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Effect of environment fluctuations on biomass and allicin level in *Allium sativum* (cv. Harnas, Arkus) and *Allium ampeloprasum* var. *ampeloprasum* (GHG-L)

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(Received September 6, 2016)

Summary

Climate variables such as temperature and precipitation are the major abiotic environmental factors determining the yields in crop plants. Given the observed trends in climate change, it is important to carry out analyses aimed at description and selection of plant species characterised by not only the best performance traits but also the best adaptation to climate changes. This study focused on phenological-morphological-biochemical investigations, comparing *Allium sativum* with *A. ampeloprasum* var. *ampeloprasum* GHG-L. We present analyses of economically important traits (biomass and allicin levels) in garlic and GHG-L grown in ecological system and effect of environment fluctuations on these traits. Comparative analysis of the biomass and allicin level in the underground part of garlic and GHG-L revealed not only substantial differences between the species and cultivars, but also great impact of the climate variables on these traits. It was found that garlic and GHG-L cultivated in adverse conditions, exhibited lower yielding rates, but the content of allicin was inversely proportional to the biomass. It should be emphasised that, irrespective of the climate fluctuations, GHG-L produced higher biomass and exhibited higher allicin level than garlic grown in the same conditions, indicating that GHG-L is well adapted to adverse climate changes.

Keywords: *Allium sativum*; *Allium ampeloprasum* var. *ampeloprasum*; allicin; temperature; drought; precipitation; morphological traits.

Abbreviations: DAD – diode array detector; DW – dry weight; HPLC – high-performance liquid chromatography; IS – internal standard; LSDs – least significant differences; PCA – principal component analysis; s.d. – standard deviations.

Introduction

Changing environmental conditions are essential for the process of evolution, as they can contribute to expansion of some species and extinction of others. Seasonal fluctuations in air temperature and precipitation rates are part of a natural cycle of growth and reproduction in plants. These climate variables exert an impact on the transpiration rate, which determines plant growth and productivity through the drought stress effect (PELTER et al., 2004). Currently, trends towards an increase in the mean annual temperature are observed in Europe (CHRISTENSEN and CHRISTENSEN, 2007) and additionally, periodic precipitation deficits, with lower water availability than plants' requirements, are one of the most frequent environmental stresses (BRAY, 1997; KALBARCZYK and KALBARCZYK, 2014). These atmospheric fluctuations can contribute to loss of crop plant yields,

which is of particular importance in view of the human population growth worldwide (BITA and GERATS, 2013).

Plants representing the genus *Allium* are widely used by humans, due to their medicinal properties determined by the presence of numerous sulphur compounds, especially *Allium sativum* (garlic) cultivars have been grown for hundreds of years at all latitudes (BLOCK, 2010). One of the most important and best-known bioactive sulphur compounds is allicin, the content of which may account for up to 70% of all thiosulfates present in garlic (BLOCK, 1992; LAWSON, 1998; ARIGA and SEKI, 2006). Allicin is beneficial to human health due to its antimicrobial, anticancer, antiinflammatory, antithrombotic, and antiatherosclerotic activities (BLOCK, 1985, 1992; KOCH and LAWSON, 1996; SINGH and SINGH, 2008). The centuries-long cultivation of the species in varying environmental conditions has resulted in emergence of hundreds of garlic cultivars, whose traits have been conserved through vegetative propagation (SIMON and JENDEREK, 2003). Therefore, it can be stated that a majority of currently grown *A. sativum* cultivars are a result of spontaneous point mutations rather than genetic recombinations taking place via sexual reproduction (VOLK et al., 2004). Interestingly, such plants differ from each other, sometimes to a substantial degree, in their phenological and morphological traits (POOLER and SIMON, 1993; PARDO et al., 2007; VOLK and STERN, 2009). Clove wrapper colour, bulb size, yield, and flavour of garlic are greatly dependent on the growth environment (WATERER and SCHMITZ, 1994; VOLK and STERN, 2009), which indicates high plasticity of the species in relation to the environment. Particularly noteworthy is the process of *A. sativum* flowering, which is extremely sensitive to changing environmental conditions. POOLER and SIMON (1994) showed that appropriate day length and temperature are essential factors initiating formation of inflorescence shoots. Similarly, other authors emphasise a significant impact of specific temperature and photoperiod regimes on the florogenesis and bulbing processes (MATHEW et al., 2011). Therefore, it can be concluded that garlic is a labile plant responding to varying environmental conditions especially by changes in flowering processes, but the morphological characteristics of vegetative organs might be affected as well. It should be noted, that the ability of species to adapt to environmental changes highly depends on genetic heterozygosity, which facilitates generative propagation. Given the advantages of sexual reproduction, which ensures unlimited genetic fluctuations, extensive investigations are carried out to elucidate the causes of garlic sterility (KONONKOV, 1953; NOVAK, 1972; KONVICKA, 1973; ETOH and SIMON, 2002; KAMENETSKY et al., 2004; WINIARCZYK, 2009; SHEMESH MAYER et al., 20013, 2015). Although studies on generatively reproducing *A. sativum* ecotypes from Central Asia and Caucasia (ETOH, 1986; ETOH et al., 1988; ETOH and SIMON, 2002) have resulted in emergence of fertile lines (INABA et al., 1995; JENDEREK, 1998; KAMENETSKY et al., 2003, 2005), there are no reports about a wider use of this type of plants. Furthermore, the emergence of fertile lines does not guarantee a possibility of mass production, since fertile garlic grown in a different climate gradually loses its ability to produce seeds (ETOH et al.,

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1988). Therefore, irrespective of the ongoing research on overcoming garlic sterility, it is important to search for new *A. sativum* cultivars, that will be best adapted to changing environmental conditions and have the best economically important traits.

The climate changes observed currently in Europe reflected in a constantly increasing trend in the mean monthly temperatures and decreased precipitation rates, justify the need for systematic analyses, aimed at description and selection of garlic cultivars with not only the best performance traits, but also the best adaptation to climate changes. So far, there have been no reports of the impact of the environment on such performance traits in garlic as yielding and the content of bioactive compounds in the underground parts of the plant. This paper focuses on the so far undescribed relationships between climatic factors, biomass growth, and allicin content in *A. sativum* and *A. ampeloprasum* var. *ampeloprasum*. The study was carried out on garlic cultivars commonly grown in Eastern Europe, i.e. Harnas and Arkus, and *A. ampeloprasum* var. *ampeloprasum* – Great Headed Garlic (GHG-L) described recently. GHG-L belongs to a separate clade within the genus *Allium*; it is phylogenetically related to *A. sativum*, which was described by NAJDA et al. (2016). The analyses carried out for the first time, were performed in an ecological cultivation system in the natural environment. The investigations highlight both the climatic-phenological-morphological-biochemical relationships in garlic and GHG-L and the degree of their adaptation and productivity to changing climatic conditions.

