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Diversity in fertility potential and organo-sulphur compounds among garlics from Central Asia

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Abstract. Extending the collection of garlic (*Allium sativum* L.) accessions is an important means that is available for broadening the genetic variability of this cultivated plant, with regard to yield, quality, and tolerance to biotic and abiotic traits; it is also an important means for restoring fertility and flowering. In the framework of the EU project 'Garlic and Health', 120 garlic accessions were collected in Central Asia – the main centre of garlic diversity. Plants were documented and thereafter maintained in field collections in both Israel and The Netherlands. The collection was evaluated for biological and economic traits. Garlic clones vary in most vegetative characteristics (leaf number, bulb size and structure), as well as in floral scape elongation and inflorescence development. A clear distinction was made between incomplete bolting and bolting populations; most of the accessions in the latter populations produced flowers with fertile pollen and receptive stigma. Wide variations were recorded with regard to differentiation of topsets, their size, number and rapidity of development. Furthermore, significant variation in organo-sulphur compounds (alliin, isoalliin, allicin and related dipeptides) was found within garlic collections and between plants grown under differing environmental conditions. Genetic fingerprinting by means of AFLP markers revealed three distinct groups within this collection, differing also in flowering ability and organo-S content.

Abbreviations: AFLP^{TM} – amplified fragment length polymorphism; HPLC-UV – high performance liquid chromatography ultra violet; PCA – principal component analysis; alliinase – S-alkyl(en)yl-L-cysteine sulphoxide lyase; alliin – (+)-S-allyl-L-cysteine sulphoxide; isoalliin – S-(*trans*-1-propenyl)-L-cysteine sulphoxide; GLUALCS – γ-glutamyl-S-allyl-cysteine; isoGLUALCS – γ-glutamyl-S-(*trans*-1-propenyl)-cysteine; allicin – diallyl thiosulphinate.

Introduction

Many *Allium* species are consumed as fresh or processed condiments, are used as ornamentals, or provide sources of natural therapeutic products. Next to onion, garlic (*Allium sativum* L.) is the second most widely used *Allium* worldwide. Garlic

leaves and young inflorescences are consumed as green vegetables, while the fresh bulbs serve as a popular condiment and are also used as a flavouring agent in many processed foods. Furthermore, large quantities of garlic are consumed as a functional food or for pharmaceutical purposes (Kik et al. 2001).

Garlic is a sterile plant, which is propagated vegetatively, therefore genetic variation can be increased only by somaclonal variation, induced mutations or genetic transformation (Novak 1990; Burba 1993; Kondo et al. 2000). Restoration of fertility and, therefore, of sexual reproduction would permit genetic studies and classical breeding of garlic. In addition, fast propagation of desired genotypes via true seeds would be expected to result in reduction of storage costs and fewer injuries caused in the production field by viruses, diseases and pests transmitted by infected propagules.

Fertility restoration in garlic has been attempted by many researchers (e.g., Kononkov 1953; Novak and Havranek 1975; Konvicka 1984; Etoh et al. 1988; Pooler and Simon 1994; Kamenetsky et al. 2004), and the presence of topsets in the inflorescence has been suggested to be one of the major causes of sterility. In 1980s, fertile garlic was collected in the Tien Shan Mountains between Kazakhstan and China (Etoh 1986; Etoh et al. 1988). Pooler and Simon (1994) improved seed set by scape detachment and removal of topsets, but seed germination was low, ranging between 10 and 12%. Later, Inaba et al. (1995), Jenderek (1998) and Kamenetsky et al. (2003) selected some fertile lines and obtained 50,000, 1.2 million, and hundreds of thousands viable garlic seeds, respectively.

A significant trait of garlic and other *Alliums* is the specific odour of organo-sulphur compounds, the composition and amount of which are strongly affected by genetic and environmental factors (for a recent review, see Randle and Lancaster 2002). The precursors of most organo-S compounds in *Alliums* are stable non-volatile odourless amino acids, including S-alk(en)yl cysteine sulphoxides and related storage dipeptides. Garlic is richer in alliin [(+)-S-allyl-L-cysteine sulfoxide] than most other plants in the genus; in fresh bulbs its concentration reaches 1.4% (Koch and Lawson 1996; Keusgen 2002). When fresh tissue is disrupted, the sulphur compounds are hydrolysed by alliinase (S-alkyl(en)yl-L-cysteine sulphoxide lyase) via fast and complicated metabolic pathway(s), to release a complex of reactive organosulphur compounds with characteristic flavour and striking bioactivity. The main compound, allicin (diallyl thiosulphinate) is unstable at room temperature. It is, however, the main source for the typical flavour of garlic (Lancaster and Boland 1990). In onion, significant differences in flavour and pungency were attributed to differences in sulphur uptake as well as in essential components of the sulphur metabolic pathway (Randle and Lancaster 2002). Similar explanations may be applicable to the significant differences between garlic cultivars.

