CHARACTERIZATION OF KENAF (*HIBISCUS CANNABINUS* L.) CULTIVARS IN SOUTH AFRICA

By

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who gave me the opportunity and trust to fulfil my dreams

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CHAPTER 1

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fibre plant native to east-central Africa where it has been grown for several thousand years for food and fibre (LeMahieu *et al*., 2003). According to Dempsey (1975), kenaf is a short-day, annual herbaceous plant cultivated for the soft bast fibre in its stem. Kenaf grows in tropical and temperate climates and thrives with abundant solar radiation and high rainfall. Under good conditions kenaf will grow to a height of five to six meters in six to eight months and produce up to 30 tonnes per hectare of dry stem material (Wood, 2003). Kenaf yields approximately three to five times as much fibre as southern pine (LeMahieu *et al*., 2003). Essentially, kenaf is a traditional, third world crop that is poised to be introduced as a new, annually renewable source of industrial fibre in the so-called developed economies (Taylor, 2003).

Kenaf is tolerant to most plant diseases. In general, anthracnose (*Colletotrichum hibisci*) is possibly the biggest potential disease problem. Nematodes are viewed in some areas as the most serious constraint to kenaf production. In cotton growing areas, the root-knot nematode/fusarium wilt complex is expected to limit yield potential of both crops. Potential insect problems could arise in the early stages of seedling emergence and development. However, the kenaf plant tolerates a fairly high population of chewing and sucking insects (LeMahieu *et al*., 2003).

The kenaf plant is composed of multiple useful components (e.g. stalks, leaves, and seeds) and within each of these plant components there are various usable portions (e.g. fibres and fibre strands, proteins, oils, and allelopathic chemicals). The combined attributes of these components provide ample potential product diversity to continue use and development of this crop (Webber and Bledsoe, 2002a).

Kenaf yields a soft fibre from the stem that is very similar to jute. Along with a closely related species called roselle (*Hibiscus sabdariffa* L.), the two species account for one-third of the world production of soft fibres used for packaging. Kenaf is rapidly replacing jute, because the crop has less intensive labour requirements, is cheaper to produce, may be grown on a wide range of soils under varied climatic conditions, and is not necessarily competitive with food crops. While kenaf is somewhat coarser than jute, it has greater tensile strength, is lighter in colour, and has greater resistance to moisture (Dempsey, 1975).

The traditional use of kenaf focuses on its fibre production, such as making ropes, sacs canvases, and carpets (Li, 1980). However, new applications of kenaf have recently been developed such as pulping and papermaking, board making, absorbents and potting media, filtration, textiles, and livestock feed. The commercial success of kenaf has important potential economic and environmental benefits in the areas of soil remediation, toxic waste cleanup, removal of oil spills on water, reduced chemical and energy use for paper production, greater recycled paper quality, reduced soil erosion due to wind and water, replacement or reduced use of fibreglass in industrial products, and the increased use of recycled plastics (Webber and Bledsoe, 2002a). The automotive industry uses the so-called biocomposites (made from kenaf bast fibre and resins) as replacement material for glass-reinforced plastic materials in the manufacture of car seats, door panels, boot trim, wheel arches and parcel shelves (Anonymous, 2005).

Kenaf seed yield edible oil that is used for first class cooking oil and margarine production. The seeds can also be used for cooking (flour) and lubrication, soap manufacture, linoleum, paints, and varnishes (LeMahieu *et al*., 2003).

The yield and composition of the stalks, leaves, and seeds can be affected by many factors including cultivar, planting date, photosensitivity, length of growing season, plant population, and plant maturity (Webber and Bledsoe, 2002a). Factors that also affect kenaf fibre yield include adaptability to the

area, rainfall, temperature, soil type and fertility, stem diameter, and plant height (Dempsey, 1975). Therefore it is necessary to understand the production factors that influence these plant components and their composition (Webber and Bledsoe, 2002a).

Identification of kenaf varieties is problematic and our understanding of genotypic characteristics and relationships between kenaf germplasm is limited, which significantly hinders their effective utilization and conservation. To date, the identification of a particular kenaf variety remains complex. Traditionally, varietal identification was based only on morphological and agronomical features, such as leaf shape, stem colour, and maturity. Since it is difficult to identify kenaf cultivars based entirely on these features, it is important to find an effective method to accurately identify kenaf varieties to meet our needs (Cheng *et al*., 2002).

Commercial kenaf was introduced to South Africa during the last five years. The Sustainable Projects Development Group (SPDG) of the UK chose South Africa for the establishment of a biocomposites project due to the excellent cultivating conditions of the crop in KwaZulu-Natal. To date, the principle site for kenaf cultivation has been Spain and South Africa will add another cropping season (per year) to their global operation. The project will not only benefit South African industry, but will also provide employment and development opportunities for rural entrepreneurs and the workforce in poor areas (Anonymous, 2005). Three cultivars are currently planted in the Winterton area, but nothing is known about other potential cultivars or stability of cultivars in the targeted production area.

Aim of this study

The aim of this study was to:

1. Determine the morphological and agronomical differences between selected kenaf varieties.
2. Compare the oil content and fatty acid composition of the kenaf varieties.

3. Employ the Amplified Fragment Length Polymorphism (AFLP) fingerprinting technique to identify and determine genetic relationships between different varieties.
4. Determine the genotype x environment interaction of nine cultivars in the targeted production area.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin, history, and production

Kenaf (*Hibiscus cannabinus* L.) is thought to have originated in sub-Saharan Africa with evidence of its domestication around 4000 BC in the Sudan region (Dempsey, 1975). Cytological data from Menzel and Wilson (1964) indicated that kenaf is of African origin. They further suggested that the Angola region might have been the centre of dispersal if not the centre of origin.

Although kenaf has been grown for several thousand years in Africa, where the fibre has been utilized for handicraft purposes and the leaves for food, kenaf was not cultivated commercially prior to World War II except in Asia, where moderate amounts of fibre were produced in India, the USSR, and in Mainland China for sacking manufacture (Dempsey, 1975).

Since 1942 many countries have begun research studies on substitute fibres. Kenaf has received the greatest attention because of its greater adaptability and ease of handling than allied fibre crops. Within the past few years much research has been carried out on kenaf stems as a raw material for pulp and paper, and the leaves as a high-protein animal food (Dempsey, 1975).

Kenaf is commercially cultivated in more than 20 countries, particularly in India, China, Thailand, and Vietnam as an important crop (FAO, 1998). China, India, and Thailand account for 90 percent of the global area sown to kenaf and more than 95 percent of global production (FAO, 2003). Other important production areas include Russia, Mozambique, Iran, Taiwan, El Salvador, Gautemala, Dahomey, Ivory Coast, and Nigeria (Dempsey, 1975). Kenaf is also planted in Africa, Latin America and some other countries of Asia. Table 2.1 shows world kenaf production over the last decade (FAO, 2003).

Table 2.1 Global production of kenaf (MT).

	1990-1992	1993-1995	1996	1997	1998	1999	2000	2001	2002
World	1093.1	869.0	753.9	811.2	545.4	452.5	412.5	433.7	426.4
Africa	10.2	11.6	13.9	14.2	13.8	14.3	12.7	12.5	12.4
Latin America	33.4	32.5	31.0	28.8	27.1	25.4	24.1	23.7	26.7
Near East	6.4	4.9	5.1	5.2	4.2	3.7	3.6	3.6	3.6
Far East	1043.1	819.9	703.9	763.0	500.3	409.1	372.1	393.9	383.7
China	619.3	465.9	364.9	429.5	248.0	164.0	126.0	136.0	130.0
India	227.6	199.8	210.4	198.7	182.2	198.2	198.0	203.4	202.1
Thailand	159.5	128.1	109.3	106.4	47.2	29.7	29.6	29.5	30.0
Vietnam	24.9	17.0	15.0	22.3	14.6	9.4	11.3	14.6	14.6

2.2 Taxonomical and botanical aspects

The name “kenaf” is of Persian origin and is used to signify both the tall annual plant (*Hibiscus cannabinus* L.) with large showy flowers, characteristic of the Mallow family, and the bast fibre obtained from the stem of that plant (Crane and Acuna, 1945; Dempsey, 1975). Kenaf belongs to the Malvaceae, a family notable for both its economic and horticultural importance. The genus *Hibiscus* is widespread, comprising some 200 annual and perennial species. Kenaf is closely related to cotton (*Gossypium hirsutum* L.), okra (*Hibiscus esculentum* L.), and hollyhock (*Althaea rosea* L.). Kenaf, together with roselle (*Hibiscus sabdariffa* L.), are classified taxonomically in the *Fucaria* section of *Hibiscus*. This section includes between 40 and 50 species (distributed throughout the tropics) that are closely related morphologically (Dempsey, 1975; Taylor, 2003). Kenaf is sometimes also referred to as Bimly, Bimlipatum, Jute and Deccan Hemp (Duke and duCellier, 1993).

The chromosome number is a multiple of 18 in all the species that have been counted. Natural species have been found with chromosome numbers of 36 (kenaf and several other species), 72, 108, 144, and 180. One hybrid between two species had 216 chromosomes (Wilson, 2003).

A technique called genome analysis (which involves determining chromosome pairing in the immature pollen of hybrids between species) has been used to determine the relationships between many of the species. For example, the hybrid between kenaf, with 36 chromosomes, and roselle with 72 chromosomes, showed 18 paired and 18 unpaired chromosomes. The conclusions from this result were that kenaf and roselle shared a common set of chromosomes (or a genome) and roselle had an additional, uncommon set. Using this method, it has been determined that there are as many as three different genomes in this group of plants (Wilson, 2003).

The diversity in numbers of chromosomes and genomes found in the section *Fucaria* is not common in the plant kingdom. This chromosomal diversity is reflected in high levels of morphological and physiological diversity in the crop. This diversity represents a rich source of material potentially useful to kenaf breeders who strive to improve the crop (Wilson, 2003).

The kenaf plant is a woody to herbaceous annual, mostly unbranched and fast growing. It has prickly stems and grows up to 4.5 m tall. Two general leaf types are produced: divided and entire (Webber *et al*., 2002a). According to Jones *et al*. (1955), the divided leaf characteristic is dominant and the entire leaf shape is recessive. The leaves are alternate, long petiolate; shallowly to deeply parted, with three to seven toothed lobes. The plant produces large showy, light yellow to creamy coloured flowers that are bell-shaped and widely open. The flowers of all cultivars have a deep red or maroon coloured centre (Webber *et al*., 2002a). The flowers are solitary, large to 10 cm in diameter, short-stalked and auxiliary. They have five sepals, five petals and numerous stamens, which are connate. The ovary is superior. The flowers open and close in a single day. They are adapted both for cross- and self-pollination (Pate and Joyner, 1958), although cross-pollination is a consequence of insect activity (Jones *et al*., 1955). The fruit is a many-seeded (20-26 seeds), hairy capsule and is about 1.9-2.5 cm long and 1.3-1.9 cm in diameter. The seeds are brown, glabrous, wedge-shaped, 6 mm long, 4 mm wide and their weight is about 35,000 to 40,000 seeds/kg (Webber *et al*., 2002a). The plant has a deep-penetrating taproot with deep-seated laterals

(Anonymous, 2003a). Freshly harvested kenaf seed have a germination percentage of about 98%; however, because of their high oil content they lose viability rapidly under conditions of high humidity. Therefore, kenaf seed should be stored in airtight containers, under refrigeration or at least in an area of low humidity (Dempsey, 1975).

2.3 Morphological and agronomical characterization

Morphological characters have long been used by plant breeders to study variability, genetic variation patterns and correlations in populations, and accessions of plants. This method involves a lengthy survey of plant growth, which is costly, labour intensive, and vulnerable to environmental conditions (Pérez de la Vega, 1993). Morphological data are affected by environmental interactions and descriptions must be made with sufficient replication. Valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1988). Management practices and human interpretation also have a strong influence on these phenotypic expressions.

Siepe *et al*. (1997) evaluated genetic variability in a collection of *Hibiscus cannabinus* L. and other *Hibiscus* spp. Characters that have been evaluated for morphological and agronomical characteristics include: Days to flowering, distribution of flower pattern, leaf form, average basal stem diameter, plant height, plant useful height, first flower height, dehiscence boll grade, *Botrytis cinerea* Pers.: Fr. susceptibility, fresh biomass yield, and total dry matter. For all the characters that have been tested, a wide variability was observed. Cheng *et al.* (2002) found that the characters, such as middle stem diameter, whole stalk weight, and days to 50% flowering, vary significantly between kenaf varieties. Morphological differences in characters such as seed character, leaf shape, stem colour, flower colour, and plant maturity are small. Most of the kenaf accessions tested had red or green stems, yellow flowers and large seeds, entire- or palmate-leaves, and four maturity types were observed. It is, however, difficult to identify individual varieties merely based on morpho-agronomic characters but clear separation of kenaf varieties can

only be achieved through RAPD and AFLP fingerprinting analyses (Cheng *et al*., 2004).

2.4 Nutritional value of kenaf seed oil

Seed composition (Duke and duCellier, 1993):

Moisture content:	9.6%
Ash:	6.4%
Fatty oil:	20.4%
Palmitic acid:	19.1%
Oleic acid:	28.0%
Linoleic acid:	44.9%
Stearic acid:	6.0%
Alpha-linolenic acid:	0.5%
Nitrogenous matter:	21.4%
Saccharifriable matter:	15.7%
Crude fibre:	12.9%
Other matter:	13.9%

The bulk of fats and oils, whether for human consumption or for industrial purposes, is presently derived from plant sources. Improvements are being made with conventional crops as well as with selected plant species that have the ability to produce unique, desirable fats and oils (Mohamed *et al*., 1995).

About 20% of the volume of kenaf seed is oil, very similar in composition to that of cotton, but having the advantage of a milder odour and being free from the toxic phenolic pigment gossypol (Dempsey, 1975). It is comparable to most common edible oils and is excellent for human consumption. A comparison of the component acids of kenaf, cotton, olive, palm, sunflower, and soybean oil is shown in Table 2.2.

Table 2.2 A comparison of the component fatty acids (%) of kenaf, cotton, olive, palm, sunflower, and soybean oil (Gunstone *et al.*, 1986; Mohamed *et al.*, 1995; Salunkhe *et al.*, 1992).

	Kenaf	Cotton	Olive	Palm	Sunflower	Soybean
Total lipids	20.0-26.0	15.2-22.0	6.0-25.0	20.0-24.0	25.0-50.0	18.0-23.0
Saturated fatty acids						
Palmitic ($C_{16:0}$)	20.1	17.0-31.0	7.5-20.0	32.0-47.0	5.0-7.2	7.0-14.0
Stearic ($C_{18:0}$)	3.2	1.0-4.0	0.5-3.5	1.0-9.0	2.0-6.5	1.4-5.5
Unsaturated fatty acids						
Oleic ($C_{18:1}$)	29.2	13.0-44.0	53.0-86.0	40.0-53.0	14.7-36.7	19.0-30.0
Linoleic ($C_{18:2}$)	45.9	13.0-59.0	3.5-20.0	2.0-11.0	60.0-70.0	44.0-62.0
Linolenic ($C_{18:3}$)	0.7	0.1-2.1	0-1.5.0		<0.3	4.0-11.0

Kenaf oil is used for first class cooking and salad oil, margarine production and lubricant oil (Anonymous, 2003b; Duke, 2003). The oil is also used in the manufacture of soap, linoleum, paints and varnishes, and for illumination (Duke, 2003).

Palmitic, oleic, and linoleic acids were reported as major fatty acids in kenaf oil (Hopkins and Chrisholm, 1959; Mohamed *et al.*, 1995). Palmitic acid ($C_{16:0}$) is the dominant saturated fatty acid followed by stearic acid ($C_{18:0}$). Kenaf oil also contains a high percentage of polyunsaturated fatty acids (PUFA) and monones. Linoleic acid ($C_{18:2}$) is the dominant PUFA, followed by oleic acid ($C_{18:1}$). Linolenic acid ($C_{18:3}$) is present in minor amounts. A high concentration of linoleic and linolenic acids is undesirable in terms of oil stability because they are readily oxidized. The PUFA are essential fatty acids for normal growth and health. Furthermore, they are important for reducing cholesterol and heart diseases (Mohamed *et al.*, 1995).

Kenaf oil is also characterized by a high concentration of phospholipids (Mohamed *et al.*, 1995). Kenaf seed has a higher total phospholipid content than cotton and soybean seed oil (Gunstone *et al.*, 1986). Lysophosphatidyl

choline, phosphatidyl choline, sphingomyelin, phosphatidyl serine, phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl glycerol and cardiolipin were identified in kenaf seed (Mohamed *et al*., 1995). Lysophosphatidyl choline and phosphatidyl choline are known to be important in membrane properties such as synthesis of lipid bilayer and liposome formation. They are also useful as emulsifiers in food and pharmaceutical applications. In kenaf oil, phospholipids, particularly those with free amino groups, may interact as a natural antioxidant and consequently increase oil stability and shelf life (Mohamed *et al*., 1995).

The sterol percentage is reported to be similar to that for soybean and cottonseed oil (Gunstone *et al*., 1986; Mohamed *et al*., 1995). Three plant sterols have been identified in selected kenaf genotypes: β-sitosterol, campsterol, and stigmasterol (Mohamed *et al*., 1995).

The potential for mass production of oil as a by-product of kenaf appears to be excellent. The relatively high oil content, the unique fatty acid composition, and the reasonable amounts of phytosterols and phospholipids suggest that kenaf oil can be used as a source of edible oil. Although kenaf is mainly used for its fiber, the seeds, as a by-product, would provide oil and meal for feed and food. Such uses could significantly increase the economic value of this crop. In addition, kenaf seed can be a source of phospholipids for several industries. The variation among genotypes indicates a potential for genetic improvement in yield and quality of both oil and phospholipids (Mohamed *et al*., 1995).

2.5 Genetic identification based on AFLP fingerprinting

Cultivar identification can be achieved accurately using DNA fingerprinting data, especially in materials characterized by high levels of genetic variation between cultivars and no variation within (Nybom, 1994).

Over the past 10 years a number of DNA fingerprinting techniques have been developed to provide genetic markers capable of detecting differences among

DNA samples across a wide range of scales ranging from individual or clone discrimination to species level (Vos *et al.*, 1995).

Examples of available techniques are: restriction fragment length polymorphism (RFLPs), polymerase chain reaction (PCR) based random amplified polymorphic DNAs (RAPDs), microsatellites, and amplified fragment length polymorphism (AFLPs) (Thottappilly *et al.*, 2000).

RFLP analysis requires relatively large amounts of very pure DNA. Prior sequence information is necessary if PCR products are to be analysed. Although this technique is labour intensive and expensive (it generally uses isotopes), it is highly repeatable and produces many polymorphic bands (Thottappilly *et al.*, 2000).

RAPD is a simple, sensitive and relatively cheap PCR-based technique in comparison to RFLP (Thottappilly *et al.*, 2000). The DNA fragment patterns generated by this technique depend on the sequence of the primers and the nature of the template DNA. No prior sequence characterization of the target genome is needed and PCR is performed at low annealing temperatures to allow the primers to hybridize to multiple loci. Due to their sensitivity to template and reaction conditions, extraordinary care must be taken to ensure repeatability across multiple reactions. The need to repeat each PCR reaction multiple times and the inability to obtain identical banding patterns in different laboratories have limited the use of this technique (Blears *et al.*, 1998). RAPD markers were used to determine genetic diversity in kenaf varieties. RAPD analysis was an effective tool in identifying kenaf varieties and determining their genetic relationships, particularly when combined with the analysis of morpho-agronomic characters (Cheng *et al.*, 2002).

Microsatellite markers offer many advantages, but the high costs and time that are generally required for the development of primers specific for any given application, have limited their use in many laboratories (Blears *et al.*, 1998).

The AFLP technique is one of many DNA fingerprinting procedures that uses PCR analysis to amplify a limited set of DNA fragments from a specific DNA sample. The technique represents a combination of RFLP and PCR, resulting in highly informative fingerprints. The resemblance with the RFLP technique was the basis in choosing the name AFLP. In contrast to the RFLP technique, AFLPs will display the presence or absence of restriction fragments rather than length polymorphisms. The technique is robust and reliable because stringent reaction conditions are used for primer annealing: the reliability of the RFLP technique is combined with the power of the PCR technique (Vos *et al.*, 1995).

The AFLP technique can be used for DNA samples of any origin and complexity. Small sequence variations can be detected using only small quantities of genomic DNA (0.005-0.5 µg). The capacity to reveal many polymorphic bands in one lane is a major advantage of AFLP markers. The numerous bands on a gel are analyzed simultaneously making AFLP an extremely efficient technique. AFLP has the capacity to inspect a much greater number of loci for polymorphism than other currently available PCR-techniques, such that the number of polymorphisms detected per reaction is much higher. AFLP is superior in terms of the number of sequences amplified per reaction and their reproducibility. The markers produced are reliable and reproducible within and between laboratories, and are relatively easy and inexpensive to generate. A virtually unlimited number of markers can be generated by simply varying the restriction enzymes, and the nature and number of selective nucleotides (Blears *et al.*, 1998).

Since the AFLP technique can be applied to any DNA sample including human, animal, plant and microbial DNAs with no prior sequence information, this technique has the potential to become a universal DNA fingerprinting system (Blears *et al.*, 1998).

2.5.1 Basic steps of AFLP fingerprinting

The AFLP technique involves five steps:

a) Digestion of genomic DNA

Restriction fragments are generated using two restriction endonucleases: a "rare"-cutting enzyme with six- to eight-base recognition (*Eco*R I) and a "frequent"-cutting enzyme of four-base recognition (*Mse* I). Three types of restriction fragments are generated: 1) fragments cut by the rare-cutting enzyme on both ends, 2) fragments cut with the frequent-cutting enzyme on both ends, and 3) fragments that have been cut by both the rare-cutter and frequent-cutter. Using *Eco*R I and *Mse* I restriction enzymes, *Eco*R I- *Eco*R I, *Mse* I-*Mse* I and *Eco*R I-*Mse* I fragments would be produced during restriction enzyme digestion (Blears *et al*., 1998).

The frequent cutter will generate small DNA fragments, which will amplify well and are in the optimal size range for separation on sequence gels. The number of fragments to be amplified is reduced by using the rare-cutter, since only the rare-cutter/frequent-cutter fragments are amplified. This limits the number of selective nucleotides needed for selective amplification. The use of two restriction enzymes makes it possible to label one strand of the double stranded PCR products, which prevents the occurrence of "doublets" on the gels due to unequal mobility of the two strands of the amplified fragments. By using two different restriction enzymes the greatest flexibility in "tuning" the number of fragments to be amplified is found (Vos et *al.*, 1995).

In complex genomes, the number of restriction fragments that may be detected by AFLP is virtually unlimited. A single enzyme combination (a combination of a specific six-base and four-base restriction enzyme) will already permit the amplification of 100 000s of unique AFLP fragments, of which generally 50-100 are selected for each AFLP reaction (Vos *et al.*, 1995).

b) Ligation of oligonucleotide adapters

Double-stranded nucleotide adapters (10-30 base pairs long), complementary to the sticky ends of the corresponding restriction site, are ligated to the restriction fragments using T4 DNA ligase (Figure 2.1). The sequence of the adapters and the adjacent restriction half-site serve as primer binding sites for subsequent PCR amplification. Adapters are composed of two synthetic oligonucleotides that are in part complementary to each other and form a double-stranded structure in solution under appropriate conditions. Ligation does not restore the original restriction enzyme site because of a base change incorporated into the adapter sequence (Figure 2.1). This change in the recognition site prevents restriction from taking place after ligation has occurred, enabling restriction and ligation reactions to be performed in the same tube. With these reactions occurring simultaneously, any fragment-to-fragment product is restricted. In addition, adapter-to-adapter ligation is prevented by using nonphosphorylated adapters. Both of these features ensure that adapters are ligated to virtually all restriction fragments (Blears *et al.*, 1998).

