Characterization of cactus pear germplasm in South Africa

BY

BARBARA KEITUMETSE MASHOPE

A thesis submitted in fulfilment of the requirements for the degree of Philosophiae Doctor

May 2007

In the Faculty of Natural and Agricultural Sciences
Department of Plant Sciences (Plant Breeding Division)
University of the Free State

Promoter: Prof. M. T. Labuschagne

Co-promoters: Prof. W. J. Swart

Dr. L. Herselman

TABLE OF CONTENTS

CHAPTER 5

Resistance of cactus pear varieties to three fungal pathogens and an option for biocontrol using yeasts

DECLARATION

“I hereby declare that the thesis submitted by me for the degree of Philosophiae Doctor at the University of the Free State is my own independent work and has not previously been submitted by me at another University/Faculty.

.....

Barbara Keitumetse Mashope

ACKNOWLEDGEMENTS

I would like to express and convey my sincere gratitude to all who assisted and contributed to the successful completion of this study. In particular, I would like to thank the following persons:

To my parents Mr. and Mrs. G. M. Mashope for their tireless support and assistance throughout my studies.

Many thanks to my promoter Prof. M.T. Labuschagne for her continuous support, encouragement and enthusiasm. I am also grateful to my co-promotor Dr. L. Herselman for her meticulous scrutiny of the many drafts that have led to the completion of this thesis and Prof. W.J. Swart for his expert advice and insightful comments.

The Limpopo Department of Agriculture for the research and weather data used in this study. I am also grateful to Mr. J. Potgieter for his profound insight, support and lively discussion in addition to supplying photos, data and articles used in this study.

My friends Drs. P.D. Gqola, E. Koen, M. Tesfaendrias, Mrs K. Lehloenya, and Ms. L. Chetty for support and encouragement throughout this study. Mr. S. Sithole and Dr. S.G. Mhlongo for collection of data for disease screening trials and Mr. L.L. Sehurutshi for his assistance. Ms. K. Ngesi and Dr. J Bahta for their assistance with the statistical analysis. Many thanks also to my dear friend Mr. O. Philippou for his gracious support and enthusiastic encouragement.

Dr. Patrick Griffith from the Rancho Santa Anna Botanic Garden, Claremont, CA and Dr. C. Mondragón-Jacobo from the Instituto Nacional de Investigaciones Forestales y Agricolas y Pecuarias, Queretaro, MEXICO for their rich discussions and assistance in obtaining journal articles on the molecular aspects of this study.

Dr. H. Fouche and Mr. P. Avenant from the Agricultural Research Council Pasture and Rangeland Institute, Department of Soil-, Crop- and Climate Sciences, UFS, Bloemfontein for supplying nutritional quality and weather data used in Chapter 4.

Prof H.O. de Waal for his rich discussions on nutritional quality of fodder and encouraging comments and Mr. W. Combrink for his technical assistance.

Dr. M. de Wit from the Department of Microbial Biochemical and Food Biotechnology for supplying photos of the pulp of the cactus pear varieties.

Mr. T Unterpertinger, Consolata Estates, Limpopo Province, for supplying the cactus pear fruit used for the biocontrol studies.

Finally I would like to thank the Andrew Mellon Foundation (UFS) and the NRF for funding this resesarch.

ABBREVIATIONS AND ACRONYMS

°C	Degree Celsius
µg	Microgram(s)
µl	Microlitre(s)
µM	Micrometer(s)
ADF	Acid detergent fibre
AFLP/s	Amplified Fragment Length Polymorphism/s
AIDS	Acquired Immuno Deficiency Syndrome
AMMI	Additive Main Effects and Multiplicative Interactions Analysis
ANOVA	Analysis of variance
ARC-ISCW	Agricultural Research Council Institute for Soil, Climate and Water
ATP	Adenine triphosphate
bp	Base pair(s)
BSA	Bovine serum albumin
CACTUSNET-FAO	FAO International Technical Co-operation Network on Cactus Pear
CAM	Crassulacean acid metabolism
cm	Centimetre(s)
CP	Crude protein
cpDNA	Chloroplast DNA
cpSSR	Chloroplast simple sequence repeat
CTAB	Hexadecyltrimethylammonium bromide
CU	Chill Units
DM	Dry matter
DNA	Deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'-triphosphate
DTT	1,4 dithiothreitol
EDTA	Ethylene-diaminetetraacetate
ESTs	Expressed sequence tags
ETo	Evapotranspiration
FAO	Food and Agricultural organisation of the United Nations
FDP	Fruit Development Period
FFR	50% fruit ripening

FM	Fresh matter
g	Gram (s)
g	Centrifugal force
G X E	Genotype x environment interaction
GSF	Genotype specific fragments
GTM	Gene Targeted Markers
ha	Hectare
hr	Hour(s)
HU	Heat units
INIFAP	Instituto Nacíonal de Investígaciones Forestales, Agríocola y Pecuarías
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter simple sequence repeat
kg	Kilogram(s)
km	Kilometre(s)
LSU	Large Subunit
m	Metre(s)
M	Molar
MDH	Malate dehydrogenase
mg	Milligram(s)
$MJ/m^2/s$	Mega Joules/Square Metre/Second
ml	Millilitre(s)
mM	Millimolar(s)
mm	Millimetre(s)
Mt	Metric tonne(s)
mtDNA	Mitochondrial DNA
NA	Nutrient agar
NDF	Neutral detergent fibre
ng	Nanogram(s)
NIH	National institute of Health
nm	Nanometre(s)
NPF	Number of polymorphic fragments
NRF	National Research Foundation
nr ITS	Nuclear ribosomal internal transcribed spacers
nt	Nucleotide
NTYSYS	Numerical taxonomy and multivariate analysis system
OM	Organic matter

PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PGI	Phosphoglucoisomerase
PGM	Phosphoglucomutase
PIC	Polymorphic Information Content
pmol	Picomole(s)
QTL	Quantitative Trait Locus
RAPD	Random Amplified Polymorphic DNA
RBB	Reproductive Bud Break
RBC	Rose Bengal Chloramphenicol
RDM	Random DNA markers
rDNA	Ribosomal DNA
REGW	Ryan Einot Gabriel and Welsch Test
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RNA	Ribonucleic acid
Rs	Solar Radiation
RUE	Rain-Use Efficiency
s	Second(s)
SNP	Single Nucleotide Polymorphisms
sp.	Species
spp.	Plural abbreviation of species
SPSS	Statistical Package for the Social Sciences
S_{SM}	Simple Matching coefficient
SSR	Simple sequence repeat
STS	Sequence tagged sites
Taq	*Thermus Aquaticus*
TBE	Tris-borate/EDTA
TE	Tris-HCl/EDTA
TFPGA	Tools for Population Genetic Analyses
TNF	Total Number of Fragments
Tris-HCl	Tris (hydroxymethyl) aminomethane Hydrochloride
TSS	Total Soluble Solids
TTA	Total titratable acidity
U	Unit(s)
UNCCD	United Nations Convention to Combat Desertification
UPGMA	Unweighted Pair-Group Method of Arithmetic Averages

v	Volume
V	Volt
v/v	Volume/volume
W	Watt
w/v	Weight/volume
YM	Yeast malt
yr	Year(s)

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Semi-arid and arid regions are a challenge to conventional cropping systems because of limited or erratic rainfall, poor soils, and high temperatures (Le Houérou, 1996). Hence, the cultivation of conventional crops such as maize, rice, and wheat in these areas has proven to be agriculturally unproductive. However, productivity in these areas can be increased by the cultivation of adapted crops such as *Opuntia* species, especially cactus pear (Pimienta-Barrios and Muñoz-Urias, 1995).

Opuntias can tolerate water-limited conditions, high temperatures, and poor soils. Consequently, cactus pear (*Opuntia* spp.) is increasingly being cultivated in semi-arid areas around the world, including South Africa, which according to the United Nations Convention to Combat Desertification (UNCCD) index for the classification of dry lands, is 80% semi-arid to arid (FAO, 2005).

Opuntia species are crassulacean acid metabolism (CAM) plants that convert water to biomass four fold more efficiently than either C_4 or C_3 plants. They are a source of dry matter in water-limited areas when fed to animals as green feed, hay, or silage. Opuntias meet the most important criteria for fodder crops in drought prone regions, drought tolerance and palatability (Tegegne, 2001). However, on its own as feed, cactus pear does not fill the dietary requirements of livestock since cladodes are low in crude protein and should be supplemented (Nefzaoui and Ben Salem, 2001).

In South Africa, *Opuntia* species were first reported in the 18th century, and grown in the Western Cape Province as a fodder crop (Van der Merwe, 1931). In 1914, 22 spine-less varieties were imported from the Burbank nursery (Wessels, 1988) and established at the Grootfontein Agricultural College, Middelburg, Eastern Cape Province. Plant material from Grootfontein was distributed to farmers in the Karoo area to be used as a drought tolerant fodder crop (Potgieter, 2002).

In cactus pear fruit plantations in South Africa terminal cladodes are used to vegetatively propagate varieties. However, the varieties have not been fully characterised, hampering research and breeding efforts directed at the development of improved varieties. In addition, few published records of varietal fruit quality traits are readily available.

Commercially, cactus pear is mainly cultivated in summer rainfall areas, most of which are prone to hail. Physical damage caused by hail facilitates the entry of pathogenic fungi. Varieties currently being cultivated have not been screened for resistance to

fungal diseases. Reports of new diseases and associated financial losses due to post-harvest fruit rot are thus increasing (Swart *et al*., 2003).

Post-harvest problems of fruit are directly related to physical damage at harvest that facilitates decay at the stem-end caused by *Fusarium* spp., *Alternaria* spp., *Chlamydomices* spp., and *Penicillium* spp. (Rodriguez-Felix, 2002). Although fungicides are the conventional method of controlling post-harvest disease, public concern over food safety and the development of fungicide resistant pathogens has increased the search for less harmful alternative methods (Spotts and Cervantes, 1986).

Biological control (biocontrol) using antagonistic microorganisms is amongst the methods being explored to replace and/or reduce the use of fungicides. Biocontrol has been endorsed as the preferred alternative to synthetic fungicides with considerable success. In particular, a host of yeast genera have been extensively used for the biological control of post-harvest diseases of fruits and vegetables (Wilson and Wisniewski, 1989; Punja, 1997).

Given the aforementioned problems confronting the rapidly expanding cactus pear industry in South Africa, a study was undertaken to investigate the specific goals presented in this thesis.

REFERENCES

FAO, 2005. Fertilizer use by crop in South Africa, Rome, Italy. http://www.fao.org/docrep/008/y5998e/y5998e06.htm#bm06

Le Houérou, H.N., 1996. Climate change, drought and desertification. Journal of Arid Environments 34: 133-185.

Nefzaoui, A. and H. Ben Salem, 2001. Opuntia: A strategic fodder and efficient tool to combat desertification in the WANA region. In : Mondragón-Jacobo, C. and S. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage, pp 73-89. FAO, Rome, Italy.

Pimienta-Barrios E. and A. Muñoz-Urias, 1995. Domestication of Opuntias and cultivated varieties. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 58-63. FAO Plant production and protection paper 132. Rome, Italy.

Potgieter, J.P., 2002. Conservation of cactus pear germplasm in South Africa. Cactus Pear News 1: 5-7.

Punja, Z.K., 1997. Comparative efficacy of bacteria, fungi, and yeasts as biological control agents for diseases of vegetable crops. Canadian Journal of Plant Pathology 19: 315-323.

Rodriguez-Felix, A., 2002. Post-harvest physiology and technology of cactus pear fruits and cactus leaves. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 191-199.

Spotts, R.A. and L.A. Cervantes, 1986. Population pathogenicity and benomyl resistance of botrytis spp., *Penicillium* spp. and *Mucor piriformis* in packinghouse. Plant Disease 70: 106-108.

Swart, W.J., M.R. Oelofse and M.T. Labuschagne, 2003. Susceptibility of South African cactus pear varieties to four fungi commonly associated with disease symptoms. Journal of the Professional Association for Cactus Development 5: 86-97.

Tegegne F., 2001. Nutritional value of *Opuntia ficus-indica* as a ruminant feed in Ethiopia. In: Mondragón-Jacobo, C. and S. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage. FAO, CACTUSNET. Rome.

Van der Merwe, C.R., 1931. Prickly pear and its eradication. Department of Agriculture Science Bulletin 93: 5-32.

Wessels, A.B., 1988. Spine-less Prickly Pear. First Perskor Publishers, Johannesburg.

Wilson, C.L. and M.E. Wisniewski, 1989. Biological control of post-harvest diseases of fruits and vegetables: An emerging technology. Annual Review of Phytopathology 27: 425-441.

Chapter 1

Characterisation and evaluation of *Opuntia* spp.

1.1 INTRODUCTION

Numerous crops previously deemed of little importance, and thus not collected and researched, are being recognised by international research organisations as necessary for agricultural sustainability and food security. The increased interest in these crops stems from the recognition of their potential contribution to agricultural diversification, their application to the exploitation of marginal lands and changing environments, and their utility as additional income sources for farmers (Padulosi, 1998).

New crops being introduced to arid and semi-arid areas include *Opuntia* spp. and the apple cactus *Cereus peruvianus* (L.) Mill (Weiss *et al*., 1993). Opuntias, in particular, have developed phenological, physiological, and structural adaptations that have enabled them to thrive in arid areas characterised by drought, erratic rainfall and poor soils. Asynchronous reproduction (Nerd and Mizrahi, 1995), CAM, structural adaptations typified by increments in water-storage tissues, and thickened cuticles (Salgado and Mauseth, 2002) have enabled the highly efficient growth of cacti under water-limited conditions (Nobel, 1995). Furthermore, the development of rhizosheaths reduces water loss to dry soil and a shallow root system assists cacti to absorb limited rainfall (Dubrovsky and North, 2002).

Opuntia ficus-indica (L.) Miller (cactus pear), a member of the *Opuntia* genus has been introduced and used in developing countries for various purposes. This crop serves as an emergency source of feed for animals. It is an efficient water utilising xerophyte, and both the young cladodes (nopalitos), and fruits (tunas) are suitable for human consumption. The multi-functionality of this crop identifies it as a plant that developing countries in arid and semi-arid regions will benefit from. If developed further, this crop could contribute to sustainable food production in countries with large areas of semi-arid and arid land (Felker and Inglese, 2003). However, one of the major obstacles in the development of cactus pear fruit and fodder varieties is the lack of adequate characterisation and evaluation of the available germplasm.

Characterisation and evaluation of germplasm accessions are the two main functions of genebanks (germplasm collections). Firstly, germplasm accessions representative of the available genetic diversity of a particular crop are collected, conserved and characterised. Secondly, germplasm material is evaluated for agronomically useful traits required by breeders. These traits are often subject to strong genotype by environment (G x E) interactions.

While a few germplasm collections of cactus pear are maintained at several locations around the world (Chapman *et al*., 2002), their maintenance is difficult and costly because of its perennial habit and large plant size. Additionally, the difficulty in genotype identification hinders the systematic collection and evaluation of *Opuntia* germplasm material (Chessa and Nieddu, 1997). This is evidenced by the scarcity of published accounts of the breeding history, characterisation and evaluation data of this crop (Chapman *et al*., 2002).

Characterisation and evaluation of the available cactus pear gene pool is, however, essential for future breeding programmes. This review focuses on the advancements made in the application of molecular markers in germplasm characterisation. The potential for the application of functional marker based molecular tools in the evaluation of germplasm for agronomically important traits will also be reviewed. In addition, the use of yeasts as biological control (biocontrol) agents to lengthen the post-harvest life of fruits will be highlighted briefly.

1.2 GENERAL BACKGROUND

Although cactus pear originates from arid and semi-arid areas in Mexico, it is presently cultivated worldwide, specifically *O. ficus-indica* which is cultivated in over 20 countries for its fruit (Inglese *et al*., 2002). Its dispersal around the world was facilitated by the inclusion of fresh cladodes on European ships in the late 15th century (Casas and Barbera, 2002).

Early European botanists called this cactus *Ficus indica*, because of its resemblance to the then already known Indian fig (possibly *Ficus bengalensis* L.) (Anderson, 2001). Linnaeus published it under a new name, *Cactus ficus-indica*, in the group *Cactus opuntia* in Species Plantarum. In 1978 Miller combined the above mentioned names into *Opuntia ficus-indica* (Griffith, 2004). Currently, cactus pear is grouped in the genus *Opuntia* in the Cactaceae family (Gibson and Nobel, 1986). The classification of cactus pear is briefly summarised below:

Order: Caryophyllales

Suborder: Portulacineae

Family: Cactaceae

Subfamily: Opuntioideae

Genus: *Opuntia*

Subgenus: *Opuntia*

Species: *ficus-indica* (L.) Mill., Gard. Dict. Abr. ed. 8. No. 2. 1768 (Scheinvar, 1995).

The taxonomic evaluation of Opuntias is complicated by variations in phenotype with changing ecological conditions, polyploidy, vegetative and sexual reproduction, and the occurrence of many hybrids between species (Scheinvar, 1995). Phenotypic variability is most frequently observed in fruit size and colour, cladode size, morphology, and phenology (fruit ripening time) (Pimienta-Barrios and Muñoz-Urias, 1995). Variability of both wild and domesticated cactus pear populations is thought to have occurred via natural hybridisation associated with polyploidy and geographic isolation (Gibson and Nobel, 1986). Natural hybrids are hypothesised to have arisen via natural crossing between different *Opuntia* species and F_1 hybrid progeny. Hybridisation was encouraged by artificial sympatric conditions in Mexican backyards where diverse species were grown in close proximity creating an environment conducive to increased gene flow between cultivars (Grant *et al.,* 1979; Pimienta-Barrios and Muñoz-Urias, 1995).

Variation in ploidy level has played an important role in the domestication of cactus pear as Mexican residents preferentially selected, and vegetatively propagated cultivars with larger fruit and cladodes. High ploidy levels are phenotypically expressed as increased vegetative (cladode size), and reproductive vigour. Different ploidy levels of 2x, 3x, 4x, 5x, 6x, 8x, 10x, 11x, 12x, 13x, 19x, and 20x have been reported amongst wild and cultivated cactus pear populations (Yuasa *et al.,* 1974; Pinkava *et al.,* 1992). Varieties with the high chromosome numbers of 2n = 6x = 66 and 2n = 8x = 88 are mostly found within cultivated populations, with the exception of wild populations of *O. streptacantha* Lemaire. Cultivars with lower chromosome numbers of 2n = 2x = 22 and 2n = 4x = 44 occur mostly in wild populations (Pinkava *et al.,* 1992).

The species *O. ficus-indica* has diffused into Argentina, California, Chile, Israel, and South Africa where naturalised stands and commercial plantations for fruit occur. Plantations of cactus pear also occur in Brazil, Colombia, Peru, Spain, Greece, Turkey, Italy, Jordan, Egypt, Tunisia, Algeria, and Morocco (Inglese *et al.,* 2002). To

develop improved cultivars from the varieties being grown in these countries, accurate germplasm characterisation is required.

1.3 GERMPLASM CHARACTERISATION

Germplasm characterisation involves the compilation and maintenance of accurate records of the identifying traits of accessions. Characterisation facilitates the classification of accessions and the estimation of the genetic diversity within a collection. To facilitate and standardise characterisation of genebank accessions globally, the International Plant Genetic Resources Institute (IPGRI) published descriptor lists for various crop species (FAO, 1996). As such, a descriptor for cactus pear was developed by scientists who participated in the Food and Agricultural Organisation of the United Nations' International Technical Co-operation Network on Cactus Pear (CACTUSNET-FAO), specifically by members of the working group for Plant Genetic Resources Collection, Evaluation and Conservation. The cactus pear descriptor follows the international format currently endorsed by the IPGRI (Chessa and Nieddu, 1997).

Mexico hosts the greatest genetic diversity of edible Opuntias and is the main source of cactus pear germplasm in the world. The largest number of entries is held at Instituto Nacíonal de Investígaciones Forestales Agrícolas y Pecuarías (INIFAP) in Mexico, and other germplasm collections are maintained at several locations around the world (Chapman *et al*., 2002). Mexican institutions engaged in cactus pear research are involved in germplasm collection and characterisation, a very costly effort. Collection of accessions is largely based on morphological traits, and often leads to duplication (Chapman *et al*., 2002).

1.3.1 Morphological markers

Morphological markers/traits are the oldest and most widely used genetic markers for germplasm characterisation. Their popularity stems from their simplicity, speed and inexpensive nature (Bretting and Widrlechner, 1995). Previously, morphological descriptors for characters that are highly heritable, easily observable, and expressed in all environments formed the core constituents of characterisation data. The cactus pear plant is unique in morphology with cladodes (pads), modified photosynthetic stems, that resemble leaves. Cladodes have numerous aereoles with glochids, short leaf spines that are easily dislodged. The descriptor for cactus pear examines plant, growth, cladode, flower, and fruit descriptors (Chessa and Nieddu, 1997).

However, Weniger observed that spininess, cladode shape and size, fruit characteristics, and plant productivity were influenced by the environment (Chapman *et al*., 2002). These characters constitute a major portion of the data collected following the descriptor format. In contrast, Chessa *et al*. (1995) found that the number of spines allowed the classification of biotypes of cactus pear according to their territorial distribution. Plants with an average or high number of thorns were concentrated in areas that were ecologically different from areas where thornless plants grew. In South Africa there are, however reports of the reversion to spininess of commercially cultivated spine-less cactus pear varieties (Figure 1.1).

The classification of commercial *O. ficus-indica* fruit types based on traditional, phenotypic taxonomic approaches is being contested by findings from molecular data. Previously, Sheinvar used spines to group taxa as either, spine-less *O. ficus-indica*; or spiny *O. hyptiacantha* Web*, O. streptacantha,* and *O. megacantha* Salm-Dick (Sheinvar, 1995). In contrast, random amplified polymorphic DNA (RAPD) patterns grouped a spiny *O. hyptiacantha* clone (1287) as similar to a spine-less *O. ficus-indica* clone (1281). The *O. ficus-indica* clone (1281) showed a greater genetic similarity to the spiny *O. hyptiacantha* clone (1287) than to other spine-less *O. ficus-indica* clones (Wang *et al.,* 1999).

FIGURE 1.1 A SPINE-LESS CACTUS PEAR PLANT WITH CLADODES THAT HAVE REVERTED TO SPININESS

Although morphological markers are easily monitored, they are inadequate in characterising germplasm, since they can be influenced by the environment, and some markers such as flower colour, appear late in plant development (Andersen and Lübberstedt, 2003). In addition, the exclusive use of morphological traits for the collection of accessions has often led to duplication, complicating subsequent evaluation and utilisation (Chapman *et al*., 2002). As a result, generally, germplasm characterisation has advanced with the evolution of genetic markers from morphological traits, through isozyme to DNA markers (Bretting and Widrlechner, 1995; Andersen and Lübberstedt, 2003).

Confusion regarding species classification within the *Opuntia* genus has hindered the characterisation of germplasm accessions. The delineation of the 250 species of the Opuntioidae subfamily based on morphology alone has resulted in taxonomic confusion because of the high level of phenotypic plasticity within its members (Wallace and Gibson, 2002). The large morphological variation of the 181 species has led Labra *et al*. (2003) to the conclusion that phenotypic traits alone will not allow a stable classification within the *Opuntia* genus.

Consequently, molecular techniques are being used to clarify classification within the *Opuntia* genus. DNA sequences of the nuclear ribosomal internal transcribed spacers (nrITS) were phylogenetically analysed, and demonstrated that the taxonomic concept of *O. ficus-indica* should be considered as polyphyletic, deriving from multiple lineages (Griffith, 2004). Labra *et al*. (2003) have suggested that the *Opuntia* genus be re-classified with the inclusion of molecular data. Their findings based on molecular data [chloroplast simple sequence repeat (cpSSR) and amplified fragment length polymorphism (AFLP)], morphological traits and biogeographic distribution, suggest that *O. ficus-indica* be considered as a domesticated form of *O. megacantha*.

Resolution of the taxonomic classification of *Opuntia* species using molecular markers will facilitate the characterisation of germplasm accessions, especially of the hybrid Burbank varieties used for commercial fruit production in South Africa. The classification of *Opuntia* x *rooneyi* M.P.Griffith and *Opuntia* x *spinosibacca* M.S. Anthony as hybrids of *O. aureispina* (S.Brack & K.D.Heil) and *O. macrocentra* Engelm, and *O. camanchica* Engelm and *O. aureispina*, respectively, was achieved using RAPD markers (Griffith and Porter, 2003).

1.3.2 Isozymes

Isozymes are the earliest molecular markers developed. They occur as a result of variations in nucleotide sequence that result in the substitution of one amino acid for another. Such a substitution may result in the alteration of the net electrical charge on a protein. The charge difference is subsequently detected as an alteration in the migration rate of a protein through an electrical field. Electrophoretic separation is then used to measure protein mobility variation within a population (Klug and Cummings, 2000). Thus, electrophoretically distinct forms of a protein (isozymes) could imply that they are encoded by different alleles, i.e., genetic variation.

The first molecular marker technique used in cactus pear to investigate genetic diversity was isozymes (Uzun, 1997). An investigation of seven enzyme systems in three Italian cultivars, and 15 Turkish cactus pear ecotypes showed no variation in isozyme banding patterns for a given enzyme system in the same plant organ. However, differences were observed between fruit and cladode isozymes for a given cultivar (Chessa *et al*., 1997; Uzun, 1997). In 1997, Chessa *et al*. demonstrated that isozyme analysis of pollen produced the best results compared to root, cladode, and petal tissues. Malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and phosphoglucomutase (PGM) isozyme banding patterns allowed grouping of different varieties and biotypes. However, unique cultivar identification using isozymes was not possible (Chessa *et al*., 1997).

Although isozymes were used in the past in various other fruit species, for example for identification of apple cultivars (Weeden and Lamb, 1985), to verify the parentage of presumed peach x almond hybrids (Carter and Brock, 1980), and as genetic markers in peach (Durham *et al*., 1987), they have been surpassed by DNA markers because of the low number of markers they generate. Additionally, because isozymes are the products of gene expression they are often affected by environmental conditions, tissue type and the developmental stage of a plant. Proteins are also subject to post-translational modifications that may alter their electrophoretic mobility (Kumar, 1999). In addition, since not all substitutions change the net electrical charge on the molecule, approximately 30% of the actual variation due to amino acid substitutions are electrophoretically detected (Klug and Cummings, 2000).

1.3.3 DNA markers

DNA polymorphisms represent differences in the DNA sequence of two individuals and are the desired markers for the identification and characterisation of plants. Given that DNA is an integral part of plants and is not subject to environmental modification (Bachmann *et al*., 2001), nuclear and cytoplasmic (chloroplast DNA [cpDNA], and mitochondrial DNA [mtDNA]) DNA can be analysed for polymorphisms using various techniques.

DNA marker techniques have progressed from hybridisation-based methods such as restriction fragment length polymorphisms (RFLPs), to more rapid polymerase chain reaction (PCR)-based DNA methods such as RAPDs, simple sequence repeats (SSRs) or microsatellites, sequence-tagged sites (STS), AFLPs, inter-simple sequence repeat amplifications (ISSR) and single nucleotide polymorphisms (SNPs) (Gupta *et al*., 1999).

RAPD markers have been used widely in fruit crops. RAPD patterns are PCR derived markers obtained by the random amplification of DNA using short nucleotide primers of arbitrary nucleotide sequence (Williams *et al*., 1990). They have been used for the characterisation of peach species and cultivars (Sharifani and Jackson, 2000), to estimate the genetic diversity of apricot (Zhebentyayeva and Sivolap, 2000) and to classify jujube cultivars (Mengjun and Zhao, 2003).

Initially, the application of molecular marker techniques was hampered by the difficulty in extracting genomic DNA from mucilaginous tissues (De La Cruz *et al.,* 1997; Wang *et al.,* 1998b; Tel-Zur *et al.,* 1999; Griffith and Porter, 2003). However, researchers have demonstrated that RAPD patterns can be obtained from cacti using primers OPA-11 (De La Cruz *et al.,* 1997), and OPA-12 (Tel-Zur *et al.,* 1999). RAPD profiles have been used to verify the maternal origin of apomictic seedlings in cactus pear (Mondragón-Jacobo , 2002).

In South Africa, preliminary studies by Potgieter and Carstens (1996) employed six RAPD primers that produced specific banding profiles for 18 accessions tested. Arnholdt-Schmitt *et al.* (2001) also found that RAPD patterns for the cactus pear cultivars tested provided reproducible banding patterns. Amongst the eight clones tested using RAPDs, reproducible and distinct differences were observed. Of the detected bands, 75% were polymorphic, and allowed for unique cultivar identification. The fruit accessions tested were closely related to each other, and the groupings

based on RAPD banding profiles agreed with those obtained from morphological and physiological data (Arnholdt-Schmitt *et al*., 2001).

Although RAPDs have the advantage of generating numerous markers, the resolution of RAPD profiles on agarose gels is poor (Gupta *et al*., 1999). This shortcoming has been circumvented by coupling RAPDs to denaturing gel electrophoresis (Dweikat *et al*., 1994), and temperature sweep gel electrophoresis (Penner and Bezte, 1994).

AFLP is another DNA-based marker technique that has been used in fruit crops for genetic diversity analysis (Hagen *et al*., 2001), and cultivar identification (Boritzki *et al*., 1999; Geuna *et al*., 2003). This technique involves the digestion of genomic DNA with two endonucleases, followed by the ligation of site specific adaptors to the DNA fragments. Primers designed with selective nucleotides added at the 3' ends and complementary to the adaptors and the restriction sites are used for amplification. Thereafter DNA fragments are resolved on standard sequencing gels (Vos *et al*., 1995). This technique has the advantages of being highly sensitive, reproducible and widely applicable. Its limitations, however, are that it is relatively expensive, technically demanding, and a dominant marker system (IPGRI, 1996).

DNA-based marker analysis techniques such as AFLP, RAPD, and RFLP are dependent on gel electrophoresis and associated with difficulties in correlating fragments on gels with allelic variants (Jaccoud *et al*., 2001), and thereby characterised as low-throughput. As a result high-throughput hybridisation techniques of nucleic acids immobilised on solid states (DNA chips) were developed to replace gel-based analysis systems.

Non-gel based high-throughput genotyping technologies such as DNA microarrays (Chee *et al*., 1996; Lipshutz *et al*., 1999) allow the simultaneous analysis of many hundreds of thousands of oligonucleotides attached to a solid silicon surface in an ordered array to create a microarray. The DNA or RNA sample of interest is PCR amplified to incorporate fluorescently labelled nucleotides and subsequently hybridised to the array. Each oligonucleotide or cDNA on the array acts as an allele specific probe. Perfectly matched sequences hybridise more efficiently, giving off a stronger fluorescent signal than mismatched oligonucleotide-target combinations. The fluorescent signals are quantified by high resolution fluorescent scanning and analysed electronically This allows the identification of heterozygous base pair mutations, insertions and deletions (Chee *et al*., 1996; Lipshutz *et al*., 1999).

DNA chips (microarrays) have been developed to genotype SNPs in germplasm (Wang *et al*., 1998a). SNPs are single base variations in the nucleotide sequence at a unique physical location. SNPs have the advantage of ease of automation because they can be screened in a digital format analysing the presence or absence of a sequence, enabling high-throughput analysis (Wang *et al*., 1998a).

Initially, DNA chips developed to analyse SNPs, required prior DNA sequencing. To circumvent sequencing, Diversity arrays (DArT™) have been developed for the detection of specific DNA fragments derived from the total genomic DNA of an organism or a population of organisms (Jaccoud *et al*., 2001). Given the progress made in other fruit crops, and a proposal for the development of a genetic map for *O. ficus-indica* using molecular sequence data (Chapman and Paterson, 2000), modest progress has been made in the application of molecular marker techniques to cactus pear germplasm characterisation.

1.4 GERMPLASM EVALUATION

The evaluation of germplasm for useful traits is the stage where the most value is added to germplasm collections. It is at this stage when it is determined whether an accession harbours genes of utility to breeders and to agriculture in general (FAO, 1996). Agronomic traits required by breeders are too genetically complex to be screened in the preliminary characterisation stages, as they may be subject to strong G x E interactions.

In order to exploit the genetic variability in the different cactus pear-producing countries it was recognised that a thorough understanding of the characteristics of *Opuntia* germplasm, and of the variability in its horticultural and pomological traits, was necessary. Consistency in the methodology used for data collection and terminology would be essential to meet this goal, as it would allow better utilisation of germplasm within and between countries for agronomic purposes, and to develop programmes for genetic improvement (Chessa *et al.,* 1995).

1.4.1 Evaluation for fruit quality

Fruit quality is complex, but the simplest definition thereof is, 'whatever the consumer desires' (Barritt, 2001). In general, the consumer assesses quality on the appearance of the fruit at the point of sale, and thereafter by its taste (Kader, 2002). Appearance, in turn, is determined by fruit size and colour (Callahan, 2003). In cactus pear, fruit

quality is based on sugar content, peel colour, fruit weight, pulp weight, and seed content (Cantwell, 1991).

The cactus pear fruit is an oval shaped berry fruit with an average weight of 100-200 g. Cactus pear fruits are appreciated for their characteristic taste and aroma, and dietetic properties. Fruits have a thick fleshy skin that contributes 30-40% of the total fruit weight. The juicy pulp contributes 60-70% of the total fruit weight, and contains many hard-coated seeds that contribute 5-10% of the pulp weight. Each variety produces fruits of different shapes, colours and flavours. The main components of the fruit pulp are water (85%), carbohydrates (10-15%) and vitamin C (25-30 mg/100g) (Cantwell, 1995).

In general, high ploidy levels are phenotypically expressed as increased reproductive vigour (fruit size). Similarly, variation in ploidy level has played an important role in the domestication of cactus pear. Mexican people preferentially selected and vegetatively propagated cultivars with larger fruit. Different ploidy levels have been reported amongst wild and cultivated cactus pear populations from cytogenetic studies (Yuasa *et al.,* 1974; Pinkava *et al.,* 1992). Varieties with the high chromosome numbers of 2n = 6x = 66 and 2n = 8x = 88 are mostly found within cultivated populations (Pinkava *et al.,* 1992).

Currently, cactus pear fruit size is evaluated based on fruit mass, length and equatorial width (Chessa and Nieddu, 1997), and edible and skin fresh matter content. Italian germplasm was evaluated using an abridged version of the descriptor list. Six accessions with high yield and fruit qualities were selected as parental types for the development of new varieties (Nieddu *et al*., 2002). In South Africa, varietal evaluation for fruit production is based on the following minimum criteria: fruit mass > 140.0 g, total soluble solids (TSS) > 13°Brix, %pulp > 50% and peel thickness < 6 mm (Potgieter and Mkhari, 2002).

The cactus pear fruit contains many hard coated seeds that are completely wrapped by a stalk that becomes hard and bony (Rebman and Pinkava, 2001) and contribute 5-10% of the pulp weight (Cantwell, 1995). The seed content in cactus pear fruits varies from 2.8-7.5 g per fruit depending on cultivar and size (Mondragón-Jacobo and Perez, 1995). The high seed content is an apparent deterrent to its introduction into new markets. High seed content is however, positively correlated with fruit size. It has therefore been suggested that a fruit of ideal size should have a high ratio of aborted to normal seed (Mondragón-Jacobo and Bordelon, 1996). Additionally, normal seed

number and matter were found to be positively inter-correlated and found to account for 57.4% of the variation in fruit size. This variation mainly affects fruit weight and size variation, suggesting that normal seed number and matter controlled fruit weight, and size (Gutiérrez-Acosta *et al*., 2002).

In general, actual fruit size is governed by G x E interactions whilst potential fruit size is genetically determined (Zhang *et al*., 2006). Fruit size is a function of cell number, volume, and density (Scorza *et al*., 1991), and is largely genetically controlled (Janick and Moore, 1996). Similarly, cactus pear researchers are reporting that fruit size is not exclusively determined by environmental or edaphic factors and that genetic factors are important determinants of fruit size (Felker *et al*., 2005).

Little is known about the molecular properties of the genes that determine fruit size. Fruit size is a complex trait governed by a number of genes or quantitative trait loci (QTL) as well as by environmental factors (Nesbitt and Tanksley, 2001). A fruit size QTL *fw2.2* responsible for a 30% difference in fruit size between large domesticated tomatoes (*Lycopersicon esculentum* Mill.) and their small-fruited wild relatives has been described. The gene underlying this QTL was cloned and found to be associated with fruit size and altered cell division in ovaries (Frary *et al*., 2000).

In permanent crops, with a medium length of juvenility such as cactus pear, evaluation for desired fruit quality traits is only possible after a few years. It is at this point that accessions to be used as parental types in breeding programmes can be selected.

1.4.2 Evaluation for fodder quality

When cactus pears plants begin fruiting, they are pruned to facilitate cultural practices and to renew fertile cladodes (Inglese, 1995). Pruning generates huge amounts of cladode waste material. Cladodes, are however very nutritious and can be used as fodder. In addition, cladodes are highly digestible and contain sufficient water and minerals that in combination with a protein source constitute a complete feed for livestock (Kueneman, 2001).

It is well established that Opuntias meet most of the requirements for fodder crops in drought prone regions (Nefzaoui and Ben Salem, 2002). Drought-tolerance of *O. ficus-indica* in the Mediterranean basin is comparable to that of olive, almond, pistachio, pomegranate, and fig tree. Yields of between 20-60 metric tons (Mt) fresh matter (FM)/ha/yr (equivalent to 3-9 Mt dry matter (DM)/ha/yr) on arid lands with a mean annual rainfall of 200-400 mm, under poor cultivation practises and no fertilization were recorded (Le Houérou, 2002). Under a mean annual rainfall of 400-

600 mm the yield in extensively managed conditions rose to 60-100 Mt FM/ha/yr (i.e., 9-15 Mt DM/ha/yr) (Le Houérou, 2002). These yields correspond to Rain-Use Efficiency (RUE) of 15-25 kg of above ground DM/ha/yr/mm. These RUEs are 3-5 times higher than the best rangelands under good management in the same areas where the RUE is seldom above 5 kg of above ground DM/ha/yr/mm (Felker, 1995).

The nutrient content of *Opuntia* spp. depends on the genetic characteristics of the species or clones, the cladode's age, the cladode sampling location, the pad harvesting season and the growing conditions such as soil fertility and climate (Nefzaoui and Ben Salem, 2001). DM content, the component in feed after drying, depends on the season in which cladodes are harvested. Significant differences in DM content among clones of *O. ficus-indica* (L) f. *inermis* Weber, *O. robusta* Wend., *O. paraguayensis* K. Schum., and *O. spinulifera* Salm-Dyck have been reported for *Opuntia* spp. In addition, a positive linear relationship between DM content and age ($p<0.05$) was established for these clones (Guevara *et al.*, 2004).

Season affects the chemical composition of cladodes. The DM content of one to three year old cladodes ranged from 10-15% in the rainy season to 15-25% in the dry season (Le Houérou, 2002). Organic matter (OM) content among *Opuntia* spp. clones varied significantly, but was not considerably different for clones of different ages. Different researchers have reported different values for OM content of cladodes, ranging from 74.6-86.9% (Guevara *et al.,* 2004).

Cladode crude protein (CP) content varied amongst clones and between cladodes of different ages and it is thought to be sensitive to changes in soil N (Guevara *et al.,* 2004), which may explain high CP contents of 8.5% previously reported by other researchers (Gregory and Felker, 1992). A negative linear relationship exists between CP content and age (Guevara *et al.,* 2004) although the rate of decrease in CP content differs between clones (Nefzaoui and Ben Salem, 2001). Crude protein content during flowering decreased from the basal to the apical area of cladodes (Gugliuzza *et al.,* 2002). With regard to sampling location, it has been shown that the central-basal zone of a cladode comprised of 40 sampling locations grouped in a rectangular manner, represented the average CP content of the entire cladode (Guevara *et al.,* 2006).

Neutral detergent fibre (NDF) denotes the insoluble portion of fodder and typically contains cellulose, hemicellulose, lignin and silica. NDF is negatively correlated with DM intake. Therefore, livestock will consume less forage, with increasing NDF

content. In addition to a positive linear relationship ($p < 0.05$) between NDF content and age, significant differences in NDF content amongst clones of *O. ficus-indica* f. *inermis*, *O. robusta*, *O. paraguayensis*, and *O. spinulifera*, have been reported (Guevara *et al.,* 2004). The NDF values reported by Guevara *et al.* (2004) were in the range previously reported as 21.8%, and 25.5% by Ben Salem *et al.* (2002). Higher NDF values of 33.8% have also been reported (Ben Salem *et al.,* 2004).

The acid detergent fibre (ADF) fraction of fodder includes cellulose, lignin, and silica. ADF is an important indicator of fodder digestibility, and is negatively correlated with digestibility. A positive linear relationship was found between ADF content and cladode age. Significant differences were observed amongst ADF content of different clones of *O. fiucs-indica* f. *inermis*, *O. robusta*, *O. paraguayensis*, and *O. spinulifera* (Guevara *et al.,* 2004). ADF contents reported for these clones (14.3-16.0%) were consistent with those previously reported as 14.7% and 16.8% by Ben Salem *et al.* (2004).

On its own as feed, cactus pear does not fill the dietary requirements of livestock. Cladodes are low in crude protein and supplementation with a protein source is recommended. The nutritional value of cladodes of different varieties (genetic characteristics), ages, at different locations, during different seasons, and under diverse growing conditions such as soil fertility and climate have been studied by various authors (Nefzaoui and Ben Salem, 2001). These factors influence the nutritional content of cladodes resulting in incomparable literature reports (Felker *et al.*, 2006). The nutritional value of cactus pear cladodes pruned annually in commercial orchards for use as fodder has however not been researched that extensively.

1.4.3 Evaluation for resistance to fungal disease

Evaluation of fruit tree germplasm for disease resistance is conventionally done with bioassays, where plants are cultured seven to ten years without fungicide application. Infected material is often brought into the orchard to increase infection pressure (Kemp and van Dieren, 2000; Kirby *et al.*, 2001). Fungal pathogens penetrate the host tissue via mechanical perforation of the cuticle and underlying cell wall, or through enzymatic activity (Granata, 1995). However, the structural nature of cladodes limits pathogen entry. The artificial inoculation of cladodes using colonised toothpicks has been described (Swart *et al.*, 2003) for bioassays in cactus pear.

Fungal pathogens naturally gain entry to cacti through wounds such as those sustained during hailstorms. Cactus pear fungal pathogens belong to the genera

Armillaria, *Dothiorella*, *Phytophthora*, *Alternaria*, *Fusarium*, *Phyllosticta*, *Sclerotina*, and to a lesser extent to the genera *Colletotricum*, *Capnodium*, *Macrophomina*, *Cercospora*, *Aecidium*, *Phoma*, *Cytospora*, *Gleosporium*, *Mycospherella*, and *Pleospora* (Granata, 1995). Reports on the screening of cacti for resistance to fungal diseases have not been widely published (Kim and Kim, 2002; Swart *et al*., 2003). Abscission layer formation in stem disk cells from a two year old resistant *Cereus peruvianus* plant limited colonisation of *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk whilst the susceptible *C. tetragonus* (L.) Miller became extensively colonised (Kim and Kim, 2002).

Glasshouse and field evaluation of the susceptibility of ten commercially important South Africa cactus pear varieties to four fungal pathogens [*Phialocephala virens* Siegfried and Siefert, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl, *Fusarium oxysporum* (Schltdl), and *F. proliferatum* (Matsush) Nirenberg ex Gerlach and Nirenberg] showed variations in susceptibility to fungal colonisation. The varieties Nudosa, and Algerian were the most susceptible, whilst Gymno Carpo, Zastron, and Malta were the most resistant to fungal disease (Swart *et al*., 2003).

Although cladodes are not highly susceptible to fungal pathogen attack, the cactus pear fruit is. As fresh produce, cactus pears are susceptible to damage in the period between harvest and consumption (Rodriguez-Felix, 2002). In general, the deterioration rate of harvested produce is proportional to respiration rate. However, cactus pears are non-climacteric fruits with low respiration rates (20 ml CO_2/kg/hr) and low ethylene production (0.2 µl C_2H_4/kg/hr) at 20°C (Rodriguez-Felix, 2002). Although cactus pear fruits produce very little ethylene, the application of Ethrel to fruits has been used experimentally to hasten abscission zone formation, reducing harvest injury at the stem end (Cantwell, 1986).

Cactus pears are highly perishable, and under marketing conditions [20°C, 60–70% relative humidity (RH)] have a shelf life of only a few days (Rodriguez-Felix, 2002). The main post-harvest problems are directly related to physical damage incurred at harvesting. This leads to water loss and stem end rots, or both (Cantwell, 1986).

Post-harvest losses vary depending on cultivar, stage of maturity, environmental conditions, and harvesting method (Schirra *et al.,* 1999). Peel thickness and toughness affect shelf life as some cactus pear varieties have been reported to be

more resilient to handling than others (Mondragón-Jacobo and Bordelon, 1996). Other factors that influence shelf life include decay at the stem end caused by *Fusarium* spp., *Alternaria* spp., *Chlamydomices* spp., and *Penicillium* spp. (Rodriguez-Felix, 2002). Stem end rots are highly prevalent in cactus pear. Pathogenic fungi have resulted in huge losses in the fresh fruit industry (Sommer, 1985).

Previous studies by Swart and Swart (2003) found fungi from the following genera associated with healthy cactus pear fruits (cv. Algerian) in South Africa; *Rhizopus* sp., *Mucor* sp., *Epicoccum* spp., *Cladosporum* sp., *Fusarium* spp., *Phoma* sp., *Aspergillus* spp., *Stemphyllium* sp., *Alternaria* spp., *Rhizoctonia* sp. *Rhizopus* spp., and *Penicillium* spp. Some bacteria were associated with post-harvest rot of cactus pear fruit (cv. Algerian) in South Africa. In addition, the yeasts *Hanseniaspora ovarum* (Niehaus) Shehata, Mrak & Phaff, *Pichia kluyveri* Bedford ex Kudryavtsev, *P. membranaefaciens* E.C. Hansen, and various *Candida* spp. were associated with diseased fruits (Swart and Swart, 2003).

Fruit shape affects harvesting as oval or barrel-shaped fruits are easier to harvest than elongated fruits and therefore undergo less harvest damage to the stem end (Cantwell, 1991). Farmers are advised to cut off a small piece of the mother cladode with the fruit to reduce damage during harvesting and thus limiting possible decay. Holding the crop at ambient conditions for one or two days at increased airflow is subsequently used to dry up the cladode piece (Rodriguez-Felix, 2002).

Cold storage increases post-harvest life of most horticultural crops (Wang, 1994) by retarding respiration, ethylene production, ripening, senescence, undesirable metabolic changes, and decay (Rodriguez-Felix, 2002). However, Chessa and Barbera reported that cactus pears are susceptible to chilling injury when stored at temperatures below 9°C or 10°C, depending on the cultivar (Inglese *et al*., 2002). Due to its sensitivity to chilling injury, various innovative techniques aimed at increasing shelf life have been developed for cactus pear. These include intermittent warming, controlled atmospheres, film wrapping, and heat treatments with hot air or water (Rodriguez-Felix, 2002).

Fungicides have been principally used to control post-harvest decay of fruits and vegetables (Sommer, 1985). However, public concern over food safety and the development of fungicide resistant pathogens has increased the search for less harmful alternative methods. Biological control using antagonistic microorganisms has

been popularised as an alternative to the use of synthetic fungicides with considerable success. Numerous studies have demonstrated the potential of biological control of post-harvest diseases using microbial antagonists (Sugar, 1999; Leverentz *et al*., 2000; Tian *et al*., 2002; Yu *et al*., 2006). In particular, a variety of yeast genera have been extensively used for the biological control of post-harvest diseases of fruits and vegetables (Wilson and Wisniewski, 1989; Punja, 1997), to protect moulding of stored grains (Petersson *et al.,* 1999), and to control foliar diseases (Urquhart and Punja, 1997). Decay caused by *Botrytis cinerea* Pers. and *Penicillium expansum* Link on pome fruits has been controlled at laboratory and pilot stage trial by bacterial and yeast antagonists (Roberts, 1990; Janisiewicz and Marchi, 1992; Janisiewicz *et al*., 1994; Chand-Goyal and Spotts, 1996). Furthermore, formulated biocontrol product such as Aspire and Bio-Save 11 are available internationally.

1.5 CONCLUSIONS

South Africa hosts one of the largest collections of genetic diversity of cultivated *Opuntia* spp. in the world, and various initiatives are now in place to facilitate a consolidated effort towards cultivar development. However, cultivar development requires accurate genotype identification that cannot be confidently achieved using phenotypic traits since cactus pear expresses significant G x E interactions. Thus, DNA marker techniques such as RAPDs, AFLPs and SSRs can be used in combination with phenotypic characterisation to increase the accuracy of genotype identification. This approach will support the identification of cactus pear varieties that can be used as parental types in future breeding programmes.

Many challenges remain in conventional breeding and the application of marker-assisted selection in cactus pear. Breeding requires the production of seeds, and cactus pear is renowned for slow seed germination (Bregman and Bouman, 1983) and apomixis (Mondragón-Jacobo and Pimienta, 1995). However, chemical scarification of seeds in concentrated H_2SO_4 or with Schweizer reagent followed by incubation in H_2O_2 under photoperiodic conditions has been shown to increase the percentage of germinated seeds in the shortest time (Altare *et al*., 2006).

Apomixis, the asexual production of seeds from maternal tissues (Koltunow, 1993), complicates breeding as it hinders the screening of progeny from crosses. Additionally, in cactus pear, it has been shown that artificial crossing in species naturally prone to this phenomenon, and the germination of seeds in the greenhouse increases apomixis (Mondragón-Jacobo, 2001a). Late emergent seedlings have been

shown to display RAPD patterns similar to that of the maternal entries (Mondragón-Jacobo, 2001b).

Subsequent to parental type selection and crossings, individuals from crosses in cactus pear are presently selected based on morpho-agronomic traits. This selection process is time consuming, especially in cactus pear due to its long juvenile phase, estimated to be between four to six years (Mondragón-Jacobo, 2001a). Currently, however, functional markers (Andersen and Lübberstedt, 2003) can be developed to screen for genes of agronomic importance before they are expressed in the mature plant, hence shortening the time required to select progeny with desirable traits and ultimately produce new cultivars.

Unlike DNA-based marker techniques such as AFLP, RFLP, SSR, and RAPD that generate markers derived from arbitrary regions of the genome, and as such are described as random DNA markers (RDMs) (Andersen and Lübberstedt, 2003), molecular markers from the transcribed region of the genome, known as gene targeted markers (GTMs) (Andersen and Lübberstedt, 2003; Gupta and Rustgi, 2004) and functional markers derive from polymorphic sites within genes responsible for phenotypic trait variation (Andersen and Lübberstedt, 2003). The development of functional markers however, requires functionally characterised genes, allele sequences from these genes, the identification of polymorphic, functional motifs that affect plant phenotype within the genes and the corroboration of the association between DNA polymorphisms and trait variation (Lübberstedt *et al*., 2005).

The progress made in genetics and genomics has improved the understanding of structural and functional aspects of plant genomes in ways that can increase the ability to improve crop plants. The complete genome sequences of *Arabidopsis thaliana* (L.) Heynh., poplar, and rice, and an enormous number of expressed sequence tags in plants (ESTs) are now available. This has made available many strategies for developing functional molecular markers such as SNP (Rafalski, 2002), SSRs (Varshney *et al*., 2005a), conserved orthologous sets of markers (Rudd *et al*., 2005), and comserved intron scanning primers (Feltus *et al*., 2006).

The transfer of QTLs of agronomically important traits from wild species into crop varieties can now be achieved via advanced backcross QTL analysis (Tanksley and Nelson, 1996). In addition, allele mining can be performed to gather information for all the alleles of a fully characterised gene in a germplasm collection. Allele mining proceeds via a strategy based on targeting induced local lesions in genomes, known

as EcoTILLING that allows the natural alleles at a locus to be characterised over many germplasm collections (Comai *et al*., 2004). This will enable the discovery of SNPs that can be used as functional markers. Nonetheless, these newly developed genetic and genomics tools will only enhance but not replace conventional breeding and evaluation (Varshney *et al*., 2005b) as the successful implementation of these tools and strategies in plant breeding programmes requires extensive and precise phenotyping of agronomic traits of breeding material (Varshney *et al*., 2005b).

REFERENCES

Altare, M., S. Trione, J.C. Guevara and M. Cony, 2006. Stimulation and promotion of germination in *Opuntia ficus-indica* seeds. Journal of the Professional Association for Cactus Development 8: 91-100.

Andersen, J.R. and T. Lübberstedt, 2003. Functional markers in plants. Trends in Plant Sciences 8: 554-560.

Anderson, E.F., 2001. The cactus family. Timber Press, Oregon, USA.

Arnholdt-Schmitt, B., L.C. Girão and R.M. Zárate, 2001. Genome characterisation of *Opuntia ficus-indica*: A simple and efficient micromethod. Journal of the Professional Association for Cactus Development 4: 57-65.

Bachmann K., F. Blatter, D. Fischer, N. Friesen, R. Fritsch, M. Klaas, T. Mes, and S. Poller, 2001. Molecular markers in *Allium*: Range of application, reliability and taxonomic implications. Proceedings of the International Symposium On Molecular Markers. Acta Horticulturae 546: 159-163.

Barritt, B.H., 2001. Apple quality for consumers. International Dwarf Fruit tree Association 34: 46-54.

Ben Salem, H., A. Nefzaoui and L. Ben Salem, 2002. Supplementation of *Acacia cyanophylla* Lindl. foliage-based diets with barley or shrubs from arid areas (*Opunita ficus-indica* f. *inermis* and *Atriplex nummularia* L.) on growth and digestibility in lambs. Animal Feed Science and Technology 96: 15-30.

Ben Salem, H., A. Nefzaoui and L. Ben Salem, 2004. Spine-less cactus (*Opunita ficus-indica* f. *inermis*) and oldman saltbush (*Atriplex nummularia* L.) as alternative supplements for growing Barbarine lambs given straw-based diets. Small Ruminant Research 51: 65-73.

Boritzki, M., J. Plieske and D. Strauss, 1999. Cultivar identification in Sweet Cherry (*Prunus avium* L.) using AFLP and Microsatellite markers. In: Geibel, M., M. Fischer and C. Fischer (Eds.), Proceedings of the EUCARPIA Symposium on Fruit Breeding and Genetics. Acta Horticulturae 538: 505-510.

Bregman, R. and F. Bouman, 1983. Seed germination in Cactaceae. Botanical Journal of the Linnean Society 86: 357-374.

Bretting, P.K. and M.P. Widrlechner, 1995. Genetic markers and horticultural germplasm management. Horticultural Science 30: 1349-1356.

Callahan, A.N., 2003. Breeding for fruit quality. In: Janick, J. (Ed.), Proceedings of the 26th International Horticultural Congress: Genetics and breeding of tree fruits and nuts. Acta Horticulturae 622: 295- 302.

Cantwell, M., 1986. Post-harvest aspects of prickly pear fruits and vegetable cladodes. In: Perishables handling, post-harvest technology of fresh horticultural crops. Cooperative extension, University of California, 59: 6-9.

Cantwell, M., 1991. Quality and post-harvest physiology of 'nopalitos' and 'tunas'. In: Proceedings of the Second Annual Texas Prickly Pear Conference. McAllen, Texas.

Cantwell, M., 1995. Post-harvest management of fruits and vegetable stems. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 120-130. FAO Plant production and protection paper 132. Rome, Italy.

Carter, G.E. and M.M. Brock, 1980. Identification of peach cultivars through protein analysis. Horticultural Science 15: 292-283.

Casas. A. and G. Barbera, 2002. Mesoamerican domestication and diffusion. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses, pp 143-183. University of California Press, California, USA.

Chand-Goyal, T. and R.A. Spotts, 1996. Control of postharvest pear diseases using natural saprohytic yeast colonies and their combination with a low dose of thiabendazole. Postharvest Biology and Technology 7: 51-64.

Chapman, B. and A.H. Paterson, 2000. Bioinformatic approaches to developing molecular tools for cactus. In: Nefzaoui, A and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal, October 22-28, Hammamet, Tunisia.

Chapman, B., C. Mondragón-Jacobo , R.A. Bunch and H. Paterson, 2002. Breeding and Biotechnology. In: Nobel, P.S. (Ed.) Cacti: Biology and Uses, pp 255-271. University of California Press, California, USA.

Chee, M., R. Yang, E. Hubbell, A. Berno, X.C. Huang, D. Stern, J. Winkler, D. J. Lockhart, S.M. Morris and P.A.S. Fodor, 1996. Accessing genetic information with high-density DNA arrays. Science 274: 610-614.

Chessa, I. and G. Nieddu, 1997. Descriptors for Cactus pear (*Opuntia* spp.) CACTUSNET- FAO Newsletter Special Issue.

Chessa, I., G. Nieddu and M. Mulas, 1995. Variability of *Opuntia ficus-indica* in a Mediterranean area: definition of a descriptor list and germplasm characterization. In: Proceedings of the Second International Congress on Cactus Pear and Cochineal, Guadalajara, Mexico.

Chessa I., G. Nieddu, P. Serra, P. Inglese and T. La Mantia, 1997. Isozyme characterization of *Opuntia* species and varieties from Italian germplasm. In: Inglese, P. and M.O. Brutsch (Eds.), Proceeding of the Third International Congress on Cactus Pear and Cochineal. Acta Horticulturae 438: 45-52.

Comai, L., K. Young, B.J. Till, S.H. Reynolds, E.A. Greene, C.A. Codomo, L.C. Enns, J.E. Johnson, C. Burtner, A.R. Odden and S. Henikoff, 2004. Efficient discovery of DNA polymorphisms in natural populations by EcoTILLING. The Plant Journal 37: 778-786.

De La Cruz, M., F. Rumirez and H. Hernandez, 1997. DNA isolation and amplification from cacti. Plant Molecular Biology Reporter 15: 319-325.

Dubrovsky, J.G. and G.B. North, 2002. Root structure and function. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses, pp 41-56. University of California Press, California, USA.

Durham, H.E., G.A. Moore and W. B. Sherman, 1987. Isozyme banding pattern and their usefulness as genetic markers in peach. Journal of the American Society for Horticultural Science 112: 1013-1018.

Dweikat, I.H., H. Ohm, S. MacKenzie, F. Patterson, S. Cambrom and R. Ratcliffe, 1994. Association of a DNA marker with the Hessian fly resistance gene *H9* in wheat. Theoretical and Applied Genetics 89: 964-968.

FAO, 1996. Report on the State of the world's Plant Genetic Resources for Food and Agriculture. Prepared for the International Conference on Plant Genetic resources. Liepzig, Germany.

Felker, P., 1995. Forage and fodder production and utilization. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 144-154. FAO Plant production and protection paper 132. Rome, Italy.

Felker, P. and P. Inglese, 2003. Short-term and long-term research needs for *Opuntia ficus-indica* (L.) Mill. Utilization in arid areas. Journal of the Professional Association for Cactus Development 5: 131-152.

Felker, P., A. Paterson and M. Jenderek, 2006. Forage potential of *Opuntia* clones maintained by the USDA, National Plant Germplasm System (NPGS) Collection. Crop Science 46: 2161-2168.

Felker, P., S. del C. Rodriguez, R.M. Casoliba, R. Filippini, D. Medina and R. Zapata, 2005. Comparison of *Opuntia ficus indica* varieties of Mexican and Argentine origin for fruit yield and quality in Argentina. Journal of Arid Environments 60: 405-422.

Feltus, F.A., H.P. Singh, H.C. Lohithaswa, S.R. Schulze, T.D. Silva and A.H. Paterson, 2006. A comparative genomics strategy for targeted discovery of single-nucleotide polymorphisms and conserved-noncoding sequences in orphan crops. Plant Physiology 140: 1183-1191.

Frary, A., T.C. Nesbitt, S. Grandillo, E. Knaap, B. Cong, J. Liu, J. Meller, R. Elber, K.B. Alpert and S.D. Tanksley, 2000. *fw*2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science 289: 85-88.

Geuna, F., M. Toschi and D. Bassi, 2003. The use of AFLP markers for cultivar identification in apricot. Plant Breeding 122: 526-531.

Gibson A.C. and P.S. Nobel, 1986. The Cactus Primer. Harvard University Press, Cambridge, Massachusetts.

Granata, G., 1995. Biotic and Abiotic diseases. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 109-119. FAO Plant production and protection paper 132. Rome, Italy.