Modern taxonomy subdivides the world's garlic germplasm into five distinct groups: *Sativum*, *Ophioscorodon*, *Longicuspis*, Subtropical and Pekinense (Fritsch and Friesen 2002). The *Longicuspis* group from Central Asia is recognised as the most primitive, the one from which the other groups were derived (Maaß and Klaas 1995; Etoh and Simon 2002; Fritsch and Friesen 2002). Central Asia was hypothesised to be the primary centre of garlic evolution and diversity (Fritsch and Friesen 2002), and recent studies on primitive garlic types in the Tien-Shan Mountains strongly support this assumption (Etoh 1986; Kotlinska et al. 1991;

Figure 1. Collecting sites in Uzbekistan, Kazakhstan, Kirgizia and Tadjikistan in 2000. The original records on collection sites and local environments are kept in Bet Dagan, Israel.

Kamenetsky et al. 2003). It is to be expected that considerable variation in garlic germplasm is present in this region, and that it should be manifested in resistance/tolerance to pests and diseases, better adaptation to abiotic stresses, as well as traits related to sexual reproduction and quality.

In order to develop a highly varied garlic collection, which is of pivotal importance for the future development of the crop, missions to Central Asia were undertaken. In the present paper we report on the assessment of a collection in Central Asia by means of amplified fragment length polymorphism (AFLP) markers, developmental traits, and contents of various organo-S-compounds.

Materials and methods

Collection

Garlic bulbs of 120 accessions were collected in May–September 2000 in local village markets in Central Asia (Figure 1). All details of collection sites and local environments were recorded in compliance with IPGRI plant passport regulations (Astley et al. 1982). Original records are kept by R.K., Bet Dagan, Israel.

Evaluation for vegetative and generative traits

For evaluation, the collection was planted both in The Netherlands and in Israel. Photoperiod and air temperatures during the respective growing periods are presented in Figure 2. In The Netherlands, individual cloves were planted in

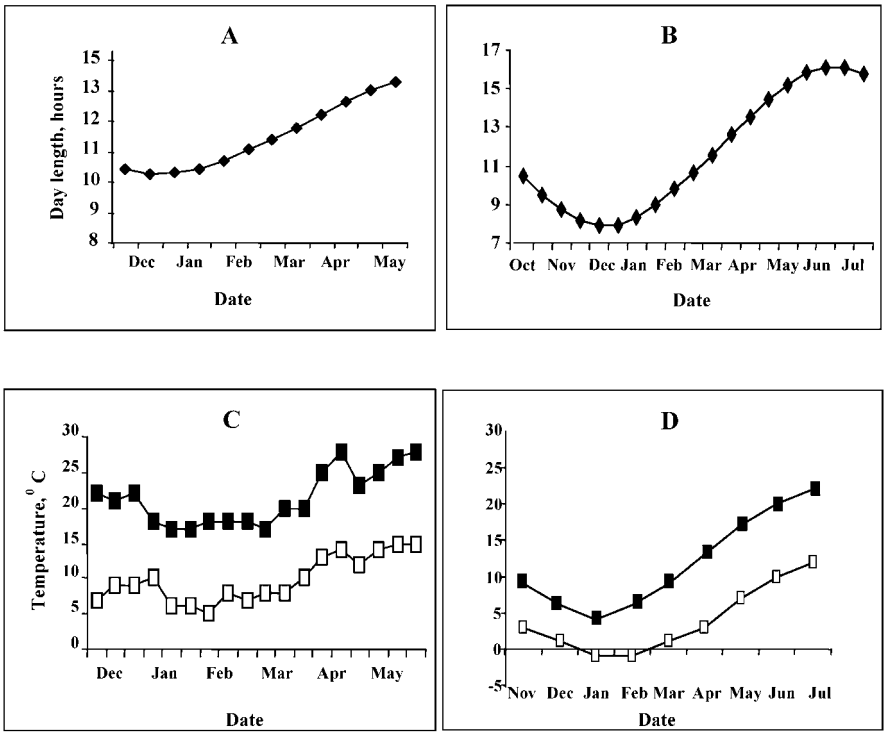


Figure 2. Daylength in Israel (A) and The Netherlands (B) and averaged weekly mean maximum and minimum temperatures in Bet Dagan, Israel (C) and Wageningen, The Netherlands (D) during the respective garlic growing seasons.

