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Variability of pollen grains in clones of *Dioscorea cayenensis* - *D. rotundata* complex grown in centre of Benin

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Abstract

The classical genetic breeding improvement of the species is through gene flows, based on pollen grains flow between individuals. The aim of this study is to evaluate the pollen variation between some clones of the complex *Dioscorea cayenensis* - *D. rotundata* of three villages of Centre of Benin. Unopened and mature male flowers were dissected and the anthers stained with acetic carmine. The preparations were observed with an optical microscope. Data on the shape, length, width of pollen were collected and subjected to descriptive analyse and pollen surface to an analysis of variance. Two forms of pollen grains were observed; the ovals shape percentage vary from 38 to 88.23%, and that of the rounds shape vary from 11.77 to 70%. The major axis has an average length of 0.49 ± 0.16 µm and that of the minor 0.32 ± 0.14 µm for oval shapes; the average diameter of round pollen was 0.36 ± 0.09 µm. These data are statistically very different ($P<0.0001$). The pollen grains of *Dioscorea cayenensis* - *D. rotundata* complex are oval or round shapes. Both shapes are present in all cultivars and the dominance of one shape of pollen grains is under genetic program. Size of the pollen grains is conserved within the species. These results are important for the classical genetic improvement of this vegetative species, where pollen grains would be the vehicles of interesting traits between cultivars identified in farmer's fields.

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Introduction

In Africa, quantity and quality of agricultural products sufficient to satisfy the dietary needs for an active and healthy life and the capacity of adaptation of seeds to current climatic conditions as ways to tackle hunger and poverty conditioned food security or sovereignty. The selection of successful cultivars that exist in farmer's fields is the essential step, as the starting point for genetic breeding by sexual reproduction. In West Africa, yam (*Dioscorea spp*) is one of the staple food crops and in Benin it's an important source of income for more than 75% of the rural population (Loko *et al.*, 2013). Facing climate change, challenges such as the delays of the beginning of rainy seasons, and the temporary's droughts or floods, the yield of this crop is decreasing. Moreover, the actions of pests, nematodes and viral diseases are threaten the production of yams. Recent works revealed the existence of high-yielding cultivars that are resistant to bio-aggressors and climate hazards (Loko, 2013). These types of cultivars that exist naturally will serve as basis for gene flow studies in order to improve or create new varieties. Method to regenerate yam seedlings from cultured yam seeds has been developed (Akoroda 1984; Zoundjihékpon, 1993; Yolou *et al.,* 2015b, Assaba *et al.,* 2018). It's an opportunity to improve cultivated yams by sexual way and this method has already been proven on several vegetatively propagated species. Thus, new potato varieties have been set up in Europe (Ducreux, 1986) and the selection of high yielding varieties of cassava with better nutritional qualities has been created by the International Institute of Tropical Agriculture (IITA) of Ibadan (Nigeria), (Agriculture, 1992). The existence of hybrid yam between cultivated and wild species, or between *Dioscorea cayenensis and Dioscorea rotundata* (Dumont *et al.*, 2010), attest the exchange of traits by gene flow, though their main mode of reproduction being vegetative. Improve or create news varieties using this technique, requires knowledge of the biology of sexual reproduction of the species. This study aims to evaluate the morphological variability of pollen grains of clones of the *Dioscorea cayenensis - D. rotundata* complex cultivated in centre of Benin.

Material and methods

Materials

The plant material consists of male flowers of nine clones *of Dioscorea cayenensis - D. rotundata* complex, these are Agatou A, Laboko I, Kpakala I, Agatou I, Laboko S, Gnalabo S, Kodjewé S, N'Kenni S and Kablètounan S.

Study sites and collect method

The study material consists of male flowers of cultivated yams from the *Dioscorea cayenensis - D. rotundata* complex cultivated by farmers in centre of Benin. The flowers were collected at three (03) different stages of floral development: at the beginning of male flowering (June), at the beginning of female flowering (August), and at the beginning of fruiting (October) of the year 2018. Samples were collected in yam's fields in three (03) villages of the Centre of Benin: Agoua Village of Bantè Township and Idaho and Soclogbo villages of Dassa-Zoumé Township. The letters A, I and S at the end of each clone name provide information on their place of origin: A = Agoua; I = Idaho and S = Soclogbo.

