

# Chapter 19

## Cuphea

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### 19.1 Introduction

Temperate plant species whose seed oils are rich in medium-chain fatty acids (MCFAs) are relatively rare (Wolf et al. 1983). One species of particular interest is cuphea, a temperate annual oilseed crop with high levels of MCFAs such as capric and lauric acid (Graham et al. 1981; Graham and Kleiman 1985; Graham and Kleiman 1992). These fatty acids are highly valued as feedstocks in manufacturing cosmetics, soaps and detergents, pharmaceuticals, and industrial lubricants (Wolf et al. 1983; Thompson et al. 1990; Cermak and Isbell 2004). Additionally, new uses for MCFAs have the potential to significantly replace petroleum-based products like motor oil, hydraulic fluid, and diesel fuel (Geller et al. 1999; Geller and Goodrum 2000; Leroux et al. 2006). This chapter primarily covers the advances of cuphea research since the previous reviews of cuphea breeding presented by Knapp (1990a, 1993) with the main focus on oilseed production in the cuphea species of *C. lanceolata*, and *C. viscosissima* which are targeted for commercialization in the US.

Research interests have centered on cuphea primarily for its seeds which have a high content of unique fatty acids (Earle et al. 1960; Graham et al. 1981; Wilson et al. 1960). The predominant fatty acids include: caprylic acid (C8), capric acid (C10), lauric acid (C12) and myristic acid (C14). Lauric acid has been the primary fatty acid of interest in US breeding programs. Lauric acid is used in foods, mostly vegetable shortenings, and in soaps and detergents as a defoaming agent and booster (Thompson 1984; Babayan 1981). Traditionally, the tropical oil crops such as coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.) commercially supply these acids. Currently the US soap and detergent industry gets half of these fatty acids from the petroleum industry and the other half from imported coconut and palm kernel oils (Hardin 1991). Coconut oil is 45–50% lauric acid, while some undeveloped lines of cuphea can produce oil that contains nearly 80% lauric acid (Graham

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1989b). To date, there are no temperate oilseed crops that can supply these lipids (Ignacio 1985; Arkcoll 1988). Many of the fatty acid-rich species of cuphea are summer annuals and through crop development programs could potentially become domestic sources of fatty acids.

The cuphea breeding line, 'PSR23' (*Cuphea viscosissima* Jacq. × *C. lanceolata* W.T. Aiton) is the only domesticated cuphea in the US and is the current focus of production and breeding programs in Illinois, Iowa, North Dakota, and Minnesota. Although rich in capric acid, PSR23 serves well as an agronomically sound plant for agronomic and physiological studies and as a foundation for breeding programs in the US.

Breeding and research efforts on other *Cuphea* spp. continues throughout the world. Research in India is focused primarily on *C. procumbens* for seed oil production (Pandey et al. 2000; Rameshkumar et al. 2002; Singh and Singh 2002; Singh and Rameshkumar 2003; Singh et al. 2007). South American researchers are looking at *C. carthagenesis* (Mathioni et al. 2005; Dezanet et al. 2007) and *C. glutinosa* (Yagueddu et al. 2006) for medicinal purposes. Japanese researchers are investigating food uses with *C. leptopoda* (Saikusa et al. 2001).

## 19.2 Domestication and Breeding History

The genus *Cuphea* (Lythraceae) contains approximately 260 species that are native to the area from Mexico through Brazil, with one species native to the eastern United States (Graham and Kleiman 1985). Several are adapted to temperate agriculture and have seed oils rich in capric and lauric acid. Initial domestication programs began in Germany to evaluate different species and to determine the feasibility of domesticating cuphea (Hirsinger 1980; Hirsinger and Röbbelen 1980; Graham et al. 1981; Hirsinger and Knowles 1984; Hirsinger 1985; Röbbelen and von Witzke 1989). At Oregon State University (OSU), Steven Knapp began an intensive breeding program to domesticate cuphea for US production. Knapp worked extensively with *C. viscosissima*, the only species native to the United States, and *C. lanceolata*, a species native to the Sierra Madre of Mexico (Graham 1988). The essential agronomic traits and seed oil yields of these species were sufficient for competition as oilseed crops in the US. Seed shattering and seed dormancy were the major domestication barriers within the genus at the time (Knapp 1990a,b).

