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IMPROVEMENT OF NEW OIL CROPS FOR KENTUCKY

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ABSTRACT OF DISSERTATION

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The Graduate School

University of Kentucky

2010

IMPROVEMENT OF NEW OIL CROPS FOR KENTUCKY

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
Watchareewan Jamboonsri

Lexington, Kentucky

Director: Dr. David Hildebrand, Professor of Plant and Soil Science

Lexington, Kentucky

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Three oil crops, chia (*Salvia hispanica* L.), flax (*Linum usitatissimum* L.), and castor (*Ricinus communis* L.), were studied because of their nutritional and industrial values. Chia and flax are rich in an ω3 fatty acid, α-linolenic acid, and castor is a very high oil producer and high in a hydroxy fatty acid. Ethyl methanesulfonate (EMS) and gamma rays were employed to mutagenize chia seeds to produce early flowering mutants. The M_1 population was grown and induced to flower by short-day photoperiods. The M_2 population was planted in the field in Lexington, KY in 2008. Early flowering plants were found 55 days after planting while non-mutagenized plants did not produce any flower buds until the 7th of October, 82 days after planting, at a daylength of 11 hours and 32 minutes. 0.012% of the EMS-treated M_2 population and 0.024% of the gamma radiation-treated population flowered much earlier than the controls. M_3 early flowering mutant lines were able to flower at photoperiods of 12-15 hours in a greenhouse. Selected lines produced flower buds on the 7th of July, 47 days after planting, at a daylength of 14 hours and 41 minutes in the field in Lexington, Kentucky.

Different varieties of flax were evaluated for seed yield and field performance in Kentucky. Plant height and yield data were collected from three growing seasons. Yields from 2006 trial varied from 368-1,267 kg/ha. Yields from 2007 and 2008 were much lower due to drought. The variety 'Carter' gave the highest yield every season. Flax can be grown in Kentucky but yields are low.

Two high-yield castor varieties, 'Carmencita' and 'TTU-LRC', were crossed in greenhouse. The F_1 population was grown in the field. Inflorescences were covered to ensure self-pollination. The F_2 population showed a high degree of segregation for plant height, stem color, capsule color and seed yield in the following growing season. Data on plant height, number of branches, color, and yield was collected from 89 F_2 individuals. Fifteen lines with the highest yield were selected to plant in the field in spring of 2009. New high-yield castor varieties are being developed for production in Kentucky.

KEYWORDS: Chia (*Salvia hispanica* L.), Flax (*Linum usitatissimum* L.), Castor (*Ricinus communis* L.), Breeding, Oil Crops

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May 07, 2010

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IMPROVEMENT OF NEW OIL CROPS FOR KENTUCKY

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To my mom, Pratoom Jamboonsri; my dad, Jane Jamboonsri;

my sister, Jantima Jamboonsri and my brother, Navee Jamboonsri

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CHAPTER 1

INTRODUCTION

Kentucky has land area of 25,388,000 acres. Livestock makes approximately 68% of cash receipts from farm marketing while crops contribute only 32%. Major crops grown in Kentucky are corn, tobacco, soybeans and wheat. Kentucky cash receipts from 2006 farm marketing for corn, tobacco, soybeans and wheat were 319,655,000; 339,260,000; 325,729,000 and 85,394,000 respectively. In 2007, the value of crop production of corn, tobacco, soybeans and wheat were $719,304,000; $331,792,000; $300,456,000 and $70,438,000 respectively. The only major oil crop grown in Kentucky is soybeans which contributes about 8% of farm income in KY (USDA, 2008).

Besides soybeans, there are many oil crops listed by USDA as alternative crops for small farms to grow. These crops include borage, broomcorn, canola, comfrey, crambe, cuphea, flax, guayule, jojoba, kenaf, Lesquerella, lupine, meadowfoam, milkweed, perilla, safflower, sesame, sunflower and Vernonia (USDA, 2009). Farmers are able to adopt new alternative crops suitable for their areas and their purposes. Flax and castor have great potential to be alternative oil crops. Flax can be grown for dual use as a source of edible oils from seeds and as a source of fiber from stems. Castor is a source of industrial oil, rich in ricinoleic acid, one of the specialty oils in the market. Approximate U.S. market size for ricinoleic acid in 2002 was about 30 million kilograms or $60 million (Schultz and Ohlrogge, 2002). Like flax, Chia is also a source of edible oil. It is not in the USDA alternative crop list because seed production cannot be done in most of the U.S.

The overall goal of the studies in this dissertation is to develop and to evaluate alternative oil crops suitable for production in Kentucky. Three crops of interest are chia, flax and castor.

Chia has long been cultivated and domesticated (Cahill, 2005). It was widely used in pre-Columbian Mesoamerica as a major commodity and its seeds were valued for food, medicine and oil. Cultivation of chia was drastically reduced after Spanish colonization due to beliefs and religious conflicts. Several *Salvia* species including *Salvia hispanica* L. have been introduced in the U.S. as potential new crops (Gentry et al., 1990). Chia and chia oil is used as human food, animal feed, drying oil in paints, ingredient in cosmetics (Athar and Nasir, 2005). Chia has recently been revived as a new crop opportunity fueled particularly due to its high oil and highest ω-3 fatty acid content among productive oilseeds (Cahill, 2003; Cahill, 2004). There is growing evidence that, similar to flax, chia can have considerable health benefits to humans (Anderson, 1998; Cahill, 2003; Taga et al., 1984). Adding whole chia seed to animal diets greatly increases the nutritive value of the resulting eggs and poultry meat (Ayerza and Coates, 1999; Ayerza and Coates, 2000; Ayerza and Coates, 2001; Ayerza and Coates, 2002; Ayerza et al., 2002). Chia may be the best source of healthful soluble fiber known (Ayerza and Coates, 2004; Cahill, 2003). Chia is a diploid with only 12 chromosomes (n = 6) which will facilitate rapid genetic improvement of this new crop (Estilai et al., 1990) for Kentucky. Chia is a short-day plant and commercially grown in Australia, Argentina, Colombia, Mexico and Peru. Chia grows well in Kentucky and has low requirement for pesticides, fertilizer and irrigation. It produces flower buds in short days of October and is killed by frost before seeds set. Seed production cannot be done in Kentucky. There was no known source of natural long day chia germplasm available. Regulation of flowering time in chia is not known. Mutagenesis is a powerful tool to introduce desired traits into organisms. The objective of this research was to produce long-day or day-neutral flowering chia.

Flax or linseed is one of the most promising new crops for meeting the increasing consumer demand for healthier diets that could readily be grown by Kentucky farmers. The industrial oils are another market sector that is looking more at seed oils especially those with double bonds. Flax is among the highest among crop plants in this respect (highest in the ω-3 fatty acid, α-linolenic acid which has three double bonds) (Bell et al., 2004). Flax is increasingly recommended as a component of healthy diets (Oliff, 2004). Evidence is rapidly accumulating that flaxseed consumption can have remarkable benefits to human health with significant reduction of heart disease and a number of types of cancer (Bhatia et al., 2007; Bloedon and Szapary, 2004; Spensor et al., 2003).

There is also evidence that it can improve cognitive ability in mammals (Hartvigsen et al., 2004). An increasing number of foods containing flaxseed have been developed (Shearer and Davies, 2005; Warren et al., 2005). Flax could have a direct market in Kentucky‘s animal industries by improving consumer products. The addition of whole flax seed into animal diets increases the nutritive value of resulting eggs (Bean and Leeson, 2003; pork (Hoz et al., 2003), milk (Petit et al., 2002) and fish (Bell et al., 2004). Flax also has industrial values as a source of biodegradable plastics (Wrobel et al., 2004) and as a drying oil in paints (Lazzari and Chiantore, 1999). Flax may also provide benefits to wheat production. When used as a rotation crop with wheat, flax reduces leaf diseases of the wheat more than the wheat was rotated with soybeans (Krupinsky et al., 2004). Parker and Jeffrey (1992) reported on flax production in western Kentucky. The report focused on social aspect and importance of flax to Shakers community in Kentucky where flax is grown primarily as a source of fiber for clothing. None of experimental data on seed production was provided. The objective of this study was to evaluate field performance of different flax varieties in Kentucky.

Oil with hydroxy and epoxy fatty acids have immediate promise as valuable new crops for industrial uses. The most promising oil crop in this group for Kentucky farming is castor (*Ricinus communis* L.). Castor oil contains as much as 90% of the industrial valuable hydroxy fatty acid known as ricinoleic acid. Major limitations of commercial production of castor were 1) concern about the toxicity of ricin found in castor seeds and 2) inefficiency of harvesting by hand or poorly developed machines. However, low ricin castor and improved harvesting machines have been developed (Pinkerton et al., 1999; Auld et al., 2001; Auld et al., 2003). The reduced ricin line TTU-LRC was registered in 2003 and released. This line was developed by Texas Tech University. The dwarf-internode cultivar, ‘Hale’ developed by Texas A&M University in 1970 was crossed with two Plant Introductions from the Soviet Union (PI 258368 and PI 257654) which were previously selected for reduced levels of ricin. In subsequent segregating generations, individual plants were selected for dwarf-internode growth habit (33 to 119 cm) and reduced levels of ricin and *Ricinus communis* agglutinin (Lowery et al., 2007). For future growth opportunities for Kentucky farmers, castor could easily be bred or genetically engineered to accumulate other high value oil derivatives such as epoxy fatty acids. It could also be used as a platform for production of lipophilic medicinal compounds in molecular farming. Castor can be grown in Kentucky, however new varieties with high

yield and low ricin should be developed for seed production in Kentucky. The objective of this project was to produce high yield castor by a conventional breeding program.

CHAPTER 2

OBJECTIVES

1. To develop chia lines that flower under longer daylengths.
2. To evaluate field performance of different flax varieties in Kentucky.
3. To produce a high yield castor variety suitable for production in Kentucky by a conventional breeding program. Low ricin and semi-dwarf characters are preferred for seed production.

CHAPTER 3

LITERATURE REVIEW

Chia (*Salvia hispanica* L.)

Plant Descriptions

Chia (*Salvia hispanica* L.) is a member of the Labiateae or Lamiaceae or mint family. The center of genetic diversity of chia is in the highlands of western Mexico (Cahill, 2004). This annual seed crop has chromosome number of 2n = 12 (Estilai et al., 1990). Stems are about 1-2 m and obtusely quadrangular. Leaves are opposite, ovate, tapered and sharply serrated. The flowers are produced in terminal and axillary four-cornered spikes protected by small bracts with long sharp points. The blue corolla is tubular with four stamens, two of them larger and sterile. Seeds are present in groups of four. Mean seed mass of domesticated chia is about 15 mg/100 seeds (Cahill and Ehdaie, 2005).

Agronomic Characteristics

Currently chia is commercially grown in tropical and subtropical areas. Ayerza and Coates (2005) reported that chia is cultivated in Argentina, Bolivia, Colombia, Mexico and Peru where latitudes range from 20°55'N to 25°05'S but in higher latitude like Choele-Choele, (39°11'S) Argentina and Tucson (32°14'N), Arizona, USA, the plant cannot produce seeds since it is killed by frost before seeds mature. The crop cycle varies from 90 to 150 days depending on the latitude where it is planted. Chia is sown at a rate of 6-8 kilograms per hectare, with row spacing of 0.70 or 0.80 m in Argentina and Bolivia and of 0.75 m in Mexico. Chia seeds require wet soil to germinate, but once the seedlings are established, chia grows well with limited water. The first 45 days of growth are critical since weeds can compete for light, nutrients and water. No herbicide has been approved for chia. Growers control weeds manually and/or mechanically. Weeds

become a minor problem once the canopy is closed. Pests and diseases are not well documented. Observations in commercial fields have not shown many problems. Ants were found to be the biggest problem in Argentina, Bolivia and Columbia. Chia is often harvested mechanically. The main difficulty is that the central flower head matures and dries out while inflorescences on side braches remain green. Waiting until all seeds are dry increases the risk of seed loss to rain, wind or birds. Chia grows well on soils containing widely varying levels of nutrients. Low nitrogen content appears to be a significant barrier to good seed yield. Commercial chia seed yields are generally 500-600 kilograms per hectare (Coates and Ayerza, 1996), however some growers have obtained up to 1,260 kg/ha. Some experimental plots yielded 2,500 kg/ha when irrigation and nitrogen fertilizer were applied.

Nutritive and Medicinal Values

Chia seed is a source of natural lipid antioxidants. Flavonol glycosides, cholorogenic acid and caffeic acid are found in chia extracts (Taga et al., 1984). The antioxidant activity of the fiber-rich fraction of chia flour was found to be higher than many cereals and similar to drinks such as wine, tea, coffee and orange juice (Vazquez et al., 2009). Chia seed coat is high in fiber which becomes mucilagenous and expands considerably when soaked in water. The fiber consists of xylose, glucose and glucuronic acid (Lin and Daniel, 1994). Vasquez et al. (2009) reported that the fiber-rich fraction of chia flour has 56.5 g/100 g total dietary fiber content. The fiber-rich fraction water-holding capacity is 15.4 g/g.

Chia seed contains about 20% protein. Chia protein digestibility was evaluated by Torres et al (2008). The digestibility was 79.8%, 34.2%, 29.1%, 24.3%, 10.9% in flour, toasted flour, raw seed, soaked seed, and toasted seed respectively. Chia flour was shown to have a low digestibility score which could be the influence of chia fiber. Chia oil content ranges from 28.5 - 32.7% (Ayerza and Coates, 2004; Ayerza and Coates, 2007). Chia is high in the omega-3 fatty acid, α-linolenic acid, 18:3. Chia diets dramatically decreased triacylglycerol levels and increased high density lipoprotein cholesterol and ω-3 fatty acid content in rat serum (Ayerza and Coates, 2005). Dietary chia seed also improves adiposity and insulin resistance in dyslipeamic rats (Chicco et al., 2009). Diets supplemented with chia have been found to decrease risks from some

types of cadiovascular diseases, cancers and diabetes. It has been reported that chia diet decreased the tumor weight and metastasis number and also inhibited growth and metastasis in a murine mammary gland adenocarcinoma (Espada et al., 2007). Long-term supplementation with chia attenuated a major cardiovascular risk factor and emerging factors safely beyond conventional therapy, while maintaining good glycemic and lipid control in people with well-controlled type 2 diabetes (Kreiter, 2005; Vuksan et al., 2007 and Vuksan et al., 2009). Omega-3 fatty acids are reported to have benefit in psychiatric disorders. A report showed that omega-3 fatty acids have significant benefit in prevention and/or treatment of unipolar and bipolar depression (Freeman, 2006).

