CHARACTERIZATION OF *Opuntia ficus-indica* CULTIVARS IN SOUTH AFRICA

RACHEL MERCIA OELOFSE

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Faculty of Natural and Agricultural Sciences
Department of Plant Science (Plant Breeding)
University of the Free State
BLOEMFONTEIN

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Supervisor: Prof. M.T. Labuschagne

Co-Supervisors: Prof. W.J. Swart

Dr. C. D. Viljoen

This work is dedicated to my father,

Mr. Piet Oelofse

and my mother,

Mrs. Marina Oelofse.

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Table of Contents

CHAPTER 1

ASPECTS PERTAINING TO THE EXPLOITATION THE GENUS *OPUNTIA* WITH SPECIFIC REFERENCE TO CACTUS PEAR (*O. FICUS-INDICA*).

1. Introduction

Cactus pear (*Opuntia ficus-indica* L. Mill.) has great potential to improve productivity in semiarid regions (Brutsch, 1979; De Kock, 1967; Pimienta-Barrios, 1993, Felker and Russel, 1985) the main reason being that it has a Crassulacean Acid Metabolism (CAM) pathway (Nobel, 1995) which is about four times more efficient in water requirement than C-4 plants such as maize (Felker and Russel, 1985). The roots of cactus pear, which are extensive and dense near the soil surface also have a high capacity to store water where they can take up moisture from light rain showers. The leaves of cactus pear are rudimentary and ephemeral and the flattened cladodes, which are modified stems, fulfill the function of photosynthesis. They consist of water-storage tissue which contain cell mucilage with a high water-binding ability. The cuticle is thick and waxy with sunken stomata, which are normally closed during the day (Burbank, 1913; Mizrahi and Nerd, 1997). Brutsch (1979) outlined the morphological features and xerophytic adaptions of the cactus pear. Although it is well adapted to arid and semi-arid conditions it will nevertheless respond favourably in arid regions to light supplementary irrigation (Guigliuzza *et al.*, 2000). This has been clearly demonstrated for conditions in the Karoo by trials at Middelburg (Cape) in South Africa (Brutsch, 1984).

The value of cactus pear in subsistence agriculture has been well documented by Barbera (1995); Brutsch (1979); Brutsch and Zimmerman (1990); Pimienta-Barrios *et al.* (1993) and Russel and Felker (1987). Because *O. ficus-indica* is regarded as a minor fruit crop well defined standards for varieties are limited. However, with renewed interest in this plant there is a growing demand for better selected plant material (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Attempts to breed cactus pear date back to the early 1900`s by Luther Burbank (1913) in California. Systematic collection and characterisation of germplasm from the native as well as naturalised

populations, and continued efforts at breeding are needed to find new selections and to develop new cultivars for desertified areas (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

Increasing knowledge of environmental influences on fruit productivity and quality will also allow more profitable production (Brutsch 1979; Le Houèrou, 1992; Mizrahi and Nerd, 1999; Pimienta-Barrios *et al.*, 1993). The recent upsurge of interest in cacti as fruit crops may be attributed to the need to relieve famine in poor socio-economic and arid countries. In particular, the high productivity and fruit quality of some species, such as the *O. ficus-indica* are important factors promoting their cultivation (Mizrahi and Nerd, 1997). Worldwide estimates of cactus pear range from 687000 to 2.3 million ha, the latter figure includes low-density stocks scattered across Northern Mexico (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

Cactus pear when introduced to non-native habitats have become serious weeds (Zimmerman, 1989; Middleton, 2000). The spiny, or naturalised plant in South Africa is referred to as the prickly pear to distinguish it from the cultivated spineless (Burbank) cactus pear forms, although both are varieties of *O. ficus-indica* (Brutsch and Zimmerman, 1993). Literature stressing the value of cactus pear tends to make scant reference to the potential for invasiveness. Furthermore literature on biodiversity conservation tends to downplay the important economic uses of *O. ficus-indica* (Middleton, 2000). Although cactus pear can become invasive and very difficult for people with poor technology and limited resources to control (Middleton, 2000), it can be of extraordinary use in arid regions as a source of food for people and their livestock (Brutsch, 1979).

This review will examine the classification and characteristics of cactus pear, while also giving an overview of the historical development and current status of the cactus pear industry in the main cactus pear producing countries.

2. Taxonomical aspects

2.1 The order Cactaceae

The cactus family (Cactaceae) comprises about 130 genera and more than 1500 species. A study of the literature reveals a certain amount of confusion about

classification and nomenclature of the cacti, a situation that reflects problems associated with the development of cactus systematics (Russel and Felker, 1987; Mizrahi and Nerd, 1997; Wang *et al.*, 2002). The classification of cacti in general, and *Opuntia* specifically, therefore requires further work (Wang *et al.*, 2002). There are approximately 11 000 binomials published, many of which are considered incorrect. Reasons for improper classification include the size of the Cactaceae family, poor representation or poor quality of specimens in herbaria and convergent evolution of morphological features in independent taxa (Wang *et al.*, 2002). The Cactaceae contain many economically promising species primarily in the genus *Opuntia*, but only a few species are known as food crops.

2.2 The Genus *Opuntia*

The name *Opuntia* comes from an ancient Greek village, Opus or Opuntia, where Tournefort found a spinious plant that reminded him of the American opuntias. It includes 11 subgenera: *Opuntioidae, Consolea, Austrocylindropuntia, Brasiliopuntia, Corynopuntia, Cylindropuntia, Grusonia, Marenopuntia, Nopalea, Stenopuntia and Tephrocactus* (Scheinvar, 1995; Wang *et al.*, 2002). The subgenus *Opuntioidae* contains 150-300 species, depending upon the taxonomic system used (Russel and Felker, 1987, Arba *et al.*, 2000). The majority of *Opuntia* species, including the cactus pear, originate from the dry, interior plateaus of Mexico and the south- western U.S.A. Some, however, are believed to be natives of Canada, and others are from Patagonia (Brutsch, 1979). According to Burbank (1913), the genus *Opuntia* is surprisingly variable, even in its wild state. It is one of the most difficult plant genera to classify, since new forms are constantly appearing and older ones gradually and imperceptibly merge together.

2.2.1 Species and cultivar aspects

While cactus pear taxonomy has been studied extensively, there are still misconceptions regarding the classification of species and cultivars (Pimienta-Barrios, 1993). The taxonomy of *Opuntia* is complicated for a variety of reasons. Firstly, the

phenotype varies greatly according to ecological conditions. Polyploidy exists in numerous populations that reproduce vegetatively and sexually. Numerous hybrids exist since almost all the species blossom during the same period of the year and there are no biological barriers preventing them from cross-pollinate. There is also considerable confusion when one compares the names of species and varieties from different localities. This is due to subjective observations of yield, agronomic performance and other characteristic descriptions that can be seen with the naked eye. Orchards use material obtained from plants bearing large fruit of high colour quality. Taxonomic characters may therefore have been based on morphological characters that could simply be inherited as single genes and thus not of value in separating species. Confusion is also due to the fact that most descriptions were made from small samples. Many of the published taxa are therefore merely varieties, geographic forms, hybrids etc. In one locality, however, the name used for one variety is reasonably constant (Pimienta-Barrios *et al.*, 1993; Scheinvar, 1995).

According to Mizrahi and Nerd (1997), there are almost 300 species of the genus *Opuntia* from Canada to Patagonia (Chile and Argentina). They provide a summary on the most important *Opuntia* species and their use. Bravo-Hollis (1978b) recorded 104 species and varieties. Bravo-Hollis (1978b) described more than 30 new species of *Opuntia* from Mexico. Some are considered synonyms and others require further study. The species are in the herbarium of the Smithsonian Institution (USA) and the New York Botanical Garden (New York) (Scheinvar, 1995). Only a few studies have described cultivated species, which include shrubs with flattened stem joints (cladodes) of the genus *Opuntia* (subfamily *Opuntioidae*) (Burbank, 1913). There is therefore an urgent need for biosystematic research on cactus pear in order to establish a more coherent classification of both wild and cultivated taxa that should not be vased on morphological characters (Pimienta-Barrios *et al.*, 1993).

2.2.2 Genetic variability

Propagation of the genus *Opuntia* is possible through seed and vegetative means, although it is mainly propagated vegetatively by using terminal cladodes. The success rate and ease of this technique makes it an obvious technique to use. However,

observations made in many commercial orchards have revealed a high degree of variability despite the use of vegetative propagation (Wessels *et al.*, 1997). Information concerning the causes of genetic and phenotypic variation is lacking despite being critical in order to define future breeding strategies (Pimienta-Barrios, 1993). Only a few studies have investigated the genetic variability of the genus and distinguished varieties in the same environment (Nobel *et al.*, 1987; Brutsch, 1993; Mizrahi and Nerd, 1997). Natural hybridization of *Opuntia*, which is related to polyploidy, is common and it appears to be one of the major causes of diversity (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Partial and total cross-pollination is found in cultivated accessions (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). In their areas of origin in Central and South America, the numerous *Opuntia* species, varieties and natural hybrids have great genetic variability (Nieddu and Chessa, 1997). All Mexican cultivars are reportedly products of hybridization of *O. ficus-indica* with different wild cactus pear forms (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). In other regions, such as the Mediterranean basin where *Opuntia* spp. were introduced only a few centuries ago, the germplasm has a lower level of genetic variation although several similarities in its morphological and genetic traits have been observed. This homogeneity within populations is attributed to interaction between plants and local climate. The presence of high summer temperatures favour asexual reproduction of biotypes, while relatively low temperatures during the fall, just after fruit ripening, limit seed germination (Nieddu and Chessa, 1997).

Barbera *et al.* (1992), Brutsch (1984), De Kock (1967) and Munoz *et al.* (1995), described the different ploidy levels occurring in *Opuntia* species. Wild species have been reported to have low ploidy levels (4x and 6x) (Mondragon-Jacobo and Pérez-Gonzàlez, 2000) and are mostly tetraploid (2n=4X=44) (Munoz *et al.*, 1995), in contrast with the cultivated species, which have higher ploidy levels (Munoz *et al.*, 1995). *O. ficus-indica* is an octaploid plant, 2n=8x=88, and cytogenic studies have described it as an auto-allo-octoploid of two species of 44 somatic chromosomes each or as a segmental allo-octaploid (Mizrahi and Nerd, 1997). The chromosome number of the species and cultivars are: *Opuntia ficus-indica* L. (Mill.) 2n=88, *O. amyclea* 2n=88; *O dillenii* 2n=66 (Barbera *et al.,* 1992). High ploidy levels are associated with anatomical traits which are related with aridity: thick cuticle, sunken stomata in stomatal crypt, and

with morphological variables: increase in areole number and areole number per central line in arborescent growth habit. Although the "green-leaved" cultivars are 2n=88, the "blue-leafed" cultivars, such as Chico, Robusta and Monterey, are 2n=44 (Brutsch, 1984).

2.2.3 The Burbank cultivars

No perfectly thornless types of cactus pear were known and very little interest was shown in these cacti regarding their agricultural or horticultural value, until they were selected and improved by Burbank (1913). A great number of species and varieties of *Opuntia*, were compared and experimented with. Some of the species Burbank used were: arborescens, basilaris, engelmanni, fragilis, lurida, senilis en triacantha. He used the varieties Amarillo, Blanca, Colorado, Morado, Tapuna, Vulgaris among many others and of the *O. ficus-indica* varieties he used Gymno Carpo, Mission, Myers, Watson and numerous others. He selected the best of these, crossed them, raised numerous seedlings and selected the best of these. He made thousands of crosses and raised thousands of seedlings. Burbank found that by crossing some cultivars, the thorns increased rather than diminished while a few had even less thorns than their so-called thornless parents. He secured the best thornless *Opuntia spp.* from Mexico, America, Africa, Australia, Japan, Hawaii and the South Sea Islands. He also included the hardy wild species from Maine, Iowa, Missouri, Colorado, California, Arizona, New Mexico, Dakota, Texas and other states. Burbank was very familiar with many species and varieties of the thornless cactus and he stated that the *tuna* and *ficus-indica* classes are without doubt from the same original source. Apparantly the *ficus-indica* class is more thoroughly domesticated, having been more carefully cultivated and selected (Burbank, 1913). Five of the cultivars developed by Burbank were aggressively marketed by him. Today, four of these cultivars remain in the South African collection (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

3. *Opuntia ficus-indica* (L.) Miller

3.1 General

The most widely cultivated cactus is *O. ficus-indica*, which includes both thornless or thorny cultivars (Mizrahi and Nerd, 1997). This species is endemic to Mexico but is becoming an interesting alternative fruit and forage crop in North Africa and other semiarid areas of the world. *O. ficus-indica*, was introduced to Spain by the Spanish conquerors and later taken to northern and southern Africa and to countries of the Mediterranean basin (Pimienta-Barrios *et al.*, 1993). The common name used for *O. ficus-indica* is Tuna de Castilla or spineless cactus pear (Mizrahi and Nerd, 1997).

The most important economical use of cactus pear is as a fruit crop and it is cultivated for this purpose in Africa, Italy, Israel, Spain, the United States, Mexico, Colombia, Brazil, Peru, Bolivia, Argentina and Chile (Brutsch, 1984; Pimienta-Barrios *et al.*, 1993). In these areas, yields of 10-15 ton ha^{-1} are easily obtained. This species has also become increasingly important for fodder in many parts of the world, in both natural and cultivated populations, in order to assure emergency stock-feed during drought (Mondragon-Jacobo and Pérez-Gonzàlez, 2000; Pimienta-Barrios *et al.*, 1993). A few varieties that have been selected from germplasm of Mexican origin, support the world market. New varieties with better fruit, vegetable or forage quality, and adapted to local needs and specific climatic restrictions, are a common goal of cactus pear breeders (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Inglese (1995) outlined the representative areas cultivated for *Opuntia* fruit production, the time course of ripening and the different orchard management systems in the most important *Opuntia* producing countries.

3.2 Characteristics of species and cultivars commonly utilised

3.2.1 Fruit

California:

Production of cactus pear in the USA is concentrated in certain areas of California and New Mexico (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). In California, cultivated cactus pears cover an area of 120 ha. Here the species provide two yields of fruit per year - one large crop between February and April and a smaller crop between July and

September. Fruit is thus available from January to August and is mostly destined for internal consumption.

Mexico:

In the south western United States, cactus pear has been considered both as a weed and forage plant. Although a vast array of species is found in the wild, small holdings support mostly cultivars of *Opuntia ficus-indica* selected by farmers (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). The first modern plantations were established in Mexico. There are, in Mexico, many varieties of cactus pear, especially with the spineless forms, which are only cultivated in the central semiarid highlands. Production at commercial level started in the 1960`s and is based on exceptional plants selected during the 1940`s and 1950`s from plantings belonging to rural households (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). Mexico`s production is also the largest in the world arising from the cultivation of 44000 ha of *O. ficus-indica* and other species (Barbera, 1995; Basile and Foti, 1997; Bravo-Hollis and Sanchez-Mejorada, 1978a). Official figures report that Mexico has 42000 ha of cactus pear distributed in the central highland (2000m) semi-arid part of the country, all of them under rainfed conditions (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). According to Rodriguez-Felix *et al.* (1992), the main producing areas in Mexico are Las Piramides and Zacatecas. Six cultivars called Reyna, Cristalina, Naranjona, Chapeada, Amarilla Montes and Roja Pelona, share about 90 % of the production area (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). The Las Piramides area relies mostly on a single cultivar, called Reyna or Alfajayucan, while in the north central area (Guanajuato, Zacatecas and San Luis Potosi states) it is common to observe mixed orchards of several commercial cultivars (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). According to Barbera (1995), the orchards in the north Central American regions are less productive than the orchards in Hidalgo and Puebla in Mexico, due to low rainfall and poor cultural intensification in these areas. Small orchards northwest of Mexico City obtain 8-10 ton ha^{-1}, with minimum effect of alternate bearing. However, growers in semi-arid regions obtain less than 5 ton ha^{-1}, with low labor inputs and low crop management conditions (Mondragon-Jacobo and Pérez-Gonzàlez, 1995).

Due to the varying climate in Mexico, the commercial cultivars produce fruit in June through to October, but rural plantings yield fruit from May through December, which is a considerably longer production season. Due to the dependence of the market in Mexico on a narrow base of cultivars, there is a temporary (three months) market saturation, associated with falling prices and low returns. It is thus necessary to extend the harvest season by means of early or later ripening cultivars or specialised crop management practices (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). Thus, there is considerable genetic diversity from the rural plantings in Mexico that can be exploited in future breeding programs in order to incorporate new cultivars in the commercial market, which are able to produce fruit earlier or later than the current production season. In some locations, fruit of *O. ficus-indica* can even be produced year-round, for example, in the Huonta Valley, Peru, and Njoro, Kenya (Mizrahi and Nerd, 1997). Cultivars such as Charola and Fafayuco are appropriate to extend the end of the season, but they are not commercially important yet (Mondragon-Jacobo and Pérez-Gonzàlez, 1995). Exploitation of the genetic diversity by breeding programmes in the future is a very important way to manipulate the time of marketing, breeding of cold-hardy plants and fruit of a higher quality. In 1985, a project was initiated to study and preserve cactus variability in the semiarid lands of the Potosino-Zacataecano plateau. A total of 65 phenotypes were collected and established in the field as a germplasm bank. From this material 25 outstanding cactus pear phenotypes were selected with desirable fruit production characteristics (Luna and Pimienta, 1995). Initiatives are also under way in Texas where Texas A&M University-Kingsville is screening 130 *Opuntia* clones with the goal of developing cold hardy fruit and forage varieties. Ecophysiology studies on cactus in the United States are primarily being conducted at UCLA and UC Riverside (Felker, 1995a; Mondragon-Jacobo and Pérez-Gonzàlez, 1995; Basile and Foti, 1997).

Chile:

South American countries have few specialised cactus pear areas, with the exception of Chile (some 1000 ha). Saunder (1992) states that the fruit of the cactus pear is the most important fruit in Northern Central American regions. This underlines the potentially important role which cactus pear could play in other arid areas in the world even though

production is lower than cactus pear producing regions (Mondragon-Jacobo and Pérez-Gonzàlez, 1995).

Italy:

Italy is the second largest cactus pear producing country in the world after Mexico. About 10-20 % of Italian cactus pear production is exported, while the remainder is sold on domestic markets. The cactus pear industry in Italy has flourished, with over 90 % of the Italian cactus pear cultivation, in terms of surface area and production, concentrated in Sicily. Italian fruit has become very common in the markets of northern Europe and North America (Pimienta-Barrios *et al.*, 1993; Basile and Foti, 1997).

Due to an increasing demand for a diversified diet and unusual products, the consumption of cactus pear in Europe has become increasingly large during the last decade. Over the last 12 years, cultivation in Italy has been marked by large increases in terms of surface area and production. In 1990, intensively managed cultivation of cactus pear in Sicily covered up to 2500 ha. In 1994, the production of cactus pear area in Italy covered 7713 ha and in Sicily 7520 ha, while the island covered about 4000 ha (Barbera *et al.*, 1990).

There are three main growing areas: San Cono Hills, Mount Etna and the south western regions in Belice Valley. The spineless form of *O. ficus-indica* offer numerous cultivars which are important for their fruit, of whom about 20 are recognised in Sicily (Le Houèrou, 1992). There is a clear preference for unpacked, medium sized fruit with yellow, rather than red or white pulp and with only a few seeds (Barbera *et al.*, 1990, 1995; Basile and Foti, 1997). The leading fruit cultivar is Gialla, while Sanguigna and Bianca are irregularly scattered within orchards. Gialla (yellow) is most abundant in plantations as it is considered to be the most productive, easily handled and liked by consumers. Some 20 cultivars of fruit clones have been developed in Sicily (Barbera *et al.*, 1992; Pimienta-Barrios *et al.*, 1993). A seedless variety is also known, but its commercial cultivation has never been attempted because of the poor fruit quality. Other cultivars are Bianca, Rossa, Femminella, Papiterra and Leucosarca (Le Houèrou, 2000).

In Sicily, the fruit yield can reach from 12 ton ha^{-1} to 30 ton ha^{-1}, depending on orchard management and rainfall (Le Houèrou, 2000). The production calendar of

cactus pear is similar to that of some big producing countries like Mexico. The domestic market season opens in mid-August and closes in December, when prices are notably higher because of waning supplies and the delivery of higher quality fruits. This harvest is called "agostani" as they mature in August (Le Houèrou, 2000). The second harvest is usually during October to November and the result of what is referred to as "scozzolatura". This is a special management technique which involves the removal of flowers and cladodes that appear towards the end of spring. This causes a flush of new floral and vegetative buds that lead to a `late` crop about two months later in the season (Asciuto *et al.*, 1995; Barbera, 1995; Brutsch, 1993; Schirra, 1996). The fruits of the second harvest are called "scozzolati". They constitute luxury fruits sold around Christmas time and therefore also called 'Christmas Figs' (Le Houèrou, 2000).

Israel:

O. ficus-indica was imported to the Mediterranean region a few hundred years ago by the Spaniards, who took the species with them when Columbus returned from the American continent. The specific name, *"ficus-indica"* (Indian fig) derives from the fact that Columbus thought he had landed in India (Mizrahi and Nerd, 1999). Cactus lovers in Israel have their own society where plant material is exchanged, but no commercial activities exist. Some nurseries offer cactus plants as ornamentals and gardening plants. In the past few decades, harvesting and selling of the fruit was carried out by children for pocket money in their school vacation (July and August) (Mizrahi and Nerd, 1999).

Weeding and minimal irrigation was the horticultural practice until the mid-eighties. After this, the Agricultural Research Organisation (ARO) released a low-in-spine clone, which was also a precocious yielder, with high quality orange fruits (cv. Ofer) and the species became an orchard crop. In the north of Israel, in areas receiving over 350 mm of annual winter rain, farmers irrigate with an additional 60-80 mm water a year to produce 30-35 ton ha^{-1} fruits in one season. In areas with lower rainfall, the amount of supplementary annual irrigation is often higher (Mizrahi and Nerd, 1999).

Local market prices are often equivalent to other summer fruits such as peaches, plums, grapes, etc. High prices have promoted more intensive planting. In order to introduce farmers to the commercial cactus industry, Mizrahi and Nerd (1999)

selected eight clones from 350 seedlings that seemed suitable for cultivation and planted them in the Besor region. At first there were numerous problems to convince the farmers of the potential of the cactus pear. However, by mid-September in 1996 the farmers were convinced that producing the fruit was worthwhile. According to Mizrahi and Nerd (1999) an Israeli company started to sell cactus pear fruit in Europe under the name Koubo, where it was well accepted. In 1997 and 1998, only a few tons were sold, both in Europe and the local market, with the demand far exceeding the available supply.

Most cultivars in Israel currently ripen between 15 July and 15 August, resulting in lower prices, because of a glut in the market. Only in the very hot Arava and Jordan valleys (average in July and August is 38-39 °C) do fruits ripen from mid-June to mid-July, thus yielding higher prices. In an attempt to solve the problem of a short season, Mizrahi and Nerd (1999) introduced over 120 clones, selected from all over the world, with the hope that they would ripen at different times. Unfortunately most of them ripened from mid-July to mid-August.

South Africa:

Precisely when cactus pear was introduced to southern Africa is not clear, although it is believed that it was during the early seventeenth century. Today, *O. ficus-indica* is one of at least 14 *Opuntia* species that have become naturalised in southern Africa (Brutsch and Zimmerman, 1990). In South Africa, cactus pear is normally cultivated under dry land conditions. Whereas spineless cactus pear is widely planted in the semi-arid areas of southern Africa, notably the Karoo, as a fodder or forage crop. It is only in the last decade or so that its potential as a commercial fruit crop has been better appreciated by farmers (Brutsch, 1979; Brutsch and Zimmerman, 1990).

Spineless cactus pear is utilized in commercial plantings in South Africa for fresh fruit production and is clonally propagated by means of terminal cladodes. Significant numbers of plantings were established since the middle eighties and are continuing (Wessels *et al.*, 1997). Commercial exploitation occurs over a wide range of climates in South Africa, and the marketing season is usually from December to March. Indications are that the season could be extended by several weeks following the removal of spring flush in certain localities.

Naturalised cactus pear is most abundant in the Eastern Cape. The main production areas, however, are in the summer rainfall region, despite unfavourable climatic conditions like dry spells even during the rainy season, late spring rains and dry winter conditions occur from time to time. Increasingly, they are being cultivated as a source of fresh fruit for local and export markets. One of the targets of the South African industry is to reach the northern hemisphere market during December to April, a highly favourable period when high prices could be attained (Brutsch, 1979; Brutsch and Zimmerman, 1990). Although distances for export to European markets are relatively large, they are nevertheless worth exploiting (Brutsch, 1997; Brutsch and Zimmerman, 1990; 1993; Van der Merwe *et al.*, 1997).

Research on cactus pear as a fruit crop is in its infancy in South Africa, but it has also enjoyed considerable attention as a fodder crop (Brutsch, 1979; Brutsch and Zimmerman, 1990). Considerable research has been done on cactus pear in South Africa, especially at Grootfontein, near Middelburg and the University of Fort Hare Rustenburg is representative of areas in Gauteng and neighbouring Bophuthatswana, where spineless cactus pears are grown commercially. In the Eastern Cape Province of South Africa the more invasive cactus pear was brought under control with successful biological control programmes, to the point that it generally is no longer considered to be invasive but more or less stable (Brutsch, 1984, 2000).