Because primers with three selective bases tolerate a low level of mismatch amplification, a two-step amplification strategy was developed for AFLP fingerprinting of complex DNAs. The first PCR amplification, called preamplification, utilizes primers having a single or no selective nucleotide. The PCR products of the preamplification are diluted and used as template for the second amplification reaction using primers both having three selective nucleotides.

The two-step amplification strategy results in two important differences when compared to direct amplification: 1) back ground “smears” in the fingerprint patterns are reduced, and 2) fingerprints with particular primer combinations lacks one or more bands compared with fingerprints generated without preamplification. An additional advantage of the two-step amplification strategy is that it provides a virtually unlimited amount of template DNA for AFLP reactions (Vos *et al.*, 1995).

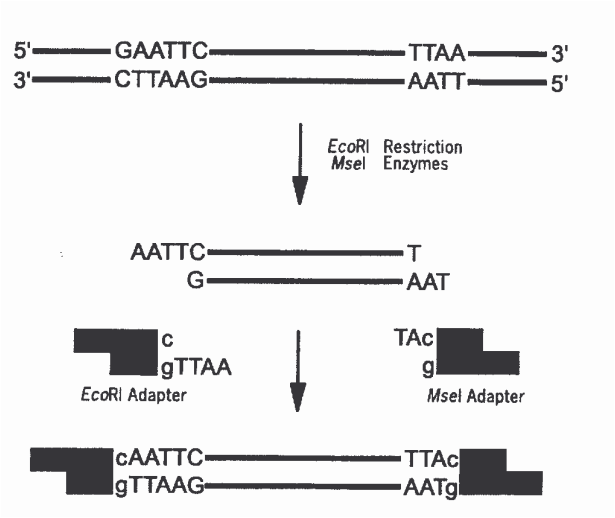

Fig. 2.1 A schematic outlining of the ligation of adapters to the ends of a restriction fragment. Genomic DNA is first restricted by *Eco*R I and *Mse* I. Double-stranded adapters, complimentary to the short single-strand extension generated by the restriction enzymes, are ligated to the DNA fragment. The *Eco*R I and *Mse* I recognition sites are not restored by ligation because of a base change in the adapter sequence shown in lower case) (Blears *et al.*, 1998).

c) Preselective amplification

Primers used in this step consist of a core sequence, the restriction site sequence and a selective single-base extension at the 3'-end. The sequences of the adapters and restriction sites serve as primer-binding sites for preselective PCR amplification. Each preselective primer has a selective nucleotide that will recognize the subset of restriction fragments having the matching nucleotide downstream from the restriction site. The primary products of the preselective PCR are those fragments having one *Mse* I cut and one *Eco*R I cut, and also having the matching internal nucleotide. This results in a 16-fold decrease in the complexity of the restriction-ligation products (Blears *et al.*, 1998).

d) Selective amplification

AFLP primers for the selective amplification contain three types of DNA sequences: the 5' region complimentary to the adapter, the restriction site sequence and two additional 3' selective nucleotides. Selective primers are either radiolabelled or fluorescently labelled. One primer is complimentary to the adapter and adjacent rare-cutter restriction site sequence with three

selective nucleotides at the 3'-end (e.g. *Eco*R I primer 3'-XXX, where X denotes the selective nucleotides), and the second primer is complimentary to the adapter and frequent-cutter recognition site sequence with a three-base extension (e.g. *Mse* I primer 3'-XXX). As the number of selective nucleotides is increased, the complexity of the DNA fingerprint decreases. The number of amplified fragments is reduced approximately four-fold with each additional selective base. From the huge number of fragments generated by the two restriction enzymes, only a subset of the template fragments, with complementary nucleotides at all three positions will be amplified under stringent annealing conditions (Blears *et al.*, 1998).

e) Gel-based analysis of the amplified fragments

Labelled fragments are resolved by gel electrophoresis on a Perkin-Elmer/Applied Biosystems Inc. automated sequencer. Only the *Eco*R I primer is labelled and therefore only the *Eco*R I-site containing strands will be labelled and detected. This ensures unambiguous detection of the single strand amplified fragments in denaturing gels by eliminating doublets.

The GeneScan software analyses four different fluorescent labels that are visualized as blue, green, yellow and red. Multiple samples (amplified with separate primer sets, each labelled with a fluorescent dye) can be loaded in a single gel lane along with an internal DNA size standard (also labelled). Such "multiplexing" reduces the cost of the analysis.

The GeneScan results are displayed as a reconstructed gel image, electropherograms, or tabular data. GeneScan results can be imported into the Genotyper programme for subsequent data analysis. This software identifies and measures bands ranging in size from 50 to 500 base pairs. The bands (alleles) are scored as present/absent, and a binary matrix is constructed. The matrix is then analysed using phenetic methods such as unweighted pair-group method using arithmetic averages (UPGMA) and cluster analysis. This technique provides numerous informative bands and can be accurately sized using fluorochrome-labelled primers and an automated sequencing gel scanner for electrophoresis and data analysis.

2.6 Agronomy

2.6.1 Adaptation and climate

Kenaf plant has a wider range of adaptation to climate than other fibre plants grown for commercial use. It is found growing wild in Africa from the Equator to a limit of latitude 30°N and 30°S and at altitudes up to 1.250 m. In some major kenaf growing areas, kenaf grows in a latitude range of 16°S to 41°N with a mean relative humidity range of 68-82%. The mean growing temperature during the season ranges from 22.6°C to 30.3°C. Kenaf is frost sensitive and therefore the growth cycle must end before the first frost occurs. The mean rainfall per month during the growing season ranges from 100-329 mm (Dempsey, 1975), but 500-625 mm over a period of 5 to 6 months is essential for the successful production of kenaf fibre (Crane, 1947). During the growing season, a well-distributed rainfall of 100-125 mm per month is necessary for proper kenaf growth (Dempsey, 1975).

2.6.2 Soil

Kenaf grows well and will produce high fibre yields on the following soil types: acid peats, alluvial and colluvial silty loams, sandy loams, sandy clay loams, clay loams, alkaline and saline desert soils, latasols, and many other soils. Good soil drainage is, however, highly required (Dempsey, 1975). Prolonged periods of standing water, particularly during the seedling stage, can severely inhibit growth (LeMahieu *et al*., 2003). The plant will, however, tolerate flooding in the late stages of growth. Kenaf is better adapted to poor soils and soil alkalinity than jute. Because the soil origin, composition, and colour do not affect kenaf, the crop will grow on a wide range of soil types; however, there should not be limiting factors such as a trace deficiency, alkalinity, or a hard pan. Deep, fertile, and well drained soils are recommended (Dempsey, 1975).

2.6.3 Photoperiod

Most kenaf varieties photoperiodic, and are influenced by the length of daylight period. Regardless of the time of planting, kenaf remains vegetative until the daylight hours decrease approximately below 12.5 hours when flowering occurs (Crane *et al*., 1946; Dempsey, 1975). It is therefore important

that all plantings for fibre should be made early in the growing season to allow the crop to produce maximum growth before the critical daylight period is reached. When planted late, the plants will have short stems with poor fibre, but high seed yields (Dempsey, 1975).

2.6.4 Varieties and cultivars

Kenaf comprises of five basic varieties and eight agricultural types (Dempsey, 1975):

1) Variety “simplex”

Type 1: Stems purple; leaves entire with purple petioles.

2) Variety “virdis”

Type 2: Stems green; leaves entire with green petioles.

3) Variety “rubber”

Type 3: Stems red below, greenish above; leaves divided with green petioles.

4) Variety “purpureus”

Stems purple; leaves divided with purple petioles.

Type 4: Late; stems very tall and slender; leaves with narrow lobes of a diffused purple colour; petals purplish.

Type 5: Early; stems short and robust; leaves green with broad lobes.

5) Variety “vulgaris”

Stems green; leaves divided with green petioles.

Type 6: Plants very early.

Type 7: Plants late; seedlings with reddish stems.

Type 8: Plants late; seedlings with green stems.

The flowers of the above mentioned varieties have red or dark maroon throats. The most valuable varieties from an economic standpoint are “virdis” and “vulgaris”. These varieties were used to develop the high-fibre-yielding,

disease resistant strains. They have also been crossed to some degree with “simplex” and “purpureus” to produce hybrids that are late maturing or, in some cases, insensitive to photoperiod and possessing resistance along with excellent hybrid vigour. Under Philippine conditions the “virdis” and “vulgaris” varieties yielded the highest fibre percentages (Dempsey, 1975).

In general, kenaf varieties may be divided into three categories based upon maturing time under normal growing conditions. These include:

1) Ultra-early types: When grown at latitudes exceeding 37°N or 37°S, these types require 70-100 days to reach maturity. They have little resistance to diseases and are especially susceptible to *Fusarium* sp. Their fibre yield is small and their height seldom exceeds 2.0-2.5 m. They usually have high seed yields.

2) Early to medium-maturing types: They require 100-120 days to mature and will reach a height of 2.5-3.5 m under optimum conditions. Their dry fibre yield is highly variable and ranges from 1000 to 4000 kg/ha. The percentage of fibre in the green plant ranges from 3.5% to 7.5%. The improved medium-maturing types generally do not have high seed yields, unless they are planted late for seed production. Among these types are “Cubana”, “Cuba 108”, the Everglades series, the Indian types and other.

3) Late-maturing types: These hybrids are highly insensitive to photoperiod. They are obtained by crossing either “simplex” or “purpureus” varieties with the earlier-maturing “virdis” or “vulgaris” varieties. The late-maturing types require 140 days or more to reach maturity and during this extended vegetative period the plants may attain a height of 3.5-4.5 m. Their wet yield is generally high and the fibre yield ranges from good to excellent. The plants do not give high seed yields when they are grown over a long vegetative period, but they produce higher seed yields when planted late (Dempsey, 1975).

Commercial kenaf varieties include: Everglades 41, Everglades 71, Tainung 1, Tainung 2, Cuba 2032, SF 459, Gregg, and Dowling (Anonymous, 2004; Taylor, 2003). The photo insensitive Guatemala 4 variety can be obtained in small quantities. Tainung 2 is by far the most popular commercial kenaf variety to date. It has demonstrated superior raw fibre production in trial plots and commercial production across many different latitudes and growing conditions (Anonymous, 2004a).

2.6.5 Cultivation

a) Seedbed preparation

Thorough soil preparation is necessary for good plant growth and therefore early ploughing to a good depth should be done. Deep cross ploughing, followed by several diskings at intervals are usually desirable for thorough tillage, and to prevent weeds and grasses from germinating (Dempsey, 1975).

A final dragging or laddering should also be included to break up small soil lumps that might hinder uniform germination. Good soil preparation provides highly desirable soil aeration and permits the taproot and deep lateral roots to grow normally without restriction (Dempsey, 1975).

Irregular plant growth, plants that differ in height, and stems that are not uniform in diameter are caused by both improperly prepared new land and a moisture deficiency during the early growth stages. This results in irregular retting and inferior fibre (Dempsey, 1975).

b) Fertilization

The nutritional requirements of kenaf can be evaluated from the nutritional uptake of the crop based upon its average growth and yield at maturity. This may be determined by a chemical analysis of dry kenaf plant components. It is estimated that a kenaf crop of 50 MT green plants/ha would withdraw about 175 kg N, 15 kg P, 75 kg K, 105 kg Ca, and 30 kg Mn from the soil (Duke and duCellier, 1993).

Kenaf is a heavy feeder and is soil depleting. Soils may be treated with manure or green manure. Rotation with green manure crops is also recommended. Kenaf generally respond the greatest to nitrogen, followed by potassium and phosphorus. While kenaf is a heavy feeder of calcium, the crop seldom shows response to calcium applications on low-calcium-content soils. Kenaf will respond best to balanced N-P_2O_5-K_2O applications on typical loam soils. The recommended fertilizer application is about 35-70 kg/ha N, 40-60 kg/ha P_2O_5, and 45-65 kg/ha K_2O. For South African sandy soils, a ratio of 85-60-60 is preferred (Dempsey, 1975; Duke, 2003).

c) Time of planting

On rain-fed soils kenaf generally is planted at the onset of the rains. This permits sufficient time for the plants to remain reproductive so that the fibre content and thus yield increase. The time of planting is extremely important to the yield of retted fibre (Crane and Acuna, 1945; Dempsey, 1975). At higher latitudes (30° to 38°N), the soil temperatures may inhibit rapid and uniform germination if kenaf is planted too early. Growers should therefore have an intimate knowledge of the variety to be planted and its reaction to photoperiod at planting time (Dempsey, 1975).

Most kenaf plantings show a dramatic and progressive drop in plant height, stem diameter, green weight tonnage, and retted fibre yield when planted late, indicating the lack of time for proper development (Dempsey, 1975).

d) Seed rate and spacing

Seed is broadcasted or planted with a grain drill in rows with about 6-30 kg seed/ha drilled 15 cm by 15 cm on dry soils or 12.5 cm by 12.5 cm on wet soils. Often two seeds are drilled and one seedling is removed if germination has been good to ensure an even stand for the production of uniform stalks. The planting depth is 0.5-3.2 cm (Duke, 2003).

e) Plant population

Plant populations can have a tremendous effect on kenaf yield and plant composition. For most fibre production situations, final populations of 185 000

to 370 000 plants/ha are desirable for maximum stalk yields and the production of single stalk plants with little or no branching (Webber and Bledsoe, 2002a). Considerable branching occurs in low-density plantings, whereas in very dense stands many plants are small and tend to lodge badly near the end of the season (Crane and Acuna, 1945). As plant populations increase, the basal stem diameter decreases. (Higgins and White, 1970; Williams, 1966). Although basal stalk diameter may vary greatly within a given kenaf field, at satisfactory populations the average stalk diameters will be in the range of 1.9 to 3.8 cm (Webber and Bledsoe, 2002a).

2.6.6 Harvesting

a) Time of harvesting

The highest quality fibre is obtained when plants are harvested during the flowering period (Duke, 2003). The fibre content does not increase significantly after this time and it is easily separated from the wood by mechanical means (Anonymous, 2003b). The period between planting to harvest ranges from 90-125 days (Duke, 2003). If the kenaf drying and defoliation process is dependent on a killing frost, the harvest date will vary according to environmental conditions of the area, including time of the killing frost and time required for kenaf to dry. Higgins and White (1970) reported that stem yield from harvests that are made several weeks before frost would be markedly lower than those from harvests immediately before or after a killing frost. Furthermore, stem yields are gradually reduced because of loss of plants by lodging as harvest is delayed after frost. Actively growing kenaf can be cut and then allowed to dry in the field. Once dried, the kenaf can then be chopped, baled, or transported as full-length stalks (Webber *et al*., 2002b).

b) Harvest method

The harvest method depends on the production location, equipment availability, processing method, and final product use.

Hand harvesting and retting

Over the last 6000 years, since its first domestication, kenaf has consistently been hand-harvested for use as a cordage crop (rope, twine, and sackcloth).

The bast fibre strands, located in the kenaf bark, are the source of cordage products. When hand-harvested, the tall, cylindrical-shaped stalks were cut at or near ground level with a curved blade or machete. Hand-harvested plants were then prepared for the retting process (Dempsey, 1975).

Retting is the process usually involving moisture and bacteria or chemicals, to remove unwanted bark material from the kenaf fibre strands within the bark. Kenaf is retted by natural processes that use primarily aerobic (air loving) bacteria, unlike water-retting of flax, that is carried out primarily by anaerobic bacteria and various fungi. The whole kenaf stalk (bark and core still attached), or only the bark portions, are tied in bundles and placed in ponds, canals, or slow-moving streams to allow the bacteria to digest the plant material around the bark's fibre strands (Dempsey, 1975).

The plant material status prior to retting influences the water-retting efficiency for kenaf. Removing the upper, non-fibrous portion of the plant, prior to the retting process, increases the retting rate by decreasing the amount of leaf and plant material to be digested. Even if the upper portion of the plant is not removed, the retting process can be increased if plants are allowed to dry for 24 to 48 hours after harvesting to promote defoliation (Webber *et al.*, 2002b). Dempsey (1975) reported that when kenaf bark material is retted at its ideal temperature, 34°C, dry ribbons of bark took 70 hours to ret, compared to green, moist ribbons of bark that took 29 hours.

Although the natural water-retting (bacterial) process is still used throughout many portions of the world, newer chemical retting processes have been studied, developed, and implemented to produce fibres of greater chemical and physical uniformity (Dempsey, 1975; Chen *et al.*, 1995; Ramaswamy, 1999). Research has determined that hand-stripped green bark ribbons and mechanically separated bark material could be successfully retted chemically using 7% and 1% sodium hydroxide, respectively, to produce good textile quality fibres from kenaf (Ramaswamy, 1999).

Ribboners and decorticators

Ribboning is the process of removing the bark from the core material. The same process is also referred to as decorticating. The original objective of the ribboners/decorticators was to harvest the bark for its valuable bast fiber and discard the unwanted core material (Webber *et al*., 2002b).

Newer ribboners/decorticators have been developed specifically for the kenaf industry (Chen *et al*., 1995) or adapted from other fiber industries (hemp and jute). The newer equipment was built specifically for kenaf and actually intended to be an in-field harvest-separator. The advantages of these newer ribboner/decorticator harvesters over other types of kenaf harvesters, such as sugarcane-type or forage-type harvesters, include the ability to produce a cleaner separation between the bark and the core components, quicker drying of the separated components, and greater flexibility in determining the cutting length of the fibre strands (Chen *et al*., 1995).

Whole stalk harvesters

Following the successful evaluation of kenaf bast and core fibres as a combined cellulose fibre source for paper products (newsprint, bond paper, and corrugated liner board) in the 1950s and early 1960s, the development of whole stalk harvesters has taken two major approaches; sugarcane-type harvesters and forage-type harvesters. In both approaches, scientists and industry have concentrated on using or adapting existing equipment, rather than developing a totally unique kenaf harvester (Webber *et al*., 2002b).

Forage-type harvesters and baling equipment

Forage-type harvesting and baling systems have been widely evaluated for use in kenaf production, harvesting, and processing systems. It has been demonstrated that standard forage cutting, chopping, and baling equipment can be used for harvesting kenaf as either a forage or fiber crop (Webber and Bledsoe, 1993). Forage harvesters are generally used for the harvesting of kenaf, because of their high efficiency and low cost. However, forage harvesters cut kenaf stems into too short fragments (Kobayashi *et al*., 2003).

Sugarcane-type harvesters

The unmodified or slightly modified sugarcane harvesters cut kenaf stems to a length of 22 cm, regardless of the travelling speed and it can be used for harvesting from the flowering stage to the withering stage of kenaf (Kobayashi *et al*., 2003).

When harvesting kenaf for fibre use, moisture content and equipment availability are important considerations. Kenaf can be harvested for fibre when it is dead, due to a killing frost or herbicides, or when it is actively growing. The moisture content of actively growing plants at harvest is normally about 75%. When the moisture content of kenaf is lower than 50%, the separation of the fibres (without retting) becomes difficult (Chen and Pote, 2004). Dry standing kenaf can be cut, chopped, baled, or transported as full-length stalks (Webber *et al*., 2002b).

2.6.7 Fibre yield and quality

The kenaf plant contains moderately long fibres in its outer stem and short fibre in its core (Fig. 2.1). The outer stem (bark) fibres make up about 35-40% of the stem weight, and are 3-4 mm long and slender. The inner stem (core) contains the remaining 60-65% and these fibres are short, from 0.5-0.7 mm (Kaldor *et al*., 1990). The fibre content of kenaf bark is about 50-55%, increasing with plant population density, while the less valuable short fibres make up about 45-50% of the inner core (Roseberg, 1996).

The yields of kenaf fibre, as percentage retted fibre in the green plant and kilograms per hectare of retted fibre, are highly variable. The fibre content of fresh green stems is 5-6% and this equates to 18-22% of the dry weight. Dry yield on average is 1-2 tonnes fibre/ha, rising to 3-3.5 t/ha under favourable conditions (Anonymous, 2003b).

Many factors influence the specific fibre yield of a given area. These include varietal characteristics such as adaptability to the area, rainfall, temperature, soil type and fertility, plant spacing, time of planting, time of harvest, stem diameter, and plant height (Dempsey, 1975).

Fig. 2.2 Kenaf stalk with core and bark material.

Factors that influence the fibre quality include soil type, harvesting, and fibre processing (method of fibre extraction):

Soil type. Pate *et al*. (1954) reported that fibre quality was significantly affected by soil type. Fibre quality of plants grown on sand was better than that of plants grown on peat soil. However, under satisfactory levels of fertility, the soil type did not significantly influence fibre yield.

Harvesting. Crane *et al.* (1946) reported that harvesting should be done at the time of blossoming to obtain the best fibre quality. Frost kill may not be the appropriate method for harvesting kenaf for quality fibres, because frost kill is often associated with fungal growth or rot that may affect fibre quality (Ramaswamy *et al*., 1999).

Retting and fibre processing. Fibre processing also affects the quality and chemical composition of fibres (Ramaswamy *et al*., 1999). The quality of fibre produced by field retting was inferior to that produced by tank retting or mechanical decortication (Pate *et al*., 1954).

Kenaf varied widely in fibre percentage in the green plant of the same variety grown under similar conditions in the same area over a period of years. However, the variation did not greatly influence fibre yield per hectare, which

may remain fairly constant when the plant is grown in the same area (Dempsey, 1975).

Kenaf varieties vary widely in the fibre percentage in the green plant. In the green plant, the fibre of better kenaf varieties varies from 4.0-5.0% (Dempsey, 1975).

In most countries yields are variable depending on the grower and the growing area. The retted fibre yield may range from less than one to more than three MT/ha (Dempsey, 1975).

The following characteristics are criteria used to determine fibre quality (Ramaswamy and Boyd, 1994):

- Reed length
- Bundle breaking tenacity
- Elongation at break
- Colour and lustre
- Gum content

Reed length is the total length from the base to tip of the decorticated kenaf stalk before and after processing. This criterion may be important for fibre yield, and when the intended use is products such as ropes and cordage (Ramaswamy and Boyd, 1994).

Bundle breaking tenacity is defined as the load required to break a fibre bundle of fixed length and weight. The flat bundle method is believed to be a good indicator of yarn strength and has a high correlation with yarn quality index. Bundle breaking tenacity, as a measure of fibre quality could provide quick, accurate results depending on linear density of the bundle. It establishes the possibility of extracting fibres for large-scale production of fibres (Ramaswamy and Boyd, 1994).

Elongation at break is the amount of stretch of a fibre bundle at break and is an important measure to indicate the ability to stretch (Ramaswamy and Boyd, 1994).

Colour and lustre are important properties depending on the fibre end use; lustre is positively correlated with strength (Ramaswamy and Boyd, 1994).

Gum content refers to the total wax, oil, lignin, and other hemicellulosic material. Residual gum content, the amount of gum left after processing, affects the fineness of fibres, which ultimately determines the success of using these fibres in a fine, woven textile structure. Since fibre quality also depends on the molecular structure, the effect of gum on crystallinity of fibres needs to be evaluated (Ramaswamy and Boyd, 1994).

Pate *et al*. (1954) measured four fibre quality factors of kenaf varieties:

- Tensile strength
- Knot strength
- Abrasion resistance
- Flexual endurance

For brevity, these are referred to as strength, shear, wear and flex, respectively. The four varieties studied did not differ significantly in fibre quality (Pate *et al*., 1954).