October 2000 and 2001 in an experimental plot in Plant Research International, Wageningen. Mature bulbs were harvested in August–September. Standard agricultural practice was employed throughout.

In Israel, in October–November 2000 and 2001, bulbs were stored at 5 °C, RH 65–70%, for 8 weeks. Thereafter, individual cloves were planted in a designated plot in the Experimental Farm of the Faculty of Agricultural, Food and Environmental Quality Sciences of the Hebrew University of Jerusalem, in Rehovot. Standard agricultural practice was employed throughout. Phenological records were taken in Israel between November and July, in 2001 and 2002; they included leaf number prior to bolting, maximal scape length at bolting; date of spathe opening; topset/flower ratio in the inflorescence, and bulb structure. Statistical analysis of morphological and phenological characteristics was based on one-way ANOVA, and Tukey's test and Student's *t*-test were used for mean separation.

Pollen germination ability was assessed in Israel in May–July 2001 according to Hong and Etoh (1996). Stamens were collected at anthesis, and crushed in Petri dishes containing 1% agar + 15% sucrose, and pollen germinability was determined

using a light microscope, following a 2- to 3-h incubation in daylight at 23–26 °C. Stigma receptivity was determined according to Dafni and Maues (1998). Macherey-Nagel Peroxtesmo Ko peroxidase test paper (15 mm × 15 mm) was soaked in 1 ml distilled water, and 1–2 droplets of the solution were applied directly onto freshly harvested mature stigmas. The development of blue colour indicated stigma receptivity.

Seed viability

Seeds were harvested upon maturation, dried out, threshed and stored in tightly sealed plastic bags at 20 °C. In October, garlic seed viability was assessed using the tetrazolium staining test. Seeds were imbibed overnight in a beaker containing tap water at 20–25 °C, in the dark. The flat side of the seed coat was carefully scratched with a sharp scalpel, avoiding the endosperm and the embryo. Incised seeds were placed in 1% water solution of 2,3,5-triphenyl tetrazolium chloride, and incubated in the dark at 30 °C for 12 h. Thereafter, the embryos of treated seeds were exposed, and the intensity of the red staining of the entire embryo, root-tip and endosperm correlated with viability (Peters 2000).

DNA isolation and AFLP fingerprinting

To assess the diversity of the collected clones, AFLP fingerprinting based on one primer enzyme combination (E36M52A, E36 = GAC TGC GTA CCA ATT CAC C, M52 = GAT GAG TCC TGA GTA ACC C) was performed in The Netherlands. The assay resulted in amplification of 80–120 fragments in each of the tested garlic accessions. The 20 clearest markers were analysed with the PHYLIP (Phylogeny Inference Package) software package, Version 3.5c. The complete AFLP-fingerprint comparison was based on the presence or absence of each of the 20 selected bands.

In order to compare the grouping of the garlic germplasm by means of AFLP markers with the groupings previously determined with isozyme and RAPD markers (Maaß and Klaas 1995), an initial AFLP fingerprinting (van Heusden et al. 2000) was made on 30 accessions from the garlic collection in Gatersleben, Germany. This comparison resulted in a similar classification of garlic accessions to that made by Maaß and Klaas (1995). Therefore, the unique primer enzyme combination was used to assess the variability in the garlic accessions collected in Central Asia.

Organo-S compound analysis

A recently developed high performance liquid chromatography (HPLC) method for the simultaneous measurement of organo-S compounds was employed (Arnault

et al. 2003) in September 2002 on freshly harvested bulbs of 15 accessions from The Netherlands. In addition, comparison was made between five identical accessions, grown in parallel in Israel and The Netherlands. The mature, freshly harvested bulbs were sent to France in July and September 2002, respectively. S-compound analysis was performed 4 weeks after harvest. Five bulbs of each accession served as replications for the analysis of the following organo-S compounds under alliinase-inhibiting condition: alliin [(+)-S-allyl-L-cysteine sulphoxide], isoalliin (S-(trans-1-propenyl)-L-cysteine sulphoxide), GLUALCS (γ-glutamyl-S-allyl-cysteine), isoGLUALCS (γ-glutamyl-S-(trans-1-propenyl)-cysteine), and allicin (diallyl thiosulphinate).