Owing to the great range of agro-ecological regions in South Africa and the incomplete understanding of cultivar performance under these varied conditions, there is still a definite need for cultivar evaluation. Extremes of temperatures are problematic in some regions, as is hail and sunburn of mature fruits. Research is therefore in progress to obtain a better understanding of the response of spineless cactus to different climatic conditions and irrigation in South Africa (Brutsch, 1979; 1984; 1997). Brutsch (1997) presented climate diagrams for representative localities in the different climatic zones across South Africa where spiny cactus pears have become naturalised or where spineless cactus pears are cultivated commercially.

3.2.2 Fodder

Texas and Mexico:

Cactus is an important source of cattle feed for Texas ranchers and the practise of feeding dairy cows to increase the flow of milk is very common, especially in south-western Texas. It is mainly used as a supplementary ration to a more concentrated feed (Felker, 1995b). In the semi-arid central part of Mexico farmers use cactus pear extensively as an emergency-forage that is harvested from both wild and cultivated populations. Numerous *Opuntia* species are utilised as forage, reportedly 10-18 species, 15 of which are platyopuntias. The most frequently used are *O. engelmanii* and *O. lindheimerii*. These species are thorny and have to be processed for more efficient use (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Although wild cactus is generally prepared for stock feed by removing the thorns with fire, this never destroys the numerous bundles of innumerable needles imbedded in the leaves and cannot even always remove all of the larger thorns. The animals` throats and tongues thus become inflamed and hard (Pimienta *et al.*, 1993). *O. ellisiana* is valued in south Texas and used as a fodder source since it is a 100% spineless cultivar (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

The COPENA cultivars were developed by the Colegion de Postgraduados. CPF1, CPF2 and CPF3, all belonging to *O. ficus-indica* were selected for fodder production regions. At present, only CPF1 is found planted in small plots in Central Mexico. ANF1 and ANV1 were obtained during the 1960`s by the UAAAN. They are spineless and suitable for fodder production. Plantations were promoted in Northern Mexico with limited success, probably due to the abundance of the nature resource. (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

Mediteranean regions:

In the early 1930`s under the colonial land allotment of Gamouda near Sidi Bouzid, in Central Tunisia (250 mm MAR), land ownership was only granted by the government under the condition that contracting beneficiaries planted 10 % of the land allocated to spineless cacti as an emergency fodder crop reserve. This proved to be a very wise governmental decision as those farmers went through a devastating drought in 1946 to 1948 virtually without livestock loss, while the small stock in the arid lands was

decimated at a rate of 70-75 % (Le Houèrou, 2000). It is only from 1920-1930 onwards that cultivation for fodder production was established, essentially based upon *O. ficus-indica f. inermis* (Le Houèrou, 2000), in Sicily and the Mediteranean areas. Today spineless cactus is also present in Sardinia, where orchards occur without any cultural management and climatic conditions except for Sicily (Mulas and D`hallewin, 1997).

Before the expansion of irrigated farming in the 1950`s and 1960`s the production of dairy operations around Tunis and other cities of North Africa, was largely based on fresh cactus as green feed, particularly in summer and fall time, as well as hay and cereal grain. Such cactus pear plantations, heavily manured, produced enormous yields (Le Houèrou, 1965). Fodder plantations were systematically developed, especially in Tunisia, following the research carried out in Texas by Griffiths and his associates (Hare and Griffiths, 1907). The main areas of *Opuntia* spp. along the western part of the Mediterranean Sea are in Sicily and Tunisia, with approximately 100000 ha and 300000 ha respectively. The figure of 106000 ha in Calabria and Sicily was reported in the early 1960`s (Le Houèrou, 2000).

South Africa:

O. ficus-indica was introduced to South Africa at least 250 years ago. This country accounts for the oldest record of cactus pear introduced as a fodder crop. In 1914, 22 cultivars were obtained from Luther Burbank in California from material collected in Central America, 19 "green-leaved" and three "blue-leaved" accessions by the way of "true-to-type-seed". From this initial collection, and assuming cross pollination, numerous crossbred cultivars have been developed (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

In the arid and semi-arid regions of South Africa where annual rainfall ranges from 150-300 mm, a scarcity of fodder often occurs and the commercial cultivation of cactus pear for fodder and fruit is practised in the cooler interior plateau. Expansion of commercial plantings has occurred in warm temperate to warm to hot sub-tropical climates stretching from Gauteng Province to Mpumalanga and Northern Province, respectively (Brutsch, 1997). Planting along the contours is common as supplementary browsing in times of drought, or in plantations from where it is harvested and fed in combination with lucerne, hay and maize. Brutsch (1984) came to the conclusion that

for fodder production the so-called "blue leaf" spineless cactus pear (2n=44), with cultivars such as Robusta and Monterey, are best. Chico, Monterey and Robusta are also extensively used and recommended because of their resistance to cold and cochineal *(Dactylopius opuntia)*, although they are not the highest yielding cultivars. Although cultivars Chico, Monterey and Robusta are well adapted to a wide range of climatic and soil conditions, they are unable to endure temperatures lower than –12 °C. It is therefore not advisable to plant spineless cactus on low-lying terrain where temperatures even lower than –12 °C are often recorded.

Spineless cactus reacts very favourably to manure. It is evident that more fodder and digestible nutrients can be produced per unit of irrigation water from spineless cactus than from lucerne. The "green leaf" spineless prickly pear (2n=88) is also suitable but is preferred for its fruit and is generally less tolerant to drought. The varieties, Chico, Monterey and Robusta also produce less fodder although they are more resistant to cochineal and cactoblastis (Brutsch, 1984).

In South Africa, it has often been demonstrated that spineless cactus can play an important role in providing stock feed. The Ministry of Agriculture and Forestry planted drought-resistant crops such as saltbush (*Atriplex nummularia*), spineless prickly pear (*O. ficus-indica, O. fusicaulis, O. robusta*) and American aloe (*Agave americana*) in order to increase production from veld and marginal rainfed lands in the Ciskei (Brutsch and Zimmerman, 1990; Le Houèrou, 2000). Species are never mixed in a given field but established as seperate and complementary entities. These Karoo plantations represent about 0.8 million hectares (0.1 Agave, 0.2 cacti, 0.5 saltbushes); they are not only utilised by the sheep industry, but also by the cattle industry, as well as by the expanding game farms and ostrich ranches (Le Houèrou, 2000). Experiments have shown that sheep fed on spineless cactus, do not require drinking water. With limited irrigation water available, spineless cactus also produce more digestible nutrients per unit of water than lucerne. The percentage moisture in the pads of spineless cactus (cultivar Fusicaulis) in winter exceeds that in summer when picked for feeding purposes. Mature sheep were kept alive for 200 days on a diet of cactus alone, although they gradually lost condition, which suggests that a spineless cactus diet must be supplemented with other fodder plants (De Kock, 1967).

3.2.3 Diseases

Most diseases of cactus pear are caused by fungi (Granata and Sidoti, 2000). The diseases often result in severe damage to the cladodes, roots and fruit. As some of these diseases can jeopardize the entire crop, stricter control should be exercised in the transfer of propagative material locally and on importation from other countries. Prevention is often the best way to control diseases and keep them from spreading into areas that are not affected (Granata and Sidoti, 2000).

Mexico:

Field observations of wild and cultivated cactus pear populations in the arid and semi-arid highland zones of Mexico have revealed a considerable variability in resistance and susceptibility of cactus pear to biotic and abiotic factors that affect its development and productivity (Pimienta-Barrios *et al.*, 1993). *Agrobacterium tumefaciens* (Crown Gall), which attacks the stem of the cactus plant, has been reported in Mexico (Granata and Sidoti, 2000). Felker (1995a) reported that *Botryodiplodia theobromae* (Pat.) Griffon & Maubl.has been tentatively identified as a pathogen causing gum exudate under cool wet conditions in the winter.

Cactus pear fruits are also highly perishable and begin to show spotting and rotting nine days after harvest (Rodriguez-Felix *et al.*, 1992), injured fruits being more easily infected. *Fusarium* spp., *Alternaria* spp. and *Chlamydomices* spp. were identified as the cause for stem end rot on cactus pear fruits *Fusarium oxysporum* f.s. *opuntiarum* is the casual agent of *Fusarium* wilt. This fungus invades the tracheids and causes wilting on cladodes and fruits wilt. Elevated soil acidity, poor permeability and elevated humidity favour the onset and development of this disease (Granata and Sidoti, 2000). Prestorage dipping of fruit in water at 55 °C for 5 minutes or conditioning at 38 °C under saturated humidity for 24 h appears to be effective in improving fruit quality for marketing and could reduce the need for postharvest fungicides, thus improving shelf quality (Schirra, 1996; Schirra *et al.*, 1997).

A *Colletotrichum* sp. which causes anthracnose of cladodes and fruits was reported in Mexico (Granata and Sidoti, 2000). The fungus *Alternaria alternata* (Fr.) Keissl. is present in many cactus pear growing countries, such as Italy, Mexico and

South Africa. The fungus is also known as *mancha de oro* in Mexico (Granata and Sidoti, 2000). The basidiomycetous fungus *Armillaria* rot and stem rot is caused by the fungus *Armillaria mellea* (Vahl:Fries) that colonises the crown and large roots. Infection can be avoided using fungus free soil for cactus pear growing, but to date no chemical control measures are known for this disease (Granata and Sidoti, 2000). The fungus *Macrophomina sp.*, or black putrefaction, has not been classified yet. The disease has only been reported in Mexico where it is called "*Pudricion nigra*" and in some areas it results in the death of 50 % of the cactus pear plants. Control treatments consist of three or four treatments with benomyl, captan or zineb (Granata and Sidoti, 2000).

Italy and Sicily:

Very few pests and diseases affect cactus pear in Sicily. The lack of alternate hosts during the reproductive cycle of the Mediterranean fruit fly (*Ceratitis capitata* Weid) is a problem in favourable years (Barbera *et al.*, 1990). It is fairly easy to control with chemicals, however (Le Houèrou, 2000). The cactus moth *Cactoblastis cactorum* Berg. and the scale insects *Dactylopious opuntiae* Cackerell and *D. tomentosus* Lam. are not very serious and never have severe impact (Barbera *et al.*, 1990). Fungal diseases, mildews such as *Phytophtorae cactorum* Schr. and *P. omnivora* De Bary are more serious, depending on soil and irrigation management. *Phyllosticta opuntiae* also has greater impact on cactus pear (Le Houèrou, 2000) and has been steadily spreading in the area, limiting the light absorption ability of the affected plant's cladodes (Barbera *et al.*, 1990).

A soft rot, caused by the yeast *Candida boidimi* has also been reported in Italy (Granata and Varvaro, 1990). In Chile, *Sclerotinia sclerotiorum* causes a cottony rot on cactus pear cladodes. Sclerotia must be prevented from touching the soil, where they may remain alive for many years (Granata and Sidoti, 2000). Bacterial spot or *Erwinia carotovora* subsp. carotovora results in a characteristiclly dry cladode rot. It is a serious disease affecting all cultivars of *O. ficus-indica*. There are no reports of tolerance in commercial varieties (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). The disease is present in Italy, Chile, Mexico and Argentina. Symptoms appear on the cladodes as water-soaked spots during spring and subseqently turn black. The disease

may be controlled by removing or destroying the infected cladodes. Two treatments of copper fungicide are recommended (Granata and Sidoti, 2000).

South Africa:

Few surveys in South Africa have focused on pests and diseases (Swart and Swart, 2000), but the need was realised after members of the South African Cactus Pear Growers` Association (SACPGA) had experienced significant disease problems. Cactus pear farmers have previously been few and far between and have seldom devoted more than 20 ha on average to cultivation of the crop. The use of fertilizers to boost fruit production has generally not been the rule. This is fast changing, however, with an increase of farmers resorting to cactus pear as an alternative source of income (Swart and Swart, 2000). It is therefore important that cactus pear growers in South Africa be made aware of the importance of crop protection. The abnormality "cladode enlargement" was first reported in Mexico but is present in Argentina, Chile, Italy and South Africa. Only in South Africa it does represent a real threat to cactus pear cultivation. Characteristic symptoms are stunted growth, cladode enlargement and loss of colour. Different varieties present different sensitivity to the abnormality (Granata and Sidoti, 2000). In South Africa the "blue-leaved" cultivars such as Robusta, Chico and Monterey, are relatively resistant to cochineal but susceptible to *Cactoblastis*. The "green-leaved" cultivars are susceptible to cochineal and *Cactoblastis* (Brutsch, 1984).

The first systematic investigation by Swart and Swart (2000) has revealed numerous species of fungi associated with cactus pear. Before this, only one fungal pathogen has been formally reported on the genus *Opuntia* in South Africa. The report is on *O. stricta* Haw., however, and not on *O. ficus-indica* (Crous *et al.*, 2000).

Table 1. Major diseases of *O. ficus-indica* (Granata and Sidoti, 2000).

Disease's type	Disease	Location
Bacterial diseases	Bacterial spot (*Erwinia carotovora*)	Argentina, Chile, Italy, Mexico
	Crown gall (*Agrobacterium tumefaciens*)	Mexico
Yeast disease	Soft rot (*Candida boidimi*)	Italy
Fungal diseases	Alternaria golden spot (*Alternaria alternata*)	Italy, Mexico, South Africa
	Anthracnose (*Colletotrichum sp.*)	Mexico
	Armillaria rot and stem rot (*Armillaria mellea*)	Argentina, Italy
	Black putrefeaction (*Macrophomina sp.*)	Mexico
	Chlorotic spot (*Aecidium sp.*)	Bolivia, Peru
	Clorotic spots (*Gleosporium hervarum*)	Argentina, Italy, Mexico
	Cottony rot (*Sclerotinia sclerotiorum*)	Argentina, Chile
	Foot rot induce by Phytopthora (*F.cactorum* and *P. nicotinae*)	Italy, United States
	Fusarium wilt (*Fusarium oxisporum*)	Italy
	Grey mould (*Botrysi cinerea*)	Argentine, Chile, Italy, Mexico, South Africa, United States
	Gummosis canker (*Dothiorella gummosis*)	Italy
	Necrotic spots (*Cytospora* sp.)	Italy, Mexico
	Necrotic spots (*Phoma sorghina*)	Argentina
	Necrotic wounds (*Cercospora* sp.)	Bolivia, Peru
	Scab (*Phyllosticta opuntiae* and *P. concava*)	Italy, Mexico
Phytoplasma-like and virus-like diseases	Cladode enlargement	Argentina, Chile, Mexico Italy, South Africa
	Flower proliferation	Italy, South Africa Mexico
Disorder caused by Environmental factors	Scurf	Chile, Italy, Mexico South Africa

3.2.4 General characteristics

In addition to the use of *Opuntia* for fruit and forage, its immature cladodes are harvested as a vegetable, which is very popular in Mexico and the southern part of the United States. In Mexico, the joints, known as "nopalitos", are harvested from both wild and cultivated populations. The fresh young pads are used in salads and marinades or cooked with meat and eggs, providing excellent supplement to dried grains. The consumption of nopalitos as a vegetable is unknown to South Africans. Efforts to introduce the vegetable to mainly the lower-income groups has unfortunately failed (Brutsch and Zimmerman, 1990).

3.2.4.1 Cold tolerance:

The low tolerance of cactus pear to low temperatures is a major limiting factor for the cultivation of *O. ficus-indica* in the United States and other cold regions (Nobel, 1995). This problem is easily solved, in principle, via breeding of local strains selected from stands lying above 1 000 m of elevation; or via the import of frost-tolerant species, cultivars or strains (Le Houérou, 2000). Hybridisation of native, cold-tolerant species and highly productive, but cold sensitive commercial species, should be a major objective of breeding programmes (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

Tolerance of low temperatures and low-temperature acclimation of the cactus pear plant are related to the water content of the cladodes. Cladodes with less water tend to tolerate lower temperatures. When temperatures fall below 0 °C, water freezes and the ice that forms between the cells extracts water from cells and kills them in a way similar to cellular death caused by prolonged drought. The most important nopal cultivars are irreversibly injured at –5 °C to –10 °C. Selection for cold hardiness was initiated in 1963 at Universidad Antonio Narro where 31 individuals which survived frost of –16 °C were selected (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). The development of hybrids with improved cold hardiness was also undertaken at Kingsville, Texas using the spiny *O. linheimerii* as a source of cold tolerant genes. *O. lindheimerii* and *O. ellsiiana* can tolerate temperatures of –20 °C (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Formal breeding with the aim of cold tolerance was also initiated in Mexico in the 1960`s. The Univida Autonoma Agraria Antonio Narro (UAAAN) also

initiated research selecting for cold hardy *Opuntias*. During the same decade the late Dr. Barrientos of the Colegio de Postgraduados de Chapingo pioneered the first hybridizations of cactus pear in Mexico (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

Nobel (1990) noted that some strains of the Vulgaris species will grow readily in Alaska and several of the thorny species will survive below zero without injury. The Tapuna strain of the semi-tropical *Opuntias* bears superior fruit in the greatest profusion and when quite young. By hybridizing these species Nobel (1990) produced improved, perfectly thornless rapid-growing varieties with high levels of cold resistance. A spineless strain of *O. spinulifera*, of good fodder value, was also recently discovered by Le Houérou (2000) in Argentina which, on several occasions, withstood –14 to –16 °C.

Species and cultivars more resistant to cold temperatures than *O. ficus-indica*, have also been imported in South Africa and introduced to Northern Africa: *O. robusta* Wendl, cvs Robusta, Chico and Monterrey and also *O. fusicaulis* Griffiths. These clones are supposed to tolerate an absolute minimum temperature of –12 °C. Investigations on more cold-tolerant species and clones for the arid steppic highlands of Northern Africa were carried out in Taadmit, Algeria, in North Africa. Certain *Opuntia* species were totally destroyed by low temperatures, for example, *O. ficus-indica. O. maxima* and *O. vulgaris*. Others were severely damaged, such as *O. megacantha* and *O. streptacantha*. *O. amyclaea, O. engelmanni, O. robusta* cvs Chico, Monterrey, Robusta and *O. fusicaulis* were only slightly damaged. Only *O. robusta* var. *undulata* (Tucson), *O. helvetica, O. linguiformis*, and *O. lindheimeri* var. *wineriana* were not affected by frost (Le Houèrou, 1992). More breeding efforts are required to develop frost tolerant *Opuntia* strains.

3.2.4.2 Breeding programmes and future research

During the last two decades, several Mexican Institutions made efforts to collect wild, backyard and cultivated genotypes of cactus pear, because an understanding of phenotypic and genetic variation is critical to cultivars or varietal development by classical or biotechnological approaches, as well as for future germplasm collection and eventual setting of priorities for germplasm maintenance (Gutièrrez-Acosta *et al.*, 2000).

Breeding is underway in the U.S., South Africa, Italy and Mexico, based on the utilisation of local and introduced germplasm. This interest is encouraged by the Food and Agriculture Organization of the United Nations and has resulted in the collection of wild and semidomesticated accessions in regions (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Texas A&M University has since 1982 been involved in collection and introduction of nopal to the U.S. The program focused on the development of frost tolerant cultivars since 1996. In 1998, the program was transferred to the Universidad Nacional Santiago del Estero in Argentia where the work continued.

Cactus pear breeding started in Brazil in 1985, with 85 clones derived from open pollination of "Palma Redonda", plus 17 other clones from several Brazilian locations. Continuous introduction of genetic material from Mexico, South Africa, Algeria and U.S. has increased the number of entries up to 1400 at the Institut Pernambucano do Pesquisa Agropecuaria, completing the highest number of fodder clones in evaluation anywhere in the world. Higher productivity and better nutritional value, as well as adaptation to more humid and warmer environments, are the goals of this program (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). D`Arrigo Brothers, a produce company based in California, supports a private breeding program aimed to improve fruit quality of their spineless cultivars "Andy Boy" (Mondragon-Jacobo and Pérez-Gonzàlez, 2000).

In the past few years many different national and international symposia and meetings dealing with cactus pear cultivation and utilisation have been held in various countries. For instance, in 1988 the First National Symposium on "Fruit Production from the Spineless Prickly Pear" was held in Pretoria from the second to fourth February. In the same year the first booklet in English on spineless prickly pears was published. Both events indicated an increasing awareness of the potential of this fruit as a commercial crop for local and export markets (Brutsch and Zimmerman, 1990). At an informal meeting held during the International Symposium on Cactus Pear held in October 1991, in Lagos de Moreno, Jalisco, Mexico, the efforts of a group of researchers from Italy, Mexico, United States, and Chile have led to the formation of an International Cactus Pear Network. The objectives of the Network are to assist the participating institution researchers in promoting an exchange of information, material, and experimental data, as well as to establish effective cooperation in research and development. The

participation of researchers and growers from less developed countries is one of the most important goals. The proposal was presented in a special session of the Second International Cactus Pear Congress held in Chile September 1992, attended by researchers from 10 countries (Argentina, Chile, Italy, United States, Bolivia, Brazil, South Africa, Spain, Israel, and Peru) and officials from the FAO. Representatives of these countries formed a committee (Pimienta-Barrios *et al.*, 1993). The Third International Congress on cactus pear and Cochenille was held at Midrand (South Africa) from January 30 to the first of February 1999. The congress was attended by 40 participants from South Africa and representatives from nine countries (Inglese and Brutsch, 1997). As a result of these symposiums, co-operation among researchers all over the world was greatly improved (Pimienta-Barrios *et al.*, 1993).

4. Conclusion

It is clear from the literature that *O. ficus-indica* is a multifunctional crop, which can be of great value in both developed and underdeveloped countries and is ideal for planting in arid areas. Most development on *O. ficus-indica* has been done in Mexico. In South Africa the full potential of cactus pear has not been realised yet. This could only be solved by "marketing" the cactus pear industry to prospective growers and research of the problems experienced in the specific climates. More research on the selection and breeding of disease resistant cultivars and cultivars higher horticultural or fodder characteristics is required.

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CHAPTER 2

EVALUATION OF 10 COMMERCIALLY IMPORTANT CACTUS PEAR [*OPUNTIA FICUS-INDICA* (L.) Mill.] CULTIVARS IN SOUTH AFRICA ON GENERAL HORTICULTURAL AND FEED CHARACTERISTICS

Keywords: acid-detergent fibre, cactus pear, cladode shape, cladode yield, crude protein, flower end depth, fruit yield, fruit mass, growth habit, habitus, neutral-detergent fibre, *Opuntia ficus-indica* (L.) Miller, organic matter, peel colour, plant shape, plant height, plant width, pulp colour, pulp mass, total soluble solids

Abstract

Spineless cactus pear [*Opuntia ficus-indica* (L.) Mill.] in South Africa is increasingly commercialised and there is a need to establish a database to assist the South African farmers in the selection of cultivars for production. The aim of this study was to contribute to such a database on horticultural and fodder level. The most important phenology stages like reproductive bud break, vegetative bud break, 50% anthesis and 50% bud break were recorded. Shape, habitus, growth habit and cladode shape were classified. Peel and pulp colour at 50% colour break were evaluated. Plant width, plant height, fruit yield and fruit mass were measured. Pulp mass was determined. Fruits were tested for peel thickness, total soluble solids and flower end depth. The cladodes were tested and compared in terms of the most important requirements for the use as fodder crop in animal diets. Cladode yield per plant, cladode mass and number of cladodes pruned per plant were measured. The crude protein and the organic matter were compared. Acid-detergent fibre of each cultivar was measured. The data was statistically analysed by using simple ANOVA's. The fodder crop data was added to the horticultural data in order to get a more informative dendrogram. The cultivars were grouped using Unweighted Pair Group Mean arithmetic Analysis (UPGMA) into two different clusters.

The cultivars were distinctly different in some characteristics and similar in others. Zastron had the earliest reproductive and vegetative budbreak, 50% anthesis and 50% fruit ripening. Zastron also had a significantly stronger vegetative growth rate than the rest of the cultivars. Skinners Court, Zastron and Meyers required the

widest plant distances, while Zastron had the highest plants. Roedtan required the closest plant distance of all the cultivars. Gymno Carpo and Turpin had the highest fruit yield. Nudosa had a significantly higher fruit mass than the rest of the cultivars. Nudosa had a significantly higher pulp mass than the rest of the cultivars. Malta, Gymno Carpo, Nudosa, Meyers, Roedtan and Morado had a significantly higher pulp percentage than the rest of the cultivars. Malta and Gymno Carpo had significantly thinner peels than the rest of the cultivars. Skinners Court had significantly thicker peels than the rest of the cultivars. Morado, Roedtan and Algerian had a significantly higher total soluble solid (TSS) than the rest of the cultivars. There were no significant differences in flower end depth between the cultivars. Turpin and Meyers had a significantly higher cladode yield than the rest of the cultivars, while Skinners Court had a significant higher cladode mass than the rest of the cultivars. Turpin had a significantly higher number of cladodes than the rest of the cultivars, except for Meyers. The cladodes of Roedtan, Morado and Turpin had a significantly higher crude matter content and acid detergent fibre than the rest of the cultivars. Morado had the highest organic matter content, although not significantly higher than most of the cultivars.

Introduction

The cultivation of cactus pear, which requires little or no irrigation, may assume greater agronomic importance in the future, since a larger fraction of the land surface is destined to become arid or semi-arid. The cultivation of cactus pear requires a low input and is therefore capable of establishing a sustainable system that will increase the efficiency and economic viability of small and medium sized farms of low-income farmers (Pimienta-Barrios *et al.*, 1993).

