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DISCRIMINANT ANALYSIS OF MORPHOLOGICAL DESCRIPTORS TO DIFFERENTIATE THE *OPUNTIA* GENOTYPES

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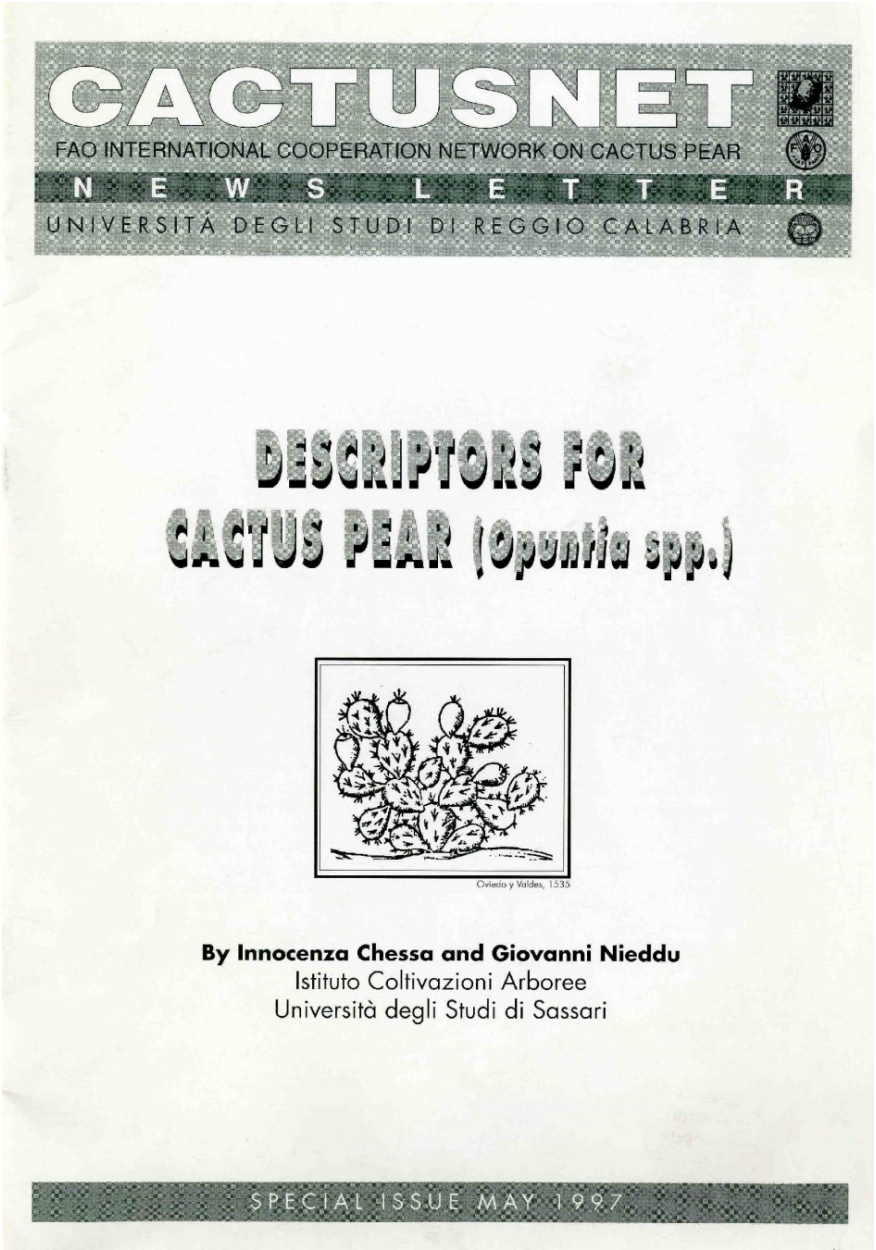
Research background

The usefulness of samples held in germplasm collections is dependent upon the degree and quality of information connected to the samples, collected using all available markers

