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# Benchmarking Bulb Yield, Medicinal Sulfur Compounds, and Mineral Nutrition of Garlic Varieties

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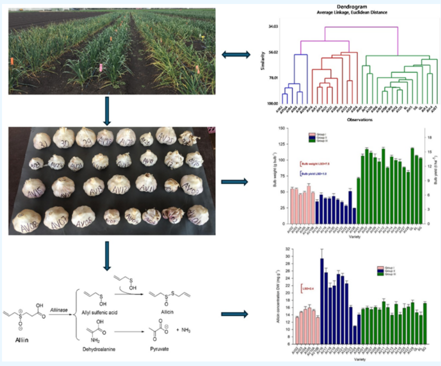
**ABSTRACT:** Garlic is widely cultivated for both its culinary and medicinal properties. Bulb yield, organosulfur compounds, and plant mineral nutrition of 32 garlic varieties were characterized. Across genotypes, there was large variability in bulb weight and alliin and allicin concentrations. The mineral concentration of a reference leaf was strongly positively correlated with bulb weight (for Zn, N, and K) and bulb allicin concentration (for S, Mo, and Cu). Alliin represented ∼61 and 88% of the total clove S content. *In situ* synchrotron-based X-ray absorption near-edge structure (XANES) analysis identified two dominant S compounds, γ-L-glutamyl-*S*-allyl-L-cysteine and alliin. Stoichiometric studies showed the ratio of conversion of alliin to pyruvate was consistently below the theoretical 1:1 ratio, while the ratio for conversion of alliin to allicin was consistently higher (∼1.9:1 to 3.5:1). A combination of optimized fertilizer management and genotype selection for high alliin can potentially substantially increase the yield and medicinal quality of garlic.



## ■ SIGNIFICANCE STATEMENT

In garlic, alliin is the precursor to the medicinal compound allicin, but the relationship between S-metabolism, mineral nutrition, and allicin concentration is poorly understood. This work identifies that garlic mineral nutrient status is an indicator for both yield potential and allicin concentration, while *in situ* synchrotron-based X-ray absorption near-edge structure (XANES) analysis identified alliin and γ-L-glutamyl-*S*-allyl-L-cysteine as the dominant sulfur compounds, which explains the observed deviation from the theoretical 2:1 stoichiometry in the conversion of alliin to allicin.

## 1. INTRODUCTION

Garlic has been cultivated over millennia and is rich in sulfur (S)-containing phytonutrients, particularly allicin, that confer its unique and highly valued medicinal and culinary properties.[1] Allicin is not present in intact garlic cloves but forms under crushing when the precursor compound alliin, present in the parenchyma cells of the bundle sheath, is combined with the spatially separated alliinase enzyme, which resides in the bundle sheath cells.[2,3] With further transformation, this gives rise to a range of volatile S compounds (including allicin)[4] that give garlic its characteristic odor. Allicin is a thiosulfinate compound, which comprises 70−80% of the total thiosulfinates in crushed garlic.[5] The production of allicin is dependent upon several factors, including the concentration of alliin and the efficiency of conversion of alliin to allicin.[6] Thus, an understanding of alliin and allicin chemistry in garlic is critical, given the many pharmacological benefits of garlic.[7]

Of the various organosulfur compounds in intact garlic cloves, approximately 95% are in the broad S groups of γ-L-glutamyl-*S*-alkyl-L-cysteine compounds [i.e., R-S-R] (∼40%) and the *S*-alk(en)yl-L-cysteine sulfoxides (ASCO) compounds [i.e., R-S(═O)-R] (∼55%).[1] For the ASCO compounds, alliin (S-allyl-cysteine sulfoxide) is the most important, accounting for 80% of the ASCO compounds (with this alliin being critical for the formation of allicin upon crushing of garlic),[8] with methiin (∼16%) and isoalliin (∼1%) also contributing to the ASCO compounds.[1,9] Although γ-L-glutamyl-*S*-alkyl-L-cysteine (GSAC) has recently been identified as a potential inhibitor of SARS-CoV-2,[10] it does not appear to have a well-established direct pharmacological effect but acts as a precursor in the formation of alliin. The ASCO compounds are odorless and are formed either by the alkenylation of the cysteine in glutathione, which is subsequently cleaved and oxidized to form the sulfoxides, or by alkenylation of cysteine or O-acetyl serine.[4] The precursor for alliin synthesis is considered to be glutathione, which is synthesized from γ-glutamyl cysteine in

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the chloroplast using $SO_4^{2-}$ as the S substrate and subsequently transferred freely into the cytoplasm.[11] In the cytoplasm, the glycyl moiety of glutathione is removed to form γ-glutamyl-S-allyl-L-cysteine (GSAC), belonging to the R-S-R group. The synthesis of alliin from GSAC can proceed via either of two related pathways that both consist of S-oxygenation and deglutamylation but in a different sequence[5,12,13] (Figure S1). In one, GSAC is deglutamylated to form S-allyl-L-cysteine, which then undergoes S-oxygenation to form alliin. In the second, GSAC undergoes S-oxygenation to form γ-glutamyl-S-allyl-L-cysteine sulfoxide, which is subsequently deglutamylated to form alliin. However, the regulation and importance of the reaction order between S-oxygenation and deglutamylation remains unclear. The organosulfur chemistry in garlic is complex, and the speciation of S compounds in garlic can provide a better understanding of the critical role of S compounds in garlic metabolism and subsequent medicinal properties across garlic genotypes.

While the pharmaceutical industry requires a high allicin concentration within the bulb, the fresh market industry prefers garlic with a large bulb size and with good conformation (shape and skin coverage). Therefore, improving both the garlic yield and allicin concentration is important in meeting the demand of these two markets. An obvious strategy for increasing the yield and allicin concentration is the identification and selection of superior varieties. Globally, there are approximately 300 garlic varieties, which are adapted to a broad range of climatic and growing conditions,[14] in particular temperature and daylength. Several studies have shown that garlic bulb weight (and yield) varies considerably, from 2 to 136 g $bulb^{-1}$.[15−17] Although only limited information is available, allicin concentration has also been reported to vary substantially, with allicin concentrations ranging from 0.5 to 9.0 g $mg^{-1}$ fresh weight (FW) across 93 varieties.[18−20] However, there is limited documented information that benchmarks the S compounds, bulb yield, and mineral nutrition of garlic varieties.

The aim of the present study was to improve our knowledge of genotypic differences in mineral nutrition profiles (across 32 varieties) and to examine the relationship between bulb yield and allicin concentration, with this providing an objective basis for agronomic improvement. In addition, to better understand the genetic potential for increasing allicin production, we evaluated the stoichiometry in the conversion of the dominant S compounds (alliin and allicin) using high-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector. Finally, to characterize the full profile of S compounds, we also examined speciation *in situ* using synchrotron-based X-ray absorption spectroscopy (XAS) across eight varieties.