Spineless cactus was introduced into South Africa for livestock feed following Burbank`s work in California (Burbank, 1913; Felker, 1995). An estimated area of 73 000 ha are under cactus pear cultivation in South Africa (Potgieter - Personal communication). According to Pimienta-Barrios and Muñoz-Urias (1995), all the varieties currently grown in South Africa, were developed from the original material, either as clones, or as artificial or natural hybrids. The main production areas in South Africa are in the summer rainfall are where unfavourable climatic

conditions like dry spells even during the rainy season, late spring rains and dry winter conditions occur from time to time. These problems result in late and poor flower induction, which in turn leads to late and lower yields. Fruit tend to be small and could be of poor quality and fruit cracking becomes serious in certain selections (Van der Merwe *et al.*, 1997). At Grootfontein, near Middelburg and at the University of Fort Hare (Cape), considerable research has been done on the cultivated cactus pear. Rustenburg is representative of areas in Gauteng where spineless cactus pear is grown commercially. Research on spineless cactus pear has been in progress at the University of Fort Hare since the 1989/90 season. One of the specific aims of this research was to further evaluate the promising cultivars and to look at the potential of crop manipulation (Brutsch, 1994). According to Pimienta-Barrios and Muñoz-Urias (1995), fruit morphology, size, colour, ripening time and quality vary among cultivated varieties in Mexico, Italy and South Africa. It is therefore also very important for each country, and even climate zone within an area, to find the specific cultivar that would perform best in the specific climate.

In many arid areas the cactus pear fruit is an important food source for satisfying the nutritional need of the local, mainly poorer populations, for about three to four months of the year. The knowledge of its chemical composition, nutritional value and effects on human health have determined, in the world, a recent increase in the consumption of cactus pear. For the small farmers the cultivation of cactus pear can be profitable, due to the low investment needed. Recently there has been increased interest in the cultivated spineless (Burbank) prickly pear as a fruit crop. Traditionally it has been appreciated as a drought-tolerant fodder crop, but it is increasingly grown as a fruit crop in its own right. There is a scope for increased production on a commercial scale for local and export markets of cactus pear fruit. Prices obtained for prickly pear fruits on the national fresh produce markets of South Africa compare very favourable with those of more common fruits, such as apple, peach and orange (Brutsch, 1994; Nieddu *et al.*, 1997).

Fruit yield. A mature cactus pear tree produces new fruits and cladodes every year at a ratio of 4:1. Most of the flowers occur on one-year-old terminal cladodes. Vegetative and reproductive buds appear contemporarily in spring or early summer when the spring flush is removed to induce reflowering. Young developing cladodes

compete with fruits, but they become a source of carbohydrates at an early stage of their development (Inglese *et al.*, 1997). Several studies concluded that fruit obtained from a second, induced crop, are of better quality. This is strongly related to more favourable environmental conditions during the second flush (Nieddu *et al.*, 1997; Inglese *et al.*, 1997).

Fruit mass and pulp mass. Final fruit mass of cactus pear depends on cultivar, seed number, fruit load and horticulural managemant such as thinning and irrigation. One-year-old fruiting cladodes are the main source of photosynthate for fruit growth in cactus pear. The ability of the cladode to support fruit growth depends on the fruit load and its light interception during the season. The orientation and location of the cladode on the plant influences light interception (Barbera *et al.*, 1997). Fruit mass is affected by the time of bud emergence, by cladode fruit load and environmental factors (Mizrahi and Nerd, 1995). Early flush buds were found to produce heavier fruits than late flush buds. A fruiting cladode entirely supports the growth of five fruits, but to support the growth of 10-15 fruits on a cladode, extensive assimilate import is needed, particularly during the last three to four weeks of fruit development. Where six fruit are left on a fruiting cladode their size significantly increase and their seed to flesh ration increase since the percent flesh does not change (Mizrahi and Nerd, 1995).

The fruit size at harvest is related to the fruit growth rate attained early during the fruit development period, where photoassimilate supply from the fruiting cladode becomes crucial to support fruit growth, probably because of the competitive demand of different and actively growing vegetative and reproductive sinks. Cladodes that have been shaded for 75 days, starting from the day of bloom, attained a fruit fresh weight of 95.24 g, while sunlit cladodes attained 133.85 g (Barbera *et al.*, 1997). In grading fruits for size, a mass of 80 g or more is considered acceptable for fruit production. This is purely an arbitrary figure in the absence of standard regulations for the grading and marketing of this crop. Fruits of 100 g and more are considered to be of a good size. The percentage of marketable fruit is therefore primarily determined by the incidence of fruit-cracking and by fruit size (Brutsch, 1979).

Pulp mass seems to be related to fruit mass, because higher fruit mass in cases of irrigation is a result of a bigger pulp portion. Irrigation of cactus pear plantations in drier regions with low rainfall can have a positive influence on yield and size, but in areas with a higher rainfall the cost of an irrigation system will have to be compared to higher income from an increased yield and larger fruit in dry years (Van der Merwe *et al.*, 1997).

Peel colour. The beginning of peel colour change is a common index for fruit harvest and may be supplemented by measurement of total soluble solids (TSS). Fruits have to be harvested for marketing 75-90 days after flowering, at the beginning of change in peel colour when the peel becomes very smooth. Fruits harvested prior to this stage (a common mistake) have an inferior taste and do not ripen properly in storage. From 90 days after flowering onwards the peel is fully coloured, but the fruits become tender and unsuitable for processing and storage. The fruit is non-climateric and can be stored for 14 days, provided that it is harvested at the proper stage of ripening. In many cultivars, TSS values indicate maturity. Even at the pre-ripening stage, pulp acid content is very low (0.02-0.06 % as citric acid). Acidity is a less useful indicator for harvest than other indices (Barbera *et al.*, 1992; Mizrahi and Nerd, 1999). Sáenz-Hernández (1995) stated that the fruit colour is undoubtedly an important parameter for determining the attractiveness of both the fruit and its products. In contrast to this, Brutsch (1979) notes that cultivar differences and consumer preferences or prejudices regarding fruit colour may sometimes seem trivial, but are worth nothing.

Total soluble solids. Ripening time and sugar content are cultivar-dependant and show wide within tree variability. The high pH value of cactus pear (pH = more or less 5.8 – 7.2) classifies this fruit within the low acid group (pH>4.5), which, together with the high content of soluble solids, make the cactus pear's pulp a very attractive microbiological medium (Barbera *et al.*, 1997; Sáenz-Hernández, 1995). TSS sharply increases 50 days after flowering and fruits reach the optimum maturity stage when TSS reach a steady state over 13% Brix. This stage corresponds to the period of peel colour breakage (Barbera *et al.*, 1992). According to Sáenz-Hernández (1995), cactus pear has a similar nutritive value to that of other fruits

(prune, apricot, cherry, peach, apple, and melon), but its soluble solids content is greater than that of other fruits. Cactus pears are non-climateric fruits with low respiration rates in comparison to those of other common fruits and non-climateric fruits are also characterised by a lack of starch as a carbohydrate reserve. There is, therefore, no significant increase in sugar content in non-climateric fruits after harvest.

Nieddu *et al.* (2000) described the main horticultural characters of 14 genotypes. Ten accessions were selected within the Sardinian local germplasm and four were introduced from Sicily. Among the 14 accessions, five had white fruit colour, three were yellow and five had red fruits, all belonging to the species *O. ficus-indica*, while only one genotype was identified as *O. amycleae* with yellow fruit.

The measurements in Table 2.5 were taken from plants planted in an extensive spacing (500 plants per hectare). Brutsch (1979) concluded that Algerian, Gymno Carpo, Malta and Blue Motto were the more promising cultivars at Fort Hare, based on the data in Table 2.5 and previous performance. He noted that Morado, apart from being a shy bearer, is a promising cultivar with the popular light green colour and white pulp.

Table 2.1 Minimum criteria for cactus pear varieties evaluated for fruit production potential that Potgieter, J.P. and Mkhari, J.J. developed from results and observations from their cactus pear research programme from 1989. The information was presented in a Combined congress at Cedara, KwaZulu Natal on the 15-17 January 2002 (unpublished).

CHARACTERISTIC	MINIMUM CRITERIA
Fruit yield potential:	
year 2	1 $t.ha^{-1}$
year 3	2.5 $t.ha^{-1}$
year 4	4 $t.ha^{-1}$
year 5	7 $t.ha^{-1}$
year 6	10 $t.ha^{-1}$
year 7	15 $t.ha^{-1}$
year 8	20 $t.ha^{-1}$
Fruit mass	>140.0g
TSS	>13 ^{0} Brix
Pulp percentage	>50%
Peel thickness	<6 mm

Table 2.2 Desirable characteristics of cactus pear varieties grown for fruit production purposes that Potgieter, J.P. and Mkhari, J.J. developed from results and observations from their cactus pear research programme from 1989. The information was presented in a Combined congress at Cedara, KwaZulu Natal on the 15-17 January 2002 (unpublished).

PLANT CHARACTERISTICS	FRUIT CHARACTERISTICS
High yield potential	Large fruit size
Short juvenile phase (early bearing)	Attractive internal and external colour
Produce good yield year after year	Long shelf-life
Moderate vegetative vigour	Low seed number
Require little pruning and fruit thinning	Seeds should be small
High tolerance to pests and diseases	Fruit should not bruise easily
Wide climatic adaptability	Acceptable peel thickness
Natural tendency to bear out-of-season	High TSS contents
Few thorns and glochids	Pleasant taste and aroma
Easily manipulated (winter production and Scozzolatura)	High juice content
	High percentage edible portion
	Crack resistant
	Easy peeling
	Suitable for processing

Table 2.3 Fruit characteristics of five of the more promising cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars in trial plantings at the University of Fort Hare (Brutsch, 1994).

Cultivar	Main harvest period	Yield potential	Fruit size	External colour of ripe fruit	Internal colour of ripe fruit	General remarks
Algerian	End of January to end of March	Very good	Medium to large	Red	Red	-Very susceptible to cochineal
Blue Motto	Early of February to end of March	Very good	Large	Light green	Yellow/ khaki	-Upright growth habit -Very few 'spines' -Less susceptible to cochineal -Except for colour it could be regarded as the best
Gymno carpo	End of January to end of March	Very good	Large	Yellow	Yellow	-Fairly susceptible to cochineal -Attractive fruit
Malta	Early February to end of March	Good	Small to medium	Yellow	Yellow	-Fruit rather small -Very susceptible to cochineal
Mexican	Mid January to early February	Fair	Medium to large	Light green	white	-Thick peel -Round fruit -Early -Susceptible to cochineal -Tendency of fruit to split

Table 2.4 Fruit characteristics of five of the more promising cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars in trial plantings at the University of Fort Hare (Brutsch, 1979).

Cultivar	**Main harvest period**	**Mean fruit mass (g)**	**External colour**	**Internal colour**
Algerian	End January – end March	88	Red	Red
Blue Motto	Early February – end March	109	Light green	Yellow/khaki
Gymno Carpo	End January - end March	102	Yellow	Yellow
Malta	Early February – end March	85	Yellow	Yellow
Morado	End January - mid March	97	Light green	White
Skinners Court	Early February - end February	107	Light green	Light green/white

Table 2.5 Fruit yield for two seasons of five of the more promising cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars in trial plantings at the University of Fort Hare (Brutsch, 1979).

Cultivar	Mean yield in the 1977 season (kg plant $^{-1}$)	Mean yield in the 1978 season (kg plant $^{-1}$)	Mean yield for the two seasons (kg plant^{-1})	Mean for the two seasons (ton ha^{-1})
Algerian	20.97	9.23	15.10	7.50
Gymno Carpo	17.65	6.44	12.04	6.00
Malta	17.65	5.84	11.69	5.80
Blue Motto	9.63	10.15	9.89	4.90
Morado	6.76	5.09	5.93	3.00
Skinners Court	4.59	7.09	5.84	2.90

Table 2.6 Results of some cultivars grown under dry land conditions (Mulas and D`hallewin, 1997).

Cultivar	Plant height (cm)	Fruit mass (g)	Pulp percentage (%)	Total Soluble Solids (°Brix)	Peel thickness (mm)
Gialla sarda	119	124	54.3	12.7	3.7
Gialla	132	103	52.9	11.9	3.4
Bianca	125	112	53.6	12.8	4.2
Sanguigna	144	121	53.6	13.9	4.4

Table 2.7 Fruit yield, fruit mass and pulp mass reported in the seasons of 1992/93, 1993/94 and 1994/95 without irrigation by Van der Merwe *et al.* (1997).

Selection	Season	Yield (ton ha^{-1})	Fruit mass (g $fruit^{-1}$)	Pulp mass (g $fruit^{-1}$)
Algerian	1992/93	12.0	117.7	69.5
	1993/94	10.5	127.0	65.9
	1994/95	13.2	113.8	65.2
Gymno Carpo	1992/93	8.4	163.7	87.8
	1993/94	6.7	152.0	80.4
	1994/95	12.6	158.5	89.7
Morado	1992/93	8.7	139.4	79.1
	1993/94	-	-	-
	1994/95	10.4	156.3	87.7
Malta	1992/93	8.2	145.1	82.6
	1993/94	4.8	142.2	81.0
	1994/95	13.2	146.9	85.3
American Giant	1992/93	8.8	119.8	66.1
	1993/94	4.3	143.7	71.0
	1994/95	9.5	142.8	73.7

Table 2.8 Different authors` results on certain horticultural characteristics for cactus pear (Sáenz-Hernández, 1995).

Parameter	1	2	3	4
Ph	5.80	5.30 - 7.10	5.75	6.37
Acidity (% citric acid)	0.05	0.01 - 0.12	0.18	0.06
Pulp and seeds	-	-	48.00	49.60
Peel thickness (mm)	-	-	5.20	5.04
°Brix (TSS)	13.20	12.00 -17.00	14.20	14.06

Authors:

1 - Askar and Al-Samahy (1981)

2 - Pimienta (1990)

3 - Sawaya et al.(1983)

4 - Sepúlveda and Sáenz-Hernández (1990)

Table 2.9 Horticultural characteristics of 20 *Opuntia ficus-indica* (L.) Mill. cultivars (Wessels, 1989).

Area of origin or common name	Fruit colour	Fruit weight (g)	Flesh percentage (%)	Ripening time	TSS (%)
Algerian	Dark-pink	110	56	Jan.-Feb.	12
Bakenslip	Light-green	84	37	Jan.-Feb.	13
Blue Motto	Pale-yellow	140	51	Feb.-Mar.	12
Castillo	Light-brown	114	52	Feb.-Mar.	14
Corfu	Pale-green	91	35	Jan.-Feb.	14
Direkteur	Light-yellow	152	50	Jan.-Mar.	13
Fresno	Yellow-white	147	55	Jan.-Feb.	14
Jusicaulus	Pale-green	104	45	Jan.-Feb.	14
Jusicaulus O.P.	Light-green	144	52	Feb.-Mar.	12
Guaya Quil	Yellow-white	89	41	Jan.-Feb.	14
Gymno Carpo	Yellow	126	52	Feb.-Mar.	12
Hardy Bred	Green-white	102	26	Jan.-Feb.	12
Mexican	Light-green	139	54	Jan.-Feb.	14
Morado	Pale-yellow	114	48	Jan.-Feb.	13
Muscatel	Pale-green	161	42	Jan.-Feb.	13
Niagra	Yellow-white	147	47	Jan.-Feb.	13
Nudosa	Brownish-green	175	47	Jan.-Feb.	11
Nos Kaap	White-green	151	49	Jan.-Mar.	14
Santa Rosa	Pink	131	52	Jan.-Feb.	12
Signal	Red	111	34	Jan.-Feb.	14

Feed characteristics. Flattened photosynthetic stems (cladodes) of the *Opuntia* subgroup known as platyopuntias, have long been used as a cattle fodder in arid and semiarid regions (Nobel *et al.*, 1978), because the utilisation of cactus has the following advantages: It is a drought resistant crop with easy management requirements and high water-use efficiency. It is easy to establish and adapts easily to a wide range of soils. Cactus pear has a high tolerance to high and low temperatures and is highly digestible by animals. It also has a high water, energy and calcium content. *Opuntia* has been successfully fed to dairy and beef cattle, oxen, sheep and hogs. There are a number of reviews covering the uses of cactus pear as forage (De Kock 1980; Wessels, 1989; Ben-Salem *et al.*, 1996). Felker (1995) states that protein and mineral supplementation ratios for feeding cactus to cattle have been developed that give excellent results.

According to Nobel *et al.* (1978) most dairy and many beef cattle in the Mexican states of Coahuila and Nuevo Leon are fed cactus pear cladodes in the wintertime when water is scarce. Despite the overall favourable use of cacti for stock, there have nevertheless been some problems. If the fruits or cladodes are fed without the spines being burned off, the spines and glochids become lodged in the gastrointestinal tracts of the animals and bacterial infections of these lesions may follow (Felker, 1995). According to Felker (1995) and Nobel *et al.* (1978), the spines of the spiny types must be singed off with a flame thrower before being fed to cattle or sheep, but this is not essential in the case of the spineless types. In South Africa, the cladodes are chopped into strips and fed to sheep. The strips could be dried and ground in a hammermill to facilitate storage during droughts. A mature cactus pear tree produces new fruits and cladodes every year at a ration of 4:1. New cladodes develop on two-year old or even older cladodes (Inglese *et al.*, 1997).

Mizrahi and Nerd (1997) stated that the consumption of cladodes could improve the flavour and colour of milk, but if fed at high rates, the cladodes have a laxative effect on the animals (Felker, 1995). According to Mizrahi and Nerd (1997), the laxative properties of cladodes can be avoided by gradually increasing the cladode portion in the feed.

The nutritional quality of *Opuntia* in animal diets has been examined (Griffiths and Hare, 1906, Flachowsky and Yami, 1985, Gregory and Felker, 1992). In general, *Opuntias* are high in moisture content (about 85%), but low in protein (Felker, 1995). Gregory and Felker (1992) examined eight forage clones and found that forage clones from Brazil had significant higher protein and phosphorus content than the Texas clones. With regards to the United States National Research Council cattle requirements, wild spiny cactus does not meet the protein, but exceeds the calsium, requirement. Cactus has a high percentage of total digestible nutrients (about 60-70%), vitamin A and water. The water requirements of cattle and sheep are greatly decreased when cladodes constitute a major part of the diet. Mizrahi and Nerd (1997) warn that the growth conditions affect the quality of cladodes as an animal feed. The water content of growing plants is related to the stage of growth, younger plants containing more water than older plants. Cladodes consist mainly of water (85-90% by fresh weight), but during water stress the water content may drop to 60%. Mizrahi and Nerd (1997) found the nutritional value of the cladodes to be similar to immature maize silage on a dry matter basis. Given the low percentage of crude protein and certain mineral nutrients in cactus pear, protein and a micro- and macro nutrient supplementation should be considered (Felker, 1995, Mizrahi and Nerd 1997).

Much of the existing information about the composition of foods is based on a system of analysis described as the proximate analysis of foods, which was devised over 100 year ago by two German scientists, Henneberg and Stochmann. This system of analysis divides the food in to six fractions: moisture, ash, crude protein, ether extract, crude fibre and nitrogen-free extractives (MacDonald *et al.*, 1995). In recent years the proximate analysis procedure has been severely criticised by many nutritionists as being imprecise. Goering and Van Soest (1970) (Table 2.10) have developed alternative procedures for fibre. The determination of acid-detergent fibre (ADF) is particularly useful for forages as there is a good statistical correlation between it and the extent to which the food is digested (digestibility) (MacDonald *et al.*, 1995).

Table 2.10 Classification of forage fractions using the detergent methods of Goering and Van Soest (MacDonald *et al.*, 1995).

Fraction	Components
Cell contents:	Lipids
(soluble in neutral detergent)	Soluble protein
	Sugars, organic acids, and water
	Soluble matter
	Pectin, starch
	Non-protein nitrogen
Cell wall constituents:	
(fibre insoluble in neutral detergent)	
1. Soluble in acid detergent	Hemicellulose
	Fibre-bound protein
2. Acid-detergent fibre	Cellulose
	Lignin
	Lignified nitrogen
	Silica

Table 2.11 Chemical composition of spineless cactus pear (cultivar Chico) as presented by de Kock (1965).

Nutrients	Percentage
Dry material	10.44
Moisture	89.56
Protein	0.38
Fat	0.16
Ash	2.03
Nitrogen-free extract	6.62

Table 2.12 Typical range of values for cladode composition for use in animal feed (Felker, 1995).

Measurement	Typical range
Moisture Content (%)	85 - 90
Crude Protein (%)	5 - 12
Crude Fibre (%)	43
Organic Matter (%)	67
Protein Digestibility (%)	72

Materials and methods

Plant material was established in a field genebank. The genebank is situated near Potgietersrus in the Northern Transvaal (23° 50`S), South Africa. This area has a subtropical climate with predominantly summer rainfall. Single cladodes were established at a plant spacing of 2 m x 5 m (1000 plants/ha) in rows orientated N/S. Standard orchard practices according to "Recommendations for the cultivation of thornless cactus pears with the aim on fruit production" (available from the South African Prickly pear Union) were commonly followed in this germplasm block. The gene block was not planted in a statistical test trial layout.

Phenology:

Reproductive bud break, vegetative bud break, 50 % anthesis and 50 % bud break were noted as a certain week of the month (week 1,2,3 or 4) when the characteristic in the majority of plants were clearly visible. Reproductive and vegetative buds are clearly distinguishable when the buds are 5 mm long.

Plant shape, habitus, cladode shape, growth habit:

Ten plants per entry were measured. Plant shape was determined by dividing plant width by plant height. This value was an index value as recommended in "Descriptors for cactus pear (*Opuntia* spp.) " - FAO plant descriptors, p.24.

Flat: $W > H$

Round: $W = H$

Elongate: $W < H$

Habitus was determined by looking at the plant shape. Cladode shape was visually determined as in FAO publications. Growth habit was determined as recommended in Wessels (1989). These measurements were all done in wintertime when the plants did not grow actively.

Peel and pulp colour:

The fruit colour has a great influence on the marketing potential of a specific variety. The peel and pulp colour was classified by visual evaluation at 50 % colour-break. Observation was done at the point where colour break of the peel is 50 % visible. This is also the point of best eating quality of the fruit.

Plant width and plant height (cm):

The plant width and plant height (cm) was measured in wintertime (May to June) just before pruning of the plants. The plants were measured with a five meter steel measuring tape. Plant width was measured in order to monitor specific plant distances for certain cultivars in this specific climate. Plant height (cm) was measured in order to get an indication of the vertical vegetative growth rate of the different cultivars in this specific climate.

Fruit yield per plant (ton ha^{-1}):

The fruit of 10 plants of each cultivar was counted in September, after fruit thinning. Fruit was thinned until there was more or less 40-50 mm space between the fruit. Pruning was done during May to July. The plants are pruned each year, so this has an influence on several characteristics of the plant, for instance plant height and shape. To calculate fruit yield, the amount of fruit per plant was multiplied by the fruit mass. Of the total yield 70-90 % is suitable for marketing.

Harvest of fruit in order to determine quality:

When 30-60 % of all the fruit on the plant reached optimal ripeness (50 % peel colour break), the samples for laboratory analysis were picked. Fruit with the same colour shade were harvested. To simplify analysis, the fruit was mechanically de-thorned before analysis.

Fruit mass (g) and pulp mass (g):

Ten fruits of each cultivar were weighed individually by using a Mettler PM 3000-K electronic laboratory scale. In order to determine the eatable amount in the fruit, the pulp mass (g) was measured using the same scale. This was done to determine whether a cultivar passes the minimum criteria for fruit production or not.

Pulp percentage (%):

The pulp mass of each individual fruit was divided by its specific fruit mass in order to get a percentage value.

Peel thickness (mm):

To measure peel thickness, 20 cactus pear fruits of each cultivar were peeled with a common kitchen knife. The peels were sliced "longitudinally". Both ends of the peel were measured with a Mitotoyo CD 8 digital shuffle "compass". This took place in January.

Total soluble solids (TSS):

In order to determine the sugar content of the fruit, total soluble solids in % Brix was measured with an Atago DBX 55 digital refractometer. A spatula was used, liquidizing a small area in the middle of the fruit. One drop of this liquid was then placed on the lense of the refractometer to determine the TSS. Ten fruits of each cultivar were measured.

Flower end depth:

Although flower end depth (mm) is a genetical character, it is also influenced by environmental factors as the length of the period of fruit development. The longer the fruit development period, the shallower the flower end depth (mm). A shallower flower end depth (mm) also indicates a higher percentage pulp mass. A Mitoyo CD 8 digital shuffle compass was used to measure the flower end depth (mm). This was done by sticking the shuffle of the compass as deep as possible in the calyx of the flower, measuring the distance up to the end-sides of the flower end. Ten fruits of each cultivar were measured.

Cladode yield per plant (kg):

A Mascot electronic digital platform scale was used to weigh the cladodes during pruning (from May to July). All the pruned leaves of an individual plant were measured together.

Cladode mass (kg):

The cladode mass (kg) was calculated by dividing the cladode yield (kg) by the cladode number (n).

Number of cladodes pruned (n) per plant:

The leaves that were pruned during May to July were counted in order to determine how many leaves will be removed when feeding it to animals. The leaves of 10 plants were counted.

Chemical analysis on a dry matter basis:

Crude Protein (CP):

The whole cladode was dried and milled. A sample was taken from this. Nitrogen of the protein and other nutritional component were firstly converted to ammonium sulfate through acid digestion (H_2SO_4). The end product of acid digestion was then cooled down and diluted with sterilised water. This was then turned into a strong basis with sodium hydroxide (NaOH). These steps were followed by letting the ammonia (NH_3) free and catching it up in a boric acid dilution. The ammonia in the boric acid was titrated with diluted HCl.