Fibre processing and characterization research has significant implications. It establishes criteria for selection and improvement of kenaf varieties for breeders and growers because breeders usually need to establish quality within a single plant (Ramaswamy and Boyd, 1994).

2.7 Genotype by environment interactions and stability statistics in cultivar assessment programmes

2.7.1 Concepts and importance

Successful cultivars need to possess high performance for yield and other essential agronomic characters. Besides, their superiority should be reliable over a wide range of environmental conditions. The basic cause for differences in yield stability between genotypes is a wide occurrence of genotype by environment (G x E) interactions. Such phenotypic stability is often used to refer to fluctuations in yield across environments. In other words, genotype by environmental interaction is a differential genotypic expression across environments. Genotypes refer to the set of genes possessed by individuals that are important for the expression of traits under investigation. The environment is usually defined as all non-genetic factors that influence the expression of traits. It may include all sets of biophysical factors like water, nutrition, temperature, disease etc. that influence the growth and development of individuals and thereby influence the expression of traits (Basford and Cooper, 1998).

According to Romagosa and Fox (1993), G x E interaction reduces association between phenotypic and genotypic values, and may cause selections from one environment to perform poorly in another, forcing plant breeders to examine genotypic adaptation. Its measurement is also important to determine an optimum breeding strategy for releasing genotypes with adequate adaptation to targeted environments. It is particularly relevant for countries that have very diversified agro-ecologies. Under such conditions breeders should be able to select desirable cultivars without losing valuable germplasm and other vital resources. Hence, agro-ecological diversity could complicate breeding and testing of improved varieties with adequate adaptation, but it could also permit identification of extreme environmental conditions that might offer selection pressure from different stresses (Romagosa and Fox, 1993).

The knowledge of G x E interaction can help to reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites and by fine tuning the programmes (Shafii *et al*., 1992; Kang and Magari, 1996). The presence of a large G x E interaction may necessitate establishment of additional testing sites, thus increasing the cost of developing commercially important varieties. Thus, G x E interaction relates to sustainable agriculture as it affects efficiency of breeding programmes and allocation of limited resources (Shafii *et al*., 1992). According to Kang and Magari (1996), G x E interaction is a major concern in plant breeding since it can reduce progress from selection and it may make cultivar recommendation difficult as it is statistically impossible to interpret the main effects. G x E interaction occurs in both short-term (less than five years testing at a location) and long-term (several years at various locations) crop performance trials.

G x E interaction is considered quantitative (Baker, 1988) if the ranking of genotypes does not change from one environment to another (i.e. non-crossover). Qualitative interactions (crossover) complicate selection and identification of superior cultivars. For variety trials, which are conducted at the same locations (L) and genotypes over years (Y), G x E analysis of variance may be partitioned into components due to G x L, G x Y and G x L x Y. If G x L is the important portion of the G x E, then the specific adaptation is exploitable by sub-dividing the regions into homogenous sites that minimise G x E within regions. When G x Y and G x L x Y values dominate, no simplification to sub-divide the testing sites are required (Baker, 1988).

In general, the common variety testing strategy is to test over a representative range of environments. Therefore, breeders aim to cover a representative sample of spatial and temporal variation. Accumulation of tolerances to a number of stresses is the key to wide adaptation and consequently selection in multiple environments is the best way to breed stable genotypes (Eisemann, 1981; Getinet and Balcha, 1989; Romagosa and Fox, 1993). These authors indicated that the success of wheat breeding in combining high yield potential and wide adaptation involved large numbers of crosses, testing advanced lines internationally, and continuous alternating selection cycles in various

environments. These environments, which differed in altitude, latitude, photoperiod, temperature, rainfall, soil-type, and disease situations allowed the expression of high yield potential. Choice of selection sites is particularly relevant in case of production areas with variable levels of abiotic stress. Research stations can be adjusted based on the study of genetic correlations between breeding sites and an extensive, and more commercially representative network of recommendation trials (Romagosa and Fox, 1993).

Different concepts and definitions of stability have been developed and applied to crop breeding programmes and evaluation of yield trials (Lin *et al*., 1986; Becker and Leon, 1988; DeLacy *et al*., 1996). According to Becker and Leon (1988), two different concepts of stability exist, the static and dynamic. Both concepts were said to be useful although their application depended on the traits under consideration. According to the former concept, stable genotypes possess unchanged or constant performances regardless of any variation of environmental conditions. That means its variance among environments is zero. In contrast, dynamic concept allows a predictable response to environments and a stable genotype has no deviation from this response to environments. The interest of most plant breeders in this regard is to develop well-buffered cultivars. The term stability, thus, refers to the character of a crop that withstands fluctuations of environments. Most breeders are interested in developing cultivars that are stable across a range of environments. In this case, environment refers to locations, years or the combination of both. In earlier years, one of the major concerns of agricultural research was to develop high yielding crop cultivars. Lately, however, stable and sustainable yields under varying environmental conditions have been gaining importance over increased yields. Stable yields are the key to sustainable food production. Farmers are basically interested in a constantly superior performance of cultivars on their own farms, specifically adapted to their conditions and needs, and which have a high degree of stability over time (Ceccarelli, 1989). Response to selection is maximised when selection is conducted in the environment where the future varieties will be grown (Becker and Leon, 1988).

DeLacy *et al*. (1996) indicated that many statistical methods have been developed for the analysis of G x E interactions. Nevertheless, better methods that more effectively describe the data for predicting performance to selection (i.e. optimising selection among genotypes) are of greater interest to the breeders. In fact, each analytical alternative seems to have some merit and thus looking into their inter-relationships appears to be a sound approach.

2.7.2 Broad versus specific adaptation of genotypes

Generally, the larger the relative size of the interaction components, the more complex the problem of identifying broadly adapted genotypes. Distinguishing and identifying repeatable and non-repeatable interactions (Jalaluddin and Harrison, 1993) is very important. If the interaction is repeatable, specific adaptation strategies should be followed; non-repeatable interactions need to be accommodated by selection for broad adaptation (Basford and Cooper, 1998). According to Romagosa and Fox (1993), if the agronomic stability (well yielding in productive and potential environment) of a genotype prevails over a wide range of environments, it is referred to as having general or wide adaptation. On the contrary, if this manifests over a limited range, that genotype has specific or narrow adaptation.

2.7.3 Analytical approaches to measure stability of genotypes

Lin *et al*., (1986) have reviewed and classified basic stability parameters into three types. Type one stability is analogous to homeostasis where a genotype is stable if its among-environment variance is small. It is based on deviations from the average cultivar effect whereas in type two, a genotype is considered stable if its response to environment is parallel to the mean response of all genotypes in the trial. The type three stability parameters are derived from the regressions on the environmental index and are measured by the residual mean squares from the regression model. Several authors (Lin *et al*., 1986; Westcott, 1986; Shafii *et al*., 1992) agree that all three concepts have problems in interpretations and usefulness to the breeders.

Type one is often associated with poor response and low yield in environments that are high yielding for other cultivars while type two is highly dependent on

cultivars involved in the test which is subsequently used as the environment index although it does not necessarily represent the actual environmental factors. Likewise, type three is generated from regression on environmental index and measures stability due to unpredictable or uncontrollable factors that may not be valid (Lin *et al*., 1986). Nevertheless, the interpretations and statistics of Eberhart and Russel (1966), that involve both type two and three parameters, are commonly used in studies of many crops (Lin *et al*., 1986; Westcott, 1986; Becker and Leon, 1988; Romagosa and Fox, 1993).

Becker and Leon (1988) have suggested two different approaches to assess stability. The first was the static, which Lin *et al*. (1986) named as Type 1 statistics. On the other hand, according to the dynamic concept (Type 2 statistics of Lin *et al*., 1986), a genotype is regarded as stable if its performance in different environments is close to what can be expected from the potentials of those environments. Maximum stability occurs if the difference between the yield of a genotype and the environmental index (mean of all tested genotypes) is constant across environments. If this difference is not the same in all environments, that genotype is said to interact with environments. Hence, if a breeder prefers the dynamic concept, the goal of breeding stable genotypes may be translated as the goal of minimising G x E interactions. The dynamic approach regards interactions as random unpredictable fluctuations or noise (Becker and Leon, 1988). However, sometimes one may be interested to further analyse the interactions and extract predictable information from it. This leads to the regression approach, which was first suggested by Yates and Cochran (1938) and further elaborated by Finlay and Wilkinson (1963), Eberhart and Russel (1966) and Perkins and Jinks (1968).

According to Romagosa and Fox (1993), there are two major approaches for studying G x E interaction and adaptation. The first is the parametric (empirical and statistical one) approach, which is more common and involves relating observed genotypic responses, in terms of yield, to a sample of environmental conditions. The second is the non-parametric (analytical clustering) approach, which defines environments and phenotypes in terms of biotic and abiotic factors. In practice, however, most breeding programmes

incorporate some elements of both approaches (Becker and Leon, 1988; Romagosa and Fox, 1993).

Recent developments comprise application of a multiplicative interaction model, which was first introduced by other biometericians, and has been introduced in the agricultural context as Additive Main Effects Multiplicative Interaction, AMMI (Piepho, 1996). These models are appropriate if one is interested in predicting genotypic yields in specific environments, for which yield trials are available. A further advantage of these models is that they may be used for modelling and understanding interaction. However, where there are sufficient funds and economic justifications are available to breed for a particular environment, stability becomes irrelevant and yield in that environment could be paramount. But if cultivars are being selected for a large group of environments, stability and mean yield across all environments are of major importance and yield for a specific environment is of less importance (Piepho, 1996).

Numerous methods have been proposed (Lin *et al*., 1986; Becker and Leon, 1988) to analyse G x E interactions or to estimate phenotypic stability and thereby to exploit positive outcomes. However, these authors indicated that parametric and non-parametric methods are the major statistical tools employed to study stability.

a) Parametric approach

According to Huehn (1996), the classical parametric stability statistics include ecovalence, environmental variance, regression coefficient, and sum of squared deviations from regression. Likewise, Lin *et al*. (1986) have described the following nine parametric stability statistics:

1. Environmental variance (variance of genotypes across environments);
2. Coefficient of variability (CV% of each genotype);
3. Mean variance (mean of estimated variance components of G x E for all pairs of genotypes);
4. Variance component for G x E interaction;
5. Wricke's (1962) ecovalence;

6. Shukla's (1972) stability variance;
7. Finlay and Wilkinson's (1963) regression coefficient;
8. Perkins and Jink's (1968) regression coefficient; and,
9. Eberhart and Russel's (1966) deviation parameter.

According to Becker and Leon (1988), the parametric approach gives only the individual aspects of stability but cannot provide an overall picture of the response. The basic reason for this difficulty is that a genotype's response to environments is multivariate, which the parametric approach tries to transform to a univariate problem via stability index. To escape this problem a different line of thought has emerged, namely to cluster genotypes according to their responsive structure (i.e. non-parametric method) (Becker and Leon, 1988).

Analysis of variance

In a conventional variety assessment trial in which the yield of G genotypes is measured in E environments over R replicates, the classic model to analyse the total yield variation contained in GER observations is the analysis of variance (Fisher, 1918; 1925, cited by Purchase, 1997). After removing the replicate effect when combining data, the G x E observations is partitioned into two sources: (1) additive main effects for genotypes and environments and (2) non-additive effects due to G x E interaction. The analysis of variance of the combined data expresses the observed (Y_{ij}) mean yield of the i^{th} genoype at the j^{th} environment as:

$$Y_{ij} = u + G_i + E_i + GE_{ij} + e_{ij}$$

Where u = overall mean, G_i, E_i and GE_{ij} represent the effect of the genotype, environment and genotype x environment interaction, respectively, and e_{ij} is the average of random errors associated with the r^{th} plot that receives the i^{th} genotype in the j^{th} environment. The non-additive interaction, GE_{ij} implies that an expected value of Y_{ij} depends not only on the levels of G and E separately, but also on the particular combination of levels of G and E (Purchase, 1997; Crossa, 1990). According to these authors, the most important limitation in the analysis of variance is that error variances over environments need to be

homogeneous to test for genotype differences. In other words, significant tests from a combined analysis of variance are valid if error terms from different environments are homogenous (Romagosa and Fox, 1993).

Cultivar superiority or performance measure (Lin and Binns, 1988), is the squares of the differences between an entry mean and the maximum mean at a location, summed and divided by twice the number of locations. Genotypes with the smallest values tend to have larger yields and are more stable than other genotypes. According to stability variance of Shukla (1972), however, the stability values are estimates of an entry's variance across environments and stable varieties have smaller estimates.

Ecovalence (W_i)

Wricke (1962) proposed using the G x E interaction effects for each genotype, squared and summed across all environments, as stability measure. As the ecovalence value increases, the genotype's contribution to the total G X E sum of squares also increases It was simpler to compute and more directly related to the G x E interactions than other statistics proposed by biometricians (Becker and Leon, 1988). According to these authors, ecovalence measures the contribution of a genotype to G x E interactions and a genotype with zero ecovalence is regarded as stable (i.e. low values indicate high ecovalences).

b) Non-parametric approach

This approach groups genotypes according to their similarity of response to a range of environments (Lin *et al*., 1986). According to Huehn (1990), non-parametric methods have the following advantages over parametric stability statistics:

- Reduction or avoidance of the bias caused by outliers.
- No assumptions are needed about the distribution of the phenotypic values.
- Stability parameters based on ranks are easy to use and to interpret.

- Additions or deletions of one or a few genotypes or another group of the material do not cause much variation of estimates unlike the parametric approach.
- For many applications (e.g. selection in breeding and testing programmes) the rank orders of the genotypes are the most essential information. Therefore, this method appears to be the appropriate method.

However, as suggested by Huehn (1990), for an efficient use of stability estimation techniques in practical applications, knowledge of the following aspects are essential: relations between different statistical measures of phenotypic stability (parametric and non-parametric); consistency of relationships among stability parameters; and repeatability of stability parameters. Lin *et al*. (1986) reported that the non-parametric or cluster method has two major sub-divisions, univariate and multivariate stability statistics. They further sub-divided univariate into four, viz. Euclidian distance, standardised distance, dissimilarity index and correlation coefficient.

According to Romagosa and Fox (1993), analysis of ranks (stratified ranking) evaluates the proportion of sites where any genotype ranks in the top, middle or bottom third of the entries. A genotype found in the top third of the entries across sites can be considered relatively well adapted. As indicated above, the advantages of non-parametric techniques include: freedom from assumptions concerning additivity of main effects, homogeneity of variances and linear response to increasing environmental yield potentials; insensitivity to error of measurements; and, measurements of adaptation are not unduly affected by genotypic performance in extreme environments. A genotype is considered stable if its ranking is relatively consistent across environments. Clustering environments or grouping genotypes is also possible. Similarly, Huehn (1990) has concluded the following from his investigations on non-parametric measures of stability.

- Corrected or transformed data should be used to perform analysis of phenotypic stability, if one wants to estimate the phenotypic stability independent from yield level effects.
- For quantitative estimation of phenotypic stability the non-parametric measure (mean rank difference) is preferable, as it is easy to calculate and interpret.
- If one is interested in a simultaneous consideration of both stability and yield the non-parametric stability parameter (sum of deviations) can be applied, measuring stability in units of yield by using the original non-corrected yield data.

c) Univariate stability statistics

Becker and Leon (1988) indicated that univariate stability statistics measure uncertainty in respective biometerical analysis. An overview of these most commonly used univariate stability parameters and their underlying stability concepts are presented in Table 2.3, as summarised by Becker and Leon (1988).

Univariate non-parametric stability statistics have also been proposed, based on rank orders of genotypes and do not need any assumptions about distribution of observed values or variance of homogeneity. These include the stability statistics such as mean rank difference and variance of ranks, which are based on corrected values (i.e. to make linear relationships). As mentioned in the above discussions, they provide a meaningful interpretation of results if ranks are based on corrected values. According to Becker and Leon (1988), they are distribution-free and no assumption on the distribution of values is necessary. As a result, they are said to be less sensitive to errors of measurements than the parametric statistics.

Rank differences (S1) and variances (S2) are non-parametric tests based on ranks of the genotypes across locations. They give equal weight to each location or environment. Genotypes with less change in rank are expected to be more stable. The S1 estimates are all possible pair wise rank differences

Table 2.3 Summary of univariate stability statistics (Becker and Leon, 1988).

Statistics	Symbol	Stability concept
Parametric:		
Environment variance	s^2_{xi}	static
Ecovalence	W_i	dynamic
Regression coefficient	b_i	Static / dynamic
Deviation mean square	s^2_{di}	dynamic
Coefficient of determination	r^2_i	dynamic
Non-parametric:		
Mean rank difference	S1	dynamic
Variance of ranks	S2	dynamic

across locations for each genotype. The S1 estimates are simply the variances of the ranks for each genotype across locations (Nassar and Huehn, 1987; Huehn, 1990). For S1, entries may be tested as significantly less stable or more stable than the average stability/instability. For S2, smaller estimates may indicate relative stability. Often, S2 has less power for detecting stability than S1. S1 may lose power when genotypes are similar in their interactions with the environments.

2.8 Characterization of kenaf cultivars in South Africa

Since kenaf is a new and unknown crop to South Africa and will in future be planted on commercial scale for fibre production, it was necessary to do an initial screening of available commercial cultivars. Cultivars needed to be evaluated for morphological and agronomic characters to determine differences between them. It is, however, difficult to identify kenaf cultivars based on these characters alone, but clear separation of cultivars can be achieved through DNA fingerprinting analysis. Since the AFLP technique was proved to be effective in identifying kenaf varieties (Cheng *et a*l., 2004), AFLP analysis was used to fingerprint and determine genetic relationships between commercial cultivars that will be planted in the Winterton area.

Kenaf seeds as a by-product, can potentially be used as a source of edible and industrial oil (Mohamed *et al*., 1995). Therefore, it was necessary to determine and compare oil yield and fatty acid composition of the seed oil of different commercial cultivars. The level of genetic variation for fatty acid composition was assessed with a view to improve oil quality through breeding programmes in South Africa.

Since kenaf will be planted commercially in South Africa, it was necessary to plant commercial trials in the Winterton area to determine the genotype x environment interactions. Three commercial cultivars are currently planted in Winterton, but further studies were needed to explore other potential high yielding cultivars that are stable in the targeted production areas.

CHAPTER 3

EVALUATION OF 10 KENAF (*H. CANNABINUS* L.) CULTIVARS ON AGRONOMIC AND MORPHOLOGICAL TRAITS

3.1 Introduction

Kenaf (*H. cannabinus* L.), an alternative fibre crop for paper pulp production, is normally grown during the entire summer growing season to maximize fibre production (Webber and Bledsoe, 2002b). Kenaf requires similar conditions to cotton for successful cultivation and matures in approximately 120-150 days from sowing. The critical factor is successful germination and establishment of a dense population of seedlings (Dempsey, 1975). Plant population has a tremendous effect on kenaf yield and plant composition. At low plant populations the crop produces plants with multiple branches with a greater percentage of leaves, rather than the more desirable single-stalk plants that favour mechanical harvesting. High plant populations result in shorter and thinner plants (Alexopoulou *et al*., 2000). Although basal stalk diameters may vary greatly within a given kenaf field, at satisfactory populations the average basal stalk diameters will be in the range of 1.9-3.8 cm (Webber and Bledsoe, 2002a).

As the plant is photoperiod sensitive and has little or no resistance to frost, sowing time is critical. Kenaf typically remains vegetative until daylight hours decrease to below approximately 12.5 hours when flowering occurs (Dempsey, 1975). Kenaf varieties can be divided into early (photosensitive) and late-maturing (day-neutral) varieties. Cultivars that belong to the early to medium maturity group includes “Everglades 41”, “Everglades 71”, “Tainung 2”, “SF 459”, and “Cuba 108” (Webber and Bledsoe, 2002a). Petrini *et al*. (1994) reported that most of the late-maturing varieties resulted in higher yields compared to the early ones. The growth rate is high until the flowering phase and thereafter it decreases gradually. Late-maturing varieties grow taller, exhibit higher growth rates, and develop larger stem diameters compared to the early-maturing ones (Alexopoulou *et al*., 2000).

Kenaf plants produce large, light yellow to creamy coloured flowers with deep red centres (Fig. 3.1). The flowers are bell-shaped and widely open (Webber *et al*., 2002a).

Kenaf plants produce two general leaf types, divided (Fig. 3.2) and entire (Fig. 3.3). The divided cultivars have deeply lobed leaves with three, five or seven lobes per leaf. Everglades 71, Tainung 2 and SF 459 are examples of divided leaf cultivars. The entire leaf cultivars (Everglades 41 and Cuba 108) produce leaves that are shallowly lobed, that are basically cordate (Webber *et al*., 2002a).

Kenaf was recently introduced to South Africa, and no screening of available germplasm has been done to date. The aim of these trials was to conduct an initial screening of 10 commercial kenaf cultivars in the Bloenfontein area, under glasshouse and field conditions, to observe agronomic and morphological differences between the cultivars.

Fig. 3.1 The kenaf flower.

Fig. 3.2 The divided leaf shape.

Fig. 3.3 The entire leaf shape.

3.2 Materials and methods

The study was conducted at the University of the Free State, in Bloemfontein, South Africa during the 2002/3 cropping season. Ten kenaf cultivars (Table 3.1) were used in this study. Seeds were obtained from the ARC Institute for Industrial Crops (Rustenburg, South Africa). All cultivars were planted in a randomised complete block with four replications in the glasshouse. They were planted in red soil (Bainsvlei soil type) in 8 ℓ pots with four seeds per pot. The plants were thinned to two plants per pot 21 days after planting. Fertilizer (6.5 N, 13.0 P, 7.0 K) was applied two weeks after emergence and thereafter every second week.

Plants were evaluated for eight agronomic traits: germination percentage of seed, plant height, basal stem diameter, branching, days to flowering, leaf shape, flower colour, weight of the dry stalk, and the stalk height.

The germination percentage was calculated 14 days after planting. Plant height and basal stem diameter were measured with a tape measure every 21 days. The number of branches of each plant was visually counted. The leaf shape and flower colour were visually observed. The number of days to flowering was counted as the time to first flower appearance of each cultivar.

All cultivars were harvested 123 days after planting. Plants were cut at ground level and left to dry. After 142 days plants were weighed to determine their dry mass and plant heights were also measured.

A field trail was planted in December 2002, 25 km west of Bloemfontein in the central Free State. The same 10 cultivars were used (Table 3.1). Two plots that consisted of five rows each were planted. Each row consisted of 10 plants of the same cultivar. Seeds were sown 10 cm apart in the row and the rows were spaced 50 cm apart. Seeds were planted 2.5 cm deep by hand. No fertilizer was applied. Plants were irrigated once a week.

Table 3.1 Cultivars used for evaluating eight agronomic and morphological traits.

Entry	Name
1	Cuba 108
2	Dowling
3	El Salvador
4	Endora
5	Everglades 41
6	Everglades 71
7	Gregg
8	SF 459
9	Tainung 2
10	Whitten

Three plants in each row were harvested 136 days after planting. Plants were cut at ground level and left to dry. After 170 days after planting, plants were weighed to determine their dry mass. The plant heights and basal stem diameters were measured and the number of branches was also counted.

3.2.1 Statistical analysis

a) Analysis of variance (ANOVA)

ANOVA for the agronomical traits was performed. The cultivar means for all traits were used to compare the performance of genotypes. Cultivar differences were determined by means of least significant differences (LSD). The AGROBASE 2000 computer programme was utilized to perform the ANOVA.