In cactus pear the high phenotypic variability and plasticity makes difficult accurate identification, lowering the reliability of morphological markers for characterization

Morphological characterization is needed to provide users valuable information on individual accessions, relationship among traits, and the structure of collections

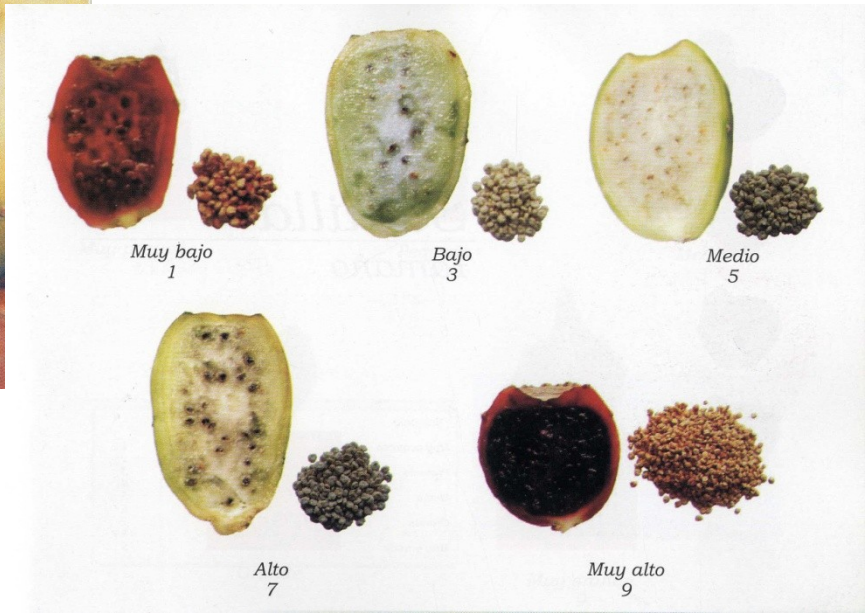
In 1997 the Cactusnet published a standard Descriptors List



	Descriptors (n)
Passport:	15
Management, Environment and Site	83
Characterization:	**63**
Plant	5
Cladode	22
Flower	8
Fruit	28
Evaluation: Plant, Abiotic stresses, Biotic stresses, Postharvest, Markers	37

Other descriptors catalogues have been proposed to facilitate collections utilization (Reyes-Agüero et al., 2005; Potgieter and Mashope, 2009)

	Descriptors (n)
Plant	3
Cladode	24
Flower	5
Fruit	29
Total	**61**



Aims

To develop a list of a minimum number of characters of highest priority based on statistical analyses, in order to provide greater standardization between collections and improved utilization of accessions.

Identify the characters of differential diagnostic value in *Opuntia* species, cultivated and wild accessions

Materials and methods

71 accessions of *Opuntia* spp. (3 plant per accessions)
38 descriptors of plant, cladode and fruits were selected from the Descriptor List for Cactus Pear (*Opuntia* spp.), and labelled

For each plant, an information file was recorded that included data referring to the collection location

Data were collected over three years (2004 - 2006)

Methods - Descriptors

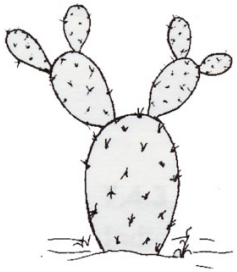
List of 38 descriptors

Plant descriptors

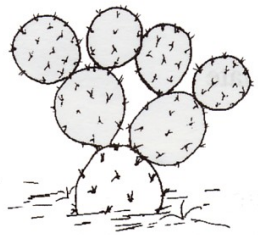
Vigour
Size
Shape
★ Habitus

Cladode descriptors

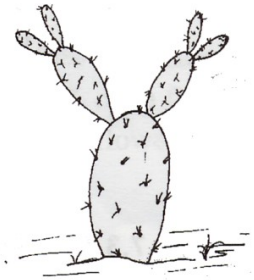
★ Cladodes shape
★ Cladodes colour
★ Spines
Spine shape
★ Glochides



