



## Sesame (*Sesamum indicum* sp.) seed security and breeding programme to improve resilience of small holder farmers in Somalia

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### Abstract

This work tested on field 5 African sesame (*Sesamum indicum*) accessions to identify the most appropriate GAP and have a phenotypic description of the most relevant morpho-productive traits in different ecosystems. In parallel the genetic variability of the African germplasm, represented by 11 accessions, has been computed by the SSR molecular markers. The most appropriate breeding program has been identified by integrating the morpho-productive information with genetic characterization.

A profitability analysis based on current practises, cost of production and market prices in Somalia revealed that those farmers in riverine along Lower Shabelle who adopted the GAP identified through this work and best performing varieties increased their income to 1.04 USD/day/person from the actual 0.4-0.6 USD/day/person.

**Key words:** *Sesamum indicum*, SSR, genomic analysis, profitability analysis

### Introduction

Sesame (*Sesamum indicum* L.) notwithstanding is cultivated since more than 5,000 years still challenges the basic domestication criteria: grows indeterminately and its scalar fruit ripen causes abundant losses at harvest time. Sesame is grown in Somalia (one of the most social, political economic and environmental fragile country in the world) by small holder farmers living below the threshold of extreme poverty in response to the market demand and the need to mitigate risk of drought during the short rain season.

In recent years, regional and international markets showed interest in Sesame produced in Somalia and regular trade flows started offering an opportunity of income growth to small holder farmers.

### Materials and methods

**Experimental design.** Two experiment plans have been set, one in Somalia during Deyr 2011 (based on experiences earned in 2010) and one in Kenya (Kitui, in the central region of semiarid land, Kilifi, on the coast where the climate is more similar to the one of Somalia) one in 2012 and a third one in Busia (near Victoria Lake, where the temperatures are lowest but rain water is more available) in 2013.

In 2011 in Somalia the trials aimed to identify the best cropping method, comparing the traditional and the improved system reported in Table 1. The experimental design was a

complete randomized block with three replications applied in three localities (Barire, Deresalam and Jannale).

Table 1:cropping protocol and trial design adopted in Deyr 2011 In Somalia

	Traditional cropping	Improved cropping
Ploughing	Yes	Yes
Spacing	Broadcasting	60cm *25 cm
Flooding	Yes	No
Arrowing	No	Yes
Furrowing	No	Yes
Sowing	5-6 seeds per pocket (about 6-7 kg/ha) or broadcasting (following the most common practice among farmers)	5-6 seeds per pocket (about 6-7 kg/ha)
Irrigation	1 before sowing (this is the flooding mentioned above) and 1 within 25 days after sowing, depending on rains	1 after land preparation 1 within 25 days after sowing, depending on rains
Weeding	About 3 times (according to needs)	3 times
Thinning	Only if necessary	Yes
Fertilization	No	Yes (100 kg ha in two moments sowing and flowering)
Spraying	Only for very hard pest attacks	When necessary

The trial’s design applied in Somalia along Deyr 2011 in each site is reported as follow:

1. each experimental area was divided in the two levels of crop factor: traditional and improved.
2. Three different varieties were tested in each site;
3. Each thesis per each site was replicated in three blocks.
4. Each thesis, in a 20 m x 20 m plot,contained 33 rows and in each row there were 80 plants ( $20/0.6=33$  and  $20/0.25=80$ ). In traditional cropping seeds were broadcasted or sown following the tractor according to the most common local practice.

The varieties tested during two season in Somalia are: Duniyar Clean (i.e.: white seeds only as the brown have been manually separated), Duniyar Dirty (using seeds withe and brown –i.e.: grain from previous season not cleaned-), Humera (provided by a local grower).

Data were collected from 10 plants taken from the two central rows per thesis. The data collected are reported in the Table 2: traits of data collected.

Table 2: traits of data collected

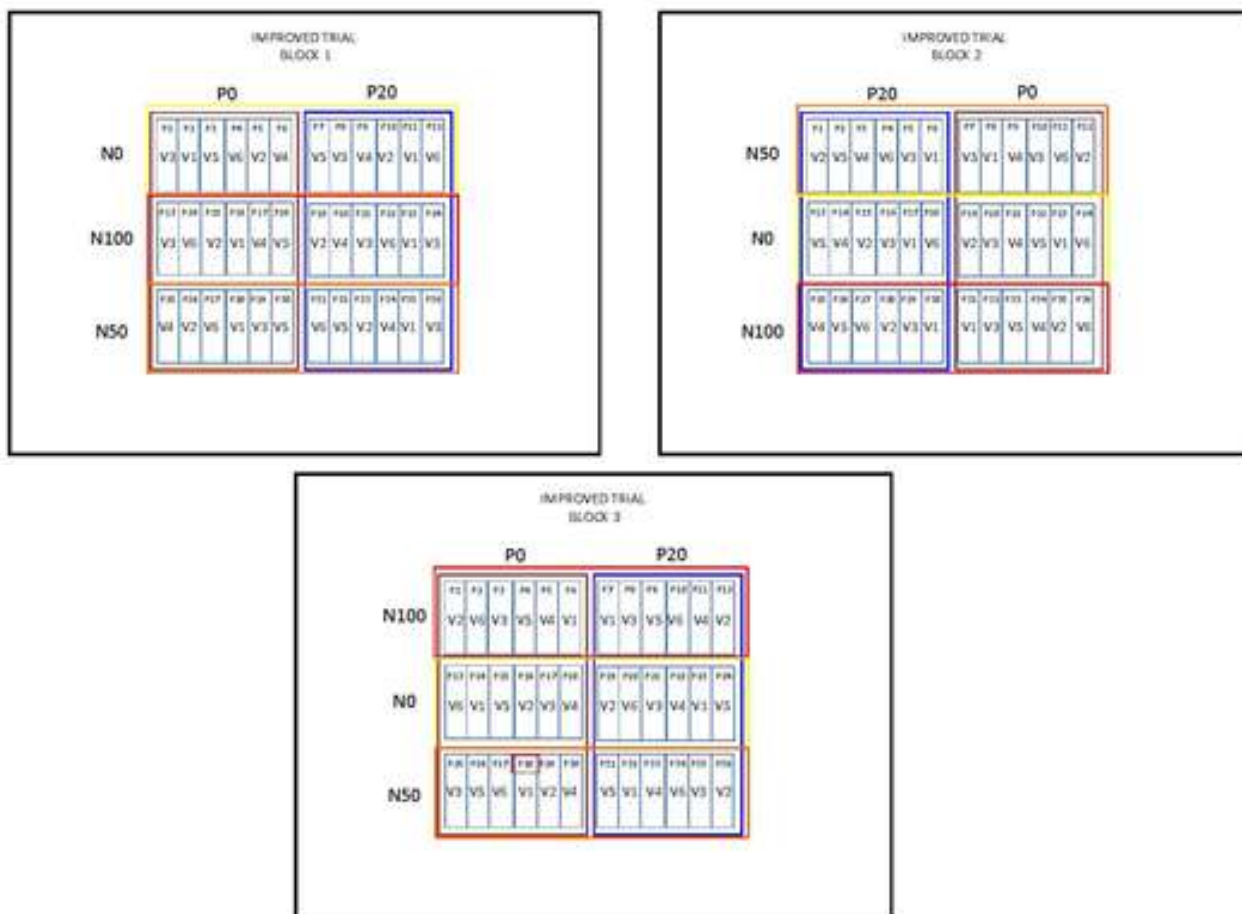
Plot No.	Plant No.	Plant height (cm)	Days to 50% flowering	No. of capsule per plant	No. of seeds per capsule	1000 seed weight (gram)	Yield per plant (gram)	Yield of all plants in the plot (kg)
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In Kenya the purpose was to identify the more appropriate level of fertilization for the 5 varieties tested. In Kenya the adopted design was more complex than in Somalia and during three following seasons sesame was grown in: Kitui, Kilifi and Busia. The first two sites were selected considering the environmental conditions closest to those of Somalia. The third one, Busia, was selected because during the two previous seasons the cropping of sesame was severely affected by the drought and data collected only partially complete. Thus, to minimize risks, Busia was selected for the third and last season. This is a site where farmers traditionally grow sesame, where water is available and rains are normally sufficient to complete a cropping cycle.

In Kitui during the season of the trials there was a drought and no irrigation scheme was in place. The results from this season have not been considered reliable and significant and thus not considered in this work. In Kilifi and in Busia, during Deyr 2011 and Deyr 2012 respectively, 6 varieties were tested: Dunyar, Ethiopian D2, Ethiopian IS, ICEASE00019, Humera, Tanzania.

The adopted experimental design was a split plot, where: nitrogen and phosphorous are the main plot (row and column) and in the sub plot are randomized the six levels of variety (Figure 1).

Figure 1: Experimental design split strip plot with three blocks



Due to lack of appropriate facilities, irrigation has not been performed apart from some emergency irrigation in Kilifi in one block only.

The improved trials too are implemented as a Strip Plot, where the nitrogen and phosphorus are the main factors and varieties are organised in sub-plot.

Below is a summary of the schema adopted for the sesame improved trials conducted in 2012

Table 3: Sesame improved Trials 2012, (STRIP PLOT)

1	Three Blocks
2	Two levels of Phosphorous (P0 = 0 kg/ha; P20 = 20 kg/ha)
3	Three levels of Nitrogen (N0 = 0 kg/ha; N50 = 50 kg/ha; N100 = 100 kg/ha)
4	Six varieties <sup>1</sup>

The sources of variance with the corresponding degree of freedom are reported below together with the factor effect per each of the considered variable.

<sup>1</sup> One variety, ICEASE, has not been considered in the final analysis as the seeds were not available for planting at the beginning of the following cropping season in Busia.

Table 4: Source of variance

Source of variance	d.f.	Factor effect
Between Block	2	Random effect
Between Nitrogen (N)	2	Fixed effects
Between Phosphorous (P)	1	Fixed effect
Interaction N x P	2	Fixed effect
Error a	10	
Between Varieties (V)	5	Fixed effect
Interaction V x N	10	Fixed effect
Interaction V x P	5	Fixed effect
Error b	70	
Total	107	

The varieties utilized in the evaluation are:

Table 5: Varieties tested and corresponding code

Denomination	Name of the variety
V <sub>1</sub>	Ethiopian d2
V <sub>2</sub>	Humera from Somalia
V <sub>3</sub>	Dunyar from Somalia
V <sub>4</sub>	Tanzania
V <sub>5</sub>	Ethiopian IS
V <sub>6</sub>	ICEASE

One variety, ICEASE, has not been considered in the final analysis as the seeds were not available for planting at the beginning of the following cropping season in Busia thus the final analysis considered only 5 out of the initial six varieties.

**Morpho-productive characterization**

Fifteen morphological and productive characters have been recorded on 5 randomly selected plants for each of the considered sesame varieties, collected in the combination thesis (site, block, levels of N and levels of P).

The traits measured were: days at 50% flowering, height at 50% flowering (cm), height at physiological maturity (cm), weight of capsules per plant (g), grain yield (weight of seeds per plant g), number of capsules per plant, average number of seeds per capsule, colour of seeds<sup>2</sup>, number of branches per plant, stem thickness at physiological maturity (mm), average capsule length calculated on the basis of three randomly chosen capsules in the plant, average of capsule thickness (mm) calculated on the basis of three measures of the thickness of the previously selected capsules, number of capsules per axil, average internode length in cm measured based on the average length of 1st, 2nd and 3rd internode length), and the weight of 100 seeds.

