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Self-fertility of Four Female Parent Clones of *Ananas comosus* L., Involved in a 6x6 Complete Diallel Mating System with Selfings using the Typological Approach

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ABSTRACT

To determine the cropping system that applies to four clones of *Ananas comosus* in on-farm trials, their behaviour under hand selfings was analysed on-station. Hybrid female clones 103-104-6, 410-106-33, 410-200-15 and Queen Victoria clone RE43 as well as clones HA10 and HA25 as controls were involved in a 6 x 6 complete diallel crossing system with selfings. The total seeds number derived from hand self-pollinations per week, mean seeds number obtained per self-pollinated flower per week, ripe fruit weight and bloomed flowers number per week were measured. The Anova, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were successively run. Results showed that 410-200-15 revealed self-incompatibility, while RE43 is found to be self-sterile. In the same way, 410-106-33 expresses self-sterile behaviour, whereas 103-104-6 showed more self-fertile characteristics. The behaviour under selfings of both clones 410-200-15 and 410-106-33 comes from their Smooth Cayenne female parent HA10 which was previously characterised as self-incompatible. The structurings provided by the Anova and HCA was globally comparable. The hybrid clone 410-200-15 can already subject to on-farm trials via single or mixed crop before authorising its popularisation. The clones 410-106-33 and 103-104-6 must be first subjected to successive back crosses before on-farm trials then their popularisation. The RE43 clone must be cultivated in on-farm trials via single crop. The morphological relatedness of four clones was discussed.

INTRODUCTION

Ananas comosus, fruits' king, is a perennial, monocotyledonous, herbaceous, diploid (2n=2x=50) plant of the Bromeliaceae (Guyot, 1992). Its cultivation covers all tropical and subtropical areas. It is used as textile, ornamental plant, among others. Its edible fruit is the object of a very active international trade with an annual world production over 14.6 million tons (Faostat, 2004; Malezieux, 2000). In Côte d'Ivoire, the cultivar Smooth Cayenne is the most cultivated. Sure enough the varieties HA10 and HA25 of Smooth Cayenne are the pillar of world pineapples industry. It is the same for variety RE43 of cultivar Queen Victoria that is also highly valued and marketed. This country is the third world exporter of fresh fruits with 188,000 tons in 2001 (http://en.wikipedia.org/wiki/Pineapple). Furthermore, the seedy fruits are not appreciated by the consumers and traders. Indeed the presence of seeds in fruit depreciates its quality (Py *et al*., 1984). A breeding programme of pineapple was initiated and conducted at the Anguededou Station / DFA (Département des Fruits et Agrumes) from the IDEFOR (Institut des Forêts for the first time) and CNRA (Centre National de Recherche Agronomique at the present time; Cabot, 1988). This programme aims to create some varieties intended for export of either fresh fruits or canning. Apart from the traditional criteria of yielding, quality and spineless, in the fist time, this programme also aims to prevent the internal fruit browning in increasing its content in ascorbic acid. In the second phase, it concerns adaptation at warm and dry climates, resistance to diseases and pests, especially nematodes and *Phytophthora* sp., as well as resistance to yellowing. The latter is a physiological disorder of the ripening. This programme consists of hybridising two cultivars : Smooth Cayenne and Mordilona and achieving the multi-characters selection of 40,000 hybrids created (Coppens d'Eeckenbrugge *et al*., 1997). From these created hybrids, nineteen were selected of which three 410-106-33, 410-200-15 and 103-104-6 were identified as the best (Cabot, 1988). Until now, we do not know the behaviour of these three hybrids in relation to the self-fertility or self-sterility. It seems that the three created hybrids are self-incompatible like HA10 and HA25 (Cabot, 1989). The knowledge of their either self-fertility or self-sterility could help to envision the cropping system of either single or mixed which could suit them.

The study aimed to determine the cropping system that applies to four female clones 103-104-6, 410-106-33 and 410-200-15 and Queen Victoria RE43 involved in a 6 x 6 complete diallel crossing system with selfings.