3.3 Results and discussion

3.3.1 Analysis of variance

In the glasshouse trial the analysis indicated highly significant ($P< 0.01$) or significant ($P<0.05$) differences among genotypes for germination percentage, plant height, basal stem diameter, dry material plant height, dry material stem

mass, and days to flowering. It also indicated significant differences among replications for dry material plant mass and days to flowering (Table 3.2).

In the field trial the analysis indicated significant ($P<0.05$) differences among replications for plant height (dry material). There were no significant differences among genotypes for plant height (dry material), basal stem diameter, dry material stem mass, and branching (Table 3.5).

3.3.2 Cultivar means in the glasshouse trial

Results of the ANOVA indicated significant differences among cultivars. Table 3.3 indicates the cultivar means for each of the agronomic traits.

a) Germination percentage

Dowling had significantly higher germination percentages than Gregg, SF 459, Cuba 108, Everglades 71, and Whitten, but did not differ significantly from Endora, El Salvador, Everglades 41, and Tainung 2.

b) Plant height

Whitten grew significantly higher than the other cultivars, and was followed by SF 459, Everglades 71, Everglades 41, Endora, El Salvador, Gregg, Tainung 2, and Dowling that did not differ significantly from each other. Cuba 108 differed significantly from SF 459.

c) Basal stem diameter

Everglades 71 had a significantly thicker basal stem diameter than SF 459, Endora, Gregg, El Salvador, Everglades 41, Tainung 2, and Dowling, but did not differ significantly from Whitten and Cuba 108. There were significant differences between Whitten and Cuba 108, Cuba 108 and SF 459, SF 459, Endora, Gregg and El Salvador, El Salvador and Everglades 41, and Everglades 41, Tainung 2 and Dowling. Whitten differed significantly from Endora, Gregg, El Salvador, Everglades 41, Tainung 2, and Dowling. SF 459 differed significantly from Tainung 2 and Dowling.

d) Plant height of the dry stalks

Whitten was the highest and differed significantly from all the cultivars. There were no significant differences between SF 459, Tainung 2, Gregg, Everglades 71, Everglades 41, Endora, and Cuba 108, but SF 459 was significantly higher than El Salvador and Dowling.

e) Mass of the dry stalks

Whitten had a significantly higher dry stalk mass than Everglades 71, SF 459, Endora, El Salvador, Tainung 2, Gregg, Everglades 41, and Dowling, but there were no significant differences between Cuba 108, Everglades 71, SF 459, Endora, and El Salvador, between Tainung 2 and Gregg, Everglades 41, and Dowling. Dowling differed significantly from Cuba 108, Everglades 71, and SF 459. There were significant differences between Cuba 108, Tainung 2, Gregg, Everglades 41, and Dowling and between Everglades 71 and Everglades 41.

f) Branching

Whitten differed significantly from Everglades 71, Gregg, El Salvador, and Tainung 2. There were no significant differences between Dowling, Cuba 108, SF 459, Everglades 41, Endora, Everglades 71, and Gregg but Dowling differed significantly from El Salvador and Tainung 2.

g) Days to flowering

The differences between the genotypes were highly significant ($P<0.01$). Endora differed significantly from SF 459, Everglades 41, Gregg, Tainung 2, Dowling, and Whitten. There were no significant differences between El Salvador and Cuba 108, Dowling, Whitten, and Tainung 2, Cuba 108 and Everglades 71, Everglades 71, SF 459, and Everglades 41, Everglades 41 and Gregg, Gregg, and Tainung 2. El Salvador and Cuba 108 differed significantly from Everglades 41, Gregg, Tainung 2, Dowling, and Whitten, and Everglades 71, SF 459, and Gregg from Tainung 2, Dowling and Whitten. There was no difference between the cultivars for flower colour. All the cultivars had light yellow flowers with maroon centres.

The cultivars differed in their leaf shapes. From Table 3.4 it is clear that the cultivars can be divided in two groups according to their leaf shapes. El Salvador, Endora, Everglades 71, Gregg, and SF 459 had the divided leaf shape and Cuba 108, Dowling, Everglades 41, and Whitten had undivided leaves. The juvenile or young leaves on all kenaf seedlings were simple, entire, and cordate. As the plants matured and additional leaves were produced, the newer leaves started to differentiate into the leaf shape characteristic of that particular cultivar. Divided leaf cultivars produced three to 10 entire juvenile leaves prior to producing the first divided leaf.

Table 3.2 Analysis of variance for seven agronomical traits of the 10 kenaf cultivars grown in the glasshouse.

Source	Mean squares						
	Germination %	Plant height (cm)	Stalk basal diameter (cm)	DM Stalk plant height (cm)	DM Stalk mass (g)	Branching (per plant)	Days to flowering
Genotype	2154.514**	1223.567*	0.180*	1780.858**	2068.170*	29.847	34.403**
Reps	182.292	553.100	0.017	965.025	3927.362**	14.025	17.225*
CV (%)	31.260	13.350	19.740	8.560	34.540	15.220	2.050

*,** = significant at 0.05 and 0.01 probability levels respectively; DM=dry material.

Table 3.3a Mean values and LSD for five agronomic traits of the 10 kenaf cultivars grown in the glasshouse.

Rank	Germination %		Plant height (cm)		Stalk basal stem diameter (cm)		DM Stalk height (cm)		DM Stalk mass (g)	
	Cultivar	Mean*	Cultivar	Mean*	Cultivar	Mean*	Cultivar	Mean*	Cultivar	Mean*
1	Dowling	93.75a	Whitten	196.75a	Everglades 71	1.60a	Whitten	318.50a	Whitten	125.00a
2	Endora	87.50ab	SF 459	156.75b	Whitten	1.53ab	SF 459	275.50b	Cuba 108	97.00ab
3	El Salvador	87.50ab	Everglades 71	154.75bc	Cuba 108	1.38abc	Tainung 2	270.50bc	Everglades 71	92.25bc
4	Everglades 41	81.25ab	Everglades 41	152.25bc	SF 459	1.33bcd	Gregg	269.35bc	SF 459	90.63bcd
5	Tainung 2	81.25ab	Endora	149.25bc	Endora	1.15cde	Everglades 71	263.25bc	Endora	75.63bcde
6	Gregg	62.50bc	El Salvador	148.50bc	Gregg	1.15cde	Everglades 41	259.25bc	El Salvador	66.63bcde
7	SF 459	62.50bc	Gregg	146.25bc	El Salvador	1.15cde	Endora	257.50bc	Tainung 2	62.13cde
8	Cuba 108	43.75cd	Tainung 2	143.25bc	Everglades 41	1.08de	Cuba 108	251.75bc	Gregg	60.05cde
9	Everglades 71	43.75cd	Dowling	139.75bc	Tainung 2	1.03e	El Salvador	246.00c	Everglades 41	59.50de
10	Whitten	25.00d	Cuba 108	131.00c	Dowling	0.98e	Dowling	246.00c	Dowling	52.13e
LSD	25.18		24.41		0.29		27.40		32.48	

* Means followed by the same letter in the same column did not differ significantly at P=0.05; DM=dry material.

Table 3.3b Mean values and LSD for two morphological traits of the 10 kenaf cultivars grown in the glasshouse.

	Branching (branches per plant)		Days to flowering	
Rank	**Cultivar**	**Mean***	**Cultivar**	**Mean***
1	Whitten	33.50a	Endora	114.50a
2	Dowling	32.00ab	El Salvador	113.75ab
3	Cuba 108	30.50abc	Cuba 108	113.50ab
4	SF 459	29.50abcd	Everglades 71	113.00abc
5	Everglades 41	29.25abcd	SF 459	111.25bc
6	Endora	29.00abcd	Everglades 41	110.75c
7	Everglades 71	27.50bcd	Gregg	110.50cd
8	Gregg	27.00bcd	Tainung 2	108.00de
9	El Salvador	26.00cd	Dowling	107.75e
10	Tainung 2	24.50d	Whitten	105.75e
LSD		4.08		2.11

* Means followed by the same letter in the same column did not differ significantly at P=0.05.

Table 3.4 Separation of the cultivars based on leaf shape.

Divided (palmate)	**Undivided (entire)**
El Salvador	Cuba 108
Endora	Dowling
Everglades 71	Everglades 41
Gregg	Whitten
SF 459	
Tainung 2	

3.3.3 Cultivar means for the field trial

Table 3.6 indicates the cultivar means for each of the agronomic traits.

a) Dry stalk height

El Salvador was the highest, but did not differ significantly from Gregg, Whitten, Tainung 2, and Endora. There were significant differences between El Salvador, SF 459, and Dowling. Gregg, Whitten, Tainung 2, Endora, and SF 459 were significantly higher than Dowling. There were no significant differences between Gregg, Whitten, Tainung 2, Endora, SF 459, Cuba 108, and Everglades 41, and between Cuba 108, Everglades 41, Everglades 71, and Dowling.

b) Dry stalk mass

Whitten had the highest dry stalk mass and differed significantly from all the other cultivars except Dowling. Dowling differed significantly from Endora. There were no significant differences between El Salvador, Everglades 41, Tainung 2, Gregg, SF 459, Everglades 71, and Cuba 108.

c) Basal stem diameter

Whitten and Everglades 41 had significantly higher basal stalk diameters than Gregg. There were no significant differences between Whitten and Everglades 41, between Tainung 2, Dowling, Everglades 71, El Salvador, Cuba 108, Endora, SF 459, and Gregg.

d) Branching

Cuba 108 had the highest number of branches per plant, and differed significantly from SF 459, Everglades 41, Everglades 71, Dowling, Gregg, and Endora. There were no significant differences between Cuba 108, Tainung 2, Whitten, and El Salvador, and between all of the nine cultivars, except Cuba 108.

Table 3.5 Analysis of variance for four agronomic traits of the 10 kenaf cultivars grown in the field.

Source	Mean squares			
	DM Stalk height (cm)	DM Stalk mass (g)	Stem basal diameter (cm)	Stalk branching (per plant)
Genotype	376.652*	34539.041	0.282	69.070
Reps	46.800	7268.133	0.030	6.700
CV (%)	6.890	42.480	15.370	19.970

*,** = significant at 0.05 and 0.01 probability levels respectively; DM=dry material.

Table 3.6 Mean values and LSD for four agronomic traits of the 10 kenaf cultivars grown in the field.

Rank	DM Stalk height (cm)		DM Stalk mass (g)		Stem basal diameter (cm)		Stalk branching (per plant)	
	Cultivar	Mean*	Cultivar	Mean*	Cultivar	Mean*	Cultivar	Mean*
1	El Salvador	187.67a	Whitten	560.00a	Whitten	3.90a	Cuba 108	51.33a
2	Gregg	182.67ab	Dowling	382.00ab	Everglades 41	3.60a	Tainung 2	43.00ab
3	Whitten	181.00ab	El Salvador	320.67bc	Tainung 2	3.50ab	Whitten	42.67ab
4	Tainung 2	177.00abc	Everglades 41	314.67bc	Dowling	3.50ab	El Salvador	42.67ab
5	Endora	171.33abc	Tainung 2	300.00bc	Everglades 71	3.40ab	SF 459	39.00b
6	SF 459	170.67bc	Gregg	275.33bc	El Salvador	3.30ab	Everglades 41	39.00b
7	Cuba 108	166.67bcd	SF 459	270.67bc	Cuba 108	3.20ab	Everglades 71	37.00b
8	Everglades 41	166.33bcd	Everglades 71	266.33bc	Endora	3.20ab	Dowling	36.33b
9	Everglades 71	160.33cd	Cuba 108	233.67bc	SF 459	3.20ab	Gregg	36.00b
10	Dowling	150.33d	Endora	151.00c	Gregg	2.80b	Endora	36.00b
LSD	16.72		184.90		0.73		11.39	

* Means followed by the same letter in the same column did not differ significantly at P=0.05; DM=dry material.

3.4 Conclusions

There were not many morphological differences between the commercial kenaf cultivars. Leaf shape was the only morphological feature that could help to identify cultivars. Data from this study indicated that morphological and agronomic characters alone still cannot be regarded as critical indicators to identify individual kenaf cultivars.

In the glasshouse and field trial, Whitten was the best performing cultivar with regards to plant height, basal stem diameter, dried plant height, dried stalk mass, branching, and it was the first cultivar to mature as soon as the day length was less than 12.5 hours. Whitten had, however, the lowest germination percentage. Pearson (2004) who conducted a field variety test in the USA also found that Whitten had the highest dry matter yield of the varieties tested and that Whitten was followed by Tainung 2, Dowling, and Gregg.

In literature it was stated that Tainung 2 is by far the most popular commercial kenaf variety. Under similar growing conditions, Tainung 2 gave the highest stalk yields (Ching *et al*., 1992; Webber, 1997). Webber (1993) examined the yield components of five kenaf varieties in the USA and found that Tainung 2 had the greatest plant height, stalk yield, and stalk diameter of the varieties tested. However, Tainung 2 did not perform that well in the glasshouse trial conducted at the University of the Free State. It ranked seventh with regards to dried stalk weight and it had a thin stem diameter. The cultivar performed better in the field trial (ranked fifth with regards to dried stalk weight), but it was still not the best performing cultivar.

CHAPTER 4

LIPID AND FATTY ACID ANALYSIS OF KENAF (*H. CANNABINUS* L.) SEED OIL

4.1 Introduction

Naturally occurring fatty acids can be grouped according to the presence of double or triple bonds in two broad classes termed *saturated* and *unsaturated*. The unsaturated fatty acids may contain one or more double or triple bonds and can be separated into monounsaturated and polyunsaturated fatty acids (PUFA) (Lobb, 1992). PUFAs can be classified into two broad categories termed “essential” and “nonessential” fatty acids. Essential fatty acids are necessary fats that humans cannot synthesize, and must be obtained through diet (Bruckner, 1992). These fatty acids are long-chained polyunsaturated fatty acids derived from linolenic, linoleic, and oleic acids. There are two families of essential fatty acids: omega-3 and omega-6. Omega-9 is necessary yet “non-essential” because the body can manufacture a modest amount on its own, provided essential fatty acids are present. The number following “omega-” represents the position of the first double bond, counting from the terminal methyl group on the molecule (Gunstone *et al*., 1986).

Omega-3 fatty acids are derived from alpha-linolenic acid, which is found primarily in dark green leafy vegetables, flaxseed, canola and soybean oils, and certain vegetable oils (Table 4.1). Alpha-linolenic acid is particularly beneficial for protection against heart and vessel disease, and for lowering cholesterol. Although linolenic acid is an essential fatty acid, in high percentages it gives the oil a rapid drying quality and this result in oxidative instability, which shortens the shelf life of the oil by causing rancidity and off flavours. Less than 3% linolenic acid is preferred for oil stability. Omega-6 fatty acids, derived from linoleic acid, are abundant in safflower, sunflower, soybean and corn oils (Table 4.1). Together, omega-3 and omega-6 fatty acids play a crucial role in brain function as well as normal growth and development (Anonymous, 2004b).

Table 4.1 Fat constituents as percentage of total fat for selected food crops (Best, 2004; White, 1992; Mohamed *et al.*, 1995).

Food	Palmitic ($C_{16:0}$)	Stearic ($C_{18:0}$)	Oleic ($C_{18:1}$)	Linoleic ($C_{18:2}$)	A-Linolenic ($C_{18:3}$)
Flaxseed Oil	3.0	7.0	21.0	16.0	53.0
Canola Oil	5.0	2.0	53.0	22.0	10.0
Soybean Oil	11.0	4.0	23.0	51.0	7.0
Palm Oil	45.0	5.0	38.0	10.0	0.0
Olive Oil	14.0	3.0	71.0	10.0	0.0
Sunflower seed Oil	6.0	4.0	24.0	65.0	0.0
Kenaf seed Oil	20.1	3.5	29.2	45.9	0.7
Safflower seed Oil	7.0	3.0	15.0	75.0	0.0
Cottonseed Oil	24.7	2.3	17.6	53.3	0.3

Omega-9 fatty acid is derived from monounsaturated oleic acid. High oleic acid is in demand for commercial food-service applications due to a long shelf life and cholesterol-reducing properties. Monounsaturated fat is the most desirable type of fat in the diet because it helps to decrease the Low Density Lipoprotein (LDL) or “bad” cholesterol in the blood and helps to increase the High Density Lipoprotein (HDL) or “good” cholesterol. Monounsaturated fatty acids also lower heart attack risk and arteriosclerosis, and aids in cancer prevention. Sources of monounsaturated fat are olive oil, canola oil, peanut oil, and most nuts. Olive oil has the highest percentage (about 77%) of monounsaturated fat of any edible oil (Table 4.1) (Anonymous, 2004b).

Saturated fat is the least healthy type of fat. Saturated fat raises the level of LDL cholesterol, which causes numerous health problems if consumed in large quantities (Anonymous, 2004b). Foods from plants that contain high amounts of saturated fatty acids include coconut oil, palm oil and palm kernel oil (often called tropical oils), and cocoa butter. Saturated fatty acids with 12, 14, and 16 carbons (lauric acid, myristic acid, and palmitic acid, respectively) are the primary contributors to elevated LDL cholesterol. On the other hand, the 18 carbon stearic acid may not raise LDL cholesterol (White, 1992).

The potential for using kenaf seeds as a source of edible oil is often overlooked when considering kenaf as a fibre and feed crop. Research was done by Mohamed *et al.* (1995) to determine the quantity and quality of kenaf seed oil from nine varieties. Kenaf seeds contained a variety of oil, fatty acids, phospholipids, and sterols. Differences occurred in percentage composition among the nine kenaf varieties.

The oil content ranged from 21.4-26.4% with a mean of 23.7%. Palmitic, oleic, and linoleic were the major fatty acids, and palmitoleic, linolenic, and stearic were the minor components (Table 4.1). Medium (C_{12}-C_{14}) and long (C_{22}-C_{24}) chain fatty acids were less than 1%. Total phospholipids ranged from 3.9-10.3% of the oil, with a mean of 6.0% while the mean sterol content was 0.9% and ranged from 0.6% of the total oil to 1.2% (Mohamed *et al*., 1995).

According to Mohamed *et al.* (1995), the relatively high seed oil content and similarity to cottonseed oil suggested that kenaf seed oil might be used as a source of edible oil. The aim of this research was to study and compare the fatty acid composition and oil content of eight commercial kenaf varieties, with a view to identify suitable parents for use in hybridization programmes aimed at improving oil quality.

4.2 Materials and methods

4.2.1 Plant materials

A total of eight kenaf cultivars were used in this study. Seeds were obtained from the ARC Institute for Industrial Crops (Rustenburg, South Africa). A description of the materials used in this study is shown in Table 4.2.

4.2.2 Lipid extraction

Total lipid was extracted in duplicate for each cultivar with chloroform-methanol (2:1 v/v) as described by Folch *et al*. (1957). Butylated hydroxy toluene (20 mg) was placed in a 2000 ml volumetric flask. Chloroform (1333.33 ml) and methanol (666.67 ml) were added. In the lower phase,

Table 4.2 Kenaf cultivars used for determining oil content and fatty acid composition.

Code	Cultivar
1	El Salvador
2	Dowling
3	Gregg
4	Cuba 108
5	SF459
6	Tainung 2
7	Everglades 41
8	Endora

chloroform (1700.97 ml) was placed in 2000 ml volumetric flask; methanol (277.23 ml) and water (19.8 ml) were added with 86:14:1 (v/v) proportions respectively. About 200 g of seed from each sample were ground with a mortar and pestle. A ±0.5 g ground seed sample was used for lipid extraction in a 250 ml round bottom flask. A 30 ml aliquot of chloroform:methanol (2:1 v/v) was added and left overnight in a refrigerator at 4℃.

The sample was transferred into a round bottom flask through a pre-weighed 18.5 cm Whatman No. 1 filter paper on top of the separating funnel. The round bottom flask was washed three times with 12.33 ml of chloroform: methanol (2:1 v/v) and transferred each time to filter paper. After allowing everything to run through, the filter paper was removed. Then 16.5 ml distilled H_2O was added to each separating funnel, which was recapped, shaken thoroughly, and allowed one hour for separation. After this the lower phase was drained into a 500 ml round bottom flask and 50 ml of the lower phase was added to each separating funnel and allowed 15 min for separation. After 15 min the lower phase was drained into the same 500 ml round bottom flask and the upper phase discarded. Contents from the 500 ml of round bottom flask were evaporated under vacuum in a rotary evaporator at 60℃ for 20 min at 15 psi. The remaining water was removed by the addition of 50 ml methanol and then by evaporating each round bottom flask at 60℃ at 15 psi.

Contents of each 500 ml round bottom flask were washed six times with 5 ml portions of diethyl ether into a pre-weighted polytop. Diethyl ether was removed from each polytop by evaporation under a stream of nitrogen on a heating block at 60℃ for 20 min. Filter papers and capped polytops were placed in a vacuum oven, dried at 50℃ overnight and weighed the next morning. The oil-solvent mixture was dried over anhydrous sodium sulphate, the solvent evaporated under nitrogen, and the oil percentage determined by weighing. A ±10 mg of the lipid was transferred to another polytop for methylation.

4.2.3 Methylation

A modification of the procedure described by Slover and Lanza (1979) was used. An amount of ±10.0 mg of lipid was washed with 6 x 1 ml hexane into a test tube with a Teflon-lined cap. Hexane was removed by N_2 evaporation without application of heat. Methanolic 0.5 N NaOH (1 ml) was added and the tube was capped and heated in a boiling water bath for 15 min. After the tube had cooled, 2 ml of BF_3/CH_3OH (14%) was added; the tube was then recapped and heated in the boiling water bath for an additional 15 min. The tube was cooled, and 1 ml of hexane and 2 ml of saturated aqueous NaCl were added. The tube was shaken vigorously for 1 min, and allowed to stand for 10 min until the phases had separated. The upper 70% of hexane layer was transferred with a Pasteur pipette to a 45x11 mm autosampler vial containing a 1 mm layer of anhydrous Na_2SO_4. The vial was capped, shaken, and allowed to stand for at least 20 min, to remove traces of water. The 100 µl hexane from each vial was transferred to a clean, labelled autosampler vial and 900 µl hexane was added to each vial stored below freezing point for gas chromatography analyses.

4.2.4 Determination of fatty acid composition by gas chromatography

After methylation, individual fatty acid composition was determined by gas chromatography for all eight cultivars. Fatty acids were quantified using a Varian GX 3400 flame ionisation gas chromatograph, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 µm ID, 0.2 µm thickness). Column temperature was 40-230ºC (hold 2 min 4ºC/min; hold 10

min). Fatty acid methyl esters in hexane (1 µl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250ºC. Hydrogen was used as a carrier gas at 45 psi and nitrogen was used as the makeup gas. Chromatograms were recorded with Varian Star Chromatography Software version 4.0. Identification of sample fatty acids was done by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards obtained from SIGMA (cat. 189-19).

4.2.5 Statistical analyses

a) Correlations

Correlation coefficients between oil and fatty acid content were obtained by using the software program AGROBASE 2000 (Agronomix, Canada) to examine the degree of association among oil content and fatty acids.

b) Genetic distances for oil and fatty acid content

Three classes for fat and fatty acid percentage were distinguished using the following formulas:

n = (highest percentage – lowest percentage) ÷ 3

Class	**Formula**
Low =	Lowest percentage + n
Medium =	Lowest percentage + 2n
High =	> Medium

A binary matrix reflecting, either low, medium or high oil or fatty acid content as present (1) or absent (0), was generated for each cultivar (Addendum A). Pairwise genetic distances were expressed as the complement of Dice's coefficient (Dice, 1945) and a cluster analysis was performed using UPGMA (unweighted pair-group method using arithmetic averages; Sokal and Michener, 1958) clustering. Statistical analysis was performed using NTSYS-pc version 2.02i.

