

# Garlic Biodiversity and Genetic Resources

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## ABSTRACT

Garlic clones exhibit a wide variation in vegetative traits, flavor and pungency; bolting capacity, and fertility. Cultivar characteristics differ considerably with the location of cultivation, and climate has a significant impact on garlic bulbing, florogenesis and flavor. All cultivated garlic clones are sterile, thus an increase in genetic variation is possible only via random or induced mutations, somaclonal variation and introduction of new genetic variation using modern molecular techniques. Conservation of garlic germplasm began about 30 years ago. Since then, field genebanks were established in Germany, Israel, Poland, Czech Republic, Russia, and the USA. Yet, only limited effort has been invested in systematic collection and preservation of wild *Allium* species of potential economic value. Isozyme and molecular analyses show considerable genetic diversity within the garlic species complex, and AFLP technology facilitates the evaluation of genetic diversity in garlic collections. Biochemical and molecular studies suggest that the highest level of heterogeneity occurs within the Central Asian gene pool, which may contain genes of interest for future use in genetic studies, as well as for plant improvement programs. Until recently, however, this gene pool did not attract much attention by researchers from outside the region. An international effort aimed at immediate collection and preservation of this heritage is imperative. It should be followed by an intensive evaluation effort and accompanied by substantial preservation projects, to halt the rapid and irreversible erosion of the Central Asia gene pool of garlic landraces and wild populations.

**Keywords:** *Allium sativum*, *Allium longicuspis*, germplasm

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## BOTANICAL AND HORTICULTURAL DIVERSITY

Modern *Allium* taxonomy includes garlic (*Allium sativum* L.) in the subgenus *Allium*, which is comprised of 15 sections and about 300 species (Friesen *et al.* 2005). Most of these species grow wild in the Irano-Turanian and Mediterranean phytogeographical regions, and are thus exposed to cold winters and hot, dry summers. For many years, *A. longicuspis* was considered to be the closest relative or ancestor of garlic (Regel 1875; Vvedensky 1968; Hanelt 1990), but recent studies on developmental morphology (Etoh 1985; Kamenetsky *et al.* 2004a), isozyme profiles, and randomly amplified polymorphic DNA (RAPD) (Pooler and Simon 1993a; Maaß and Klaas 1995; Hong 1999; Ipek *et al.* 2003) have confirmed that the two plants are closely related and that both are members of the *A. sativum* species complex (Maaß and Klaas 1995; Fritsch and Friesen 2002). Consequently, Fritsch and Friesen (2002) subdivided the *A. sativum* species complex into three major groups: the common garlic, the *Longicuspis*, and the *Ophioscorodon*, plus two subgroups: the Subtropical and the *Pekinense*. A number of other *Allium* species also possess garlic-like flavor and are consumed locally as condiments and/or green vegetables (Table 1).

Linnaeus (1753) and Don (1832) proposed that *A. sativum* originated in the Mediterranean basin and, only in the

20<sup>th</sup> century Vavilov (1926) and Kazakova (1971) concluded that Central Asia was its primary center of evolution. This hypothesis was further supported by the discoveries of several primitive garlic types in the Tien-Shan Mountains (Etoh 1986; Kotlinska *et al.* 1991; Kamenetsky *et al.* 2004a), and by biochemical and molecular studies (Pooler and Simon 1993a; Maaß and Klaas 1995; Hong 1999). Engeland (1991) suggested that wild garlic was cultivated by seminomadic tribes throughout Central Asia as long as 10,000 years ago. About five thousand years ago, garlic reached India, China and the Mediterranean basin (Etoh and Simon 2002), and evidence is available to the fact that in ancient Egypt garlic was used for cooking, embalming, and medicinal purposes. The Romans disliked the strong flavor, but provided laborers and soldiers with it, to make them strong and brave. Later, European traders distributed garlic more widely, so that it became an important vegetable and condiment throughout the world, a status it still holds. From the Mediterranean region, explorers and colonists introduced garlic to sub-Saharan Africa and to the Americas (Etoh and Simon 2002), and today, garlic is grown all over the world. Global annual production is estimated at 14 million tons with ca. 75% of the total being produced in China. The other leading countries are India (3.55% of global production) and the Republic of Korea (2.69%) (FAOSTAT data 2006).

**Table 1** List of wild and domesticated *Allium* spp. used as substitute for garlic (van der Meer 1997; Fritsch and Friesen 2002).

