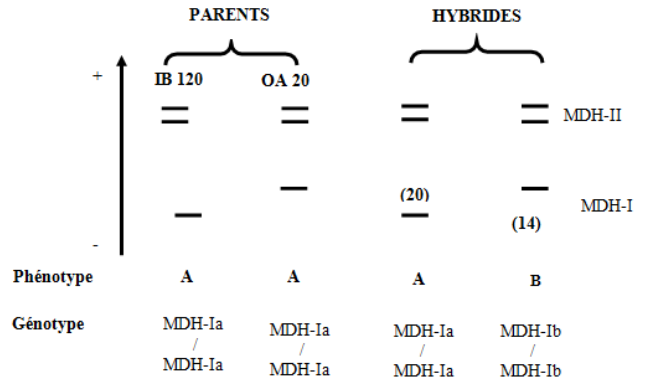

Figure 8. Enzymatic pattern of Malate dehydrogenase system (MDH) compared between parents and hybride

IB120 : female parent ; OA20 : male parent

NB : Numbers in parenthesis are individuals recorded per phenotype

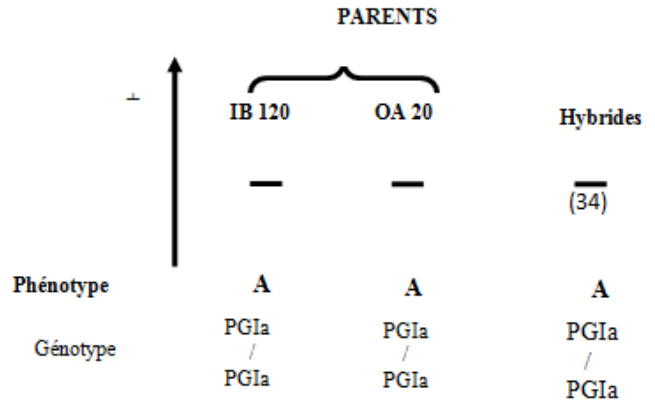

Figure 9. Enzymatic pattern of Phosphoglucose Isomerase system (PGI) compared between parents and hybrids

IB120 : female parent ; OA20 : male parent

NB : : Numbers in parenthesis are individuals recorded per phenotype

genotype of the male parent. As hybrids with phenotype and genotype different from two parents are concerned, they had a frequency of 5.88%. The proportion of these latter is 1/16 against 3/16 for hybrids with similar phenotype and genotype to male parent. Dealing with hybrids presenting identical phenotype and genotype to female parent, they had a proportion of 12/16. With regard to the MDH system (Figure 8), hybrids had phenotypes and genotypes similar only, either to the female parent, or to the male parent. Hybrids had proportions close to ½ for those resembling to female parent and ½ for those similar to the male parent. In the case of EST and PGI systems, hybrids presented phenotypes and genotypes similar to those of the two parents (Figures 9 and 10).

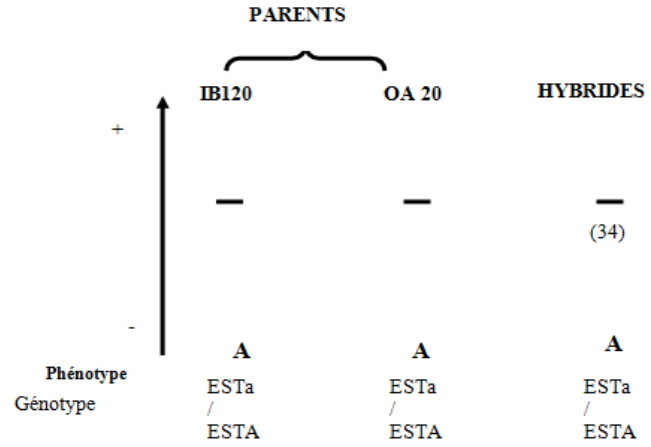

Figure 10. Enzymatic pattern of Esterase system (EST) compared between parents and hybrides

IB120 : female parent ; OA20 : male parent

NB : : Numbers in parenthesis are individuals recorded per phenotype

DISCUSSION

Segregation 1/4, 3/4 (in the case of system SKDH) and 1/16, 3/16, 12/16 (with the PGD system) allow to assert that the enzyme system SKDH is governed by one gene while the PGD system is under the control of two independent genes. The patterns observed in the current study on these systems are those of monomeric enzyme structure. These results are consistent with those of Zoundjihekpon *et al.* (1994); Dansi *et al.* (1999) and Tostain (1998). However, these authors revealed the presence of two areas of activities for PGD, in contrast to only highlight in our case. It's the quick migration zone that presents one to two bands. Indeed, epigenetic changes could be the basis for the deletion of the expression of the locus responsible of the biosynthesis of isozymes of this not readable zone on the gel. Segregation 1/2, 1/2 obtained for the MDH system lets say that this enzyme is synthesized by one gene. Furthermore, MDH presented two areas of activity within the study population: one slow and polymorphic and the other monomorphic. These results are at odds with those of Zoundjihepon (1993) obtained with progeny from the cross between species belonging to the complex D. cayenensis/ rotundata. The divergence of the results may be due to the difference of the plant material used.

Conclusion

The current study based on the use of enzyme markers showed the existence of a low genetic diversity in the population of

hybrids analyzed. However, two homogeneous classes were identified. These works also allowed identify three (3) polymorphic MDH, SKDH and PGD loci. These loci giving altogether 8 different alleles are heterozygous that were retained. Moreover, among obtained hybrids, there are also homozygous with phenotypes and genotypes of each of the parents.

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