Ovate



Round



Elliptic

★ Minimum highly discriminating descriptors

Methods - *Descriptors*

List of traits characters

Fruit descriptors

★ Shape
★ Size
★ Weight (g)
Volume (cc)
Length (mm)
Width (mm)
★ Fruits /cladode
★ Recepticular scar diameter (mm)
★ Recepticular scar diameter depth (mm)
★ Recepticular scar position
Peel thickness (mm)
★ Peel weight (g)
Peel medium weight (g)
Peel weight/ fruits weight
★ Peel colour

★ Pulp weight (g)
Fruit Pulp weight (g)
Seed number (n/100 g fruit)
Abortive seed (n/100 fruit)
★ Seed weight (g/100 fruit)
Seed weight (g)
★ Seed number (n/fruit)
Abortive seed (%)
★ Glochides on fruit peel
Pulp colour
Pulp firmness
Tritable acidity (%)
STS
pH

Methods - *Statistics Analyses*

For each of the **quantitative parameters** the means and standard deviations were calculate

A stepwise discriminant analysis (SDA) was carried out using the SPSS 17.0 program

For the **qualitative variables**, statistical comparison were done by means of the chi-square (χ^2) test, being the 5%, 1% and 0,01% the adopted significance levels.

Results

Quantitative descriptors

Opuntia spp mean values

	Weigth (g)	Volume (cc)	Length (mm)	Width (mm)	Recepticular scar diameter (mm)	Recepticular scar depth (mm)	Peel thickness (mm)	Peel medium weigth (g)
O. ficus indica	919,6	903,5	74,1	47,5	20,7	5,4	4,2	41,2
O. amyclaea	823,7	844,6	77,9	47,4	19,1	6,2	4,0	37,3
O. dillenii	172,0	190,0	35,0	25,0	19,0	10,0	7,0	0,0
O. rastrera	673,0	720,0	85,0	52,0	24,0	1,0	7,0	33,0
O. polyacantha	42,0	40,0	10,0	6,0	20,0	3,0	4,0	12,0
O. basilaris	65,0	70,0	22,0	17,0	17,0	8,0	7,0	10,0
G. L. (ni)	816,0	880,0	163,0	73,0	19,0	7,0	6,0	27,0
O. litoralis	88,0	80,0	22,0	17,0	20,0	2,0	5,0	10,0
O. soherensis	104,0	100,0	39,0	28,0	13,0	7,0	6,0	9,0
O. sulphurea	32,0	35,0	21,0	11,0	12,0	4,0	3,0	4,0
Senorbì	416,0	400,0	76,0	46,0	19,0	3,0	5,0	38,0
Total	*818,1*	*812,8*	*72,1*	*45,4*	*20,1*	*5,5*	*4,3*	*37,2*

Quantitative descriptors

Opuntia spp mean values

	Peel-fruits weigth ratio	Fruits pulp weigth (g)	Seed number (n/100 g fruit)	Abortive seed number (n/100 g fruits)	Seed weight (g/100 seeds)	Seeds weigth (g)	STS	Tritable acidity (%)	pH
O. ficus indica	40,4	64,4	358,2	198,1	30,9	36,4	13,57	0,050	6,19
O. amyclaea	38,5	58,2	308,8	167,1	22,4	23,1	13,71	0,044	6,25
O. dillenii	0,0	0,0	372,3	189,6	29,0	31,0	13,58	0,049	6,21
O. rastrera	63,0	19,0	1020,0	48,0	16,0	31,0	13,58	0,049	6,21
O. polyacantha	60,0	8,0	372,3	189,6	29,0	3,0	13,58	0,049	6,21
O. basilaris	94,0	1,0	372,3	189,6	29,0	0,0	13,58	0,049	6,21
G L (NI)	69,0	12,0	1467,0	294,0	44,0	31,0	13,58	0,049	6,21
O. litoralis	67,0	5,0	372,3	189,6	29,0	2,0	13,58	0,049	6,21
O. soherensis	86,0	1,0	372,3	189,6	29,0	1,0	13,58	0,049	6,21
O. sulphurea	67,0	2,0	372,3	189,6	29,0	2,0	13,58	0,049	6,21
Senorbì	46,0	74,0	196,0	131,0	29,0	31,0	12,00	0,051	6,29
Total	*42,7*	*56,7*	*372,3*	*189,6*	*29,0*	*31,0*	*13,58*	*0,049*	*6,21*

