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GENETIC EVALUATION OF SEED TRAITS FROM INTRASPECIFIC CROSSING OF GENETICALLY DISTINCT WATERMELON VARIETIES

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ABSTRACT

Citrullus lanatus (Thunb.) Matsumura and Nakai (Cucurbitaceae) is an important cucurbit crop worldwide. Global production of watermelon is about 90 million metric tonnes per *annum*, making it among the top five most consumed fresh fruits. The objective of this study was to evaluate seed variability in different segregating populations, and determine heritability of traits of watermelon. Interspecific crosses were made between two cultivars of *C. lanatus* (Bebu and Wlêwlê Small Seeds (WSS)) were performed at Research Station of Nangui Abrogoua University in Abidjan, Côte d'Ivoire. There was wide variability between parental, F_1 , BC_1 (first generation of back-crossing) and F_2 seeds. Seeds of all hybrid populations were intermediate versus those of the parents. Also, crossing did not affect F_1 and F_2 seed characters, but affected those of BC_1 because of maternal effects. Thus, back-crossing on Bebu cultivar produced seeds which looked like those of Bebu; while back-crossing on WSS cultivar produced seeds similar to those of WSS. Principal Component Analysis (PCA) and individuals repartitioning revealed that Bebu and WSS cultivars were genetically distinct and showed three main groups: two groups from each parental line and one from a recombinant line (hybrids). F_2 population had a wide individual's dispersion, and contained seeds of all other populations. High heritability was observed for all evaluated characters.

Key Words: *Citrullus lanatus*, Côte d'Ivoire, heritability, hybrids

RESUME

Citrullus lanatus (Thunb.) Matsumara et Nakai (Cucurbitacée) est une importante cucurbité dans le monde entier. La production annuelle globale est d'environ 90 million de tonne, ce qui place cette culture parmi les cinq fruits les plus consommés crus. L'objectif de cette étude est d'évaluer la variabilité des graines de différentes populations en ségrégation et de déterminer l'héritabilité des caractères chez l'espèce *Citrullus lanatus*. Pour cela, un croisement interspécifique entre deux cultivars de *Citrullus lanatus* (Bebu et Wlêwlê à petites graines (WSS)) a été effectué à la station de recherche de l'Université Nangui Abrogoua d'Abidjan (Côte d'Ivoire). Il existe une variabilité importante entre les graines parentales, F_1 , BC_1 (première génération de back-cross) et F_2 . Les graines de toutes les populations hybrides sont intermédiaires à celles des parents. Aussi, le sens de croisement n'affecte pas les caractères des graines F_1 et F_2 mais affecte ceux des graines BC_1 à cause de l'effet maternel. Ainsi, un backcross effectué sur le cultivar Bebu produit des graines semblables aux graines de Bebu tandis qu'un backcross effectué sur le cultivar WSS produit des graines semblables aux graines de WSS. L'Analyse en Composantes Principales et la répartition des individus dans le plan permettent d'observer que les cultivars Bebu et WSS sont génétiquement distincts et montrent trois principaux groupes : deux groupes de chaque type parental et un type recombinant (hybride). La population F_2 a une large dispersion et se compose des graines de toutes les autres populations. Une

forte héritabilité a été observée pour tous les caractères évalués. En somme, la variabilité des graines observées dans les populations en ségrégation chez *C. lanatus* pourrait être due à des effets génétiques

Mots Clés : *Citrullus lanatus*, Côte d'Ivoire, héritabilité, hybride

INTRODUCTION

Citrullus lanatus (Thunb.) Matsumura and Nakai (Cucurbitaceae) is an important cucurbit crop globally. World production of the crop is about 90 million metric tonnes annually, making it one of the top five most consumed fresh fruits. *Citrullus lanatus* can be found growing wild in various areas of the world, particularly in India and Mediterranean region, including Iran and Egypt (Kumar and Wehner, 2011).

In west-Africa, this traditional vegetable is perceived as potentially useful for income generation, food security (Achigan-Dako *et al.*, 2008; Dos Santos *et al.*, 2012) and medicinal uses (Abdel and Bamerni, 2011). Despite these numerous agronomic, social, health and economic potentials, along with its good adaptation to extremely divergent agro-ecosystems, *C. lanatus* cultivars are either missing in inbreeding programmes, neglected or underutilised (also called orphan crops). Consequently, there is lack of informations about genetics and breeding of this important crop in many African countries. Also, the volume supplied is often far short of market demand. There is need for cultivar improvement, especially for increased seed traits, particularly for countries such as Côte d'Ivoire.

In general, the traditional varieties are less productive than hybrids and improved cultivars, although they are highly adapted to specific cultivation conditions, and also a very significant repository of important genes for drought and pests resistance (Dos Santos *et al.*, 2012).