**Analysis of morpho-productive traits**

The differences of the means of each agronomic trait between varieties, locations and fertilization levels, were compared through the analysis of variance using Systat 9. The same SW has been used for Multivariate discriminant analysis, to analyze the phenotypic relationships between the different varieties landraces/populations. To visualize the phenotypic relationships existing between the varieties analyzed, the Euclidean distance matrix and the cluster analysis based on Sahn algorithm were performed using the software NTSYSpc 2.2.

In order to make comparable data from Kilifi and Busia, only five varieties (available during both years) and all the variables whose data were complete have been considered (i.e.: variable

<sup>2</sup> This trait eventually has not been analysed due to the subjective variability related to the field technicians belonging to different groups (site and years).

with data lost due to the drought or too subjective –as seed color or plant vigor- have been excluded).

### **Genetic characterization**

In this work 11 accessions from 6 African countries with wide geographical coverage have been examined in lab by genomic SSR markers as described below.

The varieties observed in this study are:

1. Humera (originary from Ethiopia)
2. Icease 00019 (provided by ICRISAT)
3. Dunyar (from South Central Somalia)

And 8 accession from different African countries most of them bought in the local markets

1. Etiope D2 (kindly provided by the Department of Agricultural and Forest Sciences of Palermo University)
2. Etiope is (kindly provided by the Department of Agricultural and Forest Sciences of Palermo University)
3. Tanzania (grown in Nyanza region close to Victoria lake kindly provided by CEFA NGO)
4. Somaliland (from Burao)
5. Uganda Local 1 (bought close to Kampala)
6. Uganda common (bought at the border with Kenya)
7. Uganda Local (bought in a local market)
8. Sudan local (bought in a local market)

The seeds of the different sesame varieties/accessions were initially sterilized in a solution of hypochlorite of sodium 2%, stirring during 2 minutes and washed several times and then germinated in autoclave sterilized tissue paper, inside disinfected germination trays in an incubator at 30° C. This methodology sometimes caused mildew and the germination process needed to be repeated but the alternative, i.e.: sowing outdoors in the greenhouse would have implied a longer time for germination and reaching the necessary size of the plants for the successive phase of the DNA extraction.

About one week after the germination the seedlings reached about 10 cm height. From the bud and young leaves of these seedlings about 20-30 mg of DNA have been extracted. The limited green material available from such young seedlings caused low yield of DNA.

Per each variety 6 seedlings have been selected. DNA has been extracted following the protocol indicated in the Machery-Nagel kit with the exception of the grinding of the samples: being the available material per each seedling so little, grinding took place in eppendorf containing the buffer in the kit, using glass rods made on purpose rough at the tip.

Once the DNA has been extracted, the visualization of the bands by electrophoresis has been conducted. Samples and marker 1kb (Fermentas) have been loaded on Agarose gel (0.32 g of Agarose and 40 µl of TBE. The solution has been warmed in the microwave in order to solve the Agarose. The gel once cooled down to room temperature has been loaded with 0.4 µl of ethidium bromide to allow the visualization of the bands to ultraviolet rays. The gel obtained, still in liquid form, was poured inside the cot where were placed the combs, which will form the wells within which the samples will eventually be loaded. Finally 8 µl of DNA mixed with 2 µl of dye (bromophenol blue) and 2 µl of markers have been loaded. The electrophoresis has been

set at 50 volts and 50 minutes. At the end of the run the gel was examined using the BIORAD Gel Doc XR + Gel Imaging to verify the presence and the quality of the DNA (Fig. 9).

The primers used for this work have been synthesized by Invitrogen, following the sequences developed by Dixit et al. and found in the literature (Dixit et al. 2005).

The primers used are listed below:

Table 6: list of primers

locus	sequenza	repeat	range bp
GBssr-sa-05F	TCATATATAAAAGGAGCCCAAC	(CT) <sub>13</sub>	158-172
GBssr-sa-05R	GTCATCGCTTCTCTTCTTC		
GBssr-sa-08F	GGAGAAATTTTCAGAGAGAAAAA	(AG) <sub>17</sub>	150-164
GBssr-sa-08R	ATTGCTCTGCCTACAAATAAAA		
GBssr-sa-09F	CCCAACTCTTCGTCTATCTC	(CT) <sub>18</sub>	217-231
GBssr-sa-09R	TAGAGGTAATTGTGGGGGA		
GBssr-sa-33F	TTTTCTGAATGGCATAGTT	(AG) <sub>24</sub>	263-275
GBssr-sa-33R	GCCCAATTTGTCTATCTCCT		
GBssr-sa-72F	GCAGCAGTCCGTTCTTG	(CT) <sub>9</sub>	289-307
GBssr-sa-72R	AGTGCTGAATTTAGTCTGCATAG		
GBssr-sa-108F	CCACTCAAATTTTCTACTAAGAA	(GA) <sub>7</sub> ,(GA) <sub>15</sub>	204-218
GBssr-sa-108R	TCGTCTTCCTCTCTCCCC		
GBssr-sa-123F	GCAAACACATGCATCCCT	(TC) <sub>21</sub> ,(TC) <sub>15</sub>	272-282
GBssr-sa-123R	GCCCTGATGATAAAGCCA		
GBssr-sa-173F	TTTCTTCTCGTTGCTCG	((G) <sub>5</sub> CTAGT(G) <sub>3</sub> (A) <sub>2</sub> ) <sub>2</sub>	218-245
GBssr-sa-173R	CCTAACCAACCACCCTCC		
GBssr-sa-182F	CCATTGAAAAGTGCACACAA	(AT) <sub>11</sub> ,(TC) <sub>18</sub> ,(TG) <sub>12</sub>	221-259
GBssr-sa-182R	TCCACACACAGAGAGCCC		
GBssr-sa-184F	TCTTGCAATGGGGATCAG	(TC) <sub>20</sub>	179-193
GBssr-sa-184R	CGAACTATAGATAATCACTTGGA		

Successively, some PCR were performed, to test the efficiency of the primers, at different temperatures, based on the annealing temperatures of each of them.

To perform PCR was used: HotStar-TaqPlus Master Mix Kit from Qiagen company.

- The protocol per each sample is reported below:
- Taq 10 µl
- Primer Fw 0,5 µl
- Primer Rw 0,5 µl
- H2O 4 µl
- DNA 5 µl

The total final volume per sample is 20 µl.

After the initial trials, Thermocycler has been set as follows:

- 95° C for 5 minutes x 2 times (preheating to activate the polymerase and denaturing DNA)

Repetition of 30 times the following cycle):

- 95° C for 1 min (DNA denaturation),
- 55°-61° C for 1 minute (annealing – the temperature chosen was changed according to the primers-)

- 72° C during 30" (extension)

Finally the extension at 72° C for 5 mins (to complete the last cycle).

To visualize the bands electrophoreses were performed on agarose gel 1%. 5 µl of amplified material were loaded per each well and 2 µl of marker used (Fermentas 100 bp). The UV rays visualization showed the efficiency of only some of the primers used. The more efficient primers were procured to "Applied Biosystems" with the extension of the M13 sequence. This is to allow the differentiated detection through the fluorochromes: NED, FAM, PET and VIC, as indicated in literature (Schuelke, 2000).

Table 7: list of primers with tail

Locus	GeneBank	sequence	repeat	range bp	With tail
GBssr-sa-08F	AY838905	GGAGAAATTTTCAGAGAGAAAAA	(AG) <sub>17</sub>	150-164	TGT AAA ACG ACG GCC AGT GGAGAAATTTTCAGAGAGAAAAA
GBssr-sa-09F	AY838907	CCCAACTCTTCGTCTATCTC	(CT) <sub>18</sub>	217-231	TGT AAA ACG ACG GCC AGT CCCAACTCTTCGTCTATCTC
GBssr-sa-72F	AY838913	GCAGCAGTCCGTTCTTG	(CT) <sub>9</sub>	289-307	TGT AAA ACG ACG GCC AGT GCAGCAGTCCGTTCTTG
GBssr-sa-108F	AY838915	CCACTCAAATTTTCACTAAGAA	(GA) <sub>7</sub> ,(GA) <sub>15</sub>	204-218	TGT AAA ACG ACG GCC AGT CCACTCAAATTTTCACTAAGAA
GBssr-sa-123F	AY838916	GCAAACACATGCATCCCT	(TC) <sub>21</sub> ,(TC) <sub>15</sub>	272-282	TGT AAA ACG ACG GCC AGT GCAAACACATGCATCCCT
GBssr-sa-182F	AY838921	CCATTGAAAACACTGCACACAA	(AT) <sub>11</sub> ,(TC) <sub>18</sub> ,(TG) <sub>12</sub>	221-259	TGT AAA ACG ACG GCC AGT CCATTGAAAACACTGCACACAA
GBssr-sa-184F	AY838922	TCTTGAATGGGGATCAG	(TC) <sub>20</sub>	179-193	TGT AAA ACG ACG GCC AGT TCTTGAATGGGGATCAG

Successively we proceeded with marked PCR specific per each primer.

The protocol per each sample is the following:

- Taq 10 µl
- Primer Fw con coda 0,24 µl
- Primer Rw 0,5 µl
- M 13 0,5 µl
- H2O 3,76 µl
- DNA 5 µl

The samples of PCR were sized at CIBIACI through the AB 1310 of Applied Biosystems.

### Results and discussion

**Agronomic trials findings: field test year 1 (2011 Barire, Daresalam and Jannale).** The varieties tested in this trial were actually two: Dunyar and Humera. The difference between Dunyar clean and Dunyar dirty refers to the colour of sown seed: Dunyar clean is constituted by seed sorted by colour and it is all white or cream; Dunyar dirty is the natural one white and black, brown and cream seed with a ratio of around 65% (white and cream) -35% (black). By sowing the clean Dunyar, it was explored the possibility to increase the rate of white sesame in following generation by planting only white sesame (the clean Dunyar). The variables examined are reported in table 10.

Table 8: variables examined in the trials of 2011 and their relative cod

Name cod	Analysed variables
PlantHeightcm	Plant height in cm
Days50%Flow	n. of days after sowing when 50% of the plants in the plot are flowering
NCapsPlant	Number of capsules per plant
NSeedsCapsule	Number of seeds per capsule
Weight1000Seed	Weight of 1000 seeds
YieldPlantg	Yield per plant in g
YieldPlotkg	Yield per plot in kg

The most relevant observation on data gathered in 2011 in the three different sites in South Central Region of Somalia is a significant interaction between sites and between cropping

systems. Confirming the high level of variability of sesame depending on the environmental conditions reported in literature.

The summary of results is reported in the table 9, where the averages of the considered variables are referred to each level of the factors included in the ANOVA model.