## 2. MATERIALS AND METHODS

Field experiments were conducted in two consecutive years (2018 and 2019) to evaluate the bulb weight (yield), mineral nutrition, and S speciation (including alliin and allicin concentration) of 32 garlic varieties (Table S1). The varieties included 28 varieties that were sourced from the World Vegetable Centre, formerly The Asian Vegetable Research and Development Centre (AVRDC). One of these 28 varieties from AVRDC was separated into two varieties (AV10A and AV10B) based on a subtle phenotypic difference in color (purple versus mauve/white), giving 29 varieties (Table S1). The 29 varieties from the AVRDC were selected from the collection of approximately 100 available varieties on the basis of their geographical origin (a maximum latitude of ∼27°N/S) and large bulb and clove size. The selected latitude corresponds to the subtropical climate that represents the main production area for garlic in Australia. A further three Australian industry standard subtropical varieties (Glenlarge, Kenlarge and Southern Glen) were also selected, giving a total of 32 varieties included in this study.

High-quality, large, healthy seed-bulbs were selected in the preceding season (2017) for planting in the present study. Seed-cloves of all varieties were planted on 22 March 22, 2018, and the experiment was repeated in 2019 to provide data from two seasons. The experiments were conducted at the Gatton Research Facility, Queensland, Australia (27°32′S, 152°20′E), with the soil being classified as a Vertosol in the Australian Soil Classification (Vertisol in the US Soil Taxonomy). The experimental design was a randomized complete block with four replicates. The plots were 2.5 m long with a buffer of 0.5 m between plots, and the within-row plant spacing was 10 cm, giving ∼50 plants $plot^{-1}$. Each plot consisted of two rows spaced 0.55 m apart and centered on beds at 1.5 m width, giving an overall plant population of ∼133,000 plants $ha^{-1}$ (Figure S2).

In order to optimize garlic growth, the following nutrients were applied (kg $ha^{-1}$): 180 N, 120 K, 80 S, 0.8 B, 0.8 Zn, 0.45 Cu, and 0.11 Mo (Table S2). No applications of P, Mg, Fe, and Mn were made because the soil was inherently high in these nutrients. Although the soil was also inherently high in Ca, $Ca(NO_3)_2$ was applied for N nutrition. The trial was irrigated using Rivulis T-Tape 510-20-500 drip irrigation tape with an emitter spacing of 20 cm and an output rate of 1 L per emitter per hour. One line of drip tape was installed for each row of garlic. Fertilizer was applied through fertigation using a partial bypass fertigation tank. Irrigation, pesticides, and herbicides were applied when required. The trial was thinned to ensure that there were no double plants.

A series of TinyTag weather data loggers were installed at the experimental site to measure ambient temperature, relative humidity, rainfall, and soil temperature at a depth of 10 cm (1 s readings averaged at intervals of 15 min). Prior to planting in 2018 and 2019, soil samples were collected from each of the four experimental blocks, with the soil samples dried at 40 °C for 48 h and stored in plastic bags at ambient temperature. Soil samples were analyzed by the Incitec-Pivot Nutrient Advantage Laboratory for pH, organic carbon (OC), electrical conductivity (EC), nitrate ($NO_3^-$) nitrogen, Mn, Cu, Fe, P, S, Zn, Cl, B, and exchangeable K, Ca, Mg, and Na (Table S3).

Since the S compounds in garlic are volatile, a preliminary experiment compared drying regimes to identify the effect of oven drying on the total S concentration. Paired samples of bulb and leaf tissue were freeze-dried or oven-dried (at 55 and 70 °C for 7 d), with the dried samples ground and analyzed for S concentration. It was found that the S concentration was not affected by the drying regime ($P > 0.05$), with the mean S concentration of leaf tissue samples (three replicates) being 4.95 g $kg^{-1}$ (freeze-dried) and 5.05 g $kg^{-1}$ (oven-dried at 70 °C). In a similar manner, the mean S concentration of bulb samples (four replicates) was 8.09 g $kg^{-1}$ (freeze-dried), 8.14 g $kg^{-1}$ (oven-dried at 55 °C), and 7.89 g $kg^{-1}$ (oven-dried at 70 °C). Given that there were no significant differences in S concentrations between drying methods for both leaves and bulbs, oven drying at 70 °C was used for analyses of nutrient content.

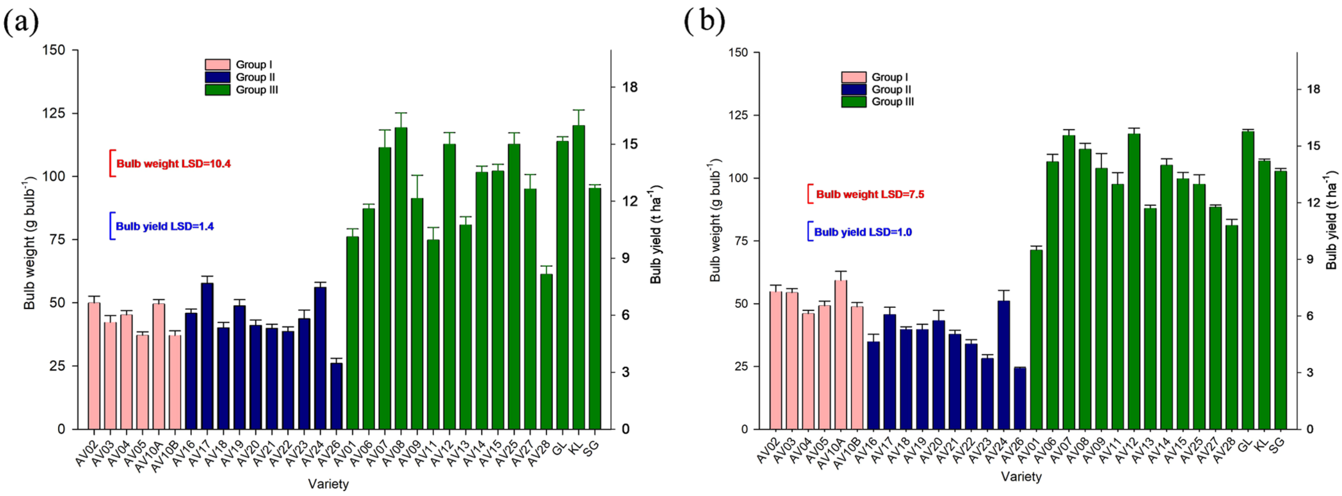

**Figure 1.** Bulb weight and bulb yield (on fresh weight basis) for 32 varieties separated into three groups (Groups I, II, and III) from 2018 (a) and 2019 (b). KL denotes Kenlarge, GL denotes Glenlarge, and SG denotes Southern Glen.

The youngest fully expanded leaf (YFEL) was collected from 20 plants of each replicate for the various varieties at the 6−8 leaf stage. The leaf sample was dried at 70 °C for 5 days prior to mineral nutrient analysis (see below). In addition, at full maturity (approximately 75% senescence), the plants in each plot were harvested and cured for 4 weeks. After curing, the plants were divided into foliage and bulbs, with the bulbs graded into size using a market specification: small (<45 mm diameter), medium (45−55 mm diameter), large (55−70 mm diameter), and extra-large (>85 mm diameter). The bulbs were counted for each market category, and the weight of the bulbs in each category was determined. The bulb yield was calculated based upon the product of average bulb weight and a plant population of 133,000 plants ha$^{-1}$. A subsample of five bulbs from the medium category of each replicate was selected, and four outer cloves from each of these bulbs were excised in a cross-shaped pattern (Figure S3) to ensure a representative sample was collected, and analyzed immediately for allicin using the method of Nguyen et al.[21] Briefly, the excised cloves were crushed, and 40 mL of deionized water was added to 1 mg of the crushed garlic. The extract was centrifuged for 10 min, filtered, and allicin was quantified using a Shimadzu HPLC-PDA following a previous study (Nguyen et al.[21]).