MATERIALS AND METHODS

Experimental site, plant material and hand self-pollinations

The experiment was conducted from September 1997 to April 1998. It was carried out in the Anguededou Research Station from DFA/IDEFOR (Département des Fruits et Agrumes /Institut Des Forêts) in Côte d'Ivoire. This Station is located in latitude 5°25', north and longitude 4°08', west as well as 25 m altitude.

Four common testors and parents 103-104-6, 410-106-33, 410-200-15 and RE43 as well as two others, namely HA10 and HA25 were used as controls . The first three are of the recently created hybrids and preselected like superior (Cabot, 1988). The clones 410-200-15 and 410-106-33 come from inter-variety crosses Smooth Cayenne x Perolera. The clone 103-104-6 comes from intra-variety cross Perolera x Perolera (Cabot, 1988). The Queen Victoria clone RE43, sweeter than cultivar Smooth Cayenne, is highly valued on the international market. The clones HA10 and HA25 belong to cultivar Cayenne. They are also well valued on the international market.

Moreover, the calcium carbide solution was applied in the leafy crown of each plant chosen as parents to force the flowering. Two months later, the inflorescences emerging from plants were bagged with the pollination bags. Every day (in the morning), the anthers surmounting the stamens were collected in Petri dish by using tongs. They were manually used either to self-pollinate or cross-pollinate the bloomed flowers. These ones were scored then marked with red oil painting. At maturity, the fruits were harvested and weighed. The seeds contained in individual fruits were also scored after dissection.

Experimental design, mating system and measured variables

All of parent clones already belonged to a pre-existent design. This consisted of plant of pineapple laid out on two rows. Six plants per clone were chosen as a function of their vigour and used as both common parents and testors. One for selfings and the five others for crossings. They were planted in two rows on the ridges. Plants had 40 x 25 cm spacings. A gap of 90 cm was maintained among ridges. Treatment consisted of a pineapple plant laid out on a ridge. In all, six treatments including crossings and selfings were tested. The clone RE43 from cultivar Queen Victoria was planted in germplasm genebank. The clones 410-200-15, 410-106-33 and 103-104-6 were set in plant multiplication fields. The control clones HA10 and HA25 were placed in monthly plantation.

The six clones were involved in a complete diallel crossing system with selfings. Sole selfings were analysed in this paper.

Four variables were measured: (1), the total seeds number derived from hand self-pollinations per week (Nbseed), (2), mean seeds number obtained per hand self-pollinated flower per week (Seedflow), (3), ripe fruit weight (Weigfruit) and (4), bloomed flowers number per week (Blooflow).

Data analysis

The data set was processed by Xlstat 2007.6 software. The Anova incorporating the means separation, Principal Component (PCA) and Hierarchical Cluster Analyses (HCA) were run to interpret the variability. The means were separate in two terms. First, Dunnett's test was applied to identify at more three classes. These are : 1) the class of female clones of which the means were on this side of the controls, 2) class of the female clones of which the means were comparable to controls and 3) that of parent clones whose means were beyond controls. Within each class the Newman-Keuls or Student t tests at 5% threshold were performed. The total seeds number derived from hand self-pollinations per week (Nbseed) and mean seeds number obtained per hand self-pollinated flower per week was subjected to the square root transformation, because they were not normally distributed. The PCA allowed the structuring of descriptors and individuals represented by the four female parent clones. The PCA also allowed the analysing of the relatedness among identified groups. Prior, the number of factors or components used for interpretation of the variability was determined basing on Kaiser and angle criteria. The most relevant descriptors were selected from their representation quality namely QLT_{kl} and Pearson's linear correlation coefficient.