Table 9: ANOVA summary of results (average) year 2011

Factors	Plant Vigour	Plant Height cm	Days 50% Flow	N Caps Plant	N Seeds Capsule	Weight of 1000 Seed	Yield Plant g	Yield Plot kg
Site	*	**	**	*	**	n.s.	**	**
Barire	3.77	86.53	40.33	53.78	65.69	4.00	8.03	23.36
DareSalaam	3.33	85.57	39.61	79.82	62.45	4.00	10.41	25.65
Jannale	3.82	120.26	40.33	83.44	68.03	3.98	14.57	29.05
Varieties	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**
DunyDirty	3.69	100.19	40.11	71.23	65.37	3.99	11.10	23.17 b
DunyImpr	3.61	97.96	40.50	72.02	64.74	3.99	10.17	24.63 b
Humera	3.61	94.21	39.67	73.78	66.07	4.00	11.74	30.27 a
Crop System	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
Improved	3.86	98.30	39.93	61.43	66.00	4.00	10.65	23.55
Traditional	3.41	96.61	40.26	83.27	64.78	3.99	11.35	28.49

The Pearson correlation analysis performed to understand the connection between the analyzed variable, shows (Tab. 10) that yield per plant resulted correlated (significance <0.01) with plant height and number of seeds per capsule. While the yield per plot resulted correlated to plant height, yield per plant, days50% flowering, the number of seeds per capsule and the number of capsules per plant. Based on the coefficients and the significance reported in the table the characters to be pursuit in order to increase the yield per plot are plant height, n. of capsules per pant and n. of seeds per capsule. The other variables even if significant, contributed only in low measure to the yield per plot. These data are in line with the literature.

Table 10: Pearson correlation matrix

	plant height cm	days 50% flow	n caps plant	n seeds capsule	weight1000 seeds	yield plant g	yield plot kg
plant height cm	1						
days 50% flow	0.205**	1					
n caps plant	0.423**	-0.018**	1				
n seeds capsule	0.463**	0.144**	0.22**	1			
weight1000 seeds	-0.096	-0.043	0.051**	-0.025	1		
yield plant g	0.558**	0.054	0.753	0.307**	0.025	1	
yield plot kg	0.19**	-0.168**	0.171**	0.087	0.01	0.243**	1

**Agronomic trials findings: field Tests year 2012 and 2013 (Kilifi and Busia)**

The variables considered in the two experiments conducted in 2012 and in 2013 in Kilifi and in Busia are reported with the relevant legend key in table 11. The ANOVA model, considers two localities as random effect factor, while the five varieties, three levels of nitrogen and two levels of phosphorous as fixed effect factors.

Table 11: examined variable in the trials of 2012 and 2013 and relative legend key

H50%Fl	Height when 50% of the plant in the plot are flowering
Plheig	Plant height at physiological maturity
Wcap	Weight of the capsules in the plant
Pyieldg	Plantyield in g
Ncap	Number of capsules perplant
NSXCap	Number of seeds per capsule
CapLeng	Length of the capsule
CapThickn	Thickness of the capsule
StThickn	Thickness of the stem
InterL	Internodelength

Table 12: Means and ANOVA summary results year 2012 and 2013

Factors	Height 50% Flow	Plant Height		Weight of Capsule	Plant Yield g	N of Capsules	N of seed X Capsule	Length Capsule	Thickness Capsule	Stem Thickness	Capsules X Axil	Internode Length			
<b>Between Sites (S)</b>	*	n.s.		*	*	**	n.s.	*	n.s.	*	*	*			
Busia	47.05	76.62		8.79	6.07	27.59	37.68	2.43	0.58	6.23	1.11	7.49			
Kilifi	71.11	87.35		17.43	14.23	99.85	46.56	2.17	0.59	2.12	1.58	4.91			
<b>Between Varieties (V)</b>	n.s.	*		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.			
Dunyar	64.89	94.27	A	16.23	12.06	74.88	41.51	2.31	0.60	5.98	a	1.00	7.49		
EtiopD2	48.23	70.83	Ab	9.48	8.37	40.73	39.46	2.40	0.59	4.83	ab	1.47	5.98		
EtiopelS	47.73	62.67	B	7.89	6.00	34.01	35.71	2.32	0.58	4.00	b	1.68	5.84		
Humera	59.64	91.28	Ab	13.64	9.16	61.19	42.01	2.27	0.57	5.31	ab	1.00	7.56		
Tanzania	50.32	81.50	Ab	9.50	6.37	31.96	41.89	2.47	0.58	5.41	ab	1.01	7.23		
<b>Between Nitrogen levels (N)</b>	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	**			
N0	56.99	80.46		12.29	9.51	53.62	39.78	2.41	a	0.59	a	5.11	1.24	6.67	ab
N050	51.61	78.80		9.75	7.68	42.32	41.39	2.33	b	0.58	b	5.23	1.20	6.55	B
N100	52.47	79.45		11.39	7.80	46.43	39.34	2.34	b	0.58	b	4.98	1.27	7.08	A
<b>Phosphorus levels (P)</b>	**	n.s.		n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.			
P0	56.04	81.48		11.51	8.60	50.54	40.91	2.38	0.58	5.18	1.26	6.87			
P20	51.42	77.76		10.85	8.05	44.57	39.38	2.34	0.58	5.02	1.21	6.70			

All varieties grew and developed well. Unfortunately, due to some losses on field mainly related to pest attacks, droughts and water logging, some plants among those selected at the beginning of the cropping cycle have been lost. Also some variables (days of 50% flowering, yield per plot and number of branches) have been lost or not considered due to discrepancies of data among different seasons and different sites (different recording teams). As well the information about the seed weight is practically uniform due to the poor scale (no decimals) available on field.

Theoretically the traits more relevant related to yield (considered in this study) are n. of capsules, n. of seeds per capsules, plant height, internodal length and number of capsules per axils and number of branches per plant (unfortunately this character cannot be considered for the reasons mentioned earlier). The interaction among these characters, contributed in the yield per plant and all of them are heavily influenced by the environment. Due to the poor quality of the scales available on field, the weight of 100 seed recorded showed a limited variability among varieties and thesis and no further analysis are possible to estimate if this variable has any impact on the variability in the yield. Also in the cases of Kilifi and Busia the most evident result from the analyses conducted is the strong interaction site per variety. This is in line with the relevant literature (Pham Duc et Al. 2010). As well it has been noted in Kilifi and Busia a limited impact of the use of fertilizer on the morphological and productive traits.

Varieties with the traits above mentioned (greater height, short internode length, high number of capsules per axil), are expected to produce more capsules per plant and potentially generate a higher seed yield. This demonstrated to be not always the case in this research and is not surprising considering the relevant literature. The low seed yield, even in presence of high performance of the other variables above mentioned, has been explained, by different researchers, by a lower number of seeds per capsules resulting in a lower yield. Baydar (2005) reported that highest yields were obtained from plants with relatively less number of capsules with highest number of seeds than from plants with a greater number of capsules having less seeds. Not always we found a positive relationship between number of seeds per capsule, capsule number per plant and seed yield per plant.

Correlation analysis has been conducted to see the connections between the considered variables and to understand the influence of these on the Plant Yield. The relevant coefficients together with their significance are reported in Table 13: Total Pearson correlations.

Table 13: Total Pearson correlations

	plant yield g	height 50% flowering	plant height cm	weight of caps	n of capsules	n seed x capsule	length capsule cm	thickness capsule cm	stem thickness	internode length cm
plant yield g	1									
height 50% flowering	,484**	1								
plant height cm	,428**	,699**	1							
weight of caps	,723**	,621**	,559**	1						
n of capsules	,690**	,674**	,482**	,841**	1					
n seed x capsule	,321**	,304**	,443**	,410**	,333**	1				
length capsule cm	-,009	-,024	,126**	,085	-,079	,264**	1			
thickness capsule cm	,081	,044	,052	,096*	,043	,110*	,069	1		
stem thickness	,288**	,438**	,714**	,408**	,326**	,322**	,092*	,069	1	
internode length cm	-,084	-,079	,367**	-,052	-,228**	,003	,230**	-,042	,348**	1

\*\* Correlation is significant at 0,01 (2-code).

\*. Correlation is significant at 0,05 (2-code)

The yield per plant resulted correlated with the height at 50% flowering, plant height (due to the behavior of this variable the second just confirms the first), number of capsules, weight of capsules and stem thickness.

The evident different behavior of all the varieties in the two sites, lead us to focus the correlation of the traits on a site by site analysis. Table 14: Pearson correlation matrix for sesame crop in Kilifi and Table 15: Pearson correlation matrix for sesame crop in Busia below report the Pearson correlation analysis in Kilifi and Busia respectively.

Table 14: Pearson correlation matrix for sesame crop in Kilifi

	plant yield g	height 50% flowering	plant height cm	weight of caps	n of capsules	n seed x capsule	length capsule cm	thickness capsule cm	stem thickness	capsules x axils	internode length cm
plant yield g	1										
height 50% flowering	0.36**	1									
plant height cm	0.376**	0.983**	1								
weight of caps	0.714**	0.487**	0.474**	1							
n of capsules	0.687**	0.487**	0.486**	0.861**	1						
n seed x capsule	0.186	0.167	0.181**	0.226	0.212	1					
length capsule cm	0.208	0.281**	0.274**	0.363**	0.348**	0.441**	1				
thickness capsule cm	0.019	-0.036	-0.066	0.003	-0.013	0.026	0.035	1			
stem thickness	0.212	0.647**	0.636**	0.355**	0.364**	0.203	0.188	0.019	1		
capsules x axils	-0.147	-0.423**	-0.417**	-0.198	-0.213	-0.08	-0.13	-0.031	-0.456**	1	
internode length cm	0.184	0.449**	0.434**	0.266**	0.243	0.004	0.102	-0.112	0.321**	-0.332**	1

In Kilifi yield per plant was highly correlated to the height of the plant (both at maturity and at 50% flowering, the number of capsules and the weight of the capsules (these two variables were also correlated among them and with the heights of the plant).

It is interesting to note a negative correlation between the heights of the plant recorded with the number of capsules per axil.

Table 15: Pearson correlation matrix for sesame crop in Busia

	plant yield g	height 50% flowering	plant height cm	weight of caps	n of capsules	n seed x capsule	length capsule cm	thickness capsule cm	stem thickness	capsules x axils	internode length cm
plant yield g	1										
height 50% flowering	0.699**	1									
plant height cm	0.484**	0.428**	1								
weight of caps	0.674**	0.482**	0.69**	1							
n of capsules	0.304**	0.443**	0.321**	0.333**	1						
n seed x capsule	-0.024	0.126	-0.009	-0.079	0.264**	1					
length capsule cm	0.044	0.052	0.081	0.043	0.11	0.069	1				
thickness capsule cm	0.438**	0.714**	0.288**	0.326**	0.322**	0.092	0.069	1			
stem thickness	-0.056	-0.171**	0.029	0.046	0.038	-0.19**	0.008	-0.127	1		
capsules x axils	-0.079	0.367**	-0.084	-0.228**	0.003	0.23**	-0.042	0.348**	-0.313**	1	
internode length cm	-0.247**	-0.116	-0.072	-0.297**	-0.071	0.119	0.098	-0.037	-0.108	0.202**	1

In Busia the same trends and correlations may be noted in what refers to plant yield but the level of correlation is in some way weaker, beside there is a negative correlation between Plant Yield and Internode length that is not observed in Kilifi.

The two sites are very different in soil and agroclimatic conditions, Kilifi is close to the coast, with relatively warm temperatures and soils sandy and loose, these conditions are more similar to those where sesame is grown in Somalia. In Busia, water availability through rain is greater, temperature is relatively lower, these conditions are more favorable for the development of diseases and weeds, while soils are heaviest and in one block water logging caused significant losses. Busia, on the shore of Victoria lake, where sesame is traditionally grown by local small holder farmers, was chosen assuming a similarity with the riverine conditions of Somalia along Shabelle river but the temperature, diseases and abundant moisture available translated in a crop depressing environment, that stressed the plants and flattened their performances reducing the manifestation of the variability as happened in Kilifi.