Grant V., A.K. Grant and D.P. Hurd, 1979. Pollination of *Opuntia lindheimeri* and related species. Plant Systematics and Evolution 132: 313-320.

Gregory, R.A. and P. Felker, 1992. Crude protein and phosphorus contents of eight contrasting *Opuntia* forage clones. Journal of Arid Environments 22: 323-331.

Griffith, M.P., 2004. The origins of an important cactus group, *Opuntia ficus-indica* (Cactaceae): New molecular evidence. American Journal of Botany 91: 1915–1921.

Griffith, M.P. and J.M. Porter, 2003. Back to the Basics: a simple method of DNA extraction for mucilaginous cacti. BRADLEYA 21: 126-128.

Guevara, J.C., J.H. Silva Colomer and O.R. Estevez, 2004. Nutrient content of *Opuntia* forage clones in the Mendoza Plain, Argentina. Journal of the Professional Association for Cactus Development 6: 62-77.

Guevara, J.C., S.O. Trione, O.R. Estevez and M. A. Cony, 2006. A sampling procedure to determine the nitrogen content in *Opuntia ficus-indica* cladodes. Journal of the Professional Association for Cactus Development 8: 63-72.

Gugliuzza, G., T. La Mantia and P. Inglese, 2002. Fruit load and cladode nutrient concentrations in cactus pear. Acta Horticulturae 581: 221-224.

Gupta, P.K. and S. Rustgi, 2004. Molecular markers from the transcribed/expressed region of the genome in higher plants. Functional and Integrative Genomics 4: 139-162.

Gupta, P.K., R.K. Varshney, P.C. Sharma and B. Ramesh, 1999. Molecular markers and their applications in wheat breeding. Plant Breeding 118: 369-390.

Gutiérrez-Acosta, F., R.D. Valdez-Cepeda and F. Blanco-Macías, 2002. Multivariate analysis of cactus pear (*Opuntia* spp.) fruits from a germplasm collection. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 111-118.

Hagen, L.S., P. Lambert, J.-M. Audergon and B. Khadari, 2001. Genetic relationships between apricot (*Prunus armeniaca* L.) and related species using AFLP markers. Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 205-207.

Inglese, P., 1995. Orchard planting and management. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 78-91. FAO Plant production and protection paper 132. Rome, Italy.

Inglese P., F. Basile and M. Schirra, 2002. Cactus pear fruit production. In: Nobel, P. (Ed.), Cacti: Biology and Uses, pp 163-183. University of California Press, California, USA.

IPGRI, 1996. Measuring Genetic Variation 6, version 1.2.

Jaccoud, D., K. Peng, D. Feinstein and A. Killian, 2001. Diversity arrays: A solid state technolgy for sequence information independent genotyping. Nucleic Acids Research 29: (4) e25.

Janick, J. and J.N. Moore, 1996. Fruit breeding, volume 1: Tree and tropical fruits, Wiley, New York.

Janisiewicz, W.J. and A. Marchi, 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae*. Plant Disease 78: 466-470.

Janisiewicz, W.J., D.L. Peterson and B. Bors, 1994. Control of storage decay of apples with *Sporobolomyces roseus*. Plant Disease 78: 466- 470.

Kader, A.A., 2002. Fruits in the global market. In: Knee, M. (Ed.), Fruit quality and its biological basis, pp 1-16. Sheffield Academic press, Sheffield, UK.

Kemp, H. and M.C. A. van Dieren, 2000. Screening of pear cultivars for resistance to fungal diseases (*Venturia pirina*, *Nectria galligena*). In: Geibel, M., M., Fischer and C. Fischer (Eds.), Proceedings of EUCARPA Symposium on Fruit Breeding and Genetics. Acta Horticulturae 538: 95-101.

Kim, Y.H. and K-H. Kim, 2002. Abscission layer formation as a resistance response of Peruvian apple cactus against *Glomerella cingulata*. Phytopathology 92: 964-969.

Kirby, M.J., C.M. Cuise and A.N. Adams, 2001. Comparison of bioassays and laboratory assays for apple stem grooving virus. In: Clark, M.F. (Ed.), Proceedings of the 18th International Symposium on Fruit Tree Virus Diseases. Acta Horticulturae 550: 281-284.

Klug, W.S. and M.R. Cummings, 2000. Concepts of genetics, 6th Edition pp 713-733, Prentice Hall International, New jersey, USA.

Koltunow, M.A., 1993. Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. The Plant Cell 5: 1425-1437.

Kueneman, E., 2001. Foreword. Cactus (*Opuntia* spp.) as forage. In: Mondragón-Jacobo, C. and G. Pérez-Gonzalez (Eds.), CACTUSNET, FAO, Rome.

Kumar, L.S., 1999. DNA markers in plant improvement: An overview. Biotechnology Advances 17: 143-182.

Labra, M., F. Grassi, M. Bardini, S. Imazio, A. Guiggi, S. Citterio, E. Banfi and S. Sgorbati, 2003. Genetic relationships in *Opuntia* Mill. genus (Cactaceae) detected by molecular marker. Plant Science 165: 1129-1136.

Le Houérou, H.N., 2002. Cacti (*Opuntia* spp.) as a fodder crop for marginal lands in the Mediterranean basin. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 21-46.

Leverentz, B., W.J. Janisiewicz, W.S. Conway, R.A. Saftner, Y. Fuch, C.E. Sams and M.J. Camp, 2000. Combining yeasts or a bacterial biocontrol agent and heat treatment to reduce postharvest decay of 'Gala" apples. Postharvest Biology and Technology 21: 87-94.

Lipshutz, R.J., S.P.A. Fodor, T.R. Gingeras and D.J. Lockhart, 1999. High density synthetic oligonucleotide arrays. Nature Genetics Supplement 21: 20-24.

Lübberstedt, T., I Zein, J.R. Andersen and G. Wenzel, 2005. Development and application of functional markers in maize. Euphytica 146: 101-108.

Mengjun, L. and J. Zhao, 2003. RAPD analysis on the cultivars, strains and related species of Chinese Jujube. In: Janick, J. (Ed.), Proceedings of the Twenty Sixth International Horticultural Congress: Genetics and breeding of tree fruits and nuts. Acta Horticulturae 622: 477-484.

Mondragón-Jacobo, C., 2001a. Verification of the apomictic origin of cactus per (*Opuntia* spp.) seedlings of open pollinated and crosses from Central Mexico. Journal of the Professional Association for Cactus Development 4: 49-56.

Mondragón-Jacobo, C., 2001b. Molecular characterisation using RAPDs of a cactus (*Opuntia* spp. Cactaceae) collection from Central Mexico as a basis for plant breeding. Revista Chapingo Serie Horticultura 9: 107-114.

Mondragón-Jacobo, C., 2002. Preliminary identification of apomictic seedlings in *Opuntia* spp. breeding populations from Central Mexico. In: Nefzaoui, A. and P. Inglese

(Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 119-124.

Mondragón-Jacobo, C. and E.B. Pimienta, 1995. Propagation. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 120-130. FAO Plant production and protection paper 132. Rome, Italy.

Mondragón-Jacobo, C. and G.S. Perez, 1995. 'Reyna' (Syn. Alfajayucan) is the leading Cactus pear cultivar in central Mexico. Fruit Varieties Journal 48: 134-136.

Mondragón-Jacobo, J. C. and B.B. Bordelon, 1996. Cactus Pear (*Opuntia* spp. Cactaceae): Breeding for fruit production. Journal of the Professional Association for Cactus Development 1: 19-35.

Nefzaoui, A. and H. Ben Salem, 2001. *Opuntia*: A strategic fodder and efficient tool to combat desertification in the WANA region. In: Mondragón-Jacobo, C. and G. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage, pp 73-89. FAO, Rome, Italy.

Nefzaoui A. and H. Ben Salem, 2002. Cacti: efficient tool for rangeland rehabilitation, drought mitigation and to combat desertification. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 295-315.

Nerd, A. and Mizrahi, 1995. Reproductive biology. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 49-57. FAO Plant production and protection paper 132. Rome, Italy.

Nesbitt, T.C. and S.D. Tanksley, 2001. *fw*2.2 directly affects the size of the developing fruit, with secondary effects on fruit number and photosynthate distribution. Plant Physiology 127: 575-583.

Nieddu, G., I. Chessa, D. Satta, L. De Pau and M. Pala, 2002. Description of six cactus pear (*Opuntia ficus-indica* Mill.) fruit cultivars from Italy. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 125-29.

Nobel, P.S., 1995. Environmental biology. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 36-48. FAO Plant production and protection paper 132. Rome, Italy.

Padulosi, S., 1998. Criteria for priority setting in initiatives dealing with underutilized crops in Europe. Paper presented at the European Symposium on Plant Genetic Resources for Food and Agriculture, Braunschweig, Germany, 29 June-5 July 1998.

Penner, G.A. and L.J. Bezte, 1994. Increased detection of polymorphism among randomly amplified wheat DNA fragments using a modified temperature sweep gel electrophoresis (TSGE) technique. Nucleic Acids Research 9: 543-547.

Petersson, S., N. Jonsson and J. Schnürer, 1999. *Pichia anomala* as a biocontrol agent during storage of high-moisture feed grain under airtight conditions. Post-harvest Biology and Technology 15: 175-184.

Pimienta-Barrios E. and A. Muñoz-Urias, 1995. Domestication of Opuntias and cultivated varieties. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 58-63. FAO Plant production and protection paper 132. Rome, Italy.

Pinkava, D.J., B.B. Purfitt, M.A. Baker and R.B. Worthington, 1992. Chromosome numbers in some cacti of Western North America VI with nomenclatural changes. Madroño 39: 93-113.

Potgieter, J. P. and J.J. Mkhari, 2002. Evaluation of cactus pear (*Opuntia* spp.) germplasm for fruit production purposes. Combined Congress of the South African Society for Crop Production and the South African Society for Horticultural Sciences, 15-17 January, Pietermaritzburg, Kwazulu/Natal.

Potgieter, J.P. and K. Carstens, 1996. The cactus pear (*Opuntia* spp.): a matter of identity. Poster presented at the Fourth International Cactus Pear Congress, 30 January – 1 February 1996, Midrand, South Africa.

Punja, Z.K., 1997. Comparative efficacy of bacteria, fungi, and yeasts as biological control agents for diseases of vegetable crops. Canadian Journal of Plant Pathology 19: 315-323.

Rafalski, A., 2002. Applications of single nucleotide polymorphism in crop genetics. Current Opinion in Plant Biology 5: 94-100.

Rebman, J.P. and D.J. Pinkava, 2001. (*Opuntia*): Cacti of North America - An overview. Florida Entomologist 84: 474-483.

Roberts, R.G., 1990. Postharvest biological control of gray mold apple by *Cryptococcus laurentii*. Phytopathology 80: 526- 530.

Rodriguez-Felix, A., 2002. Post-harvest physiology and technology of cactus pear fruits and cactus leaves. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 191-199.

Rudd, S., H. Schoof and K. Mayer, 2005. Plant markers: a database of predicted molecular from plants. Nucleic Acids Research 33: 628-632.

Salgado, T.T. and J.D. Mauseth, 2002. Shoot anatomy and morphology. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses, pp 23-40. University of California Press, California, USA.

Scheinvar L., 1995. Taxonomy of utilized Opuntias. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 20-27. FAO Plant production and protection paper 132. Rome, Italy.

Schirra, M., G. D'hallewin, P. Inglese and T. La Mantia, 1999. Epicuticular changes and storage potential of cactus pear (*Opuntia ficus-indica* Miller (L.)) fruit following gibberellic acid preharvest sprays and postharvest heat treatment. Postharvest Biology and Technology 17: 79-88.

Scorza, R., L.G. May, B. Purnell and B. Upchurch, 1991. Differences in number and area of mesocarp cell between small- and large-fruited peach cultivars. Journal of the American Society for Horticultural Science 116: 861-864.

Sharifani, M.M. and J.F. Jackson, 2000. Characterization of pear species and cultivars using RAPD primers. In: Geibel, M., M. Fischer and C. Fischer (Eds.), Proceedings of the EUCARPIA Symposium on Fruit Breeding and Genetics. Acta Horticulturae 538: 499-504.

Sommer, N.F., 1985. Role of controlled environments in suppression of postharvest diseases. Canadian Journal of Plant Pathology 7: 331-336.

Sugar, D., 1999. Control of postharvest decay in pear by four laboratory-grown yeasts and two-registered biocontrol products. The American Phytopathological Society 83: 155-157.

Swart, W.J. and Swart, V., 2003. An overview of research on diseases of cactus pear in South Africa. Journal Professional Association for Cactus Development 6: 115-120.

Swart, W.J., M.R. Oelofse and M.T. Labuschagne, 2003. Susceptibility of South African cactus pear varieties to four fungi commonly associated with disease symptoms. Journal of the Professional Association for Cactus Development 5: 86-97.

Tanksley, S.D. and J.C. Nelson, 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theoretical and Applied Genetics 92: 191-203.

Tel-Zur, N., S. Abbo, D. Myslabodski and Y. Mizrahi, 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Cactaceae) Plant Molecular Biology Reporter 17: 249-254.

Tian, S., Q. Fan, Y. Xu and H. Liu, 2002. Biocontrol efficacy of anatagonist yeasts to gray mold and blue mold on Apples and Pears in controlled atmospheres. Plant Disease 86: 848-853.

Urquhart, E.J. and Z.K. Punja, 1997. Epiphytic growth and survival of *Tilletiopsis pallescens*, a potential biological control agent of *Sphaerotheca fuliginea*, on cucumber leaves. Canadian Journal of Botany 75: 892-901.

Uzun, I., 1997. Fruit and cladode isozymes in cactus pear. In: Inglese, P. and M.O. Brutsch (Eds.), Proceedings of the Third International Congress on Cactus Pear and Cochineal. Acta Horticulturae 438: 53-56.

Varshney, R., A. Graner and M. E. Sorrells, 2005b. Genomics-assisted breeding for crop improvement. Trends in Plant Science 10: 621-630.

Varshney, R.K., A. Graner and M.E. Sorrells, 2005a. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology 23: 48-55.

Vos P., R. Hogar, M. Bleeker, M. Reijans, T. van De Lee, M. Hornes, A. Freiters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Wallace, R.S. and A.C. Gibson, 2002. Evolution and sytematics. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses, pp 1-21. University of California Press, California, USA.

Wang, C.Y., 1994. Chilling injury of tropical horticultural commodities. Horticultural Science 29: 986-988.

Wang, D., J. Fan, C. Siao, A. Berno, P. Young, R. Sapolsky, G. Ghandour, N. Perkins, E. Winchester, J. Spenser, L. Kruglyak, L. Stein, L. Hsie, T. Topaloglou, E. Hubbell, E. Robinson, M. Mittmann, M.S. Morris, N. Naiping Shen, D. Kilburn, J. Rioux, C. Nusbaum, S. Rozen, J. Hudson, R. Lipshutz, M. Chee and S. Lander, 1998a. Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science 280: 1077-1082.

Wang, X., P. Felker, M.D. Burrow and A. Paterson, 1998b. Comparison of RAPD marker patterns to morphological and physiological data in the classification of Opuntia accessions. Journal Professional Association for Cactus Development 3: 98-109.

Wang, X., P. Felker, M.D. Burrow and A. H. Paterson, 1999. Assessment of genetic diversity among *Opuntia* genotypes, reflected by DNA markers. Journal of the Professional Association for Cactus Development 3: 3-14.

Weeden, N.F. and R.C. Lamb, 1985. Identification of apple cultivars by isozyme phenotypes. Journal of the American Society of Horticultural Science 110: 509-515.

Weiss, J., A. Nerd and Y. Mizrahi, 1993. Development of *Cereus peruvianus* (Apple cactus) as a new crop for the Negev Desert of Israel, pp 486-471. In: Janick, J. and J.E. Simon (Ed.), New crops. Wiley, New York

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Wilson, C.L. and M.E. Wisniewski, 1989. Biological control of post-harvest diseases of fruits and vegetables: An emerging technology. Annual Review of Phytopathology 27: 425-441.

Yu, T., P.G. Wu, J.J. Qi, X.D. Zheng, F. Jiang and X. Zhan, 2006. Improved control of postharvest blue mold rot in pear fruit by a combination of *Cryptococcus laurentii* and gibberellic acids. Biological Control 39: 128-134.

Yuasa, H.H., S. Shimizus, S. Kashiwai and N. Kondo, 1974. Chromosome numbers and their bearing on the geographic distribution in the Subfamily *Opuntioideae* (Cactaceae) Report. The Institute for Breeding Research, Tokyo University of Agriculture 4: 1-10.

Zhang, C., K. Tanabe, S. Wang, F. Tamura, A. Yoshida and K. Matsumoto, 2006. The impact of cell division and cell enlargement on the evolution of fruit size in *Pyrus pyrifolia*. Annals of Botany 98: 537-543.

Zhebentyayeva, T.N. and Y.M. Sivolap, 2000. Genetic diversity of apricot determined by isozyme and RAPD analyses. In: Geibel, M., M. Fischer and C. Fischer (Eds.), Proceedings of the EUCARPIA Symposium on Fruit Breeding and Genetics. Acta Horticulturae 538: 525-530.

Chapter 2

Genotyping South African cactus pear (*Opuntia* spp.) varieties using AFLP markers

ABSTRACT

Cactus pear (*O. ficus-indica*) is increasingly being utilised in South Africa for fodder and fruit production. However, breeding efforts to increase productivity and fruit quality are hampered by the difficulty of varietal identification. AFLP markers were used to estimate the genetic diversity within the South African cactus pear germplasm. Estimates of genetic diversity are useful in plant breeding for organising germplasm, identification of varieties, and assisting in the selection of parents for crossings. Nine primer combinations used during AFLP analysis generated 346 fragments (per sample), of which 168 were polymorphic. A large number of the markers produced had a polymorphic information content (PIC) value between 0.3-0.5, indicative of good discriminatory value. The majority of the accessions grouped into four clusters using both the Jaccard and Simple Matching similarity coefficients. Cultivated varieties were evenly dispersed within the different clusters, with the greatest percentage clustered in group III. Varieties that originated from Botswana (R1251, R1259, and R1260) clustered together, whilst those from Israel (Sharsheret, Ofer, and Messina) were dispersed amongst groups II and III. Genotype specific fragments (GSF) were generated with the use of six primer combinations (E-A**GG** + M-C**AT,** E-A**CT** + M-C**AG,** E-A**CT** + M-C**AT,** E-A**CA** + M-C**AT,** E-A**CA** + M-C**TT**, and E-A**CA** + M-C**AG**). Genotype specific fragments allowed the unique identification of nine varieties, three of which are commercially cultivated (Meyers, Roedtan, and Santa Rosa).

2.1 INTRODUCTION

Characterisation of *Opuntia* is complicated by G x E interaction, polyploidy, the presence of vegetative and sexual reproduction, and the occurrence of many hybrids between species (Scheinvar, 1995). This complexity obstructs breeding efforts aimed at increasing productivity and fruit quality, since they require accurate and consistent cultivar identification. The difficulty experienced in identifying different *Opuntia* spp. accessions has hindered breeding and germplasm evaluation (Chessa and Nieddu, 1997).

Consequently, as with many other crops (Pejic *et al.,* 1998; Prevost and Wilkinson, 1999; Smith *et al.,* 2000; Grzebelus *et al.,* 2001; Singh *et al*., 2002; Yue *et al.,* 2002; Ferriol *et al*., 2003; Vijayan *et al.,* 2004; Zacarias *et al.,* 2004; Mba and Tohme, 2005; Wang *et al.,* 2005; Zoghlami *et al*., 2007), fingerprinting data based on molecular markers is being explored to either complement or replace morphological characters in assessing genetic diversity. Molecular markers have, for example, been investigated for varietal identification of sugar beet (De Riek *et al.,* 2001), peach (Aranzana *et al*., 2001), strawberry (Arnau *et al*., 2001), and grapevine (Regner *et al.,* 2001) varieties.

The AFLP technique is one of many molecular marker techniques being used to characterise germplasm (Ude *et al*., 2003; Nguyen *et al*., 2004; Genet *et al*., 2005). It involves the digestion of genomic DNA with two restriction enzymes, a frequent cutter such as *Mse*I, and a rare cutter such as *Eco*RI (Vos *et al*., 1995). Digestion is followed by ligation of double stranded adaptors consisting of a core sequence, and the restriction enzyme-specific sequence. A two-step procedure is subsequently used to reduce the number of fragments. The first, a PCR reaction known as pre-selective amplification, employs a primer that incorporates the adaptor sequence, the enzyme specific sequence, and an additional pre-selective single base at the 3' end for amplification, resulting in a 16-fold decrease in the number of fragments generated. The second reduction step, selective PCR amplification, uses a primer identical in sequence to the pre-selective primer with two additional nucleotide sequences at the 3' end. Amplicons are electrophoretically separated on a denaturing polyacrylamide gel, and visualised using either radio-activity or with silver staining (Vos *et al*., 1995). More recently, high-throughput genotyping has been facilitated by the use of automated sequencers and dye-labelled PCR-primers (Applied Biosystems, 2000). Scoring digital AFLP gel images, using the specifically developed software AFLP-Quantar, is now possible (Keygene, 2000).

AFLP is a highly sensitive technique that can detect polymorphisms in an entire genome, allowing the variability of unknown DNA fragments to be assayed. It has therefore been used to assess the genetic diversity in, for example, rapeseed (Lombard *et al.*, 2000), globe artichoke (Lanteri *et al.,* 2004), African daisy (Berio *et al.,* 2001), apricot (Hagen *et al.*, 2001), and the common bean (Métais *et al.*, 2001). The AFLP technique is highly sensitive, reproducible and widely applicable. Its limitations, however, are that it is a dominant marker technique, not able to discriminate between homozygous and heterozygous individuals. It is also relatively expensive and technically demanding (IPGRI, 1996).

AFLP analysis is reliable at generating hundreds of genetic markers. These markers have found the widest application in analysing genetic variation below the species level, especially in investigations into population structure and differentiation (Mueller and Wolfenbarger, 1999). Molecular markers are popularly being used for the description of genetic relationships among different germplasm in seed banks and breeding programmes, and for assessing the level of genetic diversity present in germplasm pools (Reif *et al.*, 2005).

It is thus imperative with the increasing importance and popularity of cactus pear, that its genetic diversity be evaluated to inform decision makers on crop improvement strategies, and to elucidate whether a need exists to increase South Africa's cactus pear gene pool. The AFLP technique was selected to (1) examine the level of genetic variation within the South African cactus pear germplasm, (2) determine whether AFLP markers can be used for variety identification, (3) establish genetic distances between different varieties, using two similarity coefficients, (4) compare the efficiency of different AFLP primer combinations in detecting genetic variation, and (5) compare dendrograms constructed using the Jaccard, and Simple Matching similarity coefficients.

2.2 MATERIALS AND METHODS

2.2.1 Plant material

Plant material (Table 2.1) was obtained from a field genebank near CLINVET, 20 km west of Bloemfontein in the Free State Province. This germplasm was established in 2001 by the vegetative propagation of accessions held at the Limpopo Department of Agriculture, Mara germplasm block. Thin sections of cladodes from each variety were freeze-dried (Freeze Mobile II, Virtis Inc), and stored at -20°C until further use.

2.2.2 DNA isolation

Freeze-dried material was ground to a fine powder after adding silica beads. Genomic DNA was isolated using the CTAB (hexadecyltrimethylammonium bromide) method (Saghai-Maroof *et al*., 1984). Subsequently a 250 µℓ aliquot of powder was incubated in 750 µℓ CTAB buffer, pH 8.0 [100 mM Tris-HCl [tris (hydroxymethyl) aminomethane], 1.4 M NaCl, 20 mM EDTA (ethylene-diaminetetraacetate), 2% (w/v) CTAB, and 0.2% (v/v) β-mercapthoethanol)] at 65°C for one hour. A 500 µℓ aliquot of chloroform-isoamylalcohol [24:1 (v/v)] was added prior to phase separation by centrifugation at 12 000 g for three minutes. DNA was precipitated from the aqueous phase at room temperature for 20 minutes by addition of 500 µℓ isopropanol. The pellet was collected by centrifugation at 12 000 g for five minutes, and washed with 500 µℓ ice-cold 70% (v/v) ethanol for 20 minutes at room temperature. After centrifugation at 12 000 g for five minutes, the ethanol was discarded and the pellet was air-dried at room temperature for one hour and re-suspended in TE buffer pH 8.0 (10 mM Tris-HCl, 1 mM EDTA). DNA was treated with 0.4 mg/mℓ DNase-free RNase for two hours at 37°C. DNA was further treated with 0.75 M ammonium acetate and an equal volume of chloroform-isoamylalcohol [24:1 (v/v)]. DNA was collected from the upper phase after centrifugation at 12 000 g for three minutes, and precipitated overnight at -20°C from the aqueous phase with two volumes of ice-cold absolute ethanol.

TABLE 2.1 CACTUS PEAR VARIETIES USED IN THIS STUDY

Variety number	Variety name	Commercially cultivated varieties	Country of origin
1	Direkteur	X	South Africa
2	Skinners Court	X	South Africa
3	Fusicaulis	X	South Africa
4	Nudosa	X	South Africa
5	Gymno Carpo	X	South Africa
6	American Giant	X	South Africa
7	Blue motto	X	South Africa
8	Morado	X	South Africa
10	Zastron	X	South Africa
11	Malta	X	South Africa
12	Algerian	X	South Africa
13	Turpin		South Africa
14	Roly Poly		South Africa
15	Meyers	X	South Africa
16	Roedtan	X	South Africa
17	Arbiter		South Africa
18	Ofer		Israel
20	Messina		Israel
21	Fresno		South Africa
22	Muscatel		South Africa
23	Tormentosa		South Africa
24	X 28 (Robusta x Castillo)		South Africa
25	Corfu		South Africa
26	Ficus-Indice		South Africa
27	Vryheid		South Africa
28	Mexican	X	South Africa
29	Nepgen		South Africa
30	Amersfoort		South Africa
31	Silician Indian Fig		South Africa
32	R1260		Botswana
33	R1259		Botswana
34	R1251		Botswana
35	Sharsheret		Israel
36	Rossa		Italy
37	Unknown		South Africa
38	Van As	X	South Africa
39	Cross X		South Africa
40	Berg x Mexican		South Africa
41	Santa Rosa	X	South Africa
42	Schagen		South Africa

A list of the different *Opuntia* spp. varieties used in this study, with the accompanying variety numbers. Varieties depicted in the blue font were reported by Brutsch (1979) as being of good potential for commercial fruit production

Following overnight incubation, DNA was collected by centrifugation at 12 000 g for 15 minutes, and washed twice with ice-cold 70% (v/v) ethanol. The pellet was air-dried at room temperature, and thereafter re-suspended in 50 µℓ TE buffer pH 8.0. DNA quantity and quality were estimated by measuring absorbencies at A_{260}nm and A_{280}nm using a spectrophotometer. The quality of the extracted DNA was verified by electrophoretic separation through a 0.8% (w/v) agarose gel in 1 X UNTAN (40 mM Tris-HCl, 2 mM EDTA, pH adjusted to 7.4 with acetic acid) buffer at 60 V for 45 minutes. DNA samples were diluted to working solutions of 200 ng/µℓ, and stored at -4°C until further use.

2.2.3 AFLP analysis

AFLP analysis was performed using *Mse*I- and *Eco*RI-primer pair combinations. *Eco*RI- and *Mse*I-primers were given names beginning with E and M respectively. The code following the E or M refers to the selective nucleotides at the 3'-end of the primer. This coding system will be used throughout the thesis. Different *Mse*I- and *Eco*RI-primer combinations were screened (Table 2.2). Primers and adaptors were synthesised by Integrated DNA Technologies, Inc, USA. Adaptors were prepared by the addition of equimolar amounts of both strands, heating for 10 minutes at 65 °C in a water bath, and leaving the mixture to cool down to room temperature. AFLP analysis was performed according to Vos *et al.* (1995), with minor modifications as described by Herselman (2003).

2.2.4 Restriction endonuclease digestion and ligation

Genomic DNA (1.0 µg) was digested with 4 U *Mse*I at 37°C for five hours. Thereafter, restriction fragments were further digested with 5 U *Eco*RI, in the presence of 100 mM NaCl at 37°C overnight. Ligation to adaptors was performed overnight in the presence of 0.4 mM Adenosine triphosphate (ATP), 50 pmol *Mse*I-adaptor, 5 pmol *Eco*RI-adaptor, 1 X T4 DNA Ligase buffer [(66 mM Tris-HCl, pH 7.6, 6.6 mM $MgCl_2$, 10 mM 1,4 dithiothreitol (DTT), 66 mM ATP)] and 1 U T4 DNA ligase, at 16°C.

TABLE 2.2 NUCLEOTIDE SEQUENCES OF *Eco*RI- AND *Mse*I-ADAPTORS AND PRIMERS

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adaptor-F	CTCGTAGACTGCGTACC
	Adaptor-R	AATTGGTACGCAGTCTAC
*Mse*I	Adaptor-F	GACGATGAGTCCTGAG
	Adaptor-R	TACTCAGGACTCAT
*Eco*RI	Primer +1	GACTGCGTACCAATTCA
*Mse*I	Primer +1	GATGAGTCCTGAGTAAC
*Eco*RI	Primer +3	GACTGCGTACCAATTCA**NN** E-A**NN**: A**CA**, A**CC**, A**CT**, A**GG**, A**AG**
*Mse*I	Primer +3	GATGAGTCCTGAGTAAC**NN** M-C**NN**: C**AG**, C**TC**, C**AT**, C**TT**, C**AC**

Primer+1 used for pre-selective amplification reactions, and primer+3 used for selective amplification reactions

2.2.5 Pre-selective amplification

Pre-selective reactions were performed in a total volume of 50 µℓ by the addition of 1 X Promega polymerase buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 30 ng of each pre-selective primer [*Eco*RI- and *Mse*I-primer +1 (Table 2.2)] and 0.02 U *Taq* DNA Polymerase (Promega, Madison, WI, USA), to 5 µℓ DNA template (ligation mixture). Pre-selective amplification was performed (DNA Engine DYAD™, BIO-RAD, USA) with an initial denaturation step at 94°C, followed by 30 cycles at 94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 seconds. Final elongation was performed at 72°C for five minutes. The quality and quantity of pre-selective amplification products were determined by separation through a 1.5% (w/v) agarose gel. Appropriate dilutions (1:5, 1:10 or 1:20) thereof were made in 1 X TE buffer pH 8.0 prior to selective amplification.

2.2.6 Selective amplification

Amplification reactions were performed in 20 µℓ reaction volumes containing 1 x Promega polymerase buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 30 ng *Mse*I-primer+3, 30 ng *Eco*RI+3, 0.75 U *Taq* DNA polymerase (Promega), 5 µℓ of diluted pre-selective amplification product and 100 µg/mℓ Bovine serum albumin (BSA). The cycling conditions used for amplification were initiated with denaturation at 94℃ for five minutes, followed by 10 cycles of touchdown (1℃ per cycle) PCR at 94℃ for 30 seconds, 65℃ for 30 seconds, and 72℃ for one minute, followed by 25 cycles at 94℃ for 30 seconds, 56℃ for 30 seconds, and 72℃ for one minute, with a final extension step at 72℃ for two minutes. Primers used for selective amplification were randomly selected.

2.2.7 Polyacrylamide gel electrophoresis

Amplification products were mixed with an equal volume of formamide loading buffer [98% (v/v) de-ionised formamide, 10 mM EDTA pH 8.0, 0.05% (w/v) bromophenol blue, and 0.05% (w/v) xylene cyanol], and denatured at 95℃ for five minutes. The mixtures were immediately placed on ice. Aliquots of 5 µℓ of each sample were separated through a 5% denaturing polyacrylamide gel [19:1 acrylamide: bis-acrylamide, 7 M urea, and 1 X TBE buffer (89 mM Tris-HCl, 89 mM Boric acid, 20 mM EDTA)] at a constant power of 80 W for two hours.

2.2.8 Silver staining

AFLP gels were silver stained according to the protocol of the Silver Sequence™ DNA Sequencing System supplied by Promega (Madison, WI, USA). Gels were left to air-dry overnight, and photographed by exposing photographic paper (Kodak Polymax II) positioned under the gel, to dim light for approximately 20 seconds. This produced a negative image of the same size as the gel.

2.2.9 Statistical analysis

A binary variety x marker matrix recording AFLP fragments as present (1) or absent (0) was compiled for all primer combinations used in the study. Only reliable fragments of between 150 and 700 bp were considered.

PIC measures the informativeness of genetic markers (Botstein *et al.,* 1980). PIC for dominant markers was calculated using the equation:

$$PIC = 1 - \left[f^2 + (1 - f)^2 \right]$$ (De Riek *et al*., 2001)

In the equation above, "f" is the frequency of the marker in the data set. Only polymorphic markers were used to display PIC distribution. Allele frequency for dominant markers was estimated using the method outlined by Lynch and Milligan (1994) in the programme Tools for Population Genetic Analyses (TFPGA) (Miller, 1997). PIC-values of all polymorphic fragments for a primer pair were averaged to give the PIC-value for the primer pair.

The variety x marker binary matrix was used to estimate the genetic similarity between genotypes, using the Jaccard similarity coefficient (S_J) (Jaccard, 1908) and the Simple Matching coefficient (S_{SM}) (Sokal and Michener, 1958) (Table 2.3). The NTSYS-pc programme (Version 2.02i, Rohlf, 1998) was used to calculate similarities between pairs of individuals. The unweighted pair-group method of arithmetic averages (UPGMA) was used to construct dendrograms, depicting the relationships among accessions in the germplasm.

Correlation between cophenetic distances obtained from the dendrograms and similarities calculated, using each of the similarity coefficients, was measured, using the cophenetic correlation coefficient. The size of the cophenetic correlation coefficient should be very close to one, for high quality resolution. This measure was used to compare alternative cluster resolutions.

TABLE 2.3 SIMILARITY COEFFICIENTS FOR ALLELIC NON-INFORMATIVE MARKER DATA

Variable	Similarity coefficient	Name	Range
S_{SM}	$\frac{a+d}{a+b+c+d}$	Simple matching (Sokal and Michener, 1958)	0,1
S_J	$\frac{a}{a+b+c}$	Jaccard (1908)	0,1
S_D	$\frac{2a}{2a+b+c}$	Dice (1945)	0,1

a = number of fragments in common between two operational taxonomic units i_1 and i_2,
b = number of fragments present in i_1 and absent in i_2,
c = number of fragments present in i_2 and absent in i_1,
d =number of shared absences between two operational taxonomic units i_1 and i_2 ,
(Kosman and Leonard, 2005; Reif *et al*., 2005)

2.3 RESULTS AND DISCUSSION

It was possible to obtain total genomic DNA of good quality from freeze-dried cladode sections. However, DNA could not be extracted from Zastron (variety number 10) and Cross X (variety number 39), thus they were not analysed further. Various protocols for the extraction of genomic DNA from mucilaginous cactus tissue have been developed (De La Cruz *et al*., 1997; Arnholdt-Schmitt *et al*., 2001; Griffith and Porter, 2003) in order to circumvent problems associated with mucilage and other secondary metabolites. Mucilage is a water-soluble, pectin-like polysaccharide that forms large macromolecular aggregates in solution (Cárdenas *et al.,* 1997). The above-mentioned methods were not used, as they required the use of multiple extraction buffers (De La Cruz *et al*., 1997), and the use of an expensive commercially available extraction kit (Arnholdt-Schmitt *et al*., 2001).

The use of thin epidermal cladode sections as reported by Arnholdt-Schmitt *et al*. (2001) and Griffith and Porter (2003), increased the yield of DNA extracted. The use of comparatively larger amounts of plant material 30–50 mg (Griffith and Porter, 2003) or 3 g (De La Cruz *et al*., 1997), requires larger volumes of extraction buffer than the method described here. In this study freeze-drying thin epidermal cladode sections, with subsequent grinding after the addition of silica beads, enabled DNA extraction from

250 µg plant material in 750 µℓ CTAB buffer. This is a substantial decrease in the initial amount of plant material used for the extraction of genomic DNA from cacti. Larger quantities of plant tissue have been reported in literature. The reduction in the quantity of starting material enabled the processing of more samples in one sitting since extraction could be performed in smaller microcentrifuge tubes. In addition, freeze-drying appears to have assisted in reducing the amount of mucilage co-extracted with DNA.

The nine primer combinations used for selective amplification (Table 2.4) generated 346 fragments (per sample) in total, of which 168 (48.6%) were polymorphic between samples. Five different *Eco*RI- and five different *Mse*I-primers were used in nine combinations. The average number of polymorphic fragments generated per primer combination was 19. Figure 2.1 displays AFLP markers generated using the primer combination E-A**CA** + M-C**AT**.

Primer pairs that generated the highest percentage polymorphic fragments were E-A**GG** + M-C**AT** (75%), E-A**CA** + M-C**AG** (58%), and E-A**CT** + M-C**AT** (55%) (Table 2.4). The above mentioned primer combinations can be used in future fingerprinting studies for cactus pear. They generated the highest amounts of polymorphic markers, which is essential in the assessment of genetic diversity.

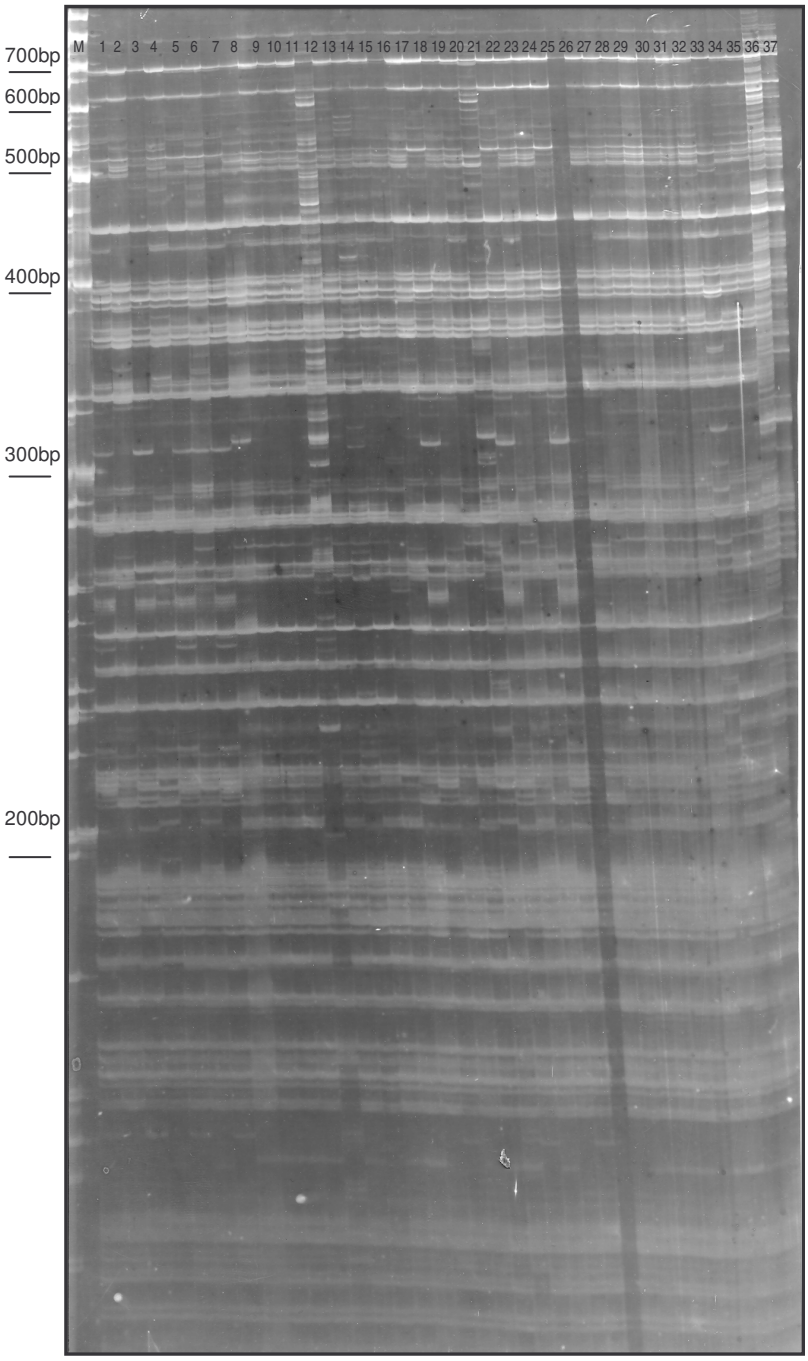
Polymorphic AFLP fragments were analysed to determine PIC (Figure 2.2). PIC measures the relative discriminatory value of a locus. It is a measure of the information content as a function of a marker system's ability to differentiate between genotypes (Weir, 1996). Monomorphic fragments have a low PIC value and thus no discriminatory power and were not included in the computation of the distribution profile.

TABLE 2.4 SUMMARY STATISTICS OF THE NINE *Eco*RI/*Mse*I-PRIMER COMBINATIONS USED FOR SELECTIVE AMPLIFICATION

Primer combination	*NPF*	*%P*	*TNF*	*GSF*	*PIC*
E-A**CA** + M-C**AG**	31	58	53	4	0.33
E-A**CA** + M-C**TC**	12	46	26	0	0.32
E-A**CA** + M-C**TT**	15	52	29	1	0.31
E-A**CA** + M-C**AT**	18	37	42	1	0.34
E-A**CT** + M-C**AT**	23	55	42	1	0.25
E-A**CT** + M-C**AG**	12	28	43	3	0.18
E-A**CC** + M-C**TC**	19	49	39	0	0.32
E-A**GG** + M-C**AT**	27	75	36	1	0.33
E-A**AG** + M-C**AT**	11	31	36	0	0.27
TOTAL	168		346	11	
Mean	19	48	38	1	0.29

NPF = number of polymorphic fragments; **%P** = percentage of polymorphic fragments; **TNF** = total number of fragments; **GSF** = genotype specific fragments; **PIC** = polymorphic information content

PIC for dominant markers has a maximum value of 0.5 for "f" = 0.5 (De Riek *et al.,* 2001). The majority of the fragments generated had a PIC value between 0.3-0.5, indicative of good discriminatory ability. Polymorphic markers with good discriminatory value occurred at a higher frequency than those with lower discriminatory value (PIC values <0.3) (Figure 2.2).



Lane	*Description*
M	100 bp DNA Ladder
1	DIREKTEUR
2	SKINNERS COURT
3	FUSICAULIS
4	NUDOSA
5	GYMNO CARPO
6	AMERICAN GIANT
7	BLUE MOTTO
8	MORADO
9	MALTA
10	ALGERIAN
11	TURPIN
12	ROLY POLY
13	MEYERS
14	ROEDTAN
15	ARBITER
16	OFER
17	MESSINA
18	FRESNO
19	MUSCATEL
20	TORMENTOSA
21	X 28
22	CORFU
23	FICUS-INDICE
24	VRYHEID
25	MEXICAN
26	NEPGEN
27	AMERSFOORT
28	SICILIAN INDIAN FIG
29	R 1260
30	R 1259
31	R 1251
32	SHARSHERET
33	ROSSA
34	UNKNOWN
35	VAN AS
36	BERG x MEXICAN
37	SANTA ROSA
38	SCHAGEN

FIGURE 2.1 PHOTOGRAPH OF A SILVER STAINED 5% DENATURING POLYACRYLAMIDE GEL AFLP fragments were amplified using the primer combination E-ACA + M-CAT

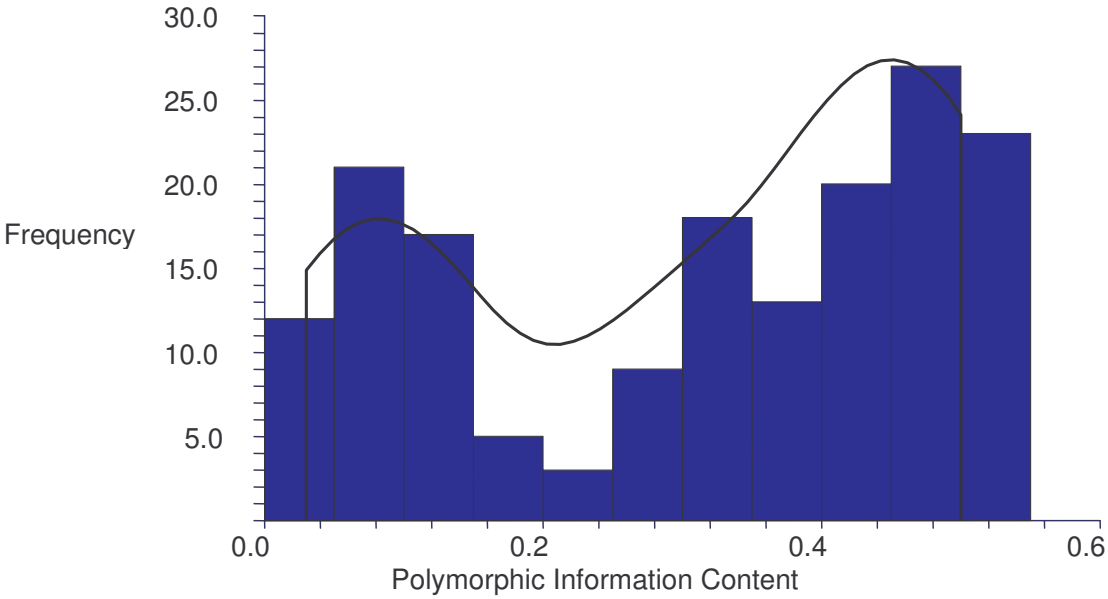


FIGURE 2.2 DISTRIBUTION OF THE POLYMORPHIC INFORMATION CONTENT OF POLYMORPHIC AFLP FRAGMENTS

The average PIC for each primer combination was computed from the PIC values generated for every polymorphic marker (Table 2.4). Six of the nine primer combinations used displayed PIC values greater than 0.3. Primer combinations that gave the highest PIC were E-A**CA** + M-C**AT** (0.34), E-A**GG** + M-C**AT** (0.33), and E-A**CA** + M-C**AG** (0.33) (Table 2.4). The average PIC (0.29) for all primer pairs used, compared well with those previously reported (Rana and Bhat, 2004).

Eleven GSF (Table 2.5) enabled the differentiation of nine varieties. Primer pairs that gave the highest GSF values were E-A**CA** + M-C**AG** (4) and E-A**CT** + M-C**AG** (3). Primer pair E-A**CT** + M-C**AG** amplified three GSF, had the lowest percentage polymorphic fragments (28), and the lowest PIC (18).

TABLE 2.5 UNIQUELY IDENTIFIED CACTUS PEAR VARIETIES

Primer combination	GSF	Variety	NUF
E-A**CA** + M-C**AG**	4	Roedtan	2
		Meyers	1
		Corfu	1
E-A**CA** + M-C**TT**	1	Roedtan	1
E-A**CA** + M-C**AT**	1	Unknown	1
E-A**CT** + M-C**AT**	1	Berg x Mexican	1
E-A**CT** + M-C**AG**	3	X 28 (Robusta x Castillo)	1
		Santa Rosa	1
		Roly Poly	1
E-A**GG** + M-C**AT**	1	Nepgen	1
TOTAL	11		11

GSF= genotype specific fragments; **NUF** = number of unique fragments

E-A**CA** + M-C**AG**, and E-A**CA** + M-C**TT** generated three specific markers in total that can be used to identify Roedtan. E-A**CT** + M-C**AG**, and E-A**CA** + M-C**AG** enabled the unique identification of five cactus pear varieties (Roedtan, Corfu, Meyers, Santa Rosa, and Roly Poly), three of which (Meyers, Roedtan, and Santa Rosa) are commercially cultivated (Tables 2.4 and 2.5). With further research, these fragments can be converted to STS markers that can be used for variety identification or to detect the presence of agronomically important traits. STS markers developed from AFLP-markers have been used by Seo *et al.* (2001) for the identification of wheat lines carrying the 2*RL* resistance gene, and for genotype identification during marker-assisted breeding for resistance to cyst nematode in soybean (Meksem *et al.*, 2001).

Commentary on the subjective choice of similarity coefficients for use as measures of genetic distance between genotypes based on molecular data is well documented in literature (Jackson *et al.*, 1989; Duarte *et al.*, 1999; Da Silva Meyer *et al.*, 2004; Kosman and Leonard, 2005). Although the coefficients under discussion are mathematically different (Table 2.3) and may give different quantitative and qualitative results of the relationship between individuals (Jackson *et al.*, 1989; Duarte *et al.*, 1999), most researchers do not offer any reasons to support their choice of coefficient (Da Silva Meyer *et al.*, 2004) in relation to the type of markers evaluated, ploidy and mating system of the organism being studied (Kosman and Leonard, 2005).

In addition, the same coefficients have been used for both dominant (RAPD and AFLP) and co-dominant (allozymes, RFLP and SSR) markers without regard for whether the species being studied are haploid, diploid or polyploid, or the degree of genetic recombination or heterozygosity expected from its mating system (Kosman and Leonard, 2005).

In response, a number of comparative studies where two or more similarity coefficients were used for data analysis (Duarte *et al.*, 1999; Da Silva Meyer *et al.*, 2004) have been published. In this study two different similarity coefficients, the Jaccard and Simple Matching coefficients, were used to estimate the genetic diversity of varieties within the cactus pear germplasm. Coefficient measures of similarity are commonly used to analyse similarity between individuals when knowledge of ancestry of all individuals in the population is not known (Kosman and Leonard, 2005). This was the case in this study as a large number of the varieties are of unknown pedigree (Table 2.1). Berg x Mexican and X 28 (Robusta x Castillo) are the only varieties of known pedigree, thus necessitating the use of similarity coefficients to estimate genetic diversity.

AFLP markers are dominant and therefore do not allow the exact determination of the genetic similarity between individuals that share a fragment at the same position (Kosman and Leonard, 2005). When using dominant markers to assess genetic diversity in diploid or polyploid individuals, one cannot distinguish fragments that represent two alleles at a homozygous locus from fragments that represent only one allele. The Jaccard coefficient was therefore used to measure genetic similarity instead of the commonly used Dice similarity coefficient (Table 2.3) which attaches more weight to shared fragments.

The Simple Matching coefficient was chosen to compare with the genetic distances generated using the Jaccard similarity coefficient. The Simple Matching coefficient computes genetic similarities by the inclusion of shared fragment absences (Table 2.3). The inclusion of joint absences has been demonstrated to give equal importance to species (fragment) presence and absence, thus giving equal importance to rare and ubiquitous species (fragments) in cluster formation (Jackson *et al.*, 1989).

All 38 varieties in this study could be separated based on AFLP fingerprints, using both similarity measures. Previously, researchers have shown that AFLP fingerprinting can be used to distinguish between *Opuntia* species (Labra *et al.*, 2003, Nilsen *et al.*, 2005). However, in contrast to our findings, Nilsen and co-workers reported that AFLP

fingerprinting failed to distinguish traditionally classified forms of *O. pilifera* F.A.C. Weber. (Nilsen *et al.,* 2005).

Previously, isozyme studies employing 13 enzyme systems on root, cladode, petal, and pollen material, failed to identify individual genotypes (Chessa *et al*., 1997). In addition, Uzun (1997) detected no differences between varieties, for the same enzyme system in the same plant organ. Using RAPD-markers, *Opuntia* spp. accessions were separated into fruit and ornamental types, but very few differences amongst fruit clones were reported (Wang *et al*., 1998). RAPD markers have also been used to investigate the genetic diversity in the Tunisian cactus pear germplasm collection (Zoghlami *et al*., 2007).

In this study, the different accessions grouped into four main clusters (Figures 2.3 and 2.4), using both similarity coefficients. In addition, varieties grouped into the same clusters using both coefficients, with the exception of Roly Poly and Schagen (Figures 2.3 and 2.4). Using the Jaccard similarity coefficient, Roly Poly clustered with Santa Rosa in cluster IV. In contrast, Roly Poly remained ungrouped, using the Simple Matching coefficient, and Santa Rosa grouped into cluster IV with Schagen.

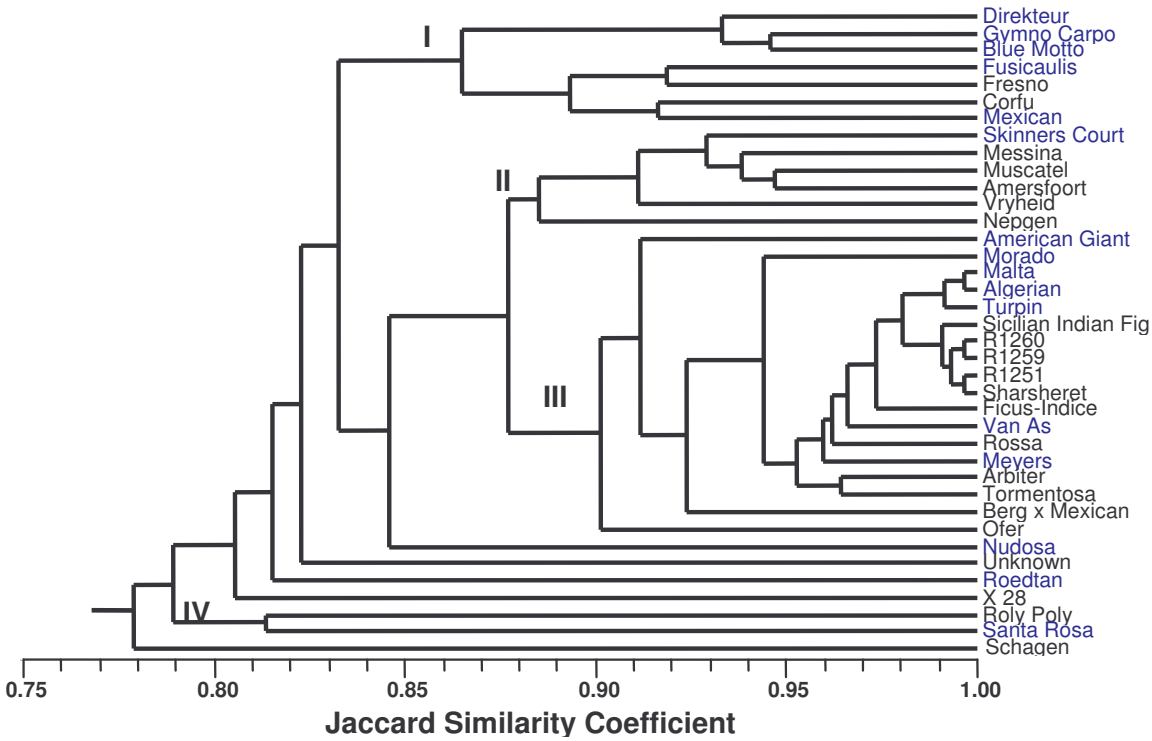


FIGURE 2.3 DENDROGRAM OF 38 SOUTH AFRICAN CACTUS PEAR VARIETIES BASED ON CLUSTER ANALYSIS (UPGMA) OF GENETIC SIMILARITY ESTIMATES USING THE JACCARD SIMILARITY COEFFICIENT

Varieties in blue are those cultivated for fruit in South Africa (Brutsch, 1979)

Cultivated varieties were dispersed amongst the different clusters, of which the highest percentage clustered in group III (Figures 2.3 and 2.4). This finding is important for both cactus pear breeders and farmers, in that it indicated that commercially cultivated varieties represent the genetic diversity present within the germplasm. Therefore, the risk of genetic homogeneity within commercially cultivated varieties in this germplasm is low.

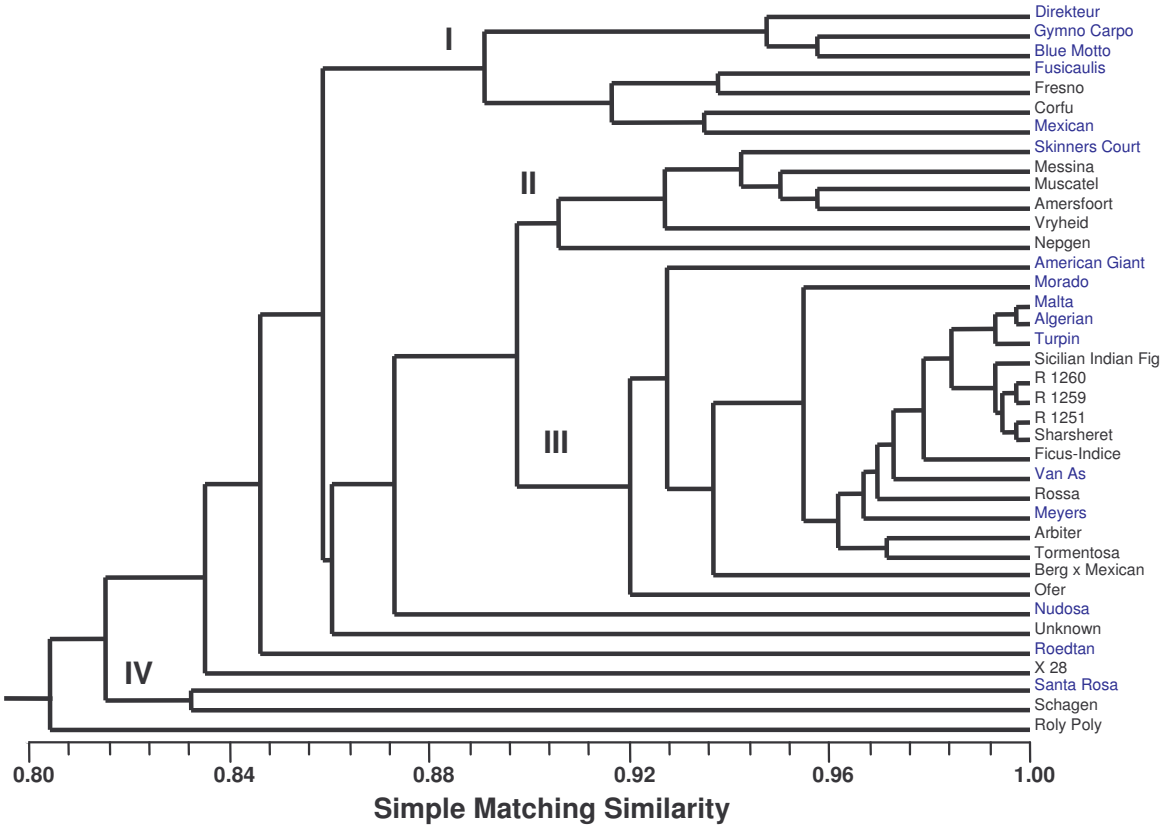


FIGURE 2.4 DENDROGRAM FOR 38 SOUTH AFRICAN CACTUS PEAR VARIETIES BASED ON CLUSTER ANALYSIS (UPGMA) OF GENETIC SIMILARITY ESTIMATES USING THE SIMPLE MATCHING COEFFICIENT Varieties in blue are those cultivated for fruit production in South Africa (Brutsch, 1979)

Sharsheret and R1251, Malta and Algerian, and R1260 and R1259 were genotypically very similar. The three varieties from Botswana (R1260, R1259 and R1251) clustered together, whilst those from Israel (Sharsheret, Ofer and Messina) were spread amongst groups II and III (Figures 2.3 and 2.4). However, Sharsheret was genotypically very similar to R1251 although from Israel and Botswana, respectively. Furthermore, in a study by Wang *et al.* (1998), cactus pear varieties used for fruit, did not group according to species or geographic origin using RAPD markers. Although one would expect accessions from different countries to differ substantially, they may have the same origin. It has been reported by Dreyer that although the majority of the South African cactus pear varieties are Burbank types obtained from the USA they were originally collected from many regions such as Mexico, Africa and Australia (Chapman *et al.,* 2002). Therefore, the origin of many of these varieties is unknown. This may also be true for Sharsheret and R1251, which are genotypically very similar even though collected from different geographic regions.

The different accessions in the germplasm were genetically similar with the greatest distance between them at 0.220 (Jaccard), or 0.195 (Simple Matching). The fact that cactus pear is commonly propagated by cloning, could be a possible explanation for the narrow genetic base observed. Plants are rarely commercially grown from seeds, limiting genetic recombination and increasing genetic homogeneity. Clustering methods will always cluster data, whether or not clusters are present in the original data (Sneath and Sokal, 1973).

Cophenetic analysis of a dendrogram computes a linear correlation coefficient between the cophenetic distances from the tree, and the original distances (similarities) used to construct the dendrogram. It verifies how accurately the dendrogram reflects the original distances (Sokal and Rohlf, 1962). It is therefore important that one confirms the existence of clusters. In this study the cophenetic correlation coefficient for the dendrogram based on the the Simple Matching coefficient was r = 0.954 (Figure 2.5) and r = 0.953 (Figure 2.6) for the Jaccard similarity coefficient. Clusters generated thus accurately represented the distances between the accessions as determined by the similarity coefficients.

