



**Full Length Article**

## Impact of Genetic Factor and Geographical Location on Allicin Content of Garlic (*Allium sativum*) Germplasm from Egypt and China

Hassan H.A. Mostafa<sup>1,2</sup>, Wang Haiping<sup>1</sup>, Liu Xinyan<sup>1</sup> and Li Xixiang<sup>1\*</sup>

<sup>1</sup>Beijing Research Station of Vegetable Crop Gene Resource and Germplasm Enhancement, Ministry of Agriculture, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, 12 Zhongguancun Nandajie, Haidian District, 100081 Beijing, China

<sup>2</sup>Central Lab. of Organic Agriculture, Agricultural Research Centre, Giza, Egypt

\*For correspondence: lixx0612@163.com; hhhalim79@yahoo.com

### Abstract

This study was conducted to explore the genetic variation of allicin content among Chinese and Egyptian garlic germplasm, the role of genetic improvement in allicin content enhancement and the combined influence of genetic and environmental factors on allicin contents in Egyptian garlic germplasm grown in Egypt and China respectively. Ultra performance liquid chromatography (UPLC) method was used for quantification of allicin in garlic bulbs. As the result of cluster analysis based on allicin content, all garlic germplasm (104 accessions) were divided into four groups. The germplasms with the highest allicin content presented in group A, comprising only two germplasm (one from Egypt and the other from China). The far distance among accessions in the cluster tree revealed the diversity in the garlic germplasm, which can selectively be used in garlic breeding programs for quality improvement. Clones selected were found to be different from their parents and had higher allicin content, which proves that clone selection is an effective way for garlic genetic improvement. Statistical analysis showed that the effects of germplasm, geographical location and interaction between germplasm and geographical location on the allicin content ( $P < 0.001$ ) in Egyptian garlic germplasm were highly significant. All Egyptian garlic germplasm grown in China had lower allicin content than those grown in Egypt. Allicin content reduction in same germplasms between two locations was 16–63.7%. It is clarified that production area selection for garlic allicin processing industry is important. © 2015 Friends Science Publishers

**Keywords:** Garlic; Allicin; Geographical location; Genetic Variation; Clonal selection; UPLC

### Introduction

Garlic (*Allium sativum*) is one of important plants in human history, which has been cultivated since 3000 B.C. in Egypt (Ipek and Simon, 2002). An Egyptian medical papyrus reported several therapeutic formulas based on garlic as useful remedy for human diseases such as heart problems, headache, bites, worms and tumours (Virginia, 2006). In China, it has been used in the kitchen and traditional Chinese medicines since 2000 B.C. (Richard, 2001). Nowadays, garlic plants are cropped world-wide and are of major economic importance being traded and consumed in most countries not only as a spice and vegetable but also as a medicinal plant (Takagi, 1990). Modern medicine researches showed that garlic has several medicinal properties such as anti-microbial (Ross *et al.*, 2001), as well as ability to lower serum lipid and glucose levels (Krest, 2001; Lawson *et al.*, 2001) and blood pressure (Ali *et al.*, 2000). Moreover, some very important epidemiological (prospective cohort) study conducted in America (Steinmetz *et al.*, 1994) and in Italy (Buiatti *et al.*, 1989), showed a decrease of gastric cancer risk proportional

to the increase of garlic intake.

Cavalitto and Bailey (1944) were the first to demonstrate that the antibacterial action of garlic is mainly due to allicin. Further research revealed that the medicinal effects of garlic were attributable to a sulfur compound known as allicin (Schulz *et al.*, 1998). And no compound outside the thiosulfinates (of which allicin is about 60–80%) were found being accounted for a significant portion of pharmacological activities of crushed garlic at levels representing normal human consumption (2–5 g/day) (Yu *et al.*, 2007).

Genetic factors can play an important role in differences of allicin content between ecotypes in Iran (Baghalian *et al.*, 2005). However, a little information is available about genetic diversity among Egyptian garlic germplasms according to their allicin content. Moreover, because garlic is mainly a vegetative plant, there is narrow diversity among garlic germplasms. A wider genetic variation will provide more opportunities to select the suitable genotypes for garlic breeding programs. Because of vegetative propagation, clonal selections are important breeding method (Lampasona *et al.*, 2003) for genetic

improvement of garlic yield (Baghalian *et al.*, 2006). Furthermore, bulbs are major product organ in garlic plants. Temperature and day-length have been confirmed as key environmental factors for bulb induction (Takagi, 1990; Nagakubo *et al.*, 1993). Along with environmental conditions change, plants may change some of their metabolic activities. Quality parameters seemed to be particularly sensitive to environmental stress such as day length, temperature, rainfall and light intensity (Ercan *et al.*, 2011). The level of allicin was not significantly affected by farming systems. There were a few reports concerning the relation between biochemical quality of garlic bulbs and environmental factors, but their results came from less different geographical environment with limited number of germplasm (Kamenetsky *et al.*, 2005; Soto *et al.*, 2010). Therefore, whether geographical location with greatly different environments (Egypt and China) will influence the allicin content of garlic germplasm should be investigated to determine suitable garlic production area targeting at high quality of garlic bulb.