For the stoichiometry study, alliin and pyruvate analyses were conducted on freeze-dried and ground samples (MM 500, Retsch ball mill). Pyruvate concentration was identified and quantified from the allicin extract using a Nexera X2 UHPLC system coupled with an LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) with electrospray ionization (ESI) in the negative ion mode.

The remaining portions of the bulbs were weighed and dried at 70 °C for 7 days. With the exception of N, mineral nutrients were analyzed following digestion using nitric acid-perchloric acid and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES). Nitrogen concentrations were determined using a LECO combustion analyzer. Samples for quantification of nitrate and ammonium concentrations were extracted using 2 M HCl solution at room temperature for 1 h and then determined using an autoanalyzer.

### 2.1. Synchrotron-Based Sulfur K-Edge X-ray Absorption Spectroscopy.

Sulfur K-edge X-ray absorption near-edge structure (XANES) spectra were collected at the Synchrotron Light Research Institute (SLRI), Thailand, at Beamline 8.[22] A InSb(111) double-crystal monochromator was utilized to select the energy of the incident beam, with a beam size of ca. 18 × 2 mm. Samples of eight garlic varieties were analyzed, being AV02, AV04, AV08, AV12, AV17, AV24, Glenlarge, Kenlarge, and Southern Glen. The bulb samples of these varieties were sliced into 3 cm pieces, oven-dried at 70 °C for 7 days, and ground into fine samples using a Retsch MM 500 ball mill. The finely crushed plant tissue samples and standards were placed on S-free Kapton tape. All spectra were recorded in fluorescence mode by using a 13-element Ge detector. The sample compartment was continually purged with He to minimize the absorption of the X-rays by air. Spectra were recorded with a dwell of 3 s per energy step across an energy range of 2372−2672 eV: 2372−2462 eV in 5 eV increments, 2462−2497 eV in 0.2 eV increments, and 2497−2672 eV in 5 eV increments. Each sample had three replicate scans with a dwell time of 3 s per energy step. In addition to the samples, we analyzed 11 standards, being reduced glutathione and cysteine (RSH), methionine and glutamyl-S-allyl-L-cysteine (RSR), cystine (RSSR), allicin [RS(═O)SR], alliin and methiin [RS(═O)R], taurine [RS(═O)$_2$OH], hypotaurine [RS(═O)OH], sodium sulfite [$SO_3^{2-}$], and sodium sulfate ($SO_4^{2-}$). The standards were prepared at an S concentration of 15 mg g$^{-1}$ diluted with boron nitride and analyzed as finely ground solids (using a Retsch MM 500 ball mill). Prietzel et al.[23] reported that self-absorption in S K-edge XANES is insignificant at an S concentration of 20 mg g$^{-1}$ or less. A sample of sodium sulfate at an S concentration of 1.5 mg S g$^{-1}$ (i.e., 10-fold dilution) was also analyzed, and there was no change in the spectrum, confirming that self-absorption was not a problem in the standards at an S concentration of 20 mg g$^{-1}$. All standards had two replicate scans. The spectra were aligned and normalized, and linear combination fitting was conducted to identify peaks using Athena Demeter version 0.9.26.[24] An example of the spectra for a sample, fit, GSAC standard, alliin standard, and residuals is presented in Figure S4.

### 2.2. Statistical Analysis.

Data were analyzed using Minitab 18.1 software for Windows (Minitab Inc., State College, Pennsylvania), using a one-way and a two-way analysis of variance (ANOVA). The differences in measured

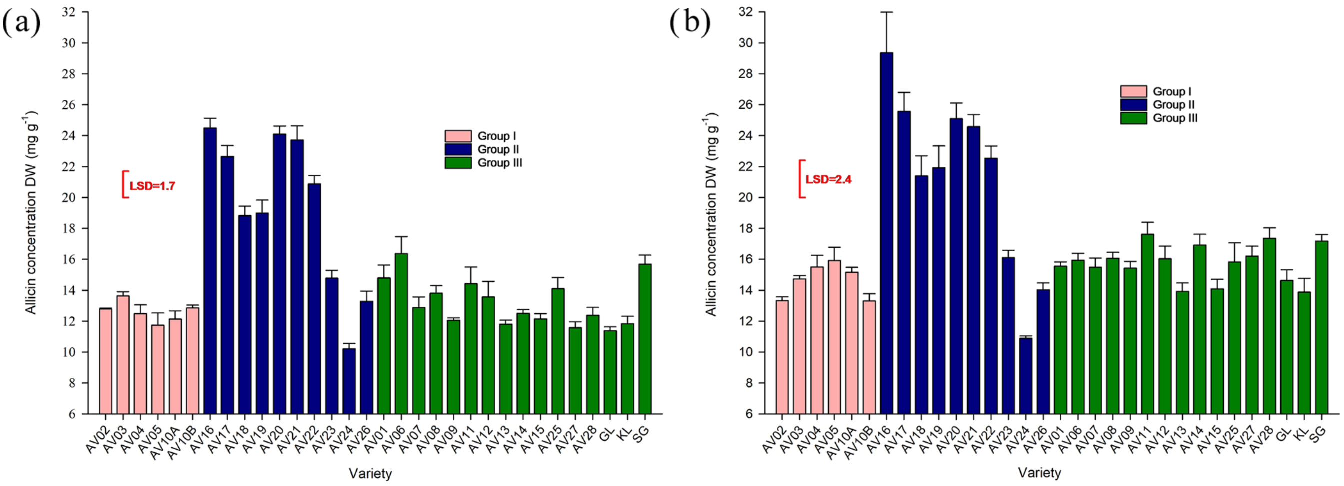

**Figure 2.** Allicin concentration on a dry weight (DW) basis of 32 garlic varieties in 2018 (a) and 2019 (b).

parameters (duration of growing period, bulb weight, allicin concentration, allicin yield, clove number, and clove weight) between varieties were determined with Tukey's HSD at $P < 0.05$. The graphs were presented as mean and standard errors using SigmaPlot 14.0 (Systat Software Inc.). The relationship between bulb yield, bulb allicin concentration, and reference leaf mineral nutrient status was analyzed using the multivariate statistical function (principal component analysis and cluster observation using Minitab) of Minitab (Minitab Inc., State College, Pennsylvania).

