
ELSEVIER
SCIENTIA
HORTICULTURAE

Scientia Horticulturae 148 (2012) 47–54

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Agro-morphological markers and organo-sulphur compounds to assess diversity in Tunisian garlic landraces

Naouel Jabbes[a,*], Ingrid Arnault[b], Jacques Auger[b], Bouthaina Al Mohandes Dridi[a], Cherif Hannachi[a]

[a] Department of Horticultural Sciences and Landscape, Higher Institute of Agronomy, 4042 Chott Mariem, Tunisia
[b] CTU-Innophyt, Université François Rabelais, Faculté des Sciences et techniques, Avenue Monge, 37 200 Tours, France

ARTICLE INFO

Article history:
Received 23 June 2011
Received in revised form 12 July 2012
Accepted 13 August 2012

Keywords:
Allium sativum L.
Morphological characteristics
Yield
Organo-sulphur compounds
Variability
Correlations

ABSTRACT

This work is aimed at studying diversity of 31 garlic landraces from Tunisia and structuring this diversity. It helped at establishing correlations between content of organo-sulphur compounds and agro-morphological characteristics. For this purpose the quantitative variability of five organo-sulphur compounds, namely: alliin, isoalliin, glutamyl allyl cysteine (GluAlCs), isoglutamyl allyl cysteine (isoGluAlCs) and allicin is studied. The diversity of the accessions for number of leaves per plant, the pseudostem length, dry weight of bulb, weight of one clove, weight of one bulb, bulb diameter, number of cloves in one bulb, the yield and number of days to dormancy release, is assessed. Particular correlations related to the yield and to the organo-sulphur compounds were demonstrated. Genetic diversity was assessed between and within accessions using statistical analyses including coefficients of variation, one way analysis of variance, hierarchical cluster analysis (HCA) and principal component analysis (PCA).

Significant agro-morphological traits and organo-sulphur contents variations were found between accessions, except for the number of days for dormancy release (DDR). There is no correlation between total measured organo-sulphur compounds and any of the agro-morphological characteristics. The yield is highly influenced by the following characteristics: weight of the clove, the weight and the diameter of the bulb, the number of leaves per plant and the stem length. Total variance was well described by the first three PCA axes which represent 80.57% of the variance. The PCA and HCA distinguished two major groups of garlic. Clustering does not correspond to the geographical origin but it distinguishes clearly between coloured and light coloured accessions.

1. Introduction

Garlic (*Allium sativum* L.) is a bulbous perennial plant of the *Alliaceae* family, genus *Allium* (Fritsch and Friesen, 2002). The centre of origin of garlic has been considered to be Central Asia, with secondary centres of diversification in China and the Mediterranean area (Etoh and Simon, 2002). Several botanical varieties of garlic are described in the literature, including *A. sativum* var. *sativum* L. which rarely or never flowers, *A. sativum* var. *ophioscorodon* (Link) Doll, which regularly flowers and *A. sativum* var. *longicuspis* including the pekinense (Prokh) Makino, which rarely flowers, like *A. sativum* var. *sativum*, but has wider leaves (Simon and Jenderek, 2003). Several other garlic subspecies names have been used. Hanelt (1990) proposed two groups of *A. sativum*. A "common garlic group" consisting of *A. sativum* var. *sativum*, *A. sativum* var. *typicum* Rgl., and *A. pekinense* Prokh., and an "*ophioscorodon* (Link) Doll group", consisting of *A. sativum* var. *ophioscorodon* (Link) Doll, *A. ophioscorodon* Link, and *A. sativum* var. *controversum* (Schrad.) Moore jr. Fritsch and Friesen (2002) added a third "longicuspis group" consisting of *A. longicuspis* Rgl.

Garlic, well-known for its health benefits, is also consumed for its flavours and taste. These properties are the result of interactions between several components, namely, allyl cysteine sulphoxides (ACSO) and their intermediate metabolites and derivatives (Randle and Lancaster, 2002). The major ACSO present in garlic are S-2-propenyl-L-cysteine sulphoxide (alliin), S-trans-1-propenyl-L-cysteine sulphoxide (isoalliin) and S-methyl-L-cysteine sulphoxide (methiin). Dipeptides γ-glutamyl-alk(en)yl-cysteine are intermediates in the pathway of the ACSO, they are considered as storage compounds of nitrogen and sulphur. Major γ-glutamyles peptides in garlic are γ-glutamyl-S-2-propenyl cysteine (GluAlC), γ-glutamyl-S-trans-1-propenyl-cysteine (IsoGluAlC) and γ-glutamyl-S-methyl cysteine (Lawson, 1996).

When plant is crushed, enzyme alliinase hydrolyses ACSOs, producing the sulfenic acids. The 2-propenesulfenic acid, first product of the reaction, condenses to form the specific thiosulphinate of garlic, allicin.

* Corresponding author. Tel.: +216 73365620.
E-mail address: naouelj@yahoo.fr (N. Jabbes).

0304-4238/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2012.08.013

The biochemical characteristics of garlic are of great importance, seen the interest growing of the concept "food–health". Garlic and its cellular components, just as allicin and various others sulphur derivatives after lyses of the cells, have great qualities preventive and curative counter the most important plagues of our time, namely the cardiovascular diseases, cancer and the diabetes (Kock and Lawson, 1996; Tatarintsev et al., 1992). The antibiotic activity of garlic has been reported (Harris et al., 2001). Li et al. (2000) and Ang-lee et al. (2001) showed that S-allyl cysteine (SAC), an intermediate compound in the formation of ACSOs, induced hypoglycemiant effects. Its administration significantly decreases the lipidemy, the glycaemia and some enzymatic activities of blood (Sheela and Augusti, 1992). Garlic and its extracts had great importance for organic agriculture, it has insecticidal, nematicidal (Gupta and Sharma, 1993) and repellent effects against birds (Hile et al., 2004) and stored product pests (Rahaman and Motoyama, 2000).

Garlic presents, in spite of its vegetative multiplication, a great diversity from agro-morphological and biochemical point of view (Abdoli et al., 2009; Burba and Gomez Riera, 1997; Hong, 1999; Lallemand et al., 1997; Messiaen et al., 1993; Ovesna et al., 2007; Stavelìkova, 2008.). This variability is required, in fact, researchers benefit from the great diversity of garlic to select best genotypes. The genetic diversity serves as a source of genotypes adapted to local conditions (Baghalian et al., 2005).

In Tunisia, which is a Mediterranean country, cultivation and consumption of garlic has a long history, still, cultivation of garlic suffers from many problems that caused a low yield of 5 T/ha. Indeed, local and most south Mediterranean garlic are not classified horticulturally. Growers use their self regenerated garlic cloves and/or cloves from neighbours and there are no local commercialized cultivars with registered names. Tunisian garlic is threatened by genetic erosion, uncontrolled introduction of foreign germplasm and is under-exploited which may lead to its loss and the extinction of the local breed.