RESULTS

Variability by descriptor of the self-sterility of four female clones

With both the total seeds number derived from hand self-pollinations per week (Nbseed) and mean seeds number obtained per hand self-pollinated flower per week (Seedflow), three groups were identified in relation to Dunnett's test. The first composed of female clone 410-200-15 was characterised by the lowest seeds production potential by hand self-pollinations per week. The second consisting of female parent clone RE43 including control clones HA10, HA25 was marked by mean production ability of seeds by hand self-pollinations per week. The third constituting of hybrid clones 103-104-6 and 410-106-33 was distinguishable by high seeds production potential derived from hand self-pollinations. Within group G2, no significant difference was noted among clones RE43, HA10 and HA25 using the Newman-Keuls' test. In the same way, within group G3, no statistical difference was evidenced between clones 103-104-6 and 410-106-33 resorting to Student's t test. In all, three groups were observed : 1) G1 constituted of female clone 410-200-15, 2) G2 composed of clone RE43 with two control clones HA10 and HA25 and 3) G3 comprised hybrid female clones 103-104-6 and 410-106-33. Variability on both sides of mean varied from 3.82 to 19.08% for Nbseed, as against from -∞ to 18.97 for Seedflow. The Untransformed means fluctuated from 00 to 21.846 seeds for Nbseed, while those of Seedflow varied from 00 to 0.943 (Table 1).

Regarding the ripe fruit weight (Weigfruit), after Dunnett's test, three groups were identified. First, composed of female clones 410-106-33 and RE43 was distinguishable by means on this side of the controls. Second, constituted of control HA10. The weight of its fruits was significantly higher than that of the two aforementioned clones. Third, consisted of female clones 410-200-15 and 103-104-6 including control HA25. They produced the heaviest fruits. Within the first group, two sub-groups were observed after Newman-Keuls test: 1) represented by clone 410-106-33 was characterised by weak fruits weight and 2) comprising clone RE43 was marked by high fruits weight. Likewise, within the third group, three sub-groups were noted. First comprising hybrid clone 410-200-15 differed from two others by low weight of fruit. The Second composed of the second control HA25. It was distinguishable by fair weight. The third consisted of hybrid clone 103-104-6, was distinguishable from the two previously mentioned as having the highest fruit weight. The gaps between the mean and each of observations oscillated from 0.05 to 0.11%. The untransformed means of this variable stretched out from 0.000 to 0.943 Kg. (Table 1).

Regarding the bloomed flowers number per week (Blooflow), two classes were evidenced according to Dunnett's test. First composed of female clones 410-106-33, 410-200-15, 103-104-6 and RE43 was characterised by weak bloomed flowers number per week on this side controls. The second consisting of control clones HA10 and HA25 recorded flowers production comparable to that of two controls. Within class C1, no significant difference was noted among clones 410-200-15, 410-106-33, 103-104-6 and RE43 after Newman-Keuls' test. Likewise, within class C2, no statistical difference was evidenced between control clones HA10 and HA25 according to Student's t test. In all, two classes were observed: 1) C1 constituted of female clones 410-106-33, 410-200-15, 103-104-6, RE43 and 2) C2 class comprised female clones HA10 and HA25. The gaps around mean oscillated from 9.15 to 16.39%. The produced flowers mean varied from 23.656 to 31.667 (Table 1).

Table 1: Classification of means of the total seeds number derived from self-pollinations per week, mean seeds number obtained per self-pollinated flower per week, ripe fruit weight and bloomed flowers number per week as a function of female clones