Stepwise discriminant analysis (SDA)

The SDA was applied using Wilk's step-wise procedure with a minimum tolerance of 0.001 and F to enter or remove 4*

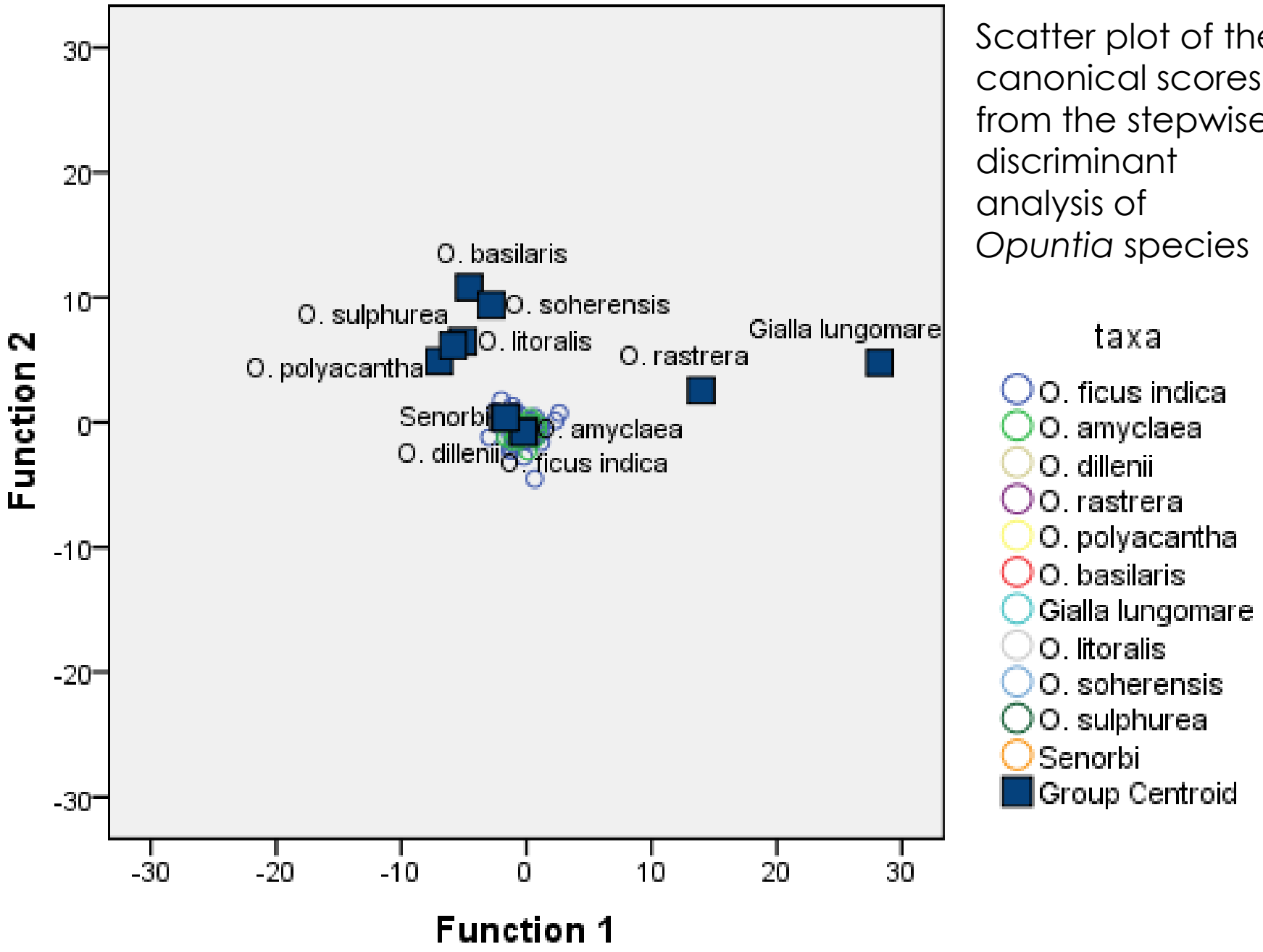
$$Yi = b_0 + b_1 X_{1i} + b_2 X_{2i} + e_i$$