This research aimed at studying genetic diversity in Tunisian garlic landraces from the agro morphological and biochemical point of view and the relationship between these characteristics. The result of this study will provide basis for the efficient use of the local germplasm.

2. Materials and methods

2.1. Plant material

Garlic bulbs of 31 local accessions were collected from 25 localities all over Tunisia (Table 1) from 15 to 31 May 2003. Gardens and traditional small farms were the target of our sampling. The collection was planted in Higher Institute of Agriculture of Chott-Mariem in the province of Sousse (Tunisian coastal city 35°55′03″N and 10°33′48″E), for 2 years (2003–2005). For each accession 40 cloves, weighing more than 1 g/clove, were planted in row plots of 2 m long and 1.4 m wide with plant to plant spacing of 10 cm on a row and 70 cm between the rows. Standard agricultural practice was employed throughout. Mature harvested bulbs were stored traditionally from collecting (mid may 2003) and harvesting dates (mid-June 2004) till planting date (25th December), at room temperature: mean day temperature 27.5 °C, mean night temperature 18.8 °C and 73% relative humidity during storage period.

2.2. Agro-morphological traits

The first observed morphological character was the skin colour of the clove. This character was observed for each accession in situ, just after sampling, by two persons. Based on IPGRI (2001) recommendations, the colour chart included: (1) white, (2) yellow and light brown, (3) pink, (4) violet, (5) purple, (6) others or mix.

The other morphological characteristics were measured during two growth seasons at the end of the growth stage, 145 days after planting as indicated by Ledesma et al. (1997). For every accession 10 plants were used as replication for recording data for the number of leaves per plant (NL), the length of the pseudostem measured from the collar to the node of the last leaf (L) and for dry weight of the bulb (DWB), obtained by drying the measured bulbs in the oven at 70 °C till constant weight. These parameters were measured at the same day of sampling.

The second set of characteristics, measured on 10 replications for each accession, was recorded 2 months after harvesting: the weight of one clove (WC), weight of one bulb (WB), diameter of the bulb (DB), number of cloves in one bulb (NC), the yield (Yi) and days to dormancy release (DDR) which was the mean number of days between harvesting time and sprouting (appearance of the first roots and leaves) during the two storage periods (2003 and 2004). The yield of each accession is obtained by this formula: [(mean weight of one bulb for year n + mean weight of one bulb for year $n+1$)/2] × plant density per hectare.

2.3. Organo-sulphur compound analysis

Chemical analysis was done one month after harvesting (July, 2005). A high performance liquid chromatography (HPLC) method for simultaneous measurement of organo-S compounds was employed (Arnault et al., 2003). The following organo-sulphur compounds were measured under alliinase-inhibiting condition: alliin (+-S-2-propenyl-L-cysteine sulphoxide), isoalliin (+-S-trans-1-propenyl-L-cysteine sulphoxide), GluAlCs (γ-glutamyl-S-propenyl-L-cysteine), isoGluAlCs (γ-glutamyl-S-trans-1-propenyl-L-cysteine) and allicin propenyl-L-cysteine), isoGluAlCs (γ-glutamyl-S-trans-1-propenyl-L-cysteine) and allicin (diallyl thiosulphinate). Fresh garlic cloves (0.13 g) were extracted in using 80 ml methanol/water (90/10) (v/v) + 0.05% formic acid (pH <3). An aliquot was diluted 10 times and filtrated (0.2 μm). Analyses were performed with a Waters 616 pump and DAD 996 diode-array detector (Waters Corporation, Milford, MA, USA). Compounds were separated on a 150 mm × 3 mm i.d. × 3 μm particle Hypurity Elite C18 column Thermo Quest, at 38 °C (Thermo Hypersil Division, Keystone, Bellefonte, PA, USA) and a UV detector operated at 208 nm. The column 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 orthophosphoric acid 85%); and B, acetonitrile-20 mM sodium dihydrogen phosphate + 10 mM heptane sulphonic acid pH = 2.1 (50/50). The elution mode is a gradient reported by Arnault et al. (2003). Data acquisition was performed using Millenium software from Waters Corporation. Reference compounds are composed of 50 μl of alliin (0.5085 mM), 40 μl of GluAlCs (0.206 mM) and 50 μl of allicin (0.500 mM). The contents of organo-sulphur compounds are calculated per fresh weight (f.w.).

2.4. Statistical analyses

For each accession, 10 plants (each year) were studied for the morphological traits, three to five bulbs for each accession served as replications for the biochemical analyses. Descriptive statistics such as averages and coefficients of variation (CV) were used to detect diversity between and within the studied accessions. Statistical analyses were based on one way ANOVA with Fisher test ($\alpha = 0.05$ and 0.001). Following correlation analysis, using Pearson correlation coefficient ($\alpha = 0.01$), was used to estimate the relationships between studied variables, particularly those related to the yield and to the organo-sulphur compounds. PCA was used to detect the characters that are most relevant to distinguish between the

Table 1
Origins of garlic accessions and agro-climatic characteristics of the prospected localities.