History and Uses

Chia was widely used in pre-Columbian Mesoamerica for different purposes such as medicinal, culinary, artistic and religious use. Ethnobotany of chia in the 16th century was extensively described by Cahill (2003). Cultivation of chia was drastically reduced after Spanish colonization due to beliefs and religious conflicts. Several *Salvia* species including *Salvia hispanica* have been introduced in the U.S. as potential new crops (Gentry et al., 1990). Chia and chia oil are used as human food, animal feed, drying oil in paints, and ingredients in cosmetics (Athar and Nasir, 2005). Chia leaf oil may be useful in flavorings or fragrances and possibly as a pesticide since white flies and other insects seem to avoid the plant (Ahmed et al., 1992). Broiler feed supplemented with chia seeds was shown to significantly lower saturated fatty acid content in white and dark meats (Ayerza et al., 2002). There have been studies on hens for the potential of chia diets as a source of ω-3 fatty acids in egg production (Ayerza and Coates, 1999; Ayerza and Coates, 2000; Ayerza and Coates, 2001; Ayerza and Coates, 2002). A recent study was done to determine fatty acid composition and nutritive value of chia seed and vegetative parts as a possible source of polyunsaturated fatty acids for ruminants (Peiretti and Gai, 2009). There is a market for chia seeds as a novelty gift known as ChiaPet, usually marketed for the Christmas season (Hershey, 1995). ChiaPets consist of an unglazed ceramic animal or tree that can be filled with water and covered with chia seeds which sprout to produce the effect of fur, hair, skin or leaves.

Flax (*Linum usitatissimum* L.)

Plant Descriptions

Flax (*Linum usitatissimum* L.) is in the *Linaceae* or flax family. This self-pollinated crop has chromosome number of 2n = 30 (Charles, 1944). Flax is also called flaxseed or linseed. In North America, it is common to use 'flaxseed' to describe flax when it is consumed by human or animal and 'linseed' when it is used for industrial purposes such as linoleum flooring. In Europe, the term flaxseed describes the varieties grown for making linen.

Flax has one main stem with slim branches. Plant height varies from 0.6 to 1.2 m. The flowers are pale blue with five petals. The fruits are round capsules containing glossy seeds. A complete capsule or boll can have up to ten seeds, averaging ~ six seeds. Flax seeds are flat and oval with a pointed tip. Seed color ranges from a deep brown to a light yellow (golden) depending on the amount of pigment in the outer seed coat. Yellow-seeded flax has a higher seed weight and oil content than brown-seeded flax (Diederichsen and Raney, 2006). Golden flaxseed is preferred in Europe, Asia, and the United States food market as it blends well in food ingredients (Berglund, 2002).

Agronomic Characteristics

Flax is an annual plant. It is usually sown on the same type of land and climate as wheat and barley production. Flax can fit into a small-grain rotation. It requires a 50-day vegetative period, a 25-day flowering period and about 35 days to mature. A planting depth of 1.9 to 3.8 cm is recommended. In general, the seeding rate is between 28-50 kg/ha. Early seeded flax generally produces the highest yield. Emerging seedlings are most susceptible to injury. Frost seldom kills flax seedlings. They tolerate temperatures down to -2 °C for a few hours. Since flax is a poor competitor with weeds, weed control is needed after flax emergence to reduce yield losses. Insects that may cause yield loss in flax are grasshoppers, armyworms, aster leafhopper, aphids and wireworm. Disease losses have been smaller in flax than many other annual crops due to the use of disease-resistant varieties. Flax is harvested when about 90 percent of the capsules turn brown (Berglund and Zollinger, 2007).

Flax is commercially grown mainly in Canada, China, India, USA, Ethiopia, Bangladesh, Russia, Ukraine, France and Argentina. In the USA, flax is mostly grown in North Dakota, South Dakota, Minnesota and Montana. North Dakota is the flax production leader with over 95% of crop area planted in 2001. Total flax area in the United States increased from 38,800 ha in 1996 to 224,600 ha in 2002. Canada produces a large amount of oilseed flax. It has approximately three times the crop area planted in the United States. In 2000 oilseed flax production in Canada was reported to be 707,000 tons (Berglund, 2002).

Flax accessions in world collections now number in the thousands and are increasingly being evaluated for agronomic and other characteristics for breeding programs (Diederichsen, 2001). The agronomic properties of flax lines and accessions have been evaluated in Ethiopia (Wakjira et al., 2004), South Africa, the Northeastern US (see http://www.sare.org/reporting/report_viewer.asp for example), the Midwestern US (Hammond et al., 2004a; Hammond et al., 2004b; Johnston et al., 2002), South Carolina (Foulk et al., 2004) and in Ontario, Canada (Bean and Leeson, 2002). Molecular genetic analysis and marker-assisted genetic resource evaluation have been described (Fu et al., 2003; Treuren et al., 2001). Flax may also provide benefits to wheat production. When used as a rotation crop with wheat, flax reduces leaf diseases of wheat more than when wheat is rotated with soybeans (Krupinsky et al., 2004).

Nutritive and Medicinal Values

Flax seed generally contains 41% lipids, 20% protein, 28% dietary fiber, and 7.7% moisture and 3.4% ash (www.flaxcouncil.ca, 2009). Flax oil has approximately 46% of linolenic acid (omega-3 fatty acid). It has long been known that flax seed hull has mucilaginous substances (Neville, 1913). Flax is a good source of fiber, both soluble and insoluble. The mucilage on flax hull comprises about 8% of the seed weight. It is primarily a mixture of polysaccharides composed of *d*-galacturonic acid, *l*-rhamnose, *l*-galactose and *d*-xylose units (Anderson and Lowe, 1947; Erskine and Jones, 1957; Fedeniuk and Biliaderis, 1994). Flax is increasingly being recommended as a component of a healthy diet (Oliff, 2004). Flax is high in lignans which appear to have anti-carcinogenic properties (Thompson et al, 1996). Evidence is accumulating that flaxseed consumption can have remarkable benefits to human health with a significant

reduction of heart disease and a number of types of cancer (Bhatia et al., 2007; Bloedon and Szapary, 2004; Spencer et al., 2003). Flax is also reported to improve mental health. There is evidence that it can improve cognitive ability in mammals (Hartvigsen et al., 2004). Joshi et al. (2006) reported that supplementation with flax oil improves the outcome of Attention Deficit Hyperactivity Disorder (ADHD). An increasing number of foods containing flaxseed are being developed (Shearer and Davies, 2005; Warrand et al., 2005).

History and Uses

Flax has been used in food in Europe, Africa and Asia since 5000-8000 BCE and its fiber has been used for linen cloth and many other uses. Flax was first brought to North America for its stem fiber to use in making linen and paper. It has been grown in the United States and Canada as a commercial oilseed crop since early colonial times and was a major crop and source of fiber for clothing of settlers from the old world (Berglund, 2002; Thomas Jefferson Agricultural Institute, 2007). It was a major crop of the Shakers in Central Kentucky in the 18th century. Currently flax seed is used as human food and animal feed and as a source of flax oil. Ground or whole flax seed can be added to almost any baked product and adds a nutty flavor to bread, waffles, cookies or other products. Flax seed flour is also used commercially in breads in the United States. Mason and Hall (1948) have reported on the use of flaxseed mucilage as an emulsifying agent for chocolate milk. Flax could have a direct market in Kentucky's animal industries by improving consumer products. The addition of whole flax seed or linseed oil into animal diets increases the nutritional value of resulting eggs (Bean and Leeson, 2003), pork (Hoz et al., 2003) and milk (Petit et al., 2002; Ward et al., 2002). Flax is increasingly being recognized as an outstanding major dietary component for healthy fish (high in ω-3 fatty acids) production (Bell et al., 2004; Kiron et al., 2004; Zheng et al., 2004). Effects of flax oil supplemented diet on sheep digestion were reported (Ikwuegbu and Sutton, 1982). Flax is even now being evaluated as a major component of horse feed (Duvaux Ponter et al., 2004; O'Neill et al., 2002). The health of consumers of animal products such as meat, eggs and butter can be improved when such animals are fed significant amounts of linseed (Weill et al., 2002).

Industrial uses of linseed are expanding (Rakotonirainy and Padua, 2001). Linseed oil has been used as a drying oil in paints (Lazzari and Chiantore, 1999). It is also epoxidized and used as reactive diluents for coating (Ashby et al, 2000 and Muturi et al., 1994). High level of linolenic acid in flax seed is important for industrial uses; however, in edible products, it can cause undesirable odors and flavor when oxidized (Graef et al., 1988; Green, 1986). A mutation program was initiated to reduce the linolenic acid content in flax (Rowland and Bhatty, 1990; Saeidi and Rowland, 1997).

Flax is also a source of biodegradable plastics (Wrobel et al., 2004) because natural fibers have the advantage of low density, low cost and biodegradability (Li et al, 2007; Barkoula et al., 2010). The main disadvantage is poor compatibility between fiber and matrix. There are studies on preparations and modifications of flax fiber to improve composite properties (Arbelaiz et al, 2005 and Liu et al, 2006). Flax fiber is processed and used for other products, for example cigarette paper, pulp and paper, and erosion control mats (Burglund, 2002).

Castor (*Ricinus communis* L.)

Plant Descriptions

Castor (*Ricinus communis* L.) is in the Euporbiaceae or spurge family. It has a chromosome number of 2n = 20 (Perry, 1943). Castor is widespread as a wild plant through East and North Africa. The diversity for many plant, fruit and seed character is enormous and this led to the assumption that the cultivated castor might be of African origin (Engels and Hawkes, 1991). As a perennial plant in the tropics, it is 10-13 m tall with the stem diameter of 7.5-15 cm. As an annual plant in the temperate regions, it is 1-3 m tall. Stems are succulent, herbaceous and very variable in all aspects. The plant has alternate, orbicular, palmately compound leaves with 6-11 toothed lobes. The flowers are in long inflorescences with male flowers at the base and female flowers at the tips. Petals are absent in both sexes and there are greenish 3-5 sepals. Stamens are 5-10 mm long with 3-celled ovary with a short style and 3 stigmas. Castor fruit is a spiny globose capsule of 2.5 cm in diameter and contains 3 seeds. Seeds are ovoid, tick-like and shiny, 0.5-1.5 cm long (Duke, 1983).

Toxins in castor

Ricin is a toxin found in castor seed. A recent study on ricin accumulation showed that ricin is found in seed only. Accumulation starts days after pollination and quickly increases until seed maturation. Ricin is degraded below levels of detection six days after germination (Barnes et al., 2009). Ricin is a glycoprotein lectin (6.6 kD). It blocks protein synthesis by altering the rRNA thus killing the cell. Ricin toxicity and mechanism of action was extensively described by Bagchi et al. (2009). The lethal oral dose in humans has been estimated to be 1-20 mg of ricin/kg of body weight (approximately 8 beans). Castor plant also contains another glycoprotein lectin, the *Ricinus communis* agglutinin. It is not directly cytotoxic but has an affinity to red blood cells, leading to agglutination and subsequent hemolysis. Ricinine is an alkaloidal toxin also found in the leaves and pericarp of the castor plant. In experimental mice models, ricinine causes convulsions and subsequent death. However, there are no reports of human ricinine poisoning (Audi et al, 2005). Castor seeds contain 2S albumins. These seed proteins are highly allergenic. Gene expression of 2S albumin has been studied to

develop and implement a genetic silencing approach to eliminate 2S albumin from castor seed (Chen, 2004).

Agronomic Characteristics

Castor was in production as early as the mid-1850s in the central part of the United States. Castor is planted when the soil is warm with ten day average of 15 °C. In Texas, planting date should be between May 5th - 25th and not later than June 10th. Emergence of the seedlings may take 7-14 days. Seeds should be planted 6.3 – 7.6 cm deep. Row spacing is usually 1 m. and plant spacing within row is between 20 – 25 cm with seeding rate of 11.2 – 15.7 kg/ha. Adequate amount of nitrogen, phosphorous and potassium must be available to produce high yield of castor seed. Yields of irrigated castor range from 2,242 – 3,363 kg/ha, and some fields have produced 3,811 kg/ha to 4,035 kg/ha (Brigham, 1993).

Castor beans are processed throughout the world to make castor oil. India currently produces the largest amount of castor oil, followed by China and Brazil (Bagchi et al., 2009). India has 0.8 million ha of area under castor and 1.0 million tonnes of production (Anjani et al, 2010). Li et al., (2010) reported that there is a tremendous demand for castor in China. There are large areas (3 million ha) along coastal saline land that could be targeted for castor plantations in the future. Castor offers immense potential for improvement for water and salt stress tolerance, disease and pest resistance, and toxin free varieties through genetic engineering. Genetic engineering technique requires a good in vitro system. Tissue culture of castor has been reported (Reddy et al, 1987) but it was not very efficient. The first success on castor transformation by *Agrobacterium* was reported in 2005 (Sujatha and Sailaja, 2005). Semilooper (*Achoea janata* L.) resistant transgenic castor was produced the next year (Malathi et al., 2006). High-frequency plant regeneration through adventitious shoot formation system has been developed for castor (Ahn, et al., 2007).

Even though castor grows naturally in the wild in Africa, these wild plants are usually too tall to harvest and seed capsules shatter when mature and dry. Improved commercial castor cultivars from the USA, Israel and South Africa were found to give good yield and were suitable for production in Zimbabwe (Tongoona, 1993).

Castor oil has a high level of ricinoleic acid content (~900 g/kg) and a low level of oleic acid (~30 g/kg). However, through an extensive screening of a world germplasm collection, the natural mutant line OLE-1 was found to have approximately 780 g/kg of oleic acid while ricinoleic acid content is as low as 140 g/kg (Rojas-Barros et al., 2004). A study was conducted to determine the inheritance of the high oleic/low ricinoleic acid content by crossing the OLE-1 line and a standard oleic/ricinoleic content line. The F_2 segregation was consistent with the action of two independent major genes which are designated *ol* and *Ml* (Rojas-Barros et al., 2005).

Castor is susceptible to climatic variations. This leads to an erratic supply and fluctuations in prices. The chemical industry would prefer to obtain hydroxylated oils from a more reliable crop such as rapeseed. The castor hydroxylase gene was cloned to make transgenic rapeseed with high ricinolic acid content (Murphy et al., 1994).

