MOLECULAR MARKERS BASED ANALYSIS FOR CROP GERMPLASM PRESERVATION

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Summary
The present paper aims to highlight the potential impact, as well as possible drawbacks, of the application of DNA marker technology to crop germplasm preservation.
Some of the issues related to crop management and use, which can be addressed by using information derived from DNA markers, are discussed with reference to case-studies.

Keywords
Crop germplasm, molecular markers, *ex situ, in situ* and *on farm* conservation

Introduction

DNA-based assays have revolutionized and modernized our ability to characterize genetic variation. The first advantage of molecular techniques is their capacity to detect genetic diversity at an higher level of resolution than other methods; furthermore, DNA-based assays are robust, speedy, information may be obtained from little amounts of plant material at any stage of development and it is not affected by environmental conditions.
However, managing biodiversity means not only genetic characterization through DNA polymorphism detection, as it requires information used to address key issues of both *ex situ* and *in situ* plant germplasm management and to assist in the process of decision making. For *ex situ* crop germplasm maintenance, molecular tools may contribute to the sampling, management and development of 'core' collections as well as the utilization of genetic diversity. For the *in situ* and '*on farm'* preservation strategies of genetic resources, molecular markers might help in the recognition of the most representative populations within the 'gene pool' of a landrace and the identification of the most suitable strategies for their managing and use.
A large number of different molecular techniques are at present available and each of them differs in its informational content. Multi-locus approaches may be convenient but have some technical and/or analytical drawbacks, such as dominance (i.e. only one allele identified, no possibility to discriminate between homozygous and heterozygous individuals). As a consequence of simultaneous visualization of many marker alleles, multi-locus data typically are analysed as pair-wise comparison of complex patterns that only have meaning relative to others in the same study, thus results are to a limited extent comparable among studies. By contrast, single-locus markers are usually characterized by co-dominance (i.e. both alleles identified in heterozygous individuals) and thus are more flexible and supply more robust and comparable data (Karp, 2002).
An appropriate use of molecular markers techniques requires to clearly define the issues addressed, what type of information will be needed (on genetic diversity) and to know what the different techniques can offer not only in terms of genetic information but also resource

requirements, reproducibility, adaptability for automation. Furthermore, it is of pivotal importance to consider how the information will be gathered and the way in which the data will be scored and analysed. For accurate and unbiased estimates of genetic diversity adequate attention has to be devoted to: (i) sampling strategies, (ii) utilization of various data sets on the basis of the understanding of their strengths and constraints, (iii) choice of genetic similarity estimates or distance measures, clustering procedures and other multivariate methods in analyses of data; and (iv) objective determination of genetic relationships (Mohammadi and Prasanna, 2003). For all these reasons, choosing the most appropriate technique may be difficult and often a combination of techniques is needed to gather the information one is interested in.

Up to now most conservation efforts have focused on agriculturally important crops and about one third of all *ex situ* accessions in gene bank represents just five species: i.e. wheat (*Triticum* sp.), barley, rice, maize and beans (*Phaseolus* spp). The relative over-representation of five species does not necessarily mean that their genetic diversity has been fully covered (Graner *et al.* 2003) but, on the other hand, there is significant lack of knowledge about the diversity and geographic distribution of less utilized crops as well as their wild relatives (Hammer *et al.* 2003).

Here we report on two case-studies addressed to assess, through molecular tools, the genetic diversity in a minor crop: i.e. *Cynara cardunculus* and in landraces of a major crop: i.e. *Zea mays*.

Case-study 1: Assessment of the genetic diversity in *Cynara cardunculus*

Cynara cardunculus L. is a diploid ($2n=2x=34$), predominantly cross-pollinated species native to the Mediterranean basin. It includes two crops: globe artichoke (var. *scolymus* L.) and cultivated cardoon (var. *altilis* DC) as well as wild cardoon [var. *sylvestris* (Lamk) Fiori], a non-domesticated perennial which has been recognised as the ancestor of both cultivated forms. Globe artichoke represents an important component of South European agricultural economy, but it is grown all over the world for its large immature inflorescences (capitula); its commercial production is mainly based on perennial cultivation of vegetatively propagated clones. Cultivated cardoon is grown for its fleshy stems and roots and is of some regional importance in Italy, Spain, and the south of France; its propagation is carried out through seed.

