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Methodological approach to indigenous fruit trees breeding: case of *Dacryodes edulis* (G. Don.) H. J. Lam.(Burseraceae) in Cameroon

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Abstract

Very little work has been done forthe varietal improvement of indigenous fruit trees. Controlled cross pollination tests were conducted on *Dacryodes edulis* to assess the influence of the origin of the male parent and the type of flower that produced pollen used for fertilization on the fruiting efficiency of 14 well-known females' accessions from three provenances. The crossbreeding test was performed following a full nested mating design. The experimental design included provenance as a fixed factor, treatment as within-subject (i.e. repeated measures) fixed factor and plant individual as a random factor (subject). The results showed that the fruiting index that determines the species' yield varies significantly ($p = 0.010$) with the combined actions of the three factors studied which were (i) the provenance of the male parent; (ii) the pollen type used for hand fertilization (pure male or hermaphrodite) and (iii) the female parent status. Six best combinations originated from Boumnyebel and Makenene provenances, characterized by high fruit-setting rate and the fruiting index (>70% and >50% respectively), then by low fruit-dropping rate after fruit set (<20%) were identified. Although we did not observe increasing in fruit size as compare to breeding in *Citrus* or *Ziziphus* species, the process of controlled cross-pollination investigated in this study significantly increased the fruit set. This could help in controlling the early fruit drop which negatively impacts the species' yield. Thereafter, control-pollinated seedlings (F_1) obtained from this study and established as progeny trials will be vulgarized within agro-ecological zones and/ormultiplied vegetatively for clonal and futurecultivars development trials.

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Introduction

Tree breeding has generally been considered an improvement of the economic value or yield by influencing the originalgenetic constitution of the regeneration material (Gil-Ariza *et al.*, 2009; Ebert 2014) Although the selection of species and provenances is consideredas tree breeding in a wide sense, the latter is often defined more strictly as consisting of selection and crossing at the individual tree level (Eriksson and Ekberg, 2001). New tools for achieving genetic improvements, based on biotechnology, are currently under development. In the wild, most tree species reach reproductive maturity after a long period of juvenility and even then, sexual reproduction appears sporadically. Hence, improvement for tree production encounters a number of difficulties particularly in relation to ageing, slow growth, long juvenile phase, fruit production variability and lack of knowledge on the silviculture of the species (Lovett and Haq, 2000). This perception has been aggravated by the limited understanding of the natural variability, reproductive biology, propagation and the lack of techniques for first cultivation, then processing. Moreover, one of the major constraints in the promotionof local species is the lack of availability of good quality germplasm (Akinnifesi *et al.*, 2007). Fortunately, the advent of reliable and robust techniques of vegetative propagation has opened up much faster approaches that allow to developf clonal cultivars (Leakey *et al.*, 2005).

However, domestication of indigenous fruit trees has received far less attention than that of annual crop plants. Domestication of perennialtrees is a multidimensional process that involves identification of superior trees, production, management, diffusion and subsequentadoption of the desired germplasm (Simons and Leakey, 2004).Thus, it is appropriate to include in the list of exotic fruit trees widely cultivated (Leipzig, 1996), indigenous species with high-commercial value, selected according to farmers' preferences and their market potential. This is the case of *Dacryodesedulis*.or African plum, an oil richfruit tree belonging to family Burseraceae in the Gulf of Guinea (Bourdeaut, 1971). In West and Central Africa, it is one of a number of indigenous fruit tree under domestication (Tchoundjeu *et al.*, 2002).It iswidely commercialized in Cameroon, Gabon, Democratic Republic of Congo, Ghana and Nigeria (Awono *et al.*, 2002). *Dacryodesedulis* is a small to medium-sized tree that grows up to 20-25 m tall with a bole up to 70-90 cm in diameter (Kengie, 1990). It has a relatively short trunk and a deep, dense crown. The bark is pale grey and rough with droplets of resin. The leaves are compound with 5-8 pairs of leaflets (Onana, 2008). The upper surface of the leaves is glossy. Leaves are alternate and imparipinnate, whereas stipules are absent. The petiole is up to 7.5 cm long with 11-19 leaflets. The species is dioecious with three types of flowers occuring on two tree types: (i) One tree type is the female tree, which bears which bears female flowers only; (ii) The second tree type is a male tree, which bears predominantly male flowers and sometimes, hermaphrodite flowers at varying proportions (Onana, 1998).

The species contributes to rural incomes, supplements the local diet and is used in traditional and modern therapies (Schreckenberg *et al.*, ,2006; Omonhinmin, 2012).The value of the fruit lies in its pulp which is a good source of proteins, fats and carbohydrates that could be used to alleviate malnutrition in children (Ajayi and Adesanwo, 2009; Duru *et al.*, 2012). Thefruits are drupepink oblong to ellipsoid (5-10 x 3-4 cm), then dark blue or purple at maturity (Kengue,1990). In this species, mature pollen is binucleate with a thick exin, and presents one vegetative nucleus and one generative nucleus (Youmbi *et al.*, 1998). In Cameroon, the World Agroforestry Centre (ICRAF) has undertaken since 1998, a domestication programme on improvement and propagation of *Dacryodes edulis* (Tchoundjeu *et al.*, 2006) based on selection, collection and vegetative propagation of improved genotypes characterized by exceptional morphological, phenological, organoleptic and/or pomological traits for domestication (Leakey *et al.*, 2002; Akamba, 2002; Waruhiu *et al.*, 2004; Tchoundjeu *et al.*,

2008). Selection pressure by farmers is strong enough in some areas of the country such as the southern region of Cameroon, where farmers selected 67% of African plum trees from the wild (Schreckenberg *et al.*, 2006). This great mass selection contributed to highlight a number of cultivars within and among *D. edulis* populations in Cameroon.

The species has been reported to be amenable to vegetative propagation methods like air-layering and leafy stem/cutting (Mialoundama *et al.,* 2002). In addition, the rooting system of vegetatively propagated trees of African plum hasbeen reported to be stable (Asaah *et al.,* 2010) and apparently less competitive for below ground resources compared to trees of seed origin (Asaah *et al.,* 2012). Studies on *in vitro*propagation (Youmbi, 2000) werenotconclusiveAs a result of African plumdioecious natureand empirical selection by farmers, unproductive male trees and trees producing fruit with sour taste are systematically eliminated.

Despite the advantages of vegetative propagation techniques which may lead to hastening sexual maturity (early fruiting), exact replication of the desired mother-tree fruit traits or characters, easy reproduction of species whose seeds are difficult to conserve , it is feared that they could also severely narrow the species' genetic diversity and increase inbreeding at farm level, ultimately leading to a decline in future production (Hollingsworth *et al.*, 2005; Duminil *et al.*, 2009; van Tassel *et al.*, 2010). Inbreeding depression is the process by which self-or related-matings lead to homozygosity, loss of heterozygote superiority and 'exposure' of deleterious mutations (Lowe *et al.*, 2005). Inbreeding depression reduces individual fitness and raises the possibilities of population and/or species extinction (Hansson and Westerberg, 2002; Reed and Frankham, 2003); Indeed, the negative effects of inbreeding in trees are well-documented and include embryo abortion, limited fruit set, reduced overall seed yield and lower germination rates for remaining seed.