Sampling procedure: 1 g of the fresh bulb was homogenised with 3 ml methanol/water (80/20) v/v + 0.05% formic acid (pH < 3) at room temperature. An aliquot was diluted 10 times and filtered through a polyvinylidene difluoride (PVDF) membrane with 0.2 µm pore diameter. A 15-µl sample of the filtrate was injected into an HPLC column. Analysis of garlic extracts was performed with a Waters 616 pump, a DAD 996 diode-array detector and a Waters 717 autosampler (Waters Corporation, Milford, MA, USA). Compounds were separated on a 150 mm × 3 mm i.d. × 3 µm particle-size Hypurity Elite C18 column (Thermo Quest), at 38 °C (Thermo Hypersil Division, Keystone, Bellefonte, PA, USA) and a UV detector was operated at 208 nm. The eluate flow was 0.4 ml/min. The mobile phase consisted of: A, 20 mM sodium dihydrogen phosphate + 10 mM heptane sulphonic acid, pH 2.1 (adjusted with 85% orthophosphoric acid); and B, acetonitrile −20 mM sodium dihydrogen phosphate + 10 mM heptane sulphonic acid, pH 2.1 (50/50). The elution programme was in gradient mode. Data acquisition was done with Millennium software (Waters Corporation). Principal Component Analysis (PCA) (Wold et al. 1987) was used for statistical data analysis to show separation among the different accessions. Statistical analyses were based on Nested ANOVA, followed by Tukey's HSD test, and on two-way ANOVA, followed by contrast tests for each accession.

Results and discussion

Assessment of variation within the collection by AFLP fingerprinting

A single AFLP primer combination was used for classification of 111 garlic accessions from Central Asia. The separation into two distinct groups, namely *Sativum* (86 accessions) and *Longicuspis* (25 accessions) was immediately obvious, and further separation of the *Longicuspis* group, by the same AFLP primer combination, into two subgroups of 15 and 10 accessions, respectively, was evident (Figure 3).

Sativum group. In addition to 82 accessions with identical and complete AFLP fingerprinting patterns, four accessions were also classified as members of the *Sativum* group, despite the facts that two of them were short of two AFLP

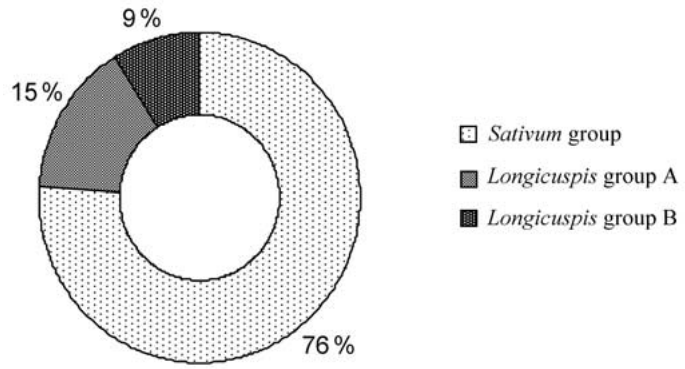


Figure 3. Classification of garlic accessions collected in Central Asia in 2000 according to AFLP fingerprinting.

fragments, a third lacked one of these two fragments, and the fourth had an additional fragment. The 86 clones were analogous to the *Sativum* group II a,b in the classification by Maaß and Klaas (1995).

Longicuspis subgroup A. Fifteen accessions exhibited identical and complete AFLP fingerprinting patterns, which differed distinctly from that of the *Sativum* group. These plants were identified as a *Longicuspis* group I or IV in agreement with the Maaß and Klaas (1995) classification, but we were not able to determine conclusively whether it was subgroup I or IV.

Longicuspis subgroup B. Ten additional accessions had identical and complete AFLP patterns, which differed from the above two, and they were identified as belonging to Longicuspis group IV according to the classification of Maaß and Klaas (1995).

Modern taxonomy sub-classifies the global *A. sativum* species complex into five groups (Fritsch and Friesen 2002), yet only two phylogenetic groups – *Sativum* and *Longicuspis* – were distinguished in the collection examined in the present study. Originally from the Mediterranean basin, selections of the *Sativum* group were adopted by growers worldwide, and thus now form the major element of the global garlic trade. The 76% representation of the *Sativum* group in the collection from Central Asia indicates the rapidly growing dominance of garlic varieties of the *Sativum* group from neighbouring China – the world's most important producer and exporter of garlic (FAO 2001). The smaller *Longicuspis* group, originated in Central Asia, is considered as a primitive and more diverse group than *Sativum* (Maaß and Klaas 1995). The *Longicuspis* gene pool is less known outside Central Asia, and it may contain genes of interest for future use in genetic studies, as well as for plant improvement programmes. In Central Asia, unique *Longicuspis* accessions are still maintained in backyard gardens and by small farmers. However, because of the inflow of cheaper garlic from China, these local landraces are being abandoned at