In the last decade, the proportion of watermelon hybrids has dramatically increased in occidental countries because of the advantages of heterosis effect, improved technology and underutilisation of controlled environments (Nerson, 2007). In these regions, watermelon breeders have contributed to the development of new cultivars and understanding of the genetics of the useful traits. In population improvement, it is imperative to determine the

extent of genetic variation for a trait to be improved (Dos Santos *et al.*, 2012).

The basic idea in the study of yield variation is its partitioning into components attributable to different causes, and the relative magnitude of these components determines the genetic properties of the population (Falconer, 1989). This led to the concept of heritability, which estimates the relative contributions of the differences in genetic and non-genetic factors, to the total phenotypic variance in a population. Determining the components of variability in yield and its components will also enable us to understand the extent of environmental influence on yield, taking into consideration the fact that yield and its components are quantitative characters and are affected by environment (Tazeen *et al.*, 2009). The objective of this research was to estimate the magnitude of the various components of variation, heritability and genetic advance in *C. lanatus* cultivars populations in Côte d'Ivoire.

MATERIALS AND METHODS

Study sites. The study was conducted in two locations; Abidjan (Research Station of Nangui Abrogoua University) and Manfla, both in Côte d'Ivoire. Abidjan is located in Lagoon District in southern Côte d'Ivoire (48°41' N, 48°00' W). The rainfall is abundant (annual mean > 2000 mm) and annual mean temperature is about 28 °C. Vegetation is mainly represented by the tropical rain forest, with mangrove on the coastal side (Zoro *et al.*, 2006).

Manfla is a village closest to Gohitafla city in the Department of Zuenoula (District of Marahou), in the west-centre of Côte d'Ivoire, between latitudes 7°00' N and 7°26' N and longitudes 6°00' W and 6°30' W (Kouassi and Zoro Bi, 2009). Rainfall is regular (annual mean of 1500 mm) and annual average temperature is about 27 °C. Vegetation is mainly represented by the tropical forest.

Plant materials. Two parental cultivars of *C. lanatus* (*Bebu* and *Wlêwlê Small Seeds*) and their offsprings (F_1 , BC_1 and F_2) obtained from crosses were used in the study. *Bebu* and *Wlêwlê Small Seeds* (WSS) were genetically distinct. Big fruits coming from *Bebu* contained few big seeds and were harvested early; while small fruits coming from WSS, contained small seeds and were harvested late (Adjoumani *et al.*, 2012). Seeds of these parental cultivars were provided for five successive self-pollination experiments, and were conserved in the Genetic Laboratory of Nangui Abrogoua University, under introduction numbers (NI) that were NI 121 for *Bebu* cultivar and NI 113 for WSS cultivar. *Bebu* and WSS were crossed and three offspring generations (F_1 , F_2 and BC_1) were produced.

Treatments. Before the crossings assessed in this study, purity of parental cultivars (*Bebu* and WSS) was obtained previously after five self-pollination generations. Then, reciprocal crosses between *Bebu* and WSS cultivars (*Bebu* ♀ x WSS ♂ and WSS ♀ x *Bebu* ♂) were done from March to June 2011. After these crosses and for each cross, F_1 plants were produced by selecting seeds from three fruits coming from these different plants. F_{1b} and F_{1w} were both families developed, respectively, from these reciprocal crosses. Then, from August to November 2011, parental, F_{1b} and F_{1w} seeds were sowed in the same plot to produce BC_1 and F_2 seeds. From F_{1b} and F_{1w} plants, self-pollination was performed to produce F_{2b} and F_{2w} plants, respectively.

Crosses between F_{1w} plants, and each parent produced BC_{1b} and BC_{1w} plants (respectively backcross on *Bebu* and WSS). All generation (parental and hybrids) seeds were sown from March to June 2012, in the same environment, at two different agro-ecological locations, namely, Manfla (savannah) and Research Station of Nangui Abrogoua University (forest). The distance between these two experimental locations (Manfla-Abidjan) was 400 Km.

In both locations, each field was 105 m x 60 m and contained 32 rows, with 3 m between them. The number of rows in each field was two for each parental line, three for each F_1 family and six for each back cross (BC_1) and F_2 families. A total of 2-3 seeds of each family were planted per hole,

with an intra-row spacing of 2 m. Ten days after sowing, seedlings were separated and the most vigorous were chosen. In each location, individuals in a family were 30, 60, and 180, respectively; for each parent, F_1 , BC_1 and F_2 family. Regular weeding of the experimental fields was done during plant vegetative cycle.

Parameters evaluated. Three fruits per plant were selected to evaluate mature seed parameters. This was done as follow:

Fresh seed mass was determined as seed mass extracted directly per fruit, weighed with precision balance (*Ohaus adventurer balance*; 0,001 g sensibility).

Dry seed mass was determined as seeds from the same fruit dried in natural condition and weighed at regular intervals of two (2) days during two weeks with precision balance (*Ohaus adventurer balance*; 0,001 g sensibility), until a constant mass. Seeds were considered dry when mass stayed constant between two consecutive interval measurements. The last measurement corresponded to dry seed mass per fruit.