This study aims at improving the availability of high quality germplasm in *D. edulis* that will serve as improved raw material for mass vegetative propagation for the establishment/enrichment of African plum plantations. More specifically, the study aims at evaluating the provenance male parent and pollen type on: (i) fruit-setting rate; (ii) fruiting index; (iii) fruit-dropping rate under cross manualcontrolled pollination, and (iv) determining, based on the combination of the three factors, the most efficient crosses to be chosen as potential candidates for breeding.Information presented, will help breeders to develop 'superior elites' which could producehigh-yielding and good quality African plum hybrids (F_1) for further breeding, clone selection and cultivar development.

Material and methods

Study sites

The present study was carried out during three productionseasons, from January 2010 to September 2012 at two experimental field trials (gene banks) established by ICRAF (former International Centre for Research on Agro-forestry, actual World Agroforestry Centre) (Fig. 1). The first living genebank is situated at Minkoa-Meyos near Yaoundé, Cameroon (3°51'N., 11°25'E and 813 m a.s.l.), with mean annual rainfall of 1400 mm with bimodal distribution, and mean annual temperature of 25°C. The soils are moderately acid,i.e pH 1:1 soil:water 5 to 6 and Al saturation 20. Nursery activities were undertaken at the ICRAF's central nursery situated at Nkolbison (3°51'N, 11°27'E and 760 m a.s.l.), with a mean annual rainfall of 1300 mm. The second living genebank is situated at 65 km from Yaoundé in Mbalmayo division (3°10'N, 11°00'E and 650 m a.s.l.), with mean annual rainfall of 1802 mm and mean annual temperature of 24°C. The soils are deep ferralitic (Ambassa-Kiki, 2000).

Plant materials used for the crossbreeding test

Bothseedling genebanks were established with participation of farmers in 2001 and first flowering took place in 2007 (Tchoundjeu *et al.,* 2002). They are comprised of five provenances from which three were chosen for this study, namely Boumnyebel

(Littoral Region), Makenene (Central Region) and Kekem (West Region). Limbe (South-West Region) is the fourth and the 5th one named Ongot (Central Region) was victim of bush fires. Limbeprovenance was left out because of insufficient trees in blooming. The threeprovenances represent the major agro-ecological zones where *D. edulis* grows naturally in Cameroon. Each provenance consists of two trees on line each of five accessions, thirty offspring per accession arranged in randomized blocks of 150 trees per block, with a maximum distance between blocks approximately 100 m. Each randomized block consisting of accessions of the same provenance, includes 15 repetitions (Appendix 1). Distance between populations was 200 km and between "plus trees" was 100 m apart. Fifty seeds were collected from each tree, seeded and grown in polyethylene bags in ICRAF's central nursery, Yaoundé, Cameroon. After six months of growth in the nursery, planting were transplanted as provenance tests. These seedlings were planted in 40 x 40 x 60 cm hole, sand filled at 30 cm depth with fertile soil at 5 x 5 m spacing. Provenance-plots are surrounded by buffer rows of unimproved and unknown *D. edulis* provenances. Trials were hand-weeded twice per year for at least the first seven years.

Agro-morphological traits and experimental design

Characteristics selected for trees include size and fruit flavor, color and thickness of pulp, pulp oil content, fruiting season, disease and pest resistance, frequency and regularity of fruiting efficiency (yield). The collected germplasm was accessions of well-known origin from home gardens, crop fields, forest fallow, cocoa (*Theobroma cacao* L.) and coffee (*coffea*spp) farms. Trees were also geo referenced using a Global Positioning System for further sites mapping (Table 1).

For the present crossbreeding test, 20 trees were used, comprising six distinct male parents including three pure male and three male-hermaphrodites, and 14 selected vigorous female trees in blooming (Table 2). Hence, experimental plant material (Fig. 2) comprised of a total of 20 selected superior genotypes of African plum (9-year-old accessions) submitted to controlled hand cross-pollination using a full nested mating design (Zobel and Talbert, 1984).

Terminology and pollination experiments

The crossbreeding test was conducted following a full nested mating design adapted from Zobel and Talbert (1984) and Nanson (2004).Pure male parents are those male trees that, since the first flowering of the tree, had never bore fruits and their essential role is the production of pollen for the fertilization of female trees. Contrary, male-hermaphrodite trees are those trees, which occasionally bore fruits compare to female trees which bear fruits during each flowering season. Three factors were studied: (i) provenance of the male parent: Boumnyebel (BUM29), Makenene (MAK33) and Kekem (KEK02); (ii) pollen type used for hand fertilization (pure male or male-hermaphrodite) and status of the female parent. The experimental design included provenance as a fixed factor, treatment as within-subject (i.e. repeated measures) fixed factor and plant individual as a random factor (subject) (Fig. 2).

Pollination experiments were performed (during three flowering seasons) between January 20 and March 3, 2010; 2011 and 2012 at the Minkoa-Meyos locality; whereas at Mbalmayo site they were carried out between March 17 and April 13, 2010; 2011 and 2012. In each provenance we marked, labeled and bagged ten healthy and vigorous panicles on five female trees and two male/hermaphrodite trees, one week prior to anthesis. Depending of treesex pre-determined by the morphology of the inflorescence, that is 8 to 40 cm in length for male and hermaphrodite panicles (Appendix 2), and 5 to 20 cm for female trees (Appendix 2), selected panicles were bagged on both sexes with fine mesh cloth bags (1 mm^2) in size of 30 cm x 15cm (Appendix 3) that allowed the passage of light and air, but not insects. By so doing, we prevented flower deformation, unwanted insect visits and possible pollen removal by bees.

Cloth bags were removed from anthesis when the first

opened flowers were in the female stage. In each established provenance, fresh pollen was harvested on two male-hermaphrodite trees between 6 and 8 a.m., located at least 10m away from the targeted female, using a pair of pliers and a fine paint brush with black hairs against which the pollen could be seen, and kept in a Petri dish. Average number of 18 flowers was used and any unopened buds were removed. Since there was a gradient of open flowers at both flowering branch and entire panicle, it was not possible to pollinate the flowers the same day. On each selected female tree, bags were removed every day at 9 a.m., recently opened flowers were pollinated. Petri dish and brush were carefully cleaned with alcohol between flowers and trees to prevent contamination. Panicles were then rebagged immediately after hand-pollination. Flowers were monitored every three to four days, recording number of fruit set per panicle and per female tree, and bags were finally removed after eight days to enable fruit to grow easily.