Previous studies have shown that all the forms within *Cynara cardunculus*: (i) are a promising source of seed oil, both with respect to quantity and quality, (ii) can be exploited for the production of lingo-cellulosic biomass for energy or paper pulp, (iii) are a source of biopharmaceuticals. The roots contain include inulin, a known improver of human intestinal flora, while the leaves are a source of antioxidant compounds, such as luteolin and di-caffeoylquinic acids (cynarin). Notwithstanding its wide possibilities of exploitation little is known on the amount and distribution of genetic diversity; in order to assess it we had to address the following questions:

1) Which area to include in the survey?
2) How can diversity of natural and cultivated populations growing *in situ* be assessed rapidly and efficiently, in order to quantify the distribution of genetic variation and gather information for both identification of populations representative of the genetic variation and application of sampling or *in situ* preservation strategies?
3) How much diversity is present in the *Cynara cardunculus* cultivated forms of different growing regions and what criteria should be adopted for the development of a core collection?
4) Can individual plants be adequately identified for future application of plant breeding programs?

Of course DNA-based methods can efficiently contribute to answer these questions but which ones are the most suitable in order to make results: (i) reproducible by different laboratories, (ii) capable of being scored and analysed using standardized methods, (iii) suitable for entry into database? Since techniques detecting heterozygotes (i.e. co-dominant markers) and providing data on allelic differences were desirable, microsatellites (SSR, simple sequence repeats) were considered the most suitable markers although not available at the time of starting the work. Our first objective was thus to develop highly informative microsatellite markers by following different strategies, as reported by Acquadro *et al.* (2003, 2005a,b). Fifty primer pairs were designed and 32 were chosen as being highly polymorphic .We also felt necessary to complement the microsatellite analysis with AFLPs (amplified fragment length polymorphisms), due to their high reproducibility and their high information content.
Two spatially isolated areas were chosen for our survey: Sardinia and Sicily Island, the latter implicated as the origin of artichoke domestication. In both Islands wild cardoon extensively colonizes dry and undisturbed areas while autochthonous globe artichoke germplasm is still the most commonly grown. The first problem we had to face was: how many populations within each area and how many plants within each population have to be sampled? Indeed, in studies aimed at analysis of population structure it is necessary to balance the need to collect as large a sample size as possible, and to get allele frequencies from as many loci as possible, against the needs to screen as many populations as possible.

Wild cardoon genetic variation

On the basis of the different pedo-climatic conditions of the growing areas, we identified three populations in Sardinia and four in Sicily (Figure 1) (Portis *et. al*. 2004, 2005a).

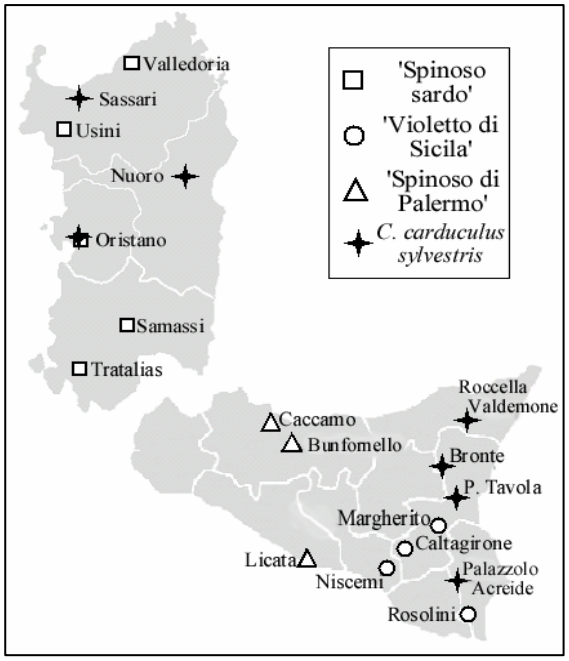

Fig.1 Geographic location of wild cardoon (C. cardunculus var. sylvestris) and globe artichoke populations.

