# Variability in seed oil content and fatty acid composition, phenotypic traits and self-incompatibility among selected niger germplasm accessions

Victoria G. Benelli<sup>1\*</sup>, Fred L. Allen<sup>1</sup> and Ming Li Wang<sup>2</sup>

<sup>1</sup>Department of Plant Sciences, University of Tennessee, 2431 Joe Johnson Drive, Knoxville, TN 37996, USA and <sup>2</sup>Plant Genetic Resources Conservation Unit, USDA ARS, 1109 Experiment Street, Griffin, GA 30223, USA

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## Abstract

Niger (Guizotia abyssinica, L.) is a desirable oilseed crop for birdseed, especially for finches (Spinus spp.) because of its high ratio of unsaturated to saturated fatty acids and relatively high oil content. In 2012, phenotypic traits, seed oil and fatty acid content measurements were made on 14 plant introductions (PIs) from the United States Department of Agriculture germplasm collection. The PIs originated in Ethiopia (ten), India (three) and USA (one). The phenotypic traits analysed included seed/plant, branches/plant, capitula/plant<sup>1</sup>, average seed/capitulum and plant height. After initial assessments of the 14 PIs, three were selected for use as parents to produce two one-way and two two-way  $F_1$  crosses for the purpose of evaluating self-incompatibility (SI). Parent and  $F_1$ progeny seeds were planted in a greenhouse and transplanted to a field site at the East Tennessee Research and Education Center (2012, 2013 and 2014). Comparisons from 2012 showed seed oil of the 14 PIs ranging from 32.9 to 37.9% (PI 508076 (Ethiopia) and PI 509436 (India), respectively). Major fatty acids included stearic, palmitic, oleic and linoleic; with linoleic acid in highest amount. PI508079 (Ethiopia) had the best combination of seed yield, seed oil and linoleic acid content. Over 2013 and 2014, SI ranged from 91.1 to 100.0%. W6 18860 (USA) had the most selfcompatibility, and the  $F_1$  plants generated from crosses between W6 18860 and other plants tended to be self-compatible when the former was used as a pollen recipient. The results obtained from this study should be useful for niger breeding and production purposes.

Keywords: breeding, Guizotia abyssinica, seed quality, self-incompatibility

## Introduction

Niger's oil with high levels of unsaturated fatty acids and low levels of saturated fatty acids makes it suitable for many purposes. Its most common purpose is as an oilseed crop for human and avian consumption, and is primarily cultivated in Ethiopia. Niger is particularly popular among subsistence farmers since it requires few inputs (Geleta and Ortiz, 2013). In the USA, niger is primarily marketed as birdseed primarily for the American goldfinch (*Spinus tristis*, L.), pine siskin (*Spinus pinus*, Wilson), common redpoll (*Acanthis flammea*, L.), finches (*Spinus* spp., Leach) and other songbirds.

Ramadan and Mörsel (2003a) reported that niger seeds had a saturated to unsaturated fatty acid ratio of 25:75. Unsaturated fatty acids are predominantly linoleic (C18:2) and oleic (C18:1), whereas primary saturated fatty acids include palmitic (C16:0) and stearic (C18:0) (Ramadan and Morsel, 2002). Linoleic acid is an essential polyunsaturated omega-6 fatty acid. Oleic acid is a monounsaturated fatty acid common in the human diet that has been associated with the reduction of low-density lipoprotein cholesterol and possibly the increase of high-density lipoprotein

<sup>\*</sup>Corresponding author. E-mail: vknapp@utk.edu

cholesterol (Mensink *et al.*, 2003). Palmitic and stearic acids are saturated fatty acids needed to maintain proper health, but if consumed in large quantities, result in negative health effects. Together, they have been linked to an increased risk of vascular disease, reduced insulin activity (Benoit *et al.*, 2009) and increased risk of Alzheimer's disease (Patil and Chan, 2005).

