Comparison of Seed Yield, Oil and Phenotypic Traits Among Selected Parents and Crosses of Niger

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Victoria Grace Benelli
August 2015

DEDICATION

I dedicate this thesis to my husband and family. Your love and encouragement is my inspiration for success.

Take up one idea. Make that one idea your life - think of it, dream of it, live on that idea. Let the brain, muscles, nerves, every part of your body, be full of that idea, and just leave every other idea alone. This is the way to success.

-Swami Vivekananda

ACKNOWLEDGEMENTS

Jesse Benelli, thank you for your constant love and support. My success would not have been possible without your advice and willingness to listen.

Mom and Dad, thank you for believing in my abilities as a student. I have no doubt that your encouragement over the years has led me here.

A special thanks to Dr. Fred Allen and Dr. Vince Pantalone for the opportunity to initially work in their labs when I transferred to the University of Tennessee as an undergraduate. That experience only confirmed my decision to pursue a career in plant breeding.

Dr. Fred Allen, thank you for guidance, kindness, and understanding over the years. I would also like to express my gratitude to my graduate committee. A special thanks to Dr. Hem Bhandari, Dr. Carl Sams, Dr. Arnold Saxton, and Dr. Dennis West for all of your time, guidance, and expertise.

To my colleagues: Matthew Bobbitt, Ali DeSantis, Eifion Hughes, and Virginia Sykes, Graduate School would certainly not have been the same without all of you. I sincerely thank you for all of your helping hands, listening ears, and friendship.

A very special thanks to Douglas Renfro, Karah Renfro, Evan Wilson, and Alex Wilson for their assistance with the field experiments. Your hard work is greatly appreciated.

Dr. Ming Li Wang and Rachel Fulton, thank you for your help with the fatty acid analysis.

Lastly, I would like to thank the Department of Plant Sciences; ETREC, HRREC, and PREC farm crews; and Agronomic Variety Testing Program and UT AgResearch for funding this research.

ABSTRACT

Niger (*Guizotia abyssinica* (L.f) Cass.) is primarily marketed as favorite seed among American goldfinches (*Carduelis tristis*) as well as pine siskin, redpoll, house finches, and ground feeding birds like quail and dove. As a part of a balanced diet, it is crucial for these species to consume a higher percentage of fats than other bird species. Fourteen niger accessions of Indian, Ethiopian, and American origin were obtained from USDA/ ARS germplasm collection at Pullman, WA and planted in August 2012 at the East Tennessee Research & Education Center. Five of these accessions were crossed to form different populations that were evaluated for genetic variation and heritability of seed yield, oil and agronomic traits to determine the feasibility of future breeding efforts in increasing total plant yield and oil content. A randomized complete block design with replication was used for this experiment. Each of four blocks consisted of 5 parent accessions (3 replications per block), 6 F_1's [filial 1] (5 replications per block), 6 F_2's [filial 2] (25 replications per block), and 8 backcrosses (5 replications per block). Two years of data were collected at the Research and Education Centers at Knoxville (2013 and 2014), Springfield (2013 and 2014), and Crossville (2014), TN (Appendix A). Traits, including seed yield, seed plant^{-1} [per plant], branches plant^{-1}, capitula plant^{-1}, average seed capitulum^{-1} [per capitulum], maturity, plant height, days to full bloom, seed oil, and fatty acid content were recorded. Parent accessions, F_1's, F_2's, and backcrosses were analyzed separately with ANOVA using SAS 9.3 (Cary, NC) statistical software to determine genetic variance and broad sense heritability estimates, and gene effects. In addition, the aforementioned traits were analyzed for correlations of seed yield with yield component traits as well as seed oil using SAS 9.3. Mean analyses resulted in significant differences among the 14 plant introductions as well as

selected parents and F_1 progeny. Evidence of high-parent heterosis and dominance gene effects suggest that hybrid breeding programs may be most appropriate. Creating inbred lines may prove to be difficult, however, due to the high degree of self-incompatibility.

TABLE OF CONTENTS

LIST OF TABLES

Chapter II:

Chapter III:

Chapter IV:

Appendix:

LIST OF FIGURES

Chapter II:

Chapter III:

Chapter IV:

INTRODUCTION

Niger (*Guizotia abyssinica* (L.f) Cass.), a member of the Asteraceae family, is a minor oilseed crop that is native to Africa from Ethiopia through Malawi (Quinn, 2012). The plant first became known to Europe in 1774 on an expedition led by explorer, James Bruce. Niger was first given the botanical description of Polymnia abyssinica L (Getinet and Sharma, 1996), but was given a taxonomic revision in 1974 by Baagoe, thus resulting in its current classification. Besides G. abyssinica, there exists five other species within the genus, including G. scabra; G. villosa; G. zavattarii; G. arborescens; and G. jacksoni (Pestros et al., 2008). Of all six species, niger is the only one to occur outside of Africa (Payasi et al., 1987).

Originally thought to have been domesticated as far back as 3000 BC in the Ethiopian Highlands, niger (Pestros et al., 2008) is believed to be the product of crossing large sized achenes of G. scabra ssp. and G. schimperi ssp. Niger is still being grown today in India, Pakistan, Nepal, Myanmar, Singapore (Yadav et al., 2012), even through parts of North America including Canada and the US (USDA, 2013).

The objectives of this study are to: (i) evaluate differences among accessions and progeny from selected crosses of niger for seed yield and phenotypic traits, (ii) determine self-incompatibility for selected parents and F1 progeny, (iii) determine differences in seed oil and fatty acid profiles for accessions and progeny from selected crosses, and (iv) determine correlations between seed yield and phenotypic traits as well as fatty acids among accessions and selected crosses of niger.

CHAPTER I
LITERATURE REVIEW

Botanical Description

Niger is a dicotyledonous herbaceous annual. After seed germination and cotyledon emergence, the first true leaves emerge. Older leaves are oppositely arranged with newer leaves in alternate arrangement. Leaf color may vary from light to dark green, as does stem color which varies from dark purple to light green. Leaf dimensions vary with lengths 10-20 cm and widths at 3-5 cm. Leaf margin morphology also vary from serrated to entire depending on accession (Getinet and Sharma, 1996).

Stems are typically 1.5 cm in diameter at the collar and are hollow, making them susceptible to breaking. According to Getinet and Sharma (1996) the number of primary branches varied from plant to plant but usually averages five to twelve. Adda et al. (1994) states total branches range from 8.5-19.3. Likewise, variation is evident in plant height. One study found height ranging between 100-197 (Sharma et al., 1994), while other sources report height ranging from 50-150 cm (Sarin et al., 2009) and 155-170 cm (Adda et al., 1994). As expected, environmental conditions, pests, and stresses play a significant role in determining an individual plant's overall performance (Getinet and Sharma, 1996).

Niger flowers are yellow and in rare cases, light yellow with heads (capitula) 15-50 mm (millimeter) in diameter. Each head, or capitulum, is hermaphroditic; containing six to eight ray florets and forty to sixty disk florets, each 5-20 mm in length (Getinet and Sharma, 1996).

The forty or so achenes, or dried fruit, are produced per capitula are black on the exterior and each contain a white embryo (Seegeler, 1983).

Uses

In Ethiopia, niger seed accounts for 50-60% of total vegetable oil production (FAO Corporate Document Repository, 2005). In India, niger accounts for 3% of the country's edible oil requirement (Riley and Belayneh, 1989). Traditionally, the oil is used for cooking whereas pressed seeds are used to make cakes for human and livestock consumption. Ethiopian methods of oil extraction include both traditional methods of warming, grinding, and mixing with hot water and centrifugation (Getinet and Sharma, 1996) as well as the utilization of mills located in major cities (Ramadan and Morsel, 2002). In Indian cities, oil is extracted from the seed using mechanized expellers and hydraulic presses. Dehulling niger seed results in higher oil and protein content and a reduction in crude fiber (Ramadan, 2012).

Niger's high Vitamin K and high linoleic and oleic fatty acid content make it not only nutritious for humans, but animals as well. In the United States, niger seed is primarily marketed as a birdseed for American goldfinch and other songbirds including house finches, pine siskins, and redpolls (Wild Bird Direct, 2011), and in the pre-flowering stage the entire plant can be used as livestock feed, green manure, as well as an attractant for bees and other pollinating insects (Ramadan, 2012).

Niger's high saponification value indicates that the oil contains acceptable levels of triglycerides and is therefore useful in the production of liquid soap and shampoo (Yadav et. al. 2012). While high linoleic acid content from Ethiopian accessions give rise to superior quality paints, other industrial purposes list niger as a constituent for perfumes (Pestros et al. 2008), cosmetics, lamp oil, and lubrication, (Hosalli, 2005) as well as a potential source of biodiesel.

In 2007, an amended version of the Clean Energy Act was passed in response to President Bush's "Twenty in Ten" challenge to reduce gasoline consumption by 20% in ten years. Known as the Energy Independence and Security Act, this bill requires an increase biofuel production from 4.7 billion US gallons in 2007 to 36 billion US gallons by 2022. Further specifications state that 21 billion gallons of the total amount of biofuel must be derived from non-corn sources (Energy Independence and Security Act, 2007). Attention has therefore been focused on production of high yielding oilseed crops including soybean, oil palm, coconut, rapeseed, canola, peanut, and sunflower. A 2012 study was conducted through the National Bureau of Plant Genetic Resources and the Division of Seed Science & Technology of New Delhi, India to test niger oil methyl ester potential as a biofuel. Results of an oil content and fatty acid profile showed that its composition is comparable to that of sunflower, safflower, and linseed. After a further investigation was conducted using Saponification value (SV), Iodine value (IV) and Cetane number (CN) as predictors of biofuel potential, it was found that niger produces a high quality fuel (Yadav et al. 2012).

Production

The main producers of niger seed are India, Ethiopia, Myanmar, and Singapore (Hosalli, 2005) though South Africa, East Africa, the West Indies, and Zimbabwe are also listed as top cultivators (Kumar, 2008). Productivity changes by location. As of 2003, Ethiopia produced between 200,000 to 250,000 tons annually. India is the next largest producer at 80,000 to 100,000 tons (Hosalli, 2005). By 2007, India as a whole was producing 281 kg ha^{-1} annually. Karnataka, India reportedly produced a large percentage of this output with yields at 182 kg ha^{-1} (Kumar, 2008), whereas the Himalayas produced yield of 1000 kg ha^{-1}.

This low production in these countries is due to the farming methods. Due to scarce resources, niger is grown under rain fed situations on infertile soils during the Asian monsoon season, kharif. Higher yields can be expected with improved production technology and breeding efforts.

In the United States, research and production efforts have been limited. Niger was grown in the late 1970s under the direction of R. Robinson from the University of Minnesota to evaluate its potential. Robinson concluded that in order to have better seed set, niger should be grown in the southern and central states (Weiss, 2000). In 1999, a trial for agronomic potential was conducted in Indiana via the Jefferson Institute. Two Ethiopian seed types were obtained through the Teff Seed Company and grown in 1999 and 2000. Low yields were reported in 1999 due to drought and other environmental factors. In 2000, management practices were altered, resulting in successful flowering and seed set (Quinn and Myers, 2002). Today, niger is grown in Ontario, Canada, California, Kansas, Maryland, Tennessee, Virginia, and most northeast states (USDA, 2013).

The demand for niger grown in the US is expected to increase due to its high cost at $1.10/1000 g in comparison to sunflower's cost at $0.26/ 1000 g and the bird food industry's annual growth rate at 5-10% annually (Quinn and Myers, 2002).

Chemical Composition

The total oil content found in niger seed has been found to be highly variable over the years. A study by Ramadan and Mörsel (2003a, 2003b) showed that dry niger seed contained 27-47% oil and with a mean of 35%. A study by Bhatnagar and Gopala Krishna revealed the cold

pressed seeds resulted in 28.3% oil, whereas hexane and ethanol extractions resulted in 38.3% and 29.7 % oil (2014). In studies comparing the variability of 35 accessions for oil quality, the total oil content ranged 35-40% with a mean of 38% (Yadav, 2012; Yadav et al., 2012).

Ramadan and Mörsel reported that total oil content consisted of 75-80% fatty acids, 97% of which was composed of linoleic, oleic, stearic, and palmitic acids. Linoleic fatty acid, an essential polyunsaturate, was found to constitute 70% of total fatty acid content. The ratio of saturated to unsaturated fatty acids was reportedly 25:75. They also reported the presence of neutral lipids, polar lipids, and sterols. Whereas neutral lipids account for 93-97% of the total lipids, glycolipids and phospholipids, which are both polar lipids, contain more saturated fat and less unsaturated fat than neutral lipids. They make up 4.9% and 0.6% total lipid content, respectively. Minor lipid components included tocopherols, a stabilizer of oil during the oxidation process, Vitamin K1, β-carotene, and phenolic compounds (Ramadan, 2012).

Previous studies have analyzed variation in fatty acid profiles. Oleic acid, a monounsaturated fatty acid, ranges 24- 53% with linoleic acid ranging from 32-58%. Palmitic acid, the primary source of saturated fat, ranged 8-9%. Stearic acid, a secondary source of saturated fat, ranged 7-9% (Yadav, 2012; Yadav et al., 2012). Ramadan and Mörsel found the major fatty acids were linoleic (up to 63%), along with oleic (11%), palmitic (17%), and stearic (7%) fatty acids (2003a). Bockisch (1998) stated that niger oil may contain up to 1% arachidic and 3% linolenic fatty acid.

In a study by Geleta, Styme, and Bryngelsson (2011), 153 niger populations were analyzed in order to determine the extent of variation among Ethiopian niger populations as well as the heritability of high oil and oleic acid contents. It was concluded that there is a highly

significant variation in both oil and fatty acid composition in niger populations. Results also showed 30-50% oil content, but only 7% of the population exceeded 50%.

Diets rich in linoleic acid are known to prevent cardiovascular disorders like coronary heart diseases, atherosclerosis, and high blood pressure. Linoleic acid also serves as a structural component of the plasma membrane and a precursor of metabolic regulatory compounds. In addition, high linoleic and oleic acid stabilizes the oil to make niger oil nutritionally valuable (Yadav, 2012).

Planting Date

A study was performed in 2004 in at the Main Agricultural Research Station, University of Agricultural Sciences in Dharwad, India to test the effect of planting date on individual plant yield. The optimal sowing date was found to fall between mid-June and the first week of July. (Kivadasannavar et al. 2007).

A similar study was performed at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad in 2007 thru 2008. Between the two varieties tested, results showed that planting niger in the first fortnight of June both resulted in significantly higher height and yield in comparison to various planting dates through the second fortnight of July (Kumar, 2008).

Priya (2007) concluded that planting niger on 1 July resulted in the highest plant height, number of primary and secondary branches, and leaf area when compared to other sowing dates.

Conditions for Optimal Growth

Environmental factors such as light duration, light intensity, soil type, and temperature have significant impacts on a plant's overall phenotype. Providing favorable growing conditions may ultimately improve total yield and reduce noise.

Getinet and Sharma (1996) reported niger's optimum seeding rates for Ethiopian and Indian accessions were 5-10 kg ha^{-1} and 5-8 kg ha^{-1}, respectively. Bhagwan (2002) recorded optimum row spacing at 15 cm. No dormancy requirements for niger seeds were reported.

A study was conducted in Thief River Falls (2003) by the University of Minnesota to test the effects of seeding rate and nitrogen fertilizer applications on niger stand, bloom, height, yield, and test weight. When seeding rates of 3kg ha^{-1}, 7kg ha^{-1}, and 10kg ha^{-1} were tested using 15.24 cm row widths, results showed that the 7kg treatment caused an earlier bloom. No significant difference in yield was recorded. Nitrogen fertilizer was added at rates of 0, 22.4, 44.8, and 67.3 kg ha^{-1}. The field was tilled in the fall after a previous crop of soybeans were grown. Results showed that adding 67.3 kg of N reduced yield when compared with the 0 kg treatment. The study concluded that niger is a low nitrogen input crop (Mehrkens and Mehrkens, 2004), thereby supporting the conclusions of other studies stating niger's ability to grow in conditions considered too harsh for other crops (Kumar, 2008; Teklewold and Almeayehu, 2002).

An experiment studying the effects of weather conditions on floral phenology showed that a maximum temperature of 24.5°C (degree Celsius) to 25.0°C and a minimum temperature of 12.5°C to 14.0°C resulted in maximum flower opening. Full sun and a higher percentage of

foliage moisture were also connected to maximum flowering (Kumari and Prasad, 2009). Sowing time exploits the full genetic potential of the plant and thereby determines the amount of time available for vegetative growth before anthesis, plant height, number of branches, flowering, and capitula habits (Kumar, 2008).

Flowering

In a study by Nayakar, the number of capitula plant^{-1} ranged between 34 and 170 (1976). Sowing dates between the first fortnight of June and the second fortnight of July resulted in the best results; 37-40 capitula plant^{-1} (Mohan Kumar et al., 2011). They also found that when seed was planted during these times it took between 37 and 67 days for plants to reach 50% flowering. Although little research analyzing the number of days to reach full flowering has been conducted, there have been multiple studies concerning days to maturity. One study from 1994 found that maturity took 90-111 days (Sharma), while another stated maturity occurred between 132 and 168 days (Alemaw and Wold, 1995). Niger is a short day plant; flowers form in conditions where daylight does not exceed 12 hours. Flowering is delayed when daylight exceeds 12 hours (Getinet and Sharma, 1996; Petros et al. 2008). In Ethiopia, flower buds open 2 months after the sowing date. According to Seegeler (1983), niger is affected by dichogamy, or a temporal difference between the maturity of stamens and pistils. This system favors cross-pollination.

Harvesting

Quinn and Myers (2002) attempted to combine (platform head) a crop of niger 133 days after planting. They found that accessions PI509436, PI511305, and W618860 were not dried

well enough and capitula were reportedly passing through the combine without releasing their seed. Also, the plant material was not passing through the combine easily. A second harvesting attempt was made at 145 days. The capitula were sufficiently dry even though the stems were green in color. The harvested plants were stored in ventilation until "cleaning" occurred two weeks later.

Yield and Quality

Yields of niger seed have been reported from 250-1255 kg ha^{-1} (Quinn and Myers, 2002; Ramadan, 2012). Bhardwaj and Gupta found seed yield between 1000-1200 kg ha^{-1} are possible when grown on Himalayan soils (1977). Still, another source states seed yield can average between 130 and 600 kg ha^{-1} (Mohan Kumar et al., 2011). Number of seed per capitulum ranges between 28 and 51 (Adda et al., 1994).

Differences in yield can be attributed to multiple factors. Limiting abiotic factors including irrigation, light duration and intensity, and humidity have been known to affect overall yield. Indeterminate growth habit, low yielding varieties, and pests are also contributing factors. Stalk boring weevil larvae, bumble flower beetle, aphids, and striped cucumber beetle are listed as pests to niger (Quinn and Myers, 2002). Lodging has also been known to affect plants during vegetative growth (Adda et al., 1994).

Self-Incompatibility

Self-incompatibility (SI) in angiosperms is defined as the inability of a fertile hermaphroditic plant containing functional male and female gametes to fertilize after pollen-pistil interactions (Shivanna and Johri 1985, Castric and Vekemans, 2004). SI systems are

typically categorized by either sporophytic SI (SSI) or gametophytic SI (GSI) and are controlled by a single gene in a region of the DNA known as the "S-locus" (Geleta and Bryngelsson, 2010). Self-incompatibility (SI) can be both an advantage and disadvantage to breeders. An advantage of self-incompatibility comes with knowing one genotype can discriminate against self-pollen. If plants from one genotype are grown in isolation from another isolated genotype, and both groups are homozygous, a hybrid will be the result of cross pollination (Hinata et al., 1994). However, if the two genotypes are not homozygous, segregation will occur in the F_1 progeny. Due to these conditions, breeders have contrasting hopes of either breaking self-incompatibility to create pure lines, or reduce self-compatibility to lower the rate of self-contamination.

Over time, many flowering plant species have evolved from being self-incompatible to predominantly self-compatible. These occur by the replacement of self-incompatible alleles at the S locus with self-compatible alleles. It has been proposed that pseudo-self-fertility, or well developed seed with aborted endosperm and embryo, is essential in the transition from self-sterility to self-fertility (Levin, 1996).

In a niger study by Nemomissa (1999), SI was thought to be, in most cases, controlled by a single S-locus. Cytology reports showed evidence that incompatible pollen grains failed to germinate on receptive stigmas, and incompatible pollen grains may germinate but fail to penetrate receptive stigma. This was thought to be due to a homomorphic sporophytic SI I system. Pseudocompatibility is common in niger. Pseudo-seeds are in most cases indistinguishable from viable seed. Self-compatibility was fairly common in parents, but further selfing revealed that the progeny were self-incompatible. It is recommended that intensive

selection procedures be implemented so as to obtain completely self-compatible quality genotypes.

Geleta and Bryngelsson (2010) conducted an experiment to analyze the population genetics of self-incompatibility in niger and develop self-compatible germplasm. Results showed that niger is a predominantly self-incompatible crop of sporophytic nature, though 9 plants out of the 340 tested showed some degree of compatibility. Geleta and Byrngelsson suggest that in breeding programs, a population of interest should be derived from parents containing different S-alleles.

It was also discovered that the mean percentage of overall compatibility within full-sib families, within populations, and between populations was 37, 74, 75%, respectively. There were no significant differences between compatibility within populations and between populations. It was also reported that the phenotype of pollen is determined by dominant (62%) and codominant (38%) allelic interactions. Plants became increasingly self-incompatible in the following generations and therefore may explain niger's low yield (Geleta and Bryngelsson, 2010). Riley and Belayneh (1989) reported that niger has a small degree of self-compatibility though the plants may not necessarily be truly self-compatible.

A study was conducted by Hosalli (2005) to understand the influence of variability among genotype on self-incompatibility. When an analysis of variance was conducted over genotypes during kharif 2004 and summer 2005, results showed significant differences among genotypes for self-fertility. Higher self-fertility was reported when pollinations were manually conducted.

Genetic Variability

Thus far genetic analysis of niger has been limited, especially where molecular markers were used (Petros et al. 2007). Despite this limitation, several studies have found high genotypic and phenotypic variability between different niger genotypes (Channarayappa, 1987; Payasi et al., 1987). In an analysis of 153 niger populations, Geleta (2008) found that within population genetic diversity is higher than among population genetic diversity. It was later found that high variation occurred in oleic acid content between populations (Geleta, 2011). A genetic divergence study of 35 niger accessions found that test weight and total oil content had low variability though high variability was found in fatty acid content (Yadav, 2012).

Despite such genetic variation, no aneuploid accessions have been discovered as of yet. All niger accessions in the diploid state contain a chromosome number of 2n=30 (Dagne and Heneen, 1992; Dagne, 1994; Dagne, 1995; Dagne at al., 2000; Hiremath and Murthy, 1988; Murthy et al., 1993).

Heritability

Heritability of a trait within a population can be simply defined as the importance of genetic and nongenetic factors in observable differences between individuals of a population. Broad sense heritability (H^2) is defined as the ratio of the genotypic variance (σ^2_g) to the phenotypic variance (σ^2_p): $h^2= \sigma^2_g/\sigma^2_p$. This equation can be further broken down, where genotypic variance is the sum of additive (σ^2_A), dominance (σ^2_D) and epistatic (σ^2_I) variances. Phenotypic variance is equal to the sum of environmental variance (σ^2_e), genotypic-environmental interaction (σ^2_{ge}), and genotypic variance. Genotypic-environmental interaction is

caused by the failure of genotypes to perform equally to each other when evaluated across different locations and/or years: $\sigma^2_{ge} = \sigma^2_{gl} + \sigma^2_{gy} + \sigma^2_{gyl}$ (Fehr, 1987).

Narrow sense heritability (h^2) is defined as the ratio of additive genetic variance to the phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_p$. Narrow sense heritability is useful for determining the resemblance of offspring to their parents, and the population's response to selection. The value therefore shows the impact of selection in altering the population (Fehr, 1987).

The equation for computing heritability on a single-plant basis when selection is based on plants of an entire population is:

$h^2 = \sigma^2_g / \sigma^2_w + \sigma^2 + \sigma^2_{ge} + \sigma^2_g$

Where

h^2= heritability

σ^2_g= genetic variance

σ^2_w=variance among plants within a plot

σ^2= variance among blocks

σ^2_{ge}= genotypic-environment interaction

(Fehr, 1987). The equation for computing heritability on a single-plant basis when selection is based on plants within a plot is:

$h^2 = \sigma^2_g / \sigma^2_w + \sigma^2_{ge} + \sigma^2_g$

Where

h^2= heritability

σ^2_g= genetic variance

σ^2_w= variance among plants within a plot

σ^2_{ge}= genotypic-environment interaction

(Fehr, 1987). The equation for computing heritability on a plot basis is:

$h^2 = \sigma^2_g / (\sigma^2_w/n) + \sigma^2 + \sigma^2_{ge} + \sigma^2_g = \sigma^2_g / \sigma^2_e + \sigma^2_{ge} + \sigma^2_g$

Where

h^2= heritability

σ^2_g= genetic variance

σ^2_w= variance among plants within a plot

n=number of plants within a plot or block

σ^2=variance among blocks

σ^2_e=experimental error

σ^2_{ge}= genotypic-environment interaction

(Fehr, 1987).

A study by Yadav (2012) estimates high broad-sense heritability (>87%) for niger test weight, total oil content, palmitic acid, stearic acid, and oleic acid.

Heterosis

The performance of hybrid relative to its parents can be expressed using mid-parent heterosis and high-parent heterosis. Mid-parent heterosis compares the hybrid with the average of its parents whereas high-parent heterosis compares the hybrid with the superior parent in the cross:

Mid-parent heterosis (%) = $(F_1\text{-MP})/MP \times 100$

High-parent heterosis (%) = $(F_1\text{-HP})/HP \times 100$

Where

F_1= performance of hybrid

MP= average performance of parents

HP= performance of best parent

(Fehr, 1987).

Generation Means Analysis

Generation means analysis is a method that estimates mean effects, additive, dominance, additive x additive, additive x dominance, and dominance x dominance gene effects of a trait of interest (Piepho and Möhring, 2010). The statistical model for generation means analysis is:

$\mu_i = m+(a)x_{i1}+(d)x_{i2}+(aa)x^2_{i1}+(dd)x^2_{i2}+(ad)x_{i1}x_{i2}$

Where

m= intercept

(a)= additive effect

(d)= dominance effect

(aa), (dd), (ad)= digenic epistatic interactions

x_{i1}, x_{i2}= corresponding coefficients

Within-plot variances can be analyzed within each generation to find estimations of genetic variance components. Generation means analysis requires six generation components from each parent combination made, thus producing P1, P2, F_1, F_2, P1 F_1 (backcross to P1), and P2 F_1 populations (backcross to P2). Estimates of the gene effects can be obtained by using the following relationships:

$m=F_2$ mean

a=(P1 F_1 mean)-(P2 F_1 mean)

d=-.5(P1 mean)-.5(P2 mean)+(F_1 mean)-4(F_2 mean)+2(P1 F_1 mean)

+2(P2 F_1 mean)

aa=-4(F_2 mean)+2(P1 F_1 mean)+2(P2 F_1 mean)

ad=-.5(P1 mean)+.5(P2 mean)+(P1 F_1 mean)+(P2 F_1 mean)

dd=(P1 mean)+(P2 mean)+2(F_1 mean)+4(F_2 mean)-4 (P1 F_1 mean)

-4(P2 F_1 mean)

Randomized complete block design is used as the experimental design, where each generation is assigned its own plot in the field. Plot size also differs between generations to optimize precision of genetic variance component estimates. P1, P2, and F_1 plots therefore contain less individual plants than P1 $F_1$and P2 F_1 plots. Likewise, the plot size for the F_2 generation is greater than all other plots. Multiple blocks are recommended where each block contains all six generations. (Gamble, 1961a, 1961b; Piepho and Möhring, 2010). Segregating generations (F_2's and backcrosses) are represented by fewer plants than non-segregating generations (parents and F_1's) to compensate for greater error variance (Hallauer and Miranda, 1988).

Generation means analysis has several advantages; one being it is relatively simple and reliable in terms of statistics. Sampling error from the means is smaller than variance error for estimating inheritance, and can be therefore be used in smaller experiments to gain the same level of precision as larger experiments. Other advantages are that estimates of epistasis can be estimated, and experiments are smaller and easier to carry out. Generation means analysis can be applied to both self and cross-pollinated species (Hallauer, 1981). A drawback to generation means analysis, however, is that it is not able to estimate heritability, which is crucial for predicting response to selection. Any interpretation from the analysis is particular to that specific set of parents. Lastly, negative effects of one locus can cancel out positive effects at another locus, thus hiding opposing effects. This may lead to underestimations of true genetic effects (Acquaah, 2007).

Correlation

Plant breeders often use parameters other than yield to identify and select parents and segregating individuals with greater yield potential. Determining correlations between primary and secondary characters is therefore important for making indirect selections. Calculating genetic correlation requires an evaluation of the genetic material across multiple environments. A definitive evaluation of genetic correlation between characters requires the use of random genotypes from segregating populations so as to estimate variance and covariance (Fehr, 1987). Indirect selection is prudent when the secondary character has a higher heritability than the primary character, or is easier to evaluate.

Panda and Sial (2012) studied correlations of yield and yield components. They found that branches and seed yield had a correlation coefficient of 0.69. Capitula plant^{-1} and yield were weakly correlated at 0.06, but seed capitulum^{-1} was strongly negatively correlated to seed yield (-0.77). Plant height and days to 50% flowering were moderately correlated to seed yield (-0.56 and 0.58, respectively).

A previous study by Alemaw and Wold (1995) found negative correlations between oleic and linoleic acid. This can be expected since oleic synthesizes linoleic acid. Likewise, a negative correlation is expected for oleic and stearic acid since oleic acid is synthesized from stearic acid. A study by Geleta, Stymne, and Bryngelsson (2011) found that seed weight and linoleic acid had a negative correlation of -0.17. Seed weight and oleic acid were correlated at 0.13, while seed weight and palmitic acid had a negative correlation of -0.15.

Literature Cited

Acquaah, G. 2007. Principles of plant genetics and breeding. Blackwell Publishing, Malden, MA.

Adda, S., T.P. Reddy, and K. Kishor. 1994. Androclonal variation in niger (*Guizotia abyssinica* Cass). Euphytica 79(1-2):59-64.

Alemaw, G. and A.T. Wold. 1995. An agronomic and seed-quality evaluation of noug (Guizotia abyssinica Cass.) germplasm in Ethiopia. Plant Breeding 114(4):175-176.

Bachlava, E., S.X. Tang, G. Pizzaro, G.F. Schuppert, R.K. Brunick, D. Draeger, A. Leon, V. Hahn, and S.J. Knapp. 2010. Pleiotropy of the branching locus (B) masks linked and unlinked quantitative trait loci affecting seed traits in sunflower. Theoretical and Applied Genetics 120, 829–842.

Bhardwaj, S.P. and R.K. Gupta. 1977. Tilangi, a potential righ yielding oil seed crop. Indian Farming 27(6):18–19.

Bhagwan, S., A.L. Rajput, and S.R. Adhar. 2002. Effect of nitrogen and row spacing on growth, yield, and economics of niger (*Guizotia abyssinica*). Indian J. of Agron 47(4):541-543.

Bhatnagar, A.S., and A.G. Gopala Krishna. 2014. Lipid classes and subclasses of cold-pressed and solvent-extracted oils from commercial Indian niger (Guizotia abyssinica (L.f.) Cass.) seed. J. Am. Oil Chem. Soc. 91:1205-2016.

Bockisch M. 1998. Vegetable fats and oils. In Fats and Oils Handbook, Bockisch M (ed.). AOCS: Champaign, IL; 301-303.

Channarayappa. 1987, Response to selection, correlation and path analysis in niger (Guizotia abyssinica Cass.). Mysore J. of Agric.Sci. 21(1):92-93.

Dagne, K. 1994. Meiosis in interspecific hybrids and genomic interrelationships in *Guizotia* Cass. (Compositae). Hereditas 121:119-129.

Dagne, K. 1995a. Karyotypes, C-banding and nucleolar numbers in *Guizotia* (Compositae). Plant Systematics and Evolution 195:121-135.

Dagne, K., B. Cheng, and W.K. Heneen. 2000. Number and sites of rDNA loci of *Guizotia abyssinica* (L.f) Cass. as determined by fluorescence in situ hybridization. Hereditas 132:63- 65.

Dagne, K. and W.K. Heneen. 1992. The karyotypes and nucleoli of *Guizotia abyssinica* (Compositae). Hereditas 117:73-83.

Dagne, K. and A. Jonsson. 1997. Oil content and fatty acid composition of seeds of *Guizotia* Cass. (Compositae). J. of the Sci. of Food and Agriculture 73:274-278.

Dempewolf, H., N.C. Kane, K.L. Ostevik, M.Geleta, M.S. Barker, Z. Lai, M.L. Stewart, E. Bekele, J.M.M. Engels, Q.C.B. Cronk, and L.H. Rieseberg. 2010. Establishing genomic tools and resources for *Guizotia abyssinica* (L.f.) Cass.- the development of a library of expressed sequence tags, microsatellite loci, and the sequencing of its chloroplast genome. Molecular Ecology Resources 10:1048-1058.

Energy Independence and Security Act of 2007. One Hundred Tenth Congress of the United States of America.

Fehr, W. 1987. Principles of Cultivar Development. Macmillan Publishing Company, New York, NY.

FAO Corporate Document Repository. 2005. Bird food grains with potential for the tropics and semi-tropics. Agriculture and Customer Protection. http://www.fao.org/docrep/008/y5831e/y5831e06.htm (accessed 12 March 2013).

Gamble, E.E. 1961a. Gene Effects in Corn (*Zea mays* L.)-Relative importance of gene effects for plant height and certain component attributes for plant height and certain component attributes of yield. Canadian Journal of Plant Science 42:349-357.

Gamble, E.E. 1961b. Gene Effects in Corn (*Zea mays* L.) Separation and relative importance of gene effects for yield. Canadian Journal of Plant Science 42:339-348.

Geleta, M. and T. Bryngelsson. 2010. Population genetics of self-incompatibility and developing self-compatible genotypes in niger (*Guizotia abyssinica*). Euphytica 176(3):417-430.

Geleta, M., T. Bryngelsson, E. Bekele, and K. Dagne. 2008. Assessment of genetic diversity of *Guizotia abyssinica* (L.f) Cass. (Asteraceae) from Ethiopia using amplified fragment length polymorphism (AFLP). Plant Genetic Resources 6:41-51.

Geleta, M., S. Stymne, and T. Bryngelsson. 2011. Variation and inheritance of oil content and fatty acid composition in niger (*Guizotia abyssinica*). J Food Comp. Anal 24(7): 95-1003.

Getinet, A. and S.M. Sharma. 1996. Niger. *Guizotia abyssinica* (L.f) Cass. Promoting the conservation and use of underutilized and neglected crops. International Genetic Resources Institute. Gatersleben, Germany.

Hallauer, A.R., and J.B. Miranda. 1981. Quantitative genetics in maize breeding. Iowa State University Press, Ames, IA, USA.

Hallauer, A.R. and J.B. Miranda, 1988. Quantitative genetics in maize breeding. Iowa State University Press, Ames, IA, U.S.A.

Hiremath, S.C. and H.N. Murthy. 1988. Domestication of niger (*Guizotia abyssinica*). Euphytica 37:225-228.

Hosalli, S. 2005. Studies on self-fertility and autogamy in niger (*Guizotia abyssinica* Cass.). M.S. thesis, Dharwad University of Agricultural Sciences, Karnataka, India.

Kivadasannavar, P., V.P. Deshpande, and B.S. Vyakarnal. 2007. Effect of sowing time, spacing and fungicidal spray on crop growth and seed yield of niger. Karnataka J. Agric. Sci. 20(4):848-850.

Kumar B.N., M. 2008. Studies on maximizing seed yield and quality in niger (*Guizotia abyssinica* Cass.) M.S. thesis, Dharwad University of Agricultural Sciences, Karnataka, India.

Kumari, V. and R. Prasad. 2009. Weather relations on floral phenology and autogamy in niger [*Guizotia abyssinica* (L.f.) Cass] under north- western Himalayas. Journal of Agrometeorology 11(1):59-63.

Mehrkens, K. and C. Mehrkens. 2004. Niger seeding rates and nitrogen evaluation-Pennington County. University of Minnesota Extension Service. http://www.nwroc.umn.edu/prod/groups/cfans/@pub/@cfans/@nwroc/documents/asset/cfans_asset_343861.pdf (accessed 12 March 2013).

Mohan Kumar, B.N., B.S. Basavegowda, B.S. Vyakaranahal, V.K. Deshpande, and P.V. Kenchanagoudar. 2011. Influence of sowing dates on production of seed yield in niger (Guizotia abyssinica Cass.). Karnataka J. Agric. Sci. 24(3):289 – 293.

Murthy, H.N., S.C. Hiremath, and S.S. Salimath. 1993. Origin, evolution and genome differentiation in *Guizotia abyssinica* and its wild species. Theoretical and Applied Genetics 87:587-592.

Nayakar. 1976. Genetic variability and heritability for six quantitative characters in niger (*Guizotia abyssinica* Cass). Mysore J. Agric. Sci. 10:553-558.

Payasi, S.K., Y. Mishra, G.K. Koutu, S.K. Bilaiya, and L.N. Yadav. 1987. Character association and component analysis in niger. J. of Oilseeds Res. 4(1):70-74.

Petros, Y. 2008. Genetic Diversity and Oil Quality of *Guizotia* Cass. (Asteraceae). Ph.D dissertation. Swedish University of Agricultural Sciences, Alnarp, Sweden.