Crude protein (CP) = N x 6.25

Protein determination:

The percentage nitrogen was determined according to the A.O.A.C. (1965) method with a few small alterations. One gram of each ovendried cladode sample was weighed and placed in a digestion tube, together with a catalytical tablet and 10 ml concentrated sulphuric acid (H_2SO_4). The digestion-oven was turned to 5 and the mixture boiled for one hour, where the sulphuric acid (H_2SO_4) digested the cladode sample to a see-through colour. Sulphuric acid (H_2SO_4) is a strong oxidant and oxidises the sample to black carbon. The sample was then left to cool down. The digestion tube was then distilled in an Erlenmeyer flask with 100 ml of boric acid, 20 ml water and 130 ml of sodium hydroxide. The sample was left to distillate until a

total volume of 200 ml was left. Three drops of a marker were then added to the Erlenmeyerflask and as the colour turned to blue, titration was started till the colour of the sample turned to a light pink.

Determination of acid detergent fibre (ADF):

The following reagents were used:

1 – Acid detergent solution (ADS) consisting of: 20 g of cetyl trimethyl ammonium bromide dissolved in one litre 1 N sulphuric acid (H_2SO_4).

2 – Pepsin-acid solution consisting of a fresh solution (prepared daily) of 4 g of pepsin (activity 1:10000) in 0.075 N hydrochloric acid (6.1 ml concentrated HCl per litre distilled water) previously heated to between 42 °C and 45 °C).

The Dosi Fiber/Fibertec system was used as recommended in Goering and van Soest (1970). The cladode samples were air dried and milled. One gram of each sample was weighed into a large glass test tube and 50 ml of pepsin-acid solution was added at 43 °C. When the sample was completely dispersed it was placed in a waterbath at 45 °C. The sample was then intermittently mixed by swirling and incubating in the waterbath for 24 hours at 45 °C. The samples were then quantitatively transferred to sintered glass crucibles in the cold extraction unit of the Tecator apparatus, using suction and rinsing with warm (50 °C) water. The crucibles with the samples were then placed in the hot extraction unit. To each crucible a 100 ml of cold acid detergent solution (ADS) was added, before each solution was boiled for 60 minutes. Each solution was then filtered through suction and three times washed with hot water and twice rinsed with acetone. The residue was then dried overnight at 105 °C, cooled down in a desiccator for 30 minutes and weighed. Ash was measured at 500 °C for four hours and the furnace was allowed to cool down to 250 °C before removing the crucibles and placing them in a desiccator for 30 minutes. Each samples was then weighed again. Corrections were made for the dry matter of the sample.

$$\% \text{ Acid detergent fibre} = \frac{RCD - RCA}{\text{Original sample mass}} \times 100$$

Where RCD = Residue in crucible after drying

RCA = Residue in crucible after ashing

Ash and organic matter content:

The ash content is important, for it is the part in the feed that does not supply energy. Thus, the higher the ash content of the feed, the lower its energy content. Two grams of each cladode sample were weighed in a silica bowl, carbonated on a hot plate and cremated overnight at 525 °C. After this each silica bowl with ash was cooled down in a dessicator and weighed.

$$\text{Percentage Moisture} = \frac{W_1 - W_2}{\text{Mass of sample}} \times 100$$

Where W_1 = Weight of bowl with ash

W_2 = Weight of bowl

Organic material was then calculated by subtracting the percentage ash from 100. The moisture content affects the energy value of the nutrition, because a low dry material content of a given ruminant feed indicates reduced nutritional value. Either drying or evaporation can determine dry matter and moisture content of ruminant feed. The results are expressed as percentage dry matter or percentage moisture.

Dendrogram:

The UPGMA method was used for cluster analysis of the pairwise distance matrix, which generated a dendogram representing the genetic distances among cactus pear cultivars. Calculation of the distance matrix and cluster analysis was carried out using NCSS 2000 software package (Hintze, 1998) (Figure 2.1).

Linear correlation matrix:

Calculation of the linear correlation matrix between all the morphological characteristics was carried out using Agrobase 2000 statistical analysis programme (Table 2.20).

Results and Discussion

Phenology:

Zastron had the earliest reproductive and vegetative bud break, 50 % anthesis and 50 % fruit ripening (Table 2.13). The ripening times more or less corresponds with the times given by Pimienta-Barrios and Muñoz-Urias (1995) (Table 2.9). All the cultivars had a slightly earlier ripening and harvesting time, than the trial plantings at Fort Hare (Table 2.3 and 2.4). This could possibly be attributed to the colder climate in Fort Hare.

Peel and pulp colour:

The results (Table 2.14) correspond with Pimienta-Barrios and Muñoz-Urias (1995) (Table 2.9). The only difference was Morado, who Pimienta-Barrios and Muñoz-Urias (1995) described as a pale-yellow colour, where we thought it to be white. This was also in correspondence with Brutsch`s (1994; 1979) descriptions of the peel and pulp colour. It is difficult to assess certain colours, since different people with different perceptions do the colour assessments, even when a colour chart is used.

Plant width and plant height (cm):

Skinners Court had the highest plant width value, significantly higher than all other entries, except for Zastron and Meyers (Table 2.15). Roedtan, had the lowest value, significantly lower than all other entries. From this, one can conclude that Roedtan requires a significantly closer planting space than the rest of the cultivars. Zastron had significantly higher plants than all other entries. The strong vegetetative growth of Zastron could possibly be attributed to its longer growing season (Table 2.15). Algerian was the entry with the lowest value. The plants were generally higher than reported by Mulas and D`Hallewin (1997), whose plant height was in a range of 119-144 cm (Table 2.6).

Fruit yield (ton ha^{-1}):

Gymno Carpo and Turpin had a significantly higher fruit yield than all the other entries (Table 2.15). This was followed by Meyers, Algerian and Roedtan, who had a significant lower fruit yield. Skinners Court was the entry with the lowest fruit yield – significantly lower than all the other entries. This corresponds with Table 4.3 where Brutsch (1994) described the yield potential of Gymno Carpo as very good. This also corresponds to Brutsch`s description of Algerian as a very good fruit bearer.

It should be noted that when comparing fruit size, Skinners Court had the second highest value. The low yield potential of Skinners Court can therefore possibly be attributed to the large tendency of this cultivar to bear large fruit. Skinners Court also had the highest plant width value, which could be a possible explanation for the low fruit yield, since vegetative growth is negatively correlated to reproductive growth because of the competition for carbohydrates in the plant.

According to the minimum criteria for cactus pear varieties for fruit production potential, all the cultivars, except Skinners Court satisfy the minimum requirement for fruit production (see Table 2.1). The cultivars had a significantly higher fruit yield (ton ha^{-1}) than reported by Brutsch (1979) (Table 2.4). The cultivars had significantly lower fruit yields for Malta, Morado and Gymno Carpo, but not for Algerian, than reported by Van der Merwe *et al.* (1997). This could possibly be attributed to different climates, rainfall, soil and orchard practices. It should be noted that pruning has a negative influence on reproductive parameters like amount of fruit per plant, but it has a positive influence on vegetative parameters like cladode yield. Climatic differences between seasons cause great variation, especially in reproductive measurements. Therefore one seasons` data is not always a good indication of the production potential of a certain cultivar.

Fruit mass (g):

Nudosa had a significantly higher fruit mass than the rest of the entries (Table 2.15). Morado and Zastron had a significantly lower fruit mass than all the entries. There were no significant differences between the rest. According to the minimum criteria for cactus pear varieties for fruit production potential, all the cultivars, except Zastron satisfy the minimum requirement for fruit production (Table 2.1). This corresponds with data from Brutsch (1994) who stated that the fruit of Algerian is medium to large

and the fruit of Gymno Carpo large (Table 2.3). Also in correspondence with this, Brutsch (1994) found that Malta had small to medium fruit. Van der Merwe *et al.* (1997) reported lower fruit masses on Algerian, Malta and Gymno Carpo, but a higher fruit mass on Morado (Table 2.7). Brutsch (1979) and Pimienta-Barrios and Muñoz-Urias (1995) had lower fruit masses for all the same cultivars they described (Table 2.4 and 2.9). This could possibly be attributed to different climates, rainfall, soil and orchard practices. There is a strong positive correlation between fruit mass and peel thickness, fruit mass and flower end depth and fruit mass and pulp mass (Table 2.6). In all three these characters Zastron ranked low, thus the correlation matrix confirms the fact that small fruit is expected from Zastron.

Pulp mass (g):

Pulp mass varied extensively between the entries, from 78.2 to 143.8 (Table 2.17). Nudosa had a significantly higher pulp mass than all other entries. Morado and Zastron had a significantly lower pulp mass than the rest of the cultivars, but with no significantly difference between them. There was, as expected, a high correlation between pulp and fruit mass (Table 2.20). This was clearly demonstrated in the entries of Morado and Zastron, where both had low fruit and pulp masses. Nudosa was, in both cases, the highest ranking entry.

Pulp percentage (%):

Malta, Gymno Carpo, Nudosa, Meyers, Roedtan and Morado had the highest pulp percentage, significantly higher than the poorest three entries (Table 2.17). According to the minimum criteria for cactus pear varieties for fruit production potential, all the cultivars satisfy the minimum requirement for fruit production (Table 2.1). The specific cultivars had a higher pulp percentage than summarised by Sáenz-Hernández (1995) (Table 2.8) and Pimienta-Barrios and Muñoz-Urias (1995) (Table 2.9).

Peel thickness (mm):

Malta and Gymno Carpo had a significantly thinner peel than all other entries (Table 2.17). Skinners Court had the thickest peel, a significantly higher value than the rest. This more or less corresponds to the peel thickness reported by Sáenz-Hernández

(1995) (Table 2.8). According to the minimum criteria for cactus pear varieties for fruit production potential, all the cultivars satisfy the minimum requirement for fruit production (Table 2.1). There is a strong negative relationship between peel thickness and fruit yield. This is clearly demonstrated by example Gymno Carpo and confirmed by the correlation matrix (Table 2.20).

Total soluble solids (TSS):

Morado, Roedtan and Algerian had a significantly higher TSS than all the other entries (Table 2.17). There were no significant differences between the rest. There is a strong positive relationship between TSS and the vegetative growth characteristics (Table 2.20). This is clearly demonstrated by Nudosa, who ranked low when comparing TSS, as well as when measuring plant height, amount of cladodes yielded and cladodes pruned. Algerian and Morado had a higher and Gymno Carpo and Nudosa a lower TSS than stated by Pimienta-Barrios and Muñoz-Urias (1995) (Table 2.9). The TSS of the cultivars also more or less corresponded with the TSS given by Sáenz-Hernández (1995) and Mulas and D`Hallewin (see Table 2.8 and 2.6). According to the minimum criteria for cactus pear varieties for fruit production potential, Skinners Court, Nudosa, Gymno Carpo and Malta do not satisfy the minimum total soluble solid requirement for fruit production (Table 2.1).

Flower end depth (mm):

There were no significant differences between the cultivars (see Table 2.17). Environmental changes in climate between seasons cause large variation, especially in reproductive measurements. Therefore the data of one season will not always be a good indication of the production potential of a specific cultivar.

Cladode yield per plant (kg):

Turpin and Meyers had a significantly higher cladode yield than all other entries (Table 2.18). Morado had the next highest yield, significantly higher than the poorest five entries. Malta had the lowest yield.

Cladode mass (kg):

Skinners Court had the highest cladode mass, significantly higher than all other entries, followed by Nudosa, which had a higher mass than all entries except Skinners Court. There were no significant differences between the rest (Table 2.18). There is an inverse relationship between cladode mass and fruit yield, this is clearly demonstrated in the case of Skinners Court, who had the lowest fruit yield, but the highest cladode mass of all the entries (Table 2.18).

Number of cladodes pruned (n) per plant:

The number of cladodes per plant varied extensively between the entries, from 22.9 to 107.6 (Table 2.18). Turpin had the largest number of cladodes, significantly more than all entries except Meyers, which was second. Skinners Court had the lowest value, significantly lower than all other entries except Meyers, which was second. Skinners Court had the lowest value, significantly lower than all other entries. For Skinners Court and Turpin there was an inverse relationship between cladode yield and number of cladodes, and cladode mass, but this was not the case for a cultivar like Morado which had a good rank for all three the characteristics (Table 2.18). The dendrogram puts Turpin and Meyers in the same cluster, confirming the fact that they ranked high for the measured cladode characteristics, except for cladode mass.

Chemical analysis:

Crude Protein:

Roedtan had a significantly higher crude protein content than the other entries except for Morado and Gymno Carpo (Table 2.19). Nudosa had the lowest crude protein content. This corresponds with Felkers` (1995) findings that all the cactus pear cultivars has a crude protein percentage in the range of 5-12% (see Table 2.12)

Acid detergent fibre:

The acid detergent fibre varied extensively between the entries, from 22.4 to 11.3 (Table 2.19). Roedtan had the highest value, significantly more than all the entries except for Gymno Carpo and Morado. Skinners Court had a significantly lower value than all the other entries. All the cultivars had an acid detergent fibre much lower than the 43% Felker (1995) found (Table 2.12).

Organic matter:

The organic matter content did not vary extensively between the entries (Table 2.19). Morado had the highest organic matter content, followed by Gymno Carpo. Nudosa had a significantly lower value, except for Skinners Court and Turpin. The mucilage complicated the neutral detergent fibre measurements to such an extent that it could not be determined.

Dendrogram:

A dendrogram constructed using the phenotypical traits is given in Figure 2.1. In this dendrogram, the range of dissimilarity of all the cultivars ranged from 0.00-2.00, and the different clusters were formed within this range. Results from the cluster section indicated two clusters among which all of the cultivars were divided. Cluster I consisted of two cultivars, Nudosa and Skinners Court. These cultivars possessed some common traits such as identical plant width, plant shape, peel colour at 50% break, organic matter content, acid detergent fibre content, TSS and flower end depth. Cluster II comprised of eight cultivars, Roedtan, Meyers, Turpin, Zastron, Morado, Aglerian Malta and Gymno Carpo. Cluster II can be divided into two main groups, group A and B. Group A consisted of three cultivars, Algerian, Malta and Gymno Carpo. The cultivars in group A had resemblance in characters like plant shape, habitus, growth habit type, cladode shape, peel colour. The cultivars also had identical plant width, fruit mass, pulp mass, organic matter content and more or less the same phenology stages. Group B consisted of five cultivars, Roedtan, Meyers, Turpin, Zastron and Morado. The cultivars in this cluster possessed equal fruit mass, identical cladode shape, growth habit type and plant shape. None of the cultivars was placed out of any cluster. This indicates the presence of a relationship within the cultivars mentioned, or between the cultivars and the other clusters, based on the 12 phenotypic traits recorded. The presence of phenotypic characters is sometimes very different from those of the mother plant when seedlings are used. This could be related to a different expression of the genes encoding these characters or a result of the effect of the environment (Nieddu and Chessa, 1997). Morphological traits could be used for cultivar identification although it cannot give accurate estimates of genetic distances (Scheinvar, 1995). However, since the

expression of morphological markers is not reproducible over a range of environments, the results for morphological data should be supplemented with biochemical or DNA markers. The rest of the discussion and comparison follows in Chapter 3.

Conclusions

It should always be remembered with the selection of a cultivar according to its fruit and plant characteristics that not all the characteristics are equally important to a producer and therefore will definitely not carry the same weight in cultivar selection. For example, high production potential and high resistance against pests and diseases is of great importance for every producer, but not necessarily cladode mass. The recommended cultivar for fruit production is Turpin and Roedtan, who scored above average in most of the horticultural characteristics, except for peel thickness. Since the peel thickness of Turpin and Roedtan still satisfies the minimum criteria for production (Table 2.1), it is not regarded as a negative characteristic.

Both these cultivars had a high TSS, which is a very important characteristic. It is clear that Skinners Court is a cultivar who favours vegetative growth, rather than reproductive growth. This makes Skinners Court an unsuitable cultivar for fruit production. Gymno Carpo would also be a good cultivar choice for fruit production, but its TSS does not meet the minimum requirement for fruit production. It is clear that Zastron has great earning potential, because of its early bearing tendencies. Skinners Court, Malta, Algerian and Turpin can also compete with this early market. The fruits of Zastron, however, do not satisfy the minimum requirement for fruit production. It is difficult to decide which fruit colour is most attractive, because this is a very subjective preference. It depends on personal taste, what the market is familiar with and how the fruit is going to be consumed, thus, in a salad, as edible fruit or as a processed product, like jam. Some South African farmers feel that the local markets prefer red to orange internal flesh colour, while the international market prefers green. It is interesting that pulp mass and pulp percentage is not correlated (Table 2.20). Nudosa performed excellently when comparing fruit mass, pulp percentage and pulp mass between the cultivars. The

fruit yield of Nudosa was very low, however, and this could be a problem, since fruit yield is a very important character. Emphasis should rather be given to cultivars that have a competitive fruit mass, a fair pulp mass and pulp percentage, but with excellent fruit yield, for example Turpin. Some of these cultivars may also perform differently in other areas, indicating why variety trials in different climatic areas are essential before variety recommendations can be made. Because of the big environmental influence on cactus pear, it is difficult to compare results with existing literature for they have different enviroments.

The cultivars investigated in this study have definite potential as ruminant feed and compared well to other varieties in literature. At this stage the farmers in South Africa either use cactus pear cladodes as animal feed as by-product, with the main aim on fruit production, or the farmers use these cladodes as ruminant feed on such a small scale, compared to the other feed they use, that the establishment of a certain cultivar solely because of its fodder qualities, could not be justified.

Turpin and Meyers, which were the best yielders, ranked low for the chemical characteristics. Morado, which ranked good for the measured yield characteristics, also performed well for the measured chemical characteristics. Morado seems to be the best cultivar in terms of all measured characteristics, and should be considered as a foraging cultivar. The dendrogram puts Nudosa and Skinners Court in the same cluster, confirming the fact that they ranked low for all measured characteristics except cladode mass. Although Gymno Carpo, Morado and Roedtan proved to be outstanding concerning their chemical composition, the rest of the cultivars also satisfied the requirements concerning chemical composition for marketing. It must be remembered that on a basis where fruit production is first priority and cladodes are merely used as a by-product, the establishment of a cultivar, solely on its cladode characteristics is not possible or practical. Therefore one should rather look for a cultivar with both good fodder and fruit production qualities, thus a bilateral crop. It is therefore important to consider both horticultural and ruminant feed qualities, but to keep in mind that not all the qualities are equally important for every producer - it depends on the specific application and aim of the producer. Roedtan meets the expectations of such a cultivar, since its cladode yield were above average, while his chemical analysis of the cladodes was ranked very high. It also had a good fruit yield and fruit mass, and very important, a very good

TSS. Although Turpin and Meyers prove to have the highest fruit yield, fruit mass, cladode yield and number of cladodes pruned per plant, their chemical analysis of the cladodes was poor. Since their chemical analysis still meet the minimum requirement for fruit production, Turpin and Meyers are also considered a good bilateral crop.

Table 2.13 Dates of the basic phenology stages of the 10 cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars evaluated in the 1999/2000 season at Potgietersrus in the Northern Transvaal (23° 50`S), South Africa.

Cultivar	Reproductive budbreak	Vegetative budbreak	50% Anthesis	50% Fruit ripening
Skinners Court	Week 2 in July	Week 3 in August	Week 3 in October	Week 2 in January
Nudosa	Week 2 in August	Week 2 in August	Week 4 in October	Week 4 in January
Gymno Carpo	Week 2 in August	Week 2 in August	Week 3 in October	Week 3 in January
Morado	Week 2 in August	Week 3 in August	Week 4 in October	Week 3 in January
Castron	Week 2 in July	Week 4 in July	Week 4 in September	Week 1 in January
Malta	Week 1 in August	Week 3 in August	Week 3 in October	Week 2 in January
Algerian	Week 1 in August	Week 4 in August	Week 3 in October	Week 2 in January
Burpin	Week 1 in August	Week 3 in August	Week 3 in October	Week 2 in January
Meyers	Week 3 in August	Week 3 in August	Week 4 in October	Week 3 in January
Roedtan	Week 2 in August	Week 2 in August	Week 4 in October	Week 3 in January

Table 2.14 Peel and pulp colour of the 10 cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars evaluated from the 1999/2000 season.

Cultivar	Peel colour at 50 % break	Pulp colour at 50 % break
Skinners Court	Light yellow/green	White/green
Nudosa	Pink/yellow/green	Red/orange
Gymno Carpo	Light orange/green	Orange
Morado	Light yellow/green	White
Zastron	Yellow/green	White
Malta	Light orange/green	Orange
Algerian	Pink/green	Dark pink
Turpin	Light orange/green	Orange
Meyers	Pink/green	Dark pink
Roedtan	Light orange/green	Orange

Table 2.15 Plant width, plant height, fruit yield and fruit mass of the 10 cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars evaluated from the season of 1999/2000.

Cultivar	Plant width (cm)	Plant height (cm)	Fruit yield (ton/ha)	Fruit mass (g)
Skinners Court	251	203	4.97	185.08
Nudosa	224	175	14.58	235.84
Gymno Carpo	217	175	30.67	170.81
Morado	223	207	19.60	145.68
Zastron	242	223	17.96	136.68
Malta	222	175	23.22	169.75
Algerian	221	173	24.98	161.88
Turpin	223	186	28.67	181.19
Meyers	231	194	26.72	176.39
Roedtan	208	181	23.66	171.74
Average	226.2	189.2	21.50	173.50
LSD (p=0.05)	**25.8360**	**10.8508**	**3.3766**	**24.6436**

Table 2.16 Plant shape, habitus, growth, habit type and cladode shape of the 10 cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars evaluated from the season of 1999/2000.

Variety	Shape	Habitus	Growth habit type	Cladode shape
Skinners Court	Flat	Medium	Large cladode	Elongated
Nudosa	Flat	Spreading	Bush	Round
Gymno Carpo	Flat	Spreading	Bush	Ovate
Morado	Flat/Round	Spreading	Bush	Ovate
Zastron	Flat/Round	Upright	Bush	Ovate
Malta	Flat	Spreading	Bush	Ovate
Algerian	Flat	Spreading	Bush	Ovate
Turpin	Flat	Spreading	Bush	Ovate
Meyers	Flat	Spreading	Bush	Ovate
Roedtan	Flat	Spreading	Bush	Ovate

Table 2.17 Pulp mass, peel thickness, total soluble solids and flower end depth of the 10 commercial cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars in South Africa as measured in the 1999/2000 season.

ivar name	Pulp percentage (%)	Pulp mass (g)	Peel thickness (mm)	Total Soluble Solids (°Brix)	Flower end depth (mm)
ners Court	54.94	101.52	5.91	12.47	6.03
osa	60.84	143.81	4.95	11.21	6.60
no Carpo	61.68	105.57	3.64	11.15	6.41
ado	60.02	87.34	4.35	14.36	5.24
ron	57.22	78.20	4.65	13.52	2.89
a	64.47	109.55	3.73	11.62	5.38
rian	59.40	96.13	4.41	13.91	6.20
in	55.04	99.86	5.17	13.62	7.12
rs	60.67	107.18	4.51	13.58	5.79
tan	60.67	104.41	4.76	14.24	4.94
(p = 0.05)	**4.6514**	**15.6124**	**0.5660**	**0.5089**	**4.7062**

Table 2.18 Mean cladode yield per plant, mean cladode mass and mean number of cladodes that needed to be pruned per plant of the 10 cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars evaluated from the 1999/2000 season.

Cultivar	Cladode yield per plant (kg)	Cladode mass (kg)	Number of cladodes pruned (n) per plant
Skinners Court	63.36	2.80	22.90
Nudosa	63.61	1.36	47.00
Gymno Carpo	63.02	1.16	56.60
Morado	78.84	1.18	72.50
Zastron	71.18	1.17	65.60
Malta	51.95	1.10	53.00
Algerian	63.48	1.23	56.30
Turpin	107.01	1.05	107.60
Meyers	102.05	1.14	91.90
Roedtan	77.09	1.09	72.00
LSD	**14.922**	**0.135**	**16.045**

Table 2.19 Chemical analysis of the 10 commercial cactus pear cultivars in South Africa.

Chemical Analysis (DM Basis)			
Cultivar	**Crude Protein (%)**	**Organic Matter (%)**	**Acid Detergent Fibre (%)**
Skinners Court	7.01	76.70	11.33
Nudosa	5.06	76.34	14.87
Gymno Carpo	8.17	84.74	20.95
Morado	8.20	87.80	22.30
Zastron	7.49	83.31	17.49
Malta	7.52	82.61	16.85
Algerian	6.22	82.88	18.67
Turpin	5.48	80.24	16.89
Meyer	6.09	83.03	14.66
Roedtan	9.25	84.16	22.42
LSD	**1.323**	**5.640**	**3.172**

Table 2.20 Linear correlation matrix of the *O. ficus-indica* cultivars, based horticultural and fodder characteristics.