A previous study by Alemaw and Tekle-Wold (1995) found negative correlations between oleic and linoleic acids. This can be expected since linoleic acid is synthesized from oleic acid. Likewise, a negative correlation is expected for oleic and stearic acids since oleic acid is synthesized from stearic acid. A study by Geleta *et al.* (2011) found that seed weight and linoleic acid had a negative correlation of -0.17. No significant correlations were found between seed weight and oleic acid nor seed weight and palmitic.

Niger, along with other members of the Asteraceae family, is influenced by sporophytic self-incompatibility (SSI) (Nemomissa *et al.*, 1999). SSI is a complex system that occurs when the outcome of the interaction between the pollen tube and the style is determined by the sporophytic dipoid tissue. Here, pollen grains fail to germinate on receptive stigmas. The pollen–stigma reaction results in callose production (Takayama and Isogai, 2005). In dicots, self-incompatibility (SI) is mapped to a single genetic locus called the S-locus (Geleta and Bryngelsson, 2010).

Quantitative traits such as yield are either the direct or indirect result of many physiological processes. Selecting for variation among genotypes via indirect selection may therefore enhance selection for yield. Greater variation indicates larger allelic diversity to make selections (Fehr, 1987). Determining the adaptability and genetic variation among accessions is important if new and improved varieties of niger are to be released in the USA. This is especially important for states such as Tennessee that are located in a transition zone between the Northern and Southern USA.

## Materials and methods

A preliminary field trial was conducted in 2012 with seed obtained from United States Department of Agriculture/ Agricultural Research Service (USDA/ARS) germplasm collection at Pullman, WA, USA. Fourteen accessions (60 seeds each) were planted in a greenhouse at the University of Tennessee, Knoxville, TN, USA on 20 July 2012. Three of these accessions were from India (PI 422242, PI 509436 and PI 511305), nine were from Ethiopia (PI 508070, PI 508072–PI 508080) and one accession was from the USA (W6 18860). Ten seedlings were randomly selected from each accession and transplanted to a field site at East Tennessee Research and Education Center (ETREC) (35.53°N, 83.57°W) in Knoxville, TN, USA on 21 August 2012 in a completely randomized design. The soil type at the Knoxville location was classified as an Etowah loam (fine-loamy, siliceous, semiactive and thermic Typic Paleudults). The field was tilled, but no herbicide was applied prior to planting.

## Phenotypic traits

On 26 September 2012, five plants of each accession were selected and transplanted into a greenhouse located at ETREC in order to avoid a frost. The selections were based on height, number of branches and overall plant vigour. At plant maturity, plant height, capitula/plant, seed/capitulum, primary branches/plant and estimated seed/plant were recorded. Estimated total seed/plant were determined by multiplying capitula/plant by the average seed/ capitulum by the average percent of mature flowers/ plant. Harvesting occurred between 10 December 2012 and 21 January 2013. Phenotypic data of niger accessions were analysed with mixed model analysis of variance and Fisher's least significant difference (LSD) (P<0.05) using SAS 9.3 (Cary, NC, USA). (Table 1)

#### Seed oil content and fatty acid composition

Fourteen accessions were planted but two accessions (PI 508078 and PI 508080) did not produce enough seeds for chemical analysis due to poor germination and plant survival. Seeds from all other plants were bulked within each accession due to seed scarcity and sent to the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA, USA for analysis of seed oil and fatty acid composition using time-domain nuclear magnetic resonance (TD-NMR) and Gas Chromatography (Agilent 7890A) using protocol explained by Jarret et al. (2011). The operating resonance frequency of the NMR analyser was set at 9.95 MHz and maintained at 40°C. For each signal acquisition, spin echo parameters consisted of a 90 pulse of 10.44 µs and reading at 50 µs followed by a 180 pulse of 21.38 µs and reading at 7 ms. A 2 s recycle delay between scans was used, and a total of 16 scans were collected for each sample. Bulk seed measurements were made in a 40 mm glass sample tube, and NMR signals were compared with oil and moisture calibration curves, generated by sample weight. Each of the 14 accessions was measured twice, and then averaged to report the value of oil content in this study. Oil standards were generated using extracted oils.