In the mid to late 1990s, important breakthroughs were made towards eliminating seed shattering and seed dormancy by exploiting interspecific diversity. Non-shattering phenotypes within the *C. viscosissima* × *C. lanceolata* f. *silenoides* population and an autofertile, non-dormant, and non-shattering *C. viscosissima* × *C. lanceolata* f. *silenoides* cultivar have all been developed (Knapp and Crane 2000a,b,c). Knapp was also successful at developing other

elite lines of cuphea that are non-shattering, auto-fertile, and have reduced levels of sticky hairs.

Sticky or glandular hairs covering stems, leaves, and flowers are characteristic of most cuphea species (Graham 1988; Amarasinghe et al. 1991). These glandular hairs have been cited as a negative trait primarily due to the difficulty in harvesting the crop (Hirsinger 1980; Hirsinger and Röbbelen 1980; Thompson 1984). The stickiness of cuphea is certainly difficult when working by hand, but it is not a limitation to the commercialization of cuphea. Recent large scale production of several hundred acres in 2005–2007 in Minnesota and Illinois were not impeded by the stickiness when production combines were used (Fig. 19.1). Although some of the sticky residue from cuphea chaff accumulates in harvesting equipment, it does not seem to hinder harvesting. The indeterminate nature, high levels of moisture, and shattering levels of cuphea are seen as the current barriers to successful and consistent harvests.



Fig. 19.1 Direct combining of PSR23 cuphea in Illinois

### 19.2.1 Oil Crop Breeding

Studies conducted in the early 1980s on cuphea focused primarily on characterizing fatty acid profiles of various cuphea species collected from the wild (Graham et al. 1981; Wolf et al. 1983; Hirsinger 1985). These studies helped direct early breeding programs to focus on lauric acid accumulating species, *C. wrightii*, for the soap and detergent industry (Thompson and Kleiman 1988). Unfortunately, domestication of this species proved to be extremely difficult

due to seed shattering, open pollination, and poor agronomic traits. However, recent efforts with the high capric acid species of *C. viscosissima* and *C. lanceolata* have been successful in improving many of the agronomic traits necessary for cuphea domestication (Knapp 1993). Several early breeding programs employed mutation breeding to address fatty acid accumulation (Hirsinger 1980; Hirsinger and Röbbelen 1980; Campbell 1987; Röbbelen and von Witzke 1989; National Botanical Research Institute 2003); however, they all experienced limited success. No newer reports of mutation breeding have been reported in the literature. Non-shattering and determinacy still pose difficult hurdles in cuphea species. To date, variety trails of cuphea accessions have yet to identify a completely non-shattering or determinate accession. With efforts continuing on improving agronomic traits, altering total oil and fatty acid content will soon become a priority once again.

### 19.3 Genetic Resources

The USDA-ARS National Plant Germplasm System (NPGS) cuphea collection is currently maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA under the curation of L. Marek. As of January 2008, 649 accessions of cuphea are in the NCRPIS collection; however, only 499 are available through the Germplasm Resources Information Network (GRIN) for distribution (a few as vegetative cuttings only, the rest as seed only). The accessions represent 67 different taxa, plus seven accessions that have not been identified to species, and 30 accessions that are hybrids of various combinations. The available accessions represent 38 different taxa, plus 2 unidentified to species, and 26 that are hybrids.

Over the past 10 years, the NCRPIS has distributed approximately 4,250 cuphea items in 167 requests. Nearly 80% of all requests were sent within the US. International distributions were to Japan, the Netherlands, Germany, France, China, England, Brazil, Greece, and Canada. Cuphea seed requests are distributed evenly between ornamental and oil related uses (breeding, enzymes, development, and evaluation) (L. Marek, personal communication).

