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Genetic analysis of a garlic (*Allium sativum* L.) germplasm collection from Argentina

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ABSTRACT

Although garlic spreads asexually, it shows a wide diversity for physiologic, morphologic and agronomic characters, due to the accumulation of mutations. Our objective was to examine the genetic diversity of a collection of 43 national and foreign introductions using fluorescent Amplified Fragment Length Polymorphism (fAFLP) markers for characterizing previously phenotypical. 351 bands were obtained, of those 251 were polymorphic. The obtained genotypes were analyzed by means of the program Structure v2.2 according to a mixture model of frequencies correlated by allelic population. Twenty simulations of 55,000 steps defaulting from two to eight different genetic populations were carried out. The solutions with more likelihood were obtained by conforming five groups of clones. Most of the individuals showed compound genotypes for portions of two or more populations and duplications were identified. If the phenotypical classifications are correct, the phenotype can respond to different genetic origins, including individuals genetically atypical in a uniformly phenotypical population. Some clones were isolated after multiple selections on populations of garlic, what is not surprising, is that some are more genetically related than others. The genetic relationships found in the collection of garlic belong together with the classification of the ecophysiological groups, although not in a definitive way, additional analysis being necessary. The conservation of the genetic diversity is of vital importance because it guarantees a broader background with which to practice artificial selection assuring the success of future programs of improvement.

1. Introduction

Garlic (*Allium sativum* L.) is one of the oldest known horticultural crops. There is clear historical evidence of its use. Nowadays, garlic grows wild only in Central Asia, but in early times garlic grew wild over a much larger region. This region is considered as its "center of origin" since this is the geographic region where the crop originated and the only place where it flourished in the wild. The center of origin is also referred to as its "center of diversity", since it is where the broadest range of genetic variation can be expected (Simon, 2001).

We know almost nothing about the early types of garlic once it was cultivated by the first garlic farmers outside its center of origin (Volk et al., 2004). No designation of garlic varieties was made in early research studies, and the first descriptions were only phenotypical (Simon, 2001).

Throughout history, humans migrating and traveling through Central Asia and surrounding areas have been collecting wild garlic and carrying it with them for later consumption and cultivation. In contrast to wild garlic, garlic in cultivation throughout history has only been propagated asexually by vegetative cloves, bulbs, and bulbils or topsets (Volk et al., 2004). Without an opportunity for interpollination and/or sexual reproduction, new genotypes have been obtained through the selection of spontaneous mutations expressing traits of horticultural interest (Simon, 2001; Volk et al., 2004).

Relatively a small number of garlic clones are in the hands of growers around the world. The clones that are held by growers are maintained as separate entities, but a system to confirm or refute the identity of a given clone often remains unsolved (Simon, 2001). We can also expect to find variations due to mutations that accumulated over time (Simon, 2001). In the case of species like garlic ($2n = 2x = 16$) with an enormous content of DNA measured as 2C in the nuclei of 32.7 pg, very similar to *Allium cepa* 33.5, means an unusually large genome size of approximately $>3 \times 10^{10}$ base pairs (Ranjekar et al., 1978) which results in the characterization by molecular markers being highly difficult. Some studies on the molecular organization of the genome demonstrate that 0.05% of

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DNA is complementary to ribosomal RNA and 30% of DNA is repetitive and interspersed with repetitive fractions.

A DNA fingerprint analysis of garlic today will serve as a useful foundation for assessing new clones coming to growers in the future (Simon, 2001). This methodology not only tells us that one clone is different from another, but it also states how closely related clones are, with each other. In this way DNA fingerprints provide modern insights into historical events for which no other historical record is available. Comparative analysis of DNA fingerprints have provided important insights into the origins and movements of human populations, cultivation and domestication histories (Simon, 2001). AFLP was applied to evaluate the genetic diversity of different garlic clones collected in different parts of the world and compared to AFLP groups with the studies of isoenzymes of Pooler and Simon (1993), Ipek and Simon (1998) and García-Lampasona et al. (2003), generating some information about the genetic identity of these materials.

The software Structure (Pritchard et al., 2000) employs a model-based clustering method for using multilocus genotype data to infer population structure and assign individuals to populations (Falush et al., 2003a,b), and estimates the probability or proportion of membership in each population for each individual (Falush et al., 2007). It has been applied for identifying cryptic population structure, detecting migrants or admixed individuals, and inferring historical population admixture (Pritchard et al., 2000; Rosenberg et al., 2002; Albert et al., 2006; Lecis et al., 2006; Ostrowski et al., 2006). Dominant markers such as Amplified Fragment Length Polymorphisms (AFLPs) provide an economical way of surveying variation at many loci (Falush et al., 2007), and a rapid and affordable approach of collecting polymorphism data on a genomic scale (Ostrowski et al., 2006; Campbell et al., 2003). However, the presence of null alleles and the limitations of genotype calling, present a problem for statistical analysis. These markers are typically ambiguous about the genotypes that underlie them. In particular, in diploids, a band will be obtained if either or both of the homologous chromosomes contain an amplifiable sequence (Falush et al., 2007).