Afterwards, two replications of the same seed were analysed for each of the 12 accessions using both the Agilent 7890A at Griffin, GA, USA and Agilent 6890 at Knoxville, TN, USA, gas chromatographs (GCs). When using the Agilent 7890A, protocol from Morris *et al.* (2013) was used. About 3–5 niger seeds from each sample were

ground to a fine powder using a mortar and pestle. Oil from a small amount ( $\sim$  50–75 mg) of meal was extracted in 5.0 ml of heptane (Fisher Scientific) and converted to fatty acid methyl esters with 500 µl of 0.5 N sodium methoxide (NaOCH<sub>3</sub>) in methanol. Water was added to separate the organic layer containing fatty acids from the niger meal, and a portion of this layer was transferred to a vial for injection. Fatty acid composition was determined on an Agilent 7890A GC system with a flame ionization detector. Peak separation was performed on a DB-225 capillary column  $(15 \text{ m} \times 0.25 \text{ mm i.d.}$  with a 0.25 µm film) from Agilent Technologies. The inlet and detector temperatures were set to 280 and 300°C, respectively. Helium, the carrier gas, was set to a flow rate of 1.0 ml/min. A sample of 1 µl was injected at a 60:1 split ratio onto the column with the following thermal gradient: 195°C for 3 min, 195-200°C at 2.5°C/min, 200-230°C at 5°C/min and 230-235°C at 1.5°C/ min for a total run time of 14 min. A FAME standard mix RM-3 plus four additional FAMES (all from Sigma) were mixed and used to establish peak retention times. Fatty acid composition was determined by comparison with a standard curve constructed from oil of the same species.

When using the Agilent 6890, fatty acid analyses were performed according to the modified AOCS Ce 1-62 (American Oil Chemists Society, 1999) protocol. Two samples of approximately 117 seeds from each of the 12 accessions were crushed and transferred to a test tube where 2.5 ml extraction solvent (eight parts chloroform (CHCl<sub>3</sub>), five parts hexane (C<sub>6</sub>H<sub>14</sub>) and two parts methanol (CH<sub>3</sub>OH)) was added. Original protocol requires 1 ml of extraction solvent. The tubes were capped and left in the hood overnight. The next day, 100 µl of extract from each tube was transferred to a corresponding vial to which 750 µl of hexane and 75 µl of methylation reagent was added. The vials were capped and transferred to autosampler trays and analysed via the Agilent 6890 (Hewlett Packard) GC. Oil and fatty acid data of niger accessions were analysed with mixed model analysis of variance and Fisher's LSD (P < 0.05) using SAS 9.3 (Cary, NC).

## Self-incompatibility

Of the original 14 niger accessions, three (PI 422242, PI 511305 and W6 18860) were selected for use as parents in crosses to evaluate SI. Choices of parents were based on plant height, number of branches and plant vigour. Four  $F_1$ 's populations were also included in the study: PI 422242/W6 18860 and reciprocal W6 18860/PI 422242; PI 511305/W6 18860 and reciprocal W6 18860/PI 511305. This study was conducted in 2013 and 2014 at the ETREC.

Fifty capitula of each parent and selected  $F_1$  progeny were hand pollinated with pollen from the same plant, and covered with transparent pollination bags. Viable seed from each capitulum was contrasted with total potential seed bearing achenes of the capitulum. Potential achenes were determined by counting the total number of ovaries of each capitulum. SI was calculated as: 100–((average number of seeds/capitulum/average number of potential achenes/capitulum) × 100) (Benelli, 2015).

### Results

## Seed oil content and fatty acid composition

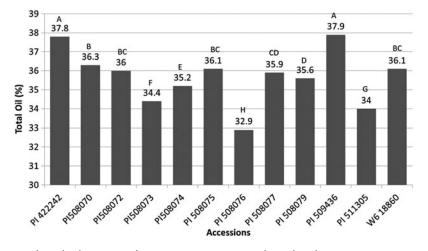
Total oil content and fatty acids composition showed significant differences among the 12 accessions for the study in 2012 (P < 0.0001). Total oil content was highest in PI 422242 (38%) and PI 509436 (38%), and lowest in PI 508076 (33%) (Fig. 1). Total oil content of seeds with Indian origin (PI 422242, PI 511305 and PI 509436) was more varied than seeds from US (W6 18860) or Ethiopian origin (PI 508070, PI 508072–PI 508077 and PI 508079) (Fig. 1).