Data recording and analysis

To assess fertilizing capacity and fruiting efficiency of tested superior/"plus-trees", depending on provenance of male parent and pollen type used for hand-pollination, four variables were observed during the study: (i) number of flowers pollinated, (ii) number of fruits set, (iii) number of mature fruits, and (iv) number of fruits dropped. Fruit began to develop with enlargement of an ovary (Palanichamy *et al.,* 2011), turning from green to pink at physiological maturity stage, then dark-blue, whitish green or purple at maturity within 3-5 months. After manual-pollination, the number of flowers effectively pollinated per panicle and per female accession was counted immediately. Young fruits were counted four days after pollination and continued every two days till eight days on each female accession. At the end of this observation period, all unopened flowers on the labelled panicles were removed. Upon physiological maturity stage (17-21 weeks after hand-cross-pollination), control-pollinated fruits were collected in open-weave collection bags, labelled and transported to ICRAF's laboratory at Nkolbison for characterization as soon as possible (within 2-3 days). Depending on the number of flowers pollinated for each crossing, fruit-setting rate (FSR), fruiting index (FI) and fruit-dropping rate (FDR) were calculated using equations 1, 2 and 3 below:

$$FSR\ (\%) = \frac{\text{number of fruits effectively set}}{\text{number of flowers pollinated}} X\ 100 \quad \text{Eq. 1.}$$

$$FI = \frac{\text{number of mature fruits}}{\text{number of flowers pollinated}} \quad \text{Eq. 2.}$$

$$FDR\ (\%) = \frac{\text{number of mature fruits, number fruits set}}{\text{number of flowers pollinated}} X\ 100 \quad \text{Eq. 3.}$$

The degree of relationship between fruit-setting rate, fruit-dropping rate and fruiting index was assessed using an analysis of correlation. Then, data were subjected to analysis of variance using procedure 'General Treatment Structure' Genstat version 14.0.0 software (Feb 16, 2011). For factors showing a significant effect on the observed variables (fruit-setting rate, fruiting index and fruit-dropping rate), means were calculated and separated using the more significant small difference (Allan *et al.,* 2006) with a threshold of 5% probability. Finally, to determine the most efficient crosses on the basis of the three evaluated criteria used in this study (fruit-setting rate, fruit-dropping rate and fruiting index), means obtained were grouped into similar performance class using the ascending hierarchical classification (cluster analysis). Average linkage cluster between provenances was analyzed. Data were processed under SPSS version 17.0.0 (Aug 23, 2008).

Results

Fruit-setting rate and fruiting index of African plum female accessions

Fruit-setting rate (FSR)

A highly significant effect (p *=0.044*) of parent provenance and a significant interaction (p*=0.005*) of pollen type and female parent status on the fruit-setting rate of *D. edulis*flowers pollinated manually was observed. Pollen type hadno significant effect (p*=0.15*) on the percentage of fruit set (Table 3).

Average fruit set per provenance was significant (p=

0.010) high in MAK33 (76.65%) than in BUM29 (70.18%) and KEK02 (69.90%) provenances respectively (Table 4).

In Boumnyebel provenance, fertilization with pollen from male-hermaphrodite accession indicated that BUM/DE/25_Seedling026 accession displayeda loweststatistically fruit-setting rate (50.94%) whereas this rate exceeded 75% for the other four accessions (BUM/DE/25 Seedling114 (78.44%), BUM/DE/37 Seedling111 (79.23%), BUM/DE/26 Seedling122 (76.06%) and BUM/DE/26 Seedling015 (75.38%)) of this provenance. Moreover, fertilization with pollen from pure male accession indicated the same trend, except that three groups couldbe distinguished: (i) BUM/DE/25 Seedling114 hadthe highest fruit-setting rate (76.14%), (ii) Seedling026 showed the lowest rate (55.93%) while BUM/DE/37 Seedling111, BUM/DE/26 Seedling122 and BUM/DE/26 Seedling015 occupy intermediate positions respectively 72.45%, 68.56% and 68.69% of fruit-setting rates.

Table 1. List of the 20 *D.edulis*selected accessions called "plus trees" collected from the agro-ecological zones where the species naturally grow in Cameroon. ACC= Accession.

N°	Acc. number	Code in genebank	Collection sites	Acc. sex	Latitude	Longitude	Altitude (m)
1.	ACC-01	BUM/DE/26 Seedling 015	Boumnyebel	Female	3°52'58.34"N	10°50'57.62"E	358
2.	ACC-02	BUM/DE/25 Seedling 026	Boumnyebel	Female	3°52'58.34"N	10°50'57.62"E	358
3.	ACC-03	BUM/DE/37 Seedling 111	Boumnyebel	Female	3°52'58.34"N	10°50'57.62"E	358
4.	ACC-04	BUM/DE/25 Seedling 114	Boumnyebel	Female	3°52'58.34"N	10°50'57.62"E	358
5.	ACC-05	BUM/DE/26 Seedling 122	Boumnyebel	Female	3°52'58.34"N	10°50'57.62"E	358
6.	ACC-06	MAK/DE/04 Seedling 078	Makenene	Female	4°53'03.84"N	10°47'41.44"E	696
7.	ACC-07	MAK/DE/28 Seedling 104	Makenene	Female	4°53'03.84"N	10°47'41.44"E	696
8.	ACC-08	MAK/DE/01 Seedling 116	Makenene	Female	4°53'03.84"N	10°47'41.44"E	696
9.	ACC-09	MAK/DE/04 Seedling 144	Makenene	Female	4°53'03.84"N	10°47'41.44"E	696
10.	ACC-10	KEK/DE/18 Seedling 050	Kekem	Female	5°09'05.91"N	10°01'16.07"E	715
11.	ACC-11	KEK/DE/18 Seedling 070	Kekem	Female	5°09'05.91"N	10°01'16.07"E	715
12.	ACC-12	KEK/DE/07 Seedling 074	Kekem	Female	5°09'05.91"N	10°01'16.07"E	715
13.	ACC-13	KEK/DE/13 Seedling 079	Kekem	Female	5°09'05.91"N	10°01'16.07"E	715
14.	ACC-14	KEK/DE/07 Seedling 142	Kekem	Female	5°09'05.91"N	10°01'16.07"E	715
15.	ACC-19	BUM/DE/29 Seedling 070	Boumnyebel	Pure male	3°52'58.34"N	10°50'57.62"E	358
16.	ACC-20	BUM/DE/29 Seedling 050	Boumnyebel	Male-hermaphrodite	3°52'58.34"N	10°50'57.62"E	358
17.	ACC-21	MAK/DE/33 Seedling 106	Makenene	Pure male	4°53'03.84"N	10°47'41.44"E	696
18.	ACC-22	MAK/DE/33 Seedling 126	Makenene	Male-hermaphrodite	4°53'03.84"N	10°47'41.44"E	696
19.	ACC-23	KEK/DE/02 Seedling 088	Kekem	Pure male	5°09'05.91"N	10°01'16.07"E	715
20.	ACC-24	KEK/DE/02 Seedling 102	Kekem	Male-hermaphrodite	5°09'05.91"N	10°01'16.07"E	715

For crosses with pollen from hermaphrodite parent in Kekem provenance, accession KEK/DE/07 Seedling074 displayeda lowestand significant fruit-setting rate (51.35%), while KEK/DE/13 Seedling079 and KEK/DE/18 Seedling070 accessions showed the highest fruit-setting rates (74.89 and 72.22%) respectively. Meanwhile, KEK/DE/18 Seedling050 and KEK/DE/07 Seedling142 accessions occupy an intermediate position with fruit-setting rates of 67.15% and 63.33% respectively. For crosses with pollen from pure male accessions, the percentage of fruit set per female accession after hand-pollination vary from 65 to 80%, but the differences observed are not significant at 5%.