## 3. RESULTS AND DISCUSSION

**3.1. Varietal Differences in Bulb Weight.** For the two experiments (2018 and 2019), garlic bulb weight differed significantly across the 32 varieties and between years ($P < 0.05$) (Figure 1a,b and Table S4), ranging from approximately 25 to 120 g bulb$^{-1}$ [on fresh weight (FW) basis]. These bulb weights were substantially higher than most previous studies, including Wang et al.[17] (~4−59 g bulb$^{-1}$ across 212 varieties), Baghalian et al.[25] (~16−62 g bulb$^{-1}$ across 24 varieties), and Jabbes et al.[16] (~11−48 g bulb$^{-1}$ across 31 varieties). However, the present results are in agreement with those reported by Panthee et al.[15] who examined 179 garlic varieties and also found a wide variation in garlic bulb weight (2−136 g bulb$^{-1}$), with the maximum bulb weight (~120 g bulb$^{-1}$) being similar to that found in the present study. There was minimal difference (<~2.5%) in the cultivar's growth response over two seasons (Figure 1).

During the growth of the garlic varieties, it was apparent these varieties could be divided into three broad groups (Groups I, II, and III) based on morphological and phenotypic traits. Varieties in Group I had pale green flaccid foliage (Figure S5a) and shallot-shaped bulbs (Figure S5b). Varieties in Group II had yellowish green foliage that was thinner and narrower (Figure S5c) and loose, silky-skinned bulbs (Figure S5d). Varieties in Group III had dark-blue-green foliage that was thicker and wider (Figure S5e), with bulbs that were tight and thick-skinned (Figure S5f). The highest bulb yield was recorded in the Group III varieties (~15−16 t ha$^{-1}$), while yield was considerably lower in Group I varieties (~5 to 6.5 t ha$^{-1}$) and Group II varieties (~3.5 to 7.5 t ha$^{-1}$) (Figure 1a,b).

**3.2. Varietal Differences in Allicin Concentration.** Allicin concentration was determined following crushing of the cloves (with deionized water added to the crushed garlic), with the allicin concentration differing significantly between varieties ($P < 0.05$). Specifically, the allicin concentration was higher in the Group II varieties than in Groups I and III (Figure 2a,b). In 2018, across all varieties, the allicin concentration ranged from ~10.2 to 24.5 mg g$^{-1}$ dry weight (DW) (~3.5−6.5 mg g$^{-1}$ FW), and 21 of the 32 varieties had allicin concentrations below the nominated minimum pharmaceutical standard of 4.5 mg g$^{-1}$ FW (Figure 2a).[26] In contrast, in 2019, the allicin concentration for each variety was 5−40% higher than in 2018 ($p < 0.05$), with the exception of AV10B and AV06 (Figure 2b), which were the same in the two years. In 2019, the allicin concentration ranged from 10.9 to 29.4 mg g$^{-1}$ DW (3.8−7.0 mg g$^{-1}$ FW), and only four of the 32 varieties (AV02, AV10B, AV24, and AV26) had allicin concentrations lower than 4.5 mg g$^{-1}$ FW (Figure 2b).

The difference in allicin concentrations between 2018 and 2019 highlights the importance of environmental conditions on allicin production. The identified variation in allicin concentration on both a FW and dry weight (DW) basis is in agreement with other studies such as Sterling and Eagling,[20,26] Khar et al.,[18] and Wang et al.[17] For example, the study of Khar et al.[18] examined 93 garlic varieties and found that the allicin concentration ranged from ~9.0 to 12.0 mg g$^{-1}$ DW, with this being considerably lower than in the present study. In the study of Sterling and Eagling,[20,26] 200 garlic samples were collected from markets around Australia, with the allicin concentration ranging from 0.5 to 9.0 mg g$^{-1}$ (FW), with ~50% of samples having an allicin concentration in the range of 4.0−6.0 mg g$^{-1}$ FW. Interestingly, the allicin concentration of variety Glenlarge reported by Sterling and Eagling[20,26] (5.6 mg g$^{-1}$ FW) was similar to that found in the present study (~4.5 mg g$^{-1}$ FW).

**3.3. Stoichiometry of the Alliin to Allicin Reaction across Varieties.** The conversion of alliin to allicin is a two-step reaction where first one alliin molecule reacts with alliinase to produce one allyl-sulfenic-acid molecule and one dehydroalanine molecule. From this, two molecules of allyl-sulfenic acid combine to form allicin, while dehydroalanine is further metabolized to form ammonium and pyruvate (Figure S1). Therefore, the theoretical stoichiometry ratio of conversion of alliin to allicin and pyruvate to allicin is 2:1, while the ratio of alliin to pyruvate is 1:1.[6] No studies are documented that examine the stoichiometry between alliin, allicin, and pyruvate in garlic. The stoichiometry in the

Table 1. Variation in Alliin, Pyruvate, and Allicin Concentration and Ratios of Conversion from Alliin to Pyruvate, Pyruvate (pyr) to Allicin, and Alliin to Allicin of Eight Garlic Varieties[a]

| | | | concentration ($\mu$mol g$^{-1}$ FW) | | | micromolar ratios | | |
|---|---|---|---|---|---|---|---|---|
| variety | (group) | DM% | pyruvate | alliin | allicin | alliin:pyruvate | pyruvate:allicin | alliin:allicin |
| AV02 | I | 33.3$^{b}$ | 96.2$^{ab}$ | 62.2$^{a}$ | 25.0$^{a}$ | 0.65 | 3.9$^{ab}$ | 2.5$^{bc}$ |
| AV17 | II | 26.8$^{a}$ | 189.5$^{d}$ | 133.0$^{d}$ | 37.9$^{c}$ | 0.73 | 5.0$^{c}$ | 3.5$^{d}$ |
| AV24 | II | 34.8$^{bc}$ | 110.7$^{abc}$ | 65.7$^{ab}$ | 24.5$^{a}$ | 0.60 | 4.5$^{bc}$ | 2.7$^{c}$ |
| AV01 | III | 36.0$^{c}$ | 125.3$^{c}$ | 72.7$^{abc}$ | 38.5$^{c}$ | 0.59 | 3.2$^{a}$ | 1.9$^{a}$ |
| AV08 | III | 32.5$^{b}$ | 114.6$^{abc}$ | 77.9$^{bc}$ | 32.5$^{b}$ | 0.69 | 3.5$^{a}$ | 2.4$^{bc}$ |
| Glenlarge | III | 34.6$^{bc}$ | 103.0$^{abc}$ | 70.0$^{abc}$ | 27.0$^{a}$ | 0.71 | 3.9$^{ab}$ | 2.7$^{c}$ |
| Kenlarge | III | 34.4$^{bc}$ | 92.3$^{a}$ | 63.7$^{a}$ | 26.4$^{a}$ | 0.69 | 3.5$^{a}$ | 2.4$^{bc}$ |
| Southern Glen | III | 34.5$^{bc}$ | 122.7$^{bc}$ | 79.6$^{c}$ | 36.0$^{bc}$ | 0.66 | 3.4$^{a}$ | 2.2$^{ab}$ |
| F test prob. | | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $P = 0.54$ | $p < 0.001$ | $p < 0.001$ |

[a]Within a column, treatment means with the same letter are not significantly different at $P = 0.05$.