Results for linoleic, oleic, palmitic and stearic acids showed a significant variation among the niger accessions (P < 0.05). PI 508079 contained the most linoleic acid (82%) and W6 18860 had the lowest (53%) (Fig. 2). Conversely, W6 18860 had the most oleic acid (30%), while PI 508079 contained the lowest (3%). Palmitic acid was highest in W6 18860 (9%) and lowest in PI 508073 (8%). Results showed that PI 508072 contained the most stearic acid (8%), whereas PI 508079 had the lowest (5%). Across all 12 accessions, arachidic acid was <1%, and linolenic acid was <0.40% (data not shown). Additional fatty acids included sapienic (<0.20%), behenic (1%) and lignoceric (<1%) (data not shown).

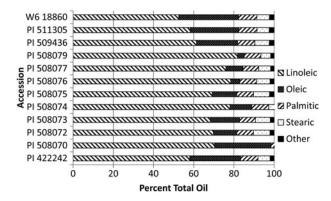
#### Phenotypic traits

Parent height, number of seeds/capitulum, number of primary branches/plant, estimated seed/plant and capitula/ plant were significantly different (P < 0.05) among niger accessions (Table 1). PI 508079 had a significantly greater number of capitula (678), seed/capitulum (24) and estimated seed/plant (16417) than other accessions. Ranges of traits for all other accessions were 354–654 capitula/ plant, 4–21 seed/capitula and 1800–12,486 seed/plant (Table 1). The accessions of Ethiopian origin performed better as a whole for every trait than the three accessions from India and the accession from the USA.

With the exception of height, the accession from the USA (W6 18860) performed similarly to the Indian accessions. The three accessions from India, PI 422242, PI 509436 and PI 511305, had the fewest number of branches. Ethiopian accessions accounted for the greatest and least



**Fig. 1.** Mean comparison of seed oil content of 12 niger accessions evaluated at the East Tennessee Research and Education Center, Knoxville, TN, USA in 2012. Seed were analysed at the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA, USA (Dr Ming Li Wang) using a Bruker mq10 minispec NMR analyser (Resonance Instruments, Whitney Oxfordshire, UK). Bars with a letter in common are not significantly different based on Fisher's LSD (P < 0.05).



**Fig. 2.** Linoleic, oleic, palmitic and stearic acid percentages of 12 niger accessions for seed collected from the East Tennessee Research and Education Center, Knoxville, TN, USA in 2012. Bars represent means of seed analysed at the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA, USA, using an Agilent 7890A GC instrument and at the University of Tennessee using an Agilent 7683 GC instrument. Significant differences were found among accessions for all fatty acids (P < 0.05).

estimated seed/plant (1755–17,102), capitula/plant (354–678) and average number of seeds/capitulum (4–24).

#### Self-incompatibility

Data from 2013 revealed that the SI of the three parents and  $F_1$  plants from the three crosses ranged from 91.1% (W6 18860) to 96.9% (PI511305/W6 18860) (Table 2). The other two parental accessions in this study, PI 422242 and PI 511305, resulted in 95.4 and 95.0% SI, respectively. The  $F_1$  progeny from PI 422242/W6 18860 (95.4%) had similar SI to PI 422242 (95.4%). PI 511305/W6 18860 (96.9%) and

its reciprocal W6 18860/PI 511305 (93.7%) had similar SI values (Table 2). The level of self-compatibility varied between the years as well as within each parent line and  $F_1$  crosses (Table 2). Each of the three parent lines exhibited a range of self-compatibility during both years. However, W6 18860 tended to produce a broader range of selfcompatibility in the  $F_1$  progeny when used as the female parent in crosses (Table 2). This may mean that there is opportunity to select for greater self-compatibility, or even stronger SI, depending on the breeding objective. Further research is needed to determine if there are some cytoplasmic effects on SI.