In Makenene provenance, fertilization with pollen from male-hermaphrodite accessions induced the highest fruit-setting rate on MAK/DE/04 accession and the lowest fruit-setting rate was observed on MAK/DE/04 Seedling 116 (64.40%). Meanwhile,

these accessions indicated the highest fruit-setting rate (80%) when fertilized with pollen from pure male accessions. MAK/DE/04 Seedling078 with only 49% of fruit-setting rate was theleast efficient female accession. With a fruit-setting rate of 78%, MAK/DE/28 Seedling104 occupies an intermediate position irrespective ofthe type of pollen used for its fertilization.

Table 2. Provenance and accession of *D.edulis*parents (selected plus trees) used for the crossbreeding test.

Provenance	Pure male tree	Male-hermaphrodite tree	Female tree
Boumnyebel (BUM29)	BUM/DE/29 Seedling 070	BUM/DE/29 Seedling 050	BUM/DE/26 Seedling 015 BUM/DE/25 Seedling 026 BUM/DE/37 Seedling 111 BUM/DE/25 Seedling 114 BUM/DE/26 Seedling 122
Makenene (MAK33)	MAK/DE/33 Seedling 106	MAK/DE/33 Seedling 126	MAK/DE/04 Seedling 078 MAK/DE/28 Seedling 104 MAK/DE/01 Seedling 116 MAK/DE/04 Seedling 144
Kekem (KEK02)	KEK/DE/02 Seedling 088	KEK/DE/02 Seedling 102	KEK/DE/18 Seedling 050 KEK/DE/18 Seedling 070 KEK/DE/07 Seedling 074 KEK/DE/13 Seedling 079 KEK/DE/07 Seedling 142

Fruiting index (FI)

The average fruiting index of 14 female accessions tested through controlled manual-pollination in this study (Table 5) varieddepending on the provenance of male parents, type of pollen used and status of female parent crossed. Analysis of variance (Table 2) showed that variation of fruiting index was related only to combined action of the three factors studied: provenance of the male parent, status of the female parent in the provenance and type of pollen used for the crossing ($p=0.01$). None of these three studied factors taken individually showed a significant effect on the number of mature fruits ($p=0.21$ and 0.37 respectively) for pollen type used and provenance of male parent.

Table 3. ANOVA of fruit-setting rate, fruit-dropping rate and fruiting index of *D.edulis* based on the male parent provenance, the type of pollen used for fertilization and the female parent status in each provenance.

Source of variation		Fruit-setting rate		Fruiting index		Fruit-dropping rate	
	D.F.	SCE	F pr.	SCE.	F pr.	SCE	F pr.
Parent provenance	2	125.7	0.044	0.042	0.369	282.6	0.479
Provenance. Pollen type	3	1050.4	0.151	0.095	0.214	485.6	0.470
Provenance. Pollen type. Female parent	22	9265.9	0.005	0.909	0.010	8254.5	0.012
Residue	112	21752.1		2.323		21371.5	
Total	139	33319		3.368		30394.2	

Thus, in MAK33 provenance, MAK/DE/04 Seedling144 and MAK/DE/28 Seedling 104 showed a statistically identical fruiting index (0.68) when fertilized by pollen from male-hermaphrodite trees. This value is higher than those obtained from MAK/DE/04 Seedling078 (0.41) and MAK/DE/04 Seedling116 (0.37) accessions. Meanwhile, when fertilized with pollen from pure male trees, three of

the four studied females had a similar fruiting index (0.54) and significantly higher than that obtained from MAK/DE/04 Seedling116 (0.37). Thethree females are MAK/DE/04 Seedling144, MAK/DE/28 Seedling104 and MAK/DE/04 Seedling 078 respectively.

Regarding BUM29 provenance, all the five accessions tested (BUM/DE/25 Seedling114, BUM/DE/37 Seedling111, BUM/DE/26 Seedling122, BUM/DE/26 Seedling015 and BUM/DE/25 Seedling026) had fruiting index ranging from 0.38 to 0.53. The differences observed between averages were not significant at 5%, irrespectively offemales crossed with pollen from a male-hermaphrodite or a pure male tree.

Table 4. Fruit-setting rate of *D.edulis* under controlled hand-pollination conditions.

Provenance	Female parent	Type of flower which produced pollen used		
		Hermaphrodite	Male	Rate
BUM29	BUM/DE/25 Seedling114	78.44 a	76.14 a	
	BUM/DE/37 Seedling111	79.23 a	72.45 ab	70,18 b
	BUM/DE/26 Seedling122	76.06 a	68.56 ab	
	BUM/DE/26 Seedling015	75.38 a	68.69 ab	
	BUM/DE/25 Seedling026	50.94 b	55.93 b	
	Mean	72.01	68.35	
KEK02	KEK/DE/13 Seedling079	74.89 a	74.10 a	
	KEK/DE/18 Seedling070	72.22 a	75.13 a	69,90
	KEK/DE/18 Seedling050	67.15 ab	77.05 a	b
	KEK/DE/07 Seedling142	63.33 ab	78.50 a	
	KEK/DE/07 Seedling074	51.35 b	65.26 a	
	Mean	65.79	74.01	
MAK33	MAK/DE/04 Seedling144	88.20 a	86.51 a	
	MAK/DE/28 Seedling104	78.03 ab	77.77 ab	76.65 a
	MAK/DE/04 Seedling116	64.40 b	82.80 a	
	MAK/DE/04 Seedling078	72.01 ab	63.49 b	
	Mean	75.66	77.64	

Means followed by a common letter within a column are not significantly different at P< 0.05 (Student-Newman-Keuls test).

In KEK02 provenance, the same trend was observed but only in female accessions crossed with pollen from the male-hermaphrodite tree. It has been observed that in this provenance, all female accessions had a statistically similar fruiting rate (0.38-0.54). Meanwhile, when crossed with pollen from a pure male tree, three groups were identified: (i) the best accession KEK/DE/18 Seedling050 with a fruiting index of 0.62; (ii) the least efficient accessionKEK/DE/07 Seedling142 (0.42) and intermediate accessions KEK/DE/13 Seedling079, and KEK/DE/07 Seedling074, KEK/DE/18 Seedling070 with indices between 0.45 and0.60respectively.

