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Garlic Cultivation for High Health-Value

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ABSTRACT

Garlic (*Allium sativum* L.) is well-known for presenting numerous health benefits linked to the high amount of organo-sulfur compounds, especially aliin, produced in the bulbs. The accumulation of alliin and its precursors in garlic is dependent on both genetic factors and environmental conditions in which the plants are cultivated. Indeed, different organo-sulfur compounds profiles were obtained for several accessions originating from Central Asia when grown in natural conditions in two different climatic contexts. Field trials carried out on three commercial varieties grown under two different climatic conditions of Western Europe confirmed this observation and suggested an important role of the cropping temperature, soil status and water stress conditions. Experiments performed under fully controlled conditions, *in vitro* and in the greenhouse, showed that sulfur fertilisation as well as light conditions could also have an impact on the organo-sulfur composition of garlic bulbs. However, the interaction with the genotype has to be considered as spring-varieties and winter-varieties did not react the same way to variations in fertilising and environmental conditions. In the mean time, the effect of increasing mineral sulfur should be considered in relation to other mineral fertilising components, like nitrogen and selenium, as well as to other sulfur sources, from the soil and from the atmosphere, as garlic seems to be able to use atmospheric sulfur. Multiple factors affect alliin accumulation in garlic, so its quality for human health. These factors should be considered when growing garlic for flavour or therapeutic value.

Keywords: *Allium sativum*, production, quality, organo-sulfur compounds

Abbreviations: AlCSOs, *S*-alk(en)yl-L-cysteine sulphoxides; **GLUACs**, γ-glutamyl-*S*-alk(en)yl-L-cysteine; **GLUPeCs** or **IsoGLUAlCs**, γ-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine; **H_2S**, hydrogen sulfide

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INTRODUCTION

Garlic is cropped world-wide and is well-appreciated for its flavour properties and health benefits. Numerous therapeutic properties have been attributed to garlic, like antifungal, antibacterial, antiviral, antithrombotic, antitumor, hypotensive, hypoglycemic and hypolipidemic properties (Keusgen 2002). More recently, therapeutic value related to cardiovascular diseases (Kik *et al*. 2001; Collin 2004) and cancer (Le Bon and Siess 2000) have been reported. These benefits are related to the organo-sulfur compounds stored in the bulbs, particularly the alk(en)yl cysteine sulphoxides (Block 1985). When the bulb is cut or crushed, enzyme alliinase (EC 4.4.1.4) is released and transforms *S*-allyl-L-cysteine sulfoxide, or alliin, into diallyl thiosulfinate, or allicin, a volatile compound which produces characteristic garlic odour and flavours. Alliin derives from related γ-glutamyl dipeptides, including γ-glutamyl-*S*-alk(en)yl-L-cysteine (GLUAlCs), and accumulates in high quantity in garlic (Block 1985; Block *et al*. 1993; Randle and Lancaster 2002). Trace, or low quantities of other alkyl cysteine sulphoxides can also be found in garlic, like isoalliin, the major flavour precursor of onion, which derives from γ-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GLUPeCs or IsoGLUAlCs; Hughes *et al*. 2005). Alliin is the component often considered as a specific flavour quality trait related to the health-value of garlic. Since recent investigations showed that the chemo-preventive properties of garlic against cancer could be improved with a higher concentration of alliin in garlic powders diets (Bergès *et al*. 2004), it is important to carry out research which enables growers to cultivate garlic in the most appropriate conditions to produce high health-value garlic material. This review provides information on the complexity of environmental factors that influence flavour and therapeutic compounds in *Allium*, especially garlic, and on the influence of the mineral fertilisation and genetic factors interacting with the environmental factors. It refers to many experiments performed under controlled and field conditions.

