Developing high oleic acid *Guizotia abyssinica* (L.f) Cass. by plant breeding

Yohannes Petros[1] , Anders Carlsson[1], Sten Stymne[1], Habtamu Zeleke[2], Ann-Sofie Fält[1] Arnulf Merker[1]

[1] Swedish University of Agricultural Sciences, Box 101, Alnarp, Sweden
[2] Haramaya University, P.O.Box 138, Dire Dawa, Ethiopia
Corresponding author, E-mail: yohannes_petros@yahoo.com

Abstract

Oleic acid content was increased from approximately 5-11% to 80-86% in *Guizotia abyssinica* (L. f.) Cass. materials from Ethiopia after repeated selection and breeding. Achenes collected from Ethiopia were screened for elevated oleic acid content by half seed technique. The starting materials for breeding were nine plants selected from among 272 seeds analyzed and having an average oleic acid content of approximately 21% which is considered to be high compared to the 5-11% reported earlier for niger materials of Ethiopian origin. It was observed that the oleic acid content steadily increased after each round of selection and breeding. The percent oleic acid in the seed oil increased to an average of 35.2% and 53.4% after the first and second round of breeding respectively and ultimately to over 80% after the third round of breeding. It was also observed that the percent oleic acid in the oil stabilizes and the plants breed true when the oleic acid content in the parental seeds was above 79%.

Key words: *Guizotia abyssinica* — niger — high oleic acid.

Introduction

Guizotia abyssinica (niger in English) is a little known oilseed crop. The major niger growing countries of the world are Ethiopia and India. Niger is also reported to be grown in West Indies, East Africa and the United States (Kandel and Porter 2002). In Ethiopia 50-60% of the edible oil requirement of the country is met by niger seed oil, whereas only about 2% of the edible oil requirement is provided by niger seed in India (Riley and Belayneh 1989, Dutta et al. 1994, Hiremath and Murthy 1988).

The oil content of niger is variously reported as 29-39% (Dutta et al 1994), 30-35% (Kandel and Porter 2002) and 42-44% (Dagne and Johnsson 1997). Dutta e*t* al. (1994) reported that the Ethiopian niger seed oil contains more than 70% linoleic acid, whereas, Dagne and Johnsson (1997) reported 66-69% linoleic acid. In all the works so far done on the fatty acid composition of niger, linoleic acid is unequivocally the dominant fatty acid present in niger seed oil followed by palmitic, oleic and stearic acids (Dutta et al. 1994, Ramadan and Morsel 2003, Dagne and Johnsson 1997). The percentage of oleic acid in the Ethiopian niger seed oil was reported to be in the range of 6-11% (Dutta et al. 1994), 5.4-7.5% (Dagne and Jonsson 1997). It is indicated that the oil content and the fatty acid profile may vary depending on the origin of the material and the maturity level of the seeds (Riley and Belayneh 1989, Stymne and Appleqvist 1980).

The quality of oil and its suitability for a particular purpose, be it for industrial use or for human consumption depends on the proportion of the different fatty acids it contains. Oils where linoleic acid is the predominant fatty acid are reported to have poor shelf life whereas those with high oleic acid content are more stable. For cooking oils, it becomes imperative that oils be suitable for the kind of cooking they are intended for (Mugendi et al. 1998, Warner and Knowlton 1997, Fehr 2007). It is also reported that the presence of high proportions of linoleic acid in oils positively contributes to the frying flavor intensity, a fact which also renders its keeping quality poorer (Warner et al. 1997). Thus, in deep fat frying, which has now become a common way of preparing food, the oil should be optimal for the frying flavor intensity of the food and the frying time of the oil (Mugendi et al. 1998, Warner and Knowlton 1997, Fehr 2007).

The high oleic sunflower oil with about 78% oleic acid is shown to have greater frying stability of the oil and oxidative stability of the food (Warner