4.3 Results and discussion

4.3.1 Description of variability for oil content and fatty acid composition

The fatty acid profile, composition, and oil content of the kenaf cultivars are shown in Table 4.3. Considerable variation was observed among the fatty acid profiles for the eight cultivars. A total of 16 different fatty acids were identified in percentages of the total fatty acid of the seed oil, but not all cultivars had all fatty acids.

The predominant fatty acids found were linoleic acid ($C_{18:2c9,12}$), oleic acid ($C_{18:1c9}$), palmitic acid ($C_{16:0}$), and stearic acid ($C_{18:0}$). To a lesser extent palmitoleic acid ($C_{16:1c9}$), arachidic acid ($C_{20:0}$), alpha-linolenc acid ($C_{18:3c9,12,15}$), behenic acid ($C_{22:0}$), lignoceric acid ($C_{24:0}$), and nervonic acid ($C_{24:1c15}$) were also found in all cultivars. Myristic acid ($C_{14:0}$), heptadecenoic acid ($C_{17:1c10}$), heneicosanoic acid ($C_{21:0}$), eicosadienoic acid ($C_{20:2c11,14}$), eicosatrienoic acid ($C_{20:3c11,14,17}$), eicosatrienoic acid ($C_{20:3c8,11,14}$), and eicosopentaenoic acid ($C_{20:5c5,8,11,14,17}$) were rarely detected in some cultivars. Fatty acid profiles of the seed samples revealed a large variation for most of the fatty acids examined. Linoleic acid was the predominant fatty acid in Gregg (49.75%), Tainung 2 (47.41%), Everglades 41 (45.51%) SF 459 (44.06%), and Dowling (43.19%), and had the highest percentage of total fatty acids (41.15%). These cultivars may be useful in developing cultivars yielding good quality cooking oils. Oleic acid was the predominant fatty acid in El Salvador (43.4%) and Endora (36.34%) and had the second highest percentage of total fatty acids (32.79%). Oleic acid is important for cooking and salad oils (Green and Marshall, 1981), whereas a high level of palmitic acid is required for the production of margarine, shortening, and other fat products (Ntiamoah *et al*., 1995).

Large variation was found in linoleic and oleic acid percentages among the cultivars examined. Less variation was found in palmitic and stearic acid. The predominance of linoleic, oleic and palmitic acid was in agreement with the findings of other researchers (Hopkins and Chrisholm, 1959; Mohamed *et al*.,

1995). The percentages of these three fatty acids were more or less the same as found by Mohamed *et al*. (1995).

All the cultivars contained small amounts of the nutritionally important α-linolenic acid (0.23-0.83%). α-Linolenic acid is an essential fatty acid that is metabolised to eicosapentaenoic acid, a precursor of eicosanoids with anti-inflammatory and antithrombotic activity (Ruiz *et al*., 2002). However, because of the low α-linolenic acid content, kenaf seed oil has good keeping quality for cooking purposes.

Large variation was found in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids among the cultivars examined. Dowling (43.52%), Gregg (50.30%), Cuba 108 (37.72%), SF 459 (44.63%), Tainung 2 (47.78%), and Everglades 41 (46.34%) had higher polyunsaturated than monounsaturated percentages. El Salvador (44.45%) and Endora (37.40%) had higher monounsaturated than polyunsaturated percentages. The predominant fatty acids were found to be polyunsaturated. The ratio of PUFA:SFA was approximately 1.7:1 for all cultivars examined.

Fatty acids identified in this study were composed of approximately 21.59-28.17% saturated, 71.83-78.26% total unsaturated, 27.96-44.45% monounsaturated, and 28.83-50.30% polyunsaturated fatty acids.

Oil content ranged from 19.02% to 21.08% with a mean of 19.83% for the eight cultivars examined. El Salvador (21.08%) had the highest oil content. Only a small variation was found for oil content among cultivars and oil content was lower than the findings of Mohamed *et al*. (1995).

Table 4.3 Fatty acid profile of eight kenaf cultivars determined by capillary gas chromatography.

No	Cultivar	%Fat	C14:0	C16:0	C16:1	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:2	C20:3	C20:5	C21:0	C22:0	C24:0	C24:1	SFA	MUFA	PUFA	UFA	PUFA/ SFA
1	El Salvador	21.08	0.00	21.28	0.45	0.00	4.30	43.42	28.6	0.23	0.75	0.00	0.00	0.00	0.00	0.28	0.12	0.57	26.73	44.45	28.83	73.27	1.08
2	Dowling	19.96	0.00	18.22	0.55	0.06	2.44	33.86	43.19	0.33	0.48	0.00	0.00	0.00	0.00	0.32	0.12	0.42	21.59	34.89	43.52	78.41	2.02
3	Gregg	20.36	0.00	18.92	0.39	0.12	2.05	26.94	49.75	0.40	0.42	0.00	0.00	0.15	0.00	0.29	0.05	0.51	21.74	27.96	50.30	78.26	2.31
4	Cuba 108	19.62	0.12	21.36	0.43	0.05	3.75	34.91	37.24	0.28	0.60	0.00	0.00	0.19	0.00	0.30	0.08	0.68	26.22	36.07	37.72	73.78	1.44
5	SF 459	19.94	0.07	20.64	0.50	0.00	2.94	29.57	44.06	0.29	0.51	0.00	0.00	0.28	0.00	0.31	0.07	0.76	24.53	30.83	44.63	75.47	1.82
6	Tainung 2	19.21	0.06	18.36	0.47	0.00	2.58	29.65	47.41	0.38	0.47	0.00	0.00	0.00	0.00	0.31	0.07	0.25	21.85	30.37	47.78	78.15	2.19
7	Everglades 41	19.50	0.08	20.72	0.55	0.00	2.90	27.63	45.51	0.39	0.51	0.06	0.06	0.32	0.06	0.34	0.06	0.79	24.68	28.98	46.34	75.32	1.88
8	Endora	19.02	0.14	22.64	0.35	0.14	4.22	36.34	33.42	0.83	0.68	0.00	0.00	0.19	0.00	0.35	0.14	0.56	28.17	37.40	34.44	71.83	1.22
	Average	19.84	0.06	20.27	0.46	0.05	3.15	32.79	41.15	0.39	0.55	0.01	0.01	0.14	0.01	0.31	0.09	0.57	24.44	33.87	41.70	75.26	1.75

SFA=saturated fatty acids, MUFA=mono unsaturated fatty acids, PUFA=polyunsaturated fatty acids, UFA=unsaturated fatty acid.

4.3.2 Relationship between oil content and fatty acids

Correlations of oil content and different fatty acid compositions as well as correlations between fatty acids are given in Table 4.4. Oil content was significantly positively correlated to the essential oleic and linoleic acids as well as stearic acid. Significantly positive correlations between oil content and other unwanted saturated fatty acids (palmitic, arachidic, and behenic acid) was also observed (Table 4.4).

Palmitic acid was significantly positively correlated with stearic, oleic, linoleic, and other saturated fatty acids (arachidic, and behenic acid). Stearic acid is considered a neutral fatty acid because it is a saturated fatty acid but does not increase the level of LDL cholesterol in the blood. Significantly positive correlations were observed for stearic acid with palmitic, linoleic, and the saturated fatty acids arachidic and behenic (Table 4.4).

Linoleic acid showed a significantly positive correlation with palmitic, palmitoleic, and behenic fatty acid. α-Linolenic acid was not significantly correlated to either oil content, or the other fatty acids (Table 4.4). A positive, but not significant correlation was found between linoleic and α-linolenic acid. Positive correlations indicate that selection directed towards increased linolenic acid, would also increase linoleic, palmitic, and stearic acids.

Table 4.4a Correlations between oil, fatty acids, and other characters for kenaf cultivars.

	PERC OIL	C14:0	C16:0	C16:1	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0
C14:0	0.2860									
C16:0	0.9700**	0.4812								
C16:1	0.9157**	0.2040	0.8567*							
C17:1	0.2567	0.2550	0.3030	-0.0187						
C18:0	0.7986*	0.5497	0.8933**	0.6360	0.2314					
C18:1	0.9167**	0.3069	0.9276**	0.7874*	0.2436	0.9338**				
C18:2	0.8795**	0.2255	0.8025*	0.8803**	0.2293	0.4588	0.6276			
C18:3	0.5483	0.6294	0.6540	0.3644	0.7117	0.5994	0.5338	0.4837		
C20:0	0.8720*	0.4374	0.9305**	0.7200	0.2390	0.9857**	0.9759**	0.5533	0.5909	
C20:2	0.1055	0.1888	0.1466	0.3130	-0.276	0.0292	-0.0471	0.2184	0.0725	0.0333
C20:3	0.1055	0.1888	0.1466	0.3130	-0.276	0.0292	-0.0471	0.2184	0.0725	0.0333
C20:5	0.3342	0.6238	0.4543	0.3426	0.1326	0.3033	0.1412	0.4072	0.3718	0.2602
C21:0	0.1055	0.1888	0.1466	0.3130	-0.276	0.0292	-0.0471	0.2184	0.0725	0.0333
C22:0	0.9583**	0.4668	0.9674**	0.9048**	0.3203	0.7906*	0.8628*	0.8810**	0.7063	0.8430*
C24:0	0.6883	0.3184	0.7364	0.5688	0.3981	0.8359*	0.8716*	0.3661	0.6572	0.8544*
C24:1	0.7509	0.4866	0.8209*	0.7234	0.1077	0.7169	0.6623*	0.6153	0.3929	0.7214
SFA	0.9548**	0.5024	0.9969**	0.8323*	0.2956	0.9259**	0.9430**	0.7550*	0.6569	0.9545**
UFA	0.9919**	0.2991	0.9524**	0.9291**	0.2649	0.7429	0.8758**	0.9252**	0.5668	0.8206*
MUFA	0.9221**	0.3130	0.9337**	0.7955*	0.2438	0.9344**	0.9998**	0.6369	0.5264	0.9766**
PUFA	0.8807**	0.2377	0.8072*	0.8795**	0.2373	0.4650	0.6294	0.9999**	0.4958	0.5579
UFA/SFA	0.0023**	0.1236	0.8362*	0.9128**	0.2365	0.5455	0.7529	0.9666**	0.4646	0.6527

SFA=saturated fatty acid, UFA=unsaturated fatty acid, PUFA=polyunsaturated fatty acid,

*,** = significant at 0.05 and 0.01 probability levels respectively.

Table 4.4b Correlations between oil, fatty acids, and other characters for kenaf cultivars.

	C20:2	C20:3	C20:5	C21:0	C22:0	C24:0	C24:1	SFA	UFA	MUFA	PUFA
C20:3	1.0000										
C20:5	0.5642	0.5642									
C21:0	1.0000	1.0000	0.5642								
C22:0	0.2191	0.2191	0.4423	0.2191							
C24:0	-0.165	-0.165	-0.0236	-0.165	0.7142						
C24:1	0.4235	0.4235	0.7944*	0.4235	0.7542	0.4274					
SFA	0.1309	0.1309	0.4349	0.1309	0.9526**	0.7669*	0.8148*				
UFA	0.1209	0.1209	0.3342	0.1209	0.9693**	0.6525	0.7127	0.9307**			
MUFA	-0.0340	-0.0340	0.1591	-0.0340	0.8699*	0.8676*	0.6761	0.9482**	0.8817**		
PUFA	0.2244	0.2244	0.4179	0.2244	0.8857**	0.3708	0.6220	0.7601*	0.9262**	0.6389	
UFA/SFA	0.0899	0.0899	0.2190	0.0899	0.8976**	0.5224	0.5644	0.7985*	0.9632**	0.7585*	0.9647**

SFA=saturated fatty acid, UFA=unsaturated fatty acid, PUFA=polyunsaturated fatty acid,

*,** = significant at 0.05 and 0.01 probability levels respectively.

4.3.3 Genetic distances for oil and fatty acid content

A dendrogram constructed using Dice's coefficient of similarity and the UPGMA clustering method is given in Figure 4.1. Two clusters and four subgroups were revealed according to their relationship for oil and fatty acid content.

Cluster A was subdivided into two subgroups, 1 and 2, and these two subgroups both consisted of sub-subgroups a and b respectively. Subgroup 1 contained the two cultivars Everglades 41 and SF 459 respectively. According to genetic distances, Everglades 41 and SF 459 were the most closely related of all the cultivars with a genetic distance of 0.762 (Table 4.5).

Sub-subgroup 2a contained Gregg and sub-subgroup 2b contained Tainung 2 and Dowling. Dowling and Tainung 2 were 67% similar and were closely related to Gregg with a genetic distance of 0.667 (Table 4.5). The cultivars in subgroup 2 had the lowest palmitic acid values (±18%) as well as the lowest saturated acid values (±21%). These cultivars had the highest total unsaturated fatty acid values (±78%). Cultivars Gregg and Tainung 2 had the highest linoleic (49.75 and 47.41% respectively) and polyunsaturated fatty acid (50.3 and 47.78% respectively) values.

Cluster B was divided into two subgroups, 1 and 2, and only subgroup 2 consisted of sub-subgroups a and b. Subgroup 1 contained Cuba 108, while subgroup 2 contained Endora and El Salvador. El Salvador and Endora were 57% similar and were closely related to Cuba 108 (with genetic distances of 0.524 and 0.571 respectively) (Table 4.5). The cultivars in cluster B had the highest palmitic acid (21-23%) and oleic acid (35-43%) values. They also had the highest total saturated fatty acid (26-28%) and monounsaturated fatty acid (36-44%) values.

Table 4.5 Genetic distances for kenaf cultivars obtained using Dice similarity coefficient.

	El Salvador	Dowling	Gregg	Cuba 108	SF 459	Tainung 2	Everglades 41
Dowling	0.333						
Gregg	0.333	0.619					
Cuba 108	0.524	0.286	0.333				
SF 459	0.238	0.476	0.524	0.333			
Tainung 2	0.333	0.667	0.667	0.381	0.619		
Everglades 41	0.095	0.238	0.333	0.238	0.762	0.476	
Endora	0.571	0.286	0.333	0.571	0.143	0.191	0.095

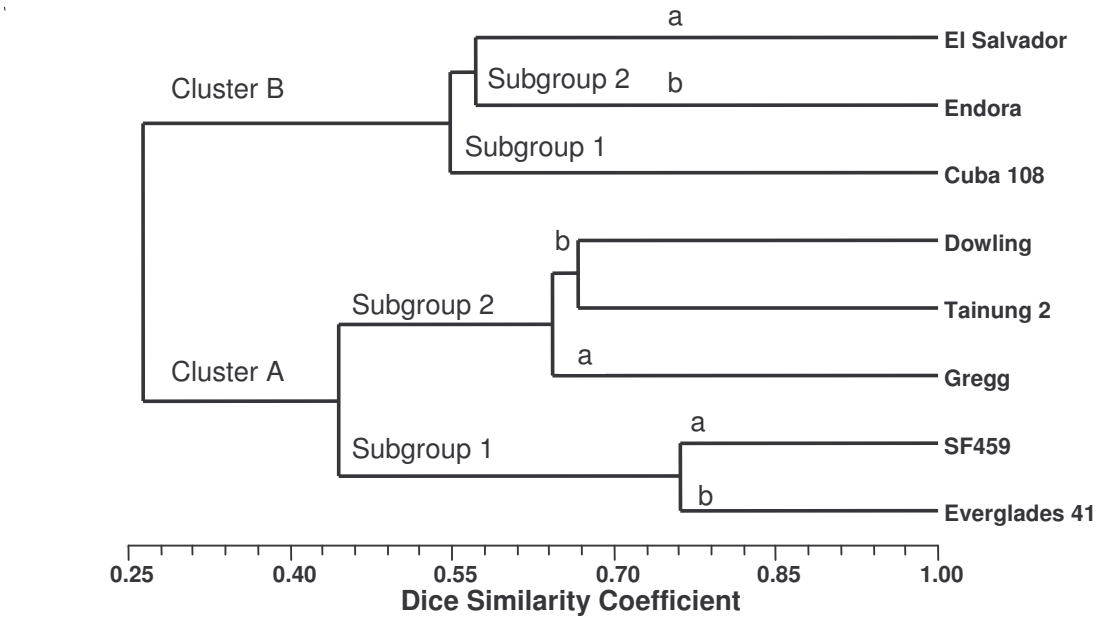

Fig. 4.1 Dendrogram showing genetic relationships for oil and fatty acid content of eight kenaf cultivars obtained, using Dice's coefficient of similarity and UPGMA clustering using the NTSYS-pc version 2.02i computer programme.

4.4. Conclusions

Linoleic, oleic, and palmitic were the predominant fatty acids in all eight cultivars. Percentages of each fatty acid varied greatly among different kenaf varieties. Gregg had the highest percentage linoleic acid whilst El Salvador had the lowest. El Salvador had the highest percentage oleic acid and Endora had the highest percentage palmitic acid. The percentage oil was relatively high and there was not much difference between different varieties. El Salvador had the highest oil content.

The relatively high oil content and the unique fatty acid composition (high oleic, linoleic and palmitic acid content) suggested that kenaf oil could be used as a source of edible oil. Kenaf oil can be considered nutritionally healthy because of the relatively high amount of monounsaturated and polyunsaturated fatty acids. It is, however, desirable to develop varieties with reduced palmitic (saturated) fatty acids. Stearic acid and palmitic acid were positively correlated, as well as stearic and linoleic acid. Breeding for increased stearic and linolenic acid and reduced palmitic acid at the same time might be difficult through conventional breeding.

Because of the great variation among the different varieties, there is a potential for genetic improvement of oil quality. Although kenaf is mainly used for its fibre, the seeds, as a by-product, would provide oil and meal for food and feed. Such uses could significantly increase the economic value of this crop.

CHAPTER 5

GENETIC IDENTIFICATION AND ANALYSIS OF KENAF (*H. CANABINUS* L.) GENOTYPES USING AFLP FINGERPRINTING

5.1 Introduction

Identification of kenaf varieties based on morphological and agronomical characters is always problematic (Siepe *et al*., 1997). In addition, the understanding of relationships between kenaf germplasm is still very limited. These factors have significantly hindered the effective utilization and conservation of valuable genetic resources (Cheng *et al*., 2004). Previous studies by Cheng *et al*. (2002) indicated that RAPD analysis was able to identify kenaf varieties and determine their genetic relationships to a certain extent, but the sources of kenaf accessions used in their study were narrow, and the number of DNA polymorphic fragments was relatively low. In order to accumulate more indicative molecular data and to determine diversity and genetic relationships of kenaf germplasm worldwide, Cheng *et al*. (2004) analysed kenaf varieties by morphological characterization and AFLP fingerprinting. Results indicated that the AFLP technique was a more powerful tool than RAPD analysis in distinguishing kenaf varieties.

The AFLP technique is a DNA fingerprinting technique that combines both PCR and RFLP strategies. It is based on the selective PCR amplification of a subset of genomic restriction fragments from a total digest of DNA (Vos *et al*., 1995). AFLP is a robust and rapid technique for displaying large numbers of DNA polymorphisms and is used extensively for genetic mapping and fingerprinting of plants at species and subspecies level (Russell *et al*., 1997). AFLP detects genomic restriction fragments similar to the RFLP technique, with the major difference that PCR amplification instead of Southern hybridization is used for detection of restriction fragments (Vos *et al*., 1995).

AFLP involves the amplification of small restriction fragments, obtained by cleaving genomic DNA with restriction enzymes, to produce high-resolution

DNA fingerprinting patterns on denaturing polyacrylamide gels. The rationale of the AFLP technique is based on the use of specially designed PCR primers that selectively amplify a small subset of restriction fragments, or "markers", out of a complex mixture comprising as many as several million fragments. Products of the reaction can be visualised by conventional DNA staining or DNA labelling procedures using either radioactive or non-radioactive methods (Thottappilly *et al*., 2000). The aim of this research was to fingerprint 19 kenaf genotypes using AFLP analysis and to determine genetic relationships between them.

5.2 Materials and methods

5.2.1 Plant material

A total of 19 genotypes (Table 5.1) were used in this study. Two plants per genotype were grown in 8 ℓ pots, containing red soil (Bainsvlei soil type), under standard glasshouse conditions at the University of the Free State, during January through May 2003. The temperature was set at 20℃ night and 26℃ day respectively.

5.2.2 DNA extraction

DNA was extracted from fresh young leaves using a modified monocot extraction protocol (Edwards *et al*., 1991). Young leaf tissue was collected on ice and single-plant samples were ground to a fine powder in liquid nitrogen using a mortar and pestle. Extraction buffer (10 ml) (0.5 M NaCl, 0.1 M Tris-HCl [pH 8.0], 0.05 M EDTA, and 1.25% (w/v) SDS), 1 ml Cetyltrimethyl-ammonium bromide (CTAB) buffer (0.2 M Tris-HCl [pH 8.0], 0.05 M EDTA, and 10% (w/v) CTAB) and 2 ml NaCl (5 M) were added. The homogenate was vortexed and incubated at 65℃ for one hour, with mixing every 10 min. Chloroform extraction was performed to remove cellular debris and proteins by the addition of 10 ml chloroform:isoamyl alcohol (24:1, v/v) followed by centrifugation for 15 min at 10 000 rpm. After centrifugation, the supernatant was retained and DNA was precipitated by adding two volumes (v/v) of cold absolute ethanol followed by overnight incubation at 4℃. The precipitated DNA was spooled with a sterile pasteur pipette and washed three times in 1

ml 70% (v/v) ethanol (EtOH). DNA was dissolved in 250 µl sterile distilled water.

Table 5.1 Genotypes used for genetic analysis.

Entry	Cultivar / Genotype	Line	Source	Country of origin
1	Cuba 108	PI 244859	ARC-IIC[3]	Cuba
2	Dowling	PI 603071	ARC-IIC[3]	Texas, USA
3	El Salvador	PI 207883	ARC-IIC[3]	Java, Indonesia
4	Endora		ARC-IIC[3]	Spain
5	Everglades 41	PI 532873	ARC-IIC[3]	Florida, USA
6	Everglades 71	PI 532874	ARC-IIC[3]	Florida, USA
7	Gregg	PI 603072	ARC-IIC[3]	Texas, USA
8	SF 459	PI 586657	ARC-IIC[3]	Texas, USA
9	Tainung 2	PI 532872	ARC-IIC[3]	Taiwan
10	Whitten		ARC-IIC[3]	
11	*Hibiscus* sp. (485)		NGB[2]	South Africa
12	K503		ARC-IIC[3]	Russia
13	K124		ARC-IIC[3]	Russia
14	*Hibiscus* sp. a		SPDG[4]	
15	K258		ARC-IIC[3]	Russia
16	*Hibiscus* sp. b		SPDG[4]	
17	Guatemala 4 (G4)		SPDG[4]	Guatemala (via USA)
18	KY33		ARC-IIC[3]	Russia
19	K521		ARC-IIC[3]	Russia

[1]Kruger National Park / Pilanesberg, [2]National Gene Bank, Department Agriculture, Pretoria, [3]Agricultural Research Council, Rustenburg, [4]Sustainable Projects Development Group, UK.

5.2.3 DNA concentration determination

DNA concentrations were determined spectrophotometerically (U-2000), by measuring absorbances at 260 nm and 280 nm. The DNA concentration was calculated using the formula, [DNA = optical density (OD_{260}) x dilution factor x constant (50 µg/ml)]. DNA samples were diluted to a working concentration of 250 ng/µl in sterile distilled water and stored at 4°C. The integrity and concentration of the DNA was confirmed by 1% (w/v) agarose gel electrophoresis for 45 min at 80 V with visualisation under UV light after staining with ethidium bromide.