Petros Y., A.S. Carlsson, S. Stymne, H. Zeleke, A.S. Falt, and A. Merker. 2009. Developing high oleic acid in *Guizotia abyssinica* (L.f.) Cass. by plant breeding. Plant Breeding 128:691–695.

Petros Y., A. Merker, and H. Zeleke. 2007. Analysis of genetic diversity of *Guizotia abyssinica* from Ethiopia using inter-simple sequence repeat markers. Hereditas 144:18–24.

Piepho, H.P. and J. Möhring. 2010. Generation means analysis using mixed models. Crop Sci.50:1674-1680.

Quinn, J. and R.L. Myers. 2002. Nigerseed: Specialty Grain Opportunity for Midwestern US. ASAH Press, Alexandria, VA.

Ramadan, M.F. 2012. Functional properties, nutritional value, and industrial applications of niger oilseeds (*Guizotia abyssinica* Cass.). Critical Rev. in Food Sci.and Nutr. 52(1):1-8.

Ramadan, M.F. and J.T Mörsel. 2002. Proximate neutral lipid composition of niger. Czech J.of Food Sci. 20:98-104.

Ramadan, M.F. and J.T. Mörsel. 2003a. Determination of the lipid classes and fatty acid profile of niger (*Guizotia abyssinica* Cass.) seed oil. Phytochemical Analysis 14:366-370.

Ramadan, M.F. and J.T. Mörsel. 2003b. Phospholipid composition of niger (Guizotia abyssinica Cass.) seed oil. LWT- Food Sci. and Tech. 36(2):273-276.

Riley, K.W. and H. Belayneh. 1989. Niger – In: Oil crops of the world. McGraw-Hill Publishing Company, New York. pp 394-403.

Sarin, R., M. Sharma, and A.A. Khan. 2009. Studies on *Guizotia abyssinica* L. oil: Biodiesel synthesis and process optimization. Bioresource Technology 100(18):4187-4192.

Sharma, S.M., G. Nagaraj and R. Balakrishnan. 1994. Niger genetic resources: Evaluation and analysis. Directorate of Oilseeds Research (ICAR), Rajendranagar, Hyderabad.

Seegeler, C.J.P. 1983. Oil plants in Ethiopia - Their taxonomy and agricultural significance. Centre for Agricultural Publication and Documentation, PUDOC, Wageningen.

Teklewold, A. and N. Alemayehu. 2002. Studies on the floral characteristics of niger seed (*Guizotia abyssinica* (L.f.) Cass.). Journal of Applied Botany 76:163-167.

USDA. 2013. *Guizotia abyssinica* (L. f.) Cass. ramtilla. Plants Profile. http://plants.usda.gov/java/profile?symbol=GUAB (accessed 12 March 2013).

Weiss, E.A. 2000. Oilseed crops (2nd ed.). Blackwell Science, Inc. Malden, MA. p. 259–273.

Wild Bird Direct. 2011. Niger Seed. http://www.wildbirddirect.com/products/niger-seed/ (accessed 12 March 2013).

Yadav, S. 2012. Genetic divergence studies in niger (*Guizotia abyssinica*) germplasm. Biomass and Bioenergy 44:64-69.

Yadav, S., S. Kumar, Z. Hussain, P. Suneja, S.K. Yadav, M.A. Nizar, and M. Dutta. 2012. *Guizotia abyssinica* (L.f.) Cass.: An untapped oilseed resource for the future. Biomass and Bioenergy 43:72-78.

CHAPTER II
COMPARISON OF YIELD, PHENOTYPIC TRAITS, AND SELF-INCOMPATIBILITY WITHIN AND AMONG NIGER POPULATIONS

Abstract

In the United States, niger (*Guizotia abyssinica* (L.f) Cass.) is primarily marketed as a seed of choice for American goldfinch (*Spinus tristis*) as well as other song and ground feeding birds because of its high oil content. In countries such as Ethiopia and India, niger is grown as an edible oilseed crop. The objectives of this study were to determine: (i) the phenotypic trait variation that exists among niger germplasm accessions from the USDA/ARS collection and (ii) determine the degree of self-incompatibility of selected parents and F_1 [filial 1] crosses. The traits analyzed included seed yield plant^{-1} [per plant], seed plant^{-1}, branches plant^{-1}, capitula plant^{-1}, average seed capitulum^{-1} [per capitulum], plant maturity, plant height, lodging, and full bloom. Fourteen niger plant introductions (PIs) of Indian, Ethiopian, and American origin were obtained from USDA/ARS germplasm collection at Pullman, WA. Ten replications of the fourteen PIs were planted under field and greenhouse conditions at the East Tennessee Research & Education Center in Knoxville in August, 2012 using a completely randomized design. After initial assessments were made, five of the PIs were then selected for further evaluation, and crosses were made to produce six F1, eight BC [backcross], and six F_2 [filial 2] populations. Seed were planted at the East Tennessee (2013 and 2014), Highland Rim (2013 and 2014), and Plateau (2014) Research and Education Centers. Results showed significant differences ($P<.05$) among the fourteen PIs for all traits. Results from 2013 and 2014 showed that P2 (parent accession PI 511305) was the highest yielding parent accession. F_1(25) produced greater seed yield (g) (n) and reached greater maturity than other F_1's. F_2(15) and was highest yielding when measured in grams, but F_2(25) was greater than other F_2's when measuring in number of seed.

Correlations between yield traits and capitula (2013) resulted in stronger correlations (0.64-0.83) than other agronomic traits.

Introduction

Niger, along with other members of the Asteraceae family, is influenced by sporophytic self-incompatibility (SI) (Nemomissa et al., 2004). Sporophytic self-incompatibility (SSI) occurs when the outcome of the interaction between the pollen tube and the style is determined by the sporophytic dipoid tissue. Here, pollen grains failed to germinate at receptive stigmas. The pollen-stigma reaction results in callose production (Takayama ans Isogai, 2005). Evidence from cytology reports also show pollen grains germinate but fail to penetrate receptive stigmas (Nemoomissa et al. 1999).

In dicots, SI is mapped to a single genetic locus called the S-locus. The S-locus consists of multiple, tightly linked genes that depend on a kinase–mediated signaling cascade. Thus far, a minimum of ten SI alleles and one self-compatibility allele have been reported on the S-locus. It was also found that approximately two thirds of the allelic interactions between the pistil and stamen were dominant-recessive, while one third was codominant. This along with dominant-recessive allelic interaction makes SSI a complex system (Geleta et al., 2010).

An advantage of self-incompatibility comes with knowing one genotype can discriminate against self-pollen. If plants from one genotype are grown in isolation from another group of genotypes, and both groups are homozygous, a hybrid will be the result of cross-pollination (Hinata et al., 1994). If the two genotypes are not homozygous, segregation will occur in the F1 progeny. Due to these conditions, breeders have contrasting hopes of either breaking self-incompatibility to create pure lines, or reduce self-compatibility to lower the rate of inbreeding.

This is not the only problem that breeders face with SI species. As long as there are many pollinators, seed yield is not a problem, but where there are few pollinators, yield suffers greatly.

It is theorized that this is the problem with niger seed production. For these reasons, it is critical to manage SI in niger in order for crop improvement to occur (Getinet and Sharma, 1996).

Over time, many flowering plant species have evolved from being self-incompatible to predominantly self-compatible. These occur by the replacement of self-incompatible alleles at the S locus with self-compatible alleles. It has been proposed that pseudo-self-fertility, or well developed seed with aborted endosperm and embryo, is an essential step in the transition from self-sterility to self-fertility (Levin, 1996). Since pseudocompatible seed is commonly found in niger, it is likely that breeding efforts could propel its natural evolution towards self-compatibility.

It is clear that more research a need to be conducted that aims at reducing SI in niger. Only then, will it be possible to pursue hybrid cultivar development. The objectives of this study were to determine the variation that exists among the USDA niger germplasm collection, within and among families of selected parents for seed yield, yield components, and phenotypic traits, and to determine self-incompatibility among selected parents and F_1 crosses.

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 of accessions is important if new and improved varieties of niger are to be released in the United States. This is especially important for states like Tennessee that are located in a transition zone between the northern and southern U.S.

When evaluating quantitative traits in an outcrossing species, a representative sample is analyzed from a population. Since not all plants in the sample are genetically identical, inferences should only apply to the population the plants were sampled from. It is also important to note that any selections of genotypes within a population will thereby change the performance of the population (Fehr, 1987). For these reasons, it is the breeder's prerogative to choose an effective population size that shows the same dispersion of allelic frequencies as the original population.

Plant breeders often use parameters other than yield to identify and select parents and segregating individuals with greater yield potential. Determining correlations between primary and secondary characters is therefore important for making indirect selections. Calculating genetic correlation requires an evaluation of the genetic material across multiple environments. A definitive evaluation of genetic correlation between characters requires the use of random genotypes from segregating populations so as to estimate variance and covariance (Fehr, 1987). Indirect selection is prudent when the secondary character has a higher heritability than the primary character.

Panda and Sial (2012) studied correlations of yield and yield components in and found that branches and seed yield had a correlation coefficient of 0.69. Captiula plant^{-1} and yield were weakly correlated at 0.06, but seed capitulum^{-1} was strongly negatively correlated to seed yield (-0.77). Plant height and days to 50% flowering had were moderately correlated to seed yield (-0.56 and 0.58, respectively).

Making selections in the field based on phenotype may expedite future niger breeding programs. Since little research has been conducted on this matter, more research is needed to

compare findings. Correlations between seed yield and yield component traits as well as seed yield, seed oil and fatty acid traits are needed.

Materials and Methods

Comparison of plant introductions

A preliminary field trial was conducted in 2012 with seed obtained from USDA/ARS germplasm collection at Pullman, WA. Fourteen accessions (60 seeds each) were planted in a greenhouse at the University of Tennessee, Knoxville 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 508077, PI 508079, PI 508080), and one accession was from the U.S. (W 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 on 21 August 2012. The soil type at the Knoxville location was classified as an Etowah loam (Fine-loamy, siliceous, semiactive, thermic Typic Paleudults). The field was tilled, but no pesticide was applied prior to planting.

On 26 September 2012, five plants of each accession were selected and transplanted into a greenhouse located at ETREC in order to avoid a killing frost. The selections were based on height, number of branches, and overall plant vigor. At plant maturity, plant height, capitula plant^{-1}, seed capitulum^{-1}, primary branches plant^{-1}, and estimated seed plant^{-1} were recorded. Harvesting occurred between 10 December 2012 and 21 January 2013.

Self-incompatibility

Of the original fourteen niger parent accessions, three (PI 422242, PI 511305 and W6 18860) were selected for use as parents in crosses. Choices of parents were based on plant height, number of branches and plant vigor. Four F_1's (progeny of crosses of selected parents) 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. Self-incompatibility (SI) was calculated as: 100 – [(average number of seed capitulum^{-1}/average number of potential achenes capitulum^{-1}) x 100].

Comparison of selected parents and crosses

Based on evaluations of the fourteen accessions in 2012, five were chosen for use as parents in crosses. Parent accessions, PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, were coded as P1, P2, P3, P4, and P5; respectively. The F_1's and F_2's from the six cross combinations, were also coded. For example, F_1(15) and F_1(51), are reciprocal F_1's from crosses of P1 and P5. In addition, F_2(15), for example, is an F_2 created from crossing different plants of F_1(15). All parents, crosses, reciprocals, and F_2 progeny evaluated in this study are listed in Table 2.1. Choice of parents was based on plant height, number of branches, and plant vigor. The U.S. accession, W6 18860, was used either as a female or male parent in all six cross combinations.

This study was conducted over 2013 and 2014 at the three locations across Tennessee. The first field location was ETREC in Knoxville, TN (35.53°N 83.57°W) (2013 and 2014). The soil at this location was classified as an Etowah loam (Fine-loamy, siliceous, semiactive, thermic Typic Paleudults). The second field location was at the Highland Rim Research & Education Center (HRREC) in Springfield, TN (36.28°N 86.51°W) (2013 and 2014). The soil type at

HRREC is classified as either a Dickson silt loam (2013) (Fine-silty, siliceous, semiactive, thermic Glossic Fragiudults) or a Sango silt loam (2014) (Coarse-silty, siliceous, semiactive, thermic Glossic Fragiudults). The third site was at the Plateau Research & Education Center (PREC; 2014 only) in Crossville, TN (36.01°N 86.13°W) on a soil type classified as Ramsey-Rock outcrop complex (loamy, siliceous, active, mesic Lithic Dystrudepts).

Seed of F_1 and F_2 progeny for each of the six crosses evaluated were produced in a greenhouse at the University of Tennessee, Knoxville. Crosses among the five parents (USDA source) were made from October thru November, 2012. F_1 seed were harvested December thru January, 2013 and a portion was planted in the greenhouse to produce F_2 and backcross (BC) progeny (BC results are presented in Chapter IV, page 103). Pollen was collected and bulked among plants within each cross [e.g. F_1(15)], and the bulked pollen was used to pollinate individual capitula (flowers) on several plants within the cross [e.g. F_1(15), F_1(51), etc.]. The BC seed were produced by crossing each F_1 [F_1(15), F_1(51), F_1(25), and F_1(52)] and its reciprocal to both respective parents [e.g. BC151, BC155, etc.]. Mature F_2 and BC seed were harvested in June, 2013. Several F_1 border plants were used to produce F_2 seed for the 2014 harvest season. In order to produce F_2 progeny for the 2014 season, two or more F_1 plants of the same cross from border plots were covered with an insect-proof mesh netting cage and a colony of honey bees (*Apis mellifera*) was placed inside each cage to allow for cross pollination between plants. Once the seed began to mature, the colonies of bees were removed. BC seed for the 2014 growing season were produced by covering F_1 and parent plants with the netting and allowing bees to cross pollinate.

Seed of parents, F_1, F_2, and BC progeny were planted in a greenhouse at the University of Tennessee on 5 July 2013 and 21 April 2014, for all locations, respectively. Seed were sown in germination trays (50 cells $tray^{-1}$) filled with Faford^{TM} germination mix. Germination occurred 3 to 5 days after planting, and on 22 July 2013 and 19 May 2014, once the seedlings reached approximately 12-18 cm in height, they were transplanted to their respective field locations.

A space planted nursery utilizing a randomized complete block design with replication was used at each location each year. Each location contained four blocks. In 2013, the space planted nurseries at ETREC and HRREC consisted of measured 2 m between rows and 2.5 m within rows. In 2014, the space planted nursery at ETREC consisted of 3 m between rows and 3.5 m within rows. At HRREC and PREC in 2014, the spacing was 4 m between rows and 4 m within rows. Within each block, each parent was replicated 3 times, and F_1's and BC's were replicated five times. Each block contained 25 F_2 plants in sets of five for each cross. Thus at each location there was a total of 12 plants of each parent, 20 F_1 and BC plants per cross, and 100 F_2 plants per cross for a grand total of 940 plants per location.

Prior to transplanting the plants to each field location, 75g of Peter's 20N-20P_2O_5-20K_2O was applied to all plants in the greenhouse on 18 July 2013 and 19 May 2014. Prior to transplanting at ETREC, the site was tilled and sprayed with 1.680 kg a.i. (active ingredient) ha^{-1} of Treflan (Trifluralin 4EC) on 6 July 2013. On 15 August 2013, the field at ETREC was sprayed with Select (Clethodim) at 0.053 kg a.i. ha^{-1}. On 3 July 2014, the site at ETREC was sprayed with .336 kg a.i. ha^{-1} of Basagran (sodium salt of bentazon) and 1.228 kg a.i. ha^{-1} of Roundup Max (Glyphosate). Prior to transplanting at HRREC, sites were plowed and treated

with 1.754 kg a.i. ha^{-1} of Treflan (Trifluralin 4EC) on 24 July 2013 and 19 May 2014. Prior to transplanting, the site at PREC was sprayed with 0.028 kg a.i. ha^{-1} of Sandea on 17 May 2014. On 21 June 2014, the site at PREC was sprayed with .355 kg a.i. ha^{-1} of Basagran (Bentazon) and 1.169 kg a.i. ha^{-1} of Roundup Max (Glyphosate).

Yield was estimated from plants collected in the 2013 growing season at ETREC and HRREC due to seed immaturity at frost and threshing problems. These estimations were determined by multiplying total capitula $plant^{-1}$, average seed $capitulum^{-1}$, plant maturity (%), and 100 seed weight, then dividing by 100. In 2014, actual yield was only recorded at ETREC and PREC due to widespread plant loss to disease at HRREC. A hand thresher, air separator (Seed Tech Systems), and electronic seed counter (Seedburo 801 Count-A-Pak) were used to determine actual yield. Whole plants were placed into the thresher, where seed and chaff were separated from the branches. The seed-chaff material was then separated using sifting pans and a vacuum air separator. Once the seed was thoroughly cleaned, it was counted using the electronic seed counter. Paired observation t-tests were run on 10 parents, 10 F_1s, 10 F_2s and 10 backcrosses in both 2013 and 2014 to determine whether the aforementioned estimations were statistically different from actual seed yield. Simple linear regression (SAS 9.3) was also run on the same 80 samples to test the associations between estimated and actual seed yield.

Full bloom (days after planting) was recorded at ETREC in 2013, and at ETREC, HRREC, and PREC in 2014. Capitula and average seed $capitulum^{-1}$ data were recorded in 2013 only at ETREC and HRREC. All other traits were recorded in 2013 at ETREC and HRREC, and in 2014 at ETREC, HRREC, and PREC.

Statistical Analyses

Data were collected on: total seed yield plant^{-1} (g) (2013 and 2014), total seed plant^{-1} (n) (2013 and 2014), total branches plant^{-1} (2013 and 2014), total capitula plant^{-1} (2013), average seed plant^{-1} (2013), maturity (%)(2013), height (cm) (2013 and 2014), lodging (2013 and 2014), and days to full bloom (days after planting) (2013 and 2014). Seed yield, total seed, branches, flowers, average seed capitulum^{-1}, maturity, height, and lodging were collected from ETREC and HRREC in 2013, whereas full bloom was recorded at ETREC only. In 2014, branches, average height, lodging, and full bloom were collected from ETREC, HRREC, and PREC, whereas seed yield and total seed number data were only collected from ETREC and PREC.

Mixed model analysis of variance and Fisher's LSD ($P<0.05$), SAS 9.3 (Cary, NC) were used to evaluate selected parents, F_1, and F_2 progeny across all years and locations. Parents and F_1 data were analyzed together, while F_2 data were analyzed separately. For traits analyzed across multiple years and locations, plant material (parents and F_1; F_2), plant material x year interaction, and plant material x location interaction were included as fixed effects. If significant plant material x year interaction was found, years were analyzed separately. Likewise, if significant plant material x location interaction was found, locations were analyzed separately. For traits analyzed by individual years and multiple locations, plant material, and plant material x location interaction were included as fixed effects. In all analyzes, year, location, block and plant material x block interaction were included as random effects. If an analysis showed insignificant plant material differences, years/and or locations were pooled.

Correlations

Correlation analyses were run on five parents (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860,) six F_1 progeny [(F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45)], and six F_2's [F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45)] in 2013 and 2014. This study analyzed correlations between yield traits (seed yield plant^{-1} (g) and seed number plant^{-1} (n)) and agronomic traits (capitula plant^{-1} (n), plant maturity (%), plant height (cm), average seed capitulum $^{-1}$ (n), branches plant^{-1} (n), and full bloom (days after planting)). Correlations between traits were analyzed using SAS 9.3 (Cary, NC) software.

Results

Comparison of plant introductions

Parent height, number of seeds capitulum^{-1}, number of primary branches plant^{-1}, estimated seed plant^{-1}, and capitula plant^{-1} were significantly different ($P<0.05$) among niger accessions (Table 2.2). PI 508079 had a significantly greater number of capitula (678), seed capitulum^{-1} (24), and estimated seed plant^{-1} (16417) than other accessions. Ranges of traits for all other accessions were 354–654 capitula, 4-21 seed capitula^{-1}, and 1,800-12,486 seed plant^{-1} (Table 2.2). The accessions of Ethiopian origin performed better as a whole for every trait than the three accessions from India and the single accession from the United States. W6 18860 from the United States was the shortest in height, while 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 estimated seed plant^{-1} (1,755-17,102), capitula plant^{-1} 354-678), and average number of seed capitulum^{-1} (4-24). Adda et al. (1994) reported slightly higher results that seed capitulum^{-1} ranged between 28 and 51.

Self-incompatibility

Data from 2013 revealed that the self-incompatibility 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 2.3). The three parents in this study, PI 422242, PI 511305, and W6 18860 resulted in 95.4%, 95.0%, and 91.1% 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%) very similar SI values (Table 2.3).

Data from 2014 showed that SI ranged from 93.6% (PI 422242) to 100.00% (W6 18860) (Table 2.3). PI 422242, PI 511305, and W6 18860 resulted in 93.6%, 99.9%, and 100.0% SI, respectively. Furthermore the F_1's tested exhibited a high level of SI (96.8-99.6%). 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 self-compatible. A previous study by Geleta and Bryngelsson (2010) found that niger is a strictly self-incompatible crop of sporophytic nature, though 9 plants out of the 340 tested showed some degree of compatibility.

Comparison of selected parents and crosses

Roughly 85% of the plants at the HRREC location were affected by Southern Blight (*Sceroltium rolfsii*) in 2014. This soil-borne disease first infects the lower stems, then caused dieback and death. The blight was most active in the middle of the season (July and August), causing the capitula to senesce before they had time to pollinate and produce seed. Total seed

yield (g) and total seed number were thereby affected, and the entire location was not included for these traits.

Seed number per plant is an indirect reflection of seed mass yield. Paired observation t-tests from 2013 and 2014 show that estimated seed number and actual seed number were not significantly different ($P<0.05$) when averaged across ten plants each of parents, F_1, F_2, and backcross progeny (Table 2.4). The number of seed for parents and F_2's were overestimated by 12% and 35%, respectively; whereas F1's were underestimated by 15%. The BC's were overestimated by 30% in 2013, and underestimated by 37% in 2014. Overall, seed number was overestimated by 21%. Simple linear regression between estimated seed yield and actual seed yield showed a slope of .90 ($P<0.05$) at ETREC in 2013 (Fig. 2.1A) and 0.59 ($P<0.05$) at PREC in 2014 (Fig. 2.1B). Although generally higher, the estimated seed numbers were reasonable predictions of actual seed numbers, but year/location and type of progeny affected the accuracy.

Each year at each location several plants did not survive after transplanting to the field. Many others did not survive the growing season due to damage by wildlife, weed pressure, and disease of individual plants. In 2014 at HRREC, except for measurements on plant height, branches per plant, and full bloom the experiment was lost due to a widespread infestation of Southern Blight that occurred near the onset of flowering.

Seed Yield Plant^{-1} (g)

Since seed yields could not be obtained from HRREC in 2014, and because PREC was not included in 2013, data from each year was analyzed across only two locations each year. In both years, the plant materials (parents and F_1) x location interaction was significant ($P<0.05$); therefore the data are presented by location in 2013 and 2014 (Fig. 2.2A and B). In general, the

yields of F1's tended to be higher than that of the parents, except for P2 (PI 511305) in 2013 and P1 (PI 422242) in 2014 (Fig. 2.2A and B). The average yields of the parents at ETREC in 2013 and 2014 were 7.0 and 3.1 g plant^{-1} respectively; whereas the yields of the F1's at ETREC for the same years were 17.6 and 7.2 g plant^{-1} respectively (Fig. 2.2A and B). The average yields of the parents compared to the F1's at HRREC in 2013 were 4.3 and 10.2 g plant^{-1} respectively (Fig. 2.2A). The yield values for the same comparisons at PREC in 2014 were 3.7 and 11.2 g plant^{-1} respectively (Fig. 2.2B). Yield at ETREC tended to be greater than HRREC and PREC. Previous studies of seed yield have reported 250-1255 kg ha^{-1} (Quinn and Myers, 2002; Ramadan, 2012). Bhardwaj and Gupta found seed yield between 1000-1200 kg ha^{-1} are possible when grown on Himalayan soils (1977). Still, another source states seed yield can average between 130 and 600 kg ha^{-1} (Mohan Kumar et al., 2011).

F_1(15) and F_1(51) showed high-parent heterosis across all years and locations. F_1(25) showed high parent heterosis in 2013 at both locations (Fig. 2.2A). There was evidence for reciprocal effects on seed yield. In the cross of P1 (P1 422242) x P5 (W6 18860), using P5 as the female parent [F_1(51)] gave better yields (Fig. 2.2A and B); whereas for the cross of P2 (PI 511305) x P5 in 2013, using P5 as the male parent gave better results (Fig. 2.2A).

Because of location inconsistency, the F_2 seed yield data for the six crosses were analyzed separately by year. In 2013, F_2 results showed significant cross effects but cross x location interaction was not significant ($P<0.05$); therefore the data were combined across locations (Fig. 2.3A). F_2(35) and F_2(45) had the lowest yields and distributions in 2013. The other crosses (including reciprocal) had similar means and distributions. In 2014, F_2 results revealed that cross and cross x location interaction were significant (Fig. 2.3B). ETREC resulted

in greater yield and distributions of progeny than PREC. Average seed yield across all F2 populations was slightly greater in 2013 (8.6g) than 2014 (6.7g) (Fig. 2.3A and B). From these results, it appears that the cross of parent 1 (PI 422242) and parent 5 (W6 18860) had the highest yield and produced the greatest diversity from which selection for yield could be made.

Seed Plant^{-1} (n)

Since seed plant^{-1} data were not recorded at HRREC in 2014, and because PREC was not included in 2013, data were analyzed separately by year. Results from 2013 showed that plant material (parents and F_1) x location interactions were not significant ($P<0.05$), therefore data from ETREC and HRREC were pooled together (Fig. 2.4A). In 2014, the plant materials x location interaction was significant ($P<0.05$); therefore the data are presented by location (Fig. 2.4B). In general, seed plant^{-1} was greater in 2013 (6827 seeds) than in 2014 (2092 seed) (Fig. 2.4A and B), and F_1's tended to have greater number of seed plant^{-1} than that of the parents, except for P2 in 2013 (Fig. 2.4A). In 2014, ETREC showed that F_1's produced 2,776 seeds while parents only produced 1,494. At PREC, F_1's produced 1,948 seed while parents produced 1,227. In 2013, the average across all F_1 crosses was greater than the average across all parents (5,125 and 3,069 seeds plant^{-1} respectively) (Fig. 4A). ETREC resulted in a greater number of seed plant^{-1} than PREC in 2014 (Fig. 2.4B).

The F_2 progeny data for seed plant^{-1} were analyzed separately by year because of locations being different each year. In 2013, F_2 results showed that cross x location interactions were insignificant ($P<0.05$), therefore data from ETREC and HRREC were pooled (Fig. 2.5A). In 2014, F_2's resulted in significant cross effects and cross x location interaction (Fig. 2.5B). In general, F_2 progeny at ETREC performed better than at PREC; having an average of 4,711 and

1,410 seeds, respectively. The mean number of seed plant^{-1} in 2013 (averaged across ETREC and HRREC) and 2014 (averaged across ETREC and PREC) were 9,398 and 2,140, respectively (Fig. 2.5A and B). In 2013, F_2(25) had the greatest amount of variation, whereas all other were similar (Fig. 2.5A). In 2014, E-F_2(15) and E-F_2(51) had the greatest amount of variation (Fig. 2.5B). Reciprocal distribution effects were observed between F_2(15) and F_2(51) in 2014 (Fig. 2.5B). All other F2 populations were similar in both years.

Total Branches Plant^{-1} (n)

Since plant material (parents and F_1) x year interactions were significant ($P<0.05$), 2013 and 2014 were analyzed separately. In both years, plant material x location interaction was significant; therefore data were presented by location separately. ETREC resulted in greater number of branches plant^{-1} in 2013 (27 branches) and HRREC resulted in a greater number of branches in 2014 (27 branches) (Fig. 2.6A and B). PREC resulted in higher number of branches plant^{-1} than ETREC (24 vs. 21 branches) in 2014 (Fig. 2.6B). Parents averaged 25 branches in each year at ETREC, and 15 branches in each year HRREC (Fig. 2.6A and B). At ETREC, F_1's averaged 28 branches in 2013 and 19 branches in 2014. At HRREC, F_1's averaged 20 branches in 2013 and 27 in 2014 (Fig. 2.6A and B). The average branches of the parents compared to the F_1's were 20 and 29 (Fig. 2.6A). In 2014, the same comparison resulted in 25 and 23 branches, respectively (Fig. 2.6B). The cross F_1(45) produced more branches than the two parents in 2013 but not in 2014 (Fig. 2.6A). Getinet and Sharma claim the number of primary branches varied from plant to plant but usually averages 5 to 12 (1996). Adda et al. (1994) reported that another source states total branches range from 8.5-19.3.

The F_2 population results from 2013 showed that neither cross effects nor cross x location were significant. After pooling, cross was still insignificant. The F_2(25) and reciprocal F_2(52) tended to produce the least number of branches compared to the other crosses (23 vs. 22 branches) (Fig. 2.7). Populations F_2(15) and F_2(35) exhibited the largest distributions for branches plant^{-1}. The mean number of branches among F_2's was 26, and no reciprocal effects were evident (Fig. 2.7).

Total Capitula Plant^{-1} (n)

Data for total number of capitula plant^{-1} were only collected in 2013 (ETREC and HRREC) for seed yield estimation purposes. Results showed significant differences for plant material (parents and F_1) x location interaction ($P<0.05$, Fig. 2.8). Both the parents and the F_1 progeny produced more capitula plant^{-1} at ETREC consistently produced more capitula plant^{-1} than at HRREC. The mean capitula for parents and F1's at ETREC were 392 and 650, respectively; whereas HRREC resulted in 204 and 371, respectively. F_1(25), F_1(35), and F_1(45) produced more capitula plant^{-1} than their respective parents at ETREC (673, 533, and 529 capitula, respectively) (Fig. 2.8). These numbers are considerably higher than some reports in the literature. In a study by Nayakar (1976), the number of capitula plant^{-1} ranged between 34 and 170. Similarly, Mohan Kumar et al. (2011) reported that capitula plant^{-1} range from 37-40 capitula plant^{-1}.

Average Seed Capitulum^{-1} (n)

Data for number of seeds capitulum^{-1} were only collected in 2013 for seed yield estimation purposes. The plant material (parents and F_1) x location interaction was significant ($P<0.05$), therefore data are presented for each location. On average, parents and F_1's at ETREC

produced more seed capitulum^{-1} than at HRREC with the exception of F_1(15) (Fig. 2.9). The mean seed capitulum^{-1} of parents and F1's at ETREC were 14 and 17, respectively. The mean seed capitulum^{-1} of parents and F1's at HRREC were 11 and 16, respectively. A study by Adda et al. (2011) showed the number of seed capitulum^{-1} ranging between 28 and 51. A reciprocal effect was observed for F_1(15) vs. F_1(51) at HRREC only. When P1 (PI 422242) was used as the female and P5 (W6 18860) was used as the male parent, seed capitulum^{-1} was greater than the reciprocal arrangement (Fig. 2.9)

Plant maturity (%)

Plant maturity (%) data were only collected in 2013 (for seed yield estimation purposes. Due to late transplanting (early July) not all plants of parents and F_1's were mature prior to a killing frost. Thus individual plants were rated for full maturity just prior to a killing frost at each location, and harvest followed within a week. Maturity was determined as the average percent of the plants within each parent line or cross that were fully mature (ready for harvest) at the time of rating. Except for F_1's (35) and (45), approximately 80+% of the F_1's were mature at harvest at ETREC and HRREC (Fig. 2.10). The parents exhibited greater variability. Whereas parents P1, P2, and P5 were about 80% mature at both locations at harvest time, P3 was only 22% and P4 was 34% prior to killing frosts. The influence of the lateness of P3 and P4 was evident in the crosses involving them [F_1(35) and F_1(45)]. Plant material x location interaction was significant ($P<0.05$) therefore data are presented by location. The average maturity of parents and F_1's at ETREC was 67 and 79%. Parents and F1's were 56% and 80% mature at HRREC. A higher percentage of plants reached maturity by harvest at HRREC than at ETREC (Fig. 2.10).

The F_2 population results showed that cross x location interaction was significant ($P<0.05$) thus data are presented by location (Fig. 2.11). The overall F_2 mean across locations was 77% mature. E-F_2(35) and E-F_2(45) had significantly later maturity than other F_2 populations and had a greater distribution than later maturing plants.

Plant height (cm)

Since plant material x location interaction was significant in 2013 but not in 2014 ($P<0.05$); data are presented by location in 2013 and averaged across location in 2014 (Fig. 2.12A and B). In 2013, the average heights of parents and F_1's at ETREC were 69 and 70cm, whereas at HRREC, they measured 42 and 41cm, respectively. In 2014, heights were even lower at 38 and 37cm, respectively. No reciprocal effects or hybrid vigor were evident in either year, nor did the plant heights of the parents or F_1's collectively clearly differ either year. In 2013, plant materials at ETREC were taller than populations grown at HRREC. The plant heights at HRREC in 2013 (42 cm), closely parallel the averages across the three locations in 2014 (37 cm) (Fig. 2.12A and B). Results for 2014 data showed that P5 (W6 18860) was significantly shorter than other parents.

Since cross x year interaction was significant ($P<0.05$), F_2's were analyzed separately by year. In 2013 and 2014, F_2 data were pooled by location due to there being no significant cross x location interaction (Fig. 2.13A and B). The overall mean for 2013 was greater (66 cm) than the overall mean from 2014 (36 cm). The F_2(15) population and its reciprocal F_2(51) tended to have taller plants than the other F2 populations in both years (Fig. 2.13).

Plant lodging score (1-5)

Analyses for plant lodging showed no significance for parents, F_1's, or F_2's, or plant material x location interaction ($P<0.05$). Lodging was determined by the position of the plant's growth habit in relation to the ground, and given a score between one and five. A one was recorded if the plant was completely upright, and a five was given if the plant lay prostrate on the ground. Lodging among parents, F_1's, and F_2's averaged between 3.5 and 4.9 (data not shown). A study by Adda et al. (19945) states that lodging is common in niger during vegetative growth.

Fullbloom (days after planting)

Full bloom data for P's and F_1's were analyzed together by year because plant material x year interaction was significant ($P<0.05$). In 2013, locations were pooled because there was no significant plant material x location interaction. In 2014, this interaction was significant; therefore data are presented by location. In general, it took less time for plants to reach full bloom in 2013 than in 2014 (Fig. 2.14A and B). In 2013, both parents and F_1's averaged 91 and 88 days to reach full bloom, whereas the same comparison resulted in 134 and 129 days respectively, in 2014 (Fig. 2.14A and B). Less rainfall in 2014 than 2013 could have stressed the plants and lengthened days until full bloom. In 2014, plants grown at ETREC took longer to reach full bloom than those grown at PREC or HRREC (Fig. 2.14B). Likewise, plants grown at PREC took longer to reach full bloom than those from HRREC (Fig. 2.14B). Mohan Kumar found that when seed was planted between June and July, plants took between 37 and 67 days to reach 50% flowering (2011).

F_2's were analyzed separately by year due to the significant cross x year interaction. In 2013, no significant cross x location interactions were found ($P<0.05$); therefore locations were

pooled (Fig. 2.15A). In 2014, significant cross x location interaction was detected ($P<0.05$); therefore data are shown by individual locations (Fig. 2.15B). The overall mean in 2013 was 88 days, but 125 days in 2014; further illustrating the differences in full bloom between years. In general, F_2 populations at ETREC took longer to reach full bloom than those at PREC or HRREC (Fig. 2.15B). This could be the result of greater weed pressure at ETREC than PREC or HRREC. Similarly, plants grown at PREC took longer to reach full bloom than those grown at HRREC. F_2(35) and F_2(45) took the longest amount of time to reach full bloom in 2013 (Fig. 2.15A). In 2014, reciprocal effects are shown (Fig. 2.15B). When P2 was used as the female and P5 was used as the male parent, it took longer to reach full bloom than if P5 was used as the female and P2 was used as the male.

Correlations

Seed yield

Branches plant^{-1}, capitula plant^{-1}, seed capitulum^{-1}, plant height, and maturity were all positively correlated with seed yield plant^{-1}across all plant material (parents, F_1, and F_2 crosses) (0.25, 0.72, 0.57, 0.25, and 0.32 respectively) (Table 2.5). The same were positively correlated among the F_1's and the F_2 populations. Correlations between seed yield and full bloom were negative and significant among the parents and among F_1's but not among the F_2 progeny (-0.33, -0.29 and -0.03, respectively) (Table 2.5). This indicates that plants which reached full bloom earlier, tended to yield less than those that took longer to reach full bloom.. Overall, correlations involving capitula plant^{-1} and seed capitulum^{-1} were stronger than those of other agronomic traits studied. Correlations tended to be stronger when the plant materials were analyzed separately, but trends were similar.

Seed number plant^{-1}

As expected, the correlations between seed number plant^{-1} and the morphological traits mimicked those of seed yield plant^{-1}. Correlations between seed number and branches were weak (0.08-0.31) (Table 2.5). Seed number with capitula plant^{-1}(0.65-0.83) and seed number with seed capitulum^{-1} (0.61-0.69) resulted in moderate to strong correlations. Whereas correlations between full bloom and seed number were moderately negative (-0.31to -0.44), correlations of seed number with height and seed number with maturity were moderately positive (0.37-0.45 and 0.32-0.40, respectively). Overall, correlation between seed number and capitula was stronger than correlations between seed number and the other agronomic traits studied. Similar to correlations involving seed yield, correlations involving seed number were stronger when plant material was analyzed separately.

Conclusion

Self-incompatibility

Overall, the SI was very high in both 2013 and 2014 (Table 2.4). 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-compatability than both parents, which could indicate cytoplasmic effects.