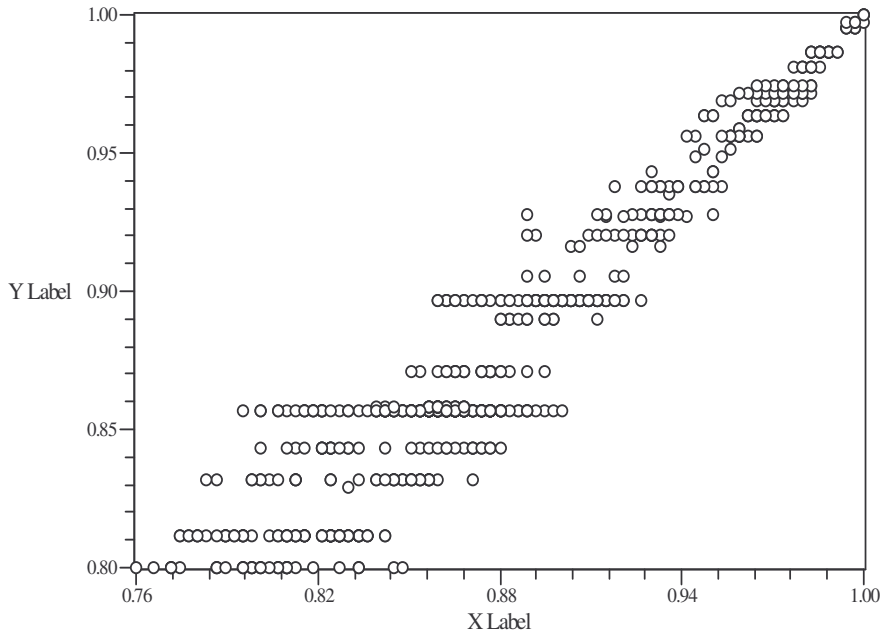


FIGURE 2.5 COPHENETIC CORRELATION MATRIX FOR SIMPLE MATCHING COEFFICENT DATA

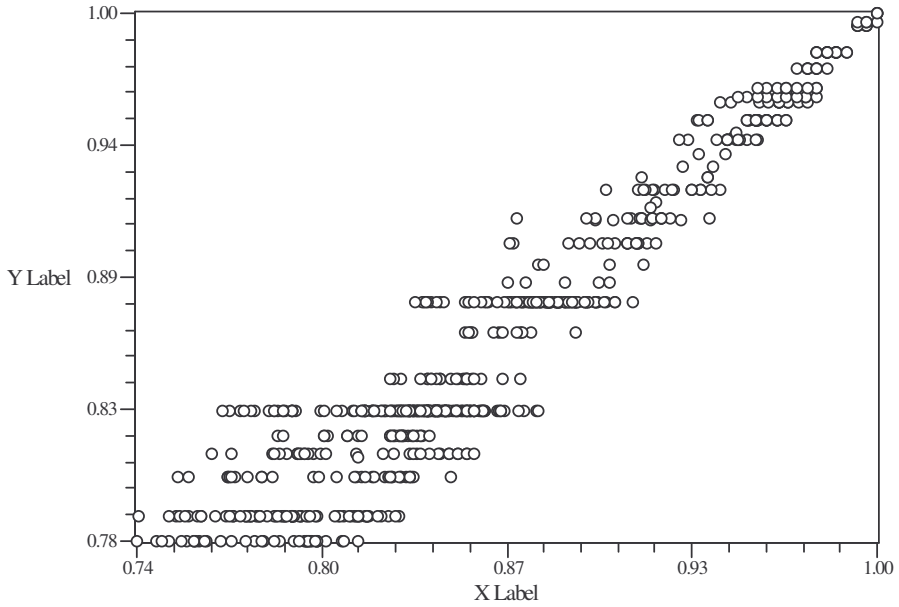


FIGURE 2.6 COPHENETIC CORRELATION MATRIX FOR JACCARD COEFFICIENT DATA

The dendrograms and clusters (Figures 2.3 and 2.4) generated by the two similarity coefficients were almost identical. This is in contradiction with various arguments, that given the different mathematical formulas used for each coefficient, different clustering patterns should result. In addition, the Simple Matching coefficient is computed using fragment absences; thus, one would expect a greater difference between the two dendrograms. The similarity matrices obtained using the Jaccard and Simple Matching coefficients, were compared by the Mantel test statistic for matrix comparison. Mantel (1967) developed a test that enables one to compare two matrices. The test gives a product-moment correlation (r), and a statistic (Z) to measure the degree of relatedness between two matrices. Rohlf (1990) further suggests that the degree of fit can be subjectively interpreted as being very good when $r \geq 0.9$. The matrices generated by the Jaccard and Simple Matching coefficients were highly correlated at $r = 0.929$. This furthermore confirmed the observed similarity of the dendrograms generated, using the different similarity coefficients. This finding suggested that these similarity coefficients give similar estimates of genetic relationships among these accessions of cactus pear.

What is known of the ancestry of the 39 South African cactus pear varieties is that they were developed either as clones or as natural or artificial hybrids from 21 spine-less types from the Burbank nursery imported into the country. These varieties were collected by researchers at the Limpopo Department of Agriculture and distributed to commercial and emerging farmers. Investigations into the reticulate evolution, (occasional hybridisation and combination of two species), in *Opuntia* spp. using

molecular markers can be applied to elucidate the ancestry of these varieties. RAPD markers were used by Griffith (2003) to complement morphological data (Griffith, 2001a) and observed interfertility of parental taxa (Griffith, 2001b) to phylogenetically identify putative parental taxa of two hybrid *Opuntia* taxa, *O*. x *rooneyi* and *O*. *x spinosibacca*.

Varieties classified as *O. fusicaulis* (Direkteur, Blue Motto, and Fusicaulis) grouped into cluster I with the exception of Gymno Carpo, which also grouped in this cluster, but is classified as an *O. ficus-indica* type. The rest of the varieties classified as *O. ficus-indica* (Morado, Malta, Algerian) grouped into cluster III. Varieties classified as hybrids between the different Opuntia species in South Africa, and hence denoted as *Opuntia* spp. (Nudosa, American Giant, and Skinners Court) were dispersed over clusters II and III (Figures 2.3 and 2.4). Variety 37, an unknown accession, was shown not to be identical to any other variety even though it was thought to be a duplicate.

2.4 CONCLUSIONS

AFLP markers were successfully applied to genotype South African cactus pear germplasm. Primer combinations that resulted in the highest percentage polymorphic fragments were described and are recommended for future fingerprinting efforts of cactus pear. Genotype specific fragments were generated with the use of six primer combinations (E-A**GG** + M-C**AT,** E-A**CT** + M-C**AG,** E-A**CT** + M-C**AT,** E-A**CA** + M-C**AT,** E-A**CA** + M-C**TT,** and E-A**CA** + M-C**AG**). These GSF allowed the unique identification of nine varieties, three of which are commercially cultivated (Meyers, Roedtan, and Santa Rosa). These fragments can be converted into STS markers for more rapid identification of cultivated varieties.

A large number of the varieties are of unknown pedigree (Table 2.1). Berg x Mexican, and X 28 (Robusta x Castillo) are the only varieties of known pedigree. Berg x Mexican clustered in group III and did not cluster in the same group as one of its progenitors, Mexican, which was classified in cluster I. For future research, the various known progenitors of such varieties should be included into AFLP marker studies in order to assist in the elucidation of their pedigrees.

Comparative analysis of dendrograms constructed, based on the Simple Matching and Jaccard similarity coefficients displayed negligible differences. The widest genetic distance within the germplasm based on the Jaccard similarity coefficient was 0.220. This value was found to be comparable to that deduced from the dendrogram based on the Simple Matching coefficient (0.195). The only differences in clusters based on the

two similarity coefficients were the grouping of Roly Poly with Santa Rosa in cluster IV, using the Jaccard similarity coefficient. In contrast, Roly Poly remained ungrouped using the Simple Matching coefficient, and Santa Rosa grouped into cluster IV with Schagen.

Cophenetic correlation analysis confirmed the goodness of fit between cophenetic values and the original similarity estimates as being high for both dendrograms. Visual similarity between the dendrograms was confirmed using the Mantel test. The Mantel test gave a high correspondence ($r = 0.9291$) for the two similarity matrices.

AFLP fingerprinting data revealed differences between the accessions currently cultivated in South Africa. The amount of polymorphic fragments between different accessions varied with varying AFLP-primer combinations, suggesting that sufficient detectable genetic differences exist within the germplasm for the use of DNA fingerprinting for varietal identification and parental selection. The genetic similarity values developed in this study will provide breeders with a starting point for increasing diversity in their crosses.

REFERENCES

Applied Biosystems, 2000. AFLP™ Plant Mapping Protocol. USA.

Aranzana, M.J., M.C. de Vicente and P. Arús, 2001. Comparison of fruit and leaf DNA extracts for AFLP and SSR analysis in peach (*Prunus persica* (L.) Batsch). Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 297-300.

Arnau, G., J. Lallemand and M. Bourgoin, 2001. Are AFLP markers the best alternative for cultivar identification? Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 301-306.

Arnholdt-Schmitt, B., L.C. Girão and R.M. Zárate, 2001. Genome characterisation of *Opuntia ficus-indica*: A simple and efficient micromethod. Journal of the professional Association for Cactus Development 1: 57-65.

Berio, T., G. Morreale, A. Giovannini, A. Allavena, L. Arru, P. Faccioli, V. Terzi and N. Pecchioni, 2001. RAPD and AFLP genetic markers for the characterisation of *Osteospermum* germplasm. Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 171-176.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32: 314-331.

Brutsch, M.O., 1979. The prickly pear (*Opuntia ficus-indica* (L.) Mill.) as a potential fruit crop for the drier regions of the Ciskei. Crop Production 8: 131-137.

Cárdenas, A., I. Higuera-Ciapara and F.M. Goycoolea, 1997. Rheology and aggregation of cactus (*Opuntia ficus-indica*) mucilage in solution. Journal of the professional Association for Cactus Development 2: 152-159.

Chapman, B., C. Mondragón-Jacobo, R.A. Bunch and A. H. Paterson, 2002. Breeding and Biotechnology. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses, pp 255-271. University of California Press, California, USA.

Chessa, I. and G. Nieddu, 1997. Descriptors for Cactus pear (*Opuntia* spp.) CACTUSNET- FAO Newsletter Special Issue.

Chessa, I., G. Nieddu, P. Serra, P. Inglese and T. La Mantia, 1997. Isozyme characterization of *Opuntia* species and varieties from Italian germplasm. Proceedings of the third International Conference on Cactus Pear and Cochineal. Acta Horticulturae 438: 45-52.

Da Silva Meyer, A., A.A.F. Garcia, A.P. De Souza and C.L. De Souza, 2004. Comparison of similarity coefficients used for cluster analysis with dominant markers in maize (*Zea mays* L). Genetics and Molecular Biology 27: 83-91.

De La Cruz, M., F. Rumirez and H. Hernandez, 1997. DNA isolation and amplification from cacti. Plant Molecular Biology Reporter 15: 319-325.

De Riek, J., E. Calsyn, I. Everaert, E. Van Bockstaele and M. De Loose, 2001. AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. Theoretical and Applied Genetics 103: 1254-1256.

Dice, L.R., 1945. Measures of the amount of ecologic association between species. Ecology 26: 297-302.

Duarte, M.C., J.B. Dos Santos and L.C. Melos, 1999. Comparison of similarity coefficients based on RAPD markers in the common bean. Genetics and Molecular Biology 22: 427-432.

Ferriol, M., B. Picó and F. Nuez, 2003. Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theoretical and Applied Genetics 107: 271-282.

Genet, T., C.D. Viljoen and M.T. Labuschagne, 2005. Genetic analysis of Ethiopian mustard genotypes using amplified fragment length polymorphism (AFLP) markers. African Journal of Biotechnology 4: 891-897.

Griffith, M.P., 2001a. A new Chihuahuan Desert prickly pear, *Opuntia* x *rooneyi*. Cactus and Succulent Journal 73: 307-310.

Griffith, M.P., 2001b. Experimental hybridization in northern Chihuanhuan Desert region *Opuntia*. Aliso 20: 37-42.

Griffith, M.P., 2003. Using molecular evidence to elucidate reticulate evolution in *Opuntia* (Cactaceae). Madroño 50: 162-169.

Griffith, M.P. and J.M. Porter, 2003. Back to the basics: a simple method of DNA extraction for mucilaginous cacti. BRADLEYA 21: 126-128.

Grzebelus, D., R. Baranski, B. Jagosz, B. Michalik and P.W. Simon, 2001. Comparison of RAPD and AFLP techniques used for the evaluation of genetic diversity of carrot breeding materials. Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 413-416.

Hagen, L.S., P. Lambert, J.-M. Audergon and B. Khadari, 2001. Genetic relationships between apricot (*Prunus armeniaca* L.) and related species using AFLP markers.

Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 205-207.

Herselman, L., 2003. Genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. Euphytica 133: 319-327.

IPGRI, 1996. Measuring Genetic Variation 6, version 1.2.

Jaccard, P., 1908. Nouvelles rescherches sur la distribution florale. Bull.Soc.Vaud.Sci.Natl. 44: 223-270.

Jackson, A.A., K.M. Somers and H.H. Harvey, 1989. Similarity coefficients: measures for co-occurrence and association of simply measures of occurrence? American Naturalist 133: 436-453.

Keygene, 2000. AFLP-Quantar® 1.0 fingerprint analysis software.

Kosman, E. and J.K. Leonard, 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploidy species. Molecular ecology 14: 415-424.

Labra, M., F. Grassi, M. Bardini, S. Imazio, A. Guiggi, S. Citterio, E. Banfi and S. Sgorbati, 2003. Genetic relationships in *Opuntia* Mill. genus (Cactaceae) detected by molecular markers. Plant Science 165: 1129-1136.

Lanteri, S., E. Saba, M. Cadinu, G.M. Mallica, L. Baghino and E. Portis, 2004. Amplified fragment length polymorphism for genetic diversity assessment of globe artichoke. Theoretical and Applied Genetics 108: 1534-1544.

Lombard, V., C.P. Baril, P. Dubreuil, F. Blouet and D. Zhang, 2000. Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: consequences for varietal registration. Cop Science 40: 1417-1425.

Lynch, M. and B.G. Milligan, 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology 3: 91-99.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209-220.

Mba, C. and J. Tohme, 2005. Use of AFLP markers in surveys of plant diversity. Methods in Enzymology 395: 177-201.

Meksem, K., E. Ruben, D. Hyten, K. Triwitayakorn and D.A. Lightfoot, 2001. Conversion of AFLP fragments into high-throughput DNA markers. Molecular Genetics and Genomics 265: 207-214.

Métais, L., C. Aubr, B. Hamon, R. Jalouzot and D. Peltier, 2001. Assessing common bean genetic diversity using RFLP, DAMD-PCR, RAPD and AFLP markers. Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 459-461.

Miller, M.P., 1997. Tools for population genetic analysis (TFPGA) 1.3: A windows program for the analysis of allozyme and molecular population genetic data.

Mueller, U.G. and L.L. Wolfenbarger, 1999. AFLP genotyping and fingerprinting Tree 14: 389-394.

Nguyen, T.T., P.W.J. Taylor, R.J. Redden and R. Ford, 2004. Genetic diversity estimates in *Cicer* using AFLP analysis. Plant Breeding 123: 173-179.

Nilsen, L.B., S.S. Dhillion, S.L. Camargo-Ricalde, B. Rendón-Aguilar and M. Heun, 2005. Traditional knowledge and genetic diversity of *Opuntia pilifera* (Cactaceae) in the Tehuacán-Cuicatlán valley, Mexico. Economic Botany 59: 366-376.

Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplick, P. Castiglioni, G. Taramino and M. Motto, 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theoretical and Applied Genetics 97: 1248-1255.

Prevost, A. and M.J. Wilkinson, 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics 98: 107-112.

Rana, M.K. and Bhat K.V., 2004. A comparison of AFLP and RAPD markers for genetic diversity and cultivar identification in cotton. Journal of Plant Biochemistry and Biotechnology 13: 19-24.

Regner, F., A. Stadbauer and C. Eisenheld, 2001. Molecular markers for genotyping grapevine and for identifying clones of traditional varieties. Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546: 331-335.

Reif, J.C., A.E. Melchinger and M. Frisch, 2005. Genetical and mathematical properties of similarity and dissimilarity coefficients applied in plant breeding and seed bank management. Crop Science 45: 1-7.

Rohlf, F.J. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology 47: 147-158.

Rohlf, F.J., 1990. Fitting curves to outlines. In: Rohlf, F.J. and F.L. Bookstein (Eds.), Proceedings of the Michigan morphometrics workshop, pp 167-177. Museum of Zoology special publications (2), University of Michigan, USA.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen and R.W. Allard, 1984. Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences of the United States of America 81: 8014-8019.

Scheinvar, L., 1995. Taxonomy of utilized Opuntias. In: Barbera G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear. FAO Plant production and protection paper 132, pp 20-27. Rome, Italy.

Seo, Y.W., C.S. Jang and J.W. Johnson, 2001. Development of AFLP and STS markers for identifying wheat-rye translocations possessing 2RL. Euphytica 121: 279-287.

Singh, A., A. Chaudhury, P.S. Srivastava and M. Lakshmikumaran, 2002. Comparison of AFLP and SAMPL markers for assessment of intra-population genetic variation in *Azadirachta indica* A. Juss. Plant Science 162: 17-25.

Smith, J.S.C., S. Kresovich, M.S. Hopkins, S.E. Mitchell, R.E. Dean, W.L. Woodman, M. Lee and K. Porter, 2000. Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Science 40: 226-232.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy: the principles and practice of numerical classification, pp 252-290. W.H. Freeman and Company, San Francisco, USA,.

Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. University of Kansas Scientific Bulletin 38: 1409-1438.

Sokal, R.R. and F.J. Rohlf, 1962. The comparison of dendrograms by objective methods. Taxon 11: 33-40.

Ude, G., M. Pillay, E. Ogundiwin and A. Tenkouano, 2003. Genetic diversity in an African plantain core collection using AFLP and RAPD markers. Theoretical and Applied Genetics 107: 248-255.

Uzun, I., 1997. Fruit and cladode isozymes in cactus pear. Proceedings of the third International Conference on Cactus Pear and Cochineal. Acta Horticulturae 438: 53-56.

Vijayan, K., P.P. Srivastava and A.K. Awasthi, 2004. Analysis of phylogenetic relationship among five mulberry (*Morus*) species using molecular markers. Genome 47: 439-448.

Vos, P., R. Hogar, M. Bleeker, M. Reijans, T. Van De Lee, M. Hornes, A. Freiters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Wang, B., W. Li and J. Wang, 2005. Genetic diversity of *Alternanthera philoxeroides* in China. Aquatic Botany 81: 277-283.

Wang, X., P. Felker, M.D. Burow and A.H. Paterson, 1998. Comparison of RAPD marker patterns to morphological and physiological data in the classification of *Opuntia* accession. Journal of the professional Association for Cactus Development 3: 98-109.

Weir, B., 1996. Genetic data analysis II: methods for discrete population genetic data. Sunderland, Mass: Sinauer Associates.

Yue, G., Y. Li, F. Chen, S. Cho, L.C. Lim and L. Orban, 2002. Comparison of three DNA marker systems for assessing genetic diversity in Asian arowana (*Scleropages formosus*). Electrophoresis 23: 1025-1032.

Zacarias, A.M., A.-M. Botha, M.T. Labuschagne and I.R.M. Benesi, 2004. Characterization and genetic distance analysis of cassava (*Manihot esculenta* Crantz) germplasm from Mozambique using RAPD fingerprinting. Euphytica 138: 49-53.

Zoghlami, N., I. Chrita, B. Bouamama, M. Gargouri, H. Zemni, A. Ghorbel and A. Mliki, 2007. Molecular based assessment of genetic diversity within the Barbary fig (*Opuntia ficus indica* (L.) Mill.) in Tunisia. Scientia Horticulturae (in press).

Chapter 3

Fruit quality of South African cactus pear (*Opuntia* spp.) varieties

ABSTRACT

South Africa hosts one of the largest cactus pear germplasm collections in the world at the Mara Germplasm bank, Limpopo. However, the available gene pool within the conserved accessions is not fully exploited. A study was undertaken to evaluate 23 cactus pear varieties for use in fruit production in the Mokopane district of the Limpopo Province. Data were collected over two seasons (1999-2000, 2000-2001), and subjected to analysis of variance (ANOVA) using the general linear model. The Gower distance coefficient was used as a measure of diversity, and the UPGMA for cluster analysis. Varieties grouped into four main clusters. Commercially cultivated varieties were evenly dispersed among the different clusters, with the greatest percentage grouped into cluster IIa. Varieties from Botswana (R1251, R1259) clustered separately. Varieties recommended for use as fruit crops grouped into cluster IIa. Gymno Carpo, Malta, and Algerian grouped into cluster IIa. Cluster I varieties had the highest TSS content (14.26°Brix) with a pulp content of 55.36%. The majority of the varieties had a fruit development period (FDP) within the 120–130 days range. Varieties with the longest FDP over both seasons were Nepgen (148 days for season 1, and 193 days for season 2), Skinners Court (141 days in season 1, and 148 days in season 2), and Zastron (148 days in season 1, and 162 days in season 2). All the varieties underwent an extended FDP during the second season as a result of chillier conditions.

3.1 INTRODUCTION

The potential of cactus pear as a commercial fruit crop in South Africa is increasingly being exploited by farmers. In South Africa, cactus pear is usually cultivated under dry-land conditions. Commercial fruit plantations make use of spine-less Burbank varieties that are clonally propagated using terminal cladodes (Brutsch, 1979; Wessels, 1988). A number of plantations were established in the middle eighties and are increasing in number (Wessels *et al*., 1997). Commercial plantations of spine-less cactus pear are well established and the Limpopo Province contains the largest cactus pear plantations for fruit production in South Africa (Potgieter, 2002).

The cactus pear is an oval shaped “false” berry (Hills, 1995) with an average weight of between 100-200 g. Cactus pear fruits are appreciated for their characteristic taste and aroma as well as their dietetic properties. It has a thick fleshy skin that contributes 30-40% of the total fruit weight. The juicy pulp contributes 60-70% of the total fruit weight, and contains many hard coated seeds that contribute 5-10% of the pulp weight (Griffiths and Hare, 1906; Cantwell, 1991; Barbera, 1995). Each variety produces fruits of different shapes, colours, and flavours. The primary components of the fruit pulp are water (85%), carbohydrates (10-15%), and vitamin C (25-30 mg/100 g) (Cantwell, 1995). Fruits are mainly produced on mature terminal cladodes, and require 110-120 days to develop (Cantwell, 1986).

The choice of variety is primarily governed by a variety's suitability to the climatic conditions of the region chosen for cultivation, and by the intended market to be supplied (i.e. local or international). Other factors that influence choice of variety include the cultivar’s yield potential, ripening time, and quality characteristics (Potgieter, 1997). Desirable characteristics of varieties to be used for fruit production in South Africa have been described (Table 3.1).

TABLE 3.1 DESIRABLE CHARACTERISTICS OF CACTUS PEAR VARIETIES IN SOUTH AFRICA

Plant characteristics	Fruit characteristics
High yield potential	Large fruit size (>140.0 g)
Short juvenile phase (early bearing)	Attractive internal and external colour
Consistent good yield	Long shelf-life
Moderate vegetative vigour	Low seed content
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 (< 6 mm)
Natural tendency to bear out of season	High TSS content (>13°Brix)
Few thorns and glochids	Pleasant taste and aroma/flavour
Easily manipulated (winter production and scozzollatura)	High juice content
	High percentage pulp (> 50%)
	Crack resistance
	Easy peeling
	Suitability to processing

TSS = Total soluble solids (Potgieter and Mkhari, 2000)

For commercial handling, the maturity or ripeness stage of fruit at harvest is important (Cantwell, 1995). Fruit quality characteristics for cactus pear include percentage pulp, thickness and ease of removal of the peel, and peel resistance to physical handling (Wessels, 1988). Kader (2000) included uniformity and intensity of colour, size, and freedom from defects and decay as indices for grading cactus pear fruits. Large differences occur among cultivars in TSS (12-17°Brix), titratable acidity (0.03-0.12%), pH (6.0-6.6), and ascorbic acid content (20-40 mg/100g fresh weight) (Kader, 2000).

Internationally a few studies have reported on the characterisation of cactus pear varieties for fruit production (Chessa and Nieddu, 1997; Arba *et al.*, 2002; Felker *et al.*, 2002a; Nieddu *et al.*, 2002; Valdez *et al.*, 2002). Characterisation of cactus pear varieties is further complicated because cactus pear, unlike most fruit crops, is not monospecific. It derives from a number of species from the genus *Opuntia* (Chessa *et al.*, 1995), hence many researchers refer to commonly cultivated varieties as *Opuntia* spp.

Although South Africa hosts one of the largest germplasm collections of cactus pear in the world (Chapman *et al.*, 2002) limited research into this emerging crop has been published. Of the work being done few publications have reported on the evaluation of the fruit quality of different varieties that occur in South Africa. It thus became the aims

of this study to (1) examine the differences in fruit quality of 23 South African cactus pear varieties and, (2) determine which varieties produce fruit of a higher quality.

3.2 MATERIALS AND METHODS

3.2.1 Trial site and layout

Trial site

Evaluation was carried out at the Gillemberg cactus pear germplasm block in the Mokopane (previously Potgietersrus) district of the Limpopo Province. This area is characterised by warm summers, cool winters and a mean annual rainfall of 450 mm that predominantly falls in summer. A brief list describing some of the climatic and soil characteristics of the trial site is provided in Table 3.2.

TABLE 3.2 CLIMATIC AND SOIL CHARACTERISTICS OF THE GILLEMBERG CACTUS PEAR GERMPLASM BLOCK

Character	Name/ Value
Farm name	Gillemberg
Magisterial district	Potgietersrus
Latitude	23º 50 'S
Longitude	28º 58 'E
Altitude (m)	1 100
Average annual rainfall (mm)	450
Average daily maximum air temperature in December (°C)	27.92
Average daily minimum air temperature in June (°C)	6.04
Accumulated positive C.U. (May-Aug) (°C)	245.5
Accumulated H.U. (Oct-Mar) Growth Degree Day (°C)	2 367
Average daily solar radiation (MJ/m^2/s)	19.10
Average wind speed (m/s)	2.38
Average daily evaporation (mm)	6.04
Average daily maximum R.H. (%)	84.18
Average daily minimum R.H. (%)	41.98
Soil texture	Loamy sand
Clay percentage	15
Silt percentage	5
Sand percentage	80
Veldt type (Acocks, 1952)	Mixed bushveld

C.U. = chill units

H.U. = heat units

R.H. = relative humidity

Trial layout

Data gathered for this study were collected from the Gillemberg cactus pear germplasm block. The trial was thus not statistically laid out for the purposes of this study. Only those varieties that showed signs of fruit bearing were evaluated and those that formed few or no flowers, were excluded from this study. Data was gathered for 23 of the varieties (Table 3.3) that were deemed promising for commercialisation (Brutsch, 1979).

The orchard consisted of 20 plants per variety planted in a single row orientated in an East/West direction. Plants were spaced 5 m between rows and 2 m in a row (1000 plants/ha). Three plants on each end of each row, and four in the middle of the row were used as border plants. The remaining 10 were used as data plants. Data collected over two seasons (Season 1: 1999-2000, Season 2: 2000-2001) were used for fruit quality evaluation.

Data for 10 fruit quality traits were captured for each variety (Table 3.4). Quantitative characters were collected as an average value of the 10 central plants per variety at two harvest times. Two harvesting times were used, one at 30-40% total fruit ripening and again at 50-60% total fruit ripening. Two harvesting times were used because flower bud burst was unsynchronised, therefore harvesting twice during fruit development allowed for a more representative sample of a particular variety. The method for quantitative trait data collection is given in Table 3.4. Phenological stages were recorded as described in Table 3.5. The fruit development period was deduced as the time period between reproductive bud break (RBB) and 50% fruit ripening (FFR).

3.2.2 Climatic data

Climatic data was captured via an automatic weather station (Mike Cotton Systems) installed 50 m from the site (Appendix I). Mean daily values for temperature (°C), rainfall (mm), heat units (HU), chill units (CU), evapotranspiration (ETo), and solar radiation (Rs) were summarised to mean monthly values.

TABLE 3.3 CACTUS PEAR VARIETIES EVALUATED FOR FRUIT QUALITY

Variety number	Variety name	Commercially cultivated varieties	Country of origin
12	Algerian	X	South Africa
40	Berg x Mexican		South Africa
39	Cross X		South Africa
26	Ficus-Indice		South Africa
5	Gymno Carpo	X	South Africa
11	Malta	X	South Africa
15	Meyers	X	South Africa
8	Morado	X	South Africa
29	Nepgen		South Africa
4	Nudosa	X	South Africa
18	Ofer		Israel
34	R1251		Botswana
33	R1259		Botswana
16	Roedtan	X	South Africa
41	Santa Rosa	X	South Africa
42	Schagen		South Africa
31	Silician Indian Fig		South Africa
2	Skinners Court	X	South Africa
23	Tormentosa		South Africa
13	Turpin		South Africa
38	Van As	X	South Africa
24	X 28 (Robusta x Castillo)		South Africa
10	Zastron	X	South Africa

A list of the different *Opuntia* spp. varieties used in this study, with the accompanying variety numbers. The cultivated varieties, depicted in the blue font, were reported by Brutsch (1979) as being of good potential for commercial fruit production

3.2.3 Cultural practices

The germplasm block was maintained as a commercial fruit orchard and generally accepted orchard practises such as pruning and pad thinning were performed. No supplementary irrigation was given, and orchard practices followed were as described in Potgieter (1997) with the following modifications:

Pruning

Varieties were pruned more severely than outlined in Potgieter (1997). Additional terminal cladodes were removed and distributed to farmers to use as planting material, especially of varieties number 13 to 42 as they were considered new varieties at the time.

TABLE 3.4 LIST OF FRUIT QUALITY TRAITS AND THEIR DESCRIPTOR STATES

Character name	Fruit quality trait and descriptive value
PEEL THICKNESS (mm)	Two measurements were taken of peel thickness at 180 degrees from one another for 20 fruits of the same variety
FRUITSHAPE	Index indicative of fruit shape derived as, fruitshape = fwidth / flength: 0.45-0.55 = oblong, 0.56-0.60 = elliptic, 0.70-0.79 = ovoid 0.80-0.89 = round
FMASS (g)	Fruit mass of 20 plants per variety
TSS (°Brix)	Total soluble solid content, was determined for 20 fruits of the same variety
%PULP	The edible portion of the fruit expressed as a percentage of the whole fruit for 20 fruits of the same variety
FRUITNO (n)	Number of fruits per plant was deduced from the number of reproductive buds counted subsequent to fruit thinning to allow 40-50 mm between buds
PEELABILITY	Peelability index, the ease with which the peel is removed from the pulp, given an arbitrary value from 1 to 5 depending on the ease of removal; 1 = difficult to remove, 5 = easy to remove
FWIDTH (mm)	Equatorial diameter of 20 fruits per variety
FLENGTH (mm)	Longitudinal length of 20 fruits per variety
PC	Pulp colour: 1 = khaki/yellow, 2 = green/white, 3 = white, 4 = orange/red, 5 = orange, 6 = pink, 7 = white/red, 8 = purple 9 = pink/purple, 10 = red, 11 = unknown (Appendix III)

Fertilisation

Fertilisation was carried out based on soil analysis results obtained from the Agricultural Research Council Institute for Soil, Climate and Water (ARC-ISCW) laboratory, Tshwane (Tables 3.6 and 3.7). Top soil samples (0-300 mm) were taken from within the drip area of the plants during late winter/spring. Five sub-samples were taken over the entire orchard.

TABLE 3.5 LIST OF PHENOLOGICAL AND QUALITATIVE TRAITS USED FOR CLUSTERING OF CACTUS PEAR VARIETIES

Character name	Phenological trait descriptive value
RBB	Reproductive bud break: week of the month when reproductive buds are clearly visible
FFO	50% flower opening (anthesis): week of the month when 50% of all flower buds are showing petals
FFR	50% fruit-ripening: week of the month during which 50% of all fruit on a variety are ripe
FDP	Fruit development period: total number of days from the first working day of the week during which reproductive bud break was recorded until and inclusive of the first day of the week during which 50% fruit ripening was recorded
PULPMASS (g)	Pulp mass: measured for ten fruits of the same variety
FPC	Flower petal colour: 1 = dark yellow, 2 = yellow, 3 = orange, 4 = unknown (Appendix II)
CLADSHAPE	Cladode shape; 1 = elliptic, 2 = ovate, 3 = large diamond, 4 = round (Appendix V)
PH	Plant habitus: 1 = bush/shrubby, 2 = spreading, 3 = upright, 4 = arborescent (Apppendix V)
PA (yrs)	Plant age: number of years since plant was established

TABLE 3.6 SOIL ANALYSIS RESULTS FOR GILLEMBERG GERMPLASM BLOCK (1999-2001)

Season	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Resistance (ohm)	pH (H_2O)	TTA (cmol (+)/kg)
1999-2000	37.3	93	732	115	12	1400	5.76	0
2000-2001	20.9	68	519	100	14	2400	5.74	0

TTA = Total titratable acidity

Soil analysis was determined for exchangeable and water soluble nutrients using the following techniques: P = Bray 1; K, Ca, Mg, and Na = ammonium acetate method, and electrical resistance = soil paste technique.

TABLE 3.7 FERTILISATION RECOMMENDATIONS AND APPLICATION FOR GILLEMBERG GERMPLASM BLOCK

Season	Element	Product	Quantity	Time of application	*Method
1999/2000	N (100 kg/ha)	LAN (28%N)	375 kg/ha	Two equal split dressings (November, February)	By hand
	K (60 kg/ha)	Potassium chloride (50% K)	120 kg/ha	December	By hand
2000/2001	N (100 kg/ha)	LAN (28%N)	375 kg/ha	Two equal split dressings (November, February)	By hand
	K (100 kg/ha)	Potassium chloride (50% K)	200 kg/ha	December	By hand
	Lime	Dolomite lime	2000 kg/ha	December	By hand

*Fertiliser was applied by hand in drip area

3.2.4 Data collection and statistical analysis

In assessing fruit quality traits of cactus pear varieties a fully randomised experimental design with random sampling of all data points was carried out. During the course of the study, data for fruit quality traits were collected for each variety, and entered into the Statistical Package for the Social Sciences (SPSS Inc, 1997). Mean values for each of the traits were calculated for each variety for both seasons. Data for quantitative characters were subjected to analysis of variance using the general linear model in SPSS. The Tukey multiple range test was used to detect significant differences between means at $p \leq 0.05$.

The Gower distance was used as a measure of diversity between different varieties. Gower similarity measure between the i th and j th individual, Sij, can be used with continuous, ordinal, binary, and nominal variables. This similarity measure was used to compute distances between varieties (Gower, 1971).

Gower distance [dij = $(1 - Sij)^{1/2}$] between two individuals is Euclidean metric. For k variables (k = 1,2,….,p), Gower similarity measure between two individuals i and j is:

$$S_{ij} = \frac{\sum_{k=1}^{p} W_{ijk} S_{ijk}}{\sum_{k=1}^{p} W_{ijk}}$$

Where:

w_{ijk} : is a weight given to the ijk th comparison, 1 is assigned to valid comparisons, and 0 for invalid comparisons (when the value of the variable is missing in one or both individuals)

S_{ijk} is the contribution of the k th variable to the total similarity between individuals i and j, and it takes values between 0 and 1 for nominal variables, if the value of the k th variable is the same for both individuals i and j then $S_{ijk} = 1$; otherwise it equals 0 for a continuous variable $S_{ijk} = 1 - |x_{ik} - x_{jk}| / R_k$ where x_{ik} and x_{jk} are the values of the k th variable for the i and j individuals respectively, and R_k is the range of the k th variable in the sample (Franco *et al.*, 2005).

Gower distances were used to compute a dissimilarity matrix, and the UPGMA used for dendrogram construction using the NTYSYS-pc programme (Version 2.02i, Rohlf, 1998).

3.3 RESULTS AND DISCUSSION

3.3.1 Fruit quality: Season1

Varieties from Botswana (R1251 and R1259) did not produce any fruit during the first season of the trial, as they had not reached the productive age for fruit production.

3.3.1.1 Peel thickness

The peel of the cactus pear fruit develops from the receptacle that surrounds the ovary (Gibson and Nobel, 1986). The peel is thick and must be removed before the tasty pulp can be consumed. Varieties that had the highest peel thickness were Nepgen (7.11 mm), Ficus-Indice (5.79 mm) and Skinners Court (5.43 mm). Varieties that had a low peel thickness were Malta (3.86 mm), Gymno Carpo (4.02 mm) and Morado (4.05 mm) (Table 3.8). Potgieter and Mkhari (2002) recommended a peel thickness of less than 6 mm for cactus pear fruit. All varieties evaluated had a peel thickness less than 6 mm, except for Nepgen which had a peel thickness of 7.11 mm. With regards to peel thickness, all varieties evaluated, except for Nepgen, meet the requirements for fruit production in South Africa.

TABLE 3.8 FRUIT QUALITY OF CACTUS PEAR VARIETIES (SEASON 1)

Variety name	Peelthick	F shape	F mass	TSS	%Pulp	Fruit no	Peelability	Fruit Width	Fruit Length	Pulp colour
Algerian	4.21abcdefh	0.77efghi	160.85abcdefg	12.89bcde	60.59defg	155.30ghi	3.20defg	59.24cdef	77.73abc	Dark pink
Berg x Mexican	4.18abcde	0.72abcdefg	158.38abcdefg	12.58abcde	59.37bcdefg	42.30bcd	4.20hij	58.70cdef	81.50abcde	Dark pink
Cross X	4.26abcdef	0.75cdefghi	159.85abcdefg	12.49abcde	60.70efg	36.60abc	4.75ij	59.41cdef	80.00abcd	Orange
Ficus-Indice	5.79j	0.68abc	173.55cdefg	12.93bcde	55.00ab	60.60bcde	2.95cde	59.80cdef	87.75def	Orange
Gymno Carpo	4.02abc	0.77ghi	173.79cdefg	11.37a	61.55fg	176.50hi	2.85cde	61.81f	80.35abcde	Orange
Malta	3.86ab	0.79hi	165.48bcdefg	11.94abc	63.62g	140.30gh	2.85cde	60.40def	77.09abc	Orange
Meyers	4.42bcdefgh	0.77fghi	170.61bcdefg	12.55abcde	61.70fg	156.60ghi	3.80gh	61.64ef	80.13abcd	Dark pink
Morado	4.05abcd	0.77ghi	146.46abcde	13.15bcde	60.38defg	133.80g	2.40bc	57.81abcde	75.01a	White
Nepgen	7.11k	0.68ab	141.31ab	12.80bcde	50.35a	77.90cde	2.60cd	54.02a	79.90abcd	White
Nudosa	4.98ghi	0.70abcdef	231.10h	11.34a	58.79bcdef	63.10bcde	1.55a	66.00g	94.53f	Red/Orange
Ofer	4.74defghi	0.80i	153.36abcdefg	13.77e	58.97bcdefg	183.00i	4.20hij	60.64def	76.05ab	Orange
R1251	0.00	0.00	0.00	0.00	0.00	0.00a	0.00	0.00	0.00	-
R1259	0.00	0.00	0.00	0.00	0.00	0.00a	0.00	0.00	0.00	-
Roedtan	4.57cdefgh	0.75defghi	163.75bcdefg	12.91bcde	61.18fg	144.50ghi	4.85j	60.08def	80.33abcde	Orange
Santa Rosa	4.06abcd	0.69abcd	157.34abcdefg	12.93bcde	59.49bcdefg	40.50abcd	3.75fgh	56.91abcd	82.96abcde	Orange
Schagen	4.43bcdefgh	0.66a	174.04cdefg	12.32abcd	57.19bcdefg	37.40abc	4.55ij	58.30bcdef	88.89ef	White
Sicilian Indian Fig	4.86efghi	0.73bcdefgh	167.60bcdefg	13.46de	56.28bcde	81.20de	3.10def	59.47cdef	81.95abcde	Dark pink
Skinners Court	5.43ij	0.73bcdefghi	186.91g	13.67de	55.37bc	26.60ab	1.50a	62.21gf	85.61cde	White/ green
Tormentosa	4.58cdefgh	0.71abcdefg	183.55fg	12.79bcde	58.98bcdefg	66.80bcde	2.80cde	60.44def	85.53cde	Orange
Turpin	4.89fgh	0.69abcd	177.96efg	12.66abcde	55.94bcd	161.10ghi	3.00cde	60.48def	87.54def	Orange
Van As	4.30bcdefg	0.70abcde	164.19bcdefg	12.83bcde	59.87cdefg	32.50ab	4.65ij	58.36bcdef	83.89bcde	White
X 28	5.09hij	0.73bcdefghi	176.40defg	11.98abc	60.49defg	91.80ef	2.70cde	60.90ef	83.50abcde	Orange
Zastron	4.82efghi	0.74bcdefghi	142.88abc	13.15bcde	56.27bcde	125.70fg	1.85ab	56.09abc	75.69ab	White
Grand mean	**4.64**	**0.73**	**165.51**	**12.68**	**58.78**	**88.44**	**3.29**	**59.30**	**81.80**	

Within column values with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple range test. **Peelthick** = peelthickness, **F shape** = fruit shape**, F mass** = fruit mass, **TSS** = Total soluble solids content, **%Pulp** = percentage pulp content, **Fruit no** = number of reproductive buds remaining per plant after thinning. The cultivated varieties, depicted in the blue font, were reported by Brutsch (1979) as being of good potential for commercial fruit production

3.3.1.2 Fruit shape

In cactus pear, fruit shape index is deduced as the ratio of equatorial fruit width and longitudinal fruit length. This index is used to determine fruit shape and did not show large variation between varieties (Table 3.8). The majority of fruits had a fruit shape index in the range of 0.70-0.79, indicative of ovoid shaped fruits. Cactus pear fruits are classified according to four shapes namely, round, elliptic, ovoid, and oblong (Chessa and Nieddu, 1997; Ochoa, 1997). Fruit size and shape are important considerations when choosing a variety for cultivation because oval or barrel-shaped fruits are easier to handle than elongated fruits. In addition, oval shaped fruits undergo less damage to the stem end during harvesting (Cantwell, 1991). Therefore, in terms of shape, the majority of the varieties would qualify for commercialisation.

Recent findings have shown that shape attributes have a genetic basis (Van der Knaap and Tanksley, 2003). Additionally, trait terms and mathematical descriptors of shape attributes have been developed to improve phenotypic analyses. A software programme, Tomato analyser, which performs semi-automated, objective and quantitative measurements of fruit shape has been developed. It is envisioned that this programme will accelerate phenotypic characterisation, and eliminate subjective scoring of many fruit shape traits (Brewer *et al.*, 2006). This development will be of great value for cactus pear researchers since evaluating fruits for shape attributes is time consuming and requires manual measurement of widths and lengths of countless fruits over many seasons.

3.3.1.3 Fruit mass

Significant differences in fruit mass were observed between varieties at $p \leq 0.05$ (Table 3.8). Cactus pear fruit mass is affected by the number of seeds (Barbera *et al.*, 1994), cladode load (Wessels, 1988; Brutsch, 1992; Inglese *et al.*, 1995b), water availability (Barbera, 1984) and ripening time (Brutsch and Scott, 1991; Nerd *et al.*, 1991, Barbera *et al.*, 1994). Nudosa (231.10 g), Skinners Court (186.92 g), and Tormentosa (183.56 g) produced the heaviest fruits during season 1. The mean fruit mass of the varieties evaluated was 165.51 g, which is higher than the minimum acceptable mass for cactus pears destined for exportation (120.00 g) (Inglese *et al.,* 2002) and that of 140.0 g recommended for commercial fruit production in South Africa (Potgieter and Mkhari, 2002). The fruit mass of Nudosa (231.10 g) and Algerian (160.58 g) were higher than previously reported as 180 g and 100 g, respectively (Wessels, 1988).

'Scozzolatura' (the removal of initial blooms in order to delay fruit ripening and harvest) has been shown to increase fruit mass (Barbera *et al*., 1990; 1991; Mulas, 1997), and induce fruit of a better quality (Nieddu *et al*., 1997). Scozzolatura can be used in conjunction with the recommended cultural practices for fruit production to enhance fruit size for varieties with low fruit mass, however, some negative effects are associated with this practise, such as lower TSS, acids, and sugar content (Mulas, 1997).

3.3.1.4 Total soluble solids content

TSS measured as °Brix, is an indication of sugar content. Sugar content is an important criterion of fruit quality for consumers since they prefer sweet fruits (Inglese *et al*., 1995a). In general, as fruits ripen, levels of soluble solids in the cell vacuoles increase as acidity decreases. Ofer (13.77 °Brix), Skinners Court (13.67 °Brix), and Morado (13.16 °Brix) were found to have high TSS content (Table 3.8). The variety Morado is amongst the sweeter varieties and is commercially cultivated in South Africa. Nudosa (11.34 °Brix), Gymno Carpo (11.37 °Brix), and X 28 (11.98 °Brix) had the lowest recorded TSS content. The mean TSS content for the varieties tested was 12.68 °Brix. This TSS level compares well with that recommended for cactus pear fruits (13-15 °Brix) (Barbera *et al*., 1992; Kuti, 1992).

3.3.1.5 Percentage pulp

Cactus pear fruits are of the berry type with a juicy pulp that contains many hard-coated seeds (Barbera *et al*., 1992). The percentage pulp should not be lower than 55-60% in fruits destined for export markets (Inglese *et al.* 1995a). Varieties with the highest percentage pulp content were Malta (63.62%), Meyers (61.70%), and Gymno Carpo (61.55%).

Nepgen, Ficus-Indice, and Skinners Court produced fruit with the lowest percentage pulp at 50.35%, 54.97%, and 55.37%, respectively (Table 3.8). The range of percentage pulp content within all varieties tested (63.62-50.35%) is higher than that previously reported (30-60%) for South African cactus pear varieties (Wessels, 1988). Nepgen was the only variety with a percentage pulp content lower than required for commercial cultivation in South Africa.

3.3.1.6 Number of fruit

Significant differences were observed in the number of fruit remaining after thinning, in September, within varieties (Table 3.8). The number of fruit remaining after thinning is an indication of the fertility of a particular variety in a specific area. Varieties that had the

highest number of fruit after thinning were Ofer (183.00 fruit/plant), Gymno Carpo (176.50 fruit/plant), Turpin (161.10 fruit/plant) and Algerian (155.30 fruit/plant). Skinners Court had a low fertility in this area and produced 26.20 fruit/plant. The ability to produce fruit is influenced by cladode position and orientation and can be related to dry matter accumulation relative to cladode surface area (Garcia de Cortazar and Nobel, 1992). The average fertility of the varieties evaluated was 88.44 fruit/plant for the first season.

3.3.1.7 Peelability

Peelability, the ease with which the peel is removed from the pulp, varied significantly between varieties (Table 3.8). Skinners Court, Nudosa, and Zastron were varieties that were difficult to peel as reflected by very low peelability indices of 1.50, 1.55, and 1.85 respectively. Varieties that allowed easy removal of the peel, were Roedtan (4.85), Cross X (4.75) and Van As (4.65).

3.3.1.8 Fruit width

Fruit width had a low variability within varieties tested (Table 3.8). Varieties that had the widest equatorial diameter were Nudosa (66.00 mm), Skinners Court (62.21 mm), Gymno Carpo (61.81 mm), and Meyers (61.64 mm). Varieties that had the lowest diameter were mainly of the white pulp colour type, namely Nepgen (54.02 mm), Zastron (56.09 mm), Santa Rosa (56.91 mm), and Morado (57.81 mm).

3.3.1.9 Fruit length

There was a low variability of fruit length within varieties tested. Varieties that had the highest fruit length were Nudosa (94.53 mm), Schagen (88.89 mm), and Ficus-Indice (87.75 mm). Varieties with the lowest length were Morado (75.01 mm), Zastron (75.69 mm), and Ofer (76.05 mm).

3.3.1.10 Pulp colour

Varieties within the germplasm block had a wide array of pulp colour (Appendix III). Pulp colour is a determinant of the market to be supplied. Local consumers prefer a white/green pulp whilst overseas consumers prefer a red/orange or purple coloured pulp (Inglese *et al.*, 2002). The majority of the varieties had a dark pink or orange pulp colour, and would thus be suitable to be sold overseas (Table 3.8). Varieties with a white or white/green pulp colour that would suite the preference of the local market were Meyers, Morado, Schagen, Skinners Court, Van As, and Zastron (Table 3.8).

3.3.2 Fruit quality: Season 2

3.3.2.1 Peel thickness

Varieties that had the thickest peels were Nudosa (5.54 mm), Roedtan (5.22 mm), and Van As (5.21 mm) (Table 3.9). Varieties that had the thinnest peels were Cross X (4.13 mm), R1251 (4.28 mm), and Sicilian Indian Fig (4.46 mm). The overall peel thickness of varieties during the second season (4.80 mm) did not differ significantly from that recorded for all varieties in the first season (4.64 mm). Thick peeled dark purple fruit varieties have been found to have low percentage pulp content (Felker *et al*., 2005). Similarly, in this evaluation varieties that had the thickest peels (Nudosa, Roedtan, and Van As) had low percentage pulp content.

3.3.2.2 Fruit shape

Varieties that had the highest values for fruit shape index were Gymno Carpo (0.73), Malta (0.72), and Algerian (0.72) (Table 3.9). This shape index is indicative of an ovoid shape, which is the preferred shape for cactus pear fruit. Varieties with the lowest shape indices were Nepgen (0.60), Zastron (0.62), and Ficus-Indice (0.64). One of the attributes of the perfect cactus pear fruit is glochids that are easily removable by mechanical brushing (Felker *et al*., 2005). Glochids located in the receptacle area are difficult to remove with mechanical brushing techniques. The degree of difficulty in removing these glochids could be influenced by fruit shape. Ovoid shaped fruits are preferred since glochid removal from the receptacle area is easier than for elliptical fruits.

3.3.2.3 Fruit mass

Varieties that had the highest fruit mass were Nudosa (223.10 g), Tormentosa (186.68 g), and X 28 (181.95 g) (Table 3.9). Varieties that had the lowest fruit mass were Nepgen (138.07 g), Ficus-Indice (141.78 g), and Malta (145.85 g). In South Africa fruits meant for the export market must exceed 120 g (Wessels, 1988), thus even these low ranking varieties would still be suitable for exportation based on fruit mass. Using the cultural practices recommended for commercial fruit cultivation (Potgieter, 1997) and under rain fed conditions, all varieties in this genebank produced fruits of an adequate mass for exportation.

TABLE 3.9 FRUIT QUALITY TRAITS OF CACTUS PEAR VARIETIES (SEASON 2)

Variety name	Peelthick	F shape	Fmass	TSS	%Pulp	Fruit no	Peelability	Fruit width	Fruit length	Pulp colour
Algerian	5.11cde	0.72de	155.24abc	12.50a	52.87 abcd	45.50cde	4.35defg	58.38abcde	81.65ab	Dark pink
Berg x Mexican	4.59abcd	0.69cde	170.46bcd	14.29bcd	56.47 cde	29.10abcd	4.00cde	59.28bcde	85.92abcd	Dark pink
Cross X	4.13a	0.67abcde	162.24abcd	12.25a	53.56 abcde	19.30abcd	4.50efg	58.24abcde	87.99abcd	Orange
Ficus-Indice	4.91bcde	0.64abc	141.78ab	14.63cde	49.96 a	46.60cde	4.90g	55.09a	86.39abcd	Orange
Gymno Carpo	4.48abcd	0.72e	158.00abcd	13.09ab	57.69 de	4.20ab	4.70efg	59.86cde	82.84abc	Orange
Malta	4.56abcd	0.72de	145.85ab	15.00cde	57.60 de	18.60abc	4.65efg	56.67abcd	78.99a	Orange
Meyers	4.81abcde	0.69cde	153.80abc	14.12bc	57.39 de	12.30abc	4.90g	57.77abcde	83.39abc	Dark pink
Morado	4.52abcd	0.69bcde	148.60ab	14.51cde	58.63 e	11.10abc	4.50efg	56.75abcd	82.81abc	White
Nepgen	4.67abcd	0.60a	138.07a	15.53de	52.18 abc	42.30cde	1.60a	54.58a	91.61cde	White
Nudosa	5.54e	0.69cde	223.09e	12.00a	53.94 abcde	2.90a	2.70b	66.49f	96.26e	Red/Orange
Ofer	5.05cde	0.68bcde	158.53abcd	15.48de	53.73 abcde	38.50bcde	4.15cdef	58.67abcde	86.19abcd	Orange
R1251	4.28ab	0.69cde	147.17ab	12.77a	54.02 abcde	18.10abc	3.75cd	56.99abcd	82.99abc	Orange
R1259	4.95bcde	0.66abcde	148.84ab	14.68cde	52.14 abc	13.10abc	4.25cdefg	55.58ab	84.73abcd	Orange
Roedtan	5.22de	0.70cde	161.81abcd	14.31bcd	55.89 cde	24.90abcd	4.45defg	58.55	84.13abc	Orange
Santa Rosa	4.56abcd	0.65abcd	157.58abcd	15.06cde	56.28 cde	32.70abcde	4.15cdef	56.54abcd	87.30abcde	Orange
Schagen	5.16cde	0.67abcde	169.28bcd	15.21cde	52.88 abcd	16.50abc	4.75fg	58.62abcde	88.38bcde	White
Sicilian Indian Fig	4.46abc	0.66abcde	152.50abc	15.64e	55.57 bcde	66.00e	3.60c	56.37abc	85.72abcd	Dark pink
Skinners Court	4.81abcde	0.70cde	157.53abcd	15.26cde	53.79 abcde	21.60abcd	1.75a	58.45abcde	86.06abcd	White/ green
Tormentosa	4.69abcd	0.66abcde	186.67d	15.17cde	57.17 cde	54.20de	3.75cd	61.43e	93.71de	Orange
Turpin	5.06cde	0.68bcde	156.39abc	14.28bcd	54.29 abcde	23.90abcd	4.40defg	57.75abcde	85.57abcd	Orange
Van As	5.21cde	0.65abcde	153.40abc	14.99cde	50.45 ab	18.30abc	4.80fg	56.23abc	86.34abcd	White
X 28	4.91bcde	0.67abcde	181.94d	15.08cde	57.09 cde	30.80abcde	4.60efg	60.52de	91.73cde	Orange
Zastron	4.78abcd	0.62ab	153.82abc	14.38cde	54.85 abcde	116.90f	1.70a	56.22abc	91.73cde	White
Grand mean	**4.80**	**0.67**	**160.11**	**14.36**	**54.72**	**30.76**	**3.95**	**58.04**	**86.55**	

Within column values with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple range test. **Peelthick** = peelthickness, **F shape** = fruit shape**, F mass** = fruit mass, **TSS** = Total soluble solids content, **%Pulp** = percentage pulp content, **Fruit no** = number of reproductive buds remaining per plant after thinning. The cultivated varieties, depicted in the blue font, were reported by Brutsch (1979) as being of good potential for commercial fruit production

3.3.2.4 Total soluble solids content

Varieties that had the highest TSS content as measured in °Brix were Sicilian Indian Fig (15.65), Nepgen (15.54), and Ofer (15.48). Varieties that had the lowest TSS content were Nudosa (12.01), Cross X (12.26), and Algerian (12.50) (Table 3.9). The vast majority of the varieties had a higher TSS content over the second season (Table 3.9) compared to the first season (Table 3.8). TSS content, an indication of the sugar content, increased from 12.68°Brix during the first season to 14.36°Brix in the second season (Table 3.9). Similar findings of year-to-year variation in the mean TSS of cactus pear clones had been reported (Wang *et al*., 1997).

3.3.2.5 Percentage pulp

Varieties that had the highest percentage pulp content were Gymno Carpo (57.70), Malta (57.60), and Meyers (57.40). Varieties with the lowest percentage pulp content were Ficus-Indice (49.96), Van As (50.46), and R1259 (52.14) (Table 3.9).

3.3.2.6 Number of fruit

Varieties with the highest number of fruit were Zastron (116.90 fruit/plant), Sicilian Indian Fig (66.00 fruit/plant), and Tormentosa (54.20 fruit/plant). Varieties that produced the lowest number of fruit in the second season (Table 3.9) were Nudosa (2.90 fruit/plant), Gymno Carpo (4.20 fruit/plant), and Morado (11.10 fruit/plant).

3.3.2.7 Peelability

Varieties that were difficult to peel as indicated by the peelability index, were Nepgen (1.60), Zastron (1.70), and Skinners Court (1.75). Of the varieties evaluated (Table 3.9) those that were easy to peel were Ficus-Indice (4.90), Meyers (4.90), and Van As (4.80).

3.3.2.8 Fruit width

Varieties that had the widest diameter (Table 3.9) were Nudosa (66.49 mm), Tormentosa (61.43 mm), and X 28 (60.52 mm). Varieties that had the narrowest diameter were Nepgen (54.58 mm), Ficus-Indice (55.09 mm), and R1259 (55.58 mm).

3.3.2.9 Fruit length

Varieties that had the longest length (Table 3.9) were Nudosa (96.26 mm), Tormentosa (93.71 mm), and X 28 and Zastron (91.73 mm). Varieties that had the shortest lengths were Malta (78.99 mm), Algerian (81.66 mm) and Morado (82.81 mm). Varieties that were the longest also had the widest diameters.

3.3.2.10 Pulp colour

Varieties from Botswana, R1251 and R1259, that did not produce fruit during the first season produced fruits with an orange coloured pulp in the second season (Table 3.9). Pulp colour of the remainder of the varieties remained the same as for season 1.

3.3.3 Phenological and qualitative traits

SEASON 1

The length of the FDP and the ripening time in cactus pear are cultivar dependant, but show large within-plant variability (Inglese *et al*., 1995a). Varieties that had the longest FDP during the first season were Zastron (148 days), Nepgen (148 days), and Skinners Court (141 days) (Table 3.10). The variety with the shortest FDP for this season was Cross X at 113 days. The majority of varieties had a FDP within the 120-130 day range (Table 3.10). Zastron, Nepgen, and Skinners court had the longest FDP as a result of early bud burst.

Reproductive bud break (RBB) of all the varieties was spread over a period of several weeks as has been reported for cactus pear varieties (Wessels and Swart, 1990). The varieties that displayed early reproductive bud break were Zastron and Nepgen during the 2nd week of August, and Skinners Court during the 4th week of August (Table 3.10). The ripening period was more concentrated in the 1st, 2nd and 3rd weeks of January for all the varieties, except for Nudosa which reached FFR during the 4th week of February (Table 3.10).

Fruit mass is influenced by the time of bud emergence, cladode fruit load and environment. Early flush buds (and thereby a longer FDP) were found to produce heavier fruits than late flush buds (Wessels and Swart, 1990). However, Zastron and Nepgen (FDP of 148 days) did not produce the heaviest fruits during the first season (Table 3.8). On the contrary Nepgen, which had a FDP of 148 days (Table 3.10), produced fruits of low mass (141.31 g) within the varieties tested (Table 3.8). Skinners Court, however, had a long FDP (141 days), but produced fruits of high mass (186.92 g). These findings could indicate that some aspects of fruit mass are genetically controlled.