In 2014, PI 422242, PI 511305 and W6 18860 had SI values 93.6, 99.6 and 100.0%, respectively (Table 2). Furthermore the  $F_1$ 's tested exhibited a high level of SI (96.8–99.6%).

### Discussion

Of the 14 niger accessions evaluated in 2012, ten originated from Ethiopia, three from India and one originated from the USA. Total oil ranged from 33 (PI 508076) to 38% (PI 422242 and PI 509436). Ethiopian accessions produced seeds containing 33–36% oil. A study analysing Ethiopian niger populations reported a broader range of results; 27–56% (Geleta *et al.*, 2011). A study by Ramadan and Mörsel (2003a, b) showed that dry niger seed contained 27–47% oil and with a mean of 35%.

Cultivation condition (environment) and extraction methods may affect the results reported from different laboratories. A study by Bhatnagar and Gopala Krishna (2013) revealed the cold pressed seeds resulted in 28% oil, whereas hexane and ethanol extractions resulted in **Table 1.** Mean comparisons<sup>a</sup> (n = 5) of phenotypic traits among 14 niger accessions evaluated at the East Tennessee Research and Education Center, Knoxville,TN in 2012

Accessions	Height (cm)	Branches/plant (number)	Capitula/plant (number)	Seed/capitulum (number)	Est. total seed/plant
Ethiopia					
PI 508070	125 ab	28 ab	520 a–d	8 e–g	4025 e–g
PI 508072	122 ab	26 а-с	593 ab	18 a–c	10,571 bc
PI 508073	121 ab	26 а-с	354 d	5 fg	1717 g
PI 508074	128 ab	28 ab	597 a	21 ab	12,232 b
PI 508075	111 bc	29 ab	647 a	19 a–c	12,147 b
PI 508076	124 ab	24 bc	552 а–с	14 с–е	8606 b–d
PI 508077	135 a	30 ab	548 a–d	15 b–d	8354 b–е
PI 508078	121 a–d	25 a–d	409 a–d	n/a <sup>b</sup>	n/a <sup>b</sup>
PI 508079	121 ab	24 bc	678 a	24 a	17,102 a
PI 508080	117 а–с	31 a	514 a-d	4 g	1755 g
India					Ū.
PI 422242	81 e	17 d	654 a	11 d–f	6331 c–f
PI 509436	97 с–е	15 d	387 b–d	9 d–g	4002 d–g
PI 511305	90 de	17 d	386 cd	10 d–g	3454 fg
USA				-	Ū.
W6 18860	58 f	21 cd	555 a–c	6 fg	3966 fg

<sup>a</sup>Means within a column followed by a letter in common are not significantly different based on Fisher's LSD (P < 0.05). <sup>b</sup>n/a, not available in 2012.

	2013 Mean ( <i>n</i> = 50)	Range	2014 Mean ( <i>n</i> = 50)	
Parent or $F_1$	(%)	(%)	(%)	Range
PI 422242	95.4	84.6-100.0	93.6	47.1–100.0
PI 511305	95.0	65.3-100.0	99.9	97.3-100.0
W6 18860	91.1	58.7-100.0	100.0	100.0
PI 422242/W6 18860	95.4	82.8-100.0	n/a <sup>b</sup>	n/a <sup>b</sup>
W6 18860/PI 422242	93.2	65.0-100.0	n/a <sup>b</sup>	n/a <sup>b</sup>
PI 511305/W6 18860	96.9	84.9-100.0	99.6	91.7-100.0
W6 18860/PI 511305	93.7	50.0-100.0	96.8	75.0–100.0

**Table 2.** Self-incompatibility  $(SI)^a$  among selected niger parents and  $F_1$  progeny (including reciprocals) evaluated at the East Tennessee Research and Education Center, Knoxville, TN in 2013 and 2014

<sup>a</sup>Each year, 50 random capitula from each parent or  $F_1$  combination were chosen and self-pollinated. Multiple plants were used within each parent of cross. SI = 100 – ((average number of seed/capitulum/number of potential achenes/capitulum) × 100). <sup>b</sup>n/a = not available in 2014.