The EEA La Consulta INTA Germplasm Bank maintains a significant collection of garlic accessions in Mendoza, Argentina. The diversity of these clones is described by a set of phenotypical and morphological descriptors known to be plastic, and varying according to environmental conditions (Burba, 1991; Ipek et al., 2003; Al-Zahim et al., 1997). The purpose of this study is to examine the diversity of a collection of 43 accessions using a set of amplified fragment length polymorphism markers and equate the fAFLP technique to characterize garlic accessions with the aim of improving the disadvantages of the traditional AFLP technique.

The analyzed data gathered was subjected to several analytical techniques that allowed us to compare the molecular diversity of these accessions, a phenotypic classification scheme, and to identify both the structure of diversity and the extent of duplication within the collection. This data will be valuable in prioritizing representative accessions for further cryopreservation, breeding and research.

2. Materials and methods

2.1. Plant material and DNA isolation

All the 43 accessions of *A. sativum* var. *sativum* used in this study (Table 1) were provided by the Germplasm Bank of INTA La Consulta, Mendoza, Argentina. Twelve cloves from each clone were sown in pots containing sand and grown for 30 days in a greenhouse. Young leaves were harvested from six plants of each accession. DNA was extracted immediately from fresh leaves by the CTAB-based method described by Murray and Thompson (1980).

Table 1
Garlic accession numbers, names and origins of clones.

Accessions	Argentina ecophysiological group (*)	Names	Origins
AR-I-030	III	Thermidrome	FR
AR-I-032	III	Fructidor	FR
AR-I-034	III	Germidour	FR
AR-I-035	III	Messidrome	FR
AR-I-080	III	Ariqueño	CH
AR-I-097	III	Cazapava	BR
AR-I-100	III	Santacruceño	AR
AR-I-101	III	Blanco A	AR
AR-I-111	III	INCO 283	AR
AR-I-112	III	Nieve INTA	AR
AR-I-113	III	INCO 207	AR
AR-I-114	III	Unión	AR
AR-I-118	III	Licán INTA	AR
AR-I-119	III	Perla INTA	AR
AR-I-131	III	Blanco Mendoza SA 90	AR
AR-I-135	III	Blanco Mendoza SA 94	AR
AR-I-136	III	Trevelin	AR
AR-I-138	III	Rincon chico	AR
AR-I-152	III	Quilmes	AR
AR-I-156	III	Norteño INTA	AR
AR-I-160	III	Blanco IFFIVE	AR
AR-I-044	IVa	Italiano	AR
AR-I-051	Iva	Don Rafael	AR
AR-I-052	IVa	Colorado Español	AR
AR-I-055	IVa	Colorado A-1	AR
AR-I-056	IVa	Colorado A-2	AR
AR-I-102	IVa	Español A	AR
AR-I-106	IVa	Español 542	AR
AR-I-107	Iva	Payén	AR
AR-I-108	IVa	El Nevado	AR
AR-I-115	Iva	INCO 30	AR
AR-I-116	IVa	Fuego INTA	AR
AR-I-121	IVa	Colorado Malvinas 1	AR
AR-I-122	IVa	Colorado Malvinas 2	AR
AR-I-124	IVa	Colorado FCA 19	AR
AR-I-125	IVa	Colorado FCA 10	AR
AR-I-132	IVa	Colorado URO21-1A2	UR
AR-I-133	Iva	Colorado UR017–22	UR
AR-I-145	IVa	Colorado L.	AR
AR-I-147	Iva	Colorado LV 6	AR
AR-I-155	Iva	Sureño INTA	AR
AR-I-033	IVb	Ruso	AR
AR-I-120	IVb	Castaño INTA	AR

Number of accessions corresponds to the Active Collection of the Garlic Germplasm Bank at INTA La Consulta, Mendoza, Argentina.
(*) Ecophysiological groups are according to Burba (2008).

DNA concentrations were quantified using Pharmacia Gene Quant spectrophotometer (Pharmacia, Biotech, Columbus, OH) and by visual comparison with lambda DNA standard of known concentration on ethidium bromide stained agarose gels.

2.2. AFLP reactions

The AFLP procedure (Vos et al., 1995) was performed as described by Berres (2001) with minor modifications (García-Lampasona et al., 2010). The modifications consisted of the use of fluorescent dyes linked to primers and the use of ABI Prism 3130, Applied Biosystems used for AFLP DNA analysis.

In short, 250 ng of genomic bulked DNA was double digested for 2 h at 37 °C with 1.25 unit of EcoRI and MseI restriction enzymes (New England Biolabs) respectively, in 12.5 μl of the buffer composed by 10 mM Tris–HCl (pH 7.5), 10 mM Mg-acetate and 50 mM K-acetate. Then, restriction enzymes were heat-inactivated for 15 min at 70 °C. To generate template DNA for amplification, the genomic DNA fragments were ligated to EcoRI and MseI adapters in a total volume of 12.5 μl using 0.25 unit of T4 DNA ligase (Promega) at 20 °C ± 2 °C for 2 h. An aliquot of digested and ligated product was diluted 1:10 with TE buffer. The template DNA was pre amplified