Hence, the study presented here aims to explore the genetic variation of garlic germplasm resources from Egypt and China based on allicin content, the role of genetic improvement in allicin content enhancement and the combined influence of genetic and environmental factors on allicin contents in Egyptian garlic germplasm grown in Egypt and China.

## Materials and Methods

### Plant Material

In the present investigation, 104 garlic germplasm were included, 21 accessions from Egypt (collected from four geographical areas) and 83 accessions from nineteen provinces in China provided by Department of Vegetables Germplasm Resource, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, China (Table 1).

### Experimental Design

In 2011, Egyptian garlic bulb samples grown in Egypt were collected and stored at 4°C and transported to Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China. Half bulbs of each accession were used to analyze their allicin content directly and half were grown at Beijing research station of vegetable crop gene resource and germplasm enhancement, Langfang Hebei, China for their bulb harvest in order to understand the effect of different geographic environments on allicin content. For comparing the allicin contents of different genotypes from both locations, all garlic germplasms from China and Egypt were cultivated under the same agronomic conditions, with one plot (400 cm × 90 cm) for one germplasm, three rows per plot and 10 cm between plants. In general, 15 garlic bulbs were collected from each germplasm, five bulbs, one

bulb from each bulb were sampled for allicin determination. Climatic data in both locations (Egypt and China) has been collected and summarized in Table 2.

### Sample Preparation

The garlic cloves from each germplasm were peeled for removal of the dry protective leaves and kept in -20°C for 3-4 h and then chopped into small slices immediately. Soon the garlic slices were put in a thin layer and kept in -80°C for 3-7 h and then frozen to dry and finally ground to powder. Uniform garlic powders were prepared from each accession by passing through a sieve size 400 mesh. 400 mg of powder was added into 50 mL centrifuge tube and 15 mL distilled cold water was added into the centrifuge tube. Instantaneously, the tube was capped and vortex for 30 s and then 15 mL of distilled cold water was added again and vortex for 30s. Then the supernatant was filtered through a 0.22 µm Millipore filter into a vial after the sample was centrifuged at 8000 rpm for 10 min at 4°C. The filtered solution was then used for injection in the Waters Acquity UPLC analysis system.

### Reagents and Chemicals

Allicin standard solution (1020 mg L<sup>-1</sup>) with 84% purity was purchased from ChromaDex (ChromaDex, USA) and stored at -80°C until used, UPLC-grade methanol was purchased from Honey well (Shanghai, China), acetonitrile and formic acid were purchased from Dikma Technologies INC, USA.

### Allicin Determination

Allicin content analysis was done at supervision and testing center for vegetable quality, Ministry of Agriculture, China. The determination and calculation of allicin content based on two replicates for each sample was performed using Ultra performance liquid chromatography (UPLC) method described by Wang *et al.* (2010).

### Data Analysis

A cluster analysis of all the germplasms was performed based on allicin content using the un-weighted pair group method with arithmetic averages (UPGMA). ANOVA for the effects of different germplasm, geographical locations and their interaction was analyzed by SAS statistical software (SAS Institute, 2000). Mean difference values among geographical locations (mean allicin content in Egypt minus mean allicin content in China) and allicin reduction percentage were compared using Duncan's test. Mean allicin content of each clone selected were compared with that of its parent using ANOVA and T test. MS-Excel has been utilized for data summarization and percentage calculations.