TABLE 3.10 REPRODUCTIVE BUD BREAK, FIFTY PERCENT FRUIT RIPENING AND FRUIT DEVELOPMENT PERIOD FOR SEASON 1

VARIETY	RBB	FFR	FDP
Algerian	1/9	2/1	127
Berg x Mexican	2/9	2/1	120
Cross X	3/9	2/1	113
Ficus-Indice	2/9	3/1	127
Gymno Carpo	2/9	3/1	127
Malta	1/9	2/1	127
Meyers	3/9	3/1	120
Morado	2/9	3/1	127
Nepgen	2/8	1/1	148
Nudosa	2/9	4/1	134
Ofer	1/9	2/1	127
R1251	-	-	-
R1259	-	-	-
Roedtan	2/9	3/1	127
Santa Rosa	2/9	2/1	120
Schagen	2/9	3/1	127
Sicilian Indian Fig	3/9	3/1	120
Skinners Court	4/8	2/1	141
Tormentosa	1/9	3/1	134
Turpin	1/9	2/1	127
Van As	2/9	3/1	127
X 28	1/9	3/1	134
Zastron	2/8	1/1	148
Mean FDP			128

RBB: reproductive bud break
FFR: 50 % fruit ripening
FDP: Fruit development period
Cultivated varieties, depicted in the blue font, were reported by Brutsch (1979) as being of good potential for commercial fruit production
- : Varieties did not produce fruit during this season

SEASON 2

The same varieties that had the longest FDP over the first season (Table 3.10) had the longest FDP in the second season: Nepgen (193 days), Zastron (162 days), and Skinners Court (148 days) (Table 3.11). The FDP were longer for these three varieties during the second season. Similar findings were observed with the effect of time of bud emergence and fruit mass. Contrary to the findings of Wessels and Swart (1990), varieties with shorter FDP, Nudosa (134 days) and Tormentosa (120 days), produced

heavier fruits, 223.09 g and 186.67 g respectively, than those with a longer FDP, Nepgen (138.10 g) and Zastron (153.82 g).

TABLE 3.11 REPRODUCTIVE BUD BREAK, FIFTY PERCENT FRUIT RIPENING AND FRUIT DEVELOPMENT PERIOD FOR SEASON 2

VARIETY	RBB	FFR	FDP	PA
Algerian	3/9	2/1	113	9
Berg x Mexican	2/9	3/1	127	5
Cross X	2/9	3/1	127	5
Ficus-Indice	2/9	2/1	120	5
Gymno Carpo	3/9	2/1	113	9
Malta	3/9	3/1	113	9
Meyers	3/9	2/1	113	8
Morado	3/9	3/1	120	9
Nepgen	4/7	4/2	193	5
Nudosa	4/9	1/2	134	9
Ofer	3/9	2/1	113	7
R1251	1/9	3/1	134	5
R1259	1/9	3/1	134	5
Roedtan	3/9	3/1	120	8
Santa Rosa	4/8	3/1	141	5
Schagen	2/9	3/1	127	5
Sicilian Indian Fig	4/8	2/1	134	5
Skinners Court	1/8	1/1	148	9
Tormentosa	2/9	2/1	120	5
Turpin	2/9	2/1	120	8
Van As	4/8	2/1	127	4
X 28	2/9	3/1	127	5
Zastron	4/7	1/1	162	9
Mean FDP			131	

RBB: Reproductive bud break

FFR: 50% fruit ripening

FDP: Fruit development period

PA : Plant age in 2001 (yrs)

Cultivated varieties, depicted in the blue font, were reported by Brutsch (1979) as being of good potential for commercial fruit production

Cladode load influences fruit mass (Nerd and Mizrahi, 1995), thus one would expect that cladodes with fewer fruit buds will have heavier fruit. This has not been consistent with the present study. This study was carried out in a low-rainfall area therefore not more than eight fruits per cladode were left on a cladode after fruit thinning (Potgieter, 1997). During the first season Cross X had fewer fruit buds per plant (36.60 fruit/plant) than Nudosa (63.10 fruit/plant), but Nudosa produced heavier fruit (231.10 g) compared to that of Cross X (159.85 g). Similarly during the second season Morado, which had been established for nine years, produced lighter fruit (148.60 g) at 11.10 fruit/plant than Tormentosa which had only been established for five years (Table 3.11) and produced heavier fruit (186.67 g) at 54.20 fruit/plant, suggesting that certain varieties naturally

produced larger fruit regardless of cladode load, time of bud emergence or the number of years since establishment. However, a limitation of this study is that the number of seeds of the varieties was not determined. Fruit size also depends on the number of seeds (Barbera *et al*., 1994). Therefore, the number of seeds could be responsible for larger fruits despite cladode load and early bud emergence.

The overall FDP during the second season (131 days) was longer than that recorded for the first season (128 days). Low temperatures during the FDP delay fruit ripening and result in an extended fruit harvest period at both plant and orchard level (Inglese *et al*., 2002). The accumulated heat units during the second season (484.84 HU) were higher than during the first season (461.90 HU). However, the accumulated chill units during the second season (305.50 CU) were also higher than those recorded for the first season (101.50 CU) (Table 3.12). This could indicate that the overall effect of chilling had a greater effect on the length of the FDP than increments in temperature. Varieties that had the shortest FDP of 113 days were Gymno Carpo, Malta, Algerian, Meyers and Ofer (Table 3.11). Knowledge of the FDP is important in crop forecasting, providing farmers with information for harvest planning, price policy and stock management (Moriondo *et al*., 2001).

3.3.4 Effect of microclimatic conditions during fruit development on fruit quality

Variations in TSS content of cactus pear varieties were observed within and between seasons in this study. The mean TSS content of the varieties was 12.68 in season 1, and increased to 14.36 in season 2 (Tables 3.8 and 3.9). The mean rainfall during the FDP was lower during the second season (76.67 mm) as compared to that of the first season of 106.06 mm (Table 3.12). Similar findings have been reported by Wang *et al*. (1997), who reported a mean TSS of 14.0 in 1996, 11.8 in 1997, and 13.6 in 1998 for 24 clones of cactus pear. The total rainfall recorded was 250 mm in 1996, 909 mm in 1997 and 23 mm 1998. High TSS content in cactus pear seems to be associated with drier periods. Various reports of correlations between cladode mineral content and TSS content have been reported. It has been reported that TSS concentration is positively correlated with cladode Mg concentration (Karim *et al*., 1997). In contrast Galizzi *et al*. (2004) have observed a significant negative correlation between TSS and cladode P and Zn concentrations. Future studies on South African varieties in local environments should include determination of whether relationships exist between TSS and Mg, P and Zn concentrations in cladodes.

TABLE 3.12 MEAN CLIMATIC CONDITIONS OVER TWO SEASONS

Parameter	Season 1	Season 2
Average rainfall (mm)	106.06	76.67
Average maximum air temperature (Tmax, °C)	26.26	27.26
Average minimum air temperature (Tmin, °C)	15.41	15.20
Average temperature (Tave, °C)	20.54	20.90
Accumulated heat units (HU)	461.90	484.84
Accumulated positive Richardson chill units (CU)	101.50	305.50
Average maximum relative humidity (RHx,%)	88.22	89.26
Average minimum relative humidity (RHn,%)	53.49	51.50
Average daily evapotranspiration (ETo, mm)	5.18	4.20
Average solar radiation (Rs, MJ/m^2/s)	1074.59	655.81

Fruit shape shifted from being ovoid (shape index 0.70-0.79) in the first season to elliptic (shape index 0.56-0.60) in the second season (Table 3.13). This change in shape can be attributed to an increase in length of the fruit from 81.80 mm in the first season to 86.54 mm in the second season, whilst the equatorial diameter remained constant (59.30 mm in the first season and 58.04 mm in the second season). These changes decreased the fruit shape index and thereby the general shape of the fruit in the second season.

Fruit mass decreased over the second season, from 165.51 g in the first season to 160.11 g in the second season (Table 3.13). The decrease in fruit mass can be attributed to a significant decrease in rainfall during the FDP over the area in the second season (Table 3.12). Fruit development in this area starts with reproductive bud burst in August and ends with 50% fruit ripening the following year in February.

The genebank under study was maintained under dry-land conditions, with rain as the only source of water. Previous reports stated that irrigation and thinning to six fruits per cladode during the FDP significantly increased fruit size. Since water constitutes 85% of the fruit pulp (Cantwell, 1995) periods of less water availability will cause a decrease in pulp content. Higher rainfall, particularly in the last two months of fruit maturation, has been observed to cause an increase in fruit size and higher percentage pulp content (Felker *et al.*, 2002b). Combined thinning and irrigation were reported to increase the frequency of fruits with a mass greater than 100 g (Gugliuzza *et al.*, 2002). However, irrigation without thinning, and vice versa, do not produce a significant increase in fruit size, but only an increase in the frequency of fruits with a mass greater than 100 g (Gugliuzza *et al.*, 2002). In addition, early thinning, taking into consideration the natural crop load of a variety, is required to have a significant effect on fruit size (Inglese *et al.*, 1995b).

TABLE 3.13 MEAN FRUIT QUALITY TRAITS OVER COMBINED SEASONS

Fruit Quality Trait	Season	Value
Peel thickness	1	4.64 mm
	2	4.80 mm
Fruit shape	1	0.73
	2	0.68
Fruit mass	1	165.51 g
	2	160.11 g
%TSS	1	12.68 °Brix
	2	14.36 °Brix
%Pulp	1	58.78%
	2	54.72%
Fruit no	1	88.44 fruit/plant
	2	30.76 fruit/plant
Peelability	1	3.29
	2	3.95
Fruit width	1	59.30 mm
	2	58.05 mm
Fruit length	1	81.80 mm
	2	86.55 mm

TSS = Total soluble solids content, **%Pulp** = percentage pulp content, **Fruit no** = number of reproductive buds remaining per plant after thinning

During the trial period chill units registered for the second season (305.50 CU) were significantly higher than for the first season (101.50 CU) (Table 3.12). Low temperatures during the fruit development period encourage an increase in peel thickness (Nerd *et al*., 1993). There was a slight increase in the overall peel thickness of the varieties evaluated from 4.64 mm during the first season to 4.80 mm during the second season (Table 3.13).

Barbera and Inglese (1993) reported that low temperatures led to a decrease in pulp content. Similar results were obtained during this study since overall percentage pulp content of the varieties decreased from 58.78% during the first season to 54.72% during the second season (Table 3.13). Even though there was a decrease in percentage pulp content during the second season, it remained within the recommended level of between 50-60%, required to maintain the post-harvest quality of fruit (Kader, 1999).

3.3.5 Combined analysis

The ideal cactus pear variety would have the following traits: spine-less cladodes, glochids easily removable by mechanical brushing, tolerance to -9°C, pulp percentage > 55%, °Brix > 13, pulp firmness > 1 kg, mature yield > 20 000 kg/ha, post-harvest shelf life at 2°C > 4 weeks, and seediness < 3 g seeds per 100 g pulp (Felker *et al*., 2002a). In addition it should produce fruit of a variety of colours (yellow, orange, pink, and purple) (Felker *et al*., 2005). With the above mentioned goal in mind the means of the various fruit quality traits were combined for each variety to give the overall performance of individual varieties over both seasons (Table 3.14). In terms of peel thickness Nepgen (5.89 mm), Ficus-Indice (5.35 mm), and Nudosa (5.26 mm) ranked the highest within varieties evaluated. These peel thickness values are still within the recommended (< 6 mm) for fruit varieties in South Africa. Varieties that had the thinnest peels were Cross X (4.20 mm), Malta (4.21 mm), and Gymno Carpo (4.25 mm).

In terms of varieties with high fruit mass, Nudosa (227.10 g), Tormentosa (185.11 g), and X 28 (179.17 g) performed best. Varieties that produced fruits low in fruit mass were Nepgen (139.69 g), R1251 (139.34 g), and R1259 (147.30 g).

Concerning TSS content, varieties that had the highest TSS content were Ofer (14.63°Brix), Sicilian Indian Fig (14.56°Brix), and Skinners Court (14.47°Brix). Varieties that had the lowest TSS content were Nudosa (11.67°Brix), Gymno Carpo (12.23°Brix), and Cross X (12.37°Brix). In terms of percentage pulp content, varieties that had the highest pulp content were Malta (60.61), Gymno Carpo (59.62), and Meyers (59.55). Those with a low percentage pulp were Skinners Court (54.58), Ficus-Indice (52.46), and Nepgen (51.26).

TABLE 3.14 FRUIT QUALITY TRAITS OVER COMBINED SEASONS

Variety	Peelthickness	Fruit shape	Fruit mass	TSS	%Pulp	Fruitno	Peelability	Fruit width	Fruit length
Algerian	4.66 ± 0.63	0.74 ± 0.03	158.05 ± 3.96	12.70 ± 0.28	56.73 ± 5.45	100.40 ± 77.64	3.78 ± 0.81	55.87 ± 0.61	77.75 ± 5.52
Berg x Mexican	4.39 ± 0.29	0.71 ± 0.02	164.42 ± 8.54	13.44 ± 1.21	57.92 ± 2.05	35.70 ± 9.33	4.10 ± 0.14	45.04 ± 0.41	83.81 ± 3.33
Cross X	4.20 ± 0.10	0.71 ± 0.06	161.05 ± 1.69	12.37 ± 0.17	57.13 ± 5.05	27.95 ± 12.23	4.63 ± 0.18	43.77 ± 0.82	83.27 ± 9.82
Ficus-Indice	5.35 ± 0.62	0.66 ± 0.03	157.67 ± 22.47	13.78 ± 1.20	52.46 ± 3.54	53.60 ± 9.90	3.93 ± 1.38	49.66 ± 3.33	85.32 ± 1.52
Gymno Carpo	4.25 ± 0.33	0.75 ± 0.03	165.90 ± 11.16	12.23 ± 1.22	59.62 ± 2.73	90.35 ± 121.83	3.78 ± 1.31	56.75 ± 1.38	83.24 ± 0.56
Malta	4.21 ± 0.50	0.76 ± 0.05	155.66 ± 13.88	13.47 ± 2.16	60.61 ± 4.26	79.45 ± 86.05	3.75 ± 1.27	53.92 ± 2.63	77.76 ± 1.74
Meyers	4.61 ± 0.28	0.73 ± 0.05	162.20 ± 11.89	13.34 ± 1.11	59.55 ± 3.05	84.45 ± 102.04	4.35 ± 0.78	55.50 ± 2.73	80.09 ± 4.67
Morado	4.29 ± 0.33	0.73 ± 0.06	147.53 ± 1.51	13.84 ± 0.96	59.51 ± 1.23	72.45 ± 86.76	3.45 ± 1.48	49.41 ± 0.74	82.39 ± 0.59
Nepgen	5.89 ± 1.72	0.64 ± 0.06	139.69 ± 2.29	14.17 ± 1.93	51.26 ± 1.29	60.10 ± 25.17	2.10 ± 0.71	44.93 ± 0.40	87.48 ± 5.84
Nudosa	5.26 ± 0.40	0.70 ± 0.01	227.10 ± 5.66	11.67 ± 0.47	56.36 ± 3.43	33.00 ± 42.57	2.13 ± 0.81	55.83 ± 0.38	96.22 ± 0.06
Ofer	4.90 ± 0.22	0.74 ± 0.08	155.94 ± 3.66	14.63 ± 1.21	56.35 ± 3.71	110.75 ± 102.18	4.18 ± 0.04	56.67 ± 1.40	83.06 ± 4.42
R1251	4.35 ± 0.10	0.70 ± 0.01	139.33 ± 11.08	12.99 ± 0.30	56.63 ± 3.69	9.05 ± 12.80	3.95 ± 0.28	35.80 ± 1.68	83.89 ± 1.27
R1259	4.26 ± 0.98	0.70 ± 0.05	147.30 ± 2.18	13.24 ± 2.04	56.43 ± 6.08	6.55 ± 9.26	3.80 ± 0.64	37.75 ± 0.28	80.32 ± 6.25
Roedtan	4.89 ± 0.46	0.73 ± 0.04	162.78 ±1.37	13.62 ± 0.99	58.53 ± 3.74	84.70 ± 84.57	4.65 ± 0.28	54.33 ± 1.08	80.62 ± 4.96
Santa Rosa	4.31 ± 0.36	0.67 ± 0.03	157.46 ± 0.17	14.00 ± 1.50	57.88 ± 2.27	36.60 ± 5.52	3.95 ± 0.28	45.07 ± 0.26	82.30 ± 7.07
Schagen	4.79 ± 0.52	0.66 ± 0.00	171.66 ± 3.37	13.77 ± 2.04	55.04 ± 3.05	26.95 ± 14.78	4.65 ± 0.14	45.85 ± 0.23	86.93 ± 2.04
Sicilian Indian Fig	4.66 ± 0.28	0.70 ± 0.05	160.05 ± 10.68	14.56 ± 1.54	55.93 ± 0.50	73.60 ± 10.75	3.35 ± 0.35	52.62 ± 2.19	83.20 ± 3.57
Skinners Court	5.12 ± 0.44	0.72 ± 0.02	172.22 ± 20.78	14.47 ± 1.12	54.58 ± 1.11	24.10 ± 3.54	1.63 ± 0.18	47.07 ± 2.65	87.31 ± 1.77
Tormentosa	4.64 ± 0.08	0.69 ± 0.04	185.11 ± 2.21	13.98 ± 1.68	58.08 ± 1.28	60.50 ± 8.91	3.28 ± 0.67	53.35 ± 0.70	89.11 ± 6.50
Turpin	4.98 ± 0.12	0.68 ± 0.01	167.18 ± 15.26	13.47 ± 1.14	55.12 ± 1.17	92.50 ± 97.02	3.70 ± 0.99	57.24 ± 1.93	87.29 ± 2.44
Van As	4.76 ± 0.64	0.68 ± 0.03	158.79 ± 7.63	13.91 ± 1.52	55.16 ± 6.66	25.40 ± 10.04	4.73 ± 0.11	44.01 ± 1.50	86.43 ± 0.13
X 28	5.00 ± 0.13	0.70 ± 0.04	179.17 ± 3.92	13.53 ± 2.20	58.79 ± 2.40	61.30 ± 43.13	3.65 ± 1.34	52.40 ± 0.27	88.47 ± 2.74
Zastron	4.81 ± 0.03	0.68 ± 0.09	148.35 ±7.73	13.77 ± 0.87	55.57 ± 1.01	121.30 ± 6.22	1.78 ± 0.11	56.00 ± 0.10	82.22 ± 13.44

Mean fruit quality traits over two seasons with their standard deviations. **F mass** = fruit mass, **TSS** = Total soluble solids content, **%Pulp** = percentage pulp content, **Fruit no** = number of reproductive buds remaining per plant after thinning, **Fwidth** = fruit width, **Flength** = fruit length. Cultivated varieties depicted in the blue font, were reported by Brutsch (1979) as being of good potential for commercial fruit production

The overall means of fruit quality traits were combined with phenological and qualitative traits to determine the relatedness between varieties in terms of fruit quality. Varieties grouped into two main clusters (Figure 3.1). Two varieties, Nepgen, and Nudosa clustered separately (Figure 3.1). Nudosa was the most dissimilar of the varieties in terms of fruit quality.

The two most similar varieties were R1251 and R1259, followed by Tormentosa and X 28. Varieties from Botswana (R1251, R1259) clustered together (cluster IId) in a group that had the lowest fruit quality traits of the deduced clusters. This cluster was characterised by a mean fruit mass of 138.63 g, TSS content of 13.11 °Brix, and a 56.53% pulp content. Malta, Algerian, Morado, and Meyers grouped into cluster IIa, consistent with clustering deduced from AFLP data (Figure 2.4) where these varieties grouped together in the same cluster.

The majority of varieties grouped together into cluster II, which can be subdivided into four sub-clusters (Figure 3.1). Ofer, a variety from Israel, although closely related to the South African varieties, clustered separately from them in cluster IIc. Cluster IIb varieties produced the heaviest fruits (168.42 g) with an acceptable TSS content (13.60°Brix) (Table 3.15). Cluster I varieties had the highest TSS content (14.26°Brix) with a percentage pulp content of 55.36 (Table 3.15). Gymno Carpo, Malta, and Algerian grouped into cluster IIa. These varieties were identified by Brutsch (1979) as varieties of promising potential for commercial cultivation in South Africa (Table 3.3).

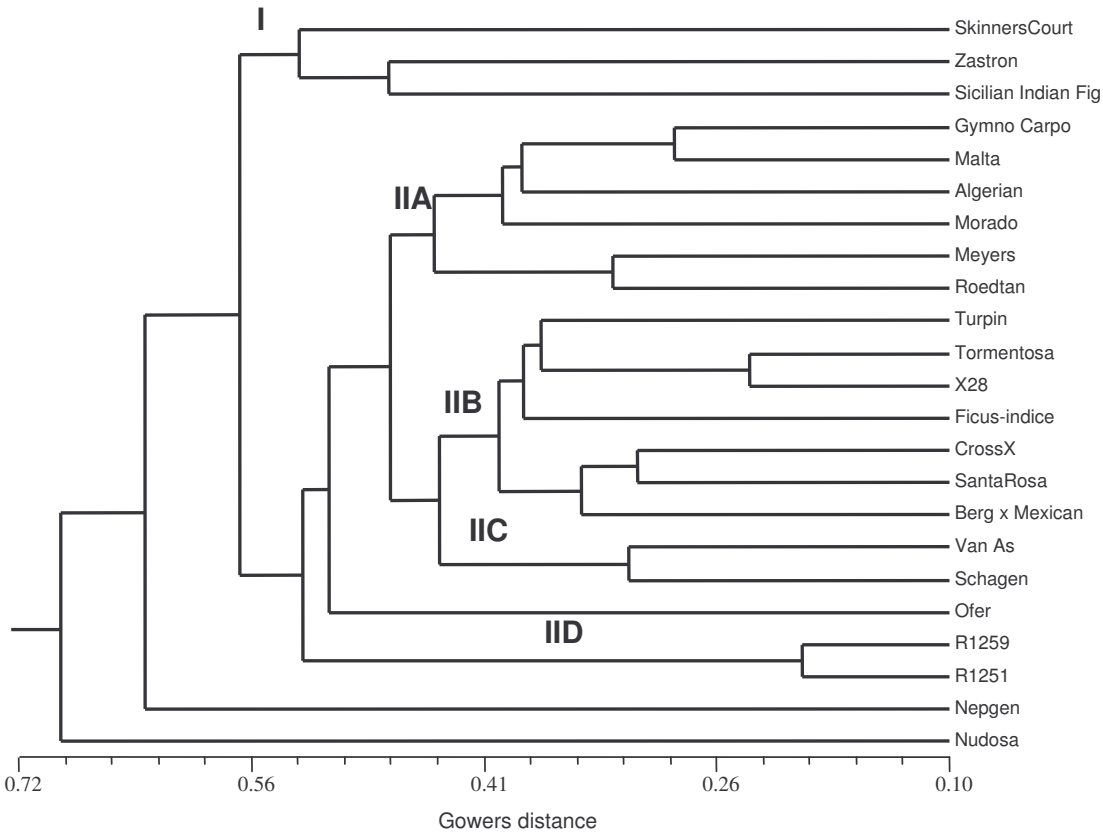


FIGURE 3.1 DENDROGRAM CONSTRUCTED FROM FRUIT QUALITY AND MORPHOLOGICAL TRAITS USING THE GOWER DISSIMILARITY COEFFICIENT

TABLE 3.15 MEAN FRUIT QUALITY TRAITS FOR DENDRGOGRAM CLUSTERS

Cluster	Fmass	Peelthickness	Peelability	%TSS	%Pulp	F shape
I	165.80	4.86	2.25	14.26	55.36	0.70
IIa	165.56	4.56	3.92	13.24	58.52	0.73
IIb	168.42	4.68	4.11	13.60	56.56	0.68
IIc	138.63	4.30	3.88	13.11	56.53	0.70

F mass = fruit mass, **TSS** = Total soluble solids content, **%Pulp** = percentage pulp content, **F shape** = fruit shape

The number of years of establishment played a significant role in the grouping of varieties into clusters. All varieties in cluster I were established for nine years except for Sicilian Indian Fig which had only been established for five years by 2001 (Table 3.11). Similarly all varieties that grouped into cluster IIa were established for eight or nine years in 2001. Other commonly cultivated varieties in South Africa (Morado, Meyers, and Roedtan) also clustered in this group. All the varieties in cluster IIb had been established for five years except for Turpin which was established for eight years, and Van As which had been established for four years in 2001.

Cultivated varieties (Table 3.3) were dispersed among the different clusters, with the greatest percentage grouped in cluster IIa (Figure 3.1). Other commercially cultivated varieties were dispersed throughout the germplasm collection. This finding is important for both cactus pear breeders and farmers as it signifies that commercially cultivated varieties represent the fruit quality diversity present within the germplasm. The dendrogram indicated that based on fruit quality traits, cultivated varieties did not all cluster into one group of closely related varieties. Therefore, the risk of genetic homogeneity within commercially cultivated varieties in South Africa is low. From the findings of this study it is evident that no single variety outperforms all others for all the fruit quality traits evaluated.

Hybrid cactus pear varieties, classified as *Opuntia* spp. (Skinners Court and Zastron) grouped together in cluster I. However, another hybrid variety (Nudosa) did not cluster with the varieties evaluated in this study (Figure 3.1). Varieties classified as *O. ficus-indica* types (Gymno Carpo, Malta, Algerian, and Morado) grouped together in cluster IIa (Figure 3.1). Potgieter and Smith (2006) found that *O. ficus-indica* varieties merged into one group of yields above the mean using the Additive Main Effects and Multiplicative Interactions Analysis (AMMI). Based on the AMMI findings these varieties were recommended for cactus pear fruit production in the Lowveld and Middleveld agro-climatic zones of the Limpopo Province (Potgieter and Smith, 2006).

3.4 CONCLUSIONS

The majority of fruit quality traits investigated in this study can be altered using recommended cultural practises. Adequate irrigation increases plant growth and as a result, fruit yield (Mulas and D'Hallewin, 1997). Harvest size can be increased with thinning of the number of fruits per plant. In South Africa, Wessels (1988) recommended not more that 9-12 fruits per cladode to increase fruit harvest size. Irrigation has been demonstrated to increase fruit size and percentage pulp content (Barbera, 1984; La Mantia *et al*., 1998). Application of nitrogen increases biomass production, fruit mass and TSS content (Potgieter and Mkhari, 2000) while application of phosphorous increases fruit production (Gathaara *et al*., 1989). Fruit quality traits are therefore amiable to manipulation using cultural practices.

Varieties that are recommended for commercial cultivation in the Mokopane district of the Limpopo Province, South Africa, are those grouped in cluster IIa. These varieties are Gymno Carpo, Malta, Algerian, Morado, Meyers, and Roedtan. These varieties meet the minimum requirements for cactus pear fruit production in South Africa

(Potgieter and Mkhari, 2002). However, because most of the traits that govern fruit quality in cactus pear are influenced by environmental conditions, cultivar recommendations will have to be determined for each of the different climatic regions in South Africa.

Previous studies have shown that fruit size and shape are affected by seed number and weight (Barbera, 1995). New consumers dislike seedy cactus pears and low pulp firmness (Felker *et al*., 2005) which have not been investigated in this study. A study investigating the seed content of the varieties in clusters IIa and IIb are recommended in order to determine the extent to which they affect fruit size.

REFERENCES

Acocks, J.P.H., 1952. Veld types of South Africa 57, pp 146. Department of Agriculture and Water Supply, Government printer. Pretoria, South Africa.

Arba, M., M.C. Benismail and M. Mimoun, 2002. The cactus pear (*Opuntia* spp.) in Morocco: Main species and cultivar characterization. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Conference on Cactus Pear and Cochineal. Acta Horticulturae 581: 103-109.

Barbera, G., 1984. Ricerche sull'irrigazione del ficodindia. Frutticoltura 8: 49-55.

Barbera, G., 1995. History, economic, and agro-ecological importance. In: Barbera G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear. FAO Plant production and protection paper 132. Rome, Italy. pp 19-35.

Barbera, G. and P. Inglese, 1993. La Coltura del Ficodindia. Calderini Edagricoe, Bologna.

Barbera, G., F. Carimi and P. Inglese, 1991. The re-flowering of prickly pear *Opuntia ficus-indica* (L.) Miller: Influence of removal time and cladode load on yield and fruit ripening. Advances in Horticultural Science 2: 77-80.

Barbera, G., F. Carimi, P. Inglese and M. Panno, 1992. Physical, morphological and chemical changes during fruit development and ripening in three cultivars of prickly pear, *Opuntia ficus-indica* (L.) Miller. Journal of Horticultural Science 67: 307-312.

Barbera, G., F. Carimi, P. Inglese and P. Sajeva, 1990. The intensive growing of prickly pear *Opuntia ficus-indica* Mill. for fruit production in Sicily. Actas del Congreso sobre "El nopal su conocimiento y aprovechamiento". Buenavista (Mexico), Diciembre: 127-144.

Barbera, G., P. Inglese and T. La Mantia, 1994. Seed content and fruit characteristics in cactus pear (*Opuntia ficus-indica* Mill.). Scientia Horticulturae 58: 161-165.

Brewer, M.T., L. Lang, K. Fujimura, N. Dujovic, S. Gray and E. Van Der Knaap, 2006. Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. Plant Physiology 141: 15-25.

Brutsch, M.O., 1979. The prickly pear (*Opuntia ficus-indica*) as a potential crop for the drier regions of the Ciskei. Crop Production 8: 131-136.

Brutsch, M.O., 1992. Crop manipulation in spine-less prickly pear (*Opuntia ficus-indica*) in South Africa. In: Proceedings of the Second International Conference on Cactus Pear and Cochineal. Santiago, Chile, pp 40-47.

Brutsch, M.O. and M.B. Scott, 1991. Extending the fruiting season of spine-less prickly pear (*Opuntia ficus-indica*). Journal of the South African Society of Horticultural Science 1: 73-76.

Cantwell, M., 1986. Post-harvest aspects of prickly pear fruits and vegetable cladodes. In: Perishables handling, post-harvest technology of fresh horticultural crops. Cooperative extension, University of California, 59: 6-9.

Cantwell, M., 1991. Quality and post-harvest physiology of 'nopalitos' and 'tunas'. In: Proceedings of the Second Annual Texas Prickly pear Conference, McAllen, Texas, pp 50-66.

Cantwell, M., 1995. Post-harvest management of fruits and vegetable stems. In: Barbera, G., P. Inglese and E. Pimienta-Barrios (Eds.), Agroecology, Cultivation and Uses of Cactus pear, FAO Plant Production and Protection Paper 132. FAO, Rome, pp 120-136.

Chapman, B., C. Mondragón-Jacobo, R.A. Bunch and A.H. Paterson, 2002. Breeding and Biotechnology. In: Nobel, P.S., (Ed.), Cacti: Biology and Uses, pp 255-271. University of California Press, California, USA.

Chessa, I. and G. Nieddu, 1997. Descriptors for cactus pear (*Opuntia* spp.). CACTUSNET, FAO – Universitá degli Studi di Reggio Calabria. Rome, Italy.

Chessa, I., G. Nieddu and M. Mulas, 1995. Variability of *Opuntia ficus-indica* in a Mediterranean area: definition of a descriptor list and germplasm characterization. In:

Proceedings of the Second International Congress on Cactus Pear and Cochineal, Guadalajara, Mexico.

Felker, P., C. Soulier, G. Leguizamon and J. Ochoa, 2002a. A comparison of the fruit parameters of 12 *Opuntia* clones grown in Argentina and the United States. Journal of Arid Environments 52: 361-370.

Felker, P., F. Galizi and C. Soulier, 2002b. Variability within *Opuntia ficus-indica* Mill. cv. "Yellow Fruit without spines" in Santiago del Estero, Argentina and individual plant selection for improved yield and fruit characteristics. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 95-101.

Felker, P., S. del C. Rodriguez, R.M. Casoliba, R. Filippini, D. Medina and R. Zapata, 2005. Comparison of *Opuntia ficus-indica* varieties of Mexican and Argentine origin for fruit yield and quality in Argentina. Journal of Arid Environments 60: 405-422.

Franco, J., J. Crossa, S. Taba and H. Shands, 2005. A sampling strategy for conserving genetic diversity when forming core subsets. Crop Science 45: 1035-1044.

Galizzi, F.A., P. Felker, C. González and D. Gardiner, 2004. Correlations between soil and cladode nutrient concentrations and fruit yield and quality in cactus pears, *Opuntia ficus-indica* in a traditional farm setting in Argentina. Journal of Arid Environments 59: 115-132.

Garcia de Cortazar, V. and P.S. Nobel, 1992. Biomass and fruit production for the prickly pear cactus, *Opuntia ficus-indica*. Journal of the American Society of Horticultural Science 117: 568-572.

Gathaara, G.N., P. Felker and M. Land, 1989. Influence of nitrogen and phosphorous on *Opuntia engelmannii* tissue N and P concentrations, biomass production and fruit yield. Journal of Arid Environments 16: 337-346.

Gibson, A. and P. Nobel, 1986. The cactus primer. Harvard University Press, Cambridge.

Gower, J.C., 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Griffiths, D. and R.F. Hare, 1906. Prickly pear and other cacti as food for stock. II. New Mexico College of Agriculture and Mechanic Arts. Agriculture Experiment Station Bulletin 60: 125.

Gugliuzza, G., P. Inglese and V. Farina, 2002. Relationship between fruit thinning and irrigation on determining fruit quality of cactus pear (*Opuntia ficus-indica*) fruits. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Conference on Cactus Pear and Cochineal. Acta Horituclturae 581: 205-211.

Hills, F.S., 1995. Anatomy and physiology. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear. FAO Plant production and protection paper 132. Rome, Italy. pp 34.

Inglese, P., F. Basile and M. Schirra, 2002. Cactus pear fruit production. In: Nobel, P.S. (Ed.), Cacti: Biology and Uses, pp 163-183. University of California Press, California, USA.

Inglese, P., G. Barbera and T. La Mantia, 1995a. Research strategies for the improvement of cactus pear (*Opuntia ficus-indica*) fruit quality and production. Journal of Arid Environments 29: 455-468.

Inglese, P., G. Barbera, T. Le Mantia and S. Protoleuo, 1995b. Crop production, growth and ultimate size of cactus pear fruit following fruit thinning. Horticultural Science 30.

Kader, A.A., 1999. Produce facts: Cactus (prickly) pear. Perishable Handling Quartely 100: 15-16.

Kader, A.A., 2000. Cactus (prickly) pear: Recommendations for maintaining post-harvest quality. Post-harvest Technology research and Information Centre, California, U.S.A.

Karim, M.R., P. Felker and R.L. Bingham, 1997. Correlations between cactus pear (*Opuntia* spp) cladode nutrient concentrations and fruit yield and quality. Annals of Arid Zones 37: 159-171.

Kuti, J.O., 1992. Growth and compositional changes during the development of prickly pear fruits. Journal of Horticultural Science 67: 861-868.

La Mantia, T., G. Barbera, G. Gugliuzza and P. Inglese, 1998. Interazione tra irrigazione ed entitá di frutti-ficazione e qualitá dei frutti di ficodindia (*O. ficus-indica* (L.) Mill.). Atti Giornate Scientifiche SOI. San Remo, Italy, pp 241-242.

Moriondo, M., S. Orlandini, P. De Nuntiis and P. Mandrioli, 2001. Effect of agrometeorological parameters on the phenology of pollen emission and production of olive trees (*Olea europa* L.). Aerobiologica 7: 225-232.

Mulas, M., 1997. Flower removal time and fruit quality in cactus pear (*Opuntia ficus-indica* Mill). In: Inglese, P. and M.O. Brutsch (Eds.), Proceedings of the Third International Congress on cactus pear and Cochineal. Acta Horticulturae 438: 123-128.

Mulas, M. and G. D'Hallewin, 1997. Fruit quality for four cactus pear (*Opuntia ficus-indica* Mill.) cultivars as influenced by irrigation. In: Inglese, P. and M.O. Brutsch (Eds.), Proceedings of the Third International Congress on cactus pear and Cochineal. Acta Horticulturae 438: 115-120.

Nerd, A. and Y. Mizrahi, 1995. Reproductive biology. In: Barbera, G., P. Inglese and E. Pimienta-Barrios (Eds.), Agroecology, Cultivation and Uses of Cactus pear, FAO Plant Production and Protection Paper 132. FAO, Rome, pp 49-57.

Nerd, A., A. Karady and Y. Mizrahi, 1991. Out-of season prickly pear: fruit characteristics and effect of fertilization and short droughts on productivity. Horticultural Science 26: 527-529.

Nerd, A., R. Mesika and Y. Mizrahi, 1993. Effect of N fertilizer on the autum floral flush and cladode N in prickly pear *Opuntia ficus-indica* (L.) Mill. Journal of Horticultural Science 68: 337-342.

Nieddu, G., I. Chessa, D. Satta, L. De La Pau and M. Pala, 2002. Description of six cactus pear (*Opuntia ficus-indica* Mill.) fruit cultivars from Italy. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Conference on Cactus Pear and Cochineal. Acta Horticulturae 581: 125-129.

Nieddu, G., L. De Pau, M. Schirra and G. D'Hallewin, 1997. Chemical composition of fruit and seeds of cactus pears during early and late-induced crop ripening. In: Inglese, P. and M. Brutsch (Eds.), Proceedings of the Third International Conference on Cactus Pear and Cochineal. Acta Horticulturae 438: 105-111.

Ochoa, J., 1997. Cactus pear (*Opuntia* spp.) varieties main characteristics at Republica Argentina. Inglese, P. and A. Nefzaoui (Eds.). CACTUSNET-FAO.

Potgieter, J.P., 1997. Guidelines for the cultivation of spine-less cactus pears for fruit production. Second Edition. Group 7 Trust Publishers, Sinoville, SA.

Potgieter, J.P., 2002. Potential of cactus pear (*Opuntia* spp.) as a multi-purpose crop for emerging farmers in semi-arid regions of the Limpopo Province. Third SANCRA Congress, 25-28 June 2002, Nelspruit, South Africa.

Potgieter, J.P. and J.J. Mkhari, 2000. The effects of N, P, K and lime on cactus pear (*Opuntia* spp.) fruit yield and quality. Fifth International Cactus Pear and Cochineal Congress, October 2000, Hammamet, Tunisia.

Potgieter, J.P. and J.J. Mkhari, 2002. Evaluation of cactus pear (*Opuntia* spp.) germplasm for fruit production purposes. Combined Congress, 15-17 January 2002, Pietermaritzburg, Kwazulu/Natal.

Potgieter, J. P. and M. Smith, 2006. Genotype X Environment interaction in cactus pear (*Opuntia* spp.), Additive Main Effects and Multiplicative interaction of fruit yield. Acta Horticulturae 728: 97-104.

Rohlf, F.J., 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology 47: 147-158.

SPSS for Windows, 22 December 1997. Copyright© SPSS Inc.

Valdez, C.R., C. Gallegos and M. Blanco, 2002. Agrupamiento Jeraquico de veintinueve genotipos de *Opuntia* spp. Mediante caracteristicas del fruti (tuna). In: Nefzaoui, A. and P. Inglese (Eds.), Congreso Nacional y 4 th Congreso Internacional sobre el conocimiento y Aprovechamiento del Nopal. Acta Horticulturae 581: 3-7.

Van der Knaap, E. and S.D. Tanksley, 2003. The making of a bell pepper-shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer tomato. Theoretical and Applied Genetics 107: 139-147.

Wang, X., P. Felker and A. Paterson, 1997. Environmental influences on cactus pear fruit yield, quality and cold hardiness and development of hybrids with improved cold hardiness. Journal of the Professional Association for Cactus Development 2: 48-59.

Wessels, A.B., 1988. Spine-less prickly pear. Perskor Publishers, Johannesburg, pp 21-24.

Wessels, A.B. and E. Swart, 1990. Morphogenesis of the reproductive bud and fruit of the prickly pear *Opuntia ficus-indica* (L.) Mill. cv. Morado. Acta Horticulturae 275: 245-253.

Wessels, A.B., L.L. van der Merwe and H. du Plessis, 1997. Yield variation in clonally propagated *Opuntia ficus-indica* (L.) Miller plants when terminal cladodes are used. In: Inglese, P. and M.O. Brutsch (Eds.), Proceedings of the Third International Congress on cactus pear and Cochineal. Acta Horticulturae 438: 73-76.

Chapter 4

Evaluation of South African cactus pear (*Opuntia* spp.) varieties for specific use as fodder

ABSTRACT

Spine-less cactus pear varieties were originally introduced to South Africa in 1914 for use as fodder. However, they are increasingly being cultivated for fruit, and the trees are pruned annually. Cladodes removed with pruning can be used as fodder, yet they are considered a waste product and destroyed at huge cost to the farmer. Hence, a study was undertaken to evaluate the nutritional quality of cladodes removed with pruning from a commercially maintained orchard, (Waterkloof) outside Bloemfontein. Crude protein (CP), organic matter (OM) and dry matter (DM) content were determined. In addition, vegetative yield under commercial orchard practices were evaluated for the Mokopane district of the Limpopo Province. Vegetative yield was assessed from data collected over two seasons (1999-2000, 2000-2001) at the Gillemberg germplasm block. The Gower distance was used as a measure of diversity between the different varieties. Two varieties, Turpin and Meyers, performed well in terms of vegetative vigour, with the mean number of cladodes removed by pruning being 107.60 ± 43.91 and 91.90 ± 37.12 cladodes per plant respectively. The variety Morado had a high number of cladodes removed by pruning, with 72.50 ± 25.88 cladodes removed per plant. The varieties Sicilian Indian Fig (89.35 ± 22.31 kg fresh material), Turpin (88.89 ± 25.62 kg fresh material), and Meyers (84.67 ± 24.58 kg fresh material) produced the highest biomass yield. The varieties Malta (109.24 g CP/kg DM), Gymno Carpo (108.23 g CP/kg DM), and American Giant (102.83 g CP/kg DM) had the highest CP content and are recommended for use as fodder. Analysis of variance showed significant ($p \leq 0.05$) differences in CP and OM content amongst the varieties tested, indicating the presence of substantial variability. Animal performance testing to measure the digestibility and palatability of these promising varieties is recommended to assess their effectiveness under practical feeding conditions.

4.1 INTRODUCTION

South Africa has been subject to severe droughts since the 1990s. It is predicted that the Western and Northern Cape regions will experience more droughts, whilst Limpopo, Gauteng, Mpumalanga and KwaZulu-Natal will suffer long dry spells followed by torrential rain and flooding (Kigotho, 2005). This change in climate will have a drastic negative effect on farming in the drier, drought prone regions of South Africa. This has led to an increasing number of South African livestock farmers planting cactus pear for use as feed during dry spells (Le Houérou, 1992; Martin, 1993).

Internationally, cactus pear (*Opuntia* spp.) has found huge application as a drought tolerant feed in arid and semi-arid regions of the world (Cordeiro Dos Santos and Gonzaga De Albuquerque, 2001; De Kock, 2001; Nefzaoui and Ben Salem, 2001; Tegegne, 2001). It is more efficient at converting water to dry matter (digestible energy) than C_3 grasses and C_4 broadleaves (Nobel, 1995; Han and Felker, 1997). It responds well to fertilization (Nobel *et al*., 1987; Nobel, 1988), can withstand pruning (Inglese *et al*., 2002), and can be fed as forage, or stored as silage (FAO, 2000; Nefzaoui and Ben Salem, 2002).

Opuntias are highly digestible (Nefzaoui and Ben Salem, 2002) and contain sufficient water and minerals that in combination with a protein source constitute a complete feed for livestock (Kueneman, 2001). Cactus pear can therefore be used to substitute grass hay for up to 20% for the maintenance of livestock live weight (Tegegne, 2002). In addition, *Opuntia* spp. meet most of the requirements for fodder crops in drought prone regions (De Kock, 1980), drought tolerance and palatability for animals. Other important requirements include adaptability to marginal land, ease of propagation, persistency, DM yield, digestibility and nutrient content (Tegegne, 2001).

In South Africa, cactus pear trees in commercial fruit plantations are pruned annually during winter and the cladodes discarded as waste. This pruned material can alternatively be used as a valuable source feed for livestock (Oelofse *et al*., 2006). These varieties have however, not been evaluated for use as fodder for livestock. It thus became the aims of this study 1) to assess the nutritional quality of different cactus pear varieties, 2) to evaluate the varieties for differences in cladode yield, and 3) to determine which varieties are suitable as fodder crops with good vegetative yield and nutritional composition.

4.2 MATERIALS AND METHODS

4.2.1 Nutritional quality analysis

4.2.1.1 Trial site 1

Plant material of 39 cactus pear varieties was collected from a five year old cactus pear orchard (Waterkloof), outside Bloemfontein (29º 06' S, 26º 18' E). The germplasm was maintained by weeding thrice annually. Five plants per variety were planted at a spacing of 3 m x 5 m (667 plants/ha) in single rows oriented in an East/West direction. No supplementary irrigation was given, and standard orchard practices as recommended by Potgieter (1997) were followed throughout the duration of the trial. The germplasm block was maintained as a commercial fruit orchard and all generally accepted orchard practises such as pruning, and pad thinning were performed. Cladodes were sampled in September 2006.

4.2.1.2 Climatic data

Climatic data was captured via an automatic weather station (Mike Cotton Systems) installed 50 m from the site (Appendix IV). Mean daily values for temperature (°C), rainfall (mm), heat units (HU), chill units (CU), evapotranspiration (ETo), and solar radiation (Rs) were summarised to mean monthly values.

4.2.1.3 Dry matter content

Three cladodes of each variety were selected; the middle third of the cladode was cut out and dried at 80℃ for 48 hours. Sections were pre-cut into strips to facilitate quick drying. The weight of the fresh as well as dried samples was recorded and the dry matter content calculated. The dried cladode material was milled through a 1 mm sieve, to facilitate further analysis. The following equation was used to determine the dry matter content (g/kg fresh material):

$$Dry\ matter\ (g\ DM\ /\ kg\ wet\ weight) = \frac{Weight\ before\ drying - Weight\ after\ drying}{Weight\ before\ drying}\ X\ 1000$$

4.2.1.4 Organic matter content

Two grams of milled cladode material was dried overnight at 100°C, cooled in a desiccator, and the weight of the crucible and dried sample recorded. Samples were incinerated at 550°C for three hours. The weight of the ash contained in the silica bowls were recorded after cooling in a desiccator for 20 minutes. Organic matter (OM) was calculated by subtracting the percentage ash from 100. The following equation was used to determine the OM content:

$$OM\,(g/kg\ DM) = 100 - \%ASH$$

4.2.1.5 Crude protein content

Approximately 0.2 g DM material was weighed into a foil cup and inserted into the Leco® Nitrogen analyser (Leco® Corporation, 2001). The total nitrogen (N) content was determined on combustion in oxygen, and a factor of 6.25 used to convert N content to CP content. All CP determinations were done in duplicate.

4.2.2 Evaluation of vegetative growth

4.2.2.1 Trial site 2

The trial site description is as given in section 3.2.1. Climate and soil characteristics can be found in Table 3.2. Trial layout and data collection methods are given in section 3.2.1.

During the course of the study, data for 14 morpho-agronomic characters were captured for each variety (Table 4.1). Data for nine quantitative characters were collected as an average value of the 10 central plants per variety. The method for quantitative trait data collection is described in Table 4.1. Data for five qualitative characters were collected according to a simplified version of the cactus pear (*Opuntia* spp.) descriptors list (Chessa and Nieddu, 1997). Each accession was scored for the most frequent character state.

TABLE 4.1 MORPHO-AGRONOMIC TRAITS AND SHORT DESCRIPTIONS

Character number	Character and descriptive value
	Quantitative traits
1	Water content **WC (g/kg)**
2	Organic matter content **OM (g/kg DM)**
3	Crude protein content **CP (g CP/kg DM)**
4	Total number of cladodes pruned from each plant for 10 plants per variety **cladno**
5	Total number of cladodes remaining on 10 plants per variety after pruning **cladleft**
6	Cladode yield per plant: the weight of the total number of cladodes pruned from 10 plants per variety **cyieldp (kg)**
7	Cladode mass: derived by dividing the number of cladodes pruned by the cladode yield per variety **cmass (kg)**
8	Plant age: number of years since the variety had been established in 2001 **pa**
	Qualitative traits
9	Vegetative bud break **(VBB)**; week of a particular month during which vegetative buds are clearly visible: (1) 1-7 August, (2) 1-7 September, (3) 1-7 October:
10	Flower petal colour **(FPC)**: (1) dark yellow, (2) yellow, (3) orange, (4) unknown
11	Country of origin **(COO)**: (1) South Africa, (2) Botswana, (3) Israel, (4) Italy
12	Cladode shape **(CS)**: (1) elliptic, (2) ovate, (3) large diamond, (4) round
13	Plant habit **(PH)**: (1) bush/shrubby, (2) spreading, (3) upright, (4) aborescent

4.2.3 Statistical analysis

Data for quantitative nutritional traits were subjected to analysis of variance using the SPSS (SPSS Inc, 1997) statistical package. The Tukey multiple range test was used to identify varieties that were significantly different from each other. The Gower distance (Gower, 1971) was used as a measure of diversity between the different varieties as described in section 3.2.4. Gower distances were used to compute a dissimilarity matrix, and used for dendrogram construction using UPGMA and the NTSYS-pc programme (Version 2.02i, Rohlf, 1998).

4.3 RESULTS AND DISCUSSION

4.3.1 Nutritional quality

4.3.1.1 Dry matter content (DM)

Cladode nutritional quality of varieties obtained from a commercially maintained orchard in Bloemfontein, South Africa was assessed in early September 2006. The climatic conditions prevalent during the preceeding winter months (Appendix IV) were characterised by low rainfall and chilly conditions (Table 4.2). Cladodes were sampled during this time as it is recommended that pruning be performed when the plants are dormant, during winter in South Africa (Potgieter, 1997). In this study substantial differences amongst the varieties tested for DM content (Table 4.3) were detected. DM is the component left in feed after drying and is strongly influenced by many factors including species, genotype, variety, soil, climate, and season (López-García *et al*., 2001). Varieties that produced the highest amount of DM were Messina (79.80 g DM/kg fresh material), Nepgen (78.62 g DM/kg fresh material), and Cross X (74.14 g DM/kg fresh material). Varieties that produced the lowest amount of DM were Gymno Carpo (52.89 g DM/kg fresh material), Ficus Indice (55.29 DM/kg fresh material), and Fusicaulis (55.97 g DM/kg fresh material) (Table 4.3).

TABLE 4.2 MEAN CLIMATIC CONDITION PRIOR TO NUTRITIONAL QUALITY ASSESSMENT

Parameter	June	July	August	September
Solar radiation (Rs, MJ m^2/s)		12.08	14.58	21.02
Average temperature (°C)	8.60	10.48	10.50	15.41
Average humidity (%)		45.74	59.72	41.29
Average rainfall (mm)	1.30	1.51	2.34	1.86
Total rainfall (mm)	0.51	0.00	86.34	0.76
Average maximum temperature (°C)	17.90	19.27	17.73	23.83
Absolute maximum temperature (°C)	23.23	25.29	25.05	29.38
Average minimum temperature (°C)	0.46	2.29	3.79	7.02
Absolute minimum temperature (°C)	-4.08	-6.21	-3.24	0.81
Average evapotranspiration per day (mm)		2.52	2.76	4.38

4.3.1.2 Crude protein content (CP)

Analysis of variance (ANOVA) results indicated that there were significant differences in CP content between varieties at $p \leq 0.05$. Cactus pear alone, as feed, is not complete to fill the dietary requirements of livestock (Nefzaoui and Ben Salem, 2001). Cladodes are low in CP and should be supplemented with protein sources.

Guevara *et al.* (2004) reported that the mean CP determined for the clones they tested was below the requirements for maintaining 40 kg goats (7.7%) and for dry pregnant mature cows (5.9%) as recommended by the National Research Council.

Protein content varies with plant age, cladode age, and variety. In addition, crude protein content is strongly influenced by soil fertility and crop management (Mondragón-Jacobo and Pérez-González, 2001), but can be increased by the application of nitrogen fertiliser. The CP increased from 5.5% for unfertilised varieties up to 9.9% with the application of 224 kg N/ha (Gonzalez, 1989). Varieties with the lowest CP content were Messina (44.13 g CP/kg DM), Cross X (62.95 g CP/kg DM) and Algerian (60.97 g CP/kg DM). Interestingly varieties Messina and Cross X produced the highest dry matter amongst the varieties tested. Varieties that produced the highest CP content were Malta (109.24 g CP/kg DM), Gymno Carpo (108.23 g CP/kg DM), and American Giant (102.83 g CP/kg DM) (Table 4.3).

4.3.1.3 Organic matter content (OM)

The majority of the varieties were significantly different in terms of OM content (Table 4.3). Varieties with low OM content were Gymno Carpo (746.30 g OM/kg DM), Fresno (750.50 g OM/kg DM), and Blue Motto (757.50 g OM/kg DM). Similarly, low OM content values have been reported for cactus pear by other researchers, 74.6% by Ben Salem *et al.* (2002) and 76.2% by Ben Salem *et al*. (2004). In contrast, the mean OM content for the varieties tested was 785.72 g OM/kg DM and lower than previous reports of an OM content of 84.4% (844 g OM/kg DM) for cactus pear (Guevara *et al.*, 2004). The highest OM within the germplasm was recorded for the varieties Cross X (833.03 g OM/kg DM), Muscatel (818.07 g OM/kg DM), and Algerian (816.80 g OM/kg DM), and Sicilian Indian Fig (816.37 g OM/kg DM) (Table 4.3).

TABLE 4.3 NUTRIENT COMPOSITION OF DIFFERENT CACTUS PEAR VARIETIES (DRY MATTER BASIS)

VARIETY	Dry matter content (g DM/kg fresh material)	Composition (g/kg DM) [a]CP	[a]OM
ALGERIAN	72.82	60.97 b	816.80 o
AMERICAN GIANT	72.45	102.83 s	798.67 jkl
AMERSFOORT	73.80	73.60 cdef	800.53 kl
ARBITER	59.36	100.51 rs	775.93 def
BERG X MEXICAN	63.83	82.54 hijk	796.03 ijkl
BLUE MOTTO	63.02	85.35 ijklmn	757.50 abc
CORFU	59.91	77.72 fgh	784.70 efghi
CROSS X	74.14	62.95 b	833.03 o
DIREKTEUR	63.30	83.43 ijkl	759.53 bc
FICUS-INDICE	55.29	87.91 lmno	788.80 ghijk
FRESNO	60.23	90.85 op	750.50 ab
FUSICAULIS	55.97	89.01 nop	757.13 abc
GYMNO CARPO	52.89	108.23 t	746.30 a
MALTA	60.96	109.24 t	774.67 de
MESSINA	79.80	44.13 a	804.93 lmn
MEXICAN	61.42	85.49 ijklmn	760.37 bc
MEYERS	63.95	85.38 ijklmn	789.33 hijk
MORADO	64.47	95.86 qr	788.93 ghijk
MUSCATEL	64.10	68.81 c	818.07 o
NEPGEN	78.62	72.23 cde	814.63 mno
NUDOSA	69.66	86.48 jklmno	772.50 d
OFER	63.07	81.27 ghi	784.57 efghi
R1251	64.00	83.00 ijkl	791.03 ijk
R1259	67.78	91.34 opq	790.43 hijk
R1260	63.29	93.16 opq	777.27 defg
ROBUSTA X CASTILLO	66.32	70.22 c	786.53 fghi
ROEDTAN	68.60	80.67 ghi	778.93 defgh
ROLY POLY	66.92	76.81 efg	759.73 bc
ROSSA	69.53	90.59 op	767.83 cd
SANTA ROSA	59.59	75.37 def	787.03 fghij
SCHAGEN	65.50	87.03 klmno	785.23 efghi
SHARSHERET	61.00	71.61 cd	789.07 ghijk
SICILIAN INDIAN FIG	64.36	62.57 b	816.37 no
SKINNERS COURT	64.33	81.92 hij	794.33 ijkl
TORMENTOSA	64.69	82.56 hijk	760.17 bc
TURPIN	71.95	88.67 mnop	803.83 lm
VAN AS	69.47	87.31 klmno	796.03 ijkl
VRYHEID	58.51	83.95 ijklm	799.43 kl
ZASTRON	66.50	89.23 nop	786.33 efghi

[a] Within column values with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple range test. **DM** = Dry matter content; **CP** = Crude protein content; **OM** = Organic matter content

4.3.2 Vegetative growth over combined seasons

4.3.2.1 Number of cladodes removed with pruning

It is common practise to prune cactus pear plants to maintain plant height at 1.8 m and improve fruit size (Potgieter, 1997). Cladodes that are removed in this way are usually discarded by farmers. These cladodes are however, a useful by-product of fruit production that can be used as fodder for livestock. The mean number of cladodes pruned per variety per year is a good indication of the amount of pruned material that can be used as fodder. Plants can be pruned from the first year after establishment.
It is important to prune plants in order to allow sufficient sunlight into the plant for the cladodes to be productive, to facilitate the early detection and control of cochineal, and to facilitate harvesting (Potgieter, 1997).

There was a higher mean number of cladodes pruned (cladno) from all the varieties in 1999–2000 (season 1) (53.02 cladodes pruned/plant) as compared to (33.83 cladodes pruned/plant) the following season 2000–2001 (season 2) (AppendixVI). Turpin (107.60 cladodes pruned/plant), Meyers (91.90 cladodes pruned/plant), and Morado (72.50 cladodes pruned/plant) had the highest mean number of cladodes pruned in season 1. The following season the highest mean number of cladodes removed with pruning was recorded for Sicilian Indian Fig (55.30 cladodes pruned/plant), Zastron (50.70 cladodes pruned/plant) and Turpin (45.50 cladodes pruned/plant).

Varieties with the lowest number of cladodes removed with pruning for season 1 were Skinners Court (22.90 cladodes pruned/plant), Tormentosa (23.30 cladodes pruned/plant), and Nepgen (23.90 cladodes pruned/plant). Van As (16.30 cladodes pruned/plant), Cross X (22.00 cladodes pruned/plant), and Skinners Court (22.90 cladodes pruned/plant) had the lowest mean number of cladodes pruned for season 2 (Appendix VI).

4.3.2.2 Number of cladodes remaining after pruning

The mean number of cladodes remaining per variety annually after pruning were recorded. Turpin (88.3), Gymno Carpo (87.30), and Meyers (85.60) had the highest mean cladodes left in season 1 (Appendix VI). Turpin and Gymno Carpo presented the highest number of cladodes remaining after pruning for the following season, (95.60) and (94.80) respectively (Appendix VI). The mean number of cladodes left on plants of a certain variety gives a good indication of pruning intensity. Turpin and Gymno Carpo were consistently high in cladodes remaining after pruning over the duration of the trial

(Figure 4.1). The production of fodder requires incomplete or total removal of the vegetative material. Thus, the ability to recover after pruning is important (Mondragón-Jacobo and Pérez-González, 2001). Turpin and Gymno Carpo consistently ranked high with regards to the number of cladodes removed, and remaining after pruning. This indicates good vegetative growth, therefore, these varieties are recommended as fodder types for the Mokopane district of the Limpopo Province.