	PEEL THICK	TSS	FED	PULP PERC	PULP MASS	PLANT WIDTH	PLANT HEIGHT	FRUIT YIELD	FRUIT MASS	CLAD MASS	CLAD PRUNE
TSS	0.2292 0.0232										
						*P=0.001					
FED	0.0961 0.3465	-0.1959 0.0532									
PULP PERC	-0.3446* 0.0005	-0.2261 0.0252	-0.0628 0.5393								
PULP MASS	0.2575 0.0105	-0.1702 0.0939	0.2442 0.0154	0.2443 0.0154							
PLANT WIDTH	0.2428 0.0160	-0.0373 0.7157	0.1347 0.1861	-0.2423 0.0162	-0.0839 0.4116						
PLANT HEIGHT	0.2086 0.0392	0.3633* 0.0002	-0.2791* 0.0054	-0.1865 0.0660	-0.2124 0.0358	0.4518* 0.0000					
FRUIT YIELD	-0.3764* 0.0001	0.0663 0.5165	-0.0080 0.9374	0.0985 0.3347	-0.1002 0.3264	-0.2833 * 0.0047	-0.2933* 0.0034				
FRUIT MASS	0.3597* 0.0003	-0.2034 0.0445	0.3379* 0.0007	-0.0029 0.9772	0.8686* 0.0000	-0.0141 0.8905	-0.1964 0.0526	-0.1755 0.0839			
CLAD MASS	0.4128* 0.0000	-0.0989 0.3325	0.1182 0.2462	-0.2245 0.0262	0.0540 0.5972	0.2171 0.0317	0.1900 0.0610	-0.6147* 0.0000	0.1496 0.1414		
CLAD PRUNE	0.0695 0.4962	0.3086* 0.0020	-0.0567 0.5793	-0.0712 0.4859	0.0190 0.8529	-0.0193 0.8501	0.0607 0.5524	0.4617 0.0000	0.0181 0.8595	-0.5217* 0.0000	
CLAD YIELD	0.1915 0.0590	0.3557* 0.0003	0.0972 0.3412	-0.2496 0.0132	0.0193 0.8502	0.1527 0.1333	0.2420 0.0164	0.2303 0.0225	0.0472 0.6445	-0.1470 0.1485	0.4628* 0.0000

TSS=Total soluble solids, FED=Flower end depth, PULP PERC= Pulp percentage, CLAD MASS=Cladode mass, CLAD PRUNE=Cladodes pruned, CLAD YIELD=Cladodes yielded

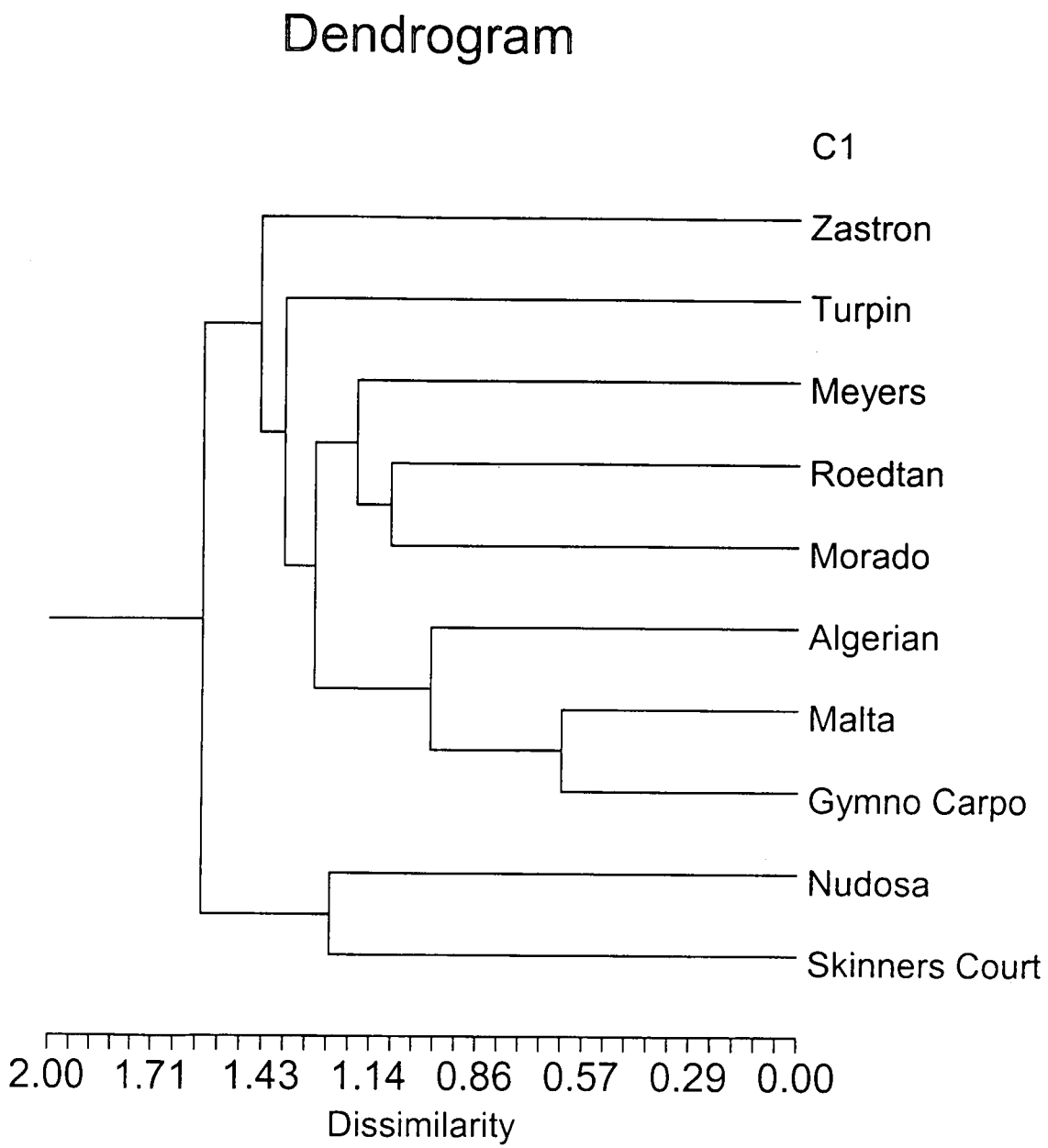

Figure 2.1 Dendogram generated by UPGMA analysis of morphological data collected from the 1999/2000 season.

Table 2.21 Actual distance between the cultivars in the dendrogram (Fig.2.1).

First Row	Second Row	Actual Distance
Skinners Court	Nudosa	1.25
Skinners Court	Gymno Carpo	1.79
Skinners Court	Morado	1.83
Skinners Court	Zastron	1.46
Skinners Court	Malta	1.79
Skinners Court	Algerian	1.68
Skinners Court	Turpin	1.54
Skinners Court	Meyers	1.73
Skinners Court	Roedtan	1.95
Nudosa	Gymno Carpo	1.28
Nudosa	Morado	1.74
Nudosa	Zastron	1.62
Nudosa	Malta	1.28
Nudosa	Algerian	1.43
Nudosa	Turpin	1.26
Nudosa	Meyers	1.50
Nudosa	Roedtan	1.49
Gymno Carpo	Morado	1.33
Gymno Carpo	Zastron	1.62
Gymno Carpo	Malta	0.63
Gymno Carpo	Algerian	0.88
Gymno Carpo	Turpin	1.54
Gymno Carpo	Meyers	1.33
Gymno Carpo	Roedtan	1.16
Morado	Zastron	1.11
Morado	Malta	1.33

Morado	Algerian	1.33
Morado	Turpin	1.49
Morado	Meyers	1.27
Morado	Roedtan	1.09
Zastron	Malta	1.49
Zastron	Algerian	1.49
Zastron	Turpin	1.51
Zastron	Meyers	1.41
Zastron	Roedtan	1.42
Malta	Algerian	1.08
Malta	Turpin	1.54
Malta	Meyers	1.33
Malta	Roedtan	1.16
Algerian	Turpin	1.27
Algerian	Meyers	1.33
Algerian	Roedtan	1.31
Turpin	Meyers	1.2
Turpin	Roedtan	1.19
Meyers	Roedtan	1.09

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CHAPTER 3

SUSCEPTIBILITY OF SOUTH AFRICAN CACTUS PEAR VARIETIES TO FOUR FUNGI COMMONLY ASSOCIATED WITH DISEASE SYMPTOMS.

Keywords: cactus pear, disease resistance, fungi, *Opuntia ficus-indica* (L) Mill.

Abstract

Ten of the most commercially important cultivars of spineless cactus pear [*Opuntia ficus-indica* in South Africa were screened in the glasshouse and field for their susceptibility to four fungal pathogens (*Phialocephala virens*, *Lasiodiplodia theobromae*, *Fusarium* sp1 and *Fusarium* sp2) commonly associated with cladode and fruit diseases in South Africa during surveys conducted over the course of the past three years. The fungi were artificially inoculated by means of toothpicks that had been colonised by the respective fungi prior to being inserted into the cladode or fruit. Control treatments consisted of sterile toothpicks. One trial was conducted with detached cladodes in the glasshouse and a second trial with cladodes of mature plants in a cactus pear orchard. A trial was also conducted with mature fruit of each cultivar in the laboratory. Following inoculation, cladodes and fruit were incubated at room temperature for 14 days to allow for lesion development around the inoculation site whereafter the diameters of lesions that resulted from inoculation were measured. In all three trials, considerable variation among cultivars in their susceptibility to each of the four pathogens was evident. Cladode inoculations in the glasshouse and field revealed that Nudosa and Algerian were generally the two most susceptible cultivars while Gymno Carpo, Zastron and Malta were generally the most resistant. These results were consistent with those of inoculations conducted on fruit. Control treatments in all three trials did not develop lesions around inoculation wounds. Cluster analysis grouped cultivars into two clusters with Nudosa in its own cluster and the remaining cultivars in a second cluster.

Introduction

Spineless cactus pear (*O. ficus-indica*) is an important crop in arid and semi-arid regions of the world (Brutsch, 1979; Pimienta-Barrios, 1994, Russel and Felker, 1987; 1985). Despite the fact that it is a native of regions with very hot and dry climates, cactus pear harbours many fungal pathogens, which attack the moisture rich fruit and cladodes. Apart from a few reports (Varvaro *et al.*, 1993; Granata, 1995; Granata and Sidoti, 2000) surprisingly few publications pertaining to diseases of *Opuntia* spp. have appeared worldwide.

The most comprehensive reports of infectious dieases (bacterial and fungal as well as other agents) of spineless cactus pear are by Granata (1995) and Granata and Sidoti (2000). A severe blight on cladodes and fruit caused by *Alternaria alternata* (Fries: Fries) von Keissler was identified by Granata and Sidoti (1997). The natural penetration site for the pathogen seems to be lesions caused by such as hail since the first symptoms are usually found in October, especially after heavy rains (Granata and Sidoti, 1997). Infected plants display chlorotic spots around the spines, which developed into yellow scabs characterised by a necrotic centre surrounded by a yellowish halo. A fruit rot caused mainly by *Alternaria* spp. was also reported by Chessa and Schirra (1992). Saad *et al.* (1998) described a bacterium causing wet rot and necrosis in cactus pear as *Erwinia carotovora* subsp. *carotovora* and Varvaro *et al.* (1993) also reported a wet rot caused by *Erwinia* sp. on cactus pear in Italy. Cactus pear is very vulnerable to root rot. One of the earliest reports of root disease is from Mexico where the causal agent was identified as *Fusarium solani* (Pettinari, 1951) but *Phytophtora nicotianae* has also been associated with root rot (Cacciola and Magnano, 1988). Scab caused by *Phyllosticta opuntiae* is reportedly on of the most aggressive and noxious diseases of *O. ficus-indica* (Barbera *et al.*, 1992).

The commercial cultivation of spineless cactus for its fruit is a relatively recent undertaking in South Africa but has been shown to possess huge export potential. Until recently, only one fungal pathogen, *Didymosphaeria opulenta* (De Not.) Sacc. was reported on the entire genus *Opuntia* in South Africa. The report however, is from *Opuntia stricta* Haw. and not *O. ficus-indica* (Crous *et al.*, 2000). More recently, three fungal pathogens associated with diseases of cladodes of *O. ficus-indica* were reported (Swart and Kriel, 2002). The need for research on the

diseases of *O. ficus-indica* in South Africa has recently become very important since local growers are increasingly reporting disease related yield losses. The aim of the present study was to systematically evaluate South African cactus pear varieties to four fungi, *Phialocephala virens* Siegfried & Siefert (Siegfried and Siefert, 1992), two unidentified *Fusarium* species, *Fusarium* sp1 and *Fusarium* sp2, and *Lasiodiplodia theobromae* (Pat.) Giff. & Maubl (Latham and Dozier, 1989) that have regularly been isolated form diseased cladodes obtained from various parts of the country over a period of three years.

Materials and Methods

Cladode inoculations. Mature cladodes from 10 commercially important cultivars of *O. ficus-indica* were obtained from Potgietersrus, Limpopo Province, South Africa (23° 50`S) and maintained at room temperature for five days prior to artificial inoculation. Single spore isolates of the four fungi were maintained on potato dextrose agar (PDA) at 5 °C. Wooden toothpick sections (20mm long) were autoclaved for 20 minutes in 250 ml distilled water, removed, blotted, and re-autoclaved in additional water to remove inhibitory substances. Toothpick pieces were then cooled in a sterile petri dish and transferred individually to margins of fast growing colonies of the four chosen fungi on potato-dextrose agar (PDA). After incubation for 72 hours, infested toothpick tips were removed and used as inoculum.

Toothpick pieces were inserted up to 10 mm deep into cladodes in the top older portion of the cladode. Insertion holes were covered with masking tape to prevent desiccation and contamination. Six detached cladodes of each cultivar (replications) were inoculated with each isolate. Control treatments consisted of autoclaved toothpick tips that were inserted into cladodes as described previously. Cladodes were incubated at room temperature for 14 days before the diameter of necrotic lesions that had developed around each inoculation site was measured. In field inoculations, three mature cladodes of each cultivar (replications) were similarly inoculated with toothpicks infested by the four respective fungal isolates. The diameter of the lesion around each inoculation wound was measured and data subjected to statistical analyses. Koch's postulates were confirmed by reisolation of the original pathogens from artificially inoculated cladodes.

Fruit inoculations. In the second trial, three mature fruit of each cultivar (replications) were inoculated with each isolate. To prevent accidental contamination, fruit were wiped with a cloth dipped in ethanol prior to the toothpicks being inserted. Control treatments consisted of autoclaved toothpick tips. Fruit were incubated at room temperature for 14 days before the diameter of the resulting lesion around each insertion wound was measured and the data subjected to statistical analyses. Koch's postulates were confirmed by reisolation of the original pathogens from artificially inoculated fruit.

Statistical analyses. A two-way analysis of variance (ANOVA) was performed on the data of each inoculation trial and treatment means were separated using the software program, NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA). Cluster analysis of data from all three trials by means of the UPGMA method was conducted to generate a dendogram showing realtionships between cultivars with regard to their susceptibility to the four pathogens using the NCSS 2000 software package (Hintze, 1998). Linear regression analyses between the treatment means of each cultivar for the three trials was performed using Agrobase 2000 (Agronomix Software Inc.)

Results

Tissue decay and mycelium growth became visible at most inoculation sites on cladodes and fruit 60 hours after inoculation (Figures 3.3-3.8). Control treatments showed very little necrosis on either cladodes or fruit. Virulence of the four fungal pathogens to each of the 10 cultivars differed significantly ($P < 0.05$) and there were also significant interactions ($P > 0.05$) between cultivars and the four isolates (Table 3.1; Figures 3.2-3.4). The results of the three respective trials with regard to the relative susceptibility of the 10 cultivars corresponded reasonably to very well. Although correlations between detached cladode and fruit inoculations ($r = 0.692$; $P< 0.05$) and detached cladode and cladode inoculations in the field ($r = 0.727$; $P < 0.05$) were significant, a highly significant correlation ($r = 0.964$; $P < 0.01$) existed between fruit inoculations and cladode inoculations in the field.

In artificial inoculation of detached cladodes in the glasshouse, *L. theobromae* and *Fusarium* sp1 caused the largest lesions in all 10 varieties while *P. virens* generally caused the smallest lesions (Fig. 3.2). Lesions caused by *L. theobromae* on detached cladodes varied between 14.32 and 32.95 mm while those for *P. virens* varied between 3.79 and 15.45 mm. Nudosa was generally the most susceptible cultivar to all four pathogens followed by Algerian, Morado and Meyers (Table 3.1). In field inoculations, lesions measured after 14 days were considerably smaller than for detached cladodes. *L. theobromae* was also not the most virulent isolate in field inoculations as was the case with inoculation of detached cladodes (Fig. 3.4). *Fusarium* sp1, although causing smaller diameter lesions than in detached cladodes, was equally virulent to *L. theobromae* in field inoculations. Nudosa and Algerian did not differ significantly ($P < 0.05$) in their overall susceptibility to the four pathogens in field inoculations. They were followed by, Morado Meyers, Turpin and Roedtan whose overall susceptibilities did not differ significantly ($P < 0.5$).

In fruit inoculations, Nudosa was overall the most susceptible cultivar to both *L. theobromae* and *Fusarium* sp1 (Fig. 3.3). The latter isolate displayed a far higher level of virulence to most cultivars, relative to *L. theobromae*, than it did in detached cladode inoculations. Both *Fusarium* sp1 and *P. virens* were significantly more virulent in fruit inoculations than in detached cladode inoculations. Algerian was the most susceptible cultivar to *P. virens*. Malta, Zastron and Gymno Carpo were the three most resistant cultivars to all four pathogens.

Dissimilarity between cultivars in the dendrogram that was generated following cluster analysis ranged from 0.50-2.59 (Fig. 3.9). The dendrogram represented two clusters, cluster A and B. Cluster A only comprised of one cultivar, Nudosa. Cluster B could be divided onto two groups, group I and II. Group I included three cultivars, Zastron, Malta and Gymno Carpo. Group II included the rest of the cultivars. Malta and Gymno Carpo and Turpin and Skinners Court were clustered closely together. The mean distance between group I and II in cluster B is 0.73 and 0.91, respectively.

Discussion

Results of the present study represent the first time that South Afrian cultivars of *O. ficus-indica* have been compared with each other regard to their susceptibility to fungal pathogens. Significant differences were observed between cultivars with regard to their susceptibility to each of the four fungal pathogens for each of the three trials. Significant cultivar-pathogen interactions for both cladode and fruit inoculations were also clearly evident. For example, in field inoculations, *Fusarium* sp1 was more virulent than *L. theobromae* on the cultivars Skinners Court, Nudosa, Morado, Malta and Turpin, but the opposite was true for Algerian and Meyers. *L. theobromae* was generally the most virulent isolate followed by *Fusarium* sp1. *P. virens* was generally the least virulent isolate in cladode inoculations but in fruit inoculations it caused lesions on some cultivars, especially Morado and Algerian that were almost equivalent to *L. theobromae*.

Despite the interactions there were nevertheless definite similarities between the three trials with regard to the overall relative susceptibilities of respective cultivars (Fig. 3.1). The fact that Nudosa and Algerian were consistently the most susceptible cultivars and Malta and Zastron the most resistant is encouraging as far as the credibility of these results are concerned. The fact that lesions in field inoculations were smaller is probably related to active host resistance of attached cladodes as opposed to detached cladodes. Another factor could be lower mean temperatures during the 14 day period from inoculation than that present in the glasshouse for the detached cladode inoculations. Varying levels of resistance among cultivars is probably also related to some inherent physiological characteristic of a particular cultivar and the ability of a particular pathogen isolate to overcome that resistance. It is unlikely that morphological characteristics such as thickness of the epidermis could have played a role since wounds were made during artificial inoculations.

Although there are options for chemical control, the search for tolerant cultivars is the safest and most economical alternative for cactus pear growers (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Despite the four different pathogens against which the 10 *O. ficus-indica* cultivars were screened in the present study yielding varying results for each different cultivar, the results are

nevertheless useful for the control of cactus pear diseases in South Africa. For example, Malta and Zastron, the two most consistently resistant cultivars in all three inoculation trials are probably also relatively resistant to other possible fungal pathogens that may be encountered in South Africa. Fortunately Morado and Gymno Carpo, two of the most popular cultivars in South Africa, also seem to be relatively disease resistant. By the same token, Nudosa and Algerian are probably the most susceptible of the 10 cultivars and cultivation of these cultivars should probably be avoided when disease pressure is high. It is therefore hoped that the results of the present study will enable growers to either plant or breed cultivars that are more resistant to disease based on the information provided here.

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Table 3.1. Diameter (mm) results of artificial inoculation of detached cladodes in the glasshouse, mature cladodes in the field and fruit inoculations in the laboratory. Values within each column followed by different lowercase letters are significantly different at $P < 0.05$.

DETACHED CLADODE INOCULATIONS

Cultivar	*P. virens*	*L.theobromae*	*Fusarium* sp2	*Fusarium* sp1	Control	CV mean
kinners Court	5.06 cb	20.03 def	11.64 b	17.60 bc	2.32	**13.58bcd**
Nudosa	15.45 a	31.04 ab	22.15 a	30.57 a	2.75	**24.80a**
ymno Carpo	4.86 bc	21.73 de	10.88 b	10.19 ef	1.97	**11.91d**
Morado	5.12 bc	28.69 abc	8.33 b	15.53 bcde	2.51	**14.42bcd**
Zastron	3.79 c	14.32 f	10.88 b	7.57 f	2.77	**9.14d**
Malta	5.93 bc	17.39 ef	9.04 b	16.75 bc	2.28	**14.55d**
Algerian	13.93 a	27.88 abc	10.60 b	13.28 cd	2.73	**16.42bc**
Turpin	6.97 b	25.25 bcd	11.75 b	12.05 cde	2.38	**14.00bcd**
Meyers	7.22 b	24.68 cd	8.94 b	19.17 b	2.14	**14.87bcd**
Roedtan	5.46 bc	32.95 a	9.27 b	12.19 cdef	2.46	**12.46bcd**
Pathogen mean	**7.38**	**24.40**	**11.35**	**15.49**	**2.43**	

FRUIT INOCULATIONS

	P. virens	*L.theobromae*	*Fusarium* sp2	*Fusarium* sp1	Control	CV mean
inners Court	14.33 bc	21.12 b	15.82b	17.21c	2.80	**17.12 c**
dosa	13.00 cd	27.11 a	22.00a	28.77a	2.71	**22.72 a**
mno Carpo	2.98 f	14.56 cd	8.00c	8.79d	2.61	**8.295 d**
rado	16.20 b	19.24 bc	19.92a	20.46c	3.00	**18.96 b**
stron	4.41 f	13.30 d	8.61c	7.00d	2.36	**8.33 d**
lta	7.70 e	9.51 d	7.92c	8.91d	3.60	**8.51 d**
gerian	27.00 a	27.55 a	15.66b	24.78b	4.10	**23.99 a**
rpin	12.17 cd	18.18 bcd	15.30b	19.23c	2.00	**16.22 bc**
yers	11.14 d	29.92 a	15.63b	18.74c	3.20	**19.42 b**
edtan	14.00 bc	20.00 b	16.02b	18.82c	3.00	**17.21 bc**
thogen an	**12.29**	**20.05**	**14.49**	**17.27**	**2.92**	

FIELD INOCULATIONS

	P. virens	*L.theobromae*	*Fusarium* sp2	*Fusarium* sp1	Control	CV mean
inners Court	4.36 c	11.33 abc	9.8 c	13.17 b	2.12	**9.66cdefg**
dosa	4.4 c	16.8 a	11 b	18.21 a	2.53	**12.6efg**
mno Carpo	5.12 ab	9 f	3 e	8.3 c	2.4	**6.36a**
rado	6.24 a	10.34 bc	8.7 d	11.94 b	2.96	**9.3cdef**
stron	3.22 d	8.14 ef	4.3 e	7 c	2.31	**5.67ab**
lta	4.81 b	7 e	5.51 f	8.48 c	3.06	**6.45b**
gerian	5.76 a	19.51 cd	8.32 a	18 a	3.3	**12.89cde**
rpin	4.73 b	9.91 d	8 d	12.37 b	2.12	**8.75cde**
yers	4.71 b	18.9 ab	9.9 abc	13.91 b	2.11	**11.88cdefg**
edtan	4.51 c	13.96 a	10.81c	13.42 b	2.25	**10.68defg**
thogen an	**4.78**	**12.49**	**7.94**	**12.48**	**25.16**	

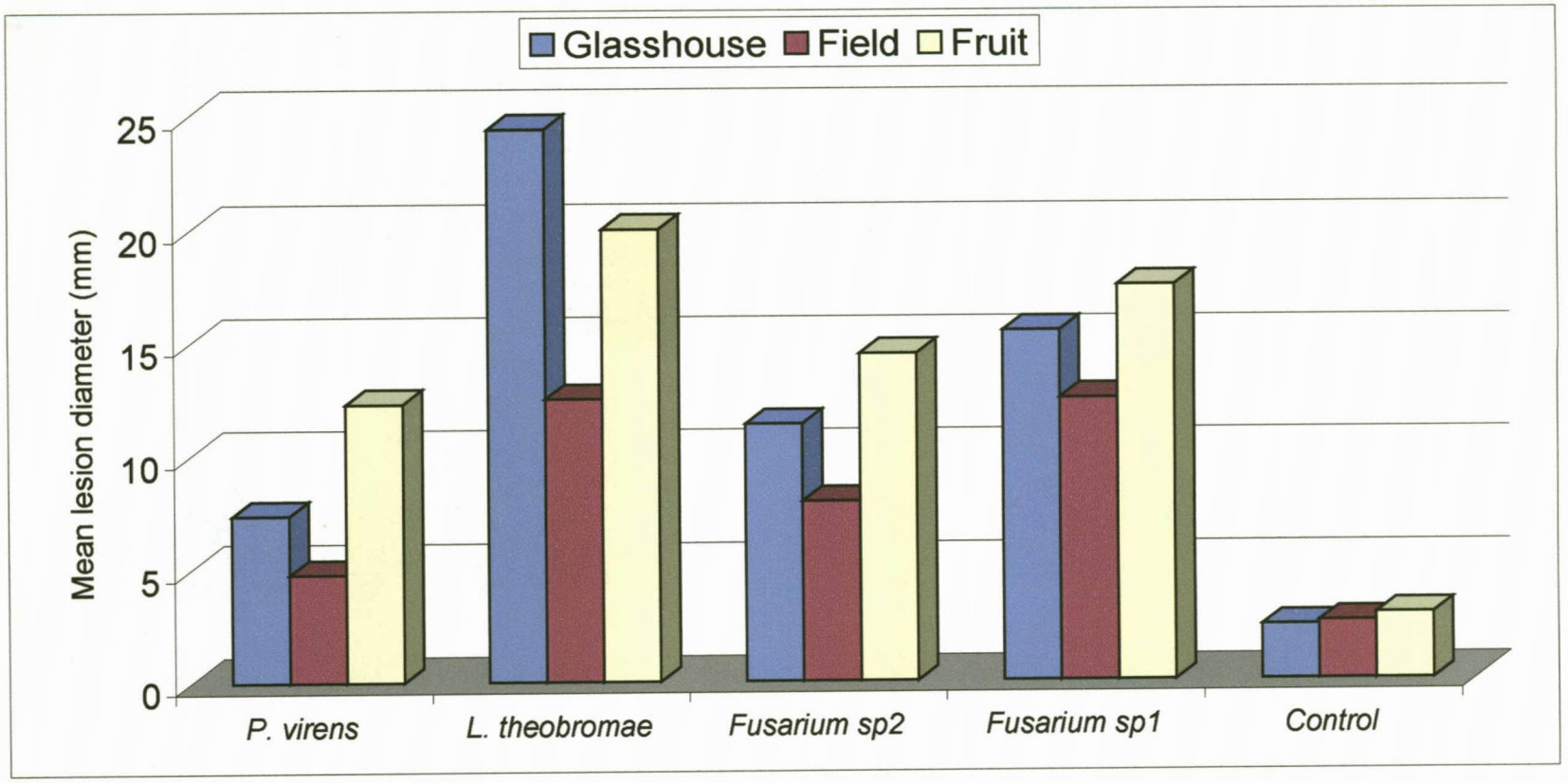

Fig. 3.1 Mean lesion diameters following artificial inoculations on cladodes in the glasshouse and field and fruit inoculations of all ten cultivars in the laboratory.