**Table 1:** Geographical origin of garlic germplasms in this study

Accession number	Origin	Accession number	Origin	Accession number	Origin
8N002A	China-Sichuan	8N141C	China-Shandong	8N260	China-JiangSu
8N002B	China-Sichuan	8N141*	China- Shandong	8N261	China-JiangSu
8N004	China-Sichuan	141 <sup>o</sup>	China- Shandong	261 <sup>o</sup>	China-JiangSu
8N016	China-Sichuan	8N145	China-SiChuan	261*	China-JiangSu
8N017	China-Sichuan	8N149	InnerMongolia	8N263	China-JiangSu
8N017*	China-Sichuan	8N155	China- Shandong	8N264	China- Shandong
8N024	China-Hubei	8N156	China- Shandong	8N273	China-JiangSu
8N026B	China-Hubei	8N172	China- Yunnan	8N274	China-JiangSu
8N027	China-Jiangxi	8N173	China-HeiLongjiang	8N275	China-JiangSu
8N028	China-Jiangxi	8N180	China-Hebei	8N306	China- Yunnan
8N032	China-Shanxi	8N188	China-Guizhou	8N321	China- Yunnan
8N034	China-Beijing	8N197	China-Gansu	8N326	China- Yunnan
8N035	China- Shandong	8N200	China-He´nan	8N362	China-ZheJiang
8N036	China- Shandong	8N202	China-Ningxia	8N494	Egypt-ElMinia
8N036-1*	China- Shandong	8N205	China-Gansu	8N495	Egypt-ElMinia
8N36-2*	China- Shandong	8N206A	China-Hebei	8N496	Egypt-ElMinia
8N043	China- Shandong	8N206B	China-Hebei	8N497	Egypt-ElMinia
8N045	China- Shandong	8N207	China-Hebei	8N498	Egypt-ElMinia
8N047	China- Shandong	8N209	China-Liaoning	8N499	Egypt-ElMinia
8N060	China-Shanxi	8N212	China-JiangSu	8N500	Egypt-ElMinia
8N066A	China-Shanxi	8N218	China-JiangSu	8N501	Egypt-ElMinia
8N066B	China-Shanxi	8N219	China- Shandong	8N510	Egypt-ELMinia
8N069	China-Shanxi	8N232	China-Hubei	8N511	Egypt-Assiut
8N078A	China-Shanxi	8N236	China-Hebei	8N512	Egypt-Assiut
8N099	China-Tibet	8N238	China-JiangSu	8N513	Egypt-El Mansoura
8N102A	China-HeiLongJiang	8N239	China-JiangSu	8N514	Egypt-Qena
8N113	China - Hubei	8N241	China- Yunnan	8N515	Egypt-Qena
8N118	China - Shaanxi	8N242	China- Yunnan	8N516	Egypt-Assiut
8N120	China - Shaanxi	8N245	China- Yunnan	8N517	Egypt-Assiut
8N122	China - JiangSu	8N248	China- Yunnan	8N518	Egypt-Assiut
8N124	China - JiangSu	8N254A	China- Yunnan	8N519	Egypt-Assiut
8N128	China - JiangSu	8N254B	China- Yunnan	8N520	Egypt-Assiut
8N129	China - JiangSu	8N257	China-JiangSu	8N521	Egypt-ElMinia
8N141A	China- Shandong	8N258	China-JiangSu	8N522	Egypt-ElMinia
8N141B	China- Shandong	258*	China-JiangSu		

\*Clones obtained using single plant selection

<sup>o</sup>Clones gotten using bulk selection**Table 2:** The climatic conditions for garlic growth in Egypt (means for 4 collection locations) and China

Month	Temperature (°C)				Photoperiod (h)	Relative humidity (%)	Wind speed (m/sec)	Rainfall (mm)				
	Egypt		China									
	Max	Min	Max	Min								
Oct.	38.06	13.14	17.30	5.80	10.26	8	45	59	1.63	2	0	64.40
Nov.	32.05	5.35	7.80	-1.90	9.30	6	56	56	1.05	2	0.8	32.4
Dec.	23.86	2.75	-0.10	-9.00	8.41	6	66	51	0.83	2	0.4	16.2
Jan.	24.90	-0.15	-1.90	-11.50	8.68	7	65	50	0.67	2	0.4	8.10
Feb.	27.54	4.85	2.30	-8.20	8.72	7	46	50	0.97	2	0	18.6
Mar.	39.43	4.51	7.80	-2.30	10.07	8	49	48	0.93	2	0	27.1
Apr.	33.02	8.87	15.10	4.20	10.47	8	40	46	1.15	3	0	54.9
May	38.57	16.65	20.50	10.30	11.31	9	24	49	2.01	2	0	93.5

## Results

### Genetic Variation of Allicin Contents among Garlic Germplasm of Different Origins

UPLC analysis showed that there was a wide range of genetic variation in allicin content among germplasm accessions (Table 3). The allicin content of 104 accessions ranged from 2.05 in 8N499 to 0.75% in 8N511. Cluster