For *100 seeds mass*, a batch of 100 dry seeds per fruit weighed with precision balance. As for *percentage seed integument*, for each fruit, a batch of 20 undecorticated dry seeds (seed with integument, M_{20UD}) was weighed. Seeds were then decorticated and weighed again to determine the mass of the batch of 20 decorticated dry seeds (seed without integument, M_{20D}). Seed integument percentage was then calculated.

Seed length was determined as the distance between the seed base and its opposite apex measured on 20 seed samples extracted in three fruits per plant.

Seed width was determined as the lateral diameter measured on 20 seed samples extracted in three fruits per plant.

Statistical test. Two way analysis of variance [ANOVA 2: families (parents, F_{1b} , F_{1w} , F_{2b} , F_{2w} , BC_{1b} and BC_{1w}) and locations (Manfla and Abidjan)] were used to evaluate parental and hybrid performance, following seed traits with SAS software (SAS, 2006). This test evaluated variability between families, the main effects of location on family production and the interaction between families-locations. Also, Principal

Components Analysis (PCA) was performed in order to seek variability factors and to observe individuals regrouping.

Estimating heritability and predicting selection response were then made. A design based on the measure of variance from parent and offspring was used to estimate environmental, genetic, and additive variances (Warner, 1952; Wright, 1968).

RESULTS

Seed size (length and width), seed mass and seed integument thickness varied significantly ($P < 0.001$) among families (Table 1). Bebu yielded the biggest seeds, with at least 18 cm and 10 cm length and width, respectively; while WSS yielded the smallest seeds with at least 11 cm and 4 cm for the same characters. The hybrids (F_1 , BC_1 and F_2) seed size were intermediate to parents. Indeed, backcross on Bebu yielded big seeds neighbouring Bebus' seeds size; while backcross on WSS yielded small seeds neighbouring the seed size of WSS. F_2 seeds contained a wide distribution of seed sizes. None of them looked like parental seed, others like F_1 and BC_1 and others again like a new recombinants (Fig. 1).

Bebu big seeds were the heaviest; while WSS small seeds were the lightest (Table 1). Thus, spawn seed yielded by Bebu weighed 54 g; while those of WSS weighed 24 g; representing less than half the mass of these first ones. Upon drying, they weighed, respectively, 28 and 8 g.

The means of offspring (F_1 , BC_1 and F_2) seed mass were intermediate to the one of parents. Partial dominance (heavy seed phenotype/light seed phenotype) was observed because F_1 hybrid seeds were intermediate to the parents and had average mass approximating the mid-parent average. BC_{1b} seeds were heaviest due to the maternal effect. In fact, seed mass yielded in BC_1 reflected the one of maternal parent. So, backcrossing on Bebu cultivar (BC_{1b}) produced heavy seeds like the Bebu's seeds; and backcross on WSS cultivar (BC_{1w}) produced light seeds similar to WSS's seeds.

Bebu seed integument was quite thick compared to that of WSS. Integument thickness of offspring seeds was intermediate to the one of parents. In the hybrid populations, BC_{1b} seed

integument was the thickest due to the maternal effects. So, backcrossing on Bebu cultivar (BC_{1b}) produced thick integuments (34 to 35% of BC_{1b} total seed mass). Bebu's seeds integuments (36 to 37% of Bebu total seed mass); and back cross on WSS cultivar (BC_{1w}) produced thin seed integuments (19 to 20% of BC_{1w} total seed mass) as WSS's seeds (18 to 19% of WSS total seed mass). Seeds produced in F_1 had thicker integuments than F_2 seeds.

Cross direction did not affect seed size, seed mass and seed integument thickness in both F_1 and F_2 generations, but it affected BC_1 seed traits due to the maternal effects. So whatever cross direction, F_{1b} and F_{1w} , and F_{2b} and F_{2w} had statistically the same seed traits. According to cross directions, seed integument thickness represented, respectively, 28.33 to 32.57% and 23.86 to 28.69% of F_1 and F_2 total seed mass according to locations. Seed integument thickness represented 34 to 35 % and 19 to 20% of BC_{1b} and BC_{1w} total seed mass, respectively.

There were significant genetic distances between populations (Table 2). Genetic distances between parental cultivars were greater than those between them and their offsprings; and between offsprings. Bebu and WSS were far genetically, and the majority of their offsprings were located between them. Besides, the genetic distance between Bebu and BC_{1w} was superior to that between Bebu and BC_{1b} . Likewise, the genetic distance between WSS and BC_{1b} was superior to that between WSS and BC_{1w} .

Table 3 presents relative individuals repartition from genetic distance on both sites, showing difference between Bebu and WSS individuals, BC_{1b} and WSS and between BC_{1w} and Bebu. However, some BC_{1b} and BC_{1w} individuals looked like, respectively, Bebu and WSS. Maternal effects were observed in those BC_1 populations. All hybrid (F_1 , BC_1 and F_2) populations had some common individuals. F_1 phenotypes were observed only in BC_1 and F_2 populations; while those of F_2 reflected all populations (parental, F_1 and BC_1).