The stepwise approach begins by choosing the single best discriminating variables. The initial variable is then paired with each of the other independent variables one at a time. The variables are best able to improve the discriminating power of the function in combination with the first variable chosen.

*indicating that a variable would be entered if the ratio between group variance to within group variance for that variable was >4

Stepwise model - *variables in the analysis*

Step		Tolerance	F to remove	Wilks' Lambda
1	**Seed number (n/100 g fruit)**	1,000	36,011	
2	Seed number (n/100 g fruit)	,844	36,615	,179
	Fruit length (mm)	,844	28,108	,143
3	Seed number (n/100 g fruit)	,839	31,245	,063
	Length (mm)	,844	24,707	,052
	Peel-fruits weigth ratio	,989	8,799	,025
4	Seed number (n/100 g fruit)	,839	19,538	,022
	Length (mm)	,516	16,212	,019
	Peel-fruits weigth ratio	,874	10,345	,014
	Peel medium weigth (g)	,520	5,762	,010
5	Seed number (n/100 g fruit)	,608	24,473	,014
	Length (mm)	,512	15,063	,009
	Peel-fruits weigth ratio	,559	16,146	,010
	Peel medium weigth (g)	,426	7,249	,006
	Abortive seed number (n/100 g fruits)	,473	5,159	,005
6	**Seed number (n/100 g fruit)**	,600	22,519	,007
	Fruit length (mm)	,487	15,582	,005
	Peel-fruits weight ratio	,554	15,962	,005
	Peel medium weight (g)	,408	7,683	,003
	Abortive seed number (n/100 g fruits)	,449	5,333	,003
	Peel thickness (mm)	,831	5,097	,003



Scatter plot of the canonical scores from the stepwise discriminant analysis of *Opuntia* species

Stepwise model - *variables in the analysis*
only *O. ficus indica* and *O. amyclaea*

variables in the analysis

Step		Tolerance	F to Remove	Wilks Lambda
1	**Recepticular scar diameter (mm)**	1,000	7,905	
2	Recepticular scar diameter (mm)	0,981	8,990	0,919
	Seed number (n/fruit)	0,981	6,240	0,881
3	**Recepticular scar diameter (mm)**	0,978	8,764	0,833
	Seed number (n/fruit)	0,803	10,751	0,858
	Pulp weight (g)	0,819	5,848	0,796

Lower variability within groups correspond to higher Wilks' λ values

Qualitative descriptors – *descriptive statistics*

	Mean	SD	Minimum	Maximum
Plant Size	4,18	1,522	3	7
Plant Shape	2,08	,277	2	3
Habitus	2,80	1,681	1	6
Plant Vigour	5,66	1,515	2	8
Cladodes Shape	2,11	1,002	1	3
Cladodes Colour	3,20	1,123	1	5
Spines	1,54	2,867	0	7
Spines' shape	,90	1,729	0	5
Glochides	2,25	1,700	0	5
Fruits number	9,25	1,748	3	10
Fruits shape	1,26	,728	1	4
Fruits size	1,95	,498	1	3
Recepticular scar position	2,80	,401	2	3
Seed number (n/fruit)	3,63	,876	0	5
Peel colour	6,67	2,561	2	11
Abortive seed (%)	5,00	,000	5	5
Glochides on fruit peels	6,95	,100	7	7
Pulp colour	5,64	2,530	0	10
Pulp firmness	2,52	,698	1	3