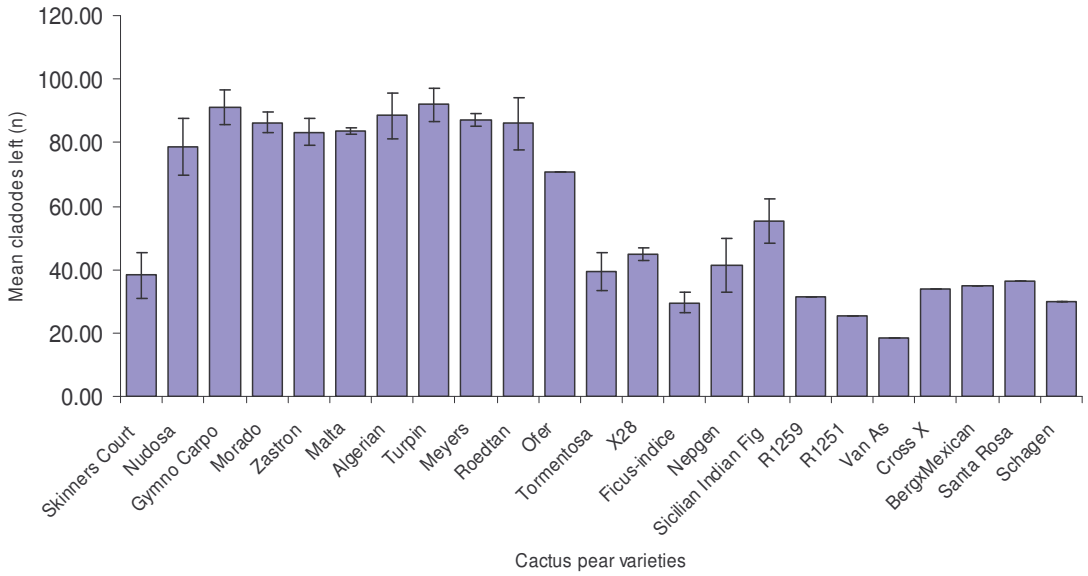


FIGURE 4.1 NUMBER OF CLADODES REMAINING ON CACTUS PEAR VARIETIES AFTER PRUNING OVER COMBINED SEASONS Bars represent the standard deviation for data recorded over two years, charts with no bars only had data for one year

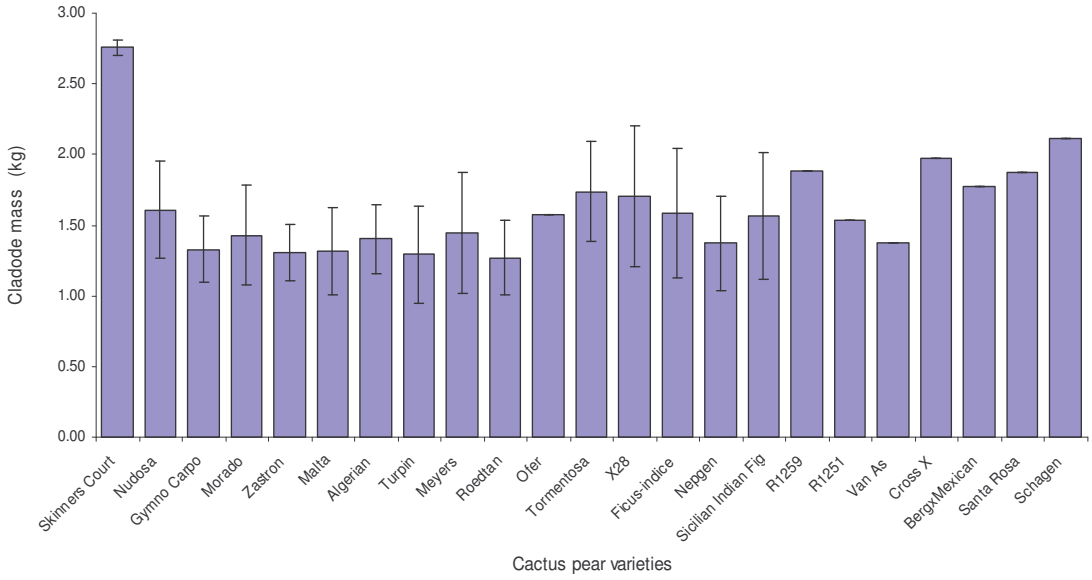


FIGURE 4.2 AVERAGE MASS (KG) OF CLADODES OF EACH CACTUS PEAR VARIETY OVER COMBINED SEASONS Bars represent the standard deviation for data recorded over two years, charts with no bars only had data for one year

4.3.2.3 Mass of cladodes

Skinners Court consistently produced the heaviest cladodes over the trial period (2.80 kg, 2.71 kg) (Appendix VI, Figure 4.2). Tormentosa (1.49 kg) and Nudosa (1.36 kg) had heavy cladodes for the period 1999–2000. Schagen (2.11 kg) and Ficus Indice (2.06 kg) gave the second and third highest values for individual cladode mass for the 2000–2001 season. Turpin (1.05 kg), Roedtan (1.09 kg), and Malta (1.05 kg) gave the lowest cladode mass for the period 1999–2000. Van As (1.37 kg), Zastron (1.45 kg), and Roedtan (1.45 kg) produced the lowest cladode mass for the period 2000–2001 (Appendix VI).

4.3.2.4 Cladode yield

Turpin (107.01 kg fresh material), Meyers (102.05 kg fresh material), and Morado (78.84 kg fresh material) produced the highest cladode yield during 1999–2000 period (Appendix VI). Sicilian Indian Fig (105.13 kg fresh material), Zastron (72.97 kg fresh material), and Turpin (70.77 kg fresh material) had the highest cladode yield for the period 2000–2001. Van As (24.89 kg fresh material), Cross X (44.46 kg fresh material), and Algerian (47.64 kg fresh material) gave the lowest cladode yield for the period 2000–2001 (Appendix VI). Of the varieties evaluated in the study, Turpin performed well in terms of vegetative yield (Figure 4.3).

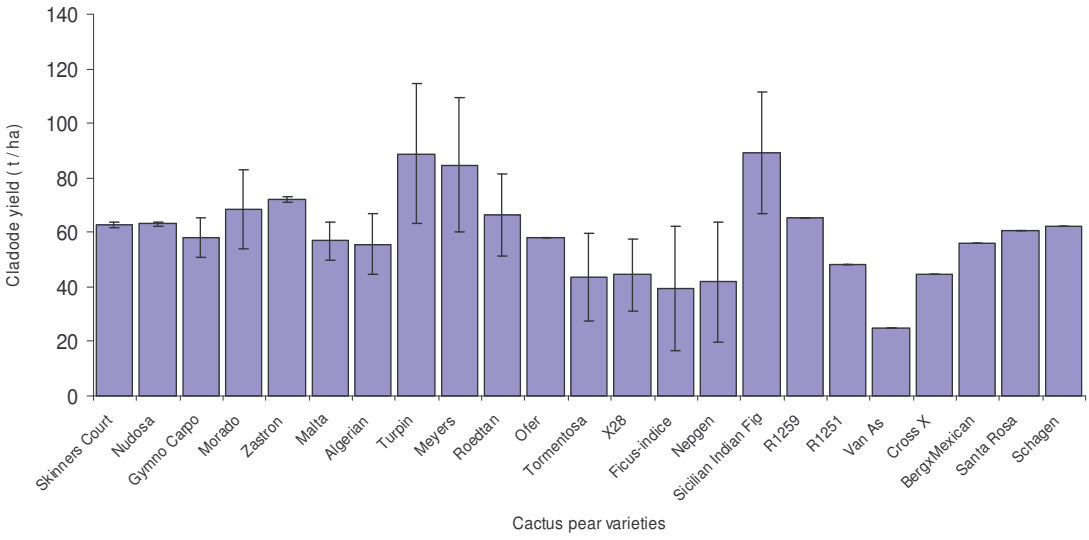


FIGURE 4.3 MEAN PRUNED CLADODE YIELD (kg) FOR CACTUS PEAR VARIETIES MEASURED OVER COMBINED SEASONS Bars represent the standard deviation for data recorded over two years, charts with no bars only had data for one year

Mean vegetative measurements for the germplasm were lower during the second season (2000–2001) as compared to the first (1999-2000) (Appendix VI). This could indicate that climatic conditions during the second season limited vegetative growth. The average rainfall recorded for season 2 (76.67mm) was lower than that recorded for season 1 (106.06mm) and the average solar radiation recorded for season 2 (655.81 MJ/m^2/s) was lower than that recorded for seaon 1 (1074.59 MJ/m^2/s) (Table 3.12).

4.3.3 Cluster analysis

Cluster analysis was used to group the 23 varieties into homogenous clusters using five qualitative and nine vegetative traits. Based on vegetative and morphological traits the 23 varieties grouped into four clusters (Figure 4.4). Skinners Court was dissimilar to the majority of the varieties analysed, and did not group into the designated clusters. The two varieties from Botswana (R1259, R1251) grouped together in cluster IV (Figure 4.4). Interestingly, these varieties grouped very closely to a variety from Israel (Ofer). The same trend was observed in the previous chapter (Figure 3.1), when the dendrogram was constructed on the basis of fruit quality and morphological traits.

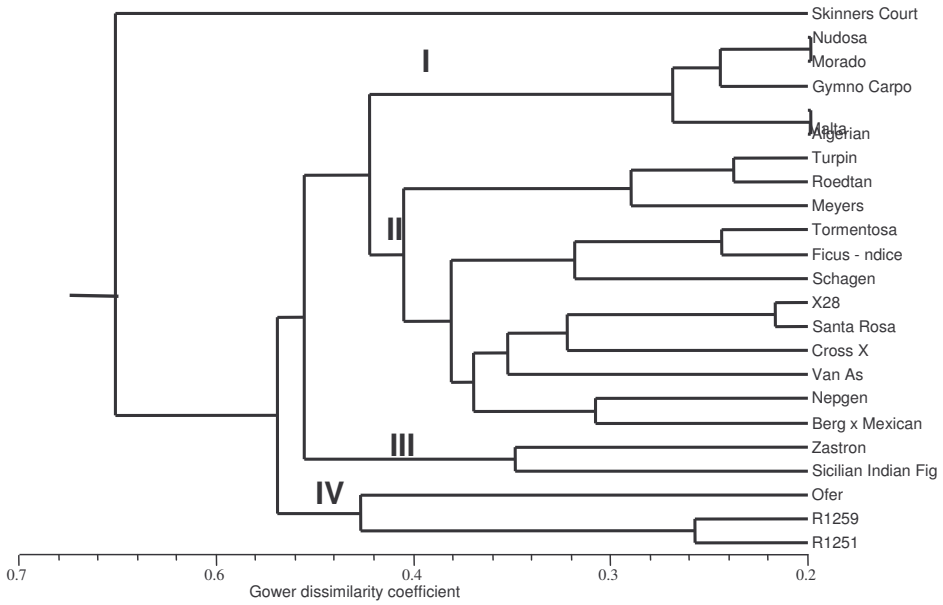


FIGURE 4.4 DENDROGRAM CONSTRUCTED FROM VEGETATIVE AND MORPHOLOGICAL TRAITS OF 23 CACTUS PEAR VARIETIES BASED ON THE GOWER DISSIMILARITY COEFFICIENT OVER COMBINED SEASONS

The majority of the varieties grouped into cluster II. This cluster is characterised by a mean number of pruned cladodes of 36.32 cladodes pruned/plant, average cladode mass of 1.63 g, cladodes remaining after pruning of 47.79, and cladode yield per plant of 54.79 kg fresh material. All the varieties in this cluster had ovate cladodes, and a shrubby plant habitus except for Nepgen that was upright (Appendix V). The varieties Turpin, Roedtan, and Meyers are recommended for use as fodder varieties in the Mokopane district of the Limpopo province and areas with similar environmental conditions because of their superior vegetative vigour in this area.

The varieties Malta and Algerian, and Nudosa and Morado were not clearly separated using morphological and vegetative traits (Figure 4.4). In chapter two Malta and Algerian were illustrated to be genotypically very similar using AFLP markers. This relationship was further confirmed by these findings.

Based on vegetative and morphological traits varieties classified as *O. ficus-indica* species (Morado, Gymno Carpo, Malta, and Algerian) grouped in cluster I (Figure 4.4). Interestingly, Nudosa, which is classified as a hybrid variety (*Opuntia* spp.) was closely associated with Morado, an *O. ficus-indica* species. The hybrid variety, Skinners Court did not cluster with the rest of the varieties evaluated in this study, whilst the other hybrid varieties, Zastron and Nudosa clustered within the same groupings with the rest of the other varieties (Figure 4.4).

4.4 CONCLUSIONS

The nutritional value of cladodes is affected by variety (genetic characteristics), age, location, season and growing conditions such as soil fertility and climate. It is, however, possible to supplement nutritional components that are found to be limited in cladodes or to apply relevant cultural practices such as fertilisation to improve the nutritional value of cladodes. Chicken litter is being favourably used in South Africa to supplement the low CP content of cladodes. Recently it was shown that dried cladode material (cv Algerian) can be successfully included into a balanced feed for sheep (De Waal *et al*., 2006).

Varieties that ranked the highest for vegetative yield fodder crops in Mokopane district of the Limpopo Province are Gymno Carpo and Turpin. It is important that further trials be performed to maximise potential productivity and nutritional quality of these varieties by manipulating cultural practises. In addition it must be highlighted that this germplasm was as per normal for orchards maintained for fruit production, and that pruning was done to maximise fruiting.

The most accurate measure of feed quality is animal performance. However, due to the arduous nature of animal trials, the screening of large numbers of varieties of feeds for genetic improvement trials is not common. Therefore, models to predict forage quality from feed attributes are being developed. This would, however, require considerable collaboration amongst researchers (Coleman and Moore, 2003). It has, however, been highlighted that the use of a single parameter or technique to aid plant breeders in selecting varieties for feed quality without animal-feed interaction will not provide an accurate estimate of animal performance under practical feeding situations (Mould, 2003).

Further research involving measures of palatability, intake, and digestibility trials are required. Research into cactus pear cladodes harvested as pruned material for use as fodder in South Africa is in its infancy. It is hoped that the information presented in this study will aid researchers in further investigations. Many of the quality and productivity traits are influenced by the environment. If is further suggested that G X E interaction studies be explored, to determine which varieties are best suited to the vastly different agro-ecological regions of this country.

REFERENCES

Ben Salem, H., A. Nefzaoui and L. Ben Salem, 2002. Supplementation of *Acacia cyanophylla* Lindl. foliage-based diets with barley or shrubs from arid areas (*Opuntia ficus-indica* f. *inermis* and *Atriplex nummularia* L.) on growth and digestibility in lambs. Animal Feed Science and Technology 96: 15-30.

Ben Salem, H., A. Nefzaoui and L. Ben Salem, 2004. Spine-less cactus (*Opunita ficus-indica* f. *inermis*) and oldman saltbush (*Atriplex nummularia* L.) as alternative supplements for growing Barbarine lambs given straw-based diets. Small Ruminant Research 51: 65-73.

Chessa, I. and G. Nieddu, 1997. Descriptors for cactus pear (*Opuntia* spp.). CACTUSNET-FAO, Universitá degli Studi, Rome, Italy.

Coleman, S.W. and J.E. Moore, 2003. Feed quality and animal performance. Field Crops Research 84: 17-29.

Cordeiro Dos Santos, D. and S. Gonzaga De Albuquerque, 2001. Fodder nopal use in the semi-arid Northeast of Brazil. In: Mondragón-Jacobo, J.C. and S. Pérez-González (Eds.), Cactus (*Opuntia* spp.) as forage, pp 37-49. CACTUSNET, FAO, Rome.

De Kock, G.C., 1980. Drought resistant fodder shrub crops in South Africa. In: H.N., Le Houérou (Ed.), Browse in Africa. The current state of knowledge. International Livestock Centre for Africa, pp 399-408. Adis Ababa, Ethiopia.

De Kock, G.C., 2001. The use of *Opuntia* as a fodder source in arid areas of Southern Africa. In: Mondragón-Jacobo, C. and S. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage, pp 101-105. CACTUSNET, FAO, Rome.

De Waal, H.O., D.C. Zeeman and W.J. Combrinck, 2006. A perspective on the wet faeces produced by dried and coarsely ground *Opuntia ficus-indica* cladodes in sheep diets. In: Louw, S. and T. Labuschagne (Eds.), Proceedings of the International Cactus pear Conference, pp 25, Centre for Plant Health Management, SA, Bloemfontein.

FAO, 2000. New on the menu for livestock.
http://www.fao.org./ag/magazine/0009sp2.htm

Gonzalez, C.L., 1989. Potential of fertilization to improve nutritive value of prickly pear cactus (*Opuntia lindheimeri* Engelm.) Journal of Arid Environments 16: 87-94.

Gower, J.C., 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Guevara, J.C., J.H. Silva Colomer and O.R. Estevez, 2004. Nutrient content of Opuntia forage clones in the Mendoza Plain, Argentina. Journal of the Professional Association for Cactus Development 6: 62-77.

Han, H. and P.Felker, 1997. Field validation of water-use efficiency of the CAM plant *Opuntia ellisiana* in south Texas. Journal of Aric Environments 36:133-148.

Inglese, P., F. Basile and M. Schirra, 2002. Cactus pear fruit production. In: Nobel, P.S. (Ed.), Cacti, biology and uses. pp 163-183. University of California Press, Los Angeles, USA.

Kigotho, W., 2005. Decades of drought predicted for Southern Africa. All africa Global Media. Scientific Development Network: http://allafrica.com/stories/printable/200506020713.html

Kueneman, E., 2001. Foreword. In: Mondragón-Jacobo, C. and S. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage. CACTUSNET, FAO, Rome.

Leco Cooperation, 2001, 3000 Lakeview Ave, St Joseph, MI 49085.

Le Houérou, H.N., 1992. The role of *Opuntia* cacti in the agricultural development of Mediterranean arid zones. Proceedings of the Second International Congress on Prickly pear and cochineal, Santiago, Chile, pp 186-198.

López-García, J.J., J.M. Fuentes-Rodríguez and R.A.R. Rodríguez, 2001. Production and use of Opuntia as forage in Northern Mexico. In: Mondragón-Jacobo, C. and S. Pérez-González (Eds.), Cactus (*Opuntia* spp.) as forage, pp 29-36. FAO, Rome, Italy.

Martin, F., 1993. Drought feed provides handy income. Farmers weekly, January 15, pp 31-32.

Mondragón-Jacobo, C. and S. Pérez-González, 2001. Germplasm resources and breeding Opuntia for fodder production. In: Mondragón-Jacobo, C. and S. Pérez-González (Eds.), Cactus (*Opuntia* spp.) as forage, pp 21-28. FAO, Rome, Italy.

Mould, L.F., 2003. Predicting feed quality-chemical analysis and *in vitro* evaluation. Field Crops Research 84: 31-44.

Nefzaoui, A. and H. Ben Salem, 2001. *Opuntia*: A strategic fodder and efficient tool to combat desertification in the WANA region. In : Mondragón-Jacobo, C. and S. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage, pp 73-89. CACTUSNET, FAO, Rome.

Nefzaoui A. and H. Ben Salem, 2002. Cacti: efficient tool for rangeland rehabilitation, drought mitigation and to combat desertification. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 295-315.

Nobel, P.S., 1988. Environmental Biology of Agaves and Cacti. Cambridge University Press, New York, USA.

Nobel, P.S., 1995. Environmental biology. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 36-48. FAO Plant production and protection paper 132. Rome, Italy.

Nobel, P.S., C.E. Russell, P. Felker, M. Galo and E. Acuna, 1987. Nutrient relations and productivity of prickly pear cacti. Agronomy Journal 79: 550-555.

Oelofse, R.M., M.T. Labuschagne and J.P. Potgieter, 2006. Plant and fruit characteristics of cactus pear (*Opuntia* spp.) cultivars in South Africa. Journal of the Science of Food and Agriculture 86: 1921-1925.

Potgieter, J.P., 1997. Guidelines for the cultivation of cactus pear for fruit production purposes. Second revised Edition. Group 7 Trust Publishers. Pretoria.

Rohlf, F.J., 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology 47: 147-158.

SPSS for Windows, 22 December 1997. Copyright© SPSS Inc.

Tegegne F., 2001. Nutritional value of *Opuntia ficus-indica* as a ruminant feed in Ethiopia. In: Mondragón-Jacobo, C. and S. Pérez-Gonzalez (Eds.), Cactus (*Opuntia* spp.) as forage. CACTUSNET, FAO, Rome.

Tegegne F., 2002. *In vivo* assessment of the nutritive value of cactus pear as a ruminant feed. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 323-328.

Chapter 5

Resistance of cactus pear varieties to three fungal pathogens and an option for biocontrol using yeasts

ABSTRACT

Increased cactus pear farming in South Africa has been accompanied by escalating reports of new diseases and financial losses due to post-harvest fruit rot especially in shipments destined for overseas markets. A study was designed to screen 38 South African cactus pear varieties for resistance to three important fungal pathogens (*Phialocephala virens, Fusarium oxysporum* and *F. proliferatum*) previously isolated from diseased cactus pear in South Africa. Disease progression was monitored on mature cladodes in the field over 52 days following artificial inoculation. There were no substantial differences in the virulence of the two *Fusarium* pathogens tested across all varieties. The varieties most susceptible to all three fungal pathogens were Roly Poly, Zastron, and Direkteur. The varieties most resistant to all three pathogens were Amersfoort, Algerian, and Meyers. In addition, yeast isolates with potential antagonistic activity against these pathogens were isolated and screened *in vitro*. Yeast isolates with the highest antagonistic activity against these pathogens were screened by means of dual challenge tests on agar plates. Statistically significant differences between mean colony diameters of the pathogens were determined (ANOVA) and means were separated using the Ryan Einot Gabriel and Welsch Test at $p \leq 0.05$. Of the ten antagonistic yeast isolates selected, 60% belonged to the genus *Cryptococcus*. An isolate of *Rhodotorula mucilaginosa* however, displayed the greatest degree of inhibition to all three fungal pathogens *in vitro*. Of the three fungal pathogens challenged *in vitro*, *P. virens* was least inhibited by the antagonistic action of the yeasts.

5.1 INTRODUCTION

The increasing interest in cactus pear as a source of fruit and fodder in arid and semi-arid regions has led to the initiation of many fruit improvement programmes (Inglese *et al*., 1995). The most important aim of fruit improvement programmes around the world is to increase resistance to pests and diseases by classical breeding and/or genetic engineering. Breeding disease resistant fruit cultivars involves combining the best fruit quality traits with disease resistance traits. This requires the assessment of resistance to disease as part of crop improvement programmes in breeding stocks or germplasm (Momol *et al*., 1996).

The introduction of cactus pear into new geographic areas has resulted in the appearance of new diseases. Relatively few reports on diseases of *Opuntia* spp. have, however, been published (Varvaro *et al*., 1993; Granata, 1995; Granata and Sidoti, 2000; Zimmermann and Granata, 2002). Fungal pathogens of cactus pear usually belong to the genera *Armillaria, Dothiorella, Phytophthora, Alternaria, Fusarium, Phyllosticta*, *Sclerotinia,* and to a lesser extent *Colletotricum, Capnodium, Macrophomina, Cercospora, Aecidium, Phoma, Cytospora, Gleosporium, Mycospherella,* and *Pleospora* (Granata, 1995). Dry rot of cladodes in South Africa is associated with *Alternaria tenuissima* (Kunze) Wiltshire, *Stemphyllium* sp., *Fusarium* spp., and various *Phoma* spp. Superficial necrosis of cladodes is associated with *A. alternata* (Fr.) Keissl, *Cylindrocarpon* sp., and *F. sporotrichoides* Sherb (Swart and Kriel, 2002).

Commercial cultivation of cactus pear for fruit production in South Africa is growing at a steady pace. However, many farmers are increasingly reporting disease-related losses of fruit. Cactus pears are highly perishable, and under marketing conditions [(20°C, 60–70% relative humidity (RH)] have a shelf life of only a few days (Rodriguez-Felix, 2002). The main post-harvest problems experienced are directly related to physical damage incurred during harvesting. Factors affecting shelf life include decay at the stem end caused by *Fusarium* spp., *Alternaria* spp., *Chlamydomices* spp., and *Penicillium* spp. (Rodriguez-Felix, 2002). Previous studies by Swart and Swart (2003) found isolates from the following fungal genera associated with decayed cactus pear fruits (cv. Algerian) in South Africa: *Rhizopus* sp., *Mucor* sp., *Epicoccum* spp., *Cladosporum* sp., *Fusarium* spp., *Phoma* sp., *Aspergillus* spp., *Stemphyllium* sp., *Alternaria* spp., *Rhizoctonia* sp. *Rhizopus* spp., and *Penicillium* spp.

Cold storage increases the post-harvest life of most horticultural crops (Wang, 1994). Cactus pear however, is sensitive to chilling injury when stored at temperatures below 9° C (Chessa and Barbera, 1984) or 10° C, depending on the cultivar. Fungicides are therefore the principal method to control post-harvest disease. Although cactus pear fruits produce low levels of ethylene, Ethrel application to fruits has been used experimentally to hasten abscission zone formation, reducing harvest injury at the stem end (Cantwell, 1986). However, public concern over food safety and the development of fungicide resistant pathogens has increased the search for alternative methods less harmful to man and the environment.

Biological control using antagonistic microorganisms has been endorsed as an alternative to the use of synthetic fungicides with considerable success. In particular, a host of yeast genera have been extensively used for the biological control of post-harvest diseases of fruits and vegetables (Wilson and Wisniewski, 1989; Punja, 1997) to protect moulding of stored grains (Petersson *et al.,* 1999), and to control foliar diseases (Urquhart and Punja, 1997).

Research into the pathogens causing diseases of cactus pear (*Opuntia* spp.) in South Africa and the relative susceptibilities of varieties of *O. ficus-indica* being cultivated to the most virulent pathogens is of the utmost importance. Specific attention directed towards establishing methods for the prevention of post-harvest fruit rot using suitable biological control agents will contribute greatly to the success of the emerging industry. The aims of this study were to (1) evaluate 38 South African cactus pear varieties for susceptibility to three common fungal pathogens (*Phialocephala virens* Siegfried and Siefert*, F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg and *F. oxysporum* Schltdl), (2) determine the relative virulence of these three pathogens to commercially important cactus pear varieties, and (3) isolate and identify yeasts displaying antagonistic activity *in vitro* against these fungal pathogens from the surface of cactus pear fruits.

5.2 MATERIALS AND METHODS

5.2.1 Trial site and layout

The trial site description is as given in section 2.2.1. The 38 cactus pear varieties used in this study (Table 2.1) were not arranged in a statistical layout. Five plants per variety were planted at a spacing of 3 m x 5 m (667 plants/ha) in single rows orientated in an East/West direction.

5.2.2 Pathogenicity studies

5.2.2.1 Inoculum preparation

Single spore isolates of three fungal pathogens (*P. virens, F. oxysporum* and *F. proliferatum*) were obtained from the New Crop Pathology Programme fungal collection and incubated at room temperature on potato dextrose agar (PDA)-streptomycin [0.03% (v/v)] plates until sizeable colonies were visible. Sterilised toothpicks (Swart *et al*., 2003) were transferred to the margins of fungal colonies and plates were further incubated at room temperature. The colonised toothpicks were used as an inoculum source.

5.2.2.2 Cladode inoculation

Inoculum coated toothpicks were inserted up to 10 mm into one year old terminal cladodes at three positions, the apical, medial and basal sections, for each isolate. Insertion points were sealed with masking tape to prevent desiccation. Nine sterile toothpicks inserted at different locations (apical, median, and basal) on three cladodes per variety were used as controls.

Lesion diameters were measured at various intervals (2, 7, 14, 21, 28, 38, and 52 days) using an electronic digital caliper to follow disease progression. Data were subjected to statistical analyses. Koch's postulates were confirmed by the re-isolation, and identification of the original pathogens from 20% of the resultant lesions.

5.2.3 Statistical analysis

Lesion diameter readings after 52 days were entered into SPSS (SPSS, 1997) and subjected to analysis of variance using the general linear model. The Tukey multiple range test was used to detect significant differences between means at $p \leq 0.05$. The Gower distance (Gower, 1971) was used as a measure of diversity between different varieties as described in section 4.2.4. Gower distances were used to compute a dissimilarity matrix and the UPGMA used for dendrogram construction using the NTYSYS-pc programme (Version 2.02i, Rohlf, 1998).

5.2.4 *In vitro* inhibition studies

5.2.4.1 Yeast isolation

Yeasts were isolated from the surfaces of fruits purchased from a commercial outlet in Bloemfontein, South Africa. The varieties used were Skinners Court, Gymno Carpo, Morado, and Fusicaulis. Fruits were rinsed in 100 ml sterile distilled water and a 2 ml aliquot of the suspension was serially diluted with 18 ml sterile 1% peptone water [1% (w/v) peptone, 0.5% (w/v) NaCl in distilled water]. A 0.1 ml aliquot of the suspension was plated in duplicate onto rose bengal chloramphenicol (RBC) agar plates and incubated at 25°C for seven days.

Visually distinguishable yeast colonies were isolated from plates of the highest dilutions with between 30-300 colony forming units. Single colonies were sub-cultured onto yeast malt extract agar (0.4% yeast extract, 0.4% glucose, 1% malt extract, 2% agar) (YM) until cultures were pure. After incubation at 25ºC for five days, cultures were stored at 4°C on YM slants for further characterisation. For long-term preservation, cultures were stored in 35% glycerol at -70°C (Henry and Kirsop, 1989).

5.2.4.2 *In vitro* inhibition screening

Fungal isolates previously associated with disease in cactus pear fruits and cladodes, *P. virens*, *F. oxysporum*, and *F. proliferatum* (Swart *et al.,* 2003) were used to assess the *in vitro* inhibition capacity of the various yeast isolates. Dual cultures were prepared with the fungal plugs at the centre of nutrient agar (NA) plates and yeast isolates from two day old cultures streaked approximately 3 cm from the pathogen plug. Control plates containing only the fungus were similarly prepared. Control and treatment reactions were prepared in triplicate for each fungal pathogen. Plates were incubated at room

temperature for seven days, followed by measurement of the diameter of the fungal colonies. The experiment was performed twice.

5.2.4.3 Molecular identification of yeast isolates

Yeast isolates were identified using the method of Kurtzman and Robnett (1998). Briefly, the variable D1/D2 domain of the large subunit (LSU) ribosomal DNA (rDNA) was amplified using primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5'-GGTCCGTGTTTCAAGACGG). Amplicons were sequenced and DNA sequences queried for nucleotide sequence alignment against all genetic sequence databases, held in GenBank® of the National institute of Health (NIH), (http://www.ncbi.nlm.gov) (Altschul *et al.,* 1997).

5.2.4.4 Statistical analysis

The percentage inhibition caused by each yeast isolate was calculated as the difference in mean colony diameter between the control (pathogen) and the mean colony diameter of the pathogen in the presence of the yeast isolate. Mean colony diameter (mm) data were analysed using ANOVA to determine significant differences between treatments. Means were separated into homogeneous groups using the Ryan Einot Gabriel and Welsch Test (REGW) (SPSS, 1997) at $p \leq 0.05$.

5.3 RESULTS AND DISCUSSION

5.3.1 Pathogenicity studies

5.3.1.1 Susceptibility of cactus pear varieties to *Fusarium oxysporum*

There were statistically significant ($p \leq 0.05$) differences between varieties in terms of mean lesion diameter (Table 5.1 and Figure 5.1). The control treatments developed very small lesions as compared to inoculated tissue. Mean lesion diameters 52 days after inoculation with *F. oxysporum* showed that Roly Poly (8.53 mm), Direkteur (8.05 mm), and Zastron (8.03 mm) produced the largest lesions and thus, were the most susceptible varieties (Figure 5.1). Varieties that were the most resistant to *F. oxysporum* infection were Algerian (5.17 mm), Amersfoort (5.32 mm), and American Giant (5.35 mm).

TABLE 5.1 MEAN LESION DIAMETER OF CACTUS PEAR CLADODES 52 DAYS POST-INOCULATION

Variety	[a] *F. oxysporum*	[a] *F. proliferatum*	[a] *P. virens*
Algerian	5.17 a	5.42 abc	5.16 a
American Giant	5.35 a	5.55 abcd	5.41 abcd
Amersfoort	5.32 a	4.82 a	5.11 a
Arbiter	6.38 abcde	6.57 abcde	6.47 abcdefgh
Blue Motto	7.61 cdef	7.16 cdefg	7.49 efghi
Corfu	7.03 abcdef	6.66 abcdef	6.83 abcdefgh
Cross X	6.79 abcdef	6.96 bcdef	6.67 abcdefgh
Direkteur	8.05 ef	7.74 efg	7.67 ghi
Ficus-Indice	6.50 abcde	6.04 abcde	6.43 abcdefgh
Fresno	6.37 abcde	7.01 cdef	5.67 abcdef
Fusicaulis	6.64 abcdef	6.06 abcde	6.44 abcdefgh
Gymno Carpo	5.99 abc	6.66 abcdef	6.24 abcdefgh
Malta	6.06 abcd	6.62 abcde	5.81 abcdefg
Messina	5.83 abc	5.46 abcd	5.58 abcde
Mexican	7.38 bcdef	6.37 abcde	7.23 cdefghi
Meyers	5.75 abc	4.90 ab	5.31 abc
Morado	6.91 abcdef	6.10 abcde	6.49 abcdefgh
Muscatel	5.50 ab	5.48 abcd	5.10 a
Nepgen	7.06 abcdef	6.61 abcde	7.58 fghi
Nudosa	6.00 abc	6.26 abcde	6.27 abcdefgh
Ofer	7.12 abcdef	6.21 abcde	6.91 abcdefgh
R1251	6.09 abcde	5.85 abcde	5.72 abcdef
R1259	5.78 abc	6.22 abcde	5.74 abcdefg
R1260	5.81 abc	6.59 abcde	5.47 abcd
Roedtan	5.98 abc	5.81 abcde	5.26 ab
Roly Poly	8.53 f	8.75 fg	8.91 i
Rossa	6.97 abcdef	6.22 abcde	6.49 abcdefgh
Santa Rosa	6.57 abcdef	6.50 abcde	7.11 bcdefghi
Schagen	5.58 ab	5.42 abc	5.46 abcd
Sharsheret	6.05 abcd	6.32 abcde	6.35 abcdefgh
Sicilian Indian Fig	6.45 abcde	7.53 defg	6.29 abcdefgh
Skinners Court	6.13 abcde	5.69 abcde	6.18 abcdefgh
Tormentosa	6.69 abcdef	6.12 abcde	5.88 abcdefg
Turpin	6.61 abcdef	6.57 abcde	5.84 abcdefg
Van As	7.08 abcdef	7.75 efg	7.27 defghi
Vryheid	7.08 abcdef	6.50 abcde	6.10 abcdefgh
X 28	6.55 abcdef	6.30 abcde	6.08 abcdefgh
Zastron	8.03 def	9.14 g	7.91 hi
Grand Mean	**6.50**	**6.42**	**6.31**

[a] Within column values with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple range test.

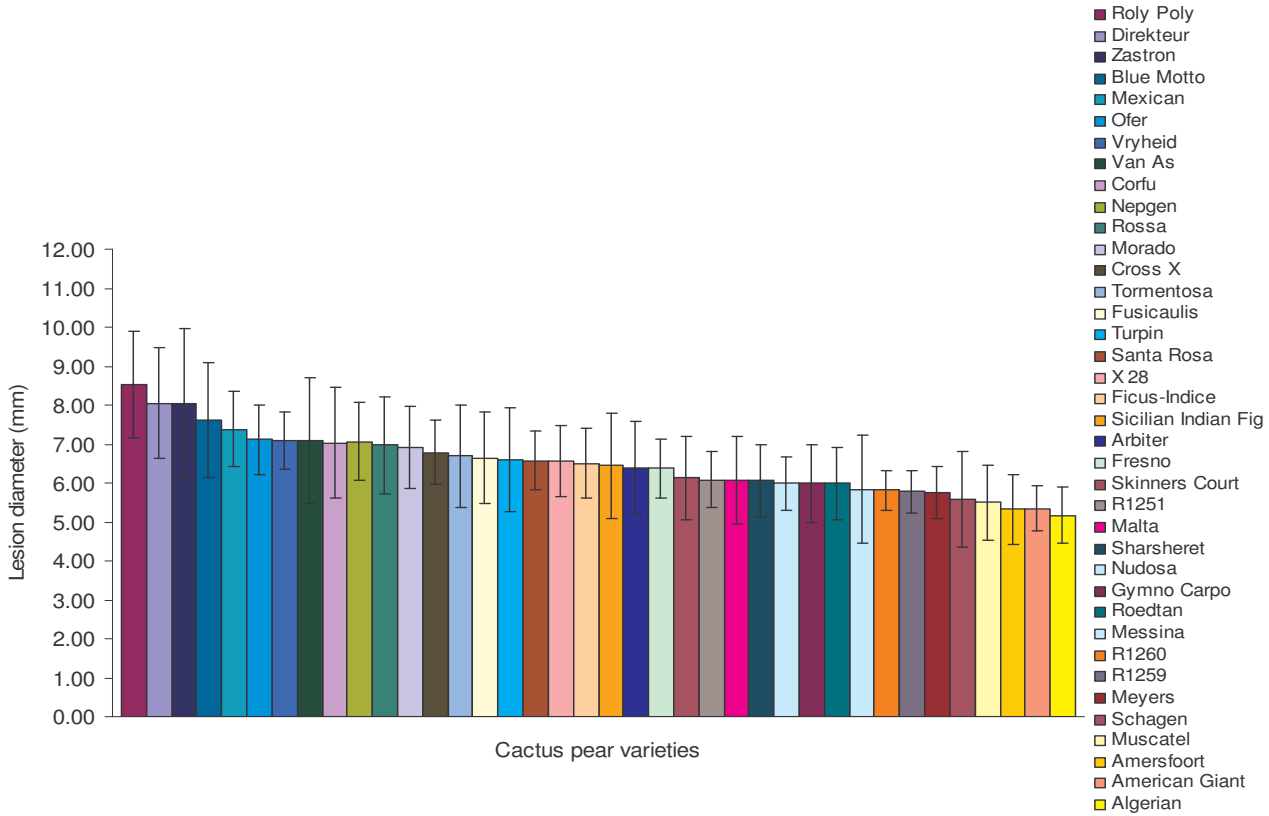


FIGURE 5.1 MEAN LESION DIAMETER OF CACTUS PEAR VARIETIES 52 DAYS AFTER INOCULATION WITH *FUSARIUM OXYSPORUM*

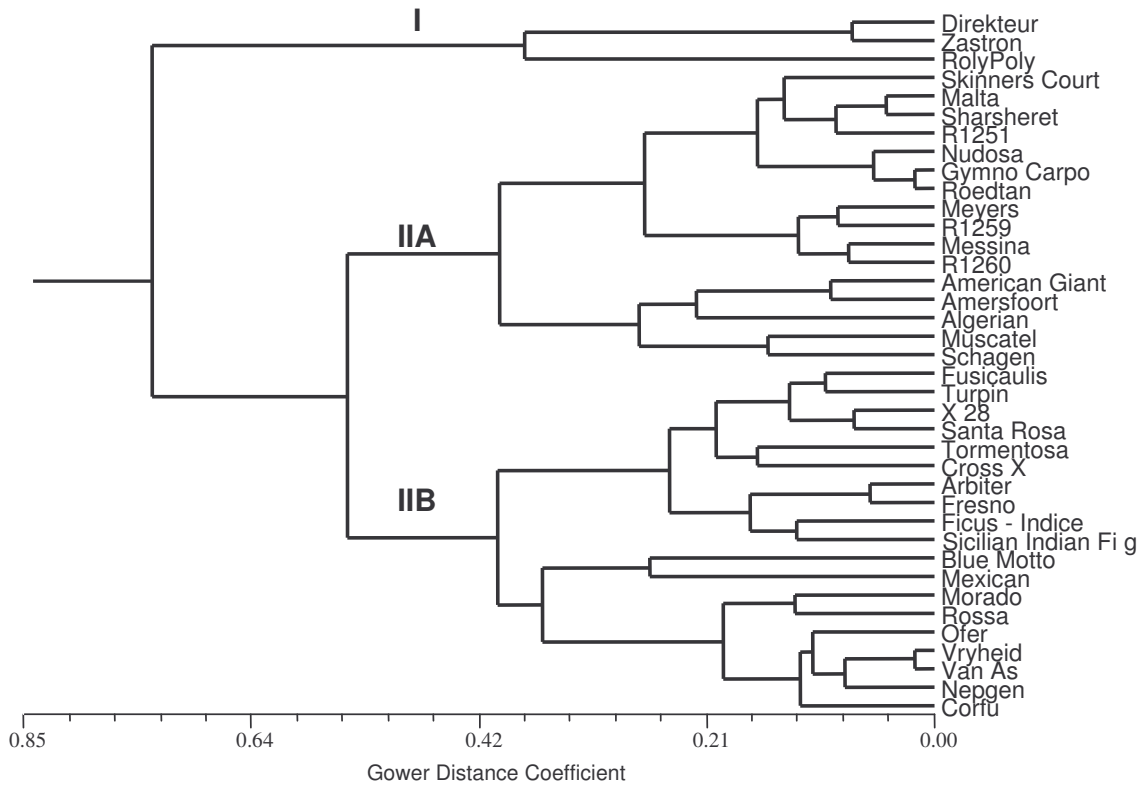


FIGURE 5.2 DENDROGRAM OF 38 CACTUS PEAR VARIETIES CONSTRUCTED ON THE BASIS OF SUSCEPTIBILITY TO *FUSARIUM OXYSPORUM* The Gower dissimilarity coefficient was used to estimate dissimilarity between varieties

Using cluster analysis, varieties Gymno Carpo and Roedtan, and Vryheid and Van As clustered closely together (Figure 5.2). Variety R1260, from Botswana clustered close to Messina from Israel. Varieties clustered into two main groups I and II. The most susceptible varieties (Direkteur, Zastron, and Roly Poly) clustered separately into cluster I (Figure 5.2). The majority of the varieties grouped into cluster II that can be sub-divided into two sub-clusters IIA and IIB (Figure 5.2). The most resistant varieties (Amersfoort, Algerian, and American Giant) clustered within a subgroup of IIA along with Muscatel and Schagen which also produced the smallest lesions with this pathogen. Commercially cultivated varieties were evenly dispersed within the dendrogram (Figure 5.2).

5.3.1.2 Susceptibility of cactus pear varieties to *Fusarium proliferatum*

Based on mean lesion diameter, the following varieties were the most susceptible to infection with *F. proliferatum*: Zastron (9.14 mm), Roly Poly (8.75 mm), Van As (7.75 mm) and Direkteur (7.74 mm). Roly Poly, Direkteur, and Zastron were also amongst the most susceptible to infection with *F. oxysporum* (Table 5.1). Varieties that

were the most resistant to *F. proliferatum* were Amersfoort (4.82 mm), Meyers (4.90 mm), Algerian, and Schagen (5.42 mm) (Table 5.1 and Figure 5.3).

Cluster analysis grouped the varieties into two main clusters I and II. Varieties that were the most similar were Algerian and Schagen, and Vryheid and Santa Rosa (Figure 5.4). Zastron and Roly Poly clustered separately in cluster II from the rest of the varieties. Zastron and Roly Poly were the varieties most susceptible to infection with *F. proliferatum*. Zastron and Roly Poly also grouped together in cluster I (Figure 5.2) based on resistance to *F. oxysporum*.

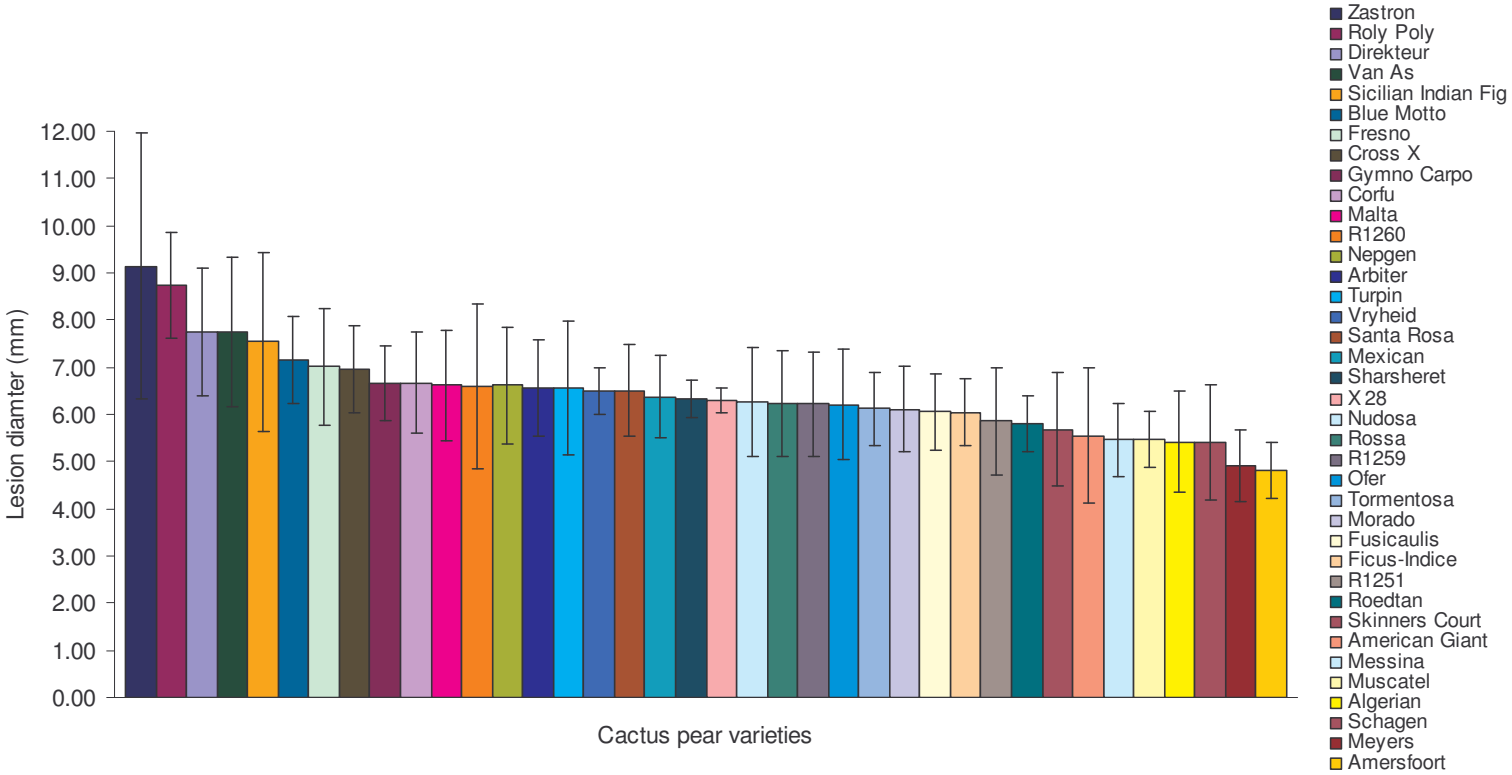


FIGURE 5.3 MEAN LESION DIAMETER OF CACTUS PEAR VARIETIES 52 DAYS AFTER INOCULATION WITH *FUSARIUM PROLIFERATUM*

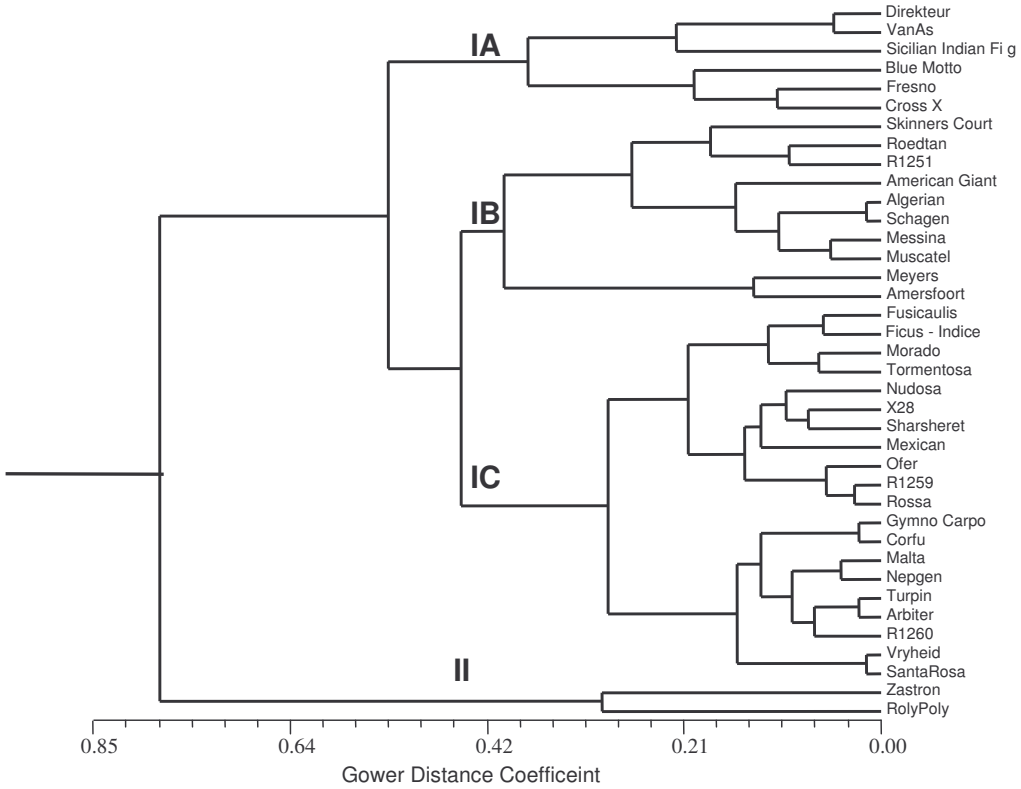


FIGURE 5.4 DENDROGRAM OF 38 CACTUS PEAR VARIETIES CONSTRUCTED ON THE BASIS OF SUSCEPTIBILITY TO *FUSARIUM PROLIFERATUM* The Gower dissimilarity coefficient was used to estimate dissimilarity between varieties

5.3.1.3 Susceptibility of cactus pear varieties to *Phialocephala virens*

Varieties that were the most susceptible to infection with *P. virens*, based on mean lesion data were Roly Poly (8.91 mm), Zastron (7.91 mm), and Direkteur (7.67 mm). The most resistant varieties to *P. virens* were Muscatel (5.10 mm), Amersfoort (5.11 mm), and Algerian (5.16 mm) (Figure 5.5 and Table 5.1).

Based on cluster analysis, Roly Poly, the most resistant to infection with *P. virens*, clustered separately from the rest of the varieties in the germplasm (Figure 5.6). The remainder of the varieties grouped into two clusters I and II. The most susceptible varieties (Zastron, Direkteur, Nepgen, and Blue Motto) were grouped in cluster I (Figure 5.6). Cluster II was further sub-divided into IIA IIB, and IIC. The most resistant varieties (Algerian, Roedtan, Muscatel, and Amersfoort) grouped into cluster IIB. Varieties Morado and Rossa could not be clearly separated from one another (Figure 5.6). Two varieties from Botswana (R1259 and R1251) grouped together in cluster IIC.

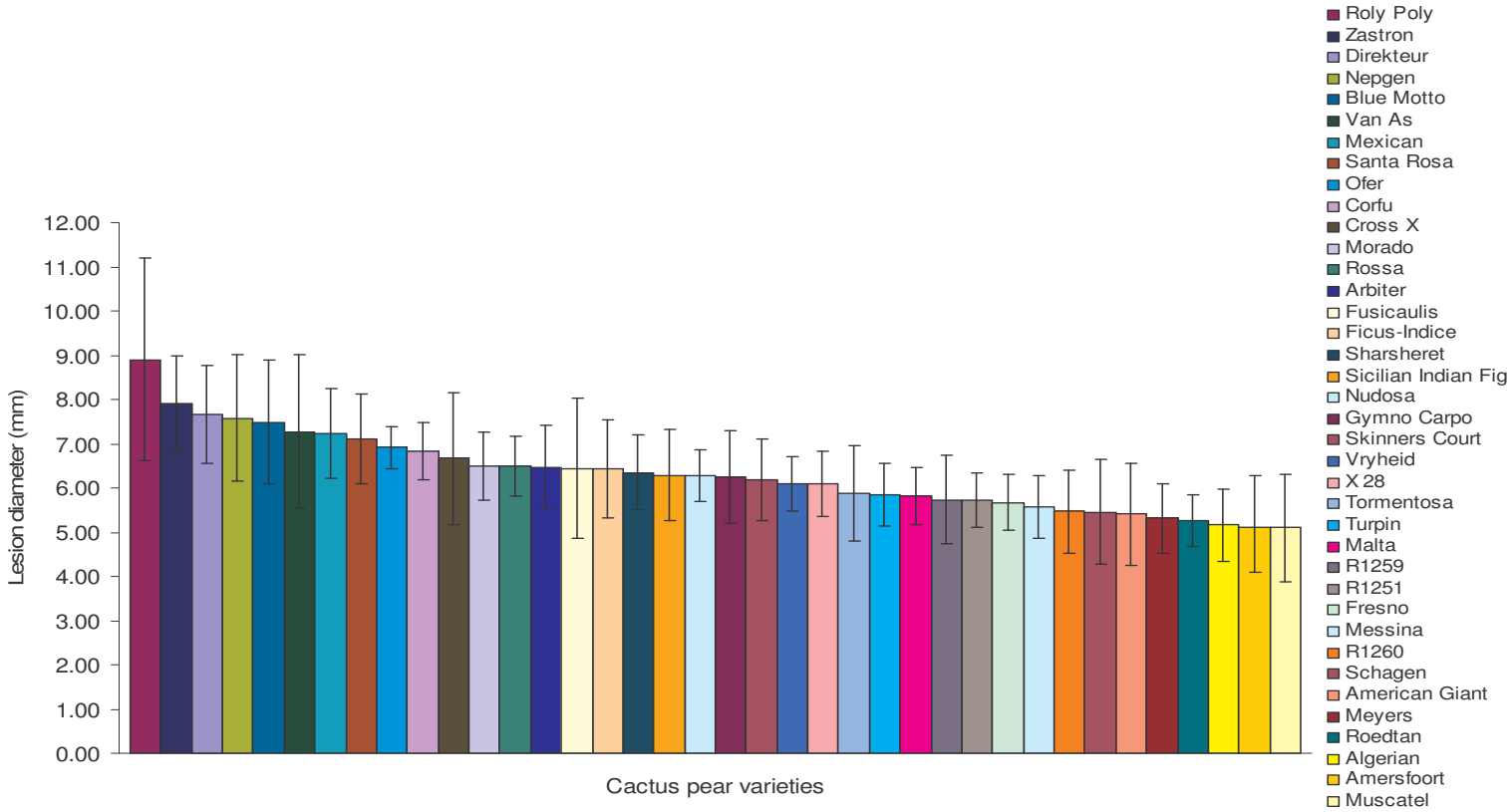


FIGURE 5.5 MEAN LESION DIAMETER OF CACTUS PEAR VARIETIES 52 DAYS AFTER INOCULATION WITH *PHIALOCEPHALA VIRENS*

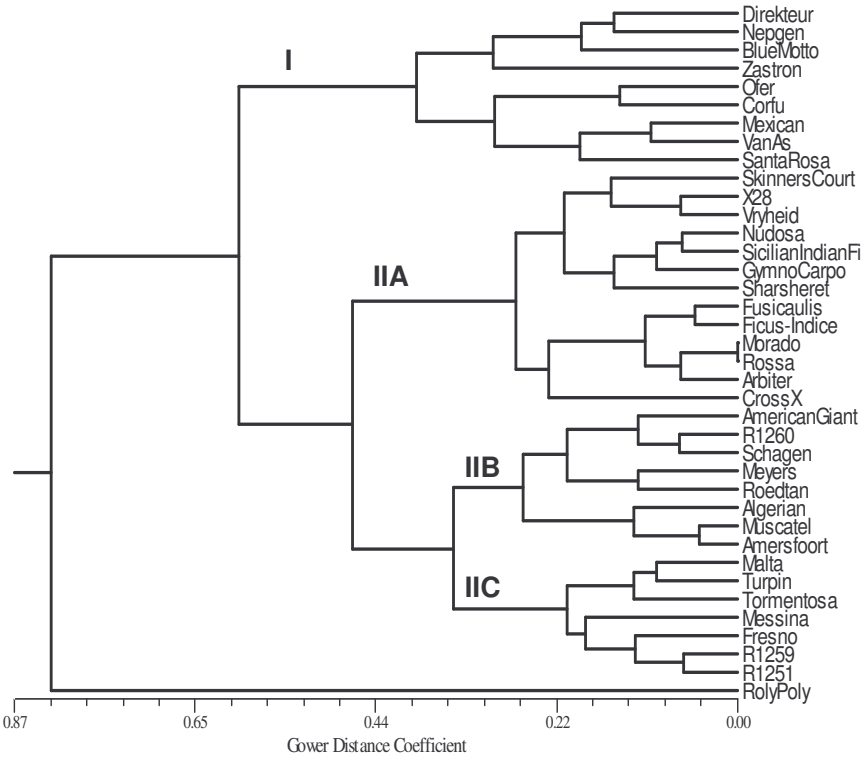


FIGURE 5.6 DENDROGRAM OF 38 CACTUS PEAR VARIETIES CONSTRUCTED ON THE BASIS OF SUSCEPTIBILITY TO *PHIALOCEPHALA VIRENS*
The Gower dissimilarity coefficient was used to estimate dissimilarity between varieties

5.3.1.4 Overall susceptibility of cactus pear varieties to fungal pathogens

There was no substantial difference in virulence between the different *Fusarium* pathogens across all varieties evaluated (Table 5.1). The overall lesion diameters across all varieties for infection with *F. oxysporum* were 6.50 mm and *F. proliferatum* 6.42 mm (Table 5.1). The overall mean lesion diameter across all the varieties for *P. virens* (6.31 mm) was considerably lower than that observed for the two *Fusarium* isolates (Table 5.1).

Using cluster analysis, varieties were grouped into two distinct clusters I and II. The most susceptible varieties (Direkteur, Blue Motto, Van As, Zastron, and Roly Poly) grouped into cluster I, separate from the rest of the varieties in cluster II. Cluster II was further sub-divided into clusters IIA, IIB, and IIC (Figure 5.7). All varieties from Botswana (R1251, R1259, and R1260) grouped together in a subgroup of IIA along with Skinners Court. Varieties Rossa and Vryheid could not be separated from each other based on disease response data. The most resistant varieties (American Giant, Algerian,

Muscatel, Amersfoort, and Roedtan) grouped together with Schagen, Meyers and Roedtan in cluster IIC. The three varieties from Israel (Sharsheret, Ofer, and Messina) were dispersed into clusters IIA, IIB, and IIC respectively (Figure 5.7).

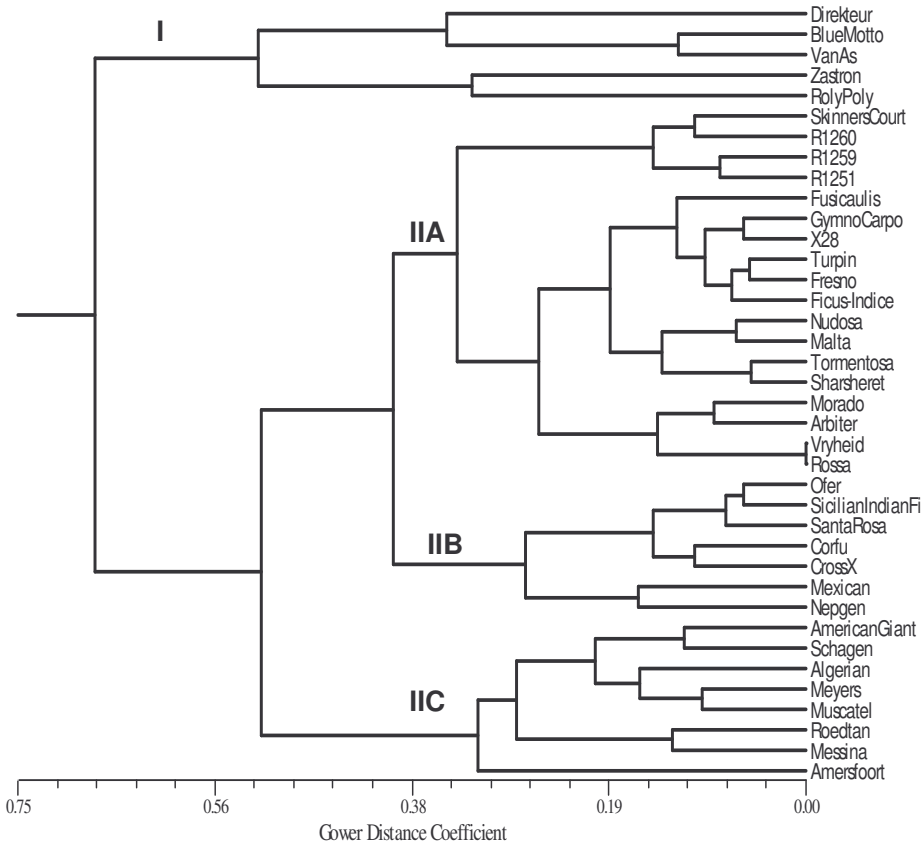


FIGURE 5.7 DENDROGRAM OF 38 CACTUS PEAR VARIETIES CONSTRUCTED ON THE BASIS OF OVERALL SUSCEPTIBILITY TO FUNGAL PATHOGENS The Gower dissimilarity coefficient was used to estimate dissimilarity between varieties

Based on the overall resistance to fungal pathogens tested, varieties that are classified as *O. ficus-indica* and *Opuntia* spp. (hybrids of unknown origin) clustered in group II and its sub-groupings. Of the known species delineated South African cactus pear varieties, Fusicaulis was the only *O. fusicaulis* type that grouped in cluster IIb (Figure 5.7). High disease resistance is important in preventing disastrous crop losses when climatic conditions favour disease (Dayton *et al*., 1983). Intact cactus pear cladodes are not susceptible to fungal pathogens since they are structurally protected by a thick waxy cuticle. Nonetheless, mechanical injury sustained during hailstorms facilitates considerable access for pathogens, highlighting the importance of selecting varieties with higher disease resistance that will sustain lower crop losses than susceptible types.