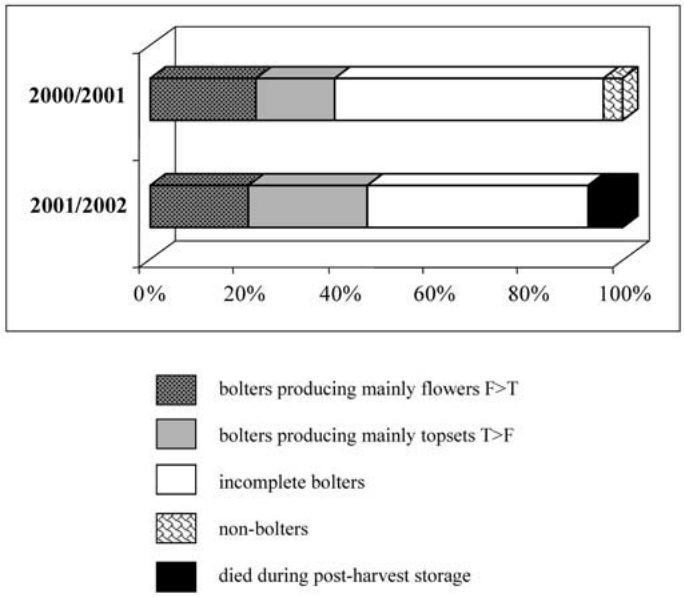


Figure 4. Subdivision of the garlic collection from Central Asia according to the blooming habit in Israel in 2000/2001 and 2001/2002. Group separation was based on scape elongation and on inflorescence morphology, as follows: bolters producing mainly flowers; bolters producing mainly topsets; incomplete bolters; non-bolters; died during post-harvest storage.

an alarming rate, and the world richest treasure of garlic diversity is being lost – forever. Immediate action should be taken to collect, protect and evaluate the remains of the genetic diversity of this taxon before it is completely lost.

Evaluation of the garlic collection for vegetative and generative traits

In Israel, phenological studies of 120 garlic accessions have revealed four subpopulations: (a) non-bolters; (b) incomplete bolters, producing short scapes with aborted inflorescence; (c) bolters that produce scapes and mainly topsets in the inflorescence ($T > F$); and (d) bolters that produce scapes and mainly flowers in the inflorescence ($F > T$).

In 2000/2001, five accessions produced neither scapes nor bulbs under the Israeli environmental conditions and died at the end of the growing season (Figure 4). Four additional accessions were lost during post harvest storage. During the 2 years of evaluation in Israel, the distribution of blooming performance within the collection was rather stable (Figure 4). Most accessions were characterised as incomplete bolters ($n = 68$ and 56 in 2000/2001 and 2001/2002, respectively). In the second season the number of bolters with prolific production of topsets increased from 20 in 2000/2001 to 30 in 2001/2002. All accessions with fertile flowers maintained their blooming habit in the second season ($n = 27$ of 120 and 25 of 111 accessions

Table 1. Phenotypic expression of garlic accessions collected in Central Asia in 2000, and grown in Israel in 2000/2001. Means followed by the same letter do not differ at 5% significance.

Blooming habit	Number of accessions	Leaf number prior to bolting	Bulb diameter (cm)	Number of cloves in the outer whorl
Incomplete bolters	68	10.4 ± 0.1a	5.7 ± 0.1a	9.4 ± 0.1a
Bolters, producing mainly topsets	20	10.6 ± 0.3a	5.7 ± 0.2a	9.2 ± 0.2ab
Bolters, producing mainly flowers	27	14.1 ± 0.2b	5.1 ± 0.1b	8.8 ± 0.2b