History and Uses

Entries in the Ebers papyrus (16th Century BC) record use of the *Ricinus* plant by the early Egyptians for medicinal purposes. For instance, seeds were chewed with beer to relieve constipation. For Aztecs, *Ricinus* seeds were chewed for hiccups or used as a purgative, and the oil was applied locally to sores and hemorrhoids (Gaginella and Phillips, 1975). Castor has been used as a medicinal plant and as a producer of oil for lighting in Africa (Engels and Hawkes, 1991).

Medicinal uses

Scarpa and Guerci (1982) summarized uses of castor in about fifty countries worldwide, relating different fields such as chemistry and pharmacognosy; pharmacology; respiratory apparatus; cardiovascular apparatus; digestive apparatus; articulations, bones and muscles; urogenital apparatus; infectious diseases; oncology; puericulture pediatrics; dermatology; venereal diseases; otorhinolaryngology; ophthalmology; obstetrics and gynecology; nervous system and miscellaneous applications. The information on uses were gathered in this report are real and presumed so further research should be carried out to determine the active compounds present in the various parts of castor plant. Some aspects were later proved real, for example in the field of obstetrics and gynecology, the report stated that some women in

India, Algeria and Mexico take castor beans to prevent pregnancy. A study was conducted to investigate the effects of castor beans on the pregnancy of rabbits. The rabbits were orally treated with 7.5 mg of castor bean/kg of body weight for 10 days. The treated rabbits showed a 4.3-fold decrease in pregnancy (Salhab et al., 1997). Castor bean has an action as a contraceptive. Castor is also used to induce labor (Skidmore-Roth, 2006). In the field of ophthalmology, low-concentration homogenized castor oil has been developed to make eye drops which are effective and safe for the treatment of patients with noninflamed obstructive meibomian gland dysfunction (MGD), a major cause of lipid-deficiency dry eye (Goto et al., 2002). Castor oil has a laxative action (Gaginella et al.,1977) as its ability to increase fluid in the colon and stimulate peristalsis, which results in increased propulsion of stool through colon. It can be used to empty the colon completely of stool as is necessary to expel worms (Skidmore-Ruth, 2006). Castor oil has been used to induce diarrhoea in many studies (Mascolo et al., 1993; Shoba and Thomas, 2001; Uddin et al., 2005).

Externally, castor oil is used to treat boils, abscesses, carbuncles, tumors, inflammation of the middle ear, and migraine headache. It may be used topically to stimulate the resolving of toughened tissue and wound healing (Vieira et al., 2000; Skidmore-Roth, 2006).

Polyanhydrides synthesized from pure ricinoleic acid half-esters with maleic and succinic anhydrides possess desired physiochemical and mechanical properties for use as drug carriers (Teomim et al., 1999).

Industrial uses

Castor seeds contain about 46-55% oil by weight. Castor oil is a viscous, pale yellow non-volatile and non-drying oil with a bland taste. It has good shelf life and does not turn rancid easily. Oil extraction can be done from castor seeds by mechanical pressing and/or solvent extraction. Ricinoleic acid comprises over 89% of the fatty acid of the oil. Other major fatty acids are linoleic (4.2%), oleic (3.0%), stearic (1%), palmitic (1%) and linolenic acid (0.3%). Trace amount of ricinoleic acid is also found in common vegetative oils such as cotton seed, soybean, rice bran, corn, sesame, rapeseed, sunflower, safflower and coconut oils (Yamamoto et al., 2008). Castor oil is used as a natural emollient in lip cosmetics, makeup removers, moisturizers (Engasser, 2000; Biebl and Warshaw, 2006). However, ricinoleic acid in cosmetics have accounted for

allergic contact dermatitis (Goh, 2003; Taghipour et al., 2008). Ricinoleic acid (12-hydroxy-9-octadecenoic acid) has a hydroxyl group and a double bond which makes castor oil suitable for many chemical reactions. Suthar et al. (1991) and Ogunniyi (2006) reported that castor oil is used in isocyanate reactions to make polyurethane elastomers, polyurethane millable, castables, adhesives and coatings, interpenetrating polymer network from castor oil-based polyurethane and polyurethane foam. The oil is useful as a component in blending lubricants because it has high viscosity.

In spite of its high protein content, castor cake is not used as livestock feed due to the presence of toxic factors, ricin, ricinine and allergen. A study was conducted in an attempt to detoxify ricin in castor cake. It has been found that autoclaving and lime treatment completely destroyed the toxin (Anandan et al., 2005). Aqueous extracts of castor cake can be used as a substrate for mass production of biological control agent *Trochoderma harzianum* for the management of *Meloidogyne incognita* on eggplant (Rao et al., 1998). Castor stalks can be used as a lignocellulosic resource for particleboard industry (Grigoriou and Ntalos, 2001).

In several southern countries, castor oil is used as a fuel. Regarding the fuel-related properties, advantages of castor oil are the high calorific value, the high cetane number, the low content of phosphorous and carbon residues. The disadvantages are that it has significantly higher viscosity at temperatures under 50°C and possibly higher compressibility. Its hygroscopicity causes relatively high water content and thereby possibly algae growth, filtration and corrosion problems. These properties complicate the use of castor oil as straight vegetable oil in engines (Scholz and da Silva, 2008). There are research and development efforts to improve castor oil as fuel. There have been many studies on optimization of castor oil to use as biodiesel (de Oliveira et al., 2004; Albuquerque et al., 2009; Jeong and Park, 2009).

Warfare uses

Ricin is a potential warfare or terrorist attack agent since it is available throughout the world, is easy to make, and causes extreme pulmonary toxicity when inhaled. It has so far been used only as a weapon of assassination, but when used as an aerosol it could possibly lead to widespread illness and death among victims (Cashman, 2008). The US War Department considered ricin for chemical warfare as early as 1918. During World War I, it was being considered for use either as a toxic dust or as a coating for

bullets and shrapnel. However the war ended before it was weaponized (Bagchi et al., 2009). In the 1940s in the United States and in the late 1980s in Iraq, weapon-grade ricin (i.e., purified and inhalable particles that can be aerosolized for a mass attack) was manufactured and tested in animal experiments and in artillery shells in field testing (Audi et al., 2005).

Mutagenesis

Chemical and physical mutagens have been used to obtain different desired traits. Ethyl methanesulfonate (EMS) is most commonly used among chemical mutagens (Sega, 1984). EMS induces point mutations in DNA via a transition from guanine to adenine. The methyl group of EMS reacts with an oxygen atom on the base guanine which disrupts one of the three H-bonds between guanine-cytosine pairing (Reiner and Zamenhof, 1957). Instead of cytosine, thymine is paired with guanine during DNA replication. Thus guanine-cytosine pairing is transitioned to adenine-thymine in the next round of replication. Point mutations induced by EMS may have a variety of effects on gene function, including complete loss of gene function, partially reduced function, qualitative altered function and constitutive function. EMS is an excellent mutagen and also a strong carcinogen. It must be carefully handled and properly discarded (Weigel and Glazebrook, 2002). EMS mutagenesis has been used in large-scale mutant generation in different organisms (Krieg, 1963 and Greene et al., 2003). Protocols for EMS mutagenesis in *Arabidopsis* have been developed (Kim et al., 2006). It has also been successfully used to gain different traits in different species; for example, fatty acid changes in flax (Rowland, 1991), early flowering in spring rape (Thurling and Depittayana, 1992), shoot apical meristem mutants in *Arabidopsis* (Leyser and Furner, 1992) and herbicide resistance in *Arabidopsis* (Jander et al., 2003).

Physical mutagens such as X-rays, gamma-rays (γ-rays), ultraviolet (UV), fast neutrons and thermal neutrons have also been used to create mutations in different organisms. Soft X-rays have higher linear energy transfer (LET) values and are more likely to break chromosomes and produce deletions. Fast neutrons represent high energy particulate radiation, whereas thermal neutrons have much lower energy and are more destructive. UV has a low penetration. High energy radiation like hard X-rays and γ-rays are more penetrating and have low LET and are more likely to induce intramolecular changes than gross destruction of the genetic material (Redei, 1992). Recommended doses for radiation treatments on some common crops have been reported (Redei, 1992 and Harten, 1998). Gamma radiation doses for seed treatments range between 100 - 600 grays (Gy). For uncommon species, different types and doses of irradiation should be tested for optimal dose. Lately, ion beams have been found to be very effective mutagens. They have much higher LET and relative biological

effectiveness than those of gamma-rays. Comparison of $^{12}C^{5+}$, $^{4}H^{2+}$ ion beams and gamma ray on gymnomonoecious spinach was reported (Naoki et al., 2006).

CHAPTER 4

Development of long-day flowering chia (*Salvia hispanica* L.) by mutagenesis

Materials and Methods

Seed materials

Chia seeds from two commercial varieties and five accessions used in this experiment were obtained from different sources as shown in Table 4.1. Chia Pinta was purchased online from Arizona Chia and Chia Seed and Oil, www.chiaseedandoil.com, and was shipped from Tucson, Arizona. Chia seeds from Yoli Inc., Chicago, USA were purchased from a local store in Lexington, Kentucky. The accessions were obtained through the personal contact with Dr. Joseph Cahill. They were from the National Germplasm of Mexico stored at Chapingo and collections made by Howard S. Gentry. Plant characteristics of these accessions can be found in the study of variation and heritability of seed mass in chia (Cahill and Ehdaie, 2005).

Table 4.1, Chia varieties and sources

Varieties/accessions	Type	Source
Chia Pinta	Cultivated	ACCSO[a]
Chia Pinta	Cultivated	Yoli Inc
JC 15001	Wild/cultivated	Germplasm
JC 16001	Wild	Germplasm
JC 17001	Domesticated	Germplasm
JC 18001	Wild/cultivated	Germplasm
JC 19001	Domesticated	Germplasm

[a]Arizona Chia and Chia Seed and Oil, www.chiaseedandoil.com.

Chia seeds were planted on the May 23, 2006 at Spindletop Farm, Lexington, KY, USA (38° 1′ 47″ N, 84° 29′ 41″ W, 298 m above sea level). The plot size was 4.57 m by 6.1 m, 5 rows per plot. Seed rate was 2.5 g/row. The plot was not irrigated.

Seed germination test

Two replications of 50 chia seeds were placed into Petri dishes containing germination paper moistened with water (International Seed Testing Association, 1999). Petri dishes were wrapped with Parafilm and placed in a germination chamber at 30 °C for 8 hours in fluorescent light and 20 °C for 16 hours in the dark. Germination percentage was observed at 3, 7 and 14 days.

Oil and protein content in chia seed

Three replications of chia 'Pinta' seeds were analyzed for oil and protein content at the Division of Regulatory Services, College of Agriculture, University of Kentucky.

Lipid extraction

20 mg of chia 'Pinta' seeds were crushed in 0.5 ml of sodium methoxide in a 13x100 mm glass tube. Samples were shaken at room temperature for 45 min. 0.5 ml of hexane was added into the tube and vortexed. The top layer was transferred into a new tube. 0.5 ml of 0.9% KCl was added into the tube and vortexed until mixed well. The top layer was transferred into a vial. This experiment was done in three replications.

Samples were analyzed by gas chromatography, Varian CP-3800 equipped with Varian 1177 capillary column injector, FID detector and CP-8400 autosampler. The column used in this study was Varian Select FAME: a proprietary high percent cyanopropyl formulation tuned for separation of cis-, trans-fatty acid methyl esters; closest equivalent standard column is Agilent DB-23 or Varian VF-23. Column dimensions were 25 m x 0.25 mm. Typical oven program were 90 °C for 1 min, then to 150 °C at 20 deg/min, then to 175 °C at 3.3 deg/min, then to 245 °C at 16 deg/min with final hold of 4 min; total program run time is 20 min. Injection port temperature is 250 °C. FID detector temperature is 260 °C. Carrier gas is Helium held at constant flow of 0.9 ml/min (~ 23 cm/sec linear velocity) using electronic pressure programming. Typical injection volume ranges from 0.5 to 1.5 µl using a 5 µl syringe.

Mutagenesis

Chia seeds used in this experiment were obtained online from www.chiaseedandoil.com.

EMS mutagenesis: Ethyl methanesulfonate (EMS) used in this experiment was obtained from ACROS Organics, Morris Plains, NJ. Seeds were soaked in 45 ml of EMS diluted with deionized water in 50ml falcon tubes at different concentrations for six hours in an incubator shaker at room temperature. EMS concentrations used in the first experiment were 0, 2.5, 5 and 10 mg/ml. EMS concentrations used in the second experiment were 0, 30, 50 and 70 mg/ml. Seeds were then washed four times in 1L of deionized water for three minutes.

Gamma ray mutagenesis: Dry chia seeds were irradiated in a Shepherd Mark I-30 Irradiator at the Department of Radiation Medicine, University of Kentucky. The irradiator was loaded with 8,000 curies of Cs-137 in June 1983. Gamma energy was 0.662 MeV. Gamma constant was 0.0033 Gy/hr – Ci at 1m. Dose rate potential in the event of exposure to the unshielded source was 163,337.6 Gy/hr at 1cm. Seed samples were irradiated at 0, 50, 100, 300 and 500 Gy. One gray is the absorption of one joule of energy, in the form of ionizing radiation, by one kilogram of matter. Seed samples were irradiated at 0, 50, 100, 300 and 500 Gy. The optimal concentration was determined by seedling abnormalities such as leaf twisting and variegation.

Mutagenized (EMS 60 mg/l; 500 Gy) chia seeds were then pipetted onto Promix® potting mix. Seed germination was observed at 7, 14 and 21 days. M_1 seedlings were grown in a greenhouse at 14/10 h of light/dark provided by extending the daylength with lamps. The greenhouse temperature was set for 29°C high and 21°C low. The M_1 population number was calculated to give a probability of finding early flowering mutation. The M_1 population must be greater than 5,000 plants to yield at least one mutation/gene. At the 4-node stage, over 6,000 EMS-mutagenized M_1 seedlings and 5,000 gamma-mutagenized M_1 seedlings were transferred to another greenhouse under natural photoperiod of winter in Lexington, KY without additional light. The greenhouse temperature was set for 29°C high and 13°C low. M_1 plants were induced to flower by natural short days between 9 hours and 36 minutes and 10 hours and 47 minutes. All M_2 seeds were harvested and cleaned.