Accessions	Province	Locality	Coordinate points	Cultivation system	Annual minimal temperature (°C)	Annual maximal temperature (°C)	Photoperiod (h/day)[a]	Existence of F.S[b]
K_1	Kairouan	El Hajeb	35°22′37″N 9°32′44″E	Irrigated	13.3	26.1	8 h 31 min	1
SBZ_2	Sidi Bouzid	Oum Laâdham	34°50′46″N 9°07′24″E	Irrigated	11.6	24.7	7 h 45 min	2
SBZ_3	Sidi Bouzid	El Haria	34°58′25″N 9°12′02″E	Irrigated	11.6	24.7	7 h 45 min	2
SBZ_4	Sidi Bouzid	Garaât Bouzid	34°50′55″N 9°30′53″E	Irrigated	11.6	24.7	7 h 45 min	1
G_5	Gafsa	El Kasba	34°24′42″N 8°46′57″E	Oasis	12.8	25.6	9 h 13 min	3
KB_6	Kébili	Steftimi	33°48′18″N 9°00′36″E	Oasis	14.6	28.2	8 h 35 min	2
KB_7	Kébili	Steftimi	33°48′08″N 9°00′47″E	Oasis	14.6	28.2	8 h 35 min	2
KB_8	Kébili	Béni Mhamed	33°38′29″N 8°59′39″E	Oasis	14.6	28.2	8 h 35 min	2
SBZ_9	Sidi Bouzid	Zannouch	34°30′50″N 9°03′47″E	Irrigated	11.6	24.7	7 h 45 min	2
KS_{10}	Kasserine	Errakhmet	35°02′57″N 9°13′08″E	Irrigated	10.2	23.5	8 h	2
KS_{11}	Kasserine	Errakhmet	35°02′57″N 9°13′08″E	Irrigated	10.2	23.5	8 h	2
JND_{12}	Jendouba	Jleïlia	36°27′14″N 8°33′21″E	Dry	10.9	24.7	7 h 45 min	2
JND_{13}	Jendouba	Souadguia	36°27′35″N 8°32′41″E	Dry	10.9	24.7	7 h 45 min	2
JND_{14}	Jendouba	Oueslatia	36°27′36″N 8°47′05″E	Dry	10.9	24.7	7 h 45 min	1
JND_{15}	Jendouba	Riabna	36°37′12″N 8°58′59″E	Irrigated	10.9	24.7	7 h 45 min	1
BJ_{16}	Béja	Bir Lahmar	36°43′07″N 9°12′47″E	Dry	10.7	23.8	7 h 15 min	2
BJ_{17}	Béja	Hammam Siala	36°51′09″N 9°11′32″E	Dry	10.7	23.8	7 h 15 min	2
BJ_{18}	Béja	Hammam Siala	36°51′17″N 9°11′27″E	Dry	10.7	23.8	7 h 15 min	1
BJ_{19}	Béja	Nefza	36°58′26″N 9°03′47″E	Dry	10.7	23.8	7 h 15 min	2
NB_{21}	Nabeul	Bhira	36°34′44″N 10°50′56″E	Dry	15.4	22.7	7 h 50 min	2
NB_{22}	Nabeul	Bhira	36°34′24″N 10°50′42″E	Dry	15.4	22.7	7 h 50 min	2
NB_{23}	Nabeul	Oued Khataf	36°51′26″N 11°06′14″E	Irrigated	15.4	22.7	7 h 50 min	2
NB_{24}	Nabeul	Ebène	37°02′43″N 11°01′38″E	Irrigated	15.4	22.7	7 h 50 min	2
NB_{25}	Nabeul	Ebène	37°02′47″N 11°00′34″E	Dry	15.4	22.7	7 h 50 min	2
BZT_{26}	Bizerte	Ghézala	37°07′43″N 9°30′13″E	Dry	13.2	22.8	8 h 31 min	3
BZT_{27}	Bizerte	Ghézala	37°07′43″N 9°30′13″E	Dry	13.2	22.8	8 h 31 min	2
BJ_{28}	Béja	Sidi Boutefaha	36°42′56″N 9°06′44″E	Irrigated	10.7	23.8	7 h 15 min	2
KF_{29}	Kef	Bir Salah	35°58′04″N 9°05′45E	Dry	9.7	22.8	8 h	2
GAB_{30}	Gabès	Local market	33°52′55″N 10°06′07″E	Irrigated	15.4	23.9	9 h 18 min	2
KF_{31}	Kef	Téjrouine	35°52′57″N 8°32′53″E	Dry	9.7	22.8	8 h	2
MH_{32}	Mahdia	El Baghdadi	35°34′03″N 11°01′02″E	Irrigated	14.4	23.4	7 h 44 min	2

[a] Average photoperiod during growth season (November–June).
[b] F.S: flower scape; (1) some F.S in the same accession; (2) F.S non existent; (3) high frequency of the F.S in the same accession.

accessions. Clusters of accessions were formed on the basis of these factors using HCA, the graph representing classification is a dendrogramme of dissimilitude with standardized distances representing the closest accessions in homogeneous groups. Statistical analyses are done using the software XLSTAT 7.5.2.

3. Results

3.1. Contents variability of organo-sulphur compounds

The accessions vary significantly in their contents of alliin and GluAlCs and were highly different for their content of isoalliin, IsoGluAlCs and allicin. The highest coefficients of variation were found for the GluAlCs and the isoalliin (Table 2). The average content of the collection of alliin is 73.9 nmol/mg of fresh weight (f.w.); it varies from 37.6 nmol/mg (BJ_{17}) to 97.2 nmol/mg of f.w. (JD_{13}). Accessions whose average contents of alliin are higher than the average are 17 (K_1, $SBZ_{2,3,4,9}$, G_5, KB_6, $KS_{10,11}$, $JND_{12,13}$, $BJ_{16,18}$, $NB_{21,24}$, $KF_{29,31}$) representing 55% of the collection. The average content of isoalliin is 1.1 nmol/mg of f.w. Certain accessions ($JND_{12,14,15}$ and BZT_{26}) seem to be deprived of this compound or they have so negligible contents that they are undetectable. The content of isoalliin can reach high values within the collection (4 nmol/mg of f.w. for NB_{21}). The average contents of GluAlCs and isoGluAlCs are 34.5 and 27.2 nmol/mg of f.w., respectively. For GluAlCs the lowest average, 14.6 nmol/mg, was found in accession MH_{32} whereas the highest average content is at accession JD_{14}, it is of 68.2 nmol/mg of f.w. The average content of isoGluAlCs varies from 19.2 to 39.3 nmol/mg of f.w. present respectively at the accessions BJ_{28} and NB_{24}. The level of allicin is in average of 14.6 nmol/mg of f.w., the highest content is at accession SBZ_3 (23 nmol/mg of f.w.) and the lowest at the accession JND_{14} (6.6 nmol/mg of f.w.).

3.2. Agro-morphological characteristics

The 31 Tunisian accessions are differently coloured (Table 2), 42% of the collection is purple, 16% is pink, 13% is violet, two accessions have white clove skin and only one accession is light brown. 20% of the accessions are not homogenous for this character.

The variability between accessions is statistically significant for the quantitative agro-morphological characters, except for IDS (Table 2). Number of leaves/plant varies from 6.4 (GAB_{30}) to 14.2 (KS_{11}). The length of the pseudostem oscillates between 16.4 cm, measured at JND_{12}, and 43.5 cm, measured at SBZ_3. The average of the collection is of 25.8 cm. Mean dry weight of bulb, 145 DAP, is of 2.1 g, varying between 0.6 g and 6.9 g. The number of days necessary for the dormancy release (DDR) is in average 179.4 days, almost 6 months after harvest. Certain accessions reach this stage 130 days after harvest ($SBZ_{2,3}$ and $KB_{7,8}$), whereas others (KS_{10}, JND_{15}, $BJ_{18,28}$ and NB_{25}) need 223 days for the elongation of the adventitious roots and sprouting.

The average number of cloves (NC) by bulb is 15.6. Accession NB_{23} has the lowest NC (10.7) whereas KB_6 has the highest NC (38.7). The accessions of which the NC is higher than the average account for 26% of the collection. The clove average weight (WC) is 1.23 g; it ranges from 0.30 g (KB_6) to 2.90 g (MH_{32}). Accessions having a WC lower than 1 g account for 34% of the collection. On the other hand, the accessions characterized by WC higher than 1.5 g are SBZ_2, KS_{11} and MH_{32}. Accessions KB_6 and BJ_{19} had WC lower than 0.5 g. The mean weight of the bulb was found of 19.45 g, it varies from 10.92 g (JND_{13}) to 48.14 g (MH_{32}).