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Invited Mini-Review

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE QUALITY OF GARLIC BULBS

Environmental factors are known to induce and influence bulb formation in garlic. Temperature and day-length are key factors for bulb induction (Takagi 1990; Nagakubo *et al*. 1993). Storage temperature of planting material was described to determine the bulb size and division rate of most of garlic accessions (Messiaen *et al*. 1993). It was also stressed that day-length and trophic factors such as carbohydrate source affect *in vitro* bulb formation frequency (Kahane *et al*. 1997). Whereas light spectral quality is well documented for its role in onion bulb formation (Kato 1963; Lercari 1982; Kahane *et al*. 1992a), it has been emphasised that the bulbing process of some garlic cultivars was also responsive to the light spectrum composition (Kahane *et al*. 1997). Only spring accessions, belonging to group II according to Messiaen's classification (Messiaen *et al*. 1993), were highly responsive to additional far-red radiation to the light conditions and depended on light spectrum quality to complete bulbing. This was confirmed *in vitro* with the varieties 'Morasol', a Mediterranean variety from group I, 'Printanor', a temperate variety from group II, and 'Messidrôme', an autumn variety from group III, micropropagated following cyclic multiplication previously reported for onion (Kahane *et al*. 1992b). No effect of light intensity could be observed. For 'Printanor', bulb frequency was increased from 13.3% to 96.7% *in vitro* when the light spectrum was enriched with far-red light, and as a consequence, 'Printanor' bulbs were significantly bigger under this light condition (**Table 1**). This spring-planted variety showed a similar reaction to incandescent light to that of long-day onions, which suggests the implication of the High Irradiance Reaction (HIR) of the phytochrome (Lercari 1984). Over-wintering material (group III) only depended on low temperatures (Kahane *et al*. 1997).

Furthermore, environmental factors not only influence bulb formation, but also affect bulb flavour quality. Water stress affects the biosynthesis of organo-sulfur compounds of watercress, cabbage and onion, a close relative of garlic (Freeman and Mossadeghi 1973). Besides, Coolong and Randle reported that the average growing temperature (2003) as well as the root zone temperature (2006) could strongly affect the flavour composition of onion. In garlic, distinct cropping temperatures produced different organo-sulfur compounds profiles among several accessions originating from Central Asia when grown in natural conditions in two different climatic contexts, namely in Israel and in the Netherlands (Kamenetsky *et al*. 2005). Commercial varieties were also compared in experiments carried out in field conditions under two climatic conditions of Western Europe, in France and in Spain (Huchette *et al*. 2007b). An ion-pairing method, using HPLC, was used to quantify S compounds from the harvested bulbs according to a protocol formely reported (Kamenetsky *et al*. 2005; Huchette *et al*. 2007a). This technique without derivatisation (Arnault *et al*. 2003) allows the quantification of allicin, alliin, isoalliin and their peptide intermediates (γ-glutamyl-*S*-allyl-cysteine (GLUAlCs) and γ-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GLUPeCs). In both studies, the influence of cropping year and geographic location emphasised the influence of environmental and climatic factors on garlic flavours. Differences in soil sulfur could partially explain the location differences between France and Spain but climatic data suggested also a possible role of other environmental factors, such as the temperature, water availability and light radiation (Huchette *et al*. 2007b).

In vitro studies helped to confirm the effect of growing temperatures and light spectrum on the garlic bulb composition in organo-sulfur compounds, especially alliin (Huchette *et al*. 2005). They were also used to establish the role of the light spectrum and light intensity on the organo-sulfur compounds composition of garlic bulbs (O. Huchette, unpublished data). *In vitro* plants have proven to be a useful model system for studying the influence of environmental or trophic factors on *Allium* physiology (Kahane *et al*. 1997; Le Guen-Le Saos *et al*. 2002) and carbohydrate biochemistry (Kahane *et al*. 2001). In these experimental conditions, increasing intensity level of fluorescent light did not affect significantly the bulb composition in organo-sulfur compounds of any of the tested garlic cultivar. However, additional far-red light dramatically increased the organo-sulfur content of spring-planted varieties (**Fig. 1**). No influence was observed for the autumn variety, suggesting a cultivar dependent effect of the light spectrum on the organo-sulfur compounds of garlic. Anyway, the far-red enriched light condition appeared as optimal to favour a high alliin accumulation in plants grown *in vitro*.