Materials and methods

Plant materials and growth conditions

The experimental material included garlic (*A. sativum* cultivars Harnas and Arkus) obtained from Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN and *A. ampeloprasum* var. *ampeloprasum* (GHG-L – GenBank number: KT809295-KT809296) deposited in the collection of the Botanical Garden of Maria Curie-Skłodowska University in Lublin under catalogue number 105/2013. All experiments were performed at the Botanical Garden of Maria Curie-Skłodowska University in Lublin situated in the NW part of the town, latitude 51° 16'N and longitude 22° 30'E. The terrain is highly diversified with

altitudes from 217.0 m to ca. 178.0 m a. s. l. Brown soils formed on loess (33% of fractions < 0.02 mm) with 2.72% humus content and 7.1 alkaline pH in 1 mol KCl·dm⁻³ prevail in the area (Index Seminum 2014 Hortus Botanicus Universitatis Marie Curie-Skłodowska). The experiment was established in a univariate randomised block design with 4 replications. After drying, single cloves of *A. sativum* (both cultivars) and GHG-L were planted in autumn 01.10.2013 and 2014. Thus, no herbicide, fungicide, or any chemical inputs were incorporated, and manual weeding methods were used in each case before and throughout the investigated plant cultivation periods. Data on weather conditions (temperature and rainfall) prevailing during the growing seasons 2013/14/15 as well as the multiyear means (1951-2005) are presented in Fig. 1; numerical data are presented in Tab. S1 (supplementary data).

Phenology and morphology

Observations of the developmental phases and morphological traits of the analysed species were carried out in the period between clove germination and anthesis of single flowers in the inflorescence. The length of the inflorescence shoot was measured from the base, and the inflorescence composition was analysed after growth and maturation of the analysed species (15.08.2014 and 2015). Morphological observations of the underground parts were conducted in the post-harvest period (30.07.2014 and 2015). Approximately 50 plants from each species were analysed. Macroscopic images were taken with a Nikon D300 camera equipped with an AF MICRO NIKKOR 60 mm objective. Single flowers from the inflorescences (Arkus and GHG-L) were examined under a stereoscopic microscope Olympus SZX16 equipped with a DP 72 camera.

Biochemical analyses

The analysis of the allicin content in the underground parts of *A. sativum* (both cultivars) and GHG-L were performed immediately after the harvest period. For evaluating the allicin content, we used the methods of LAWSON et al. (1991) and BAGHALIAN et al. (2005). This analysis was performed in the following steps: distilled water was added to 5 g of fresh cloves of *A. sativum* cultivar Harnas and Arkus

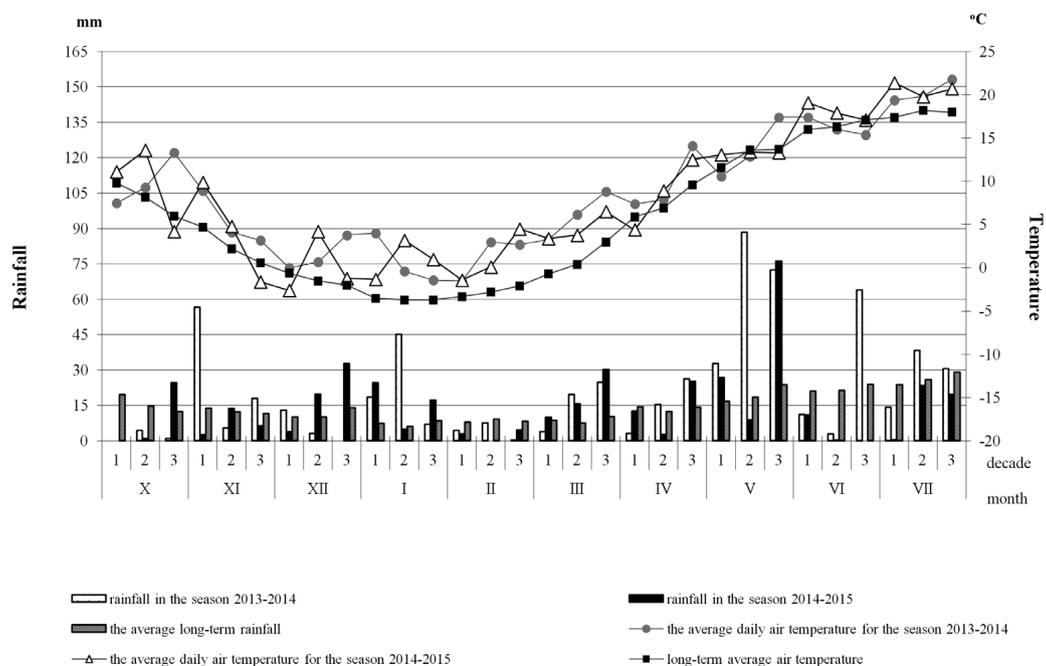


Fig. 1: Meteorological data from 2013/2014/2015 and multiyear average (1951-2005) rainfall (mm) and temperature (°C). The meteorological station in Lublin Litewski Square (Index Seminum 2013, 2014, and 2015 Hortus Botanicus Universitatis Marie Curie-Skłodowska).

and *A. ampeloprasum* var. *ampeloprasum* (10 ml per g), mixed, kept for 10 min at room temperature, and centrifuged at 14000 rpm for 5 min. Next, 600 µl of the supernatant was added to methanol (1:1 v/v).

The HPLC method with butyl parahydroxybenzoate as an internal standard (IS) was used for quantification of allicin. The 1 mg of IS corresponds to 8.65 mg of allicin. The HPLC conditions were as follows: LaChrom-Merck type equipped with a diode array detector DAD (L-7450), quaternary pump (L-7100), degasser (L-7612), injection loop 20 µl, thermostat (L-7360), Rheodyne injector, steel column LiChrospher 100 RP C 18 (250 mm × 4 mm dimensions) filled with the stationary phase (dp = 5 µm). The mobile phase was methanol: water (50% v/v) at a flow rate of 0.8 ml/min. Peaks were detected at 254 nm and recorded by a LaChrom-Merck recorder model L-7210. The content of allicin was calculated by the D-7000 HPLC System Manager program. Each sample was measured at least twice. Values were expressed as mg allicin 100g⁻¹ DW.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed in order to verify the zero hypotheses on a lack of differences between species, between years as well as the hypothesis on a lack of an effect of species × years interaction of the values of the observed traits, i.e. bulb size, bulb weight, clove size, clove weight, and allicin.