5.2.4 AFLP procedure

The AFLP analysis system from Life Technologies, Inc (GIBCO BRL, 1996) was used according to the manufacturer's protocol, using commercial adapter and primer sequences (Table 5.2).

a) Restriction endonuclease digestion and ligation of adapters

Genomic DNA (250 ng) was double digested with two restriction endonucleases (*Eco*R I and *Mse* I) at 37°C for two hours. Following heat inactivation of the restriction endonucleases at 70°C for 15 min, the digested DNA fragments were ligated to *Eco*R I and *Mse* I adapters (Table 5.2) using T4 DNA ligase (10 U) for two hours at 20°C. The reaction mixture was diluted 10-fold in TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) buffer and used as template for subsequent PCR amplification.

b) Polymerase chain reaction

A 50 µl pre-selective PCR reaction was performed using 5 µl (1:10) diluted ligation product, 40 µl pre-amp primer mix, 10x PCR buffer and 1 U of Ampli *Taq* DNA polymerase (GIBCO BRL, 1996). A touchdown Hybaid thermal cycler was used to perform the reaction for 20 cycles with the following profile: a 30 s denaturing step at 94°C, a 60 s annealing step at 56°C and a 2 min extension step at 72°C. Pre-selective PCR products were diluted 50 fold in TE buffer.

Selective PCR-reactions were performed in 20 µl reactions containing 5 µl of the diluted pre-selective reaction, 4.5 µl of the *Mse*+3 primer (Table 5.2), 1 µl *Eco*+3 primer, 2 µl of 10x PCR buffer and 1 U of Ampli *Taq* DNA polymerase. The following cycling programme was used for selective amplification: denaturation at 94°C for 5 min followed by one cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temperature was lowered by 1°C per cycle during the next eight cycles after which 25 cycles were performed for 30 s at 94°C, 30 s at 56°C and 60 s at 72°C followed by one last elongation of 5 min at 72°C. *Eco*R I primers (PE Biosystems) were labelled with NED and FAM respectively. After amplification, 5 µl of each of the selective reactions were added to a new tube containing 24 µl of formamide and 1 µl of ROX-

1000 standard size marker, denatured at 94℃ for 10 min, cooled on ice and resolved on a Perkin Elmer ABI Prism 310 Automated capillary sequencer (PE Biosystems).

Table 5.2 Adapters and primers used for AFLP analysis.

Name of adapters/primers	Sequences (5'-3')
Adapters	
*Eco*R I adapters	CTCGTAGACTGCGTACC
	AATTGGTACGCAGTCTAC
Mse I adapters	GACGATGAGTCCTGAG
	TACTCAGGACTCAT
Selective primers	
*Eco*R I-AAC-NED	GACTGCGTACCAATTCAAC
*Eco*R I-ACA-FAM	GACTGCGTACCAATTCACA
Mse I-CAG	GATGAGTCCTGAGTAACAG
Mse I-CAC	GATGAGTCCTGAGTAACAC
Mse I-CTC	GATGAGTCCTGAGTAACTC

5.3 Statistical analysis

AFLP data were scored into a binary matrix as discrete variables ("1" for presence and "0" for absence) (Addendum B). All reproducible fragments, above a threshold fluorescence intensity of 35, were scored using a minimum peak height of 100. Genotypic data were used to calculate pairwise genetic distances. Pairwise genetic distances were expressed as the complement of Dice coefficient (Dice, 1945). Estimates of similarity between genotypes were based on the probability that an amplified fragment from one genotype will also be present in another. Associations among the 19 genotypes were determined from cluster analysis based on the genetic estimates. The UPGMA clustering method was used for hierarchical clustering, and the necessary computations were performed using NTSYS-pc version 2.02i (Exeter software, USA).

5.4 Results and discussion

5.4.1. Genetic distances and cluster analysis

Using six primer combinations (each primer contained three selective nucleotides), unique AFLP DNA fingerprints were generated for each primer combination. The six primer combinations generated a total of 406 fragments of which 229 (56.4%) were polymorphic. A minimum number of 32 (primer combination *Eco*R I-AAC/*Mse* I-CAG) and a maximum of 49 (*Eco*R I-ACA/*Mse* I-CAG) polymorphic fragments per primer combination were observed. The *Eco*R I-ACA/*Mse* I-CAG primer combination amplified the highest rate of polymorphisms (66.2%) of the six primer combinations. All 19 kenaf genotypes were easily distinguished by any one primer combination.

A dendrogram constructed using Dice's coefficient of similarity and the UPGMA clustering method is given in Fig. 5.1. Guatemala 4, *Hibiscus* sp. no. 485 and one of the wild *Hibiscus* sp.a clearly grouped separately from the rest of the genotypes. The other 16 genotypes grouped into two main groups.

The first main group consisted of six commercial cultivars (commercial group A, Fig. 5.1). According to Dempsey (1975) Cuba 108 was developed in Cuba and is a less spiny selection of Cubano (a spiny selection from cultivar El Salvador). Everglades 41 and Everglades 71 were 91% similar (Table 5.3). Both cultivars were developed in Florida (USA) and are selections from the cultivar El Salvador (USDA, 2004). SF 459 and Gregg were 90% (Table 5.3) similar and both cultivars have their origin in Texas (USA). SF 459 was developed during mass selection from the strain 45-9 (Cook and Scott, 1995). Gregg was developed from a cross between SF 459 and germplasm line 15 (Cook and Scott, 2000b). Dowling was 90% (Table 5.3) similar to Gregg and also has its origin in Texas. Dowling was developed through a cross between Everglades 41 and the germplasm line 15 (Cook and Scott, 2000a). Similarity coefficients ranged from 0.88 to 0.92 for this group (Table 5.3).

The second main group consisted of two subgroups. The first subgroup consisted of four commercial cultivars and one wild type (commercial group B,

Fig. 5.1), while the second subgroup consisted of five Russian breeding lines (Fig. 5.1). El Salvador is probably the oldest commercial cultivar and was developed in Java (Indonesia) in 1942. It is a heterogeneous mixture of at least two varieties, about 75% “vulgaris” (palmate leaf) and 25% “virdis” (cordate leaf). El Salvador is the parent to most Cuban and Taiwanese types (Dempsey, 1975). Tainung 2 was developed in Taiwan and is a strain selected from Tainung 1 (selected from cultivar El Salvador) (USDA, 2004). Tainung 2 was 93% (Table 5.3) similar to Whitten, a selection from the segregating array of a cross of Everglades 41 and a selection of Guatemala 45 (N. Sinclair, personal communication).

The Russian breeding lines formed the second subgroup with genetic distances that ranged from 0.91 to 0.94 (Table 5.3). No pedigree information could be found for Endora and these breeding lines.

Guatemala 4 was genetically the most distinct genotype with a genetic similarity of 85% to the other 18 genotypes. The cultivar was developed in Guatemala from relatively simple crosses. One parent was the somewhat photo-insensitive, entire leaf, red stem “Tingo Maria” (var. “simplex”) and the other parent was El Salvador (Dempsey, 1975).

The genetic similarity over all varieties ranged from 0.84 to 0.94. The closest related varieties using Dice’s coefficient were KY33 and K521 with a genetic similarity of 0.942 (Table 5.3).

Table 5.3a Genetic distances for 19 kenaf genotypes obtained using Dice similarity coefficient.

	Cuba 108	Dowling	El Salvador	Endora	Everglades 41	Everglades 71	Gregg	SF 459	Tainung 2
Dowling	0.875								
El Salvador	0.885	0.884							
Endora	0.882	0.879	0.911						
Everglades 41	0.877	0.870	0.911	0.908					
Everglades 71	0.914	0.878	0.904	0.893	0.913				
Gregg	0.894	0.899	0.886	0.872	0.914	0.906			
SF 459	0.883	0.872	0.899	0.900	0.918	0.901	0.900		
Tainung 2	0.903	0.904	0.908	0.915	0.899	0.910	0.899	0.897	
Whitten	0.881	0.881	0.887	0.907	0.884	0.885	0.868	0.889	0.923
***Hibiscus* sp. 485**	0.864	0.865	0.875	0.886	0.867	0.868	0.866	0.883	0.893
K503	0.851	0.849	0.868	0.876	0.857	0.854	0.866	0.866	0.883
K124	0.851	0.875	0.888	0.892	0.870	0.861	0.874	0.869	0.899
***Hibiscus* sp. a**	0.847	0.851	0.880	0.877	0.885	0.874	0.880	0.875	0.883
K258	0.854	0.864	0.889	0.898	0.863	0.857	0.867	0.867	0.895
***Hibiscus* sp. b**	0.878	0.880	0.891	0.911	0.871	0.895	0.871	0.883	0.905
Guatemala 4	0.832	0.822	0.835	0.871	0.830	0.840	0.821	0.834	0.856
KY33	0.870	0.858	0.874	0.914	0.871	0.867	0.861	0.880	0.895
K521	0.847	0.864	0.881	0.914	0.860	0.854	0.854	0.865	0.898

Table 5.3b Genetic distances for 19 kenaf genotypes obtained using Dice similarity coefficient.

	Whitten	*Hibiscus* sp. 485	K503	K124	*Hibiscus* sp. a	K258	*Hibiscus* sp. b	Guatemala 4	KY33
***Hibiscus* sp. 485**	0.899								
K503	0.875	0.872							
K124	0.901	0.866	0.912						
***Hibiscus* sp. a**	0.892	0.863	0.887	0.874					
K258	0.894	0.856	0.908	0.912	0.880				
***Hibiscus* sp. b**	0.913	0.884	0.878	0.888	0.884	0.915			
Guatemala 4	0.854	0.836	0.847	0.859	0.856	0.863	0.855		
KY33	0.893	0.848	0.898	0.907	0.883	0.914	0.900	0.876	
K521	0.890	0.861	0.911	0.920	0.887	0.938	0.900	0.871	0.942

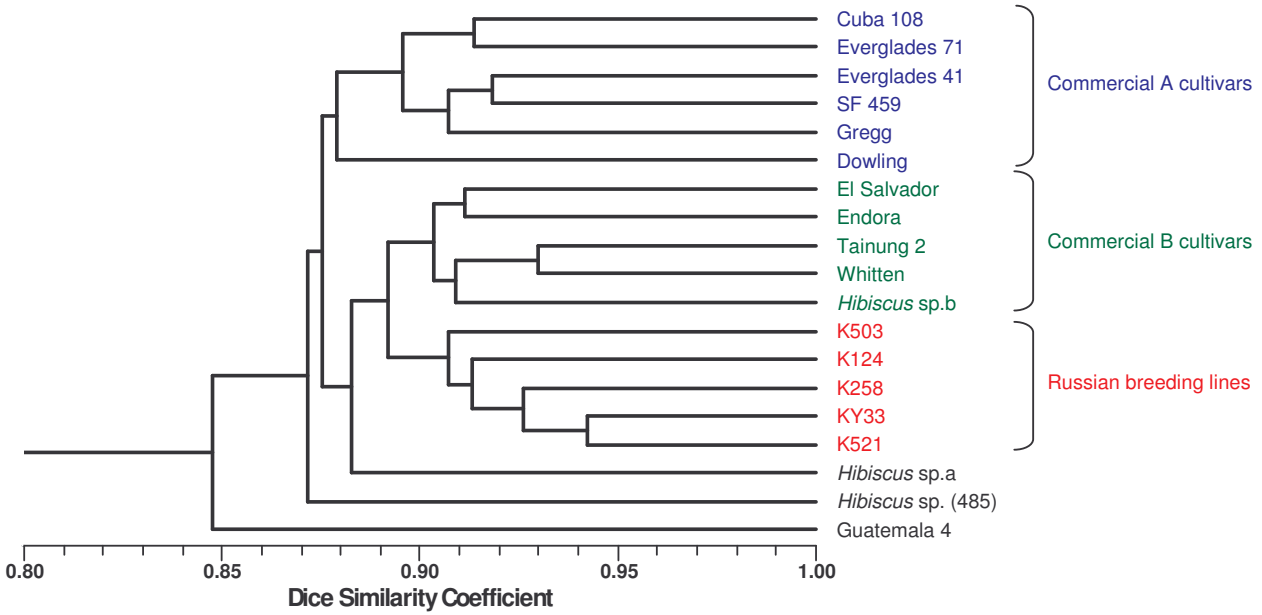

Fig. 5.1 Dendrogram based on AFLP data constructed for 19 kenaf genotypes, using Dice's coefficient of similarity and UPGMA clustering using the NTSYS-pc version 2.02i computer programme.

5.5 Conclusions

AFLP analysis was successful in detecting genetic diversity and determining genetic relationships within 19 selected kenaf genotypes. All 19 genotypes could be uniquely distinguished from each other.

A low level of genetic diversity was detected, even though three wild type genotypes were included. The most distinct genotype, Guatemala 4, was still 85% similar to all other genotypes.

The genotypes clustered more or less according to known pedigree and/or origin data. Two of the three wild types (*Hibiscus* sp. no. 485 and *Hibiscus* sp. a) clustered separately from the commercial cultivars and Russian breeding lines. One of the wild types, *Hibiscus* sp. b clustered with some of the commercial cultivars. This might be due to the fact that kenaf is a relatively new breeding crop and some of the cultivars could recently have been developed from the wild types. The ploidy number of the cultivated and wild types is the same, enabling cross-pollination between cultivated and wild types.

The commercial A cultivars in the first subgroup all originated from central and North America, and surrounding islands (Texas, Cuba, and El Salvador). The Russian breeding lines all grouped together. The commercial B cultivars subgroup was the only group that contained genotypes from different geographic origins.

Traditionally, morphological characters provided very limited information for varietal identification of kenaf germplasm (Deng *et al*., 1994; Siepe *et al*., 1997; Cheng *et al*., 2002). On the contrary, AFLP analysis in this study provided a reliable molecular tool for the identification of kenaf genotypes, although relatively low genetic diversity was found in the selected kenaf genotypes. Results from this study confirmed results obtained by Cheng *et al*. (2004) that also detected low levels of genetic diversity.

CHAPTER 6

GENOTYPE X ENVIRONMENT INTERACTION IN KENAF (*H. CANNABINUS* L.) YIELD

6.1 Introduction

The basic cause for differences between genotypes in their yield stability is a wide occurrence of genotype x environment (G x E) interactions. G x E interaction is a differential genotypic expression across environments. Genotypes refer to the set of genes possessed by individuals that are important for the expression of traits under investigation. The environment is usually defined as all non-genetic factors that influence expression of traits. It may include all sets of biophysical factors including water, nutrition, temperature, and diseases that influence the growth and development of individuals and thereby influencing expression of traits (Basford and Cooper, 1998).

When the effects of environmental differences are large, it may be expected that the interaction of G x E will also be large. As a result it is not only average performance that is important in genotype evaluation programmes, but also the magnitude of interactions, i.e. one cultivar may have the highest yield in some environments, while a second cultivar may excel in other environments (Fehr, 1991; Gauch and Zobel, 1997).

According to Romagosa and Fox (1993), G x E interaction reduces association between phenotypic and genotypic values, and may cause promising selections from one environment to perform poorly in another, forcing plant breeders to examine genotypic adaptation. Its measurement is also important to determine an optimum breeding strategy for releasing genotypes with adaptation to target environments.

The study of G x E interaction is particularly relevant for countries that have diversified agro-ecologies. Under such diversified agro-ecological conditions,

the breeder should be able to select desirable genotypes without losing valuable germplasm and other vital resources. Hence, agro-ecological diversity could complicate breeding and testing of improved varieties with adequate adaptation, but it could also permit identification of extreme environmental conditions that might offer selection pressure from different stresses (Romagosa and Fox, 1993).

Changes in relative rankings appear to be the inevitable consequence of growing a set of plant genotypes in more than one location or season. Thus, for plant breeders large G x E interaction impedes progress from selection and has important implications for testing and cultivar release (Smithson and Grisley, 1992).

Performance tests over a series of environments give information on G x E interactions at population level, but from a practical point of view, it is important to measure the stability of performance of an individual genotype. Variation in genotypic yield response in different environments (location and/or years) in multi-environment yield trials is known as G x E interaction. The effects of genotypes and environments are statistically non-additive, which means that differences between genotypes depend on the environment. For data sets with more than two genotypes and more than two environments, G x E interactions are commonly calculated by analyses of variance (ANOVA) techniques, leading to an estimated variance component for G x E interactions (Eberhart and Russel, 1966).

G x E interaction is a major concern in plant breeding for two main reasons: it reduces progress from selection, and secondly, it makes cultivar recommendation difficult, because it is statistically impossible to interpret the main effect. G x E interaction occurs both in short-term (three to four years testing at a location) and long-term (several years at several locations) crop performance trials (Eberhart and Russel, 1966). Several methods have been proposed to analyze G x E interaction (Lin *et al.*, 1986; Becker and Leon, 1988; Kang, 1990).

An understanding of environmental and genotypic causes of G x E interaction is important at all stages of plant breeding, including ideotype design, parent selection based on traits, and selection based on yield. Understanding of the causes of G x E interaction can be used to establish breeding objectives, identify ideal test conditions, and formulate recommendations for areas of optimal cultivar adaptation (Jackson *et al*., 1998; Yan and Hunt, 1998).

Kenaf is a tropical crop with high water use requirement. Crane (1947) stated that 500-625 mm of rainfall over a period of 5-6 months is essential for the successful production of kenaf fiber and a well-distributed rainfall of about 125 mm for each month during the growing season leads to an optimum yield (Mambelli and Grandi, 1995). Little information is available on the water requirements for growing kenaf under irrigated conditions, particularly with regard to increasing their vegetative growth. Kenaf requires 780-1200 mm of irrigation for optimal growth and production (Dempsey, 1975) and therefore, the large water requirement of kenaf could be a problem in areas where irrigation water is limited and the rainfall low. Most irrigation studies have focused on improving fiber production in kenaf (Bañuelos *et al*., 2002). The aim of this study was to determine the genotype x environment interaction of nine kenaf cultivars in two environments in the Winterton area. Since kenaf is a new crop to South Africa, it is necessary to determine the best performing and most stable cultivars for commercial production in the targeted production area.

6.2 Materials and methods

Nine kenaf cultivars were used in this study (Table 6.1). Trials were planted on 4 November 2003 in the Winterton area. Plots consisted of six rows, 9 m in length with a 25 cm inter-row and 10 cm intra-row spacing. Trial 1 was planted in canola stubble, and trial 2 in prepared seedbeds. Four replications of nine cultivars were planted in a randomised block design. After a month, seeds that did not germinate were replanted. Trial 1 was planted under irrigation, and trial 2 under dry land conditions.

Table 6.1 A list of kenaf cultivars used for G x E interaction and stability analyses.

Entry	Name
1	Cuba 108
2	Dowling
3	El Salvador
4	Endora
5	Everglades 41
6	Everglades 71
7	Gregg
8	SF 459
9	Tainung 2

The middle four rows were harvested, after 1 m at each end was removed. Final plot size was therefore 7 m x 1 m = 7 m^2. A factor of 1.4286 was used to calculate ton per hectare from yield per plot. Ten plants of each plot were weighed, then defoliated and weighed again. These 10 defoliated stalks per plot were used to measure plant length, and base and middle stem diameter. One meter of two stalks from each plot was taken from the middle of the plant down. They were weighed immediately. They were dried for 12 hours at 100℃ and then weighed again. Percentage dry mass was calculated. Dry mass per hectare was calculated as the percentage dry mass of defoliated ton per hectare.

6.2.1 Statistical analyses

Analyses of variance were done on genotypes, locations and G x E interactions for the measured characteristics. The following statistical analyses were performed:

1. Separate trial analysis for each location
2. Combined analysis across locations for one year
3. Stability analyses by using:
    a) Ecovalence (Wricke, 1962)

b) Cultivar superiority (performance) measure (Lin and Binns, 1988)
c) Variance of ranks (Nassar and Huehn, 1987)

4. Correlations between measured traits

All analyses were done using AGROBASE (AGROBASE, 2000).

6.3 Results and discussion

6.3.1 Separate analysis of the trials

ANOVA

Separate analysis of variance for location 1 (irrigated conditions) indicated significant ($P<0.05$) differences among entries for plant length only (Table 6.2). However, highly significant ($P<0.01$) differences among entries for plant length, stem base diameter, and middle stem diameter occurred for location 2 (dry land conditions). With the exception of plant length, there were no significant differences between blocks (Table 6.2).

Mean values

Wet material and defoliated stem yield

El Salvador gave the highest wet material and defoliated stem yield for both locations. Tainung 2 and Dowling performed second and third best for location 1, while Endora and Everglades 71 performed second and third best for location 2. El Salvador performed significantly better than Gregg, Endora, Cuba 108, and SF 459 at location one as well as Dowling, SF 459, and Gregg at location 2 (Table 6.3).

Dry stem yield

Tainung 2 had a significantly higher dry stem yield than all other cultivars at location 1. Dowling and El Salvador performed second and third best. There were no significant differences among the rest of the cultivars. Endora gave the highest dry material yield at location 2, but the yield was not significantly higher than Everglades 71 and El Salvador that performed second and third

best. Endora performed significantly better than Cuba 108, Dowling, SF 459, and Gregg at location 2 (Table 6.3).

Stem base diameter

Endora and Everglades 41 performed the best at location 1; however, no significant differences occurred between cultivars for stem base diameter. At location 2, Tainung 2 gave the highest stem base diameter and it was significantly higher than all cultivars, except for Endora (Table 6.3).

Middle stem diameter

At location 1, Endora gave the highest middle stem diameter, but it was not significantly higher than Everglades 41 and El Salvador that performed second and third best. Endora performed significantly better than Cuba 108, Dowling, Tainung 2, and Gregg. At location 2, Endora again gave the highest middle stem diameter and it was significantly higher than all other cultivars, except for Tainung 2 that performed second best (Table 6.3).

Plant length

Tainung 2 gave the highest plant length at location 1, but it was not significantly higher than El Salvador and Endora that performed second and third best. However, Tainung 2 performed significantly better than Dowling, Cuba 108, SF 459, and Gregg. At location 2 Endora gave the highest plant length, but it was not significantly higher than Tainung 2, Gregg, and El Salvador. Significant differences occurred between Endora and the rest of the cultivars (Table 6.3).

Table 6.2 Mean squares of analysis of variance for six measured traits of nine kenaf cultivars evaluated across two environments.

	Source	Mean squares					
		Wet t/ha	**Defol. t/ha**	**Dry t/ha**	**Base diameter**	**Middle diameter**	**Length**
Loc 1	Entry	797.303	466.947	106.552	0.218	0.356	677.361*
	Block	679.785	445.005	83.753	1.227	0.079	1478.250**
Loc 2	Entry	525.185	338.611	29.466	2.225**	1.023**	1256.611**
	Block	457.289	402.830	35.743	0.215	0.214	176.741

*,** = significant at 0.05 and 0.01 probability levels respectively; Defol = defoliated.

Table 6.3 Mean values and LSD for six measured traits of nine kenaf cultivars evaluated across two environments.