Generic name	Botanical name	Section	Origin	Cultivation
Naples garlic	<i>A. neapolitanum</i> Cirillo	Molium	Mediterranean basin	Mexico
Ramsoms	<i>A. ursinum</i> L.	Ophioscordon	Europe	Central and North Europe
Long-rooted garlic	<i>A. victorialis</i> L.	Anguinum	Europe to Caucasus and Siberia	East Asia
Canada garlic	<i>A. canadense</i> L.	Amerallium	North America	Cuba
Ramp	<i>A. tricoccum</i> Solander	Anguinum	North America	
Djusai	<i>A. ramosum</i> L.	Butomissa	Central and East Asia	Central and East Asia
Rakkyo	<i>A. chinense</i> G. Don	Sacculiferum	East Asia	China, Korea, Japan, Indonesia
Chinese or Japanese garlic	<i>A. macrostemon</i> Bunge	Pallasia	Far East, Mongolia, China	East Asia
Great-headed garlic or elephant-garlic	<i>A. ampeloprasum</i> L.	Allium	Mediterranean basin; Iran, Caucasus	Asia Minor, Iran, Caucasus, California, Europe
Rocamboles	<i>A. scorodoprasum</i> L.	Allium	Northern, Central and Eastern Europe	Formerly cultivated
-	<i>A. obliquum</i> L.	Petroprason	East Europe to Siberia and China	Siberia, China, Europe

Cultivated garlic is propagated only vegetatively, but exhibits wide variations in bulb size, shape and color; number and size of cloves; maturity date; flavor and pungency; bolting capacity; numbers and sizes of topsets and of flowers in the inflorescence (McCollum 1976; Astley *et al.* 1982; Astley 1990; Pooler and Simon 1993b; Lallemand *et al.* 1997; Hong *et al.* 2000; IPGRI 2001; Kamenetsky *et al.* 2004a). The increase in genetic variation most probably resulted from random or induced mutations (Burba 1993) and/or somaclonal variation (Novak 1990), followed by clonal selection in a variety of growing environments (Jones and Mann 1963; Rubatzky and Yamaguchi 1997). The strong interaction between the genotype and the environment led to a variety of phenotypic expressions (Lallemand *et al.* 1997; Portela 2001; Kamenetsky *et al.* 2004b). Evidently, recent restoration of garlic fertility, seed production and evaluation of variation between seedlings' populations has demonstrated that a great part of the variation known today between garlic clones is available in the garlic genome and can be reproduced by seed propagation (Kamenetsky *et al.* 2004a).

Genetic transformation enables the incorporation of gene(s) of interest into the genome of desired plants. Recently, *Agrobacterium*-mediated gene-transfer systems have been developed (Kondo *et al.* 2000; Zheng *et al.* 2003, 2004) for garlic, and the first transgenic Bt garlic was generated (Zheng *et al.* 2004). This application is undoubtedly a great addition to the tools currently available to breeders and will open the way to a further increase in the variation in garlic, especially with regard to traits not available in this plant's genome.

## GENETIC RESOURCES

N.I. Vavilov and H.V. Harlan (Pistorius 1997) were the first to warn about the adverse impact of genetic erosion on crop plants. However, worldwide preservation of plant genetic resources (PGR) became an important issue for the sustainability of crops only in the late sixties of the 20<sup>th</sup> century,

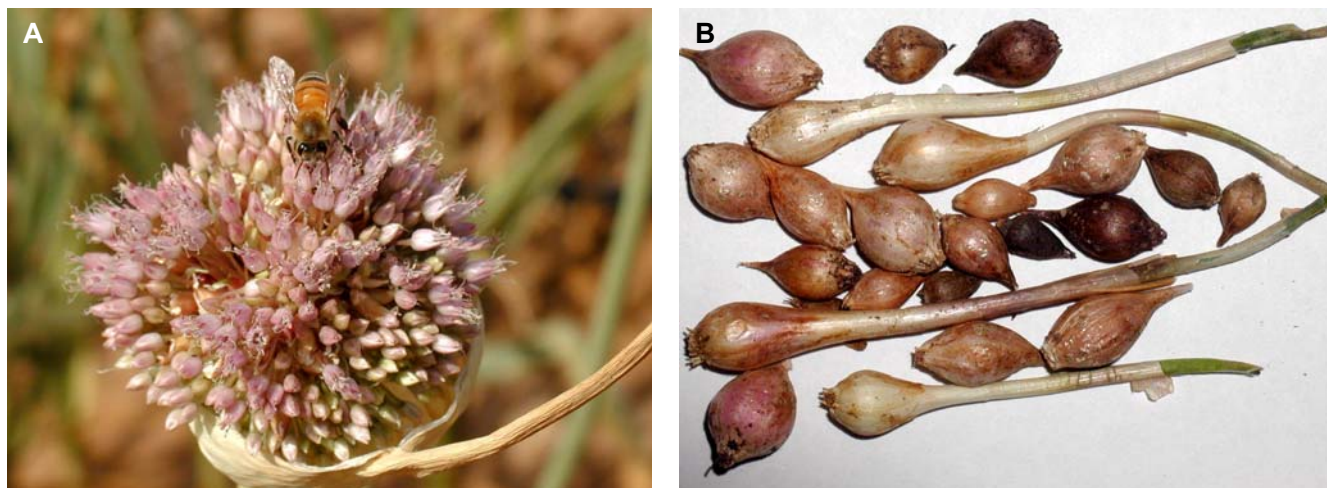
and little effort was invested in the collection and conservation of *Allium* species until about 30 years ago (Astley *et al.* 1982, Astley 1990). Only then did the conservation of *Allium* germplasm begin, with special emphasis on the bulb onion. Later garlic, leek and other alliaceous crops and their wild relatives were also included.