Principal Components Analysis (PCA) indicated that variations between populations were bound to one principal factor, regardless of experimental site (Table 4). This first and main factor contributed 77.31% for Abidjan

TABLE 1. Means \pm standard deviation of each population according to traits and locations

Traits	Sites	Populations								Statistical tests	
		Bebu	BC _{1b}	F _{1b}	F _{1w}	F _{2b}	F _{2w}	BC _{1w}	WSS	F	P
Seed length (mm)	Abidjan	18.3 \pm 1.01 ^a	17.61 \pm 1.17 ^b	15.41 \pm 1.29 ^c	14.7 \pm 1.17 ^c	14.72 \pm 1.28 ^d	14.24 \pm 1.66 ^d	13.23 \pm 1.10 ^e	11.37 \pm 0.75 ^f	228.27	<0.001
	Manfla	18.4 \pm 0.91 ^a	17.71 \pm 1.31 ^b	16.2 \pm 1.23 ^c	16.14 \pm 0.97 ^c	15.6 \pm 1.45 ^d	15.57 \pm 1.72 ^d	14.49 \pm 1.34 ^e	12.17 \pm 0.68 ^f	142.94	<0.001
Seed width (mm)	Abidjan	10.36 \pm 0.29 ^a	9.2 \pm 0.71 ^b	7.99 \pm 0.95 ^c	7.6 \pm 0.67 ^c	6.94 \pm 0.87 ^d	7.18 \pm 1.28 ^d	5.54 \pm 0.95 ^e	4.13 \pm 0.36 ^f	189.39	<0.001
	Manfla	10.52 \pm 0.74 ^a	9.26 \pm 0.87 ^b	8.01 \pm 1.12 ^c	7.98 \pm 0.62 ^c	7.36 \pm 1.08 ^d	7.39 \pm 1.54 ^d	6.09 \pm 1.14 ^e	4.09 \pm 0.33 ^f	230.72	<0.001
Fresh seed mass (g)	Abidjan	53.91 \pm 9.92 ^a	46.32 \pm 19.91 ^b	45.08 \pm 10.84 ^{bc}	42.02 \pm 0.17 ^c	31.85 \pm 17.45 ^d	28.9 \pm 20.67 ^d	29.33 \pm 10.91 ^d	22.05 \pm 4.92 ^f	35	<0.001
	Manfla	54.06 \pm 9.74 ^a	48.04 \pm 17.72 ^b	45.91 \pm 11.01 ^{bc}	42.45 \pm 10.01 ^c	37.34 \pm 14.68 ^d	33.88 \pm 19.96 ^{de}	30.61 \pm 13.10 ^e	25.47 \pm 6.23 ^f	34.63	<0.001
Dry seed mass (g)	Abidjan	27.35 \pm 5.92 ^a	22 \pm 11.88 ^b	24.31 \pm 5.47 ^b	22.66 \pm 5.01 ^b	13.87 \pm 9.74 ^c	13.86 \pm 8.45 ^c	9.81 \pm 5.29 ^d	6.75 \pm 1.52 ^e	49.42	<0.001
	Manfla	29.48 \pm 7.31 ^a	25.08 \pm 9.93 ^b	25.13 \pm 5.94 ^b	25.12 \pm 5.48 ^b	20.39 \pm 10.43 ^c	19.82 \pm 8.45 ^c	14.79 \pm 6.29 ^d	9.97 \pm 3.47 ^e	35.18	<0.001
Hundred seeds mass (g)	Abidjan	16.78 \pm 1.53 ^a	13.85 \pm 2.53 ^b	9.76 \pm 2.26 ^c	9.54 \pm 1.92 ^c	8.2 \pm 2.08 ^d	7.99 \pm 3.20 ^d	5.69 \pm 2.42 ^e	3.5 \pm 0.35 ^f	293.38	<0.001
	Manfla	17.7 \pm 3.77 ^a	14.76 \pm 2.92 ^b	11.52 \pm 2.12 ^c	11.85 \pm 1.24 ^c	10.43 \pm 2.29 ^d	10.42 \pm 3.09 ^d	7.2 \pm 2.20 ^e	4.52 \pm 0.59 ^f	185.87	<0.001
Seed integument thickness	Abidjan	36.64 \pm 4.00 ^{a*}	34.89 \pm 8.09 ^b	29.46 \pm 5.59 ^c	28.33 \pm 3.94 ^c	24.23 \pm 6.87 ^d	23.86 \pm 10.9 ^d	19.64 \pm 9.23 ^e	18.37 \pm 3.50 ^e	79.35	<0.001
	Manfla	37.07 \pm 4.11 ^a	35.03 \pm 7.92 ^b	32.57 \pm 4.98 ^c	32.39 \pm 4.44 ^c	28.69 \pm 8.30 ^d	27.51 \pm 9.60 ^d	19.04 \pm 7.75 ^e	18.84 \pm 3.79 ^e	58.92	<0.001