Cultivar	**Wet t/ha**		**Defol. t/ha**		**Dry t/ha**		**Base diameter**		**Middle diameter**		**Length**	
	Loc 1	**Loc 2**	**Loc 1**	**Loc 2**	**Loc 1**	**Loc 2**	**Loc 1**	**Loc 2**	**Loc 1**	**Loc 2**	**Loc 1**	**Loc 2**
Cuba 108	100.36	84.47	79.72	69.26	20.38	13.49	7.65	8.15	6.25	6.53	317.25	335.75
Dowling	126.08	74.82	102.12	64.23	28.09	13.08	7.78	6.63	6.08	5.35	319.75	305.00
El Salvador	135.36	102.40	108.14	83.88	26.25	17.04	7.78	8.03	6.60	6.58	341.75	349.25
Endora	104.11	98.25	84.38	80.91	23.62	18.37	8.20	8.90	6.68	7.10	335.50	357.75
Everglades 41	123.75	90.08	95.30	73.59	26.07	14.52	8.20	7.68	6.63	6.40	334.25	336.00
Everglades 71	115.54	95.00	90.88	78.52	23.99	18.26	7.88	7.58	6.50	5.90	334.75	327.75
Gregg	110.54	71.61	87.44	59.60	22.78	11.09	7.63	8.03	5.88	6.20	309.00	351.50
SF 459	98.75	71.97	81.31	57.95	22.50	11.53	7.80	7.65	6.33	6.23	313.00	320.00
Tainung 2	134.65	87.72	106.92	70.05	38.06	14.95	7.55	9.15	5.98	6.75	344.00	355.00
Mean	116.570	86.256	92.910	70.887	25.747	14.701	7.828	7.975	6.322	6.336	327.694	337.556
C.V. (%)	16.60	19.09	17.35	19.72	27.24	25.57	8.75	6.84	6.83	5.45	4.84	4.18
LSD (0.05)	23.4141	19.9163	19.5040	16.9072	8.4843	4.5471	0.8282	0.6600	0.5227	0.4176	19.1919	17.0549

Defol = defoliated.

6.3.2 Combined analysis of variance across two locations for one year

ANOVA

The combined analysis of variance, which was carried out across two locations for one year, showed highly significant ($P<0.01$) differences among locations for the three measured traits (Table 6.4). Influences of entry and entry x location were not significant.

Mean values

Wet material yield

El Salvador gave the highest wet material yield in combined analysis of variance, but the yield was not significantly higher than Tainung 2 and Everglades 41 that performed second and third best. El Salvador performed significantly better than Dowling, Cuba 108, Gregg, and SF 459 (Table 6.5 and Fig. 6.1).

Defoliated stem yield

El Salvador gave the highest defoliated stem yield in combined analysis, but the yield was not significantly higher than Tainung 2 and Everglades 71 that performed second and third best. However, El Salvador performed significantly better than Cuba 108, Gregg, and SF 459 (Table 6.5 and Fig. 6.2).

Dry stem yield

Tainung 2 gave the highest dry stem yield in combined analysis, but the yield was not significantly higher than El Salvador, Everglades 71, and Endora. Significant differences occurred between Tainung 2 and the rest of the cultivars (Table 6.5 and Fig. 6.3).

Table 6.4 Mean squares of the combined analysis of variance for three measured traits of nine kenaf entries evaluated across two environments.

	Mean squares		
Source	Wet t/ha	Defol. t/ha	Dry t/ha
Entry	892.239	534.646	74.908
Locations	16541.380**	8729.961**	2196.166**
Entry x location	430.262	270.919	61.110

*,** = significant at 0.05 and 0.01 probability levels respectively; Defol = defoliated.

Table 6.5 Mean values for three measured traits and rank of nine kenaf entries evaluated across two environments.

	Mean values					
Cultivar	Wet t/ha	Rank	Defol. t/ha	Rank	Dry t/ha	Rank
Cuba 108	92.41	7	74.49	7	16.94	8
Dowling	100.45	6	83.17	5	20.58	5
El Salvador	118.88	1	96.01	1	21.64	2
Endora	101.18	5	82.65	6	21.00	4
Everglades 41	106.91	3	84.44	4	20.29	6
Everglades 71	105.27	4	84.70	3	21.12	3
Gregg	91.07	8	73.52	8	16.93	9
SF 459	85.36	9	69.63	9	17.01	7
Tainung 2	111.18	2	88.48	2	26.50	1
Mean	101.413		81.899		20.224	
C.V. (%)	17.72		18.42		27.82	
LSD (0.05)	18.062		15.167		5.656	

Defol = defoliated.

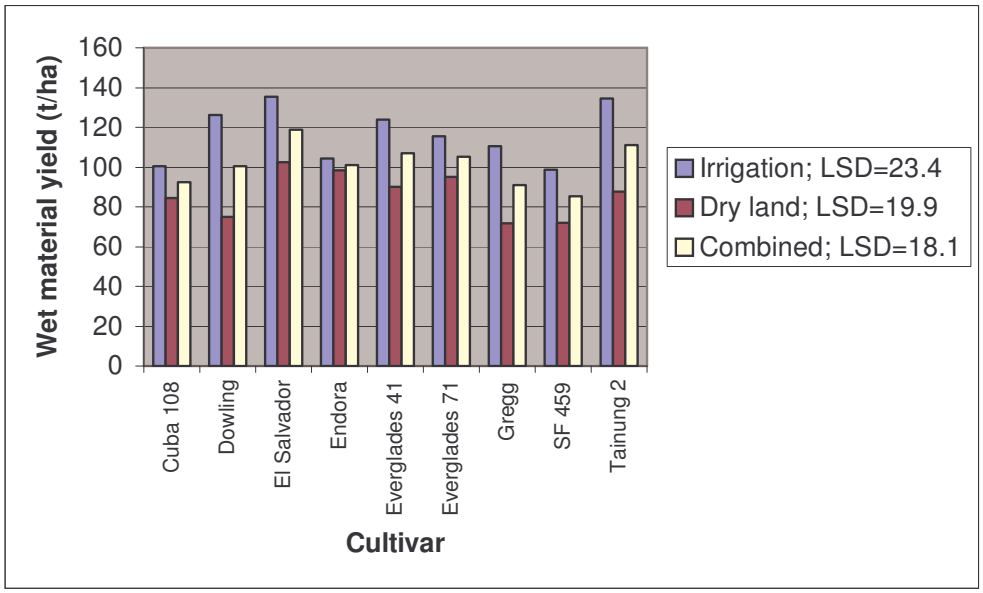

Fig. 6.1 Wet material yield of nine kenaf cultivars for irrigated, and dry land conditions and combined analysis.

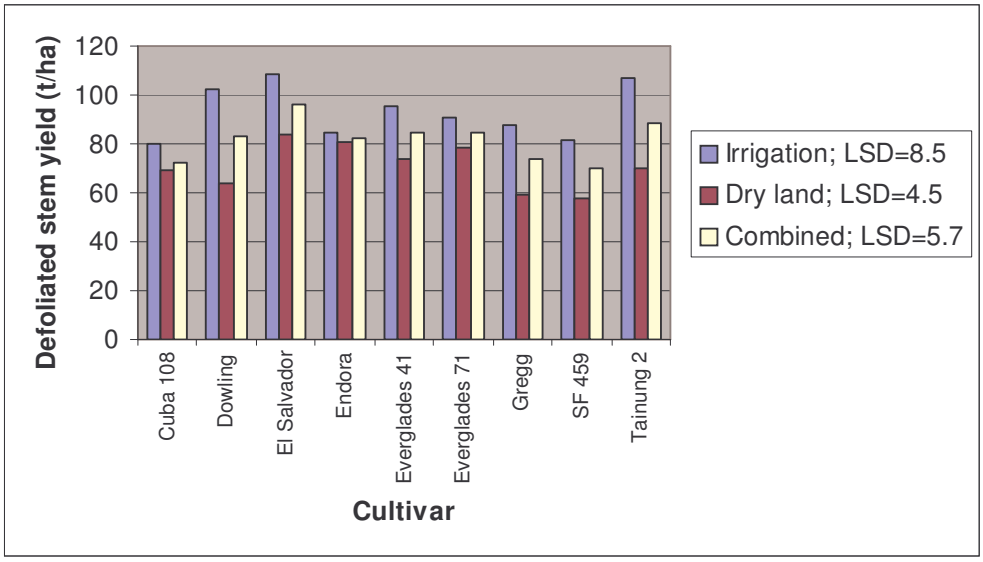

Fig. 6.2 Defoliated stem yield of nine kenaf cultivars for irrigated, and dry land conditions and combined analysis.

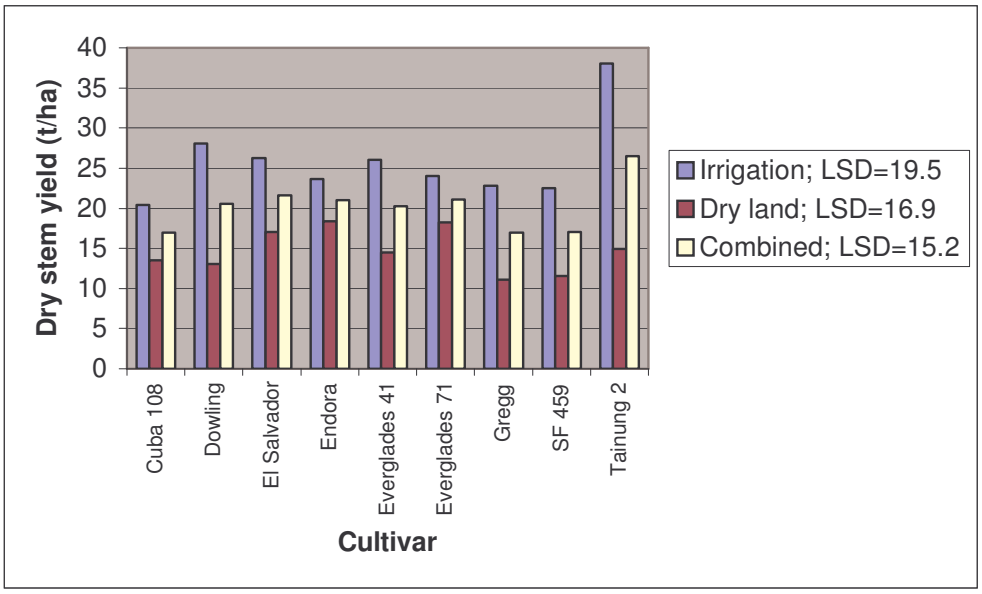

Fig. 6.3 Dry stem yield of nine kenaf cultivars for irrigated, and dry land conditions and combined analysis.

6.3.3 Stability analyses

a) Wricke's ecovalence analysis

Wricke's ecovalence (1962) is one of the methods frequently used to determine stability of genotypes based on G x E interaction effects. It indicates the contribution of each genotype to G x E interaction. Consequently, genotypes with small ecovalence will have small deviations from the mean across environments and thus will be considered more stable (Purchase, 1997).

Ecovalence was computed for nine entries of kenaf and results are summarized in Table 6.6. With regards to wet material yield, El Salvador followed by Everglades 41 and SF 459 were the most stable cultivars. Dowling and Endora were unstable cultivars. Everglades 41 was the most stable cultivar for defoliated stem yield, followed by SF 459 and El Salvador. Dowling and Endora were unstable cultivars. SF 459, followed by Everglades 41 and Gregg were the most stable cultivars for dry stem yield. Endora and Tainung 2 were unstable cultivars.

Table 6.6 Wricke's ecovalence value and ranks for nine kenaf genotypes tested in two environments.

Cultivar	Ecovalence					
	Wet t/ha	Rank	Defol t/ha	Rank	Dry t/ha	Rank
Cuba 108	103.96	6	66.88	6	8.65	6
Dowling	219.19	8	125.81	8	7.86	5
El Salvador	3.50	1	2.50	3	1.69	4
Endora	299.01	9	172.05	9	16.82	8
Everglades 41	5.66	2	0.05	1	0.13	2
Everglades 71	47.82	5	46.64	5	14.13	7
Gregg	37.13	4	16.87	4	0.21	3
SF 459	6.22	3	0.89	2	0.00	1
Tainung 2	138.04	7	110.15	7	72.74	9

Defol = defoliated.

b) Lin and Binns' cultivar superiority measure

According to Lin and Binns (1988), the superiority measure (Pi) of cultivars is estimated by the squares of differences between an entry mean and maximum entry mean, summed and divided by twice the number of locations. Cultivars with the lowest Pi values are considered most stable and tend to have higher yields than other genotypes.

Table 6.7 represents the cultivar superiority measure (Pi) for three traits of nine kenaf entries tested in two environments. Accordingly, El Salvador was the most stable cultivar with regards to wet material yield as well as defoliated stem yield and was followed by Tainung 2 and Everglades 41. SF 459, Cuba 108, and Gregg were unstable cultivars. With regards to dry stem yield, Tainung 2, followed by Dowling and El Salvador were the most stable cultivars. Cuba 108, SF 459, and Gregg were unstable cultivars. In most cases, the ranks of cultivar superiority measure were in harmony with that of the overall mean yield of measured traits.

Table 6.7 Lin and Binns' (1988) cultivar superiority measure (Pi) and ranks of nine kenaf genotypes tested in two environments.

Cultivar	Cultivar superiority measure					
	Wet t/ha	Rank	Defol t/ha	Rank	Dry t/ha	Rank
Cuba 108	396.61	7	255.30	8	84.06	9
Dowling	211.64	5	105.58	5	31.87	2
El Salvador	0.00	1	0.00	1	35.30	3
Endora	248.41	6	143.28	6	52.11	6
Everglades 41	71.63	3	67.71	3	39.61	4
Everglades 71	111.88	4	81.62	4	49.47	5
Gregg	390.99	8	254.47	7	71.64	7
SF 459	566.52	9	348.13	9	72.19	8
Tainung 2	54.00	2	48.18	2	2.93	1

Defol = defoliated.

c) Nassar and Huehn's variance of ranks

Table 6.8 presents Nassar and Huehn's (1987) non-parametric measures of stability for yield of three traits of kenaf entries evaluated in two environments. Both S1 (mean absolute rank differences) and S2 (variance of ranks) of genotypes over test environments are measurements of stability (Huehn, 1990). However, the use of S1 was more preferred than S2 for many practical applications. S1 was reported to be easy to calculate, interpret and has efficient tests of significance, according to Huehn (1990).

Wet material yield

El Salvador had the smallest changes in ranks (S1) and was considered the most stable cultivar. El Salvador also had the highest mean wet material yield. The next most stable cultivars were Everglades 41 and SF 459. Dowling and Endora were significantly unstable cultivars (Table 6.8a).

Defoliated stem yield

SF 459 had the smallest changes in ranks (S1) and was considered the most stable cultivar. However, SF 459 had the lowest mean defoliated stem yield. The next most stable cultivars were El Salvador and Everglades 41. El Salvador gave the highest mean yield and was followed by Tainung 2. Dowling and Endora were significantly unstable cultivars (Table 6.8b).

Dry stem yield

SF 459 had the smallest changes in ranks (S1) and was considered the most stable cultivar. However, SF 459 was ranked seventh for dry stem yield. The next most stable cultivars were El Salvador and Everglades 41. Endora and Tainung 2 were significantly unstable (Table 6.8c).

Table 6.8a Mean absolute rank difference (S1) and variance of ranks (S2) of Nassar and Huehn (1987) for wet material yield (ton/ha) of nine kenaf entries tested in two environments.

	Nassar-Huehn Rank Test: Wet material yield					
Entry	**S1**	**Z1**	**S2**	**Z2**	**Overall mean yield**	**Rank**
Cuba 108	6.00	2.03	9.00	0.09	92.41	7
Dowling	8.00	5.57*	16.00	1.42	100.45	6
El Salvador	0.00	1.93	0.00	0.72	118.88	1
Endora	8.00	5.57*	16.00	1.42	101.18	5
Everglades 41	2.00	0.20	1.00	0.52	106.91	3
Everglades 71	4.00	0.24	4.00	0.12	105.27	4
Gregg	4.00	0.24	4.00	0.12	91.07	8
SF 459	2.00	0.20	1.00	0.52	85.36	9
Tainung 2	6.00	2.03	9.00	0.09	111.18	2

Overall chi-square for stability = 18.00, 9 df individual Z1 distributed as single df chi-squares; overall chi-square for stability = 5.00, 9 df individual Z2 distributed as single df chi-squares; *,** significantly different at 0.05, and 0.01 probability levels, respectively.

Table 6.8b Mean absolute rank difference (S1) and variance of ranks (S2) of Nassar and Huehn (1987) for defoliated stem yield (ton/ha) of nine kenaf entries tested in two environments.

	Nassar-Huehn Rank Test: Defoliated stem yield					
Entry	**S1**	**Z1**	**S2**	**Z2**	**Overall mean yield**	**Rank**
Cuba 108	6.00	2.03	9.00	0.09	74.49	7
Dowling	8.00	5.57*	16.00	1.42	83.17	5
El Salvador	2.00	0.20	1.00	0.52	96.01	1
Endora	8.00	5.57*	16.00	1.42	82.65	6
Everglades 41	2.00	0.20	1.00	0.52	84.44	4
Everglades 71	4.00	0.24	4.00	0.12	84.70	3
Gregg	4.00	0.24	4.00	0.12	73.52	8
SF 459	0.00	1.93	0.00	0.72	69.63	9
Tainung 2	6.00	2.03	9.00	0.09	88.48	2

Overall chi-square for stability = 18.00, 9 df individual Z1 distributed as single df chi-squares; overall chi-square for stability = 5.00, 9 df individual Z2 distributed as single df chi-squares; *,** significantly different at 0.05, and 0.01 probability levels, respectively.

Table 6.8c Mean absolute rank difference (S1) and variance of ranks (S2) of Nassar and Huehn (1987) for dry stem yield (ton/ha) of nine kenaf entries tested in two environments.

	Nassar-Huehn Rank Test: Dry stem yield					
Entry	**S1**	**Z1**	**S2**	**Z2**	**Overall mean yield**	**Rank**
Cuba 108	4.00	0.24	4.00	0.12	16.94	8
Dowling	6.00	2.03	9.00	0.09	20.58	5
El Salvador	2.00	0.20	1.00	0.52	21.64	2
Endora	8.00	5.57*	16.00	1.42	21.00	4
Everglades 41	2.00	0.20	1.00	0.52	20.29	6
Everglades 71	6.00	2.03	9.00	0.09	21.12	3
Gregg	4.00	0.24	4.00	0.12	16.93	9
SF 459	0.00	1.93	0.00	0.72	17.01	7
Tainung 2	8.00	5.57*	16.00	1.42	26.50	1

Overall chi-square for stability = 18.00, 9 df individual Z1 distributed as single df chi-squares; overall chi-square for stability = 5.00, 9 df individual Z2 distributed as single df chi-squares; *,** significantly different at 0.05, and 0.01 probability levels, respectively.

6.3.4 Comparison of stability parameters

Wricke's ecovalence analysis, Lin and Binns's cultivar superiority measure and Nassar and Huehn's variance of ranks were harmonious in sorting out the most stable genotype, El Salvador with regards to wet material yield.

According to Wricke's stability parameter and Nassar and Huehn's ranks test, El Salvador, Everglades 41, and SF 459 were the most stable cultivars. According to Lin and Binns's stability parameter, El Salvador, Tainung 2 and Everglades 41 were the most stable cultivars (Table 6.9). In comparison with the mean yields for the three measured traits of nine kenaf entries in the combined analysis (Table 6.5), El Salvador, Tainung 2, and Everglades 41 were the cultivars that tended to have the highest yields. Although SF 459 was stable according to Wricke's and Nassar and Huehn's stability analyses, the cultivar performed poorly with regards to the three measured traits. The cultivar was ranked last for both wet and defoliated stem yield and seventh for dry stem yield in the combined analysis (Table 6.5).

Table 6.9 Comparison of three stability parameters.

Stability parameter	Rank	Wet t/ha	Defol t/ha	Dry t/ha
Wricke	1	El Salvador	Everglades 41	SF 459
	2	Everglades 41	SF 459	Everglades 41
	3	SF 459	El Salvador	Gregg
Lin and Binns	1	El Salvador	El Salvador	Tainung 2
	2	Tainung 2	Tainung 2	Dowling
	3	Everglades 41	Everglades 41	El Salvador
Nassar and Huehn	1	El Salvador	SF 459	SF 459
	2	Everglades 41	El Salvador	El Salvador
	3	SF 459	Everglades 41	Everglades 41

Defol = defoliated.

6.3.5 Correlations between measured traits

Correlations between seven measured traits for trial 1 (irrigation) are shown in Table 6.10. There were highly significant ($P<0.01$) positive correlations between wet yield and defoliated yield, wet yield and dry mass yield, defoliated yield and dry mass yield, dry mass yield and plant length, stem base diameter and middle stem diameter. Significantly ($P<0.05$) positive correlations occurred between wet yield and plant length, defoliated stem yield and plant length, stem base diameter and plant length, and middle stem diameter and plant length. Dry mass yield was negatively affected by foliar percentage, indicating that dry mass yield and therefore fiber yield, increases with reduced foliar growth.

Correlations between seven measured traits for trial 2 (dry land conditions) are shown in Table 6.11. There were highly significant ($P<0.01$) positive correlations between wet yield and defoliated yield, wet yield and dry mass yield, defoliated yield and dry mass yield, stem base diameter and middle stem diameter, base stem diameter and plant length, middle stem diameter and plant length, foliar percentage and base stem diameter, and foliar percentage and middle stem diameter.

Correlations between seven measured traits for the combined analysis are shown in Table 6.12. Highly significant ($P<0.01$) positive correlations occurred between wet yield and defoliated yield, wet yield and dry mass yield, defoliated yield and dry mass yield, stem base diameter and middle stem diameter, base stem diameter and plant length, and middle stem diameter and plant length. Significantly ($P<0.05$) positive correlations occurred between foliar percentage and base stem diameter, and foliar percentage and middle stem diameter.

Significantly positive correlations between wet material yield and dry stem yield implicated that, as wet material yield increases, dry stem yield also increases. However, foliar percentage was negatively correlated with dry mass yield and therefore, as foliar percentage increased, dry stem yield decreased. In order to improve dry stem yield (ton per hectare) and therefore

fiber yield, vegetative growth of the stem can be increased through cultural practices including optimum irrigation (Amaducci *et al*., 2000; Bañuelos *et al*., 2002), fertilization (Kaldor *et al*., 1990; Williams, 1966), and higher plant populations (Crane *et al*., 1946).

Under irrigated conditions, as plant length increased, wet material and dry stem yields increased significantly. However, mean plant heights were slightly higher for most cultivars under dry land conditions than for irrigation.

Table 6.10 Simple correlations among eight traits of kenaf cultivars tested under irrigation.

	Wet t/ha	Defol t/ha	Dry t/ha	Base dm	Middle dm	Plant length
Defol t/ha	0.982**					
Dry t/ha	0.778**	0.807**				
Base dm	0.066	0.033	-0.02			
Middle dm	0.180	0.149	0.00	0.632**		
Plant length	0.432*	0.409*	0.476**	0.339*	0.421*	
Fol perc	0.024	-0.161	-0.186	0.133	0.111	0.101

*,** = significantly correlated at 0.05 and 0.01 levels respectively, defol = defoliated, dm = diameter, fol perc = foliar percentage.

Table 6.11 Simple correlations among eight traits of kenaf cultivars tested under dry land conditions.

	Wet t/ha	Defol t/ha	Dry t/ha	Base dm	Middle dm	Plant length
Defol t/ha	0.980**					
Dry t/ha	0.823**	0.842**				
Base dm	0.077	-0.024	0.066			
Middle dm	0.147	0.054	0.069	0.857**		
Plant length	0.325	0.267	0.285	0.603**	0.560**	
Fol perc	-0.085	-0.275	-0.244	0.508**	0.470**	0.276

*,** = significantly correlated at 0.05 and 0.01 levels respectively, defol = defoliated, dm = diameter, fol perc = foliar percentage.