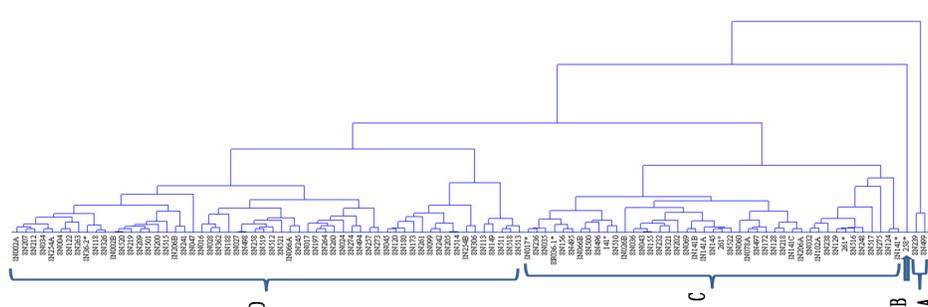
analysis of all 104 garlic germplasms using UPGMA, based on their allicin content in one environment, has categorized them into four groups (Group A, B, C and D), comprising 2, 1, 43 and 58 accessions respectively (Fig. 1). The ranges of allicin content for cluster group A, B, C and D were 2.05~2.02, 1.61, 1.84~1.32 and 1.31~0.75, respectively. Meanwhile, the highest allicin content was recorded from group A, which included only two germplasm (8N499 from Egypt and 8N239 from China) with a mean of 2.035%.

**Table 3:** Alliin contents of all garlic germplasms used in this study

Accession number	Alliin content (% dry matter)	Accession number	Alliin content (% dry matter)	Accession number	Alliin content (% dry matter)	Accession number	Alliin content (% dry matter)	Accession number	Alliin content (% dry matter)
8N002A	1.27	8N066B	1.45	8N172	1.41	8N245	1.12	8N495	1.52
8N002B	1.23	8N069	1.36	8N173	0.88	8N248	1.60	8N496	1.46
8N004	1.23	8N078A	1.40	8N180	0.91	8N254A	1.28	8N497	1.39
8N016	1.14	8N099	0.95	8N188	1.14	8N254B	0.97	8N498	1.17
8N017	1.16	8N102A	1.64	8N197	1.07	8N257	1.02	8N499	2.05
8N017*	1.54	8N113	0.78	8N200	1.21	8N258	1.17	8N500	1.44
8N024	1.03	8N118	1.30	8N202	1.48	258*	1.61	8N501	1.22
8N026B	1.36	8N120	0.91	8N205	0.95	8N260	1.05	8N510	1.49
8N027	1.17	8N122	1.23	8N206A	1.32	8N261	0.97	8N511	0.75
8N028	1.11	8N124	1.82	8N206B	1.29	261°	1.41	8N512	1.17
8N032	1.63	8N128	1.34	8N207	1.28	261*	1.60	8N513	0.75
8N034	1.28	8N129	1.60	8N209	1.22	8N263	1.24	8N514	0.95
8N035	1.51	8N141A	1.42	8N212	1.29	8N264	1.07	8N515	1.19
8N036	1.47	8N141B	1.36	8N218	1.33	8N273	1.00	8N516	1.59
8N036-1*	1.56	8N141C	1.32	8N219	1.24	8N274	1.05	8N517	1.70
8N36-2*	1.37	8N141*	1.84	8N232	1.43	8N275	1.72	8N518	0.76
8N043	1.44	141°	1.52	8N236	1.52	8N306	0.99	8N519	1.15
8N045	0.92	8N145	1.39	8N238	1.62	8N321	1.45	8N520	1.23
8N047	1.21	8N149	0.76	8N239	2.02	8N326	1.31	8N521	1.13
8N060	1.37	8N155	1.44	8N241	1.27	8N362	1.12	8N522	1.40
8N066A	1.13	8N156	1.53	8N242	0.96	8N494	1.03		

\*Clones obtained using single plant selection

°Clones gotten using bulk selection

**Fig. 1:** Cluster analysis of 104 garlic germplasms from Egypt and China based on alliin content by UPGMA

The alliin contents in all germplasms were higher than pharmacopeia level (4.5 mg/g, ~0.45%). The accessions 8N499 and 8N239 (group A) had higher alliin content which will be useful for garlic quality improvement.

As for the relation of germplasm origin and alliin content, 21 Egyptian garlic accessions were presented separately in three groups (A, C and D). Cluster C consisted of 8 Egyptian garlic germplasm but they were grouped in different subgroups. Similarly, twelve Egyptians garlic were placed on cluster D but not on the same subgroup.