Means with same letters along rows are not significantly different (P< 0.05)

Seed variability in *C. lanatus*



Figure 1. Parental (band w) and hybrids seeds. b: bebu, w: *Wléwlé Small Seed*, F_{1w} : F_1 from *Wléwlé* ♀ × *Bebu* ♂ cross, F_{1b} : F_1 from *Bebu* ♀ × *Wléwlé* ♂ cross, BC_w : back cross on *WSS* cultivar; BC_b : back cross on *Bebu* cultivar, g: recombinant form which look like “*wléwlébig seed*”, m: recombinant form which look like “*wléwlémedium seed*”

TABLE 2. Mahalanobis genetic distance between populations and asterisk steady statistical test on Manfla (Abidjan)

Populations*	Bebu	WSS	BC_{1w}	BC_{1b}	F_{2w}
WSS	67.45*** (72.35***)				
BC_{1w}	34.06*** (44.28***)	6.18*** (4.62***)			
BC_{1b}	4.47*** (6.06***)	30.61*** (40.93***)	15.28*** (21.30***)		
F_{2w}	21.34*** (23.22***)	15.40*** (16.28***)	2.77*** (4.98***)	4.23*** (7.06***)	
F_{1w}	19.97*** (24.53***)	16.99*** (20.51***)	6.33*** (7.45***)	3.84*** (7.19***)	1.83*** (1.13***)

WSS: *Wléwlé Small Seed*, BC_{1w} : back cross on *WSS* cultivar using F_{1w} male flowers, BC_{1b} : back cross on *Bebu* cultivar using F_{1w} male flowers, F_{1w} : F_1 from *WSS* ♀ × *Bebu* ♂ cross, ♂: F_{2w} from self-pollination of F_{1w} plants; *** significant test at 0,1%

TABLE 3. Individuals repartition according to population on both locations

Locations	Families	Percentage of well classified individuals (%)	Number of well classified individuals per population					
			Bebu	WSS	BC_{1w}	BC_{1b}	F_{2w}	F_{1w}
Manfla (Abidjan)	Bebu	54.55 (69.05)	24 (29)	0 (0)	0 (0)	16 (12)	4 (3)	0 (0)
	WSS	96.49 (72.73)	0 (0)	55 (24)	2 (9)	0 (0)	5 (5)	0 (0)
	BC_{1w}	57.01 (88.00)	0 (0)	9 (2)	61 (176)	4 (5)	33 (18)	6 (4)
	BC_{1b}	80.35 (90.48)	5 (4)	0 (0)	1 (2)	184 (247)	31 (16)	8 (6)
	F_{2w}	66.27 (51.06)	2 (2)	2 (3)	28 (39)	38 (36)	165 (62)	14 (14)
	F_{1w}	41.25 (55.75)	0	0	4 (3)	8 (7)	39 (40)	33 (63)
	Total	68.15 (74.01)	31 (35)	66 (29)	96 (229)	250 (307)	277 (144)	61 (87)

WSS = *Wléwlé Small Seed*, BC_{1w} = back cross on *WSS* cultivar using F_{1w} male flowers, BC_{1b} = back cross on *Bebu* cultivar using F_{1w} male flowers, F_{1w} : F_1 from *WSS* ♀ × *Bebu* ♂ cross, ♂: F_{2w} from self-pollination of F_{1w} plants

experimental site, and 77.50 % for Manfla. This factor, which expressed more than 77 % of total variability, had been used for result interpretation. Correlation matrix between characters and axis 1 and 2 showed that factor 1 was defined by all variables on both sites (Table 5). Seed characters were positively correlated to axis 1. This axis can be used as production axis.

The projection of the individuals in plan 1 and 2 showed 3 main groups on both experimental sites (Fig. 2). The first group contained individuals, which looked like WSS individuals. It regrouped all WSS individuals and some BC_{1w} and F₂ individuals. The second group contained hybrid individuals, which looked like F₁ hybrids. It regrouped all F₁ individual, some BC_{1w}, BC_{1b} and F₂ individuals. The third group contained individuals which looked like Bebu individuals. It regrouped all Bebu individuals and some BC_{1b} and F₂ individuals.

PCA revealed that Bebu cultivar was different from WSS cultivar. F₁ hybrids were intermediate to both parents; while F₂ population had a wide individual dispersion. Those individuals were present in all groups. BC_{1w} individuals were close to the ones of WSS; while BC_{1b} individuals were close to the ones of Bebu.

Bebu was more heterogeneous than WSS because Bebu's genetic variance was higher than one of WSS (Table 6). Generally, genetic variance from F₂ population was higher than for BC₁ population, which was also higher than those of parental and F₁ populations. This shows that, F₂ population was more heterogeneous, while parental and F₁ populations were more homogeneous.