The cuphea genetic resources collection was recently assisted by the identification of protocols to help remove dominance issues of wild type accessions (Widrechner and Kovach 2000; Crane et al. 2006). It was also found that the current storage protocols were detrimental to seed germination (Volk et al. 2006). *C. wrightii* A. Gray, *C. laminuligera* Koehne, *C. carthagenensis* (Jacq.) J.F. Macbr., and *C. aequipetala* Cav are considered sensitive to low temperature storage. The seeds of these species have triacylglycerols that are crystalline at  $-18^{\circ}\text{C}$  and melt when the seeds are warmed to  $>35^{\circ}\text{C}$  (Volk et al. 2007). Cuphea seeds imbibed while the triacylglycerols are crystalline fail to germinate and exhibit visual damage. However, germination proceeded normally when

dry seeds were warmed adequately to melt any crystalline triacylglycerols before imbibition.

Although most of the available accessions have basic descriptors, the majority of the collection has limited information regarding fatty acid content and yield. Several studies have screened portions of the collection, but very little consistency exists between them (Graham et al. 1981; Wolf et al. 1983; Hirsinger 1985). Extraction method, location and year where seeds were produced, and gas chromatography analysis have all led to variations in total oil and fatty acid composition being reported for individual accessions. Many of the early studies often involved complicated and difficult extraction and analysis procedures requiring hours of preparation. These methods are impractical for supporting high throughput breeding programs aimed at developing new varieties. A recent study by Phippen et al. (2006) developed a reliable and efficient method for determining total oil and fatty acid content in cuphea seed and evaluated 185 cuphea accessions.

## 19.4 Advances in Cuphea Production

Knapp, while at OSU, essentially maintained the only cuphea breeding program in the US from the mid 1980s to 2004. Many of his breeding advancements were summarized in Knapp (1993) and more recently in Knapp et al. (2004).

Knapp's more recent efforts were directed towards: the development of fully self-pollinated, partially non-shattering cultivars; breeding for high oil, high capric acid, and semi-determinant flowering; and strategies for developing high lauric and fully non-shattering cultivars (Knapp et al. 2004). The development of high capric cultivars is underway using high capric acid (85–89% C10) germplasm sources. As reported by Knapp et al. (2004), the molecular breeding program was restarted to build the foundation for genetic analyses and marker-assisted selection in cuphea. More than 200 sequence-tagged-site (STS) markers have been developed. The molecular genetic diversity in *C. viscosissima* and *C. lanceolata* has already been surveyed, along with the development of segregating populations and near-isogenic lines for mapping and manipulating phenotypic loci. Quantitative trait loci for seed dormancy, seed shattering, self-pollination, and other economically important traits continue to be identified. This advancement effort is aided by a genetic map of cuphea developed by Webb et al. (1992).

### 19.4.1 'PSR23' Cuphea

One of the first successful releases of a cuphea breeding line was 'PSR23' (PI 606544) released by Knapp and Crane (2000c). PSR23 was the first line to

introduce the critical 'Partial Shatter Reduction' (PSR) trait into cuphea. This trait inhibits the rotation of the placenta from the capsule thereby leading to an increase in seed retention (Fig. 19.2). Typical wild type cuphea populations have a near 100% seed loss due to shattering. The PSR trait reduces this seed loss to only 20–30%. Other traits of interest in PSR23 include relatively high oil content of  $295 \text{ g kg}^{-1}$ , high capric acid content of 72%, self-compatibility, and non-dormant seed. Although still largely open-pollinated, PSR23 was selected as the first cuphea line to begin commercialization.