Sampling and microscopic observations

Inflorescences of unopened mature male flowers were collected from five male plants per clone. Samples collected were labeled, put into cotton bags transported to the laboratory and kept in the refrigerator. Two or three flowers are isolated, dissected and the anthers stained with acetic carmine on a slide with a glass stem and covered by a microscope slide. Acetic carmine, used as a pollen grain mounting solution, is a dye that gives only to alive pollen grains pink color. Five microscopic preparations are made per clone and morphological parameters (large and small axis) were taken on fifty (50) pollen grains, picked randomly in the optical fields. An optical microscope (*motic BA 210*) has been used to observe pollen grains, with amplification 10X for eyepiece and 40X for objective. Once microscope's eyepieces is equipped with a micrometer, which was used to take measures directly in the optical field. Pictures of pollen grains were taken using a camera (*Perfex Science Camris*) embedded to the microscope and connected to a computer that allows to visualize and recording pollen grains pictures. For the measurement of pollen grains size, two axes have been defined, the major axis (L) which represents the length and the minor axis (l) corresponding to the width.

When the axis L is greater than the l axis, we have the oval shape, and round shape, when L = 1; in the latter case, characteristic axis is the diameter D. Measure unit is the micrometer (µm).

Statistical analysis

Descriptive statistics were used to calculate the different averages. The dominant pollen grain form is the one with the highest frequency on the 50 grains of pollen per clone. The surfaces (S) of the pollen grains were calculated from the following formulas: $S = \pi r^2$ ***(1);*** for round shape, with r = ray of pollen grain; π = 3.14 and for oval shape, S = L*l ***(2),*** with L = major axis and l = minor axis.

The Newman-Keuls mean comparison test was carried out with the software Xlstat 2014 to show the length variations between the axis, the diameter and the surface of pollen grains of the same shape, and to establish a relationship between shape and size. The Wilcoxon test was performed to evaluate the difference of size between round and oval pollen grains within a clone. The Kruskal Wallis test was used to compare the size of same-shape pollen grains between the clones.

Results

The different results obtained for the different parameters analysed (shape, size and surface of the pollen grains) didn't show a significantly difference at the 5% threshold between different collection periods. Therefore, in this section, the period of collection is not considered.

Table 1. Variability of the surface of pollen grain forms by clones.

Yam's clones name	Round shaped pollen grain		Oval shaped pollen grain		Probability
	Mean (µm²)	Standard deviation	Mean (µm²)	Standard deviation	
Laboko S	0,094	0,080	0,132	0,077	0,01*
Agatou A	0,113	0,084	0,166	0,106	0,54
Laboko I	0,168	0,086	0,301	0,235	0,01*
Kpakala I	0,069	0,031	0,086	0,065	0,77
Kabletounan S	0,051	0,035	0,061	0,031	0,33
Kodjewé S	0,091	0,065	0,114	0,069	0,42
Agatou I	0,105	0,058	0,132	0,088	0,50
Gnalabo I	0,081	0,045	0,140	0,058	0,08
N'Kenni S	0,093	0,095	0,134	0,060	0,69

Form of the pollen grains

Two forms of pollen grains, oval and round were observed in the clones. Fig. 1 shows the proportions of different forms of pollen grains per clone. The percentage of oval-shaped pollen grains varies from 30 to 88.23%, respectively in the clones Kabletounan S and N'kenni S and that of round-shaped of pollen grains varies from 11.77 to 70% respectively in the clones N'kenni S and Kabletounan. The clones N'kenni S, Gnalabo S, Agatou I, Kpakala I, Laboko S, and Kodjewé S have more oval-shaped pollen grains with respective percentages of 88.23%, 71.92%, 66.66%, 64.46%, 62.26%, 60% and 58.93%.Kabletounan S is the only clone which havemore round pollen grainsat rate reaching 70%. Both forms of pollen grains are found in identical percentage in the clone Agatou A, with 50% of each form of pollen grains. The two forms of pollen grain are found in all clones studied and the oval shape has the highest frequency.