Both *in situ* and *ex situ* management strategies can be applied in PGR preservation. The former has not been employed for garlic and its wild relatives. Field genebanks of garlic were established in Germany, Israel, Poland, Russia, and the USA (Astley 1990), as well as in Turkey and Syria. However, little efforts have been invested in the systematic collection and preservation of wild *Allium* species with potential economic value (Rabinowitch and Zeltzer 1984; Kamenetsky 1993; Baitulin *et al.* 2000; Fritsch 2001; Keller and Senula 2001). Currently the largest collections of wild relatives of garlic are maintained in Germany (IPK, Gatersleben), the UK (RBG, Kew), Poland (Skiermiewice) and USA (Pullman, Washington). Both, preservation *in vitro* (Novak, 1990; Keller and Lesemann 1997; Pateña *et al.* 1998; Senula *et al.* 2000; Volk *et al.* 2004b) and cryopreservation (Keller 2002; Keller *et al.* 2006) were proposed as alternatives to *ex situ* garlic germplasm maintenance, but neither were applied widely in genebanks worldwide due to the costs involved and the uncertainty of regeneration.

Genetic diversity in garlic field collections has been revealed by morphological, developmental, biochemical, and molecular differences (Pooler and Simon 1993b; Lallemand *et al.* 1997; Hannan and Hellier 1998; Hong *et al.* 2000; Bradley *et al.* 2001; Xu *et al.* 2001; Volk *et al.* 2004a; Kamenetsky *et al.* 2005). Isozyme and molecular analyses showed considerable genetic diversity within the garlic species complex (Pooler and Simon 1993a; Maaß and Klaas 1995; Al-Zahim *et al.* 1997; Jenderek *et al.* 1997; Hong 1999; Ipek *et al.* 2003), but similar zymograms were obtained for various morpho-physiological groups. Thus, several cultivars from Japan and northern China, with differing growth characteristics, display a similar enzymatic pattern (Messiaen *et al.* 1993). In Brazil, a great morphological var-



**Fig. 1** Garlic collection sites of garlic in Central Asia. (From: Kamenetsky R, London Shafir I, Khassanov F, Kik C, van Heusden AW, Vrieling-van Ginkel M, Burger-Meijer K, Auger J, Arnault I, Rabinowitch HD (2005) *Biodiversity and Conservation* 14, 281-295, with kind permission of Springer Science and Business Media).



**Fig. 2** Reproductive potential of garlic fertile accessions from Central Asia. (A) Formation of numerous fertile flowers in the inflorescence, accessions #3028; (B) Bulbs formed by garlic plants grown from seeds (Photo: R. Kamenetsky).

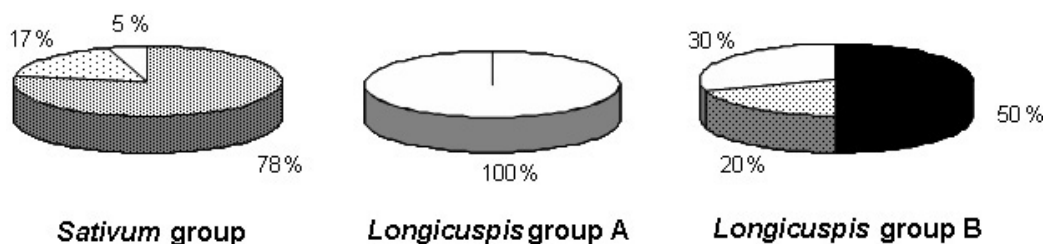
iation was recorded among 73 clones, but isozyme analysis revealed that they can all be clustered into two groups (Siqueira *et al.* 1985). It has become evident that the assessment of garlic diversity with isozymes and RAPD markers agrees only with the general morphological observations, but fails to discriminate individual clones. Later, the introduction of AFLP techniques further improved our ability to identify genetic diversity in plants. Comparing results from AFLP, RAPD and isozymes techniques showed that the former analyses exhibit abundant polymorphism in garlic, and demonstrated genetic diversity among closely related clones, which could not be differentiated by isozymes and RAPD markers (Ipek *et al.* 2003). Hence, AFLP evaluation of 211 garlic accessions from the USDA's National Plant Germplasm System (NPGS) and from commercial growers showed 64% and 41% duplications, respectively, and yet revealed substantial diversity in the collections, which is largely consistent with the major phenotypic classes (Volk *et al.* 2004a). This information is important for garlic researchers, germplasm collections, and growers, interested in garlic diversity.