38 and 30% oil. In studies comparing the variability of 35 accessions for oil quality, results showed that the total oil content ranged 35–40% with a mean of 38% (Yadav *et al.*, 2012a, b).

For fatty acid composition, PI 508079 had a high level of linoleic acid (82%) but low levels of oleic (3%) and stearic (5%). Conversely, W6 18860 had lower levels of linoleic

acid (53%) but highest levels of oleic (30%) and palmitic (9%) acids. This result is expected since Yaun and Bloch (1961) found that oleic acid is the precursor to linoleic acid. Ethiopian accessions showed a wide range of oleic acid (3–28%). Geleta *et al.* (2011) reported a similar range of 3–31% oleic acid in Ethiopian populations. Other studies have found linoleic and palmitic acids to be negatively

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correlated to oleic acid and positively correlated to each other across all plant materials (Benelli, 2015; Geleta et al., 2011). Previous studies have analysed variation in fatty acid profiles in niger and reported that oleic acid ranged from 24 to 53% with linoleic acid ranging from 32 to 58% (Yadav et al., 2012a, b). Palmitic acid, the primary source of saturated fat, ranged 8-9%, and stearic acid, a secondary source of saturated fat, ranged 7-9%. Ramadan and Mörsel (2003a) found the major fatty acids in niger were linoleic (up to 63%), along with oleic (11%), palmitic (17%) and stearic (7%). Bockisch (1998) stated that niger oil may contain up to 1% arachidic and 3% linolenic acid. These studies, along with the comparison of niger accessions in this study, indicate the possibility of future breeding efforts to increase oil content, and alter fatty acids profiles for niger grown in the USA.

According to Miller *et al.* (1987), seed quality is retained longer in seeds with higher oleic acid versus linoleic acid. They state that this is due to oleic acid's lower susceptibility to oxidation, thus plant breeding efforts to improve oil composition should focus on increasing oleic over linoleic acid, especially if oil for human consumption is the desired end use. The syntheses of unsaturated fatty acids are controlled by several genes and are a part of a complex metabolic pathway. Breeding efforts might therefore be most effective using marker assisted selection, when appropriate markers are available.

Ethiopian accessions produced taller plants with more branches than Indian and US accessions. W6 18860 (US) had lower estimated total seed yield than most other accessions in the study, whereas PI 508072, PI 508074 and PI 508075 (Ethiopian) resulted in the greater estimated total seed yield. In 2012, seed/capitulum ranged between 4 and 24, whereas other studies found greater results. Adda *et al.* (1994) reported that seed/capitulum ranged between 28 and 51. Aleminew *et al.* (2015) reported that seed/capitulum ranged from 26 to 29.

Overall, the SI was very high in both 2013 and 2014. Countries of origin did not appear to have an effect on the outcome of the SI values. There were also no visible trends between parents and  $F_1$  progeny in the data. Still, in 2013 greater amounts of self-compatibility were present when self-pollination was performed on W6 18860, W6 18860/PI 422242 and W6 18860/PI 511305. In 2014, W6 18860/PI 511305 also had greater self-compatibility than both parents, which could indicate cytoplasmic effects. When W6 18860 was used as a male, the  $F_1$  populations resulted in greater SI. This could also point towards cytoplasmic effects.

Hosalli (2005) reported that higher self-fertility was obtained when manual pollinations were made; however, manual pollinations of the individual plants in the study herein did not appear to reduce the SI. These findings are consistent with Riley and Belayneh (1989) when they reported that niger has a small degree of self-compatibility though the plants may not necessarily be truly selfcompatible. A previous study by Geleta and Bryngelsson (2010) found that niger is a strictly self-incompatible crop of sporophytic nature, though nine plants out of the 340 evaluated showed some degree of compatibility.

For future breeding programs involving cross-pollination of niger, it may be beneficial to choose parents with a range of SI so as to increase the diversity of the progeny for selection. Continued study of W6 18860 is recommended because of the large difference between 2013 and 2014 outcomes.

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