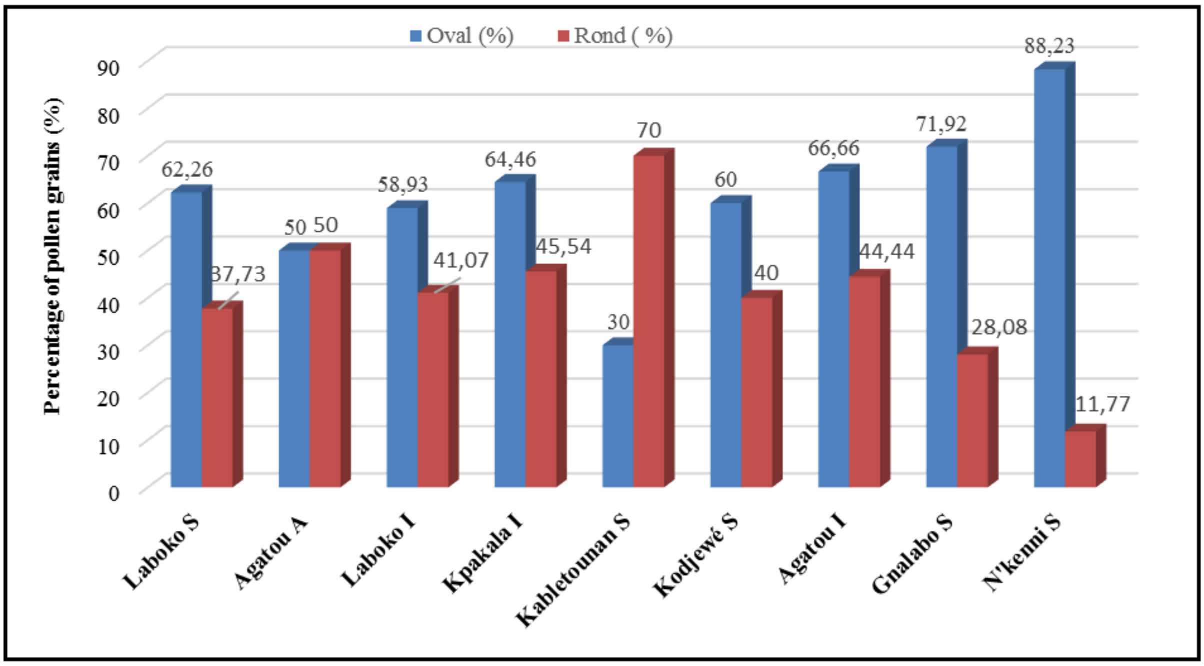

Fig. 1. Percentage of pollen forms per clone.

Morphological characteristics of pollen grains

The morphological characteristics of the different shape of pollen grains are shown in Fig. 2. For oval shaped pollen grains (Fig. 2a), the length of the major axis is between 0.2 and 1.5 µm with an average of 0.43 µm, obtained in the clones Kabletounan S and Laboko I. The average length of the major axis of the pollen grains varies between 0.28 ± 0.06 µm and 0.70 ± 0.27 µm, obtained for the clones Kabletounan S and Laboko I. This fluctuation of the size of the major axis is significantly different (P <0.0001) between the pollen grains of the clones. The length of the small axis of the oval-shaped pollen grains (Fig. 2b) varies between 0.1 µm and 1.2 µm, obtained in the clone Kabletounan S and Laboko I.

The variation of the mean length of the small axis of the pollen grains is between 0.20 ± 0.06 µm and 0.45 ± 0.22 µm, respectively for the clones Kabletounan S and Laboko I. This length variation of the small axis of the pollen grains between clones is significantly different (P <0.0001) at the 5% threshold.