For individual traits, mean values and standard deviations (s.d.) were calculated. Moreover, Fisher's least significant differences (LSDs) were also estimated at the significance level $\alpha = 0.05$.

The results were also analysed using multivariate methods. The possibility of graphic distribution of the species in the particular years of the study described by the bulb size, bulb weight, clove size, and clove weight together was obtained with the use of the principal component analysis. Principal component analysis (PCA) is a mathematical procedure that uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (NOWOSAD et al., 2016). All the analyses were conducted using the GenStat v. 17 statistical software package.

Results

Climate parameters

The investigations were conducted in the growing seasons of 2013/14 and 2014/15, which were characterised by substantial fluctuations in the climate variables (temperature and precipitation). The precipitation rates in the analysed period were considerably lower in 2015 than in 2014. The differences ranged from 80% to 120%, as noted in May (2014 - 175.7 mm/2015 - 101.7 mm), June (2014 - 62.7 mm/2015 - 16.3 mm), or July (2014 - 50.0 mm/2015 - 21.9 mm). The meteorological data from the two analysed seasons were compared with the multiyear sum means. The observations of the weather variables indicate that 2014 and 2015 were warmer by 3.1 °C and 2.7 °C, respectively, than the multiyear temperature means. In turn, the atmospheric precipitation rates in the analysed seasons were highly varied. The total precipitation sum in 2014 was by 166.5 mm higher and that in 2015 by 84 mm lower than the multiyear totals (Fig. 1). Detailed numerical meteorological data are presented in Tab. S1 (supplementary data). In the period of intensive biomass growth and accumulation of reserve substances in the *Allium* species, the average air temperature in 2014 was comparable to that in 2015, and the precipitation rate in 2014 was substantially higher than that in 2015. A comparison of the two seasons with the multiyear means revealed a growing trend in air temperature; in turn, precipitation varied widely with clear periodic droughts in 2015.

Phenology analyses

In this study, we analysed *A. sativum* cv. Harnas and Arkus as well as *A. ampeloprasum* var. *ampeloprasum* GHG-L, plants exhibiting genetic uniformity, investigated by our research team. The material used for reproduction (cloves) was considered to be of high initial quality based on participant surveys. *A. sativum* had been cultivated for many years in the Botanical Garden and the yields were monitored every year. Harnas, Arkus, and GHG-L have to be exposed to low temperatures before the growing season. Single cloves of each species were planted in autumn (2013 and 2014) and thus vernalised. In both 2014 and 2015, the first leaves appeared in spring between March 1 and 15 (1-6.03. Harnas and Arkus, 9-15.03. GHG-L). The inflorescence shoot in Harnas, Arkus, and GHG-L was formed in late May and early June in 2014 and 2015 (1.05-7.05. Harnas, 1.05-5.05. Arkus, 20.05-27.05. GHG-L). The observations of the development of the inflorescence shoot in the analysed species showed that anthesis proceeded in the period from 25.07 to 5.08 in Arkus and from 2.07 to 12.08 in GHG-L, whereas no anthesis was noted in the Harnas cultivar in 2014 and 2015. The development of the aboveground part of the investigated species was synchronous in 2014 and 2015 (Fig. 2).

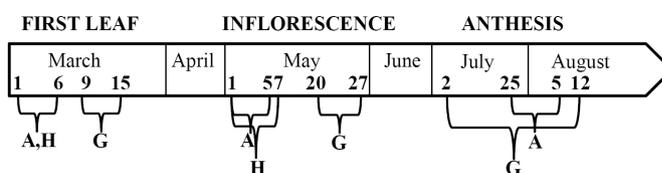


Fig. 2: Period of formation of the first leaf, inflorescence shoot, and anthesis in the species analysed in 2014 and 2015. *A. sativum* cv. Harnas (H), Arkus (A), *A. ampeloprasum* var. *ampeloprasum* GHG-L (G).

Morphology analyses

The comparative analysis consisted in morphological observations of the aboveground parts in terms of the foliage attitude, leaves width, initiation of the inflorescence, and formation of its components (flowers and bulbils). The underground part was assessed for its shape and the size of the bulb and its components – cloves and bulbils. The *A. sativum* cultivars, i.e. Harnas and Arkus, and GHG-L had scaly assimilating leaves, which initially did not differ in their shape and attitude. In contrast, they clearly differed in the width of the leaf blade, which was substantially broader in GHG-L from the beginning of the growth of the aboveground part. Furthermore, after emergence of a greater number of leaves, the foliage attitude in GHG-L changed from erect to semi-erect (Fig. 3a, e, i). A permanent and characteristic feature of genus *Allium* species is the amount of biomass (number of leaves) indispensable for formation of the inflorescence shoot. Prior to the emergence of the inflorescence shoot, Harnas and Arkus produced 7 leaves, while GHG-L had from 8 to 11 leaves. There were also differences in the shape of the inflorescence shoot; in *A. sativum*, it was initially curved, but it slightly straightened during the inflorescence maturation, reaching a length of 1000-1200 mm in Harnas and 1200-1500 mm in Arkus (Fig. 3b, f). In turn, the inflorescence shoot in GHG-L was erect from the beginning to the end of its growth and reached a considerable length of 1500-1700 mm (Fig. 3j). The inflorescence in the Harnas and Arkus cultivars and in GHG-L was initially covered by a broad transformed bract called the spathe (Fig. 3c, g, k). After spathe dehiscence, an umbel-like inflorescence was visible; in Harnas and Arkus, it was composed of tiny single flowers and numerous bulbils (Fig. 3d, h). In contrast, GHG-L formed numerous flowers but no bulbils in the inflorescence (Fig. 3l). The perianth of the flower was green in all the analysed species. During the maturation period until the anthesis stage, it changed colour into purple in Arkus and GHG-L. In turn, there was no anthesis in Harnas