**Fig. 19.2** Partial shatter reduction trait in PSR23 cuphea with reduced placenta rotation (A). Wild type shattering trait with increased placenta rotation (B)

PSR23 was first distributed to research programs in Illinois (IL) and Minnesota (MN) in the summer of 2000. The original population was heterogeneous and clearly demonstrated potential for environmental selection. Over the next 7 years, selections were increased in IL and MN, remained separated and developed unique phenotypes for each region. Under the drier and longer IL growing season, a larger, erect, and less vegetatively pronounced phenotype was favored. Unfortunately, this phenotype lost the essential PSR trait. In the cooler shorter season of the northern corn belt, a smaller, more vegetatively pronounced, and more compact phenotype prevailed. With this phenotype, the reduced shattering trait remains intact.

A recent environmental adaptation study of the PSR23 ecotypes was conducted in IL and MN in 2007. Preliminary results from the Illinois field studies indicate that IL grown PSR23 selected in 2006 produced the least amount of seed in 2007 ( $210 \text{ kg ha}^{-1}$ ), which is not surprising due to the lack of the PSR trait. However, due to the small plant size of the MN grown PSR23 selected in 2006 its seed production under IL growing conditions was limited to  $240 \text{ kg ha}^{-1}$ . When compared to the original PSR23 with a seed yield of  $350 \text{ kg ha}^{-1}$ , both the MN and IL ecotypes fared worse under IL conditions. Under MN growing conditions, all cuphea lines performed much better than in IL with the original PSR23 reporting  $830 \text{ kg ha}^{-1}$ . Interestingly, only the first year selection in MN performed as well as the original line. After 7 years of selection at both

locations, most lines significantly decreased in seed yield when compared to the original line. This suggests the original PSR23 is still heterogeneous and that the current selection protocols are not adequate for maintaining high seed yields.

### **19.4.2 Commercialization**

With PSR23 being the only cuphea line available in adequate volumes, many of the more recent agronomic, plant physiological, and product development research reports in the US are based on this line. Although two ecotypes of PSR23 exist, they do not differ significantly in total oil yield and fatty acid constituents.

This first major breakthrough in producing cuphea on a large scale was the identification of herbicides that could control broadleaf weeds in production fields. Fortunately, cuphea demonstrates tolerance to several soil-applied herbicides including ethalfluralin, isoxaflutole, and trifluralin, and one postemergence herbicide, mesotrione (Forcella et al. 2005a). Repeated studies in IL and MN have enabled researchers to secure a registered 24C herbicide label for the mesotrione herbicide for application on cuphea in 2005. This helped facilitate large scale seed increases by contracted producers. To help control biennial wormwood (*Artemisia biennis*) and Canada thistle (*Cirsium arvense*) in MN, clopyralid could also be used safely in conjunction with soil-applied isoxaflutole (Papiernik et al. 2006).

The summer of 2004 marked the first year for an experimental commercialization trial focused on developing an agricultural management strategy for cuphea utilizing conventional technologies to minimize the need for specialized equipment. Technology Crops International, in cooperation with the USDA Agricultural Research Service, grew 18.6 ha of PSR23 within a 32 km radius of Morris, Minnesota (45.35°N, 95.53°W). Although not all hectareage was successful, the harvestable plantings produced seed yields ranging from approximately 78–744 kg ha<sup>-1</sup> at 12% moisture (Gesch et al. 2006). Being the first large scale production of a partially domesticated breeding line, valuable knowledge was learned through this experience that might not have been gained by plot-scale experiments alone. PSR23 remains indeterminate displaying a wide range of seed maturities at time of harvest. Gesch et al. (2006) indicated post-harvest management of seed on a large-scale (e.g., drying, cleaning, and storing) was problematic, suggesting further need for introducing determinacy into the current cuphea breeding lines.

Much of the success of the 2004 commercialization trial was due to the many research projects focused on improving the cultural practices of producing cuphea. Recent studies have addressed seed germination response to temperature (Berti and Johnson 2008), row spacing (Gesch et al. 2003; Sharratt and Gesch 2004), sowing dates (Gesch et al. 2002; Forcella et al. 2005b), temperature sensitivity (Gesch and Forcella 2007), nutrient requirements

(Olness et al. 2005), irrigation studies (Gesch et al. 2004), seed physiological maturity (Berti et al. 2007) seed drying (Cermak et al. 2005) and harvesting methods (Berti et al. 2005; Forcella et al. 2007). Tisserat et al. (2008) have indicated that applications of ultra-high CO<sub>2</sub> treatments accelerated cuphea PSR23 growth and development and aided in seedling establishment. The basic knowledge gained by the previously mentioned experiments has been the single greatest advancement towards breeding and ultimately commercializing cuphea. Although significant progress has been made in production protocols for cuphea, the lack of any significant breeding effort to develop auto-fertile, non-shattering, and determinate plant lines still limit the success of cuphea as a commercial crop.