With regard to the diameter of the bulb, the general average is 3.64 cm; 35% of the accessions have a bulb diameter higher than this average. It is to be noticed, as well for the weight as for the diameter of the bulb, extremes are present at the same accessions. Thus,

Table 2
Agro-morphological and organo-sulphur characteristics of Tunisian garlic landraces.

Accession	Colour of the clove[a]	Alliin (nmol/mg)	Isoalliin (nmol/mg)	GluAlCs (nmol/mg)	IsoGluAlCs (nmol/mg)	Allicin (nmol/mg)	Total OSC[b] (nmol/mg)	NL[c]	DDR[d] (days)	DWB[e] (g)	L[f] (cm)	WC[g] (g)	WB[h] (g)	BD[i] (cm)	NC[j]	Yi[k] (T/ha)
Significance degree		*	**	*	**	**	*	**		**	**	**	**	**	**	*
K_1	5	85.9	0.2	64.1	34.7	15.2	200.1	8.7	177	1.42	20.67	1.57	23.64	4.03	14	3.36
SBZ_2	3	81.8	0.8	28.8	28.3	17.7	157.3	11.7	130	6.88	40.33	2.38	33.93	4.80	13.3	6.40
SBZ_3	1	86.7	1.3	24.2	28.8	23.8	164.9	14	130	2.98	43.50	1.31	23.62	4.03	18.3	5.15
SBZ_4	1	91.3	0.7	18.6	36.0	21.9	168.6	11.3	145.5	3.78	31.93	1.54	26.72	4.13	16.0	3.51
G_5	2	92.4	1.6	44.8	29.0	19.6	187.4	13.3	169.5	2.18	27.83	1.10	17.74	3.17	15.3	4.36
KB_6	3	87.5	1.9	16.9	33.4	13.7	153.4	13.3	154	3.55	35.40	0.32	12.74	3.52	38.7	5.06
KB_7	6	64.9	0.9	15.7	36.5	23.0	140.9	10.7	130	6.06	43.17	1.57	26.00	4.48	16.3	3.73
KB_8	6	60.5	0.4	21.1	30.1	17.2	129.3	12.7	130	5.06	37.00	1.31	19.83	3.78	15.0	4.57
SBZ_9	5	86.9	0.8	35.8	29.2	11.2	163.9	10.7	192	0.94	23.33	1.19	19.63	3.43	15.3	4.37
KS_{10}	4	74.9	1.0	42.3	23.1	12.9	154.3	10.7	223	1.15	23.00	1.19	19.85	3.63	15.0	5.21
KS_{11}	6	82.0	1.6	38.0	24.6	15.8	162.0	13.3	145.5	4.45	40.00	1.85	22.51	4.32	11.7	8.05
JND_{12}	5	96.7	0.0	54.0	21.3	12.6	184.7	9	194	0.77	16.40	1.00	15.61	3.58	14.7	2.81
JND_{13}	5	97.2	1.7	65.3	20.9	13.0	198.0	9.7	193	0.71	20.57	0.82	10.93	2.83	12.3	4.47
JND_{14}	5	51.1	0.0	68.2	21.5	6.6	147.4	8.5	179	0.96	20.83	0.82	19.37	3.70	22.3	3.27
JND_{15}	6	45.1	0.0	65.4	19.8	8.7	139.0	8.3	223	1.23	20.83	1.30	16.83	3.43	12.3	3.53
BJ_{16}	5	74.5	2.5	36.9	23.6	14.3	151.8	10	208	0.60	17.67	1.31	19.15	3.43	13.7	2.09
BJ_{17}	5	37.6	0.9	45.4	24.9	12.1	120.8	11	194	1.47	21.00	1.39	16.55	3.48	12.0	2.85
BJ_{18}	5	93.5	0.9	30.6	27.5	12.8	165.2	9.3	223	0.66	21.00	1.40	18.68	3.62	12.3	3.64
BJ_{19}	3	59.7	0.7	17.9	31.7	13.5	123.5	10.7	171.5	4.90	39.00	0.51	15.45	3.52	27.7	3.07
NB_{21}	5	79.3	3.9	33.8	27.0	10.0	154.1	9.7	194	1.12	22.17	0.90	10.96	3.07	11.7	2.51
NB_{22}	4	69.4	1.0	32.2	20.8	12.9	136.2	8.3	177	1.08	18.67	0.89	13.26	3.08	14.0	3.12
NB_{23}	4	56.0	1.1	36.8	23.3	12.2	129.4	8	177	0.83	19.67	1.25	14.21	3.30	10.7	2.87
NB_{24}	5	82.0	1.8	19.3	39.3	21.2	163.4	8.5	177	0.72	18.00	0.94	13.97	3.15	13.0	2.94
NB_{25}	5	66.8	1.5	24.2	25.2	10.7	128.3	8.5	223	1.64	18.00	0.97	13.31	3.17	13.0	2.16
BZT_{26}	4	55.9	0.0	35.5	22.0	12.2	125.7	8.7	177	1.22	21.67	1.16	14.61	3.23	11.7	3.21
BZT_{27}	6	73.5	0.7	30.3	26.3	12.8	143.4	8	192	0.95	20.33	1.32	17.40	3.47	12.3	4.11
BJ_{28}	6	64.4	0.7	15.7	19.2	10.6	110.6	8.3	223	0.74	18.97	1.18	18.79	3.40	14.0	3.38
KF_{29}	5	92.0	2.0	33.5	28.4	11.2	167.1	11	177	1.02	23.83	0.76	12.63	3.23	17.3	3.42
GAB_{30}	3	64.7	1.2	33.5	27.8	21.2	148.3	8.7	147.5	0.93	19.33	1.12	23.42	4.07	23.0	5.83
KF_{31}	5	80.1	0.8	26.5	37.0	16.8	161.2	12.5	192	3.13	30.00	1.55	23.53	4.02	13.7	3.59
MH_{32}	3	58.0	0.8	14.6	23.1	16.4	112.9	9	192	1.86	21.00	2.90	48.14	5.55	15.5	6.69
Average		73.9	1.1	34.5	27.2	14.6	151.4	10.2	179.4	2.1	25.6	1.3	19.5	3.7	15.7	4.0
SD		15.9	0.8	15.6	5.5	4.3	23.1	1.8	29.3	1.8	8.5	0.5	7.4	0.6	5.6	1.4
CV%		21.6	76.4	45.2	20.3	29.5	15.3	18.0	16.3	84.0	33.2	39.6	38.2	15.5	35.9	34.5

[a] (1) white; (2) yellow and light brown; (3) pink; (4) violet; (5) purple; (6) heterogeneous population with bulbs coloured differently.
[b] Organo-sulphur content.
[c] Number of leaves.
[d] Days for dormancy release.
[e] Dry weight of the bulb.
[f] Length of the pseudostem.
[g] Weight of the clove.
[h] Weight of the bulb.
[i] Bulb diameter.
[j] Number of the cloves in one bulb.
[k] Yield.
[*] Significant difference at $\alpha = 0.05$.
[**] Difference very highly significant at $\alpha = 0.001$.