It is recommended for this study to be replicated in a greenhouse setting. Despite using pollination bags to exclude insects from cross-pollinating the capitula, several insects were found feeding through the mesh of the bag, most likely affecting the results. Wind and rain also

affected the outcome of the study by reducing the amount of pollen that was produced. With fewer pollen grains available to hand-pollinate, it is likely that some stigmas received less pollen than others.

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

Comparisons of selected parents.

Results showed that P2 (PI 511305) was the best performing parent for total seed yield (g), seed number, average seed capitulum $^{-1}$, and maturity. P2 lodged more than other parents. P3 (PI 508075) and P4 (PI 508076) resulted in lower seed yield (g) (n); fewer branches, capitula plant^{-1}, seed capitulum $^{-1}$; and required more days than other parents to reach full bloom. P5 (W6 18860) was shorter than other parents.

F_1(25) produced greater seed yield (g) and had a higher percentage of plants reach maturity than other F_1's. F_1(15) was taller and produced the most capitula plant^{-1} and average seed capitulum $^{-1}$, but lodged more than other F_1's. F_1(45) produced the lowest amount of seed and average seed capitulum $^{-1}$, but produced more branches than other F_1's. F_1(35) was most upright of all F_1's. F_1(35) and F_1(45) had the latest maturity and took more time than other F_1's to reach full bloom.

F_2(15) and was highest yielding (g), but F_2(25) was greater than other F_2's when measuring number of seed. F_2(45) was consistently the lowest yielding and had the longest maturity ratings.

2013 appeared to result in greater total seed yield than 2014. Plants also took longer to reach full bloom in 2014 than in 2013. ETREC produced greater yield and taller plants than HRREC and PREC. Interestingly, plants at ETREC lodged with greater frequency and took longer to mature than the other two locations. Plants grown at HRREC reached full bloom in the shortest time.

Reciprocal effects were observed among F_2(15) and its reciprocal F_2(51) in seed number and seed capitulum^{-1}. More seed was observed when P5 (W6 18860) was used as the female, whereas more seed capitulum^{-1} was observed when P5 was used as the male. F2(25) and F2(52) also exhibited reciprocal effects. It took significantly fewer days for plants to reach full bloom when P5 was used as the female and P2(PI 511305) was used as the male, than if P5 was used as the male and P2 used as the female.

Correlations

Overall, seed yield and seed plant^{-1} were comparable in their correlations to agronomic traits, though correlations between agronomic traits components and total seed were slightly greater that of seed plant^{-1}. Of all agronomic traits analyzed, capitula plant^{-1} consistently had the strongest correlations. Selections based on this trait could be decided earlier in the season than agronomic traits like seed capitulum^{-1}, full bloom, and maturity.

Findings from this study differed from that of Panda and Sia (2012). Their results showed stronger correlations for branches plant^{-1} (0.69), days to flowering (0.58), seed capitulum^{-1} (-0.77), and height (-0.56) than the findings of this study. Correlations between yield traits and branches in this study ranged roughly between 0.25 and 0.50. Correlations for yield traits with days to flowering ranged roughly between 0.30 and 0.50, while correlations involving seed

capitulum^{-1} ranged 0.57 and 70. Correlations involving height ranged between 0.20 and 0.50. Correlations of yield traits with capitula plant^{-1} (0.65-0.80) were greater than the aforementioned study (0.06). Other differences between these two studies involve the direction of the correlations. Panda and Sia found that positive correlations between yield and days to flowering while this study resulted in negative correlations. The longer it took for a plant to reach full bloom, the more likely it is that the plant will not reach maturity by the end of the growing season. This will ultimately reduce the number of mature plants, and therefore reduce seed yield. Seed capitulum^{-1} and plant height both showed negative correlations in Panda and Sia's research, but were positive in this study.

Literature Cited

Adda, S., T.P. Reddy, and K. Kishor. 1994. Androclonal variation in niger (*Guizotia abyssinica* Cass). Euphytica 79(1-2):59-64.

Bhardwaj, S.P. and R.K. Gupta. 1977. Tilangi, a potential righ yielding oil seed crop. Indian Farming 27(6):18–19.

Fehr, E.L. and H.J. Jessen. 1987. Principles of cultivar development. Vol. 1. Macmillan Pub. Co., NY.

Geleta, M. and T. Bryngelsson. 2010. Population genetics of self-incompatibility and developing self-compatible genotypes in niger (*Guizotia abyssinica*). Euphytica 176(3):417-430.

Getinet, A. and S.M. Sharma. 1996. Niger (Guizotia abyssinica (L.f.) Cass. Promoting the conservation and use of underutilized and neglected crops. 5th ed. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.

Hosalli, S. 2005. Studies on self-fertility and autogamy in niger (*Guizotia abyssinica* Cass.). M.S. thesis, Dharwad University of Agricultural Sciences, Karnataka, India.

Mohan Kumar, B.N., B.S. Basavegowda, B.S. Vyakaranahal, V.K. Deshpande, and P.V. Kenchanagoudar. 2011. Influence of sowing dates on production of seed yield in niger (Guizotia abyssinica Cass.). Karnataka J. Agric. Sci. 24(3):289 – 293.

Nayakar. 1976. Genetic variability and heritability for six quantitative characters in niger (*Guizotia abyssinica* Cass). Mysore J. Agric. Sci. 10:553-558.

Nemomissa, Sileshi, Endashaw Bekele, and Kifle Dagne. Self-incompatibility system in the Ethiopian populations of Guizotia abyssinica (LF) Cass.(niger). *Sinet: Ethiopian J. of Sci.* 22.1 (2004): 67-88.

Panda, S. and P. Sial. 2012. Assessment of existing genetic variability and yield component analysis in niger (Guizotia Abyssinica Cass). Indian J. Innovations Dev., Vol. 1(7):511-514.

Quinn, J. and R.L. Myers. 2002. Nigerseed: Specialty Grain Opportunity for Midwestern US. ASAH Press, Alexandria, VA.

Ramadan, M.F. 2012. Functional properties, nutritional value, and industrial applications of niger oilseeds (*Guizotia abyssinica* Cass.). Critical Rev. in Food Sci.and Nutr. 52(1):1-8.

Riley, K.W. and H. Belayneh. 1989. Niger – In: Oil crops of the world. McGraw-Hill Publishing Company, New York. pp 394-403.

Sarin, R., M. Sharma, and A.A. Khan. 2009. Studies on *Guizotia abyssinica* L. oil: Biodiesel synthesis and process optimization. Bioresource Technology 100(18):4187-4192.

Sharma, S.M., G. Nagaraj and R. Balakrishnan. 1994. Niger genetic resources: Evaluation and analysis. Directorate of Oilseeds Research (ICAR), Rajendranagar, Hyderabad.

Takayama, S. and A. Isogai. 2005. Self-incompatibility in plants. Ann. Rev. Plant Biol. 56:467-489.

Yadav, S., S. Kumar, Z. Hussain, P. Suneja, S.K. Yadav, M.A. Nizar, and M. Dutta. 2012. *Guizotia abyssinica* (L.f.) Cass.: An untapped oilseed resource for the future. Biomass and Bioenergy 43:72-78.

Appendix

Tables

Table 2.1. List of niger parents, F_1's, and F_2's with codes† used to designate each in this study.

P Code	F_1 Code	F_2 Code
P1†=PI 422242	F_1(15) †=PI 422242/W6 18860	F_2(15) †=PI 422242/W6 18860 // PI 422242/W618860
P2=PI 511305	F_1(51)=W6 18860/PI 422242	F_2(51)= W6 18860/PI 422242 // W6 18860/PI 422242
P3=PI 508075	F_1(25)=PI 511305/W6 18860	F_2(25)= PI 511305/W6 18860 // PI 511305/W6 18860
P4=PI 508076	F_1(52)=W6 18860/PI 511305	F_2(52)= W6 18860/PI 511305 // W6 18860/PI 511305
P5=W6 18860	F_1(35)=PI 508075/W6 18860	F_2(35)=PI 508075/W6 18860 // PI 508075/W6 18860
	F_1(45)=PI 508078/W6 18860	F_2(45)=PI 508078/W6 18860 // PI 508078/W6 18860

† Example: F_1(15) is a cross between parent 1(PI 422242) and parent 5 (W6 18860). F_1(51) is the reciprocal cross.

Table 2.2. Mean comparisons† (n=5) of phenotypic traits among 14 niger accessions evaluated at the East Tennessee Research and Education Center, Knoxville in 2012.

Accessions	Height	Branches plant $^{-1}$	Capitula plant $^{-1}$	Seed capitulum^{-1}	Est. Total Seed plant^{-1}
	cm	----------	number	----------	
Ethiopia					
PI 508070	125 ab	28 ab	520 a-d	8 efg	4,025 efg
PI 508072	122 ab	26 abc	593 ab	18 abc	10,571 bc
PI 508073	121 ab	26 abc	354 d	5 fg	1,717 g
PI 508074	128 ab	28 ab	597 a	21 ab	12,232 b
PI 508075	111 bc	29 ab	647 a	19 abc	12,147 b
PI 508076	124 ab	24 bc	552 abc	14 cde	8,606 bcd
PI 508077	135 a	30 ab	548 a-d	15 bcd	8,354 b-e
PI 508078	121 a-d	25 a-d	409 a-d	n/a‡	n/a
PI 508079	121 ab	24 bc	678 a	24 a	17,102 a
PI 508080	117 abc	31 a	514 a-d	4 g	1,755 g
India					
PI 422242	81 e	17 d	654 a	11 def	6,331 c-f
PI 509436	97 cde	15 d	387 bcd	9 d-g	4,002 d-g
PI 511305	90 de	17 d	386 cd	10 d-g	3,454 fg
United States					
W6 18860	58 f	21 cd	555 abc	6 fg	3,966 fg

† Means within a column followed by a letter in common are not significantly different based on Fisher's Least Significant Difference ($P< 0.05$)

‡ n/a = not available

Table 2.3. Self-Incompatibility (SI) † among selected niger parents and F_1 progeny evaluated at the East Tennessee Research and Education Center (ETREC), Knoxville in 2013 and 2014.

	2013		2014	
Parent or F_1	**Mean (n=50)**	**Range**	**Mean (n=50)**	**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	n/a
W6 18860/PI 422242	93.2	65.0-100.0	n/a	n/a
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

† Each year, 50 random capitula from each parent or F_1 combination were chosen, and pollinated with pollen from the same source plant. SI= 100- [(Average number of seed capitulum^{-1}/ number of potential achenes capitulum^{-1}) x 100].

‡ n/a = not available in 2014.

Table 2.4. Comparison of average estimated versus actual seed number values for 10 random plants each of niger parents (P), F_1's, F_2's, and backcrosses (BC) at East Tennessee Research and Education Center (ETREC) in 2013 and the Plateau Research and Education Center (PREC) in 2014.

Parent or Progeny	Year	Actual Seed Number	Estimated Seed Number	Difference
P	2013	50,623	53,887	-3,264ns†
	2014	16,388	21,170	-4,782ns
F_1	2013	31,133	28,322	2,811ns
	2014	20,785	15,788	4,997ns
F_2	2013	19,851	23,902	-4,051ns
	2014	21,818	32,216	-10,398ns
BC	2013	10,701	11,026	-3,254ns
	2014	20,957	13,101	7,855ns
All (n=40)	2013	28,077	29,284	-7,758ns
(n=40)	2014	19,987	20,568	-2,328ns

† ns= Not significant based on Paired t-test ($P< 0.05$)

Table 2.5. Correlation coefficients between seed yield and agronomic traits as well as seed number and agronomic traits for parents, F_1's, and F_2's combined across East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013, and ETREC, HRREC, and Plateau Research and Education Center (PREC) in 2014

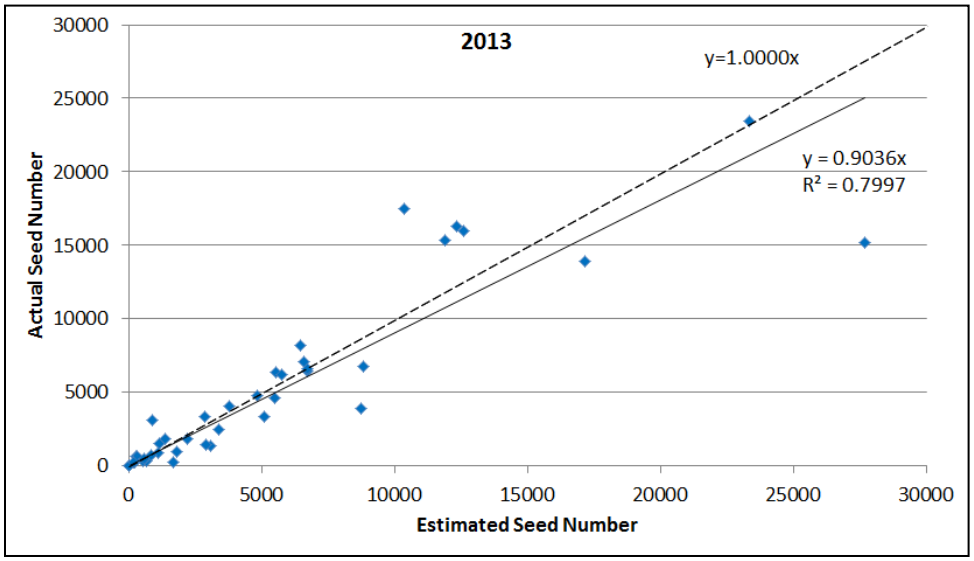
Year	**Plant material**	**Yield Traits**	**Agronomic traits**					
			Branches	Capitula§	Seed Capitulum^{-1}§	Full bloom (DAP)	Height	Maturity§
2013-2014	Parents, F_1's, and F_2's	Yield	0.25**	0.72**	0.57**	-0.13**	0.25**	0.32**
	Parents	Yield	0.14	0.64**	0.70**	-0.33**	0.30**	0.39**
	F_1's	Yield	0.30**	0.74**	0.63**	-0.29**	0.39**	0.33**
	F_2's	Yield	0.27**	0.80**	0.61**	-0.03	0.20**	0.38**
	Parents, F_1's, and F_2's	Seed Number	0.29**	0.79**	0.61**	-0.33**	0.41**	0.34*
	Parents	Seed Number	0.08	0.65**	0.69**	-0.44**	0.37**	0.40**
	F_1's	Seed Number	0.30**	0.73**	0.65**	-0.35**	0.45**	0.32**
	F_2's	Seed Number	0.31**	0.83**	0.61**	-0.31**	0.41**	0.37**

† Significant at P<0.01 and P<0.05 is indicated by ** and *, respectively.

‡ Data from 2013 only.

Figures

A



B

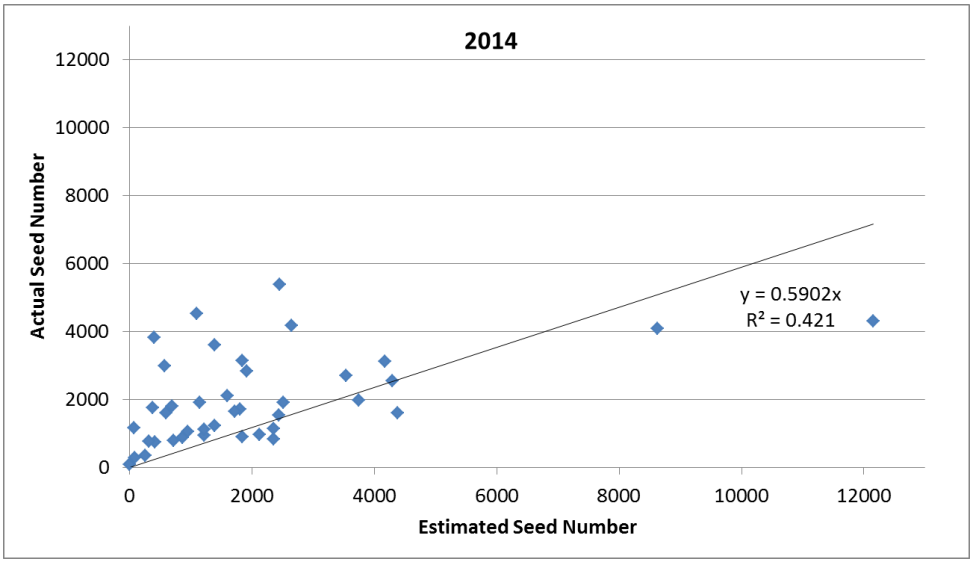
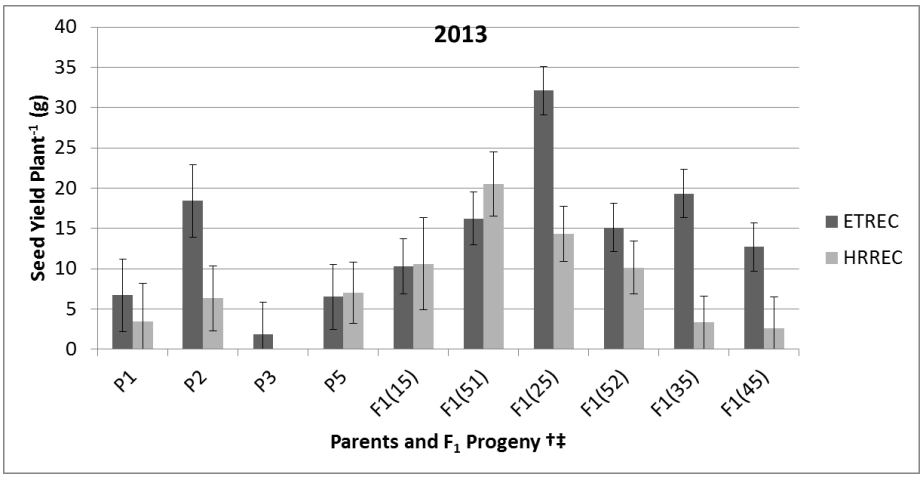


Fig. 2.1. Scatter plot of estimated versus actual seed number of niger plants obtained at the East Tennessee Research and Education Center, Knoxville (2013) (r=0.90; P<0.05) (A) and Plateau Research and Education Center, Crossville, TN (2014) (r=0.49; P<0.05) (B). Seed yield was estimated for ten parents, ten F_1's, ten F_2's, and ten backcrosses, and compared to actual seed yields for each plant. Estimated seed number was calculated by multiplying the total number of capitula x the average number of seeds capitulum^{-1} x the percent of capitula that bore seed. Fig. 2.1A and 2.1B show that the slope is significantly different from 1.

A



B

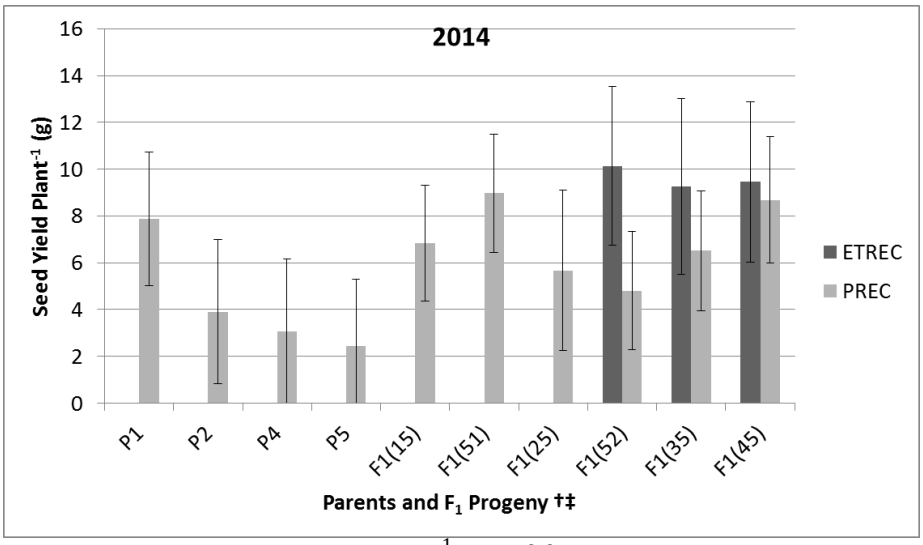


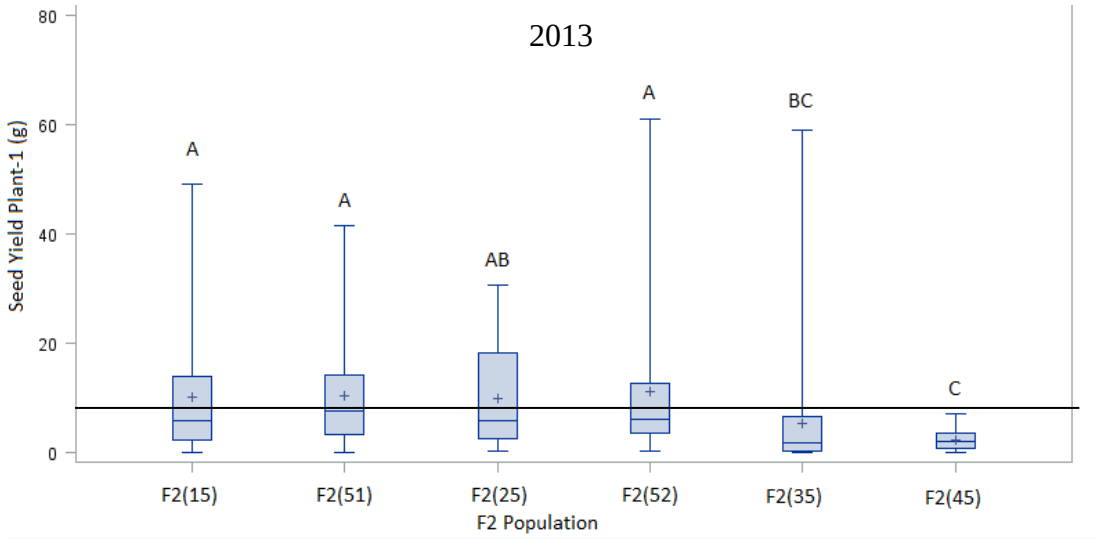
Fig. 2.2. Average seed yield plant^{-1} (g) of five niger parents (P) and six F_1 crosses in 2013 at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers (A), and in 2014 at ETREC and Plateau Research and Education Center (PREC) (B). There was significant plant material x location interaction in both years; therefore the data are shown for each location. P1, P2, P3, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 6, 10, 16, 16, 18, 18, 19, and 19 observations, respectively, at ETREC, and 7, 10, 0, 11, 5, 10, 14, 16, 11, and 7 at HRREC in 2013 (A). P1, P2, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 0, 0, 0, 0, 0, 0, 0, 7, 5, and 6 observations, respectively, at ETREC, and 10, 8, 8, 10, 16, 15, 6, 15, 14, and 12 at PREC in 2013.

† Parents and F_1's differ significantly at $P< 0.05$.

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

§ P4 was missing from (A) and P3 was missing from (B) due to poor germination and disease.

A



B

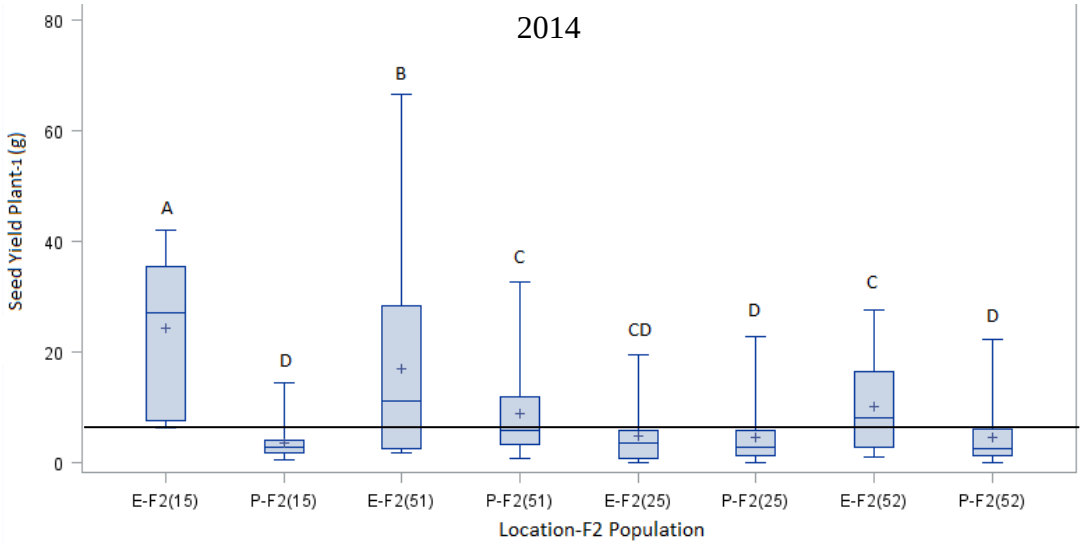
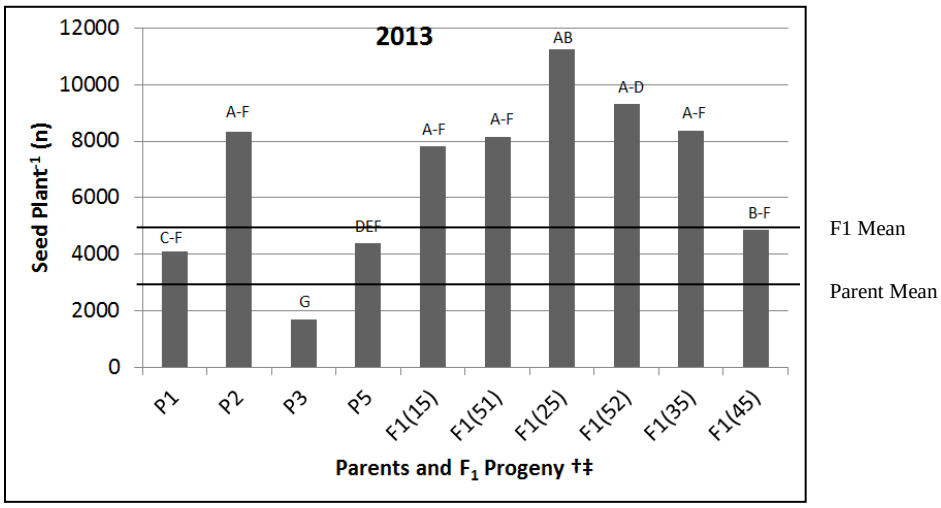


Fig. 2.3. Boxplots of seed yield plant^{-1} (g) for F_2 progeny of six niger crosses pooled across the East Tennessee (E) and Highland Rim Research and Education Centers in 2013 (A), and in 2014 at ETREC and the Plateau (P) Research and Education Center (B). There was a significant plant material x location interaction in 2014; therefore the data are shown for each location. F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 87, 95, 17, 77, 64, and 42 observations, respectively (A). F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 6, 15, 16, and 23 observations, respectively, in 2014 at ETREC, and 24, 24, 72, and 88 at PREC (B).
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_2(15) and F_2(51), for example, are reciprocal crosses. F_2(15) indicates that P1 was the maternal and P5 was the paternal parent.
§ F_2(35) and F_2(45) from PREC were missing in (B) due to poor germination and disease.

A



B

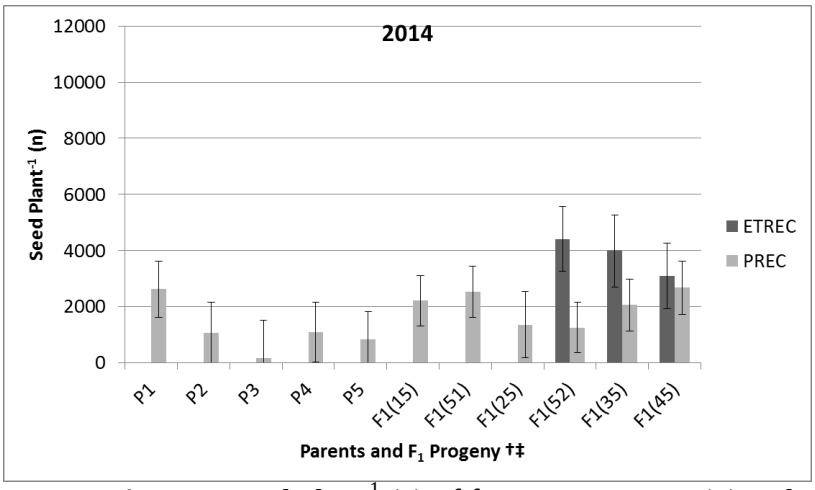


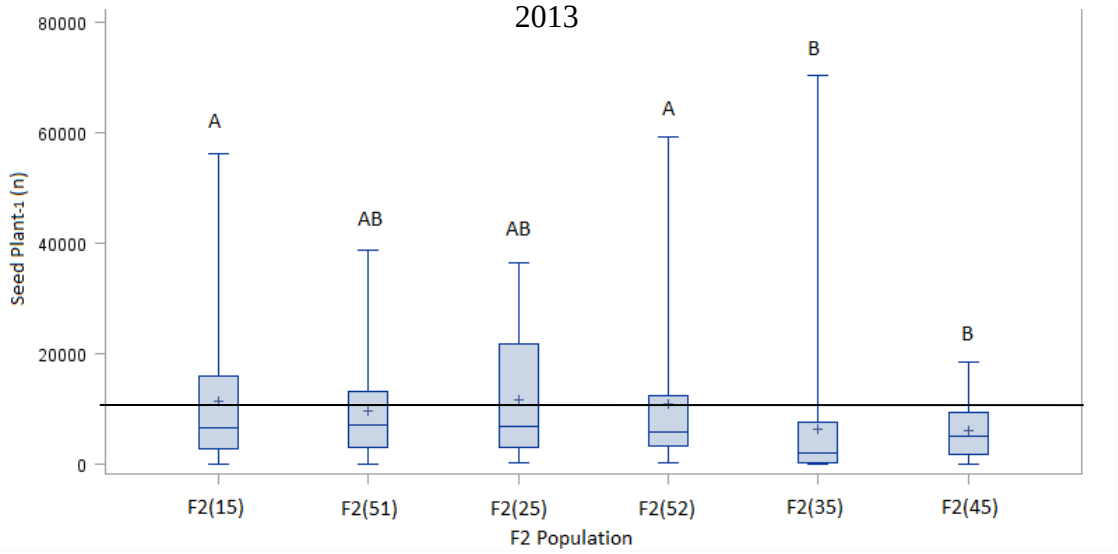
Fig. 2.4. Average seed plant^{-1} (n) of five niger parents (P) and six F_1 crosses at East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013 (A), and in 2014 at ETREC and Plateau Research and Education Center (PREC) (B). There was a significant plant material x location interaction in 2014; therefore the data are shown for each location. P1, P2, P3, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 6, 10, 16, 16, 18, 18, 19, and 19 observations, respectively, at ETREC, and 7, 10, 2, 11, 5, 10, 14, 16, 11, and 7 at HRREC in 2013 (A). P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 0, 0, 0, 0, 0, 0, 0, 0, 7, 5, and 6 observations at ETREC, and 10, 8, 8, 10, 16, 15, 6, 15, 14, and 12 at PREC in 2014 (B).

† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

§ P4 was missing from (A) due to poor germination and disease.

A



B

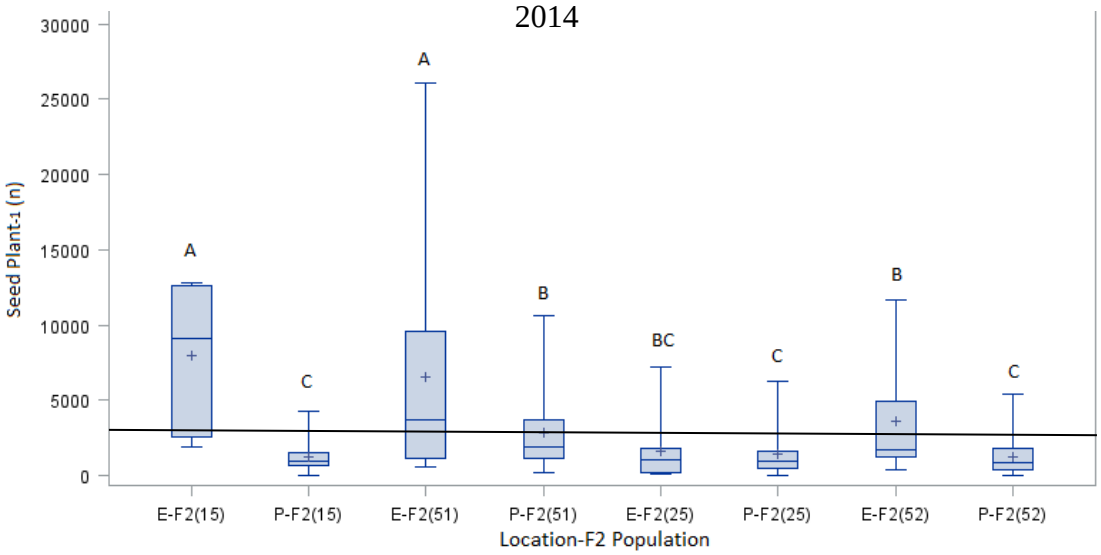
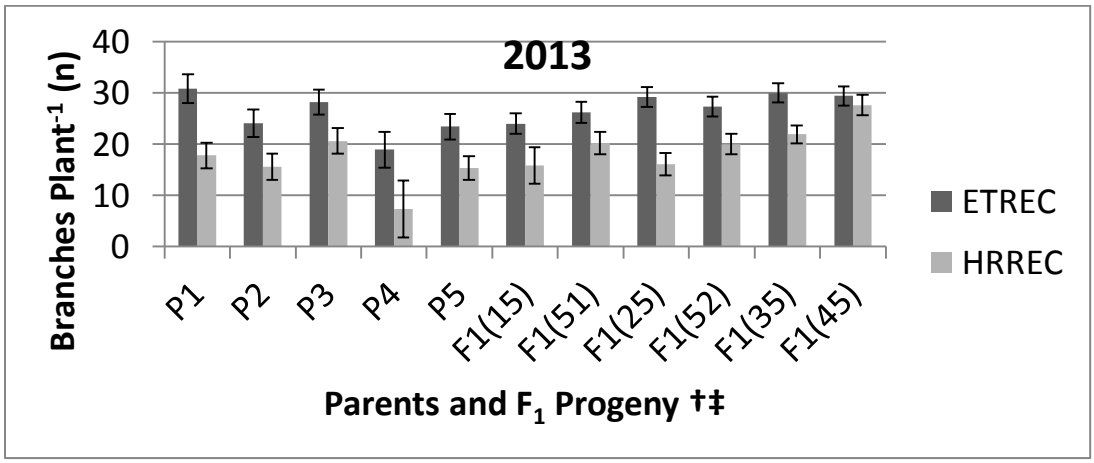


Fig. 2.5. Boxplots of seed plant^{-1} (n) for F_2 progeny of six niger crosses pooled across the East Tennessee (E) and Highland Rim Research and Education Centers in 2013 (A), and in 2014 at ETREC and Plateau Research and Education Center (P) (B). There was a significant plant material x location interaction in 2014; therefore the data are shown for each location. F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 87, 95, 17, 77, 64, and 42 observations, respectively, in Figure 4A. F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 6, 15, 16, and 23 observations, respectively, in 2014 at ETREC, and 24, 24, 72, and 88 at PREC (B).
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_2(15) and F_2(51), for example, are reciprocal crosses. F_2(15) indicates that P1 was the maternal and P5 was the paternal parent.
§ F_2(35) and F_2(45) from ETREC and PREC were missing in (B) due to poor germination and disease.