Central Asia is recognized to be the main center of origin, and is home to the richest genetic diversity of many *Allium* species. It is therefore of paramount importance that germplasm from this area be preserved (Etoh 1986; Hanelt 1990; Etoh 1997; Simon 2003; Kamenetsky *et al.* 2004a). In the early 1980s, expeditions to Uzbekistan, Tadjikistan, Kirgizistan, and Kazakhstan collected a number of garlic bulbs (Etoh *et al.* 1988). The collection was grown in Kagoshima, Japan, and following topsets' excision, 17 clones developed fertile flowers. During 1995-2001, a number of collecting missions to Central Asia gathered over 300 garlic accessions from landraces and natural populations (Baitulin *et al.* 2000; Kamenetsky *et al.* 2004a; Kik *et al.* 2004; Kamenetsky *et al.* 2005; **Fig. 1**). Evaluation of the collections for horticultural and economic traits revealed a great inter-

clonal variation in most vegetative (e.g. leaf number, bulb size and structure), and reproductive traits (e.g. scape elongation and inflorescence development). A clear distinction was made between incomplete-bolting and bolting clones; most of the latter produced flowers with fertile pollen and receptive stigma (Kamenetsky *et al.* 2004a, 2005; **Fig. 2**). Large variation was recorded with regard to a differentiation of topsets, their size, number and rapidity of development. Furthermore, significant variation in organo-sulphur compounds (alliin, isoalliin, allicin and related dipeptides) was detected within the garlic collections and between plants grown under different environmental conditions (Kamenetsky *et al.* 2004a, 2004b; Kik *et al.* 2004; Kamenetsky *et al.* 2005). AFLP fingerprinting revealed three distinct groups within these collections, differing also in flowering ability and organo-sulphur content (Kamenetsky *et al.* 2005; **Fig. 3**).

Biochemical and molecular studies confirmed the highest level of heterogeneity within the Central Asian group (Pooler and Simon 1993a; Maaß and Klaas 1995; Hong 1999), which is the most important source of garlic genetic variation (Etoh and Simon 2002; Simon 2003; Kamenetsky *et al.* 2004a, 2005). The *Longicuspis* gene pool is less known outside Central Asia, but may contain genes of interest for future use, both in genetic studies and in plant improvement programs. In Central Asia, unique landraces are still grown in backyard gardens and small commercial plots. However, the increasing imports of cheap garlic from China has resulted in the elimination of local production, the abandoning of landraces at an alarming rate, and the consequent disappearance of the vegetatively propagated garlic diversity, one of the world's richest treasure, which is being lost forever.

There is no point in trying to reverse the economic wheel, however, an immediate international effort for the collection and preservation of this heritage is imperative. This program should be followed by a concerted evaluation,



**Fig. 3** Distribution of garlic accessions with different blooming performance within phylogenetic groups, as delimited by AFLP fingerprinting. Plant material was collected in Central Asia in 2000 and grown in Israel. Separation was made into non-bolters, incomplete bolters; bolters producing mainly topsets (T>F) and bolters producing mainly flowers (F>T). (From: Kamenetsky R, London Shafir I, Khassanov F, Kik C, van Heusden AW, Vrieling-van Ginkel M, Burger-Meijer K, Auger J, Arnault I, Rabinowitch HD (2005) *Biodiversity and Conservation* 14, 281-295, with kind permission of Springer Science and Business Media).

carried out by researchers in a variety of disciplines, aiming at a complete morphological, developmental, molecular and neutraceutical characterization, for the benefit of growers and consumers alike. Concomitantly, progress in the restoration of garlic fertility, seed production and the understanding of garlic seed physiology will enable to preserve the diversity in seed genebanks.

Utilization of both classical and novel genetic tools, including molecular markers for yield and quality traits and gene transfer (Ipek *et al.* 2003, 2005; Zheng *et al.* 2004; Zewdie *et al.* 2005), deepening our genetic and physiological knowledge (Etoh and Simon 2002; Kamenetsky and Rabinowitch 2002, 2006; Rotem *et al.* 2007) and that of inherent control mechanisms of vital processes in garlic, such as growth and development, florogenesis, pollination, fertilization and seed production and seed germination, are essential for the improvement of this important crop, as well as its adaptation to a wider range of climatic conditions and introduction of tolerance traits to biotic and abiotic stress.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the partial financial support of the European Union as this research has been carried out in the context of the EU-FP5 project Garlic & Health (project number QLK-CT-1999-00498; overall project coordinator C. Kik, plant theme coordinator R. Kahane and health theme coordinator R. Gebhardt). We also acknowledge kind permission of Springer Science and Business Media for figure publication.

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