#### The Effect of Genetic Improvement on Alliin Contents by Single Plant Selection and Bulk Selection

By single plant selection or bulk selection, most offspring

clones from tested germplasm had significantly higher alliin content ( $P < 0.01$ ) than their parent materials. Compared with bulk selection, the effect of single plant selection was more obvious. It has been evidenced that the alliin content in an offspring clone (8N017\*) obtained as a result of a single plant selection from parent 8N017 was observed to be 1.54%, while its parent (8N017) gave only 1.16% (Fig. 2). Also, significant difference in alliin content were observed between offspring clone (258\*, 1.61%) obtained by a single plant selection and its parent 8N258, which yielded 1.17%. Similarly, the offspring clone (8N036-1\*, 1.56%) selected by single selection from parent 8N036 yielded, while its parent was 1.47%. Likewise, alliin content in the clones by single plant selection (8N141\*, 1.82%) and bulk selection (141°, 1.52%)

**Table 4:** The difference value (%), reduction percentage (%) and cluster group of allicin in Egypt garlic germplasm grown in Egypt and China

Accession number	Difference value of allicin content between two countries (%)	Allicin reduction % over two locations	Group by clustering
8N499	0.39 <sup>i</sup>	16.12 <sup>l</sup>	A
8N496	0.44 <sup>i</sup>	23.24 <sup>k</sup>	B
8N510	0.52 <sup>i</sup>	25.88 <sup>k</sup>	B
8N512	0.41 <sup>i</sup>	25.91 <sup>k</sup>	B
8N495	0.63 <sup>hi</sup>	29.41 <sup>jk</sup>	B
8N517	0.82 <sup>gh</sup>	32.37 <sup>j</sup>	B
8N500	1.04 <sup>efg</sup>	42.08 <sup>i</sup>	C
8N515	0.91 <sup>fg</sup>	43.17 <sup>hi</sup>	C
8N522	1.21 <sup>c</sup>	46.56 <sup>ghi</sup>	C
8N501	1.16 <sup>c</sup>	48.59 <sup>gh</sup>	C
8N519	1.21 <sup>c</sup>	51.35 <sup>fg</sup>	C
8N516	1.72 <sup>bc</sup>	52.01 <sup>fg</sup>	C
8N514	1.07 <sup>ef</sup>	52.81 <sup>efg</sup>	C
8N497	1.79 <sup>ab</sup>	56.19 <sup>def</sup>	D
8N520	1.75 <sup>abc</sup>	58.72 <sup>cde</sup>	D
8N513	1.12 <sup>ef</sup>	60.18 <sup>bcd</sup>	D
8N498	1.91 <sup>ab</sup>	62.00 <sup>abcd</sup>	D
8N494	1.78 <sup>ab</sup>	63.45 <sup>abc</sup>	D
8N521	1.98 <sup>a</sup>	63.58 <sup>abc</sup>	D
8N518	1.47 <sup>d</sup>	66.00 <sup>ab</sup>	D
8N511	1.53 <sup>cd</sup>	67.25 <sup>a</sup>	D

**Note:** Accession numbers were arranged according to cluster group based on allicin reduction (%) over two locations  
The different superscript letters are indicating statistically significant differences among germplasms

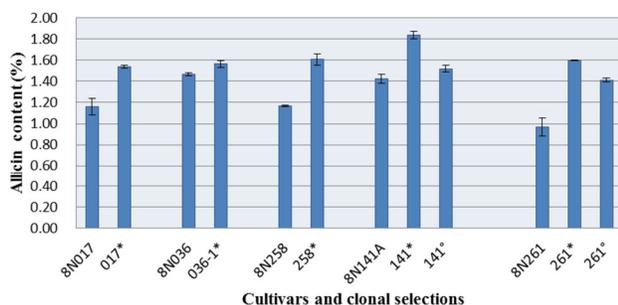
were found to be higher, respectively than their parent (8N141A), which give 1.42%. In the same way, the offspring clone (261<sup>o</sup>) selected by bulk selection from parent 8N261 yielded 1.41% of allicin content and another offspring clone (261<sup>\*</sup>) obtained by a single plant selection from the same parent gave 1.60% allicin content, which were much higher than their parent (8N261) itself, which yielded only 0.97%.

The cluster analysis (Fig. 1) has also showed that even if they were clones selected from a same parent, the clone 8N017<sup>\*</sup> from 8N017 was grouped in a different group (Group C) from its parent (Group D). Similarly, the selected clone offspring 8N036-2<sup>\*</sup> was placed in Group D, while its parent (8N036) and its sister clone (8N036-1<sup>\*</sup>) were grouped in different subgroups of Group C. Even more, accessions 8N141<sup>\*</sup> and 141<sup>o</sup> which were clones selected from the same material (8N141A) located at different nodes of Group C. Likewise, Parent genotype 8N258 was placed in a different group in the cluster from its clone offspring (258<sup>\*</sup>) by single plant selection. Clone 261<sup>\*</sup> and 261<sup>o</sup> from parent 8N261 were divided into different subgroups of Group C, while parent 8N261 fell in Group C. All these cases proved that clone selection is an effective way for high allicin content improvement.