Qualitative descriptors

Results of the chi-square (χ^2) test

Frequencies

Two descriptors examples

Seed number (n/fruit)

	Observed N	Expected N	Residual
1 very few	2	12,2	-10,2
2 few	2	12,2	-10,2
3 medium	12	12,2	-,2
4 many	43	12,2	30,8
5 very many	2	12,2	-10,2
Total	61		

Fruits shape

	Observed N	Expected N	Residual
1 ovoid	52	15,3	36,8
2 round	5	15,3	-10,3
3 elliptic	1	15,3	-14,3
4 oblong	3	15,3	-12,3
Total	61		

Qualitative descriptors

Results of the chi-square (χ^2) test between *O. ficus indica* and *O. amyclaea*

	Plant Size	Habitus	Plant Vigour	Cladodes Shape	Cladodes Colour	Spines
Chi-Square	4,429	7,714	4,857	10,286**	8,857	7,143*
df	2	3	3	1	4	1
Asymp. Sig.	,109	,052	,183	,001	,065	,008

	Spines' shape	Glochides	Fruits number	Fruits size	Recepticular scar position
Chi-Square	4,429	,571	11,714**	9,571**	10,286**
df	2	2	3	2	1
Asymp. Sig.	,109	,751	,008	,008	,001

*p<0,05; **p<0,01; ***p<0,001

Qualitative descriptors

Results of the chi-square (χ^2) test between *O. ficus indica* and *O. amyclaea*

	Seed number (n/fruit)	Peel colour	Glochides on fruit peels	Pulp colour	Pulp firmness
Chi-Square	1,143	21,429***	1,143	16,286***	1,857
df	1	3	1	3	2
Asymp. Sig.	,285	,000	,285	,001	,395

*p<0,05; **p<0,01; ***p<0,001

Conclusions

3 quantitative fruit descriptors (Recepticular scar diameter, Seed number and Pulp weight) and

7 qualitative descriptors (cladodes shape, spines, fruits number, fruits size, recepticular scar position, peel and pulp colour) have been identified as differential parameters in *Opuntia ficus indica* and *O. amyclaea*.

Most of the differential traits are marked with a star (★) in the Descriptors List, as minimum discriminating descriptors

Conclusions

The most important variables that differentiate species were determined by discriminant analysis and the success efficiency of these techniques in accessions identification was observed.

Collections utilization is improved by the use of differential parameters, for quick and easy identification.

The method, including also descriptors of other type, such as molecular, biochemical and geographical, could be applied to develop a "core collection". The core subset in turn analysed could be used as a reference collection to evaluate newly acquired accessions for their similarity or novelty