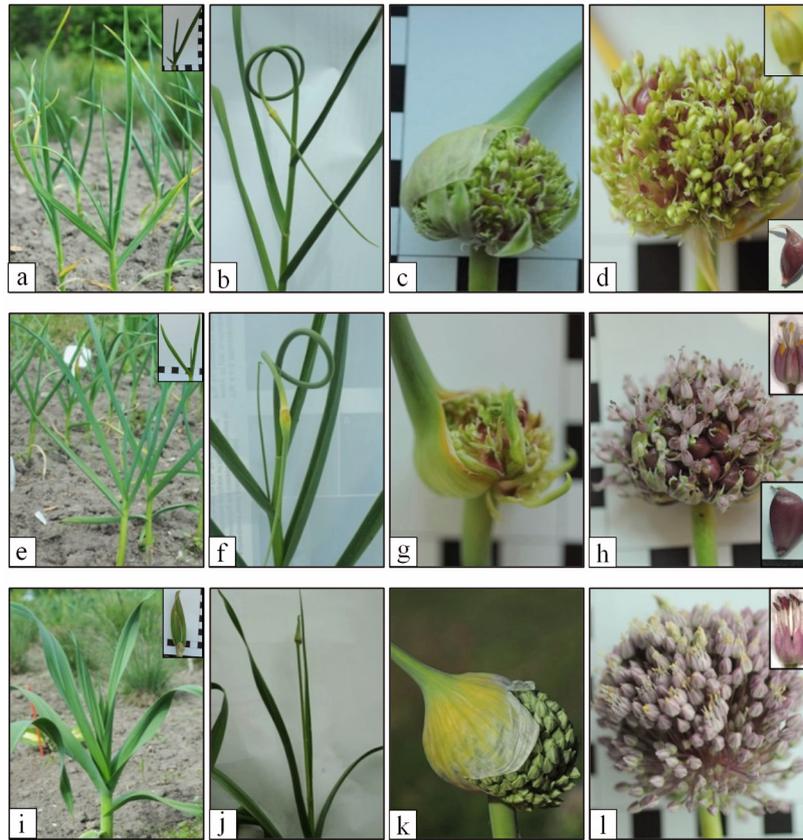


Fig. 3: Morphology of the aboveground part of a flowering plant of *A. sativum* cv. Harnas: **a, b** – inflorescence shoot, **c** – inflorescence partially covered by a spathe, **d** – inflorescence devoid of a spathe and a single flower and single topset; cv. Arkus: **e, f** – inflorescence shoot, **g** – inflorescence partially covered by a spathe, **h** – inflorescence devoid of a spathe and a single flower during anthesis and single topset; *A. ampeloprasum* var. *ampeloprasum* GHG-L: **i, j** – inflorescence shoot, **k** – inflorescence partially covered by a spathe, **l** – inflorescence devoid of a spathe and a single flower during anthesis.

and the colour of the perianth remained green until the end of the vegetation period.

In the analysed plants, the underground part, i.e. the bulb composed of cloves, did not differ in its shape of the base and distribution of cloves. Harnas, Arkus, and GHG-L had a flat shape of the bulb base (Fig. 4a, d, g) and radial distribution of bulb cloves (Fig. 4b, e, h). The plant differed in the bulb colour (Harnas, GHG-L – yellowish and Arkus – purple), the number of bulb cloves (Harnas 6-15, Arkus 5-8, GHG-L 3-11), as well as the presence of bulbils on the peduncles in GHG-L (Fig. 4g – small picture) and the absence of bulbils in the Harnas and Arkus bulbs. Tab. 1 presents a summary of the morphological traits of the analysed species. The analysis of the underground biomass was based on measurements of the size of the bulbs and cloves (Fig. 4a, c – bar) and the weight of the bulbs and cloves of the analysed species. The data obtained in 2014 indicate that the diameter/weight of bulbs in both Harnas (503.2 mm/36.13 g) and Arkus (437.9 mm/21.91 g) was significantly lower than that in GHG-L (821.4 mm/95.25 g). This trend was also observed in 2015 (Harnas – 436.4 mm/27.65 g; Arkus – 366.6 mm/18.82 g; GHG-L – 691.1 mm/84.65 g). Similarly, the values of the diameter/weight of cloves in the analysed Harnas and Arkus cultivars were lower than in GHG-L: Harnas – 179.8 mm/2.838 g; Arkus – 207.1 mm/3.86 g; GHG-L – 296.7 mm/18.722 g in 2014. While in 2015, Harnas – 126.0 mm/2.658 g; Arkus – 175.6 mm/3.817 g; GHG-L – 272.4 mm/14.064 g. The results of the analysis of variance indicated that the main effects of the species and years as well as the effect of the species × years interaction were statistically significant for all observed traits (Tab. S2, supplementary data). The multidimensional analysis of the tested traits comparing the species and years in terms of bulb size,

bulb weight, clove size, and clove weight simultaneously is shown in Fig. 5A, B, C, and D. Numerical data are presented in Tab. S3 (supplementary data). GHG-L produced greater biomass amounts than garlic; furthermore, the diameter/weight of bulbs and cloves was shown to be greater in 2014 than in 2015 in all the species. The multivariate analysis of variance (ANOVA) allowed discarding the tested hypotheses about the lack of multi-trait variability between the species, years and species × years interaction ($P < 0.001$). Individual traits are of different importance and have a different share in the joint multivariate variability. A study on the multivariate variability for treatments also includes identification of the most important traits in the multivariate variation of treatments. Principal components analysis PCA is a statistical tool making it possible to solve this problem. It showed that the analysed *A. sativum* cultivars formed separate groups in relation to GHG-L, and their variability ranges did not overlap. All traits with the exception of the clove size, which differentiated the cultivars best, were correlated with the first principal component (PC1). In turn, the clove size was correlated with the second principal component (PC2). PC1 explained as much as 97.67% of the variability, whereas PC2 explained only 2.23% of the total variability of the analysed morphological traits (Fig. 6).

Alliin level analyses

The phenological and morphological analyses were extended with biochemical assays, which revealed the alliin level in the underground part (cloves) of *A. sativum* cv. Harnas and Arkus as well as *A. ampeloprasum* var. *ampeloprasum* GHG-L grown in an ecological cultivation system. The analyses showed differences in the content of



Fig. 4: Morphology of the underground part of *A. sativum* and *A. ampeloprasum* var. *ampeloprasum*. Single bulb (the site of the bulb diameter measurement - bar): **a** – Harnas, **d** – Arkus, **g** – GHG-L (bulbils located on a peduncle - small picture). Cross section across the bulb shows the distribution of cloves: **b** – Harnas, **e** – Arkus, **h** – GHG-L. Single cloves (the site of the clove diameter measurement - bar): **c** – Harnas, **f** – Arkus, **i** – GHG-L.