Table 3
Correlation matrix between the agro-morphological characteristics and organo-sulphur contents.

	Alliin	Isoalliin	GluAlCs	IsoGluAlCs	Allicin	NL[a]	DDR[b]	DWB[c]	*L*[d]	WC[e]	WB[f]	BD[g]	NC[h]	Yi[i]
Alliin	1													
Isoalliin	0.334	1												
GluAlCs	−0.019	−0.221	1											
IsoGluAlCs	0.331	0.118	−0.436	1										
Allicin	0.291	0.058	−0.458*	0.635*	1									
NL[a]	0.351	0.221	−0.254	0.411	0.465*	1								
DDR[b]	−0.123	0.031	0.295	−0.482*	−0.684*	−0.552*	1							
DWB[c]	0.007	−0.110	−0.429	0.467*	0.497*	0.625*	−0.719*	1						
L[d]	0.127	−0.007	−0.408	0.471*	0.540*	0.786*	−0.752*	0.911*	1					
WC[e]	−0.111	−0.228	−0.153	0.004	0.324	0.064	−0.151	0.315	0.177	1				
WB[f]	−0.084	−0.273	−0.272	0.138	0.439	0.151	−0.283	0.401	0.296	0.886*	1			
BD[g]	−0.085	−0.279	−0.295	0.220	0.462*	0.241	−0.422	0.547*	0.453	0.826*	0.950*	1		
NC[h]	0.048	0.034	−0.233	0.280	0.082	0.315	−0.320	0.261	0.347	−0.437	−0.040	0.082	1	
Yi[i]	0.154	−0.054	−0.130	−0.003	0.361	0.459*	−0.448	0.425	0.482*	0.518*	0.584*	0.638*	0.159	1

[a] Number of leaves.
[b] Days for dormancy release.
[c] Dry weight of the bulb.
[d] Length of the stem.
[e] Weight of the clove.
[f] Weight of the bulb.
[g] Bulb diameter.
[h] Number of the cloves in one bulb.
[i] Yield.
* Very significant correlation with Pearson test at $\alpha = 0.01$ using XLSTAT 7.5.2. Software.

accession JND_{13} has the lowest bulb diameter (2.83 cm) and accession MH_{32} the largest (5.55 cm). The mean yield of the collection is almost 4 T/ha, 39% of the accessions have a yield higher than this average. According to Table 2, accession KS_{11} produced the highest yield (8.04 T/ha) whereas accession BJ_{16} gave the lowest one (2.1 T/ha).

3.3. Correlation between characters

Table 3 shows that IsoGluAlCs and allicin are positively related to length of the pseudostem, to dry weight of the bulb, and negatively correlated to the intensity of the dormancy stage. On the other hand, the two peptides GluAlCs and iso-GluAlCs are related respectively positively and negatively to the allicin content. Their correlation coefficients are significant at 1% as shown in Table 3. Neither the weight nor the diameter of the bulb is correlated to the number of cloves/bulb. Thus, having high number of cloves by bulb does not lead to heavy bulb or large diameter. On the other hand, a high weight of the clove guarantees higher weight and diameter upon the positive and highly significant correlation between these three parameters. In the same way, a positive and highly significant correlation was observed between the weight and the diameter of the bulb. As for the yield, it is highly influenced, according to the matrix of correlation (Table 3), by the weight of the clove and the weight and the diameter of the bulb, in addition to number of leaves per plant and stem length.

3.4. Multivariate analyses

Multivariate analyses were used to visualize the correlations between the organo-sulphur compounds and the agro-morphological traits. For this purpose we used the total content of the five analysed elements as one set of variables called total organo-sulphur content (Total OSC). The first three PCA axes described 80.57% of the total variance, thus indicating that these three axes represent the majority of the variation for the agro-morphological and organo-sulphur characteristics. The first axe accounts for 46% of the variability, it is correlated to the morphological traits of the plant and of the bulb after harvesting and to the yield (Table 4). The second axe represents 23.3% of the variation, it represents the characteristics of the clove (weight of one clove and number per bulb) and the third axe separates the accessions on the basis of their total sulphur contents. The illustrations of the three axes are provided in Fig. 1(a) and (b), representing two distinct groups comprising the same accessions in both cases. The same two groups are distinguished also by HCA (Fig. 2). The first group is compact showing low variability. The second one is composed of K_1, $SBZ_{2,3,4}$, G_5, $KB_{6,7,8}$, KS_{11}, BJ_{19}, KF_{31} and MH_{32}, it is less compacted indicating higher heterogeneity than the first group.

4. Discussion

The present study assesses variability of the local Tunisian garlic varieties. Variability between accessions is significant for the yield, for the level of alliin and for the GluAlCs and highly significant for

Table 4
Correlation matrix between PCA axes and analysed characters.

	F1	F2	F3
Eigen value	4.597	2.338	1.123
% variance	45.970	23.376	11.228
% cumulated	45.970	69.347	80.574
Total OSC[a]	0.00	−0.28	**0.89**
NL[b]	**0.65**	−0.53	0.24
DDR[c]	**−0.74**	0.40	−0.02
DWB[d]	**0.84**	−0.28	−0.16
L[e]	**0.83**	−0.45	−0.06
WC[f]	0.61	**0.76**	0.13
WB[g]	**0.74**	0.60	−0.04
BD[h]	**0.84**	0.46	−0.09
NC[i]	0.23	**−0.61**	−0.43
Yi[j]	**0.75**	0.12	0.18

Numbers in bold indicate the characters that are most relevant to distinguish between accessions on the respective axe. Fi: principal component representing a PCA axis.
[a] Organo-sulphur content.
[b] Number of leaves.
[c] Days for dormancy release.
[d] Dry weight of the bulb.
[e] Length of the pseudostem.
[f] Weight of the clove.
[g] Weight of the bulb.
[h] Bulb diameter.
[i] Number of the cloves in one bulb.
[j] Yield.