A



B

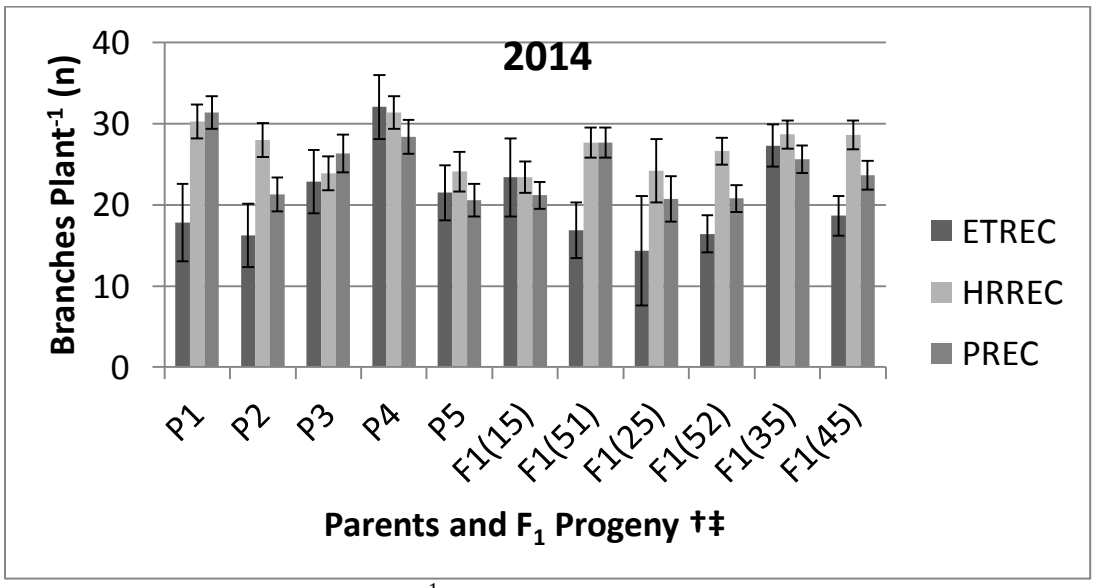


Fig. 2.6. Average branches plant^{-1} (n) for five niger parents (P) and six F_1 crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013 (A), and in 2014 at ETREC, HRREC, and Plateau Research and Education Center (PREC) (B). There was a significant plant material x location interaction in 2013 and 2014; therefore the data are shown for each location. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 11, 5, 10, 17, 16, 18, 17, 19, and 18 observations, respectively, at ETREC, and 10, 10, 10, 1, 12, 5, 13, 14, 16, 21, and 16 at HRREC in 2013 (A). P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 2, 3, 3, 3, 4, 2, 4, 1, 10, 7, and 8 observations, respectively, at ETREC, 11, 11, 11, 12, 8, 12, 14, 3, 18, 16, and 16 at HRREC, and 12, 11, 9, 11, 12, 18, 18, 6, 18, 18, and 16 at PREC in 2014 (B).
† Parents and F1's with overlapping error bars are not significantly different at $P< 0.05$.
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

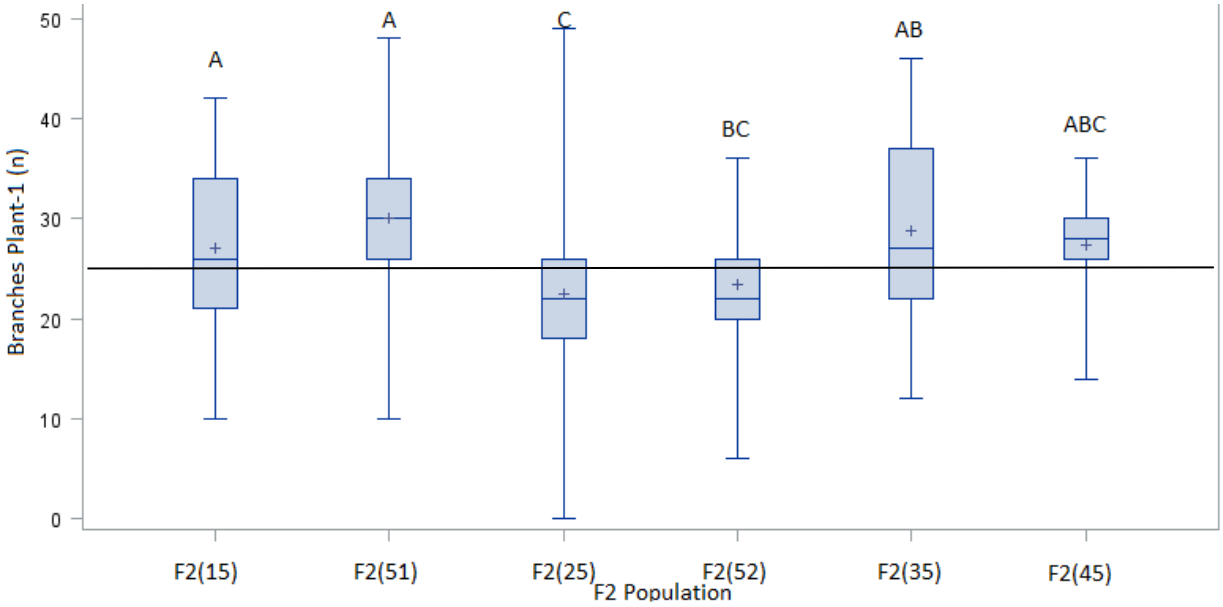


Fig. 2.7. Boxplots of branches plant^{-1} (n) for F_2 progeny of six niger crosses combined across the East Tennessee (ETREC), Highland Rim (HRREC), and Plateau (PREC) Research and Education Centers in 2014. F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 86, 96, 35, 101, 77, and 49 observations, respectively.
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_2(15) and F_2(51), for example, are reciprocal crosses. F_2(15) indicates that P1 was the maternal and P5 was the paternal parent.

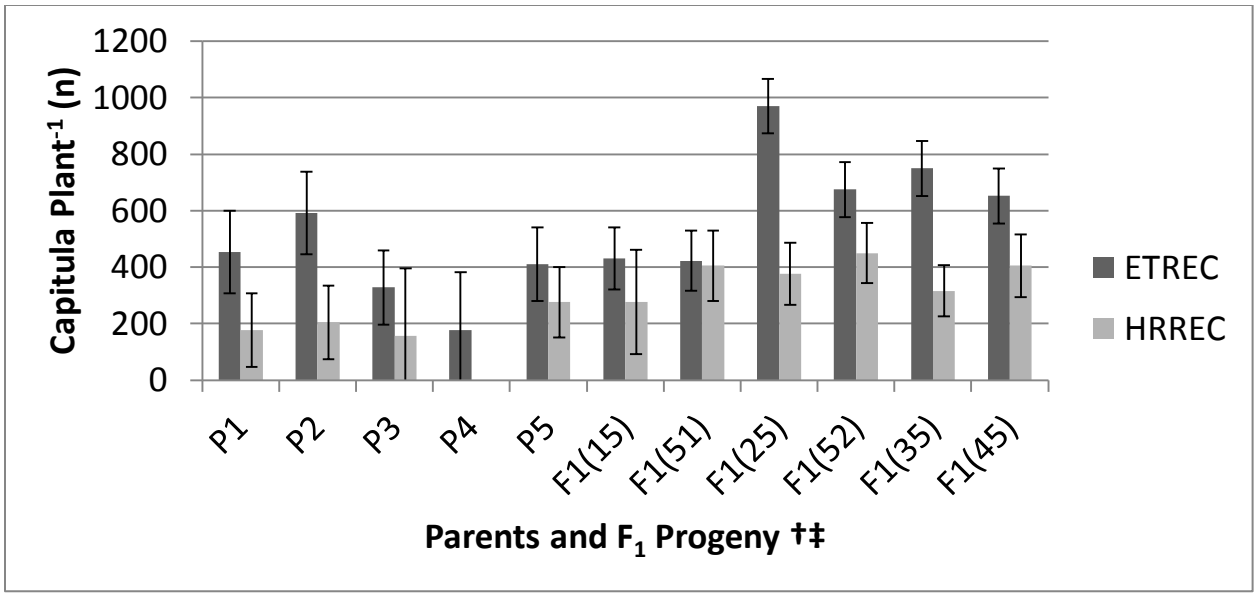


Fig. 2.8. Average capitula plant^{-1} (n) of five niger parents (P) and six F_1 crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013. There was a significant plant material x location interaction; therefore the data are shown for each location. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 11, 4, 10, 17, 16, 18, 18, 19, and 19 observations, respectively, at ETREC, and 10, 10, 3, 0, 12, 5, 12, 14, 16, 21, and 16 at HRREC.

† Parents and F1's with overlapping error bars are not significantly different at $P<0.05$.

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are the reciprocal F_1 crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

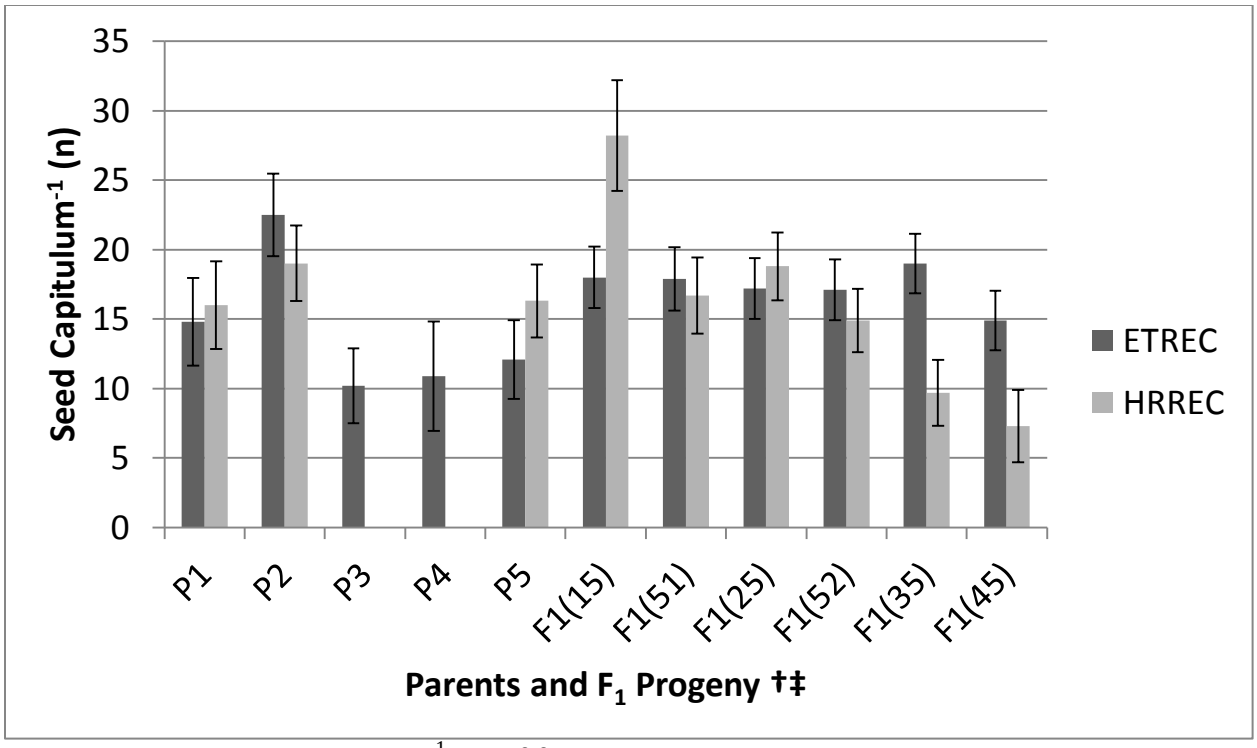


Fig. 2.9. Average seed capitulum^{-1} (n) of five niger parents (P) and six F_1 crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013. There was a significant plant material x location interaction; therefore the data are shown for each location. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 6, 4, 10, 16, 16, 18, 18, 19, and 19 observations, respectively, at ETREC, and 8, 11, 0, 0, 11, 5, 10, 14, 16, 11, and 8 at HRREC.

† Parents and F1's with overlapping error bars are not significantly different at $P<0.05$.

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

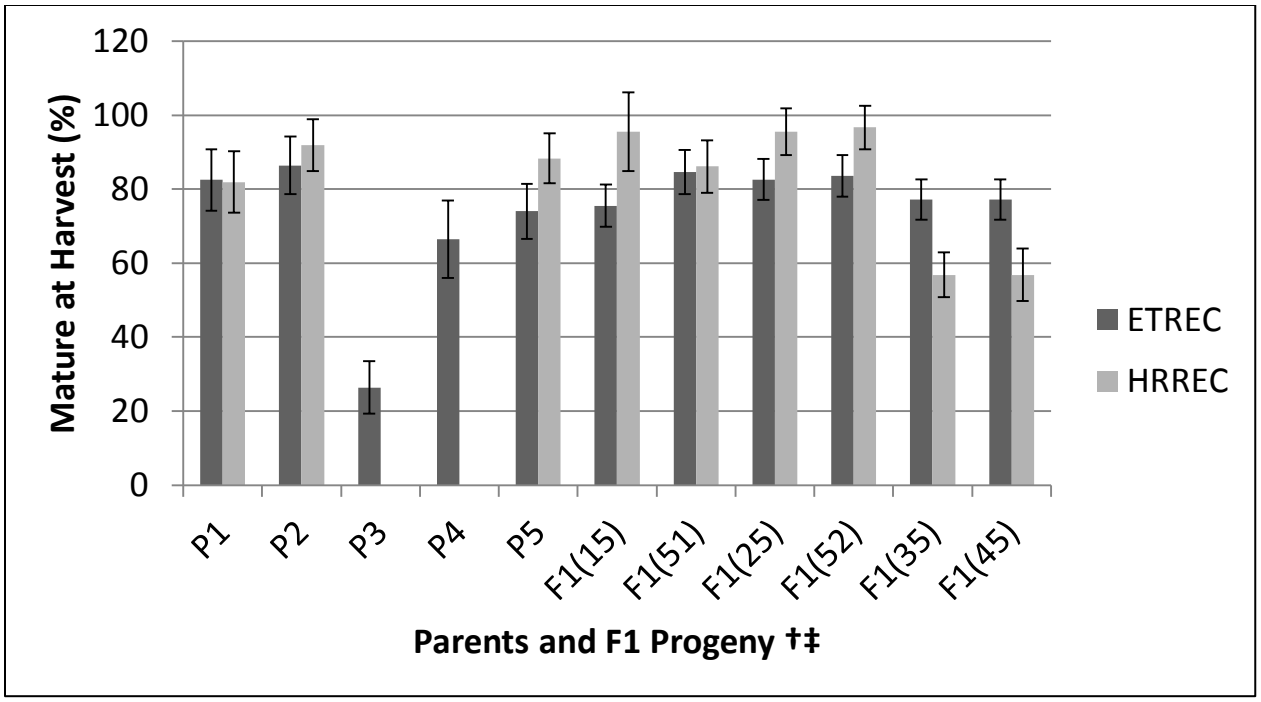


Fig. 2.10. Average maturity by harvest (%) of five niger parents (P) and six F_1 crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013. There was a significant plant material x location interaction; therefore the data are shown for each location. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 6, 4, 10, 16, 16, 18, 18, 19, and 19 observations, respectively, at ETREC, and 8, 11, 0, 0, 11, 5, 10, 14, 16, 11, and 7 at HRREC.

† Parents and F1's with overlapping error bars are not significantly different at $P<0.05$.

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

§ Harvest occurred in mid to late October in 2013 following a killing frost.

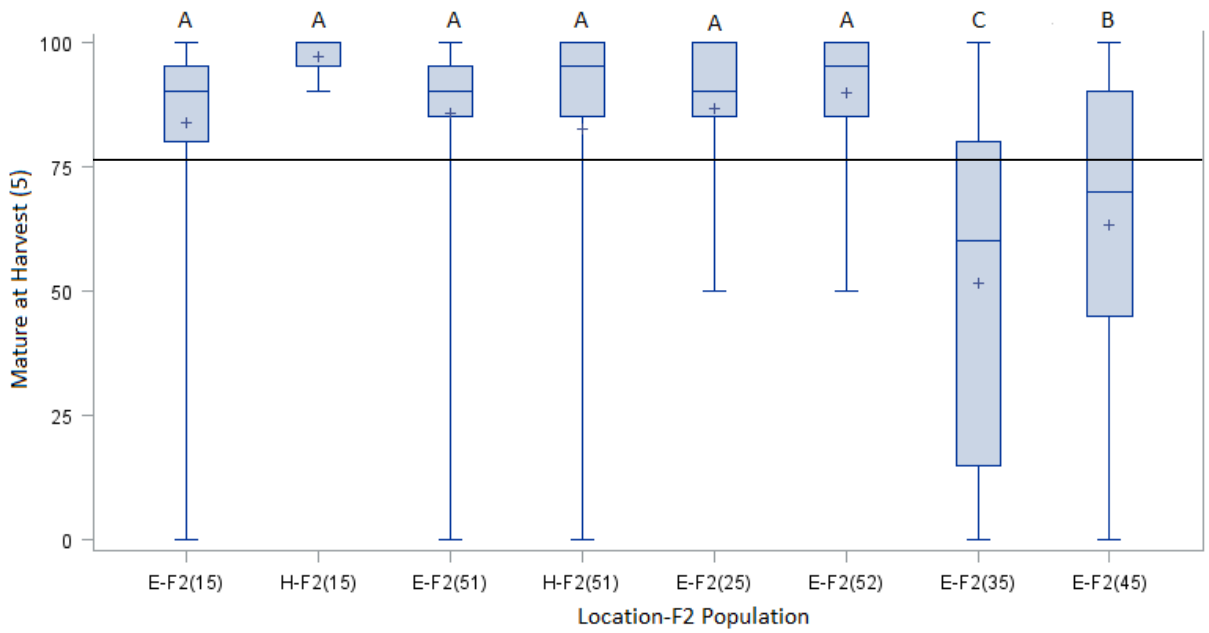


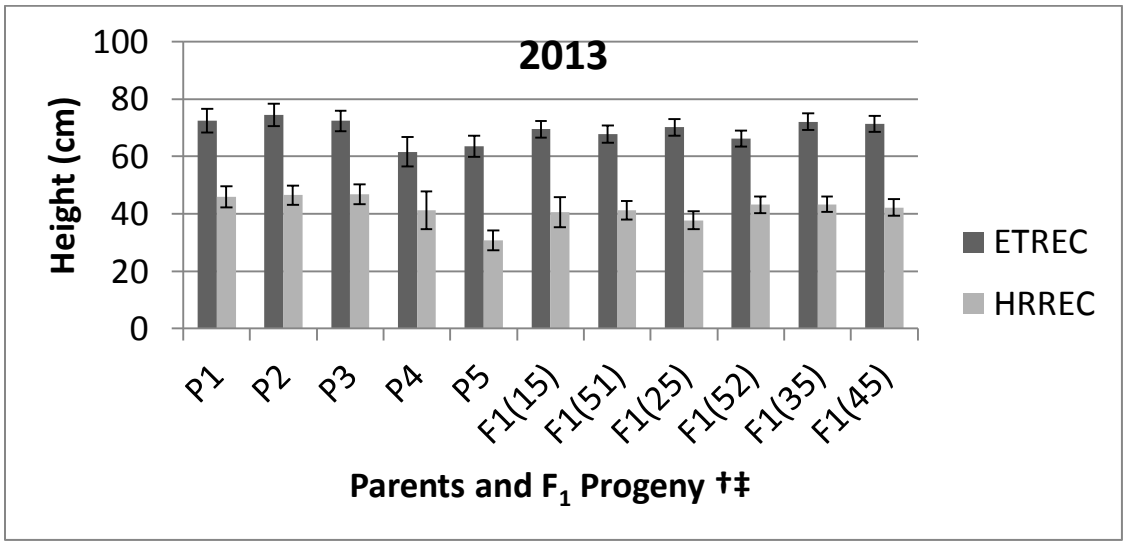
Fig. 2.11. Boxplots of maturity by harvest (%) for F_2 progeny of six niger crosses in 2013 at the East Tennessee (E) and Highland Rim (H) Research and Education Centers. There was a significant plant material x location interaction; therefore the data are shown for each location. F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 81, 80, 15, 74, 63, and 42 observations, respectively, at ETREC, and 7, 15, 0, 0, 0, and 0 at HRREC.

† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_2(15) and F_2(51), for example, are reciprocal crosses. F_2(15) indicates that P1 was the maternal and P5 was the paternal parent.

§ F_2(45) from HRREC was missing from the figure due to poor germination and disease.

A



B

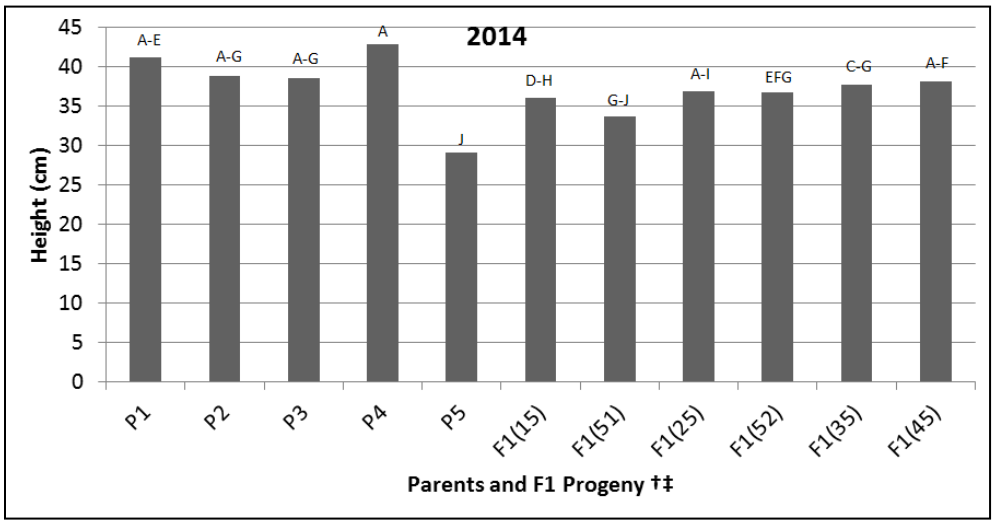
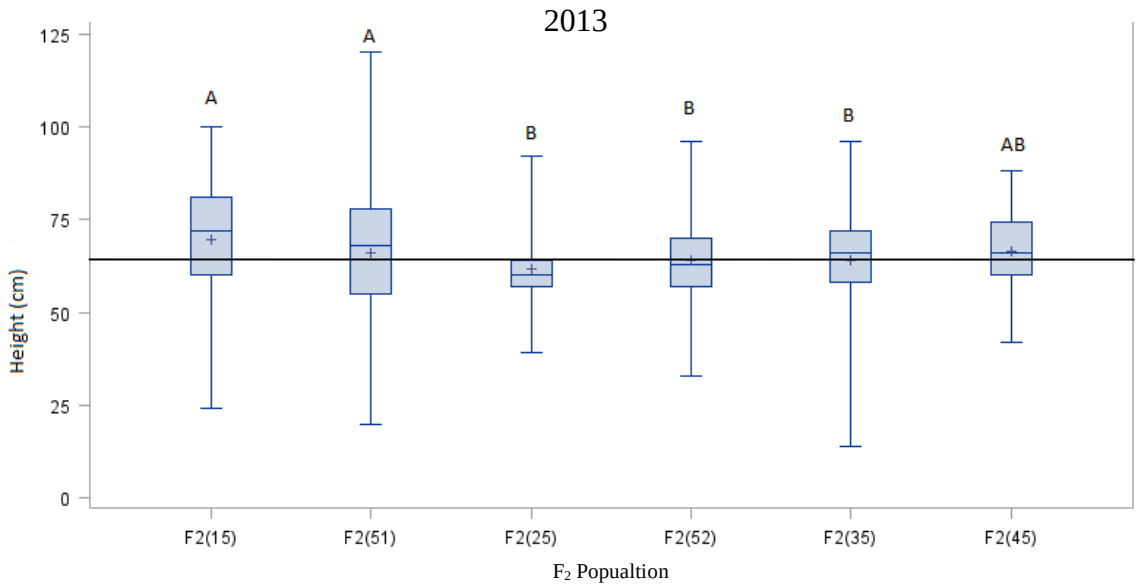


Fig. 2.12. Average height of five niger parents (P) and six F_1 crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013 (A), and in 2014 at ETREC, HRREC, and the Plateau Research and Education Center (B). There was a significant plant material x location interaction in 2013; therefore the data are shown for each location. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 8, 9, 11, 5, 10, 17, 15, 18, 18, 18, and 19 observations, respectively, at ETREC, and 10, 12, 12, 3, 12, 5, 13, 15, 17, 21, and 16 at HRREC in 2013 (A). P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 25, 25, 23, 26, 24, 33, 36, 10, 46, 41, and 40 observations, respectively, in 2014 (B).

† Parents and F1's with overlapping error bars or shared letters (based on Fisher's LSD) are not significantly different at $P<0.05$.

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) shows that P1 was the maternal and P5 was the paternal parent.

A



B

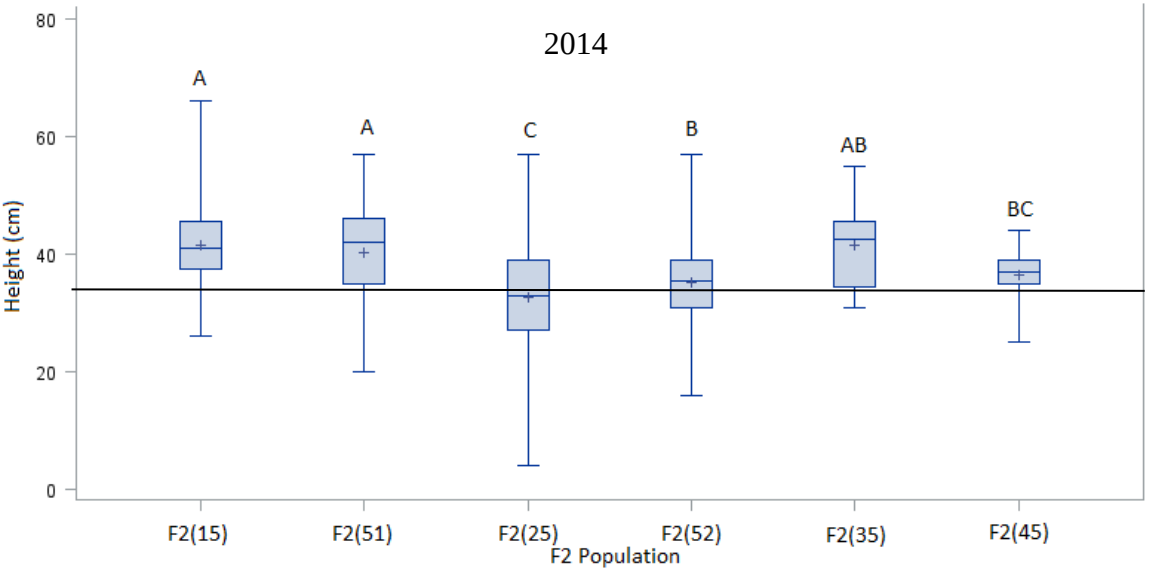
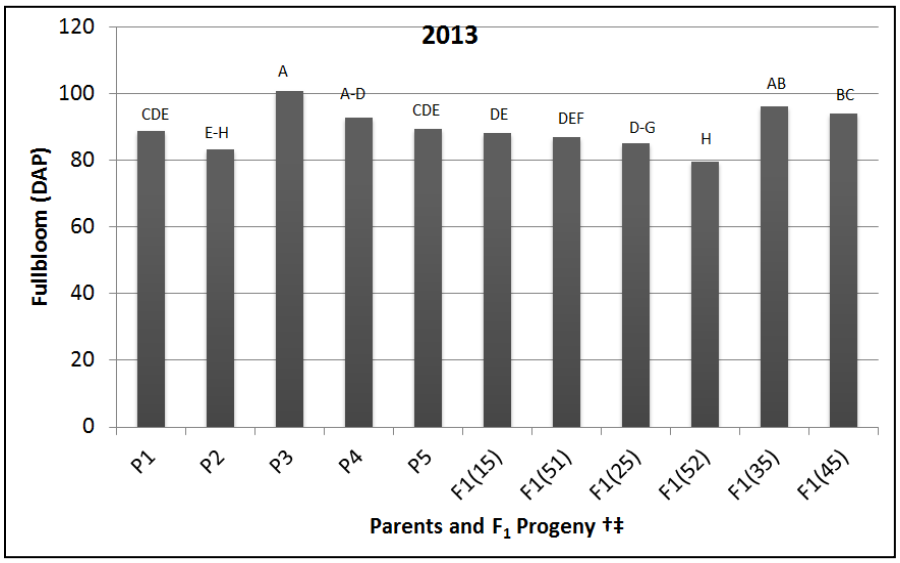


Fig. 2.13. Boxplots of height for F_2 progeny of six niger crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013 (A), and in 2014 at ETREC, HRREC, and Plateau Research and Education Center (PREC) (B). F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 87, 100, 17, 76, 83, and 48 observations, respectively, in 2013 (A). F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 44, 65, 175, 212, 8, and 9 observations, respectively, in 2014 (B).
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_2(15) and F_2(51), for example, are reciprocal crosses. F_2(15) shows that P1 was the maternal and P5 was the paternal parent.

A



B

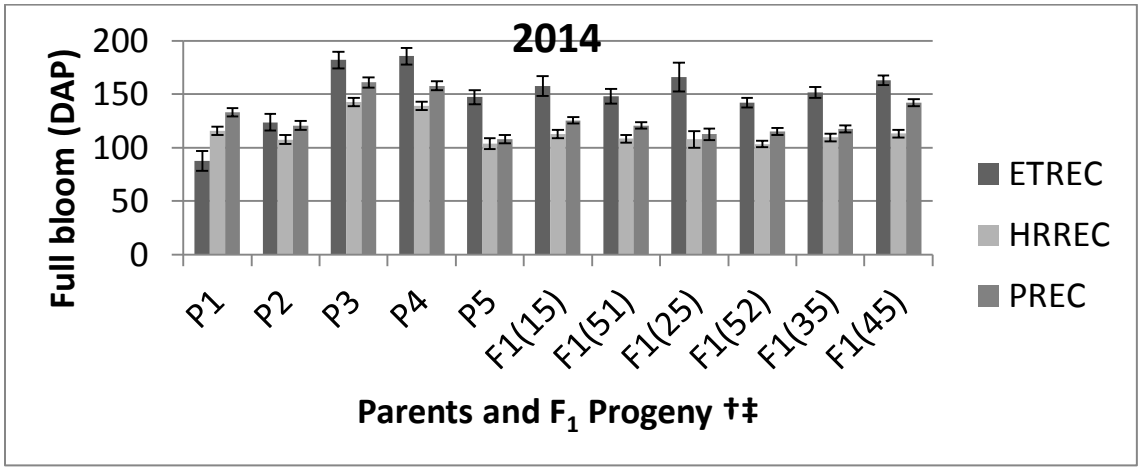
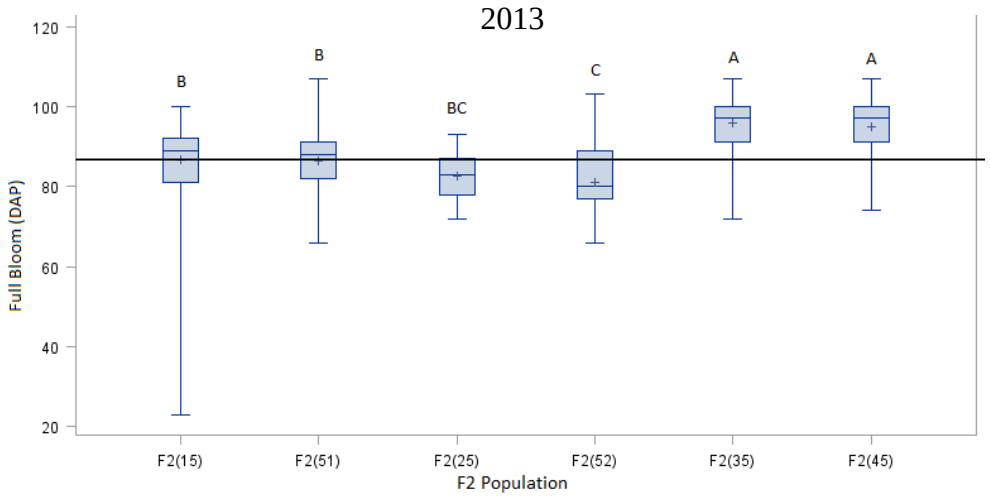


Fig. 2.14. Average full bloom measured in days after planting (DAP) showing variation of five niger parents (P) and six F_1 crosses at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013 (A), and in 2014 at ETREC, HRREC, and the Plateau Research and Education Center (B). There was a significant plant material x location interaction in 2014; therefore the data are shown for each location. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 11, 12, 13, 5, 9, 18, 17, 18, 18, 18, and 19 observations, respectively, in 2013 (A). P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 1, 3, 3, 3, 4, 2, 4, 1, 10, 7, and 9 observations, respectively, at ETREC, 11, 11, 11, 12, 7, 12, 14, 3,18, 16, and 15 at HRREC, and 12, 11, 8, 11, 12, 18, 18, 6, 18, 18, and 16 at PREC in 2014 (B).

† Parents and F_1's with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are reciprocal crosses. F_1(15) indicates that P1 was the maternal and P5 was the paternal parent.

A



B

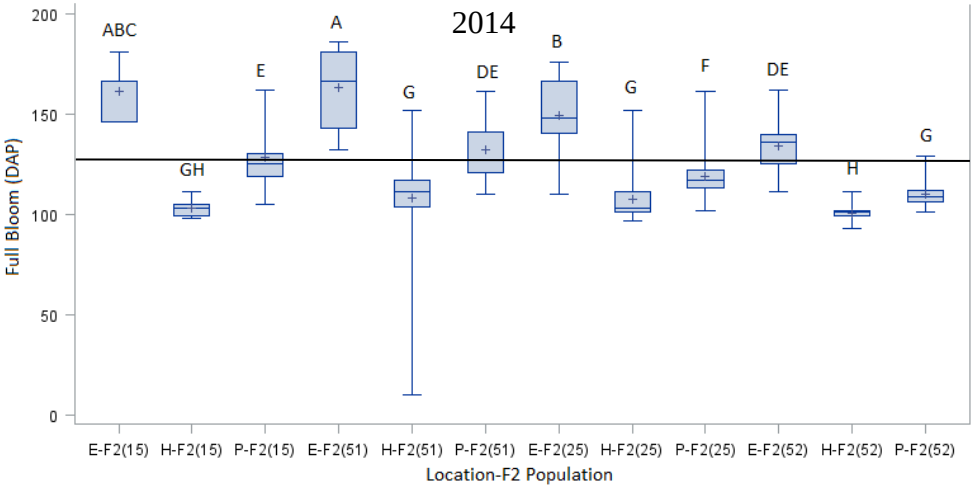


Fig. 2.15. Boxplots of full bloom measured in days after planting (DAP) for F_2 progeny of six niger crosses at the East Tennessee (E) and Highland Rim (H) Research and Education Centers in 2013 (A), and at E, H, and the Plateau (P) Research and Education Center in 2014 (B). There was a significant plant material x location interaction in 2014; therefore the data are shown for each location. F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 89, 92, 15, 79, 87, and 51 observations, respectively, in (A). F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45) had 7, 15, 20, 27, 4, and 2 observations, respectively, at ETREC, 11, 22, 71, 89, 3, and 5 at HRREC, and 26, 28, 82, 96, 1, and 2 at PREC in 2014.
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_2(15) and F_2(51), for example, are reciprocal crosses. F_2(15) indicates that P1 was the maternal and P5 was the paternal parent.

CHAPTER III

COMPARISON OF OIL AND FATTY ACIDS AMONG NIGER ACCESSIONS AND SELECTED CROSSES

Abstract

American Goldfinches and other songbirds such as house finches, pine siskins, and redpolls have a preference for niger seed for its high oil (energy) content. The objective of this study was to determine total seed oil (%) [percent] and fatty acid composition on plant introductions (PIs) and progeny of crosses from selected parents from the USDA/ARS germplasm collection at Pullman, WA. Ten replications of the fourteen PIs were planted under field and greenhouse conditions at the East Tennessee Research & Education Center in Knoxville in August of 2012 using a completely randomized design. After initial assessments, five of these PIs were then selected for further evaluation, and crosses were made to produce six F_1's [filial 1], eight backcrosses (BC's), and six F_2 [filial 2] populations. Seed were planted at the East Tennessee (2013 and 2014), Highland Rim (2013 and 2014), and Plateau (2014) Research and Education Centers in a randomized complete block design with replication. Comparisons showed seed oil ranging from 32.87 to 37.89% (PI508076 and PI509436, respectively). Major fatty acids included stearic, palmitic, oleic, and linoleic; with linoleic acid in greatest amount. PI508079 had the best combination of seed yield, seed oil, and linoleic acid content. Results from 2013 and 2014 showed PI 422242 and PI 508076 had the greatest linoleic and lowest oleic fatty acid, whereas PI 511305 and W6 18860 had the lowest linoleic but greatest oleic fatty acid. W6 18860/PI 422242//W6 18860/PI 422242 had the greatest linoleic acid but lowest oleic fatty acid, whereas W6 18860/PI 511305//W6 18860/PI 511305 had the greatest oleic but lowest linoleic fatty acid. Moderate correlations were found between yield traits and palmitic acid (0.38-0.50) whereas correlations of linoleic acid (-0.15 and 0.34) and oleic acid (-

0.27 and 0.01) with yield traits were weak. Oleic and linoleic acid had a strong negative correlations (-0.84- -0.87).

Introduction

Niger's excellent oil quality makes it suitable for many purposes. Its most common purpose is as an oilseed crop for human and avian consumption. In Ethiopia, niger accounts for roughly 50-60% of the country's edible oil requirement (Quinn and Myers, 2002). In the United States, niger is primarily marketed as birdseed primarily for the American goldfinch, pine siskin, common redpoll, finches, and other songbirds (Wild Delight, 2015). Niger has been used as silage for livestock; has been incorporated in paints, perfumes, and soaps; and has the potential for use as a biodiesel (Demirbas, 2008). Fatty acid methyl esters from niger oil have been touted as an environmentally safe, nontoxic, and suitable biodiesel source from its saponification value, cetane number, and iodine value (Yadav et al., 2012). Ramadan and Mörsel (2003a) reported that total oil content consisted of 75-80% fatty acids, 97% of which was composed of linoleic, oleic, stearic, and palmitic acids. Linoleic fatty acid, an essential polyunsaturate, was found to constitute 70% of total fatty acid content. The ratio of saturated to unsaturated fatty acids was reportedly 25:75. They also reported the presence of neutral lipids, polar lipids, and sterols. Whereas neutral lipids account for 93-97% of the total lipids, glycolipids and phospholipids, which are both polar lipids, contain more saturated fat and less unsaturated fat than neutral lipids. They make up 4.9% and 0.6% total lipid content, respectively. Minor lipid components included tocopherols, a stabilizer of oil during the oxidation process, Vitamin K1, β-carotene, and phenolic compounds (Ramadan, 2012).

Fatty acids are major components of cellular membranes, and therefore have the ability to influence cellular processes such as transporter, receptor, and other membrane bound protein activities (Ruthig and Mechling-Gill, 1999). The majority of fatty acids comprising niger seed oil

are unsaturated; predominantly linoleic (C18:2) and oleic (C18:1). Primary saturated fatty acids include palmitic (C16:0) and stearic (C18:0) (Ramadan and Morsel, 2002). Linoleic fatty acid is a polyunsaturated omega-6 fatty acid found in abundance in many nuts and oilseeds. This essential fatty acid must be consumed for proper health. Without such, research has discovered negative effects such as hair loss, mild scaling of skin (Cunnane and Anderson, 1997), and poor wound healing (Ruthig and Mechling-Gill, 1999). Oleic fatty acid is monounsaturated fat common in the human diet that has been associated with the reduction of LDL cholesterol and possibly the increase of HDL cholesterol (Mensink et al., 2003). Oleic acid is also associated with the reduction of adrenoleukodystrophy (ALD), blood pressure, and risk of breast cancer. Palmitic and stearic fatty acids are saturated fats needed to maintain proper health, but if consumed in large quantities, result in negative health effects. Together, they has been linked to an increased risk of vascular disease, reduced insulin activity (Benoit et al., 2009), and increase risk of Alzheimer's disease (Patil and Chan, 2005).