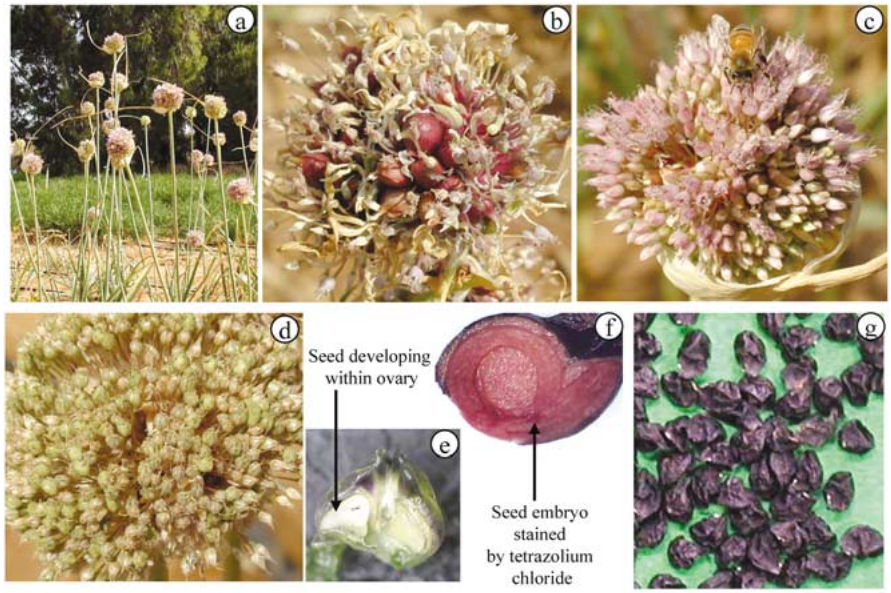
in 2000/2001 and 2001/2002, respectively), thus indicating the dominance of the genetic control on flowering in garlic.

Leaf number prior to the transition from the vegetative to the reproductive state was significantly higher in bolters with fertile flowers than in the other plants, yet the bulbs of the former were smaller and contained fewer cloves in the outer whorl than the latter (Table 1). In bolting plants (Figure 5a), the formation of flowers and topsets was almost complete when the stalks reached about 30 cm in length, and spathe break occurred when the scapes were 35–50 cm long. At this point, the differentiated flowers were visible to the naked eye. In the 20 bolting accessions, in which inflorescences were characterised as $T > F$, developing topsets intermingled with and physically strangled the young flower buds, thus causing their degeneration (Figure 5b). In 27 accessions, umbels were almost topset-free (Figure 5c). Most of the flowers in these inflorescences produced viable pollen and receptive stigmas, and seed setting was evident (Figure 5d,e). Seed viability was confirmed using the tetrazolium chloride staining test. Most plants produced between 100 and 500 seed per umbel without the removal of topsets (Figure 5 f,g).

In The Netherlands, most accessions were characterised as bolters with high ratios of topsets to flowers ($T > F$) (data not shown), thus emphasising the effect of environment on floral expression (Kamenetsky et al. 2004).

In garlic, flower differentiation occurs in plants exposed to short photoperiod and low temperatures (Kamenetsky et al. 2004), a set of environmental conditions common in the main growing season in Israel, which is the winter. However, conditions in The Netherlands, where plants are grown during summer under a long photoperiod, are not conducive to flowering of garlic. This accounts for the main differences in performance of the same genotypes in Israel and in The Netherlands, respectively.

We have tested the relationship between phylogenetic affiliation as determined by AFLP fingerprinting, bolting ability and flower development (Figure 6). Most accessions in the *Sativum* group were characterised as incomplete bolters (Figure 6a), yet 19 out of 86 produced scapes and umbels. Incomplete bolters were not found among two *Longicuspis* subgroups, and all plants of the subgroup A produced only inflorescence with viable flowers (Figure 6b), whereas half of the accessions in subgroup B were categorised as non-bolters under Israeli conditions



a - bolting garlic plants at full flowering, May 2001

b - developing topsets intermingled with the young flower buds, thus causing their degeneration

c - topset-free umbels with normal flowers, producing viable pollen and receptive stigmas

d -seed setting in garlic inflorescence

e - seed development inside the enlarged ovary

d - seed viability confirmed using the tetrazolium chloride staining test, October 2001

g – matured garlic seeds after harvest, July 2001

Figure 5. Inflorescence and seed development in bolting garlic of the *Longicuspis* group in Israel.

(Figure 6c). We thus conclude that the unique AFLP primer-enzyme combination may be used for mass selection of garlic with flowering ability.

Assessment of organo-S compounds

The contents of five organo-S compounds were analysed in bulbs of 15 accessions grown in The Netherlands in 2001/2002. Six of these accessions belonged to the *Sativum* group and nine to the *Longicuspis* group. The results of a principal component analysis (PCA) demonstrated a clear distinction in organo-S contents between the two phylogenetic groups (Figure 7). The first two PCA axes described 84% of the total variance, thus indicating that these two axes represent the majority of the variation for the analysed organo-S compounds in the garlic germplasm.