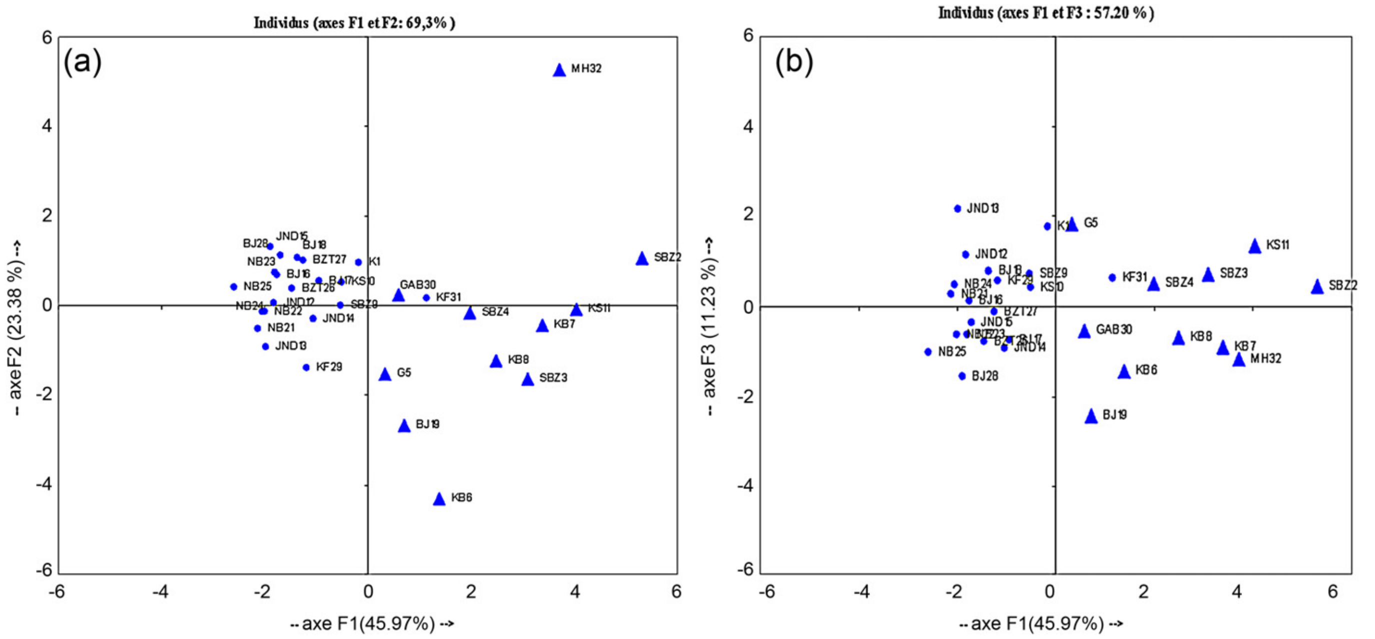

Fig. 1. (a) Two dimensional principal component analysis representing the axes F1 and F2 illustrating the variation of the agro-morphological and organo-sulphur compounds characters of 31 Tunisian garlic (*Allium sativum* L.) accessions. The variability shown accounts for 69.3% of total variability. (b) Two dimensional principal component analysis representing the axes F1 and F3 and illustrating the variation of the agro-morphological and organo-sulphur compounds characters of 31 Tunisian garlic (*Allium sativum* L.) accessions. The variability shown accounts for 57.2% of total variability. (▲) White, light brown or pink accessions; (●) violet or purple accessions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

all the other quantitative parameters, except for the intensity of the dormancy stage. Baghalian et al. (2005), report that Iranian garlic ecotypes were significantly different for the weights of bulbs and cloves, but non different for the number of cloves/bulb. This result is neither in agreement with ours nor with the results of Frasca et al. (1997) who showed that the majority of the variation resides in the number of cloves. These differences in results can be explained, in addition to the environmental conditions and cultivation practices, by the differences in garlic genotypes.

The significant difference between the accessions for the dry weight of the bulb measured 145 DAP can be the result of different bulbing abilities, affected by environmental conditions and genotypes. Since all the accessions of our collection are planted for 2 years in the same environmental conditions then this difference might be of genetic origin.

4.1. Organo-sulphur compounds

Knowing that total and individual ACSOs have been used to separate onion cultivars for flavour quality and intensity (Block, 1992), and that alliin is a specific amine of garlic, the ACSOs could be used to characterize and study the genetic diversity in garlic.

A broad variability intra-accession and inter-accessions was observed for the analysed organo-sulphur compounds. This variability was observed in onions accessions, for volatile sulphur content, total bulb sulphur, γ-glutamyl peptides and

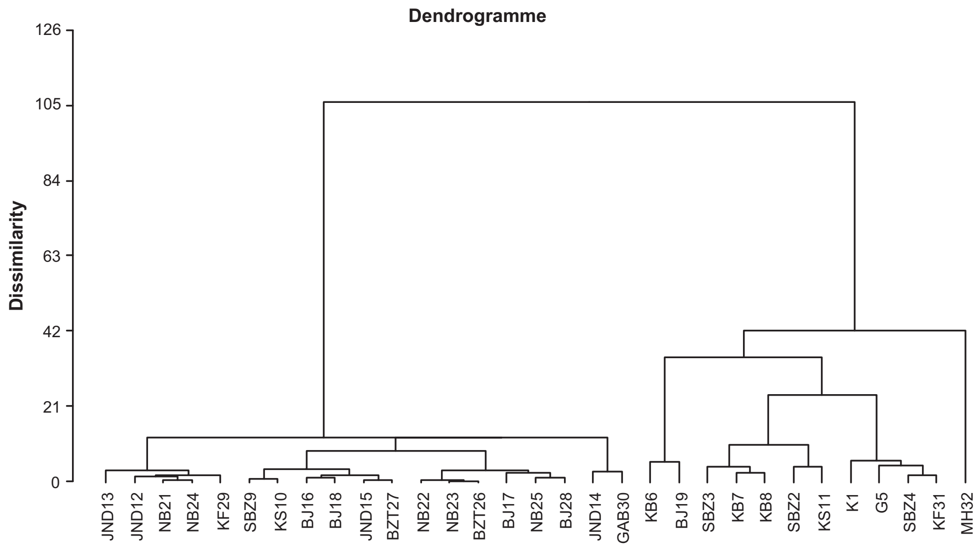

Fig. 2. Dissimilarity dendrogramme generated by HCA using XLSTAT 7.5.2. Software showing two major clusters on the basis of agro-morphological and organo-sulphur compounds characters of 31 Tunisian garlic (*Allium sativum* L.) accessions.

flavour-precursor content (Lancaster and Shaw, 1991). While differences in mean flavour intensity or quality exist among onion cultivars, significant bulb-to-bulb variation occurs for measured flavour components. In a heterogeneous species, such as onion, inherent variability of any cultivar can be expected (Dowker, 1990). This explanation might be applicable in the case of garlic, since the measured variability is high in spite of its vegetative propagation. In the development of onion cultivars, the lack of active selection for flavour coupled with severe inbreeding depression, has probably contributed to this variability.

According to this analysis, alliin is the major organo-sulphur component present in Tunisian accessions, also indicated by Block (1992). This compound is present with an average of 73.9 nmol/mg of f.w. equivalent of 1.28% of f.w., value comparable with that indicated by Kock and Lawson (1996) and higher than those mentioned by Block et al. (1993) and Kamenetsky et al. (2005). The sum of the content (alliin + isoalliin) represents 1.32% of the f.w. for the 31 accessions, alliin counts of 84.8% and isoalliin accounts for only 15.2% of them. These values are close to those indicated by Yoo and Pike (1998). GluAlC is in a majority compared to the isoGluAlC, Block (1992) also brought back this predominance. These contents indicate that the local accessions are rich in precursors of allicin, which might be due to the genetic origin or to the environmental conditions, especially temperature. Cultivars differ in total plant sulphur compounds; differences in flavour intensity and quality probably arise due to variability in sulphur uptake and its metabolism through the flavour biosynthetic pathway. Considering the complexity of sulphur uptake, its reduction and requirement for healthy plant growth and development, its use in the flavour biosynthetic pathway and the fact that garlic have been cultivated by different civilization for millennia, its understandable that continuous variation exists for flavour intensity among garlic cultivars (Randle and Lancaster, 2002).