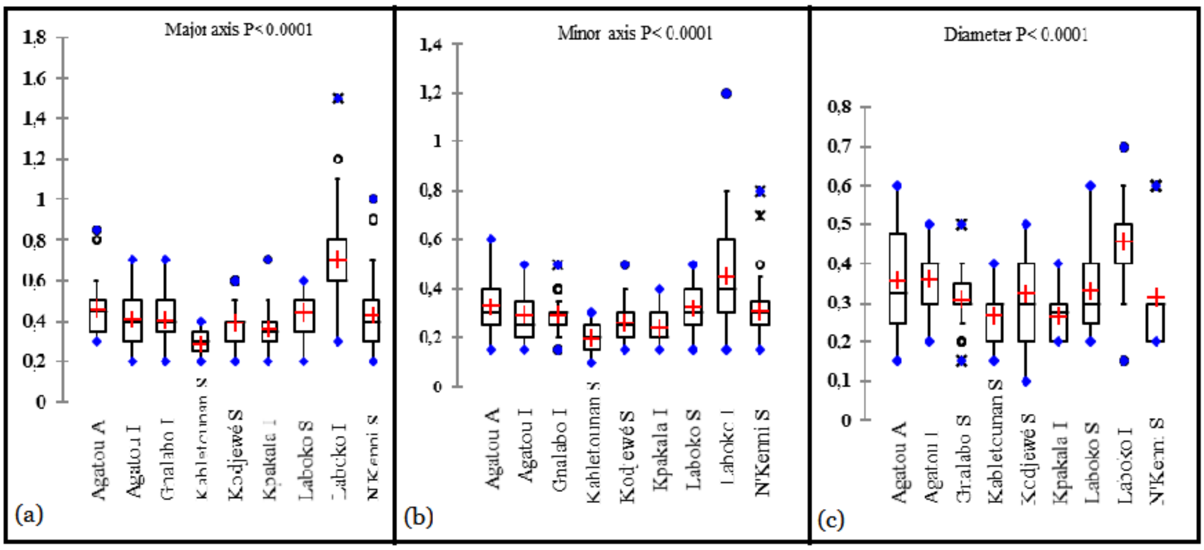

Fig. 2. Characteristics of the different pollen grains. (a) Major axis and (b) Minor axis of oval shape and (c) Diameter of round shape.

The shortest pollen grains were observed in the clones Kabletounan S and Kpakala I and those, which had largest pollen grains, were Agatou A and Laboko I. The standard deviation of the mean of the different axis reveals considerable variability in the size of the pollen grains within the same clone and between clones.

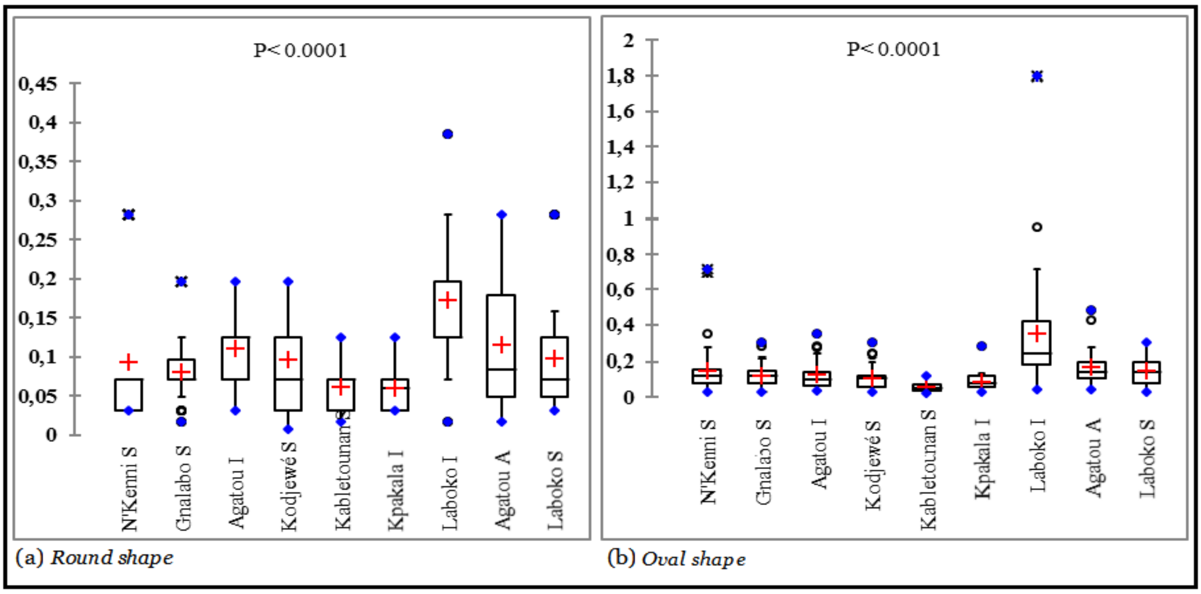

Fig. 3. Surfaces of intra clone pollen grains.

The diameter of round pollen grains (Fig. 2c) varies from 0.1 µm to 0.7 µm, obtained in the clones Kodjewe S and Laboko I, with an average of 0.33 µm. The mean length of the diameter fluctuation between the clones varies from 0.27 ± 0.07 µm (Kpakala I) to 0.45 ± 0.12 µm (Laboko I). This fluctuation of the mean diameter of the pollen grains is significantly different (<0.0001) in the clones. The mean of standard deviations range from 0.07 to 0.14; shows a large variation between the diameters of the round form of pollen grains between clones, but this variation is not significantly different in the clones N'Kenni S and Gnalabo I.