Tab. 1: Morphological traits of *A. sativum* (cv. Harnas, Arkus) and *A. ampeloprasum* var. *ampeloprasum* (GHG-L). IS – inflorescence shoot.

| Morphological traits | Harnas | Arkus | GHG-L |
|------------------------------|-----------|-----------|------------|
| Leaf width | Narrow | Narrow | Broad |
| Foliage attitude | Erect | Erect | Semi-erect |
| Leaves before IS (number) | 7 | 7 | 8-11 |
| Shape of IS | Curved | Curved | Erect |
| Length of IS (mm) | 1000-1200 | 1200-1500 | 1500-1700 |
| Flower colour | Green | Purple | Purple |
| Bulbils in the inflorescence | Present | Present | Absent |
| Bulb colour | Yellowish | Purple | Yellowish |
| Shape of bulb base | Flat | Flat | Flat |
| Distribution of bulb cloves | Radial | Radial | Radial |
| Number of bulb cloves | 6-15 | 5-8 | 3-11 |
| Bulb with bulbils | Absent | Absent | Present |

this substance between garlic and GHG-L. Particularly noteworthy is the fact that there were differences in the alliin level between the vegetation seasons in all the analysed species. The cloves of the Harnas cultivar exhibited lower alliin content in 2014 (on average 1.262 g 100g⁻¹ DW) than in 2015 (1.813 g 100g⁻¹ DW). Similarly, Arkus had lower amounts of alliin in 2014 (1.081g 100g⁻¹ DW) than in 2015 (1.335 g 100g⁻¹ DW). The same tendency in the alliin level in cloves was noted in GHG-L (2014 – 1.343 g 100g⁻¹ DW, 2015 – 2.097 g 100g⁻¹ DW). The results of the analysis of variance indicated that the main effects of the species and years as well as the effect of the species × years interaction were statistically significant for the alliin level (Tab. 2 and 3). To sum up, the alliin content was lower in 2014 than in 2015 in all the analysed species. Noteworthy, GHG-L

was found to be richer in alliin in comparison with the garlic cultivars in both growing seasons.

Discussion

Climate variables, e.g. air temperature and atmospheric precipitation are major abiotic factors affecting plant growth and development. They induce ontogenetic development of plants, in particular bulb plants, which can be observed upon vernalisation thereof (KAMENETSKY and OKUBO, 2012). Crop plants have been observed to have relatively low adaptability to climate variables, such as periodic drought; hence, the genetic yield potential cannot be fully exploited in changing weather conditions (BRAY, 1997). Therefore, the search for species able to adapt efficiently to adverse environmental conditions is desirable.

Plants representing the genus *Allium* are widely distributed in cultivation and used as a valuable source of bioactive compounds; therefore, maintenance of the high level of yielding is extremely vital. This can be achieved by production of new cultivars with better adaptation to climatic variables or by selection of existing varieties that can adapt to climate fluctuations. Since, *A. sativum* has lost the capability of sexual reproduction, many researchers have focused their attention on the impact of the environment on the formation of generative organs in this plant, and on the process of gametogenesis. This research is important in view of restoration of the capability of generative reproduction and, consequently, effective production of new varieties (INABA et al., 1995; JENDEREK, 1998; ETOH and SIMON, 2002; KAMENETSKY et al., 2003, 2004, 2005; SHEMESH MAYER et al., 20013, 2015). On the other hand, there is no detailed information on the relationship between the climate impact and garlic yielding and the content of bioactive substances such as alliin.

These investigations focus on assessment of biomass and the level of alliin in two species, garlic and GHG, in response to adverse climate changes. The *A. sativum* cultivars Harnas and Arkus analysed in this study are widely grown in central and eastern Europe. In turn,

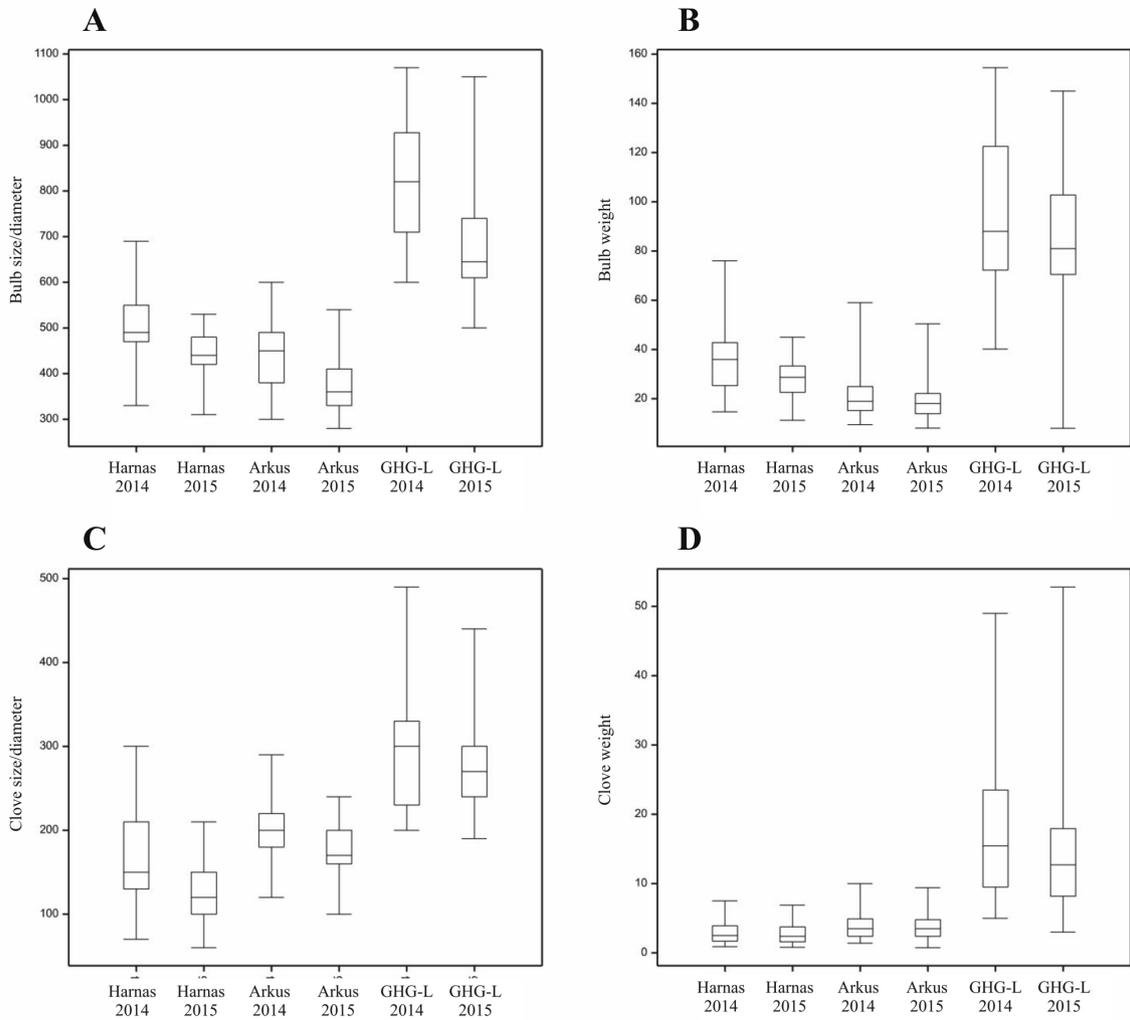


Fig. 5: Box-and-whisker diagram of the values of **A** bulb size (mm), **B** bulb weight (g), **C** clove size (mm), **D** clove weight (g); classified by the *A. sativum* cv. Harnas, Arkus and *A. ampeloprasum* var. *ampeloprasum* GHG-L and the years of the study.