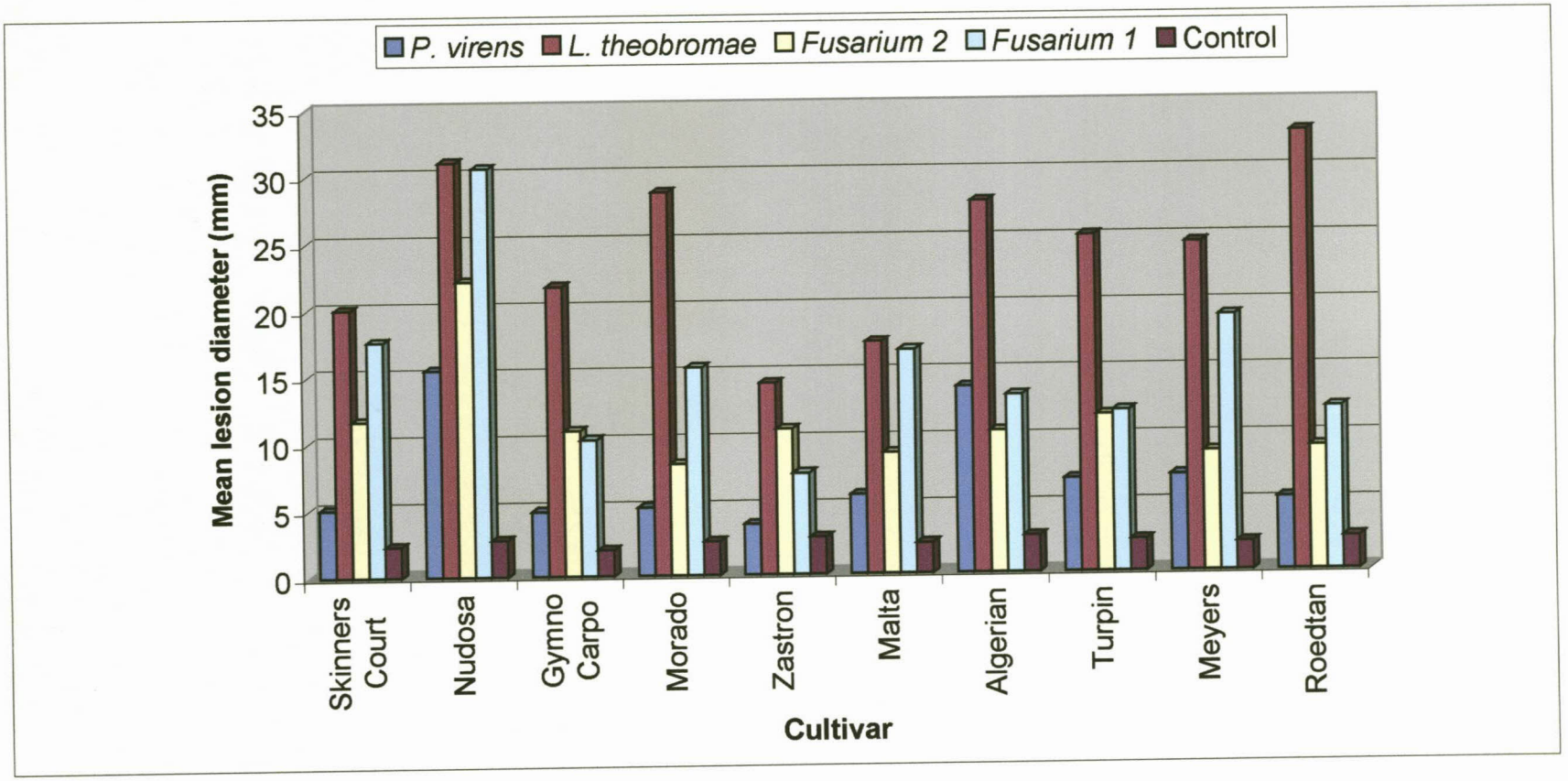

Fig. 3.2 Results of artificial inoculations in the glasshouse on detached cladodes of 10 *O. ficus-indica* cultivars with four fungal pathogens.

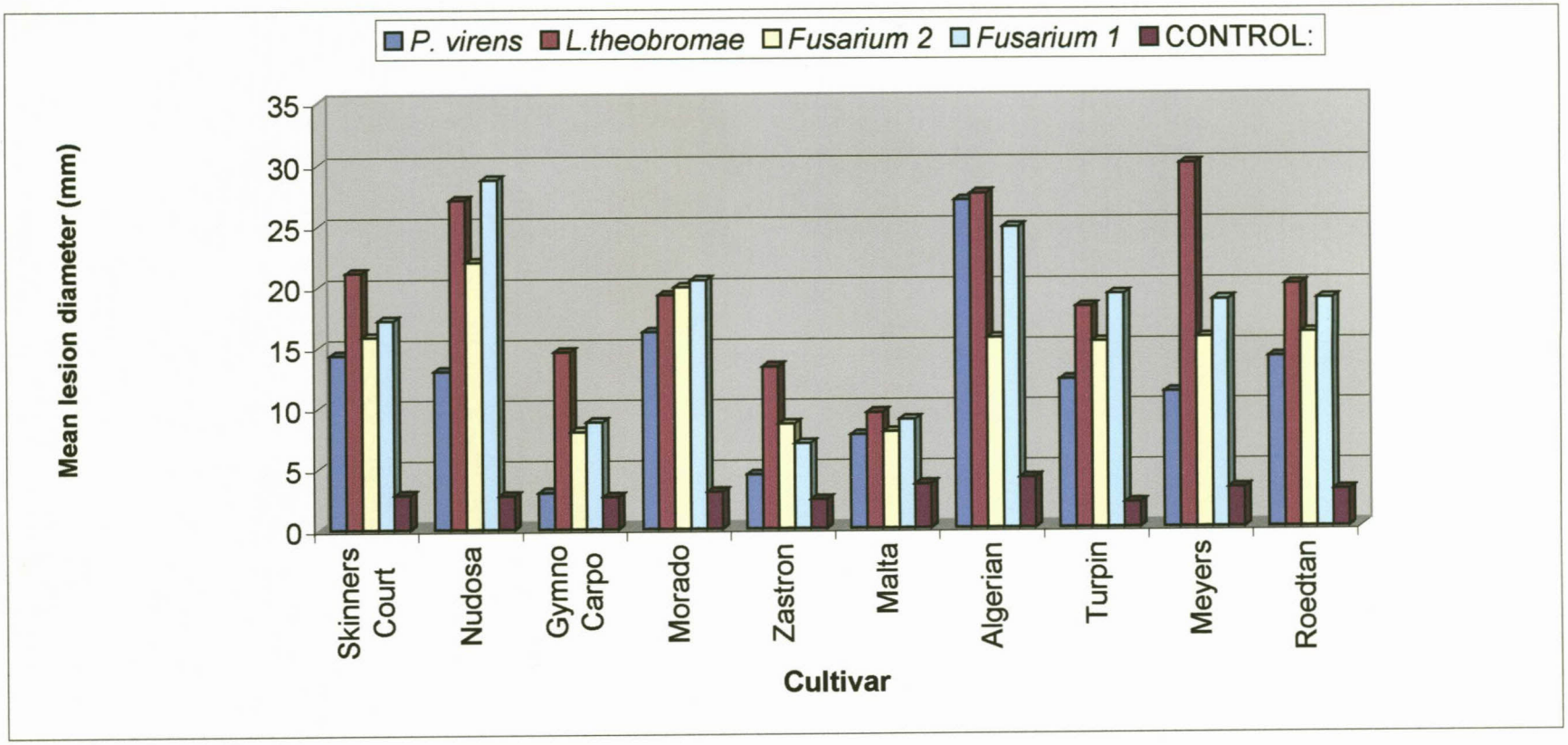

Fig. 3.3 Results of artificial inoculations in the laboratory on fruit of 10 *O. ficus-indica* cultivars with four fungal pathogens.

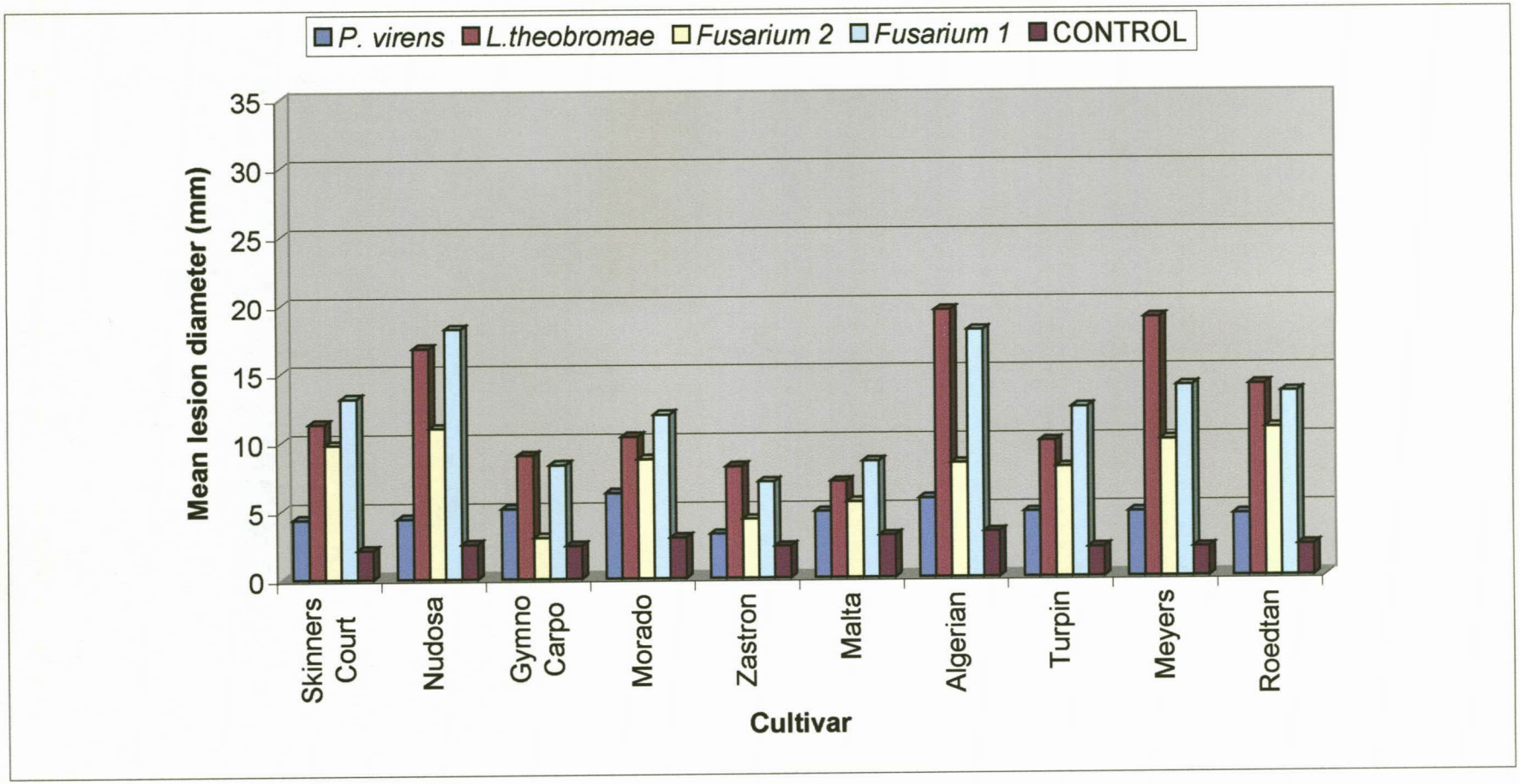

Fig. 3.4 Results of artificial inoculations in the field on cladodes of 10 *O. ficus-indica* cultivars with four fungal pathogens.

Fig. 3.5 Detached cladodes of *O. ficus-indica* inoculated with toothpick colonised by four fungal pathogens.

Fig. 3.6 Lesion caused by the toothpick inoculation technique on a detached cladode. Arrow indicates the diameter of the lesion.

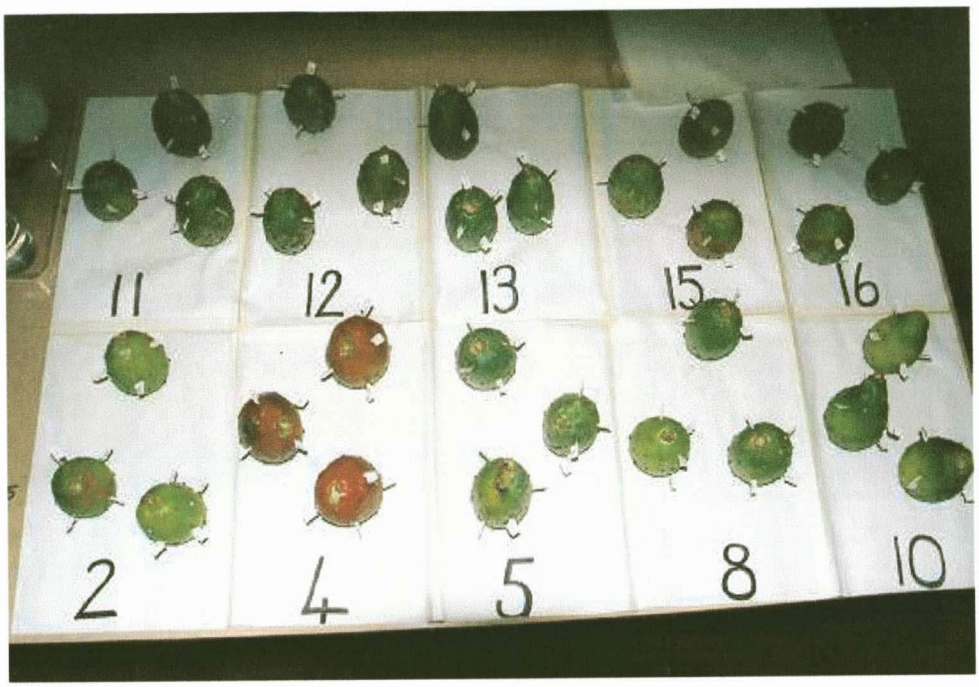

Fig. 3.7 Fruit inoculations using the toothpick technique.

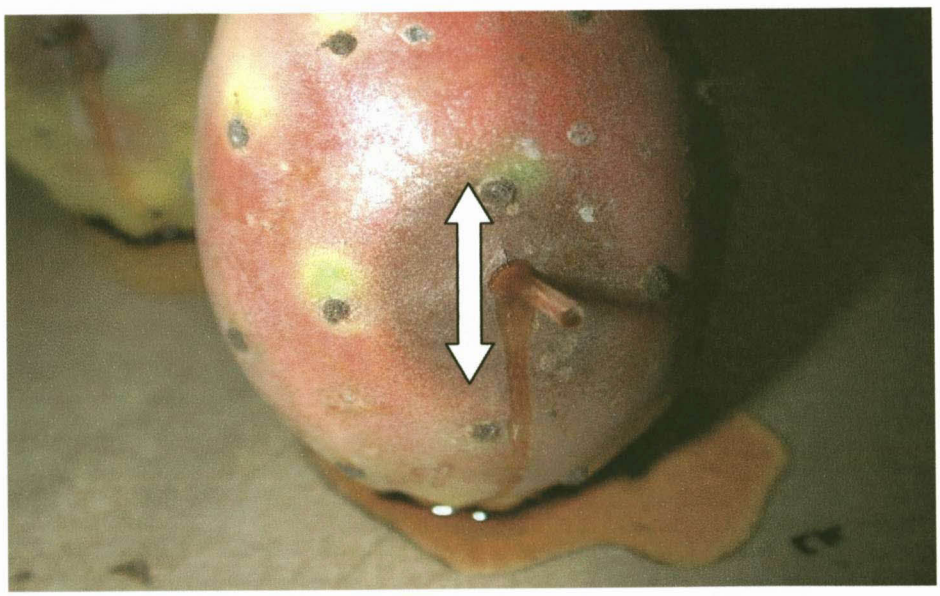

Fig. 3.8 Artificial inoculation of cactus pear fruit using the toothpick technique. Arrow indicates the diameter of the resulting lesion.

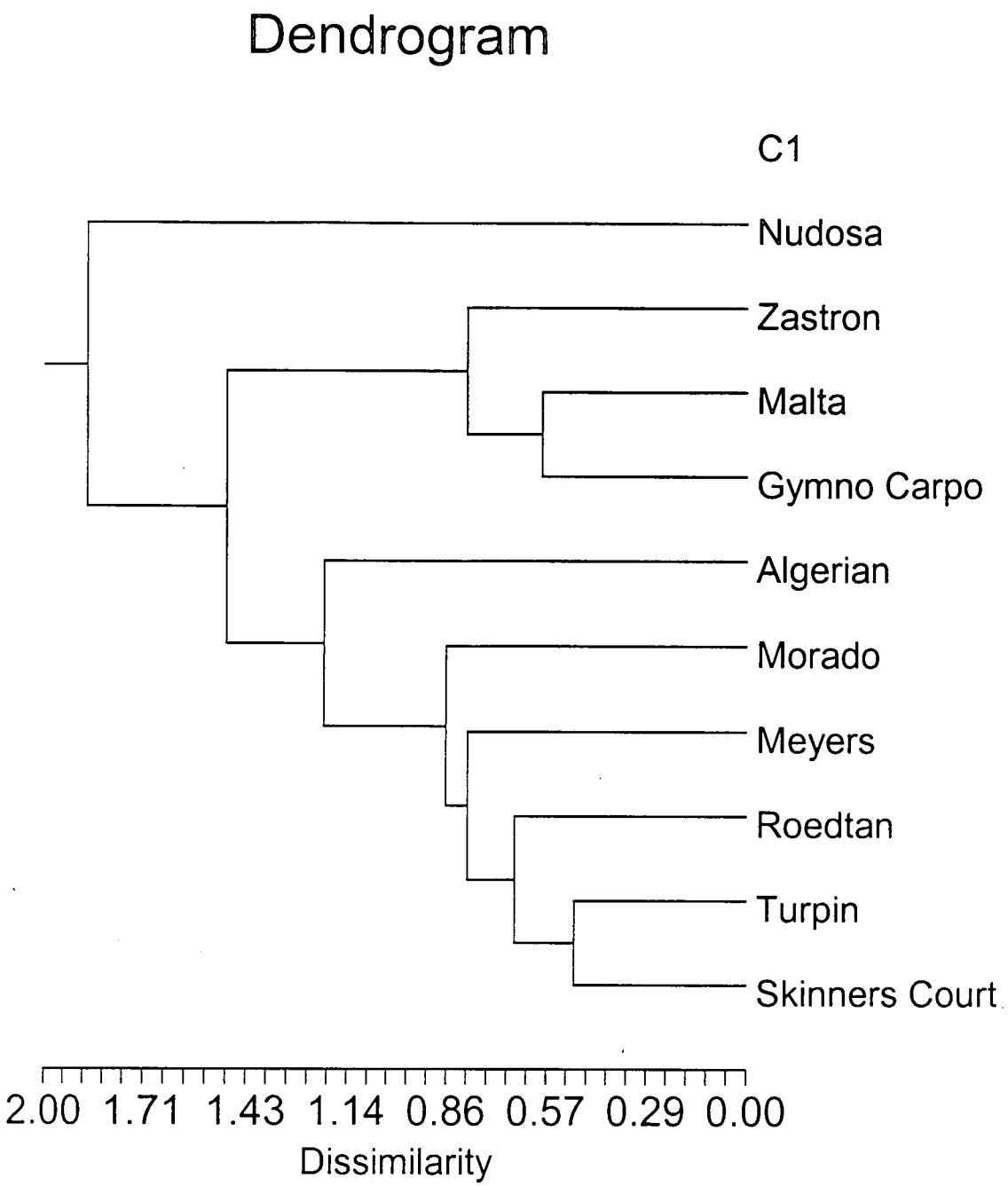

Figure 3.9 Dendogram generated by UPGMA analysis of the combined data collected from all three inoculation trials.

CHAPTER 4

GENETIC IDENTIFICATION AND ANALYSIS, OF COMMERCIALLY GROWN CACTUS PEAR [*OPUNTIA FICUS-INDICA* (L.) Mill.] SELECTIONS IN SOUTH AFRICA, USING AFLP FINGERPRINTING.

Keywords: Amplified fragment length polymorphism, cactus pear, DNA fingerprinting, *Opuntia ficus-indica* (L.) Mill.

Abstract

The amplified length polymorphism (AFLP) technique was used to assay 10 commercial cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars in South Africa. AFLP markers are genomic restriction fragments detected after selective amplification using the polymerase chain reaction (PCR). To prepare the AFLP template, genomic DNA was isolated and digested with two restriction endonucleases simultaneously, in order to generate the required substrate for ligation and subsequent amplification. The restriction fragments for amplification were generated by two restriction endonucleases: *Eco*RI and *Mse*RI. Following heat inactivation of the restriction endonucleases, the digested genomic DNA fragments were then ligated to *Eco*RI and *Mse*I adapters. PCR was performed in two consecutive reactions using the complimentary adapter sequence as primer. The product from the selective amplification was then analysed for polymorphisms by using an automatic sequencer and analytical software. Considerable variation was detected among the cactus pear entries using two primer combinations. AFLP data was scored into a binary matrix with AFLP fragments coded as present (1) or absent (0) across all the genotypes. Calculation of the distance matrix and cluster analysis was carried out using NCSS III. UPGMA clustering of the AFLP data identified two clusters, consisting of seven and three cultivars respectively.

Introduction

Cultivation of cactus pear is gaining popularity as an alternative crop for semiarid areas (Barbera, 1995; Mondragon-Jacobo and Pérez-González, 2000). Systematic collection and characterisation of germplasm from the native as well as naturalised populations, and continued efforts at breeding are needed to find new selections and to develop new cultivars for desertified areas (Mondragon-Jacobo and Pérez-González, 2000).

Current cultivar identification is mainly based on morphological traits in horticultural crops. However ,these traits are often affected by environmental factors and many characteristics fluctuate (Izun, 1997). Existing germplasm used in breeding programs should be characterised in terms of ecological adaptation, fertility, productivity, ripening time and fruit quality. Nieddu *et al.* (2000) did a variety description that represents the first systematic approach to evaluate and characterise selected accessions for their potential utilisation in the fruit industry. The propagation of genetically characterised varieties for plantation establishment is an important tool to improve the management of cactus pear cultivation (Nieddu *et al.*, 2000).

For breeding selecion it is also important to have a reliable, genetic method of identification of existing commercial varieties (Mondragon-Jacobo and Pérez-González, 2000; Wang *et al.*, 2002). Traditional breeding may be facilitated by analysing the genetic variation in existing cultivars and wild populations using molecular marker technology (Mondragon-Jacobo and Pérez-González, 2000). Due to the power and speed of many molecular techniques, cactus breeders would like to be able to make use of these techniques to study physiology and breeding. The maintaining of extra individuals in nurseries diminishes the output of breeding programmes (Chapman and Paterson, 2000; Mondragon-Jacobo and Pérez-González, 2000). While groups have had success applying molecular techniques to cactus research, their work is in part hindered by the lack of molecular tools available to work with cactus. A major challenge will be to develop tools for cactus to allow researchers to produce improved varieties. The development of these tools can be accelerated by utilising the methodology applied in well-studied plant species (Chapman and Paterson, 2000).

DNA fingerprinting is used to visualise DNA polymorphisms between samples (in this case, cultivars). These fingerprints may be used as a tool for determining the identity of a specific DNA sample or to assess the relatedness between samples. Fingerprints are also used as the source for genetic markers to generate linkage maps or to identify molecular markers linked to phenotypic traits and/or genetic loci. The application of DNA fingerprinting has proven valuable in the identification of cultivars and species (Kuhnlein *et al.,* 1990), and could help to create more efficient breeding programs through the detection of genetic linkages between DNA fingerprinting band and agricultural important quantitive trait loci (QTL) (Russel *et al.*, 1995). The high variability of DNA fingerprinting described in humans, animals and plants allows the identification of different individual genotypes and species (Lin *et al.,* 1993).