Phenotypic ($\sigma^2(P)$), environment ($\sigma^2(E)$), genotypic ($\sigma^2(G)$) and additive ($\sigma^2(A)$) variances, large and strict heritabilities and genetic advance were grouped in Table 7. Environmental variance was weak; while phenotypic, genotypic and additive variances were high. Environment effects on seed variability were very weak, which led to high heritabilities (large and strict) ($H^2 > 0.50$ and $h^2 > 0.50$) for all characters on both site. More than 50% of seed variability between populations was imputable to genetic variance. All of these characters can be transferred exactly to offsprings whatever the environment percentage. Genetic advance was high for seed mass on both sites.

The number of effective factors for each character on both sites, is summarised in Table 8. The effective factor estimation method used

TABLE 4. Matrice of principales composantes on both experimentales sites

Locations	Axes	Value	Variances	Cumulated value	Cumulated Variances
Manfla (Abidjan)	1	8.52 (8.50)	77.50 (77.31)	8.52 (8.50)	77.50 (77.31)
	2	0.63 (0.78)	5.73 (5.73)	9.15 (9.28)	83.24 (84.43)

TABLE 5. Correlation matrix between traits and axes at Manfla (Abidjan)

Traits	Factor 1	Factor 2
Seed length (SL)	0.87 (0.96)	-0.32 (0.24)
Seed width (SW)	0.91 (0.94)	-0.37 (0.30)
Fresh seed mass (FSM)	0.93 (0.90)	0.18 (0.00)
Dry seed mass (DSM)	0.94 (0.95)	0.17 (-0.06)
Hundred seeds mass (HSM)	0.93 (0.93)	-0.30 (0.31)
Percentage of seed integument (PSI)	0.90 (0.87)	-0.25 (0.22)
Variance expliquée (%)	22 (14)	17 (-37)

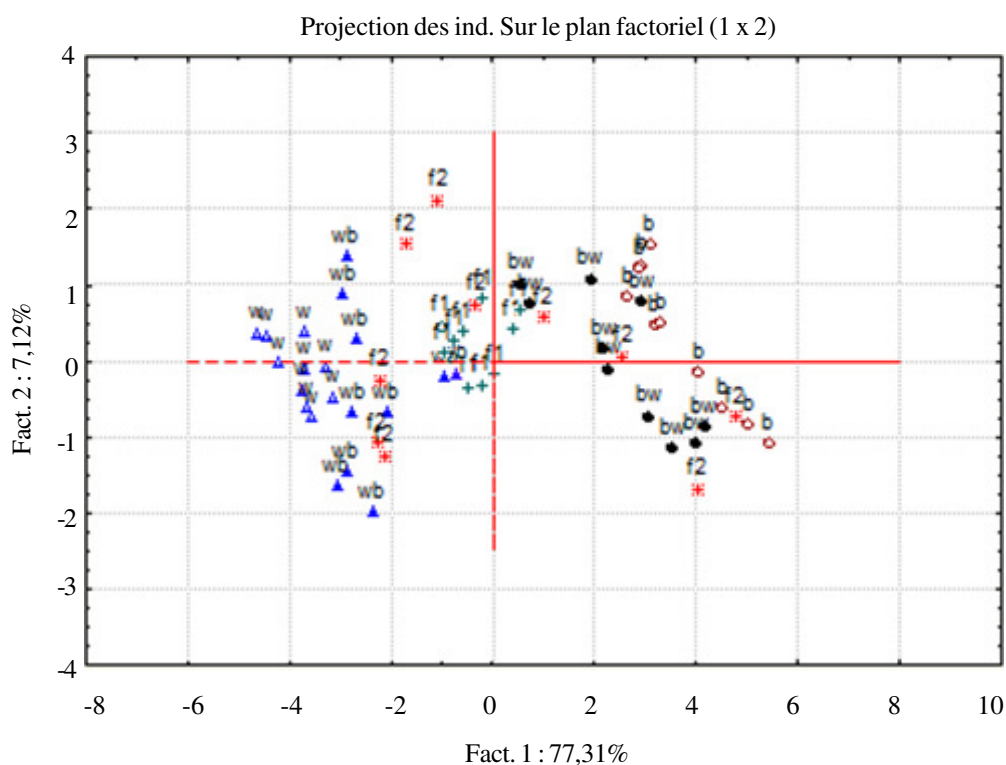


Figure 2. Individuals repartition in first plan of PCA. Individuals repartition is the same on both sites. So, here we represent for one site (Abidjan). b: bebu, WSS: *Wléwélé Small Seed*, wb: back cross on WSS cultivar using F_{1W} male flowers, bw: back cross on *Bebu* cultivar using F_{1W} male flowers, F_1 , F_1 from WSS ♀ × *Bebu* ♂ cross, ♂ F_2 from self-pollination of F_1 plants.

provided similar result on both experimentation sites. The effective factor calculated was statically identical to unity (1) for spawn and dry seed mass, and seed integument thickness. Thus, these traits were governed by only one gene pair. However, for 100 seeds mass, length and width seeds, effective factor calculated turn around three. These characters were governed by at least 3 effective genes.

