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Polymorphic microsatellite DNA markers in *Opuntia* spp. collections

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Genetic diversity among *Opuntia* spp

Knowledge of genetic diversity and of relationship among species and varieties in the cactus pear is useful:

- in the documentation of genetic resources collections
- to clarify the taxonomy still questioned regarding the delineation of the various species within the *Opuntia* genus
- to acquire information about the ploidy level of *Opuntia* species not yet known

The codominant nature of SSRs have make them the marker of choice for DNA fingerprinting

Aims

Evaluate the degree of polymorphism in:

- a novel set of microsatellite loci isolated by the University of Sassari in different species and varieties of *Opuntia*
- microsatellite previously identified by Hensen *et al.* (2007) in the *O. echios* varieties (var. *echios* and var. *gigantea*)

Identify and characterize genetic diversity at species and intra-species level in *Opuntia*

Provide information on the current state of *Opuntia* genetic resource conservation in the germplasm collections, identify duplicates, synonymies and homonymies

Establish common protocols

Materials and methods

The genomic DNA was isolated from young cladodes of 29 *Opuntia* accessions from 2 collections

On field *ex situ* collections:

- Italy, hosted by the University of Sassari,
- Argentina, hosted by the UNSE

Analyzed genotypes and accessions

Collection IT

O. robusta
O. stricta
O. dillenii
O. lindheimeri
O. polyacantha
O. rastrera
O. soherensii
O. sulphurea

O. amyclaea (BB)
Nopalea cochenillifera
Gialla Lungomare (not identified)
O. ficus indica Achefri
O. ficus indica (BSC)
Rossa Armerina (not identified)
O. basilaris

Collection AR

O. megacantha
O. crassa verde
O. crassa rosa
O. ellisiana
O. sulphurea
O. streptacantha
O. matudae (xoconostle)

O. ficus indica 1294
O. ficus indica 1282
O. ficus indica 7
O. ficus indica verde
O. ficus indica 11
O. ficus indica Rojo
O. ficus indica 1281
O. ficus indica 1321

Materials and methods

Five SSRs from a novel set of 10 microsatellite loci were analyzed in this study and compared with 8 SSRs from those previously isolated by Hensen *et al*. 2007

Ten microsatellites were developed within a pool of *Opuntia* DNA samples (Erre et al., in press).