Little research has investigated fatty acid nutrition in birds; however, the importance of maintaining a healthy omega-3/omega-6 dietary balance in humans is well documented (Simopoulos, 2002). Omega-3/omega-6 ratios ranging between 1/2 and 1/5 are linked to a reduction in frequency of cardiovascular diseases, cancer, and inflammatory and autoimmune diseases. However, seeds high in omega-3 fatty acids are susceptible to oxidation instability, and therefore shorter shelf-life. Trace amounts of linolenic acid, an omega-3 fatty acid, has been reported in niger. If birds were affected by the omega3/omega-6 ratio in the same way that humans are, this would create a conundrum for future breeders; however, more research is certainly needed to determine these effects.

A previous study by Alemaw and Wold (1995) found negative correlations between oleic and linoleic acid. This can be expected since oleic can be synthesized from linoleic acid. Likewise, a negative correlation is expected for oleic and stearic acid since oleic acid can be synthesized from stearic acid. A study by Geleta, Stymne, and Bryngelsson (2011) (2011) found that seed weight and linoleic acid had a negative correlation of -0.17. Seed weight and oleic acid were correlated at 0.13, while seed weight and palmitic acid had a negative correlation of -0.15.

While a previous study indicated that the fatty acid differentiation between taxa was insignificant (Dagne and Jonsson, 1997), it is possible that the USDA/ARS germplasm collection contains plant introductions that are significantly different. The objective of this study is to determine variation of total oil as well as four major fatty acids in seed of niger accessions and progeny (F_1 and F_2) from crosses of selected accessions used as parents.

Materials and Methods

Preliminary evaluation of plant introductions

The 14 accessions described in Chapter 2, were planted under field and greenhouse conditions at the East Tennessee Research & Education Center in Knoxville in August of 2012. Seed from all available plants were bulked within each accession due to seed scarcity and sent to the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA for analysis of seed oil and fatty acid composition using Time Domain Nuclear Magnetic Resonance (TD-NMR) and Gas Chromatography (Agilent 7890A). Afterwards, the same seed were analyzed University of Tennessee's gas chromatograph (Agilent 6890). Two replications of were analyzed with both the Agilent 7890A (Griffin, GA) and Agilent 6890 (Knoxville, TN) Gas Chromatographs. The operating resonance frequency was set at 9.95 MHx 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 second 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 to oil and moisture calibration curves, generated by sample weight. Each of the fourteen accessions was measured twice then averaged to report the value of oil content in this study. Oil standards were generated using extracted oils. Seed of the five selected parent accessions as well as the F1 and F2 progeny from the six crosses among the selected parents (described in Chapter 2) produced at the East Tennessee and Plateau Research and Education Centers in in 2014 were analyzed for different fatty acids.

Fatty acid content was estimated using Gas Chromotography. Three to five niger seed were ground to a fine powder using a mortar and pestle. Oil from a small amount (~50−75 mg) of meal was extracted in 5.0 mL of heptane (Fisher Scientific) and converted to FAMEs with 500 µL of 0.5 N sodium methoxide (NaOCH3) 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 (Hewlett Packard) gas chromatograph (GC) with a flame ionization detector (FID). Peak separation was performed on a DB-225 capillary column (15 m × 0.25 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. The carrier gas was helium set to a flow rate of 1.0 mL/min. One microliter of sample was injected at a 60:1 split ratio onto the column with the following thermal gradient: 195 °C for 3 min, 195 to 200 °C at 2.5 °C min, 200 to 230 °C at 5 °C min, and 230 to 235 °C at 1.5 °C min for a total run time of 14 min. A fatty acid methyl ester (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 identifying and calculating relative peak areas. The O/L ratio was determined by % oleic acid /% linoleic acid.

Fatty acid analyses were performed according to modified AOCS Ce 1-62 (American, 1999) protocol. Two samples of approximately 117 seed from each of the 12 accessions were crushed and transferred to a test tube where 2.5mL extraction solvent [8 parts chloroform ($CHCl_3$), 5 parts hexane (C_6H_{14}), and 2 parts methanol (CH_3OH)] was added. Original protocol requires 1 mL of extraction solvent. The tubes were capped and left in the hood overnight. The next day, 100µL of extract from each tube was transferred to a corresponding vial to which

750µL of hexane and 75µL of methylation reagent was added. The vials were capped and transferred to autosampler trays and analyzed via the Agilent 6890 (Hewlett Packard) Gas Chromatograph.

In 2014, parent, F_1, F_2, and backcross (BC) seed from ETREC and PREC were analyzed separately by location. Each sample was dried for 5 hours, and was lightly crushed using a mortar and pestle. The samples were transferred to a 15 ml (16x100 mm) test tube where the sample was weighed. 5 ml of extraction solvent (5:8:2 mixture of hexane, chloroform, methane, respectively) was added to the crushed seed. The mixture was allowed to sit for at least 6 hours so the oil fraction would separate from the solvent solution and niger hulls. The oil sample was derivitized by transesterification to find the fatty acid profiles. 150 uL of extract was transferred to vial to which 75uL methylation reagent and 0.75 mL Hexane was added to each vial. The samples were analyzed via an Agilent 7683 Series gas chromatograph.

Statistical Analyses

The preliminary oil and fatty acid data of niger accessions and the fatty acid analysis of parents, F_1 and F_2 progeny from 2014 were analyzed with mixed model analysis of variance and Fisher's LSD ($P<.05$) using SAS 9.3 (Cary, NC). The preliminary evaluation of oil and fatty acids used a completely randomized design, where different sub-samples of seed were used as replications. The 2014 fatty acid analysis used a randomized complete block design with replication. Parents and F_1's were analyzed in the same data set, while F_2's were analyzed separately. Location, plant material (parents and F_1; F_2), and plant material x location interaction were considered fixed effects, while block and block x plant material interaction were considered

random effects. Since plant material x location interaction was insignificant, data were pooled across locations.

Correlation

Correlation analyses were run on five parents (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860,) six F_1 progeny [(F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45)], and six F_2's [F_2(15), F_2(51), F_2(25), F_2(52), F_2(35), and F_2(45)] in 2014. This study analyzed correlations between yield traits (seed yield plant^{-1} (g) and seed plant^{-1} (n)) and fatty acid traits (linoleic fatty acid (%), oleic fatty acid (%), and palmitic fatty acid (%)). Correlations between traits were analyzed using SAS 9.3 (Cary, NC) software.

Results

Total oil content and all fatty acids showed significant differences among the 12 accessions for the study in 2012 (P<.0001). Total oil was greatest in PI 422242 (38%) and PI 509436 (38%), and lowest in PI 508076 (33%) (Fig. 3.1). A study by Ramadan and Mörsel (2003a, 2003b) showed that dry niger seed contained 27-47% oil and with a mean of 35%. A study by Bhatnagar and Gopala Krishna (2014) revealed the cold pressed seeds resulted in 28% oil, whereas hexane and ethanol extractions resulted in 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, 2012; Yadav et al., 2012).

Significant differences (P<0.05) were found between testing methods for determining mean separation for linoleic, oleic, and palmitic fatty acids (Fig. 3.2), but not for stearic acid. Still, results between both methods were very similar, so the methods were pooled. Results for linoleic, oleic, palmitic, and stearic fatty acid compositions showed significant variation among

the USDA niger accessions ($P<0.05$). PI 508079 contained the most linoleic acid (82%) and W6 18860 had the least (53%). W6 18860 had the most oleic acid (30%) while PI 508079 contained the least (3%). Palmitic fatty acid was greatest 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 least (5%). Arachidic fatty acid was less than 1%, and linolenic fatty acid was less than 0.40% (data not shown). Additional fatty acids included sapienic (less than 0.20%), behenic (1%), and lignoceric (less than 1%) (data not shown).

Previous studies have analyzed variation in fatty acid profiles. Oleic acid, a monounsaturated fatty acid, ranged from 24- 53% with linoleic acid ranging from 32-58% (Yadav, 2012; Yadav et al., 2012). 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%) fatty acids. Bockisch (1998) stated that niger oil may contain up to 1% arachidic and 3% linolenic fatty acid. These studies, along with the preliminary evaluation of niger accessions in this study, indicate the possibility of future breeding efforts to improve seed yield, increase oil content, and increase various fatty acids content for niger grown in the United States.

Significant differences ($P<0.05$) were found among parents and F_1's for palmitic fatty acid for data collected in 2014. Since there were no significant location or plant material (parents and F_1) x location interaction, data from each fatty acid was pooled across locations. F1(45) had the greatest amount of palmitic fatty acid (16%) (Fig. 3.3). Parents and F1's showed very similar average percent palmitic fatty acid, 11% and 12%, respectively. No significant differences were

found for linoleic, oleic, or stearic acid ($P<0.05$); however, reciprocal effects were apparent in F_1(15) and its reciprocal F_1(51) as well as F_1(25) and its reciprocal F_1(52). Linoleic acid tended to be greater when P5 was used as the males (58% and 58%, respectively), and P1 and P2 were used as the females (50% and 50%, respectively). P1 and P4 contained the greatest amount of linoleic fatty acid (60% and 61%, respectively), whereas P2 and P5 contained the least (47% and 49%, respectively) (Fig. 3.3). Parents and F_1's both averaged 54% percent linoleic fatty acid (Fig. 3.3). P1 and P4 had the least oleic fatty acid (15%), whereas P2 and P5 had the most (25% and 26%, respectively) (Fig. 3.3). Parents and F_1's both averaged 20% oleic fatty acid. Stearic acid ranged from 77% (P1) to 88% [F_1(45)] (Fig. 3.3). F_1(35) and F_1(45) resulted in high parent heterosis (Fig. 3.3).

Significant differences ($P<0.05$) were found among F_2's in linoleic, oleic, and palmitic fatty acids, but not in stearic acid. Since plant material (parents and F_1) x location interaction was not significant, data were pooled across locations for linoleic, oleic, and palmitic acids (Fig. 3.4-3.6). The mean for linoleic acid across all F2 crosses was 53% (Fig. 3.4). Boxplots show that F_2(51) contained the greatest amount of linoleic acid (61%), whereas F_2(52) contained the least (49%) (Fig. 3.4). Contrastingly, F_2(51) contained the least amount of oleic fatty acid (13%), and F_2(52) contained the greatest (26%) (Fig. 3.5). The mean for oleic fatty acid was 22% across all F_2 crosses (Fig. 3.5), whereas palmitic fatty acid averaged 11% (Fig. 3.6). F_2(25) and F_2(52) showed significantly lower palmitic fatty acid (11%) than F_2(15) and F_2(51) (13%). F_2(35) and F_2(45) were missing from the analyses due to poor germination and disease. The mean for stearic acid was 7 % across all F_2 crosses.

Correlations

Seed Yield

Correlation coefficients were only significant ($P<0.05$) when all plant material was analyzed together and when F_2's were analyzed separately. Correlations between seed yield and linoleic acid were not significant ($P>0.05$) (0.06-0.34) except for the analysis exclusively involving F_2 plant material, which was weakly positive (0.12) (Table 3.1). Correlations between seed yield and oleic acid were negative (-0.17 to -0.24) except for the correlation of F_1 plant material (0.01). Palmitic acid and seed yield resulted in weak to moderate correlations (0.02-0.38).

Seed Number

There was no correlation between seed number and linoleic acid; a negative correlation with oleic acid (-0.21); and positively correlated with palmitic acid (0.38) (Table 3.1)when all plant materials were analyzed together, or when only F_2's were analyzed separately (-0.25 and 0.50, respectively) (Table 3.1).

Fatty acids

Correlations between fatty acids resulted in strong negative correlations between oleic and linoleic acid (-0.84- -0.86) (Table 3.2). Comparisons of palmitic and linoleic acid resulted in weak to moderate positive correlation coefficients (.07-0.55). Palmitic acid and oleic acid resulted in weak negative correlations (-0.12- -0.27). Correlations between oleic and linoleic acid as well as palmitic and linoleic were significant among all plant material with the exception of F_1 correlations involving palmitic and linoleic acid.

Conclusions

Of the 12 accessions analyzed in 2012, total oil was greatest in PI 422242 and PI 509436 (38% and 38%, respectively) and lowest in PI 508076 (33%). Linoleic acid was greatest in PI 508079 (82%) and lowest in W6 18860 (53%); whereas PI 508079 had the least (3%) and W6 18860 had the greatest (30%) amount of oleic acid. PI 508073 resulted in the least amount of palmitic acid (8%); whereas W6 18860 resulted in the greatest (9%). Stearic acid was greatest in PI 508072 (8%) and lowest in PI 508079 (5%).

Correlations were moderate (palmitic) to strong (stearic, oleic, and linoleic) between both instruments when analyzing fatty acid content of niger seeds. Since the Agilent 7890A Chromatograph had a standard error of 0.081 and the Agilent 6890 Chromatograph had a standard error of 0.084, it can be assumed that either technique will report similar results.

Seed quality is retained longer in seeds with high oleic fatty acid content than seeds high in linoleic fatty acid. This is due to oleic acid's lower susceptibility to oxidation (Miller et al., 1987). 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, if appropriate markers are available.

In 2014, no significant differences were found among parents and F1 progeny for linoleic, oleic, or palmitic acid (Fig. 3.3). Linoleic acid of parents ranged from 47% (P2) to 61% (P4). F_1 progeny ranging from 50% [F_1(52)] to 58% [F_1(15) and F_1(25)] (Fig. 3.3), suggests that reciprocal effects exist. Linoleic acid is greater when P5 (W6 18860) is used as the male parent and P1(PI 422242) and P2 (PI 511305) are used as female parents. F_2 progeny ranged from 49% [F_2(52)] to 61% [F_1(51)] (Fig. 3.4). Much variation was found for linoleic acid. Box and

whisker plots show that linoleic acid was highly variable in F_1(51) (Fig. 3.4). Overall, there was not much variation in the F_2 progeny for oleic, or palmitic acid (Fig. 3.5 and 3.6). F_1(25) and F_1(52) resulted in high parent heterosis in linoleic acid (Fig. 3.3).. Oleic acid of parents ranged from 15 (P1 and P4) and 26% (P5) (Fig. 3.3). F_1 progeny ranged from 17% [F_1(45) and F_1(25)] to 24% [F_1(52)] (Fig. 3.3), while F_2 progeny ranged from 16% [F_2(15)] to 26% [F_2(52)] (Fig. 3.5). F_2(25) and F_2(52) showed high-parent heterosis in oleic fatty acid (Fig. 3.5).

These results are expected because oleic acid is the precursor fatty acid to linoleic acid (Alemaw and Wold, 1995). A previous study also found a strong negative correlation of -0.83 (Geleta et al., 2011). Strong negative correlations found between oleic and linoleic acid were also found in this study. Correlations ranged between -0.84 and -0.86 (Table 3.2).

Palmitic acid for parents ranged from 11% (P2 and P5) to 13% (P4) while F_1 progeny ranged from 10% [F_1(52)] to 16% [F_1(45)] (Fig. 3.3). F_1(15), F_1(25), and F_1(45) showed high parent heterosis for palmitic fatty acid (Fig. 3.3). F2 progeny ranged from 11% [F_2(25) and F_2(52)] to 13% [F_2(15) and F_2(51)] (Fig. 3.6). Stearic acid ranged from 7% (P1) to 8% [F_1(45)] (Fig. 3.3). F_1(35) and F_1(45) resulted in high parent heterosis (Fig. 3.3). Trace fatty acids made up the rest of the fatty acid composition.

Alemaw and Wold (1995) found weak correlations of seed yield and linoleic (-0.17), oleic (0.13), and palmitic acid (-0.15). These findings were similar to those of this study, with the exception of moderate correlations found for yield traits and palmitic acid (0.38-0.50). Correlations involving linoleic acid ranged between -0.15 and 0.34 while correlations involving oleic acid ranged between -0.27 and 0.01.

Literature Cited

Alemaw, G. and A.T. Wold. 1995. An agronomic and seed-quality evaluation of noug (Guizotia abyssinica Cass.) germplasm in Ethiopia. Plant Breeding 114(4):175-176.

American Oil Chemists Society (AOCS) 1999. Fatty acid composition by gas chromatography. Offical methods and recommended practices of the Americal Oil Chemists Society, 5. AOCS, Champaign Method Ce 1-62.

Benoit, S., C. Kemp, C.F. Elias, W. Abplanalp, J.P. Herman, S. Migrenne, A. Lefevre, C. Cruciani-Gulielmacci, C. Magnan, F. Yu, K. Niswender, B.G. Irani, W.L. Holland, and D. Clegg. 2009. Palmitic acid mediates hypothalamic insulin resistance by altering PKC-θ subcellular localization in rodents. J. Clin. Invest., 119(9):2577-2589.

Cunnane, S.C. and M.J. Anderson. 1997. Pure linoleate deficiency in the rat: influence on growth, accumulation of n-6 polyunsaturates, and [1^{-14} C] linoleate oxidation. J. of Lipid Research, 38:805-812.

Dagne, K. and A. Jonnson. 1997. Oil content and fatty acid composition of seeds of Guizotia Cass (Compositae). J Sci Food Agric, 73:274-278.

Demirbas, A. 2008. Biodiesel: A realistic fuel alternative for diesel engines. Spring, London, UK.

Geleta, M., S. Stymne, and T. Bryngelsson. 2011. Variation and inheritance of oil content and fatty acid composition in niger (Guizotia abyssinica). J Food Comp. Anal. 24(7): 95-1003.

Mensink R., P. Zock, A. Kester, and M. Katan. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nutr., 77:1146-55.

Miller, J. F., D. C. Zimmerman, and B. A. Vick. 1987. Genetic control of high oleic acid content in sunflower oil. Crop Sci., 27:923–926.

Patil, S. and C. Chan. 2005. Palmitic and stearic fatty acids induce Alzheimer-like hyperphosphorylation of tau in primary rat cortical neurons. Neurosci. Lett., 384(3):288-293.

Quinn, J. and R.L. Myers. 2002. Nigerseed: Specialty Grain Opportunity for Midwestern US. ASAH Press, Alexandria, VA.

Ramadan, M.F. 2012. Functional properties, nutritional value, and industrial applications of niger oilseeds (*Guizotia abyssinica* Cass.). Critical Rev. in Food Sci.and Nutr. 52(1):1-8.

Ramadan, M.F. and J.T. Morsel. 2002. Proximate neutral lipid composition of niger (Guizotia abyssinica Cass.) seed. Czech J. Food Sci., 20: 98–104.

Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56(8):365-79.

Wild Delight. 2015. Nyjer Seed. D & D Commodities Ltd. http://www.wilddelight.com/products/nyjer-seed/ (accessed 23 March 2015).

Appendix

Tables

Table 3.1. Correlation coefficients between seed yield and fatty acid traits as well as seed number and fatty acid traits for parents, F1's, and F2's at the East Tennessee (ETREC) Plateau (PREC) Research and Education Centers in 2014.

Year	Plant Material	Yield Traits	Fatty Acid Traits		
			Linoleic	Oleic	Palmitic
				%	
2014	Parents, F_1's, and F_2's	Yield	0.06	-0.17**	0.27**
	Parents	Yield	0.26	-0.17	0.04
	F_1's	Yield	-0.15	0.01	0.02
	F_2's	Yield	0.12*	-0.24**	0.38**
	Parents, F_1's, and F_2's	Seed Number	0.07	-0.21**	0.38**
	Parents	Seed Number	0.34*	-0.27	0.11
	F_1's	Seed Number	-0.03	-0.1	0.12
	F_2's	Seed Number	0.09	-0.25**	0.50**

† Significant at $P<0.01$ and $P<0.05$ is indicated by ** and *, respectively.

Table 3.2. Correlation coefficients between fatty acid traits for parents, F1's, and F2's at the East Tennessee (ETREC) Plateau (PREC) Research and Education Centers in 2014.

Year	Plant Material		Fatty Acid Traits	
			Linoleic	Oleic
			%	
2014	Parents, F_1's, and F_2's	Oleic	-0.84**	-
		Palmitic	0.15**	-0.22**
	Parents	Oleic	-0.86**	-
		Palmitic	0.55**	-0.22
	F_1's	Oleic	-0.85**	-
		Palmitic	0.07	-0.12
		Oleic	-0.84**	-
	F2's	Palmitic	0.16**	-0.27**

† Significant at P<0.01 and P<0.05 is indicated by ** and *, respectively.

Figures

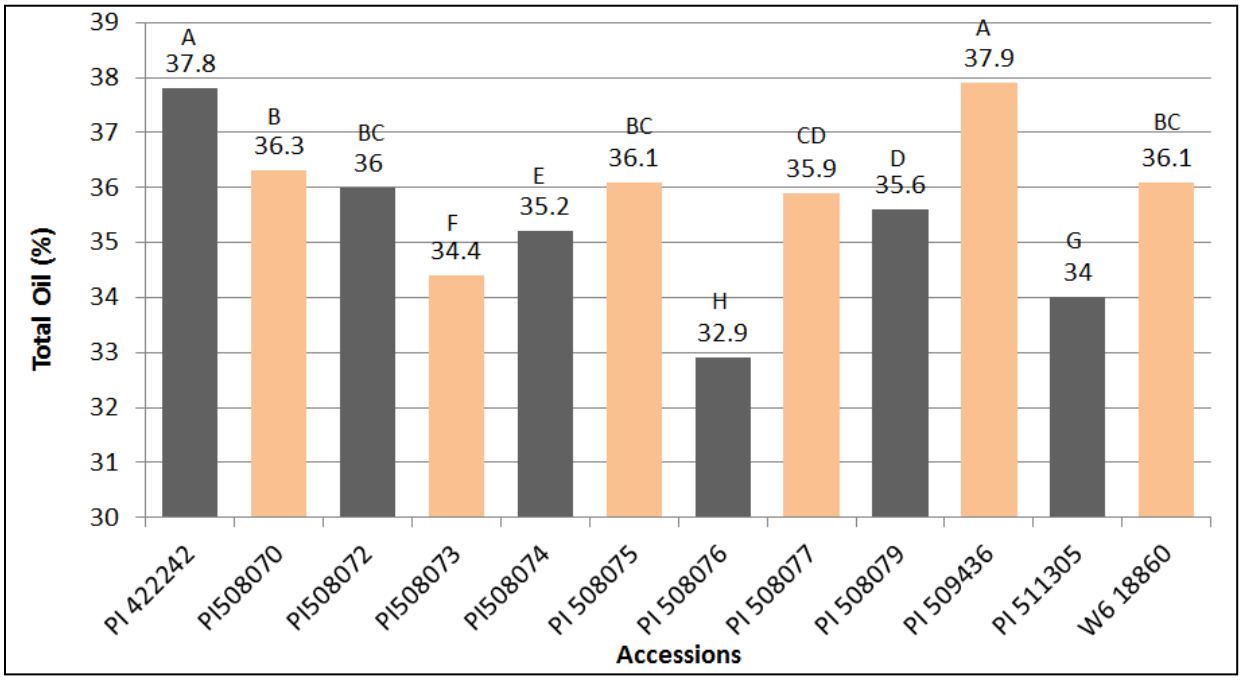


Fig. 3.1. Mean comparison of seed oil content of 12 niger accessions evaluated at the East Tennessee Research and Education Center, Knoxville in 2012. Seed were analyzed at the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA (Dr. Ming Li Wang) using a Bruker mq10 minispec NMR analyzer (Resonance Instruments, Whitney Oxfordshire, UK).
† Bars with a letter in common are not significantly different based on Fisher's LSD (P< 0.05).

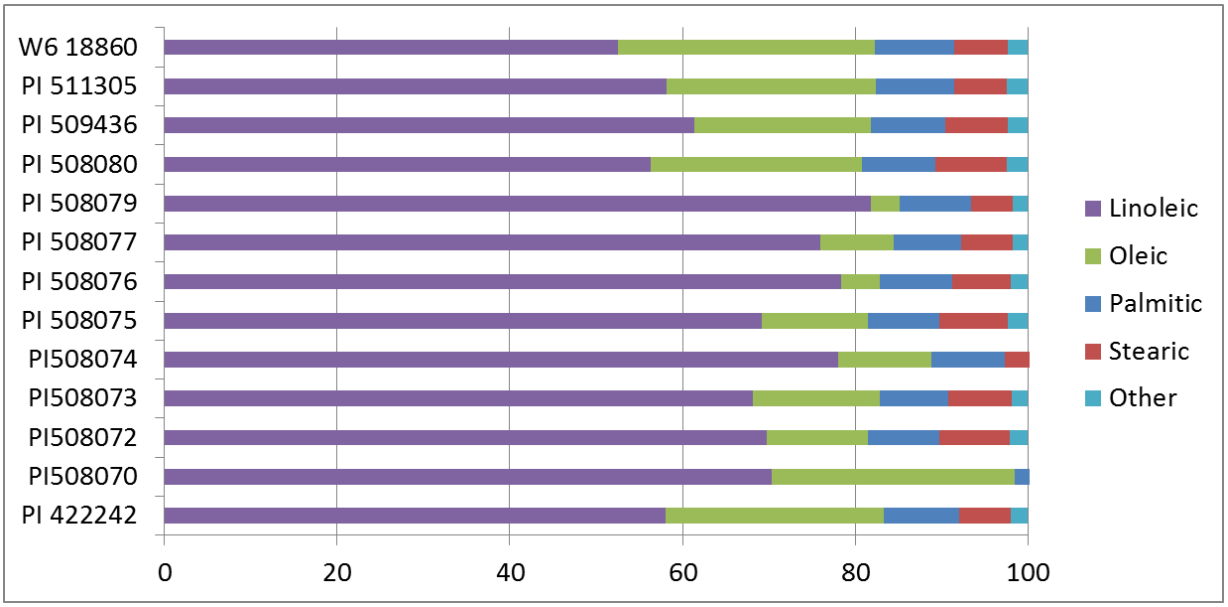


Fig. 3.2. Linoleic, oleic, palmitic, and stearic fatty acid percentages using the mean of two different gas chromatograph models for seed collected from the East Tennessee Research and Education Center, Knoxville in 2012. Seed was analyzed at the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA (Dr. Ming Li Wang) using Agilent 7890A. Different samples of the same seed were later analyzed at the University of Tennessee using Agilent 7683. Means were averaged from both tests and ranked by accession according. Significant differences were found among accessions.

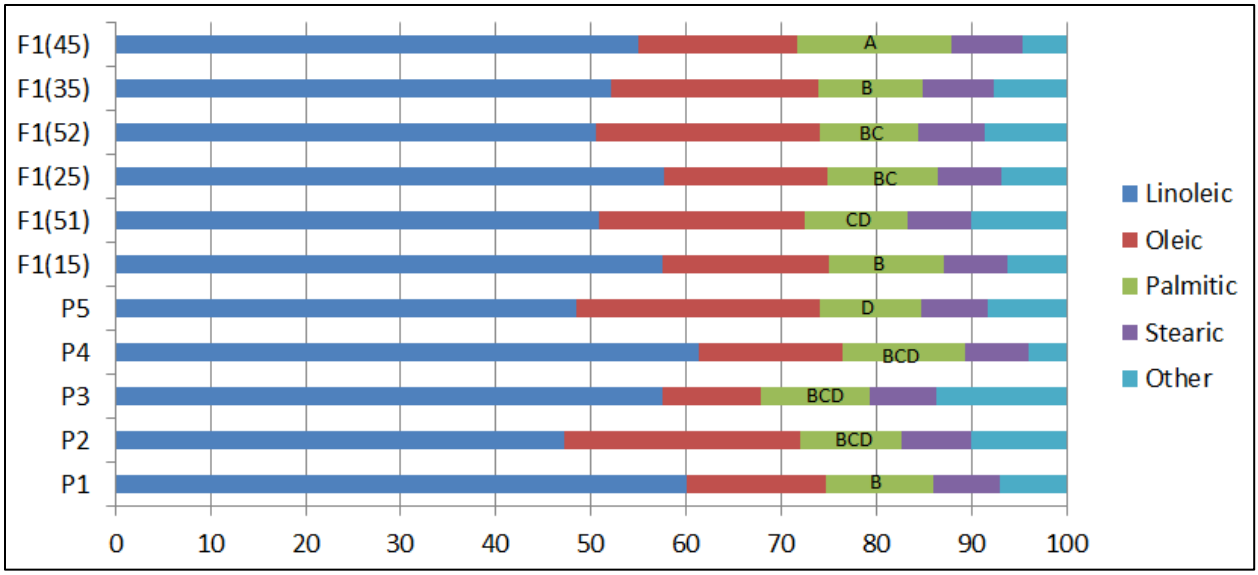


Fig. 3.3. Average linoleic, oleic, palmitic, and stearic fatty acid (%) for parents (P) and F_1 progeny in 2014 at the East Tennessee (ETREC) and Plateau (PREC) Research and Education Centers. P1, P2, P3, P4, P5, F_1(15), F_1(51), F_1(25), F_1(52), F_1(35), and F_1(45) had 12, 10, 7, 12, 20, 16, 7, 22, 23, and 22 observations for linoleic acid, 12, 10, 7, 12, 20, 16, 7, 22, 23, and 21 observations for oleic acid; and 12, 10, 7, 12, 20, 16, 7, 22, 23, and 22 observations for palmitic and stearic acid, respectively. Palmitic acid was the only fatty acid with significant differences. Plant material was significantly different for palmitic acid only.

† Parents and F_1's with a letter in common are not significantly different based on Fisher's Least Significant Difference ($P< 0.05$).

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are the reciprocal F_1 crosses. F_1(15) shows that P1 was the maternal parent plant and P5 was the paternal parent plant.

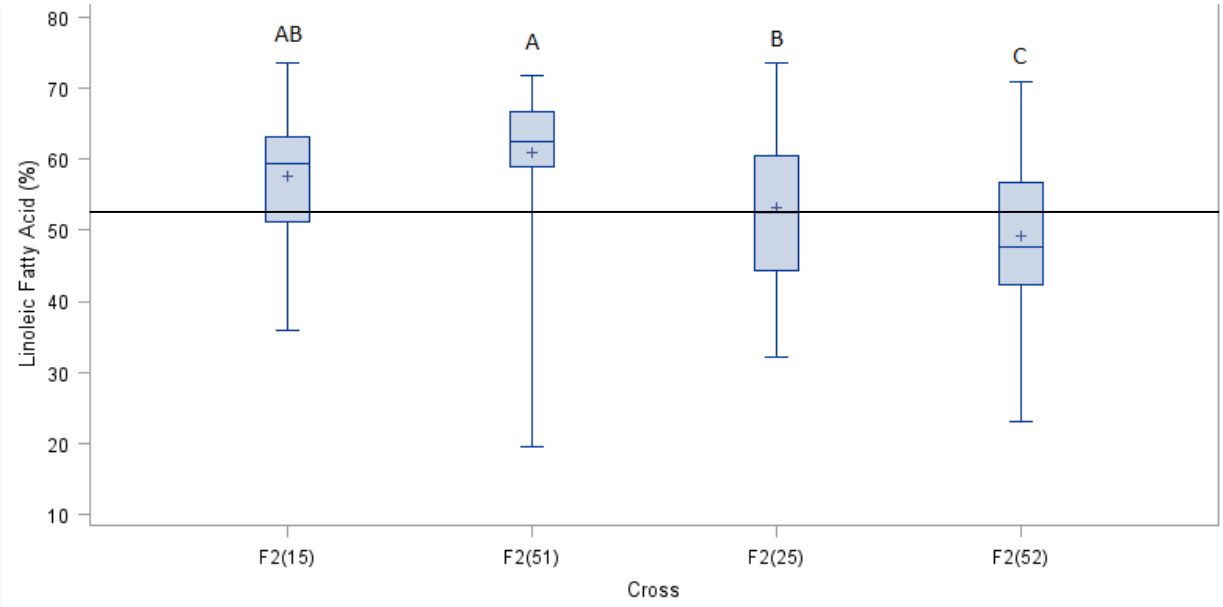


Fig. 3.4. Boxplots showing linoleic fatty acid (%) variation of F_2's in 2014 at the East Tennessee (ETREC) and Plateau (PREC) Research and Education Centers. F2(15), F2(51), F2(25), F2(52), F2(35), and F2(45) had 29, 41, 89, and 114 observations, respectively.
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are the reciprocal F_1 crosses. F_1(15) shows that P1 was the maternal parent plant and P5 was the paternal parent plant. F_2(15) shows that two F_1(15) plants were crossed with one another.
§ F_2(35) and F_2(45) from were missing due to poor germination and disease.

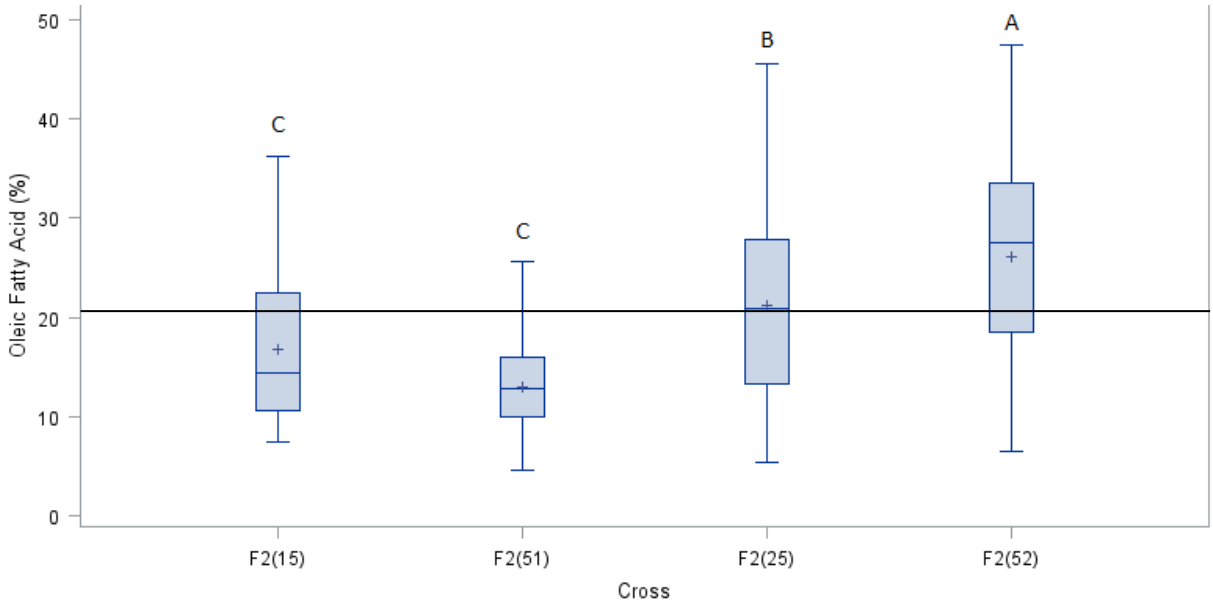


Fig. 3.5. Boxplots showing oleic fatty acid (%) variation of F_2's in 2014 at the East Tennessee (ETREC) and Plateau (PREC) Research and Education Centers. F2(15), F2(51), F2(25), F2(52), F2(35), and F2(45) had 29, 41, 89, and 114 observations, respectively.
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are the reciprocal F_1 crosses. F_1(15) shows that P1 was the maternal parent plant and P5 was the paternal parent plant. F_2(15) shows that two F_1(15) plants were crossed with one another.
§ F_2(35) and F_2(45) from were missing due to poor germination and disease.

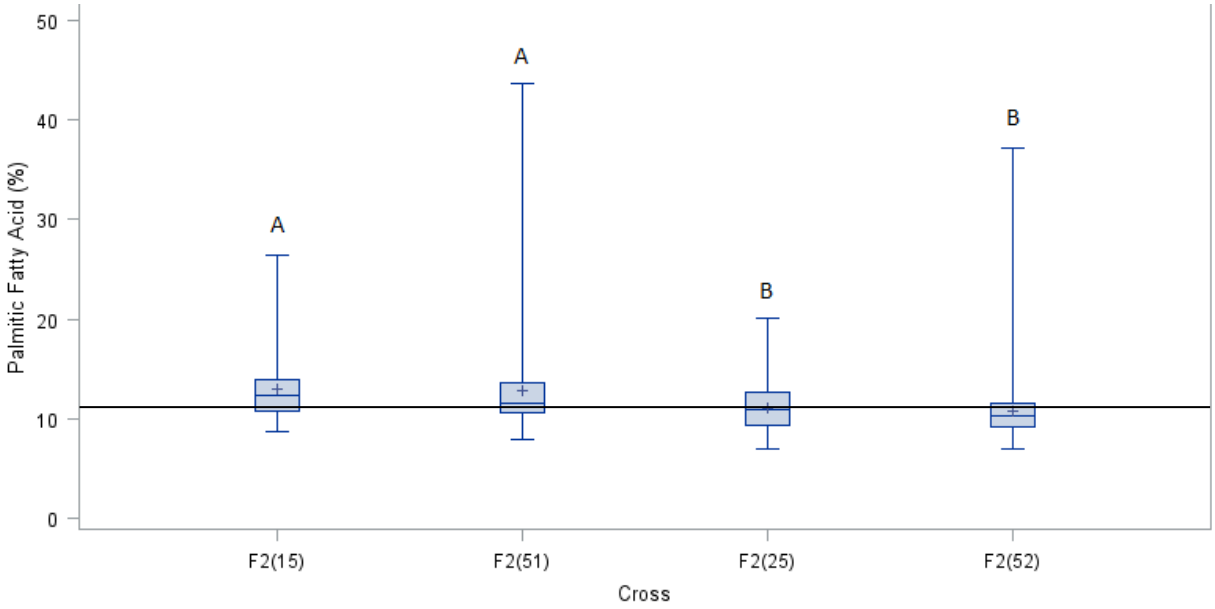


Fig. 3.6. Boxplots showing palmitic fatty acid (%) variation of F_2's in 2014 at the East Tennessee (ETREC) and Plateau (PREC) Research and Education Centers. F2(15), F2(51), F2(25), F2(52), F2(35), and F2(45) had 29, 41, 89, and 114 observations, respectively.
† Bars with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) and F_1(51), for example, are the reciprocal F_1 crosses. F_1(15) shows that P1 was the maternal parent plant and P5 was the paternal parent plant. F_2(15) shows that two F_1(15) plants were crossed with one another.
§ F_2(35) and F_2(45) from were missing due to poor germination and disease.