DISCUSSION

Seed size, seed mass and seed integument thickness varied significantly among populations (Table 1). This inter-population variability had been confirmed by genetic distance and PCA tests and must be amputated to difference between parental seeds. PCA revealed a difference between *Bebu* and WSS seeds. All analysed parameters discriminated them clearly and showed seed genetic diversity in *C. lanatus* as had

mentioned by Guner and Wehner (2004) and Gusmini and Wehner (2005).

This phenotypic difference between parental seeds increased the heterozygote index in their offsprings and yielded different seeds types. This heterozygote index produced wide seed variability in BC1 and especially in F_2 where there was character segregation according to Mendel laws. Gusmini *et al.* (2004) also observed seeds character segregation in BC₁ and F_2 populations during crossing between two *C. lanatus* cultivars: *Charleston Gray* 3 PI 560006 and *Calhoun Gray* 3PI490383w.

This character segregation was the evidence that some individuals can be identical to one of the parents; while others must be recombinants exhibiting different proportions of parental characters (Vedele and Loudet, 2001). So, the projection of the individuals in first PCA plan confirmed ANOVA test, and permitted to class all populations' seed into 3 main groups on both

TABLE 6. Phenotypic variances for seeds traits

Locations	Families	SL	SW	FSM	DSM	HSM	PSI
Manfla (Abidjan)	Bebu	0.83 (1.02)	0.55 (0.08)	94.84 (98.44)	53.49 (35.01)	14.25 (2.34)	16.87 (15.98)
	WSS	0.47 (0.56)	0.11 (0.13)	38.75 (24.20)	12.01 (2.31)	0.35	14.34 (12.25)
	BC _{1b}	1.73 (1.37)	0.76 (0.50)	314.15 (396.40)	98.63 (141.10)	-0.13	62.69 (65.50)
	BC _{1w}	1.79 (1.20)	1.31 (0.91)	171.63 (119.10)	39.63 (127.99)	-6.39	60.13 (85.29)
	F _{2w}	2.95 (2.75)	2.36 (1.65)	398.30 (427.07)	109.19 (219.97)	4.83	92.22 (118.79)
	F _{1w}	0.94 (1.37)	0.39 (0.45)	100.27 (103.42)	30.04 (25.14)	-5.86	19.71 (15.51)
						-3.69	

FSM = Fresh seed mass, DSM = Dry seed mass, HSM = hundred seeds mass, PSI = Percentage of seed integument: SL = Seed length, SW = Seed width, WSS = Wléwé Small Seed, BC_{1w} = back cross on WSS cultivar using F_{1w} male flowers, BC_{1b} = back cross on Bebu cultivar using F_{1w} male flowers, F_{1w}, F₁ from WSS ♀ × Bebu ♂ cross, ♂: F_{2w} from self-pollination of F_{1w} plant

TABLE 7. Variance, heritability and genetic gain to selection estimates for families for seeds traits

Locations	Traits	Variances				Heritability		Genetic advance	
		σ^2 (P)	σ^2 (E)	σ^2 (G)	σ^2 (A)	H ²	h ²	5%	10%
Manfla (Abidjan)	SL	2.95 (2.75)	0.80 (1.08)	2.15 (1.67)	2.07 (1.62)	0.73 (0.61)	0.70 (0.59)	0.07 (0.09)	0.14 (0.18)
	SW	2.36 (1.65)	0.36 (0.28)	2.01 (1.37)	1.66 (1.09)	0.85 (0.83)	0.70 (0.66)	0.09 (0.07)	0.17 (0.14)
	FSM	398.30 (427.07)	83.53 (82.37)	314.77 (344.71)	310.83 (338.66)	0.79 (0.81)	0.78 (0.79)	0.78 (0.82)	1.56 (1.64)
	DSM	109.19 (219.97)	31.40 (21.90)	77.79 (198.07)	70.11 (170.85)	0.71 (0.90)	0.64 (0.78)	0.38 (0.58)	0.77 (1.15)
	HSM	9.56 (10.23)	4.42 (2.46)	5.15 (7.77)	4.78 (6.81)	0.54 (0.76)	0.50 (0.66)	0.09 (0.13)	0.19 (0.26)
	PSI	92.22 (118.79)	17.66 (14.81)	74.56 (103.98)	61.62 (86.79)	0.81 (0.88)	0.67 (0.73)	0.32 (0.40)	0.64 (0.80)

FSM = Fresh seed mass, DSM = Dry seed mass, HSM = hundred seeds mass, PSI = Percentage of seed integument: SL = Seed length, SW = Seed width, Phenotypic (P), environmental (E), genotypic (G), additive (A) variance effects, broad (H²) and narrow (h²) sense heritability and genetic advance (Gs) of each character were estimated from generation variances as follows (Warner, 1952; Wright, 1968)