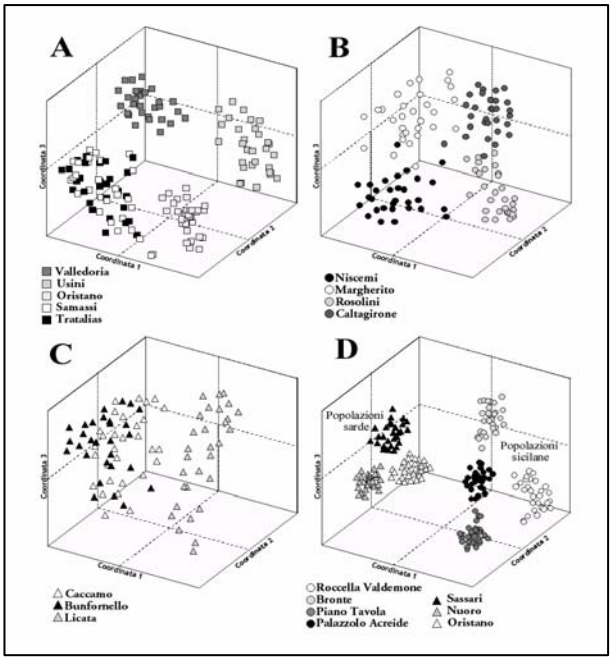

Fig.2 PCO plot of first three principal coordinates depicting the genetic relationship among accessions from populations of globe artichoke: 'Spinoso sardo '(A), 'Violetto di Sicilia'(B), Spinoso di Palermo (C), and wild cardoon (D).

It is well known that the minimum sample size for detecting alleles at a given frequency is greater in inbreeding than in out-breeding species (Gregorius 1980); being *Cynara cardunculus* an allogamous species we randomly sampled 30 individuals per population,

	N° polymorphic bands		N° locally common alleles		N° private alleles	
Population	SSR	AFLP	SSR	AFLP	SSR	AFLP
BRO	35	342	7	22	3	6
PAL	40	347	10	25	5	5
ROC	34	302	6	15	3	5
TAV	24	328	4	13	2	3
ORI	34	286	6	16	1	5
SAS	25	269	3	12	1	4
NUO	22	250	2	13	0	4
Sicilian populations	60	419	26	70	2	22
Sardinian populations	42	333	10	36	3	19

Table 1 Number of bands, and common and rare alleles within a location, detected with SSR and AFLP. Roccella (ROC), Bronte (BRO), Piano Tavola (TAV), Palazzolo (PAL), Sassari (SAS), Nuoro (NUO), Oristano (ORI).

which ensured (P< 0.95) the detection of alleles present at relative frequencies between 0.08 to 0.09 (Gillet, 1999). Plants were genotyped using the developed SSRs and seven AFLP primer combinations which generated more than 400 polymorphic bands. Genetic divergence between populations was found to be consistent between the two marker systems. As a result of the geographical isolation, the Sardinian and Sicilian populations were clearly differentiated, forming two distinct gene-pools (Figure 2D). Both marker systems show that the Sardinian and Sicilian populations possess a remarkable number of unique (private) alleles (Table 1) so that the two gene pools must have evolved independently.

Several criteria have been suggested to identify priority population for sampling the maximum possible genetic variation. (Maguire *et al.* 2002): (i) allelic richness, or the number of alleles per locus; (ii) the evaluation of the 'locally common, i.e. alleles that are common in one to several populations, but not in the species as a whole; (iii) the identification of unique (private) alleles. Based on any one of these criteria and for both AFLP or the SSR data sets, (Table 1) the same priority populations were identified: Palazzolo and Bronte in Sicily and Oristano in Sardinia.

In both Islands most of the AFLP and SSR genetic variation was present within rather than between populations, which is consistent with data reported by Gaudel *et al.* (2000) for outbreeding species. Nevertheless, we detected a remarkable extent of within-population clustering. We could not identify any overlap between populations and thus, despite the high ratio of within-to-between population genetic variance, the AFLP banding pattern of each genotype was found a relatively reliable predictor of its parental population.