CHAPTER IV
GENERATION MEANS ANALYSIS OF SEED YIELD, MORPHOLOGICAL TRAITS, AND FATTY ACIDS AMONG CROSSES OF NIGER

Abstract

In the United States, niger (*Guizotia abyssinica* (L.f) Cass.) is primarily marketed as a seed of choice for American goldfinch (*Spinus tristis*) as well as other song and ground feeding birds because of its high oil content. In countries such as Ethiopia and India, niger is grown as an edible oilseed crop. The objectives of this study were to determine: (i) genetic variance, (ii) broad sense heritability, (iii) and gene effects using generation mean analysis of selected crosses. The traits analyzed included seed yield plant^{-1} [per plant], seed number plant^{-1}, branches plant^{-1}, capitula plant^{-1}, linoleic acid, oleic acid, and palmitic acid content. Fourteen niger plant introductions (PIs) of Indian, Ethiopian, and American origin were obtained from USDA/ARS germplasm collection at Pullman, WA. Ten replications of the 14 plant introductions were planted under field and greenhouse conditions at the East Tennessee Research & Education Center in Knoxville in August, 2012 using a completely randomized design. After initial assessments were made, 3 of the PIs (plant introductions) were then selected for further evaluation, and crosses were made to produce four F_1 [filial 1], eight BC [backcross], and four F_2 [filial 2] populations. Seed were planted at the East Tennessee (2013 and 2014), Highland Rim (2013 and 2014), and Plateau (2014) Research and Education Centers. With the exception of seed yield, all traits had the greatest genetic variance in the F_2 population. Results showed zero to moderate (0.00-0.44) broad sense heritability for all traits. Dominance gene effects were most influential on all traits. Epistatic gene effects were significant and had equal influence on traits as additive gene effects.

Introduction

Quantitative traits such as yield are controlled by multiple genes. This makes it difficult to separate individuals of a population into discreet classes. Populations can be expressed by the amount and type of genetic variability it contains. These components include: phenotypic, genotypic, additive, dominance, interaction, and environmental (Fehr, 1987). Estimating the relative magnitude of these components determines the genetic properties of the population as well as the degree of relatedness between relatives. Genetic variance among individuals of a population is the function of the additive, dominance, epistatic interaction, and gene frequencies. The level of genetic variance is therefore unique to the population from which the components were obtained (Falconer, 1960). Generation means analysis is useful because it allows for the separation and estimation of gene effects involved in the inheritance of quantitative traits along with their relative importance (Gamble, 1961). Generation means analysis is reliable and has smaller sampling errors than variance component analysis. Estimates of epistasis are more progressive because experiments are smaller and easier to carry out. A study involving 35 niger accessions analyzed the heritability for linoleic, oleic, palmitic, and stearic fatty acid resulted in high broad sense heritability (>0.90) for all traits except linoleic (0.79) (Yadav et al., 2014). Marinkovic et al. (2006) studied the gene effects on biomass of sunflower and showed significant additive, dominant, and epistatic interaction. Dominance effects were most influential of all gene effects in both years of their study. No gene effect research has been conducted thus far on niger. The objective of this study is to determine genetic variance and gene effects for seed yield and yield components (height, capitula plant^{-1}, seeds capitulum^{-1}, and number of branches

plant^{-1}) as well as fatty acids (linoleic, oleic, palmitic, and stearic) among niger accessions as well as selected crosses among 3 accessions.

Materials and Methods

Information on the five selected parents and the F_1 and F_2 progeny from six crosses is presented in Chapter 2, page 31. BC progeny were also produced so that mean information could be included in the generation means analysis to estimate gene effects. Parent accessions used to produce BC progeny were PI 422242, PI 511305, and W6 18860; coded as P1, P2, and P5, respectively. F_1 seed were harvested December thru January, 2013 and a portion was planted in the greenhouse to produce backcross (BC) progeny. Pollen was collected and bulked among plants within each cross [e.g. F_1(15)], and the bulked pollen was used to pollinate individual capitulum (flowers) on several plants within the cross [e.g. F_1(15), F_1(51), etc.]. The BC seed were produced by crossing four F_1 crosses [F_1(15), F_1(51), F_1(25), and F_1(52)] and its reciprocal to both respective parents (F_1's were used as the maternal parents). The eight resulting backcrosses were used in the generation means analysis [e.g. BC151, BC155, etc.]. Mature F_2 and BC seed were harvested in June, 2013. The BC progeny were included along with the parents, F1 and F2 progeny in the space planted nurseries at the ETREC (2013 and 2014), HRREC (2013) and PREC (2014) described in Chapter 2.

Statistical Analysis

Data were collected on: seed yield plant^{-1} (g) (2013 and 2014), seed plant^{-1} (n) (2013 and 2014), total branches plant^{-1} (2013 and 2014), total capitula plant^{-1} (2013), and linoleic, oleic, palmitic, and stearic fatty acids (2014). Seed yield, seed plant^{-1}, branches, and capitula were collected from ETREC and HRREC in 2013. In 2014, data for branches were collected from ETREC, HRREC, and PREC, whereas seed yield, seed plant^{-1}, and fatty acid data were only collected from ETREC and PREC.

Backcross Means

Mixed model analysis of variance and Fisher's LSD ($P<0.05$), SAS 9.3 (Cary, NC) were used to evaluate selected backcrosses across all years and locations using a randomized complete block design with replication (BC plants were planted at the same locations as parents, F_1's, and F_2's). Branch data were analyzed across multiple years and locations, year, location, plant material (backcross), plant material x year interaction, and plant material x location interaction were included as fixed effects. If significant plant material x year interaction was found, years were analyzed separately. Likewise, if significant plant material x location interaction was found, locations were analyzed separately. For traits analyzed by individual years and multiple locations, location, plant material, and plant material x location interaction were included as fixed effects. In all analyzes, block and plant material x block interaction were included as random effects. If an analysis showed insignificant plant material differences, analyses were conducted across locations and/or years.

Genetic Variance

A mixed model analysis of variance using SAS 9.3 (Cary, NC) determined genetic variance for combined parents, combined F_1's, combined F_2's, combined BC's, as well as all populations combined together. Genetic variance was reported directly from the covariance parameter estimate output, "cross", using PROC Mixed (SAS 9.3). Other random terms in the model included location and cross x location interaction since years were analyzed individually. The "cross" covariance parameter estimate was divided by the sum of all covariance parameter estimates in the model to calculate broad sense heritability for combined F_2's:

$H^2=V_G/V_P$

where:

H_2=Broad Sense Heritability

V_G=Genetic Variance

V_P=Phenotypic Variance

Gene Effects

Generation means analysis was used to estimate gene effects based on the work of Gamble (1961). Estimates were taken from contrast statements using mixed model analysis of variance in SAS 9.3, and recorded for each F_1 and related generations (P1, P2, F_2, $P2F_1$, and $P2F_1$) to obtain estimates of gene effects of the mean (m), additive (a), dominance (d), and epistasis (aa, ad, dd). Estimates of the above effects were computed for each cross as follows:

$m= F_2$

$a=P_1F_1-P_2F_1$

$d=-1/2P_1-1/2P_2+F_1-4F_2+2P_1F_1+2P_2F_1$

$aa=-4F_2+2P_1F_1+2P_2F_1$

$ad=-1/2P_1+1/2P_2+P_1F_1-P_2F_1$

$dd=P_1+P_2+2F_1+4F_2-4P_1F_1-P_2F_1$

where:

F_2= F_2 mean

P1= Female parent one mean

P2= Male parent two mean

F_1= F_1 mean

$P1F_1$= Backcross mean (backcross F_1 to female parent)

$P2F_1$= Backcross mean (backcross F_1 to male parent)

(Gamble, 1961).

Results

Roughly 85% of the plants at the HRREC location were affected by Southern Blight (*Sceroltium rolfsii*) in 2014. This soil-borne disease first infects the lower stems, then caused dieback and death. The blight was most active in the middle of the season (July and August), causing the capitula to senesce before they had time to pollinate and produce seed. Total seed yield (g) and total seed number were thereby affected, and the entire location was not included for these traits. Results for parents, F_1's, and F_2's can be found in Chapter 2 starting on page 38.

Backcross Means

Seed Yield Plant^{-1} (g)

Backcrosses were analyzed separately by year. In 2013, BC results showed no significant cross or cross x location effects, but did show significant location differences. After pooling, cross remained insignificant. In 2014, backcross results showed that cross and location were significant, and cross x location interaction was not (Fig. 4.1). Yield ranged from 5.9 g (BC525) to 20.3 g plant^{-1} (BC511). BC515 and BC255 were not included in the figure due to poor germination and disease. The average backcross seed yield plant^{-1} was 12.7 g. Seed yield was lower for BC155 and BC525 (7 and 6, respectively) where F_1(15) and F_1(52) were pollinated with P5 (W6 18860) instead of the other parent (16 and 11, respectively).

Seed Plant^{-1} (n)

Backcrosses were analyzed separately by year. In 2013, BC results showed significant location and cross x location effects. Cross effects were insignificant (Fig. 4.2A). Backcross populations at ETREC resulted in a greater number of seed than populations at HRREC. BC255-E was the highest yielding population. In 2014, backcrosses resulted in significant cross, location, and cross x interaction effects (Fig. 4.2B). Plants grown at ETREC resulted in more seed (7,268 seed) than those grown at PREC (2,014 seed). BC511-E had significantly more seed (14,996) than all other backcross populations. BC151 resulted in more seed when F_1(15) was crossed to P1 rather than P5 at ETREC in 2014 (8205 vs. 2679).

Branches Plant^{-1} (n)

Backcrosses were analyzed separately by year. In 2013, backcrosses showed that cross and cross x location were not significant, but location was. After pooling, cross was still insignificant. In 2014, backcrosses resulted in significant cross and location effects (Fig. 4.3). BC525 had significantly fewer branches than the other backcrosses. Backcrosses were analyzed with year (2013 and 2014) and location (ETREC and HRREC) effects. Backcrosses showed that cross was the only significant fixed effect (Fig. 4.3). BC522 had the greatest number of branches.

Capitula Plant^{-1}(n)

Capitula notes were only taken in 2013. Backcrosses resulted in no significant fixed effects (cross and cross x location). After pooling, cross was still insignificant. Overall, means ranged from 279 (BC151) to 883 (BC515) capitula plant^{-1}.

Linoleic, Oleic, Palmitic, and Stearic Fatty Acid (%),

Results showed no significant differences among backcrosses for any of the fatty acids. There were no significant location or cross x location effects. Linoleic and oleic acid ranged from 47-57% and 17-25%, respectively. Palmitic and stearic acid ranged from 11-13% and 7-8% respectively.

Genetic Variance

The genetic variance and broad sense heritability estimates for the F_2 populations varied by year and location and were generally very low for all of the traits. This illustrates the large environmental effects on these traits, and the small and slow genetic gain that could be made from selection in the crosses studied. In 2014, all F_1's showed no genetic variance for seed yield plant^{-1} (Table 4.1) across locations. Data combined over both years showed no genetic variance or broad sense heritability for parents, F_1's, backcrosses, and F_2's. The greatest genetic variance existed in 2013 among the parent generation at ETREC (38.7). In 2014, broad sense heritability of F_2's ranged from 0 to 0.19. In 2014, broad sense heritability was greatest at ETREC in comparison to the other locations.

Results for total seed plant^{-1} (n) can be seen in Table 4.2. Parents showed the greatest genetic variance. In 2013, all backcrosses resulted in no genetic variance. Genetic variation was greatest overall in 2013 from the parent population at ETREC (17,019,745). The F_2 population had the greatest genetic variance overall in both years, ranging from 291,483 to 4,519,130. In 2013, broad sense heritability ranged from 0.03 to 0.15, and ranged from 0.04 to 0.17 in 2014. Broad sense heritability was greatest at HRREC in 2013, and ETREC in 2014.

The genetic variance results for total branches plant^{-1} (n) are presented in Table 4.3. The parents exhibited the greatest genetic variance of all plant materials. Genetic variance among parents was 0 in when years were combined. Genetic variance was overall greatest in the parent population in 2014, ranging from 6.4 to 17.3 (Table 4.3). Broad sense heritability ranged from 0 to 0.22. Broad sense heritability was greatest at PREC in 2014.

Results for total capitula plant^{-1} (n) can be seen in Table 4.4. The parents, F_1's, backcrosses, and F_2's exhibited no genetic variance for capitula plant^{-1} when combined across locations. Genetic variance was greatest in the F_1 population at ETREC (24,956). Broad sense heritability in 2013 was 0 at ETREC and 0.44 at HRREC, (Table 4.4).

The genetic variance and heritablilty estimates for the linoleic, oleic and palmitic acids follow similar trends to the above traits in that the values are generally low and vary by year and location (Tables 4.5-4.7). For linoleic acid, the F_1's showed a lower genetic variance than P's, F_2's, and backcrosses (Table 4.5). Broad sense heritabilities among F_2's at E+P (0.03) and E (0.01) were relatively lower than at P (0.27). Similarly for oleic acid, the F_1's showed a lower genetic variance than P's, F_2's, and backcrosses (Table 4.6). Broad sense heritabilities among F_2's were lower at E+P (0.11) and E (0.01) than at P (0.32). Genetic variance was lower for palmitic acid (Table 44.7) than oleic and linoleic acids. Broad sense heritabilities ranged from 0 (E) to 0.10 (P).

Gene Effects

Evaluations of the gene effects in this study indicate that dominance gene effects tended to be the most important in the inheritance for most of the traits. Epistatic interactions, especially additive x additive, tended to have a positive effect whereas additive gene effects were

often negligible or negative. These findings support those of Gamble (1966) in which dominance > epistatic > additive gene effects for yield in corn. In the study herein, with the exception of P5xP1, additive gene effects were negative; having a diminishing effect on seed yield; whereas dominance gene effects were positive (Table 4.8). Of all three types of digenic epistasis, additive x additive gene effects were most positive.

Dominance gene effects were also the most important in the inheritance for total seed plant^{-1} (Table 4.9). Epistatic gene effects were also important in seed numbers. Additive x additive gene effects appeared to be more important than additive x dominance and dominance x dominance gene effects.

Additive, dominance, and epistatic gene effects have roughly equal positive and negative estimates for total branches plant^{-1} (n) inheritance (Table 4.10). Dominance gene effects appear to have a greater magnitude than additive gene effects. Additive x additive and dominance x dominance epistatic gene effects appear to be equally important in branch inheritance.

Dominance gene effects appear to be more important than additive gene effects in the inheritance of total capitula plant^{-1} (Table 4.11). Additive x additive gene effects were the most influential of all three digenic epistatic gene effects.

Relative to fatty acid composition, the cross, P1xP5, had the greatest mean effects of all crosses for linoleic fatty acid (Table 4.12). P2xP5 had the greatest additive effects. Values for d and dd were not estimable. F_1 crosses using P5 as the male parent resulted in greater additive gene effects than their reciprocals. Means for P1xP5 and P5xP2 were greater than P5xP1 and P2xP5, respectively. Contrastingly, additive x additive gene effects were greater in P5xP1 and P2xP5 than P1xP5 and P5xP2, respectively. The cross, P2xP5, had the greatest mean effects of

all crosses for oleic acid (Table 4.13). The cross, P5xP2, had the greatest additive effects. Again, values for d and dd were not estimable. Means for P1xP5 were lower than P5xP1, and means for P5xP2 were lower than P2xP5 with the exception of the Plateau location. Additive x additive gene effects were greater for P1xP5 and P5xP2 than P5xP1 and P2xP5, respectively. P1xP5 and P5xP2 had the greatest mean effects of all crosses for palmitic fatty acid (%) (Table 4.14). Values for d and dd were not available.

Conclusion

Seed yield ranged between 5.9g (BC525) and 20.3g (BC511), and averaged 12.7g (Fig. 4.1). Seed yield was lower for BC155 and BC525 (7 and 6, respectively) where F_1(15) and F_1(52) were pollinated with P5 (W6 18860) instead of the other parent (16 and 11, respectively) (Fig. 4.1). Seed plant^{-1} was greater at ETREC than HRREC in 2013 (Fig. 4.2A) and greater than PREC in 2014 (Fig. 4.2B). BC255-E and BC511-E resulted in the greatest number of seed plant^{-1} in 2013 and 2014, respectively (Fig. 4.2A and B). Reciprocal effects were evident between BC151 and BC155 when P1 was used as the male parent. Seed plant^{-1} was greater than when P5 was used as the male parent (8205 and 2679, respectively) in 2014 (Fig. 4.2B). BC525 resulted in significantly fewer branches than other backcrosses in 2014 (Fig. 4.3). When data from 2013 and 2014 were combined, BC522 resulted in the greatest number of branches (Fig. 4.3).

The genetic variance for total seed yield plant^{-1} was relatively low over all year combinations (Table 4.2). The relative magnitude of genetic variance for total seed plant^{-1} (Table 4.3) and total branches were also low in 2013 and 2014. Total capitula at HRREC showed greater broad sense heritability (0.44) than any other trait (Table 4.4). This indicates moderate

presence of genetic variance for capitula. F_1's showed lower genetic variance than P's, F_2's, and BC's for linoleic and oleic fatty acid (Table 4.6 and 4.7).

F_1(52) appeared to have the greatest mean performance and dominance effects for seed yield (Table 4.8), seed plant^{-1} (Table 4.9), branches (Table 4.10), capitula (Table 4.11), linoleic acid (Table 4.12), oleic acid (Table 4.13), and palmitic acid (Table 4.14). P1xP5 and P5xP2 crosses means were greater than the respective reciprocal crosses for linoleic acid, but lower for oleic acid. Similar contrasting results were seen for additive x additive gene effects, where P5xP1 and P2xP5 were greater than the reciprocal crosses for linoleic, but lower for oleic. Reciprocal effects are evident where F_1 crosses using P5 as a male parent result in greater additive effects if the other parents were used as the male parent.

Estimates of genetic variance and heritability may be used to assist plant breeders with answering critical questions about their program. The first question that should be addressed by breeders interested in making improvements in niger is what type of varieties to develop. Results from generation means analysis indicate the presence of dominance, additive x dominance, and dominance x dominance gene effects. This, along with the high-parent heterosis seen among F_1 progeny for seed yield, seed plant^{-1}, capitula, and seed capitulum^{-1} in the mean analyses, encourages the use of a semi-hybrid breeding program (Brummer, 1999). Due to self-incompatibility in niger, parents should be separated by heterotic groups, accessed by test crossing for combining ability, and allowed to cross pollinate within each group. Improvements may be made to each heterotic group by using recurrent selection. Crossing heterotic groups should result in substantial heterosis.

Another question is how extensively (in terms of years, locations, replications) must the breeding material be tested to: a) identify superior genetic populations or b) identify superior parents within these populations (Dudley, 1969)? When using generation means analysis, smaller experiments are possible due to smaller sampling errors than variance estimates. Even so, there was a great deal of missing data from dominance, additive x dominance, and dominance x dominance gene effects. It is recommended for the entire study to be carried out in at least two more locations, where full sets of data prevent missing gene effect values.

Another important question that should be answered is whether genetic variance is great enough within the germplasm pool to allow primary character improvement (Dudley, 1969). Gene effect estimates of the plant materials indicate that dominance makes up a considerable amount of genetic variance.

Literature Cited

Brummer, C. 1999. Capturing heterosis in forage crop cultivar development. Crop Sci. 39(4):943-954.

Dudley, J.W. and R.H. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9(3):257-262.

Falconer, D.S. 1960. Introduction to quantitative genetics. Robert MacLehose and Company Limited, Glasgow.

Fehr, E.L. and H.J. Jessen. 1987. Principles of cultivar development. Vol. 1. Macmillan Pub. Co., NY.

Gamble, E.E. 1961. Gene effects in corn (Zea Mays L.)- II. Relative importance of gene effects for plant height and certain component attributes of yield. Canadian J. of Plant Sci. 42:349-358.

Marinković, R., D. Jovanović, and J. Joksimović. 2006. Gene action for hectoliter mass in sunflower (Helianthus annuus L.). Helia 29(44):95-100.

Wright, S.1968. Evolution and genetic of population. *Volume I. Genetic and Biometric Foundation*. University of Chicago Press, Chicago. pp. 371-420.

Yadav, S. Z. Hussain, P. Suneja, M.A. Nizar, S.K. Yadav, and M. Dutta. 2014. Genetic divergence studies in niger (Guizotia abyssinica) germplasm. Biomass and Bioenergy 44:64-69.

Appendix

Tables

Table 4.1. Estimates of genetic variance and broad sense heritability for seed yield plant^{-1} (g) among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) (2013 and 2014), Highland Rim (H) (2013), and Plateau (P) (2014) Research and Education Centers.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material †**				
Year	**Location**	Parents	F_1	F_2	BC	F_2
2013	E+H	8.8	14.9	11.8	18.3	0.09
	E	38.7	38.0	10.7	22.5	0.09
	H	0.8	35.8	1.6	0	0.10
2014	E+P	4.6	0	0	1.6	0
	E	6.6	0	32.6	0	0.19
	P	4.5	0	2.3	0	0.07

† Number of observations:
2013; E+H; Parents= 67; F_1=169; F_2=381; BC=118.
2013; E; Parents= 37; F_1=106; F_2=354; BC=83.
2013; H; Parents= 30; F_1=63; F_2=27; BC=35.
2014; E+P; Parents= 44; F_1=101; F_2=274; BC=103.
2014; E; Parents= 4; F_1=23; F_2=65; BC=31.
2014; P; Parents= 40; F_1=78; F_2=209; BC=72.

Table 4.2. Estimates of genetic variance and broad sense heritability for total seed plant^{-1} among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) (2013 and 2014), Highland Rim (H) (2013), and Plateau (P) (2014) Research and Education Centers.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material †**				
Year	**Location**	Parents	F_1	F_2	BC	F_2
2013	E+H	3,305,165	0	4,519,130	0	0.03
	E	17,019,745	4,578,166	4,099,575	0	0.03
	H	0	6,330,327	2,862,336	0	0.15
2014	E+P	585,917	42,592	465,409	2,086,820	0.04
	E	1,855,584	0	3,999,983	4,218,523	0.17
	P	451,374	8293	291,483	0	0.11

† Number of observations:
2013; E+H; Parents= 67; F_1=169; F_2=381; BC=118.
2013; E; Parents= 37; F_1=106; F_2=354; BC=83.
2013; H; Parents= 30; F_1=63; F_2=27; BC=35.
2014; E+P; Parents= 44; F_1=101; F_2=274; BC=103.
2014; E; Parents= 4; F_1=23; F_2=65; BC=31.
2014; P; Parents= 40; F_1=78; F_2=209; BC=72.

Table 4.3. Estimates of genetic variance and broad sense heritability for total branches plant^{-1} among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) (2013 and 2014), Highland Rim (H) (2013 and 2014), and Plateau (P) (2014) Research and Education Centers.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material †**				
Year	**Location**	Parents	F_1	F_2	BC	F_2
2013	E+H	15.1	4.0	0	0	0
	E	11.5	1.8	2.9	0.1	0.04
	H	5.6	12.2	0	0	0
2014	E+H+P	9.8	3.2	9.8	8.1	0.17
	E	13.0	14.6	11.1	9.8	0.19
	H	6.4	0	11.9	3.5	0.18
	P	17.3	1.6	9.8	6.8	0.22
2013/2014	E+H	0	0	0.5	2.7	0.01
	E	0	2.0	2.6	2.0	0.04
	H	0	6.9	0	1.5	0

† Number of observations:
2013; E+H; Parents= 86; F_1=190; F_2=407; BC=122.
2013; E; Parents= 43; F_1=105; F_2=370; BC=82.
2013; H; Parents= 43; F_1=85; F_2=37; BC=40.
2014; E+H+P; Parents= 123; F_1=205; F_2=512; BC=194.
2014; E; Parents= 15; F_1=32; F_2=75; BC=35.
2014; H; Parents= 53; F_1=79; F_2=203; BC=66.
2014; P; Parents= 55; F_1=94; F_2=234; BC=93.
2013 and 2014; E+H; Parents= 154; F_1=301; F_2=685; BC=223.
2013 and 2014; E; Parents= 58; F_1=137; F_2=445; BC=117.
2013 and 2014; H; Parents= 96; F_1=164; F_2=240; BC=106.

Table 4.4. Estimates of genetic variance and broad sense heritability for total capitula plant^{-1} (n) among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) (2013) and Highland Rim (H) (2013) Research and Education Centers.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material**				
Year	**Location**	Parents	F_1	F_2	BC	F_2
2013	E+H	0	0	0	0	0
	E	3613.8	24956	0	0	0
	H	1035.2	0	3181.6	413.27	0.44

† Number of observations:
2013; E+H; Parents= 77; F_1=191; F_2=407; BC=121.
2013; E; Parents=42; F_1=107; F_2=373; BC=83.
2013; H; Parents= 35; F_1=84; F_2=34; BC=38.

Table 4.5. Estimates of genetic variance and broad sense heritability for linoleic fatty acid (%) among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) and Plateau (P) Research and Education Centers in 2014.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material**				
Year	**Location**	Parents	F_1	F_2	BC	F_2
2014	E+P	17.4	0	3.4	6.2	0.03
	E	0	4.3	2.08	33.3	0.01
	P	41.1	4.2	28.3	6.2	0.27

† Number of observations:
2014; E+P; Parents= 45; F_1=109; F_2=280; BC=110.
2014; E; Parents=3; F_1=23; F_2=65; BC=29.
2014; P; Parents= 42; F_1=86; F_2=215; BC=81.

Table 4.6. Estimates of genetic variance and broad sense heritability for oleic fatty acid (%) among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) and Plateau (P) Research and Education Centers in 2014.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material**				
Year	**Location**	Parents	F_1	F_2	BC	F_2
2014	E+P	10.8	0	12.2	6.8	0.11
	E	0	0	0.8	18.4	0.01
	P	35.3	3.5	35.9	6.8	0.32

† Number of observations:
2014; E+P; Parents= 45; F_1=108; F_2=280; BC=110.
2014; E; Parents= 3; F_1=22; F_2=65; BC=29.
2014; P; Parents= 42; F_1=86; F_2=215; BC=81.

Table 4.7. Estimates of genetic variance and broad sense heritability for palmitic fatty acid (%) among five niger parents and the six sets of F_1, F_2, and BC progeny evaluated at the East Tennessee (E) and Plateau (P) Research and Education Centers in 2014.

		Genetic Variance				Broad Sense Heritability
Parameter		**Plant Material**				
Year	**Location**	P	F1	F2	BC	F2
2014	E+P	0.1	2.8	0.8	0.4	0.05
	E	2.0	19.9	0	0	0
	P	0.1	0.3	0.4	0.4	0.10

† Number of observations:
2014; E+P; Parents= 45; F_1=109; F_2=280; BC=110.
2014; E; Parents= 3; F_1=23; F_2=65; BC=29.
2014; P; Parents= 42; F_1=86; F_2=215; BC=81.

Table 4.8. Mean estimates of gene effects for four crosses for seed yield plant^{-1} (g) at the East Tennessee (E) (2013 and 2014), Highland Rim (H) (2013), and Plateau (P) (2014) Research and Education Centers.

			Gene Effects					
Year	**Loc.**	**Cross**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2013	E+H	P1xP5	7.79	-14.07	18.88	16.00	-13.08	-31.15
		P5xP1	7.99*	-8.30	13.78	2.47	-9.30	9.41
		P2xP5	7.31	-6.84	52.62**	38.20**	-9.42	-38.77
		P5xP2	8.17*	-15.69**	57.87**	54.86**	-13.12**	-98.39**
	E	P1xP5	10.40**	-13.69	19.36	15.66	-13.80	-39.09
		P5xP1	11.42**	-9.09	13.34	3.80	-8.98	-7.71
		P2xP5	10.06**	-13.02*	69.27**	49.58**	-18.97**	-50.24
		P5xP2	11.46**	-17.19**	54.75**	52.14**	-11.24	-95.11**
	H	P1xP5	6.47	-8.05	n/a	0.52	-8.05	n/a
		P5xP1	0.79	12.29**	n/a	34.45	12.29**	n/a
		P2xP5	3.36	9.09	n/a	7.61	9.09	n/a
		P5xP2	6.35*	-0.03	n/a	25.08	-0.03	n/a
2014	E+P	P1xP5	4.79**	-2.57	n/a	10.53	n/a	n/a
		P5xP1	7.40**	8.42**	n/a	16.55**	n/a	n/a
		P2xP5	11.04**	-10.68**	n/a	15.52	n/a	n/a
		P5xP2	11.57**	-5.45	n/a	-11.65	n/a	n/a
	E	P1xP5	4.91	-3.39	n/a	9.64	n/a	n/a
		P5xP1	10.19**	15.58*	n/a	25.69	n/a	n/a
		P2xP5	11.04	-16.14	n/a	41.01	-16.14	n/a
		P5xP2	22.10**	-5.78	n/a	-44.45	-5.78	n/a
	P	P1xP5	4.68**	-1.75	n/a	11.43*	n/a	n/a
		P5xP1	4.61**	1.25	n/a	7.41	n/a	n/a
		P2xP5	1.04	-5.21	n/a	30.01	n/a	n/a
		P5xP2	7.57**	-5.12	n/a	-4.96	n/a	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa= additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations

Table 4.9. Mean estimates of gene effects for four crosses for total seed plant^{-1} (n) at the East Tennessee (E) (2013 and 2014), Highland Rim (H) (2013), and Plateau (P) (2014) Research and Education Centers.

			Gene Effects					
Year	**Loc.**	**Cross**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2013	E+H	P1xP5	8926.36**	-7322.51	-12156	-14529	-7072.26	13118.00
		P5xP1	7973.52**	-1041.50	5897.73	2681.40	-1291.75	-13773.00
		P2xP5	9934.40**	-4431.06	6303.43	1149.35	-6250.42	-6398.20
		P5xP2	8705.31**	-932.52	4342.31	1413.89	886.84	-6462.25
	E	P1xP5	11918**	-6692.94**	-13246**	-16122**	-7300.74**	9669.59**
		P5xP1	10670**	-1609.36**	4978.25**	2632.45**	-1001.56**	-23907**
		P2xP5	11989**	-8125.54**	14378**	7134.82**	-12208**	-14498**
		P5xP2	11107**	-2145.31**	1811.12**	-1018.38**	1937.11**	-3488.37**
	H	P1xP5	6264.70*	-6309.84*	n/a	-5908.21	-6309.84*	n/a
		P5xP1	942.37	5834.24**	n/a	17574.00	5834.24**	n/a
		P2xP5	2719.63	5520.81	n/a	2236.26	5520.81	n/a
		P5xP2	4278.74*	58.34	n/a	13776.00	58.34	n/a
2014	E+P	P1xP5	1496.70**	-525.74	n/a	3920.69	n/a	n/a
		P5xP1	2450.04**	2999.54**	n/a	4835.37*	n/a	n/a
		P2xP5	3358.67*	-6341.66**	n/a	10999.00	n/a	n/a
		P5xP2	2729.00	-1127.79	n/a	600.27	n/a	n/a
	E	P1xP5	1590.94	-551.37	n/a	4090.18	n/a	n/a
		P5xP1	3614.83**	5525.75	n/a	7308.20	n/a	n/a
		P2xP5	3358.67	-11304**	n/a	25701*	-11304**	n/a
		P5xP2	5109.00	-1025.09	n/a	-4597.03	-1025.09	n/a
	P	P1xP5	1402.47**	-500.12	n/a	3751.21**	n/a	n/a
		P5xP1	1285.26**	473.33	n/a	2362.54	n/a	n/a
		P2xP5	349.00	-1379.03	n/a	8334.94	n/a	n/a
		P5xP2	2640.10**	-1230.50	n/a	-2966.83	n/a	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa=additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations comprising the equation

Table 4.10. Mean estimates of gene effects for four crosses for total branches plant^{-1} (n) at the East Tennessee (E) (2013 and 2014), Highland Rim (H) (2013 and 2014), and Plateau (P) (2014) Research and Education Centers.

			Gene Effects					
Year	**Location**	**Crosses**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2013	E+H	P1xP5	23.39**	-10.81	-22.06	-20.26	-13.06	29.88
		P5xP1	24.09**	8.58	11.92	10.45	10.83	-27.51
		P2xP5	19.58**	-3.09	27.38**	24.06**	-3.29	-41.22**
		P5xP2	20.24**	-0.70	11.25	7.32	-0.51	-9.15
	E	P1xP5	27.49**	-10.76	-23.74	-20.45	-14.34	33.03
		P5xP1	28.69**	8.27	7.83	8.69	11.85	-25.17
		P2xP5	23.40**	0.18	26.30*	21.04*	-0.13	-29.53
		P5xP2	24.12**	-2.32	7.86	4.59	-2.01	-3.50
	H	P1xP5	19.33**	11.69*	n/a	10.04	n/a	n/a
		P5xP1	17.86**	-1.97	n/a	12.06	n/a	n/a
		P2xP5	23.00**	10.71	n/a	-10.57	n/a	n/a
		P5xP2	17.70**	0.93	n/a	6.49	n/a	n/a
2014	E+H+P	P1xP5	26.22**	3.72*	-0.37	1.24	1.69	-13.99
		P5xP1	29.93**	1.23	-24.48*	-22.20*	3.26	16.69
		P2xP5	21.95**	-6.28	17.17	19.65	-6.20	-43.92
		P5xP2	23.40**	-6.28**	-10.22*	-9.07*	-6.36**	10.33
	E	P1xP5	28.00**	-0.86	n/a	-6.29	n/a	n/a
		P5xP1	20.25**	0.00	n/a	39**	n/a	n/a
		P2xP5	22.96**	-1.71	n/a	0.72	n/a	n/a
		P5xP2	27.50**	-1.33	n/a	-32.67*	n/a	n/a
	H	P1xP5	34.17**	-3.13	n/a	-28.45**	n/a	n/a
		P5xP1	25.14**	-7.19	n/a	3.83	n/a	n/a
		P2xP5	26.00**	-2.71	n/a	10.57	n/a	n/a
		P5xP2	36.00**	-8.93**	n/a	-46.13**	n/a	n/a
	P	P1xP5	27.63**	-3.17	n/a	-14.19*	n/a	n/a
		P5xP1	20.46**	-6.67*	n/a	6.67	n/a	n/a
		P2xP5	21.25**	-4.40	n/a	10.20	n/a	n/a
		P5xP2	12.00*	-8.56**	n/a	30.46	n/a	n/a
2013/2014	E+H	P1xP5	27.69**	2.50	-6.00	-4.74	0.10	-7.85
		P5xP1	28.12**	7.87	-1.05	-1.57	10.27	-12.35
		P2xP5	22.73**	-2.88	18.14**	16.41**	-3.29	-32.35**
		P5xP2	24.57**	-4.57**	-1.04	-2.28	-4.17**	-3.30
	E	P1xP5	25.18**	2.05	7.00	9.40	-0.12	-25.64
		P5xP1	27.26**	4.79	2.66	4.14	6.96	-21.58
		P2xP5	21.87**	-0.79	22.23*	17.52*	-0.22	-25.37
		P5xP2	22.92**	-1.86	-0.46	-0.80	-2.42	-1.63
	H	P1xP5	26.40**	-2.86	n/a	-9.90	n/a	n/a
		P5xP1	20.93**	-6.88	n/a	4.41	n/a	n/a
		P2xP5	21.85**	-6.34*	n/a	17.58*	n/a	n/a
		P5xP2	24.98**	-3.46	n/a	-7.97	n/a	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa=additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations comprising the equation

Table 4.11. Mean estimates of gene effects for four crosses for total capitula plant^{-1} (n) at the East Tennessee (E) and Highland Rim (H) Research and Education Centers in 2013 only.

			Gene Effects					
Year	**Loc.**	**Cross**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2013	E+H	P1xP5	441.35**	-508.96	-383.4	-366.25	-494.28	161.34
		P5xP1	428.34**	280.85	379.69	621.38	266.18	-1509.76
		P2xP5	554.71**	-138.3	599.31	279.5	-161.54	-656.77
		P5xP2	372.65*	-50.10	819.59*	631.68	-26.86	-896.69
	E	P1xP5	629.2**	-524.24	-353.52	352.34	-546.16	-87.20
		P5xP1	599.59**	259.59	602.95	612.44	281.52	-1914.94
		P2xP5	703.20**	-155.5	868.06	399.2	-206.3	-670.69
		P5xP2	528.30**	-36.17	844.76*	670.46	54.63	-1102.73
	H	P1xP5	280.67*	127.6	n/a	242.13	n/a	n/a
		P5xP1	206.17*	126.48	n/a	691.71	n/a	n/a
		P2xP5	550**	472.83*	n/a	-1050.33	472.83*	n/a
		P5xP2	177.30**	7.61	n/a	592.43	7.61	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa=additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations comprising the equation

Table 4.12. Mean estimates of gene effects for four crosses for linoleic fatty acid (%) at the East Tennessee (E) and Plateau (P) Research and Education Centers in 2014 only.

