

## 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 type to apply to four clones of *Ananas comosus* in farms, their behaviour under hand selfings was analysed. 103-104-6, 410-106-33 and 410-200-15 hybrid female clones and RE43 Queen Victoria clone as well as HA10 and HA25 as controls were involved in a 6 x 6 complete diallel crossing system with selfings. The total seeds number derived from self hand-pollinations per week, mean seeds number obtained per self-pollinated flower and per week, weight of ripe fruit 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 was revealed self-incompatible, while RE43 is found to be self-sterile. In the same way, 410-106-33 expresses self-sterile behaviour, whereas 103-104-6 shows the self-fertile one. The behaviour under selfings of both 410-200-15 and 410-106-33 comes from their HA10 Smooth Cayenne female parent which was previously characterised like self-incompatible. The structurings provided by the Anova and HCA are globally comparable. The 410-200-15 hybrid clone can already be recommended for on-farm trials under mono-crop. Nonetheless, the multi-crop will be envisioned once panmixia results will have demonstrated its inter-sterility. The 410-106-33 and 103-104-6 must be first subjected to successive back crosses before their on-farm trials. The RE43 clone must be cultivated in one crop. The morphological relatedness of five clones is discussed.

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### 1. INTRODUCTION

*Ananas comosus* L. Merr, fruits king, is a perennial, monocotyledonous, herbaceous, diploid ( $2n=2x=50$ ) plant of the Bromeliaceae [1]. In world, 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 [2],[3].

In Côte d'Ivoire as in world 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 very prized and marketed. This country was 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 [4]. A breeding programme of pineapple was initiated and conducted at the Station of Anguededou / DFA (Département des Fruits et Agrumes) from the IDEFOR (Institut des Forêts) at the present time CNRA (Centre National de Recherche Agronomique) [5]. This programme aimed to create some varieties intended for export of either fresh fruits or canning. In the first time, apart from the traditional criteria of yielding, quality and spineless, this programme also aimed to prevent the internal fruit browning in increasing its content in ascorbic acid. In the second phase, it concerned 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 consisted in hybridizing two cultivars Smooth Cayenne and Mordilona and achieving the multi-characters selection of 40,000 hybrids created [6]. From these created hybrids, nineteen were selected of which three 410-106-33, 410-200-15 and 103-104-6 identified like the best [5]. 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 one of their parents namely HA10 clone [7]. The knowledge of their either self-fertility or self-sterility could help to envision the cropping type either one or mixed cropping which could suit them.

The aim of this to determine the cropping type to apply to four female clones 103-104-6, 410-106-33 and 410-200-15 as well as RE43 Queen Victoria involved in a 6 x 6 complete diallel crossing system with selfings.

## 2. RESEARCH METHOD

### 2.1. Experimental site, plant material and self hand-pollinations

The experiment was conducted from September 1997 to April 1998. It was carried out in the Research Station of the Anguededou at 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 above sea.

Four common tester and parent clones 103-104-6, 410-106-33, 410-200-15 and RE43 as well as two others HA10 and HA25 as controls were used. The first three are of the recently created hybrids [5]. They were identified as superior hybrids. The clones 410-200-15 and 410-106-33 come from Smooth Cayenne x Perolera inter-variety crosses. The clone 103-104-6 comes from Perolera x Perolera intra-variety cross [5]. The Queen Victoria clone RE43, more sugared than cultivar Smooth Cayenne, is very 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 after such an applying the inflorescences emerging from plant were bagged with the isolation bags. Every day, in the morning the anthers surmounting the stamens were collected in Petri dish by means of 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 counted after dissection.

### 2.2. Experimental design, mating system and measured variables

All of parent clones already belonged to pre-existent design. The latter 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 testers. 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 the collection. The 410-200-15, 410-106-33 and 103-104-6 clones were set in plant multiplication fields. The HA10 and HA25 control clones 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 deriving from self hand-pollinations per week (Nbseed), 2) mean seeds number obtained per self hand-pollinated flower and per week (Seedflow), 3) weight of ripe fruit (Weigfruit) and 4) bloomed flowers number per week (Blooflow).

## 2.2. 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 times. 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 that of controls, 2) class of the female clones of which the means were comparable to controls and 3) that of parent clones whose the means were beyond controls. Within each class the Newman-Keuls or Student t tests at 5% threshold were performed. The total seeds number deriving from self hand-pollinations per week (Nbseed) and mean seeds number obtained per self hand-pollinated flower and 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 proximity among identified groups. Prior, the number of factors or components used to interpret the variability was determined by means of Kaiser and angle criteria. The most relevant descriptors were selected from their representation quality namely  $QLT_{kl}$  and Pearson's linear correlation coefficient.