Dependent variable*	Femaclone*		Transformed mean*	Transformed mean*	CV (%)*	Untransformed mean*	Untransformed mean*
		After Dunnett	After Newman-Keuls	After Student		After Newman-Keuls	After Student
Nbseed	410-200-15	On this side of control	0.000	-	+∞	0.000	-
	HA10	Comparable to control	0.471a	-	3.82	0.222	-
	HA25		0.745a	-	19.08	0.555	-
	RE43		1.000a	-	14.10	1.000	-
	410-106-33	Beyond control	-	2.544a	16.25	6.472	-
	103-104-6		-	4.674a	12.62	21.846	-
Seedflow	410-200-15	On this side of control	0.000	-	-∞	0.000	-
	HA10	Comparable to control	0.086a	-	17.91	0.007	-
	HA25		0.136a	-	18.97	0.018	-
	RE43		0.189a	-	11.96	0.036	-
	410-106-33	Beyond control	-	0.672a	18.36	0.452	-
	103-104-6		-	0.971a	3.47	0.943	-
Weigfruit	410-106-33	On this side of control	-	-	0.11	-	513.000a
	RE43		-	-	0.11	-	538.000b
	HA10	Comparable to control	-	-	0.08	698.000	-
	410-200-15	Beyond control	-	-	0.06	939.500a	-
	HA25		-	-	0.06	959.000b	-
	103-104-6		-	-	0.05	1220.000c	-
Blooflow	410-106-33	On this side of control	-	-	16.39	23.656a	-
	410-200-15		-	-	12.78	24.677a	-
	103-104-6		-	-	11.58	25.000a	-
	RE43		-	-	10.73	26.000a	-
	HA10	Comparable to control	-	-	9.34		31.000a
	HA25		-	-	9.15		31.667a

Dependent variable*: Nbseed: Total seeds number derived from self-pollinations per week. **Seedflow*:** mean number of seeds obtained per self-pollinated flower per week. **Weigfruit:** Ripe fruit weight. **Blooflow:** Bloomed flowers number per week. **Femaclone*:** Clone used as female parent**. Transformed mean*:** Obtained mean applying √x transformation. **CV (%)*:** Coefficient of variation. **Untransformed mean*:** Obtained mean squaring the transformed mean, mainly the variables Nbseed and Seedflow. Values bearing the same letter in a column are not significantly different according to the Newman-keuls and Student t tests at 5% likelihood. Student's test was performed to compare two means.

Variability of the self-sterility of four female parent clones with the descriptors as a whole

Principal components F1 and F2 synthesised the information as a whole contained in the four initial descriptors. They were used in the course of the study

to describe and interpret the variability (Figure 1; Table 2).

From the PCA, the four initial descriptors allowed the structuring of four female parent clones in two a priori groups. The first, composed of female clone 103-104-6. The second, constituted of parent clones RE43, 410-106-33, 410-200-15 with the control ones namely HA25 and HA10. This same PCA showed that among the four initial descriptors, two were relevant. These are the mean seeds number obtained per hand self-pollinated flower per week (Seedflow) as well as ripe fruit weight (Weigfruit). Therefore, they were used in the rest of the study to cluster a posteriori studied clones via the HCA. This, is to search for relationship between the a priori and a posteriori groups. In the opposite, the total seeds number derived from hand self-pollinations per week (Nbseed) and bloomed flowers number per week (Blooflow) was dopped from the study (Figures 2 and 3; Tables 2, 3 and 4).

Footnote relating to the Principal Component Analysis

According to Kaiser's criterion, sole factorial axes F1 and F2 recorded eigenvalues higher than 1 (F1 eigenvalue = 2.424; F2 eigenvalue = 1.237; F3 eigenvalue = 0.335; F4 eigenvalue = 0.004). After angle criterion, the frequencies histogram at point (F3;3) recorded brutal falling of the eigenvalue. In figure 1, this point corresponds to inflection point. Beyond this point, there is not more information but, on this side, axes F1 and F2 contain the essential information. In sum, factorial axes F1 and F2 were retained for the rest of the study to analyse the variability. These two factorial axes described 91.54% total variation. Factorial axis F1 explained 60.61% total variation. The seeds number derived from cross-pollinations per week (Nbseed) and seeds number obtained per self-pollinated flower per week (Seedflow) were well represented there (Table 3). This axis represented the ability of tested parents to produce seeds in selfings. Factorial axis F2 accounted for 30.93% residual variation unexplained by factorial axis F1, but complementary to this one. This axis stated the rhythm of flowers issue. Therefore it represented the potential of tested parents to yield flowers (Figure 2).