Globe artichoke genetic variation

An analogous approach was applied for the analysis of genetic variation in autochthonous Sicilian and Sardinian globe artichoke germplasm, which is at risk of genetic erosion due to the recent introduction of varieties selected abroad or germplasm from other region which best fit market demand (Lanteri *et al.* 2001; Portis *et al.* 2005b). In Sicily we identified seven populations, of which three were of the spiny type 'Spinoso di Palermo (SP)', which is generally cultivated on the western side of the island, and four of the non-spiny type 'Violetto di Sicilia (VS)', which is confined to its eastern side. In Sardinia we identified five populations of the autochthonous spiny type 'Spinoso sardo (SS)' (Figure 1). Due to the limited polymorphism of SSR markers in globe artichoke, we applied AFLP and RAPD (random amplified polymorphic DNA) markers.

In Sicily a significant genetic differentiation between spiny and non spiny types was found as the PCO analysis identified two main clusters, one of which groups the three representative SP populations, while the other groups the four VS populations (data not reported). About one third of the AFLPs scored were found specific to one or other of these two types.

In both Sicilian and Sardinian populations the majority of the genetic variation was present within, rather than between populations. As the three varietal types are vegetatively propagated, the within genetic variation presumably reflects their multiclonal composition, a direct consequence of the limited selection criteria adopted by farmers. An additional source

of variation may be *via* spontaneous mutations, which maintained as they are not subject to any meiotic sieve and might not be detectable at the phenotypic level.
Despite the high level of within population genetic variation present, most of the populations could be genetically differentiated from one another due to farm fragmentation, or adaptation to local pedo-climatic conditions. In Sicily the between population differentiation was more evident in VS, allowing the PCO to define four clusters with minimal overlap (Figure 2B). Vice versa in Sicilian SP, and in Sardinian SS two of the populations were partially overlapping, presumably as a consequence of some exchange of material between farmers (Figure 2A-C). Our data resulted very informative for the implementation of 'on farm' germplasm preservation strategies. On the basis of the criteria reported above, the Rosolini (VS) and the Buonfornello (SP) populations in Sicily and the Oristano population in Sardinia are the most representative of the 'gene pool' of the varietal types, and priority should be given to them for application of 'on farm' preservation strategies.

Globe artichoke and cultivated cardoon germplasm characterization

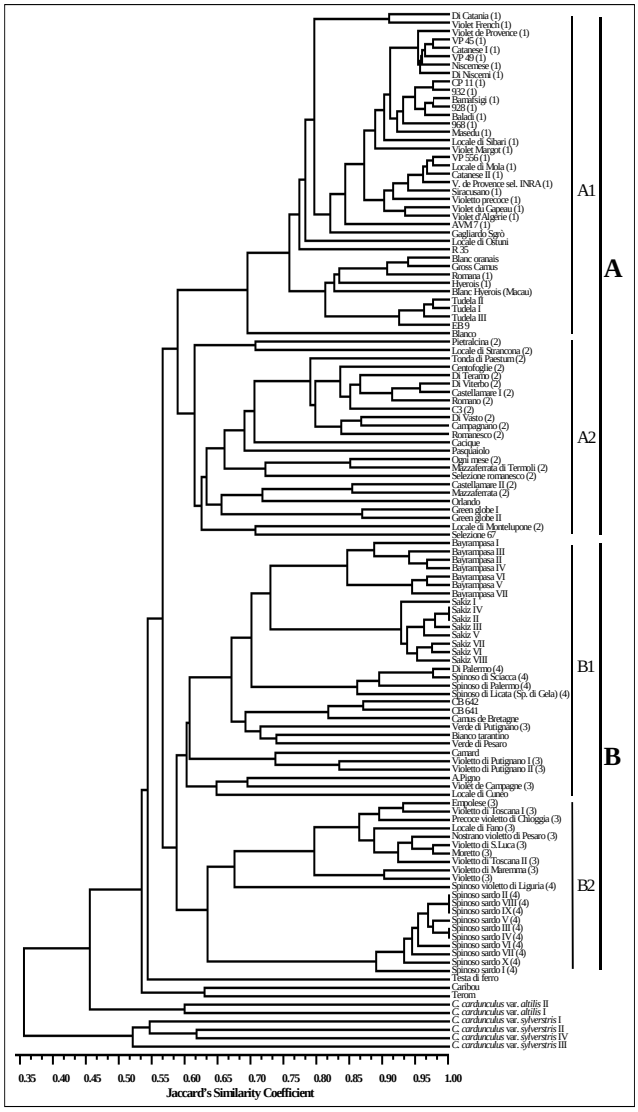

Fig. 3: Dendrogram obtained from UPGMA cluster analysis of AFLP data of 118 globe artichoke accessions. Wild and cultivated cardoon were also included in the analysis. Co-phenetic correlation coefficient = 0.92