et al. 1997). Warner et al. (1997) also reported that there is significant positive correlation between increase in the level of linoleic acid in the oil and decreasing oxidative stability of the food. Because the oxidative stability of an oil and its shelf life have an inverse relationship to the amount of polyunsaturated fatty acids it contains, it becomes necessary to lower the degree of unsaturation in order to decrease the oxidative rancidity of oils. For the most part this is done by blending the oil with one having high oleic acid content or by hydrogenation. (Mugendi et al.1998, Burton et al.1983). Hydrogenation of oils, although it increases the thermal stability and resistance to atmospheric oxidation, is however, known to produce unwanted positional isomers (Tompkins and Perkins 2000, Wilson and Rinne 1976, Fehr 2007). Trans-esterification of oils by hydrogenation is indicated to pose adverse health effects, and recently attempts have been made to genetically modify the degree of unsaturation in oils through genetic engineering (Kinney 1994, Chapman et al.2001). It has been shown that oils whose oleic/linoleic ratio has been modified through genetic engineering exhibited greater frying stability (Warner and Knowlton 1997, Tompkins and perkins 2000). Genetically engineered food crops, however, are not appealing for the most part to the public and its use for human consumption is still controversial in many countries around the world. Thus, to circumvent the ethical, public health as well as economic problems presented by the chemical and genetic modification of oils, modification of the proportion of the fatty acids towards the desired composition by plant breeding remains the best alternative to date. To this end, several oil crop varieties have been developed by plant breeders including high oleic acid sunflower, High oleic acid safflower and high oleic acid soybean (Urie 1985, Fuller et al. 1996, Wilson and Rinne 1976, Burton et al. 1983). As both oleic acid and linoleic acid are produced by the same desaturation pathways of 18:1 to 18:2 and 18:2 to 18:3 (Stymne and Appleqvist 1980, Voelker and Kinney 2001), modifying the fatty acid composition of niger by reversing the oleic/linoleic ratio towards elevated proportion of oleic acid in the seed by plant breeding is believed to increase the oxidative stability of the oil. It is also envisaged that developing high oleic varieties of niger is indeed an important objective as it is reported that intake of diet with high oleic acid in the oil would reduce the low density lipoprotein cholesterol in blood plasma in addition to increasing its shelf life and oxidative stability which same characters are the most sought after in developing any crop seed for deep fat frying (Takagi and Rahman 1996). Increasing the percentage of oleic acid in the oil of niger would inevitably lead to the reduction in the percentage of linoleic acid in the seed oil, as there is an inverse relationship in the inheritance of these two fatty acids in niger.

The present work is an attempt to increase the percentage of oleic acid in niger seed oil through selection and breeding. We are reporting, in the present paper, an increase of oleic acid >80% of the total fatty acid in niger seed oil not heretofore reported. It is believed that the study would contribute to the efforts already underway to enhance some of the desirable qualities in niger by plant breeding.

Materials and Methods

The plant material

The plant materials were collected from niger growing regions in Ethiopia during November and December 2005. Seeds from single plants were collected in farmer's fields. Bulked seeds from each of 87 plant progenies from 78 fields in four regions were analyzed for content of oleic acid. In the progenies of nine plants with elevated levels of oleic acid, the half seed method was used. The nine individual seeds with the highest levels of oleic acid (Table 1) were planted and used in further crossing. The breeding program was initiated in the Autumn of 2006 with these nine plants; four plants from Wellega, three plants from Jimma and two plants from Gojam.

Table 1. Region of origin, site coordinates and the percentage composition of oleic acid of the niger materials collected from Ethiopia.

Code	Region	Site coordinates	% oleic acid
W45-1	Wellega	9° 22'N, 36° 24'E	22
W45-2	Wellega	9° 22'N, 36° 24'E	30.6
W45-3	Wellega	9° 22'N, 36° 24'E	29.6
W46	Wellega	9° 22'N, 36° 24'E	17
G37	Gojam	11° 31'N, 37° 27'E	16.8
G65	Gojam	11° 41'N, 37° 28'E	17.4
J6-1	Jimma	7° 45'N, 37° 11'E	20.2
J6-2	Jimma	7° 45'N, 37° 11'E	19.2
J6-3	Jimma	7° 45'N, 37° 11'E	17.9

The screening procedure

Seeds from the various accessions were soaked in water on filter paper in Petri dishes. The husk was removed to expose the embryo. The cotyledons were cut in half and the part with the miniature embryo left on the Petri dish while the other half is analyzed for its oleic acid content by gas chromatography. Where the oleic acid content of the half seed turns out to be high, the other half seed is planted in the green house. Screening of the seeds for high oleic acid was done after harvest of each round of breeding.