### **19.4.3 Product Development**

With the success of the 2004 commercialization trial and the following growing seasons, several thousand pounds of seed were made available to the USDA-ARS in Peoria, IL for product development. Some of the more recent advances can be found in modifying the fatty acids of cuphea to meet current industrial needs as lubricants (Evangelista and Cermak 2007), fuels (Leroux et al. 2006) and cosmetics (Cermak et al. 2007).

A recent study even characterized the proteins in cuphea (PSR23) seed to provide fundamental information on their size, amino acid profile, solubility classes, and solubility behavior (Evangelista et al. 2006). The seed contained 32% (dry basis, db) oil and 21% (db) crude protein. Glutelins and albumins accounted for 83.5% and 15.4%, respectively, of the total protein extracted. PSR23 has been further investigated to determine the effects of oil processing conditions on functional properties of the seed proteins to evaluate their potential for value-added uses (Hojilla-Evangelista and Evangelista 2006).

## **19.5 Current Breeding Goals**

### **19.5.1 Lauric Acid Accumulation**

The original US cuphea breeding programs focused on cuphea species rich in capric acid, with the intent of crossing in lauric acid once an agronomically sound plant had been obtained. Current breeding lines are all progeny of *C. viscosissima* × *C. lanceolata* f. *silenoides*, which are diploid with six chromosomes ( $n = 6$ ) and rich in capric acid. Attempts have been made in the past to create high lauric acid accumulating cuphea types, however, much of this early work was on non-adapted cuphea species. Koehne (1903) described five cuphea interspecific hybrids that were documented from herbarium samples in Europe. Röbbelen and Hirsinger (1982) observed spontaneous outcrossing among



species in their collection and were able to produce colchicine-induced hybrids of several cuphea species. However, the only successful crosses were between species with similar fatty acid profiles. Lorey and Röbbelen (1984) were also able to create hybrids, but lacked any success in altering fatty acid content. More recently, Ray et al. (1988) developed 18 interspecific hybrids between eight different species by hand emasculation and controlled pollination. They are the first to report a successful cross between a capric and lauric acid accumulating cuphea species. Unfortunately, the crosses between *C. leptopoda* and *C. llaminuigera* were conducted on species not suitable for commercial production in the US. These results demonstrate the feasibility of developing a lauric acid accumulating interspecific hybrid using the capric species.

In 2001, a new cuphea breeding program was established at Western Illinois University (WIU) to address the introgression of lauric acid accumulation into the progeny lines for PSR23. The breeding program was initially supported by private industry and was highly encouraged to only employ traditional breeding methods for fear of losing certain commercial markets. This approach was supported by the promising results from Ray et al. (1988) which suggested a more traditional approach was warranted to help create an agronomically sound high lauric cuphea plant.

Cuphea species have a tremendous variation in chromosome numbers and ploidy levels (Graham and Cavalcanti 2001). The original PSR23 is an autogamous diploid with six chromosomes ( $n=6$ ). Selected wild accessions suitable for agronomic production are mostly diploid, but vary in number of chromosomes from 8 to 14. *C. lutea* ( $n=14$ ), *C. viscosissima* ( $n=6$ ), *C. toluicana* ( $n=12$ ), *C. wrightii* ( $n=22$ ), and *C. carthagenesis* ( $n=8$ ) are the autogamous species (Graham 1989a). *C. lutea* and *C. wrightii* are undoubtedly allotetraploids (Campbell 1987).