In our trials Sesame demonstrated to be very sensible to the origin's site and the site of cultivation. These data corresponds to those in literature where it is reported that several factors may contribute to the yield variability of the observed landraces/varieties to the final production including climatic reasons such as temperature (day/night), day length, light intensity, precipitation, altitude and latitude. Photosynthesis is influenced by various biotic and abiotic stresses during grain filling, therefore, that decreasing or increasing photosynthesis capacity is a major limiting factor for yield and all yield components (Beheshti and Fard, 2010). There are reasons to believe that the less performing varieties not adjusted to the field conditions and therefore gave a low yield in this study. Basu and coworkers (2009) reported that seed yield is known to be a complex trait governed by polygene and therefore is influenced more by environmental factors. As well our observations on sesame behavior in different environments are in agreement with previous studies, showing how sesame is highly sensitive to day length since it is a short day plant (Narayanan and Reddy, 1982). Suddhiyam and coworkers (1992) also reported about the effect of the interactions between temperature and day length on the flowering rate.

In our study the difference of the behavior of the different varieties in the different environments was confirmed by a highly significant correlation existing in Kilifi between yield per plant and plant height (height 50% flowering and plant height), number of capsule per plant and weight of capsule, while in Busia yield per plant was correlated with plant height (height 50% flowering and plant height), number of capsules, weight per capsule, capsule thickness and negatively correlated with Internode length.

Again it is evident that the ideotypes are different for the different sites and different environmental conditions.

**Discriminant analysis of morpho-productive traits**

Following the field data collection, a discriminant analysis has been conducted to identify the main sources of differences to draw the dendrograms of the phenotypic differences.

Initially the discriminant analysis was conducted considering the group as combination of Varieties cultivated in the two Sites with the three levels of Nitrogen. The phosphorous levels were omitted because of their little influence observed in the ANOVA. The new coordinates obtained by the discriminant functions have been utilized to compare the 30 identified different groups (five varieties x two sites x three level of nitrogen). After the Matrix rotation the eigenvalues have been found and are those reported in Table 16: Eigenvalue.

Table 16: Eigenvalue

Function	Eigenvalue	% of variance	% cumulated	Canonical Correlation
1	5,010 <sup>a</sup>	69,8	69,8	,913
2	,809 <sup>a</sup>	11,3	81,1	,669
3	,507 <sup>a</sup>	7,1	88,2	,580
4	,244 <sup>a</sup>	3,4	91,6	,443
5	,204 <sup>a</sup>	2,8	94,4	,411
6	,133 <sup>a</sup>	1,9	96,3	,343
7	,109 <sup>a</sup>	1,5	97,8	,314
8	,072 <sup>a</sup>	1,0	98,8	,259
9	,048 <sup>a</sup>	,7	99,5	,215
10	,036 <sup>a</sup>	,5	100,0	,187

a. for the analysis the first 10 canonical functions have been used

It is possible to note that the first three discriminant functions describe around 88% of total variance. In particular the eigenvalue of the first function describes around 70% of the total variance.

Since the first assessment it was evident that two different groups existed, each one related to the site where they belonged. The distribution of the centroids in the 3D diagram (Fig. 2) based on discriminant functions 1, 2 and 3 shows the above.

Figure 2: Tri-dimensional plot defined by the first three discriminant functions describing 88.2% of the total variance

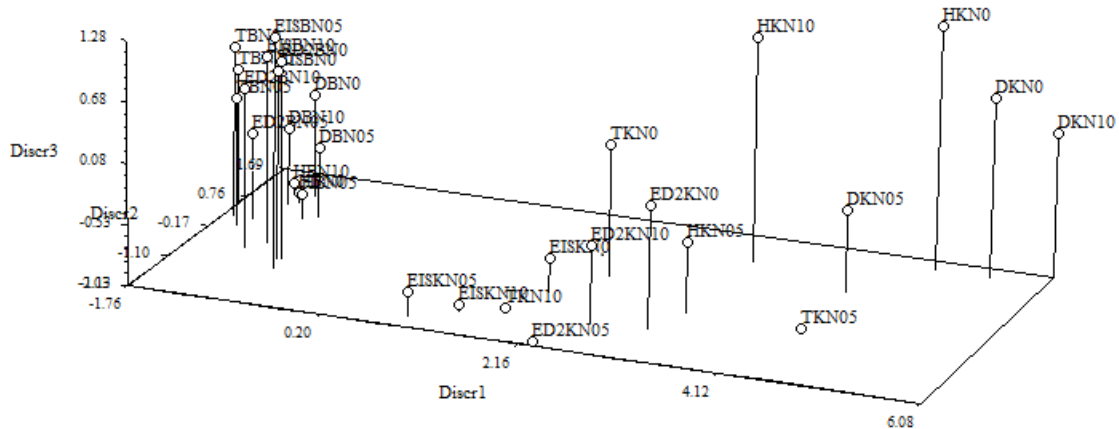


Figure 2 displays the distance between the two groups, the one in Kilifi (with K in the code) and the one in Busia (with B in the code) and the different behaviours in the two sites: high variability in Kilifi and relative concentration in Busia.

The variables associated to the discriminant functions (Tab. 17) are: number of capsules for the first function, internode length, plant height, height at 50% flowering and weight of capsule for the second function while capsule length is associated to the third discriminant function.

Table 17: Matrix of association between original variables and the discriminant functions

Matrix of structure										
Discriminant Functions										
Variables	1	2	3	4	5	6	7	8	9	10
Ncap	,530*	,381	,183	,524	-,351	-,147	,070	-,310	-,014	-,143
InterL	-,313	,805*	-,085	-,057	,034	,219	,009	-,194	,314	,242
Plheig	,197	,764*	-,105	,057	,452	-,384	,089	,000	-,002	,063
H50%Fl	,541	,545*	,379	-,295	,345	-,085	,063	,075	,194	,085
Wcap	,322	,474*	,278	,383	-,424	-,439	,044	,136	-,072	,223
CapLeng	-,196	,102	,682*	,296	,326	-,430	,201	-,191	,171	-,036
Pyieldg	,282	,269	,163	,677*	,022	,147	,029	,143	-,242	,512
NSXCap	,124	,044	-,097	,311	,201	-,576*	,021	-,141	,552	,423
StThickn	,096	,495	-,173	-,036	,138	-,233	,772*	-,079	-,034	,196
CapThickn	,011	,027	-,022	,321	,033	,055	,255	,685*	,472	-,366
Correlations common within groups between discriminating variables and standardized canonical discriminant functions										
Variables ordered by absolute size of correlation within each function.										
*: Largest absolute correlation between each variable and any discriminant function										

Based on this finding, it was then decided to proceed to two parallel discriminant analyses, one based on the data from Kilifi and one from the data from Busia analogously to what was done for the correlation analyses.

In each of the two sites then 15 groups have been identified corresponding to the combination of the 5 varieties with the three levels of Nitrogen fertilization. Based on the phenotypic

differences gathered in Kilifi the table of the eigenvalues has been obtained and it is reported below (Tab. 18).

Table 18: EigenvaluesKilifi

Function	eigenvalues	% of variance	% cumulated	Canonical Correlation
1	5,206 <sup>a</sup>	63,2	63,2	,916
2	1,272 <sup>a</sup>	15,4	78,7	,748
3	,665 <sup>a</sup>	8,1	86,7	,632
4	,384 <sup>a</sup>	4,7	91,4	,527
5	,262 <sup>a</sup>	3,2	94,6	,456
6	,206 <sup>a</sup>	2,5	97,1	,414
7	,145 <sup>a</sup>	1,8	98,8	,356
8	,065 <sup>a</sup>	,8	99,6	,247
9	,024 <sup>a</sup>	,3	99,9	,153
10	,006 <sup>a</sup>	,1	100,0	,079

In Kilifi the first three functions explain 86.7 %. We could have proceeded in this case too drawing a tri-dimensional diagram of the centroids but due to the fact that in Busia, to reach a similar level of the described variance 4 functions were needed (Tab. 20), to visualize the distribution of the 15 groups, instead of 2 tri-dimensional graphs (one per site), two dendrograms have been drafted (Fig. 5 and 6).

Table 19: Matrix of association between original variables and the discriminant functionsfor Kilifi data

Matrix of structure										
Discriminantfunctions										
Variables	1	2	3	4	5	6	7	8	9	10
Plheig	,729*	,139	,047	-,256	,336	,068	-,234	-,455	-,048	,012
H50Fl	,728*	,077	,057	-,299	,319	,125	-,340	-,295	,070	-,215
StThickn	,573*	,162	-,318	-,201	-,354	,039	,285	,527	,113	,074
InterL	,413	-,658*	,456	,164	-,097	-,331	,078	,053	-,110	,146
NSXCap	,096	,565*	,366	,277	-,379	-,476	-,219	,022	,179	-,106
CapLeng	,139	,314	,750*	-,079	-,210	,196	,370	-,068	,053	-,295
Ncap	,354	,087	,030	,600*	-,160	,431	,088	-,380	,010	-,377
Wcap	,331	,065	,174	,423	-,386	,568*	-,297	-,191	-,239	-,156
CapThickn	-,027	,076	,123	,223	,262	,338	-,132	,412	,633*	,396
Pyieldg	,229	,171	,117	,600	,254	,267	-,097	,120	-,601*	-,154

The above matrix of structure in Kilifi (Tab. 19) shows the variables associated to the 10 discriminant functions. The first function is associated to plant height, height at 50% flowering and stem thickness. The second one is associated to internode length and number of seeds per capsule. Finally the third discriminant function is associated to the length of the capsule.

The following bi-dimensional centroid’s diagram (Fig. 3) obtained showing a high level of dispersion of the 15 groups (5 varieties per 3 levels of N).

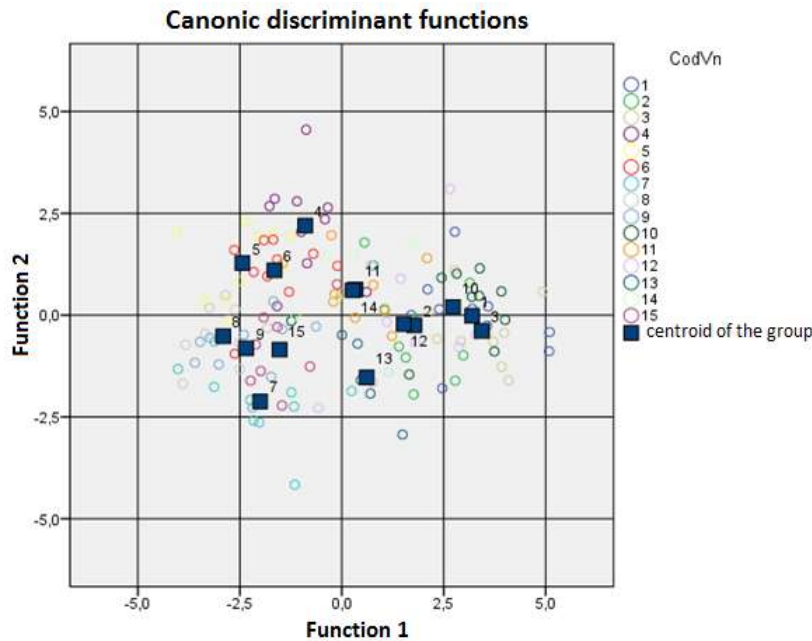


Figure 3: bi-dimensional centroid's diagram of the 15 groups in Kilifi

In Busia the discriminant analysis gave the following eigenvalues table (Tab. 20) whose first three functions explains only 77.6 of the total variability, a bi-dimensional diagram of the centroids of the 15 groups, resulting from the combination of the 5 varieties examined and the 3 levels of Nitrogen fertilization adopted, has been drafted (Fig. 4). In this case the distribution of the centroids and of the single observation shows an overlapping of the 15 groups, indicating a reduction of the variation in this locality if compared to Kilifi.