Breeding procedure

The design of the experiment was a simple one, the objective being to obtain niger progeny that are breeding true for high oleic acid. During the first round of breeding (November 2006 to March 2007), half seeds known to have relatively high oleic acid were planted in the Biotron at SLU, Alnarp, the day time temperature adjusted to 25°C and the night temperature adjusted to 18°C. Cross pollination was carried out by dusting the pollen from a plant onto the inflorescence of another plant. Mature inflorescences from all the plants were harvested in March 2007 and screened for the high oleic trait by the half seed technique. Only seven individuals which showed markedly high oleic acid percentage were selected for planting in the second round of breeding (April 2007 – Sept 2007). These had percent oleic acid content of 57-83% (Table 2). Crosses were made in all possible combinations including reciprocal crosses in all instances. These were harvested in September 2007 and the screening for the high oleic phenotype resumed by the half seed technique. Only those known to have high percentage composition of oleic acid ($\geq$ 79%) were planted for the third round of breeding (October 2007 – March 2008). These were harvested in March 2008 and analyzed the same way for the oleic acid content.

Analytical procedure

For the bulk materials samples containing 18 seeds were homogenized in 3.75ml Methanol:Chloroform (v/v 2:1) and 1ml of 0.15M acetic acid, and the homogenate transferred into a screw cap tube. The homogenizer was rinsed with 1.25ml chloroform and transferred to the screw cap tube, to which was added 1.25ml water. This was thoroughly mixed, centrifuged

and the bottom phase taken. This was evaporated under Nitrogen and methylated with 2ml of 0.1M Sodium Hydroxide in methanol and heated for 15 minutes at 90°C. To this was added, 3ml hexane, 2ml water and 100µl of the internal standard, methyl heptadecanoate and mixed thoroughly before centrifugation for 3 minutes at 2000 rpm. Seventy microliters of the top hexane phase was transferred to the GC vials. The half seeds were directly methylated by the addition of 0.1M Sodium Hydroxide in methanol and the fatty acid methyl esters extracted with hexane.

Separation of the fatty acid methyl esters was done by gas chromatography (Shimadzu GC-17A, Kyoto, Japan) with a flame ionization detector (FID). A WCOT fused Silica capillary column (50m x 0.32mm), CP-wax 58 (FFAP)-CB (Cromopack, Middelburg, the Netherlands) was used. The temperature was held at 160°C for 0.5min and increased at a rate of 3°C/min to 250°C and held there for 0.5min before coming to 265°C at a rate of 25°C/min and held there for 2.4minutes. The injection port and the detector were held at 275°C and 280°C respectively.

Results

The oleic acid percentage in the total oil was observed to steadily increase after each round of selection and breeding. The starting material consisting of nine plants had an average oleic acid content of around 21% with the individual seed's oleic acid content ranging from 17-30.6% (Table 1). It was observed that after the first round of breeding the average oleic acid content in the progeny has increased to about 35.2%. The individual seeds from these crosses, however, exhibited wide range of variation from 4-83% (Table 2.A). Whereas only 1% of the seeds analyzed after the first round of breeding had oleic acid content >80%, after the second round of breeding, however, more than 20% of the seeds were observed to have oleic acid percentage >80. The progeny seeds from all possible combinations of crosses including reciprocal crosses in the second round of breeding showed a wide range of variation with respect to the oleic acid content of individual seeds. Nevertheless, the proportion of progenies with high oleic acid content significantly increased and the average oleic acid content of seeds was found to be 53.2%, though the oleic acid content of individual seeds ranged from 14% to 86.3% (Table 2.B).

Table 2. Mean values and range of the major fatty acids in niger obtained after the first (A), second (B) and third (C) round of breeding.