			Gene Effects					
Year	**Loc.**	**Crosses**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2014	E+P	P1xP5	60.94**	2.06	n/a	-16.35	n/a	n/a
		P5xP1	56.71**	-0.32	n/a	5.26	n/a	n/a
		P2xP5	51.84**	13.31	n/a	-13.28	n/a	n/a
		P5xP2	56.99**	-7.38*	n/a	-25.12	n/a	n/a
	E	P1xP5	62.61**	8.62	n/a	-24.58	8.62	n/a
		P5xP1	56.17**	3.155	n/a	26.59	3.16	n/a
		P2xP5	56.99**	1.205	n/a	15.10	1.21	n/a
		P5xP2	65.35**	-16.07*	n/a	-44.39	-16.07*	n/a
	P	P1xP5	60.96**	3.65	n/a	-33.02**	n/a	n/a
		P5xP1	50.81**	3.60	n/a	11.83	n/a	n/a
		P2xP5	47.50**	7.12	n/a	-8.34	n/a	n/a
		P5xP2	51.86**	1.32	n/a	-18.75	n/a	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa=additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations comprising the equation

Table 4.13. Mean estimates of gene effects for four crosses for oleic fatty acid (%) at the East Tennessee (E) and Plateau (P) Research and Education Centers in 2014 only.

			Gene Effects					
Year	**Loc.**	**Crosses**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2014	E+P	P1xP5	12.80**	-1.99	n/a	15.35	n/a	n/a
		P5xP1	18.76**	-3.40	n/a	-15.18	n/a	n/a
		P2xP5	22.80**	-3.17	n/a	-6.85	n/a	n/a
		P5xP2	22.59**	2.20	n/a	1.21	n/a	n/a
	E	P1xP5	14.85**	-5.08	n/a	-1.93	-5.08	n/a
		P5xP1	17.25**	-1.96	n/a	-27.03*	-1.96	n/a
		P2xP5	22.59**	4.05	n/a	-46.78*	4.05	n/a
		P5xP2	10.28	8.28	n/a	35.26	8.28	n/a
	P	P1xP5	13.15**	-3.63	n/a	33.89**	n/a	n/a
		P5xP1	22.67**	-6.17	n/a	-7.74	n/a	n/a
		P2xP5	28.35**	3.42	n/a	-15.88	n/a	n/a
		P5xP2	28.83**	-3.89	n/a	-8.51	n/a	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa= additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations comprising the equation

Table 4.14. Mean estimates of gene effects for four crosses for palmitic fatty acid (%) at the East Tennessee (E) and Plateau (P) Research and Education Centers in 2014 only.

			Gene Effects					
Year	**Loc.**	**Crosses**	**m**	**a**	**d**	**aa**	**ad**	**dd**
2014	E+P	P1xP5	13.46**	1.13	n/a	-2.99	n/a	n/a
		P5xP1	11.81**	1.56	n/a	9.78*	n/a	n/a
		P2xP5	11.57**	2.76	n/a	-7.28	n/a	n/a
		P5xP2	12.12**	1.53	n/a	-0.12	n/a	n/a
	E	P1xP5	12.90**	-10.05**	n/a	21.74*	-10.05**	n/a
		P5xP1	12.98**	1.37	n/a	9.13	1.37	n/a
		P2xP5	12.12**	-4.98	n/a	9.94	-4.98	n/a
		P5xP2	14.17**	3.79	n/a	-0.44	3.79	n/a
	P	P1xP5	11.48**	1.00	n/a	0.01	n/a	n/a
		P5xP1	10.72**	1.97	n/a	5.10*	n/a	n/a
		P2xP5	10.16**	1.78	n/a	-3.62	n/a	n/a
		P5xP2	12.00**	-0.74	n/a	-7.54	n/a	n/a

† Significant at P≤0.01 and P≤0.05 is indicated by ** and *, respectively.

‡ P1, P2, P3, P4, and P5 code for the following accessions: PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively.

§ m= mean effects, a= additive, d= dominance, aa= additive x additive, ad= additive x dominance, and dd= dominance x dominance

¶ n/a indicates the value was not computable due to missing generations comprising the equation

Figures

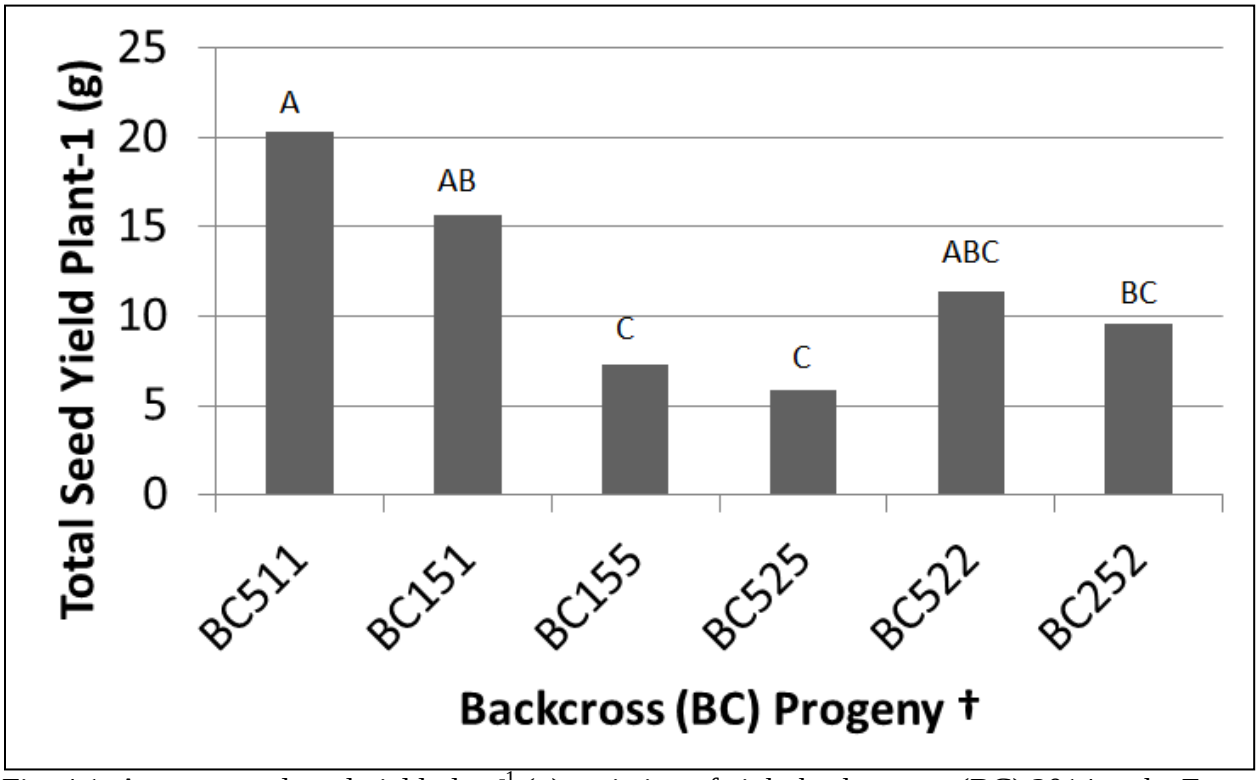
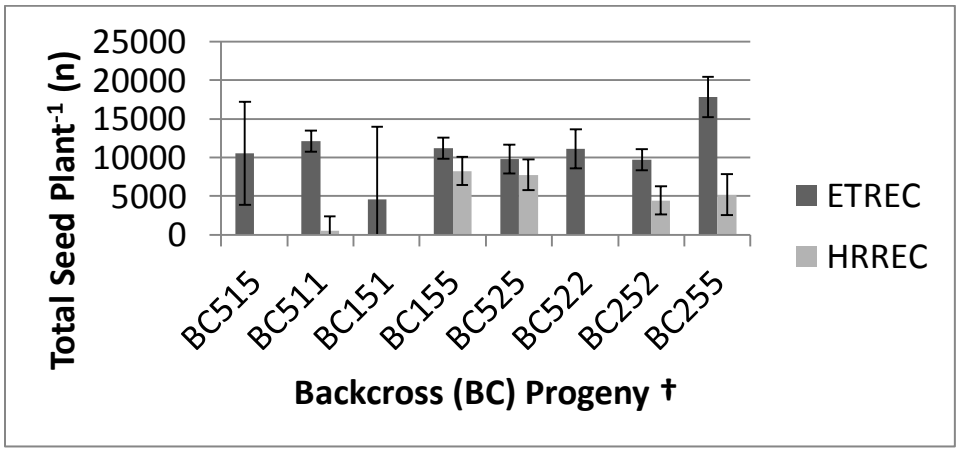


Fig. 4.1. Average total seed yield plant^{-1} (g) variation of eight backcrosses (BC) 2014 at the East Tennessee (ETREC) and the Plateau (PREC) Research and Education Centers. BC511, BC151, BC155, BC525, BC522, and BC252 had 6, 20, 19, 11 21, 25 observations, respectively.
† BC's with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) indicates that P1 was the maternal and P5 was the paternal parent. BC511 for example, indicates that F_1(51) was crossed with P1.
§ BC515 and BC255 were missing due to poor germination and disease.

A



B

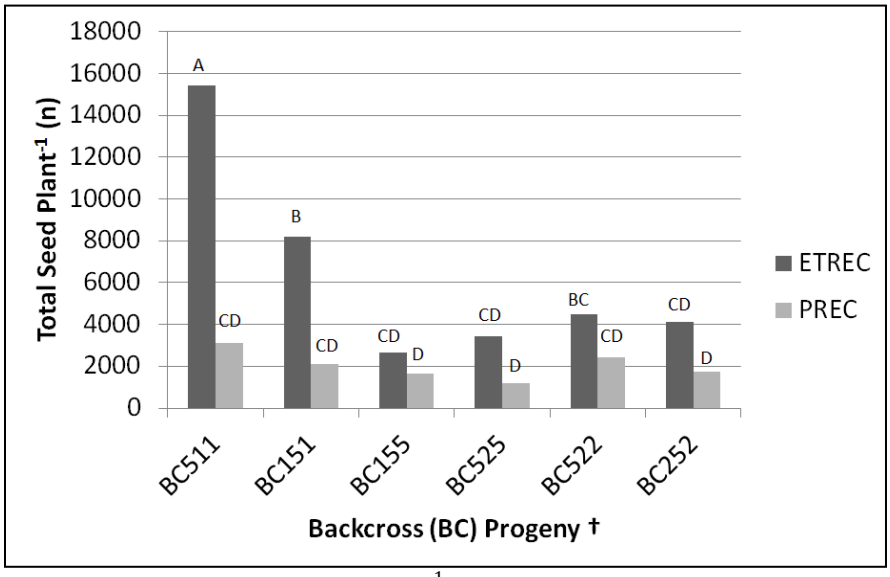


Fig. 4.2. Average total seed plant^{-1} (n) for eight backcrosses (BC) at the East Tennessee (ETREC) and Highland Rim (HRREC) Research and Education Centers in 2013 (A), and in 2014 at ETREC and the Plateau (PREC) Research and Education Center (B). There were significant cross*location interactions in both years; therefore crosses were shown for each location. BC511, BC151, BC155, BC525, BC522, and BC252 had 2, 11, 1, 17, 18, 14, 12, and 8 observations, respectively, at ETREC, and 0, 1, 0, 9, 14, 0, 5, and 6 at HRREC in 2013 (A). BC511, BC151, BC155, BC525, BC522, and BC252 had 2, 4, 6, 5, 7, 7 observations, respectively, at ETREC, and 4, 16, 13, 7, 14, 18 at PREC in 2014.
† BC's with a letter in common are not significantly different based on Fisher's LSD ($P< 0.05$).
‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) indicates that P1 was the maternal and P5 was the paternal parent. BC511 for example, indicates that F_1(51) was crossed with P1.
§ BC515 and BC255 from ETREC and PREC were missing from (B) due to poor germination and disease.

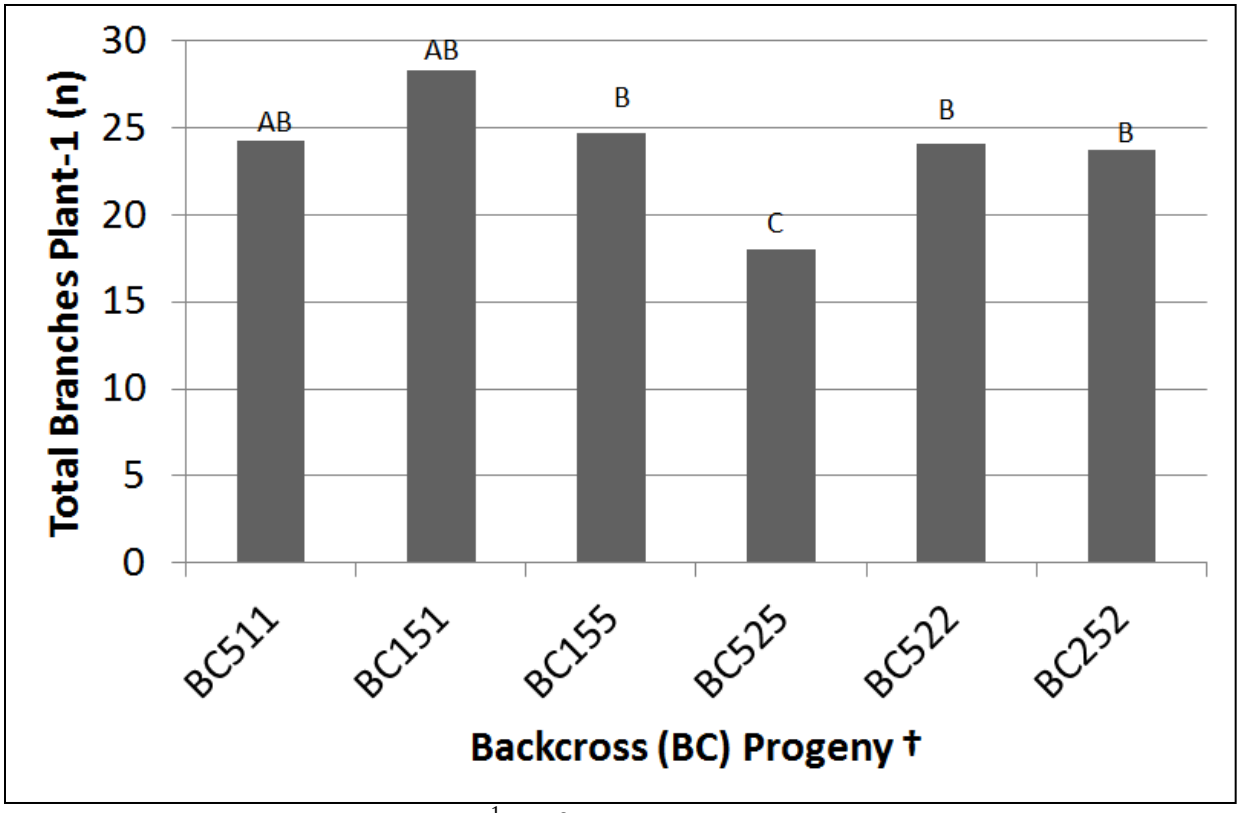


Fig. 4.3. Average total branches plant^{-1} (n) for eight backcrosses (BC) in 2014 at East Tennessee (ETREC), Highland Rim (HRREC), and Plateau Research and Education Center (PREC). BC511, BC151, BC155, BC525, BC522, and BC252 had 2, 9, 39, 40, 21, 41, 41, and 1 observations, respectively.

† BC's with a letter in common were not significantly different based on Fisher's LSD ($P< 0.05$).

‡ P1, P2, P3, P4, and P5 are parent accessions (PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, respectively). F_1(15) indicates that P1 was the maternal and P5 was the paternal parent. BC511 for example, indicates that F_1(51) was crossed with P1.

§ BC515 and BC255 from ETREC and PREC were missing from (B) due to poor germination and disease.

OVERALL SUMMARY

Fourteen plant introductions with origin from Ethiopia (PI 508070, PI 508072-PI 508080), India (PI 422242, PI 509436, and PI 511305), and the United States (W6 18860) were received from the USDA/ARS germplasm collection at Pullman, WA in 2012. Ten replications from each accession were planted at the East Tennessee Research and Education Center (ETREC) in summer 2012 in a completely randomized design. Traits included height, branches plant^{-1}, capitula plant^{-1}, seed capitulum^{-1}, estimated seed yield plant^{-1}, total oil, and fatty acid composition. Results indicated that plants from Ethiopia were the tallest (111-135 cm), whereas the W6 18860 from the United States was the shortest (58 cm). Plants from Ethiopia also had the most number of branches plant^{-1} (24-31) whereas plants from India possessed the fewest (15-17). Plants from Ethiopia displayed a great deal of variation by accounting for the greatest and least number of capitula plant^{-1}, seed capitulum^{-1}, and estimated seed yield plant^{-1}. Total oil (%) was greatest in PI 422242 (38%) and PI 509436 (38%), and lowest in PI 508076 (33%). PI 508079 contained the most linoleic acid (82%) and W6 18860 had the least (53%). W6 18860 had the most oleic acid (30%) while PI 508079 contained the least (3%). Palmitic fatty acid was greatest 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 least (5%).

Three of the fourteen accessions (PI 422242, PI 511305, and W6 18860) were chosen as parents in crosses for self-incompatibility testing. Selections were based on height, branches, and plant vigor. PI 422242/W6 18860 and reciprocal W6 18860/PI 422242 as well as PI 511305/W6 18860 and reciprocal W6 18860/PI 511305 were produced so that the U.S. accession W6 18860, could be used as either the female or male parent in every cross. All of the results for the parents

and the F1's indicated an average SI greater than 90%; however there was evidence for some self-compatability. In 2013, results indicated that F_1 crosses using W6 18860 as the female parent had greater self-compatibility than when W6 18860 was used as the male parent. In 2014, W6 18860/PI 511305 had greater self-compatibility than both parents. These results may indicate cytoplasmic inheritance of self-incompatability. Continued study of W6 18860 is recommended because of the vast difference between 2013 and 2014 performance.

Due to the possibility of cross contamination, it is suggested that future studies concerning self-incompatibility be made in a greenhouse or similar environment where wind and pollinating insects are not potential means for experimental error. Furthermore, rain events could have affected the results due to temporal differences in flowering dates among parents and F_1 progeny. Since not every plant reached full flowering on the same date, it is possible that rain events could have washed off pollen on some plants while other plants had either already undergone fertilization or had not yet reached full flowering.

Five of the fourteen plant introductions were selected for use as parents in crosses for mean analysis. Similar to the self-incompatibility study, selections were based on height, branches, and plant vigor. Parent accessions, PI 422242, PI 511305, PI 508075, PI 508076, and W6 18860, were used to produce F_1, F_2, and BC progeny (BC mean results reported in Chapter 4). All parents, crosses, reciprocals, and F_2 progeny evaluated in this study are listed in Table 2.1. The study was conducted over two years at ETREC (2013 and 2014), Highland Rim (HRREC) (2013 and 2014) and Plateau (PREC) Research and Education Centers (2014), using a space planted nursery utilizing a randomized complete block design with replication was used at each location each year. Actual yield of 40 plants (10 parents, 10 F_1's, 10 F_2's, and 10

backcrosses) were compared to 40 yield estimations in 2013 and 2014 using simple linear regression and paired t-tests. Results indicate a 0.90 slope in 2013 and a 0.59 slope correlation in 2014. Paired t-tests indicated that estimated and actual seed yield values were not significantly different.

Results showed that P2 was the best performing parent for total seed yield (g) (n), average seed capitulum $^{-1}$, and maturity; however P2 lodged more than other P's. P3 and P4 resulted in lower seed yield; fewer branches, capitula plant^{-1}, seed capitulum $^{-1}$; and required more to than other P's to reach full bloom. F_1(25) produced greater seed yield and reached greater maturity than other F_1's. F_1(15) was taller and produced the most capitula and average seed capitulum $^{-1}$, but lodged more than other F_1's. F_1(45) produced the lowest amount of seed and average seed capitulum $^{-1}$, but produced more branches than other F_1's. F_1(35) was most upright of all F_1's. F_1(35) and F_1(45) resulted in the least percentage of plants mature by frost, and took more time than other F_1's to reach full bloom. F_2(15) and was highest yielding, but F_2(25) was greater than other F_2's for number of seed plant^{-1}. F_2(45) was consistently the lowest yielding and had the lowest maturity ratings. 2013 appeared to result in greater total seed yield than 2014. Plants also took longer to reach full bloom in 2014 than in 2013. ETREC resulted in greater yield and taller plants than HRREC and PREC. Plants grown at HRREC reached full bloom in the least amount of time. Overall, there appeared to be evidence of some high parent heterosis in F1 crosses for seed yield, seed plant^{-1}, capitula plant^{-1}, average seed capitulum^{-1}, maturity, and full bloom. All parents, F_1's, and F_2's, were analyzed with correlations between yield traits (seed yield plant^{-1} (g) and seed number plant^{-1} (n)) and agronomic traits (capitula plant^{-1} (n), plant maturity (%), plant height (cm), average seed capitulum $^{-1}$ (n), branches plant^{-1}

(n), and full bloom (days after planting)). Overall, seed yield and seed plant^{-1} were comparable in their correlations to agronomic traits, though correlations between agronomic traits components and total seed were slightly greater that of seed plant^{-1}. Of all agronomic traits analyzed, capitula plant^{-1} consistently had the strongest correlations. Selections based on this trait could be decided earlier in the season than yield components like seed capitulum^{-1}, full bloom, and maturity. The longer it took for a plant to reach full bloom, the more likely it is that the plant will not reach maturity by the end of the growing season. This will ultimately reduce the number of mature plants, and therefore reduce seed yield.

Fatty acid analyses were performed on seed collected from all parents, F_1, and F_2 progeny in 2014. Seed was collected from ETREC and PREC only since disease affected seed yield at HRREC. Significant differences among plant material were only found in palmitic acid. Parents and F_1's both averaged 54% percent linoleic fatty acid, and 20% oleic acid. Parents and F_1's showed slight differences in average percent palmitic fatty acid, 11% and 12%, respectively. Stearic acid ranged from 7% (P1) to 8% [F_1(45)]. Significant differences ($P<0.05$) were found among F_2's in linoleic, oleic, and palmitic fatty acids, but not in stearic fatty acid. The mean for linoleic, oleic, and palmitic acid across all F_2 crosses were 53%, 22%, and 11%, respectively. The mean for stearic acid was 7 % across all F_2 populations.

Yield traits and fatty acids of all parents, F_1's, and F_2's, were compared using correlations. Correlations between seed yield and linoleic acid were weakly positive (0.06-0.34) except for the analysis exclusively involving F_1 plant material, which was weakly negative (-0.15). Correlations between seed yield and oleic acid were negative (-0.17to -0.24) except for the correlation of F_1 plant material (0.01). Palmitic acid and seed yield resulted in weak to moderate

correlations (0.02-0.38). Correlation coefficients were only significant ($P<0.05$) when all plant material was analyzed together and when F_2's were analyzed separately.

Strong negative correlations were found between oleic and linoleic acid (-0.84- -0.86) (Table 3.2). Comparisons of palmitic and linoleic acid resulted in weak to moderate positive correlation coefficients (.07-0.55), while palmitic acid and oleic acid resulted in weak negative correlations (-0.12- -0.27). Correlations between oleic and linoleic acid as well as palmitic and linoleic were significant among all plant material with the exception of F_1 correlations involving palmitic and linoleic acid.

Genetic variance, broad sense heritability, and gene effects were determined for seed yield plant^{-1} (2013 and 2014), seed plant^{-1} (2013 and 2014), total branches plant^{-1} (2013 and 2014), total capitula plant^{-1} (2013), and linoleic, oleic, palmitic, and stearic fatty acids (2014).

Genetic variance for seed yield and seed plant^{-1} was greatest in 2013 among the parents at ETREC (38.7 and 17,019,745, respectively). Genetic variance was greatest in parent and F_2 populations for branches, capitula, linoleic, and oleic traits. The F_1 populations resulted in the greatest genetic variance for palmitic acid. Broad sense heritability for all traits was relatively low (0-0.32), except for capitula plant^{-1}which had a broad sense heritability of 0.44 at HRREC. Gene effects indicated that dominance effects were most influential for seed yield, seed plant^{-1}, and capitula plant^{-1}. There were many missing gene effect values in all traits for dominance, additive x dominance interaction, and dominance x dominance interaction due to missing components of the equation. Gene effects for branches indicated that additive, dominance, and epistatic gene interactions had about equal effects. Additive x additive interaction had more influence over the fatty acid composition than additive gene effects.

Generation means analysis has several advantages; one being it is relatively simple and reliable in terms of statistics. Sampling error from the means is smaller than variance error for estimating inheritance, and can be therefore be used in smaller experiments to gain the same level of precision as larger experiments. Another advantage is that estimates of epistasis are more progressive than variance estimates because experiments are smaller and easier to carry out. Generation means analysis can be applied to both self and cross-pollinated species.

Several disadvantages were noted when using generation means analysis. One was the inability to estimate heritability, which is crucial for predicting response to selection. Another disadvantage was that any interpretation from the analysis was particular to those specific sets of parents. It was possible that negative effects of one loci can cancel out positive effects at another loci, thus hiding opposing effects. This may lead to underestimations of true genetic effects (Acquaah, 2007). Underestimation of heterotic effects could have occurred since the parents used in this study were heterozygous.

Given the presence of dominance, additive x dominance interaction, and dominance x dominance interaction of the aforementioned niger crosses, and given the presence of heterosis in the F_1 generation, a hybrid breeding program is recommended. Self-incompatibility is a potential issue when producing inbred lines. If this issue can be overcome, evidence from this research points to significant advances in seed yield and agronomic traits.

APPENDIX

Table 1A. Field map of niger plots at the East Tennessee Research and Education Center (ETREC) in 2013.

	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	242X242	242X242	242X242	242X242	BC1	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242
Block 4	W6XW6	W6XW6	W6XW6	FF16	W6xW6	FF15	.	FF15	W6XW6	W6XW6	FF17	BC8	BC1	FF12	FF15	P1	FF16	FF12	FF16	FF17	FF12	F11	FF13	FF12	242X242	242X242				
	305	W6XW6	W6XW6	FF16	W6XW6	FF15	FF13	W6XW6	W6XW6	BC3	FF17	BC1	242X242	FF12	W6XW6	P2	FF16	W6XW6	FF16	FF17	FF12	F8	FF13	FF12	BC4	305					
	305	W6XW6	W6XW6	FF16	W6XW6	W6XW6	FF13	W6XW6	W6XW6	W6XW6	W6XW6	BC1	BC4	FF12	W6XW6	W6XW6	FF16	FF12	FF16	W6XW6	FF12	F6	FF13	.	W6XW6	305					
	305	W6XW6	W6XW6	FF16	.	FF15	FF13	W6XW6	W6XW6	BC2	FF17	BC3	W6XW6	FF12	W6XW6	P1	FF16	FF12	FF16	FF17	FF12	F9	FF13	FF12	W6XW6	305					
	305	W6XW6	W6XW6	FF16	FF15	W6XW6	FF13	W6XW6	W6XW6	BC2	FF17	BC8	BC2	FF12	W6XW6	P3	FF16	FF12	FF16	FF17	W6XW6	F11	FF13	FF12	W6XW6	305					
	305X305	FF16	305X305	FF17	305X305	F7	305X305	BC8	FF15	FF12	FF13	F11	P5	F6	BC6	FF17	FF13	FF16	F9	W6XW6	W6XW6	FF14	F9	FF13	P2	242					
	305X305	FF16	FF17	FF17	305X305	F6	305X305	BC3	FF15	FF12	FF13	.	P2	F10	BC8	FF17	242X242	FF16	F10	W6XW6	FF14	W6XW6	F7	FF13	P1	305X305					
	305X305	FF16	FF17	FF17	305X305	F6	75X75	BC2	305X305	FF12	FF13	F11	P5	F10	305X305	FF17	FF13	FF16	F8	BC3	W6XW6	W6XW6	F11	FF13	P4	242					
	305X305	FF16	FF17	FF17	305X305	F10	305X305	BC1	FF15	FF12	FF13	F7	P5	F7	BC3	FF17	FF13	FF16	F9	BC1	FF14	W6XW6	F10	W6XW6	W6XW6	W6					
	242X242	FF16	242X242	242X242	242X242	F8	P2	242X242	FF15	FF12	FF13	F9	P3	F8	242X242	242X242	FF13	FF16	F7	BC4	FF14	305X305	F6	FF13	P3	W6					
Block 3	242X242	242X242	FF12	F8	FF12	FF13	FF12	242X242	P2	FF15	FF15	F6	BC3	242X242	F11	P3	242X242	242X242	FF16	F10	FF16	BC1	W6XW6	305X305	FF17	W6					
	242X242	242X242	FF12	F9	FF12	FF13	FF12	BC2	BC8	FF15	242X242	F9	BC1	FF17	242X242	W6XW6	242X242	242X242	FF16	F11	FF16	BC4	BC3	305X305	305X305	242					
	242X242	242X242	W6XW6	F11	FF12	FF13	FF12	242X242	BC2	FF15	FF15	F10	BC1	FF17	F7	.	242X242	FF14	FF16	F8	W6XW6	.	305X305	FF17	FF17	242					
	305X305	305X305	FF12	F11	FF12	FF13	FF12	BC1	.	FF15	P5	F10	BC6	FF17	F8	P2	305X305	305X305	FF16	F11	FF16	305X305	305X305	305X305	FF17	242					
	305X305	305X305	FF12	F10	FF12	FF13	FF12	242X242	305X305	FF15	FF15	F6	305X305	FF17	F10	P1	305X305	305X305	FF16	F7	FF16	BC2	BC2	FF17	FF17	242xW6					
	305X305	305X305	FF13	305X305	FF12	FF17	P5	P3	FF13	F7	305X305	FF15	FF12	FF13	305X305	FF16	FF16	F7	FF12	FF16	FF15	FF13	BC8	FF15	305X305	.					
	305X305	305X305	FF13	305X305	FF12	FF17	P2	242X242	FF13	F6	242X242	FF15	FF12	FF13	P2	FF16	FF16	F6	FF12	FF16	FF15	FF13	BC8	FF15	305X305	305XW6					
	75X75	75X75	.	75X75	FF12	FF17	P2	P1	FF13	P5	305X305	FF15	FF12	FF13	FF17	FF16	FF16	F9	FF12	FF16	FF15	FF13	BC6	FF15	BC1	305XW6					
	75X75	FF14	FF13	75X75	FF12	FF17	P5	P5	FF13	F9	BC4	FF15		FF13	FF17	FF16	FF16	F9	FF12	FF16	FF15	FF13	W6XW6	FF15	W6XW6	305XW6					
	75X75	75X75	FF13	75X75	75X75	242X242	P3	P4	FF13	F8	BC8	FF15	FF12	FF13	242X242	FF16	FF16	F8	FF12	FF16	FF15	FF13	BC3	FF15	305X305	W6X305					
Block 2	75X75	75X75	FF13	242X242	FF12	242X242	P3	F11	FF15	305X305	FF12	FF15	BC2	FF16	305X305	P3	FF17	F9	305X305	F6	FF12	BC3	F7	FF12	F11	W6X305					
	W6XW6	W6XW6	FF13	FF17	FF12	W6XW6	P2	F6	FF15	BC1	FF12	FF15	W6XW6	FF16	W6XW6	P5	W6XW6	F10	75X75	F11	75X75	BC8	F8	FF12	F8	W6X305					
	W6XW6	W6XW6	FF13	W6XW6	FF12	BC3	P1	F10	FF15	.	FF12	FF15	BC3	.	W6XW6	P3	FF17	F10	75X75	F7	FF12	BC1	F10	FF12	F11	75X75					
	W6XW6	W6XW6	FF13	W6XW6	FF12	BC8	P1	F6	FF15	BC2	FF12	FF15	BC5	FF16	W6XW6	P2	242X242	F8	75X75	F9	FF12	BC2	F9	FF12	F6	75X75					
	W6XW6	W6XW6	FF13	W6XW6	FF12	BC6	W6XW6	F8	FF15	BC6	FF12	FF15	242X242	FF16	W6XW6	P5	FF17	W6XW6	FF14	F11	FF12	242X242	F10	FF12	F9	75X75					
	305X305	BC1	FF13	305X305	FF15	FF13	P5	.	.	FF13	FF17	W6XW6	FF15	F6	FF16	W6XW6	FF12	W6XW6	FF15	FF16	W6XW6	FF13	FF17	W6XW6	W6XW6	W6XW6					
	305X305	305X305	FF13	305X305	FF15	FF13	FF16	305X305	FF16	FF13	W6XW6	BC6	FF15	F9	FF16	FF14	FF12	W6XW6	FF15	FF16	W6XW6	FF13	FF17	W6XW6	P5	W6X242					
	305X305	305X305	FF13	305X305	FF15	FF13	FF16	BC8	FF16	FF13	W6XW6	BC2	FF15	F8	FF16	W6XW6	.	BC2	FF15	FF16	FF14	FF13	FF17	W6XW6	P2	W6X242					
	305X305	BC6	FF13	FF14	FF15	FF13	FF16	305X305	BC1	FF13	W6XW6	BC1	FF15	F7	FF16	W6XW6	FF12	BC3	FF15	FF16	FF14	FF13	W6XW6	W6XW6	P1	W6X305					
	76XW6	BC1	FF13	FF14	FF15	FF13	FF16	BC1	FF16	FF13	305X305	BC8	FF15	F7	FF16	.	FF12	BC4	FF15	FF16	305X305	FF13	305X305	305X305	242X242	W6X305					
Block 1	76XW6	75XW6	FF16	F10	305X305	P3	305X305	BC1	FF12	FF12	F6	FF15	305X305	FF17	FF13	FF13	FF15	305X305	305X305	FF12	BC8	BC1	FF13	305X305	305X305	W6X305					
	76XW6	75XW6	FF16	F6	FF14	P4	FF17	305X305	FF12	FF12	F6	FF15	305X305	FF17	FF13	FF13	FF15	BC1	305X305	FF12	305X305	BC3	FF13	305X305	305X305	W6X305					
	76XW6	75XW6	75XW6	F11	305X305	P1	FF17	305X305	FF12	FF12	F10	FF15	305X305	305X305	FF13	FF13	FF15	BC4	305X305	.	BC6	305X305	FF13	FF14	BC6	305XW6					
	242X242	242X242	.	F8	242X242	P5	FF17	BC6	FF12	FF12	F9	FF15	242X242	242X242	FF13	FF13	FF15	BC7	242X242	FF12	242X242	BC8	FF13	FF14	BC8	305XW6					
	242X242	242X242	FF16	F11	242X242	P2	242X242	BC6	FF12	FF12	F10	FF15	242X242	FF17	FF13	FF13	FF15	242X242	FF17	FF12	BC5	BC8	FF13	242X242	242X242	242X242					
	242X242	242X242	FF15	BC4	FF16	FF16	FF13	F10	242X242	FF15	FF16	BC2	P3	FF13	F7	FF12	P1	FF15	F8	242X242	242X242	FF12	FF16	.	F8	242X242					
	242X242	242X242	FF15	.	FF16	FF16	FF13	F7	242x242	FF15	FF16	242X242	P4	FF13	F11	FF12	P2	FF15	F6	242X242	FF17	FF12	FF16	242X242	F8	242X242					
	75X75	BC2	.	BC1	FF16	FF16	FF13	F8	FF17	FF15	FF16	W6XW6	P4	FF13	F9	FF12	P5	FF15	F11	W6XW6	W6XW6	FF12	FF16	W6XW6	F11	W6XW6					
	75X75	BC4	FF15	75X75	FF16	FF16	FF13	F9	75X75	FF15	FF16	BC1	P1	FF13	F7	FF12	P3	FF15	F9	W6XW6	W6XW6	FF12	FF16	W6XW6	F7	W6XW6					
	75X75	75X75	FF15	BC8	FF16	FF16	FF13	F9	75X75	FF15	FF16	BC2	.	FF13	F7	FF12	P3	.	F10	FF14	FF17	FF12	FF16	W6XW6	F6	W6XW6					
	75X75	75X75	.	75X75	75X75	75X75	75X75	75X75	75X75	W6XW6	W6XW6	BC1	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6					

†Red font indicates that the plant was used as a fill.

‡ Yellow highlighted boxes indicate plants that were used to produce 2014 seed.

Table 1B. Field map of niger plots at the East Tennessee Research and Education Center (ETREC) in 2014.