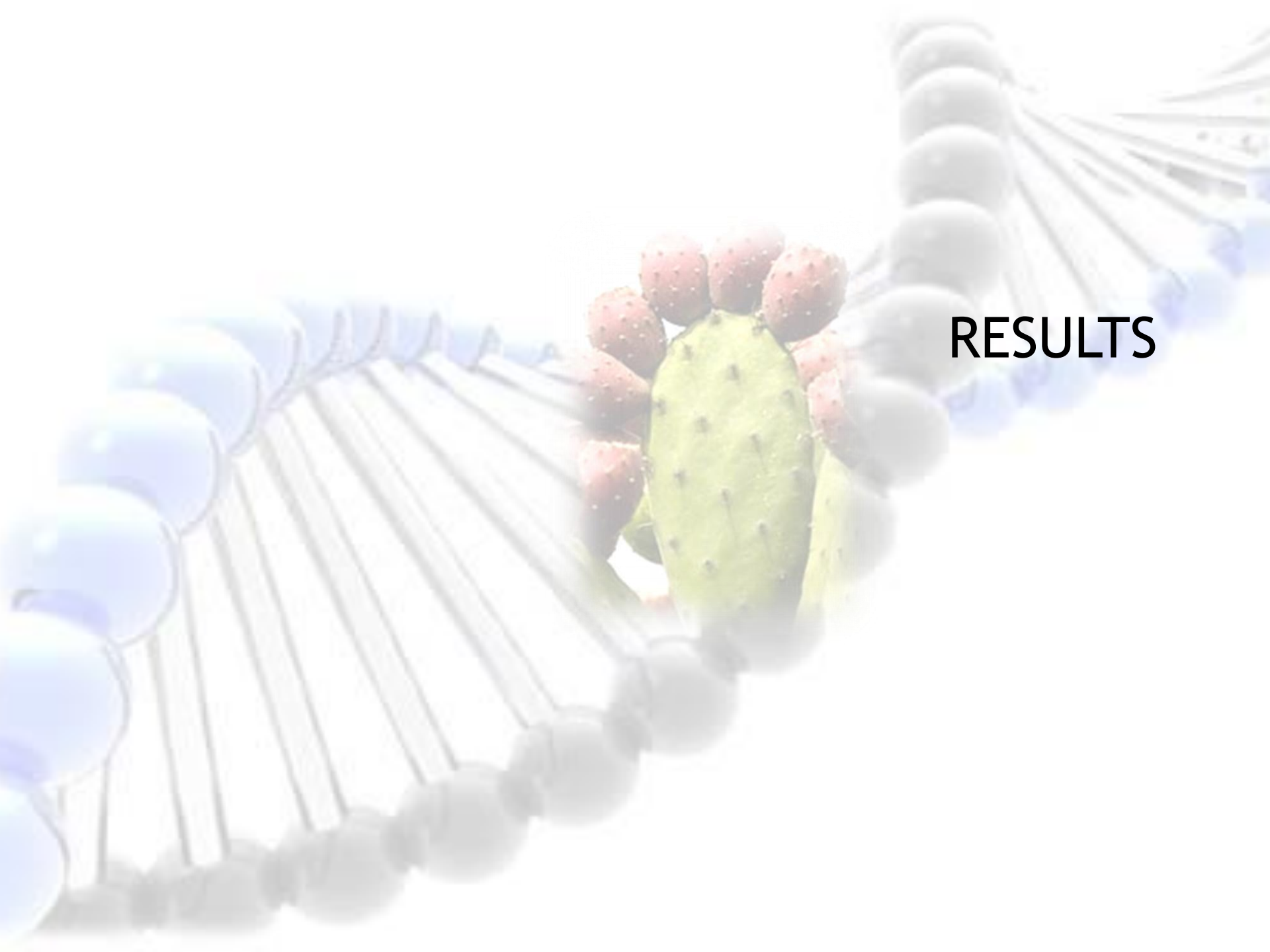
An enriched library was prepared from size selected genomic DNA ligated into SNX forward/SNX reverse-linker and enriched by magnetic bead selection with biotin-labelled $(CT)_{13}$ $(GT)_{13}$ $(AAC)_{10}$ and $(AAG)_{10}$ oligonucleotide repeats. Of the 528 recombinant colonies screened, 304 (225 GT/CT, 79 AAC/AAG) gave a positive signal after hybridization. Plasmids from 98 positive clones were sequenced and primers were designed for 16 microsatellite inserts, all of which were tested for polymorphism in the 26 samples.

Materials and methods

To analyze the genetic data, the Popgene32, Identity 1.0 and Ntsys 2.0 softwares were used

- *genetic diversity within species and cultivars population*: percentage of polymorphic loci (P%), observed mean number of alleles per locus (no), effective mean number of alleles per locus (ne), Shannon's information index (Is) and Nei's gene diversity (He)

- *Genetic distances between all pairwise combinations of the accessions:* a dendrogram was generated with the Simple Matching Coefficient (SM) and the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of the similarity using the SAHN-clustering and TREE programmes of the NTSYS-pc 2.02



RESULTS

Characteristics of polymorphic microsatellite loci

Locus		Repeat type	Size range (bp)	Alleles	Alleles per ind	PIC
Erre *et al.* 2010	Opufic01	$(CT)_{16}$	148-184	19	1-12	0,765
	Opufic03	$(TG)_{12}$	134-163	14	2-8	0,849
	Opufic04	$(TG)_{12}$	196-222	17	2-9	0,746
	Opufic13	$(TC)_{12}(AC)_{11}$	136-190	31	2-18	0,941
	Opufic14	$(CTT)_{7}...(CTT)_{10}$	143-282	43	3-21	0,849
Helsen *et al.* 2007	Opuntia2	$(AG)_{14}(CG)_{4}$	201-217	9	1-4	0,438
	Opuntia4	$(GA)_{12}$	98-124	14	1-3	0,656
	Opuntia8	$(CT)_{5}(TC)_{12}GC(TC)_{5}$	102-137	19	1-8	0,713
	Opuntia9	$(AG)_{15}$	146-173	27	1-7	0,803
	Opuntia10	$(CT)_{9}$	159-191	8	2-5	0,372
	Opuntia12	$(TC)_{4}C(TC)_{12}$	226-272	21	1-12	0,696
	Opuntia13	$(AG)_{12}$	238-262	19	1-8	0,742
	Opuntia21	$(TC)_{14}$	93-166	28	1-11	0,814