Table 2. Variation in Dry Matter Concentration (DM%) and Sulfur (S) Concentrations (concn) Including Total S, Alliin-S, and Molar Difference between Pyruvate and Total S of Eight Garlic Varieties[a]

| | | total S concn | | alliin-S concn | | pyruvate minus total S |
|---|---|---|---|---|---|---|
| variety | group | (mg g$^{-1}$ DW) | ($\mu$mol g$^{-1}$ DW) | (mg g$^{-1}$ DW) | (% total S) | ($\mu$mol g$^{-1}$ DW) |
| AV02 | I | 9.8$^{b}$ | 305$^{b}$ | 6.0$^{a}$ | 61.1$^{a}$ | −15.9$^{a}$ |
| AV17 | II | 18.1$^{c}$ | 565$^{c}$ | 15.9$^{d}$ | 88.3$^{c}$ | 141.0$^{b}$ |
| AV24 | II | 8.2$^{a}$ | 257$^{a}$ | 6.1$^{a}$ | 73.9$^{ab}$ | 61.3$^{ab}$ |
| AV01 | III | 9.8$^{b}$ | 304$^{b}$ | 6.5$^{abc}$ | 66.5$^{ab}$ | 42.4$^{ab}$ |
| AV08 | III | 9.9$^{b}$ | 307$^{b}$ | 7.7$^{c}$ | 78.4$^{bc}$ | 45.3$^{ab}$ |
| Glenlarge | III | 8.0$^{a}$ | 250$^{a}$ | 6.4$^{ab}$ | 79.3$^{bc}$ | 47.4$^{ab}$ |
| Kenlarge | III | 7.9$^{a}$ | 248$^{a}$ | 5.9$^{a}$ | 74.6$^{b}$ | 19.8$^{ab}$ |
| Southern Glen | III | 9.7$^{b}$ | 302$^{b}$ | 7.4$^{bc}$ | 76.7$^{bc}$ | 53.7$^{ab}$ |
| F test prob. | | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p = 0.016$ | $p = 0.035$ |

[a]Within a column, treatment means with the same letter are not significantly different at $P = 0.05$.

conversion of alliin and allicin was evaluated for eight of the garlic varieties selected from the three garlic varietal groups. Evaluating the stoichiometry of the transformation of S compounds in garlic bulbs can provide a basis for improving the efficiency in the conversion of alliin to allicin.

The eight varieties showed significant differences ($P < 0.001$) in alliin, pyruvate, and allicin concentrations (Table 1). Across the varieties, the alliin concentration varied from ~62 to 133 $\mu$mol g$^{-1}$ FW, with the lowest alliin concentration in varieties AV02 (62.2 $\mu$mol g$^{-1}$ FW) and Kenlarge (63.7 $\mu$mol g$^{-1}$ FW), while AV17 had the highest alliin concentration (133.0 $\mu$mol g$^{-1}$ FW) (Table 1). The allicin concentration ranged between 24.5 and 38.5 ($\mu$mol g$^{-1}$ FW) (Table 1) and is consistent with values of approximately 8−34 ($\mu$mol g$^{-1}$ FW) reported in previous studies.[27−29] The pyruvate concentrations across the eight varieties ranged from 92 to 189 ($\mu$mol g$^{-1}$ FW) (Table 1) and were higher than those reported previously (27−88 $\mu$mol g$^{-1}$ FW).[27,28,30] Consistent with the alliin results, the pyruvate concentrations were also lowest in varieties AV02 (~96 $\mu$mol g$^{-1}$ DW) and Kenlarge (~92 $\mu$mol g$^{-1}$ DW), while AV17 also had the highest pyruvate concentration (~189.0 $\mu$mol g$^{-1}$ DW) (Table 1).

Across the eight varieties in our study, the ratio of conversion of alliin to pyruvate ranged from 0.59 to 0.73 (Table 1) and was well below the theoretical 1:1 ratio for the production of allicin, indicating high relative concentrations of pyruvate. In garlic, pyruvate can also be formed from an alternate sulfoxide-S compound, i.e., S-methyl cysteine sulfoxide, which represents approximately 10% of the total sulfoxide compounds[31] that react to form a secondary S compound independent of allicin production. The elevated pyruvate concentration from this reaction contributes to an increase in the pyruvate concentration, reducing the stoichiometry ratio of alliin/pyruvate from the theoretical 1:1 ratio. However, allowing for the contribution of pyruvate from this reaction (~10%), the stoichiometry of alliin/pyruvate is still low (0.59−0.73).

In contrast to the pyruvate/alliin and allicin ratios, the ratios for conversion of alliin to allicin across the eight varieties varied from 1.9:1 to 3.5:1 (Table 1). These values are substantially higher than that in a study of Mochizuki et al.,[29] which identified alliin/allicin conversion ratios of 1.8 to 2.1 over 8 varieties. In our study, the mean ratio (~2.5:1) was approximately 20% greater than the theoretical 2:1 ratio. This anomaly could relate to the genotypic inefficiency of conversion of the intermediate allyl-sulfenic-acid to allicin or the production of other secondary S compounds from allyl-sulfenic-acid (e.g., S-methyl prop-2-ene-1-sulfinothioate[31]). The present study also demonstrated differential conversion of alliin to allicin across variety groups where the ratio of alliin to allicin was higher ($p < 0.001$) in the Group II varieties (2.7:1 to 3.5:1) compared with the Group I and III varieties (1.9:1 to 2.7:1). This indicated that the allicin concentration could potentially be increased by selecting varieties with an inherently high alliin concentration, and/or selecting varieties which are more effective in converting alliin to allicin. Furthermore, the higher ratio of conversion of alliin to allicin than the theoretical value of 2:1 would indicate that further study should be conducted to more completely understand the mechanism and the role of genetics in the conversion of alliin to allicin. Jones et al.[32] identified six different alliinase isoforms