Descriptors were chosen basing on representation quality, QLT_{kl} in abbreviate. The Nbseed, Seedflow and Weigfruit were well represented on plane 1-2 (QLT_{kl}(1-2) of Nbseed = 0.962; QLT_{kl}(1-2) of Seedflow = 0.963; QLT_{kl}(1-2) of Weigfruit = 0.901). In addition, Nbseed and Seedflow were significantly and favourably correlated (r Nbseed / Seedflow = + 0.984*). The Seedflow was better represented on 1-2 plane than Nbseed. Therefore, the former was retained for the rest of the study, while the latter was dropped. In all, the Seedflow and Weigfruit were used in the rest of the study (Figure 2; Table 3).

The projection of six female parent clones on the plane 1-2 of the factorial map with the four descriptors as a whole allowed the observing of some groups. On the principal plane, two a priori structured groups were observed. These are: 1) G1, composed of clone 103-104-6. 2) G2-G3, consisting of clones RE43, 410-106-33, 410-200-15 with controls HA25 and HA10 (Figure 3).

Hierarchical Cluster Analysis

The HCA performed with the two most relevant descriptors which are Seedflow and Weigfruit provided three a posteriori groups as against two a priori identified through the PCA. Group G1 consisting of the only clone 103-104-6, was both characterised by high mean seeds number obtained per hand self-pollinated flower per week as well as ripe fruit weight. Group G2 constituted of clones RE43 and 410-106-33 including the control one namely HA10, was both singularisable by low mean seeds number produced per hand self-pollinated flower per week as well as ripe fruit weight. Group G3 composed of clone 410-200-15 with the control one termed HA25, both stood out from the two aforementioned clones by very weak mean seeds number obtained per hand self-pollinated flower per week, but mean ripe fruit weight (Figure 4; Table 4).

Calculated genetic distance, by means of euclidian distance, showed that groups G2 and G3 would be related. It could belong to the same group. Finally, two big groups would exist from the six studied clones. The former named group G1 comprising clone103-104-6, was both distinguishable by high mean seeds number obtained per hand self-pollinated flower per week as well as ripe fruit weight (Seedflow = 1.373; Weigfruit = 1220). The latter termed group $G2_{2-3}$, consisting of clones 410-106-3, 410-200-15 and RE43 including the control ones namely HA10 and HA25, was both singularisable by low mean seeds number obtained per hand self-pollinated flower and per week as well as ripe fruit weight (Seedflow = 0.1765; Weigfruit = 766.125; Table 5).

Relatedness among the identified groups

Groups G2 and G3 would be morphologically related. In short, two big groups could be identified from the six clones parents typed with two out of four initial descriptors : 1) group G1 composed of sole clone 103-104-6 and 2) group $G2_{2-3}$, constituted of clones 410-106-3, 410-200-15 and RE43 with the control ones namely HA10 and HA25 both produced the lowest mean seeds number obtained per hand self-pollinated flower per week as well as ripe fruit weight (Table 5).

Table 2: Choice of principal components from eigenvalue according to Kaiser's criterion

	F1*	F2*	F3*	F4*
Eigenvalue*	**2.424**	**1.237**	0.335	0.004
Variability (%)	60.61	30.93	8.37	0.10
Cumulated %	60.61	91.54	99.91	100.00

F1*, F2*, F3* and F4*: factorial axes or principal components. **Eigenvalue* :** The two values in bold for factorial axes 1 and 2 fulfil Kaiser's criterion.