	Crosses	No. seeds	Mean	Palmitic Range	Palmitic Mean	Stearic Range	Stearic Mean	Oleic Range	Oleic Mean	Linoleic Range	Linoleic Mean
	Percentage composition of the major fatty acids										
A	16.8x17.9	27	6.6	5.5-7.7	4.7	3.6-6.2	38.8	22.1-53.2	47.5	32.6-64.9	
	17x17.4	21	7.1	5.8-8.5	5.7	3.5-6.6	42.3	16.2-83.0	42.0	4.0-69.7	
	19.2x22	5	6.1	5.1-6.3	4.5	4.1-5.2	43.5	26.8-62.7	43.6	24.9-59.9	
	22x20.2	21	7	5.7-8.6	4.7	3.2-6.2	29.2	5.8-59.8	56.6	25.8-78.2	
	29.6x30.6	29	7.3	5.9-10.2	6.3	4-9.8.0	30.1	4.0-57.4	53.5	25.1-77.8	
B	83x62	15	6	4.8-7.5	4.4	2.9-6.3	64.8	39.3-86.3	22.8	2.7-49.7	
	83x61	39	7	5.3-8.6	5.1	3.6-6.9	39.8	15.9-70.3	46.2	15.0-70.3	
	61x62	3	6.4	5.4-7.8	6.3	5.5-7.3	40.5	19.6-54.5	44.6	31.7-64.7	
	61x59	13	6	4.7-6.8	6.1	3.9-7.1	52.0	25.0-84.6	33.7	4.5-48.0	
	61x57	8	7.2	6.3-8.6	5.9	4.7-8.5	45.5	21.6-65.5	39.1	19.5-59.6	
	83x58	30	6	4.5-7.3	4.9	3.5-6.4	67.9	48.4-83.8	19.1	3.4-38.2	
	83x57.7	23	6.8	5.1-8.6	4.1	2.5-5.2	60.5	28.5-84.6	26.9	3.3-57.9	
	57.7x62	9	6	5.4-6.8	4.3	3.2-5.3	46.7	24.6-61.2	41.1	25.8-63.0	
	61x57.7	14	7.3	6.0-8.8	5.4	4.4-6.9	38.6	14.0-65.4	46.6	19.5-71.5	
	58x57.7	13	6.2	4.7-7.3	5.4	3.9-6.6	55.7	25.2-84.3	30.9	4.5-60.1	
	57.7x57	32	5.9	4.0-7.3	5.0	3.3-7.1	51.9	19.6-86.1	35.5	3.7-67.6	
	83x57	14	5.8	4.6-7.7	4.6	3.2-6.2	65.4	30.8-85.4	22.3	3.6-55.9	
C	82x85	16	5.2	4.6-5.9	3.4	2.9-4.2	83.6	77-87	6.0	2.9-12.2	
	85x83	8	4.9	4.6-5.6	3.4	2.3-4.7	83.1	81.1-85.2	6.6	3.3-8.9	
	84x85	6	5.3	4.9-5.8	4.1	3.2-4.7	82.7	81.5-84.8	5.9	3.9-8.2	
	86x84	5	5.6	5.2-6.3	3.8	3.1-5.2	81.8	78.1-83.9	6.9	3.9-11.0	
	85x85	2	5.2	5.1-5.3	3.5	3.1-3.8	84.3	83.6-85.0	5.3	5.1-5.5	
	82x83	3	5.6	5.2-5.9	3.9	3.4-4.5	80.9	78.1-82.5	7.4	5.3-9.7	
	82x86	3	4.6	4.1-5.3	3.5	2.8-4.3	85.2	83.6-86.3	4.7	3.7-6.4	
	81x79	2	4.3	3.9-4.6	3.4	2.8-3.9	82.0	79.7-84.2	9.0	7.9-10.1	
	82x79	3	5	4.7-5.2	3.5	3.2-3.7	83.2	82.9-83.8	6.6	6.2-6.9	
	79x85	3	5	4.9-5.2	3.6	3.1-4.1	83.4	82.8-84.2	6.1	5.0-6.7	
	79x83	2	4.1	3.9-4.3	4.1	3.6-4.5	82.3	81.9-82.6	7.4	6.4-8.3	

No significant variation was observed between the reciprocal crosses. All materials selected for the third round of breeding had oleic acid content in the range of 79-86%.There has been a dramatic increase in the average percent oleic acid after the third round of breeding. As expected, the vast majority of the progeny seeds from this round of breeding contained oleic acid >80% (Table 2.C). It was observed that progeny seeds obtained from parents having ≥79% oleic acid content were highly stable and yielded progeny seeds with >80% oleic acid content. It was also noted that as the oleic acid content in the seed oil of niger increased from approximately 8.4% in the wild type niger collections (data not shown) to approximately 80 – 86% in the high oleic material developed, there has been a corresponding decrease in the content of palmitic acid from 7.9% to 5.3% which has a significant negative correlation value of -0.994 (P 0.006). The chromatograms for the wild type niger materials from Ethiopia and that for the high oleic acid niger developed in this study are presented in figure 1.