Screening for early flowering mutants

In order to screen for early flowering or long-day mutants, M_2 seeds were planted in bulk in the field of Spindletop farm in Lexington, KY (38° 1′ 47″ N, 84° 29′ 41″ W, 298 m above sea level). The planting date was July 25, 2008 when the day length was 14 hours and 18 minutes (U.S. Naval Observatory Astronomical Applications Department, http://aa.usno.navy.mil/data/docs/RS_OneDay.php). Approximately 1,000 seeds were planted in four rows of 1.5 x 6.1 m^2 plots. There were 209 plots of M_2 EMS-mutagenized plants, 232 plots of M_2 gamma-mutagenized plants and 11 plots of non-mutagenized plants. Border rows were non-mutagenized plants. There was no herbicide or pesticide application with experimental plots. Irrigation was applied. The plot was monitored twice a week for flower bud initiation. Early flowering plants were flagged and tagged. They were then potted and transferred into a greenhouse. Chia is a self-pollinated plant. The mutants were maintained for flowering and seed maturation in the greenhouse without flower bagging. M_3 seeds were harvested, cleaned and collected for greenhouse and field studies.

Day length study

A study on the day length of early flowering mutants was done in greenhouse conditions. M_3 EMS-treated seeds and gamma-treated seeds were sown in 10.2 x 10.2 x 10.2 cm^3 plastic containers filled with Promix® potting mix on February 19, 2009. The greenhouse temperature was set for 29°C high and 21°C low. M_3 seedlings were grown under a photoperiod of 14.5 hours until having at least six nodes (one month old). The seedlings were then moved to four different daylength treatments. Each day a black-out curtain was used to cover plants to provide a natural 12-hr daylength. Daylength was extended using incandescent lamps for 1, 2 and 3 hours providing daylength of 12, 13, 14 and 15 hours with approximately the same daily light irradiance integral.

A light exclusion tent was constructed using black-out curtains (Hummert International, St. Louis, USA). It was white-coated on one side and black-coated on the other side. The sheet was cut and stapled into cubic shape of 1.2x1.2x1.2 m^3. The tents were supported by jointed plastic pipes. The extended light was given by using new 60-Watt clear incandescent bulbs. Light irradiance was measured by using the F400-VisNIR

fiber optic cable (designed for a sensor) with the instrument (EPP2000 fiber optic spectrometer from StellarNet Inc.). The bar sensor was placed diagonally in a light tent and about 0.9 m below light bulbs. A spot sensor was also used to measure light intensity on the corners of a light tent. The average photosynthetically active radiation (PAR, 400 – 700 nm) of the extended light was 13 µmol/sec/m^2. The PAR was 0.001 µmol/sec/m^2 when the light was switched off. The greenhouse temperature was set for 29°C high and 21°C low. The actual highest temperature in the light tents was 31°C and the lowest was 21°C.

Each light treatment contained 16 experimental units. One experimental unit consisted of six M_3 plants from one M_2 line. Each experimental unit was randomized for a placement in a light treatment. Flowering of mutant plants was monitored and compared with non-mutagenized plants.

Field test of mutants

Forty M_3 full sib families (20 EMS-mutagenized lines and 20 gamma-mutagenized lines) from self-pollinated M_2 plants were seeded using a small push-planter in rows with two replications. Border rows and every sixth row were planted with wild type chia. The planting date was May 20, 2009 when the day length was 14 hours and 23 minutes. The length of rows was 6.1 m and they were 1 m apart. The plot was not irrigated nor fertilized. There was no herbicide application. Weeding was done by mowing between rows when required. This field test was done to investigate flowering of mutants. However, data on seed weight, total plant dry weight above ground were collected. Harvest index was then calculated.

$$\text{Harvest index} = \frac{\text{total seed weight}}{\text{total plant dry weight}}$$

Results and Discussion

Observation on flowering on non-mutagenized chia

Two commercial varieties and five accessions of chia were planted at Spindletop Farm, Lexington, KY in spring 2006 for observation on flowering. All varieties and accessions germinated and grew well during the summer. Flower buds emerged in October and were killed by frost before seed set. There was no major damage on chia from any specific diseases or pests during the growing season.

Seed study

Commercial chia seeds are frequently a mixture of seeds with different seed coat colors which are dark charcoal, white and brown at a ratio of approximately 80:15:5. A single recessive gene, designated *scc*, is reported to govern the white seed characteristic (Cahill and Provance, 2002). The germination of each color was tested. Charcoal and white seeds had germination of approximately 80% while brown seeds were less viable (Table 4.2). Brown seeds were found to be immature or broken thus the germination was lower.

Table 4.2, Germination of chia seeds with different seed coat colors

Seed coat color	Seed germination (%)
	Mean ± SE
Charcoal	79 ± 3.0
White	83 ± 5.0
Brown	47 ± 1.0

To determine whether chia seed was endospermic, longitudinal sectioning was done on imbibed seeds. Chia seed is found to be endospermic with thin embryos embedded in the center as shown in Figure 4.1.

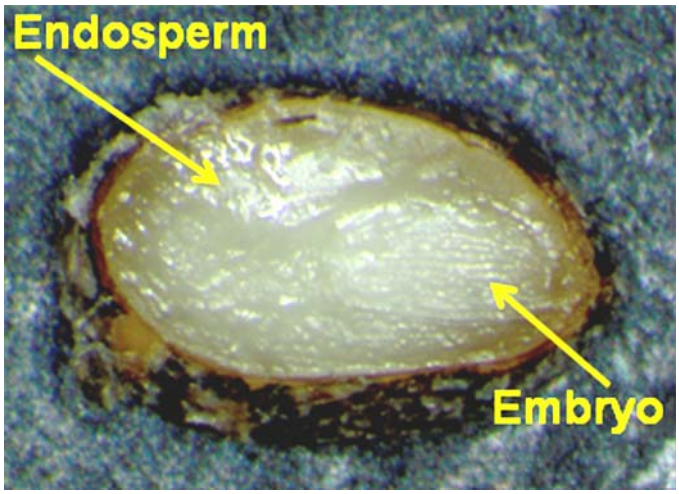

Figure 4.1, Chia seed section

Average chia oil content was found to be 31.9% and protein content was 20.5%.

Chia oil was extracted and analyzed for fatty acids by gas chromatography (Table 4.3). The results showed that white seeds contain the same amount of fatty acids as charcoal seeds. The α-linolenic acid (ALA) content of 57% is the highest when compared to other species in the *Salvia* genus (Goren et al., 2005) and considered among the highest plant species although *Dracocephalum moldavica* is reported to have 68% ALA and *Perilla frutescens* 59% ALA (Rao et al., 2008) both also of the same family as chia (Lamiaceae).

Mutagenesis

Chemical mutagenesis by ethyl methanesulfonate (EMS) was used in an attempt to produce early flowering mutants. EMS is considered effective at concentrations that reduce germination to about 50 percent (Redei, 1992). Germination was unaffected by EMS concentrations between 0 and 10 mg/ml (Figure 4.2). This range is effective for numerous species including Arabidopsis (Redei, 1992). The result showed that such EMS concentrations used were too low even though standard for many species. Another set of higher EMS concentrations were applied for mutagenesis. However, germination was reduced at EMS concentrations above 30 mg/l (Figure 4.3).

Table 4.3, Fatty acid profile of charcoal seed and white seed

Fatty acid	Fatty acid profile of chia seed oil (% of total)	
	charcoal	white
Myristic acid (14:0)	0.06	0.06
Palmitic acid (16:0)	9.20	8.90
Palmitoleic acid (16:1)	0.10	0.10
Margaric acid (17:0)	0.06	0.06
Stearic acid (18:0)	3.40	3.40
Oleic acid (18:1)	7.70	7.90
Linoleic acid (18:2)	21.00	20.70
Linolenic acid (18:3)	57.30	57.80
Nonadecanoic acid (19:0)	0.07	0.07
Gadoleic acid (20:1)	0.10	0.10
Behenic acid (22:0)	0.04	0.04
Lignoceric acid (24:0)	0.50	0.50

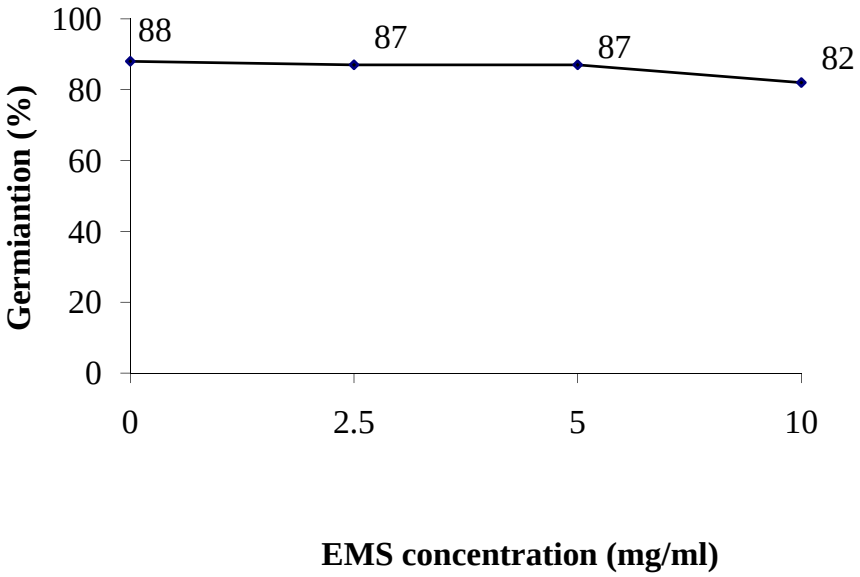

Figure 4.2, Germination of chia seeds treated with different concentrations of EMS

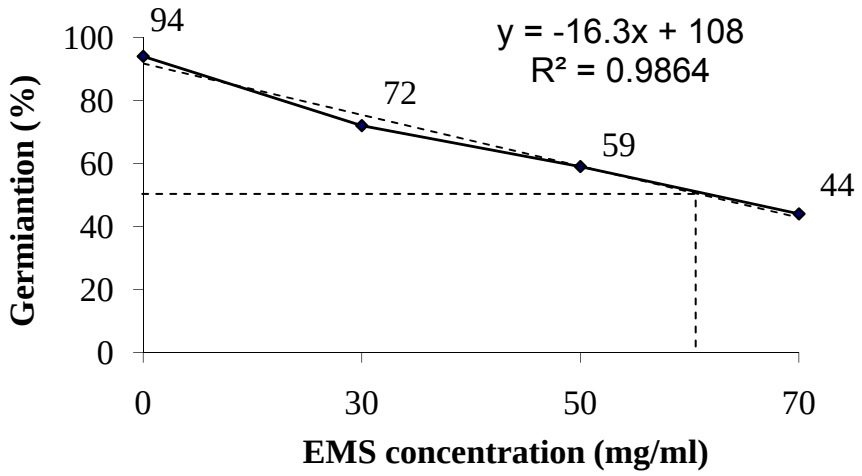

Figure 4.3, Germination of chia seeds treated with higher concentrations of EMS

Using linear regression, 50% germination could be obtained by treating seeds at approximately 60 mg/ml of EMS (Figure 4.3). This concentration was found to be very high for EMS treatment compared to recommended EMS concentrations for other crops. Fatty acid mutation in flax was achieved by treating flax seeds with 4 mg/ml of EMS (Rowland and Bhatty, 1990). Two mg/ml of EMS was used in mutagenesis in *Arabidopsis* (Jander et al., 2003). Chia seed secretes a mucilaginous substance as seeds imbibe, which might have absorbed some EMS before it was absorbed through the seed coat. The optimal dose of 60 mg/ml of EMS was applied to treat chia seeds in the chemical mutagenesis experiment.

Germination of irradiated seeds was tested in a germination chamber and in greenhouse condition. Germination was higher than 85% in all treatments and not different from controls (Figure 4.4). Different doses did not seem to have any effect on chia germination. Observations on seedlings that germinated in the greenhouse showed

many visible malformations of cotyledons and leaves (Figure 4.5) especially from the treatment of 500 Gy. Leaf twisting and leaf variegation were found the most in M_1 seedlings. This suggested that there were physical damages inside the seed after it was irradiated. It was expected that there might be genetic changes in the seed also. Irradiated seeds at 500 Gy were chosen to be planted out to build the M_1 population.

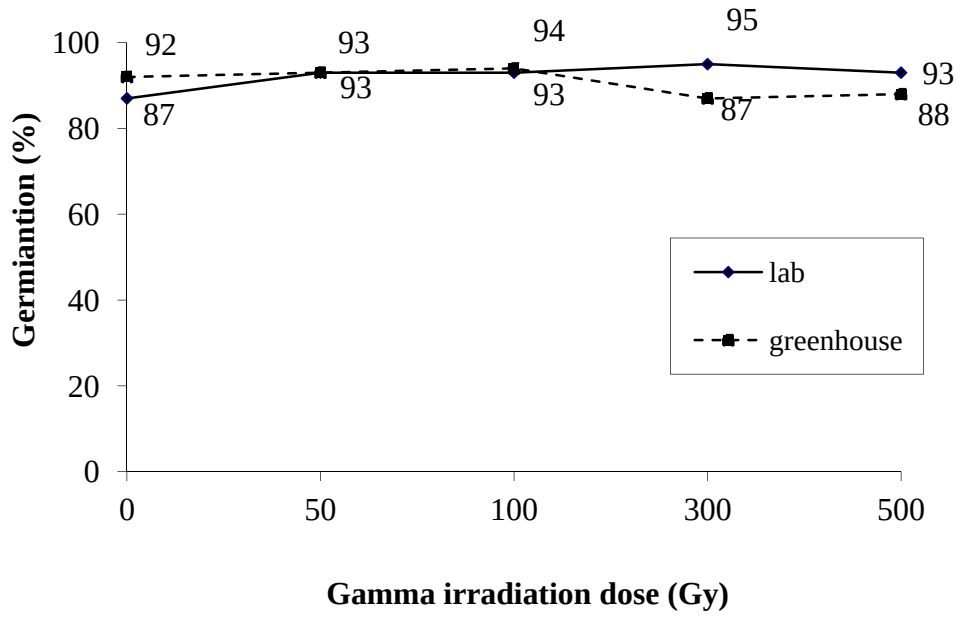

Figure 4.4, Germination of chia seeds treated with gamma irradiation

Figure 4.5, Visible abnormalities in M_1 seedlings from gamma-treated seeds

The first flower bud on M_1 plants was noticed at the fourth week of flowering induction. Physical abnormalities such as chlorophyll deficiency, fused leaf, twisted stem, double flower, variegation, accumulation of anthocyanin and different flower colors were found in the M_1 population. None of these mutations have been seen in the untreated, non-mutated source material. M_2 seeds were harvested in spring of 2008.