The use of molecular markers that quantify the genetic diversity within and between accession may significantly increase the efficiency of the assessment and management of germplasm collection by reducing redundancy. Several works have detailed the philosophy behind optimising collections to ensure diverse genetic representation through either the creation of 'core' collections or some form of hierarchical sampling. We applied eight AFLP primer combinations for characterizing a living collection of globe artichoke germplasm maintained at CRAS (Oristano, Sardinia).
We also included in the analysis accessions collected in the field in Spain, Turkey and USA for a total of 89 varietal types; for three of them different provenances were assessed for a total of 118 accessions (Lanteri *e. al.* 2004a). Figure 3 shows the dendrogram from UPGMA (unweighted pair-group method arithmetic average) cluster analysis of AFLP data. Our results suggest that traits selected by man play an important role in understanding variation and differentiation within cultivated artichoke germplasm. Within branch A two main clusters were found: A1 and A2. Cluster A1 mainly contained Catanesi types having small elongated heads, while cluster A2 included the Romaneschi types, with big spherical or sub-spherical non-spiny heads and mainly

cultivated in central Italy together with te American accessions of Green globe, which accounts more than 85% of the artichoke production in the United States. Branch B contained two additional clusters: B1 and B2, both of which included Spinosi and Violetti types with medium-small heads. Cluster B1 also included all the Turkish accessions. AFLP data furnished important information for assembling a core collection of globe artichoke germplasm, taking into account the hierarchical structure of the genepool. Our results showed that the genetic variation detected within the same varietal type was in some cases higher than that found between varietal types. The Jaccard's similarity index among clones of the same varietal type might thus be considered to be a threshold value that identifies material sharing the same genetic background. On the basis of this threshold a limited number of core subset could be identified. Genetic studies in selected crops have demonstrated that widespread and localised alleles occurring in the entire collection are usually contained in the core subset, with only rare localized alleles excluded (van Hintum *et al.* 2000). The core subset can thus provide an entry point to further study of biodiversity of the entire collection or to identify suitable material for future breeding efforts. Furthermore the results obtained in this study, as well as in another study aimed at genotyping selected clones of the varietal type 'Spinoso sardo' (Lanteri et. al. 2004b), showed that AFLP markers are useful to identify duplicates and provide evidence for the uniqueness of a particular genotype.
An analogous study was carried in cultivated cardoon (Portis *et al.*, 2005c), whose genetic variation of the material in cultivation in Spain and Italy was assessed by DNA profiling at five microsatellite loci and with eight AFLP primer combinations. The analysis of genetic similarities showed that the Spanish and Italian accessions represent two distinct gene pools. In this study we also demonstrated that a fruitful approach in characterizing germplasm collections with AFLP markers is to use a two-tiered approach: first low density profiles to compare all samples and resolve the main cluster and then high density profiles to resolve samples within clusters.

Case-study 2: Italian landraces of maize and molecular markers for their characterization and conservation

Maize (*Zea mays* L.) is one of the most important crops in Italian agriculture. The species was introduced in the national cultivation system approximately four centuries ago and grown mainly for human consumption. Since then, a number of landraces have been developed in order to meet specific needs of cultivation and utilization, and to overcome environmental constraints of different areas. As a consequence, new landraces originated from the original populations introduced, through adaptation to local conditions as well as hybridization brought about by continuous exchange and trade. These landraces were locally maintained by farmers as open-pollinated populations and thus each of them represented a collection of highly heterozygous and heterogeneous plants. Although a considerable range of variation within each population was present, a between population differentiation was detectable for several distinctive traits as a consequence of both natural and human selection pressure.
Within the last few decades, the Italian agricultural scenery has profoundly changed and the subsistence mixed farming unit is now transformed into an intensive monoculture (Bertolini *et al.*, 1998). At present, a small number of populations of flint maize (*Z. mays* var. *indurata*) can be found under very peculiar agricultural situations or in marginal areas, such as alpine valleys, on small fields traditionally managed according to low-input agronomic practices, and with production exclusively addressed to human consumption (Lucchin *et al.*, 2003). The agricultural environment together with the traditional diet of these regions allows preservation of some landraces and limits diffusion of modern hybrids. Unfortunately, many locally cultivated populations were lost before it was realized that they were important sources of