Cultivar identification can be achieved more accurately using DNA fingerprinting data, especially in materials characterised by high genetic variation between cultivars and a lack of variation, in vegetatively propagated material derived from cross pollinating species. The most closely related cultivars can be distinguished with the aid of DNA fingerprinting methods (Nybom, 1994). Many DNA fingerprinting techniques have been developed in the past few years.

The AFLP technique is a DNA fingerprinting technique that combines both RAPD and RFLP strategies. It is based on the selective PCR amplification of a subset of genomic restriction fragments from a total digest of genomic DNA. Amplified Restriction Fragment Polymorphism (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. The AFLP technique is robust and reliable because stringent reaction conditions are used for primer annealing. AFLP detects genomic restriction fragments similar to the RFLP technique; with the major difference that PCR amplification instead of Southern hybridisation is used for the detection of fragments. Thus, the AFLP technique combines the reliability of the RFLP technique with the power of PCR (Waugh, 1997). A comparison of different mapping techniques – RFLP, RAPD, SSR, and AFLP – for their relative efficiency in detecting polymorphism demonstrated that AFLP is the most efficient (Russell *et al.*, 1995).

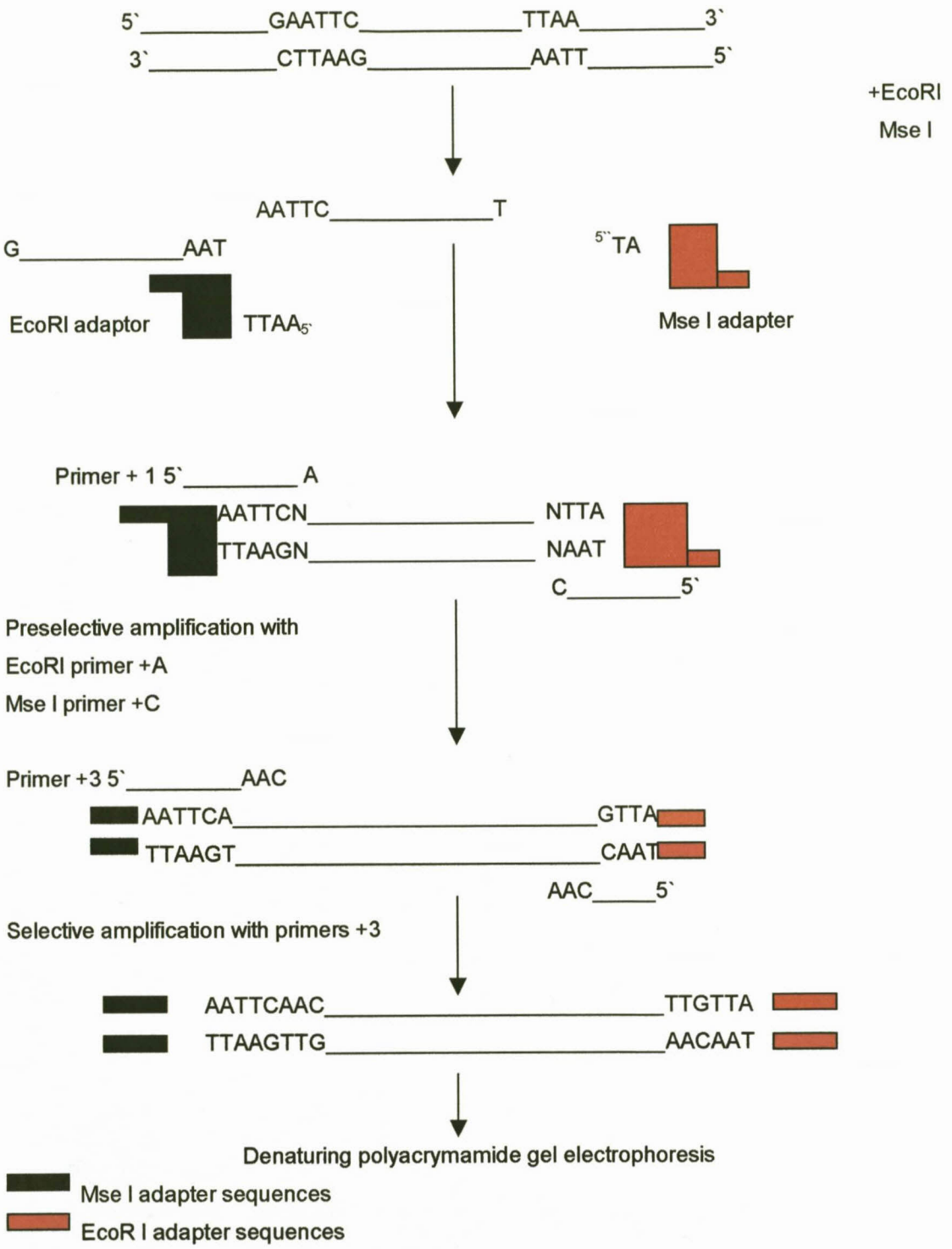

Figure 4.1 Example of the AFLP procedure using one primer pair (AFLP Analysis System I, GIBCO BRL Products).

The AFLP technique involves three major steps:

1. Restriction endonuclease digestion of the DNA and ligation of oligonucleotide adapters.
2. Selective amplification of the restriction fragments.
3. Gel analysis of the amplified fragments

Amplification of restriction fragments is achieved using the restriction site sequence as target sites for adaptor ligation. Adaptor sequences are used for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualised by PCR without prior knowledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction fragments (Russell *et al.*, 1995).

Only a few reports of DNA extraction of cacti are available. Mondragon-Jacobo (1996) reported a successful protocol for extraction of several cacti. The sticky, jelly-like water-absorbing substance called mucilage complicates the DNA extraction procedure. Mucilage is produced and secreted by specific cells that can be located at the inner side of the chlorophyll-containing tissue (chlorenchyma), and large droplets are exuded when a cladode is cut (Mizrahi and Nerd, 1997). Wang *et al.* (2002) modified a standard protocol for the DNA extraction of cactus. They report that cactus DNA often needed further purification through spin columns. They found that phenol-chloroform extraction did not improve the quality of the DNA and reduced the DNA yield. They also found that younger cladodes yielded better quality DNA (Wang *et al.*, 2002).

Recent studies on *Opuntia* genetic resources describe the genetic variability in the genus detected within the European population and the accessions most representative were *ex situ* collected from both cultivated, or natural environments. One of the largest collections is located in Sardinia (Italy) where 56 have been grown and studied since 1993 in terms of the *Opuntia* descriptor list (Nieddu *et al.*, 2000). Wang *et al.* (2002) determined that RAPD (random amplified polymorphic DNA) analysis was usefull to determine genetic variability in *Opuntia*. However, he found low levels of DNA

variation among five fruit cacti, from different countries, considered to represent three different species.

Wang *et al.* (2002), found that there were differences in the grouping of accessions based on marker vs. morphological and physiological data. He attributed this to three possible factors. Some of the phenotypic traits like fruit size and total soluble solids, have been selected during domestication. Therefore, phenotypic similarity may not reflect evolutionary relationships across the entire genome and is more likely to be detected by molecular markers. Also, phenotypic data consisted primarily of fruit and cladode measurements, and did not represent a random sampling of gene effects. Finally, phenotypic expression may be influenced by many non-genetic factors that are not expected to affect DNA marker patterns. The aim of this study was to fingerprint the ten promising cactus pear cultivars in South Africa and to determine genetic relationships between them.

Materials and Methods

Plant materials

Plant material was obtained from a field genebank near Potgietersrus, Northern Province (23°50`S), a subtropical area with predominantly summer rainfall. Single cladodes were established at a plant spacing of two meters within rows and five (1000 plants/ha) in rows orientated N/S. Two year old cladodes were harvested and brought to the University of the Free State, Bloemfontein.

DNA preparation

An altered method for extraction of high quality DNA was used, with hexadecyltrimethylammonium bromide (CTAB). Approximately 1.5 g of fresh leaf was collected from one plant representing each variety. The DNA was obtained from tender tissue on the outsides of the cladodes ("peel" or green part), cut into 1 cm pieces and stored at –20 °C. The plant material was homogenised in liquid nitrogen using a mortar and pestle. The powder was then incubated in 10 ml preheated extraction buffer pH 8 (10 ml NaCl (5 M), 20 ml Tris-HCL (0.5 M) (pH 8), 20 ml EDTA (ethylenediaminetetaacetic acid) (pH 8) (0.25 M), 6.25 ml SDS (20%) and 48 g of

ureum) at 65 °C for 30 minutes, with shaking every 10 minutes. Centrifugation for 40 minutes at 10000 rpm was used to seperate the cellular debris from the aqueous phase. The homogenate was further incubated at 65 °C for 1 hour with 1 ml of 1% CTAB (cetyltrimethyulammonium bromide) solution and 2 ml of 5M NaCl. Following incubation, chloroform-isamylalcohol (24:1) was added, mixed and centrugated to precipitate the DNA. The aqueous phase was removed to a fresh tube and two volumes of ethanol (100 %) were added, followed by centrifugation to precipitate the DNA. The precipitated DNA was spooled and washed in 70 % cold ethanol. The DNA was dissolved in 1 ml of sterile (distilled) water. The cuvet was washed between every reading with Sabax water.

The DNA concentration was calculated with the following equation:

[DNA] = optical density x dilution x constant (50 μg/ml)

Restriction Endonuclease Digestion

The AFLP analysis system from Life Technologies, Inc (GIBCO BRL) was used according to the manufacturer`s protocol. Approximately 250 ng of genomic DNA was double digested with two units restriction endonucleases at 37 °C for two hours. Following heat inactivation of the restriction endonucleases at 70 °C for 15 minutes, the digested DNA fragments were ligated to *Eco*RI and *Mse*I adapters (Table 4.1) using T4 DNA ligase (10 units) for 2 hours at 20 °C. Following ligation, the reaction mixture was diluted 10-fold in TE (10 mM Tris-HCL, 1 mM EDTA) buffer and used as template for subsequent PCR amplification. Selective nucleotides extending into the restriction fragments were added to the 3` ends of the PCR primers so that only one subset of the restriction fragments are recognised. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotieds were then amplified. The subset of amplified fragments were then analysed by using analytical software on an automatic sequencer.

Amplification reactions

PCR was performed in two consecutive reactions. In the first reaction, called preamplification, adapter ligated genomic DNA was amplified with AFLP primers each having one selective nucleotide (Table 4.1). Preamplification was performed for 20

cycles with the following cycle profile: 30 seconds denaturation at 94 °C, 1 minute annealing step 56 °C, and 2 minutes extension at 72 °C. In the preamplification step, 5 µl of the diluted ligation reaction (1:10) was used with 40 µl preamp primer mixture, 5 µl of 10X PCR buffer plus Mg, and 1 µl (1 unit/µl) *Taq* DNA polymerase (1 unit/µl). The results of preamplification was confirmed by running 12 µl of the reaction mixture on a 1 % agarose gel stained with ethidium bromide. The gel was run at a constant current (80 mA) for approximately 1 hour and the amplification products visualised under UV light. The second amplification reaction was performed using three AFLP primer combinations, each containing three selective nucleotides (Table 4.1). The *Eco*RI-AAC, *Eco*RI-ACA primers were fluorescently labelled "Ned" (black) and "Fam" (blue), respectively. Cycle conditions for selective amplification was as follow: 5 µl (1:50 preamplification product in TE, 5.5 µl of "mix 1", containing 1 µl labelled *Eco* RI primer, 4.5 µl *Mse*I primer which includes dNTP`s, and 9.5 µl of "mix 2", containg 7.4 µl AFLP grade water, 2 µl 10X PCR buffer plus Mg, 0.1 µl (5 units/µl) Taq DNA polymerase, in a total volume of 20 µl. PCR was carried out in three different cycles. The first cycle of PCR was at 94 °C for 30 seconds, followed by 65 °C for 30 seconds; and 72°C for 60 seconds. All amplification reactions were performed in a "Hybaid, Touch-down, Hot lid" thermocycler. After selective amplification, 5 µl of each selective amplification product was added to 1 µl Rox standard and 24 µl formamide. The samples were then denatured at 94 °C for 10 minutes with quick cooling on ice. Fragments were visualised on an ABI310 automatic capillary sequencer (PE Biosystems).

AFLP scoring and data analysis

The AFLP data was scored into a binary matrix as discrete variables ("1" for presence and "0" for absence) (Tabel A.9). All reproducible fragments, above a threshold fluorescence intensity of 35, were scored using a minimum peak height of 120. Genotypic data was used to calculate pairwise genetic distances. Estimates of similarity between genotypes were based on the probability that an amplified fragment from one genotype would also be present in another. Associations among the 10 cultivars were determined from cluster analysis based on the genetic distance estimates. The UPGMA clustering method was used for hierarchical clustering, and the necessary computations were performed using NCSS III (Hintze, 1997).

Table 4.1 Oligonucleotide adaptors and primers used for AFLP analysis.

Name of adapters/primers	Sequences (5'-3')
Mse primers	**Eco primers**
*Eco*RI adaptors	CTCGTAGACTGCGTACC
	CTGACGTATGGTTAA
*Mse*I adaptors	GACGATGAGTCCTGAG
	TACTCAGGACTCAT
AFLP primers:	
Pre-selective primers:	
*Eco*RI-A	AGACTGCGTACCAATTCA
*Mse*I-C	GACGATGAGTCCTGAGTA
Selective primers[a]:	
*Eco*RI-AAC-Ned	GACTGCGTACCAATTCAAC
*Eco*RI-ACA-Fam	GACTGCGTACCAATTCACA
*Mse*I-CTT	GATGAGTCCTGAGTAACTT
*Mse*I-CTC	GATGAGTCCTGAGTAACTC
*Mse*I-CAG	GATGAGTCCTGAGTAACAG

[a] *EcoR* I and *Mse* I selective primers were used in all possible combinations.

Results and Discussion

The mucilage released by the tissue was the main problem encountered during DNA extraction. The plants typically contained high levels of mucilages, complex polysaccharide compounds that bind water preventing DNA extraction by common miniprep methods, similar to the case of Mondragon-Jacobo and Pérez-González (2000). These compounds also bind to the water present in the extraction buffer, making further extraction difficult. This mucilage also complicated the determining of spectophotometric readings, for it was difficult to pipet the actual DNA and not just the surrounding mucilage. The amount of mucilage present in particular varieties was highly variable and the final sample size had to be adjusted according to variety. After centrifugation, the upper aqueous phase in these samples was still thick and slimy. Increasing the centrifugation time from 15 minutes to 40 minutes during the DNA extraction allowed separation of debris and extracted DNA.

There were 91 and 32 fragments identified when using the primer pair, MseI-CTT, EcoRI-ACA and EcoRI-AAC respectively (Table A.1 and A.2). With the combination MseI-CTC, EcoRI-ACA and EcoRI-AAC, 25 and 36 fragments were identified respectively (Table A.3 and A.4). There were 66 and 46 fragments identified when using the primer pair, EcoRI-CAG, EcoRI-ACA and EcoRI-AAC respectively (Table A.5 and A.6). Results of the AFLP markers scored as present (1) or absent (0) for the 10 cactus pear [*Opuntia ficus-indica* (L.) Mill.] cultivars are given in Table A.9 in Appendix A. There were 187 polymorphic fragments identified among the different cultivars.

The dendrogram representing the genetic distances among the cactus pear cultivars are given in Figure 4.2. In this dendrogram, the minimum and maximum range of dissimilarity of all the entries ranged from 0.49 to 0.69. Results from the cluster section indicated two clusters, cluster A and B, at a genetic distance of 0.62. Cluster A consists of seven cultivars, Roedtan, Nudosa, Skinners Court, Malta, Meyers, Gymno Carpo and Zastron. Cluster B included three cultivars, Turpin, Algerian and Morado. The range of dissimilarity within cluster A and B in the AFLP dendrogram ranges form 0.49-0.63 and 0.58-0.61, respectively (Table 4.2).

The mean range of genetic dissimilarity within cluster A in the AFLP dendrogram is 0.57 and within cluster B is 0.66, thus even though cluster A contain more cultivars, the range of dissimilarity in cluster B is great than in cluster A. From this can be concluded that the range of genetic dissimilarity in cluster A is also comparable to the genetic dissimilarity between cluster A and cluster B (0.08). The range of dissimilarity within cluster A and B of the morphological data ranges from 1.25-1.25 and 0.8-1.62, respectively. The mean range of genetic dissimilarity within cluster A in the morphogical dendrogram is 1.31 and within cluster B is 1.25, thus the range of dissimilarity in cluster A is greater than in cluster B. The range of dissimilarity within the two clusters are thus smaller than the range of dissimilarity between the two clusters.

When comparing the dendrogram constructed from the dendrogram of the morphological and pathological data to the dendrogram constructed from the AFLP data, no similarity in the overall clustering of the cultivars is found, but it is clear that some similarity in the smaller subclusterings does exist. In all three these dendrograms, Malta and Gymno Carpo are clustered together. Nudosa and Skinners Court both fall closely together within cluster B in the morphological and in cluster A in the AFLP dendrogram, but this is not the case in the pathological dendrogram, where they are clustered into two different clusters. Morado, Turpin and Algerian are also clustered together in cluster B in the AFLP and pathological dendrogram, while they are still clustered together in the morphological dendrogram, but only in cluster A. What can be concluded from the difference in clustering is that the morphological and pathological descriptors used are not as reliable as the AFLP descriptors in order to characterise a certain cultivar. This could be attributed to low correlation between random samping of the total DNA and the subset of DNA related to morphological characters and the subset of DNA related to pathology. There must be some reliable morphogical and pathological descriptors present though, responsible for the correlation within small subgroup between the two dendrograms. This corresponds with Wang *et. al* (1998) findings, who attributed the differences to the fact that morphological characters represents a smaller subset of genes. This could possibly explain the taxonomical problems experienced, since many morphological characters could be single gene controlled and taxonomic characters in the past may have been based on characters that are simply inherited as single genes and thus not of value in separating species.

It is clear from the two dendrograms that the total range of dissimilarity in the AFLP dendrogram is far smaller than the morphological and pathological dendrogram. This could be attributed to the fact that far less morphological and pathological parameters were used than in the case of the AFLP data, thus a polymorphism in the morphological and pathological data contributed a far greater deal to dissimilarity than in the case of a polymorphism in the AFLP dendrogram. From this can also be concluded that the morphological and pathological characters is over estimating genetic dissimilarity in terms of AFLP`s. This can be attributed to the fact that morphological data is environmentally influenced, because of genotype-environment interaction.

The differences in grouping of accessions based on marker vs. morphological data is in agreement with the findings of Wang *et al.* (2002), who attributed this to the fact that phenotypic expression may be influenced by many non-genetic factors that are not expected to affect DNA marker patterns, for instance, the influence of the environment. It is clear that the AFLP analysis is a direct measure of genetic similarity between individuals. Thus, compared to morphological markers, AFLP provides a more detailed coverage throughout the genome, which in turn provides a more reliable estimation of the genetic distances among genotypes. AFLP markers are, therefore, a powerful tool for measuring the genetic diversity and determining relationships within and among species (Waugh, 1997). Although low levels of DNA variation was found by previous RAPD studies, the DNA variation in AFLP analysis was high. This could possibly be attributed to the fact that the AFLP method is able to detect polymorphisms in a higher degree than what is the case with the RAPD method.

This experiment demonstrated the potential usefulness of molecular markers in classification and identification of cactus accessions and gives a good indication of the possibility of a comprehensive effort to determine the relationships among *Opuntia* species using molecular markers. Further collection, evaluation and utilisation of additional germplasm is important for *Opuntia* improvement.

Conclusions

All the genotypes had distinct AFLP profiles. A total of 296 fragments were amplified using six primer combinations with an average of 50 per primer combination. The AFLP fragments for all the different genotypes studied were 63% polymorphic. The overall clustering of the cultivars of the AFLP dendrogram do not correlate with the clusterings of the cultivars in the morphological and pathological dendrograms. From this can be concluded that in cactus pear, low correlation exist between the subset of DNA related to morphological characters, the subset of genes related to pathology and a random sampling of the total genomic DNA.

Table 4.2 Actual distance between the cultivars in the dendrogram (Fig.4.2).

First Row	Second Row	Actual Distance
Morado	Zastron	0.60
Morado	Algerian	0.61
Morado	Turpin	0.60
Morado	Meyers	0.6
Morado	Roedtan	0.66
Morado	Gymno Carpo	0.58
Morado	Malta	0.59
Morado	Skinners Court	0.6
Morado	Nudosa	0.64
Zastron	Algerian	0.54
Zastron	Turpin	0.60
Zastron	Meyers	0.54
Zastron	Roedtan	0.63
Zastron	Gymno Carpo	0.49
Zastron	Malta	0.55
Zastron	Skinners Court	0.59
Zastron	Nudosa	0.57
Algerian	Turpin	0.59
Algerian	Meyers	0.60
Algerian	Roedtan	0.70
Algerian	Gymno Carpo	0.59
Algerian	Malta	0.63
Algerian	Skinners Court	0.64
Algerian	Nudosa	0.64
Turpin	Meyers	0.662

Turpin	Roedtan	0.68
Turpin	Gymno Carpo	0.63
Turpin	Malta	0.61
Turpin	Skinners Court	0.62
Turpin	Nudosa	0.65
Meyers	Roedtan	0.59
Meyers	Gymno Carpo	0.50
Meyers	Malta	0.50
Meyers	Skinners Court	0.54
Meyers	Nudosa	0.57
Roedtan	Gymno Carpo	0.56
Roedtan	Malta	0.58
Roedtan	Skinners Court	0.64
Roedtan	Nudosa	0.60
Gymno Carpo	Malta	0.49
Gymno Carpo	Skinners Court	0.56
Gymno Carpo	Nudosa	0.59
Malta	Skinners Court	0.58
Malta	Nudosa	0.56
Skinners Court	Nudosa	0.61

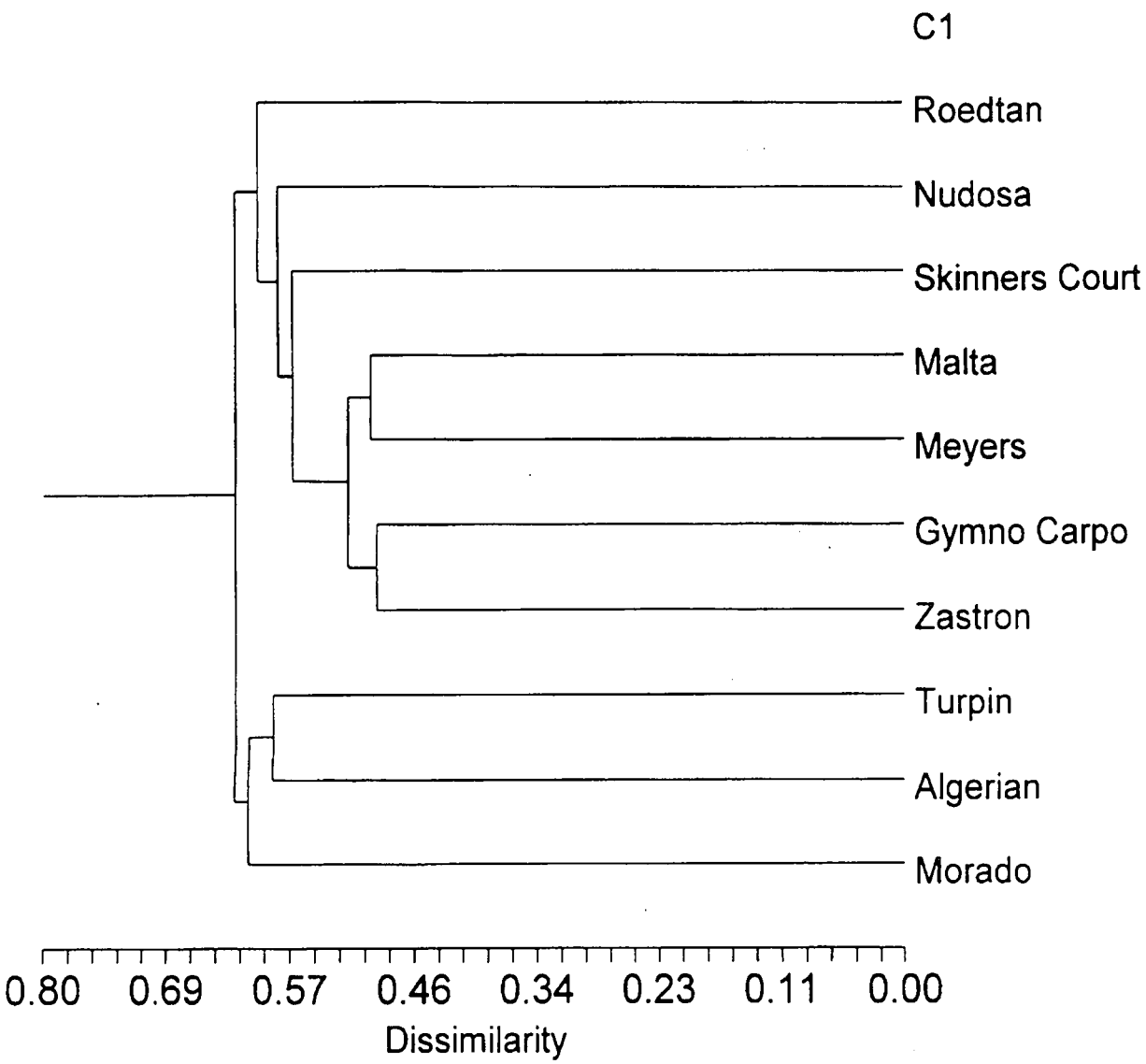

Fig. 4.2 Dendogram generated by UPGMA analysis of the AFLP markers.