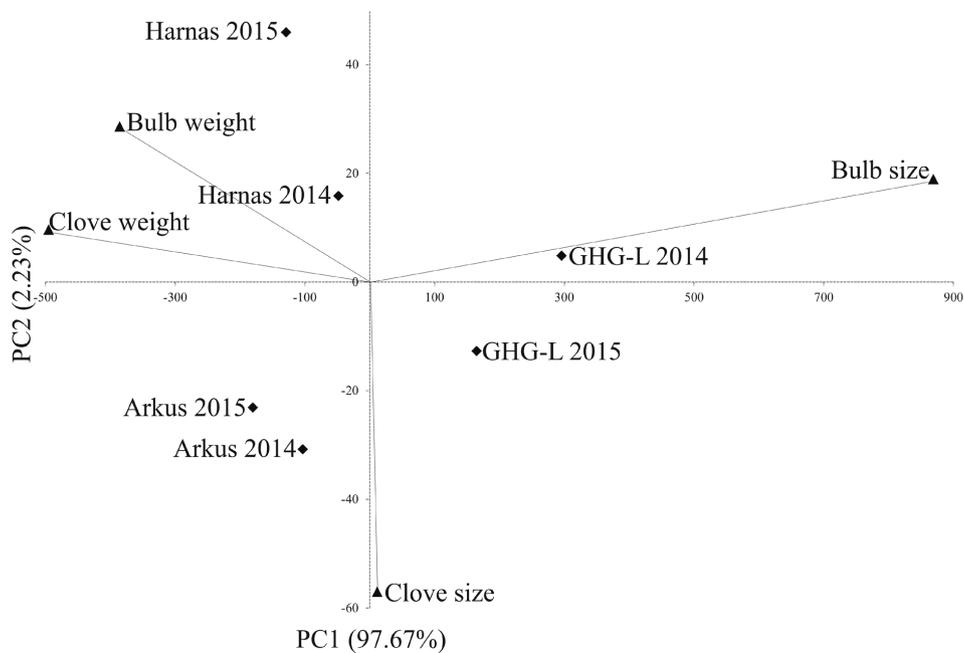


Fig. 6: Principal components analysis (PCA) – grouping coordination scheme of *A. sativum* and *A. ampeloprasum* var. *ampeloprasum* GHG-L cultivars in relation to morphological features.

Tab. 2: Mean squares from analysis of variance for allicin.

| Source of variation | Number of degrees of freedom | Mean squares |
|---------------------|------------------------------|--------------|
| Year | 1 | 1.23343*** |
| Species | 2 | 1.16741*** |
| Year × Species | 2 | 0.05522*** |
| Residual | 24 | 0.00008 |

*** P<0.001

Tab. 3: Mean values and standard deviations (s.d.) of allicin levels (dry mass) in raw *A. sativum* cv. Harnas, Arkus and *A. ampeloprasum* var. *ampeloprasum* GHG-L (g 100g⁻¹ DW).

| Year | 2014 | | 2015 | |
|-----------------------------|--------------|--------------|--------------|---------------|
| | Mean | s.d. | Mean | s.d. |
| Species | | | | |
| Harnas | 1.262 | 0.00608 | 1.813 | 0.00841 |
| Arkus | 1.081 | 0.00576 | 1.335 | 0.0092 |
| Mean for cv. | 1.172 | | 1.574 | |
| GHG-L | 1.685 | 0.00394 | 2.097 | 0.01626 |
| Mean for all species | 1.343 | 0.262 | 1.748 | 0.3259 |

LSD_{0.05} Year: 0.0069; Species: 0.0085; Year × Species: 0.01198

the *A. ampeloprasum* var. *ampeloprasum* GHG-L is cultivated non-commercially in eastern Poland. GHG-L is phylogenetically related to *A. sativum* but, as we have shown recently, has higher nutritional values than garlic (NAJDA et al., 2016). Given the significant climatic differences in the two growing seasons, the periods of 2013/2014 and 2014/2015, the biomass and level of allicin were analysed to focus more light on the relationship between climate fluctuations and biomass production among these two species. The species analysed during the two seasons were grown in an ecological cultivation system in the same area. The seasons were characterised by fluctuations in average temperatures and clearly different values of rainfall. Compared to the previous 54 years, higher values of the mean air temperature (by 3.1 °C) and higher mean precipitation rates (by 166.5 mm) were noted in 2014. In contrast, in 2015, the mean air temperature was by 2.7 °C higher, but particularly noteworthy, the mean precipitation rates were by 84 mm lower than the multiyear means. These data indicate a constant increase in the air temperature and occurrence of substantial periodic water shortages, which was also reported from Europe (MARSZ, 2005; BITA and GERATS, 2013; KALBARCZYK and KALBARCZYK, 2014). Thus, these two vegetative seasons are very suitable to compare the analyses with respect to biomass production by the analysed species.

The first stage of our study was the comparative phenological analysis of *A. sativum* cv. Harnas, and Arkus and *A. ampeloprasum* var. *ampeloprasum* GHG-L. It showed that some periodic phenomena in the development of the analysed species, such as formation of the first leaf and the inflorescence shoot, took place synchronically in Arkus and Harnas. In contrast, the first leaf and the inflorescence shoot in GHG-L were formed at a later period than in garlic, and anthesis lasted longer than in Arkus. In Harnas, there was no anthesis stage in the inflorescence. However, no significant phenological differences were observed between the analysed cultivars in both growing seasons (2014 and 2015).

Another step was the morphological analysis of the aboveground parts of the analysed plants. These observations indicate that Harnas

and Arkus produce assimilating leaves with the same size, shape, and number, and inflorescence shoots with a similar shape, size, and similar constituents. In turn, GHG-L differed from garlic in its size, habit, and inflorescence composition, as it produced numerous flowers but no bulbils. These differences are associated with the fact that GHG-L resembles leek in its aboveground parts, with which it is phylogenetically related (as well as with garlic) (NAJDA et al., 2016). It can be stated that the environmental variables (temperature and rainfall) had no effect on the morphological traits of the aboveground parts of Harnas, Arkus, and GHG-L during the two analysed growing seasons. It can be explained by the fact that, in the initial stages of analysed plant development, winter soil water resources were sufficient for effective growth of the aboveground parts and the periodic precipitation shortages did not produce a negative effect, indicating that initial water resources are important at first stages of the aboveground parts of the analysed plants.