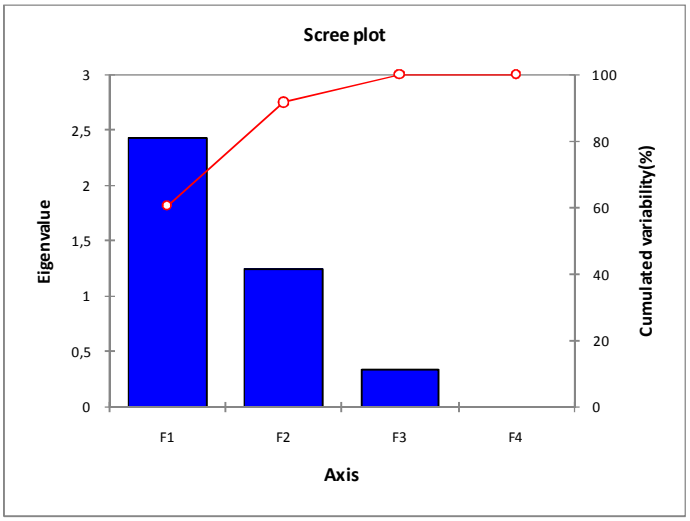

Figure 1: Scree plot associated with histogram of the eigenvalues of the PCA showing the falling of the latter, and hence, the inflection point or angle (here F3;3)

Table 3 : Choice of the most relevant descriptors from their representation quality expressed through squared cosine

	Cos^{2*}		QTL_{kl}*
Descriptors	F1	F2	1-2
Blooflow	0.293	0.572	0.865
Nbseed	0.950	0.012	0.962
seedflow	0.961	0.002	0.963
Weigfruit	0.218	0.683	0.901

Cos^{2*}: square cosine on factorial axes F1 and F2 of used descriptors. **QTL_{Kl}*: r**epresentation quality of used descriptors on the plane 1-2 of the correlation circle of the PCA. The Nbseed, Seedflow and Weigfruit better expressed representation quality. This one was greater than or equal to 0.9.

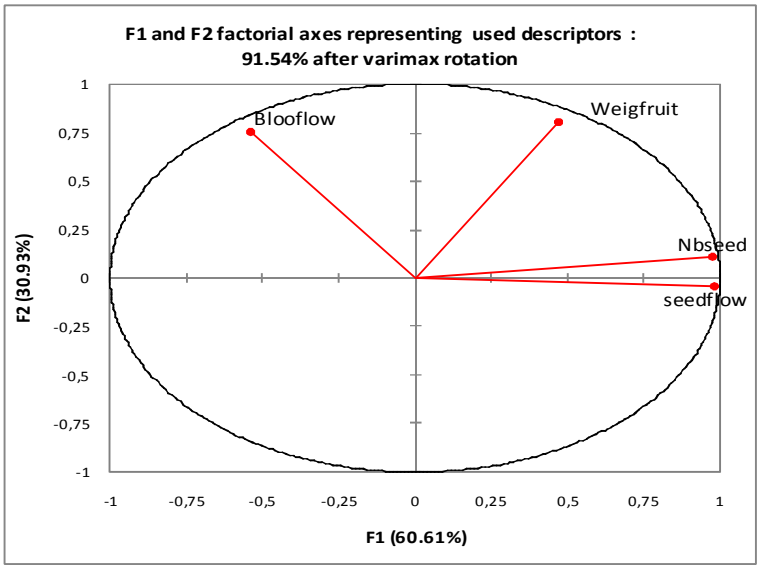

Figure 2: Relationship between the measured descriptors and principal components of parent clones through the correlation circle of the PCA