Unusual high levels of precursors, especially of GLUPeCs, have been observed for garlic plants grown *in vitro* compared to field conditions (**Fig. 1**; Huchette *et al*. 2007a). The high level of this component, which is not known to be involved in the major flavour pathway of garlic (Lawson 1996; Hughes *et al*. 2005), questions its role in the biosynthesis of aliin, especially as no isoalliin could be detected. Its proportion *in vitro* varied from about 70% to 90%. So, its detection might bring more detailed information about the sulfur status of garlic cultivars from different varietal groups. The composition in organo-sulfur compounds, including the precursors, has also proven to bring more detailed information about sulfur metabolism and sulfur status of a large number of garlic accessions grown in different climatic conditions (Kamenetsky *et al*. 2005). However, the role of the precursors in the health-value of garlic is unknown.

EFFECT OF SULFUR FERTILISATION

Numerous studies have investigated how sulfur nutrition affects flavour in onion by changing bulb pungency (Freeman and Mossadeghi 1970; Randle 1992; Randle and Bussard 1993; Hamilton *et al*. 1997), as well as the biosynthetic pathway of the organo-sulfur compounds (Randle *et al*. 1995). However, if sulfur nutrition has been investigated in garlic (Raysseguier 1995), only a few reports described its influence on garlic flavours and showed that increasing sulfur fertilisation had a more limited impact on flavour composition than in onion (Freeman and Mossadeghi 1971; Huchette *et al*. 2007a). Under very high level of mineral sulfur supply, sulfur absorption has been confirmed, but it seems that over-fertilisation is not used through the metabolism of organo-sulfur compounds by garlic plants (Huchette *et al*. 2007a). As previously observed in onion, sulfur could be stored in the form of sulphate in cell walls (Lancaster *et al*. 1998) or in cell vacuoles (Randle 2001) instead of being used through the AlCSOs (*S*-alk(en)yl-L-cysteine sulphoxides) pathway. Field trials carried out in France and in Spain in 2000 and 2001 confirmed that increasing sulfur supply had no systematic direct impact on the alliin content of garlic bulbs and was interacting with environmental factors and cultivars (Huchette *et al*. 2007b). Hamilton *et al*. (1998) showed also that bulb pungency was not affected by sulfur fertilisation, when onions were grown in soils with

Table 1 Environmental effects on bulbing frequency and bulb size of light spectrum and light intensity when applied on micropropagated garlic plants. Bulbing in variety Printanor was induced by a cold period (2 months at 3°C – 10 h photoperiod - white light) followed by bulb formation in a warm environment (22-24°C) under 16 h photoperiod.

Light condition *	Bulbing frequency (%)	Bulb fresh weight (mg)
White light, 95 µmol m^{-2} s^{-1}	13.3	165 ± 21
Enriched in far-red light, 95 µmol m^{-2} s^{-1}	96.7	516 ± 60
White light, 200 µmol m^{-2} s^{-1}	10.0	166 ± 11

* Light intensity was controlled with a radiation sensor (LI-COR, LI-189 Quantum / Radiometer / Photometer). Light spectrum was varied by exposing the plants to white light (fluorescent tubes Universal Light, OSRAM), alone or added with far-red light (incandescent tubes 185W, ARIC).