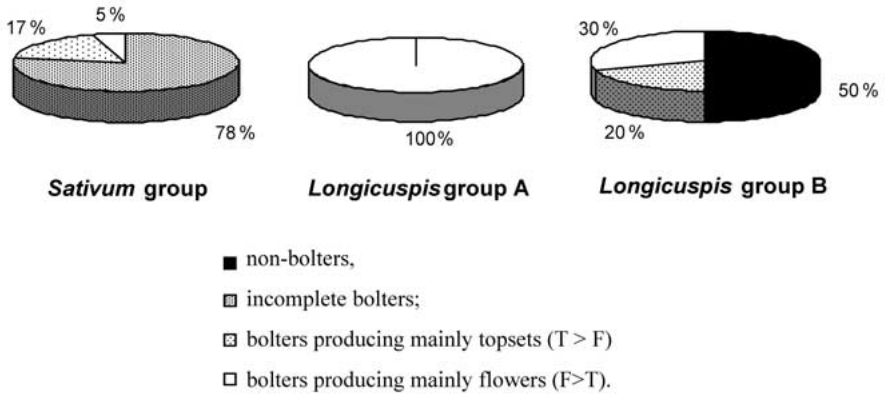


Figure 6. Distribution of garlic accessions with different blooming performance within phylogenetic groups, as delimited by AFLP fingerprinting. Separation was made into non-bolters, incomplete bolters, bolters producing mainly topsets ($T > F$) and bolters producing mainly flowers ($F > T$). Plant material was collected in Central Asia in 2000 and grown in Israel.

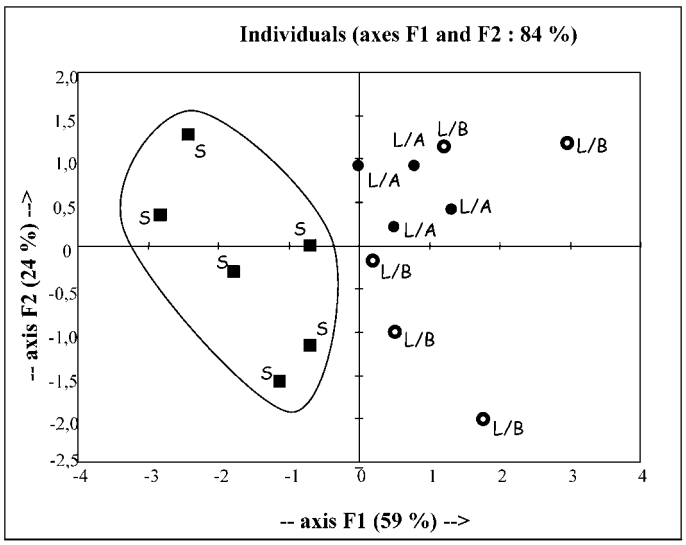


Figure 7. Principal component analysis based on the variation of five organo-S compounds for 15 garlic accessions. Bulbs were collected in Central Asia in 2000 and grown in The Netherlands in 2001/2002. Measurements were made in September, on mature, freshly harvested bulbs of the *Sativum* (S), and *Longicuspis* A (L/A) and *Longicuspis* B (L/B) subgroups.

The quantification of five organo-S compounds: alliin, isoalliin [details in Materials and methods], allicin, GLUALCS, and isoGLUALCS from 15 garlic accessions reconfirmed the separation of our collection into two distinct phylogenetic

Table 2. Quantification of five organo-S compounds in 15 garlic accessions and their separation into the *Sativum* and *Longicuspis* phylogenetic groups. Plant samples from Central Asia were grown in The Netherlands in 2001/2002. Partition into phylogenetic groups was based on AFLP fingerprinting (Figure 3). Separation of organo-S compounds in mature freshly harvested bulbs and quantification (nmol/mg fresh weight) were done in September–October 2002 by means of HPLC-UV. Statistical analysis is based on Nested ANOVA, followed by Tukey's HSD test. Means followed by similar letters do not differ at 5% significance.

Phylogenetic grouping	Alliin	Isoalliin	GLUALCS	IsoGLUALCS	Allicin
Sativum group ($n=6$)	40.5 ± 2.38a	1.6 ± 0.36a	6.7 ± 0.24a	15.0 ± 0.57a	9.6 ± 0.74a
Longicuspis subgroup A ($n=4$)	48.4 ± 3.60ab	0.3 ± 0.17b	15.3 ± 0.96b	19.8 ± 1.67b	7.1 ± 0.33b
Longicuspis subgroup B ($n=5$)	54.9 ± 2.44b	0.6 ± 0.09b	9.8 ± 0.41c	18.3 ± 0.58b	8.5 ± 0.54ab

Table 3. Environmental effects on the content of alliin, isoalliin, GLUALCS, isoGLUALCS and allicin in five garlic accessions. Bulbs from the Central Asia collection were grown in parallel in Israel and The Netherlands in 2001/2002. Assays for organo-S compounds and quantification (nmol/mg fresh weight) were made in mature bulbs four weeks after harvest, in July–September 2002.