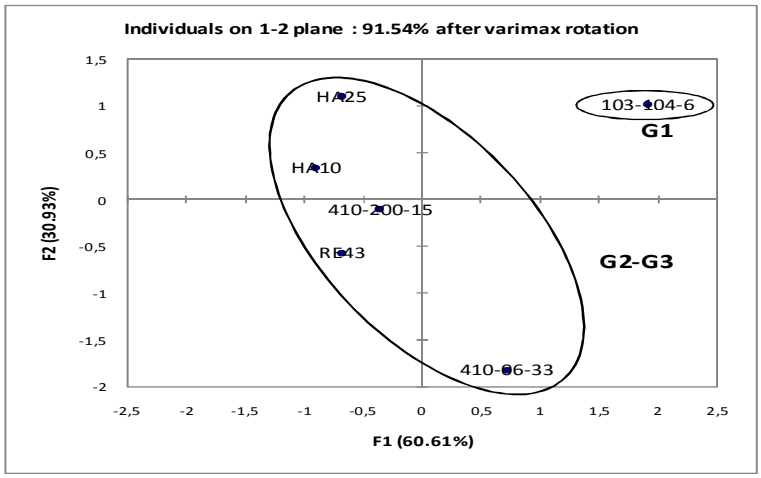

Figure 3: Clustering of the individuals on the plane 1-2 of the factorial map of the PCA

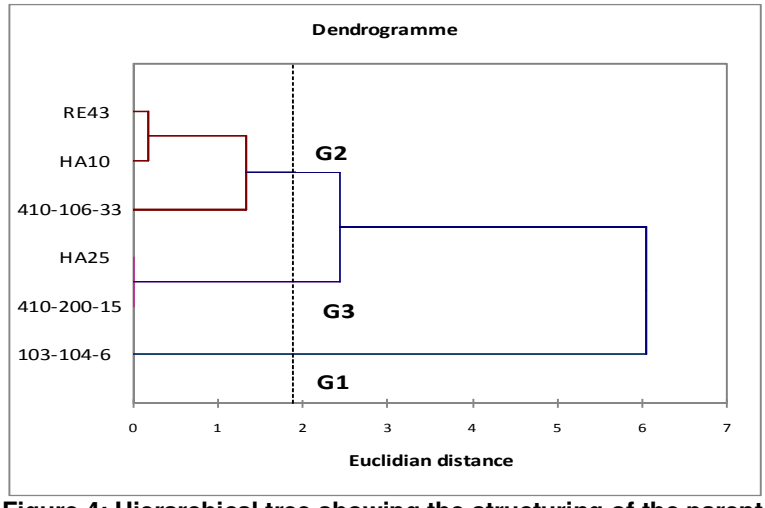

Figure 4: Hierarchical tree showing the structuring of the parent clones using the euclidian distance from the HCA

Table 4: Hierarchical classification of the parent clones by means of the relevantly identified descriptors

Group	Seedflow	Weigfruit
G1	1.373	1220.000
G2	0.327	583.000
G3	0.026	949.250
Mean	0.576	917.417

Descriptor* : G1 : Group composed of the only 103-104-6 clone. **G2 :** Group consisting of clones RE43 and 410-106-33 as well as the control HA10. **G3:** Group comprising clone 410-200-15 with the control one namely HA25.

Table 5: Relatedness among groups identified from the euclidian distance of relatedness matrix from the Cluster Hierarchical Analysis

	G1	G2	G3
G1	0	2.482	2.085
G2	2.482	0	**1.222**
G3	2.085	**1.222**	0

In bold and underlined, the value of the weakest calculated genetic distance between G2 and G3 groups indicating their genetic relatedness.

DISCUSSION

The cultivation system that applies to four female parent clones was looked at through their behaviour under hand selfings in a 6 x 6 complete diallel mating system. The self-incompatibility and self-compatibility were studied in *A. comosus* in Cardin (1990). The latter defined self-incompatible clone as the one which by hand self-pollination do not produce any seeds, but produces them by hand cross-pollination. Likewise self-sterile clone is the one which by hand selfing shows a mean seeds production per self pollinated flower null. Other authors studied the fertility in pineapple (Brewbaker and Gorrez, 1967; Chan, 1986; Chan, 1989). Our works displayed the self-incompatibility of parent clone 410-200-15, but self-sterility of parent clones HA10, HA25, 410-106-33 and RE43.