4.2. Correlations

The establishment of correlations between the parameters shows very interesting results from agronomic point of view. The components which were determining for the estimated yield have been pointed out. These components are the weight of clove, the weight of the bulb and its diameter, number of leaves/plant and length of the pseudostem. The more these variables are maximized the higher will be the yield. Choosing genotypes with high levels of these characteristics, and application of appropriate agronomical techniques will guaranty higher yield.

The number of cloves does not seem to intervene directly on the yield. Zepeda (1997), in agreement with these results, did not note direct relation between the low number cloves and high yield, for that he used these parameters as two distinct selection criteria for the creation of new varieties starting from old populations. Other authors report that the components of the yield are not only the diameter and the weight of the bulb but also the number of cloves/bulb under Nepal environmental conditions (Panthee et al., 2006). Bulb diameter and number of cloves per bulb are important characteristics of quality and calibration for garlic (Zepeda, 1997). Our results showed that the weight of cloves is higher as their number per bulb is weak. This is explained by the fact that the photo assimilates coming from the leaves are distributed between existing cloves (Ledesma et al., 1997), the lower their number is, the higher they weigh.

The absence of correlation between alliin and isoalliin contents indicates that they derive from two different pathways, as it was suggested by some chemists (Lawson, 1996; Collin et al., 2005). In their study, Ovesna et al. (2007) found significant correlation between alliin and isoalliin contents. The variability of sulphur metabolism could be the cause of the absence of correlation between the precursors and storage peptides (GluAlCs and IsoGluAlCs). This lack of correlation between the ACSO (alliin and isoalliin) and their precursory peptides was shown by several authors (Block, 1992; Arnault et al., 2005). Randle et al. (1999), explaining the origin of the variability of sulphur metabolism, claimed that cultivars differed in the way sulphur was partitioned into flavour and non flavour compounds. The significant correlations existing between allicin and isoGluAlCs on the one hand and between these components and intensity of dormancy stage, dry weight of the bulb and pseudostem length, on the other hand, can indicate that high levels of allicin and isoGluAlCs can be found in accessions characterized by short dormancy period, high pseudo stem and dry weight of bulb measured at the end of the vegetative cycle (145 DAP). These two phenotypical characteristics are interesting since that one plant with high amounts of allicin and isoGluAlCs can be detected early in season. The lack of correlation between yield and organo-sulphur compounds can allow the valorisation of genotypes having low yield but high levels of health beneficial compounds like SBZ_4, JND_{12} and KF_{29}, in addition, these characteristics can be two distinct selection criteria for the creation of varieties with high yield and high content of organo-sulphur compounds.

4.3. Diversity structuring

The two groups of accessions shown in Fig. 1(a) and (b) are distinguished mainly on the basis of their clove weight and total OSC, seen their distribution in relation to the axes F2 and F3. Giving each accession its colour, it appears that the two groups in Fig. 1 are also distinguished by the colour of the cloves. The accessions forming the light coloured (white or pink) group are characterized by short dormancy stage (between 130 and 192 days), the highest dry weight of the bulb and the longest pseudostem measured 145 DAP, high weight and diameter of the bulb, high weight of one clove and a yield medium to high. Checking the total OSC of each group, there is no difference in total content of organo-sulphur compounds between coloured and light coloured accessions.

The accessions are not separated on the basis of their geographical origins.

5. Conclusion

Significant differences are shown between and within local garlic accessions. Tunisian garlic landraces are rich in organo-sulphur compounds, but differ in the quantity and the quality of these compounds. Significant bulb-to-bulb variability for flavour quality and intensity suggests that active selection during cultivar development is needed to improve flavour quality and consistency. The content of organo-sulphur compounds can not be indicated by agro-morphological characters, but high contents of allicin and isoGluAlCs seems to be related to short dormancy period, high pseudo stem and dry weight of bulb were measured at the end of the vegetative cycle. This result needs to be confirmed under various environmental conditions. Colour of clove skin seems to influence somehow the structuring of general diversity, but more investigation is needed to study this influence.

Acknowledgement

L'Institut de la Recherche sur la Biologie des Insectes (IRBI), Université François Rabelais, Tours, France, has supported the fees of the chemical analysis.

References

Abdoli, M., Habibi-Khaniani, B., Baghalian, K., Shahnazi, S., Rassouli, H., Naghdi Badi, H., 2009. Classification of Iranian garlic (*Allium sativum* L.) ecotypes using RAPD marker. J. Med. Plants 8, 45–51.

Ang-lee, M.K., Moss, J., Yuan, C.S., 2001. Herbal medicines perioperative care. JAMA 28, 208–216.

Arnault, I., Christidès, J.P., Mandon, N., Haffner, T., Kahane, R., Auger, J., 2003. High performance ion-pair chromatography method for simultaneous analysis of alliin, deoxyalliin, allicin and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. J. Chromatogr. A 991, 69–75.

Arnault, I., Hafner, T., Siess, M.H., Vollmar, A., Kahane, R., Auger, J., 2005. Analytical method for appreciation of garlic therapeutic potential and for validation of a new formulation. J. Pharm. Biomed. Anal. 37, 963–970.

Baghalian, K., Sanei, M.R., Naghavi, M.R., Khalighi, A., Naghgi Badi, H.A., 2005. Post culture evaluation of morphological divergence in Iranian garlic ecotypes. Acta Hortic. 688, 123–125.

Block, E., 1992. The organosulfur chemistry of the genus *Allium*-implications for the organic chemistry of sulphur. Angew. Chem. Int. Ed. Engl. 31, 1135–1178.

Block, E., Naganathan, S., Putman, D., Zhao, S.-H., 1993. Organo-sulfur chemistry of garlic and onion: recent results. Pure Appl. Chem. 65 (4), 625–632.

Burba, J.L., Gomez Riera, P., 1997. Characterization, adaptation and selection of garlic germplasm (*Allium sativum* L.) through the management of dormancy in Mendoza, Argentina. Acta Hortic. 433, 151–164.

Collin, H.A., Hughes, J., Tregova, A., Milne, J.G.C., Van der Werff, G., Wilkinson, M., Jones, M.G., Tomsett, A.B., Cosstick, R., Trueman, L., Crowther, T., Brown, L., Thomas, B., 2005. Sulphur biochemistry of garlic: the biosynthesis of flavour precursors. Acta Hortic. 688 (165302), 72.