Erre P., Nieddu G. and I. Chessa, 2010. Identification of microsatellite loci in *Opuntia* spp and their characterization in cultivars and species. Acta Hort (*in press*)
Helsen P., Verdyck P., Tye A. *et al.*, 2007. Isolation and characterization of polymorphic microsatellite markers in Galapagos prickly pear (*Opuntia*) cactus species. Mol Ecol Notes 7 :454-456

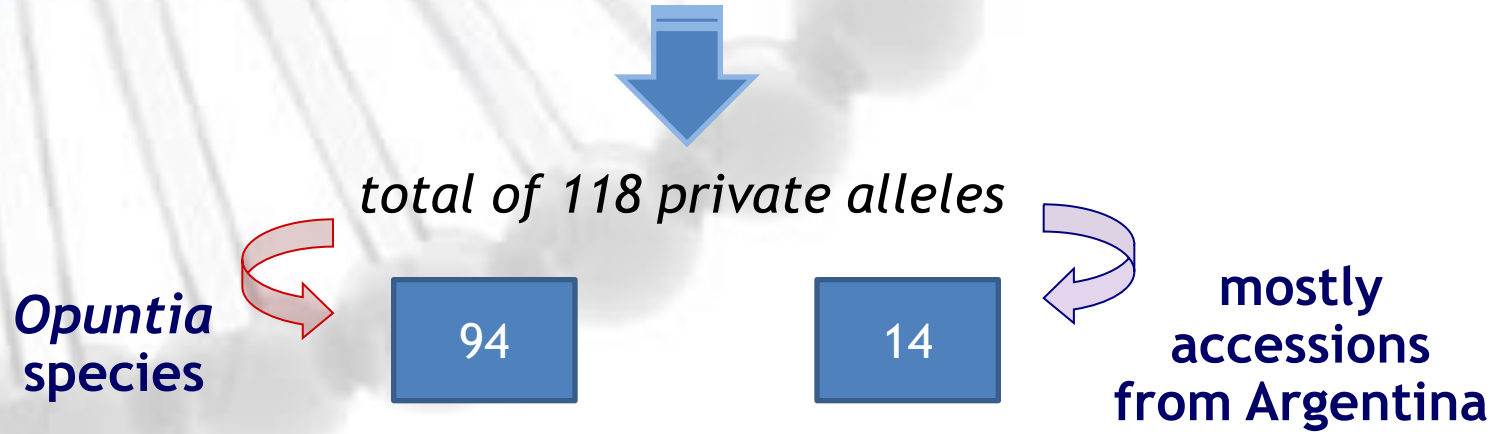
Measures of genetic diversity within groups of *Opuntia* spp.

Groups	*P%*	*no*	*ne*	*Is*	*H*
Cultivars	65,220	1,652	1,321	0,294	0,192
Species	93,480	1,930	1,251	0,302	0,178
Mean	79,350	1,791	1,286	0,298	0,185
S.D.		0,197	0,049	0,006	0,010