TABLE 8. Number of effective factor for each character on both sites

	Seed length	Seed width	Fresh seed mass	Dry seed mass	Hundred seeds mass	Percentage of seed integument
Abidjan	3.37	3.04	0.39	0.27	3.37	0.4
Manfla	2.94	2.97	0.34	0.6	2.7	0.57
Mean	3.16	3	0.37	0.44	3.04	0.49
Number of effective factor	3	3	1	1	3	1

The number of effective factors (N), an estimate of the genetic factors determining a quantitative trait (Mendelian genes or quantitative trait loci), was estimated as follows:

$$N = \frac{D^2}{8(\sigma^2(F_2) - \sigma^2(F_1))}$$

Where: D = numeric value from difference between mean (i) parental line: $D = (\mu_{\text{Bebu}} - \mu_{\text{WSS}})$

experimental sites (Fig. 2). In the parental types, plant individuals which were grouped with WSS produced numerous small light seeds per fruit, while individuals which were grouped with Bebu, produced few big and heavy seed per fruit. The recombinant type had intermediate seed characters to both parents like F_1 hybrids.

Fulks *et al.* (1979) and Zamir (2001) also observed intermediate forms resulting from natural crosses between *C. lanatus* and its wild ancestor *C. colocynthis*. F_2 individuals were present in all groups that this population had wide seeds dispersion because many seed types had been observed in this population (characters segregation according to Mendel laws). Nevertheless, majority of F_1 and F_2 hybrids looked more like WSS than Bebu according to evaluated genetic distances (Table 2). This result must be imputed to considered cross type ($WSS_{\text{B&}} \times Bebu_{\text{B&}}$) during genetic distance evaluation. So, many of F_1 and F_2 progenies looked like their female parents. However, statistical analyses showed that cross direction did not affect F_1 and F_2 seeds because F_{1b} and F_{1w} , and F_{2b} and F_{2w} were statically similar. On the other hand, cross direction affected BC_1 seeds characters due to the maternal effect. Observations showed that Bebu and BC_{1b} and WSS and BC_{1w} were similar genetically (Fig. 2). So, backcrossing on Bebu yields few, big and heavy seeds, with thick integument; while backcrossing on WSS exhibited numerous seeds, small and light seeds with thin integument (Table 1).

Phenotypical similarity between BC_1 hybrids and their maternal parents, suggests the existence of sufficient parental genes compared with F_1 hybrids during backcrossing, in expression of examined characters. These results were the evidence that cross direction is an important factor for *C. lanatus* genetic breeding. Variability in quantitative and qualitative character expressions according to cross direction had been already observed in *C. lanatus* (Henderson *et al.*, 1998; Gusmini *et al.*, 2004).

These observations suggest that Bebu and BC_{1b} , and, WSS and BC_{1w} were similar genetically. These genetic distances and statistical test between populations confirmed ANOVA results and certified wide variability between populations on both site. Similar results were reported by Bodzon (2004), Akbar *et al.* (2008) and Waqar-Ul-Haq *et al.* (2008). Thus, all characters examined admitted high large and strict heritabilities, irrespective of experimental site (Table 7). This suggests that environment effects are less important than genetic effects. More than 50% of character variability observed in populations were bound to genetic variability. This high heritability confirmed the high difference between both selected parents (Singh and Westermann, 2002). For all of these characters, genotypic and additive variances were widely superior to environmental variance on both sites. In addition, high heritability of seed mass was proportional to high genetic advance on both sites. Individuals' selection according to seed mass,

irrespective of environment could be profitable to cultivar breeding. Breeders can choose plants which yield heavy seeds. Similar observations were made on fruit characters (Gusmini and Wehner, 2005; Kumar, 2009).

The number of effective factors which controlled each character was also evaluated (Table 8). It permitted to the observation that spawn and dry seed mass and seed integument thickness had been controlled by one effective factor; these characters were monogenic. One hundred seed mass, seed length and width had been controlled by 3 effective factors; these characters were polygenic. The first group was governed by one allelic pair, while the second was governed by at least 3 effective genes. Several studies showed that number of effective factors, which governed each character, varied considerably according to crosses and experimental sites (Gusmini and Wehner, 2005; Kumar, 2009; Lou, 2009).

CONCLUSION

This study show large genetic variability between populations (parental and hybrid) seed. These populations were classed to 3 main groups: two parental types including each parental type with its offspring maternal backcross and some F_2 individuals and one recombinant type including all F_1 individuals and some BC_{1b} , BC_{1w} and F_2 individuals.

Phenotypic similarity between BC_1 hybrids and their maternal parents suggests existence of sufficient parental genes than in F_1 hybrids during backcrossing in expression of examined characters. These results are the evidence that cross direction is an important factor for *C. lanatus* genetic breeding.

All characters examined, admitted a high large and strict heritabilities, suggesting that environment effects are less important than genetic effects. None of these characters were monogenic while others were polygenic.

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