The largest lesion diameters induced with inoculation in this study were restricted (Table 5.1), and did not exceed 10mm, even when the structural protection against fungal pathogens offered by a thick waxy cuticle was pierced with toothpicks. This could be attributed to the formation of abscission layers which led to restriction in lesion diameter. Researchers have found that resistant peruvian apple cactus (*Cereus peruvianus*) formed an abscission layer upon inoculation with *Glomerella cingulata* (Stoneman) (Spauld and H. Schrenk). No such abscission layer was formed with the susceptible *Cereus tetragonus* (L.) Miller (Kim and Kim, 2002). Abscission layer formation as a physical disease response mechanism in cactus pear should be investigated in future studies of cactus pear.

5.3.2 *In vitro* inhibition studies

5.3.2.1 Yeast isolate identification

Sequence alignment allowed the unambiguous identification of nine of the 10 isolates submitted for identification (Table 5.32. One yeast isolate, 96, was identified as either *Cryptococcus albidosimilis* or *C. liquefaceins* (Table 5.2). All sequences were identified as partial sequences of the 26S/28S ribosomal gene sequence (Appendix VII). Nucleotide (nt) alignments obtained were significantly high, between 99-100%. Of the isolates identified as possible biocontrol agents, 60% belonged to the genus *Cryptococcus* (Table 5.2).

In the present study, sequence divergence analysis of the large subunit of rDNA was used to identify yeast isolates. Divergence at the variable D1/D2 domain of the LSU rDNA is generally adequate to resolve individual species (Kurtzman and Robnett, 1998), however, isolate 96 was not clearly identified. It was identified as being either *C. albidosimilis* or *C. liquefaciens.* The sequence obtained for this isolate had three mismatched nucleotides with *C. albidosimilis* with a 99% sequence homology. Four mismatched nucleotides with a 99% sequence homology were found with the sequence for *C. liquefaciens* (APPENDIX VII). This finding is similar to that reported by other researchers that have found that some *Cryptococcus* species may appear identical based on sequences of the D1/D2 domain of the large subunit of the rDNA. Fell and co-workers recommend the analysis of internal transcribed spacer sequences to resolve species within the *Cryptococcus* genus (Fell *et al*., 2000).

5.3.2.2 *In vitro* inhibition screening

Of the 270 yeast isolates obtained from the surfaces of cactus pear fruit, and screened for *in vitro* antagonistic activity, ten were chosen for further analysis using dual culture tests (Figure 5.8). All ten yeast isolates reduced hyphal growth of all three fungal pathogens, as compared to that of the control (Figure 5.8). Yeasts found on plant surfaces are thought to provide natural antagonistic protection against fungal plant diseases (Fokkema *et al*., 1979) via their killer activity (Starmer *et al*., 1987; Abranches *et al*., 1998; Golubev *et al*., 2003).

Yeast isolate 26 (*Rhodosporidium kratochvilovae*) had the largest antagonistic activity against *F. proliferatum* (Table 5.3), while isolate number 25 (*R. mucilaginosa*) was most inhibitory towards *F. oxysporum*. The yeast isolate that had the highest antagonistic activity against *P. virens* was isolate number 25 (*Rhodotorula mucilaginosa*). Isolate number 22 (*C. saitoi*) displayed the lowest antagonistic activity against the *Fusarium* fungal pathogens. The yeast isolated that was least effective against *P. virens* was isolate number 115 (*Cystofilobasidium feraegulla*). As judged by percentage inhibition (Table 5.3) of the three fungal pathogens tested, *P. virens* was the least affected by the antagonistic activity of the yeast isolates.

TABLE 5.2 YEAST ISOLATE, SPECIES NAMES AND NUMBER OF NUCLEOTIDES OF THE SEQUENCED D1/D2 DOMAIN

Isolate number	*Sequence length (nt)*	*%nt – nt Alignment*	*[a]Species*
22	505	100	*Cryptococcus saitoi* A. Fonseca, Scorzetti & Fell
25	387	100	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison
26	529	100	*Rhodosporidium kratochvilovae* Hamam., Sugiy & Komag
29	500	100	*Hanseniaspora clermontiae* Cadez, Poot, Raspor, & M.T. Sm
72	526	100	*Cryptococcus saitoi*
87	499	99	*Cryptococcus albidosimilis* Vishniac & Kurtzman
96	515	99	*Cryptococcus albidosimilis/ liquefaciens* (Saito & M.Ota) Á. Fonseca, Scorzetti & Fell
109	446	100	*Cryptococcus saitoi*
110	520	100	*Cryptococcus saitoi*
115	517	100	*Cystofilobasidium feraegula*

nt = nucleotide

[a] Yeast isolate identification was based on nucleotide sequence divergence at the variable D1/D2 domain of the large sub-unit (LSU) ribosomal DNA

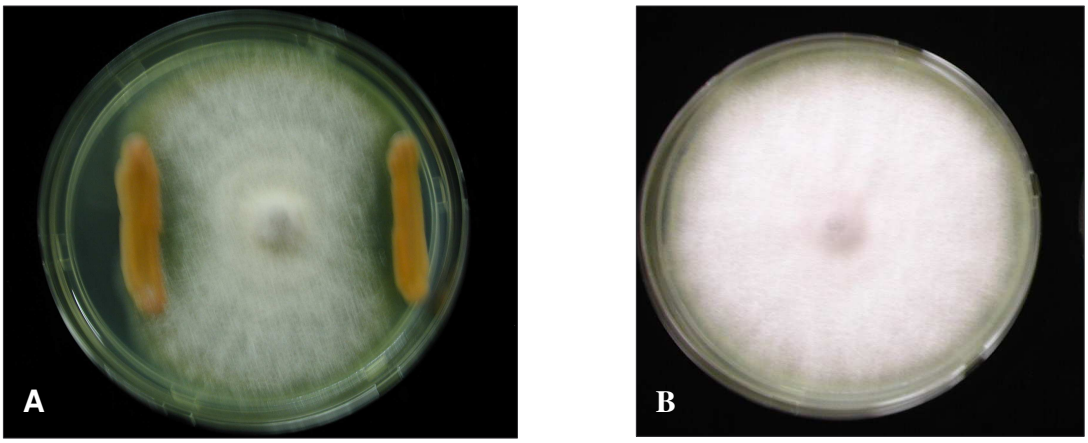


FIGURE 5.8 *IN VITRO* GROWTH INHIBITION (A) Inhibition of *Fusarium oxysporum* growth by yeast isolate number 25 as compared to growth of the yeast free control (B)

Isolate 25 (*Rhodotorula mucilaginosa*) performed well against all three pathogens, whilst the remainder of the isolates displayed inhibition at varying intensity (Table 5.3). Treatment means (Table 5.3) of each of the isolates differed significantly from each other at $p \leq 0.05$. *Rhodotorula mucilaginosa* gave the best all around performance against all pathogens reducing mycelial growth of *F. oxysporum* by 40.77%, *F. proliferatum* by 36.58% and *P. virens* by 37.13% (Table 5.3). The weakest isolate across all fungal pathogens was *Cryptococcus saitoi* (Isolate 22), only able to inhibit *F. oxysporum* mycelial growth by 15.80%, *F. proliferatum* by 9.45%, and *P. virens* by 17.40% (Table 5.3).

Most of the yeasts (60%) with potential antagonistic activity belonged to the genus *Cryptococcus*. Yeasts belonging to this genus are basidiomycetous, colonise various habitats (De Jager *et al*., 2001; Valinsky *et al*., 2002), and have a worldwide distribution (Renker *et al*., 2004). In members of this genus the killer ability is linked to the production of killer toxins, or mycocins (Golubev *et al*., 2003). Killer yeasts produce and excrete protein toxins which are lethal to sensitive yeasts. The K_1 killer toxin in yeasts is a small monomeric protein which is heat labile and only active within a pH 4.2-4.6 range (Starmer *et al*., 1987). It is recommended that in future *in vitro* inhibition studies that these culture conditions be maintained to maximise toxin activity.

TABLE 5.3 MEAN COLONY DIAMETER (MM) AND PERCENTAGE INHIBITION OF FUNGAL PATHOGENS ON DUAL CULTURES SEEDED WITH VARIOUS YEAST ISOLATES

Yeast isolate number	[a] *F. oxysporum*	%IHN	[a] *F. proliferaum*	%IHN	[a] *P. virens*	%IHN
22	67.60 *d*	15.81	68.72 *ef*	9.45	64.80 *cd*	17.40
25	47.47 *a*	40.77	48.17 *ab*	36.58	49.38 *a*	37.13
26	51.09 *abc*	36.28	43.38 *a*	42.90	52.89 *ab*	32.64
29	60.59 *bcd*	24.50	56.01 *cd*	26.22	58.34 *abc*	25.67
72	47.79 *a*	40.36	61.94 *de*	18.40	64.87 *cd*	17.31
87	66.82 *d*	16.77	58.25 *cd*	23.27	60.55 *bcd*	22.83
96	48.99 *a*	38.88	52.78 *bcd*	30.49	59.50 *bc*	24.17
109	57.07 *abcd*	28.86	50.21 *abc*	33.88	69.49 *d*	11.39
110	50.60 *ab*	36.89	51.90 *abc*	31.65	68.81 *cd*	12.27
115	61.74 *cd*	23.07	53.54 *bcd*	29.49	62.99 *bcd*	19.72
Untreated control	80.350 *e*		75.88 *f*		78.39 *e*	

[a] Within a column, values with the same letter are not significantly different at $p \leq 0.05$ according to Ryan, Einot, Gabriel, and Welsch (REGW) F test. %IHN = Percentage inhibition

In addition, members of this genus have an antibacterial capacity (*C. laurentii*) (McCormack *et al*., 1994), and some (*C. neoformans*, *C. albidus*, and *C. curvatus*) are pathogenic to humans, especially in AIDS patients (Kordossis *et al*., 1998). Further trials to determine the safety of these yeasts to humans, especially those that are immune compromised are recommended before any pilot scale experiments are attempted.

Considerable treatment (yeast) x pathogen interaction was observed indicating differing responses of the fungal pathogens to the yeast antagonistic action. Antagonistic modes of action vary from the activation of host defences, to competition for space and nutrients and or antibiosis (Droby and Chalutz, 1994). Investigations to elucidate the antagonists' (yeast isolates) mode(s) of action are required in order to optimise their performance and establish a better screening procedure. It has been shown that exocellular lytic enzymes secreted by yeasts act as depolymerases of fungal cell walls and appear to have antifungal activity (Lorito *et al*., 1994). The level of β-1, 3-glucanase activity has been correlated with the antagonistic activity of *Pichia guillermondii* Wick. against *Botrytis*

cinerea Pers. (Wisniewski *et al*., 1991). It is recommended that future screening procedures include the examination of the presence and amount of β-1, 3-glucanases, and chitinase activity in the ten yeast isolates tested in this study. In addition, further screening trials should focus on members of the *Rhodotorula* genus, as they seem to be more effective against fungal pathogens of cactus pear.

5.4 CONCLUSIONS

The expression of disease resistance within the 38 South African varieties surveyed in this study indicates a quantitative mode of resistance across all varieties evaluated for all three pathogens tested. Roly Poly, Direkteur, and Zastron were the more susceptible varieties. Zastron, one of the most susceptible varieties, is a commercially cultivated variety in South Africa. It is a white pulp variety suitable for the local market and has a high fruit yield of 121.30 ± 6.22 fruit/plant (Chapter 4). Although fruits produced by this variety are comparatively of low mass (148.35 ±7.73 g), it meets the minimum requirements for fruit production in South Africa (> 120 g). Zastron can in future be crossed with a more resistant variety such as Algerian which has acceptable fruit quality traits. It is recommended that prior to the assignment of resistance levels to these varieties that evaluation be replicated over a number of years and/or over diverse locations. Standard tests to quantify resistance of cactus pear varieties to fungal pathogens are required in order to continue with further evaluation of germplasm material. In addition resistance to other fungal pathogens is recommended in future studies.

Roly Poly and Zastron can in the future be used in disease trials as indicator varieties that can be used to quantify resistance response of susceptible varieties when challenged with different races of the same fungal pathogens used in this study. These susceptible varieties will allow estimation of the amount of inoculum present, the effect of fluctuating seasonal conditions, and aid in confirming that they are amongst the most susceptible cactus pear varieties in South Africa.

The most resistant varieties surveyed in this germplasm across all three fungal pathogens were Amersfoort, Meyers, and Algerian. Algerian is commercially cultivated for fruit production in South Africa, especially in the more humid areas in the Limpopo Province. Thus, in the event of a disease outbreak involving one of the pathogens tested in this study, farmers who have planted Algerian will sustain lower crop losses than those who have planted Zastron for example, which is more susceptible to fungal disease. However, it is recommended that disease screening trials with the pathogens tested in

this study be repeated in the humid areas of Limpopo Province since the climatic conditions in this area are different from the ones where this study was performed, and will influence disease progression.

Fusarium spp. are considered to be important pathogens for cactus pear since they flourish in hot, humid areas, and disease development is encouraged by poor soil conditions characterised by increased acidity, low permeability, and elevated humidity (Granata, 1995). *Fusarium proliferatum* is listed amongst the most important pathogens of native *Opuntia* species in Arizona (Mildenhall *et al*., 1987). *Fusarium oxysporum* f.s. *opuntiarum* causes 'Fusarium wilt' in Opuntias. It affects the vascular tissues, causing wilting of cladodes and fruit, leading to a reddening of infected tissues (Zimmermann and Granata, 2002). It is recommended that further trials be performed in the Limpopo Province with the same varieties and using the pathogens tested here to augment the reliability of these findings.

Post-harvest biological control in fruit is promising from a practical point of view because application sites are limited to the fruit and environmental conditions are defined and controlled in storage rooms (Jijakli *et al*., 1999). Products containing *Pseudomonas syringae* Van Hall, active against the genera *Botrytis*, *Penicillium*, *Mucor*, and *Geotrichum* are commercially available (Janisiewicz and Jeffers, 1997), whilst products containing antagonistic yeasts are still under development (Ippolito *et al*., 2000). The results presented in this study, are however, only preliminary. Investigations performed under field conditions or in storage facilities will be more conclusive, and are recommended to confirm these findings. In addition, the modes of antagonism of these yeast isolates against fungal pathogens of cactus pear fruit and their safety to humans require further research.

REFERENCES

Abranches, J., P. Valente, H.N. Nóbrega, F.S.A. Fernandez, L.C. Mendonca-Hagler and A.N. Hagler, 1998. Yeast diversity and killer activity dispersed in faecal pellets from marsupials and rodents in a Brazilian tropical habitat mosaic. Federation of European Microbiological Societies Microbiology Ecology 26: 27-33.

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389-3402.

Cantwell, M., 1986. Post-harvest aspects of prickly pear fruits and vegetable cladodes. In: Perishables handling, post-harvest technology of fresh horticultural crops. Cooperative extension, University of California 59: 6-9.

Chessa, I. and G. Barbera, 1984. Indagine sulla frigocon-servszione dei frutti dell acv. Gialla di ficodindia. Frutticoltura 46: 57-61.

Dayton, D.F., R.L. Bell and E.B. Williams, 1983. Disease resistance. In: Moore, J.N. and J. Janick (Eds.), Methods in fruit breeding, pp 189-215. Purdue University Press, West Lafayette, Indiana, USA.

De Jager, E.S., F.C. Wehner and L. Korsten, 2001. Microbial ecology of the mango phylloplane. Microbial Ecology 42: 201-207.

Droby, S. and E. Chalutz, 1994. Mode of action of biocontrol agents of post-harvest diseases. In: Wilson, C.L. and M.E. Wisniewski (Eds.), Biological Control of Post-harvest Diseases-Theory and Practise, pp 63-75. CRC Press, Boca Raton, USA.

Fell, J.W., T. Boekhout, A. Fonseca, G. Scorzetti and A. Statzell-Tallman, 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. International Journal of Systematic and Evolutionary Microbiology 50: 1351-1371.

Fokkema, N.J., J.G. den Houter, Y.J.C. Kosterman and A.L. Nelis, 1979. Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum*. Transactions of the British Mycology Society 72: 19-29.

Golubev, W.I., M. Gadanho, J.P. Sampaio and N.W. Golubev, 2003. *Cryptococcus nemorosus* sp. nov. and *Cryptococcus perniciosus* sp. nov., related to *Papiliotrema* Sampaio *et al*. (Tremellales). International Journal of Systematic and Evolutionary Microbiology 53: 905-911.

Gower, J.C., 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Granata, G., 1995. Biotic and Abiotic diseases. In: Barbera, G., P. Inglese and E. Pimienta-Barrios (Eds.), Agro-ecology, cultivation and uses of cactus pear, pp 109-119. FAO. Plant production and protection paper 132, Rome.

Granata, G. and A. Sidoti, 2000. Survey of diseases discovered on *Opuntia ficus-indica* in producer countries. Proceedings of the fourth International Congress on cactus pear and cochineal. Acta Horticulturae 51: 231-237.

Henry, J. and B. Kirsop, 1989. Cryopreservation of yeasts in polypropylene straws. WFCC-Education Committee, UNESCO (http://www.wfcc.info/tis/info3.html).

Inglese, P., G. Barbera and T. La Mantia, 1995. Research strategies for the improvement of cactus pear (*Opuntia ficus-indica*) fruit quality and production. Journal of Arid Environments 29: 455-468.

Janisiewicz, W.J. and S.N. Jeffers, 1997. Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. Crop Protection 16: 629-633.

Ippolito, A., A. El-Ghaouth, C.L. Wilson, and M. Wisniewski, 2000. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biological Technology 19: 265-272.

Jijakli, H.M., P. Lepoivre and C. Grevesse, 1999. Yeast species for biocontrol of apple post-harvest diseases: an encouraging case study for practical use. In: Mukerji, K.G., B.P. Chamola and R.K. Upadhyay (Eds.), Biotechnological approaches in biocontrol of plant pathogens, pp 31-49. Kluwer Academic/Plenum Publishers, New York, London, UK.

Kim, Y.H. and K-H. Kim, 2002. Abscission layer formation as a resistance response of Peruvian apple cactus against *Glomerella cingulata*. Phytopathology 92: 964-969.

Kordossis, T., A. Avlami, A. Velegraki, I. Stefanou, G. Georgakopoulos, C. Papalambrou and N.J. Legakis, 1998. First report of *Cryptococcus laurentii* meningitis and a fatal case of *Cryptococcus albidus* cryptococcaemia in AIDS patients. Medical Mycology 36: 335-339.

Kurtzman, C.P. and C.J. Robnett, 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73: 331-371.

Lorito, M., C.K. Hayes, A. Di Pietro, S.L. Woo and G.E. Harman, 1994. Purification, characterisation, and synergistic activity of a glucan 1,3-β-glucosidase and an *N*-acetyl-β-glucosaminidase from *Trichoderma harzianum*. Phytopathology 84: 398-405.

McCormack, P.J., H.G. Wildman and P. Jeffries, 1994. Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeast like fungi. Applied Environmental Microbiology 60: 927-931.

Mildenhall, J.P., S.M. Alcorn and W.F.O. Marasas, 1987. Pathogenicity of fungi isolated from *Opuntia* species in Arizona to *Opuntia aurantiaoa*. Phytophylactica 19: 485-489.

Momol, M.T., J.L. Norelli, H.S. Aldwinckle and W. Zeller, 1996. Use of the area under the disease progress curve for quantification of resistance of apple and pear varieties and rootstocks to *Erwinia amylovorsa*. Acta Horticulturae 411: 373.

Punja, Z.K., 1997. Comparative efficacy of bacteria, fungi, and yeasts as biological control agents for diseases of vegetable crops. Canadian Journal of Plant Pathology 19: 315-323.

Renker, C., V. Blanke, B. Börstler, J. Heinrichs and F. Buscot, 2004. Diversity of *Cryptococcus* and *Dioszegia* yeasts (Basidiomycota) inhabiting arbuscular mycorrhizal roots or spores. Federation of European Microbiological Societies Yeast Research 4: 597-603.

Rodriguez-Felix, A., 2002. Post-harvest physiology and technology of cactus pear fruits and cactus leaves. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 191-199.

Rohlf, F.J. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology 47: 147-158.

SPSS for Windows, 22 December 1997. Copyright© SPSS Inc.

Starmer, W.T., P.F. Ganter and V. Arberdeen, 1987. The ecological role of killer yeasts in natural communities of yeasts. Canadian Journal of Microbiology 33: 783-796.

Swart, W.J. and V.R. Swart, 2003. The current status of research on diseases of *Opuntia ficus-indica* in South Africa. In: Nefzaoui, A. and P. Inglese (Eds.), Proceedings of the Fourth International Congress on Cactus Pear and Cochineal. Acta Horticulturae 581: 239-245.

Swart, W.J. and W.M. Kriel, 2002. Pathogens associated with necrosis of cactus pear cladodes in South Africa. Plant Disease 86: 693.

Swart, W.J., M.R. Oelofse and M.T. Labuschagne, 2003. Susceptibility of South African cactus pear varieties to four fungi commonly associated with disease symptoms. Journal of the professional Association for Cactus Development 5 : 86-97.

Urquhart, E.J. and Z.K. Punja, 1997. Epiphytic growth and survival of *Tilletiopsis pallescens*, a potential biological control agent of *Sphaerotheca fuliginea*, on cucumber leaves. Canadian Journal of Botany 75: 892-901.

Valinsky, L., G. Della Vedova, T. Jiang and J. Borneman, 2002. Oligonucleotide fingerprinting of rRNA genes for analysis of fungal community composition. Applied Environmental Microbiology 68: 5999-6004.

Varvaro, L., G. Granata and G.M. Balestra, 1993. Severe *Erwinia* damage on *Opuntia ficus-indica* in Italy. Journal of Phytopathology 138: 325-330.

Wang, C.Y., 1994. Chilling injury of tropical horticultural commodities. Horticultural Science 29: 986-988.

Wilson, C.L. and M.E. Wisniewski, 1989. Biological control of post-harvest diseases of fruits and vegetables: an emerging technology. Annual Review of Phytopathology 27: 425-441.

Wisniewski, M., C. Biles, S. Droby, R. McLaughlin, C. Wilson and E. Chalutz, 1991. Mode of action of the post-harvest biocontrol yeast *Pichia guillermondi.* I. Characterisation of attachment to *Botrytis cinerea*. Physiological and Molecular plant Pathology 39: 245-258.

Zimmermann, H.G. and G. Granata, 2002. Insect pests and diseases. In: Nobel, P.S. (Ed.), Cacti, biology and uses, pp 235-254.. University of California Press, Los Angeles.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Commercial cactus pear fruit orchards in South Africa make use of the spine-less Burbank varieties, that are clonally propagated using terminal cladodes. Most of the planting material supplied to these farmers originated from the Limpopo Provincial Department of Agriculture, Mokopane genebank, and the Mara genebank (experimental farm near Makhado) that host more than 80 accessions of cactus pear. These varieties were developed from the original Burbank material either as clones, or as artificial or natural hybrids. Currently, it is likely that duplicated accessions occur under different common names in the collections. Cactus pear has very subtle morphological differences and it undergoes drastic changes in different environments in traits, such as cladode spininess, shape, and size, that are routinely used to identify different varieties. It therefore became important to determine whether a molecular marker technique such as AFLP can be used to characterise these varieties into homogeneous clusters in agreement with other agronomic traits of interest such as disease resistance, cladode nutritional quality, fruit quality, and vegetative yield.

One of the aims of the current study was to genetically fingerprint germplasm using AFLP markers to circumvent the difficulty of doing so solely on phenotypic data. Furthermore, the varieties were evaluated for disease resistance, cladode nutritional quality and fruit quality. In addition, a search to find yeasts able to limit post-harvest rot of fruit was undertaken.

Amidst the taxonomic confusion regarding the delineation of the various species within the *Opuntia* genus, AFLP markers were successfully used in this study to fingerprint 38 South African cactus pear (*Opuntia* spp.) varieties. Based on the Jaccard similarity coefficient the majority of the varieties were approximately 83% genetically similar. Phenotypic identification is unreliable for cactus pear varieties due to the high morphological similarity between cultivars, and its high plasticity. In addition, because of the possibility of partial and total hybridisation between cultivated varieties, hybrids are easily formed. In this study, cactus pear varieties were fingerprinted using AFLP markers, generating unique marker profiles for each variety. No duplicates were detected within the varieties tested. It is recommended that the AFLP technique be used to screen the remaining accessions within the South African cactus pear germplasm collection with the inclusion of more primer combinations. Genotype specific fragments generated for nine varieties can then be confirmed, since these unique markers may be present in varieties not screened in this study.

In South Africa, cactus pear can be used as a dual purpose crop for fruit and fodder production as annually pruned cladodes are discarded as waste at huge cost to farmers (Potgieter and Smith, 2006). The AFLP marker data generated in this study can assist breeders in the selection of varieties for fruit and fodder production. Traditional identification of fruit varieties relies on the assessment of pomological, morphological, and horticultural traits of the adult plant, which leads to significant time delays when surveying germplasm. Cactus pear breeding can be supplemented by direct selection at the genetic level using molecular markers that allow fingerprinting of plant germplasm that co-segregate with the plant genes of interest. AFLP markers have been applied in apricots for cultivar identification (Guena *et al*., 2003), for the identification of peach and nectarine varieties (Manubens *et al*., 1999).

Varieties recommended for commercial fruit production in the Mokopane district of the Limpopo Province, based on cluster analysis of fruit quality and yield are Gymno Carpo, Malta, Algerian, Morado, Meyers, and Roedtan. These varieties meet the minimum requirements for cactus pear fruit production in South Africa (Potgieter and Mkhari, 2002). These varieties, except for Meyers and Roedtan, are also adapted for fruit production in the Middleveld area of the Limpopo Province (Potgieter and Smith, 2006). Multi-location yield trials are therefore recommended for these varieties (Meyers and Roedtan) to assess their fruit yield in the different agro-climatic regions of South Africa.

Given that it is well established that cladodes are adequate as animal feed, provided that a protein supplement is given, the nutritional quality of annually pruned cladodes from commercial orchards for use as fodder was investigated. Malta, Gymno Carpo, American Giant, and Arbiter ranked the highest for CP content. Messina, Nepgen, and Cross X ranked the highest for DM content. Findings of this study showed that cladodes from a commercially maintained orchard had high crude protein content, and are adequate for use as fodder. In addition, since the selection of superior plants for forage production is traditionally based on plant vigour and vegetative yield, the vegetative yield was assessed. The varieties that ranked the highest for vegetative yield were Turpin and Gymno Carpo.

The expression of disease resistance within the varieties surveyed indicates a quantitative mode of resistance across all varieties evaluated for all three pathogens tested. Roly Poly, Direkteur, and Zastron were the more susceptible varieties. The most resistant varieties surveyed in this germplasm across all three fungal pathogens were Amersfoort, Meyers, and Algerian.

The overall mean pathogen lesions previously reported by Swart *et al*. (2003) were smaller than those reported in this study for *P. virens* (4.78 mm), but bigger than those reported for *F. oxysporum* (12.48 mm) and *F. proliferatum* (7.49 mm). Variation in pathogen mean lesion diameters between the two studies could be attributed to differences in climatic conditions prevailing during field trials, as the amount and occurrence of infection can be influenced by environmental conditions (Dayton *et al*., 1983). In this study the disease trial was performed during summer when humidity was low, which could have limited fungal growth. Since annual variations in climatic conditions affect the host as well as the activity of the pathogen, the reliability of field testing (Dayton *et al*., 1983) may be reduced. It is therefore recommended that subsequent screening of these cactus pear varieties be repeated over a few years, where each season is considered as a repeat test against each pathogen.

Phenotypic selection for the traits evaluated in this study (fruit quality, fruit yield, disease resistance, and vegetative yield), in future breeding studies can be altered as a result of environmental effects and by the complex genetic nature of these polygenic traits. Ideally, from the dendrograms presented in this study, individuals differing in agronomic traits of interest can be selected and hybridised to produce fertile, sexual offspring. Subsequently the offspring (mapping population) can be used to map molecular markers that are linked to agronomically important traits. These markers can then be used in future breeding programmes to shorten the time required for the selection of new varieties. The occurrence of apomixis, asexual seed production from the maternal tissues (Mondragón-Jacobo and Pimienta, 1995) is however, still an obstacle in breeding of cactus pear varieties.

The short cactus pear fruit shelf life and the continuous cold chain needed to deliver attractive, high quality fruit is not always available to farmers, especially in developing countries. Thus future research into ways of increasing the post-harvest quality of fruits is essential to minimise loss incurred by farmers (Felker and Inglese, 2003). Although post-harvest biological control of fruit rot is promising, further trials to determine optimum culture conditions of yeast killer toxins and their safety to humans are required. It is hoped that the results of this study will assist in the breeding and selection of South African cactus pear varieties for increased fruit yield and quaility which in turn would result in significant improvements in productivity.

REFERENCES

Dayton, D.F., R.L. Bell and E.B. Williams, 1983. Disease resistance. In: Moore, J.N. and J. Janick (Eds.), Methods in fruit breeding, pp 189-215. Purdue University Press, West Lafayette, Indiana, USA.

Felker, P. and P. Inglese, 2003. Short-term and long-term research needs for *Opuntia ficus-indica* (L.) Mill. Utilization in arid areas. Journal of the Professional Association for Cactus Development 5: 131-152.

Guena, F., M. Toschi and D. Bassi, 2003. The use of AFLP markers for cultivar identification in apricot. Plant Breeding 122: 526-531.

Manubens, A., S. Lobos, Y. Jadue, M. Toro, R. Messina, M. Lladser and D. Seelenfreund, 1999. DNA isolation and AFLP fingerprinting of nectarine and peach varieties (*Prunus persica*). Plant Molecular Biology Reporter 17: 255-267.

Mondragón-Jacobo, C. and E.B. Pimienta, 1995. Propagation. In: Barbera, G., P. Inglese and B.E. Pimienta (Eds.), Agroecology, cultivation and uses of cactus pear, pp 120-130. FAO Plant production and protection paper 132. Rome, Italy.

Potgieter, J. P. and M. Smith, 2006. Genotype X Environment interaction in cactus pear (*Opuntia* spp.), Additive Main Effects and Multiplicative interaction of fruit yield. Acta Horticulturae 728: 97-104.

Potgieter, J.P. and J.J. Mkhari, 2002. Evaluation of cactus pear (*Opuntia* spp.) germplasm for fruit production purposes. Combined Congress, 15-17 January 2002, Pietermaritzburg, Kwazulu/Natal.

Swart, W.J., M.R. Oelofse and M.T. Labuschagne, 2003. Susceptibility of South African cactus pear varieties to four fungi commonly associated with disease symptoms. Journal of the professional Association for Cactus Development 5 : 86-97.

SUMMARY

South Africa hosts one of the largest cactus pear germplasm collections in the world. However, not all the varieties have been fully characterised, and evaluated for fruit quality, nutritional quality for use as fodder, and disease resistance. In this study, 38 South African cactus pear (*Opuntia* ssp.) varieties were characterised using AFLP markers to circumvent G X E effect on phenotypic characterisation. With the use of nine primer combinations, the varieties were grouped into four main clusters based on 346 fragments (per sample) of which 48% were polymorphic between samples. The dendrograms generated indicated that commercially cultivated varieties were dispersed amongst the different clusters indicating that they represent the genetic diversity within the germplasm. Genotype specific fragments were generated using six primer combinations, allowing the unique identification of nine varieties, three of which are commercially cultivated (Meyers, Roedtan, and Santa Rosa). Varieties that are recommended for commercial cultivation in the Mokopane district of the Limpopo Province, based on fruit quality and yield are Gymno Carpo, Malta, Algerian, Morado, Meyers, and Roedtan. These varieties meet the minimum requirements for cactus pear fruit production in South Africa. Nutritional quality evaluation of pruned cladodes from a commercial orchard in the Free State Province indicated that the varieties, Malta, Gymno Carpo, and American Giant ranked the highest in terms of CP content. Varieties that yielded the highest DM content were Messina, Nepgen, and Cross X. Varieties that ranked the highest for OM content were Cross X, Nepgen, and Sicilian Indian Fig. Gymno Carpo and Malta are amongst the varieties recommended for cultivation for fruit, as such they can be used as dual purpose crops for the production of both fodder and fruit. Evaluation for disease resistance indicated a quantitative mode of resistance across all varieties for all three fungal pathogens tested. The most resistant varieties surveyed in this study across all three fungal pathogens were Amersfoort, Meyers, and Algerian. Roly Poly, Direkteur, and Zastron were the more susceptible varieties. Of the three fungal pathogens tested, *P. virens* was the least affected by the antagonistic activity of the yeast isolates. Isolate 25 (*Rhodotorula mucilaginosa*) performed well against all three pathogens, whilst the remainder of the isolates displayed inhibition at varying degrees.

Key words: AFLP, antagonistic yeasts, biocontrol, cactus pear, disease screening, fodder, fruit quality, fungal pathogens, genotyping, inhibition, *nutritional quality, Opuntia ficus-indica*

OPSOMMING

Suid-Afrika besit een van die grootste turksvy kiemplasma versamelings in die wêreld. Al die variëteite is egter nog nie ten volle vir vrugkwaliteit, voedingswaarde vir veevoer en siekteweerstand gekarakteriseer of geëvalueer nie. In hierdie studie is 38 Suid-Afrikaanse turksvy (*Opuntia* ssp.) variëteite suksesvol m.b.v. AFLP merkers gekarakteriseer om die effek van G X E op fenotipiese karakterisering uit te skakel. Met die gebruik van nege voorvoerder kombinasies is die variëteite op grond van 346 fragmente (per monster) waarvan 48% polimorfies was in vier groepe verdeel. Kommersiële variëteite was tussen die verskillende groepe versprei, wat aangedui het dat hulle die genetiese diversiteit binne die kiemplasma verteenwoordig. Genotipe spesifieke fragmente is met ses voorvoerder kombinasies gegenereer, wat die unieke identifikasie van nege variëteite moontlik gemaak het, insluitend drie kommersiële variëteite (Meyers, Roedtan en Santa Rosa). Variëteite wat gebaseer op vrugkwaliteit en opbrengs vir kommersiële produksie in die Mokopane distrik van die Limpopo Provinsie, aanbeveel word, is Gymno Carpo, Malta, Algerian, Morado, Meyers en Roedtan. Hierdie variëteite voldoen aan die minimum vereistes vir turksvy produksie in Suid Afrika. Voedingswaarde analise van die gesnoeide kladodes van ‘n kommersiële boord in die Vrystaat Provinsie het aangedui dat die variëteite Malta, Gymno Carpo en American Giant die beste t.o.v. ruproteïen inhoud gevaar het. Die variëteite met die hoogste droëmassa inhoud was Messina, Nepgen en Cross X. Die variëteite wat die beste vir organiese inhoud gevaar het was Cross X, Nepgen en Sicilian Indian Fig. Gymno Carpo en Malta was van die variëteite wat vir vrugproduksie aanbeveel word. As sulks kan hulle vir dubbeldoel produksie vir beide veevoer en vrugte gebruik word. Evaluasie vir siekteweerstand het ‘n kwantitatiewe model vir weerstand oor alle variëteite vir al drie fungus patogene aangedui. Die mees weerstandbiedende variëteite vir al drie patogene was Amersfoort, Meyers en Algerian. Roly Poly, Direkteur en Zastron was die mees vatbare variëteite. Van die drie fungus patogene wat getoets is, was *P. virens* die minste deur antagonistiese aksie van gis isolate beïnvloed. Isolaat 25 (*Rhodotorula mucilaginosa*) het goed teenoor al drie die patogene gereageer, terwyl die res van die isolate inhibisie van verskillende grade getoon het.

Sleutelwoorde: AFLP, antagonistiese giste, biobeheer, fungus patogene, genotipering, inhibisie, *Opuntia ficus-indica, siekte-evaluasie, turksvy, veevoer, voedingswaarde, vrugkwaliteit*

APPENDIX I: GILLEMBERG WEATHER DATA (1999-2001)																		
CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	1	18.4	27.9	19.1	21.7	13.5	-24.0	1.2	98.4	65.1	4.3	20.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	2	0.0	29.7	19.2	23.9	14.5	-24.0	1.6	96.4	58.8	4.9	21.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	3	0.0	27.7	17.9	22.7	12.8	-24.0	1.8	96.0	62.5	5.0	24.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	4	0.0	27.5	17.2	22.2	12.4	-23.0	1.8	95.1	56.3	5.1	24.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	5	8.4	29.0	16.7	21.3	12.9	-22.5	1.7	96.2	57.3	4.9	22.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	6	0.2	27.7	17.4	21.9	12.6	-23.0	1.7	94.3	62.0	5.6	28.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	7	0.0	28.2	17.5	23.1	12.9	-23.0	2.7	93.4	54.7	6.5	32.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	8	0.0	27.0	17.6	21.5	12.3	-23.0	2.8	94.2	51.9	6.0	28.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	9	0.0	28.2	16.4	21.8	12.3	-21.5	1.8	92.9	48.1	6.0	29.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	10	0.0	28.5	16.1	22.7	12.3	-22.0	2.3	91.9	49.6	6.4	31.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	11	13.4	28.0	19.3	22.0	13.7	-24.0	2.3	97.0	63.2	4.3	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	12	0.6	28.7	19.0	22.5	13.9	-24.0	2.2	95.3	61.0	5.4	25.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	13	0.0	28.8	17.4	22.8	13.1	-23.5	1.9	94.9	50.7	5.2	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	14	0.0	30.7	15.8	23.4	13.3	-22.5	1.9	96.3	37.9	6.5	28.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	15	0.0	29.1	16.0	23.0	12.6	-23.0	2.5	95.8	46.5	7.0	33.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	16	0.0	29.0	16.6	22.9	12.8	-22.5	2.1	93.8	42.9	6.9	33.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	17	0.0	32.1	15.1	24.1	13.6	-20.5	1.7	95.5	31.4	7.3	33.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	18	0.0	33.6	16.3	25.2	15.0	-22.5	1.4	87.2	40.5	7.2	32.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	19	24.2	33.4	19.0	23.9	16.2	-24.0	2.1	91.2	43.7	6.0	21.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	20	0.0	30.4	20.5	24.7	15.5	-24.0	2.7	87.2	51.6	6.5	27.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	21	7.8	28.1	19.2	21.8	13.7	-24.0	2.3	95.2	69.4	3.7	15.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	22	0.0	26.3	18.7	21.8	12.5	-24.0	2.4	89.7	62.2	4.8	22.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	23	0.0	26.1	18.3	21.2	12.2	-24.0	1.5	92.1	63.3	3.9	17.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	24	6.8	27.7	19.1	21.4	13.4	-24.0	1.3	94.2	63.0	4.1	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	25	0.2	28.9	18.2	22.9	13.6	-24.0	2.0	95.4	46.1	5.8	26.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	26	0.0	29.5	15.8	22.8	12.7	-21.5	2.0	95.4	43.2	6.3	29.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	27	0.4	26.8	15.7	20.7	11.3	-22.0	2.0	92.2	44.9	5.3	23.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	28	17.8	26.0	17.2	19.7	11.6	-20.0	1.5	94.6	59.5	4.1	19.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	29	0.0	28.8	18.0	22.5	13.4	-24.0	2.3	89.1	53.4	5.2	21.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	30	20.2	28.4	19.1	22.1	13.8	-24.0	2.3	96.2	59.7	4.2	16.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	1	31	0.0	27.7	18.7	22.6	13.2	-24.0	1.7	92.6	61.6	4.7	22.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	1	0.0	27.5	17.9	22.4	12.7	-24.0	2.3	96.6	62.4	5.1	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	2	0.8	28.3	18.7	22.3	13.5	-24.0	2.1	94.2	65.3	4.4	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	3	5.6	28.2	18.8	22.1	13.5	-24.0	1.3	94.0	67.0	4.0	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	4	0.8	29.0	19.1	22.7	14.1	-24.0	1.9	96.3	57.8	4.5	18.8

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	5	14.0	26.7	19.4	22.6	13.1	-24.0	1.6	95.5	67.1	3.4	14.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	6	0.0	32.1	19.6	24.4	15.9	-24.0	1.4	99.0	46.0	6.2	27.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	7	0.0	31.7	19.3	24.2	15.5	-24.0	2.2	97.3	49.3	6.5	28.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	8	0.0	27.7	18.1	21.9	12.9	-24.0	2.2	94.4	58.4	4.4	18.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	9	0.0	29.4	14.8	21.7	12.1	-20.0	2.3	95.2	48.7	6.4	30.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	10	0.0	27.7	15.4	21.0	11.6	-20.0	1.8	91.7	48.3	5.8	29.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	11	0.0	31.2	13.4	22.4	12.3	-17.5	1.9	92.7	42.3	6.6	30.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	12	0.0	29.4	17.9	23.2	13.7	-24.0	2.1	90.9	41.2	6.9	32.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	13	0.0	32.0	15.9	23.1	14.0	-21.0	1.8	99.9	30.1	6.9	30.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	14	0.0	29.6	14.3	21.8	12.0	-19.5	2.7	91.5	37.8	6.8	30.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	15	0.0	25.8	17.3	20.5	11.6	-23.0	2.3	89.6	65.2	4.0	17.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	16	0.0	30.1	16.9	22.3	13.5	-22.5	1.9	91.5	43.0	5.7	25.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	17	0.0	29.9	16.4	22.7	13.2	-22.5	2.4	91.5	41.7	6.4	29.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	18	0.0	31.4	15.3	23.6	13.4	-21.5	2.2	93.5	31.3	6.9	31.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	19	0.0	33.4	19.9	25.5	16.7	-24.0	1.9	91.5	36.5	6.5	27.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	20	0.0	29.8	20.1	22.8	15.0	-24.0	2.6	92.5	63.9	4.9	20.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	21	0.0	28.3	17.5	22.8	12.9	-24.0	2.3	91.5	54.8	4.8	20.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	22	0.0	31.5	17.7	23.6	14.6	-24.0	1.9	92.5	49.5	5.3	21.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	23	0.0	29.2	17.7	23.0	13.5	-24.0	2.0	91.5	48.2	4.6	18.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	24	0.0	31.0	14.9	22.9	13.0	-19.5	2.2	91.5	41.7	6.5	28.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	25	0.0	32.9	15.5	24.1	14.2	-21.0	2.5	93.5	30.0	7.5	32.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	26	0.0	33.5	15.8	24.5	14.7	-21.5	2.0	69.9	29.3	7.7	31.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	27	0.0	32.6	16.6	24.3	14.6	-23.0	2.0	91.0	24.2	7.1	28.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	2	28	0.0	35.2	14.4	24.5	14.7	-19.5	1.8	93.9	22.6	7.7	31.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	1	0.0	37.0	15.2	26.4	15.1	-21.5	2.7	75.7	19.8	9.4	31.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	2	0.0	35.7	18.0	25.2	16.5	-24.0	2.3	91.2	21.6	8.3	31.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	3	0.0	32.0	15.5	23.4	13.8	-21.5	2.8	90.8	28.5	7.8	31.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	4	0.0	34.6	14.9	24.6	14.8	-19.5	2.5	91.5	22.0	8.2	31.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	5	0.0	28.1	14.6	22.2	11.4	-19.5	2.3	92.6	50.7	4.4	17.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	6	2.0	28.0	19.5	22.5	13.8	-24.0	2.9	92.7	56.4	4.1	14.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	7	0.0	28.9	18.4	22.6	13.7	-24.0	2.8	93.6	38.7	6.1	25.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	8	0.0	28.7	16.6	22.1	12.7	-20.5	2.6	92.8	38.0	6.6	30.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	9	0.0	29.5	16.2	22.3	12.9	-21.5	2.8	90.9	38.3	6.8	30.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	10	0.0	29.7	15.9	22.6	12.8	-21.5	3.0	90.8	36.8	6.8	28.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	11	0.0	30.6	15.6	23.1	13.1	-21.5	2.2	93.8	34.2	6.8	30.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	12	0.0	33.2	16.8	24.4	15.0	-22.5	1.9	95.5	32.9	6.3	24.8

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	13	0.0	30.3	19.5	23.9	14.9	-24.0	3.1	86.7	42.7	5.8	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	14	0.0	28.1	18.4	22.5	13.3	-24.0	2.8	86.0	50.0	4.9	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	15	2.4	29.2	16.8	21.2	13.0	-22.5	2.3	92.4	50.6	5.0	21.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	16	5.0	26.4	18.6	20.3	12.5	-24.0	1.9	96.6	61.7	3.0	11.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	17	0.2	29.7	18.1	21.7	13.9	-24.0	1.9	96.6	46.3	4.5	17.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	18	0.2	27.9	17.9	21.3	12.9	-24.0	2.5	94.4	54.1	4.4	17.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	19	0.0	26.6	17.5	22.1	12.1	-18.6	2.1	97.9	53.4	4.0	17.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	20	0.0	27.3	17.8	22.5	12.5	-19.5	1.7	94.8	45.0	4.1	17.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	21	0.0	28.1	16.2	22.2	12.2	-18.8	2.1	96.8	53.4	4.4	19.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	22	2.9	25.4	19.1	22.3	12.3	-19.1	2.2	89.7	59.1	3.7	16.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	23	0.4	24.1	18.9	21.6	11.5	-13.0	1.5	94.9	71.6	2.9	14.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	24	0.0	27.7	17.6	21.7	12.7	-23.0	2.3	92.4	54.8	4.5	20.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	25	0.0	25.4	16.2	20.4	10.8	-21.5	2.5	88.2	52.2	4.9	23.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	26	0.0	28.6	14.8	21.3	11.7	-19.0	1.9	95.4	42.3	5.2	24.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	27	0.0	27.8	15.9	21.3	11.9	-20.0	2.9	88.6	44.8	5.7	25.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	28	0.0	26.6	16.7	20.5	11.7	-19.0	2.6	91.1	46.9	5.0	22.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	29	0.0	27.9	14.4	20.5	11.2	-16.5	2.1	94.1	40.9	5.2	24.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	30	0.0	25.4	12.7	19.2	9.1	-16.5	3.0	94.0	47.1	4.9	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	3	31	0.0	25.7	12.8	19.3	9.3	-16.0	2.8	91.0	56.7	4.2	19.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	1	0.0	28.1	12.9	20.3	10.5	-17.0	2.2	94.8	35.1	5.6	26.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	2	0.0	28.0	14.0	20.3	11.0	-17.5	2.0	93.3	41.6	4.8	22.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	3	0.0	27.6	14.6	20.7	11.1	-19.0	2.3	90.1	41.9	4.9	21.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	4	0.0	28.5	15.9	21.2	12.2	-20.5	2.1	89.3	40.0	4.9	20.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	5	0.0	31.1	14.7	21.8	12.9	-19.5	2.3	88.9	30.6	6.0	23.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	6	0.0	27.3	12.8	20.1	10.1	-17.0	2.1	92.8	39.9	5.1	25.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	7	0.0	26.8	13.5	19.9	10.2	-18.0	2.2	94.4	39.3	4.7	20.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	8	0.0	27.4	12.3	19.9	9.9	-17.0	2.0	95.1	43.8	4.8	24.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	9	0.0	30.5	13.2	21.8	11.9	-17.5	1.6	85.8	37.9	5.3	25.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	10	0.0	32.2	15.3	23.5	13.8	-21.0	2.0	88.4	31.7	6.0	24.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	11	1.0	26.7	16.5	20.4	11.6	-20.0	3.5	83.7	50.0	5.0	20.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	12	0.0	24.8	13.0	18.4	8.9	-14.0	2.9	82.1	49.8	4.6	21.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	13	0.0	24.0	11.0	17.1	7.5	-9.0	2.5	96.5	44.9	4.5	24.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	14	0.0	27.3	9.0	17.9	8.2	-9.5	1.8	90.5	25.9	5.2	25.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	15	0.0	29.3	9.6	19.3	9.5	-12.0	1.3	93.3	30.3	4.8	24.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	16	0.0	31.9	13.3	22.6	12.6	-18.0	1.7	58.5	25.4	5.8	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	17	0.0	31.1	15.3	23.4	13.2	-22.0	2.3	69.0	24.6	6.3	24.3

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	18	0.0	32.2	15.1	23.9	13.7	-20.5	2.3	80.2	28.2	6.1	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	19	0.0	32.9	16.7	24.2	14.8	-22.0	1.8	65.5	24.4	5.9	24.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	20	0.0	33.6	19.1	25.4	16.4	-24.0	2.1	72.8	29.7	5.9	19.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	21	1.2	30.1	19.0	23.0	14.6	-24.0	1.9	92.5	47.8	3.6	12.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	22	21.2	27.3	17.8	20.9	12.6	-23.5	3.6	94.1	63.7	3.4	11.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	23	0.0	26.7	17.6	21.0	12.2	-22.0	2.0	91.1	61.3	3.3	14.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	24	0.0	28.0	16.7	21.2	12.4	-20.0	2.8	90.9	57.6	4.5	21.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	25	0.0	25.1	16.5	19.7	10.8	-19.0	2.6	89.5	62.2	3.7	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	26	0.0	25.1	15.5	19.2	10.3	-17.0	3.4	95.3	59.1	3.7	17.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	27	0.0	24.0	13.4	17.7	8.7	-12.5	2.2	94.8	60.6	3.3	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	28	0.0	21.9	10.3	16.0	6.1	-7.5	2.9	95.5	59.2	3.4	19.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	29	0.0	21.8	9.6	14.9	5.7	-3.5	2.4	96.5	50.4	3.4	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	4	30	0.0	22.5	10.4	15.7	6.5	-5.0	1.9	93.1	56.5	3.1	16.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	1	0.0	25.9	9.4	16.8	7.7	-8.0	1.2	96.2	45.6	3.5	20.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	2	0.0	28.3	11.4	19.7	9.9	-13.0	1.3	79.6	40.2	4.0	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	3	0.0	29.4	12.5	21.1	11.0	-16.5	1.7	74.2	36.7	4.7	21.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	4	0.0	29.5	13.0	21.0	11.3	-16.0	1.4	73.1	36.8	4.3	21.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	5	0.0	30.6	13.2	20.3	11.9	-15.0	3.0	85.2	36.8	5.4	18.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	6	0.0	20.2	12.4	15.8	6.3	-7.5	3.3	88.3	66.4	3.0	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	7	0.0	21.7	11.7	15.8	6.7	-7.0	1.6	91.2	59.0	2.6	14.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	8	0.0	23.9	8.9	16.2	6.4	-7.5	1.5	96.5	54.9	3.1	19.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	9	0.0	24.7	10.6	17.8	7.7	-11.0	2.0	92.3	49.6	3.6	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	10	0.0	25.0	12.2	18.4	8.6	-13.5	1.8	90.0	50.9	3.6	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	11	0.0	24.6	13.4	18.0	9.0	-11.5	2.5	87.2	52.5	3.8	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	12	0.0	25.4	10.2	17.5	7.8	-9.0	1.5	93.2	46.3	3.5	20.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	13	0.0	27.7	10.3	19.2	9.0	-13.0	2.6	82.5	36.1	4.8	20.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	14	0.0	23.9	12.2	17.4	8.1	-12.0	2.0	95.9	56.0	3.0	15.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	15	0.0	24.6	10.2	17.6	7.4	-11.5	1.8	92.9	50.5	3.3	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	16	0.0	22.4	13.4	17.2	7.9	-11.0	3.7	80.7	56.1	3.6	14.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	17	0.0	20.8	9.8	14.9	5.3	-3.5	3.5	97.4	66.6	2.6	14.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	18	0.0	18.3	11.3	14.1	4.8	-0.5	2.6	93.7	71.4	1.9	9.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	19	0.0	22.5	8.3	15.5	5.4	-5.0	2.0	96.0	57.0	3.0	18.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	20	0.0	25.1	0.0	18.4	2.6	-14.5	2.1	92.2	53.0	3.1	13.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	21	13.6	22.9	13.2	17.6	8.1	-12.0	2.1	98.2	63.8	2.3	9.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	22	0.0	24.2	12.7	17.9	8.5	-14.5	2.1	97.6	59.5	2.9	15.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	23	0.0	22.9	12.0	17.4	7.5	-12.0	2.4	95.5	45.3	3.5	19.1

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	24	0.0	24.5	8.0	16.5	6.3	-7.5	1.8	78.7	42.7	3.5	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	25	0.0	23.9	11.8	17.1	7.9	-10.0	1.8	93.0	49.4	3.2	18.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	26	0.0	25.4	10.3	17.2	7.9	-8.5	1.3	91.4	47.8	3.1	18.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	27	0.0	28.3	11.8	19.0	10.1	-11.5	1.6	79.5	33.6	4.0	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	28	1.4	27.0	0.0	18.3	3.5	-9.5	2.3	81.5	29.3	4.6	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	29	0.0	21.7	11.9	15.5	6.8	-7.5	2.1	90.6	55.2	2.9	17.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	30	0.0	24.6	8.6	16.0	6.6	-4.5	1.3	95.8	46.2	2.9	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	5	31	0.0	24.4	9.6	16.4	7.0	-6.5	2.2	80.4	36.4	3.8	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	1	0.0	22.6	9.1	15.7	5.9	-6.5	2.7	92.3	46.8	3.4	18.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	2	0.0	19.0	7.6	13.9	3.3	1.0	2.6	94.0	57.8	2.4	12.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	3	0.2	16.5	11.3	14.1	3.9	0.0	2.5	91.4	76.0	1.5	6.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	4	0.0	22.4	8.3	14.8	5.4	-3.0	1.6	96.5	45.7	2.8	17.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	5	0.0	24.1	9.0	16.4	6.6	-5.5	2.0	86.8	40.4	3.4	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	6	0.0	24.5	8.0	15.9	6.3	-5.5	1.4	88.4	43.4	3.0	17.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	7	0.0	20.7	5.2	13.2	3.0	2.0	1.7	88.9	42.8	2.9	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	8	0.0	21.3	3.9	12.7	2.6	4.5	1.5	90.9	33.2	3.0	18.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	9	0.0	22.0	9.4	14.9	5.7	-3.5	2.4	67.1	35.6	3.8	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	10	0.0	21.4	4.9	13.3	3.2	1.0	1.3	81.1	40.4	2.8	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	11	0.0	23.3	6.3	14.0	4.8	0.5	1.7	75.5	31.6	3.4	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	12	0.0	24.2	5.8	14.7	5.0	-2.0	1.5	65.8	27.7	3.5	18.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	13	0.0	23.8	6.6	14.2	5.2	1.5	1.4	63.8	28.7	3.3	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	14	0.0	24.5	4.2	14.2	4.4	0.5	1.4	59.5	30.7	3.4	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	15	0.0	24.0	6.5	15.6	5.3	-3.5	1.4	62.8	33.7	3.3	18.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	16	0.0	23.5	12.3	16.5	7.9	-9.0	2.1	70.3	36.5	3.7	18.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	17	0.0	23.3	8.5	16.1	5.9	-8.0	2.1	75.5	37.6	3.3	13.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	18	0.0	24.4	11.6	17.5	8.0	-12.5	1.9	84.9	47.8	3.0	12.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	19	0.2	24.7	11.5	17.6	8.1	-10.5	2.3	95.4	45.0	3.3	15.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	20	0.0	18.4	8.6	13.2	3.5	4.0	2.6	88.6	53.1	2.7	15.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	21	0.0	21.3	8.7	13.5	5.0	1.0	1.7	84.2	53.5	2.7	17.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	22	0.0	25.6	6.0	15.8	5.8	-4.0	1.5	93.6	35.8	3.2	17.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	23	0.2	26.2	11.2	18.0	8.7	-9.5	1.8	68.9	34.7	3.8	17.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	24	0.0	26.8	9.8	18.2	8.3	-11.5	1.6	77.6	32.0	3.5	17.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	25	0.0	25.5	10.3	17.9	7.9	-10.5	2.3	65.9	34.0	3.9	12.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	26	0.0	20.0	10.0	14.8	5.0	-5.5	3.0	87.0	57.0	2.9	15.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	27	0.0	20.2	6.8	12.7	3.5	4.0	2.0	96.3	43.9	2.8	17.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	28	0.0	21.7	5.6	12.7	3.7	4.0	1.5	86.5	35.6	2.9	18.2

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	29	0.0	21.4	3.6	12.1	2.5	4.0	1.3	79.0	35.3	2.8	18.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	6	30	0.0	22.5	4.4	13.3	3.5	1.0	1.4	66.1	30.5	3.2	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	1	0.0	21.1	6.3	13.3	3.7	1.0	1.3	70.6	34.4	2.9	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	2	0.0	22.9	6.1	14.8	4.5	-2.0	2.5	54.5	28.1	4.3	18.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	3	0.0	20.5	9.0	13.9	4.7	0.0	2.4	94.4	37.9	3.2	16.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	4	0.0	21.6	7.6	14.1	4.6	-1.0	1.8	92.8	40.1	3.0	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	5	0.0	18.9	8.0	13.0	3.4	3.5	2.3	90.9	49.2	2.6	14.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	6	3.8	15.2	10.6	13.1	2.9	3.0	1.7	96.1	78.3	1.1	4.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	7	0.0	17.9	9.5	13.5	3.7	1.5	1.4	95.3	64.7	1.7	9.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	8	0.0	20.0	10.1	14.4	5.0	-2.5	2.3	92.1	50.8	2.8	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	9	0.0	19.0	9.0	13.4	4.0	-0.5	1.9	89.7	53.9	2.5	15.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	10	0.0	20.5	7.3	13.2	3.9	3.0	1.4	96.6	45.2	2.5	15.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	11	0.0	18.5	10.1	14.2	4.3	-1.0	2.8	82.9	49.8	2.9	13.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	12	0.0	17.1	8.4	12.2	2.8	5.5	2.1	94.3	59.2	2.1	12.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	13	0.0	19.9	7.8	13.2	3.8	2.5	1.6	89.5	49.6	2.7	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	14	0.0	21.4	7.4	14.1	4.4	1.0	1.3	97.1	44.1	2.7	18.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	15	0.0	23.3	11.9	16.8	7.6	-9.0	1.6	73.1	39.6	3.3	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	16	0.0	25.4	9.5	18.0	7.4	-10.5	2.0	72.9	30.6	4.0	18.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	17	0.0	24.7	9.8	17.0	7.2	-7.5	1.8	89.3	37.2	3.5	17.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	18	0.0	25.0	11.7	18.1	8.4	-10.5	2.6	85.3	42.7	3.8	15.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	19	0.0	16.0	10.1	12.8	3.1	4.5	4.2	84.7	53.6	2.7	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	20	0.0	17.0	4.5	10.5	0.7	11.0	2.1	93.7	47.2	2.7	19.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	21	0.0	19.3	5.4	11.8	2.3	5.5	1.7	86.6	39.4	3.0	19.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	22	0.0	22.2	5.5	13.6	3.8	1.5	1.4	91.3	39.3	3.1	19.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	23	0.0	23.2	7.0	14.6	5.1	-2.5	1.7	78.3	31.6	3.6	20.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	24	0.0	19.5	7.9	13.3	3.7	2.5	2.3	88.5	49.4	3.1	19.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	25	0.0	19.4	7.4	14.0	3.4	-0.5	2.1	94.8	51.0	2.8	17.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	26	0.0	19.6	11.2	14.8	5.4	-4.0	1.5	87.9	46.4	2.6	13.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	27	0.0	21.9	10.2	15.6	6.0	-5.5	1.4	76.0	38.5	3.2	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	28	0.0	21.9	7.8	15.5	4.8	-5.0	2.0	73.5	37.2	3.7	20.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	29	0.0	22.7	12.1	16.8	7.4	-8.5	3.0	64.6	38.4	4.4	18.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	30	0.0	24.1	9.8	17.6	7.0	-11.5	2.0	74.3	35.9	3.9	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	7	31	0.0	23.7	10.8	17.8	7.3	-12.0	1.9	72.0	40.2	3.6	15.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	1	0.0	25.6	12.9	19.0	9.2	-14.0	2.3	72.0	34.9	4.3	17.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	2	0.0	27.1	11.4	19.4	9.3	-14.5	2.0	81.3	29.6	4.5	20.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	3	0.0	29.1	14.7	20.7	11.9	-17.0	1.9	68.0	43.1	4.5	20.4