germplasm. Maize breeders have recently become more aware of the need for both maintaining genetic diversity among hybrid varieties and improving the management of genetic resources through the conservation of landraces. From this comes the renewed interest for *in situ* conservation of the landraces not only to preserve important sources of genetic material for breeding, but also to allow their valorization as essential components of sustainable agriculture. Landraces are the cultivated maize material with the highest genetic variation as well as with the best adaptation to the natural and anthropological environment where they have evolved. Then they contain locally adapted alleles and likely represent an irreplaceable bank of highly co-adapted genotypes. Knowledge of genetic diversity among local populations and breeding stocks is expected to have a significant impact on the improvement of this crop. In maize, information on both qualitative and quantitative morphological traits of existing landraces may be useful in maintaining their genetic variability and preserving them from genetic erosion. Nowadays, after years of lack of interest towards the so-called old local varieties, this valuable source of maize germplasm has been rediscovered and exploited as a niche crop suitable for the cultivation of marginal lands.
The development of molecular markers has greatly facilitated basic and applied research programs of maize genetics and breeding. DNA polymorphism assays are also known as powerful tools for characterizing gene pools and investigating germplasm resources.

Molecular characterization of field populations belonging to an Italian landrace of flint maize (*Zea mays* var. *indurata*)

A comparative characterization of farmer populations of the flint maize landrace "Nostrano di Storo" was recently carried out by Barcaccia *et al.* (2003) using different types of PCR-based markers. The inbred line B37 and three synthetics (VA143, VA154 and VA157) selected from as many landraces were used as reference standards. Genetic diversity and relatedness were evaluated with SSR and Inter-SSR markers. Nei's total genetic diversity as assessed with SSR markers was H_T=0.851 while the average diversity within populations was H_S=0.795. The overall Wright's fixation index F_{ST} was as low as 0.066. Thus, more than 93% of the total variation was found within population. Dice's genetic similarity coefficients within and between populations on the basis of Inter-SSR fingerprints were 0.591 and 0.564, respectively. The UPGMA dendrogram displayed all populations but one clustered into a distinct group, in which a synthetic variety selected from the landrace 'Marano Vicentino', was also included (Figure 4).

One population and the other two synthetics, 'Spino Bresciano' and 'Dente di Cane Piemontese', were clustered separately. Findings suggest that, although a high variability can be found among plants, most of their genotypes belong to the same landrace locally called 'Nostrano di Storo' (Barcaccia *et al.*, 2003). This result was also confirmed by a further molecular investigation carried out using AFLP and RAPD markers to fingerprint pooled DNA samples from all farmer populations.

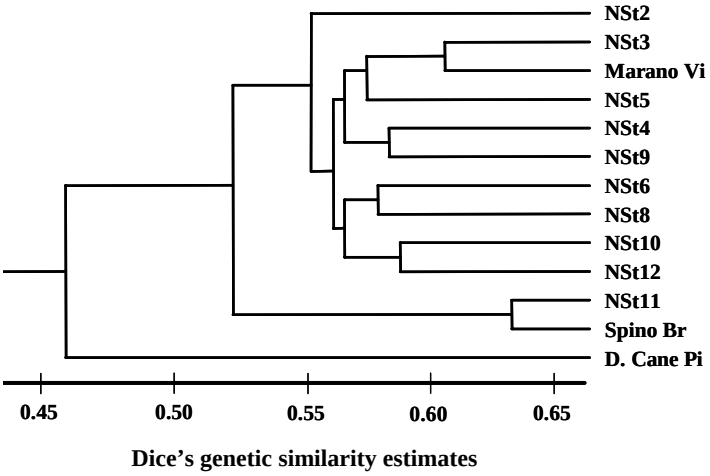

Fig. 4: UPGMA dendrogram of the maize farmer populations.