Fruit-dropping rate (FDR)

Table 6 shows the average fruit-dropping rate after fruit set at the end of themanual-pollination test in *D. edulis* depending on provenance of male parents, type of pollen used and status of female parent. Fruit-dropping rate varied in each provenance from a female accession to another depending on pollen type

used for the crossing. Analysis of variance (Table 2) showed that apart from the combined action of pollen type used and plant mother status and provenance (*p=0.012*) in a given provenance, none of the three studied factors taken individually significantly affected (p≥0.470) fruit-dropping rate after fruit set in *D. edulis*.

Thus, in BUM29 provenance, BUM/DE/25 Seedling026 accession showed the lowest dropping rate that is only 13.17%, against 34.29% for BUM/DE/26 Seedling122 accession. Fruit-dropping rate was statistically similar in BUM/DE/26 Seedling015 individuals (22.71%), BUM/DE/37 Seedling111 (26.12%) and BUM/DE/25 Seedling114 (28.44%). With pollen from pure male tree, the same trend is observed between five female accessions and values are apparently lower than in crosses involving male-hermaphrodites as males; Min 7.9% and max 30.73% for BUM/DE/25 Seedling026 and BUM/DE/26 Seedling122 respectively.

Table 5. Fruiting index of *D.edulis*under controlled hand-pollination condition.

Provenance	Female parent	Type of flower which produced pollen used		
		Hermaphrodite	Male	Rate
BUM29	BUM/DE/25 Seedling114	0.50 a	0.53 a	
	BUM/DE/37 Seedling111	0.53 a	0.51 a	0.47a
	BUM/DE/26 Seedling122	0.42 a	0.38 a	
	BUM/DE/26 Seedling015	0.53 a	0.46 a	
	BUM/DE/25 Seedling026	0.38 a	0.48 a	
	Mean	0.47	0.47	
KEK02	KEK/DE/13 Seedling079	0.42 a	0.59 ab	
	KEK/DE/18 Seedling070	0.54 a	0.48 ab	0,48a
	KEK/DE/18 Seedling050	0.38 a	0.62 a	
	KEK/DE/07 Seedling142	0.40 a	0.42 b	
	KEK/DE/07 Seedling074	0.46 a	0.51 ab	
	Mean	0.44	0.52	
MAK33	MAK/DE/04 Seedling144	0.68 a	0.54 a	
	MAK/DE/28 Seedling104	0.68 a	0.54 a	0,51a
	MAK/DE/04 Seedling116	0.37 b	0.54 a	
	MAK/DE/04 Seedling078	0,41 b	0.37 b	
	Mean	0.53	0.50	

Means followed by a common letter within a column are not significantly different at *P< 0.05* (Student-Newman-Keuls test).

Regarding KEK33 provenance, KEK/DE/07 Seedling074 accession crossed with pollen from male-hermaphrodite tree had the best fruit-dropping rate of 5.15% against 32.53% and 29.07% for KEK/DE/13 Seedling079 and KEK/DE/18 Seedling050 respectively. When crossed with pollen from pure male tree, KEK/DE/07 Seedling074 accession maintained the lowest dropping rate (14.49%) but this time with KEK/DE/13 Seedling079 (14.94%) and KEK/DE/18 Seedling050 (15.33%) accessions. The highest fruit-dropping rate was recorded on KEK/DE/07 Seedling142 (36.29%).

In MAK33 provenance, the lowest fruit-dropping rate after fruit set was recorded on MAK/DE/28 Seedling104 (10.47%) crossed with pollen from a male-hermaphrodite tree. For the other three accessions, rates were higher than 20%: MAK/DE/04 Seedling144 (20.73%), MAK/DE/04 Seedling116 (27.67%) and MAK/DE/04 Seedling078 (31.28%). It

was noticed that, in this provenance, when crossed with pollen from a pure male parent, all the six female accessions recorded a fruit dropping rate above 20%.

Ascending hierarchical classification of the studied accessions

Fig.3 shows the hierarchical classification grouping the 28 genetic crosses conducted during this study depending on the similarity of the performance based on the evaluation criteria (fruit-setting rate, fruit-dropping rate and fruiting index). It shows that crosses can be classified into five distinct clusters:

Cluster I consists of four crosses from exclusively Kekem provenance. It is characterized by a greater fruit-setting rate higher than 70%, a fruiting index higher than 50% but a fruit-dropping rate that exceeds 25% of the flowers pollinated.

Table 6. Fruit-dropping rate in *D. edulis*under controlled pollination.

Provenance	Female parent	Type of flower which produced pollen used		
		Hermaphrodite	Male	Rate
BUM29	BUM/DE/25 Seedling114	28.44 ab	22.93 ab	
	BUM/DE/37 Seedling111	26.12 ab	21.04 ab	22,97 a
	BUM/DE/26 Seedling122	34.29 a	30.73 a	
	BUM/DE/26 Seedling015	22.71 ab	22.71 ab	
	BUM/DE/25 Seedling026	13,.17 b	07.90 b	
	Mean	24.95	20.99	
KEK02	KEK/DE/07 Seedling142	23.22 ab	36.29 a	
	KEK/DE/13 Seedling079	32.53 a	14.94 b	21,68 a
	KEK/DE/18 Seedling070	18.22 ab	27.54 ab	
	KEK/DE/18 Seedling050	29.07 a	15.33 b	
	KEK/DE/07 Seedling074	05.15 b	14.49 b	
	Mean	21.64	21.72	
MAK33	MAK/DE/04 Seedling144	20.73 ab	32.10 a	
	MAK/DE/28 Seedling104	10.47 b	23.70 a	25,23 a
	MAK/DE/04 Seedling116	27.67 a	29.06 a	
	MAK/DE/04 Seedling078	31.28 a	26.80 a	
	Mean	22.54	27.92	

Means followed by a common letter within a column are not significantly different at *P< 0.05* (Student-Newman-Keuls test).

Cluster II includes six crosses mainly characterized by a high fruit-setting rate (>70%) and fruiting index (>50%) and a low fruit-dropping rate (<20%) respectively after fruit setting. These crosses belong to Boumnyebel and Makenene provenances as follow: BUM_050*015; BUM_070*111; BUM_070*114; MAK_106*104;MAK_126*104 andMAK_126 *144; with symbol * as used as 'crossed with'.

Cluster III comprises crosses with low levels of fruit-setting rate (<70%), fruiting index (<50%) and fruit-dropping rate (<25%) respectively.

With regard to crosses in cluster IV, fruit-setting rate (>70%) and fruiting index (>50%) are higher, but contrary to results described above in cluster II, dropping is abundant (>25%).

Cluster V has the same trend as cluster III unlike the fact that fruit-dropping rate is high (>25%). It includes four crosses, two with pollen from Makenenemale parent (MAK_106*078 and MAK_126*116), and one with pollen from Boumnyebel (BUM_070*122) and Kekem (KEK_088*142) male parent.