Table 20: Eigenvalues Busia

Function	Eigenvalues	% of variance	% cumulated	Canonical Correlation
1	,693 <sup>a</sup>	52,4	52,4	,640
2	,199 <sup>a</sup>	15,1	67,5	,408
3	,133 <sup>a</sup>	10,1	77,6	,343
4	,114 <sup>a</sup>	8,6	86,2	,320
5	,091 <sup>a</sup>	6,9	93,1	,289
6	,036 <sup>a</sup>	2,7	95,8	,187
7	,025 <sup>a</sup>	1,9	97,7	,156
8	,013 <sup>a</sup>	1,0	98,7	,114
9	,011 <sup>a</sup>	,9	99,6	,106
10	,006 <sup>a</sup>	,4	100,0	,075

In table 21 the matrix of structure in Busia shows the association between variables and discriminant functions.

Table 21: Matrix of association between original variables and the discriminant functions for Busia data

Matrix of structure										
Discriminant Functions										
Variables	1	2	3	4	5	6	7	8	9	10
Plheig	,659*	,199	,334	,267	-,033	,225	-,168	-,419	-,183	,234
InterL	,641*	,065	,258	-,088	,161	,078	-,362	-,200	,548	-,083
CapLeng	-,278	,711*	,389	,476	-,005	,135	-,086	-,100	,046	-,035
H50Fl	-,014	-,125	,732*	,077	,225	,291	-,296	-,398	-,049	,243
CapThickn	,159	-,044	,527*	,220	,235	-,377	,429	,453	-,129	,208
StThickn	,460	-,257	,303	,603*	-,316	,101	-,310	-,247	-,026	,018
Wcap	,216	,153	,518	,078	-,671*	,122	,012	-,031	-,021	,434
NSXCap	,157	,288	,056	,321	-,070	,620*	-,020	,274	,144	,548
Ncap	,121	-,059	,346	,239	-,451	,259	,529*	-,395	,245	,196
Pyieldg	,110	,082	,034	,255	-,277	-,375	-,110	-,230	,264	,751*

Plant height and intermodal length are associated with the first discriminant function, length of the capsule with the second and height at 50% flowering and thickness of the capsule with the third discriminant function.

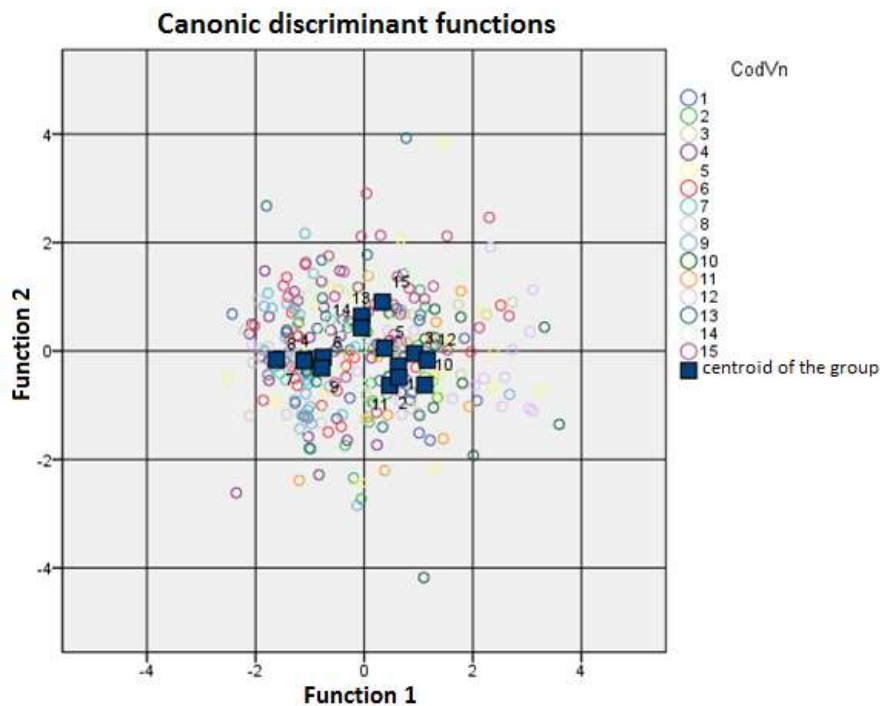


Figure 4: bi-dimensional centroid's diagram of the 15 groups in Busia

The variations observed in the African sesame accessions examined include differences in plant height, capsule length, capsule thickness and capsule weight per plant, number of capsules per plant, number of seeds per capsule, and yield per plant. These findings support previous observations about the large variability in these traits among sesame genotypes (Bedigian, 2010, Bedigian 2011c; Bedigian and Harlan, 1986; Ercan et al., 2002).

In Kilifi the dendrogram based on morpho-physiological traits consisted of five main clusters (Fig. 5). It is worth to notice that N levels of fertilization in some way create phenotypic variability in particular for the varieties Tanzania and Humera that grouped separately, for the others the nitrogen levels do not modified so much the phenotypic structure of the plants.

In Busia the dendrogram based on morpho-physiological traits consisted of four main clusters (Fig. 6) Tanzania cluster again in one single group while it is Ethiopian D2 with Ethiopian D2 and Humera at 50 kg of N per ha, separating from their respective groups at N0 and N100. Dulary in both sites has the same behaviour.

The main difference between the behaviours of the 15 groups in Kilifi and Busia is the similarity among groups belonging to the same accession. It seems that Busia's environment relatively less warm and with higher levels of water availability has a homogenizing effect and different accessions have reduced distances. Dulary, Humera, Tanzania forms homogeneous groups. The same applies, with a small exception, to Ethiopian IS while Ethiopian D2 shows certain variability. In Busia the different levels of Nitrogen fertilization do not seem to generate a significant variability for the reasons explained before.

In Kilifi the scenario changes considerably. Dulary groups in one homogeneous cluster and this behaviour is similar to the one in Busia. Ethiopian IS and Ethiopian D2 cluster in a perfectly similar way: with N50 and 100 they cluster together with a small distance from N0.

Humera with N0 and N100 cluster together while relatively distant is Humera N50. Finally Tanzania is the one showing the highest level of variability not clustering with any other phenotype in N0, and clustering with Humera at N50 and Ethiopian IS at N100.

These conclusions are coherent with the practical information of cropping sesame: high sensitivity to temperature (the highest the better for crop development), high susceptibility to water logging, and limited response to fertilization.

### Findings of the Genomic analysis

11 varieties of African sesame have been screened in the genomic analysis following the methodology described in the relevant chapter of materials and methods. Among these 11 genotypes were included the 5 accessions tested on field for morphological and productive traits. 6 genotypes per each accession have been analysed with 7 primers.

DNA has been extracted, evaluated, amplified and finally sized.

The number of alleles per primer ranged from 2 to 10 (the allele nulli is considered) with the primers 8 and 182 showing the lowest variability (only two loci) and 108 and 123 the highest (10 loci).

The value reported per each accession in the last column of the table is the average of allelic frequency (an indicator of the genetic variability) of all loci. The accessions showing the highest variability are the red ones, those with an intermediate grade of variability in black while in blue are reported those with low variability (Tab. 22).

EthiopianIS and Tanzanian are the most variable with highest levels of genetic polymorphism while Ugandan accessions the lowest.

Is a bit surprising finding high variability in Ethiopian IS and Ethiopian D2. Probably these accessions have been collected in field visits or on the local market in Ethiopia and not from some research station as assumed initially. They cluster together and are relatively close to Tanzania, which confirms the phenotypic behaviour in Kilifi.

Is as well surprising to find low variability in the Ugandan accessions: are they the results of the selection work of a research station and distributed to farmers country wide?

In the varieties Icease and Humera there is reason to believe that characters have been fixed through a breeding programme: Icease has been provided by ICRISAT and Humera is originated in Ethiopia where has been likely produced in a research station of the Ministry of Agriculture. In both cases characters have been fixed and variability limited.

The high variability of Tanzania and Dunyar is expected as both varieties come from local markets.

Based on the allelic frequencies, the Nei distance between the 11 accessions has been calculated<sup>3</sup> (Tab. 23).

With Sahn algorithm (in NTSYS pc2.2) Nei distances have been elaborated to draft the dendrogram visualizing the genetic distances between all the accessions shown in figure 7.

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<sup>3</sup>Nei index =  $1 - \sum \sqrt{\text{products of the allelic frequencies between two accessions compared per each locus}}$ .