Surface of pollen grain variation within a clone

The variability of the surface of pollen grain per shape is presented inTable 1.The average surface of the round pollen grains varies from 0.051 µm² to 0.168 µm²obtained in the clones Kabletounan S and Laboko Iand is between 0.061 µm² and 0.301 µm² obtained in the clones Kabletounan S and Laboko I for oval shape. For the variability of the size of different shapes of the pollen grain, a significant effect (P <0.0001) was observed. Within the same clone, there is no significant effect in the size of the pollen grains according to the form. This difference of size between round and oval pollen grains of the same clone is significantly different (P = 0.01) for Laboko I and Laboko S clones, regardless of their different origin. For the other clones Agatou A, Kpakala I, Kabletounan S, Kodjewe S, Agatou I, Gnalabo I and N'Kenni S, the variation of the surface of the pollen grains whatever its form is not significantly different (P> 0.05).

Variability of pollen grain surface between clones

The variation of the average surface of the pollen grains between the clones studied is presented by Fig. 3. This average surface of round pollen grains is between 0.059 ± 0.030 and 0.173 ± 0.082 µm² obtained in the clones Kpakala I and Laboko I clones. The smallest pollen grains were observed in the clones Kabletounan S and Kpakala I whereas the clones Agatou I and Laboko I have the largest pollen grains (Fig. 3a). Between clones, the average size of the oval-shaped pollen grains varies from 0.059 ± 0.031 to 0.350 ± 0.335 µm², obtained in the Kabletounan S and Laboko I clones.

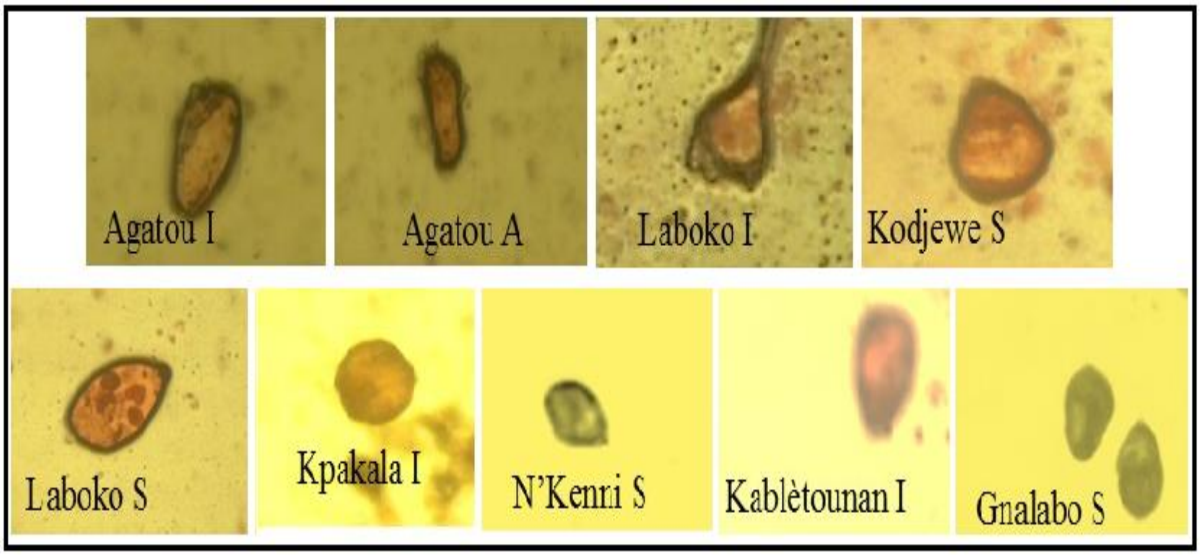

Fig. 4. Pollen grains of some male clones of *Dioscorea cayenensis - D. rotundata* complex species (magnification 400X).

The smallest pollen grains were observed in the clones Kpakala I and Kabletounan S and the clones Agatou A, Laboko I, Laboko S and N'Kenni S have larger pollen grains (Fig. 3b). Standard deviation of mean which is 0.073 for round shape pollen grains and 0.148 for oval shape pollen grains, showed a significant effect ($p < 0.0001$) of pollen grain surface between clones as showed by the Fig. 4.