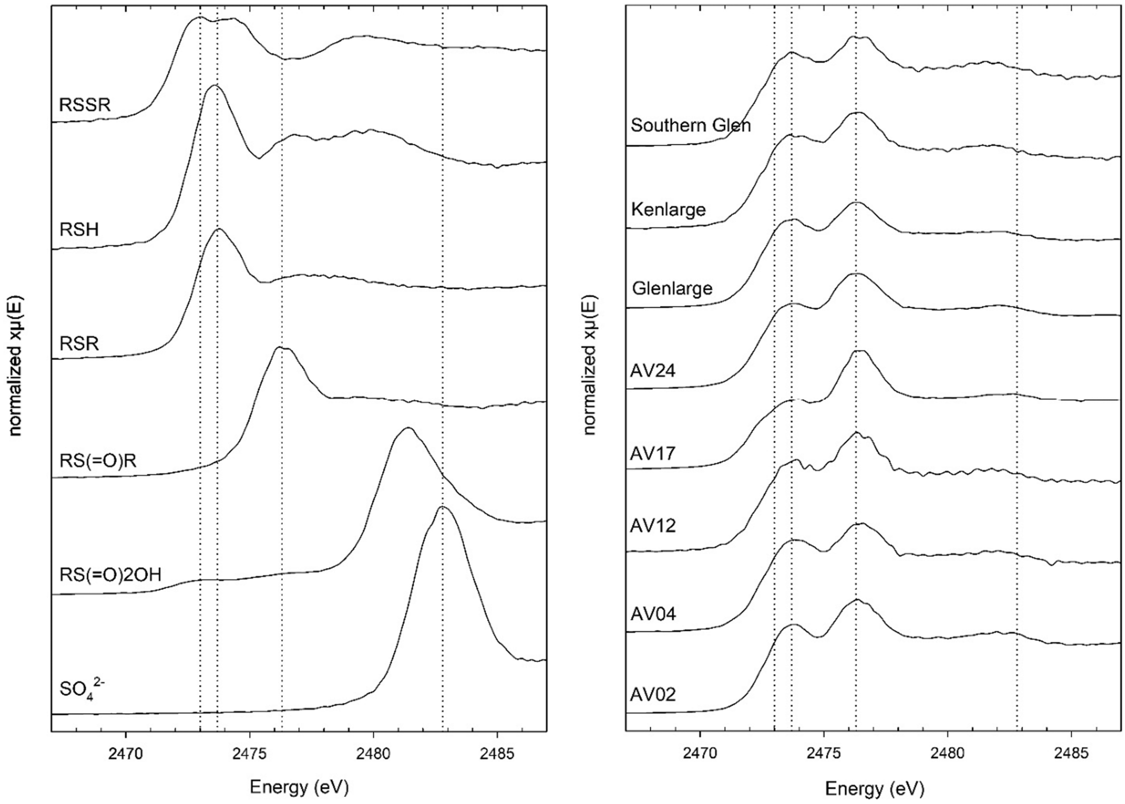

**Figure 3.** Sulfur K-edge X-ray absorption spectra of (a) standard compounds and (b) garlic varieties. The vertical dotted lines indicate the energies corresponding to the white line peaks of RSSR (2473.0 eV), RSR (2473.7 eV), RS(═O)R (2476.3 eV), and $SO_4^{2-}$ (2482.8 eV). Top to bottom: RSSR, cystine; RSH, reduced glutathione; RSR, glutamyl-*S*-allyl-L-cysteine; RS(═O)R, alliin; $RS(═O)_2OH$, taurine; and $SO_4^{2-}$, Na sulfate.

in garlic that present a genetic basis for determining efficiency in the conversion of alliin to allicin.

### 3.4. Potential Stoichiometry of Alliin to Allicin Based on Total Sulfur Concentration.

The total S concentrations varied across varieties and ranged from 7.9 to 18.1 mg S kg$^{-1}$ DW, with the bulb S concentration for AV17 (18.1 mg S kg$^{-1}$ DW) being about twice that of the other varieties (Table 2). The alliin concentration was also expressed as units of S (alliin/S mg g$^{-1}$ DW). The trend for this was consistent with that of total S, where the alliin-S concentration for AV17 (15.9 mg of alliin-S g$^{-1}$ of DW) was approximately twice as high as for other varieties (5.9−7.7 mg of alliin-S g$^{-1}$ of DW). Across the various varieties, alliin-S accounted for approximately 61−88% of the total S in the garlic cloves (Table 2).

To allow a comparison of the potential stoichiometry, the total S concentration (mg S kg$^{-1}$ DW) was also expressed as $\mu$mol S g$^{-1}$ DW (Table 2), with this being the maximum potential value for alliin and the conversion to pyruvate or allicin. In this regard, the bulb pyruvate concentration across varieties, with the exception of AV02, was greater than the maximum potential concentration based on the full conversion of all S compounds to allicin. This indicates that the latent pyruvate concentrations in the original uncrushed garlic samples were high. This is further confirmed in the pyruvate/allicin ratios that ranged from 3.2:1 to 5.0:1 across varieties and considerably higher than the theoretical 2:1 ratio. From the limited data within the published literature for pyruvate and allicin concentrations, pyruvate/allicin ratios are in the range of 0.78−2.06,[27,28] but we are unaware of any studies that have reported data regarding the ratio of conversion of alliin to pyruvate.

### 3.5. *In Situ* Analyses of S Speciation in Ground Dried Garlic Cloves Using Synchrotron-Based XANES.

Since alliin represented only 60−80% of the garlic's total S, synchrotron-based X-ray absorption spectroscopy was conducted to characterize all S compounds in garlic. First, we considered differences in the X-ray absorption near-edge structure (XANES) spectra of the various S standards, with these spectra showing pronounced differences between the different forms of S. The most apparent were the differences in the energy corresponding to the white line peak (Figure 3a). Indeed, the energy corresponding to the white line peak varied, largely depending upon the oxidation state, with energy generally increasing with the oxidation state. For example, the energy corresponding to the white line peak was 2473.6 eV for thiol functional groups (RSH, oxidation state of −2), 2476.3 eV for sulfoxide functional groups (RS(═O)R, oxidation state of 0, e.g., alliin), and up to 2482.8 eV for free sulfate (oxidation state of +6). Even for compounds that are quite similar, clear differences could be observed in the spectra. For example, cysteine (RSH) and methionine (RSR) differed by ca. 0.1 eV in their white line peaks. As expected, disulfide compounds (RSSR, cystine) showed two peaks ca. 1.4 eV apart, representing both 1s → $\sigma$*(S−S) and 1s → $\sigma$*(S−C). In a similar manner, disulfide S-monoxide compounds (thiosulfinate, RS(═O)SR, and allicin) had two peaks (2473.0 and 2476.2 eV). In addition to these differences in energy, there were also clear differences in the intensity of the white line peak. For example, the spectrum for free sulfate had a much higher white line peak than did many other forms of S. When specifically comparing the S forms potentially of most importance for garlic, we found that alliin [RS(═O)R] had

Table 3. Mineral Nutrient Concentrations in the Reference Leaf (Youngest Fully Expanded Leaf) Collected at the 6−7 Leaf Stage across Three Groups of Garlic Varieties

| mineral | units | group I | group II | group III | LSD (5%) | significance |
|---|---|---|---|---|---|---|
| N-$NO_3^-$ | mg $kg^{-1}$ | 401 | 920 | 1559 | 177 | <0.001 |
| N-$NH_4^+$ | mg $kg^{-1}$ | 2091 | 1607 | 2309 | 223 | <0.001 |
| N | g $kg^{-1}$ | 45.7 | 42.7 | 52.1 | 1.3 | <0.001 |
| K | g $kg^{-1}$ | 28.0 | 26.7 | 30.3 | 1.1 | <0.001 |
| P | g $kg^{-1}$ | 3.87 | 5.22 | 3.70 | 0.18 | <0.001 |
| S | g $kg^{-1}$ | 8.66 | 8.51 | 5.58 | 0.34 | <0.001 |
| Mg | g $kg^{-1}$ | 5.44 | 5.18 | 4.99 | 0.23 | <0.001 |
| Ca | g $kg^{-1}$ | 7.68 | 7.93 | 7.97 | 0.00 | NS ($p = 0.40$) |
| B | mg $kg^{-1}$ | 30.0 | 24.7 | 30.0 | 0.96 | <0.001 |
| Mo | mg $kg^{-1}$ | 4.3 | 4.1 | 1.6 | 0.31 | <0.001 |
| Zn | mg $kg^{-1}$ | 39.5 | 20.0 | 52.4 | 5.41 | <0.001 |
| Fe | mg $kg^{-1}$ | 70.3 | 106.7 | 88.8 | 9.89 | <0.001 |
| Cu | mg $kg^{-1}$ | 4.4 | 5.3 | 3.7 | 0.25 | <0.001 |

a white line peak at 2476.3 eV, glutamyl-*S*-allyl-L-cysteine [RSR] had a white line peak at 2473.8 eV, and allicin [RS(═O)SR] had peaks at 2472.5 and 2476.2 eV.