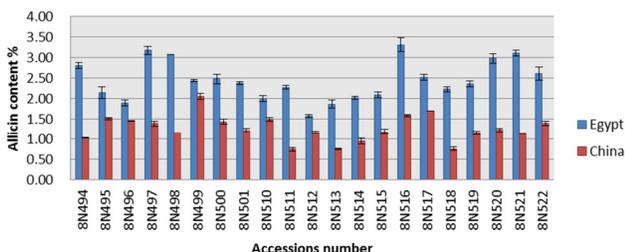
#### Combined Influence of Genotypes and Geographic Location on Allicin Content in Egyptian Garlic Germplasms

The analysis variance of allicin contents of 21 Egyptian garlic germplasm grown in Egypt and China respectively confirmed that the effects of genotype, geographical

location and interaction of genotype and geographical location were found to be significant for allicin content ( $P < 0.001$ ). The difference in allicin contents among most Egyptian garlic germplasm were found to be significant whether being grown in Egypt or in China. The variation ranges of allicin contents of the same batch of garlic germplasm grown in Egypt and China were 3.31~1.58% and 2.05~0.75% respectively (Fig. 3). All the varieties grown in China showed the lower allicin content compared with those grown in Egypt. Furthermore, different germplasm had different response to the environmental difference. The lowest difference (0.39 %) in allicin content of the same germplasm between Egypt and China was observed in 8N499 (Table 4), while, the highest difference (1.98%) in allicin content between two locations was expressed in 8N521 (Table 4). In order to understand stability of allicin content response in different germplasm to the environmental changes, cluster analysis based on allicin reduction (%) for each germplasm over two locations was used (Table 4). Accordingly the 21 Egyptian garlic germplasms were divided into four groups (Group A, B, C and D). The lowest percentage of allicin reduction over location (16.12%) was recorded from 8N499 (clustered in group A). Thus, the allicin content in 8N499 was more stable compared with the other Egyptian garlic germplasm in response to the change in environmental conditions. However, cluster D, consisted of 8 germplasm, had higher percentage of allicin reduction (67.25~56.19%) over two locations. Therefore, the germplasm in group D were found to be more sensitive to environmental changes among tested germplasm in this study.



**Fig. 2:** Alliin content (% dry matter) of germplasm and their clone selected with standard deviations



**Fig. 3:** Alliin content (% dry matter) of Egyptian garlic grown respectively in Egypt and China with standard deviations

## Discussion

Variability in alliin content of garlic germplasm from different origins could be affected by different factors (such as genetic background, geographical factors, drying and analytical methods, etc.). That is why alliin content values ranged greatly in different researches (Baghalian *et al.*, 2005; Gonzalez *et al.*, 2009 and Soto *et al.*, 2010). In our study, different germplasm collected from different countries were planted under the same agronomic conditions. Moreover, freeze drying method was used to dry garlic slices, which has been proved to get high-quality products by maintaining active components (Yu *et al.*, 2007; Mohammad *et al.*, 2009). UPLC based sensitive alliin concentration analysis method developed by Wang *et al.* (2010) was used. Some materials with high and stable alliin content can be used in quality improvement in the future. According to British Pharmacopoeia, garlic powder products with higher alliin content than 4.5 mg/g are more favourable to ensure pharmaceutical and economical activities. The alliin content of all accessions tested was higher than citation in the present study, which indicated that all tested germplasm are suitable to the pharmaceutical industry.

Garlic genetic improvement for high content of alliin is desirable for large-scale garlic cultivation and medicine production. In yield improvement, Baghalian *et al.* (2006) recommended that selection from ecotypes had higher yield

than the world mean garlic yield. In addition, Gvozdanovic *et al.* (2004) reported that some clones had largest bulb mass and dry matter yield of winter garlic. Our results disagreed with the previous studied. Most of the clones selected were found to have higher alliin content compared with their parent. This suggested that improvement of alliin content in garlic could be effective through clonal selections, especially single plant selection.