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	4	0.0	27.8	13.4	19.8	10.6	-13.0	2.2	70.8	45.4	4.3	18.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	5	0.0	26.8	11.2	18.7	9.0	-11.5	2.7	91.6	46.3	4.3	19.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	6	0.0	18.9	11.7	15.1	5.3	-5.5	3.5	94.3	71.3	2.6	17.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	7	0.0	21.0	10.1	14.9	5.6	-3.0	1.9	95.1	60.8	2.8	17.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	8	0.0	23.9	7.5	15.4	5.7	-3.0	1.5	87.7	55.7	3.4	21.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	9	0.0	23.7	11.6	17.4	7.6	-10.0	2.9	81.6	55.0	4.0	20.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	10	0.0	22.0	11.7	16.6	6.8	-9.0	2.6	86.6	63.2	3.4	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	11	0.0	22.6	12.1	16.6	7.3	-9.0	2.8	89.8	62.0	3.4	20.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	12	0.0	21.0	12.2	15.8	6.6	-7.0	2.8	92.0	61.3	3.1	17.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	13	0.0	23.8	9.9	16.8	6.8	-8.5	1.7	90.2	52.5	3.6	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	14	0.0	26.1	9.6	18.2	7.8	-11.5	2.2	86.8	45.5	4.3	22.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	15	0.0	27.4	11.7	19.2	9.6	-13.0	2.4	77.5	41.1	4.9	22.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	16	0.0	24.5	8.2	15.8	6.4	-3.5	2.2	93.4	41.9	4.3	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	17	0.0	24.7	5.6	14.7	5.2	-1.5	1.7	93.8	51.8	3.8	23.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	18	0.0	23.6	8.1	15.5	5.8	-3.0	2.2	90.7	55.6	3.9	24.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	19	0.0	22.6	8.3	15.7	5.5	-5.0	2.3	91.8	58.3	3.7	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	20	0.0	22.8	13.6	17.3	8.2	-10.5	1.7	84.8	63.8	3.1	16.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	21	0.6	25.0	11.6	17.4	8.3	-9.0	1.7	92.0	58.8	3.6	20.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	22	0.0	27.6	11.5	20.1	9.5	-15.5	1.8	83.4	52.7	4.4	23.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	23	0.0	30.3	18.2	23.7	14.2	-24.0	4.0	77.4	42.6	6.4	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	24	0.0	17.2	9.0	12.8	3.1	4.0	4.5	92.0	52.1	2.9	9.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	25	0.0	18.1	6.0	12.8	2.0	4.5	2.6	89.7	57.5	3.1	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	26	0.0	18.8	4.6	12.7	1.7	3.0	2.6	88.3	54.7	3.4	22.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	27	0.0	20.2	8.2	14.1	4.2	-1.5	2.0	93.0	70.3	3.1	22.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	28	0.0	23.1	7.8	15.9	5.4	-5.5	1.5	92.8	63.6	3.7	25.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	29	0.0	26.6	9.0	17.9	7.8	-9.0	1.6	87.2	58.9	4.2	25.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	30	0.0	28.8	11.6	18.9	10.2	-10.5	2.1	83.4	53.6	5.0	25.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	8	31	0.0	26.0	9.3	18.1	7.6	-11.0	1.9	86.5	59.4	4.3	25.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	1	0.0	25.5	12.2	18.5	8.9	-12.0	2.8	96.5	58.8	4.4	24.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	2	0.0	21.8	12.8	16.8	7.3	-10.5	3.9	95.4	69.1	3.7	23.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	3	0.0	22.0	12.9	16.6	7.5	-10.0	3.4	93.2	64.0	4.0	24.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	4	0.0	24.9	9.3	17.4	7.1	-11.0	1.8	90.5	53.4	4.3	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	5	0.0	25.9	13.5	19.4	9.7	-14.5	2.8	81.1	56.5	4.8	25.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	6	0.0	27.8	12.2	19.8	10.0	-14.5	2.4	90.7	50.7	5.0	25.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	7	0.0	24.5	13.9	18.5	9.2	-12.5	2.7	87.9	59.8	4.5	25.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	8	0.2	27.0	12.0	19.3	9.5	-14.0	1.7	90.3	48.3	4.8	25.9

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	9	0.0	29.5	13.9	22.0	11.7	-18.0	2.0	71.4	39.4	5.8	26.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	10	0.0	27.0	14.4	22.2	10.7	-21.0	3.1	55.4	42.0	5.3	15.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	11	0.0	16.8	8.2	12.9	2.5	4.0	3.6	62.9	37.4	4.9	28.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	12	0.0	22.4	7.7	14.7	5.0	-2.5	2.9	67.2	39.4	5.3	27.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	13	0.0	19.6	9.2	14.2	4.4	-2.0	4.2	83.3	50.6	4.6	26.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	14	0.0	22.5	8.8	15.7	5.7	-5.5	2.8	85.0	45.3	4.9	27.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	15	0.0	26.3	11.8	18.7	9.1	-11.5	1.9	77.3	37.1	5.4	27.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	16	0.0	29.1	12.4	21.0	10.8	-17.5	1.7	66.7	31.8	5.8	27.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	17	0.0	32.7	14.9	23.6	13.8	-19.5	2.0	51.8	28.0	6.8	26.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	18	0.0	20.3	11.5	15.7	5.9	-6.5	4.5	86.9	38.4	5.2	26.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	19	0.0	17.5	10.2	13.6	3.9	1.0	3.6	86.1	67.3	2.8	14.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	20	0.0	26.1	13.5	18.5	9.8	-13.5	1.9	78.0	43.0	5.2	25.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	21	0.8	29.0	13.0	21.3	11.0	-17.5	2.0	72.3	38.3	5.2	20.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	22	1.0	19.6	14.4	16.6	7.0	-10.0	4.7	86.5	66.4	3.5	17.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	23	0.0	24.2	12.6	18.0	8.4	-13.0	3.1	89.8	53.4	4.6	22.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	24	0.0	30.2	15.5	22.4	12.8	-18.5	3.4	81.1	39.3	6.6	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	25	0.0	34.2	18.6	26.5	16.4	-24.0	3.4	73.9	29.4	8.0	24.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	26	3.4	33.9	21.3	28.0	17.6	-24.0	3.4	74.1	28.0	7.1	15.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	27	2.2	30.0	16.6	22.9	13.3	-22.5	2.7	86.9	28.5	6.3	21.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	28	0.0	27.1	11.0	17.9	9.1	-9.5	3.7	82.9	30.9	6.9	28.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	29	0.0	17.2	10.9	13.7	4.1	2.0	3.9	82.4	52.0	3.0	7.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	9	30	2.2	15.0	9.5	11.7	2.2	8.5	2.5	83.9	59.3	2.1	6.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	1	0.0	19.5	9.7	14.1	4.6	-2.0	2.6	91.6	58.6	3.8	23.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	2	0.0	26.1	11.3	18.5	8.7	-11.5	3.3	83.9	35.3	6.5	30.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	3	0.0	19.4	12.5	16.1	6.0	-10.0	3.1	85.4	53.0	3.3	11.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	4	0.0	24.4	12.1	17.9	8.2	-11.5	3.7	83.7	45.1	6.0	30.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	5	0.0	26.2	13.2	19.1	9.7	-14.5	2.8	80.9	43.6	5.9	27.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	6	0.0	26.4	13.0	19.3	9.7	-15.0	2.8	88.0	41.4	5.8	27.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	7	0.0	23.8	11.3	17.7	7.6	-11.5	4.1	80.8	36.5	6.5	31.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	8	0.0	23.3	13.2	17.2	8.2	-10.5	2.8	79.7	33.8	6.2	32.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	9	0.0	26.8	8.9	17.7	7.9	-9.0	2.4	93.5	29.3	6.5	32.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	10	0.0	31.6	11.3	22.0	11.4	-15.5	2.5	75.2	19.6	8.1	32.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	11	0.0	32.4	14.4	23.8	13.4	-19.5	2.0	61.4	20.7	7.8	32.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	12	0.0	30.6	15.1	22.0	12.9	-18.0	3.7	76.9	28.1	8.4	31.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	13	0.0	22.4	12.9	16.4	7.6	-9.0	5.4	81.3	46.9	6.0	30.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	14	0.0	26.7	10.5	19.0	8.6	-12.5	2.3	89.0	40.6	6.1	31.2

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	15	0.0	30.9	14.9	23.0	12.9	-19.5	2.0	68.6	28.1	6.9	28.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	16	0.0	32.2	13.9	23.4	13.0	-20.0	3.2	67.1	19.4	8.9	31.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	17	0.0	23.4	13.9	18.5	8.6	-15.5	4.2	79.6	43.6	6.4	31.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	18	0.0	25.5	13.3	19.6	9.4	-16.5	3.0	86.7	38.4	6.5	32.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	19	0.0	29.3	16.2	22.3	12.7	-20.5	3.6	66.3	30.2	8.1	30.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	20	6.4	22.2	15.7	18.2	8.9	-16.5	4.8	94.7	62.6	4.3	22.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	21	1.4	23.4	14.8	18.3	9.1	-13.5	4.4	91.2	64.0	4.7	25.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	22	0.0	31.9	14.6	22.8	13.2	-18.0	2.4	93.6	31.3	7.5	32.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	23	0.0	32.1	17.0	24.8	14.5	-22.5	3.5	86.7	35.8	8.0	30.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	24	8.4	33.8	19.3	24.6	16.5	-24.0	3.7	85.0	38.7	7.8	25.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	25	1.6	26.4	15.8	20.7	11.1	-20.0	4.0	91.5	60.8	5.6	29.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	26	0.4	26.6	16.2	21.4	11.4	-20.5	3.3	90.8	58.3	5.3	25.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	27	0.0	28.7	16.5	23.2	12.6	-23.0	2.4	85.3	44.6	6.2	27.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	28	6.4	25.2	16.6	19.8	10.9	-19.5	4.0	87.6	50.5	5.8	26.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	29	0.0	24.0	14.9	19.0	9.5	-15.5	4.2	87.5	51.8	6.1	32.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	30	0.0	25.7	11.8	19.4	8.8	-15.5	2.7	85.3	32.6	6.8	33.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	10	31	0.0	29.3	16.1	22.2	12.7	-21.0	2.2	68.2	32.3	7.2	30.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	1	0.0	32.5	16.6	24.2	14.5	-22.0	2.6	79.3	21.7	8.4	32.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	2	0.0	30.6	17.9	24.1	14.2	-23.5	3.1	60.6	31.6	8.6	33.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	3	0.0	28.7	16.9	22.4	12.8	-23.0	3.6	78.4	38.8	7.8	34.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	4	0.0	34.2	16.8	25.6	15.5	-22.0	2.3	81.8	27.0	8.4	33.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	5	3.2	29.9	17.5	23.7	13.7	-22.5	3.0	88.9	38.2	6.6	25.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	6	0.2	22.4	15.6	18.6	9.0	-17.5	3.8	89.2	61.5	4.3	20.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	7	0.0	27.0	14.9	20.7	10.9	-18.0	2.4	91.7	47.3	5.5	25.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	8	0.0	31.9	17.5	24.8	14.7	-23.0	2.1	84.3	29.8	6.7	25.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	9	21.2	24.1	17.5	20.4	10.8	-21.5	3.6	93.3	57.2	5.5	28.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	10	14.6	29.1	17.4	21.7	13.2	-21.0	2.1	93.6	43.6	6.2	28.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	11	0.8	27.0	19.3	22.4	13.1	-24.0	3.3	81.8	43.2	6.1	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	12	0.0	29.2	16.5	22.9	12.9	-22.0	2.3	91.1	36.2	7.2	33.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	13	0.0	29.5	17.4	23.3	13.5	-23.0	2.7	75.7	30.2	8.0	34.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	14	0.0	29.8	16.5	23.4	13.2	-22.5	3.0	79.1	31.7	8.2	35.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	15	5.0	31.8	18.9	25.2	15.4	-24.0	2.7	65.4	29.8	8.7	34.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	16	0.0	30.4	19.9	25.0	15.2	-24.0	3.7	68.0	41.0	8.3	31.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	17	0.0	34.3	21.0	27.9	17.6	-24.0	2.7	71.3	29.7	8.6	30.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	18	3.4	33.4	22.0	25.9	17.7	-24.0	3.1	81.6	36.2	6.9	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	19	8.6	23.2	16.5	19.6	9.8	-20.5	2.4	95.4	63.9	2.5	7.7

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	20	9.8	24.4	16.4	19.1	10.4	-19.0	2.6	95.0	67.4	3.9	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	21	1.0	20.8	17.1	18.7	9.0	-19.0	1.4	93.2	78.8	1.8	6.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	22	3.0	22.1	16.3	18.8	9.2	-19.5	1.5	96.3	74.6	2.5	11.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	23	0.0	26.6	17.7	22.0	12.2	-21.5	2.2	94.5	58.0	5.4	26.8
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	24	3.8	26.7	18.5	21.7	12.6	-24.0	2.5	95.0	61.8	5.0	23.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	25	8.6	22.8	18.6	20.0	10.7	-24.0	2.3	95.4	75.7	2.7	12.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	26	20.6	25.0	18.0	20.7	11.5	-24.0	1.7	96.2	72.0	3.2	14.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	27	49.0	26.6	18.0	20.3	12.3	-24.0	2.3	96.8	66.8	3.1	11.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	28	10.2	26.2	16.9	20.8	11.6	-21.5	2.0	97.7	64.4	4.4	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	29	1.6	23.7	17.3	20.1	10.5	-23.0	3.0	92.3	76.5	3.1	14.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	11	30	4.0	20.5	16.3	18.0	8.4	-17.5	2.5	94.1	74.7	2.4	10.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	1	0.0	23.1	16.0	18.9	9.5	-18.5	2.1	91.2	66.5	3.8	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	2	0.0	24.7	15.9	20.3	10.3	-20.5	2.6	90.8	59.6	5.6	30.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	3	1.8	26.0	17.1	20.7	11.6	-21.0	2.3	94.3	61.2	5.1	25.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	4	0.4	24.5	18.6	21.2	11.5	-24.0	1.8	94.9	70.0	3.4	15.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	5	0.0	28.1	18.4	22.6	13.2	-24.0	2.2	94.5	59.0	5.8	28.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	6	5.0	29.6	17.0	22.7	13.3	-21.5	2.0	95.4	57.2	5.9	26.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	7	0.2	25.7	16.9	21.2	11.3	-21.0	2.7	95.4	59.7	5.2	26.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	8	0.0	27.3	18.3	22.3	12.8	-24.0	2.7	94.4	52.2	6.1	31.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	9	0.0	26.3	18.1	21.8	12.2	-24.0	2.7	95.4	57.2	4.9	23.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	10	0.0	25.8	18.3	21.6	12.1	-24.0	3.1	86.6	54.7	5.1	26.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	11	0.2	27.3	17.9	22.1	12.6	-23.5	2.2	94.4	64.6	5.6	27.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	12	12.4	24.0	16.9	20.5	10.4	-23.0	2.2	97.3	58.4	3.4	15.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	13	3.2	23.0	16.6	19.6	9.8	-20.0	2.9	97.3	70.9	3.7	18.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	14	0.0	26.7	13.1	20.5	9.9	-17.0	2.1	96.4	70.9	6.7	35.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	15	0.0	28.0	16.5	21.9	12.2	-21.0	1.8	95.4	38.5	5.9	28.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	16	0.0	29.3	17.2	22.9	13.2	-23.0	2.3	94.4	39.8	6.9	34.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	17	0.0	26.7	16.4	21.6	11.6	-22.5	2.3	89.5	39.8	6.6	35.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	18	0.0	29.3	16.9	22.9	13.1	-21.5	2.3	90.5	47.2	7.1	35.4
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	19	0.0	31.4	17.7	24.5	14.5	-23.0	2.0	94.4	52.2	6.6	29.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	20	0.2	33.5	20.3	26.0	16.9	-24.0	2.2	81.8	47.2	6.7	27.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	21	2.4	29.4	17.8	22.4	13.6	-23.0	2.2	95.4	46.0	4.9	19.5
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	22	2.4	27.8	18.0	22.3	12.9	-22.5	2.7	89.3	72.3	4.8	23.2
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	23	0.0	30.3	19.3	24.7	14.8	-24.0	2.7	90.6	54.4	6.4	28.1
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	24	0.0	31.9	18.6	25.9	15.3	-24.0	1.9	92.7	44.9	7.6	35.3
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	25	0.0	32.9	20.2	27.2	16.5	-24.0	2.6	84.8	40.9	8.0	32.8

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	26	0.0	31.2	19.7	25.4	15.4	-24.0	2.7	83.6	47.9	7.2	30.0
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	27	0.0	31.6	20.8	26.3	16.2	-24.0	3.2	89.0	50.4	7.4	30.6
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	28	35.6	30.6	16.9	24.7	13.7	-23.5	3.4	98.0	51.1	6.6	28.9
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	29	12.2	28.1	16.8	22.3	12.4	-21.0	1.5	98.3	64.2	5.1	25.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	30	0.0	30.1	19.8	24.8	15.0	-24.0	1.4	94.6	56.4	6.8	33.7
30090	GILLEMBERG	-23.8333	28.9667	1100	1999	12	31	3.2	30.1	20.3	24.3	15.2	-24.0	1.6	93.5	58.4	6.0	28.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	1	2.6	28.5	19.5	23.0	14.0	-24.0	2.3	95.7	66.6	5.4	26.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	2	0.2	29.0	20.2	23.4	14.6	-24.0	2.2	94.4	64.0	6.1	30.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	3	20.2	25.2	19.2	21.5	12.2	-24.0	3.5	98.4	74.8	4.2	22.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	4	0.0	26.9	19.4	22.1	13.2	-24.0	2.8	93.5	70.7	5.3	27.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	5	0.0	27.3	18.7	22.4	13.0	-24.0	1.8	92.5	65.1	4.9	23.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	6	0.0	29.7	19.6	24.4	14.7	-24.0	1.6	94.3	62.2	6.3	31.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	7	3.4	25.7	19.9	22.8	12.8	-24.0	1.5	93.5	71.7	3.6	16.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	8	17.6	30.3	18.7	23.0	14.5	-24.0	2.3	96.6	57.2	6.6	31.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	9	0.0	25.3	18.2	21.0	11.7	-24.0	2.2	93.4	71.6	4.0	19.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	10	0.2	27.6	18.7	22.6	13.1	-24.0	1.6	94.8	65.3	4.8	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	11	0.0	27.7	18.1	22.9	12.9	-24.0	2.1	97.8	50.4	6.5	33.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	12	0.0	29.3	18.2	23.5	13.7	-24.0	2.0	93.7	51.0	6.4	30.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	13	0.0	24.8	17.9	21.1	11.3	-23.5	3.9	84.0	61.3	5.2	23.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	14	0.0	23.7	15.7	19.6	9.7	-18.0	2.7	85.1	63.8	4.2	19.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	15	0.0	21.7	15.8	18.7	8.7	-18.5	3.4	88.8	69.3	3.3	14.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	16	34.2	20.8	17.4	19.0	9.1	-21.0	2.5	97.2	87.3	1.9	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	17	6.8	19.8	17.2	18.1	8.5	-17.0	4.4	95.5	77.4	2.5	12.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	18	1.0	19.6	15.5	17.4	7.5	-14.5	2.0	91.1	69.6	2.9	13.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	19	0.0	21.7	12.9	17.9	7.3	-14.5	1.8	95.1	59.5	4.0	21.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	20	0.0	24.1	14.9	19.0	9.5	-15.5	2.1	90.2	58.9	4.6	23.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	21	0.0	25.9	14.1	20.0	10.0	-17.0	1.7	93.8	55.2	6.2	34.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	22	0.0	27.7	15.2	21.6	11.5	-19.0	1.5	91.1	53.5	6.4	34.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	23	0.0	27.0	15.4	21.6	11.2	-21.0	2.1	91.9	50.8	6.4	33.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	24	0.0	26.5	17.2	21.4	11.9	-22.0	2.6	89.1	53.3	6.2	31.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	25	0.0	18.5	16.7	17.7	7.6	-3.0	1.7	83.7	75.9	0.7	0.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	26	7.8	28.6	20.1	24.3	14.3	-23.4	2.3	95.0	49.2	3.9	12.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	27	8.6	25.3	19.7	23.2	12.5	-13.0	2.3	96.9	71.8	3.8	18.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	28	0.0	28.7	18.9	22.7	13.8	-24.0	1.4	97.8	54.8	5.5	26.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	29	0.0	31.3	17.4	23.9	14.4	-22.5	1.6	92.0	37.0	7.4	35.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	30	0.0	29.5	18.6	23.9	14.1	-24.0	1.8	94.7	54.7	6.4	31.5

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	1	31	0.0	31.6	17.9	24.9	14.7	-23.5	1.8	97.4	48.8	7.2	35.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	1	0.0	30.4	20.5	24.8	15.4	-24.0	2.0	90.9	55.7	6.7	31.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	2	6.0	29.8	19.5	23.7	14.6	-24.0	2.2	95.9	61.4	5.4	24.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	3	0.0	29.1	17.0	22.8	13.1	-22.0	2.2	95.6	50.8	6.0	28.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	4	0.0	27.2	14.9	21.8	11.0	-20.5	2.4	93.8	58.4	5.6	28.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	5	0.2	26.9	17.9	22.0	12.4	-23.5	3.3	90.0	63.5	4.2	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	6	20.8	20.9	19.0	20.1	10.0	-24.0	3.0	96.3	88.7	5.7	41.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	7	22.8	25.7	19.2	22.3	12.5	-24.0	3.4	97.7	76.3	4.1	22.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	8	17.6	24.4	18.9	21.5	11.7	-24.0	2.7	97.0	80.9	2.4	10.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	9	5.2	23.6	19.9	21.2	11.8	-24.0	1.8	96.4	81.9	1.9	7.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	10	33.2	25.3	19.8	21.9	12.5	-24.0	2.6	98.1	74.4	3.7	18.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	11	26.4	26.6	19.9	22.4	13.3	-24.0	2.0	97.4	71.1	4.2	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	12	9.8	27.4	19.0	22.3	13.2	-24.0	1.6	98.2	66.0	5.0	25.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	13	0.0	26.2	20.3	22.1	13.2	-24.0	1.9	94.7	75.9	3.9	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	14	0.0	26.7	19.9	22.6	13.3	-24.0	2.4	93.8	65.9	4.9	23.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	15	17.4	28.0	19.5	22.6	13.7	-24.0	1.9	97.0	65.3	4.9	23.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	16	0.2	25.2	18.7	21.5	11.9	-24.0	3.7	97.5	71.5	4.3	23.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	17	0.0	24.1	16.0	20.0	10.0	-22.0	3.3	93.3	68.4	4.8	27.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	18	0.0	25.7	14.4	20.1	10.1	-16.5	2.6	95.7	63.7	4.8	25.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	19	0.0	26.0	16.0	21.1	11.0	-20.5	1.7	96.6	67.9	4.3	22.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	20	0.0	29.2	17.4	22.7	13.3	-23.0	1.4	96.5	56.5	4.8	22.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	21	0.4	28.9	19.8	23.9	14.3	-24.0	1.6	95.5	51.8	5.6	26.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	22	0.0	27.2	16.2	22.2	11.7	-22.0	2.1	95.2	58.7	4.7	22.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	23	0.0	25.6	19.8	22.4	12.7	-24.0	4.0	83.7	63.9	4.4	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	24	36.2	22.5	16.7	18.9	9.6	-20.0	3.9	97.1	73.9	2.3	7.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	25	33.0	23.4	17.3	19.8	10.3	-21.0	4.1	97.7	71.0	3.0	12.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	26	0.0	24.1	17.5	20.3	10.8	-21.0	2.8	91.5	74.2	2.9	12.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	27	0.0	25.0	17.2	20.9	11.1	-22.0	2.1	96.7	71.4	3.4	16.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	28	0.0	24.6	15.6	20.1	10.1	-20.0	3.0	94.5	59.1	4.8	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	2	29	0.0	25.7	17.3	21.2	11.5	-20.5	2.3	94.8	64.8	4.8	25.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	1	0.0	27.4	17.8	22.5	12.6	-23.5	1.5	95.1	59.6	5.1	26.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	2	0.0	27.9	19.7	23.0	13.8	-24.0	2.5	93.1	59.2	5.1	23.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	3	0.0	26.6	20.0	22.5	13.3	-24.0	1.9	90.9	64.7	4.5	21.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	4	0.0	27.3	19.2	22.6	13.3	-24.0	1.4	89.5	63.6	4.1	19.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	5	5.0	27.3	19.1	21.6	13.2	-24.0	1.4	94.6	61.8	4.2	19.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	6	0.2	27.5	18.4	21.8	12.9	-24.0	1.5	93.7	59.9	4.9	24.7

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	7	10.2	25.2	17.0	20.7	11.1	-22.0	1.6	97.3	69.5	3.6	18.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	8	0.0	25.6	15.4	20.3	10.5	-19.5	1.8	95.6	60.2	4.2	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	9	0.2	26.3	15.0	20.7	10.6	-18.0	1.4	99.3	56.9	4.3	22.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	10	0.0	29.4	16.0	22.3	12.7	-21.0	1.9	97.0	43.0	6.0	29.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	11	0.0	28.3	18.1	22.9	13.2	-24.0	2.7	96.4	55.0	5.5	26.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	12	0.6	26.4	17.5	21.1	11.9	-21.5	2.8	94.6	60.6	4.7	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	13	1.2	25.5	18.0	20.9	11.8	-24.0	1.9	96.1	61.9	3.6	16.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	14	0.0	27.0	16.9	21.6	12.0	-22.0	1.6	95.3	59.9	4.7	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	15	0.0	28.1	16.8	22.6	12.4	-22.0	1.3	97.7	59.5	4.4	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	16	1.6	27.3	19.0	22.6	13.1	-24.0	1.4	96.0	63.8	3.8	17.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	17	1.6	25.9	19.8	22.4	12.8	-24.0	1.4	96.1	73.7	3.5	17.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	18	4.4	26.5	19.7	21.9	13.1	-24.0	1.5	98.0	68.9	2.8	12.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	19	29.2	22.5	18.1	20.0	10.3	-24.0	1.7	98.5	76.6	2.1	9.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	20	15.6	23.6	18.8	19.9	11.2	-24.0	1.7	96.6	75.8	2.1	8.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	21	1.2	23.6	18.2	20.4	10.9	-24.0	2.4	94.7	70.9	3.5	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	22	5.8	26.3	17.5	21.3	11.9	-21.5	1.4	93.9	64.1	4.0	20.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	23	0.4	26.8	16.9	21.6	11.9	-21.5	1.7	96.9	58.4	4.6	24.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	24	0.0	26.9	18.0	21.8	12.4	-23.5	1.3	96.0	56.9	3.9	18.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	25	0.0	27.1	16.4	21.1	11.7	-22.0	1.2	97.2	57.5	4.5	24.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	26	0.2	28.1	16.8	21.2	12.4	-22.0	1.8	95.4	59.2	4.4	21.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	27	0.0	21.7	16.7	19.0	9.2	-20.5	2.1	95.8	73.3	2.1	8.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	28	0.0	22.8	14.3	18.1	8.6	-14.0	1.1	97.9	71.7	2.3	11.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	29	0.0	25.4	15.7	20.2	10.5	-20.5	2.1	94.0	59.4	3.6	16.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	30	0.0	24.3	17.2	20.0	10.7	-20.5	1.3	94.4	69.9	2.6	12.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	3	31	5.4	25.9	17.7	20.0	11.8	-21.5	1.9	95.7	63.6	3.0	12.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	1	0.0	23.6	16.2	19.3	9.9	-18.5	2.5	93.5	61.9	3.5	17.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	2	0.0	24.6	15.0	18.8	9.8	-15.0	1.5	94.4	57.0	3.9	21.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	3	0.0	26.4	13.9	19.8	10.1	-17.0	1.1	98.2	55.7	4.0	22.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	4	0.0	26.6	15.2	20.8	10.9	-18.0	1.2	96.6	54.6	4.0	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	5	58.4	18.2	13.5	15.4	5.8	-5.0	2.4	98.9	79.3	1.6	6.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	6	5.6	18.8	13.1	15.3	5.9	-7.0	1.4	98.1	73.2	2.2	12.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	7	0.2	22.1	12.5	16.5	7.3	-9.5	1.2	98.7	60.7	2.8	15.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	8	0.2	23.3	12.5	17.4	7.9	-12.0	1.2	98.3	55.8	3.3	18.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	9	0.0	25.9	12.5	18.8	9.2	-14.0	1.6	95.9	47.9	4.3	23.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	10	0.0	23.2	12.5	18.0	7.9	-14.0	1.7	96.7	57.2	3.8	22.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	11	0.0	23.8	14.0	18.7	8.9	-15.5	1.3	97.8	62.4	3.3	18.8

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	12	0.0	26.5	13.6	19.5	10.0	-15.0	1.3	97.8	47.2	4.0	21.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	13	0.0	26.6	14.1	19.9	10.3	-17.5	1.7	92.7	49.2	4.1	20.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	14	31.4	22.7	14.8	18.1	8.7	-14.0	1.9	98.7	66.1	2.7	14.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	15	15.8	18.1	13.7	15.5	5.9	-5.5	1.3	97.8	81.7	1.3	5.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	16	5.0	21.2	13.5	16.1	7.3	-7.5	1.6	96.7	67.1	2.7	15.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	17	0.2	24.4	11.5	17.0	8.0	-8.0	1.0	99.1	56.5	3.6	22.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	18	0.2	24.1	13.2	18.2	8.7	-12.5	2.1	95.0	47.8	4.0	21.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	19	0.0	21.8	13.2	16.8	7.5	-9.5	1.9	95.7	60.5	2.8	14.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	20	0.0	20.3	12.9	16.2	6.6	-9.5	1.7	94.3	64.5	2.5	13.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	21	7.2	20.0	13.1	16.1	6.6	-9.0	1.1	98.3	73.4	1.8	9.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	22	0.4	25.1	12.1	17.7	8.6	-11.0	1.0	99.1	47.8	3.7	22.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	23	0.0	26.1	11.9	18.7	9.0	-12.0	1.2	95.7	46.1	3.7	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	24	0.0	26.8	13.4	19.6	10.1	-15.0	1.7	86.6	45.1	4.3	22.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	25	0.0	25.0	13.3	19.1	9.1	-15.5	1.8	97.0	56.6	3.7	21.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	26	0.0	28.0	13.1	20.0	10.5	-14.5	1.5	95.5	44.0	4.2	22.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	27	0.0	28.3	13.7	21.1	11.0	-19.0	1.9	89.7	39.4	4.6	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	28	0.0	18.2	13.5	15.2	5.8	-5.5	3.4	88.4	67.3	2.8	16.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	29	0.0	18.8	12.8	15.3	5.8	-5.5	2.9	89.4	66.9	2.4	11.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	4	30	0.0	19.5	11.5	15.2	5.5	-5.5	1.8	95.9	61.1	2.7	17.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	1	0.0	22.9	8.7	15.4	5.8	-4.0	1.1	98.4	53.8	3.2	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	2	0.0	24.5	10.2	16.7	7.4	-7.0	1.3	95.1	38.8	3.6	21.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	3	0.0	22.1	10.3	16.8	6.2	-10.5	2.3	91.3	53.9	3.4	20.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	4	7.2	21.6	11.9	16.5	6.8	-9.0	2.1	97.2	66.9	2.4	12.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	5	38.8	20.4	10.9	14.2	5.6	-0.5	2.1	97.9	69.0	2.5	16.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	6	0.2	20.9	8.4	14.0	4.6	0.5	1.3	99.6	54.2	2.9	20.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	7	0.0	21.1	9.0	14.5	5.0	-2.0	1.9	91.1	56.7	3.2	21.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	8	0.0	20.8	8.6	14.4	4.7	-1.5	1.6	95.0	59.9	2.6	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	9	0.0	22.7	10.0	16.1	6.3	-6.5	1.6	96.0	53.8	3.0	17.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	10	0.0	20.7	9.5	14.6	5.1	-2.5	1.3	97.9	55.7	2.6	16.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	11	0.0	22.0	8.1	15.2	5.0	-4.0	1.3	96.8	56.5	2.9	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	12	0.0	20.2	11.3	14.9	5.7	-2.0	1.9	94.9	58.1	2.5	13.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	13	0.0	18.2	3.6	10.9	0.9	9.5	1.4	95.8	49.6	2.7	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	14	0.0	19.7	4.2	11.5	2.0	6.5	1.4	96.6	42.7	2.9	20.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	15	0.0	20.9	6.8	13.3	3.8	3.0	1.6	87.6	42.3	3.1	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	16	0.0	19.9	7.5	13.7	3.7	0.5	1.4	93.2	54.2	2.7	19.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	17	0.0	21.3	7.1	12.9	4.2	5.0	1.3	99.2	45.9	2.7	17.8

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	18	0.0	22.9	7.6	14.9	5.2	-1.0	1.4	96.9	46.0	2.9	17.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	19	0.0	22.3	8.7	15.2	5.5	-3.5	1.4	92.2	48.6	3.0	19.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	20	0.0	21.8	10.7	15.3	6.3	-3.5	1.3	90.9	51.6	2.8	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	21	0.0	23.2	7.7	14.7	5.4	-1.0	1.2	93.0	48.0	2.8	18.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	22	0.0	22.6	8.0	14.7	5.3	-1.5	1.2	89.0	49.5	2.8	19.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	23	0.0	22.9	7.2	15.0	5.1	-2.0	1.5	91.4	48.6	3.0	18.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	24	0.0	20.7	11.9	14.9	6.3	-4.5	2.3	91.7	55.8	3.0	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	25	0.0	21.2	10.6	15.6	5.9	-5.5	2.4	91.2	57.8	3.0	18.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	26	5.4	20.7	9.3	14.3	5.0	-3.0	1.7	95.3	63.8	2.2	12.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	27	0.0	21.6	5.8	13.0	3.7	3.0	1.4	96.0	41.1	2.9	19.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	28	0.0	18.7	7.9	12.3	3.3	5.5	1.4	96.3	61.9	1.9	11.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	29	0.0	20.5	5.2	12.3	2.9	5.0	1.0	94.3	47.5	2.5	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	30	0.0	22.9	6.4	13.2	4.6	3.0	1.1	89.0	42.2	2.8	19.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	5	31	0.0	22.9	5.7	14.3	4.3	0.5	1.6	82.5	31.9	3.4	19.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	1	0.0	20.1	5.9	13.2	3.0	2.5	0.6	95.6	51.8	2.1	18.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	2	0.0	20.3	6.7	12.6	3.5	4.5	2.1	97.6	52.8	2.7	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	3	0.0	20.4	5.4	12.6	2.9	4.0	0.8	98.1	48.0	2.0	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	4	0.2	18.8	7.7	12.1	3.2	6.0	1.5	94.2	53.0	2.3	11.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	5	0.2	16.4	9.4	13.1	2.9	2.5	1.8	93.1	71.3	1.6	8.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	6	0.0	17.0	6.3	11.2	1.7	11.0	1.9	99.2	70.4	1.4	6.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	7	0.0	18.9	6.7	12.5	2.8	4.5	2.0	98.5	61.9	2.3	16.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	8	0.2	20.0	8.3	12.8	4.1	3.0	1.7	100.0	57.1	2.5	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	9	0.0	22.2	7.2	14.2	4.7	0.0	1.9	96.4	50.7	2.9	18.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	10	0.0	19.6	8.9	13.8	4.3	-0.5	1.7	97.2	65.3	2.0	11.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	11	0.0	22.7	7.9	14.7	5.3	-2.0	1.7	94.6	43.8	3.1	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	12	0.0	24.1	9.5	15.8	6.8	-5.5	1.8	88.5	40.4	3.3	15.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	13	0.0	20.2	10.2	14.0	5.2	0.5	1.6	95.9	55.7	2.5	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	14	0.0	23.0	7.8	13.9	5.4	1.5	1.5	98.1	45.9	3.0	17.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	15	0.0	22.6	7.1	14.5	4.8	-1.0	1.7	96.1	41.2	3.1	18.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	16	0.0	20.9	9.8	15.7	5.3	-6.0	2.1	88.2	55.5	2.3	9.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	17	0.0	22.4	13.4	16.9	7.9	-9.0	2.0	90.9	49.3	2.8	13.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	18	0.0	19.7	13.3	15.6	6.5	-7.0	1.7	89.9	60.4	2.1	8.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	19	0.0	18.5	12.0	14.7	5.2	-2.5	2.2	90.4	58.8	2.3	14.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	20	9.4	13.2	11.0	11.9	2.1	9.0	1.8	96.8	87.6	1.7	19.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	21	0.2	17.1	9.5	12.9	3.3	4.5	1.6	100.0	78.8	1.3	7.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	22	0.2	19.8	8.1	13.1	4.0	3.0	1.7	99.7	62.8	2.3	17.0

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	23	8.8	23.9	8.0	15.6	5.9	-5.0	2.0	96.3	50.4	3.0	17.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	24	0.6	15.4	9.6	13.0	2.5	4.0	1.7	98.7	83.7	1.0	4.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	25	0.0	19.8	7.5	12.6	3.6	4.0	1.7	99.7	55.9	2.4	15.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	26	0.2	22.3	5.8	13.5	4.1	2.5	1.7	95.6	44.3	3.0	18.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	27	0.0	22.1	8.2	14.4	5.2	-2.0	1.5	96.8	51.0	2.9	17.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	28	0.0	20.4	6.9	13.3	3.6	3.0	1.9	98.2	50.6	2.7	17.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	29	0.0	20.9	5.5	12.8	3.2	2.5	1.8	97.2	52.9	2.7	17.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	6	30	0.0	17.2	5.8	11.2	1.5	9.5	1.7	95.8	56.4	2.2	14.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	1	0.0	17.9	6.2	11.1	2.0	10.0	1.2	97.6	53.8	1.8	11.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	2	0.0	19.8	7.1	12.4	3.5	5.5	1.3	96.1	43.8	2.5	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	3	0.0	18.6	5.6	11.2	2.1	8.5	1.3	97.1	56.7	2.1	16.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	4	0.0	18.8	4.7	10.9	1.8	9.0	1.2	97.2	52.5	2.2	17.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	5	0.0	20.1	5.4	11.9	2.8	6.0	1.9	94.5	46.4	2.8	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	6	0.0	18.2	3.9	11.2	1.0	8.0	2.0	93.7	52.1	2.5	17.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	7	0.0	18.2	5.4	11.8	1.8	7.5	1.7	96.3	54.4	2.4	18.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	8	0.0	18.0	6.5	11.3	2.3	9.5	1.6	95.8	51.0	2.3	15.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	9	0.0	19.4	6.2	11.7	2.8	7.0	1.3	97.6	53.2	2.2	16.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	10	0.0	21.0	5.8	13.0	3.4	3.5	1.4	97.1	49.2	2.6	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	11	0.0	20.9	8.9	14.7	4.9	-3.5	1.3	100.0	59.7	2.2	14.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	12	0.0	18.4	9.8	13.3	4.1	2.0	1.2	100.0	99.1	1.2	10.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	13	0.8	19.6	9.0	13.5	4.3	2.0	1.2	100.0	99.6	1.3	11.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	14	0.0	22.0	8.0	14.7	5.0	-3.0	1.3	N/A	N/A	2.7	16.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	15	0.0	23.8	7.9	16.0	5.8	-6.0	2.0	N/A	N/A	3.5	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	16	0.0	15.1	4.2	8.9	0.0	17.5	2.0	100.0	100.0	1.5	20.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	17	0.0	18.5	2.3	9.8	0.4	11.0	2.3	100.0	98.8	1.5	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	18	0.0	24.0	2.1	11.5	3.0	5.5	1.6	100.0	98.8	2.0	20.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	19	0.0	25.6	4.6	14.8	5.1	0.0	1.7	N/A	N/A	3.0	20.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	20	0.0	21.4	5.1	12.9	3.3	5.0	2.3	100.0	99.5	1.8	19.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	21	0.0	17.3	1.5	9.3	0.0	9.5	2.4	100.0	55.3	2.4	17.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	22	0.0	20.3	4.2	11.7	2.2	5.5	2.2	97.2	42.0	3.1	19.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	23	0.0	15.1	6.3	10.6	0.7	12.5	2.8	95.9	60.9	2.1	15.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	24	0.0	18.0	6.5	11.4	2.2	9.0	2.7	94.8	53.0	2.7	19.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	25	0.0	23.0	3.0	12.3	3.0	4.0	2.2	98.4	33.7	3.6	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	26	0.0	18.2	9.5	13.1	3.9	2.5	2.5	90.7	55.7	2.6	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	27	0.0	18.4	8.2	12.2	3.3	6.0	3.0	96.2	51.4	2.5	13.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	28	0.0	20.3	5.3	12.3	2.8	4.5	2.9	94.6	48.6	3.1	19.9

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	29	0.0	22.1	4.2	13.0	3.1	1.5	2.3	93.4	38.4	3.5	19.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	30	0.0	21.4	8.3	14.3	4.9	-0.5	2.4	76.1	53.3	3.3	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	7	31	0.0	22.3	6.8	14.5	4.5	-2.0	2.5	89.3	36.5	3.6	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	1	0.0	21.5	10.6	15.7	6.1	-5.5	1.7	81.1	42.0	2.8	19.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	2	0.0	19.3	9.3	13.4	4.3	0.5	1.3	93.2	48.7	2.9	21.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	3	0.0	23.0	7.2	14.6	5.1	-2.0	2.1	96.8	45.4	3.6	20.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	4	0.0	26.4	9.7	17.6	8.0	-9.0	3.8	84.5	33.1	5.5	21.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	5	0.0	21.1	12.4	15.6	6.8	-6.5	2.0	93.9	56.3	3.1	19.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	6	0.0	22.8	11.3	15.4	7.0	-4.5	2.1	95.8	52.3	3.3	18.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	7	0.0	20.1	9.6	14.1	4.8	-0.5	1.8	93.6	55.8	2.6	14.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	8	0.0	23.1	6.6	14.4	4.8	-1.0	1.4	90.9	36.0	3.5	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	9	0.0	22.5	6.8	14.1	4.7	0.0	1.6	86.7	42.6	3.6	21.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	10	0.0	22.5	6.6	14.4	4.6	-0.5	1.9	82.1	39.2	3.9	22.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	11	0.0	23.6	7.5	14.9	5.5	-1.5	2.6	85.8	38.8	4.3	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	12	0.0	21.3	9.4	15.3	5.4	-5.5	2.9	79.8	49.1	3.9	21.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	13	0.0	21.6	7.8	14.6	4.7	-1.5	1.9	96.9	40.4	3.3	16.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	14	0.0	21.0	8.0	14.0	4.5	0.0	2.0	95.9	43.8	3.6	22.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	15	0.0	21.0	5.8	12.8	3.4	3.5	3.5	94.1	46.6	4.0	23.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	16	0.0	22.3	7.1	13.6	4.7	2.0	1.8	97.3	43.2	3.7	23.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	17	0.0	24.9	5.5	15.1	5.2	-2.0	1.4	88.0	37.5	3.9	23.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	18	0.0	24.3	10.2	17.0	7.3	-9.0	3.3	73.9	38.0	5.2	23.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	19	0.0	19.7	9.5	14.6	4.6	-4.0	1.9	88.4	52.3	3.4	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	20	0.0	19.8	7.8	13.2	3.8	3.0	2.7	91.3	48.0	3.7	22.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	21	0.0	21.8	7.4	14.5	4.6	-2.0	2.5	89.1	44.0	4.1	23.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	22	0.0	25.2	8.3	16.6	6.8	-6.5	1.8	82.8	37.7	4.4	23.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	23	0.0	24.9	11.1	17.6	8.0	-8.5	1.9	81.8	40.1	4.4	22.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	24	0.0	24.9	10.1	17.4	7.5	-9.0	1.7	95.5	45.5	3.9	21.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	25	0.0	25.1	10.2	17.6	7.7	-9.5	1.6	84.3	45.2	4.1	21.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	26	0.0	25.1	11.0	17.9	8.1	-11.5	1.5	90.9	42.2	3.9	20.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	27	0.0	25.4	10.2	17.8	7.8	-11.0	3.7	94.1	41.0	5.1	22.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	28	0.0	26.4	9.6	18.1	8.0	-11.0	2.5	94.3	36.3	4.9	22.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	29	0.0	26.6	9.0	18.0	7.8	-10.0	2.3	90.8	36.2	5.0	23.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	30	0.0	29.5	10.0	19.2	9.7	-9.0	2.0	77.5	30.6	5.6	24.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	8	31	0.0	30.9	11.2	20.5	11.0	-12.0	1.4	60.6	26.6	5.4	25.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	1	0.0	31.3	12.0	22.0	11.6	-16.5	1.8	55.8	27.3	6.0	25.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	2	0.0	30.1	14.1	22.0	12.1	-19.0	2.0	82.3	30.3	5.5	22.9

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	3	0.0	31.3	13.1	21.9	12.2	-17.0	1.7	70.1	26.3	5.5	22.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	4	0.0	28.1	14.4	20.6	11.3	-15.5	2.3	86.4	36.2	4.9	18.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	5	0.0	22.7	13.3	17.6	8.0	-13.0	1.8	91.6	59.7	3.0	14.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	6	0.0	29.1	12.9	20.0	11.0	-14.0	2.3	93.7	41.6	5.0	20.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	7	0.0	30.0	11.5	21.2	10.7	-15.0	2.2	81.8	23.5	6.4	27.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	8	0.0	21.4	12.3	16.3	6.9	-9.0	1.3	87.0	46.2	4.2	25.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	9	0.0	24.8	11.0	17.6	7.9	-10.5	1.8	88.1	41.7	4.8	26.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	10	0.0	31.5	11.0	21.6	11.2	-15.0	2.6	82.5	29.9	6.5	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	11	0.0	22.3	11.5	17.2	6.9	-12.5	2.5	81.5	40.2	4.7	24.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	12	0.0	20.0	11.0	14.5	5.5	-2.5	3.0	86.8	56.0	3.7	19.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	13	0.0	29.2	9.0	19.1	9.1	-11.0	3.6	94.6	41.8	6.1	25.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	14	0.0	33.1	18.1	25.6	15.6	-24.0	1.8	72.7	29.1	6.4	25.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	15	0.0	19.8	12.4	16.2	6.1	-10.5	3.0	90.2	68.6	3.6	23.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	16	0.0	19.3	12.1	15.5	5.7	-7.0	2.3	91.2	71.7	2.4	11.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	17	0.0	28.2	14.1	19.8	11.2	-14.5	3.6	94.3	50.5	5.3	22.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	18	0.0	24.9	12.9	18.1	8.9	-12.0	3.8	84.3	51.1	5.0	21.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	19	1.0	18.8	12.7	15.0	5.8	-5.5	3.6	92.7	69.5	2.7	13.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	20	0.0	18.3	13.2	15.3	5.8	-6.5	2.9	89.5	71.8	2.1	7.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	21	0.0	21.8	10.6	15.4	6.2	-6.5	1.7	97.2	59.2	3.3	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	22	0.0	21.7	10.8	16.2	6.3	-8.0	2.4	96.0	57.9	3.7	20.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	23	0.0	24.1	12.3	17.5	8.2	-11.0	2.8	95.3	52.3	4.4	22.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	24	0.0	23.4	11.8	17.6	7.6	-12.0	2.0	92.1	49.9	4.3	22.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	25	0.0	28.9	12.0	20.9	10.5	-16.0	2.2	85.7	39.1	6.0	28.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	26	0.0	30.7	14.4	22.8	12.5	-20.0	2.0	72.4	36.0	6.3	27.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	27	0.0	22.2	14.0	17.4	8.1	-6.0	2.6	86.2	62.0	2.9	11.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	28	0.0	29.9	12.9	21.5	11.4	-17.0	3.2	94.7	38.4	4.0	10.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	29	0.0	30.3	15.0	22.3	12.6	-19.5	3.4	89.9	39.5	3.9	8.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	9	30	0.0	30.7	17.2	23.5	14.0	-23.5	2.8	73.7	37.7	4.3	9.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	1	0.2	32.8	15.6	24.5	14.2	-20.5	0.7	70.5	32.7	2.7	9.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	2	0.0	24.1	15.6	20.0	9.8	-22.0	0.6	90.5	61.2	1.9	8.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	3	0.2	24.0	14.4	18.3	9.2	-13.0	0.6	94.7	58.3	2.2	8.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	4	0.0	27.5	12.7	20.3	10.1	-17.0	0.6	93.6	50.7	1.9	6.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	5	0.0	32.2	16.1	24.8	14.2	-20.5	3.4	79.7	35.4	3.2	9.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	6	0.0	28.8	19.5	23.2	14.1	-24.0	3.7	71.7	47.0	5.0	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	7	0.0	27.3	17.1	21.5	12.2	-21.5	4.1	88.6	49.2	4.5	9.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	8	0.0	26.8	17.8	21.2	12.3	-22.5	3.6	85.4	47.0	3.9	4.9

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	9	0.0	30.1	19.1	23.5	14.6	-24.0	2.5	71.7	42.1	4.5	8.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	10	0.0	34.8	19.5	26.6	17.1	-24.0	2.6	68.0	30.0	5.8	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	11	0.0	19.9	13.9	16.9	6.9	-12.0	3.9	87.9	73.5	2.0	3.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	12	0.0	21.2	13.0	16.3	7.1	-8.5	3.5	86.7	61.2	3.1	9.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	13	0.0	29.0	11.0	19.8	10.0	-14.0	2.0	96.4	40.8	4.0	11.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	14	0.0	31.5	15.2	23.6	13.3	-21.0	2.3	78.7	33.2	5.0	10.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	15	0.0	30.9	16.2	23.2	13.6	-21.5	2.6	72.1	28.3	5.6	11.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	16	0.0	31.6	15.1	23.5	13.4	-19.5	2.3	77.6	29.8	5.3	11.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	17	0.0	33.8	16.5	25.2	15.2	-22.5	2.6	76.6	25.5	6.1	11.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	18	0.0	32.8	16.7	24.7	14.7	-22.5	2.6	88.0	31.9	5.3	10.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	19	4.4	33.7	18.0	24.4	15.9	-24.0	2.7	79.8	31.6	5.4	8.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	20	0.0	23.2	16.2	19.4	9.7	-20.0	3.9	89.5	62.7	3.2	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	21	0.8	29.3	14.8	19.6	12.0	-15.0	3.2	91.2	43.4	4.5	8.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	22	0.0	23.4	14.6	18.1	9.0	-13.5	3.0	88.9	58.9	2.9	6.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	23	0.0	25.3	14.3	19.7	9.8	-16.5	2.3	91.0	53.7	3.1	8.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	24	0.0	29.9	15.3	22.8	12.6	-20.5	2.0	86.5	40.5	4.1	9.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	25	0.0	31.8	17.6	24.9	14.7	-23.5	2.6	74.5	36.7	5.1	9.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	26	6.2	21.8	16.4	18.8	9.1	-20.0	1.8	92.5	69.4	1.5	2.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	27	24.0	19.9	14.9	16.8	7.4	-9.5	2.3	97.7	79.0	1.3	2.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	28	0.2	21.8	14.5	17.0	8.1	-9.0	2.7	92.8	65.3	2.2	4.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	29	0.0	25.7	12.5	19.4	9.1	-15.5	1.9	94.6	50.6	3.5	12.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	30	38.0	29.0	15.3	20.7	12.2	-15.5	2.7	94.3	38.4	4.6	11.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	10	31	0.2	17.1	12.8	14.9	4.9	-4.0	3.5	95.6	78.9	1.6	5.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	1	3.8	22.3	14.0	17.4	8.1	-12.0	2.5	93.0	68.6	2.0	4.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	2	0.2	26.5	16.5	21.0	11.5	-20.5	2.7	88.8	55.8	3.3	7.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	3	0.0	25.7	17.6	20.2	11.6	-12.0	2.5	93.1	59.2	2.2	5.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	4	0.0	31.0	13.8	22.4	12.4	-19.1	3.1	100.0	74.7	1.7	0.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	5	0.0	30.1	15.0	22.6	12.6	-19.4	3.2	95.1	38.6	5.6	22.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	6	0.0	27.5	18.8	23.2	13.2	-20.7	3.2	97.3	59.3	6.9	31.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	7	0.0	27.5	17.8	22.7	12.7	-19.6	3.1	99.4	67.0	6.0	26.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	8	0.0	29.5	18.8	24.2	14.2	-22.8	2.8	94.0	64.4	5.4	21.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	9	0.0	30.0	18.3	24.2	14.2	-22.8	2.7	89.7	63.1	6.4	27.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	10	0.0	32.0	18.2	25.1	15.1	-24.0	3.0	97.3	61.8	6.6	26.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	11	0.0	30.5	17.3	23.9	13.9	-22.3	2.8	100.0	56.7	6.2	26.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	12	0.0	30.0	17.0	23.5	13.5	-21.4	2.5	100.0	64.4	6.8	30.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	13	0.0	33.3	17.3	25.3	15.3	-24.0	2.5	100.0	77.3	7.5	33.3

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	14	0.0	25.1	16.8	21.0	11.0	-16.1	2.6	87.1	55.3	6.6	31.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	15	0.0	26.0	13.8	19.9	9.9	-13.8	2.2	90.4	59.1	9.2	49.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	16	0.0	26.5	16.6	21.6	11.6	-17.3	2.6	88.2	43.1	5.1	20.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	17	0.0	25.5	14.3	19.9	9.9	-13.8	2.9	91.5	55.6	4.3	16.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	18	4.0	24.5	16.3	20.4	10.4	-14.9	2.9	98.2	50.1	3.8	14.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	19	5.0	22.5	14.8	18.7	8.7	-11.2	2.7	99.7	60.7	5.6	28.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	20	0.0	25.0	12.3	18.7	8.7	-11.2	2.4	99.8	59.6	3.6	11.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	21	0.0	26.0	16.8	21.4	11.4	-17.0	1.9	100.0	67.0	3.6	11.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	22	3.0	21.5	17.3	19.4	9.4	-12.8	2.1	97.3	67.0	6.4	32.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	23	0.0	24.9	14.3	19.6	9.6	-13.2	2.2	97.3	77.3	5.4	24.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	24	0.0	31.6	12.9	22.3	12.3	-18.8	2.3	100.0	63.1	6.9	30.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	25	15.5	27.0	17.5	22.3	12.3	-18.8	2.3	91.9	38.6	5.8	23.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	26	14.0	29.7	14.8	22.3	12.3	-18.8	2.8	100.0	70.9	6.6	29.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	27	0.0	27.2	19.8	23.5	13.5	-21.4	2.7	95.1	56.7	4.1	14.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	28	0.0	27.0	19.4	23.2	13.2	-20.8	2.5	90.8	63.1	3.4	9.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	29	0.0	30.3	19.4	24.9	14.9	-24.0	2.4	97.3	70.9	4.5	15.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	11	30	0.0	25.5	19.3	22.4	12.4	-19.1	2.2	100.0	58.0	4.1	14.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	1	13.0	25.3	14.7	20.0	10.0	-14.0	2.5	91.4	77.3	5.7	29.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	2	23.0	30.4	12.8	21.6	11.6	-17.4	2.4	96.6	64.4	6.1	29.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	3	21.5	31.9	13.5	22.7	12.7	-19.8	2.6	93.5	38.6	5.8	26.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	4	7.0	26.8	14.9	20.9	10.9	-15.8	2.8	84.1	38.6	4.9	21.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	5	2.0	25.9	15.6	20.8	10.8	-15.6	2.4	98.7	63.1	4.6	20.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	6	0.0	28.8	15.2	22.0	12.0	-18.3	2.0	98.7	70.8	4.7	20.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	7	3.5	30.3	17.1	23.7	13.7	-21.9	2.0	93.5	56.7	5.8	26.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	8	2.3	31.0	19.9	25.5	15.5	-24.0	2.7	81.0	46.4	4.3	14.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	9	0.0	24.1	19.4	21.8	11.8	-17.7	2.3	95.6	64.4	1.7	1.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	10	0.0	28.3	17.9	23.1	13.1	-20.6	2.1	96.6	88.9	3.6	15.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	11	0.0	27.6	19.3	23.5	13.5	-21.3	2.2	94.5	65.7	2.4	5.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	12	2.0	23.3	17.7	20.5	10.5	-15.1	2.7	96.6	83.7	5.7	29.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	13	6.5	29.0	15.4	22.2	12.2	-18.7	2.3	90.4	77.3	8.8	47.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	14	0.0	26.3	11.9	19.1	9.1	-12.1	2.1	97.6	70.8	8.5	48.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	15	0.0	29.3	15.6	22.5	12.5	-19.2	2.4	93.5	51.5	6.1	29.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	16	4.0	30.7	11.9	21.3	11.3	-16.8	2.1	98.7	65.7	6.1	29.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	17	0.0	30.8	12.1	21.5	11.5	-17.1	2.7	95.6	39.9	4.6	18.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	18	0.0	31.7	15.9	23.8	13.8	-22.1	2.6	89.3	36.1	5.8	25.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	19	0.0	31.1	14.9	23.0	13.0	-20.4	2.5	94.5	43.8	5.4	22.4

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	20	0.0	33.9	16.1	25.0	15.0	-24.0	2.2	92.4	51.5	5.0	19.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	21	58.4	27.6	19.0	24.4	13.3	-12.0	2.8	93.8	57.7	2.2	4.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	22	8.2	24.8	17.3	20.6	11.1	-22.0	1.4	96.0	69.1	2.0	5.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	23	0.0	27.4	17.4	22.4	12.4	-22.5	1.6	94.5	55.4	3.3	11.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	24	0.2	29.1	18.2	23.7	13.7	-24.0	1.6	84.0	58.2	3.5	11.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	25	2.4	29.8	18.6	23.5	14.2	-24.0	1.7	95.3	55.3	3.3	9.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	26	0.2	23.5	17.5	20.1	10.5	-22.0	3.4	91.0	68.7	2.9	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	27	0.0	23.3	16.1	19.3	9.7	-19.0	2.0	90.2	67.5	2.4	6.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	28	0.0	29.7	14.9	22.3	12.3	-19.0	1.4	95.9	52.5	3.6	12.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	29	0.0	32.3	18.8	25.4	15.5	-24.0	1.5	86.9	46.6	4.1	12.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	30	1.2	30.6	20.3	24.2	15.4	-24.0	1.6	82.5	56.1	3.4	9.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2000	12	31	0.0	31.6	19.3	24.8	15.4	-24.0	1.8	90.4	49.8	4.1	12.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	1	1.4	26.4	17.4	22.3	11.9	-23.5	2.6	93.2	55.9	3.2	8.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	2	0.0	24.4	12.8	19.1	8.6	-16.0	2.7	80.4	54.3	3.6	10.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	3	0.0	25.9	13.4	19.6	9.6	-16.0	2.4	88.8	52.5	3.8	12.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	4	0.0	27.3	13.0	20.1	10.1	-15.5	2.3	88.7	50.6	3.9	12.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	5	0.0	30.2	11.9	21.7	11.1	-17.0	2.0	90.8	45.8	4.3	12.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	6	0.0	31.9	14.9	23.6	13.4	-20.0	1.7	88.4	42.5	4.4	12.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	7	3.4	32.0	18.9	24.0	15.5	-24.0	2.8	75.4	44.6	5.2	11.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	8	0.0	29.8	17.8	23.3	13.8	-23.0	2.1	89.2	48.2	4.2	12.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	9	0.0	31.6	19.0	24.6	15.3	-24.0	2.0	87.7	46.4	4.3	11.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	10	0.0	35.2	19.0	27.1	17.0	-24.0	1.7	86.3	38.0	4.8	12.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	11	0.0	37.0	19.4	27.9	17.2	-24.0	2.0	83.7	28.4	5.7	12.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	12	0.0	31.2	21.5	25.3	16.3	-24.0	3.4	84.8	51.0	4.7	9.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	13	0.0	30.7	19.1	24.2	14.9	-24.0	2.4	88.3	42.6	4.6	11.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	14	0.0	32.6	19.8	25.6	16.2	-24.0	2.0	69.0	41.3	4.8	11.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	15	0.0	31.7	21.1	25.6	16.4	-24.0	2.9	83.8	48.3	4.6	9.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	16	0.0	28.1	18.4	22.7	13.3	-24.0	2.8	91.5	58.9	3.2	7.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	17	0.0	26.0	18.5	21.5	12.2	-24.0	2.5	91.0	63.0	2.5	4.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	18	0.0	28.4	17.6	21.7	13.0	-22.5	1.9	86.7	51.8	3.3	8.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	19	0.0	32.3	16.0	24.7	14.2	-22.0	1.9	84.9	43.0	4.4	11.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	20	0.0	32.0	20.5	25.4	16.3	-24.0	2.2	85.2	47.2	4.3	10.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	21	4.0	32.4	21.0	25.1	16.7	-24.0	2.7	84.6	45.6	4.7	9.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	22	0.0	29.0	19.0	23.6	14.0	-24.0	2.7	88.6	47.8	4.1	9.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	23	0.0	30.4	18.6	24.1	14.5	-24.0	1.8	88.8	47.0	3.8	10.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	24	6.8	34.2	19.8	25.7	17.0	-24.0	2.4	85.7	40.3	5.0	10.7