Screening for long day flowering mutants in M_2 population

Flower buds of the earliest flowering mutants formed 55 days after planting when the day length was 12 hours and 16 minutes. No flower buds were found in control plots and in non-mutagenized border rows on that date. Figure 4.6 shows an early flowering plant in the field. There were 22 early flowering plants found in approximately 165,000 plants of M_2 EMS-mutagenized population which is 0.013%. In gamma-mutagenized population of approximately 185,000 plants, there were 46 early flowering plants which is about 0.024%. The rest of the field started to flower on the second week of October at 82 days after planting. Early flowering plants were marked and transferred to a greenhouse before the killing frost on the third week of October. Non-mutagenized plants were killed before blooming (petal opening). Early flowering M_2 plants set seeds in

the greenhouse. M_3 seeds were collected for the day length study and the field test for flowering.

Figure 4.6, An early flowering plant in M_2 gamma-mutagenized population

Chia mutant responses to daylength

Salvia is a large genus and has species that vary in their daylength requirements for flowering (Zanin and Erwin, 2006). Several horticultural species of subgenus *Calospace* including the annuals *S. splendens*, and *S. farinaceae* have been shown to be facultative long-day plants, *S. greggii,* a perennial, is a facultative short-day plant, while other perennials are obligate short day plants as seen in *S. leucantha* and *S. elegans*. The annuals utilized for seed production such as *S. hispanica* and *S. tiliaefolia* when planted in the spring of the northern hemisphere flower in the short days of September for seed harvest in fall (Gentry et al., 1990). Chia shows a short-day

response to flowering (Table 4.4). Under controlled conditions, non-mutagenized plants were only able to flower when the daylenth was less than 14 hours. This suggests a critical daylength between 12 and 13 hours. Similarly, *S. leucantha* appears to have a critical daylenth at approximately 12 hours (Armitage and Laushman, 1989).

Since, irradiance can impact flowering in several *Salvia* species (Mattson and Erwin, 2004), the current experiment with chia held total daily light integrals nearly the same by covering all plants after 12 hours of daylight and only varied the daylength with incandescent bulbs under the covering. Under these conditions, all chia mutants (except one EMS mutant) flowered under a 15-hour photoperiod (Table 4.4). In addition, all chia mutants (except one EMS mutant) flowered within three weeks of moving to the 12 hour photoperiod compared to non-mutagenized plants that required five weeks to initiate flowering. Time to flowering was unaffected in the gamma-irradiated mutants and two of the EMS mutants, which all flowered after three weeks regardless of daylength. The other four EMS mutants took longer to flower at the 14 and 15-hour photoperiods compared to the 12-hour photoperiod. In several *Salvia* species, flowering requires a critical photoperiod to initiate flowering as well as for flower development. For example, *S. leucantha* required a 12 hour photoperiod for flower induction and a 10 hour photoperiod for continued flower development (Armitage and Laushman, 1989). Although, the critical daylength for continued floral development was not evaluated in the current study for wild type chia plants, it appears that the majority of chia mutants were altered for daylength floral induction as well as floral development as flower formation appeared to be normal under the 15 hour photoperiod.

Tables 4.5, 4.6, 4.7 and 4.8 show percentage of plants in each experimental unit with flower buds from the second week to the fifth week of the experiment. Petal opening was observed at the fourth week. Some plants that grew slower (less than 6 nodes) than others in the same experimental unit were not able to flower.

Table 4.4, Flowering of different M_3 mutant lines under different photoperiods compared to non-mutagenized plants

L	TM	SC	Photoperiod											
			12 hours			13 hours			14 hours			15 hours		
			3W	4W	5W	3W	4W	5W	3W	4W	5W	3W	4W	5W
A	NMF	C			❋									
B	NMR	C			❋			❋						
C	NMR	W			❋			❋						
D	EMS	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
E	EMS	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
F	EMS	C		❋	❋		❋	❋			❋			
G	EMS	C	❋	❋	❋	❋	❋	❋		❋	❋		❋	❋
H	EMS	W	❋	❋	❋	❋	❋	❋		❋	❋		❋	❋
I	EMS	W	❋	❋	❋	❋	❋	❋	❋	❋	❋			❋
J	γ	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
K	γ	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
L	γ	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
M	γ	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
N	γ	C	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
O	γ	W	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋
P	γ	W	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋	❋

L: line; TM: type of mutagenesis; NMF: non-mutagenized family; NMR: random non-mutagenized seeds; EMS: EMS-mutagenized line; γ: gamma ray-mutagenized line; SC: seed coat color; C: charcoal seed coat; W: white seed coat; 3W: at the third week of induction; 4W: at the fourth week of induction; 5W: at the fifth week of induction; ❋: flowering

Table 4.5, Percentage of plants in each experimental unit with flower buds under different photoperiods at the second week of flowering induction

line	Type of mutagenesis	Seed coat color	Day length (h)			
			12	13	14	15
A	NMF	C	0%	0%	0%	0%
B	NMR	C	0%	0%	0%	0%
C	NMR	W	0%	0%	0%	0%
D	EMS	C	*83%	*83%	100%	0%
E	EMS	C	100%	*83%	100%	100%
F	EMS	C	0%	0%	0%	0%
G	EMS	C	*0%	67%	0%	0%
H	EMS	W	33%	0%	0%	0%
I	EMS	W	17%	83%	33%	0%
J	γ	C	100%	100%	100%	100%
K	γ	C	100%	100%	100%	100%
L	γ	C	100%	*83%	100%	100%
M	γ	C	100%	100%	100%	100%
N	γ	C	100%	100%	100%	100%
O	γ	W	100%	100%	100%	100%
P	γ	W	100%	100%	100%	100%

NMF: non-mutagenized family; NMR: random non-mutagenized seeds; EMS: EMS-mutagenized line; γ: gamma ray-mutagenized line; C: charcoal seed coat; W: white seed coat, *: some plants had less than six nodes

Table 4.6, Percentage of plants in each experimental unit with flower buds under different photoperiods at the third week of flowering induction

line	Type of mutagenesis	Seed coat color	Day length (h)			
			12	13	14	15
A	NMF	C	0%	0%	0%	0%
B	NMR	C	0%	0%	0%	0%
C	NMR	W	0%	0%	0%	0%
D	EMS	C	100%	*83%	100%	0%
E	EMS	C	100%	*83%	100%	100%
F	EMS	C	0%	0%	0%	0%
G	EMS	C	*67%	100%	0%	0%
H	EMS	W	67%	17%	0%	0%
I	EMS	W	33%	83%	33%	0%
J	γ	C	100%	100%	100%	100%
K	γ	C	100%	100%	100%	100%
L	γ	C	100%	100%	100%	100%
M	γ	C	100%	100%	100%	100%
N	γ	C	100%	100%	100%	100%
O	γ	W	100%	100%	100%	100%
P	γ	W	100%	100%	100%	100%

NMF: non-mutagenized family; NMR: random non-mutagenized seeds; EMS: EMS-mutagenized line; γ: gamma ray-mutagenized line; C: charcoal seed coat; W: white seed coat, *: some plants had less than six nodes

Table 4.7, Percentage of plants in each experimental unit with flower buds under different photoperiods at the fourth week of flowering induction

line	Type of mutagenesis	Seed coat color	Day length (h)			
			12	13	14	15
A	NMF	C	0%	0%	0%	0%
B	NMR	C	0%	0%	0%	0%
C	NMR	W	0%	0%	0%	0%
D	EMS	C	100%	100%	100%	67%
E ❋	EMS	C	100%	100%	100%	100%
F	EMS	C	100%	100%	0%	0%
G	EMS	C	100%	100%	100%	100%
H	EMS	W	100%	100%	100%	100%
I	EMS	W	100%	100%	100%	0%
J	γ	C	100%	100%	100%	100%
K	γ	C	100%	100%	100%	100%
L ❋	γ	C	100%	100%	100%	100%
M	γ	C	100%	100%	100%	100%
N ❋	γ	C	100%	100%	100%	100%
O ❋	γ	W	100%	100%	100%	100%
P ❋	γ	W	100%	100%	100%	100%

NMF: non-mutagenized family; NMR: random non-mutagenized seeds; EMS: EMS-mutagenized line; γ: gamma ray-mutagenized line; C: charcoal seed coat; W: white seed coat, *: some plants had less than six nodes; ❋: petal opening

Table 4.8, Percentage of plants in each experimental unit with flower buds under different photoperiods at the fifth week of flowering induction

line	Type of mutagenesis	Seed coat color	Day length (h)			
			12	13	14	15
A	NMF	C	100%	0%	0%	0%
B	NMR	C	100%	100%	0%	0%
C	NMR	W	100%	100%	0%	0%
D ❋	EMS	C	100%	100%	100%	67%
E ❋	EMS	C	100%	100%	100%	100%
F	EMS	C	100%	100%	67%	0%
G	EMS	C	100%	100%	100%	100%
H ❋	EMS	W	100%	100%	100%	100%
I ❋	EMS	W	100%	100%	100%	17%
J ❋	γ	C	100%	100%	100%	100%
K ❋	γ	C	100%	100%	100%	100%
L ❋	γ	C	100%	100%	100%	100%
M ❋	γ	C	100%	100%	100%	100%
N ❋	γ	C	100%	100%	100%	100%
O ❋	γ	W	100%	100%	100%	100%
P ❋	γ	W	100%	100%	100%	100%

NMF: non-mutagenized family; NMR: random non-mutagenized seeds; EMS: EMS-mutagenized line; γ: gamma ray-mutagenized line; C: charcoal seed coat; W: white seed coat, *: some plants had less than six nodes; ❋: petal opening

Field test of mutants

Forty lines of M_3 early flowering chia mutants were planted in spring 2009 (May 20, 2009 when the day length was 14 hours and 23 minutes) at Spindletop Farm. Twenty lines were EMS-mutagenized and the other twenty lines were gamma-mutagenized. Flower buds were first noticed on mutant lines on July 7, 2009, 47 days after planting at a daylength of 14 hours and 41 minutes. Petal opening was observed for the first time on July 17, 2009 (Table 4.9). Flower buds were not found on non-mutagenized plants. Most of tested mutant lines had petal opening by July 24, 2009. However, there were some lines that opened petals later and some lines that did not form flower buds until induced by much short days. Plant height was measured on September 8, 2009 and number of nodes was also recorded. Chia stops growing taller after flowers form. Mutant lines that flowered earlier were shorter than the ones that flowered later (Table 4.10). All mutant lines tested flowered after having at least eight nodes which plants already passed a juvenile stage.

Most of the new early flowering chia lines set seed and matured by early October, 2009. Some of the lines matured and produced harvestable dry seeds as early as September 16, 2009 when the daylength was 12 h and 23 min. This plot was not planted for yield evaluation, however, data on seed weight, total plant weight and harvest index of each line was collected and shown as a preliminary result in Table 4.11. The mutant line that flowered earliest may not necessary the best line for yield production. Some lines that flower later might give more biomass and yield since they have longer growth period. Field trial for yield evaluation should be done in the next growing season.

Table 4.9, Petal opening date of different early flowering mutant lines

line	Date of observation on petal opening (weekly)						
	07/17	07/24	07/31	08/07	08/14	08/21	10/09
E1 (D)							
E2 (E)							
E3 (F)							
E4 (G)							
E5							
E6							
E7							
E8							
E9							
E10							
E11							
E12							
E13							
E14							
E15							
E16							
E17							
E18							
E19							
E20							
G1 (J)							
G2 (K)							
G3 (L)							
G4 (M)							
G5 (N)							
G6 (O)							
G7 (P)							
G8							
G9							
G10							
G11							
G12							
G13							
G14							
G15							
G16							
G17							
G18							
G19							
G20							

E1 - E20: EMS mutant lines; G1 – G20: gamma mutant lines
E1 - E4, G1 - G7 tested in the greenhouse for critical day length

Table 4.10, Week† of petal opening, plant height and number of node of different early flowering mutant lines

line	week of petal opening	Plant height (cm)	Number of node
E1 (D)	3rd	171	10
E2 (E)	4th	167	9
E3 (F)	6th	187	10
E4 (G)	6th	207	10
E5	1st	138	10
E6	4th	164	10
E7	11th	*	11
E8	2nd	142	8
E9	2nd	153	8
E10	2nd	147	9
E11	2nd	174	9
E12	2nd	140	9
E13	2nd	132	10
E14	11th	*	10
E15	1st	143	9
E16	11th	*	11
E17	11th	*	11
E18	2nd	142	10
E19	4th	140	8
E20	5th	181	8
G1 (J)	1st	130	9
G2 (K)	2nd	150	9
G3 (L)	2nd	140	9
G4 (M)	2nd	158	10
G5 (N)	4th	185	8
G6 (O)	2nd	156	9
G7 (P)	2nd	155	9
G8	2nd	133	9
G9	1st	125	9
G10	2nd	155	10
G11	2nd	159	10
G12	2nd	158	9
G13	2nd	144	9
G14	1st	152	10
G15	2nd	157	9
G16	2nd	132	9
G17	2nd	128	10
G18	2nd	165	9
G19	2nd	139	9
G20	2nd	145	9

† the first week was between July 10 and July 17, 2009

* no flower buds on plants on measuring date, plant height at least 250 cm

Table 4.11, Total chia seed weight, plot weight and harvest index of early flowering mutants grown in the field in 2009

entry	row number	seed wt (g)	plot wt (g)	Harvest Index
E1	3	18.2	898.9	0.020
E1	15	19.3	808.1	0.024
E2	30	129.9	1570.8	0.083
E2	42	71.9	1116.8	0.064
E5	85	26.4	472.2	0.056
E5	97	110.7	853.5	0.130
E6	58	147.5	1534.5	0.096
E8	4	26.5	726.4	0.036
E8	16	126.4	971.6	0.130
E9	5	23.0	880.8	0.026
E9	17	81.4	962.5	0.085
E10	44	133.0	917.1	0.145
E10	32	85.2	853.5	0.100
E11	59	78.6	1135.0	0.069
E11	71	22.7	853.5	0.027
E12	86	91.6	925.3	0.010
E12	98	123.2	880.8	0.140
E15	33	73.2	962.5	0.076
E15	45	33.4	898.9	0.037
E18	34	45.1	517.6	0.087
E18	46	30.9	653.8	0.047
G1	9	45.6	790.0	0.058
G1	21	81.7	644.7	0.127
G2	36	44.4	644.7	0.069
G2	48	43.8	517.6	0.085
G3	63	267.0	1416.5	0.188
G3	75	271.5	1162.2	0.234
G4	90	57.9	980.6	0.059
G4	102	92.5	871.7	0.106
G5	91	164.0	1207.6	0.136
G5	103	117.5	1343.8	0.087