To help overcome the barriers in creating hybrids between the wild accessions and the PSR23 line, various breeding methods are being employed. Initial inter-specific crosses were conducted between PSR23 (PI 606544) and *C. lutea* accessions utilizing hand emasculation techniques and pollen mixing procedures as described by Ray et al. (1988) and Fehr (1987). Thirty two crosses were successful and were evaluated in the field in 2005. From these crosses, four progeny lines were selected for the 2006 growing season. Fatty acid profiles of these progeny indicated a slight increase in lauric acid (C12) from 2.9 to 4.9%, but a much larger increase in C14 (2.9–23.5%) and C16 (4.1–11.9%). Unfortunately, all four progeny lines were unstable and reverted to the original profiles.

To improve success of the interspecific crosses, seedlings of PSR23 were treated with a meiotic inhibitor, colchicine, to create colchiploids. The use of colchicine and other meiotic disruption chemicals have been shown to be successful in creating hybrids in cuphea and other species (Przybecki et al. 2001; NBRI 2003). Large scale plantings of PSR23 are mainly pollinated by resident bumblebees. The long floral tubes prevent honeybees from gaining access to the nectar. Commercial production of PSR23 will remain limited without solving the insect pollinator problem. It is hypothesized that by

increasing the ploidy level of the PSR23 not only will self-fertilization be increased (Barringer 2007), but the chances of developing interspecific crosses with the high lauric acid accessions would be improved. Successful ploidy level changes were identified by increased vigor and cytologically confirmed using pollen mother cell analysis techniques as described by Ray et al. (1989). Confirmed polyploid PSR23 plants were found to be fertile and were later crossed with high lauric acid accumulating accessions of *C. lutea*, *C. toluhana*, and *C. carthagenesis* in 2006. Seeds collected from each cross were excised and germinated to avoid any seed dormancy issues (Mathias et al. 1990). S<sub>1</sub> progeny were evaluated under field conditions in 2007. Fatty acid profiles indicated once again a significant increase in myristic acid (C14) with no increase of lauric acid (C12). Selection criteria for the S<sub>2</sub> progeny will be for self-pollination and increased lauric acid content. Hybrids with the highest seed and oil yield will be further selected to improve seed weight and total yield in subsequent years.

Along with developing lauric acid accumulating PSR23 progenies, the WIU program continually focuses on developing new varieties utilizing mass and recurrent selection for improving agronomic traits including increased vigor, stem rigidity, drought tolerance, and self-pollination.

### ***19.5.2 Insect Resistance***

With cuphea production limited in the US to the Midwest and upper Midwest, the adaptability of cuphea to the current crop rotation and production protocols of the region play a critical role in the success of growing this new crop. Proposing cuphea as a new broadleaf crop may provide an undesirable habitat for corn, soybean, and wheat pests. Research conducted by the USDA-ARS recently investigated the potential of utilizing cuphea in crop rotations to provide cultural control of Western corn rootworm beetles (*Diabrotica virgifera virgifera* LeConte). It was hypothesized that cuphea, because of its sticky surface, would reduce or prevent oviposition in these fields. Unfortunately after an intensive 4 year study with seven rotation programs, it was concluded that a crop rotation with cuphea would not provide any consistent, economical cultural control of corn rootworm (Behle and Isbell 2005).

From 2000–2005, no major insect pests were recorded as feeding on cuphea vegetation. The only known insect pests were flea beetles (*Altica* spp.) which could cause early season damage to young seedlings if not controlled. During the 2006 growing season, elevated corn earworm (*Helicoverpa zea* Boddie) populations were experienced in much of the Midwest including cuphea production research sites in central and west-central Illinois. Corn earworm larvae were observed feeding on cuphea leaves during August and early September and feeding shifted to flowers and seed pods as larvae counts increased through September. By crop harvest time in October, seed losses to corn earworm in IL were estimated at 90%, while sites elsewhere in the state experienced more than