Figure 5: Dendrogram of combination of varieties with N in Kilifi

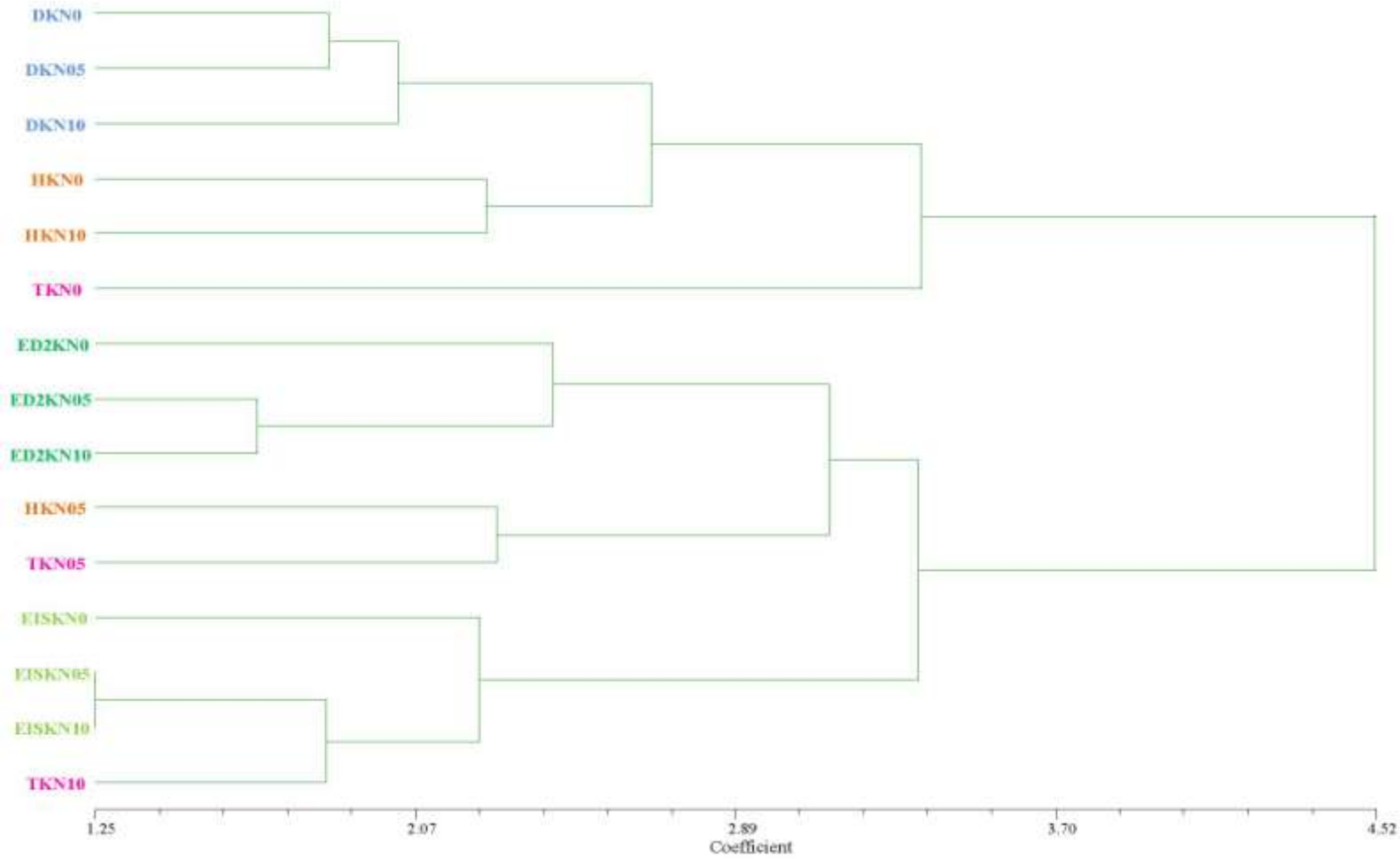


Figure 6: Dendrogram of combination of varieties with N in Busia

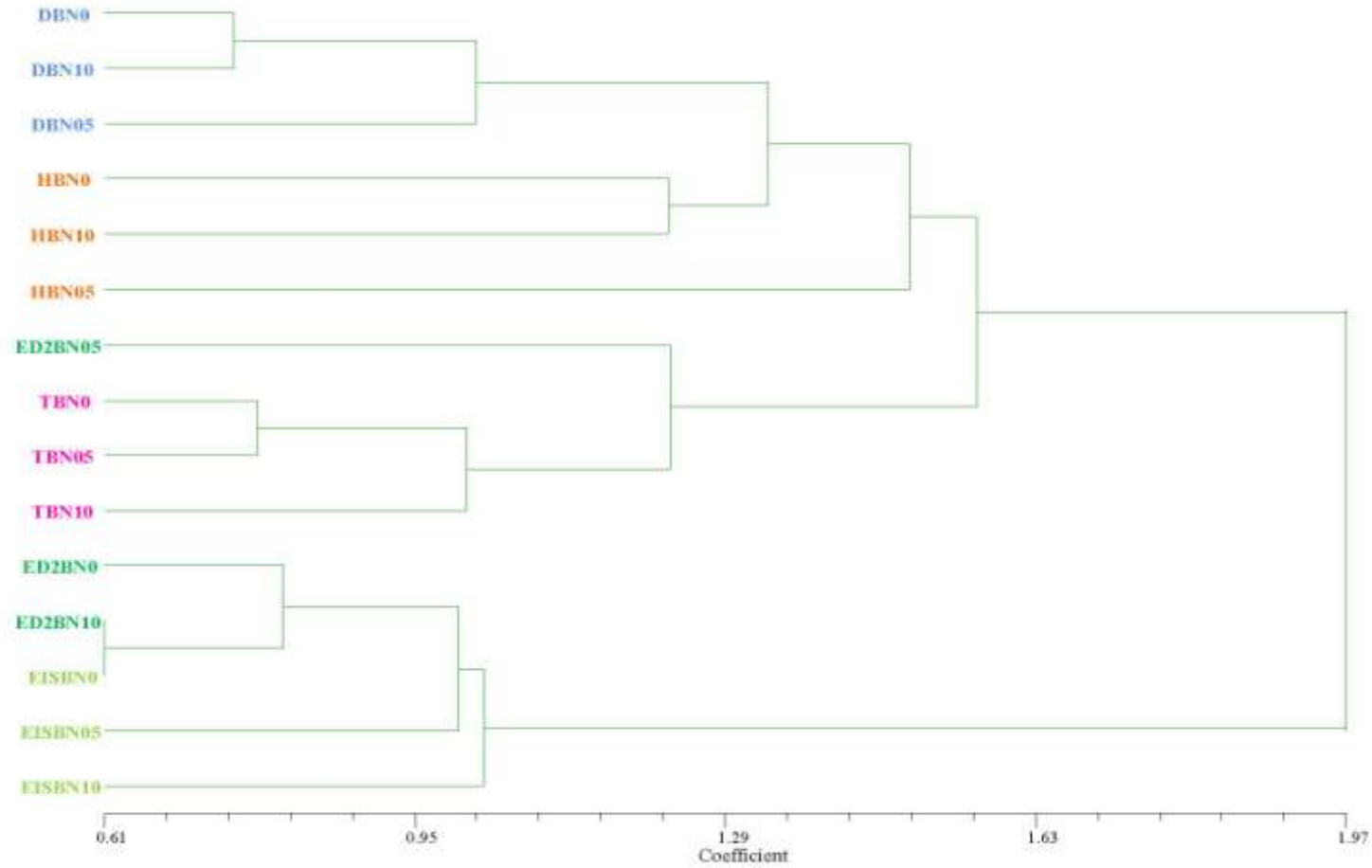


Table 22: Table of allelic frequency

Accessions	8			9			72			108		
	Na	Ne	hExp	Na	Ne	hExp	Na	Ne	hExp	Na	Ne	hExp
Uganda	1	1.000	0.000	1	1.000	0.000	1	1.000	0.000	2	1.180	0.153
Uganda C	1	1.000	0.000	1	1.000	0.000	1	1.000	0.000	3	2.323	0.569
Uganda L	1	1.000	0.000	1	1.000	0.000	1	1.000	0.000	4	3.429	0.708
Sudan	1	1.000	0.000	1	1.000	0.000	1	1.000	0.000	3	2.571	0.611
Tanzania	2	1.385	0.278	4	2.057	0.514	1	1.000	0.000	3	2.571	0.611
Icease	1	1.000	0.000	2	1.385	0.278	1	1.000	0.000	3	2.182	0.542
Humera	1	1.000	0.000	3	2.571	0.611	1	1.000	0.000	2	1.385	0.278
Somaliland	1	1.000	0.000	4	3.429	0.708	2	1.385	0.278	3	2.571	0.611
Dunyar	1	1.000	0.000	4	3.600	0.722	1	1.000	0.000	4	3.000	0.667
Etiopie D	1	1.000	0.000	4	3.600	0.722	3	2.880	0.653	2	1.800	0.444
Etiopie IS	3	2.909	0.656	3	2.667	0.625	2	1.600	0.375	3	2.667	0.625
	<b>2</b>	<b>1.209</b>	<b>0.085</b>	<b>7</b>	<b>2.119</b>	<b>0.380</b>	<b>3</b>	<b>1.260</b>	<b>0.119</b>	<b>10</b>	<b>2.334</b>	<b>0.529</b>

Accessions	123			182			184			Accessions	P	Hav
	Na	Ne	hExp	Na	Ne	hExp	Na	Ne	hExp			
Uganda	2	1.800	0.444	2	1.800	0.444	1	1.000	0.000	Uganda	3	0.149
Uganda C	3	2.000	0.500	2	1.385	0.278	1	1.000	0.000	Uganda C	3	0.192
Uganda L	3	2.571	0.611	1	1.000	0.000	1	1.000	0.000	Uganda L	2	0.188
Sudan	3	2.571	0.611	2	1.800	0.444	1	1.000	0.000	Sudan	3	0.238
Tanzania	4	3.000	0.667	3	2.571	0.611	3	2.571	0.611	Tanzania	6	0.470
Icease	2	2.000	0.500	1	1.000	0.000	2	1.180	0.153	Icease	4	0.210
Humera	1	1.000	0.000	2	1.800	0.444	3	2.000	0.500	Humera	4	0.262
Somaliland	2	1.385	0.278	2	1.385	0.278	3	2.000	0.500	Somaliland	6	0.379
Dunyar	4	3.429	0.708	2	1.800	0.444	2	2.000	0.500	Dunyar	5	0.435
Etiopie D	4	2.483	0.597	1	1.000	0.000	2	1.800	0.444	Etiopie D	5	0.409
Etiopie IS	3	2.909	0.656	1	1.000	0.000	2	1.600	0.375	Etiopie IS	6	0.473
	<b>10</b>	<b>2.286</b>	<b>0.507</b>	<b>2</b>	<b>1.504</b>	<b>0.268</b>	<b>4</b>	<b>1.559</b>	<b>0.280</b>			

Na=number of di alleles observed per each locus and each accession

Ne= effective number of the alleles based on the frequency they appear

Hexp= expected heterozygosis (1-summatory of squared frequencies)