Discussion

Microscopic observations of the pollen grains of the nine clones of the *Dioscorea cayenensis - D. rotundata* complex from central of Benin revealed two forms: oval and round shapes. These forms are encounter in all clones in different proportions. These results confirm those previous works, where the authors mentioned two different forms within the pollen grains of male clones of this species (Akoroda 1984, Zoundjihékpon 1993, Assaba *et al.*, 2015, Yolou 2016). The percentage of oval-shaped pollen grains is between 30% to 88.23%, observed in the clones Kabletounan S and N'kenni S, and that of round-shaped percentage is between 11.77% and 70% observed in the clones N'kenni S and Kabletounan S. Proportion of pollen grain forms analysis showed that some clones have in mainly oval shape of pollen grains (N'kenni S 88,23% and Gnalabou I 71,92%). Those have more round pollen grains (Kabletounan S 70%) and others clones have the two forms of pollen grains in same proportion (Agatou 50% for the both forms). These results are different from the results obtained with *Dioscorea dumetorum* whose pollen grains shape are only oval (Assaba, 2013). The pollen grain forms could be under genetic control, by an allelic combination in the genome of these clones.

The long axis (L) length of the oval-shaped pollen grains is between 0.2 µm and 1.5 µm, the minimal length is observed in all clones except Laboko I and that of the minor axis (I) is between 0.1 µm and 1.2 µm obtained in the clone Kabletounan S and Laboko I. The diameter of round pollen grains varies between 0.1 µm and 0.7 µm, in the clones Kodjewe S and Laboko I. This fluctuation of diameter length is significantly different ($P < 0.0001$). These observations confirm the results obtained by Assaba, (2013); Yolou (2016) and Denadi (2018) who worked on the cultivated yams of the complex species from Centre of Benin and in the same conditions. According to Zoundjihékpon (1993), the size of the minor axis (l) varies from 10 µm to 17 µm and the major axis (L) from 10.5 µm to 23 µm for oval-shaped pollen grains; and that the diameter of round pollen grains varies between 11 µm and 20 µm. These results are similar with results obtained by Miège, (1952) and Akoroda (1984) who observed pollen grains whose size was between 15 µm and 20 µm. This high difference of size of the pollen grains could be justified by the dyes used to carry out the microscopic preparations, because Zoundjihekpon (1993) had used glycerin-safranin, whereas in the present study acetic-carmine was used as dye, or by the quality and performance of the microscopes.

Pollen grains are conserved traits between generations, and constitute an excellent means of species identification (Iwanami *et al.,* 1988; Franssen *et al.,* 2001), they can't incur large variation in about two decades. This variation of pollen grain size was due to the type of microscope and observation magnification, and thus to the quality of the microscope, because some previous research had showed the stability of pollen grains of yams of the *Dioscorea cayenensis - D. rotundata* complex in Ivory Coast, as well as between cultivated and hybrids clones (Zoundjihékpon, 1993).

Similar results have been obtained in the *Amaranthaceae* family; with the average of pollen grain diameter of *Amaranthus dubius* Mart., observed at x400 magnification to optical microscope ranged from 7.25 µm to 10.25 µm (Soulemane, 2016). However, with magnification around x3500 or x4000 of electronic scanning microscope, or x3400 to optical microscope, the mean of *Amaranthus palmeri* S. Wats pollen grains diameter was between 21 µm and 38 µm (Sosnoskie *et al.,* 2009) and pollen grains of *Amaranthus viridis* diameter size obtained at x1000 magnification was 25 µm (Habari, 1983).

The large variation of standard deviation of the different size reflects a large variability in the size of pollen grains between clones. These results are similar with the observations of Soulemane, (2016) on *Amaranthus dubius*, who obtained a large variation between the standard deviation of the means size of pollen grains.

The fluctuation of pollen grain size showed different effect ($P < 0.0001$). This variation could be due to the genetic background of clones, nor to environmental effect, because climatic conditions haven't meanningful effect on the size of pollen grains (Arezki *et al.*, 1991).

Conclusion

This study showed two forms of pollen grains (oval and round) in the *Dioscorea cayenensis - D. rotundata* complex species. The oval shape of pollen grains is in highest proportion. The predominance of one form of pollen grain could be associated to genetic background of clones. However, pollen grain size is a stable trait in the species, which isn't influenced by the origin of clones. For each form of pollen grains, significant different effect was revealed between clones. The stability of the pollen grains size could facilitate the breeding of this species by sexual way, using the pollen grains as vectors of interest traits flowed between clones.

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