## 3. RESULTS AND ANALYSIS

### 3.1. Variability of the self-sterility of four female clones by descriptor

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

Regarding the weight of ripe fruit (Weigfruit), after Dunnett's test, three groups were identified. First composed of 410-106-33 and RE43 was distinguishable by averages on this side of control. Second constituted of HA10 control. The weight of its fruits was significantly higher than that of two above-mentioned clones. Third consisted of 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 410-106-33 was characterized by fruits of weak weight and 2) comprising clone RE43 was marked by fruits of elevated 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. Second composed of second control HA25. It was distinguishable by fair weight. Third consisted of hybrid clone 103-104-6, was distinguishable from two previous by the highest fruit weight. The gaps between the average and each of observations oscillated from 0.05 to 0.11%. The untransformed averages 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 410-106-33, 410-200-15, 103-104-6 and RE43 female clones was characterized by weak bloomed flowers number per week on this side controls. Second consisted of HA10 and HA25 control clones recorded flowers production comparable to two controls. Within C1 class, no significant difference was noted among 410-200-15, 410-106-33, 103-104-6 and RE43 after Newman-Keuls' test. Likewise, within C2 group, no statistical difference was evidenced between HA10 and HA20 control clones according to Student's t test. In all, two classes were observed: 1) C1 constituted of 410-106-33 410-200-15, 103-104-6, RE43 female clones and 2) C2 class comprising HA10 and HA25 female clones. The gaps around average oscillated from 9.15 to 16.39%. The produced flowers average varied from 23.656 to 31.667 flowers (Table 1).

Before interpreting results, self-incompatibility and self-sterility were defined. Self-incompatible clone is defined as the one which by self hand-pollination does not produce any seeds, but produces them by cross hand-pollination. Likewise the self-sterile one is the one which by hand selfing shows a mean seeds production per self pollinated flower null [8].

In sum, The HA10, HA25, RE43 and 410-200-15 female parent clones both expressed low production potential of seeds through the Anova (Table 1). Except for 410-106-33 parent clone, such an observation was confirmed by the HCA searching for proximity among three a posteriori groups (Table 5). According to previously given definitions, 410-200-15 hybrid female clone was classified like self-incompatible. Our works also showed that HA10 and HA25 control clones are self-sterile. In contrast, Cabot (1989) both ranked HA10 and HA25 clones like self-incompatible. Thereby, the self-incompatibility of 410-200-15 hybrid clone finds an explanation through self-sterility of its parent HA10. The RE43 clone was identified like self-sterile (Table 1). Moreover, the fruits weight of 410-200-15 and HA25 clones 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 410-200-15 hybrid clone can already be recommended for on-farm trials under mono-crop. Nonetheless, the multi-crop will be envisioned once panmixia results will have demonstrated its inter-sterility.

Likewise, 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 like 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 [9]. At the moment where pollen makes contact with stigma, if it does not exist no recognition between proteins of two organs, this pollen germinates and issues pollen tube. This 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. Thereby, Majumber *et al.*, (1964), Brewbaker and Gorrez (1967) and Coppens d'Eeckenbrugge *et al.* (1997) defended the hypothesis of an only one locus [6],[10],[11]. In contrast Hayman (1956), Cardin (1990) and Issali (1998) postulated the assumption of S and Z independently segregating loci [8],[12],[13].

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

Dependent variable*	Femaclone*		Transformed average*	Transformed average*	CV (%)*	Untransformed average*	Untransformed average*
			After Dunnett	After Newman-Keuls		After Student	After Newman-Keuls
<b>Nbseed</b>	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	-
<b>Seedflow</b>	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	-
<b>Weigfruit</b>	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	-
<b>Blooflow</b>	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

**Legend: Dependent variable\*:** Nbseed: Total seeds number deriving from self-pollinations per week. **Seedflow\*:** mean number of seeds obtained per self-pollinated flower and per week. **Weigfruit:** Weight of ripe fruit. **Blooflow:** Bloomed flowers number per week. **Femaclone\*:** Clone used as female parent. **Transformed average\*:** Obtained average applying  $\sqrt{x}$  transformation. **CV (%)\*:** Coefficient of variation. **Untransformed average\*:** Obtained average squaring the transformed average, 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 averages.

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.*, submitted for publication). Furthermore, fruits from 410-106-33 and 103-104-6 hybrid clones 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 use as a HA10 recurrent parent clone, whereas the latter will use its Perolera female parent.