Dowker, B.D., 1990. Onion breeding. In: Rabinowitch, H.D., Brewster, J.L. (Eds.), Onions and Allied Crops. CRC Press, Boca Raton, FL, pp. 215–232.

Etoh, T., Simon, P.W., 2002. Diversity, fertility and seed production of garlic. In: Rabinowitch, H.D., Currah, L. (Eds.), *Allium* Crop Science: Recent Advances. CAB Int., Wallingford, pp. 101–117.

Frasca, L.A., Rigoni, C., Silverster, V., Burba, J.L., 1997. Genetic variability estimation and correlations in white clonal type garlic characters. Acta Hortic. 433, 279–284.

Fritsch, R.M., Friesen, N., 2002. Evolution, domestication and taxonomy. In: Rabinowitch, H.D., Currah, L. (Eds.), *Allium* Crop Science: Recent Advances. CAB Int., Wallingford, pp. 5–30.

Gupta, R., Sharma, N.K., 1993. A study of the nematicidal activity of allicin—an active principle in garlic, *Allium sativum* L., against root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. Int. J. Pest Manage. 39, 390–392.

Hanelt, P., 1990. Taxonomy, evolution and history. In: Rabinowitch, H.D., Brewster, J.L. (Eds.), Onions and Allied Crops, I. Botany, Physiology and Genetics. CRC Press, Boca Raton, pp. 1–26.

Harris, J.C., Cottrel, S.L., Plummer, S., Lloyd, D., 2001. Antimicrobial properties of *Allium sativum* (garlic). Appl. Microbiol. Biotechnol. 57, 282–286.

Hile, A.G., Shan, Z., Zhang, S.-Z., Block, E., 2004. Aversion of European starlings (*Sturnus vulgaris*) to garlic oil extract treated granules: garlic oil as an avian repellent. Garlic oil analysis by nuclear magnetic resonance spectroscopy. J. Agric. Food Chem. 52, 2192–2196.

Hong, C.J., 1999. Fundamental Studies on Crossbreeding in Garlic, *Allium sativum* L. Kagoschima University, Kagoshima, Japan.

IPGRI, 2001. Descriptors for *Allium*. International Plant Genetic Resources Institute, Rome, Italy, 42 pp.

Kamenetsky, R., London Shafir, I., Khassanov, F., Kik, C., Van Haudsen, A.W., Vrielnk Van Ginkel, M., Burger-Meijer, K., Auger, J., Arnault, I., Rabinowitch, H.D., 2005. Diversity in fertility potential and organo-sulphur compounds among garlics from Central Asia. Biodivers. Conserv. 14, 281–295.

Kock, H.P., Lawson, L.D., 1996. Garlic – The Science and Therapeutic Application of *Allium sativum* L. and Related Species, 2nd ed. Williams and Wilkins, Baltimore.

Lallemand, J., Messian, C.M., Briad, F., Etoh, T., 1997. Delimitation of varietal groups in garlic (*Allium sativum* L.) by morphological, physiological and biochemical characters. Acta Hortic. 433, 123–132.

Lancaster, J.E., Shaw, M.L., 1991. Metabolism of γ-glutamyl peptides during development, storage and sprouting of onion bulbs. Phytochemistry 30, 2857–2859.

Lawson, L.D., 1996. The composition and chemistry of garlic cloves and processed garlic. In: Koch, H.P., Lawson, L.D. (Eds.), Garlic. The Science and Therapeutic Application of *Allium sativum* L. and Related Species. Williams and Wilkins, Baltimore, pp. 37–107.

Ledesma, A., Nunez, S.B., Argüello, J., 1997. Bulbing physiology in garlic (*Allium sativum* L.) cv. "*Rosado Paraguayo*". II. Characterization of ontogenetic stages by shoot growth dynamics and its relation to bulbing. Acta Hortic. 433, 405–416.

Li, G., Shi, Z., Jia, H., Ju, J., Wang, X., Xia, Z., Qin, L., Ge, C., Xu, Y., Cheng, L., Chen, P., Yuan, G., 2000. A clinical investigation on garlic in injection for treatment of unstable *Angina pectoris* and its actions on plasma endothelin and blood sugar levels. J. Tradit. Chin. Med. 4, 24–36.

Messiaen, C.M., Cohat, J., Leroux, J.P., Pichon, M., Beyries, A., 1993. Les Allium alimentaires reproduits par voie végétative. INRA, Paris.

Ovesna, J., Kucera, L., Kralova, J., Leisova, L., Stavelikova, H., Velisek, J., 2007. Genetic diversity among garlic clones as revealed by AFLP, phenotypic descriptors and S-amino acids level. Veg. Crops Res. Bull. 66, 105–116, http://dx.doi.org/10.2478/v10032-007-0013-9.

Panthee, D.R., KC, R.B., Regmi, H.N., Subedi, P.P., Bhattarai, S., Dhakal, J., 2006. Diversity analysis of garlic (*Allium sativum* L.) germplasms available in Nepal based on morphological characters. Genet. Resour. Crop Evol. 53, 205–212.

Rahaman, G.K., Motoyama, M.M., 2000. Repellent effect of garlic against stored product pests. Nippon Noyaku Gakkaishi 25 (3), 247–252.

Randle, W.M., Lancaster, J.E., 2002. Sulphur compounds in alliums in relation to flavour quality. In: Rabinowitch, H.D., Currah, L. (Eds.), *Allium* Crop Science: Recent Advances. CAB Int., Wallingford, pp. 329–356.

Randle, W.M., Kopsell, D.E., Kopsell, D.A., Snyder, R.L., 1999. Total sulphur and sulphate accumulation in onion is affected by sulphur fertility. J. Plant Nutr. 22, 45–51.

Sheela, C.G., Augusti, K.T., 1992. Antidiabetic effects of S-allyl-cysteine sulfoxide isolated 370 from garlic (*Allium sativum* L.). Indian J. Exp. Biol. 30, 523–526.

Simon, W.P., Jenderek, M.M., 2003. Flowering, seed production and the genesis of garlic breeding. Plant Breed. Rev. 23, 211–244.

Stavelìkova, H., 2008. Morphological characteristics of garlic (*Allium sativum* L.) genetic resources collection—Information. Hort. Sci. (Prague) 35, 130–135.

Tatarintsev, A.V., Vrzhets, P.V., Ershov, D.E., Shchegolev, A.A., Turgiev, A.S., Karamov, E.V., Kornilaeva, G.V., Makarova, T.V., Fedorov, N.A., Varfolomeev, S.D., 1992. The ajoene blockade of integrin-dependent processes in an HIV infected cell system. Vestn. Ross. Akad. Med. Nauk. 1, 6–10.

Yoo, K.S., Pike, L.M., 1998. Determination of flavour precursor compound S-alk(en)yl-L cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. Sci. Hortic. 75, 1–10.

Zepeda, A.H., 1997. Number of cloves per bulb; selection criteria for garlic improvement. I. Results with chileno type. Acta Hortic. 433, 265–267.