Environmental conditions for garlic growth in both geographical locations were considerably varied. The temperature and the photoperiod during growing season in Egypt were higher or longer than those in China, while rainfall in Egypt was lower than that in China. Accordingly, the alliin contents of most Egyptian garlic germplasm grown in China were significantly lower than those grown in Egypt. This suggested that high alliin content might be associated closely with the climatic conditions of high temperature, low rainfall, long day, etc. This suggestion could be supported by previous results demonstrated by Zofia and Zaborska (2012), who reported that thiosulfates (where alliin is the major thiosulfate) content in garlic cloves depended on conditions of plant cultivation, insolation, temperature and humidity of soil. Moreover, the significant differences in alliin content from the same cultivar in different locations were in agreement with the results drawn by Soto *et al.* (2010) when four garlic cultivars were used in different geographical areas in Argentina. Alliin content reduction in same germplasms between two locations was 16–63.7% in this study, while Kamenetsky *et al.* (2005) showed that alliin contents of five garlic cultivars from Israel were 150–300% of those in the same genotypes from Netherlands. The difference of alliin content between different locations in different studies may be due to genotypes, environmental characteristics, and analytical method at studied location. On the other hand, our result about the impacts of environmental factors on alliin content in garlic was against to the suggestions made by Baghalian *et al.* (2005, 2006) and Asili *et al.* (2010). This maybe because these studies were conducted in a very narrow range of latitudes and environments, while our study was conducted in a very wide range of environment.

## Conclusion

From the results presented in this study, we can conclude that the alliin content in garlic bulbs was influenced by both genetic and environmental factors. There was a wide range of genetic variation in alliin content among all germplasm studied, which could be classified into four groups based on alliin content. Clonal selections could obviously improve alliin content in garlic bulbs. The accessions 8N499 and 8N239 from China and 8N497, 8N498, 8N516 and 8N521 from Egypt with high alliin content could be used in future breeding programs aiming at improvement of garlic quality. All garlic varieties grown in

China had lower allicin content than those grown in Egypt with the allicin content reduction of 16~63.7%, which proved that production area choice for high allicin is important.

## Acknowledgment

The authors are grateful to Dr. Mahmoud S.S. Abdel-Rahman, Dr. Yasser M.M. Moustafa and Dr. Waled Abdel-Alim for providing us with Egyptian garlic germplasm materials. This work was supported by the Special Fund for Agro-scientific Research in the Public Interest from the Ministry of Agriculture in China (200903018-3), National Science Foundation for Young Scientists of China (31000910), Species conservation project of Ministry of Agriculture (NB2013-2130135-28), and the National Key Technology R&D Program from the Ministry of Science and Technology of China (2013BAD01B04-8). We are also thankful to the key Laboratory of Biology and Genetics Improvement of Horticultural Crops, Ministry of Agriculture and the Supervision and Testing Centre for Vegetable Quality, Ministry of Agriculture, China for the happily provision of their experimental facilities.