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CHAPTER 5

Summary

1. Spineless cactus pear [*Opuntia ficus-indica* (L.) Mill.] in South Africa is increasingly commercialised and there is a need to establish a database to assist the South African farmers in the selection of cultivars for production. The aim of this study was to contribute to such a database on horticultural, fodder, genetical and pathoglogical level.
2. Plantmaterial of the 10 cultivars were obtained from a genebank situated near Potgietersrus in the Northern Transvaal (23° 50`S), South Africa. This area has a subtropical climate with predominantly summer rainfall. The cultivars were characterised on general horticultural characteristics, characteristics for use as fodder and resistance to four pathogens obtained from infected cactus pear orchards in South Africa. The cultivars were also genetically characterised using the AFLP method.
3. All the morphological results obtained were compared with the genetical data. The results obtained showed that morphological data, whether horticultural, fodder or pathological, are inadequate to distinguish and characterise the different cultivars. DNA fingerprinting can complement this characterisation.
4. The fact that the major clustering in the morphological and pathological dendrograms does not correlate with the AFLP dendrogram proves that low correlation exist between the subset of DNA related to morphological characters, the subset of DNA related to pathology and a random sampling of the total genomic DNA. It also underlines the unreliability of using only morphological and pathological descriptors in selection. In order to solve this problem, further description and characterisation of these cultivars is needed, but in the different cactus pear production areas in South Africa in order to better understand the influence of the environment on these cultivars. Description and characterisation of the cultivars in terms of a wider set of parameters, for instance resistance to a wider range of pathogens and cold tolerance is also needed in order to make the morphological and pathological data more reliable.

5. It is clear from the literature that *O. ficus-indica* is a multifunctional crop, which can be of great value in both developed en undeveloped countries, because of its ability to utilise the full potential of arid areas, though in South Africa the full potential of cactus pear is not utilised yet. It is clear from the morphological and pathological data in this study that the 10 commercial cultivars in South Africa compare well to other cultivars described in the literature.

Opsomming

1. *O. ficus-indica* (L.) Mill. in Suid-Africa word toenemend gekommersialiseer en daar het `n behoefte ontstaan na `n databasis wat produsente kan riglyne gee vir seleksie van kultivars vir produksie doeleindes. Die doel van hierdie studie is om `n bydrae te maak tot so `n databasis.
2. Plant materiaal van die 10 kultivars is verkry van `n geenbank naby Potgietersrus in die Noord Transvaal (23° 50`S), Suid-Afrika. Hierdie is `n area met `n subtropiese klimaat en oorwegende somer reënval. Die kultivars is gekarakteriseer volgens algemene hortologiese en veevoer eienskappe. Die kultivars se weerstand teen vier patogene wat uit kommersiële boorde in Suid-Afrika versamel is, is ook getoets.
3. Al die morfologies data is vergelyk met die genetiese data wat versamel is. Die resultate het aangedui dat morfologiese data, ongeag of dit hortologies, patologies of veekundig van aard is, onvoldoende is om die cultivars genoegsaam te karakteriseer, as dit nie in kombinasie met genetiese studies gedoen word nie.
4. Die feit dat hoof indelings van die morfologies en patologiese dendrogramme nie korreleer met die AFLP dendrogram nie, is `n bewys van lae korrelasies tussen die substel DNA verwant aan morfologies karakters, die substel DNA verwant aan patologie en die toevallige monster van totale genomiese DNA. Dit beklemtoon die onbetroubaarheid van die gebruik van slegs morfolgiese en patologiese parameters in seleksie. Om hierdie probleem op te los sal verdere karakterisering van hierdie kultivars in die verskillende verbouiingsgebiede in Suid-Afrika gedoen moet word, sodat die invloed van die omgewing op hierdie kultivars beter verstaan kan word. Karakterisering van hierdie kultivars met `n wyer hoeveelheid parameters, bv. ook koue bestandheid en weerstand ten opsigte van `n wyer verskeidenheid patogene, kan die gebruik van morfologiese data vir seleksie ook meer betroubaar maak.
5. Dit is duidelik uit die literatuur dat *O. ficus-indica* `n veelsydige gewas is, wat van groot waarde kan wees in onderontwikkelde en ontwikkelde lande vanweë sy vermoë om ariede areas tot hulle volle potensiaal te benut. Die volle potensiaal

van *O. ficus-indica* in Suid-Africa is nog nie ten volle benut nie, tog is dit duidelik uit hierdie studie dat die 10 kommersïele kultivars in Suid-Afrika goed met ander kultivars wat in die literatuur beskryf word, vergelyk.

Appendix A

Table A.1 The AFLP data with primer EcoRI-ACA and MseI-CTT.

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
39	39	39	39	39	39	39	39	39	39
42	42			41	42	42	41	42	
45	45	45	45		45	45	45	45	45
47	47	47	47	47	47	47	47		
49	49			49	49	49	49		
52	52	52	52	52	52	52	52	52	52
56	56		56	56	56	56	56		56
58	58	58	58		58	58	58	58	
61	61	61	61	61	61	61	61	61	61
63			63	63	63	63	63	63	63
64	64	64	64	64	64	64	64	64	64
66	66	66	66	66	66	66	66	66	66
68	68	68	68	68	68	68	68	68	68
70	70	70	70	70	70	70	70	70	70
73	73	73	73	73	73	73	73	73	73
77		77	77	77	77	77	77	77	
78		78	78	78	78	78	78	78	78
81	81		81	81	81	81	81	81	81
83	83	83	83	83	83	83	83	83	83
87	87	87	87	87	87	87	87	87	87
	92		92	92	92	92	92	92	92
	95	95	95	95	95	95	95	95	95
96	96	96	96	96	96	96	96	96	96
99	99	99	99	99	99	99	99	99	99
	101	101	101	101	101	101	101	101	101
103	103	103	103	103	103	103	103	103	103
114	114	114	114	114	114	114	114	114	114
118	118		118	118	118	118	118		118
120	120	120	120	120	120	120	120	120	120
123	123			123	123	123	123	123	123
124	124	124	124	124	124	124	124	124	124
	126		126	126	126	126	126	126	
	129			129		129	129		129
131	131	131	131		131	131	131		131
134	134		134	134	134	134	134	134	134
135				135	135	135	135	135	135
138				138	138	138	138	138	138
	140	140	140	140	140	140	140	140	140

		142	142	142	142	142	142	142	142
146			146	146	146	146	146	146	146
147	147		147	147	147	147	147	147	147
	149	149	149	149	149	149	149	149	149
	154	154	154	154	154	154	154	154	154
158	158	158	158	158	158	158	158	158	158
	160		160	160	160	160	160	160	
164	164			164	164	164	164		164
165	165	165		165	165	165	165	165	165
169	169				169	169	169	169	169
173	173	173	173	173	173	173		173	173
177	177	177	177	177	177	177	177	177	177
180		180	180	180	180	180	180	180	180
183	183	183	183	183	183	183	183	183	183
187	187			187	187	187	187	187	187
191	191				191	191	191	191	191
195	195	195	195	195	195	195	195	195	195
202	202	202	202	202	202	202	202	202	202
205	205		205	205	205	205	205	205	205
	213	213	213	213		213		213	
215	215		215	215	215	215		215	
	225	225	225					225	225
227	227	227	227			227		227	227
228	228	228	228		228			228	228
	231			231			231	231	231
235	235			235	235	235	235		235
	238		238	238	237	237	238	238	237
240	240		240	242	240	240	240	240	240
	245			245	245	245	245	245	245
250	250		250	250	250	250	250		250
	252	252	252	252		252		252	252
255	255		255	255	255	255	255	255	
258	258		258	258			258	258	258
	265			265	265	265	265	265	265
271			271	271	271	271		271	271
	273			273			273	273	273
278			278	278	278	278	278	278	278
				279			279	279	279
	280	280	280	280	280	280	280	280	280
284	284	284		284			284	284	284
		289			289	289		289	289
		293		293			293	293	293
		301	301	301	301	301	301	301	301
315	315			315	315	315		315	315
	317	317			317	317			
321	321	321	321	321	321	321	321	321	321
	330			330	330	330	330	330	330
354		354	354		354	354		354	

357		357		357	357	357		357	
	368	368	368	368	368	368	368	368	368
	378		378	378	378	378	378	378	378
402	402	402			402	402		402	
		413	413	413	413	413	413	413	413

Table A.2 The AFLP data with primer *Eco*RIMse-AAC and MseI-CTT.

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
53	53	53		53	53	53	53	53	53
57	57	57	57		57	57	57	57	57
61	61	61	61	61	61	61	61	61	61
69	69	69	69	69	69	69	69	69	69
73	73	73	73	73	73	73	73	73	73
78	78	78	78	78	78	78	78	78	78
90			90	90	90		90		90
97	97	97	97			97	97	97	97
99	99	99	99	99	99	99	99	99	99
112	112	112	112	112	112	112	112	112	112
119		119	119	119			119	119	119
124	124	124	124	124			124	124	
129			129	129	129	129	129	129	129
138	138	138	138	138	138	138	138	138	138
147	147	147	147	147			147	147	147
150	150	150	150	150				150	150
166	166	166	166	166	166	166	166	166	
173	173	173	173	173	173	173	173	173	173
184	184	184	184				184		
192			192	192	192	192		192	192
197				197			197		197
226	226	226	226				226	226	226
235							235	235	
252	252	252	252	252		252		252	252
259			259				259	259	
262									262
280	280	280	280	280	280	280	280	280	
295	295	295		295	295				295
332				332					
340		340	340	340	340	340		340	340
353	353	353	353	353	353	353	353		
402	402	402	402			402			402

Table A.3 The AFLP data with primer EcoRI-ACA and MseI-CTC .

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
80	80	80	80	80	80	80	80	80	80
84	84	84	84	84	84	84	84	84	84
86	86	86	86	86	86	86		86	86
90	90	90	90	90	90	90	90	90	91
93	93	93	93	93	93	93	93	93	93
100	100		100	100	100		100	100	100
	104		104	104	106		104	104	106
114	114	114	114	114	114	114	114	114	114
120	120	120		120	120	120	120		120
126	126	126		126	126	126	126		126
	128	128	128	128	128	128	128	128	128
137	137	137	137	137	137	137	137	137	137
142	142	142	142	142	142	142	142	142	142
169	169	169	169	169	169	169	169	169	169
194	194	194	194	194	194	194	194	194	194
198	198	198	198	198	198	198		198	198
201	201	201	201	201		201	201	201	
	212	212	212	212	212	212	212	212	212
231	231	231	231	231	231	231	231	231	231
240	240	240	240	240	240	240	240	240	240
245	245	245	245	245	245	245	245	245	245
	273	273		273	273				273
	339	339		339	339	339	339	339	339
352				352		352	352		
386	386	386	386	386	386	386	386	386	386

Table A.4 The AFLP data with primer EcoRIMse-AAC and MseI-CTC.

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
79	79	79	79	79	79	79	79	79	79
	85	85	85					85	
89	89	89	89	89	89	89	89	89	89
94	94	94	94	94	94	94	94	94	94
98	98	98	98	98	98	98	98	98	98
107	107	107	104	107		107		107	
116	116	116	116	116	116	116	116	116	116
119	119	119		119	119	119	119		119
126	126	126		126	126	126		126	126
129	129	129	128	129	129	129	129	129	129
132	132	132	132	132	132	132	132	132	132
138	138	138	137	138	138	138	138	138	138
148	148	148	148	148	148	148	148	148	148
151	151	151		151	151	151	151	151	151
	154					154		154	
	158	158		158	158	158	158	158	158
	168		168		168	168	168		168
170	170	170	175	170	170	170	170	170	170
182	182	182		182	182	182		182	182
194	194	194	194	194	194	194	194	194	194
208	208	208		208	208	208	208	208	208
217	217	217	218	217		217	217	217	
					221	221		221	
	238	238	239	238	238	238	238	238	238
248	249	249	248		249			249	
252	252	252	252	252		252	252	252	252
	258	258		258	258	258	258	258	258
262	262	262		262	262	262	262	262	262
285	285	285		285	285	285	285	285	285
				303	303			303	
308	308	308				308	308		308
338	338	338	338	338	338	338	338	338	338
	353	353		353		353	353	353	353
364	364	364		364	364	364	364	364	364
	466	466		466		466		466	
	476	476		476	476	476	476	476	476

Table A.5 The AFLP data with primer EcoRI-ACA and Mse CAG.

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
41	41	41	41	41	41	41	41	41	41
47	47	47	47	47	47	47	47	47	47
51	51	51	51	51	51			51	51
53	53		53		53			53	53
60	60	60	60		60	60	60	60	60
63	63	63	63	63	63	63	63	63	63
66	66	66	66	66	66	66	66	66	
69	69	69	69	69	69	69	69	69	69
71	71	71	72	72	71	71	71	71	73
73	73	73		73	73	73	73	73	
76	76	76	76	76	76	76	76	76	
78	78	78	78	78	78	78	78	78	78
79	79	79	79	79	79	79	79	79	79
82	82	82	82	82		82	82	82	82
84	84	84	84	84	84	84	84	84	84
87	87	87	87	87	87	87	87	87	87
89	89	89	89	89	89	89	89	89	89
91	91	91	91	91		91	91	91	
93	93	93	93	93	93	93	93	93	93
95	95	95	95	95	95	95	95	95	95
96	96	96	96	96	96	96	96	96	96
98	98	98	98	98	98	98	98	98	
102	102	102	102	102	102	102	102	102	102
105		105		105	105		105	105	105
107	107	107	107	107	107	107	107	107	107
111	111	111	111	111	111	111	111	111	111
113	113	113	113	113	113	113	113	113	113
116	116	116	116	116	116	116	116	116	
120	120	120	120	120	120	120	120	120	120
122	122	122	122	122		122	122	122	122
129	129	129	129	129	129	129	129	129	129
131	131	131	131	131	131	131	131	131	131
134	134	134	134	134	134	134	134	134	134
136	136	136	136	136	136	136	136		
137	137	137		137		137	137	137	
140	140	140	140	140	140	140	140	140	
144	144	144	144	144		144		144	144
146	146	146	146	146		146	146	146	146
151	151	151	151	151	151	151	151	151	151
154	154	154	154	154	154	154	154		154

159	159	159	159	159	159	159	159	159	159
162	162	162	162	162	162	162	162	162	162
169	169	169	169	169		169	169	169	169
173	173	173	173	173	173	173	173	173	173
178	178	178	178	178		178	178	178	178
180	180	180	180	180	180	180	180	180	180
184	184		184		184	184	184		184
189	189	189	189	189	189	189	189		189
191	191	191	191		191			191	
195	195	195	195	195	195	195	195	195	
200	200	200	200	200					200
202	202	202	202	202	202	202	202		202
205	205	205	205	205		205	205	205	
212	212	212	212	212	212	212	212		212
				214	214	214	214	214	
225	225	225	225	225	225	225	225		225
259	259	259		259	259	259	259		259
266	266	266	266	266		266	266	266	266
321	321	321	321	321	321	321	321	321	321
360	360	360	360	360	360	360	360	360	360
390	390	390		390		390	390		390
414	414	414	414	414	414	414	414		414
425	425	425	425	425	425	425	425	425	425
476	476	476	476	476	476	476	476	476	476
486	486	486	486	485		486	486	486	486
516	516	516	516	516	516	516	516		516

Table A.6 The AFLP data with primer EcoRIMse-AAC and MseI-CAG.

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
79	79	79	79	79	79	79	79	79	79
81	81	81	81	81	81	81	81	81	81
83	83	83	83	83		83	83	83	83
86		86	86	86	86	86	86	86	86
88	88	88	88	88	88	88	88	88	88
93	93	93	93	93	93	93	93	93	93
96	96	96	96	96	96	96	96	96	95
98	98	98	98	98	98	98	98	98	98
103	103	103	103	103	103	103	103	103	102
107	107	107	107	107	107	107	107	107	107
108	108	108	108	108			108	108	108
111	111	111	111	111	111	111	111	111	111
114	114	114	114	114		114	114	114	114
116	116	116	116	116		116	116	116	115
120	120	120	120	120	120	120	120	120	120
124	124	124	124	124		124	124	124	124
125	125	125	125	125		125	125	125	
130	130	130	130	130	130	130	130	130	130
133	133	133	133	133	133	133	133	133	133
136	136	136	136	136	136	136	136	136	136
140	140	140	140	140	140	140	140	140	140
145			145					145	145
	147	147	147	147					148
150	150	150	150	150	150	150	150	150	150
158	158	158	158	158	158	158	158	158	158
162	162	162	162	162	162	162	162	162	162
173	173	173	173	173	173	173	173	173	173
178	178	178	178	178	178	178	178	178	178
	182				182				
185	185	185	185	185		185	185	185	185
190	190	190	190	190	190	190	190	190	190
193	193	193	193	193	193	193	193	193	192
	197	197	197	197			195	197	197
202	202	202	202	202	202	202	202	202	202
204	204	204		204	204	204	204	204	204
217	217	217	217	217	217	217	217	217	217
225	225	225	225	225	225	225	225	225	225
227	227	227	227	227	227	227	227	227	
	245	245	245	245	245	245	245		
247	247	247	247	247	247	247	247	247	247
		254	254	254	254		254	254	254

275	275	275	275	275	275	275	275	275	275
281	281	281	281	281	281	281	281	281	281
297	297	297	297	297	297	297	297	297	297
301	301	301	301	301	301	301	301	301	301
	458		458		458	458	458	458	

Table A.7 The AFLP data scored into a binary matrix as discrete variables ("1" for presence and "0" for absence).

Morado	Zastron	Algerian	Turpin	Meyers	Roedtan	Gymno Carpo	Malta	Skinners Court	Nudosa
1	1	0	0	1	1	1	1	1	0
1	1	1	1	0	1	1	1	1	1
1	1	1	1	1	1	1	1	0	0
1	1	0	0	1	1	1	1	0	0
1	1	0	1	1	1	1	1	1	1
1	1	1	1	0	1	1	1	0	1
1	1	1	1	1	1	1	1	1	0
1	0	0	1	1	1	1	1	1	1
1	0	1	1	1	1	1	1	1	1
1	0	1	1	1	1	1	1	1	0
1	1	0	1	1	1	1	1	1	1
0	1	0	1	1	1	1	1	1	1
0	1	1	1	1	1	1	1	1	1
0	1	1	1	1	1	1	1	1	1
1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	0	1
1	1	0	0	1	1	1	1	1	1
0	1	0	1	1	1	1	1	1	1
0	1	0	0	1	0	1	1	1	0
1	1	1	1	0	1	1	1	0	1
1	1	0	1	1	1	1	1	0	1
1	0	0	0	1	1	1	1	1	1
1	0	0	0	1	1	1	1	1	1
0	1	1	1	1	1	1	1	1	1
0	0	1	1	1	1	1	1	1	1
1	0	0	1	1	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1
0	1	1	1	1	1	1	1	1	1
0	1	1	1	1	1	1	1	1	1
0	1	0	1	1	1	1	1	1	1
1	1	0	0	1	1	1	1	1	0
1	1	1	0	1	1	1	1	0	1
1	1	0	0	0	1	1	1	1	1
1	1	1	1	1	1	1	0	1	1
1	0	1	1	1	1	1	1	1	1
1	1	0	0	1	1	1	1	1	1
1	1	0	0	0	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1
0	1	1	1	1	0	1	1	1	1
1	1	0	1	1	1	1	0	1	0

0	1	1	1	0	0	1	0	1	0
1	1	1	1	0	0	0	0	1	1
1	1	1	1	0	1	1	0	1	1
0	1	0	0	1	0	0	0	1	1
1	1	0	0	1	1	0	1	1	1
0	1	0	1	1	1	1	1	0	1
1	1	0	1	1	1	1	1	1	1
0	1	0	0	1	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1
0	1	1	1	1	0	1	1	0	1
1	1	0	1	1	1	1	0	1	1
1	1	0	1	1	0	1	1	1	0
0	1	0	0	1	1	0	1	1	1
1	0	0	1	1	1	1	1	1	1
0	1	0	0	1	0	1	0	1	1
1	0	0	1	1	1	0	1	1	1
0	0	0	0	1	0	1	1	1	1
0	1	1	1	1	1	0	1	1	1
1	1	1	0	1	0	1	1	1	1
0	0	1	0	0	1	0	1	1	1
0	0	1	0	1	0	1	0	1	1
0	0	1	1	1	1	0	1	1	1
1	1	0	0	1	1	1	1	1	1
0	1	1	0	0	1	1	0	1	1
1	1	1	1	1	1	1	0	0	0
0	1	0	0	1	1	1	1	1	1
1	0	1	1	0	1	1	1	1	1
1	0	1	0	1	1	1	0	1	0
0	1	1	1	1	1	1	1	1	1
0	1	0	1	1	1	1	1	1	1
1	1	1	0	0	1	1	1	1	1
0	0	1	1	1	1	1	0	1	0
1	1	1	0	1	1	1	1	1	1
1	1	1	1	0	1	1	1	1	1
1	0	0	1	1	1	0	1	0	1
1	1	1	1	0	0	1	1	1	1
1	0	1	1	1	0	0	1	1	1
1	1	1	1	1	0	0	1	1	0
1	0	0	1	1	1	1	1	1	1
1	1	1	1	1	0	0	1	1	1
1	1	1	1	1	0	0	0	1	1
1	1	1	1	1	1	1	1	1	0
1	1	1	1	0	0	0	1	0	0
1	0	0	1	1	1	1	0	1	1
1	0	0	0	1	0	0	1	0	1
1	1	1	1	0	0	0	1	1	1
1	0	0	0	0	0	0	1	1	0
1	1	1	1	1	0	1	0	1	1
1	0	0	1	0	0	0	1	1	0

1	0	0	0	0	0	0	0	0	1
1	1	1	1	1	1	1	1	1	0
1	1	1	0	1	1	0	0	0	1
1	0	0	0	1	0	0	0	0	0
1	0	1	1	1	1	1	0	1	1
1	1	1	1	1	1	1	1	0	0
1	1	1	1	0	0	1	0	0	1
1	1	1	1	1	1	1	0	1	1
1	1	0	1	1	1	0	1	1	1
0	1	0	1	1	1	0	1	1	1
1	1	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	0	1
0	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	0	1	1
1	1	1	1	1	0	1	1	1	0
0	1	1	1	1	1	1	1	1	1
0	1	1	0	1	1	0	0	0	1
0	1	1	0	1	1	1	1	1	1
1	0	0	0	1	0	1	1	0	0
0	1	1	1	0	0	0	0	1	0
1	1	1	1	1	0	1	0	1	0
1	1	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	0	1	1
1	1	1	0	1	1	1	1	1	1
0	1	0	0	0	0	1	0	1	0
0	1	1	0	1	1	1	1	1	1
0	1	0	1	0	1	1	1	0	1
1	1	1	0	1	1	1	0	1	1
1	1	1	0	1	1	1	1	1	1
1	1	1	1	1	0	1	1	1	0
0	0	0	0	0	1	1	0	1	0
0	1	1	1	1	1	1	1	1	1
1	1	1	1	0	1	0	0	1	0
1	1	1	1	1	0	1	1	1	1
0	1	1	0	1	1	1	1	1	1
1	1	1	0	1	1	1	1	1	1
1	1	1	0	1	1	1	1	1	1
0	0	0	0	1	1	0	0	1	0
1	1	1	0	0	0	1	1	0	1
0	1	1	0	1	0	1	1	1	1
1	1	1	0	1	1	1	1	1	1
0	1	1	0	1	0	1	0	1	0
0	1	1	0	1	1	1	1	1	1
1	1	1	1	1	1	0	0	1	1
1	1	0	1	0	1	0	0	1	1
1	1	1	1	0	1	1	1	1	1
1	1	1	1	1	1	1	1	1	0
1	1	1	1	1	1	1	1	1	0
1	1	1	1	1	1	1	1	1	0

1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	0	1	1	1	0
1	1	1	1	1	1	1	1	1	0
1	0	1	0	1	1	0	1	1	1
1	1	1	1	1	1	1	1	1	0
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	1	1	1	0	0
1	1	1	0	1	0	1	1	1	0
1	1	1	1	1	1	1	1	1	0
1	1	1	1	1	0	1	0	1	1
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	1	1	1	0	1
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	0	1	1	1	1
1	1	0	1	0	1	1	1	0	1
1	1	1	1	1	1	1	1	0	1
1	1	1	1	0	1	0	0	1	0
1	1	1	1	1	1	1	1	1	0
1	1	1	1	1	0	0	0	0	1
1	1	1	1	1	1	1	1	0	1
1	1	1	1	1	0	1	1	1	0
1	1	1	1	1	1	1	1	0	1
0	0	0	0	1	1	1	1	1	0
1	1	1	1	1	1	1	1	0	1
1	1	1	0	1	1	1	1	0	1
1	1	1	1	1	0	1	1	1	1
1	1	1	0	1	0	1	1	0	1
1	1	1	1	1	1	1	1	0	1
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	1	1	1	0	1
1	1	1	1	1	0	1	1	1	1
1	0	1	1	1	1	1	1	1	1
1	1	1	1	1	0	0	1	1	1
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	0	1	1	1	1
1	1	1	1	1	0	1	1	1	0
1	0	0	1	0	0	0	0	1	1
0	1	1	1	1	0	0	0	0	1
0	1	0	0	0	1	0	0	0	0
1	1	1	1	1	0	1	1	1	1
0	1	1	1	1	0	0	1	1	1
1	1	1	0	1	1	1	1	1	1
0	1	1	1	1	1	1	1	0	1
1	1	1	1	1	1	1	1	1	0
0	0	1	1	1	1	0	1	1	0
0	1	0	1	0	1	1	1	1	1