Table 4.11, (continued)

entry	row number	seed wt (g)	plot wt (g)	Harvest Index
G6	64	67.3	862.6	0.078
G6	76	99.1	1171.3	0.085
G7	37	49.4	862.6	0.057
G7	49	135.9	989.7	0.137
G8	10	239.8	1312.1	0.183
G8	22	144.1	681.0	0.212
G9	11	82.7	708.2	0.117
G9	23	46.4	372.3	0.125
G10	38	57.1	635.6	0.090
G10	50	64.7	535.7	0.121
G11	65	58.3	953.4	0.061
G11	77	47.2	572.0	0.083
G12	92	119.5	1026.0	0.116
G12	104	79.2	826.3	0.096
G13	93	60.3	653.8	0.092
G13	105	38.8	753.6	0.051
G14	66	73.4	681.0	0.108
G14	78	81.8	917.1	0.089
G15	39	68.1	934.4	0.007
G15	51	281.2	1434.6	0.196
G16	12	82.8	1171.3	0.071
G16	24	72.7	699.2	0.104
G17	13	69.2	735.5	0.094
G17	25	74.3	553.9	0.134
G18	40	44.6	989.7	0.045
G18	52	44.4	735.5	0.060
G19	67	42.8	671.9	0.064
G19	79	94.1	690.1	0.136
G20	94	82.2	735.5	0.112
G20	106	74.0	889.8	0.083

This is the first report on induction of early flowering chia mutants. Although genetic variation for many traits can be induced via mutagenesis (Koche and Choudhary, 2008) the genetic mechanisms of floral induction varies among plant species and it was not obvious that early flowering in chia could be achieved by mutagenesis as multiple genes could be involved. Indeed, with the range of early flowering chia lines we have produced with floral induction ranging from early July when the daylength was close to 15 h and late September when the daylength was shorter than 12 h indicates numerous early flowering genes involved in the different lines. However we see remarkable uniformity in flowering of most M_3 plants of the M_2 families with little segregation for flowering phenotype as illustrated in Figure 4.7. This might suggest that many of the mutations are dominant but further studies will need to be conducted on the inheritance of these early flowering chia mutants.

Figure 4.7, Flowering of an early flowering chia line (G8, row 10) in the field in Lexington, KY, July 2009

In plants, daylength is perceived by leaves through the influence of red, far red and blue light on phytochromes (Ishikawa et al., 2009) which induces a mobile signal known as florigen to move through the phloem to apical meristems. In the apical meristems florigen causes changes in the expression of genes which alters the developmental program of the meristems to produce flowers instead of leaves (Turck et al., 2008). Numerous genes have been reported to influence floral induction in Arabidopsis and rice (Fornara et al., 2009; Ishikawa et al., 2009; Komiya et al., 2009; Notaguchi et al., 2008; Ryu et al., 2009; Stangeland et al., 2009; Strasser et al., 2009; Yoshida et al., 2009). Hou and Yang (2009) describe two genes in orchids that regulate flowering. Thurling and Depittayanan (1992) report on the induction of early flowering mutants in spring rape (*Brassica napus*) by EMS mutagenesis although they also produced later flowering lines. Further studies are needed on the genetic control of flowering in chia and the genetic basis of the early flowering lines reported here.

CONCLUSIONS

Chia seed is endospermic which contains 31.9% of oil and 20.5% of protein. Chia oil is ~ 57% α-linolenic acid. Chia grows vigorously in Lexington, KY and normally flowers during short days of fall in late September. Known accessions are usually killed in October by frost before seed set.

Ethyl methanesulfonate (EMS) and gamma rays were employed to mutagenize chia seeds to produce early flowering mutants. Treating seeds in 6% EMS for 6 h is an optimal dose for chia. Mutants were obtained by gamma-ray irradiating at 500 Gy on dry chia seed. The M_1 population was grown and induced to flower by short-day photoperiods. The M_2 population was planted in the field in Lexington, KY in 2008. Early flowering plants were found 55 days after planting at a daylength of 12 hours and 45 minutes while non-mutagenized plants did not produce any flower buds until the October 7, 2008 at a daylength of 11 hours and 32 minutes. 0.013% of the EMS-treated M_2 population and 0.024% of the gamma radiation-treated population flowered much earlier than the controls. M_3 early flowering mutant lines were able to flower at photoperiods of 12-15 hours in a greenhouse. Selected lines produced flower buds on July 7, 2009 at a daylength of 14 hours and 41 minutes in the field in Lexington, Kentucky. They are long day mutants. These new chia lines should allow chia to be produced in much of the United States and other temperate regions.

CHAPTER 5

Evaluation of flax (*Linum usitatissimum* L.) field performance in Kentucky

Materials and Methods

Plant varieties and plot design

Eight flax varieties were used in the field trial of 2006. Seeds were obtained from North Dakota State University and Minnesota (Table 5.1). This study was done at Spindletop Farm, Lexington, KY. Flax plots were in randomized complete block design with four blocks. Plot size was 9.1 m^2, 1.5m x 6.1m. There were seven rows per plot. Row width was 19 cm. Seeds rate was 44.83 kg/ha or 36.5 g/plot. Seeding date was April 12, 2006.

Table 5.1, Flax varieties, seed colors and sources for the 2006 field trial

Variety	Seed color	Seed source
Omega	golden	North Dakota
Carter	golden	North Dakota
Rahab94	brown	North Dakota
York	brown	North Dakota
Pembina	brown	North Dakota
York	brown	Minnesota
Golden	golden	Minnesota
Linaza	brown	Yoli Inc.

Only six flax varieties were used in 2007 as shown in Table 5.2. The field trial of flax was repeated with the same plot size and spacing as in 2006 on a different location at Spindletop farm. Seeds were planted on April 02, 2007. Due to an unusual and extreme temperature decrease after planting following an unusually warm period, the seedling survival rate was very low. The study was unsuccessful. A new plot was relocated and replanted on the first week of May.

Table 5.2, Flax varieties, seed colors and sources for the 2007 field trial

Variety	Seed color	Seed source
Rahab	brown	North Dakota
York	brown	North Dakota
Pembina	brown	North Dakota
Omega	golden	North Dakota
Carter	golden	North Dakota
Linaza	brown	Yoli Inc.

Four more varieties were added to the trial in 2008 as shown in Table 5.3. Four varieties from France were obtained from the personal contact to Dr. Chad Lee, Department of Plant and Soil Science, University of Kentucky. Field trial of flax was repeated with the same plot size and spacing as in 2006 on a different location at Spindletop farm. Seeds were planted on April 02, 2008. Due to a heavy rain after planting, most of the seedlings were unable to force their way through a top soil crust. The germination rate was very low. The plot was not qualified for a trial experiment, so a new plot was relocated and replanted on May 15, 2008.

Table 5.3, Flax varieties, seed colors and sources for the 2008 field trial

Variety	Seed color	Seed source
Rahab	brown	North Dakota
York	brown	North Dakota
Pembina	brown	North Dakota
Omega	golden	North Dakota
Carter	golden	North Dakota
Linaza	brown	Yoli Inc.
Eole	brown	France
Jupiter	brown	France
Niagara	brown	France
Princess	brown	France

Weed control

The pre-emergence herbicide Spartan® 4F was applied to the soil for the control of certain broadleaf weeds, grasses and sedges. The herbicide was sprayed not later than four days after planting at the concentration of 438.5 ml/ha. The method was applied to every plot in this study. Manual weeding was done as needed on weeds that survived pre-emergence herbicide application. The post-emergence herbicide Basagran® was used for the 2008 study only. Yellow nutsedge (*Cyperus esculentus*) was a major weed in the plot. The herbicide was sprayed at the concentration of 1.75 l/ha and reapplied at the same rate 8 days later. The glyphosate herbicide Roundup® was applied as a desiccant before harvest.

Fertilizer application

Nitrogen fertilizer (ammonium nitrate) was applied to every plot at the rate of 190 kg/ha.

Lipid extraction

20 mg of flax seeds were crushed in 0.5 ml of sodium methoxide in a 13x100 mm glass tube. Samples were shaken at room temperature for 45 min. 0.5 ml of hexane was added into the tube and vortexed. The top layer was transferred into a new tube. 0.5 ml of 0.9% KCl was added into the tube and vortexed until mixed well. The top layer was transferred into a vial. This experiment was done with two replications.

Samples were analyzed by gas chromatography, Varian CP-3800 equipped with Varian 1177 capillary column injector, FID detector and CP-8400 autosampler. The column used in this study was Varian Select FAME: a proprietary high percent cyanopropyl formulation tuned for separation of cis-, trans-fatty acid methyl esters; closest equivalent standard column is Agilent DB-23 or Varian VF-23. Column dimensions were 25 m x 0.25 mm. Typical oven program were 90 °C for 1 min, then to 150 °C at 20 deg/min, then to 175 °C at 3.3 deg/min, then to 245 °C at 16 deg/min with final hold of 4 min; total program run time is 20 min. Injection port temperature is 250 °C. FID detector temperature is 260 °C. Carrier gas is Helium held at constant flow of 0.9

ml/min (~ 23 cm/sec linear velocity) using electronic pressure programming. Typical injection volume ranges from 0.5 to 1.5 µl using a 5 µl syringe.

Statistical analysis

Analysis of Variance (ANOVA) and Tukey's test on yield and plant height data were performed with SAS program, SAS Institute Inc., North Carolina. Differences in means were tested at a p-value of 0.05. Plant height was measured randomly from eight plants in a plot. Seed yield was weighed from total yield of each plot.

Results and discussion

Yield

Yield data from the 2006 trial is shown in Table 5.4. Carter gave the highest yield of 1266.6 kg/ha. The lowest yield of 367.8 kg/ha was from York. Carter and Rahab94 outperformed other varieties tested in that year. The block effect was not significant.

Yield data from 2007 trial is shown in Table 5.5. Carter again gave the highest yield of 146.4 kg/ha. The lowest yield of 54.1 kg/ha was from York. Carter and Omega outperformed other varieties tested in 2007. The yield trend for each variety in 2006 and 2007 is the same. However, the 2007 yield was much lower than the 2006 trial. The decrease in yield might be from two major causes. The first one is the late planting date. Berglund and Zollinger (2007) mentioned that early seeded flax generally produces highest yield. This plot was replanted in May to replace the one planted in April because of the late season severe and prolonged freeze after an unusually warm spell. The second reason is likely the drought in 2007. Even though the plot was irrigated later in the growing season, it might not have been sufficient for the flax to yield well. The block effect was significant for the 2007 trial. Block 1 gave the highest yield while the lowest yield was from block 4 in every variety.

Table 5.4, Yield of different flax varieties grown in Lexington in 2006

Variety	Yield (kg/ha)				
	Block 1	Block 2	Block 3	Block 4	Mean ± SE
Omega	958.7	996.2	726.9	1079.0	940.2 ± 75.4[b]
Carter	1081.6	1338.9	1329.1	1316.9	1266.6 ± 61.8[a]
Rahab94	1191.1	1170.6	1211.5	1395.1	1242.1 ± 51.7[a]
York	426.1	305.9	401.5	337.9	367.8 ± 27.8[f]
Pembina	832.4	835.2	680.7	583.5	732.9 ± 61.5[c,d]
Linaza	944.7	1072.1	796.1	941.5	938.6 ± 56.4[b]
York	753.0	482.6	659.8	337.7	558.3 ± 92.4[d]
Golden	868.8	968.9	840.4	827.4	876.4 ± 32.0[b,c]

[a] is significantly different from [b], [b] is significantly different from [c], [c] is significantly different from [d], [d] is significantly different from [e] with a p-value of 0.05

Table 5.5, Yield of different flax varieties grown in Lexington in 2007

Variety	Yield (kg/ha)				
	Block 1	Block 2	Block 3	Block 4	Mean ± SE
Omega	221.5	161.3	118.3	41.2	135.2 ± 37.9[a,b]
Carter	290.0	185.2	103.8	72.5	146.4 ± 48.6[a]
Rahab94	119.6	106.5	94.7	72.5	146.4 ± 16.2[b,c]
York	57.2	71.5	66.3	21.5	54.1 ± 11.3[c]
Pembina	31.3	72.0	67.6	23.7	48.6 ± 12.3[c]
Linaza	203.5	80.2	76.9	47.4	102.0 ± 34.6[a,b,c]

[a] is significantly different from [b], [b] is significantly different from [c] with a p-value of 0.05

Table 5.6 shows flax yield from the 2008 trial. Carter yet again was among the highest yielding varieties while the least yield was from Princess. Carter and Omega performed better than other varieties. Among varieties from North Dakota, York had the least yield as expected. Among French varieties, Eole gave the best yield. However, this is still inconclusive since the yield data on French varieties was obtained from only one growing season. In general the French varieties did not perform well in Kentucky when compared to the American varieties. 2008 yields were also low compared to 2006 yields for the same reason mentioned above for 2007. The 2008 study was replanted in May because of the soil crust limiting germination. It was also very dry in Kentucky during the growing season of 2008. The block effect was significant as block 2 generally gave the best yield while block 4 gave the lowest yield for most of the varieties.