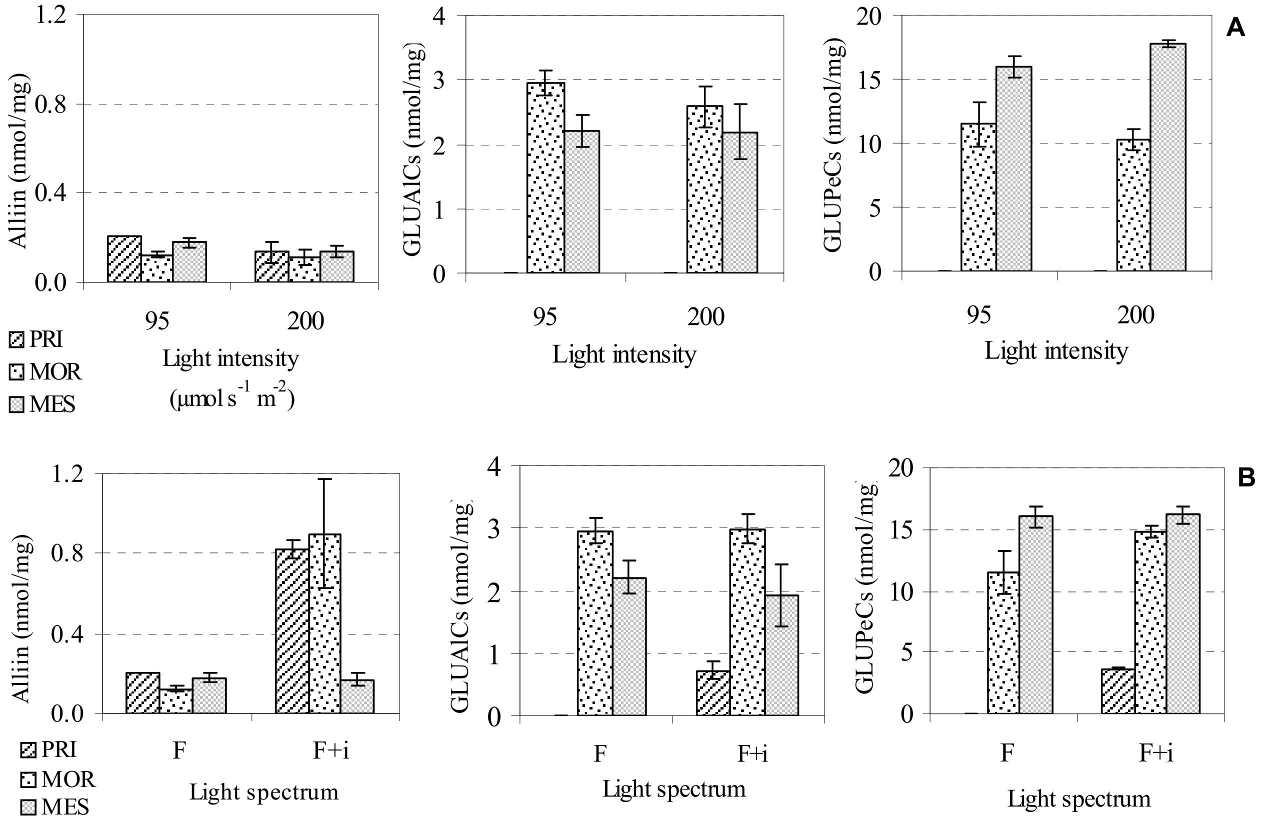

Fig. 1 Influence of light conditions on the concentration of organo-sulfur compounds in garlic. (**A**) Light intensity and (**B**) light spectrum effects on alliin and precursors (GLUAlCS, γ-Glutamyl-*S*-allyl-L-cysteine, and GLUPeCS, γ-Glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine) of garlic bulbs from variety Printanor (PRI), Morasol (MOR) and Messidrôme (MES) grown *in vitro* according to a protocol formely reported (Huchette *et al.* 2005). Ninety PRI plants, 48 MES plants and 48 MOR plants were distributed and exposed to three light conditions for bulb formation: F (white light, 95 µmol m^{-2} s^{-1}), 2F (white light, 200 µmol m^{-2} s^{-1}) and F+i (white light plus far-red light, 95 µmol m^{-2} s^{-1}). Concentrations are presented as means of values measured from harvested bulbs with their standard errors.

sufficient mineral sulfur supply, even if increased sulfur uptake and bulb yield have been reported following increasing sulfur supply, especially in the form of gypsum (Singh *et al.* 1995).

Interestingly, no symptoms of sulfur deficiency have been observed with garlic grown in the greenhouse under deficient sulfur conditions (Freemand and Mossadeghi 1971; Huchette *et al.* 2007a), while many reports describe those symptoms on onion (Freeman and Mossadeghi 1970; Randle *et al.* 1995; Chatterjee *et al.* 1999). Experiments with garlic grown *in vitro* showed, however, that garlic was susceptible to sulfur deficiency under this specific growing condition. They suggested that garlic could compensate sulfur deficiency in the mineral nutrition by another sulfur supply, such as atmospheric sulfur as already observed in different plant species (Kühn and Faller 1972). Although no measurement of absorption of hydrogen sulfide (H_2S) could confirm this hypothesis, less gaseous exchanges probably occur *in vitro* compared to growing conditions in the greenhouse or in the field. Previous studies had already speculated that garlic plants could compensate mineral sulfur deficiency by absorption of atmospheric sulfur (Freeman and Mossadeghi 1971; Raysseguier 1995). It was confirmed that onion was able to use atmospheric sulfur as sole sulfur source for growth and that hydrogen sulfide (H_2S) absorbed through the leaves was metabolised into flavour precursors (Dürenkampf and De Kok 2004).