We then examined the XANES spectra for the garlic samples that were prepared by oven drying at 70 °C for 7 days before being ground into fine powder. Across the eight genotypes examined, all spectra had two distinct peaks, being at 2473.8 and 2476.3 eV (Figure 3b). These two peaks across all eight genotypes aligned with the $\gamma$-glutamyl-*S*-allyl-L-cysteine (GSAC) standard (2473.8 eV, with GSAC being representative of $\gamma$-L-glutamyl-*S*-allyl-L-cysteine compounds, RSR) and the alliin standard (2476.3 eV, RS(═O)R). This indicated that these two forms of S dominated across all genotypes in these samples, which had been prepared under conditions that were not conducive to the conversion of alliin to allicin. This is the first study to utilize XANES to examine the speciation of S *in situ* within garlic cloves without extraction. This approach confirmed that $\gamma$-L-glutamyl-*S*-allyl-L-cysteine compounds and alliin are the dominant forms within the clove, with concentrations of other forms of S (such as RSH, $RS(=O)_2OH$, and $SO_4^{2-}$) not present in detectable quantities within the clove. However, it must also be noted that there was also a very subtle shoulder at 2472.5 eV (most noticeable in the AV17 sample), indicating minor formation of allicin (most likely during sample preparation).

Although GSAC and alliin were the dominant forms of S across all eight genotypes, differences were observed in the relative intensities of these peaks. For example, the peak at 2473.8 eV (GSAC) was lowest for AV17, indicating that the proportion of S present as GSAC relative to alliin was the least for this genotype. In contrast, the peak at 2473.8 eV corresponding to GSAC was greatest for Glenlarge, AV04, and AV02, indicating that the proportion of S present as GSAC relative to alliin was greatest for these genotypes. Using linear combination fitting (LCF), it was predicted that 40% of the S was present as GSAC compounds in AV17 (and 60% as alliin), while in Glenlarge, approximately 60% was predicted to be as GSAC with 40% as alliin. Using HPLC, Mütsch-Eckner, Sticher, and Meier[33] identified that the two dominant S compounds in uncrushed garlic cloves of four varieties were alliin and two isomers of glutamyl-*S*-allyl-cysteine. The relative proportion of alliin in their study varied between 44 and 62%, which is similar to the estimated proportions of alliin of 40−60% across varieties in this study. Similarly, in this study using synchrotron-based XANES, the dominant S compounds in intact garlic were alliin and $\gamma$-L-glutamyl-*S*-allyl-L-cysteine. In a similar manner, using synchrotron-based XANES analyses, Sneeden et al.[34] found a high proportion of S was present as sulfoxide in intact garlic, consistent with the $RS(=O)_2OH$ peak we observe at 2476.3 eV. In each variety, there was a slight signal for sulfate at 2482.8 eV, indicative of very low sulfate concentrations, which is consistent with the findings of very low sulfate in garlic by Sneeden et al.[34]

### 3.6. Variation in Mineral Nutrient Status of Reference Leaves.

The mineral nutrient concentration in garlic YFEL varied significantly across the 32 varieties ($P < 0.05$) (Table S5). This is the first in-depth study to examine concentrations of a range of essential elements in garlic reference leaf, including S (4.80−10.3 g $kg^{-1}$), B (21.0−39.3 mg $kg^{-1}$ DW), Mo (1.01−5.50 mg $kg^{-1}$ DW), Fe (59.3−125 mg $kg^{-1}$ DW), and Cu (3.1−5.7 mg $kg^{-1}$ DW) across 32 varieties. The concentrations of N (40.0−56.5 g $kg^{-1}$), P (3.30−6.05 g $kg^{-1}$), Mg (4.25−6.0 g $kg^{-1}$), and Ca (6.15−9.38 g $kg^{-1}$) were considered to be in the adequate range.[35] The foliar concentration of K was ∼23.8−32.0 g $kg^{-1}$ and considered to be in the marginal to low-adequate range.[35] However, there were no deficiency symptoms observed in the field, and the recorded yields were considerably higher than those generally reported in the literature (e.g., Wang et al.[17]). This suggests that the actual sufficiency level for K may be lower than that cited in the literature. The concentration of Zn ranged from 14.0 to 60.5 mg $kg^{-1}$ DW, which is substantially higher than the deficiency criteria (∼10 mg $kg^{-1}$).[35] The YFEL data for Mn were not included due to leaf contamination with mancozeb fungicide.

Of particular interest is the difference in mineral profiles across the groups of varieties (Table 3). The group III varieties had higher N, $NO_3^-$, and $NH_4^+$ concentrations than the group I and II varieties. In contrast, the concentrations of S, Mo, and Cu in the Group I and II varieties were considerably higher than those in the Group III varieties. The S concentration in the Group I and II varieties was similar, yet the Group 2 varieties had substantially higher concentrations of allicin, indicating leaf S status is not necessarily indicative of allicin concentration in the bulb. Since all varieties were grown under the same field conditions, the differences in mineral profiles represent a genotypic difference in either root system development or nutrient uptake capacity that requires further research.