Block 4	76X76	76X76	76X76	W6XW6	76X76	76X76	76X76	76X76	76X76	242x242	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76
	76X76	76X76	FF14	F6	F7	F6	F6	F6	BC7	F6	F6	F9	BC3	F10	F9	F9	FF12	FF12	242x242	242x242	242x242	242x242	FF15	F9	BC1	FF15	76X76
	76X76	76X76	FF14	F6	242x242	F6	FF13	F6	BC8	F6	FF14	F9	F9	242x242	F9	76X76	F9	FF12	F9	242x242	242x242	242x242	F9	F9	242x242	76X76	
	76X76	76X76	FF14	W6XW6	F10	F6	F6	F6	BC3	242x242	FF14	F9	F9	F6	F9	F9	F9	242x242	F6	BC8	242x242	FF15	F9	F9	FF15	76X76	
	76X76	76X76	FF14	F6	F11	F6	FF13	F6	F6	F6	242x242	242x242	BC2	F7	F9	242x242	F9	FF12	242x242	242x242	P5	242x242	242x242	242x242	FF15	76X76	
	76X76	76X76	FF14	F6	F11	F6	242x242	F6	BC3	F6	242x242	242x242	F9	F11	F9	F9	F9	FF12	242x242	242x242	P1	FF15	F9	242x242	FF15	76X76	
	76X76	FF14	F9	FF15	BC2	BC2	242x242	F6	BC2	242x242	FF14	242x242	FF13	242x242	242x242	P3	F10	242x242	FF15	242x242	BC1	242x242	F6	242x242	F6	76X76	
	W6XW6	FF14	F9	FF15	242x242	F6	BC8	BC8	242x242	BC3	FF14	F6	FF13	F6	P5	P2	242x242	242x242	FF15	F6	242x242	F6	242x242	F6	242x242	76X76	
	76X76	FF14	F11	FF15	BC1	BC8	BC8	BC8	BC8	BC2	FF14	F6	FF13	F6	P4	242x242	F10	F6	FF15	242x242	BC1	F9	242x242	F6	F6	76X76	
	76X76	FF14	F7	FF15	242x242	242x242	BC8	BC8	BC1	BC8	BC8	F6	FF13	F6	P2	242x242	F9	242x242	242x242	242x242	BC2	F6	242x242	242x242	242x242	242x242	76X76
	W6XW6	FF14	F7	FF15	BC7	BC8	BC8	BC8	BC7	BC6	FF14	242x242	FF13	FF17	P1	242x242	F6	242x242	FF15	F6	BC8	242x242	FF16	242x242	F6	76X76	
Block 3	76X76	76X76	BC1	BC7	P5	FF13	242x242	242x242	F11	FF14	242x242	BC7	FF15	242x242	FF14	F11	242x242	BC7	F6	242x242	242x242	FF15	BC8	242x242	242x242	76X76	
	W6XW6	76X76	BC8	W6XW6	P3	BC7	242x242	BC7	F9	BC7	242x242	BC7	FF15	242x242	242x242	F11	BC8	BC7	242x242	242x242	FF17	FF15	BC3	242x242	BC8	76X76	
	76X76	W6xW6	BC2	BC7	P1	FF13	BC7	242x242	F7	FF14	BC2	BC7	FF15	242x242	242x242	F7	BC7	242x242	F7	242x242	BC7	FF15	242x242	242x242	BC8	76X76	
	W6XW6	76X76	BC1	BC7	P5	BC7	BC7	242x242	242x242	242x242	242x242	BC7	FF15	BC7	242x242	F10	242x242	242x242	BC7	242x242	242x242	242x242	242x242	BC8	BC7	76X76	
	76X76	76X76	BC7	FF16	P4	242x242	BC7	242x242	F10	FF14	BC7	BC7	FF15	BC1	242x242	F6	BC7	BC7	242x242	BC7	242x242	242x242	BC3	242x242	242x242	76X76	
	76X76	FF13	W6XW6	FF12	FF14	76x76	P2	BC3	242x242	F10	242x242	BC3	242x242	242x242	242x242	P4	BC3	FF15	242x242	FF14	F10	242x242	BC3	FF15	FF13	76X76	
	76X76	BC3	W6XW6	BC3	242x242	242x242	P2	BC3	FF15	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	BC3	242x242	242x242	242x242	242x242	242x242	76X76
	76X76	BC3	W6XW6	FF12	BC3	BC7	242x242	BC3	FF15	BC3	242x242	242x242	242x242	BC3	242x242	242x242	242x242	FF15	242x242	FF14	BC3	242x242	242x242	FF15	242x242	76X76	
	W6XW6	BC3	W6XW6	FF12	242x242	BC3	P4	BC3	242x242	242x242	242x242	242x242	242x242	242x242	BC3	242x242	242x242	242x242	242x242	BC3	242x242	242x242	242x242	242x242	242x242	76X76	
	76X76	BC3	W6XW6	BC3	242x242	BC3	P3	BC3	FF15	242x242	BC2	242x242	242x242	242x242	242x242	P5	242x242	242x242	FF17	BC3	242x242	242x242	BC8	FF15	242x242	76X76	
Block 2	76X76	76X76	P3	W6XW6	242x242	242x242	242x242	FF14	242x242	242x242	242x242	242x242	242x242	FF13	F6	242x242	242x242	242x242	242x242	FF15	F6	242x242	242x242	BC2	242x242	242x242	76X76
	76X76	76X76	P1	W6XW6	242x242	BC8	242x242	242x242	F6	242x242	242x242	242x242	242x242	FF13	242x242	242x242	F6	F6	F6	FF15	F6	242x242	BC3	F7	BC2	242x242	76X76
	76X76	W6xW6	P4	FF15	76X76	242x242	242x242	242x242	F6	242x242	242x242	242x242	FF13	FF14	F6	242x242	F11	F6	FF15	F6	FF13	BC8	242x242	242x242	242x242	242x242	76X76
	W6XW6	76X76	P2	FF15	242x242	242x242	242x242	FF14	242x242	F6	242x242	242x242	242x242	242x242	242x242	242x242	F7	F6	242x242	242x242	FF13	BC6	F8	BC2	242x242	242x242	76X76
	W6XW6	76X76	P5	W6XW6	242x242	BC3	F6	FF14	242x242	242x242	F10	242x242	242x242	FF14	242x242	242x242	F6	F6	FF15	242x242	FF13	BC3	F10	242x242	242x242	242x242	76X76
	76X76	BC7	FF15	W6XW6	242x242	242x242	BC7	FF15	242x242	FF14	BC7	BC8	FF13	FF16	242x242	P4	242x242	BC8	BC8	242x242	242x242	F6	BC1	242x242	242x242	242x242	76X76
	W6XW6	FF14	FF15	W6XW6	242x242	242x242	242x242	FF15	242x242	FF14	242x242	242x242	FF13	FF16	BC1	P5	F6	BC8	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	76X76	FF14	FF15	W6XW6	242x242	F11	242x242	FF15	BC7	FF14	BC7	242x242	242x242	242x242	BC8	P5	F10	BC8	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	W6xW6	FF15	W6XW6	242x242	242x242	242x242	FF15	242x242	FF14	242x242	BC8	242x242	FF16	BC1	242x242	F9	242x242	242x242	FF14	FF17	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	FF14	FF15	BC6	242x242	F8	BC8	FF15	242x242	242x242	242x242	242x242	242x242	BC8	242x242	242x242	F11	242x242	242x242	FF14	242x242	242x242	242x242	242x242	242x242	242x242	76X76
Block 1	W6XW6	76X76	FF15	FF15	BC6	P1	FF12	242x242	242x242	FF15	242x242	F11	242x242	F11	242x242	F11	242x242	P4	F11	BC7	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	76X76	76X76	FF12	FF15	BC2	242x242	242x242	BC8	BC2	FF15	FF14	BC2	242x242	F11	242x242	242x242	F11	P3	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	76X76	FF12	FF15	BC1	P4	BC2	242x242	FF13	242x242	FF14	242x242	F6	FF14	242x242	FF12	F11	242x242	242x242	242x242	242x242	FF15	242x242	BC1	242x242	242x242	76X76
	W6XW6	76X76	W6XW6	BC2	242x242	242x242	BC2	242x242	FF13	FF15	FF14	242x242	242x242	242x242	F11	242x242	F11	P2	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	76X76	FF15	242X242	242x242	242x242	242x242	242x242	242x242	FF15	FF14	242x242	F11	FF14	242x242	242x242	F11	P3	242x242	BC7	FF15	242x242	BC3	242x242	242x242	242x242	76X76
	76X76	F8	BC2	242X242	242x242	242x242	FF14	BC2	242x242	F8	242x242	FF15	BC2	P5	FF13	242x242	F11	242x242	FF14	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	F7	W6XW6	242X242	BC3	242x242	FF14	BC2	BC2	F9	FF14	242x242	242x242	P5	242x242	242x242	F11	BC3	242x242	242x242	242x242	BC7	242x242	242x242	242x242	242x242	76X76
	W6XW6	W6xW6	W6XW6	242X242	242x242	242x242	FF14	BC2	BC2	F6	242x242	FF15	242x242	242x242	BC3	FF15	242x242	242x242	242x242	242x242	242x242	242x242	242x242	BC8	242x242	242x242	76X76
	W6XW6	W6xW6	BC2	FF15	242x242	242x242	242x242	242x242	242x242	242x242	FF14	.	242x242	P1	FF13	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	W6xW6	BC2	FF15	242x242	242x242	242x242	242x242	BC2	242x242	242x242	.	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	242x242	76X76
	W6XW6	W6xW6	242X242	242X242	242x242	242x242	76X76	242x242	242x242	242x242	242x242	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76	76X76

†Red font indicates that the plant was used as a fill

Table 1C. Field map of niger plots at the Highland Rim Research and Education Center (HRREC) in 2013.

	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75
Block 4	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	F8	P3	75X75	75X75	P1	75X75	75X75	75X75	FF13	F7	75X75	75X75	BC1	75X75	75X75	75X75	FF16	75X75	
	.	242X242	242X242	242X242	242X242	BC1	242X242	242X242	242X242	P2	75X75	75X75	P5	75X75	75X75	.	75X75	F9	75X75	75X75	75X75	75X75	75X75	75X75	FF16	75X75	
	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	F9	P3	242X242	75X75	P5	75X75	75X75	75X75	FF13	F9	75X75	75X75	75X75	75X75	75X75	75X75	242X242	75X75	
	.	242X242	.	242X242	242X242	BC6	242X242	242X242	F8	242X242	.	F10	P1	75X75	75X75	75X75	FF13	F9	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75	
	242X242	242X242	FF16	242X242	242X242	242X242	242X242	.	F10	P1	75X75	F8	P2	75X75	75X75	75X75	FF13	F11	75X75	75X75	BC3	75X75	75X75	75X75	FF16	.	
	305X305	BC3	305X305	305X305	FF13	305X305	305X305	305X305	305X305	305X305	F10	242X242	242X242	242X242	242X242	242X242	242X242	.	242X242	242X242	242X242	242X242	BC1	242X242	242X242	P2	BC3	242X242	242X242
	305X305	BC1	.	BC1	FF13	305X305	305X305	305X305	305X305	.	242X242	242X242	242X242	242X242	242X242	242X242	.	242X242	242X242	242X242	BC8	242X242	F10	P3	242X242	242X242	242X242
	305X305	BC3	305X305	305X305	.	305X305	305X305	305X305	305X305	305X305	F10	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	BC3	242X242	.	.	242X242	242X242	.
	305X305	BC8	305X305	305X305	FF13	305X305	305X305	305X305	305X305	305X305	F6	242X242	242X242	FF16	BC8	242X242	242X242	242X242	242X242	242X242	.	242X242	F11	P5	242X242	242X242	242X242
	W6XW6	W6XW6	.	W6XW6	.	W6XW6	FF14	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	F11	W6XW6	242X242	FF16	242X242	W6XW6	W6XW6	305X305	305X305	305X305	305X305	P4	305X305	305X305	305X305
Block 3	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	FF13	F7	W6XW6	W6XW6	W6XW6	P4	F10	W6XW6	.	W6XW6	W6XW6	P2	305X305	305X305	305X305	305X305	305X305	305X305	305X305	305X305	.
	W6XW6	W6XW6	W6XW6	.	W6XW6	W6XW6	W6XW6	F9	FF13	W6XW6	W6XW6	P1	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	P3	305X305	W6XW6	305X305	305X305	305X305	305X305	F10	305X305	
	W6XW6	W6XW6	W6XW6	.	W6XW6	W6XW6	W6XW6	F11	W6XW6	W6XW6	W6XW6	P5	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	P1	.	305X305	305X305	F10	BC1	305X305	F9	305X305	
	75X75	.	75X75	75X75	75X75	75X75	75X75	75X75	75X75	W6XW6	W6XW6	P3	F8	W6XW6	.	W6XW6	W6XW6	P5	FF12	W6XW6	W6XW6	F7	BC3	W6XW6	F8	W6XW6	
	75X75	75X75	.	.	75X75	75X75	75X75	F10	.	W6XW6	W6XW6	P5	F11	.	.	BC1	W6XW6	P1	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	F8	W6XW6	
	75X75	75X75	75X75	F11	75X75	F9	75X75	.	P3	W6XW6	W6XW6	W6XW6	W6XW6	.	W6XW6	W6XW6	.	FF12	FF13	W6XW6	.	FF13	BC1	W6XW6	W6XW6	W6XW6	
	75X75	75X75	75X75	F9	BC8	F8	75X75	75X75	75X75	W6XW6	FF16	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	.	FF12	W6XW6	W6XW6	W6XW6	.	W6XW6	W6XW6	W6XW6	W6XW6	
	305X305	305X305	.	75X75	75X75	F10	FF16	305X305	.	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	.	242X242	242X242	242X242	242X242	.	
	305X305	75X75	305X305	F7	305X305	F11	305X305	305X305	P2	242X242	242X242	.	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	.	242X242	242X242	BC8	FF12	242X242	242X242
	305X305	.	F10	F7	305X305	75X75	242X242	BC3	P2	242X242	242X242	242X242	242X242	242X242	305X305	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242
Block 2	305X305	305X305	F10	P2	F10	305X305	.	75X75	75X5	242X242	BC4	242X242	305X305	BC8	305X305	.	P3	FF17	242X242	F7	242X242	242X242	242X242	242X242	242X242	242X242	242X242
	75X75	75X75	BC4	P5	F11	75X75	75X75	75X75	BC1	305X305	.	.	.	305X305	305X305	305X305	P4	305X305	305X305	F9	75X75	305X305	305X305	305X305	75X75	305X305	
	75X75	75X75	75X75	P5	F9	75X75	75X75	75X75	75X75	305X305	BC4	.	305X305	242X242	305X305	FF13	242X242	305X305	305X305	75X75	.	305X305	305X305	305X305	305X305	305X305	
	75X75	75X75	75X75	P3	75X75	75X75	75X75	75X75	BC8	305X305	242X242	305X305	305X305	BC1	305X305	W6XW6	.	305X305	305X305	F10	75X75	.	305X305	305X305	75X75	305X305	
	75X75	75X75	75X75	P1	F10	75X75	BC3	75X75	BC6	305X305	305X305	242X242	305X305	BC1	305X305	FF13	P2	305X305	305X305	75X75	.	.	305X305	305X305	305X305	305X305	
	W6XW6	BC1	.	F10	W6XW6	W6XW6	W6XW6	F11	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	F8	W6XW6	W6XW6	W6XW6	P1	75X75	75X75	75X75	BC1	.	75X75	75X75	75X75	
	W6XW6	W6XW6	F7	F11	W6XW6	W6XW6	W6XW6	W6XW6	FF13	W6XW6	W6XW6	.	.	F9	W6XW6	.	W6XW6	P3	75X75	.	75X75	.	.	75X75	FF13	75X75	
	W6XW6	.	F10	F7	W6XW6	W6XW6	305X305	F8	FF13	W6XW6	W6XW6	W6XW6	W6XW6	F11	W6XW6	W6XW6	W6XW6	P2	75X75	.	75X75	75X75	75X75	75X75	75X75	75X75	
	W6XW6	W6XW6	F8	W6XW6	W6XW6	W6XW6	305X305	F9	FF13	W6XW6	W6XW6	W6XW6	W6XW6	F9	W6XW6	W6XW6	W6XW6	W6XW6	.	75X75	75X75	.	75X75	.	FF13	75X75	
	305X305	242X242	242X242	F7	FF14	242X242	305X305	F11	242X242	305X305	.	305X305	305X305	F8	305X305	305X305	.	P5	.	W6XW6	W6XW6	.	W6XW6	W6XW6	W6XW6	W6XW6	
Block 1	242X242	305X305	305X305	P2	305X305	242X242	BC4	305X305	305X305	.	BC1	305X305	242X242	F7	305X305	305X305	FF13	305X305	P5	W6XW6	.	W6XW6	F6	BC3	W6XW6	W6XW6	
	305X305	305X305	BC3	P3	305X305	242X242	305X305	305X305	FF15	305X305	.	.	242X242	F10	305X305	FF12	.	305X305	P1	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	W6XW6	
	305X305	305X305	242X242	P1	242X242	305X305	BC4	305X305	242X242	305X305	.	305X305	305X305	F11	305X305	.	FF13	305X305	.	W6XW6	BC1	.	F9	BC4	FF12	W6XW6	
	75X75	75X75	75X75	.	.	.	75X75	75X75	75X75	.	.	75X75	.	F8	75X75	75X75	.	75X75	.	305X305	.	.	F11	305X305	75X75	305X305	
	75X75	75X75	75X75	P5	.	75X75	BC8	75X75	FF15	.	75X75	75X75	W6XW6	F11	75X75	75X75	75X75	75X75	P3	305X305	305X305	305X305	F8	BC1	75X75	305X305	
	75X75	75X75	242X242	75X75	75X75	P5	75X75	75X75	F9	.	.	75X75	.	F7	BC4	75X75	75X75	75X75	.	75X75	305X305	F8	75X75	F11	305X305	305X305	
	75X75	.	.	75X75	.	P2	75X75	75X75	F6	.	75X75	.	75X75	F8	BC8	.	FF12	75X75	242X242	305X305	305X305	242X242	BC1	305X305	242X242	305X305	
	242X242	.	242X242	242X242	242X242	P3	242X242	242X242	F6	242X242	242X242	242X242	FF13	F10	242X242	.	242X242	FF17	75X75	FF16	75X75	F9	BC8	F9	75X75	75X75	
	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	F10	242X242	242X242	242X242	FF13	242X242	BC1	242X242	242X242	.	.	242X242	.	F7	75X75	F6	FF15	75X75	
	242X242	.	.	242X242	.	P2	242X242	242X242	F7	.	242X242	242X242	242X242	F11	242X242	242X242	242X242	242X242	75X75	242X242	.	F10	75X75	F10	75X75	75X75	
	242X242	242X242	242X242	.	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	242X242	75X75	75X75	75X75	75X75	75X75	75X75	75X75	75X75

†Red font indicates that the plant was used as a fill.

Table 1D. Field map of niger plots at the Highland Rim Research and Education Center (HRREC) in 2014.

Block 4	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79
	79X79	79X79	P5	P5	P5	BC2	P5	FF15	BC2	P4	BC7	P5	FF14	P5	P5	P5	P5	F11	P5	F10	F6	P5	P5	242X242	P2	79X79
	79X79	79X79	P5	P5	P5	BC8	P5	FF15	P5	P5	P5	242X242	FF14	P5	P5	FF13	FF14	F11	P5	F11	F8	P5	P5	P4	P4	79X79
	79X79	79X79	P5	P5	BC2	P5	P5	FF15	P5	P5	P5	P5	FF14	P5	P5	P5	FF14	P5	P5	F10	F9	FF17	P5	P3	P3	79X79
	79X79	79X79	P4	FF12	P5	P5	P4	FF15	P4	P4	P4	P5	FF14	P5	P5	P5	FF14	F7	P5	F6	F10	P5	P5	P1	P1	79X79
	79X79	79X79	P3	P3	P3	P3	P3	FF15	P3	P4	BC3	P5	FF14	P5	P5	P5	FF14	F9	P5	F9	P5	P5	P5	P1	P1	79X79
	79X79	P3	FF15	P3	P3	P3	FF14	P3	F6	BC1	P3	P3	P3	F7	P3	BC8	P3	FF14	FF14	BC3	F10	P3	FF15	FF15	FF15	79X79
	79X79	FF13	FF15	P3	FF12	FF17	FF14	P2	P3	P3	P3	P3	BC2	F6	P3	BC2	P3	FF14	FF14	BC7	F10	P3	FF15	FF15	FF15	79X79
	79X79	P3	FF15	BC4	P3	P3	FF14	P4	F11	BC3	P3	P3	P3	F11	P3	P3	P3	FF14	P3	BC7	F6	P3	FF15	FF15	FF15	79X79
	79X79	FF13	FF15	BC3	FF12	P3	FF14	P2	F9	BC7	P3	P3	BC2	F7	P3	BC7	P3	FF14	P3	P3	F9	FF15	FF15	FF15	FF15	79X79
	79X79	P3	FF15	BC3	FF12	FF17	FF14	P1	P3	P3	P3	P3	BC8	P3	P3	BC1	P3	FF14	FF14	BC8	F7	P3	FF15	FF15	FF15	79X79
Block 3	79X79	79X79	P4	P3	FF14	FF15	FF14	F8	P3	P3	FF14	P3	P3	FF15	BC8	FF14	F11	P1	P3	F9	FF13	FF15	P3	P3	P3	79X79
	79X79	79X79	P3	P3	FF14	FF15	FF14	F9	FF13	BC3	FF14	P3	P3	FF15	P3	FF14	F9	P5	P3	F6	FF13	FF15	P3	P3	P3	79X79
	79X79	79X79	P1	P3	P3	FF15	FF14	F7	P3	BC3	FF14	P3	FF13	FF15	BC7	FF14	F11	P2	P3	F11	P3	FF15	P3	P3	P3	79X79
	79X79	79X79	P1	FF13	FF14	FF15	P3	F10	P3	BC7	P3	P3	FF13	FF15	P3	FF14	F10	P4	P3	F6	P3	FF15	P3	P3	P3	79X79
	79X79	79X79	P2	P3	FF14	FF15	FF14	F7	P3	BC8	FF14	P3	P3	FF15	P3	FF14	F7	P5	P3	F10	FF13	FF15	P3	P3	P3	79X79
	79X79	P1	BC7	F9	P1	F6	P1	P1	P1	P1	P1	P3	FF14	FF13	FF15	BC2	P2	BC8	P2	P2	P2	FF15	P2	P2	F7	79X79
	79X79	P1	BC2	F9	P1	F11	P1	P1	P1	BC8	P1	P5	FF14	FF13	FF15	P2	P2	BC1	P2	P1	P1	FF15	P2	P2	F10	79X79
	79X79	P1	P1	P1	P1	F6	P1	P1	P1	P1	P1	P4	FF14	FF13	FF15	BC7	P1	P1	P1	BC1	P1	FF15	P2	BC2	F10	79X79
	79X79	P1	BC3	P1	P1	P1	P1	P1	P1	P1	FF12	P2	P1	FF13	FF15	P1	P1	BC3	P1	BC8	P1	FF15	P2	P2	P2	79X79
	79X79	F9	BC7	F9	F9	F11	F9	FF12	F9	BC2	P1	P3	FF14	FF13	FF15	P1	FF17	BC3	P1	P1	P1	FF15	P2	P2	P2	79X79
Block 2	79X79	79X79	F10	FF13	F7	FF13	F6	FF15	F10	F9	F9	FF14	P2	F10	F10	FF15	F10	F10	FF12	F10	F10	F10	F10	FF14	BC8	79X79
	79X79	79X79	F7	F9	F7	FF13	242X242	FF15	F11	BC8	F9	FF14	P4	F10	F10	FF15	F10	FF17	FF12	BC3	F10	F10	F10	FF14	BC2	79X79
	79X79	79X79	F11	FF13	F9	F9	F11	FF15	F9	BC8	F9	FF14	P5	F10	F10	FF15	F9	F10	FF12	F10	F10	F10	BC7	F10	BC7	79X79
	79X79	79X79	F10	F9	F9	FF13	F7	FF15	F8	BC7	F9	F9	P1	FF13	F9	FF15	F7	F9	F9	BC8	F10	F10	BC3	FF14	BC2	79X79
	79X79	79X79	F9	FF13	F9	FF13	F9	FF15	F11	BC3	F9	FF14	P4	FF13	F9	FF15	F6	F9	F9	F9	F9	F10	BC2	FF14	F10	79X79
	79X79	F9	F9	FF15	F9	P2	BC1	FF15	F9	F9	F9	BC8	F9	F9	FF13	BC2	FF15	F9	FF14	F9	FF13	F9	FF14	F6	P5	79X79
	79X79	BC3	F9	FF15	F9	P5	F9	FF15	FF14	BC7	F9	F9	F9	F9	FF13	F9	FF15	F9	FF14	F9	FF13	F9	FF14	F11	P3	79X79
	79X79	BC3	F9	FF15	FF16	P4	F9	FF15	FF14	F9	F9	F9	F9	F9	FF13	BC7	FF15	F9	F9	F9	FF13	F9	FF14	F6	P1	79X79
	79X79	BC6	F9	FF15	F9	P2	F9	FF15	FF14	F9	F9	BC2	F9	F9	FF13	F9	FF15	F9	FF14	F9	FF13	F9	FF14	F8	P3	79X79
	79X79	F9	F9	FF15	FF16	P1	F9	FF15	F9	BC1	F9	BC2	F9	F9	FF13	F9	FF15	F9	FF14	F9	FF13	F9	FF14	F6	P3	79X79
Block 1	79X79	79X79	P2	F9	P2	FF15	P2	P2	P2	P2	FF15	P2	P2	F11	FF15	FF14	FF15	P2	F10	P2	FF14	BC8	FF14	P3	P3	79X79
	79X79	79X79	P2	F9	P2	FF15	F11	P2	P2	P2	FF15	P2	P3	F9	P2	FF14	FF15	P2	F10	P2	P2	BC7	FF14	P3	P3	79X79
	79X79	79X79	P2	F11	P2	FF15	F9	P2	P2	P2	FF15	P2	P1	P2	P2	FF14	FF15	P2	F8	P2	FF14	BC2	P3	P3	P3	79X79
	79X79	79X79	P2	F11	P2	FF15	F7	P2	P2	P2	FF15	P2	P1	F7	P2	FF14	FF15	P2	F6	P2	FF14	P3	P3	P3	P3	79X79
	79X79	79X79	P2	F10	P2	FF15	P2	P2	P2	P2	FF15	P2	P2	F9	FF15	FF14	FF15	FF17	F11	P2	FF14	P2	FF14	P3	P3	79X79
	79X79	F10	F9	F7	P4	F9	FF15	F9	F9	BC2	P3	BC8	P1	P1	P1	P1	P1	P1	BC3	FF14	F7	P1	P1	BC8	P1	79X79
	79X79	F10	F10	F7	P4	F10	FF15	BC2	F10	F10	P4	BC2	P1	BC6	P1	P1	P1	P1	BC2	FF14	F10	P1	FF14	BC7	P1	79X79
	79X79	F10	F10	F6	P1	F10	FF15	BC1	F10	BC7	P3	BC1	P1	BC8	P1	BC3	P1	P1	P1	FF14	F6	P1	FF14	BC3	P1	79X79
	79X79	FF17	F10	F6	P5	F10	FF15	F10	F10	BC3	P2	P1	P1	P1	P1	BC6	P1	P1	P1	FF14	F6	P1	FF14	BC8	P1	79X79
	79X79	F10	FF12	F10	P5	F10	FF15	F10	F10	BC1	P5	BC3	P1	BC1	P1	P1	P1	P1	BC7	FF14	F10	P1	FF14	BC7	P1	79X79
	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79	79X79

†Red font indicates that the plant was used as a fill.

Table 1E. Field map of niger plots at the Plateau Research and Education Center (PREC) in 2014.

Block 4	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77
	77X77	77X77	BC3	FF15	FF14	FF14	77x77	F7	F8	77X77	P4	F11	W6XW6	77X77	P2	FF14	FF15	FF15	W6XW6	BC1	BC8	FF12	W6XW6	P3	77X77	77X77	77X77
	77X77	77X77	77X77	FF15	FF14	77X77	FF12	F10	F6	242	P2	F6	W6XW6	FF13	P2	FF14	FF15	W6XW6	FF12	W6XW6	P2	FF12	W6XW6	P1	77X77	77X77	77X77
	77X77	77X77	BC8	FF15	FF14	77X77	77x77	F7	F9	77X77	P1	F6	FF13	P2	W6XW6	FF14	FF15	W6XW6	W6XW6	BC3	W6XW6	FF12	W6XW6	P4	W6XW6	77X77	77X77
	77X77	77X77	77X77	FF15	FF14	FF14	FF12	F8	F9	77X77	P2	F8	FF13	P2	W6XW6	FF14	FF15	W6XW6	FF12	BC6	BC2	FF12	77X77	P5	77X77	77X77	77X77
	77X77	77X77	BC2	FF15	FF14	FF14	242	F8	F9	77X77	P2	F6	77X77	P2	P2	FF14	FF15	P2	P2	P2	P2	W6XW6	77X77	P1	77X77	77X77	77X77
	77X77	242	79X79	79X79	F7	79X79	P4	79X79	79X79	BC1	W6XW6	BC8	77X77	77X77	77X77	FF15	W6XW6	77X77	FF15	BC7	FF14	77X77	77X77	BC8	77X77	77X77	77X77
	77X77	FF15	FF13	79X79	F11	F7	P5	FF13	242	BC2	W6XW6	BC7	FF15	FF13	F9	FF15	77X77	77X77	FF15	BC3	FF14	77X77	BC2	BC1	77X77	77X77	77X77
	77X77	FF15	FF13	242	F11	F11	P5	79X79	79X79	BC7	FF14	BC7	FF15	FF15	F9	FF15	W6XW6	W6XW6	FF15	77X77	FF14	FF16	BC5	77X77	FF12	77X77	77X77
	77X77	FF15	79X79	242	F10	F10	242	242	79X79	BC3	FF14	BC3	79X79	FF13	F7	FF15	W6XW6	P3	FF15	W6XW6	FF14	77X77	BC6	77X77	FF12	77X77	77X77
	77X77	FF15	79X79	79X79	F6	F10	P3	FF13	79X79	BC7	FF14	79X79	W6XW6	W6XW6	F10	FF15	79X79	FF15	W6XW6	BC8	FF14	79X79	BC2	77X77	76X76	77X77	77X77
Block 3	77X77	77X77	FF15	242	P4	F10	76x76	FF14	BC8	F9	W6XW6	W6XW6	BC3	79X79	79X79	W6XW6	79X79	79X79	F7	FF15	W6XW6	FF14	79X79	W6XW6	79X79	77X77	77X77
	77X77	77X77	FF15	242	P3	F7	76x76	FF14	W6XW6	F7	F9	FF12	BC1	W6XW6	W6XW6	79X79	79X79	FF12	F11	FF15	79X79	W6XW6	79X79	BC2	79X79	77X77	77X77
	77X77	77X77	FF15	242	P2	F10	76x76	FF14	BC7	F10	F11	FF12	BC3	W6XW6	FF13	F13	79X79	FF12	F6	FF15	FF12	W6XW6	79X79	W6XW6	79X79	77X77	77X77
	77X77	77X77	FF15	242	P2	F9	76x76	FF14	BC2	76X76	F11	W6XW6	W6XW6	76X76	W6XW6	W6XW6	W6XW6	FF12	F8	FF15	W6XW6	W6XW6	79X79	BC2	W6XW6	77X77	77X77
	77X77	77X77	FF15	242	P4	F6	242	FF14	BC7	F7	F9	FF12	BC1	W6XW6	79X79	FF13	79X79	W6XW6	F6	FF15	W6XW6	FF14	FF16	79X79	79X79	77X77	77X77
	77X77	FF15	76X76	FF14	242	76X76	76x76	242	FF15	FF13	BC8	W6XW6	P4	FF14	W6XW6	W6XW6	W6XW6	FF15	BC2	76X76	FF17	76X76	P5	W6XW6	76X76	77X77	77X77
	77X77	FF15	BC4	FF14	F6	76X76	BC3	FF13	FF15	76X76	W6XW6	W6XW6	P1	FF14	F11	W6XW6	FF14	FF15	BC7	W6XW6	W6XW6	W6XW6	P5	W6XW6	W6XW6	77X77	77X77
	77X77	FF15	BC3	FF14	242	BC8	242	242	FF15	FF13	W6XW6	W6XW6	P5	FF14	F10	W6XW6	FF14	FF15	BC7	W6XW6	76X76	W6XW6	P3	76X76	76X76	77X77	77X77
	77X77	FF15	BC2	FF14	242	76X76	242	FF13	FF15	76X76	BC8	W6XW6	P1	FF14	76X76	W6XW6	FF14	FF15	76X76	76X76	76X76	W6XW6	P1	76X76	76X76	77X77	77X77
	77X77	FF15	BC8	FF14	F10	BC1	242	FF13	FF15	242	BC7	W6XW6	P3	FF14	F9	76X76	FF14	FF15	BC3	76X76	76X76	76X76	P2	W6XW6	76X76	77X77	77X77
Block 2	77X77	77X77	76X76	P3	242	P2	BC2	76X76	242	FF14	76X76	76X76	FF15	F7	F10	FF13	FF12	F7	FF13	FF14	W6XW6	77X77	W6XW6	FF14	BC2	77X77	77X77
	77X77	77X77	76X76	P1	76X76	P2	BC3	FF12	W6XW6	FF14	W6XW6	BC3	FF15	F7	76X76	W6XW6	W6XW6	F6	FF13	FF14	F10	W6XW6	76X76	FF14	BC3	77X77	77X77
	77X77	77X77	76X76	P1	76X76	P3	242	FF12	76X76	FF14	76X76	76X76	FF15	W6XW6	F6	76X76	FF12	F11	76X76	FF14	F9	76X76	76X76	FF14	W6XW6	77X77	77X77
	77X77	77X77	76X76	P5	76X76	P5	76x76	76X76	FF12	FF14	76X76	W6XW6	FF15	W6XW6	F10	FF13	FF12	F10	76X76	FF14	F9	W6XW6	W6XW6	FF14	BC2	77X77	77X77
	77X77	77X77	76X76	P2	76X76	P4	76x76	76X76	FF12	76X76	76X76	W6XW6	FF15	F11	F7	W6XW6	FF12	F11	76X76	FF14	W6XW6	W6XW6	W6XW6	FF14	W6XW6	77X77	77X77
	77X77	77X77	77X77	77X77	F9	FF14	FF13	242	77X77	77X77	77X77	P5	77X77	FF15	F6	77X77	BC8	FF14	FF15	FF15	BC3	W6XW6	FF15	W6XW6	W6XW6	77X77	77X77
	77X77	77X77	77X77	BC7	F6	FF14	242	BC7	77X77	77X77	W6XW6	P1	W6XW6	FF15	F7	W6XW6	77X77	FF14	FF15	FF15	W6XW6	W6XW6	FF15	77X77	76X76	77X77	77X77
	77X77	77X77	77X77	BC2	F11	FF14	FF13	BC7	W6XW6	FF13	W6XW6	P3	77X77	FF15	F9	W6XW6	BC2	FF14	FF15	FF15	BC7	W6XW6	FF15	76X76	76X76	77X77	77X77
	77X77	77X77	77X77	BC8	F8	FF14	242	BC8	W6XW6	FF13	W6XW6	P4	77X77	FF15	W6XW6	FF12	BC1	FF14	FF15	FF15	BC7	77X77	FF15	W6XW6	76X76	77X77	77X77
	77X77	77X77	77X77	BC6	F6	FF14	77x77	BC8	W6XW6	FF13	W6XW6	P4	77X77	FF15	F10	FF12	BC8	FF14	FF15	FF15	BC1	77X77	FF15	76X76	BC3	77X77	77X77
Block 1	77X77	77X77	77X77	FF15	FF15	P2	BC3	FF14	FF15	77X77	W6XW6	77X77	77X77	FF14	F9	P3	FF15	76X76	76X76	76X76	76X76	F7	77X77	FF14	77X77	77X77	77X77
	77X77	77X77	77X77	FF15	FF15	P3	BC3	FF14	FF15	BC2	77X77	77X77	77X77	FF14	F9	P2	FF15	77X77	77X77	76X76	W6XW6	F6	77X77	FF14	77X77	77X77	77X77
	77X77	77X77	77X77	FF15	FF15	P4	BC5	FF14	FF15	BC1	FF13	77X77	77X77	FF14	F10	P5	FF15	77X77	77X77	76X76	76X76	F7	77X77	FF14	77X77	77X77	77X77
	77X77	77X77	FF17	FF15	FF15	P1	77x77	FF14	FF15	BC6	W6XW6	77X77	77X77	FF14	77X77	P3	FF15	77X77	77X77	76X76	76X76	W6XW6	76X76	FF14	77X77	77X77	77X77
	77X77	77X77	242	FF15	FF15	P5	BC2	FF14	FF15	W6XW6	77X77	77X77	77X77	FF14	F6	P1	FF15	77X77	W6XW6	FF13	W6XW6	F11	76X76	FF14	W6XW6	77X77	77X77
	77X77	BC7	BC3	P5	BC7	P5	P5	P5	P5	P5	P5	P4	77X77	F10	W6XW6	77X77	FF14	77X77	77X77	FF15	W6XW6	W6XW6	BC3	F11	F9	77X77	77X77
	77X77	BC7	BC3	P5	BC7	FF13	P5	P5	P5	P5	P5	P5	FF14	F6	77X77	77X77	FF14	77X77	77X77	FF15	F9	W6XW6	BC2	F11	F6	77X77	77X77
	77X77	P5	BC8	P5	242	P5	P5	P5	P5	P5	P5	P4	FF14	F10	FF12	77X77	FF14	77X77	W6XW6	FF15	77X77	W6XW6	77X77	F9	F7	77X77	77X77
	77X77	BC8	BC8	P5	P5	P5	P5	BC2	P5	P5	P5	P1	FF14	F7	W6XW6	W6XW6	FF14	W6XW6	77X77	FF15	F6	W6XW6	W6XW6	F8	W6XW6	77X77	77X77
	77X77	BC8	BC7	P5	P5	FF13	BC8	BC2	P5	P5	P5	P2	FF14	F11	W6XW6	77X77	FF14	FF13	77X77	FF15	F10	W6XW6	BC6	F11	F7	77X77	77X77
	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77	77X77

†Red font indicates that the plant was used as a fill.

Vita

Victoria Benelli was born in 1988 in Johnson City, NY to David and Cynthia Knapp. She enrolled at Broome Community College after graduating from Harpursville Central High School in 2006. It was here that she completed an A.S. in Business Management, Marketing, and Sales in 2008. Victoria transferred to the University of Tennessee, Knoxville in 2010 to pursue a B.S. in Horticulture Science and Production. After graduation, she began work as a Graduate Research Assistant while earning a M.S. degree in Plant Breeding under Dr. Fred Allen. Soon after her enrollment in graduate school, she met her husband Jesse Benelli; also another graduate student. Victoria plans to work as an assistant plant breeder.