The subsequent comparative analysis of the underground parts revealed that such morphological traits as the shape of bulbs and distribution of cloves were identical in all the analysed species, which however differed in the colour and number of cloves in the bulb. Additionally, GHG-L had characteristic bulbils arranged on peduncles, which were not found in Harnas and Arkus. Based on the analysis of the two vegetation seasons, it can be concluded that the climate fluctuations had no impact on the aforementioned features of the underground parts in Harnas, Arkus, and GHG-L, as is formed at early stage of plant formation. These observations are consistent with previous reports (VOLK and STEARN, 2009), whose authors conclude that such traits as the colour and shape of bulbs and arrangement of cloves in the bulb are species specific and independent of environmental parameters. It has been found that the yields determined as diameter and weight of bulbs and cloves in the analysed plants revealed differences between the cultivars Harnas, Arkus, and GHG-L. Noteworthy, the differences between Harnas and Arkus were inconsiderable, whereas GHG-L was characterised by a significantly greater diameter and bulb and clove weight, compared with garlic. GHG (Great Headed Garlic, synonym “elephant garlic”) is known for the large size of its underground bulb and is cultivated at all latitudes as a garlic substitute (FIGLIUOLO et al., 2001; BOHANEK et al., 2005; HIRSCHGEGGER et al., 2006, 2010; LANZAVECHIA, 2009; NAJDA et al., 2016). It should be emphasized that the economically important trait, i.e. the bulb size, ranks GHG in the first place among the plants from the genus *Allium*. The comparative analysis of the yielding level in the examined plants in both vegetation seasons showed noteworthy dependence. Significant differences were observed between the cultivars. In 2015 with water shortage, all the plants produced bulbs with a smaller diameter and weight than in 2014, which implies that the unfavourable climate conditions led to a decrease in the yields of all the analysed cultivars. It should also be emphasised that, irrespective of the lower yields, GHG-L was characterised by greater biomass than that of garlic in both growing seasons and produced the highest yield. Our observations are in line with other authors, which reported that garlic yielding depends not only on the genetic traits of the species, but largely on climatic variables (WATERER and SCHMITZ, 1994; VOLK and STERN, 2009; WANG et al., 2014).

The yielding efficiency is especially important in crop plants, but an equally important trait in garlic is the content of bioactive compounds, e.g. allicin. The presented comparative analysis of this compound in the bulb (cloves) revealed intriguing results, which has never been reported for garlic species, ecologically cultivated in the natural environment. The level of allicin contained in the cloves differed not only between cultivars Harnas, Arkus, and GHG-L, but interesting dependence was noticed between the studied seasons, the level of allicin was higher in all the plants analysed in 2015 with seasonal droughts, although their yield was lower than that in 2014. These results are consistent with previous observations carried out in iso-

lated systems, which showed a correlation between increased alliin content and decreased bulb weight, which was associated with modulation of the metabolism of these plants by humidity and temperature (BLOEM et al., 2011). Furthermore, LEE et al. (2013) have shown a correlation between the activity of γ -glutamyl transpeptidase (GGT), i.e. an enzyme involved in the metabolism of sulphur compounds and the temperature and humidity, which may be a direct factor contributing to the increase in the alliin content in garlic heads. Additionally, a close relationship between the growing conditions (fertilisation) and the sulphur content in plants from the genus *Allium* has also been reported (WANG et al., 2014). Thus, alliin level may represent a sensitive indicator of metabolic changes upon fluctuating growth conditions.

Conclusion

This paper presents for the first time an analysis of economically important traits in garlic and GHG-L grown in an ecological cultivation system, without human interference. The comparative analysis of the development of the aboveground and underground parts of each species included an important yield-determining element, i.e. environment fluctuations during the two vegetation seasons. Analysed plants grown in adverse climatic conditions (periodic droughts) were characterised by lower mass yields, but the alliin content was inversely proportional to the increase in biomass. In the adverse soil moisture conditions in 2015, garlic cv. Harnas exhibited a substantial increase in the level of alliin contained in the underground bulbs. It should be emphasised that, irrespective of the climate fluctuations, GHG-L had a significantly higher biomass and a higher level of alliin than garlic grown under the same conditions. The correlations between the bulb size and the alliin content in Harnas, Arkus, and GHG-L in both growing seasons are presented in Fig. 7. As already reported in earlier studies and according to the data presented in this paper, it can be concluded that *A. ampeloprasum* var. *ampeloprasum* – GHG-L represents much better characteristics from the agricultural and biochemical/nutritional point of view. Thus, greater popularisation of GHG and introduction thereof to large-scale cultivation should be considered as an alternative to traditional garlic, especially in regions with significant climate fluctuations.

Acknowledgements

D.T. – conceived the study, interpreted the data, wrote the manuscript, J.B. – statistical analyses, A.N. – biochemical analyses, A.D. – provided materials, K.W. – conceived the study, interpreted the data.

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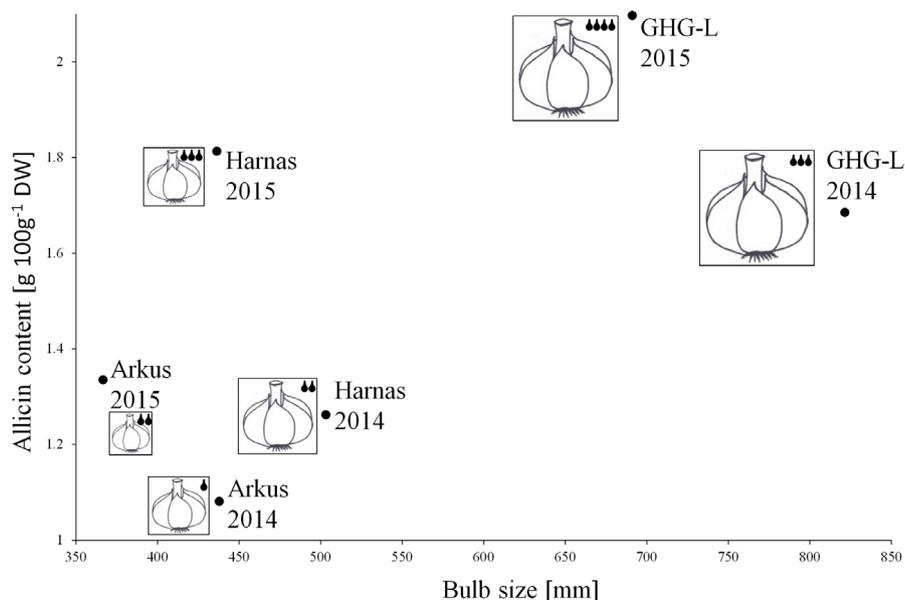


Fig. 7: Correlations between the bulb size and alliin level of the *A. sativum* cv. Harnas and Arkus and *A. ampeloprasum* var. *ampeloprasum* GHG-L in the years of the study. Graphic signs: black droplet – alliin level (number of droplets corresponds to the alliin content), bulb size – yields.