Discussion

During the crossbreeding experiments, manual pollination seemed to be difficult to conduct with pollens from pure-male trees due to their agglutinated nature and small size which renders them difficult to collect. This type of pollen withered rapidly when in contact with ambient air or when kept in aPetri dish (pers. obs.). Contrary to this observation, given the significant size of pollen from male-hermaphrodite trees, it was easy to harvest and more accurately deposed on the stigma of the flower. Given these observations, one could expect that manual-pollination performed with pollen from male-hermaphrodite tree may lead to best results (high fruit set, high fruiting index and low fruit drop). This hypothesis was confirmed for Boumnyebel and Makenene provenances, Kekem provenance having presented a reverse trend. From this study, one might think that pollen from a pure male tree, because of its small size, is more able to adhere to the micropyle of the ovule, thus reducing handling errors. This ability of pollen from pure male tree to adhere to the micropyle of the ovule because of its small size, explains the phenomenon which happens in natural conditions when the honeybee, main pollinator of the species, disperses pollen.

Fig. 1. Localization of the studied sites.

Source: National Institute of Cartography 2006 (Redrawn by Priscilla Ngaukam).

Fruit development is an exquisitely plant specific process under the control of a complex interplay of endogenous and environmental factors. Likewise, the process of fruit set is defined as the commitment of the ovary tissues to undergo transformation into a fruit (Gillaspy *et al.*, 1993). This process is gaining increasing interest also for its potential exploitation to control parthenocarpic fruit development, in the absence of pollination/fertilization. Regarding the estimation of fruit-setting rate, results of this study are almost in line with those reported previously by Kengue (1990) on *D. edulis*. Indeed, this author obtained an average fruit-setting rate of 93.62% in a manual-pollination test on a sample of only three female trees in the studied species. The difference observed between these two results may be attributed to: (i) size of the sample; (ii) genetic disposition of the sample coupled to environmental conditions wherein it is located, and/or (ii) applied methodology (studied factors).

Results from this study on *D. edulis* manual-pollination, compared to studies performed on open-pollination of some fruit tree species under natural conditions, suggest that manual-pollination process improves fruit set. These results are in line with those reported by Degani *et al.* (1990), Johannsmeier and Morudu (1999), and Omokhua and Koyejo (2009) on avocado (*Perseaamericana*Mill.); Kalinganire *et al.* (2001) on silky oak (*Grevillea robusta*A.Cum.ex R.Br.*)*; Omokhua and Ukoimah (2008) on *Teprapleuratetraptera*(Schum. and Thonn.); Koné *et al.* (2009) on *Ziziphusmauritiana*Lam. and Iqbal *et al.* (2010) on palm date (*Phoenix dactylifera* L.). Therefore, fruit set improvement through controlled manual pollination can be attributed to precision with which pollen is applied on the stigma of the opened flower.

Increasing outcross pollen from one to four did not alter fruit set, nor did increasing the quantity of eggs per flower. These results are consistent with the findings of Margriet *et al.* (2000), Bots and Mariani (2005), and De la Bandera and Trasevet (2006). These results mean that all flowers had an equal

chance of fruit set regardless of pollen quantity, pollen type or egg number. Similar results were pointed out by Vander Kloet (1983) from a study on the relationship between seed number and pollen viability of *Vacciniumcorymbosum* L., and Allison (1990) on a study related to the reproductive biology of *Taxuscanadensis*Marsh. Similarly, these results are also consistent with those reported by Winsor *et al.*(1987) on *Cucurbitapepo* L., Holland *et al.*(2004) on senitacacti (*Lophocereusschottii*Engel.) and Jorge *et al.* (2005) on *Bauhinia ungulata* L. Generally, the effective pollination period is determined by length of stigma receptivity, pollen tube growth rate, and ovule longevity.

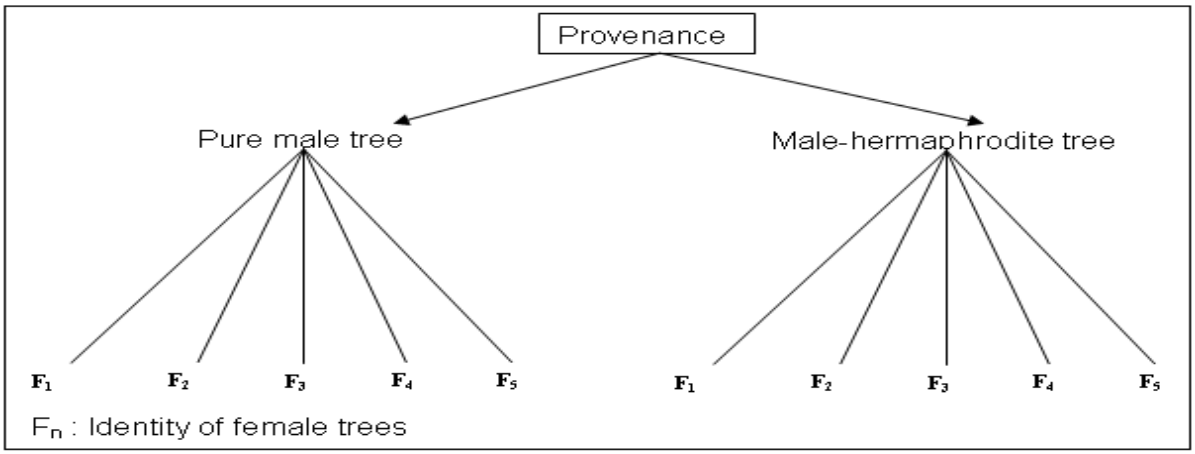


Fig. 2. Nested mating design diagram (Zobel and Talbert, 1984; Nanson, 2004). In the diagram, the chosen male is crossed with five different female accessions belonging to the same provenance, knowing that we used three provenances.

In fruit crops, the fruiting index is heavily correlated with the flowering index (Niang, 2002; Gassana-Dia *et al.,* 2003). Many proximate (ecological) and ultimate (evolutionary) hypotheses have been proposed to explain excess flower production and low fruit-to-flower ratios (De la Bandera and Traveset, 2006). Among the array of reproductive parameters in tropical forest species, fruiting efficiency is the most remarkable (important) to the commercial farmer, because it determines the overall yield of his crops per hectare. Moreover, low fruiting efficiency implies low harvest yield and vice versa (Sakai, 2002; Koenig *et al.,* 2003; Wright *et al.,* 2005). Mean fruiting efficiency of 48% recorded for *D. edulis* in this study is higher than 1.31 and 1.36% obtained by Omokhua and Koyejo (2009) respectively on *D. edulis* var. *edulis* and var. *parvicarpa* in Nigeria undernatural conditions. In parallel, this result is also higher than those obtained by Oni (1990) working on *Terminalia ivorensis* A. Chev.as well as Oni and Adedire (1987) working on *T. catappa*L. These authors reported 21% and 26% fruiting efficiency respectively for these species. This result may suggest that manual-pollination increases fruit set and fruiting index.