Although gene flow from commercial hybrids might have occurred, the large number of polymorphisms and the presence of both unique alleles and alleles unshared with B37 and synthetics are the main factors underlying the value of this flint maize landrace as a source of genetic variation and peculiar germplasm traits. Because of its exclusive utilization for human consumption, such a molecular marker characterization will be a key step to promote the *in situ* conservation and protection of the landrace.

Construction of a linkage map for a maize landrace based on a pseudo-testcross strategy using multi-locus PCR-based markers.

Genetic linkage maps based on molecular markers represent basic tools for investigating and characterizing local germplasm resources. A linkage map of the flint maize landrace 'Nostrano di Storo' based on dominant multi-locus PCR-derived markers (RAPD, I-SSR, AFLP and SAMPL) was constructed according to a one-way pseudo-testcross mapping strategy (Barcaccia *et al.*, 2000; 2005). The feasibility of such a study depended on the presence of high levels of heterozygosity in the landrace parent that was mapped and on the informativeness of the PCR-based marker systems that were used. Co-dominant single-locus SSR markers were adopted to assign each linkage group to a specific chromosome and all marker loci to specific chromosome arms. The final genetic map includes 282 marker loci and covers 1.826 cM (Table 2).

Table 2: Summary of the map statistics, including length, number of marker loci per chromosome and total.

Statistics	Chromosome										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
Map length (cM)	217	220	166	260	172	161	173	150	142	165	1.826
No. AFLP	29	27	27	20	24	19	25	14	20	17	222
No. SAMPL	1	6	6	1	0	2	2	2	0	4	24
No. RAPD	0	0	3	1	0	2	0	1	0	0	7
No. Inter-SSR	1	2	0	1	0	0	0	0	1	0	5
No. SSR	3	2	2	2	4	3	2	2	2	2	24
Total marker loci	34	37	38	25	28	26	29	19	23	23	282
Average map density	6.4	6.0	4.4	10.4	6.2	6.2	6.0	7.9	6.2	7.2	6.5

This genetic map based on multi-locus marker systems and on easily detectable molecular markers will find application and prove useful for rapidly characterizing the genetic diversity within and relatedness among farmer populations belonging to the "Nostrano di Storo" landrace maintained according to different conservation strategies.

Assessment of the optimal plant and molecular marker sample size to estimate genetic diversity in maize landraces

The genetic characterization of landraces represents an essential step for their conservation. This requires to establish the most appropriate system and type of markers (i.e. random or mapped) and the minimum number of markers and plants required to describe the genetic structure of a given population. In spite of their importance for the success of any germplasm conservation program, a few information is available on landraces because almost all studies were performed on inbred lines. A sample of plants were chosen as representative of the landrace in terms of morpho-phenological and agronomic traits, and then assayed at hundreds

of marker loci, either mapped or random. Genetic similarity and diversity coefficients computed using mapped markers proved to be significantly higher than estimates based on total markers. Moreover, no significant changes of marker allele frequency and polymorphism information content were scored when the number of sampled ears was progressively reduced from 50 to 15, even if a steady increase of standard errors was observed. The influence of the number and type of molecular markers on genetic similarity and diversity measurements was also investigated: no significant changes in terms of mean PIC values were observed, whereas the CV of standard deviations raised proportionally to the reduction of sample size. A total of 120 random markers and 80 mapped markers was needed to get CVs lower than 5%. Also when the number of ears and markers were evaluated together no significant changes of the mean PIC values were observed and an increase of the molecular data variability was confirmed (Pallottini, 2002).
Influence of marker and plant sampling on statistics used to measure genetic diversity should be useful to investigate the genetic consequences of different modes of conservation of maize landraces (*on farm*, *in situ* and *ex situ*). In conclusion, reliable and effective investigations of landrace population genetic structure with AFLP markers can be performed using at least 30 ears per population and one plant per ear and require at least 80 mapped marker loci.