Table 5.6, Yield of different flax varieties grown in Lexington in 2008

Variety	Yield (kg/ha)				
	Block 1	Block 2	Block 3	Block 4	Mean ± SE
Omega	438.2	522.3	470.5	73.6	376.1 ± 102.3[a]
Carter	268.9	459.1	282.8	434.7	361.4 ± 49.7[a,b]
Rahab94	376.7	420.4	262.8	162.8	305.7 ± 58.0[a,b]
York	211.4	440.2	230.5	101.7	245.9 ± 70.7[b,c]
Pembina	354.4	416.2	195.3	209.7	293.9 ± 54.3[a,b]
Linaza	394.6	375.5	392.1	147.7	327.5 ± 60.1[a,b]
Eole	426.5	295.9	281.0	118.3	280.4 ± 63.1[a,b]
Jupiter	367.9	366.2	159.5	93.7	246.8 ± 70.7[b,c]
Niagara	201.6	114.7	115.7	63.7	123.9 ± 28.6[c,d]
Princess	50.4	115.6	35.1	17.4	54.6 ± 21.4[d]

[a] is significantly different from [b], [b] is significantly different from [c], [c] is significantly different from [d] with a p-value less than 0.05

Yield comparison of the 2006, 2007 and 2008 years is shown in Table 5.7. Yield from 2007 and 2008 were much lower than of 2006. Carter showed the best yield performance followed by Omega, Rahab94 and Linaza. Pembina and York did not performed well. Heat and drought stress might have had impact on yield decrease in 2007 and 2008. Flax is a temperate plant. Exposure to continuous heat for a period of

time results in significant decreased seed weight (Dybing et al., 1965; Green, 1986; Casa et al., 1999). Cross et al. (2003) reported that the number of malformed, sterile seed increased three-fold after 14 days of heat stress. It was also mentioned in the study that the effect of heat stress on both pollen and ovules contributes to decreased boll formation and seed set in flax. When flax plants exposed to continuous heat of 31°C for 3-5 days, up to 64% malformed seed was observed (Kraft et al., 1963). This supports the results in this study that during the third week of flowering the temperature in Lexington had been higher than 31°C for at least three days.

Heavy rain and/or unexpected cold spell in early spring affect germination of flax seedlings. Planting too late results in seed yield decrease due to heat stress. This suggests that Kentucky's climate is not suitable for growing flax.

Average yield of Carter produced in South Dakota is 1650 kg/ha (Grady, 2005). The best yield from Carter from the best year in this study is 1266.6 kg/ha which is about only 75% of the production in the Northern States. Flax can be grown in Kentucky but does not give a very competitive yield.

Table 5.7, Yield of different flax varieties grown in 2006, 2007 and 2008

Variety	Yield (kg/ha)		
	2006	2007	2008
Omega	940.2	135.6	376.1
Carter	1266.6	146.4	361.4
Rahab94	1242.0	91.6	305.7
York	367.8	54.1	245.9
Pembina	732.9	48.6	293.9
Linaza	938.6	102.0	327.5
York	876.4	n/a	n/a
Golden	558.3	n/a	n/a
Eole	n/a	n/a	280.4
Jupiter	n/a	n/a	246.8
Niagara	n/a	n/a	123.9
Princess	n/a	n/a	54.6

Plant height

Plant height from field trial in 2006 is shown in Table 5.8. Plant height varied from 52-63 cm. Rahab was the tallest variety followed by Linaza while York from South Dakota and Minnesota were the shortest. Data from 2007 shows that plant height of every variety was not significantly different except for Omega which was shorter than others (see Table 5.9). In 2008, plant heights in different varieties ranged from 51-70 cm. Linaza was the tallest variety (see Table 5.10). Omega was the shortest American variety with the height of 61 cm. Among French varieties, Jupiter was the tallest and Niagara was the shortest. The American varieties were significantly taller than the French varieties grown in Lexington. Among French varieties, Jupiter was tallest, followed by Princess, Eole and Niagara. Table 5.11 shows the comparison of plant height from 2006, 2007 and 2008 trials. Plants grown in 2007 were taller than other years.

Table 5.8, Plant height of flax grown in 2006

Variety	Yield (kg/ha)				
	Block 1	Block 2	Block 3	Block 4	Mean ± SE
Omega	56.8	61.9	56.9	61.5	59.3 ± 1.4[b]
Carter	58.1	61.6	59.6	60.0	59.9 ± 0.7[b]
Rahab94	60.6	65.8	63.3	62.8	63.1 ± 1.0[a]
York	52.4	52.9	55.6	54.6	53.9 ± 0.7[c]
Pembina	57.0	63.4	62.1	55.5	59.5 ± 1.9[b]
Linaza	60.0	64.4	60.0	59.5	61.0 ± 1.1[a,b]
York	54.1	55.3	57.0	43.3	52.4 ± 3.1[c]
Golden	56.8	64.1	61.1	59.4	60.4 ± 1.5[b]

[a] is significantly different from [b], [b] is significantly different from [c] with a p-value of 0.05

Table 5.9, Plant height of flax grown in 2007

Variety	Yield (kg/ha)				
	Block 1	Block 2	Block 3	Block 4	Mean ± SE
Omega	66.8	68.4	67.9	63.9	66.7 ± 1.0[b]
Carter	66.0	68.9	68.5	74.4	69.4 ± 1.8[a]
Rahab94	68.4	71.5	72.6	74.6	71.8 ± 1.3[a]
York	67.5	68.6	67.8	74.3	69.5 ± 1.6[a]
Pembina	66.3	70.4	73.4	74.6	71.2 ± 1.9[a]
Linaza	65.5	68.6	69.8	75.4	69.8 ± 2.1[a]

[a] is significantly different from [b] with a p-value of 0.05

Table 5.10, Plant height of flax grown in 2008

Variety	Yield (kg/ha)				
	Block 1	Block 2	Block 3	Block 4	Mean ± SE
Omega	56.0	62.8	62.0	61.6	60.6 ± 1.6[d]
Carter	64.0	62.0	68.4	63.0	64.4 ± 2.4[b,c]
Rahab94	59.4	58.8	65.8	64.4	62.1 ± 1.7[c,d]
York	61.6	62.6	68.4	64.4	64.3 ± 1.5[b,c]
Pembina	58.8	66.0	70.0	66.0	65.2 ± 4.3[b]
Linaza	65.4	70.4	73.4	69.2	69.6 ± 1.6[a]
Eole	52.2	52.4	55.2	54.4	53.6 ± 0.7[f,g]
Jupiter	57.8	58.6	55.6	58.6	57.7 ± 0.7[e]
Niagara	47.0	53.6	54.4	49.0	51.0 ± 1.8[g]
Princess	50.8	54.0	56.2	54.6	53.9 ± 1.1[f]

[a] is significantly different from [b], [b] is significantly different from [c], [c] is significantly different from [d], [d] is significantly different from [e], [e] is significantly different from [f], [f] is significantly different from [g] with a p-value of 0.05

Table 5.11, Plant height of different flax varieties grown in 2006, 2007 and 2008

Variety	Yield (kg/ha)		
	2006	2007	2008
Omega	59.3	66.7	60.6
Carter	59.9	69.4	64.4
Rahab94	63.1	71.8	62.1
York	53.9	69.5	64.3
Pembina	59.5	71.2	65.2
Linaza	52.4	69.8	69.6
York	60.4	n/a	n/a
Golden	61.7	n/a	n/a
Eole	n/a	n/a	53.6
Jupiter	n/a	n/a	57.7
Niagara	n/a	n/a	51.0
Princess	n/a	n/a	53.9

Fatty acid profile

Fatty acid profile of flax seed oil extracted from the 2008-trial seeds is shown in Table 6.12. Major fatty acids found in flax seeds varied; palmitic acid 5.1 – 6.6%; stearic acid, 4.3 – 7.3%; oleic acid, 24.8 – 35.2%, linoleic acid, 12.1 – 17.3% and linolenic 38.5 – 48.0%. Fatty acid profiles from seed oil of different flax varieties were not much different except for the French variety, Jupiter which contained less of the polyunsaturated fatty acids, 11% linoleic acid and 38% linolenic acid and more of the unsaturated fatty acids, 7% of palmitic acid and 7% of stearic acid and 35% of the monounsaturated fatty acid, oleic acid.

Table 6.12, Fatty acid profile of flax grown in 2008

Variety	% fatty acid (mean ± SE)				
	16:0	18:0	18:1	18:2	18:3
Omega	5.4 ± 0.2	5.7 ± 0.1	30.7 ± 0.1	15.2 ± 1.2	42.7 ± 1.1
Carter	5.4 ± 0.1	4.3 ± 0.1	24.8 ± 1.7	17.0 ± 0.4	47.2 ± 0.9
Rahab 94	5.1 ± 0.1	5.6 ± 0.2	27.7 ± 1.3	17.3 ± 0.5	41.6 ± 0.5
York	5.4 ± 0.1	5.4 ± 0.3	25.2 ± 1.5	14.5 ± 0.0	48.0 ± 2.9
Pembina	5.1 ± 0.1	5.5 ± 0.5	28.3 ± 0.1	17.2 ± 0.1	41.5 ± 1.0
Linaza	5.7 ± 0.1	5.3 ± 0.1	26.8 ± 1.2	14.5 ± 0.4	45.7 ± 0.7
Eole	5.5 ± 0.4	5.8 ± 0.0	30.1 ± 1.2	15.0 ± 2.4	40.9 ± 1.0
Jupiter	6.6 ± 0.3	7.3 ± 0.3	35.2 ± 3.1	10.9 ± 0.8	38.5 ± 1.7
Niagara	5.2 ± 0.1	6.9 ± 0.9	25.1 ± 3.0	13.8 ± 0.7	47.4 ± 0.3
Princess	6.2 ± 0.1	5.7 ± 0.0	27.9 ± 0.7	12.1 ± 0.5	46.7 ± 0.2

16:0 (palmitic acid); 18:0 (stearic acid); 18:1 (oleic acid); 18:2 (linoleic acid); 18:3 (linolenic acid)

Flowering

Flowering was observed by 54 days after planting in every variety except for York which was a couple of days later than other varieties. According to reports from North Dakota and South Dakota, York flowers later than other varieties (Berglund and Zollinger, 2007; Grady, 2005). From the observation in 2008, the French varieties flowered 4 days earlier than the American varieties.

Other observations

Major pests and diseases were not found during the field trails. Butterflies, carpenter bees and some other insects were found in flax field but they did not harm the plants.

Conclusions

Field trials of flax were conducted in years 2006, 2007 and 2008. In the 2006 trial, the yield ranged between 558-1,267 kg/ha. Carter showed the highest yield performance followed by Omega, Rahab94 and Linaza. Pembina and York did not performed well. The yield trends for each variety from 2007 and 2008 were similar to the 2006 trial however the yields were much lower. The decreased seed yield might have been the results of drought and heat stress from the late planting date. The effect of heat on pollen and ovules contributes to decreased boll formation and seed set. The American varieties performed better than the French varieties in Lexington.

There was not much difference among these flax varieties in plant height. The tallest variety was not the same in each year. The 2008 data showed that American flax varieties were taller from French varieties.

For flowering time, French varieties flowered earlier than American varieties. Among American varieties, York flowered later than others. There were no major pest or diseases observed in the flax plots during the three trials.

Flax can be grown in Kentucky but it does not give competitive yields compared with North Dakota and Canada. However, it might be grown in a small scale for health food markets in local areas.

CHAPTER 6

Breeding for high yield castor (*Ricinus communis* L.) in Kentucky

Materials and Methods

Plant materials

Two castor lines were chosen to be parental plants in this breeding project. The high-yield line 'Carmencita' has a distinctive dark red color on stems and capsules. The seeds of Carmencita were obtained from Select Seed Co. in Union, Connecticut. The other line was obtained from Dich Auld at Texas Tech University, Lubbock, Texas. This line has a low ricin level hence the name 'TTU-LRC' (Texas Tech University – Low Ricin). TTU-LRC is semi-dwarf and green in stem and capsules.

Cross pollination

Cross pollination of the two parental lines was done in the greenhouse. Young female flowers on a mother plant were covered in a pollination bag to prevent undesired pollination and male flowers were discarded. At the time of pollination, female flowers were uncovered and pollen from a father plant was thoroughly dusted on stigmas. Pollinated flowers were re-covered and uncovered again two days after pollination. The cross of Carmencita x TTU-LRC (C x T) was done as well as the reciprocal TTU-LRC x Carmencita (T x C).

F_1 population

F_1 seeds were planted at Spindletop Farm, Lexington, KY on May 24, 2006. The planting was done in rows of 6.1 m. Row spacing was 0.9 m and spacing between plants was 38 cm. Flowers were bagged to ensure self-pollination.

F_2 population

F_2 seeds were planted at Spindletop Farm on May 15, 2007. Approximately 500 F_2 seeds from the C x T and 500 seeds from the T x C were seeded. Row spacing and plant spacing were the same as for the F_1 population in 2006. Some F2 individuals were selected from the visual appearance of vigor. The preferred height for seed production is approximately 1.5 m. A few very short and tall plants were also selected for breeding programs in the future. Plants with long and high number of inflorescences were preferred. Inflorescences of selected plants were bagged to prevent out-crossing. However, there was a possibility of out-crossing in some individuals in the case that bagging was done after pollination.

Small scale field test of parental lines

The parental lines Carmencita and TTU-LRC were seeded at Spindletop Farm on May 15, 2007. Row spacing and plant spacing were the same as for the F_2 population. Each line was planted in 2 replications of 5 rows. A field test was repeated again in 2008 with the same plot design and also in 2009.

Cultural practice

Herbicide was not applied to any plot in this study. Weeding was done manually as needed. No application of pesticide and fertilizer was done. Irrigation was applied as needed.

Seed weight and harvest index calculation

Castor seed harvesting and cleaning was done manually since there was no harvesting machine or combine for castor available. For the same reason, actual seed weight was not measured. However, data on seed weight was obtained indirectly by weighing 100 random capsules, cleaning seeds, weighing cleaned seed and debris compared to the whole capsule weight, and calculating approximate seed weight.

$$\text{Harvest index} = \frac{\text{total seed weight}}{\text{total plant dry weight}}$$

Results and Discussion

F_1 population

The reciprocal F_1 populations obtained from the cross pollination had intermediate stem color between two parental lines. Stems were green on the top part and red on the lower part (Figure 6.1). F_1 plants from both crosses looked alike and uniform (Figure 6.2).

F_2 population

The F_2 population showed segregation of traits including, plant height, stem color, stigma color, capsule color, branching. Figure 6.3 shows capsule colors of parental plants and Figure 6.4 shows variation of capsule colors in the F_2 population. Data on plant height, stem color, capsule color, number of branch and number of inflorescence were collected from the best looking 89 plants in the field (Table 6.1). All plants were weighed for total capsule weight. Only 50 plants were collected for harvest index calculation (see Table 6.2). Plants with more branches and inflorescences gave better single plant yields. The harvest index ranged from 0.3 – 0.7.

The F_2 plants number 26, 29, 31, 32 and 89 gave the highest total seed per plant ranging from 0.4 – 0.5 kg. These lines might give seed yields between 3,200 – 4,000 kg/ha (when calculated with recommended plant spacing of 25 cm and row spacing of 1 m). Brigham (1993) reported that some good fields produced 3,811 to 4,035 kg/ha with irrigation.