## References

- Ali, M., K.K. Al-Qattan, F. Al-Enezi, R.M. Khanafer and T. Mustafa, 2000. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 62: 253–259
- Asili, A., B. Javad, R.N. Mohammad and A. Javad, 2010. Genetic diversity of Persian shallot (*Allium hirtifolium*) ecotypes based on morphological traits, allicin content and RAPD markers. *Open Access J. Med. and Aromatic Plants*, 1: 1–6
- Baghalian, K., M.R. Naghavi, S.A. Ziai and H.A. Naghdi-Badi, 2006. Post-planting evaluation of morphological characters and allicin content in Iranian garlic (*Allium Sativum* L.) ecotypes. *Sci. Hort.*, 107: 405–410
- Baghalian, K., S.A. Ziai, M.R. Naghavi, H.A. Naghdi-Badi and A. Khalighi, 2005. Evaluation of allicin content and botanical traits in Iranian garlic (*Allium Sativum* L.) ecotypes. *Sci. Hort.*, 103: 155–166
- Buiatti, E., D. Palli, A. Decarli, D. Amadori, C. Avellini, S. Bianchi, R. Biserni, F. Cipriani, P. Cocco, A. Giacosa, E. Marubini, R. Puntoni, C. Vindigni, J. Fraumeni and W. Blot, 1989. A case-control study of gastric cancer and diet in Italy. *Int. J. Cancer*, 44: 611–616
- Cavalitto, C. and J.H. Bailey, 1944. Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. *J. Amer. Chem. Soc.*, 66: 1944–1952
- Ercan, C., K. Ali and Ö. Mustafa, 2011. Environmental Effects on Quality Parameters of Plant Products. *International Conference on Biology, Environment and Chemistry IPCBEE*, Vol. 24. IACSIT Press, Singapore
- Gonzalez, R.E., V.C. Soto, M.M. Sance, A.B. Camargo and C.R. Galmarini, 2009. Variability of solids, organosulfur compounds, pungency and health enhancing traits in garlic (*Allium sativum* L.) cultivars belonging to different ecophysiological groups. *J. Agric. Food Chem.*, 57: 10282–10288
- Gvozdanovic, V.J., M. Vasic, J. Cervenski and D. Bugarski, 2004. Genotype and environment effects on yield and quality of winter garlic. *Genetika*, 36: 161–170
- Wang, H., L. Xixiang, L. Su and J. Song, 2010. Quantitative determination of Allicin in *Allium sativum* L. bulbs by UPLC. *Chromatographia*, 71: 159–161
- Ipek, M. and P. Simon, 2002. *Evaluation of Genetic Diversity among Garlic Clones using Molecular Markers: Comparison of AFLPS, RAPDS and ISOZYMES*. Plant and Animal Genome. X Meeting, January 12-16. Available at: <http://www.intl-pag.org/> (Accessed: 15 April 2013)
- Kamenetsky, R., S.I. London, F. Khassanov, C. Kik, A.W. Van-Heusden, G.M. Vrieling-Van, K. Burger-Meijer, J. Auger, I. Arnault and H.D. Rabinowitch, 2005. Diversity in fertility potential and organo-sulfur compounds among garlics from Central Asia. *Biodiver. Conserv.*, 14: 281–295
- Krest, I., 2001. Gender may affect the action of garlic oil on plasma cholesterol and glucose levels of normal subjects. *J. Nutr.*, 131: 1471–1478
- Lampasona, G.S., L. Martinez and J.L. Burba, 2003. Genetic diversity among selected Argentinean garlic clones (*Allium sativum* L.) using AFLP (Amplified Fragment Length Polymorphism) source. *Euphytica*, 132: 115–119
- Lawson, L.D., Z.J. Wang and D. Papadimitriou, 2001. Allicin release under simulated gastrointestinal conditions from garlic powder tablets employed in clinical trials on serum cholesterol. *Planta Med.*, 67: 13–18
- Mohammad, S.R., H.A. Qassim, B.B. Gunnar, S.S. Shyam and A. Ahmed, 2009. Drying kinetics and allicin potential in garlic slices during different methods of drying. *Drying Technol.*, 27: 467–477
- Nagakubo, T., A. Nagasawa and H. Ohkawa, 1993. Micropropagation of garlic through *in vitro* bulblet formation. *Plant Cell, Tiss. Org. Cult.*, 32: 175–183
- Richard, S.R., 2001. Historical perspective on the use of garlic. *J. Nutr.*, 131: 9515–9545
- Ross, Z.M., E.A. O’Gara, D.J. Hill, H.V. Sleightholme and D.J. Maslin, 2001. Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Applied and Environ. Microbiol.*, 67: 475–480
- SAS Institute, 2000. *The SAS System for Windows, release 8.0*. Stat. Anal. Syst. Institute, Cary, North Carolina, USA
- Schulz, V., R. Hansel and V.E. Tyler, 1998. *Rational Phytotherapy*, pp: 107–127. Aphysicians’ Guide to Herba Medicine, Springer
- Soto, V.C., R.E. Gonzalez, M.M. Sance, J.L. Burba and A.B. Camargo, 2010. Genotype environment interaction on the expression of allicin and pyruvic acid in garlic (*Allium sativum* L.). *J. revista de la facultad de Ciencias Agrarias, Universidad Nacional de Cuyo*, 42: 15–22
- Steinmetz, K.A., L.H. Kushi, R.M. Bostick, A.R. Folsom and J.D. Potter, 1994. Vegetables, fruit, and colon cancer in the Iowa Women’s Health Study. *Amer. J. Epidemiol.*, 139: 1–15
- Takagi, H., 1990. Garlic (*Allium sativum* L.). In: *Onions and Allied Crops*, Vol. III, pp: 109–146. Brewster, J.L. and H.D. Rabinowitch, (eds.). CRC Press, USA
- Virginia, L., 2006. Review the analysis of onion and garlic. *J. Chromatogr. A*, 1112: 3–22
- Yu, L., X. Shi-ying and S. Da-Wen, 2007. Preparation of garlic powder with highallicin content by using combined microwave-vacuum and vacuum drying as well as microencapsulation. *J. Food Eng.*, 83: 76–83
- Zofia, O. and W. Zaborska, 2012. A Spectrophotometric assay for total garlic thiosulfates content: kinetic aspects of reaction with chromogenic thiols. *Polish J. Food Nutr. Sci.*, 62: 23–29

(Received 20 January 2014; Accepted 29 April 2014)