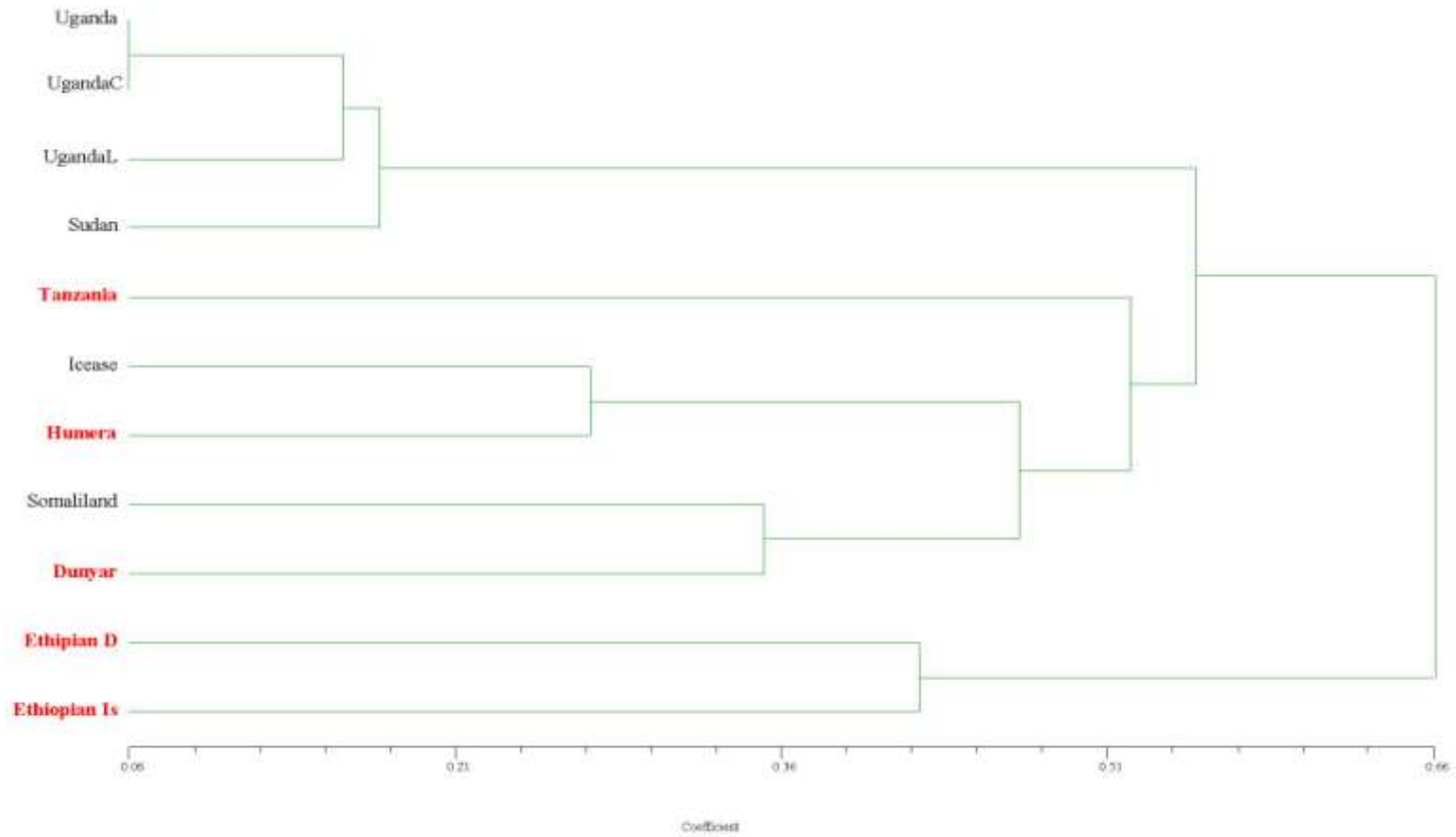
Hav= average heterozygosis

P= polymorphic alleles per each accession

Table 23: Matrix of Nei distances between 11 African accessions

	Uganda	UgandaC	UgandaL	Sudan	Tanzania	Icease	Humera	Somaliland	Dunyar	EthiopianD2	EthiopianIS
Uganda	0.000										
UgandaC	0.056	0.000									
UgandaL	0.169	0.141	0.000								
Sudan	0.209	0.102	0.204	0.000							
Tanzania	0.563	0.587	0.527	0.587	0.000						
Icease	0.515	0.543	0.500	0.598	0.468	0.000					
Humera	0.455	0.457	0.356	0.431	0.505	0.269	0.000				
Somaliland	0.705	0.692	0.547	0.664	0.526	0.426	0.395	0.000			
Dunyar	0.597	0.566	0.444	0.629	0.573	0.534	0.510	0.348	0.000		
EthiopianD2	0.720	0.723	0.727	0.712	0.763	0.555	0.567	0.431	0.495	0.000	
EthiopianIS	0.699	0.668	0.644	0.658	0.858	0.739	0.683	0.561	0.630	0.420	0

Figure 7: Nei genetic distance between the 11 African considered accessions based on allelic frequencies of the examined genes



Some interesting indications are drawn from Figure 7:

1. the three accessions from Uganda cluster together and with Sudan accession probably do to the same origin.
2. Dunyar and Somalialand cluster together, this confirming that farmers in Somaliland use the same accession used in South and called Mombasa (see Sidow Ahmed Yakub, 2011) possibly originating from Kenya and Mombasa and Dunyar are different names attributed to the same genetic material.
3. Tanzania is completely isolated and does not cluster with any other accession.
4. Ethiopian D2 and IS cluster together confirming the common origin.

The genic pool constituted by the 11 African accessions examined showed a relatively wide variability encouraging using them in further sesame breeding programs.

From a theoretical point of view it is expected that morpho-physiological and molecular data may complement and confirm each other. The former providing information on plant characteristics while the latter providing the information on genetic basis of these characteristics. It is remarkable that Tanzania, in both analysis the morphological and the genetic behaved in the same high variable way as well as Humera confirmed its medium variability, while Dunyar, from the analysis of morphological and productive traits, showed a limited variability, but from the genetic analysis showed one of the highest.

In our study, the comparison of different accessions based on the morpho-productive traits and the genomic SSR markers showed that there was only a partial correlation among the two classifications this is in line with literature as Ercan et al (2004) and Bhat et al (1999) observed the same poor correlation between molecular and morphological markers.

Nevertheless other studies (Tabatabaei et al.2011) reports that among the traits evaluated, seed yield per plant, height to the first capsule, flowering period, and number of capsules showed high correlation with molecular data, providing encouraging evidence that for traits with less complex genetic background, molecular markers can be effective in sesame breeding.

The information so far collected provides interesting guidelines for breeding programmes:

Considering the:

1. Plasticity of the sesame gene pool capable to adapt to different ecosystems;
2. The different response to fertilization and the extreme rusticity of sesame accessions;
3. The variability showed by several accessions among those examined.

The following crossing scheme may be considered for sites like Kilifi where high temperatures and light soils are available.

	Ethiopian IS	Humera
Tanzania	X	X
Ethiopian D	X	-

While in sites where temperature is lower and moisture in the soil is higher like it happens in Busia, crossing like the below may be explored.

	Tanzania	Ethiopian D2	Dunyar
Tanzania	X	-	-
Ethiopian IS	-	X	-
Dunyar	-	-	X

**Assessment of sesame production profitability in Somalia**

Data gathered at field level on the costs and benefits of production have been used to establish the sesame cropping profitability. The analysis has been conducted at household level considering the data available from FSNAU (Food Security and Nutrition Analysis Unit in FAO), field reports and personal interviews.

Keeping in mind that a continuum exists among different sesame cropping methods and ecosystems, the profitability analysis of sesame cropping (expected level of production, costs and benefits and break even point) is carried out according to the three most typical possible scenarios of sesame cropping in Somalia:

- a. rainfed traditional: 6 kg of seed per ha, sowing broad casted and minimum crop caring;
- b. rainfed improved: 8 kg of seed per ha, sown in rows (generally 60 cm x 18 cm), with at least two weedings (riverine traditional is in between the rainfed improved and the other two systems (rainfed improved and riverine traditional) with 6-8 kg of seed per ha, broadcasted and without weeding but with some additional irrigation if gravity irrigation is available or pumped irrigation if affordable by the farmer);
- c. riverine improved: 8 kg of seed per ha, sown in rows (generally 60 cm x 18 cm), with two to three weedings and some irrigation when gravity irrigation is available or pumped irrigation if affordable by the farmer.

All data refer to the unit area of 1 hectare, which is the area usually grown at smallholder farmer level. This figure may be significantly different in riverine and rainfed systems. Nevertheless, the analysis carried out can provide useful information for these systems as well and can be considered representative for the South Central region of Somalia.

**Production costs**

Here below are reported the summary of production costs (Tab. 24) computing the actual cost of production in the three different scenarios where sesame is grown in Somalia: rainfed traditional, rainfed improved and riverine improved.

Table 24: Sesame summary of production costs

Production items	Riverine improved	Rainfedimproved	Traditional
Services (land preparation, irrigation)	108	108	108
Labour	315.82	253.34	247.1
Chemical	4.5	4.5	4.5
<b>Post Harvest</b>			
Services (transport, packaging)	46	32.5	32.5
Labour	31.3	18.72	15.6
<b>Total</b>	<b>505.62</b>	<b>417.06</b>	<b>407.7</b>

The cost of production has been calculated including the cost of labour as this is the usual approach in economic analysis. However, in standard operational situation in Somalia, labour is normally provided by the household; as a result, the cost of labour should not be considered as a financial cost, considering that the household labour opportunity cost is close to zero for lack of real working opportunities. In addition considering real situation in Somalia, not all the labour activities considered in the cost analysis are *real* (e.g.: spraying). Actual labour costs really sustained are those related to mechanical power for ploughing and

harrowing and some other costs during peak time <sup>4</sup>. This implies that the actual financial cost of labour for cropping sesame is about 33%, 40% and 41% <sup>5</sup> of the total labour cost computed respectively for sesame riverine improved, sesame rainfed improved and sesame rainfed traditional of what is reported above with a consequent positive impact on the profitability of the cropping.

However, it should be noticed that his assumption in the last years may be challenged because of the ongoing urbanization process, internal migration related to lack of security or search for better life opportunities and to the fact that during the labour peaks in the cropping season (sowing, weeding, harvesting) there may be a labour shortage.

Another important consideration refers to the allocation of workload by gender (Tab. 25) (Sidow, 2011) shows the relevance of the role of the women in the sesame cropping. In Somalia the share of women work contribution to household total workload in sesame production activities is second only to that of vegetables. This is extremely important because in principle any increased income form sesame production can be in principle conducive to women empowerment, due to existing customary income sharing rules in rural areas.

Table 25: Tasks required for sesame cultivation in Somalia

Operation	Hours	Men	Women
Bund formation	54	54	
Irrigation	51	51	
Planting and seed cost	24	14	10
spraying	10	10	
Weeding	36		36
Basal dressing	5	5	
Harvesting	90	36	54
Threshing	50	25	25
Carting	2	2	
Cleaning	12		12
Subtotal	334	197	137
Miscellaneous (10%)	33.4	19.7	13.7
Total	367.4	216.7	150.7
Percent		59%	41%

### Profitability of sesame production in Somalia

A recent field data collection in Lower and middle Shabelle (riverine area) referring to 4,500 farmers belonging to two different agrosystems (riverine and rainfed) who received 8 kg of Humera seeds showed the following.

Table 26: Basic data on sesame production

		Area	Yield obtained Kg/Ha		
Sowing date	Harvesting date		Low	High	Average
<i>Nov-Dec</i>	<i>Feb-March</i>	Riverine	650	900	775
		Rain-fed	400	600	500

The value of the production is, in the case of sesame, clearly defined by the level of production and by the quality of the production. The quality of the production identified by

<sup>4</sup> Smallholder farmers supply in a form of mutual support with no money transaction, i.e. groups of 10-15 neighbouring farmers works in one of the member's farm one day and receive food and so on during the next 15 days where all the farm have received the support of all the others.

<sup>5</sup> Considering the cost for food provided during the peak time to guest the neighbouring farmers about 20 USD/day for three days (sowing, weeding and harvesting) along the cropping season and the cost of labour as computed.

the traders is based on size, taste and colour. The sesame traditionally grown in Somalia is called Dunyar and is mixed in colour (black 35% cream and white 65%), level of production are between 300 and 350 kg/ha. Humera, the tested variety in Somalia, produced between 600 and 900 kg/ha and it is white with an excellent taste.

Dunyar has a high domestic demand and is used also to produce oil. Market price of sesame in towns may reach, in retailers' shops 3 USD/kg and the oil at about 5 USD/l while the cake resulting from the oil extraction is sold at 1 USD/Kg as animal feed integrator. Very seldom this variety only rarely may have access to foreign markets.

Table 29 below summarizes the different income levels according to the cropping system.