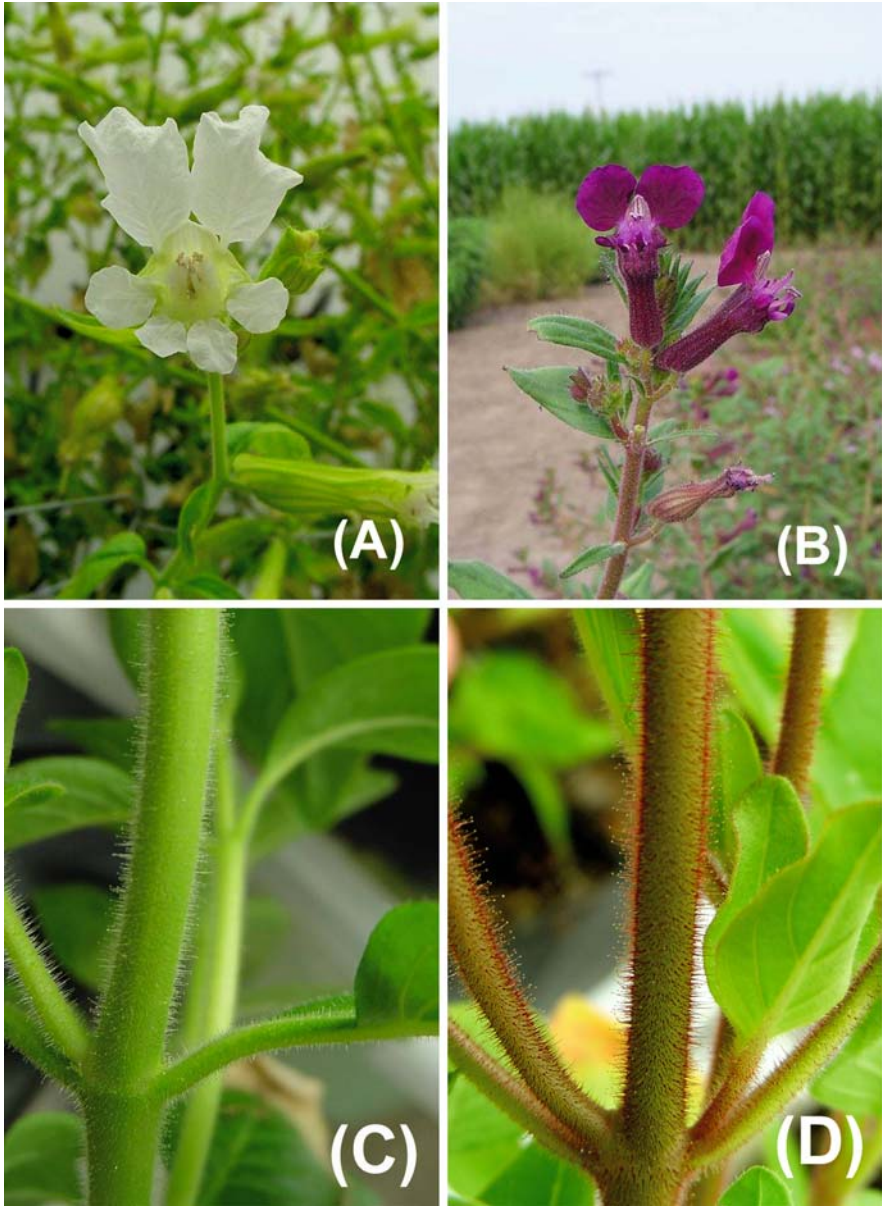
50% seed loss. Minor losses were reported in Iowa, with only occasional sightings occurring in North Dakota, and no presence was reported in Minnesota. Effective insecticides are available, but generally require repeated applications and entail substantial monetary and environmental costs. Use of resistant crop varieties is generally acknowledged as the best core strategy for avoidance of corn earworm damage.