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

F1 and F2 principal components 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. First, composed of 103-104-6 female clone. Second, constituted of RE43, 410-106-33, 410-200-15 parent clones with the control ones namely HA25 and HA10. This same PCA showed that among the four initial descriptors, sole two were relevant. These are the mean seeds number obtained per self hand-pollinated flower and per week (Seedflow) as well as weight of ripe fruit (Weigfruit). Therefore, they were used in the rest of the study to cluster a posteriori studied clones via the HCA. This, to search for relationship between the a priori groups and the a posteriori ones. In the opposite, the total seeds number deriving from self hand-pollinations per week (Nbseed) and bloomed flowers number per week (Blooflow) was eliminated from the study (Figures 2 and 3; Tables 2, 3 and 4).

In brief, F1 and F2 were the best selected principal components using Kaiser's and angle criteria (Table 2). They synthesised the essential of information contained in the four initial descriptors. Such an approach was also been used in Yao (2012) [14]. Moreover, the second a priori group consisting of RE43, 410-106-33, 410-200-15 parent clones including the control ones namely HA25 and HA10 provided the least seedy fruits. The mean of group seem to be widely influenced by self-incompatibility of 410-200-15 clone (Table 1).

Concerning descriptors choice, the mean seeds number obtained per self hand-pollinated flower and per week (Seedflow) as well as weight of ripe fruit (Weigfruit) were found to be relevant, hence selected (Table 3). They expressed better representation quality ( $QLT_{ki}$ ) on the 1-2 plane.

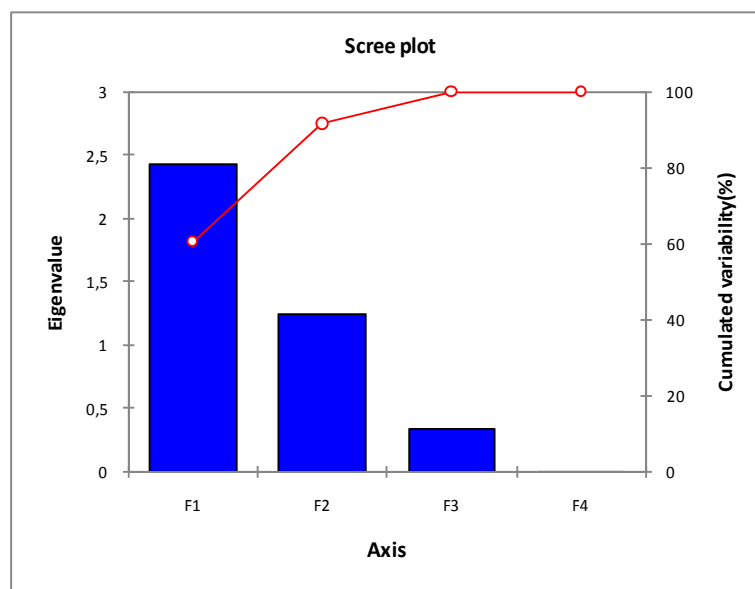


Figure 1. Scree plot associated with histogram of the eigenvalues of the PCA showing the falling of the latter, and hence, the point of inflection or angle (here F3;3)

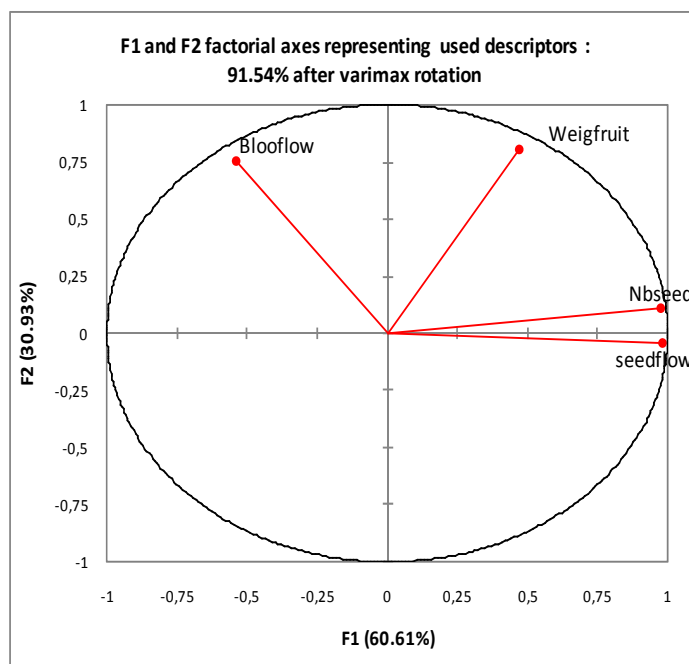


Figure 2. Relationship between the measured descriptors and principal components of parent clones through the correlation circle of the PCA.