The female parent clones HA10, HA25, RE43 and 410-200-15 both expressed low production potential of seeds through the Anova (Table 1). Except for parent clone 410-106-33, such an observation was confirmed by the HCA searching for relatedness among three a posteriori groups (Table 5). According to previously given definitions, hybrid female clone 410-200-15 was classified as self-incompatible. Our works also showed that control clones HA10 and HA25 are self-sterile. In contrast, Cabot (1989) both ranked clones HA10 and HA25 as self-incompatible. Thereby, the self-incompatibility of hybrid clone 410-200-15 finds an explanation through self-sterility of its parent HA10. The clone RE43 was identified as self-sterile (Table 1). Moreover, the fruits weight of clones 410-200-15 and HA25 ranked them in grade C (0.9 to 1.1 Kg). They could be intended to export in this grade. Weight of fruit from RE43 classes it in grade lower than D for export. Consequently, the new hybrid clone 410-200-15 must be cultivated in on-farm trials via single or mixed crop system .

As regards the two others represented by 103-104-6 and 410-106-33, the former can be considered as a self-fertile, while the latter as self-sterile (Table 2). The self-fertility is due to the lack of recognition between stigma and pollen proteins, while self-sterility is caused by the existence of such a recognition (Demarly, 1977). At the moment pollen makes contact with stigma, there is no recognizable existence between proteins of two organs this pollen germinates and issues pollen tube. One lengthens in stylar canal up to ovary. The vegetative nucleus weathers, whereas the reproductive one divides into two antherozoa. They fertilise eggs contained in embryo bag of ovule. The incompatibility in pineapple is under gametophytic control with either S or S/Z polymorphic loci according to authors. Majumber et *al*. (1964), Brewbaker and Gorrez (1967) and Coppens d'Eeckenbrugge et *al*. (1997) defended the hypothesis of an only one locus. In contrast Hayman (1956), Cardin (1990) and Issali (1998) postulated the assumption of S and Z independently segregating loci. By reason of the existence of differential compatibility from back cross ♀HA10 x ♂410-200-15, the second hypothesis would be the most plausible (Issali *et al*. accepted for publication). Furthermore, fruits from hybrid clones 410-106-33 and 103-104-6 belong to grade B (1.1 to 1.5 Kg). They will be intended to export in this grade, on condition of reducing self-fertility of 103-104-6. In brief, 401-106-33 female clone can be cultivated in only one crop. However, by reason of the presence of lots of small corms at fruit basis and multiple crowns on fruit (data not shown), 410-106-33 should be subjected to successive back-crosses with 103-104-6. The former will be used as a HA10 recurrent parent clone, whereas the latter will use its Perolera female parent.

Group G3 constituted of parent clones 410-200-15 and HA25 showed the weakest seeds production potential, but mean ripe fruit weight (Table 4). Nonetheless, this group can be added to Group G2 composed of clones RE43 and 410-106-33 as well as the control HA10. This clustered group $G2_{2-3}$ could be morphologically related. Indeed, the controls HA25 and HA10 belong to Smooth Cayenne cultivar. The latter originated from Venezuela where it was selected and cultivated by Indians. Thereafter, it was introduced from Cayenne, in French Guyana, in 1820 (http://en.wikipedia.org/wiki/Pineapple). The clone RE43 would be originated from South Africa. Queen Victoria and clones Smooth Cayenne are considered as varieties, but not as different cultivars (Coppens d'Eeckenbrugge *et al*., 1997). They come from accumulation of minor somatic mutations. Hence, they would be very genetically related. The clones 410-106-33 and 410-200-15 descended from crosses ♀HA10 x ♂Perolera. They contain genes inherited from their mother. This could explain the relatedness among clones constituting group $G2_{2-3}$. In contrast, hybrid clone 103-104-6 comes from cross ♀Perolera x ♂Perolera. It might contain infrequent alleles justifying its distance in relation to clones from group $G2_{2-3}$. Nevertheless, they belong to the same species complex (Coppens d'Eeckenbrugge *et al*., 1997), and hence are all cross-fertile.

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