Table 6.12 Simple correlations among eight traits of kenaf cultivars for the combined analysis.

	Wet t/ha	Defol t/ha	Dry t/ha	Base dm	Middle dm	Plant length
Defol t/ha	0.986**					
Dry t/ha	0.862**	0.865**				
Base dm	-0.002	-0.05	-0.052			
Middle dm	0.120	0.075	0.011	0.766**		
Plant length	0.160	0.151	0.140	0.488**	0.500**	
Fol perc	0.183	0.019	0.085	0.303*	0.303*	0.100

*,** = significantly correlated at 0.05 and 0.01 levels respectively, defol = defoliated, dm = diameter, fol perc = foliar percentage.

6.4 Conclusions

Stem yield did not differ significantly between cultivars and replications; however, highly significantly differences occurred between the two trials for wet material and dry stem yield. All cultivars had the highest yields under irrigation. Trial means for dry material yield under irrigation were almost twice that for dry land conditions. Bañuelos *et al*. (2002) reported that total dry matter production was significantly increased by irrigation of kenaf. Mambelli and Grandi (1995) also reported that irrigation affected crop growth and that no significant differences occurred between cultivars. They found that the total yield of well-watered crop was twice that of the rainfed control. Therefore, to obtain the highest dry stem yield and therefore fiber yield, it is best to plant kenaf under irrigation.

The basal and middle stem diameter was more or less the same for both trials and no significant differences occurred between cultivars and replications under irrigation. However, highly significant differences occurred between cultivars under dry land conditions.

Plant length differed significantly among cultivars under dry land conditions, and among cultivars and replications under irrigated conditions. However, plant length was on average only slightly lower under irrigation than for dry land conditions. Various studies have shown that kenaf plant height appears to be an important component as a contributor to total or stem dry matter (Mambelli and Grandi, 1995).

Under irrigated conditions all plants received the same amount of water and therefore had the ability to perform their best. Water was not a limiting factor and therefore there were no significant differences between cultivars and replications. However, under dry land conditions, water was a limiting factor and differences occurred between cultivars.

El Salvador was the best performing cultivar under dry land and irrigated conditions for wet material and dry stem yield. However, El Salvador did not

perform significantly better than Tainung 2 that performed second best for these two traits. Tainung 2 performed significantly best under irrigated conditions for dry stem yield, but the cultivar was not significantly better than El Salvador in the combined analysis.

El Salvador was stable for the three yield traits (wet material, defoliated stem and dry stem yield) and would therefore be the cultivar of choice for both irrigated and dry land conditions. Tainung 2 will perform the best under irrigated conditions for dry stem yield.

CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Morphologically, different kenaf cultivars could not be distinguished. Leaf shape was the only feature that could help to identify cultivars; however, cultivars with the same leaf shape could not be distinguished. Agronomically, some cultivars performed significantly better in plant height, wet and dry yield, and basal stem diameter than others, but most commercial cultivars were not significantly different for most measured traits. Germination percentage varied from very low to very high with a mean of 67%. This could be due to the age of the seed, and improper storage conditions of the seed. Kenaf seed lose viability rapidly under high humidity conditions because of their high oil content.

Kenaf seed have relatively high oil content and compares with cotton seed and palm oil. Percentages of each fatty acid varied greatly between different cultivars. It is, however, desirable to develop varieties with reduced palmitic acid. There is, however, a potential for genetic improvement of oil quality for different end-uses.

AFLP analysis was successful in detecting genetic diversity and determining genetic relationships within the selected kenaf genotypes. However, a low level of genetic diversity was detected and the cultivars are closely related. This might be due to the fact that kenaf is a relatively new breeding crop and some of the commercial cultivars could recently have been developed from the wild types.

Genotype x environment interactions occurred for kenaf cultivars planted in different environments. Different cultivars performed differently in the two environments planted and the ranks of the cultivars changed as expected. The cultivar that performed best under dry land conditions for dry yield, gave lower dry yields under irrigated conditions. All cultivars performed on average better under irrigated conditions than under dry land conditions. Genotype x

environment x year interactions as well as the fibre yield and quality are currently being tested for the Winterton area.

CHAPTER 8

SUMMARY

Key words: AFLP fingerprinting; fatty acids; genotype x environment interactions; genetic relationships; *Hibiscus cannabinus*; kenaf; morphology; seed oil; yield

- Kenaf (*Hibiscus cannabinus* L.) is one of the world's most economically important fibre crops. The traditional use of kenaf focuses on its fibre production, for the making of products such as ropes, sacks, canvases, and carpets. New applications of kenaf have been developed such as pulping and papermaking, board making, filtration media, potting media, animal feed, and oil absorption. The fibres are also processed for use in the automotive industry. Kenaf was introduced to South Africa during the last few years. The crop is being explored as a potential commercial crop for fibre production in South Africa.

- The objectives of this study were to conduct glasshouse and field trials on commercial kenaf cultivars to evaluate morphological and agronomic differences between them; to compare the oil content and fatty acid composition of the seed of eight cultivars; to determine genetic relationships between commercial cultivars and nine wild type varieties and to determine the genotype x environment (G x E) interactions and stability of commercial cultivars.

- Seeds were obtained from several sources and sown directly into pots. Seedlings were grown in a heat-controlled glasshouse. A field trial was also planted in another location. These plants were used in various investigations.

- Two field trials were planted in two different locations to determine the G x E interactions. The experimental designs were complete randomised blocks with four replications. Eight morphological and

agronomic characters studied were subjected to an ANOVA and significant differences were found for some traits between cultivars tested in the glasshouse. Significant differences also occurred among replications for some traits in both trials. There were not many morphological differences between cultivars and therefore it is difficult to identify individual cultivars merely by morphological characters.

- Seeds of eight cultivars were used to determine the oil content and fatty acid composition of the seed oil. The percentage oil was relatively high for all cultivars; however, there was not much difference between cultivars for oil content. The percentages of each fatty acid varied greatly among different cultivars and therefore there is a potential for genetic improvement in oil quality.

- AFLP analysis was used fingerprint commercial cultivars and three wild type varieties. Six primer combinations between *Eco*R I and *Mse* I were used to determine genetic relationships. A total of 406 fragments were generated, of which 229 (56.4%) were polymorphic. A low level of genetic diversity was detected in kenaf genotypes.

- Separate and combined analyses of variance across locations, three types of stability parameters and correlation analyses were performed.

- The separate trial analyses for two locations have shown significant differences among cultivars for three yield traits. With the exception of plant length, there were no significant differences between replications. The combined analysis of variance across locations showed highly significant differences among locations for three yield traits. There were, however, no significant differences among genotypes and their interactions with localities for three traits. All cultivars performed on average better in location 1 (under irrigated conditions) for six measured traits.

- According to Wricke's (1962) ecovalence, El Salvador, Everglades 41, and SF 459 were the most stable cultivars for the three yield traits respectively. Lin and Binns' (1988) cultivar superiority measure indicated that El Salvador and Tainung 2 were the most stable cultivars for the three traits. Nassar and Huehn's (1987) non-parametric measure of stability revealed that El Salvador and SF 459 had the smallest changes in ranks and thus were the most stable cultivars.

OPSOMMING

- Kenaf (*Hibiscus cannabinus* L.) is een van die wêreld se mees ekonomies belangrike vesel gewasse. Die tradisionele gebruik van kenaf fokus hoofsaaklik op die produksie van vesel vir die maak van tou, sakke, materiaal doeke en matte. Nuwe gebruike van kenaf is ontwikkel en sluit onder andere in: die maak van pulp, papier en bordpapier, filtrerings mediums, potgrond mediums, diere voedsel en olie absorberings materiaal. Die vesels word ook geprosesseer en vir die motor industry aangewend. Kenaf word vir die laaste paar jaar in Suid-Afrika verbou en die gewas word tans ondersoek as ‘n potensiële kommersiële gewas vir die produksie van vesel.

- Die doelstellings van die studie was om morfologiese en agronomiese verskille tussen 10 kommersiële kenaf kultivars te ondersoek; die olie-inhoud en vetsuursamestelling van die saad van agt kultivars te vergelyk; die genetiese afstande en verwantskappe tussen kommersiële kultivars en nege wilde tipes te bepaal en om die genotipe x omgewing (G x E) interaksies en stabiliteit van kommersiële kultivars te ondersoek.

- Sade afkomstig vanaf verskeie bronne is direk in potte gesaai en die saailinge is in ‘n temperatuurbeheerde glashuis gekweek. ‘n Veldproef is ook in ‘n ander lokaliteit geplant. Die volwasse plante was in verskeie ondersoeke gebruik.

- Twee veldproewe is in twee afsonderlike lokaliteite geplant om G x E interaksies te ondersoek. Die eksperimentele ontwerp was in beide gevalle ‘n volledige gerandomiseerde blok ontwerp met vier herhalings. Agt morfologiese en agronomiese eienskappe is met ‘n ANOVA ontleed en betekenisvolle verskille is tussen die kultivars in die glashuisproef vir sekere eienskappe waargeneem. Betekenisvolle

verskille tussen die herhalings is ook vir sekere eienskappe in beide die glashuis- en veldproef waargeneem. Byna geen morfologiese verskille is tussen die kultivars waargeneem nie en dus kan die kultivars nie op morfologiese eienskappe alleen onderskei word nie.

- Die olie-inhoud en vetsuursamestelling van die olie in die sade van agt kultivars is bepaal. Die olie-inhoud van al die kultivars was relatief hoog, maar daar was egter nie groot verskille tussen die kultivars vir olie-inhoud nie. Die vetsuurpersentasies het grootliks tussen die kultivars gevarieer. Daar is dus ‘n potensiaal vir die genetiese verbetering van die olie kwaliteit.

- AFLP analise is gebruik om DNA fingerafdrukke van die kommersiële kultivars sowel as van die drie wilde tipe varieteite te maak. Ses priemstuk kombinasies tussen *Eco*R I en *Mse* I is gebruik om genetiese verwantskappe te bepaal. ‘n Totaal van 406 fragmente is waargeneem waarvan 229 polimorfies was. ‘n Relatiewe lae vlak van genetiese diversiteit was vir die kenaf genotipes waargeneem.

- Afsonderlike en gekombineerde analise van variansie is oor omgewings gedoen. Drie tipes stabiliteitsparameters en korrelasie analises is gedoen.

- Die afsonderlike proefanalises vir die twee omgewings het betekenisvolle verskille tussen kultivars vir drie opbrengs eienskappe aangedui. Daar was egter geen betekenisvolle verskille tussen die herhalings nie. Die gekombineerde analise van variansie oor omgewings het hoogs betekenisvolle verskille tussen lokaliteite vir drie opbrengs eienskappe aangetoon. Daar was egter geen betekenisvolle verskille tussen genotipes en hul interaksies met lokaliteite vir die drie eienskappe nie. Al die kultivars het oor die algemeen beter gepresteer in lokaliteit 1 (onder besproeiing) vir die ses eienskappe gemeet.

- Volgens Wricke (1962) se ekovalensie, was El Salvador, Everglades 41 en SF 459 die mees stabiele kultivars vir die drie opbrengs eienskappe respektiewelik. Lin en Binns (1988) se kultivar superioriteit analise het aangetoon dat El Salvador en Tainung 2 die mees stabiele kultivars vir dieselfde drie eienskappe is. Nassar en Huehn (1987) se nie-parametriese meting van stabiliteit het aangetoon dat El Salvador en SF 459 die kleinste verskil in rangorde toon, en dus die mees stabiele kultivars was.

LIST OF ABBREVIATIONS

α	alpha
AFLP	amplified fragment length polymorphism
AMMI	additive main effects multiplicative interaction
ANOVA	analysis of variance
ARC	Agricultural Research Council, South Africa
℃	degrees Celsius
Ca	calsium
CTAB	cetyl triethyl ammonium bromide
C.V.	coefficient of variation
cm	centimetre
Defol	defoliated
DM	dry material
dm	diameter
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetra-acetate
EtOH	ethanol
FAM	*Eco* + ACA
FAO	UN Food and Agriculture Organization
Fol perc	foliar percentage
g	grams
G x E	genotype by environment interaction
ha	hectare
HCl	hydrochloric acid
HDL	high density lipoprotein
kg	kilogram
km	kilometre
KNP	Kruger National Park, South Africa
ℓ	litre
L	location(s)
LDL	low density lipoprotein
LSD	least significant difference

m	metre
M	molar
mg	milligrams
min	minutes
ml	millilitre
mm	millimetre
mM	millimolar
Mn	manganese
MT	metric tones
MUFA	monounsaturated fatty acid
N	normal
°N	degrees north
NaCl	sodium chloride
NED	*Eco* + AAC
ng	nanogram
NGB	National Gene Bank
nm	nanometre
NPK	nitrogen, phosphorus, potassium fertilizer
PCR	polymerase chain reaction
pH	acidity
Pi	superiority measure
PUFA	polyunsaturated fatty acid
RAPD	randomly amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
s	seconds
°S	degrees south
SDS	sodium dodecyl sulfate
SFA	saturated fatty acids
Sp.	species (singular)
SPDG	Sustainable Projects Development Group, United Kingdom
Spp.	species (plural)
t	ton
T*aq*	*Thermus aquaticus*

TE	Tris EDTA buffer
Tris	tris(hydroxymethyl) aminomethane
U	units
µg	microgram
µl	microlitre
µm	micrometre
UFA	unsaturated fatty acid
UPGMA	Unweighted pair-group method using arithmetic averages
USA	United States of America
USDA	United States Department of Agriculture
USSR	Old or New Russian Republic(s)
UV	ultraviolet
V	volt
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
Y	year(s)

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ADDENDUM A

Binary data matrix for oil content, fatty acids, and other characters measured.

Character	Class	El Salvador	Dowling	Gregg	Cuba 108	SF 459	Tainung 2	Everglades 41	Endora
% Fat	L	0	0	0	1	0	1	1	1
	M	0	1	1	0	1	0	0	0
	H	1	0	0	0	0	0	0	0
C14:0	L	1	1	1	0	0	0	0	0
	M	0	0	0	0	1	1	1	0
	H	0	0	0	1	0	0	0	1
C16:0	L	0	1	1	0	0	1	0	0
	M	0	0	0	0	1	0	1	0
	H	1	0	0	1	0	0	0	1
C16:1	L	0	0	1	0	0	0	0	1
	M	1	0	0	1	0	1	0	0
	H	0	1	0	0	1	0	1	0
C17:1	L	1	0	0	1	1	1	1	0
	M	0	1	0	0	0	0	0	0
	H	0	0	1	0	0	0	0	1
C18:0	L	0	1	1	0	0	1	0	0
	M	0	0	0	0	1	0	1	0
	H	1	0	0	1	0	0	0	1
C18:1	L	0	0	1	0	1	1	1	0
	M	0	1	0	1	0	0	0	1
	H	1	0	0	0	0	0	0	0
C18:2	L	1	0	0	0	0	0	0	1
	M	0	0	0	1	0	0	0	0
	H	0	1	1	0	1	1	1	0
C18:3	L	1	1	1	1	1	1	1	0
	M	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	1
C20:0	L	0	1	1	0	1	1	1	0
	M	0	0	0	1	0	0	0	0
	H	1	0	0	0	0	0	0	1
C20:2	L	1	1	1	1	1	1	0	1
	M	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	1	0
C20:3	L	1	1	1	1	1	1	0	1
	M	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	1	0
C20:5	L	1	1	0	0	0	1	0	0
	M	0	0	1	1	0	0	0	1
	H	0	0	0	0	1	0	1	0
C21:0	L	1	1	1	1	1	1	0	1
	M	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	1	0
C22:0	L	1	0	1	1	0	0	0	0
	M	0	1	0	0	1	1	0	0
	H	0	0	0	0	0	0	1	1
C24:0	L	0	0	1	1	1	1	1	0
	M	0	0	0	0	0	0	0	0
	H	1	1	0	0	0	0	0	1

C24:1	L	0	1	0	0	0	1	0	0
	M	1	0	1	0	0	0	0	1
	H	0	0	0	1	1	0	1	0
SFA	L	0	1	1	0	0	1	0	0
	M	0	0	0	0	1	0	1	0
	H	1	0	0	1	0	0	0	1
MUFA	L	0	0	1	0	1	1	1	0
	M	0	1	0	1	0	0	0	1
	H	1	0	0	0	0	0	0	0
PUFA	L	1	0	0	0	0	0	0	1
	M	0	0	0	1	0	0	0	0
	H	0	1	1	0	1	1	1	0
UFA	L	1	0	0	1	0	0	0	1
	M	0	0	0	0	1	0	1	0
	H	0	1	1	0	0	1	0	0

ADDENDUM B

Binary data matrix for AFLP data

Cuba 108

0101010001000100101100011110101001011000010011100001011011111
1011111111111110011111111010001000001101011111111001110111001
1111101111110110100110001000101011111000011111111110111101111
0010110111110111011101111011110101010010110110101011100101011
0111111101011101111111111101101110011111110110111011111111101
0111001111111111111111111101111101111111111011111111101110111101
1111111110111111100111101111111

Dowling

0100011011000101101111111111101001011011010011100101011011110 0
0011111111111100001101111100111100001101011101111001010111001 1
1111111110011111111101111111010110110000111111111111111011111
1110111111100111111101111111111101111110111011011000011011101 1
0111111111111101111111111111101110111111100111111111111111111
1111001111111111111111111101111111111111111011111111101111111111
1111111110111111110111111111111

El Salvador

0100011011000101101111111111101001011011010011100001001011110 0
1010111111111011001101111110110100001101011111111101010111111
1111110111110110101111011101101011011000011111111111011111111111
0010111111111111111111111111111011111101110101001111110011101 11
0101111101111111111111111111111100111111011110111111111111101
0110001111111111111111111110111111111101111101111111101110111111
1111111110110111100111111111111

Endora

1110010011100101111111111011101001001101010111100001101011110 0
0010111111111011011101111110111100001111011111111101110111011 1
1111100011100010101110001101101111111100011111111111011111111110
0010110111111011111110111101111011111001110111001111110011101 11
1111111101111101111111111111111111011111111011111111111111111101
0111011111111111111111111111111111111111011011111111101111101 11
1110111111111111100111111101111111

1111001111111111111111101110111111111111111101111111101111101111
1110111110111111101111111111191

Everglades 71

0000010001000100001101010000001000001000010011110001011011110 0
0010111111111110111100111000000000001101011111111001010191911 1
1111110111000110101110011101101011111000011111111111011111111110
0010110111110111111101111011110101110010101110101011100111011 1
0101111111011101111111111111101110011111110111111011111111101 1
1111001111111111111111111111011111111111111111111111111110111110101
1110111110111011110111011111111

Gregg

0100011111100101001100011100101110001001011011101101011011110 0
1011111111110100001101111000000000001101011111111100101019191 1
1111010111001110100110000001101011111000011111119911111111111 1
0010111111111111111101111011110101111111111111101101110011111111
0111111111111101111111111011101111011111111111011101111111111111
1111001111111111111111111111011111111111111111101111111101111101 01
1110101110111111100111111111111

SF 459

0110010011000100101110010110001001001000011011100001011011111 0
0010111111111011011101111010111100001101011111111110101011101 11
1111010000000000100110000001101001111000011101999101111111110
0010111111101111111101111011110101111111101111011011100111011 1
0111111111011111111111111011111110011101111111111011111111101
1111001111111111111111011101111111111111111111111111110111111111
1111111111111011100111111111191

Tainung 2

1100010011010111101111111110101101011101010111110001111011111 0
0011111111111011011100111000100100001101011111111110101011101 11
1111111010110101111100011011010111110000111111111110111111111
1010110111111111111101111011110111011011110110111011100111011 1
0101111111111101111111111101101110011111110111111111111111111
1111001111111111111111111111011111111111111111101111111101111101 11
1111111101111111101111111111111

Whitten

0110010111010111101111111110101001011101010111100001011111111 1
0010111111111011011101111100101100001101011111111111101111101 11
1111111011011010111110001101101110111001010111111111011111111011
0010110111110111111101111011110110111010101110011111100111011 1
0101011111011101011111111101101110011111100111111011111111101
1111001111111111111111111011111111111111111111111111111110111010111
1101111110111111100111011111111

***Hibiscus* sp. (no. 485)**

01100100110001011011100111101010010011010101111101011010111100
00111111111110110111011111101001000011010110111111010101110011
11111110100001101011100010011011110110000111111111011111111111
00101110111101111111011110111101111110111011101010111001010111
01110111111111011010111110111010100111011001101110019990100109
19110019111111199911110111111111101111111111111111111111111111
11011111101111111101110111001111

K503

11100111110111111011101111011011010010100101111100110110111110
00111111111110110111011111111111000011010111111111110101011011
11111101100011101001000010011010110110000111111111011111111110
00101111111111111111011110111101101100111011101011111001110111
01111111101110111111111111111011110111111111111111111011910101111
01111010111111199111111111011101001101110110101111011111010111
11011011101110111000101110111111

K124

11111111110111111011111101011001010010010101111000010110111110
01101111011110100011001111011111000011010111111111110101110111
11111100110110111011000011011011110110000111111111011111111111
00101110111111111111011111111101111110100011100111111001110111
01111111111111011011111111111011111011111111011011101111191121111
01111011111111199911111111011111111111111011011111111111011111
11111011101110111001101110111111

***Hibiscus* sp. a**

01100111110101111011110100001011100110000110111100010110111100
00101111111111011001101111010101110001101011111111111010111110111
11111110100011101011110010011010110110011111111111101111111111010
10101111111101111111011110111101101100111011100111111001010111
01111111111111111111111111111111101111111011111101111111111111
11110111111111111111111011101110100111111110101010111000111101 01
11101011100111119999999999999999

K258

11101111111011111011111110111001010011010101111100010110111111
10101111011110110111011111111111100111111011111111111111011111111
11111101110101101111100010111010110110000111111111011111111111
10111111111111111111101111111111011011011100111001111110011101 11
01111111110111111111111111111011100111111111111101111111111111
01110011111111111111111111101111111111111011111111100111101010 1
11111011100111199999999999999999

***Hibiscus* sp. b**

01101111111011111011110111010110111010101111100011010111110
00101111111111011011101111111011101011110101111111111010101011111

1111111010010110101110011101101111111000011111111101111011111
0010110111110111111101111011111111111011010111001111110011109 91
0111111111011101111111111101101100011111110111111111101111111 01
0111001111111111111111101110111111111111111111111111111110111 110101
1111101110111011101111011111111

Guatemala 4

0100011011010111111101110111010101100101110111110010111101111 10
1010111111111110111011101110101111011111111111111111111111010111
1111110010000011101110001000111011011100011111111110111111111 10
1010111111110111111101111011101111100101011100111111001110111
0111111111111111001111111101001110011111111011011111101 90101101
1111001111111111911011111110111010111110100110999999999999999 99
9999999999999999999999999999999

KY33

1110011111011111111111011111100101011101010111110001011011111 0
0010111101111011011111111111111111111011110111111111111101110111
1111110111001000100100000001101011011010011111999910111111101 0
0010110111110111111110111101111011011001010111001111110011101 11
0111111111011101111111111111111111011111111011011111110111111111
0111001111111111111111111111011111111111110110111111110111011101
1110111110111011100111101010 1199

K521

1110111011011111111111111011100101001101010111110001101011111 0
0010111101111011011101111111111100101111011111111111101110111
1111110111001010101100001001101011011000011111111101111111101 0
1010111111110111111101111011101111011001111100111111100111011 1
0111111111111101111111111111111111001111111011111111111111111
0111001111111111111111111111011111110110011011011111101011101 1001
1111101110111011100110011 0101111