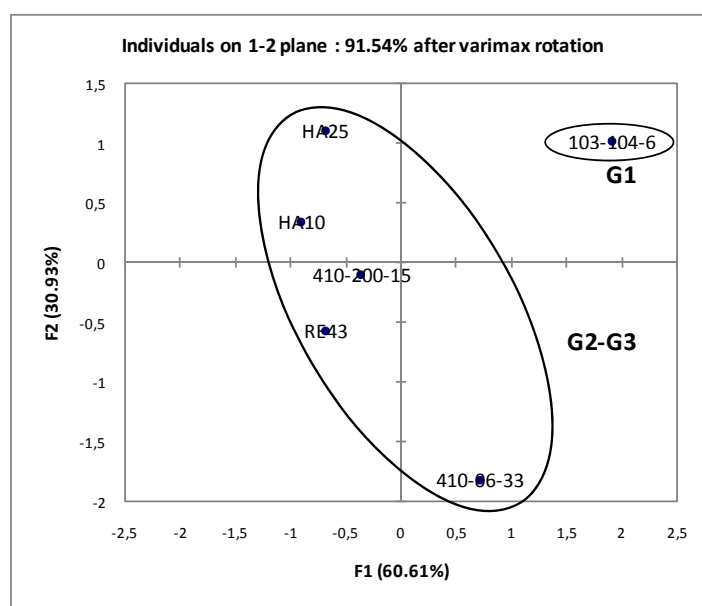


Figure 3. Projection of the individuals on the plane 1-2 of the factorial map of the PCA

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

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

**Legend:**

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

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

Descriptors	Cos <sup>2</sup> *		QTL <sub>kl</sub> *
	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

#### Legend

**Cos<sup>2</sup>\***: square cosine on F1 and F2 factorial axes of used descriptors. **QTL<sub>kl</sub>\***: representation quality of used descriptors on the 1-2 plane of the correlation circle of the PCA. The Nbseed, Seedflow and Weigfruit expressed a good representation quality. This one was greater than or equal to 0.9.

#### Footnote relating to the Principal Component Analysis

According to Kaiser's criterion, sole F1 and F2 factorial axes 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 is the point of inflection. Beyond this point, there is not more information but, on this side of this point, F1 and F2 axes contain the essential information. In sum, F1 and F2 factorial axes were retained for the rest of the study to analyse the variability. These two factorial axes described 91.54% total variation. F1 factorial axis explained 60.61% total variation. The seeds number deriving from cross-pollinations per week (Nbseed) and seeds number obtained per self-pollinated flower and per week (Seedflow) were well represented there (Table 3). This axis represented the ability of tested parents to produce seeds in selfings. F2 factorial axis was accounted for 30.93% residual variation unexplained by F1 factorial axis. This axis stated the rhythm of flowers issue. So it represented the potential of tested parents to yield flowers (Figure 2).

Descriptors were chosen basing on representation quality, QLT<sub>kl</sub> in abbreviate. The Nbseed, Seedflow and Weigfruit were well represented on 1-2 plane (QLT<sub>kl</sub>(1-2) of Nbseed = 0.962; QLT<sub>kl</sub>(1-2) of Seedflow = 0.963; QLT<sub>kl</sub>(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 eliminated. In all, the Seedflow and Weigfruit were used in the rest of the study (Figure 2; Table 3).

The projection of four female parent clones on the 1-2 plane 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 103-104-6 clone. 2) G2-G3 consisting of RE43, 410-106-33, 410-200-15 clones with HA25 and HA10 controls (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. G1 group, consisted of the only 103-104-6 clone, was both characterised by high mean seeds number obtained per self hand-pollinated flower and per week as well as weight of ripe fruit. G2 group, constituted of RE43 and 410-106-33 clones including the control one namely HA10, was both singularisable by low mean seeds number produced per self hand-pollinated flower and per week as well as weight of ripe fruit. G3 group, composed of 410-200-15 clone with the control one termed HA25, both stood out from two aforementioned clones by very weak mean seeds number obtained per self hand-pollinated flower and per week, but mean weight of ripe fruit (Figure 4; Table 4).