Thank you for your
attention

Research background

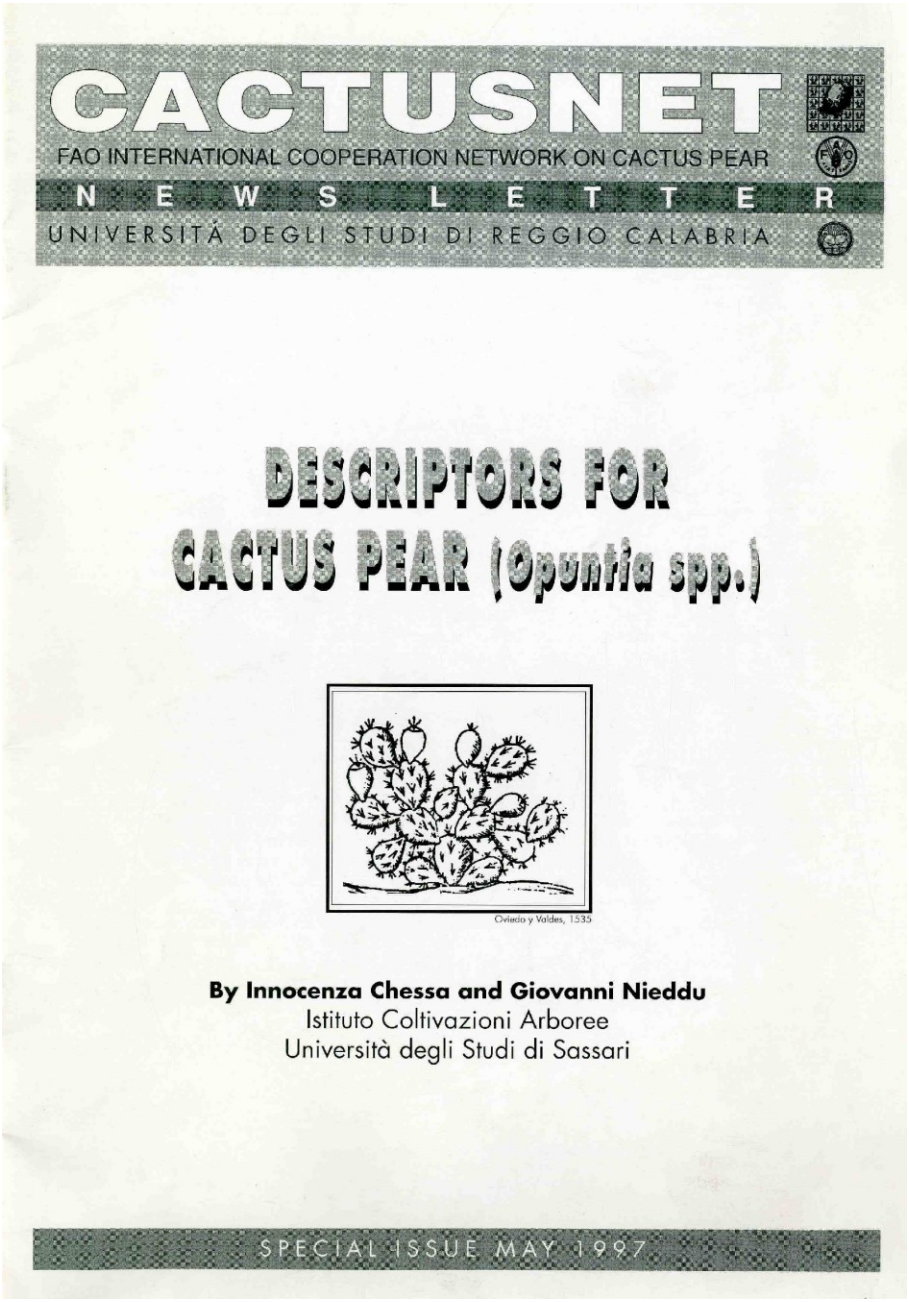
Identification of the cactus pear taxon is always difficult because of its phenotypic variability

To date no exhaustive studies addressing botanical and morphological description have been published

Aims

identify the characters of differential diagnostic value in *Opuntia* spp. and its cultivated genotypes

Efficient germplasm management requires species characterization and genetic diversity assessment using available markers



	Descriptors (n)	
Passport	15	
Collecting	22	
Management	18	98
Site	16	
Environment	27	
Characterization		
Plant	5	
Cladode	22	63
Flower	8	
Fruit	28	
Evaluation		
Plant	8	
Abiotic stresses	8	
Biotic stresses	9	37
Postharvest	4	
Markers	8	

Methods - *Statistics Analyses*

Discriminant analysis is a technique for finding functions so as to discriminate groups previously defined. It is therefore of considerable interest to those wishing to classify species, on each of which a number of measurements have been made and which are to be collected in previously defined groups.

Discriminant analysis were used to evaluate the data in order to clarify and contribute to Opuntia spp. genetic resources identification