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Tab. S1: Meteorological data from 2013/2014/2015 and multiyear average (1951-2005) rainfall (mm) and temperature (°C). The meteorological station in Lublin Litewski Square (Index Seminum 2013, 2014, and 2015 Hortus Botanicus Universitatis Marie Curie-Skłodowska)

| Month | Decade | Rainfall (mm) | | | Temperature (°C) | | |
|-------|--------|---------------|---------|-----------|------------------|---------|-----------|
| | | 2013/14 | 2014/15 | Long-term | 2013/14 | 2014/15 | Long-term |
| X | 1 | 0 | 0 | 19.4 | 7.5 | 11.1 | 9.8 |
| | 2 | 4.4 | 1 | 14.6 | 9.3 | 13.6 | 8.2 |
| | 3 | 1 | 24.6 | 12.3 | 13.3 | 4.2 | 6 |
| XI | 1 | 56.6 | 2.6 | 13.7 | 8.9 | 9.9 | 4.7 |
| | 2 | 5.4 | 13.7 | 12 | 4.1 | 4.8 | 2.2 |
| | 3 | 18 | 6.4 | 11.4 | 3.2 | -1.6 | 0.6 |
| XII | 1 | 13 | 3.9 | 9.9 | 0 | -2.6 | -0.6 |
| | 2 | 3.2 | 19.9 | 9.9 | 0.7 | 4.2 | -1.5 |
| | 3 | 0 | 32.7 | 13.9 | 3.8 | -1.2 | -2 |
| I | 1 | 18.5 | 24.6 | 7.3 | 4 | -1.3 | -3.5 |
| | 2 | 45.2 | 4.9 | 6 | -0.4 | 3.2 | -3.7 |
| | 3 | 7.1 | 17.3 | 8.3 | -1.4 | 1 | -3.7 |
| II | 1 | 4.4 | 2.9 | 7.7 | -1.5 | -1.4 | -3.3 |
| | 2 | 7.7 | 0 | 9.1 | 3 | 0.1 | -2.8 |
| | 3 | 0.2 | 4.5 | 8.1 | 2.7 | 4.5 | -2.1 |
| III | 1 | 3.8 | 9.9 | 8.4 | 3.3 | 3.4 | -0.7 |
| | 2 | 19.7 | 15.7 | 7.4 | 6.2 | 3.8 | 0.4 |
| | 3 | 24.9 | 30.2 | 10.1 | 8.8 | 6.5 | 3 |
| IV | 1 | 3.2 | 12.7 | 14.2 | 7.4 | 4.4 | 5.9 |
| | 2 | 15.3 | 2.8 | 12.3 | 7.9 | 8.9 | 6.9 |
| | 3 | 26.2 | 25.3 | 14.1 | 14.1 | 12.5 | 9.6 |
| V | 1 | 32.7 | 26.8 | 16.6 | 10.6 | 13.1 | 11.6 |
| | 2 | 88.4 | 8.9 | 18.3 | 12.9 | 13.4 | 13.6 |
| | 3 | 72.5 | 76.2 | 23.5 | 17.4 | 13.3 | 13.7 |
| VI | 1 | 11.2 | 11.1 | 20.8 | 17.4 | 19.1 | 16 |
| | 2 | 3 | 0.1 | 21.2 | 16 | 17.9 | 16.3 |
| | 3 | 63.9 | 0 | 23.8 | 15.4 | 17.1 | 17.1 |
| VII | 1 | 14.2 | 0.4 | 23.5 | 19.4 | 21.4 | 17.4 |
| | 2 | 38.4 | 23.5 | 25.7 | 19.8 | 19.8 | 18.2 |
| | 3 | 30.6 | 19.7 | 29 | 21.8 | 20.7 | 18 |

Tab. S2: Mean squares from analysis of variance for the observed traits of the studied species

| Trait | Source of variation | Species | Year | Species × Year | Residual |
|--------------|---------------------|-------------|------------|----------------|----------|
| Bulb size | d.f. | 2 | 1 | 2 | 335 |
| | m.s. | 7456640*** | 1342025*** | 68301*** | 6413 |
| Bulb weight | d.f. | 2 | 1 | 2 | 274 |
| | m.s. | 297118.6*** | 8660.8*** | 974.5* | 407.9 |
| Clove size | d.f. | 2 | 1 | 2 | 592 |
| | m.s. | 956818*** | 258135*** | 13966** | 2308 |
| Clove weight | d.f. | 2 | 1 | 2 | 692 |
| | m.s. | 11790.41*** | 288.64*** | 363.45*** | 22.32 |

* P<0.05; ** P<0.01; *** P<0.001

Tab. S3: Mean values and standard deviations (s.d.) for the observed traits of *A. sativum* cv. Harnas, Arkus, and *A. ampeloprasum* var. *ampeloprasum* GHG-L

| Year | Bulb size | | | | Bulb weight | | | |
|---------------------|--------------------------------|--------|-------|--------|-------------------------------|--------|--------|-------|
| | 2014 | | 2015 | | 2014 | | 2015 | |
| | Mean | s.d. | Mean | s.d. | Mean | s.d. | Mean | s.d. |
| Harnas | 503.2 | 73.89 | 436.4 | 51.73 | 36.13 | 13.52 | 27.65 | 8.17 |
| Arkus | 437.9 | 72.2 | 366.6 | 58.06 | 21.91 | 11.11 | 18.82 | 7.52 |
| GHG-L | 821.4 | 147.83 | 691.1 | 162.59 | 95.25 | 34.57 | 84.65 | 35.48 |
| LSD _{0.05} | S: 14.58; Y: 11.52, S×Y: 21.00 | | | | S: 3.07; Y: 2.425, S×Y: 4.423 | | | |
| Year | Clove size | | | | Clove weight | | | |
| | 2014 | | 2015 | | 2014 | | 2015 | |
| | Mean | s.d. | Mean | s.d. | Mean | s.d. | Mean | s.d. |
| Harnas | 179.8 | 62.81 | 126.0 | 36.37 | 2.838 | 1.479 | 2.658 | 1.351 |
| Arkus | 207.1 | 39.41 | 175.6 | 25.38 | 3.86 | 1.964 | 3.817 | 1.843 |
| GHG-L | 296.7 | 65.77 | 272.4 | 52.61 | 18.722 | 11.673 | 14.064 | 8.873 |
| LSD _{0.05} | S: 8.73; Y: 6.9, S×Y: 12.58 | | | | S: 8.73; Y: 6.9, S×Y: 12.58 | | | |