Effects of different conservation strategies (*on farm, in situ* and *ex situ*) on the population genetic structure of maize landraces as assessed with molecular markers
The *on farm*, *in situ* and *ex situ* conservation methods may exert a different influence on the genetic structure of populations grown by farmers. This influence should be accurately evaluated to avoid genetic erosion and conservation program failure. In fact, the loss of genetic diversity could be due to drift and shift, inbreeding that can result from either ones, natural and human selection and gene flow. Each of these factors has a different relative importance on the types of conservation methods. Molecular markers were used to investigate the influence of the conservation strategy on the genetic structure of farmer populations grown for two years with three different methods: i) *on farm* conservation by farmers, using own seeds and traditional agronomic practices; ii) *in situ* conservation in the original area but taking into account the spatial isolation from other fields cultivated with hybrid varieties; iii) *ex situ* conservation far away from the original area with no gene flow because of the total absence of fields grown with the same crop (Pallottini, 2002). Statistical tests failed to reveal any significant difference in terms of diversity/similarity absolute values among the populations conserved according to the three distinct strategies (Figure 5).
However, about 10% of the comparisons performed for the marker allele frequency parameter at the total assayed loci showed significant differences. Even the differences between genetic variation parameters computed for mapped and random marker loci were significant. In particular, some marker loci were more affected than others by changes of the marker allele frequency depending on the conservation method. These markers, distributed throughout the genome, could be related to important genes involved in the adaptation to environmental conditions or responsible for traits evaluated in the selection by farmers.

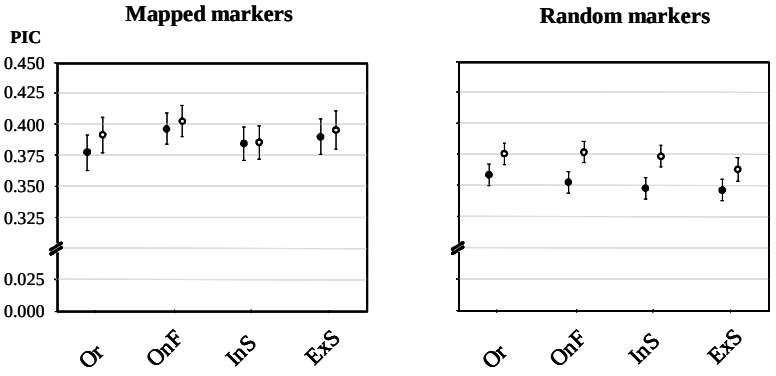

Fig. 5 PIC values and standard errors computed in the original population and in the populations obtained from *on farm*, *in situ* and *ex situ* conservation strategies, using mapped and random markers (black bullets relate to markers shared between original and final plant materials, while white bullets indicate total markers).

In conclusion, although all conservation methods studied have determined significant changes on the genetic structure of the farmer populations, the genetic variation and diversification occurred with the *ex situ* conservation was much stronger than that observed for *in situ* and *on farm* conservation. It is worth mentioning that to monitor these changes it is essential the level at which the investigation is performed. When the mean values of the more common genetic diversity and/or similarity indexes are taken into account no significant differences are highlighted because of the large set of molecular data and the occurrence of bidirectional changes of marker allele frequencies over all marker loci. Consequently, variation of the marker allele frequency has to be computed and interpreted at each single marker locus or between pairs of marker loci, but not on the whole molecular marker data set.

Conclusions

Our case-studies show how molecular marker techniques may help in characterizing and managing genetic diversity, however other questions have to be answered. Molecular tools are very informative but are generally employed in an anonymous way, they are able to detect high levels of DNA polymorphisms but are they really providing the kind of information which is required to make effective and sound judgements on diversity? What is the functional relevance of the polymorphism detected? Indeed understanding the significance or assessing the value of the diversity is still a difficult challenge.

New approaches have been recently developed for adapting the current PCR-based techniques to target functional diversity. As stated by Tanksley and McCouch (1997) ‘New findings from genome research indicate that there is a tremendous genetic potential locked up in germplasm collections that can be released only by shifting the paradigm from searching from phenotypes to searching for superior genes with the aid of molecular linkage maps’. At present the increasing information available from genome mapping means that markers known to be very closely linked to trait of interest can be better addressed for characterizing genetic diversity and help in identifying variation of use to breeders. Furthermore the identification of genes controlling a trait and the availability of their DNA sequences may facilitate the classification of variation in germplasm pools. High resolution genetic maps enable closely linked markers to be used and the increasing numbers of ESTs and SNPs provide routes for more targeted sequence-based approaches. Classification of the sequence variants at a targeted locus would substantially reduce the amount of work needed to assess their potential for breeding and lead to the identification of superior alleles (Sorrels and Wilson, 1997).

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