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	25	0.0	29.8	19.5	23.6	14.6	-24.0	3.5	85.4	48.5	4.9	11.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	26	0.0	30.5	16.3	23.4	13.4	-21.0	2.3	92.0	42.9	4.6	13.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	27	0.0	29.1	16.2	23.4	12.7	-22.5	2.2	91.0	49.2	3.6	8.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	28	0.0	31.3	17.4	24.2	14.3	-23.0	1.9	93.6	41.0	4.0	10.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	29	0.8	31.7	18.6	24.6	15.2	-24.0	2.2	87.5	44.0	4.5	11.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	30	0.2	33.0	19.3	25.9	16.2	-24.0	2.5	84.2	43.7	5.0	11.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	1	31	7.6	32.9	19.8	24.5	16.3	-24.0	2.7	94.4	42.2	4.5	8.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	1	0.2	27.5	19.5	22.5	13.5	-24.0	1.8	88.5	60.0	2.8	7.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	2	0.0	32.6	18.2	25.1	15.4	-24.0	1.8	90.3	39.0	4.5	12.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	3	0.0	33.9	21.0	27.1	17.5	-24.0	2.3	70.5	39.9	5.1	10.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	4	10.6	30.2	17.7	23.2	14.0	-23.5	3.7	90.7	51.9	4.4	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	5	0.2	25.6	15.1	20.1	10.3	-18.0	2.1	94.2	61.1	2.7	7.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	6	0.2	25.4	18.5	21.4	11.9	-24.0	2.1	89.6	68.8	2.4	6.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	7	0.0	27.2	18.4	22.0	12.8	-24.0	1.8	93.6	62.7	2.6	7.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	8	0.0	29.1	19.2	23.7	14.2	-24.0	2.1	90.0	49.9	3.5	8.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	9	0.0	26.8	17.5	22.0	12.1	-23.0	3.3	86.2	50.4	4.1	9.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	10	0.0	24.7	19.2	21.2	11.9	-24.0	2.6	84.8	57.6	3.0	6.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	11	0.0	26.0	18.4	21.6	12.2	-24.0	2.6	85.2	54.6	3.2	7.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	12	0.0	28.5	15.4	21.9	11.9	-18.5	2.0	92.9	48.4	3.7	10.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	13	0.0	31.0	17.5	24.1	14.2	-22.0	1.9	91.3	38.3	4.3	11.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	14	0.0	27.4	18.2	23.1	12.8	-24.0	2.0	85.7	58.5	2.7	5.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	15	0.0	26.6	18.2	21.4	12.4	-24.0	2.8	85.3	56.7	3.4	7.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	16	0.0	27.8	17.4	21.8	12.6	-21.0	2.0	89.0	53.0	3.3	9.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	17	0.0	32.1	18.3	24.8	15.2	-24.0	2.1	89.1	39.6	4.4	10.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	18	26.8	28.6	19.4	22.9	14.0	-24.0	2.9	94.1	54.1	3.5	7.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	19	10.6	25.5	19.6	21.1	12.5	-24.0	1.7	96.1	73.4	1.9	5.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	20	20.0	27.3	19.4	22.2	13.3	-24.0	2.0	95.9	64.1	2.6	7.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	21	9.4	22.4	19.4	20.4	10.9	-24.0	1.8	95.5	79.8	1.2	2.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	22	0.0	26.8	18.4	22.0	12.6	-24.0	2.0	91.6	62.7	2.7	7.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	23	0.0	29.3	18.6	23.6	13.9	-24.0	1.4	96.2	55.0	3.2	10.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	24	0.0	26.9	19.7	22.9	13.3	-24.0	3.0	93.9	63.1	3.0	8.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	25	0.0	26.0	18.3	21.9	12.1	-24.0	2.4	89.7	64.8	2.6	6.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	26	0.0	22.6	19.1	20.7	10.8	-24.0	2.1	88.8	74.8	1.7	3.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	27	33.8	19.0	16.6	17.8	7.8	-17.0	1.5	96.0	86.3	1.0	2.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	2	28	18.4	17.3	15.8	16.3	6.6	-10.5	1.5	96.3	91.2	0.9	2.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	1	0.0	21.8	14.5	17.3	8.2	-11.5	1.3	97.3	70.6	1.7	5.3

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	2	0.4	26.5	14.0	19.9	10.2	-16.5	1.4	98.8	56.2	2.4	7.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	3	0.0	28.6	16.5	21.7	12.5	-22.0	1.4	96.8	43.1	3.2	9.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	4	0.0	28.2	18.1	22.6	13.2	-24.0	2.3	89.0	51.4	3.7	10.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	5	0.0	27.3	17.6	21.9	12.4	-22.5	1.7	90.4	56.4	2.9	8.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	6	0.0	28.7	18.0	22.9	13.4	-24.0	1.3	94.4	57.7	2.7	8.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	7	0.0	29.8	18.5	23.8	14.1	-24.0	1.8	93.9	54.1	3.1	8.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	8	0.0	30.3	17.3	23.9	13.8	-22.5	1.6	95.2	47.3	3.4	10.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	9	10.4	29.3	19.6	22.9	14.5	-24.0	1.9	93.4	59.1	2.8	6.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	10	0.0	27.8	19.8	22.6	13.8	-24.0	1.4	93.2	65.7	2.4	7.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	11	0.0	29.3	17.6	23.5	13.5	-23.0	1.8	88.9	52.0	3.5	10.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	12	0.0	27.7	17.2	22.8	12.5	-22.0	1.2	94.4	65.1	2.3	7.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	13	0.0	24.4	17.3	21.5	10.9	-23.0	3.9	85.3	66.0	3.2	8.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	14	0.0	24.7	15.1	19.7	9.9	-16.5	2.6	91.9	60.1	2.9	7.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	15	0.0	26.7	16.4	21.0	11.5	-20.5	2.3	92.7	55.4	3.1	8.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	16	0.0	26.2	14.7	20.5	10.4	-19.0	2.0	95.9	60.0	2.6	7.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	17	0.0	26.7	15.9	20.5	11.3	-19.5	1.3	96.6	62.1	2.2	6.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	18	0.0	29.1	15.3	21.8	12.2	-19.0	1.5	95.8	54.0	2.9	8.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	19	1.2	20.7	16.4	18.3	8.6	-18.0	2.9	92.9	75.8	1.7	3.6
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	20	0.0	24.9	14.1	19.2	9.5	-16.5	1.7	91.1	61.2	2.7	9.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	21	0.0	25.2	14.3	19.7	9.8	-16.5	2.3	94.6	59.4	2.8	8.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	22	0.0	22.8	16.3	19.4	9.6	-19.5	2.8	92.1	65.8	2.4	6.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	23	0.0	23.1	17.1	20.0	10.1	-20.5	2.8	90.1	67.4	2.3	5.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	24	3.0	19.8	17.6	18.7	8.7	-22.0	2.0	93.9	84.3	1.1	1.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	25	2.0	24.7	17.3	19.8	11.0	-19.5	1.1	96.8	65.6	1.7	4.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	26	0.4	28.1	15.0	20.7	11.5	-17.0	1.0	97.8	57.2	2.4	8.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	27	0.0	28.4	16.1	22.2	12.3	-20.5	2.0	94.8	57.5	3.0	9.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	28	0.0	27.1	18.9	21.9	13.0	-24.0	1.8	92.3	63.2	2.3	5.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	29	0.0	27.7	17.5	21.6	12.6	-23.0	1.8	96.1	55.3	2.5	5.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	30	0.0	27.0	16.2	21.4	11.6	-22.5	1.8	96.1	54.8	2.9	8.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	3	31	0.0	29.4	15.8	22.1	12.6	-20.5	1.4	95.6	46.8	3.1	9.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	1	0.0	28.9	17.3	22.9	13.1	-23.0	2.3	95.9	52.0	3.2	7.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	2	0.2	28.4	20.3	23.0	14.3	-24.0	1.4	93.2	56.4	2.3	5.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	3	0.6	30.4	17.6	22.4	14.0	-23.0	1.8	94.9	52.8	3.0	7.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	4	15.0	20.8	17.4	19.1	9.1	-22.0	2.6	95.3	80.9	1.3	2.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	5	0.0	23.2	15.7	18.5	9.5	-15.5	2.3	95.6	61.8	2.4	7.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	6	0.0	21.4	13.8	17.1	7.6	-11.0	2.2	91.6	63.1	2.4	8.7

CompNo	Name	Lat	Long	Alt	Year	M	Day	Rain	Tmax	Tmin	Tave	HU	UCU	U2	RHx	RHn	ETo	Rs
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	7	0.0	21.6	12.7	16.9	7.2	-11.5	1.7	93.2	64.7	2.1	7.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	8	0.0	23.8	15.3	18.7	9.6	-15.5	1.5	88.0	59.4	2.2	5.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	9	2.8	24.3	13.2	17.6	8.7	-11.5	1.5	96.7	62.6	2.0	5.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	10	0.0	24.9	16.2	19.4	10.5	-19.0	1.4	95.8	63.5	1.9	5.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	11	0.0	25.7	15.7	19.9	10.7	-18.0	1.9	93.8	56.9	2.6	7.5
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	12	1.4	26.3	14.4	19.2	10.3	-15.5	1.3	95.4	52.8	2.4	7.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	13	0.2	26.7	13.9	19.7	10.3	-15.0	1.2	96.6	57.6	2.3	7.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	14	0.0	24.3	16.6	19.8	10.5	-20.0	3.1	87.0	61.4	3.0	7.8
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	15	0.0	20.1	14.0	17.3	7.0	-13.5	2.2	94.5	70.7	1.5	3.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	16	0.0	23.4	13.0	17.2	8.2	-11.5	1.6	95.4	61.8	2.0	6.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	17	0.0	27.1	11.2	18.5	9.1	-12.5	1.2	97.5	52.5	2.3	7.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	18	8.4	26.6	14.5	20.0	10.6	-15.0	1.8	91.8	56.4	2.5	6.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	19	0.0	26.0	15.4	19.7	10.7	-18.0	1.3	95.6	59.9	2.1	6.4
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	20	0.0	24.2	14.6	19.3	9.4	-17.0	1.4	95.2	66.5	1.7	5.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	21	0.0	24.7	15.0	19.7	9.8	-18.0	1.3	95.5	57.5	2.0	6.3
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	22	0.0	26.1	12.4	19.0	9.2	-14.5	1.1	99.3	69.6	1.9	7.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	23	0.0	27.6	13.2	19.9	10.4	-14.5	1.2	91.4	32.4	2.7	8.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	24	0.0	28.6	13.3	21.1	10.9	-19.5	1.5	92.4	33.9	3.0	8.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	25	0.0	29.9	12.9	21.6	11.4	-18.0	1.5	92.4	27.7	3.2	8.2
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	26	0.0	29.3	14.1	21.6	11.7	-18.5	2.4	87.5	33.9	3.7	8.1
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	27	1.0	22.2	15.6	19.0	8.9	-18.0	1.5	91.4	80.1	1.5	2.9
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	28	0.0	28.2	13.3	20.1	10.8	-15.0	1.3	93.4	29.3	2.8	7.7
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	29	0.0	28.2	14.1	21.4	11.2	-18.0	1.4	90.4	33.9	2.7	7.0
30090	GILLEMBERG	-23.8333	28.9667	1100	2001	4	30	0.0	25.4	17.8	21.1	11.6	-23.0	2.4	92.4	66.2	2.1	5.0

Lat	= Latitude	Tmax	= Maximum temperature	RHx	= Maximum humidity	HU	= Heat Units
Long	= Longitude	Tmin	= Minimum temperature	Rhn	= Minimum humidity		
Alt	= Altitude	Tave	= Average temperature	Eto	= Evapotranspiration		
M	= Month	CU	= Chill Units	Rs	= Solar radiation		

APPENDIX II: FLOWER PETAL COLOUR


Zastron


Algerian


American Giant


Amersfoort


Berg X Mexican


Corfu


Cross X


Direkteur


Ficus Indice


Fusicaulis


Gymno Carpo


Malta


Messina


Mexican


Meyers


Morado


Nepgen


Nudosa


Ofer


R1251


R1259


R1260


Roedtan


Roly Poly


Rossa


Santa Rosa


Schagen


Sharsheret


Sicilian Indian Fig


Tormentosa


Turpin


Van As


X 28

PHOTOS: J.P. POTGIETER

APPENDIX III: PULP COLOUR


Zastron


Algerian


American Giant


Amersfoort


Arbiter


Blue Motto


Corfu


Cross X


Direkteur


Fresno


Fusicaulis


Gymno-Carpo


Malta


Messina


Meyers


Morado


Muscatel


Nepgen


Nudosa


Ofer


R1251


R1259


R1260


Roly Poly


Rossa


Santa Rosa


Schagen


Sicilian Indian Fig


Skinners Court


Turpin


Van As


Vryheid

PHOTOS: M. DE WIT

APPENDIX IV : WATERKLOOF CLIMATIC DATA												
Date	DOY	Rn	T	RH	WS	Rain	Tx	Tn	RHx	RHn	CU	HU
2006/07/01	182	6.72	9.99	64.92	1.63	0	15.16	4.75	84	47.41	12.5	0
2006/07/02	183	10.84	9.46	60.73	1.34	0	16.67	2.56	87	32.08	13	0
2006/07/03	184	12.59	7.91	56.54	1.32	0	16.91	1.22	86	25.49	10	0
2006/07/04	185	12.73	7.9	50.81	1.06	0	17.58	-0.4	82	21.57	8	0
2006/07/05	186	12.43	10.88	42.37	1.17	0	20.35	-0.1	76	21.76	4.5	0.15
2006/07/06	187	12.56	12.7	41.17	1.81	0	21.95	6.67	62	19.81	5	4.31
2006/07/07	188	13	6.4	56.4	1.27	0	14.84	-0.4	84	24.23	11	0
2006/07/08	189	13	6.44	53.84	1.32	0	17.3	-3.3	87	23.66	5	0
2006/07/09	190	12.88	9.64	43.37	0.91	0	18.17	2.69	61	24.26	11	0.43
2006/07/10	191	13.08	8.06	54.54	0.79	0	18.39	-0.6	85	25.55	5.5	0
2006/07/11	192	13.33	9.31	44.16	1.12	0	19.49	-0.8	82	16.45	4	0
2006/07/12	193	13.14	11.58	37.26	1.27	0	20.85	4.61	53	19.97	6	2.73
2006/07/13	194	12.97	11.83	39.66	2.25	0	19.39	6.53	58	23.2	7	2.96
2006/07/14	195	7.11	11.43	55.18	3.33	0	18.15	3.15	77	36.13	7.5	0.65
2006/07/15	196	11.91	10.39	58.4	1.83	0	19.68	-0.7	89	33.96	0	0
2006/07/16	197	8.4	14.31	48.98	3.47	0	19.53	11.2	58	33.47	0	5.37
2006/07/17	198	12.22	12.22	54.94	2.04	0	19.6	4.65	74	33.87	5	2.13
2006/07/18	199	13.27	9.25	49.25	0.7	0	20.23	-1.9	88	17.28	3	0
2006/07/19	200	12.8	10.95	47.16	1.11	0	22.75	-0.6	74	22.2	0	1.06
2006/07/20	201	12.04	15.63	36.7	2.06	0	23.34	8.07	62	18.04	0	5.71
2006/07/21	202	3.67	13.88	44.76	2.03	0	17.57	7.37	79	31.85	1	2.47
2006/07/22	203	8.72	4.67	69.94	1.92	0	9.71	-3.5	88	51.31	19	0
2006/07/23	204	14.16	2.81	48.41	1.03	0	11.57	-4.8	83	22.88	10.5	0
2006/07/24	205	14.47	5.57	34.54	1.27	0	17.82	-6.2	64	11.36	6.5	0
2006/07/25	206	13.74	10.26	33.97	0.97	0	22.2	-1.3	62	13.35	2.5	0.47
2006/07/26	207	13.53	14.59	34.01	1.43	0	24.58	5.34	63	16.01	0	4.96
2006/07/27	208	13.21	16.15	30.34	1.53	0	25.29	10.8	45	16.04	0	8.05
2006/07/28	209	13.54	13.89	32.66	1.03	0	23	6.45	55	15.35	1.5	4.73
2006/07/29	210	14.08	12.02	34.01	1.07	0	22.35	1.56	67	13.49	4.5	1.96
2006/07/30	211	14.09	11.96	29.39	1.05	0	21.76	1.76	52	13.36	2.5	1.76

Date	DOY	Rn	T	RH	WS	Rain	Tx	Tn	RHx	RHn	CU	HU
2006/07/31	212	14.2	12.88	29.57	1.82	0	21.05	6.29	42	15.65	3.5	3.67
2006/08/01	213	2.38	8.06	81.83	3.22	19.3	12.89	3.58	97	43.44	20	0
2006/08/02	214	3.82	3.66	96.28	5.01	6.6	6.06	1.63	98	93.6	18.5	0
2006/08/03	215	11.99	6.5	76.88	4.47	0.25	11.78	2.78	96	43.97	21	0
2006/08/04	216	11.39	5.96	73.65	2.25	0	11.54	1.2	92	45.1	18	0
2006/08/05	217	15.66	5.03	72.72	0.98	0.25	12.8	-1.4	96	38.46	11	0
2006/08/06	218	15.97	8.01	56.26	0.86	0	17.44	-1.3	93	23.13	6	0
2006/08/07	219	16.25	12.12	34.87	1.29	0	21.7	4.31	55	13.79	4	3.01
2006/08/08	220	16.39	11.61	35.42	1.6	0	20.01	4.04	71	12.53	7	2.02
2006/08/09	221	16.37	9	53.91	0.82	0	19.02	1	86	21.69	9	0.01
2006/08/10	222	15.85	13.12	51.37	3.34	0	21.53	5.98	83	21.54	3	3.75
2006/08/11	223	15.64	14.6	44.52	2.61	0	23.08	7.34	73	19.64	0	5.21
2006/08/12	224	16.87	9.34	53.64	1.51	0	15.44	3.58	76	32.54	14.5	0
2006/08/13	225	17.01	11.63	43.68	1.63	0	22.68	-0.4	89	13.82	0	1.16
2006/08/14	226	15.59	16.17	32.07	2.7	0	25.05	9.35	56	15.28	0	7.2
2006/08/15	227	10.74	9.13	60.02	2.93	0	14.17	3.8	91	36.82	15.5	0
2006/08/16	228	17.16	8.11	65.65	1.46	0	16.69	1.85	93	31.64	13	0
2006/08/17	229	17.39	10.61	51.81	0.98	0	19.98	-0.7	88	23.99	1	0
2006/08/18	230	16.98	12.87	50.61	1.3	0	20.55	6.92	72	32.26	4.5	3.73
2006/08/19	231	17.63	13.77	44.14	1.05	0	21.77	7.75	76	16.34	1.5	4.76
2006/08/20	232	17.71	14.08	36.64	1.78	0	23.94	2.15	78	13.09	0	3.04
2006/08/21	233	14.53	15.19	50.66	6.3	0	20.9	10.1	77	31.31	0	5.5
2006/08/22	234	4.07	11.36	81.08	2.96	18.54	13.88	9.69	97	49.39	9.5	1.79
2006/08/23	235	13.18	13.42	78.2	4.76	9.4	18.79	9.51	97	51.82	0	4.15
2006/08/24	236	3.69	9.42	93.38	2.42	32	12.49	6.15	97	88.2	17.5	0
2006/08/25	237	16.22	10.12	77.79	1.41	0	17.24	4.02	97	48.93	10.5	0.63
2006/08/26	238	18.55	11.26	68.98	1.34	0	19.01	3.19	95	40.08	5.5	1.1
2006/08/27	239	16.45	13.34	65.74	2.77	0	20.28	9.24	80	45.37	0.5	4.76
2006/08/28	240	17.74	15.06	56.61	1.52	0	22.53	6.55	93	29.61	0	4.54
2006/08/29	241	18.97	11.07	53.2	3.59	0	16.41	0.34	79	26.23	5.5	0
2006/08/30	242	19.95	3.52	60.87	2.3	0	11.2	-3.2	89	29.63	10.5	0

Date	DOY	Rn	T	RH	WS	Rain	Tx	Tn	RHx	RHn	CU	HU
2006/08/31	243	19.83	8.45	48.89	1.25	0	18.71	-1.5	86	19.8	1.5	0
2006/09/01	244	19.92	12.2	41.01	1.45	0	20.4	4.82	62	19.67	4	2.61
2006/09/02	245	19.08	14.38	37.26	1.25	0	22.5	8.09	59	18.77	0	5.29
2006/09/03	246	19.42	15.04	44.31	0.96	0	25.34	3.57	84	13.22	0	4.45
2006/09/04	247	19	15.16	50.49	3.43	0	22.26	10.3	73	24.46	0	6.26
2006/09/05	248	20.11	14.97	46.3	1.88	0	22.45	9.01	69	22.83	0	5.73
2006/09/06	249	18.13	15.66	43.77	1.32	0	23.64	8.73	73	21.82	0	6.19
2006/09/07	250	19.34	17.17	47.76	3.64	0	23.79	12.1	79	25.09	0	7.93
2006/09/08	251	19.09	16.16	49.13	2.31	0	22.22	8.48	78	30.7	0	5.35
2006/09/09	252	20.49	15.26	50.7	1.24	0	24.14	6.84	85	23.96	0	5.49
2006/09/10	253	20.89	17.81	38.63	1.2	0	25.81	10.9	67	18.64	0	8.38
2006/09/11	254	21.06	19.32	30.41	1.35	0	27.14	12.2	51	14.49	0	9.66
2006/09/12	255	20.86	19.17	29.93	1.34	0	28.52	8.82	62	13.05	0	8.67
2006/09/13	256	20.66	18.48	36.49	2.59	0	26.2	12.6	56	17.88	0	9.42
2006/09/14	257	20.57	19.02	38.87	1.44	0	27.75	9.01	74	17.51	0	8.38
2006/09/15	258	12.08	17.6	50.03	1.97	0.76	24.57	12.9	80	23.23	0	8.71
2006/09/16	259	20.38	14.99	39.05	1.97	0	23.54	6.96	78	11.73	0	5.25
2006/09/17	260	15.33	10.3	57.68	2.9	0	15.68	4.33	90	26.37	15.5	0
2006/09/18	261	22.64	8.84	54.39	1.92	0	16.71	0.81	93	21.14	9.5	0
2006/09/19	262	23.11	11.47	35.67	1.85	0	20.77	3.17	61	12.86	5.5	1.97
2006/09/20	263	23.32	10.25	43.09	1.43	0	19.42	1.28	79	15.26	3.5	0.35
2006/09/21	264	23.11	12.21	38.77	1.05	0	22.42	1.39	64	17.74	0	1.91
2006/09/22	265	21.93	15.02	35.42	1.65	0	24.94	3.27	68	14.12	0	4.11
2006/09/23	266	23.62	18.03	26.8	2.17	0	27.68	7.29	56	10.47	0	7.48
2006/09/24	267	23.73	18.96	23.36	1.67	0	28.8	6.79	61	9.17	0	7.8
2006/09/25	268	23.54	17.22	25.87	3.4	0	23.92	8.52	69	10.97	0	6.22
2006/09/26	269	22.46	10.4	65.17	3.26	0	17.55	5.66	91	36.16	12.5	1.6
2006/09/27	270	23.56	13.25	55.88	1.54	0	22.58	3.42	94	23.79	0.5	3
2006/09/28	271	24.29	16.88	36.26	1.3	0	26.53	7.3	74	11.86	0	6.91
2006/09/29	272	24.39	17.76	34.54	1.24	0	28.19	4.99	67	12.79	0	6.59
2006/09/30	273	24.54	19.43	31.65	1.06	0	29.38	7.04	67	11.33	0	8.21

Date	DOY	Rn	T	RH	WS	Rain	Tx	Tn	RHx	RHn	CU	HU
2006/10/01	274	19.44	20.08	25.12	2.83	0	29	11.1	43	12.79	0	10.02
2006/10/02	275	11.21	12.42	40.82	1.15	0	18.58	4.75	74	22.61	5	1.67
2006/10/03	276	24.14	12.14	48.58	2.17	0	21.11	4.57	87	16.79	4	2.84
2006/10/04	277	25.36	9.07	57.04	1.41	0	17.21	0.61	93	23.33	8.5	0
2006/10/05	278	25.87	11.61	45.21	1.14	0	21.15	0.79	94	14.65	1	0.97
2006/10/06	279	22.95	21.2	41.14	3.97	0	30.91	11.4	72	12.69	0	11.16
2006/10/07	280	10.58	18.79	62.38	2.58	2.29	23.77	15.3	87	41.74	0	9.54
2006/10/08	281	14.11	19.17	70.61	3.02	9.14	25.75	15.2	93	34.76	0	10.5
2006/10/09	282	18.19	15.36	70.37	1.57	9.4	22.05	8.51	96	34.47	0	5.28
2006/10/10	283	25.64	15.69	62.14	1.37	0	25.89	3.97	97	26.31	0	4.93
2006/10/11	284	25.01	19.07	51.22	2.43	0	26.9	12.2	82	17.27	0	9.54
2006/10/12	285	24.59	19.56	50.41	2.45	0	26.66	14	78	25.55	0	10.34
2006/10/13	286	16.54	18.57	57.63	1.2	1.27	26.13	11.5	82	30.97	0	8.82
2006/10/14	287	23.5	21.52	43.98	1.83	0	28.8	13.1	83	19.43	0	10.96
2006/10/15	288	22.76	22.04	39	1.9	0	29.4	14.7	65	19.8	0	12.04
2006/10/16	289	24.68	21.97	44.48	2.97	0	29.39	17.4	72	20.33	0	13.39
2006/10/17	290	19.64	20.11	58.44	2.1	1.02	27.13	14.1	91	30.44	0	10.62
2006/10/18	291	25.93	19.96	59.95	2.13	0.25	28.81	10.6	96	21.56	0	9.71
2006/10/19	292	18.73	18.44	71.01	4.74	9.91	26.5	14.2	90	44.19	0	10.32
2006/10/20	293	23.36	19.22	66.71	2.74	1.02	27.64	13.7	88	37.67	0	10.65
2006/10/21	294	25.67	21.45	42.99	2.09	0	29.45	14.4	85	11.1	0	11.92
2006/10/22	295	27.93	20.25	47.13	1.37	0	29.53	10.6	94	13.39	0	10.05
2006/10/23	296	28.21	22.01	36.72	1.09	0	31.03	11.7	74	14.81	0	11.38
2006/10/24	297	28.78	24.73	22.83	2.38	0	33.86	17.6	42	9.17	0	14.79
2006/10/25	298	27.87	23.15	24.06	1.66	0	31.54	12.4	42	14.32	0	11.95
2006/10/26	299	15.14	20.35	34.83	1.66	0	26.08	13.7	68	22.83	0	9.88
2006/10/27	300	23.28	20.56	53.42	2.54	2.03	30.1	11.4	78	22.65	0	10.73
2006/10/28	301	24.61	21.44	56.68	3.31	0	28.92	15.1	86	29.46	0	11.98
2006/10/29	302	16.47	20.61	61.3	2.48	0.25	27.94	15.3	92	33.74	0	11.62
2006/10/30	303	13.76	20.13	59.29	2.25	0.51	25.72	15.4	74	42.4	0	10.54
2006/10/31	304	22.61	20.72	55.89	1.83	0	29.71	12.8	93	20.2	0	11.26

2006/11/01	305	17.98	20.68	65.57	2.85	41.16	27.53	16	98	38.31	0	11.76
2006/11/02	306	8.06	15.12	91.08	1.88	31.24	19.74	11.8	98	80.7	0	5.75
2006/11/03	307	30.16	13.93	59.38	2.71	0	19.98	8.13	95	26.76	0	4.05
2006/11/04	308	30.51	14.8	46.15	1.4	0	22.45	5.22	83	20.97	0	3.84
2006/11/05	309	30.48	13.78	52.82	2.43	0	20.2	7.25	89	21.84	0.5	3.72

APPENDIX V: PLANT HABITUS AND CLADODE SHAPE


Rossa


Santa Rosa


Schagen


Sharsheret


Sicilian Indian Fig


Skinners Court


Tormentosa


Turpin


Van As


Vryheid


X 28


Zastron


Algerian


American Giant


Amersfoort


Arbiter


Berg X Mexican


Blue Motto


Corfu


Cross X


Direkteur


Ficus Indice


Fresno


Fusicaulis


Gymno Carpo


Malta


Messina


Mexican


Meyers


Morado


Muscatel


Nepgen


Nudosa


Ofer


R1251

R1259

R1260

Roedtan

Roly Poly

PHOTOS: J.P. POTGIETER

APPENDIX V: CLADODE SHAPE


Round


Diamond


Elliptic


Ovate

PHOTOS: J.P. POTGIETER

APPENDIX VI : VEGETATIVE TRAITS SEASON 1

CLADODES REMOVED WITH PRUNING (cladno)

Variety	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Average
Skinners Court	19	16	20	35	19	24	24	17	28	27	22.90
Nudosa	43	60	72	53	44	32	36	46	37	47	47.00
Gymno Carpo	50	38	61	66	113	45	35	44	48	66	56.60
Morado	145	84	33	121	77	34	53	58	64	56	72.50
Zastron	100	68	75	101	90	56	49	36	32	49	65.60
Malta	64	72	51	40	129	29	30	42	46	27	53.00
Algerian	97	111	47	67	68	43	39	37	35	19	56.30
Turpin	111	124	115	104	168	69	72	99	136	78	107.60
Meyers	122	91	131	68	129	69	90	79	89	51	91.90
Roedtan	34	104	70	70	93	58	43	74	77	97	72.00
Tormentosa	40	34	36	20	24	12	17	18	16	16	23.30
X28	42	50	39	19	37	10	18	12	20	25	27.20
Ficus-indice	20	24	23	36	12	5	12	20	8	26	18.60
Nepgen	39	24	23	27	31	28	18	17	11	21	23.90
Sicilian Indian Fig	102	3	78	40	81	55	42	39	54	75	56.90
Seasonal Mean											**53.02**

CLADODES REMAINING AFTER PRUNING (cladleft)

Variety	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Average
Skinners Court	36	28	24	39	23	33	46	40	39	23	33.10
Nudosa	72	81	75	69	71	68	58	59	84	85	72.20
Gymno Carpo	90	86	96	93	117	87	71	74	79	80	87.30
Morado	115	101	48	99	81	71	88	64	89	85	84.10
Zastron	108	72	79	82	90	67	74	89	70	72	80.30
Malta	103	94	89	89	110	77	61	87	78	57	84.50
Algerian	111	106	93	76	92	88	84	72	66	46	83.40

Variety	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Average
Turpin	113	49	112	97	132	81	78	66	75	80	88.30
Meyers	107	101	93	81	89	84	77	85	86	53	85.60
Roedtan	66	104	87	86	75	69	66	67	90	92	80.20
Tormentosa	28	35	28	40	28	39	33	38	41	43	35.30
X28	40	49	52	42	48	35	37	37	46	47	43.30
Ficus-indice	26	34	31	28	25	14	32	27	20	34	27.10
Nepgen	26	19	42	46	34	27	26	49	34	52	35.50
Sicilian Indian Fig	47	4	58	50	52	56	46	54	61	74	50.20
Seasonal Mean											64.69
CLADODE MASS (cmass)											
Variety	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**Plant 6**	**Plant 7**	**Plant 8**	**Plant 9**	**Plant 10**	**Average**
Skinners Court	2.74	2.58	3.03	2.37	3.34	2.48	2.89	2.88	2.53	3.13	2.80
Nudosa	1.13	1.37	1.27	1.28	1.24	1.36	1.51	1.53	1.51	1.43	1.36
Gymno Carpo	1.16	1.06	1.02	0.85	0.79	1.23	1.32	1.32	1.40	1.48	1.16
Morado	0.86	0.88	1.53	0.97	1.06	1.42	1.22	1.31	1.15	1.40	1.18
Zastron	0.87	0.93	0.85	1.04	0.84	1.33	1.43	1.45	1.32	1.62	1.17
Malta	0.76	0.88	0.81	0.96	0.70	1.29	1.45	1.39	1.24	1.50	1.10
Algerian	0.96	0.88	1.06	0.95	1.05	1.37	1.65	1.68	1.49	1.19	1.23
Turpin	0.84	0.77	0.75	0.71	0.77	1.45	1.50	1.13	1.21	1.37	1.05
Meyers	0.87	0.84	0.98	0.94	0.94	1.39	1.41	1.32	1.57	1.14	1.14
Roedtan	0.92	0.95	0.89	0.88	0.80	1.20	1.40	1.45	1.26	1.12	1.09
Tormentosa	1.19	1.30	1.02	1.01	1.25	1.83	1.93	1.54	1.69	2.10	1.49
X28	1.28	0.98	1.21	1.40	1.35	1.36	1.52	1.55	1.28	1.56	1.35
Ficus-indice	1.09	1.06	1.01	0.93	0.93	1.08	1.81	1.92	1.16	1.64	1.26
Nepgen	1.05	1.14	0.87	0.82	0.96	1.18	1.51	1.28	1.27	1.28	1.14
Sicilian Indian Fig	1.04	0.45	1.16	1.15	0.84	1.50	1.30	1.65	1.67	1.76	1.25
Seasonal Mean											**1.32**

CLADODE YIELD (cyieldp)											
Variety	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**Plant 6**	**Plant 7**	**Plant 8**	**Plant 9**	**Plant 10**	**Average**
Skinners Court	52.00	41.25	60.60	82.90	63.40	59.60	69.35	48.95	70.95	84.60	63.36
Nudosa	48.75	82.10	91.60	68.10	54.40	43.40	54.35	70.15	55.85	67.40	63.61
Gymno Carpo	58.15	40.15	62.52	55.80	89.40	55.35	46.10	57.95	67.25	97.55	63.02
Morado	124.45	73.90	50.50	117.20	81.80	48.35	64.50	76.15	73.40	78.15	78.84
Zastron	86.55	62.90	63.40	105.30	75.45	74.45	69.90	52.30	42.35	79.20	71.18
Malta	48.40	63.35	41.50	38.35	90.80	37.50	43.50	58.40	57.15	40.50	51.95
Algerian	92.80	97.60	49.60	63.60	71.10	59.05	64.20	62.15	52.00	22.65	63.48
Turpin	93.30	95.75	85.90	74.35	129.50	100.10	108.25	111.65	164.65	106.60	107.01
Meyers	106.15	76.50	127.90	63.90	121.10	95.90	127.05	104.15	139.75	58.10	102.05
Roedtan	31.40	98.60	62.30	61.85	74.25	69.75	60.15	107.00	97.15	108.45	77.09
Tormentosa	47.75	44.35	36.75	20.20	30.10	21.90	32.75	27.65	27.00	33.65	32.21
X28	53.70	48.95	47.20	26.55	49.85	13.55	27.40	18.60	25.65	39.00	35.05
Ficus-indice	21.80	25.45	23.25	33.40	11.10	5.40	21.70	38.40	9.30	42.55	23.24
Nepgen	40.85	27.40	19.90	22.15	29.90	33.05	27.25	21.75	14.00	26.80	26.31
Sicilian Indian Fig	106.15	1.35	90.80	45.95	67.90	82.65	54.40	64.20	90.20	132.15	73.58
Seasonal Mean											**62.13**

APPENDIX VI : VEGETATIVE TRAITS SEASON 2

CLADODES REMOVED WITH PRUNING (cladno)

Variety	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Average
Skinners Court	19	16	20	35	19	24	24	17	28	27	22.90
Nudosa	23	40	35	24	20	30	37	42	56	35	34.20
Gymno Carpo	32	30	51	42	37	36	27	28	37	38	35.80
Morado	52	47	24	30	21	13	38	20	50	64	35.90
Zastron	43	26	40	47	35	50	66	65	57	78	50.70
Malta	43	48	43	37	45	34	35	45	36	40	40.60
Algerian	37	31	32	48	24	31	32	35	20	14	30.40
Turpin	58	15	45	28	77	38	48	51	62	33	45.50
Meyers	42	34	70	49	32	31	39	41	36	20	39.40
Roedtan	20	42	27	53	54	27	33	33	39	56	38.40
Ofer	49	30	32	37	41	34	35	33	42	37	37.00
Tormentosa	31	34	28	27	27	22	24	21	36	28	27.80
X28	31	30	32	16	33	14	32	24	25	25	26.20
Ficus-indice	24	33	36	4	23	9	53	41	11	41	27.50
Nepgen	29	27	51	42	29	32	26	40	52	39	36.70
Sicilian Indian Fig	83	2	81	55	45	59	26	52	54	96	55.30
R1259	42	60	14	30	26	31	36	29	41	31	34.00
R1251	30	65	14	17	30	30	5	43	22	14	27.00
Van As	21	29	5	5	14	16	1	4	30	38	16.30
Cross X	16	21	28	46	10	16	21	15	22	25	22.00
BergxMexican	38	25	33	21	18	30	31	40	34	54	32.40
Santa Rosa	28	35	22	32	42	39	20	40	29	36	32.30
Schagen	29	27	42	15	29	41	38	27	24	27	29.90
Seasonal Mean											**33.83**

CLADODES REMAINING AFTER PRUNING (cladleft)											
Variety	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**Plant 6**	**Plant 7**	**Plant 8**	**Plant 9**	**Plant 10**	**Average**
Skinners Court	36	42	51	50	44	45	40	23	54	47	43.20
Nudosa	91	84	71	78	76	85	69	80	116	98	84.80
Gymno Carpo	104	86	103	92	114	95	78	91	95	90	94.80
Morado	118	110	45	93	85	87	81	73	89	105	88.60
Zastron	92	73	82	71	95	95	79	100	77	101	86.50
Malta	98	87	90	74	97	84	67	84	82	65	82.80
Algerian	89	117	95	99	95	101	103	91	86	61	93.70
Turpin	123	70	114	103	132	80	71	80	99	84	95.60
Meyers	110	98	90	98	95	93	80	84	83	55	88.60
Roedtan	77	116	108	103	74	90	87	76	97	89	91.70
Ofer	89	75	55	67	77	66	72	81	58	66	70.60
Tormentosa	47	56	40	38	47	32	46	38	50	42	43.60
X28	42	45	52	49	65	40	34	39	48	50	46.40
Ficus-indice	37	34	38	26	24	20	35	39	24	40	31.70
Nepgen	42	36	49	77	57	33	27	58	35	58	47.20
Sicilian Indian Fig	64	7	78	54	55	70	53	60	74	87	60.20
R1259	48	49	23	25	32	24	25	27	33	30	31.60
R1251	27	52	16	23	31	21	22	33	20	11	25.60
Van As	22	23	16	12	21	16	13	18	24	17	18.20
Cross X	45	38	37	49	24	29	39	28	25	26	34.00
BergxMexican	39	43	31	44	22	35	34	26	19	54	34.70
Santa Rosa	42	31	40	39	43	40	33	38	29	30	36.50
Schagen	35	25	34	33	37	31	28	31	17	29	30.00
Seasonal Mean											**59.16**

CLADODE MASS (cmass)											
Variety	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**Plant 6**	**Plant 7**	**Plant 8**	**Plant 9**	**Plant 10**	**Average**
Skinners Court	2.74	2.58	3.03	2.37	2.52	2.48	2.89	2.88	2.53	3.13	2.71
Nudosa	1.93	1.72	1.70	1.71	2.17	2.09	1.85	1.85	1.62	1.92	1.86
Gymno Carpo	1.49	1.45	1.36	1.35	1.29	1.68	1.80	1.42	1.36	1.74	1.49
Morado	1.54	1.29	2.32	1.62	1.60	1.74	1.62	1.83	1.70	1.56	1.68
Zastron	1.47	1.52	1.47	1.56	1.37	1.45	1.55	1.46	1.44	1.22	1.45
Malta	1.16	1.40	1.38	1.53	1.50	1.67	1.82	1.70	1.66	1.54	1.54
Algerian	1.94	1.58	1.46	1.33	1.57	1.63	1.62	1.45	1.64	1.56	1.58
Turpin	1.44	1.08	1.22	1.29	1.34	1.70	2.12	1.77	1.56	1.84	1.54
Meyers	1.77	1.74	1.46	1.36	1.77	1.79	1.88	1.84	2.03	1.84	1.75
Roedtan	1.21	1.32	1.26	1.15	1.39	1.55	1.74	1.65	1.71	1.56	1.45
Ofer	1.57	1.66	1.54	1.40	1.69	1.42	1.36	1.98	1.22	1.90	1.57
Tormentosa	1.89	1.66	2.10	1.75	1.71	2.07	2.29	2.16	2.06	2.22	1.99
X28	1.96	1.94	1.98	1.94	1.92	2.29	2.33	2.05	1.82	2.33	2.06
Ficus-indice	1.65	1.95	1.72	1.61	1.75	1.61	2.28	2.33	2.06	2.14	1.91
Nepgen	1.42	1.44	0.85	1.15	1.15	1.92	2.72	1.73	1.67	2.03	1.61
Sicilian Indian Fig	1.73	1.58	1.53	1.73	1.81	1.92	2.14	2.13	2.10	2.21	1.89
R1259	2.01	2.13	1.73	1.65	1.96	1.64	1.99	1.90	1.90	1.89	1.88
R1251	1.94	2.14	1.13	1.52	1.62	1.89	0.24	1.90	1.55	1.38	1.53
Van As	1.65	1.52	1.13	1.06	0.75	1.37	1.40	1.29	1.93	1.62	1.37
Cross X	1.99	2.20	2.04	2.44	1.95	1.97	1.75	2.01	1.72	1.66	1.97
BergxMexican	1.87	2.16	1.69	1.82	1.83	2.09	1.78	1.60	1.38	1.49	1.77
Santa Rosa	1.98	1.78	1.96	1.88	2.14	1.55	1.88	2.18	1.56	1.82	1.87
Schagen	2.08	2.26	2.11	2.29	2.17	1.65	2.21	2.42	2.00	1.91	2.11
Seasonal Mean											**1.76**

CLADODE YIELD (cyieldp)											
Variety	**Plant 1**	**Plant 2**	**Plant 3**	**Plant 4**	**Plant 5**	**Plant 6**	**Plant 7**	**Plant 8**	**Plant 9**	**Plant 10**	**Average**
Skinners Court	52.00	41.25	60.60	82.90	47.80	59.60	69.35	48.95	70.95	84.60	61.80
Nudosa	44.30	68.80	59.65	41.05	43.40	62.80	68.30	77.55	90.90	67.20	62.40
Gymno Carpo	47.55	43.55	69.60	56.75	47.55	60.45	48.65	39.65	50.30	66.00	53.01
Morado	80.15	60.55	55.65	48.45	33.50	22.65	61.60	36.50	84.85	100.05	58.40
Zastron	63.05	39.60	58.95	73.10	47.80	72.45	102.10	95.15	81.95	95.50	72.97
Malta	49.90	67.30	59.45	56.75	67.70	56.65	63.60	76.60	59.90	61.75	61.96
Algerian	71.65	48.90	46.80	64.05	37.60	50.40	51.70	50.60	32.80	21.85	47.64
Turpin	83.60	16.15	54.95	36.05	102.95	64.70	101.70	90.20	96.70	60.70	70.77
Meyers	74.20	59.10	102.05	66.50	56.75	55.60	73.45	75.25	73.25	36.80	67.30
Roedtan	24.20	55.45	34.00	60.90	75.15	41.80	57.30	54.55	66.60	87.60	55.76
Ofer	77.10	49.85	49.40	51.80	69.40	48.35	47.65	65.35	51.05	70.20	58.02
Tormentosa	58.55	56.45	58.70	47.30	46.25	45.55	55.00	45.45	74.20	62.15	54.96
X28	60.85	58.15	63.45	31.00	63.45	32.05	74.70	49.20	45.55	58.20	53.66
Ficus-indice	39.55	64.30	61.90	6.45	40.25	14.50	120.85	95.40	22.65	87.70	55.36
Nepgen	41.15	38.75	43.45	48.50	33.25	61.45	70.60	69.25	87.05	79.30	57.28
Sicilian Indian Fig	143.25	3.15	123.85	95.20	81.60	113.00	55.60	110.55	113.20	211.90	105.13
R1259	84.35	127.55	24.25	49.50	50.95	50.90	71.70	55.15	78.10	58.60	65.11
R1251	58.10	139.40	15.80	25.90	48.55	56.70	1.20	81.70	34.10	19.25	48.07
Van As	34.65	44.15	5.65	5.30	10.50	21.85	1.40	5.15	57.85	61.60	24.81
Cross X	31.90	46.15	57.15	112.20	19.50	31.45	36.85	30.15	37.80	41.45	44.46
BergxMexican	70.90	54.05	55.80	38.15	32.85	62.75	55.30	63.80	46.80	80.55	56.10
Santa Rosa	55.45	62.25	43.05	60.00	89.95	60.55	37.55	87.25	45.25	65.45	60.68
Schagen	60.30	60.95	88.75	34.35	62.80	67.65	84.05	65.40	47.90	51.60	62.38
Seasonal Mean											**59.04**

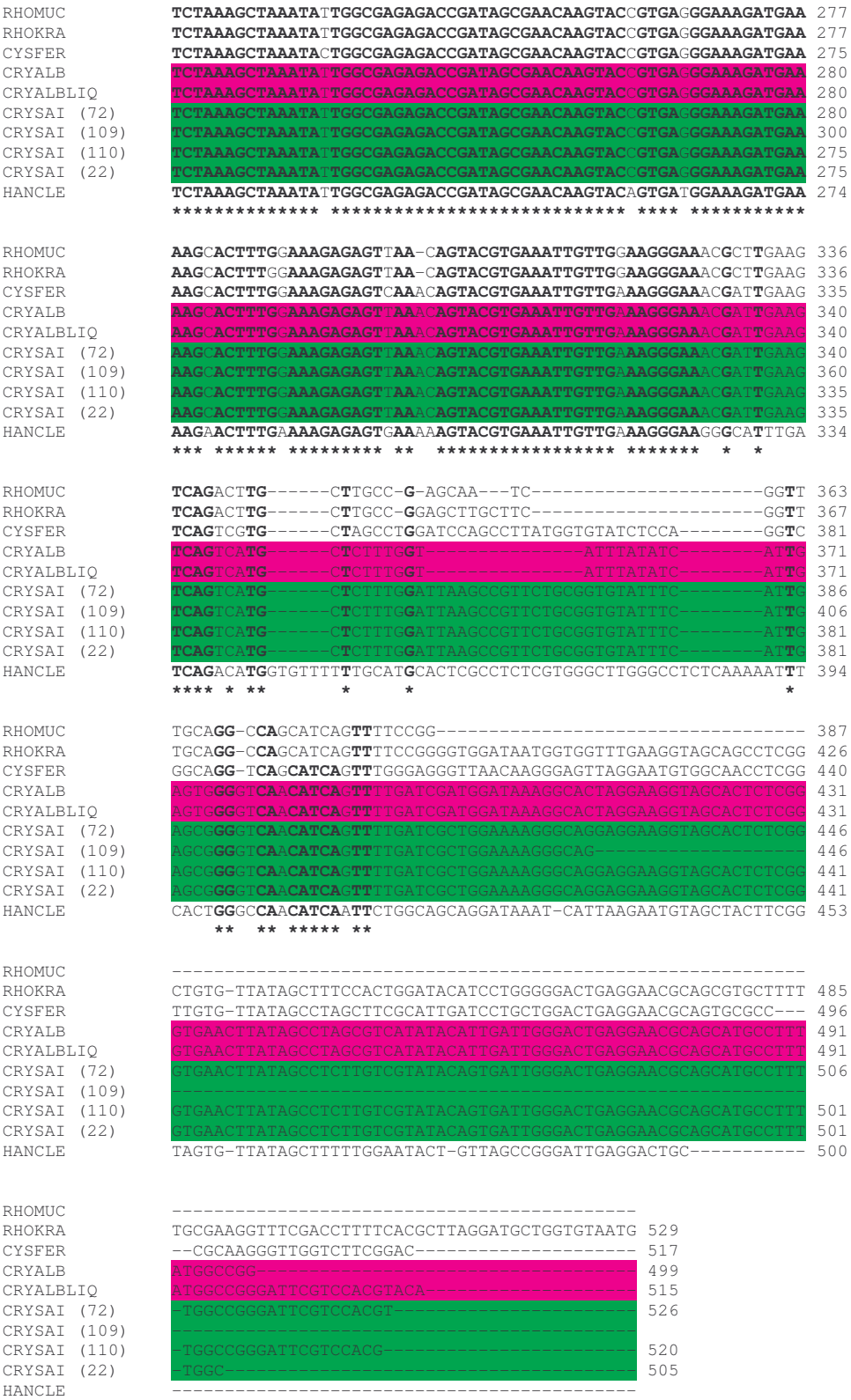
APPENDIX VII: ALIGNMENT OF D1/D2 SEQUENCE DATA OF ALL YEAST ISOLATES WITH POSSIBLE BIOCONTROL APPLICATION AGAINST CACTUS PEAR PATHOGENS

```
RHOMUC          --------------------GAAGCGGGAAGAGCTCAAATTTATAATCTGGCA-CCTTCG 39
RHOKRA          --------------------GAAGCGGGAAGAGCTCAAATTTATAATCTGGCA-CCTTCG 39
CYSFER          ------------------------GGGAAAAGCTCAAATTTAAAATCTGGCAGTCTACG 35
CRYALB          --------------------GAAGCGGGAAGAGCTCAAATTTGAAATCTGGTAGCCTTCG 40
CRYALBLIQ       --------------------GAAGCGGGAAGAGCTCAAATTTGAAATCTGGTAGCCTTCG 40
CRYSAI (72)     --------------------GAAGCGGGAAGAGCTCAAATTTGAAATCTGGTAGCCTTCG 40
CRYSAI (109)    TTCCCCTAGTAACGGCGAGTGAAGCGGGAAGAGCTCAAATTTGAAATCTGGTAGCCTTCG 60
CRYSAI (110)    ------------------------GGGAAGAGCTCAAATTTGAAATCTGGTAGCCTTCG 35
CRYSAI (22)     ------------------------GGGAAGAGCTCAAATTTGAAATCTGGTAGCCTTCG 35
HANCLE          --------------------AAGCGGTAAAAGCTCAAATTTGAAATCTGGTA-CTTTCA 38
                                       ** ** ***********  ******* *   * *

RHOMUC            GT-GTCCGAGTTGTAATCTCTAGAAATGTTTTCCGCGTTGGACCGCACACAAGTCTGTTG 98
RHOKRA            GT-GTCCGAGTTGTAATCTCTAGAAGTGTTTTCCGCGTTGGACCGCACACAAGTCTGTTG 98
CYSFER            ATTGTCCGAATTGTAATCTCGAGAAGTGTTTTCCGCGTTGGCCTGTGCACAAGTCCCTTG 95
CRYALB            GTTGCCCGAGTTGTAATCTAGAGAAGTGTTTTCCGTGCCGGCCCATGTACAAGTCCCTTG 100
CRYALBLIQ         GTTGCCCGAGTTGTAATCTAGAGAAGTGTTTTCCGTGCCGGCCCATGTACAAGTCCCTTG 100
CRYSAI (72)       GTTGCCCGAGTTGTAATCTAGAGAAGTGTTTTCCGCGTTGGCCCATGTACAAGTCCCTTG 100
CRYSAI (109)      GTTGCCCGAGTTGTAATCTAGAGAAGTGTTTTCCGCGTTGGCCCATGTACAAGTCCCTTG 120
CRYSAI (110)      GTTGCCCGAGTTGTAATCTAGAGAAGTGTTTTCCGCGTTGGCCCATGTACAAGTCCCTTG 95
CRYSAI (22)       GTTGCCCGAGTTGTAATCTAGAGAAGTGTTTTCCGCGTTGGCCCATGTACAAGTCCCTTG 95
HANCLE            GT-GCCCGAGTTGTAATTTGTAGAATTTGTCTTTGATTAGGTCCTTGTCTATGTTCCTTG 97
                   * * **** ******* *  **** *  * *  *    ** *       * **   ***

RHOMUC            GAATACAGCGGCATAGTGG-TGAGACCCCCGTATATGGTGCGGACGCCCAGCGCTTTGTG 157
RHOKRA            GAATACAGCGGCACAGTGG-TGATACCCCCGTACACGGTGCGGACGCCCAGCGCTTTGTG 157
CYSFER            GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGGCACAGACACCCAATGCTTTGTG 155
CRYALB            GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGACATGGACCCCGGTGCTTTGTG 160
CRYALBLIQ         GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGACATGGACCCCGGTGCTCTGTG 160
CRYSAI (72)       GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGACATGGACCCCAATGCTTTGTG 160
CRYSAI (109)      GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGACATGGACCCCAATGCTTTGTG 180
CRYSAI (110)      GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGACATGGACCCCAATGCTTTGTG 155
CRYSAI (22)       GAACAGGGCGTCATAGAGGGTGAGAATCCCGTCCTTGACATGGACCCCAATGCTTTGTG 155
HANCLE            GAACAGGACGTCATAGAGGGTGAGAATCCCGT--TTGGCGAGGATACCTTTTT-CTCTGTA 154
                  *** *   ** ** ** ** *** *  *****    *     **  **     ** ***

RHOMUC            ATACATTTTCGAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAATTGGGTGGTAAATTCCA 217
RHOKRA            ATACACTTTCAATGAGTCGAGTTGTTTGGGAATGCAGCTCAAATTGGGTGGTAAATTCCA 217
CYSFER            ATACACTCTCAATGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGAGGTAAATTCCT 215
CRYALB            ATACACTTTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCA 220
CRYALBLIQ         ATACACTTTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCA 220
CRYSAI (72)       ATACACTTTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCA 220
CRYSAI (109)      ATACACTTTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCA 240
CRYSAI (110)      ATACACTTTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCA 215
CRYSAI (22)       ATACACTTTCAACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCA 215
HANCLE            AGACTTTTTCGAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAGTGGGTGGTAAATTCCA 214
                  * **  * ** * ***************************** **** *** ******
```

RHOMUC = *Rhodotorula mucilaginosa*
RHOKRA = *Rhodosporidium kratochvilovae*
CYSFER = *Cystofilobasidium ferigula*
CRYALB = *Cryptococcus albidosimilis*
CRYALBLIQ = *Cryptococcus albidosimilis / liquefaciens*
CRYSAI (72) = *Cryptococcus saitoi* (isolate number 72)
CRYSAI (109) = *Cryptococcus saitoi* (isolate number 109)
CRYSAI (22) = *Cryptococcus saitoi* (isolate number 22)
HANCLE = *Hanseniaspora clermontiae*

* = all the nucleotides in that column are identical in all the sequences in the alignment
\- = signifies a gap in the nucleotide sequence

Sequences highlighted in green have been identified as *Cryptococcus saitoi*, those highlighted in pink have been identified as *C. albidodimilis or liquefaciens*.


RHOMUC TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 277
RHOKRA TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 277
CYSFER TCTAAAGCTAAATACTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 275
CRYALB TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 280
CRYALBLIQ TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 280
CRYSAI (72) TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 280
CRYSAI (109) TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 300
CRYSAI (110) TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 275
CRYSAI (22) TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA 275
HANCLE TCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAA 274
********** ********** **** **********
RHOMUC AAGCACTTTGGAAAGAGAGTTAA-CAGTACGTGAAATTGTTGGAAGGGAAACGCTTGAAG 336
RHOKRA AAGCACTTTGGAAAGAGAGTTAA-CAGTACGTGAAATTGTTGGAAGGGAAACGCTTGAAG 336
CYSFER AAGCACTTTGGAAAGAGAGTCAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 335
CRYALB AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 340
CRYALBLIQ AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 340
CRYSAI (72) AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 340
CRYSAI (109) AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 360
CRYSAI (110) AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 335
CRYSAI (22) AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGGGAAACGATTGAAG 335
HANCLE AAGAACTTTGAAAAGAGAGTGAAAAAGTACGTGAAATTGTTGAAAGGGAAGGGCATTTGA 334
*** ****** ********* ** ********** ******* * *
RHOMUC TCAGACTTG------CTTGCC-G-AGCAA---TC----------GGTT 363
RHOKRA TCAGACTTG------CTTGCC-GGAGCTTGCTTC----------GGTT 367
CYSFER TCAGTCGTG------CTAGCCTGGATCCAGCCTTATGGTGTATCTCCA--------GGTC 381
CRYALB TCAGTCATG------CTCTTTGGT---------ATTTATATC--------ATTG 371
CRYALBLIQ TCAGTCATG------CTCTTTGGT---------ATTTATATC--------ATTG 371
CRYSAI (72) TCAGTCATG------CTCTTTGGATTAAGCCGTTCTGCGGTGTATTTC--------ATTG 386
CRYSAI (109) TCAGTCATG------CTCTTTGGATTAAGCCGTTCTGCGGTGTATTTC--------ATTG 406
CRYSAI (110) TCAGTCATG------CTCTTTGGATTAAGCCGTTCTGCGGTGTATTTC--------ATTG 381
CRYSAI (22) TCAGTCATG------CTCTTTGGATTAAGCCGTTCTGCGGTGTATTTC--------ATTG 381
HANCLE TCAGACATGGTGTTTTTTGCATGCACTCGCCTCTCGTGGGCTTGGGCCTCTCAAAAATTT 394
**** * ** * * *
RHOMUC TGCAGG-CCAGCATCAGTTTTCCGG---------- 387
RHOKRA TGCAGG-CCAGCATCAGTTTTCCGGGGTGGATAATGGTGGTTTGAAGGTAGCAGCCTCGG 426
CYSFER GGCAGG-TCAGCATCAGTTTGGGAGGGTTAACAAGGGAGTTAGGAATGTGGCAACCTCGG 440
CRYALB AGTGGGGTCAACATCAGTTTTGATCGATGGATAAAGGCACTAGGAAGGTAGCACTCTCGG 431
CRYALBLIQ AGTGGGGTCAACATCAGTTTTGATCGATGGATAAAGGCACTAGGAAGGTAGCACTCTCGG 431
CRYSAI (72) AGCGGGGTCAACATCAGTTTTGATCGCTGGAAAAGGGCAGGAGGAAGGTAGCACTCTCGG 446
CRYSAI (109) AGCGGGGTCAACATCAGTTTTGATCGCTGGAAAAGGGCAG---------- 446
CRYSAI (110) AGCGGGGTCAACATCAGTTTTGATCGCTGGAAAAGGGCAGGAGGAAGGTAGCACTCTCGG 441
CRYSAI (22) AGCGGGGTCAACATCAGTTTTGATCGCTGGAAAAGGGCAGGAGGAAGGTAGCACTCTCGG 441
HANCLE CACTGGGCCAACATCAATTCTGGCAGCAGGATAAAT-CATTAAGAATGTAGCTACTTCGG 453
** ** ***** **
RHOMUC ----------
RHOKRA CTGTG-TTATAGCTTTCCACTGGATACATCCTGGGGGACTGAGGAACGCAGCGTGCTTTT 485
CYSFER TTGTG-TTATAGCCTAGCTTCGCATTGATCCTGCTGGACTGAGGAACGCAGTGCGCC--- 496
CRYALB GTGAACTTATAGCCTAGCGTCATATACATTGATTGGGACTGAGGAACGCAGCATGCCTTT 491
CRYALBLIQ GTGAACTTATAGCCTAGCGTCATATACATTGATTGGGACTGAGGAACGCAGCATGCCTTT 491
CRYSAI (72) GTGAACTTATAGCCTCTTGTCGTATACAGTGATTGGGACTGAGGAACGCAGCATGCCTTT 506
CRYSAI (109) ----------
CRYSAI (110) GTGAACTTATAGCCTCTTGTCGTATACAGTGATTGGGACTGAGGAACGCAGCATGCCTTT 501
CRYSAI (22) GTGAACTTATAGCCTCTTGTCGTATACAGTGATTGGGACTGAGGAACGCAGCATGCCTTT 501
HANCLE TAGTG-TTATAGCTTTTTGGAATACT-GTTAGCCGGGATTGAGGACTGC---------- 500
RHOMUC ----------
RHOKRA TGCGAAGGTTTCGACCTTTTCACGCTTAGGATGCTGGTGTAATG 529
CYSFER --CGCAAGGGTTGGTCTTCGGAC---------- 517
CRYALB ATGGCCGG---------- 499
CRYALBLIQ ATGGCCGGGATTCGTCCACGTACA---------- 515
CRYSAI (72) -TGGCCGGGATTCGTCCACGT---------- 526
CRYSAI (109) ----------
CRYSAI (110) -TGGCCGGGATTCGTCCACG---------- 520
CRYSAI (22) -TGGC---------- 505
HANCLE ----------