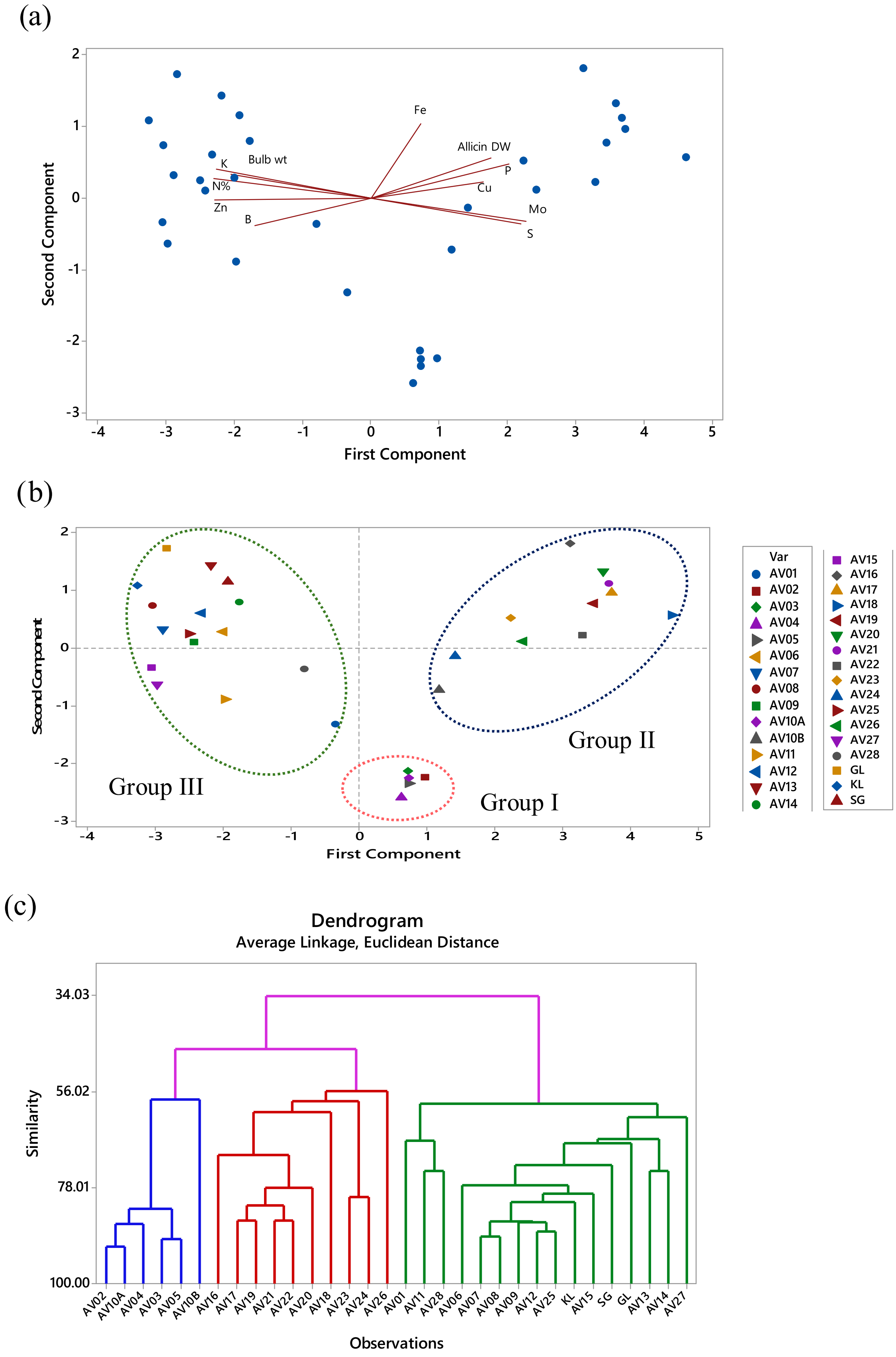

**Figure 4.** Principal component analysis, including Biplot (a), score plot (b), and dendrogram (c), showing the relationship between mineral nutrient concentration in reference leaf with bulb weight and allicin concentration in garlic bulb across 32 garlic varieties grown in 2018.

A PCA was conducted to identify the relationship between bulb weight, allicin concentration, and reference leaf mineral nutrient concentration across the varieties (Figure 4). This is the first time that the relationship between yield, allicin concentration, and leaf mineral nutrient concentration was determined. The Biplot showed that the allicin concentration was negatively correlated with bulb weight, demonstrating a trade-off between genotypic selection for allicin concentration and yield potential. In addition, bulb weight was positively correlated with the concentration of Zn, N, and K in the YFEL, while bulb allicin concentration was strongly correlated with concentrations of S, Mo, and Cu (Figure 4a).

PCA of YFEL mineral concentration, bulb weight, and allicin were made on data for 18 selected varieties from the 2018 and 2019 trials (Figure S6). The PCA from 2019 confirmed the 2018 finding and, particularly, the positive relationship between S, Mo, and Cu with allicin and total N with bulb weight. A regression analysis of the relationship between YFEL mineral concentrations between 2018 and 2019 showed strongly significant relationships for N ($p$ = 0.002, $r^2$ = 0.439), P ($p < 0.001$, $r^2$ = 0.574), S ($p < 0.001$, $r^2$ = 0.959), B ($p$ = 0.002, $r^2$ = 0.457), Cu ($p < 0.001$, $r^2$ = 0.839), and Mo ($p < 0.001$, $r^2$ = 0.843). The present findings suggest that the reference leaf mineral status during crop growth (6−7 leaf stage) is a useful diagnostic tool for maximizing yield potential and allicin concentration, particularly, for N, P, S, Cu, and Mo.

The score plot (Figure 4b) showed that the groups of garlic varieties clustered closely based on the relationship between the reference leaf mineral nutrient concentration, bulb weight, and allicin concentration. A dendrogram (Figure 4c), constructed based on bulb weight, allicin concentration, and mineral nutrient in reference leaf (except Ca and Mn), indicated that there were three main garlic groups with a similarity of ∼55% within a group. This dendrogram confirmed the result from the PCA-score plot of grouping varieties, and these groups were identical to those identified based on morphological differences (viz. Groups I, II, and III) (Figure S4). Hence, mineral nutrient and allicin concentrations and bulb weight appear to be intrinsically related to the morphology of garlic. Across the 32 garlic varieties, there were several varieties that showed greater similarity than others, such as AV07 and AV08 or AV02 and AV10A, which showed about 90% similarity (Figure 4c). There are about 300 garlic varieties that are adapted to a broad range of climatic and growing conditions.[14] The high diversity of garlic is manifested in various ways, including morphological attributes,[36−38] geographical adaptation,[39] and biochemical diversity.[17,25,37] Across 120 garlic varieties collected from five countries, the genotypes could be divided into four groups, and genetic diversity was strongly related to the geographical origin.[40] Portela et al.[41] developed a garlic classification grouping based on daylength and temperature that divided cultivars into three groups, including tropical, subtropical, and temperate to cold-temperate. Hirata et al.[36] divided a selection of 103 garlic genotypes into 4 groups based on predisposition for bolting but the extent of bolting for individual genotypes varied with latitude. Depending on the allicin or alliin concentration, garlic was divided into six groups[25] or five groups.[17] Hence, typically, garlic has been divided into a range of traits related to geography, phenology, and biochemical traits. In the present study, we identified the strong relationship between yield, reference leaf mineral nutrition, and allicin concentration that can assist in the selection of specific high-yielding high allicin garlic lines. The additional understanding of reference leaf mineral criteria can assist in developing improved nutrient management for optimizing yield. This is the first study in which garlic varieties have been classified using characteristics of nutrient status, bulb weight, allicin concentration, and morphology.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c06678.

> Graphical representation of biochemical pathway for glutathione to allicin (Figure S1); layout and planting design of field experiments (Figure S2); selection process for garlic clove samples (Figure S3); example of linear combination fitting for synchrotron-based X-ray absorption near-edge structure (XANES) analysis (Figure S4); images of variability in the morphological characteristics for foliage and bulbs of garlic varieties (Figure S5); principal component analysis of reference leaf mineral nutrient concentration and bulb weight and allicin concentration (Figure S6); World Vegetable Centre reference codes and origins of the 28 garlic varieties (Table S1); fertilizer form and application rates for field experiments (Table S2); soil properties for experimental trial sites (Table S3); seasonal comparison (2018 to 2019) for garlic bulb fresh weight, yield, dry matter yield, allicin concentration, and allicin yield (Table S4); mineral nutrient concentrations in the reference leaf for 32 garlic varieties (Table S5) (PDF)

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### Notes

The authors declare no competing financial interest.

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