F_3 population

F_3 seeds from 30 F_2 individuals (plant number 1, 5, 6, 8, 9, 10, 12, 14, 15, 16, 17, 18, 20, 21, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 38, 67, 69, 75, 76, 89) with high yield were planted in 2008. The 2008 trial was not qualified because the F_3 plants did not yield well because of drought. Irrigation was applied to the plot but the main water line to the plot was broken and it was not fixed in time. Castor is considered drought tolerant but the stress was too severe to give qualified yield. However, F_3 family seeds from 15 F_2 individuals with the best harvest index and best yield (plant number 1, 10, 14, 15, 17, 21, 26, 28, 29, 30, 31, 32, 67, 69 and 89; shaded rows in Table 6.2) were planted in 2009. Plants have been harvested. Yield data will be analyzed later.

Figure 6.1, TTU-LRC (T) seedling (left) and Carmencita (C) seedling (right)

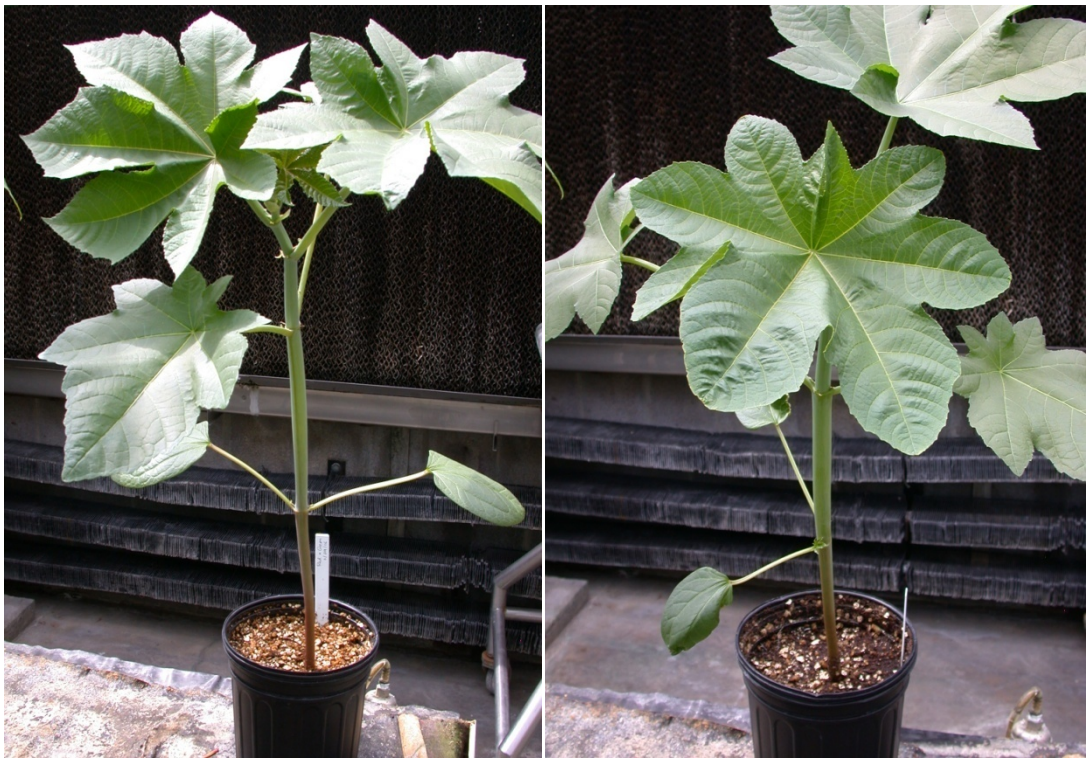

Figure 6.2, F_1 seedling from C x T (left), F_1 seedling from T x C (right)

Figure 6.3, Fruit and stem colors of the parental lines Carmencita (left) and TTU-LRC (right)

Figure 6.4, Variation of capsule and stem colors in F_2 population

Table 6.1, Plant height, stem color, capsule color, number of branches and number of inflorescences of Carmencita, TTU-LRC and F_2 individuals grown in 2007

Number	Cross	Plant height (m)	Stem color	Capsule color	No. of branches	No. of inflorescences
	C	1.7	red	red	9	15
	T	1.2	green	green	5	9
1	T x C	1.5	red	red	8	15
2	T x C	1.4	red	dark green	7	14
3	T x C	1.1	green	green	8	17
4	T x C	1.5	pink	green	4	7
5	T x C	1.0	green	green	6	12
6	T x C	1.1	green	green	3	4
7	C x T	1.2	pink	green	7	19
8	C x T	1.3	red	red	6	13
9	C x T	1.3	red	green	6	15
10	C x T	1.3	pink	green	8	20
11	C x T	1.4	pink	green	6	10
12	C x T	1.1	red	green	7	14
13	C x T	1.6	pinkish green	pale green	10	11
14	C x T	1.4	pink	pink	8	19
15	C x T	1.5	pink	pink	7	18
16	C x T	1.2	green	green	6	11
17	C x T	1.0	green	green	3	8
18	C x T	1.0	pink	green	5	13
19	C x T	1.3	red	pinkish green	6	13
20	T x C	1.2	pink	green	4	7

Table 6.1, (continued)

Number	Cross	Plant height (m)	Stem color	Capsule color	No. of branches	No. of inflorescences
21	T x C	1.1	red	green	5	11
22	T x C	1.6	green	green	7	14
23	T x C	1.4	green	green	3	4
24	T x C	1.4	red	pink	5	11
25	T x C	1.4	red	pale green	5	8
27	T x C	1.8	red	pink	7	15
28	T x C	1.4	green	green	9	21
29	T x C	1.7	red	green	11	20
30	T x C	1.8	red	green	10	19
31	T x C	1.5	pink	green	8	27
32	T x C	1.8	pinkish green	green	6	22
33	T x C	1.2	pink	green	4	9
34	T x C	1.4	red	pink	8	17
35	T x C	1.3	pink	pink	7	17
36	T x C	1.7	pink	green	7	12
37	T x C	1.4	pinkish green	green	4	7
38	C x T	1.5	red	green	8	14
39	C x T	0.9	red	pale green	6	9
40	C x T	1.6	pink	green	7	10
41	C x T	1.3	pale green	green	7	11
42	C x T	1.5	green	green	8	11
43	T x C	1.4	red	red	5	7
44	C x T	1.5	green	green	5	10
45	C x T	1.4	pink	pink	5	8

Table 6.1, (continued)

Number	Cross	Plant height (m)	Stem color	Capsule color	No. of branches	No. of inflorescences
46	T x C	1.5	pink	pink	6	11
47	T x C	1.7	pink	pale green	7	14
48	T x C	2.6	red	red	1	1
49	T x C	2.2	red	red	1	4
50	C x T	2.7	red	red	8	1
51	C x T	1.3	green	green	5	7
52	C x T	1.1	red	red	7	7
53	T x C	1.1	green	green	6	8
54	T x C	1.2	red	green	7	13
55	C x T	1.1	red	red	10	19
56	C x T	1.3	pink	green	4	7
57	T x C	1.7	pinkish green	green	5	9
58	T x C	1.4	red	red	8	17
59	T x C	1.5	green	green	7	13
60	T x C	1.5	green	green	14	22
61	C x T	1.2	red	red	10	18
62	C x T	1.0	red	pink	6	17
63	C x T	1.1	green	green	4	9
64	C x T	1.9	pink	green	5	10
65	C x T	1.0	pinkish green	green	2	4
66	T x C	1.6	green	green	11	20
67	T x C	1.7	red	red	13	27
68	T x C	1.8	red	green	5	14
69	T x C	1.8	red	green	15	27

Table 6.1, (continued)

Number	Cross	Plant height (m)	Stem color	Capsule color	No. of branches	No. of inflorescences
70	T x C	1.5	green	green	4	10
71	T x C	1.6	red	green	8	14
72	T x C	1.3	green	green	4	11
73	T x C	1.2	green	green	4	7
74	C x T	1.5	pink	green	7	10
75	C x T	1.7	red	green	7	16
76	T x C	2.1	red	pink	11	19
77	T x C	1.6	green	green	4	8
78	T x C	1.7	green	green	8	15
79	T x C	1.7	red	green	6	11
80	T x C	1.6	red	green	3	13
81	C x T	1.5	red	green	13	28
82	C x T	1.7	red	pink	4	8
83	C x T	1.6	red	green	6	22
84	C x T	0.6	pink	pink	1	2
85	C x T	0.6	red	red	1	1
86	C x T	1.5	red	green	5	8
87	C x T	0.9	pink	pale green	6	12
88	T x C	1.3	pink	green	7	16
89	T x C	2.2	green	green	11	24

Table 6.2, Weight and harvest index of F_2 individuals grown in 2007

Number	Stem wt (g)	Capsule wt (g)	Total plant wt (g)	100 capsule wt (g)	Seed wt*(g)	Total seed wt (g)	Harvest index
1	270	604	874	138	96	420	0.48
2	240	348	588	142	98	240	0.41
3	170	330	500	116	82	233	0.47
4	130	214	344	131	93	152	0.44
5	50	171	221	104	71	117	0.53
6	50	151	201	115	82	109	0.54
7	230	401	631	139	100	288	0.46
8	110	261	371	114	82	188	0.51
9	110	340	450	152	109	244	0.54
10	190	482	672	145	101	336	0.75
11	110	205	315	114	83	149	0.47
12	100	228	328	135	96	162	0.49
13	280	337	617	106	69	219	0.35
14	300	552	852	161	113	387	0.45
15	180	401	581	139	99	286	0.49
16	60	191	251	99	73	141	0.56
17	30	160	220	149	109	139	0.63
18	50	152	202	106	78	112	0.55
19	140	276	416	96	69	198	0.47
20	50	161	211	118	86	117	0.56
21	110	281	391	130	102	220	0.56
22	170	374	544	148	109	257	0.47
23	70	159	229	48	33	109	0.48
24	100	229	329	60	42	160	0.49
25	160	310	470	142	103	225	0.48

Table 6.2, (continued)

Number	Stem wt (g)	Capsule wt (g)	Total plant wt (g)	100 capsule wt (g)	Seed wt*(g)	Total seed wt (g)	Harvest index
26	450	619	1069	117	97	513	0.48
27	190	365	555	141	107	277	0.50
28	230	506	736	86	63	371	0.50
29	380	620	1000	201	144	444	0.44
30	330	550	880	82	60	402	0.46
31	280	576	856	148	107	416	0.49
32	390	735	1125	187	139	546	0.49
33	70	229	299	118	84	163	0.54
34	140	313	453	130	96	231	0.51
35	170	291	461	150	109	211	0.46
36	170	289	459	85	60	205	0.45
37	90	175	265	110	80	127	0.48
38	180	391	571	149	109	286	0.50
39	80	144	224	65	49	108	0.48
40	150	245	395	168	120	175	0.44
41	70	157	227	130	88	106	0.47
42	110	178	288	105	78	132	0.46
43	100	220	320	134	95	156	0.49
44	110	209	319	154	114	155	0.48
45	90	163	253	140	102	119	0.47
46	100	214	314	169	120	152	0.48
47	200	331	531	80	57	236	0.44
48	450	196	646	108	78	191	0.30
49	460	271	731	73	57	212	0.29
50	540	47	587	immature	n/a	n/a	n/a

Table 6.2, (continued)

Number	Stem wt (g)	Capsule wt (g)	Total plant wt (g)	100 capsule wt (g)	Seed wt*(g)	Total seed wt (g)	Harvest index
51†		222				166.5	
52		145				108.7	
53		143				107.2	
54		168				126.0	
55		381				285.7	
56		186				139.5	
57		155				116.2	
58		249				186.7	
59		214				160.5	
60		308				231.0	
61		335				251.2	
62		189				141.7	
63		160				120.0	
64		255				191.2	
65		78				58.5	
66		243				182.2	
67		509				381.7	
68		288				216.0	
69		533				399.7	
70		192				144.0	
71		368				276.0	
72		189				141.7	
73		145				108.7	
74		190				142.5	
75		281				210.7	

Table 6.2, (continued)

Number	Stem wt (g)	Capsule wt (g)	Total plant wt (g)	100 capsule wt (g)	Seed wt*(g)	Total seed wt (g)	Harvest index
76		391				293.2	
77		194				145.5	
78		304				228.0	
79		276				207.0	
80		266				199.5	
81		257				192.7	
82		197				147.7	
83		313				234.7	
84		18				13.5	
85		13				9.75	
86		201				150.7	
87		118				88.5	
88		207				155.2	
89		696				522.0	

* Seed weight of 100 capsules

† Total seed weight of plant numbers 51-89 was calculated as 75% of total capsule weight. Stem weight of plant numbers 51-89 was not measured.

Small scale yield trial of parental lines

It was found that seed weight of Carmencita comprises 75% of the capsule weight. In TTU-LRC, seed weight is 79% of the capsule. Yields of Carmencita and TTU-LRC from a small scale field test in 2007 are shown in Table 6.3. Baldwin and Cossar (2009) reported that mean yield of castor planted in four locations in Tennessee and Mississippi ranged from 89 – 1,954 kg/ha. From this trial, Carmencita gave the yield of 2,202 kg/ha and TTU-LRC 2,015 kg/ha. The yields were higher than the reported average in TN and MS but were lower than yields of 2,242 to 3,363 kg/ha in Texas (Brigham, 1993). However, this is promising considering the severe drought in 2007 and fertilizer was not applied to the plot and weed control was minimal. Castor should give competitive yield in Kentucky when irrigation, weed control and fertilizer are applied.

Table 6.3, Yield from Carmencita and TTU-LRC from a small scale trial in 2007

Variety	Yield (kg/ha)
	Mean ± SE
Carmencita	2202 ± 61
TTU-LRC	2051 ± 336

The small scale trial was done in 2008 but it was not successful because of drought.

Conclusions

The F_2 population showed a high degree of segregation for plant height, color, branching and seed yield. The data was collected from 89 F_2 individuals. Fifteen lines with highest yield were selected for planting in the future. New high-yield castor varieties are being developed in Kentucky. These new lines will be selected with the preference to low ricin level and shorter plant height.

The small scale field trial in 2007 showed promising results for Carmencita and TTU-LRC yield. With sufficient irrigation, weed control and fertilizer application, castor production in Kentucky could be competitive.

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