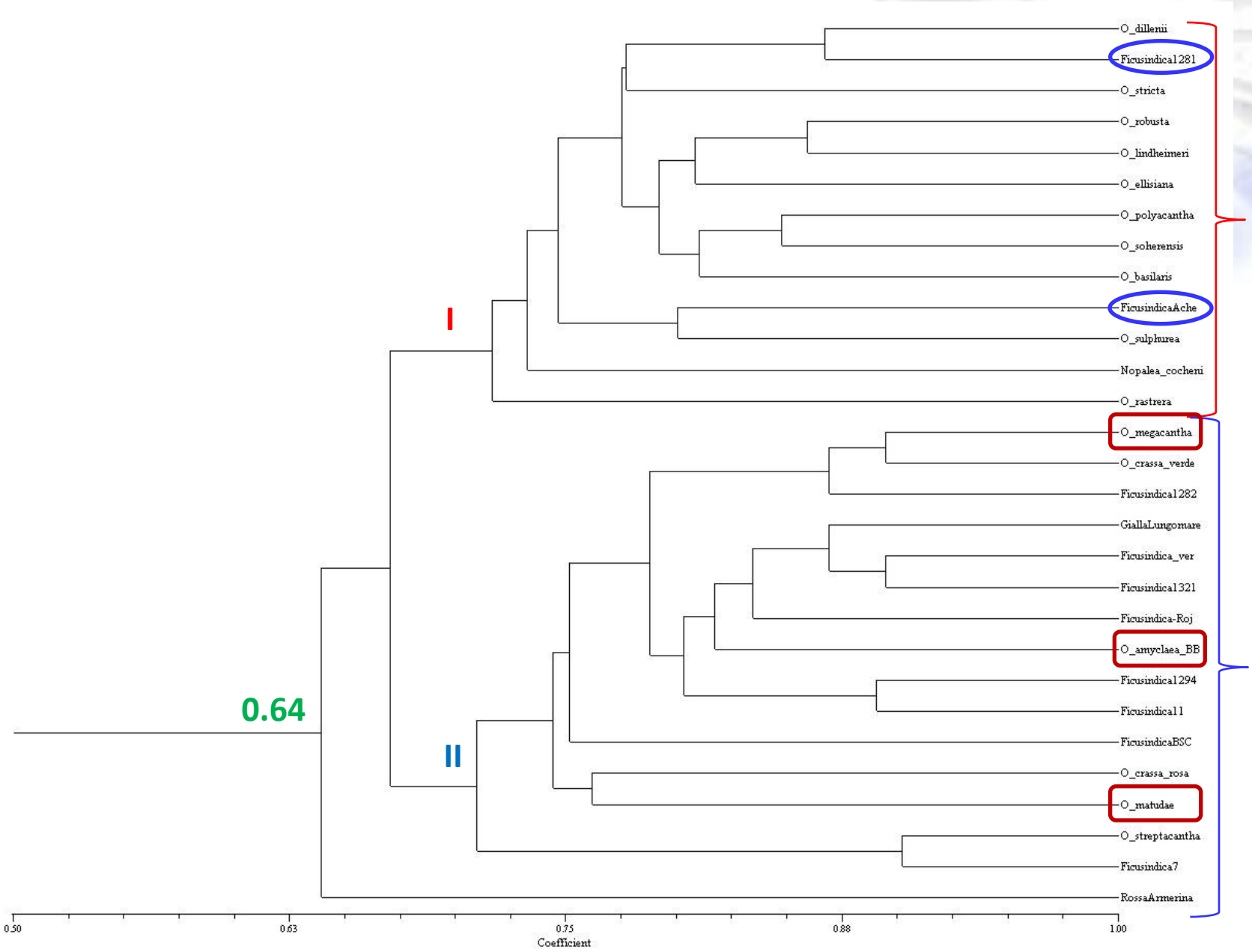
P%: percentage of polymorphism; no: observed number of alleles per locus; ne: effective number of alleles per locus; Is: Shannon's information index; H: Nei's gene diversity.

Polymorphism of SSR markers

- All SSRs produced polymorphic amplifications in the 29 accessions analysed
- The polymorphic index content (PIC) provided good discriminating ability for the assessment of genetic diversity in the genotypes studied
- The high levels of genetic variability between species and the minor levels of differentiation between cultivated accessions were recorded
- A total of 276 alleles was observed (ranging from 8 to 43 with an average of 21.46)



Genetic relationships between accessions



Conclusions

An high levels of genetic variability between species and a medium level of polymorphism between cultivated accessions were recorded.

The cultivated accessions retained more diversity than expected for domesticated species, as a result of the limited artificial selection and crop breeding in cactus pear

The level of polymorphism and the relatively high number of alleles detected suggest that these markers can be used for both inter and intra-specific studies.

The developed markers proved to enables the analysis of different species using the same SSR loci set

The information obtained can sheds some light on the classification of Opuntia species, based on their allelic profiles.

Conclusions

The SSR data combined with agronomic, qualitative, morphological, and phenological data will create a useful instrument to facilitate the management and use of Cactus pear collections

However, due to the presence of polyploidy within the *Opuntia* genus, the SSR may have a limited capacity to represent true genetic distances between cultivars, owing to the difficulty of identifying the allelic profile at a locus

A better understanding of the effectiveness of the different molecular markers is considered a priority step toward cactus pear and a prerequisite for more effective breeding program.


Thanks for your
attention