Additionally, many fruit species bear an abundance of flowers, which produce a surplus of fruits that the tree is unable to support. In *D. edulis*, a pure male or male-hermaphrodite inflorescence can carry between 300 to 500 flowers with 75-120 only, given their positions, rich at anthesis (Kengue, 1990). Contrary in female trees, flowering shoots are 5-10 inflorescences each consisting of 90 flowers. According to this physiology, it is obvious that a large number of flowers formed would not develop into fruits. This situation tends to deplete resources of the tree. Thus, for pollination experiment, wetherundernatural or controlled (manual) conditions, it would be wise to check the phenomenon of fruit set (fraction of flowers beginning fruit development) because in *D. edulis*, panicles having a certain high fruit-setting rate particularly loose prematurely much fruits. This result is in line with those reported by many other

authors whose studies were carried out on fruit trees (Anila and Radha, 2003; Basharat etal., 2008; Al-Naggar *et al*., 2009). To solve this problem, one method could be to spray, with growth inhibitors as ethephon or 2-chloroethyl phosphoric acid, gibberellic acid (AG3) and the perlagonic acid or endothall during flowering to reduce the fruit-setting rate. This technique may help reducefruit load and improve tree fruiting efficiency as earlier pointed out by Liao *et al.* (2006) and Modise *et al.* (2009).

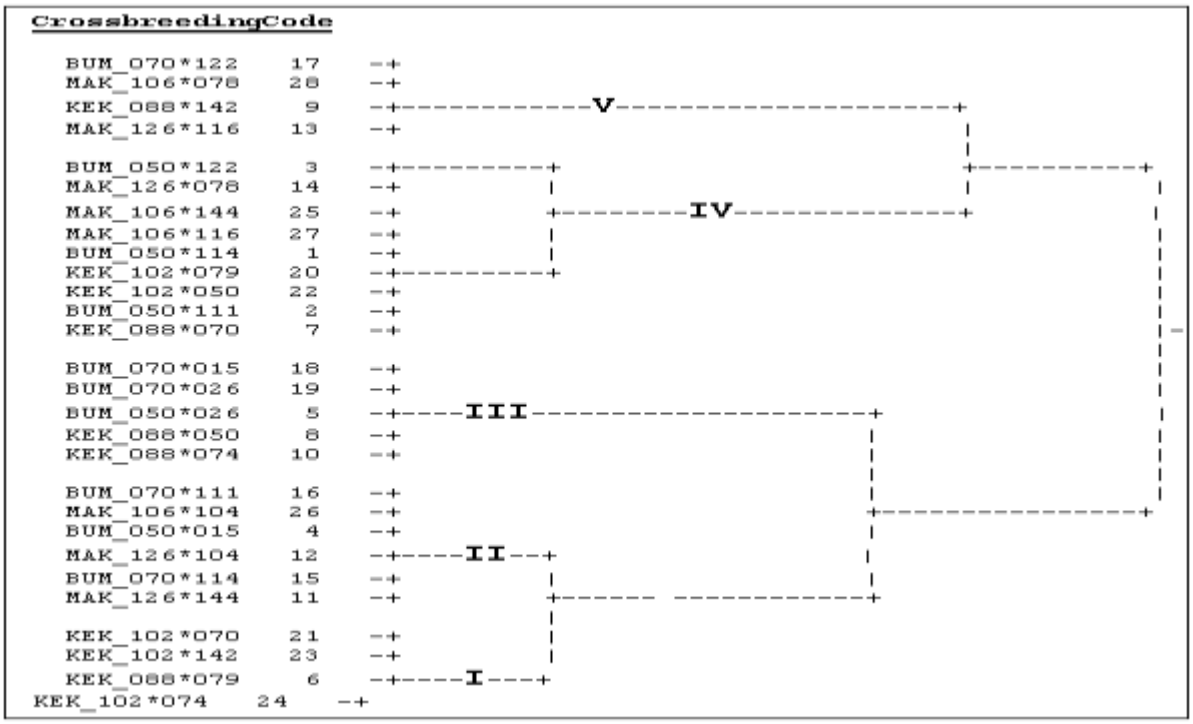


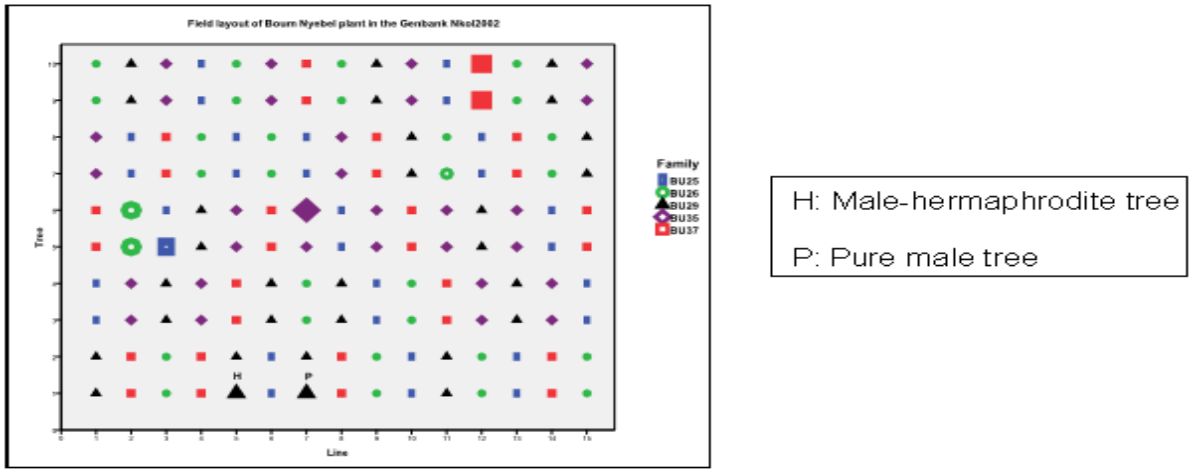
Fig. 3. Ascending hierarchical classification of 28 crosses conducted with six male and 14 female accessions of selected *Dacryodes edulis* plus-trees, from three provenances in Cameroon (MAK=Makenene ; KEK=Kekem and BUM=Boumnyebel).

Likewise, fruit trees have evolved a system to control fruit load in relation to their nutritional status, thus allowing the plant to make efficient use of resources. This is naturally achieved by a process called physiological drop, involving the abscission of young developing fruits mainly due to a correlative dominance effect of adjacent fruit and/or nearby shoots (Bangerth, 2000). To control fruit load, the major fruit species developed an immature fruit (fruitlet) physiological drop as a self-regulatory mechanism. This process is at least in part a consequence of the competition among fruits and between fruits and shoots for carbon assimilates. The phenomenon of fruit drop is very pronounced in most fruit trees (Lebon *et al*., 2004; Iqbal and Karacali, 2004). It can be a physiological fruit drop (fruit abortion) or the abscission of ripe fruit (fruit abscission), all related to factors that could be explored in further work. To avoid these effects, farmers could perform blossom or fruitlet thinning to adjust crop load and ensure a satisfactory fruit quality at harvest for commercial purposes.