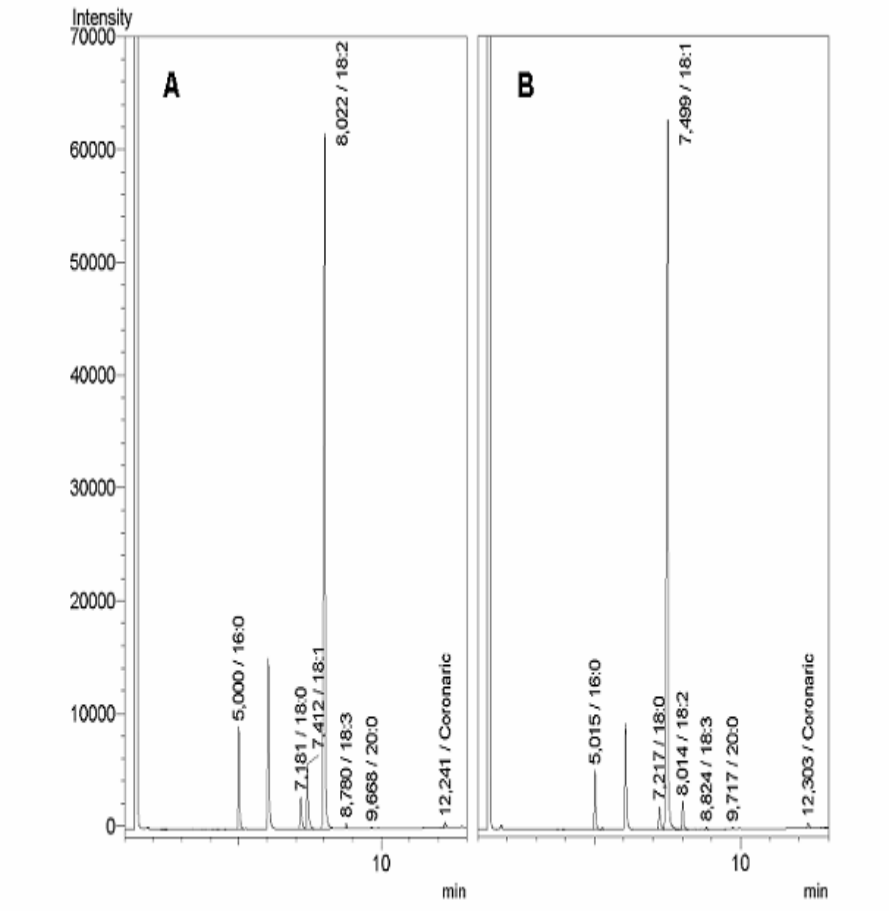

Figure 1. Chromatographic depiction of the fatty acid profile of niger seed oil. A- wild type niger. B- high oleic acid niger.

Discussion

The present study shows that oleic acid content in the seeds of niger is heritable and can be increased by repeated selection and breeding. It was observed that parental plants whose oleic acid content is approximately $\geq$79% are true breeding for the high oleic trait, whereas those with oleic acid content < 70% yield all ranges of oleic acid content (Table 2). Presently, a study is underway to determine the inheritance of high oleic trait in niger. From our present study, it may not be possible to point out what changes in the biochemical pathway led to the accumulation of 18:1 in

the seed oil of niger but Okuley et al. (1994) indicated that mutation at the FAD2 locus of *Arabidopsis* led to the reduction in the production of polyunsaturated fatty acids. As there can be all combinations of triacylglycerol (TAG) molecular species in niger seed oil namely, trilinolein, monooleyl-dilinolein, dioleyl-monolinolein and triolein in the original starting materials with relatively high oleic acid content relative to the common niger materials, it is thought that selection and breeding increased the oleic acid moieties in the TAG and ultimately rendered triolein to be the predominant TAG molecular species in the oil. Thus, selection for high oleic acid seeds and breeding led to the increase of the percent oleic acid on the average after every round of breeding until the oleic acid content stabilized at oleic acid levels of $\geq$80%. Increase in oleic acid content in niger inevitably leads to proportional decrease in the content of PUFA's particularly linoleic acid. This is also the case for many of the oil crops including *Brassica napus* (Schnurbusch 2000) and cottonseed (Chapman et al. 2001). Schnurbusch (2000) indicated that increase in the palmitic acid content of *Brassica napus* is accompanied by decrease in oleic acid and the oil content. Whether increase in the oleic acid content of niger is accompanied by an increase in its oil content needs to be investigated further.

It has been reported that the abundance of PUFA's particularly linoleic acid enhances frying flavor intensity of foods due to the presence of volatiles like 2,4-decadieenal which is the oxidation product of linoleic acid whereas an increase in the content of oleic acid in oils increases the stability of the oil (Warner et al.1997). The high oleic materials of niger developed in the present study presents ample opportunity for breeders to develop plants with optimal fatty acid composition for healthy human consumption by maintaining a balance between oleic and linoleic contents of the oil to increase the stability of the oil without unduly compromising its frying flavor intensity.

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