Evaluation plots of novel cuphea genotypes located adjacent to production fields in IL displayed a wide range of severity and timing of feeding losses; suggesting that genetic sources of elevated corn earworm feeding resistance may be available. A 2007 breeding project evaluated forty accessions representing 16 different species of cuphea for reduced larval feeding. The accessions were selected based on their agronomic potential in the Midwest, self-pollination, and diverse sticky hair structures. Preliminary results indicate larval insects have a preference for certain cuphea species and that several species demonstrated no larval feeding damage throughout the entire growing season. It is believed that the variations in the sticky hairs are contributing to the effective defense against insect pests. Aphids and many other insects are typically immobilized by the sticky hairs. A breeding program is already initiated to cross the identified resistant accessions with the original PSR23 line. In contrast, non-sticky mutants have been reported for *C. lanceolata* (Hirsinger 1980; Hirsinger and Röbbelen 1980; Knapp 1993). Although a non-sticky trait might prove useful in aiding harvest, the role of sticky hairs as a defense mechanism certainly outweighs the convenience in handling the crop.

### **19.5.3 Anthocyanin Mutants**

During the summer of 2004, variations in flower pigments were noticed in PSR23 production fields in both MN and IL. PSR23 is characterized by deep purple flowers with red pigmented stems. Several novel plants were collected including a completely all white flower and variations of pink flowers.

The all white flower phenotype is devoid of any anthocyanin or red pigments throughout the entire plant (Fig. 19.3). Even under cold and nutrient stress conditions, the all white phenotype will remain. Named as ‘Snowflake’ in IL and ‘Blizzard’ in MN, both all-white lines are exhibiting inbreeding depression. After five self-pollinated generations in a growth chamber, the anthocyaninless phenotype appears to be stable. However, this line clearly demonstrates diminished vigor and seed set after each cross. When grown under field conditions, Snowflake performs well early in the growing season but soon collapses under environmental stress. Anthocyanins have been shown to serve as antioxidants and enable plants to better deal with environmental stresses (Chalker 1999). Efforts are currently under way to backcross Snowflake to the original PSR23 to develop an all white flower plant with anthocyanin production limited to the vegetative tissue. The all white flower is not only



‘Snowflake’

‘PSR23’

**Fig. 19.3** Comparison of the all-white ‘Snowflake’ breeding line flower (A) and stem (C) to the anthocyanin rich ‘PSR23’ breeding line flower (B) and stem (D)

unique for ornamental applications but also appears to have improved seed retention and produces seed coats devoid of anthocyanin pigments. This could potentially play a role in developing seed oils with less pigment contamination, thus diminishing production costs.

## 19.6 Breeding Methods

### 19.6.1 Genetic Engineering

As with the development of any new crop, the time required to domesticate and develop new varieties utilizing traditional methods is extremely slow. Several research programs have investigated developing tissue culture methodologies for propagation and perhaps engineering new traits. Early work focused on explant propagation methodologies for *C. wrightii* (Janick and Whipkey 1986) and *C. tolucana* (Przybecki et al. 2001). Other studies began screening a wider variety of species for the potential of utilizing engineering techniques (Millam et al. 1997). However, it is currently unrealistic to pursue genetically engineering cuphea for large scale agronomic production. The most likely arena cuphea can serve is as a rich source of genes encoding enzymes specialized for seed-specific synthesis of short- and medium-chain fatty acids (Filichkin et al. 2006). If cuphea species fail to be commercialized, they can still potentially provide a diverse source of seed-specific genes for manipulating fatty acid content in other oilseed plants.

## 19.7 Concluding Remarks

Although the advancement of cuphea breeding has been slow, tremendous progress has been made in developing the basic understanding of cuphea physiology and agronomic production guidelines. The identification and development of new potential industrial products and biofuels from cuphea oils are also helping to increase the awareness of this unique oilseed crop. However, for cuphea to truly be successful as a new commercial crop grown on large scale volumes, continued industry support and the development of auto-fertile, non-shattering, and determinate plant lines are needed. I would certainly like to invite fellow breeders to take up the challenge and assist in advancing the breeding efforts to locate or create these much needed traits.

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