Moreover, it is known that from fruit set to maturity and repining, fruit may drop at different stages as it was demonstrated in other nuclei fruit trees (Alcaraz and Hormaza, 2009; Muhammad *et al.,* 2011). The physiological drop may depend on the success of fertilization, which is essential to maintaining the fruit on the tree, or on climatic conditions such as adverse effects of drought, winds and heavy rains (Holland and DeAngelis, 2002).

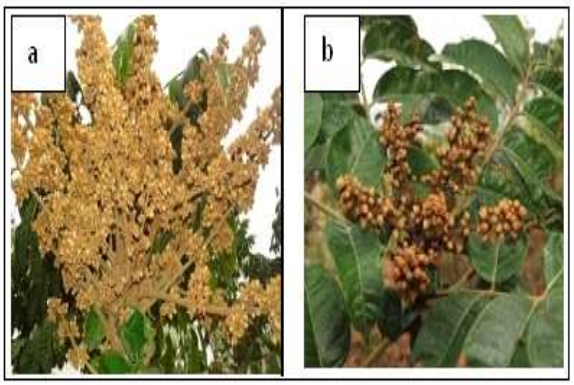
Similarly, abscission is a natural self-regulatory mechanism whereby fruit trees shed part of the fruitlets, and is an important agricultural event from

the farmer's point of view because it directly affects the final size and quality of the commodity. In spite of this self-regulatory mechanism, fruit trees set too many fruitlets negatively affecting not only the final quality, but also the returning bloom (Eccher *et al.*, 2013). With regard to fruit abscission (Eccher *et al.*, 2013), some factors can reduce the accuracy of the estimation of the pollination activity (McFadyen *et al.*, 2011). These factors can be: (i) competition both between young fruit and growth of vegetative organs, and secondly between fruits themselves for minerals, growth hormones and especially for carbohydrates. Studies related to this competition have been carried out by many authors such as Sheard (2008) or Omokhua and Chima (2009); (ii) risk of fruits predation and diseases (Bos *et al.,* 2007) and (iii) environmental conditions.



Appendix 1. Layout of Boumnyebel*D. edulis*provenance trial.

The interaction of these physiological and environmental factors could explain the selection that operates on the level of the tree between fruit drop and those thatremain on the tree as pointed out by Mehdi *et al.* (2007) and De Smedt *et al.* (2012).



Appendix 2. *D. edulis* inflorescences: (a) Male/hermaphrodite; (b) Female

Mean fruit-dropping rate of 24.89% recorded for *D. edulis* in this study is lower than 98.79 and 98.64% obtained by Omokhua and Koyejo (2009) respectively for *D. edulis* var. *edulis* and var. *parvicarpa* in open-pollination. The difference observed can be explained as result of mass selection did by farmers during many years and the fact that our study was carried out undercontrolled conditions. Indeed, mass selection may have contributed to the selection of superior genotypes with low fruit drop and controlledmanualpollination may help increase fruit set and by so doing, reduce fruit drop. This result is also lower than that reported by Omukhua and Chima (2009) working on avocado. These authors reported 99.21% fruit-dropping rate (open-pollination condition).

Furthermore, the phenomenon of fruit drop is also probably under the control of growth hormones such as ethylene (Hilt and Bessis, 2003), cytokinins (Ollat *et al.,* 2002), gibberellins and auxins (Roberts *et al.,* 2002) and finally the polyamines (Malik and Singh, 2003). Indeed, auxins and gibberellins play a pivotal role in the inductive phase of fruit set and parthenocarpic development of fruits. Like many other tropical forest fruit trees, the reasons for low

average yields of *D. edulis* are complex and need further research. Evolutionary history, stage of domestication and vegetative-reproduction competition for photosynthate at critical stages is speculated as contributory factors to the low yields of the studied species. Similar results have been obtained by McFadyen *et al.* (2011) on a study related to shoot growth post-pruning of macadamia in Australia. In addition, Blumenfeld *et al.* (1983) reported that vegetative-reproductive growth competition involving the partitioning of photosynthate is a major limitation to some cultivated fruit trees yield potential such as avocado. Similarly, Bawa and Webb (1984) as well as Allison (1990) stated that pollination limitations and fertilization failures are contributory factors to low fruiting efficiency in tropical plants. As our study was focused on controlled manual-pollination, pollen limitations could not be a limiting factor for low fruiting efficiency.

Appendix 3. *D. edulis* floral panicle isolated with fine mesh cloth bag.

From the present study, we can deduce that during the pollination process, several factors can influence fruiting efficiency of a tree in a given species. It couldinclude: (i) success of pollination guaranteed by methodology used. Indeed, during this process, pollen viability (Firmage and Dafni, 2001; Bots and Mariani, 2005; Ferreira *et al.,* 2007) and stigma receptivity may be questioned, the micropyle of the ovule may be damaged when applying pollen; (ii) amount of mineral resources available during flowering. Indeed, during flowering, competition for water and nutrients between various parts of the plant, including floral panicles initiation and the growth of vegetative organs may be a limiting factor forfruit set; (iii) genetic status of the plantand, (iv) environmental factors may also influence the process. In addition, Lahav and Gazit (1994), Lahav and Lavi (2002) as well as Klein *et al.,* (2003) believe that with the process of controlled pollination (manual or hand pollination), there is substantial evidence that cross-pollinated fruits have a better chance of maturing. This hypothesis is fairly in line with the results of the present study.

Conclusion

The present study has produced great information with regard to breeding system of *D. edulis*. Indeed, grouping crosses into clusters revealed that cluster II combined the best potential candidates for further breeding. It includes six crosses characterized by high fruit-setting rate (>70%) and high fruiting index (>50%), then a low fruit-dropping rate (<20%) respectively after fruit setting. These comprised three crosses from Boumnyebel provenance (BUM_050*015; BUM_070*111; BUM_070*114) and three others from Makenene provenance (MAK_106*104; MAK_126*104 and MAK_126 *144). Although we did not observe increasing in fruit size as compared to breeding in *Citrus* and *Ziziphus* species, the process of controlled manual-pollination investigated in this study significantly increase the fruit set. This could help in controlling the early fruit drop which negatively impacts the species' yield. Control-pollinated seedlings (F_1) obtained from this study were established as progeny trials. They will be monitored and managed till the first flowering and fruiting for genetic gain estimation. Furthermore, F_1 seedlings will be vulgarized within agro-ecological zones in Cameroon.They could be multiplied vegetatively and used to establish clonal trials for cultivars development.Nonetheless, the spatio-temporal regulation of the molecular factors involved in early steps of fruit set and development should be investigated. This information may help to understand how a plant response to endogenous/environmental perturbations.

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