Sulfur, however, should not be considered as the sole mineral factor to be varied in a fertilising program in order to influence garlic flavours. In onion, both pungency and biosynthetic pathway of organo-sulfur compounds can be affected by other mineral factors such as nitrogen, a major macroelement for the plant (Gamiely *et al.* 1991; Randle 2000), and selenium, a nonessential element (Kopsell and Randle 1997). In garlic, the effects of nitrogen fertility on the yield and bulb quality have also been reported (Bertoni *et al.* 1992). Interactions between sulfur and both of these components should be considered as influencing the biosynthesis pathway of organo-sulfur compounds. Sulfur and selenium can be antagonistic in plants (Shrift 1954), and in onion especially (Barak and Goldman 1997). Onion flavour studies have provided further evidence of a competition, which should be considered in garlic (Kopsell and Randle 1999). Besides, interactions between sulfur and nitrogen have been reported on both onion (Coolong and Randle 2003) and garlic (Huchette *et al.* 2007a) flavours.

INTERACTION WITH THE GENOTYPE

Flavour intensity measured by pyruvic acid and total bulb sulfur concentration varies among onion cultivars grown in a single and uniform environment (Randle 1997) and this trend has also been observed in garlic. Experiments carried out under controlled conditions as well as in the field showed that spring-planted varieties could present a higher potential in alliin than autumn varieties, even if these ones had a higher proportion of precursors and a higher flavour potential when considering alliin and precursors as well (Huchette *et al.* 2007a, 2007b). The influence of genetic factors on the bulbs' sulfur compound concentration and composition has been confirmed with a study on wild garlic germplasm, as significant differences were found between the *Sativum* and *Longicuspis* groups, and even between two *Longicuspis* subgroups (Kamenetsky *et al.* 2005).

However, the interaction between the genotype and environmental factors has also to be considered. While Kamenestky *et al.* (2005) showed differences in the flavour profiles of the same pattern of garlic accessions from Central Asia grown in Israel and in the Netherlands, field trials conducted in France and in Spain in 2000 confirmed that garlic cultivars were not showing the same flavour potential in different environmental and climatic conditions (Huchette *et al*. 2005, 2007b).

Interaction between the genotype and light conditions was confirmed on garlic plants grown *in vitro* as different responses were observed according to the varietal groups of the tested varieties. The organo-sulfur compounds levels of spring-planted varieties could be greatly improved by the addition of far-red light (**Fig. 1**). A similar cultivar dependent effect was observed when varying sulfur, combined or not with nitrogen, and suggested that the autumn variety Messidrôme was more efficient at accumulating sulfur for flavour than the spring-planted varieties Printanor and Morasol (Huchette *et al*. 2007a).

CONCLUDING REMARKS

To cultivate garlic with a high amount of organo-sulfur compounds, a balance should be sought between environmental and genetic factors. This balance can vary however for different varieties and climates. To maximise the biosynthesis of alliin and its organo-sulfur precursors, proper cultivar selection is required as well as growing the plants in the most conducive environment, including a good adjustment of a fertilising program with balanced sulfur, nitrogen and selenium supplies. Selenium and sulfur, especially, are both of interesting prospects for their relation to garlic value for human health, but their antagonism raises problems for growing enriched-garlic. Hydroponics and full-controlled cultivation of garlic could offer new possibilities for producing either selenium or sulfur enriched-garlic, at least for further experimental purposes (Tsuneyoshi *et al*. 2006). The role of atmospheric sulfur, temperature, light conditions and water stress should be further investigated as possible factors for explaining differences between cropping locations for the same cultivars. Recent studies even emphasised the importance of the storage conditions of garlic bulbs, as their organo-sulfur compounds level and composition were affected by a storage at 4°C (Hughes *et al*. 2006).

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