Table 27: Summary of income according to different cropping system

Income	Unit	Sesame traditional cropping system			Sesame good quality in rainfed			Sesame good quality in riverine			
		Qty	Unit price (USD)	Total (USD)	Qty	Unit price (USD)	Total (USD)	Qty	Unit price (USD)	Total (USD)	
1	Yield (average)	Kg/ha	350			500			775	0	0
2	Gross revenue at farm/village gate	USD	350	0.85	297.5	500	1	500	775	1	775
3	Gross revenue at market level	USD	350	1	350	500	1.2	600	775	1.2	930
4	Gross revenue at international trader level	USD	350	0	0	500	1.4	700	775	1.4	1085
5	Cost of production	USD			407.7			417.1			505.62
6	Net revenue at farm gate level (Output-Input)				-110.2			82.94			269.38
7	Net revenue at market level (Output-Input)				-57.7			182.94			424.38
8	Net revenue at international trader level (Output-Input)				-407.7			282.94			579.38
	<b>Man day/ha</b>		<b>54</b>			<b>57</b>			<b>69</b>		

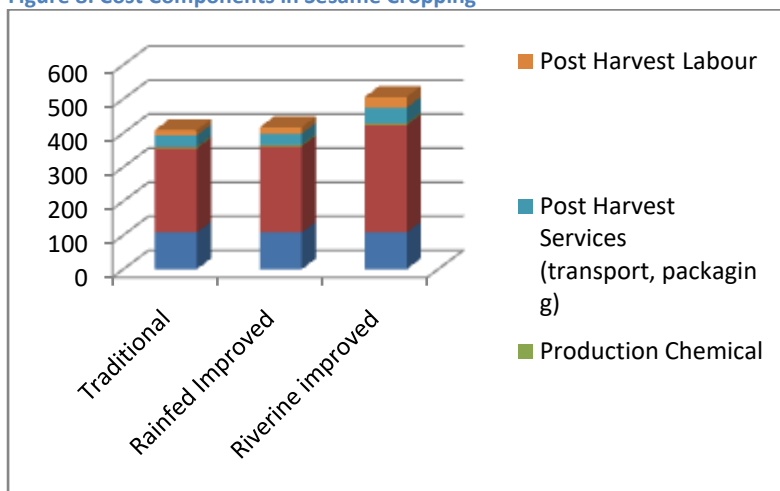
It may be noted that that

1. in a traditional cropping system wouldn't be profitable to smallholder farmers should they pay for labour, i.e. hiring casual workers.
2. in a rainfed improved cropping system would be profitable to smallholder farmers even if they should they pay for labour, i.e. hiring casual workers.
3. in a riverine improved cropping system would be very profitable to smallholder farmers even if they should they pay for labour, i.e. hiring casual workers.

**Overall assessment**

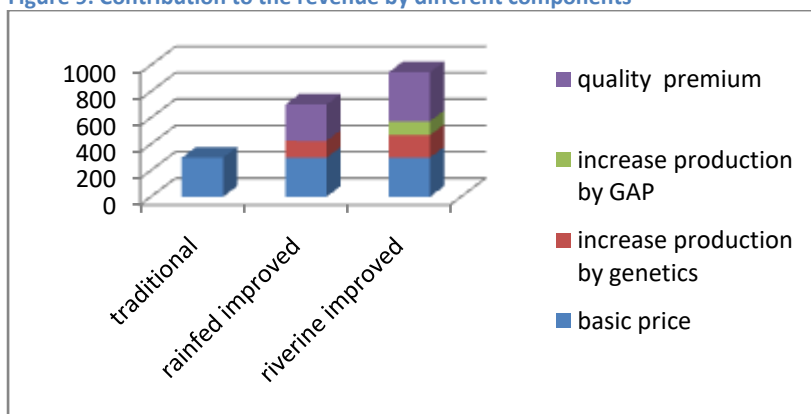
Figure 8 summarizes the costs components per each cropping system. Comparing these components it emerges that the production cost slightly increases with the level of production while the post harvest cost increases significantly with the production.

Figure 8: Cost Components in Sesame Cropping



Considering the basic price of 0.85-0.9 USD/kg and the positive impact on production due to the use of improved genetic material and of adequate GAPs, and finally considering the quality premium paid by the international trader (1.4 USD/kg – 0.85 USD/kg)/1.4 USD/kg= 39%), the total revenue for the smallholder farmers in the three different scenarios is depicted in Figure 9.

Figure 9: Contribution to the revenue by different components



The key success element is the link created among farmer organizations (receiving and stocking the production) and an international trader guaranteeing the purchase of the entire production by the end of the season paying a quality premium.

Based on the production costs summarized for the three cropping methods described so far in table 24, to conclude this analysis it is worth to assess the break even of production for the different scenarios with and without the cost of labour (Tab. 32 and 33).

Table 28: Sesame summary of production costs

Production items	Riverine improved	Rainfedimproved	Traditional
Services (land preparation, irrigation)	108	108	108
Labour	315.82	253.34	247.1
Chemical	4.5	4.5	4.5
<b>Post Harvest</b>			
Services (transport, packaging)	46	32.5	32.5
Labour	31.3	18.72	15.6

<b>Total</b>	<b>505.62</b>	<b>417.06</b>	<b>407.7</b>
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Table 29: Break even (kg/ha) considering labour cost

Break Even with labour costs	Riverine improved	Rainfed improved	Traditional
Quantity produced (kg/ha)	775	500	350
Total cost of production	505.62	417.06	407.7
Unit cost (USD/kg)	0.65	0.83	1.16
Price of sale (USD/kg)	1.4	1.2	0.9
<b>Break even (kg/ha)</b>	<b>361.16</b>	<b>347.55</b>	<b>453</b>

Table 30: Break even (kg/ha) without considering labour cost

Break Even without labour cost	Riverine improved	Rainfed improved	Traditional
Quantity produced (kg/ha)	775	500	350
Total cost of production	189.8	163.72	160.6
Unit cost (USD/kg)	0.24	0.33	0.46
Price of sale (USD/kg)	1.4	1.2	0.9
<b>Break even (kg/ha)</b>	<b>135.57</b>	<b>136.43</b>	<b>178.44</b>

In order to break even the production costs in the traditional cropping system (when the cost of labour is considered) at least 453 kg/ha of sesame should be produced. However, as in the traditional system no more than 350 kg/ha are produced, the only reason why sesame is still cropped in this agricultural system is that labour is not paid (accounted) for and is a short cycle crop minimizing crop failures during the short rain season. Vice versa, sesame production is highly profitable in the two other production systems, with a break even well below the standard operating conditions.

Furthermore, the financial analysis shows that in Somalia it is more convenient to produce sesame than maize in riverine improved conditions. In the case of maize, a similar work conducted in the past three years testing the income growth related to germplasm improvement and GAP adoption in riverine irrigated areas led to the following outcome: the gross revenue per ha computed adopting the same methodology is 1,200 USD with a production cost of 770 USD and a net revenue of 430 USD per ha. In riverine a small holder farmer growing maize in Gu and sesame in deyr will have and income of about 2,285 USD corresponding to 1.04 USD/person/day<sup>6</sup>.

However, the alternative is purely academic in Somalia where Gu, the long rain season, is used for producing maize, and Deyr the short rainy season to produce sesame and would not be politically not correct in a food insecure country like Somalia to grow sesame also in Gu. Luckily the latter matches with the technically more appropriate solution as the crop rotation with maize is in any case an ideal solution in terms of agronomic practice and in addition growing sesame in Gu also does not seem compatible with temperature and light requirements of sesame.

Another important aspect to analyse is the efficiency in the use of water, the scarcest resource in Somalia. Table 35 summarizes the most important parameters in the use of water comparing the sesame cropping systems with the other major crops in the region, namely sorghum and maize.

Table 31: Value the water in term of different production

Crop	mm water needs (min)	mm water needs (max)	yield grain MT/Ha	efficiency in water use kg grain/mm	mm of water per Ton of green matter	value of grain/ha (US\$)	value of fodder US\$/ha	total value production (US\$)	US\$ value of each mm of water
Sesame	304.8	406.4	0.6	1.18	-	840	0	840	2.36

<sup>6</sup> Household average composition is of 6 persons (FSNAU Data). Average sesame production 775 kg/ha average sesame price 1.4 USD/kg.

Sorghum	449.58	482.6	1.2	1.74	31.08	360	150	510	1.09
Maize	558.8	609.6	3	3.47	41.01	960	250	1210	2.07

In terms of technical efficiency, growing maize is the most efficient system of using water. However, in terms of economic efficiency (i.e. considering the net revenue of each crop) the most efficient crop is sesame, for which 1 mm of water produces 2.36 USD.

**Conclusions**

For a Somali farmer, daily engaged in surviving, dwelling with drought and floods occurring regularly one season after the other when not during the same season, the existential problem is how to survive. Social networks are almost non-existing (with exclusion of the clan support and the zaquat<sup>7</sup>), the state is almost absent and in the market he is the weakest ring of the chain .... He is alone and shall cope with the hardship of natural and social environment alone. No surprise if along the centuries he has developed some coping mechanisms perfectly adapted to the environment. One of these is the rotation sesame maize during Deyr and Gu respectively. In this context two consecutive genetic improvement works, one on Maize and the present on sesame have created the conditions for improving his/her quality of life through income growth.

The present study concludes the following:

1. The 11 accession of Sesame African germplasm examined showed high genetic variability and are suitable to begin new breeding programs to improve the production and the quality;
2. Sesame is sensitive to the ecosystem and high environmental impact on phenotypic expression is demonstrated in all the accessions/varieties tested on field, for this reason it is relevant to develop local breeding programs involving local farmer;
3. Several gene pools identified are promising to improve sesame cultivation in different sites with different cropping systems;
4. Sesame, under the experimental conditions adopted in this study, demonstrated a relatively limited response to N and P fertilization, in other words its requirements for these nutrients are limited. The crop rotation adopted in Somalia by small holder farmers is in line with this conclusion as fertilizer, if used, is distributed during Gu to maize and sesame in Deyr will take advantage of the residual fertility of the nutrients distributed during the previous season.
5. Due to the previous, sesame showed to be an environmental friendly crop with reduced input costs thus requesting a limited financial investment the latter providing an additional motivation for smallholder farmers to continue and expand its cultivation.
6. The question if sesame cropping would reduce food security in Somalia is purely academic; farmers will continue growing maize in Gu and sesame in Deyr. However, global sesame demand represent an opportunity at household level for income growth thus cropping sesame might reduce food sovereignty but would increase food security.
7. It has been demonstrated along this study that the adoption of improved GAPs (without the adoption of extra input –fertilizer and extra labour- but following the on-going cropping practices thus without extra costs) and improved germplasm in sesame cropping translates in an income growth exceeding 1,000 USD per year. The final result for a small holder farmer household adopting improved GAPs and germplasm in growing maize and sesame along the year is 1.04 USD/day per person (significantly higher than the 0.26-0.53 US\$/person/day estimated in rural areas of Somalia). An economic analysis not

<sup>7</sup> Charity, one of the five pillars of Islam.

considering the cost of labour provided by the farmer in his own farm would bring a more positive result.

## Acknowledgement

This research started during one of the worst humanitarian catastrophe faced by the people of Somalia: the famine of 2011. In those years a group of Somali field technicians implemented an EC funded project and supported the implementation of field plot and data collection. Successively in Kenya other professional and FAO colleagues of the Agricultural sector, among others Musungu Maureen, Julius Mwangi and Joseph Ahenda, participated in the set up of the experimental plots and in the data collection for an EC funded project. Invaluable has been the support provided by AYUUB NGO, and its staff (Xusni and Mudane in particular) in South Central Region of Somalia for the abnegation in the field work and the extraordinary effort in supporting small holder farmers. Titus Mutinga, Ahmed Nur and Stoyan Nedyalkov, helped shopping sesame seed all around Africa during their duty missions and the University of Palermo (Prof. Ettore Barone) and CEFA NGO (Flavio Braidotti) who also supported providing some sesame seed.

The authors express their gratitude to all of them and to FAO for its unique and inestimable role for a world without hunger.

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