Location	Alliin	Isoalliin	GLUALCS	IsoGLUALCS	Allicin
The Netherlands	40.5 ± 2.75	1.7 ± 0.44	6.8 ± 0.28	15.0 ± 0.66	9.7 ± 0.86
Israel	42.3 ± 2.26	0.7 ± 0.16	20.7 ± 1.60	22.3 ± 0.67	19.6 ± 0.95

groups (Table 2). The two phylogenetic groups *Sativum* and *Longicuspis* differed significantly in their contents of organo-S compounds. Bulbs of the former group had smaller amounts of alliin and of the dipeptides GLUALCS and IsoGLUALCS, and higher concentrations of isoalliin and allicin than those of the *Longicuspis* A and B subgroups. The major differences between the phylogenetic groups were determined mainly by the differences in the dipeptide and alliin contents.

Previous methods used for comparison of organo-S compounds in garlic referred to only a single component, mainly alliin (Mochizuki et al. 1989; Kubec et al. 1999). In the present study, however, we employed a novel HPLC system (Arnault et al. 2003) which allows simultaneous measurements of all the organo-S compounds in the biosynthesis chain, from the original dipeptides to the final flavour-volatile products. This method thus provides a detailed and complete account of the organo-S compounds throughout the metabolic system, and can be used in studying genetic and/or environmental effects on S-metabolism in plant tissues.

Effect of environmental conditions on organo-S content in garlic accessions

Compositions of organo-S compounds were compared in five accessions grown in parallel in Israel and in The Netherlands during two seasons (Table 3). These results

indicate the importance of the environmental conditions on the secondary metabolism in garlic. Marked differences were found in allicin, GLUALCS, and isoGLUALCS contents, all of which were higher in the Israel-grown bulbs, but alliin contents were quite similar, and the isoalliin content was slightly higher in the bulbs grown in The Netherlands.

The dipeptides and allicin contents of the bulbs from Israel were 150–300% of those in the same genotypes from The Netherlands. The alliin contents were similar in all bulbs, and the isoalliin content was about twice as great in the Dutch-grown accessions as in the ones from Israel. The data for individual accessions show a very similar pattern (data not shown). Variability at the dipeptides level between accessions and growth locations were obvious for all accessions. Unexpectedly, alliin level was relatively consistent in all accessions from both growing sites, whereas allicin contents were significantly higher in most accessions grown in Israel.

These results clearly show the major and consistent environmental effect on organo-S compounds in garlic, which should be taken into account in quality assessments of garlic genotypes, and probably of other alliaceous crops (Randle and Lancaster 2002).

Conclusions

Cultivated garlic exhibits a wide variation in many vegetative and reproductive traits. Taxonomic studies categorise the *Longicuspis* group from Central Asia as the most primitive taxon within the species, and biochemical and molecular studies suggest that this group still maintains the highest level of intraspecific variation. It is, therefore, obvious that the *Longicuspis* group is the most important source of genetic variation within the species and thus requires special attention to ensure the utilisation of its unique traits in future improvement programmes (Pooler and Simon 1993a,b; Maaß and Klaas 1995; Lallemand et al. 1997; Hong 1999; Hong et al. 2000; Etoh and Simon 2002; Kamenetsky et al. 2003). In addition, Etoh (1986), Jenderek (1998), Pooler and Simon (1993a,b), Kamenetsky et al. (2003) and others reported on various levels of fertility in garlic plants from Central Asia. Our present study (Table 1, Figure 6) indicates that these are most probably members of the *Longicuspis* group, and that under appropriate environmental conditions the expression of flowering and the production of viable pollen and of receptive stigma are rather stable. In addition to yield and quality traits, and tolerance to biotic and abiotic stress conditions, this group seems to have conserved the genes responsible for complete reproductive expression – a trait of the utmost scientific and economic importance.

This precious gene-pool is, however, currently under severe threat of extinction, due to the very rapid replacement of the traditional landraces with modern cultivars of the *Sativum* group. An international effort in the very near future is imperative. It should comprise a large-scale rescue operation involving collection *in situ*, to be followed by an evaluation and preservation project, to halt the rapid and irreversible decline of the Central Asia pool of garlic landraces.

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