Calculated genetic distance, by means of euclidian distance, showed that G2 and G3 groups would be related. It could belong to the same group. Finally, two big groups would exist from the six studied clones. The former named G1 group, comprising 103-104-6 clone, was both distinguishable by strong mean seeds number obtained per self hand-pollinated flower and per week as well as weight of ripe fruit (Seedflow = 1.373; Weigfruit = 1220). The latter termed G2<sub>2-3</sub> group, consisting of 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 self hand-pollinated flower and per week as well as weight of ripe fruit (Seedflow = 0.1765; Weigfruit = 766.125; Table 5).

### 3.3. Proximity among the identified groups

G2 and G3 groups 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) G1 group composed of sole 103-104-6 clone and 2) G<sub>2,3</sub> group, constituted of 410-106-3, 410-200-15 and RE43 with the control ones termed HA10 and HA25 both produced the lowest mean seeds number obtained per self hand-pollinated flower and per week as well as weight of ripe fruit (Table 5).

In short, G3 group constituted of 410-200-15 and HA25 parent clones showed the weakest seeds production potential, but mean weight of ripe fruit (Table 4). Nonetheless, at this group can be added G2 group composed of RE43 and 410-106-33 clones as well as HA10 control. The big G<sub>2,3</sub> group is the same than the one previously identified via the factorial map of the PCA (Figure 3). This clustered G<sub>2,3</sub> group could be morphologically related. Indeed, 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>). RE43 clone would be originated from South Africa. Queen Victoria and Smooth Cayenne clones are considered as varieties, but not as different cultivars [6]. They come from accumulation of minor somatic mutations. Hence, they would be very genetically related. 410-106-33 and 410-200-15 are descended from ♀HA10 x ♂Perolera crosses. They bring the genes inherited from their mother. This could explain the relatedness among clones constituting G<sub>2,3</sub> group. In contrast, 103-104-6 hybrid clone comes from ♀Perolera x ♂Perolera cross. It might bring infrequent alleles justifying its distance in relation to clones from G<sub>2,3</sub> group. Nevertheless, they belong to the same species complex [6], and hence are all cross-fertile.

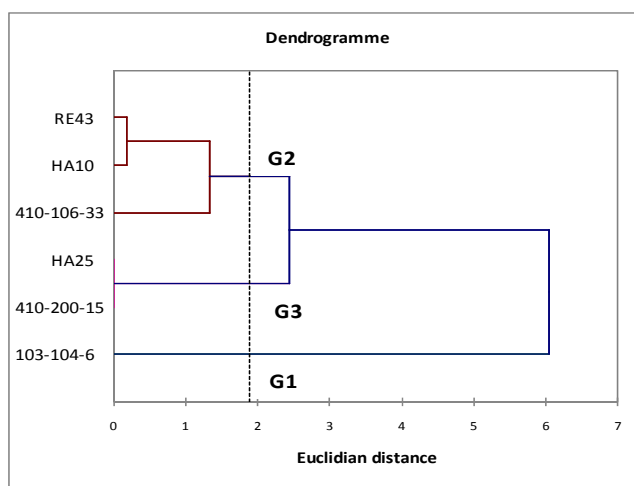


Figure 4. Hierarchical tree showing the structuring of the parent clones using the euclidian distance from the HCA

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
Average	0.576	917.417

#### Legend

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



Table 5. Proximity among groups identified from the euclidian distance of proximity matrix from the Cluster Hierarchical Analysis

	G1	G2	G3
G1	0	2.482	2.085
G2	2.482	0	<b><u>1.222</u></b>
G3	2.085	<b><u>1.222</u></b>	0

Legend is as indicated under Table 3.

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

#### 4. CONCLUSION

Our work assumption postulated that the three 103-104-6, 410-106-33 and 410-200-15 created hybrid clones and RE43 Queen Victoria clone would be self-incompatible like one of their parents namely HA10 clone. Our work showed that sole 410-200-15 is self-incompatible. Unlike Cabot (1989), who proved the self-incompatibility of HA10 and HA25 commercial varieties, we have demonstrated their self-sterility [7]. The 410-106-33 clone was revealed self-sterile, whereas 103-104-6 hybrid parent was displayed self-fertile. The RE43 Queen Victoria clone was ranked like self-sterile. Therefore, 401-200-15 can be ready tested under on-farm trials under mono-crop on condition that their assessing in panmixia with other clones confirm the one obtained here under self hand-pollination. The 410-106-33, although self-sterile, nevertheless belongs to same group than 103-104-6 clone. The two must be subjected to successive back crosses with self-sterile parent such as HA10 then under cross hand-pollinations before authorising their use under on-farm trials. Furthermore, RE43, 410-106-33 and 410-200-15 clone clones as well as HA10 and HA25 controls would be morphologically related.

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