

# Impacts of root sulfate deprivation on growth and elements concentration of globe amaranth (*Gomphrena globosa* L.) under hydroponic condition

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## ABSTRACT

Sulfur (S) regarded as the fourth key element is mainly taken by the plant roots. However, some plants can also absorb atmospheric sulfides, which may be of great importance for ameliorating the environment and for farming as a green organic S fertilizer used to balance insufficient soil S content for intensive cultivation in China; H<sub>2</sub>S and mainly SO<sub>2</sub> are emitted to air as a result of the rapid industrialized and economic development. Globe amaranth (*Gomphrena globosa* L.) might be one of the plants that can use atmospheric sulfides for its growth. Therefore the effects of sulfate deprivation from root on its growth, S status and other elements concentration under hydroponic culture were explored firstly. Based on measurements of plant growth, biomass, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), S, iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), and molybdenum (Mo) concentration, the results showed that S concentration in flower, shoot and root of plant without root sulfate supplied was increased with plant growth and development, symptoms of S deficiency disappeared and other elements concentration in plant tended to be nearly the same as the root sulfate-supplied plants. The interesting results might imply that globe amaranth may be able to live on the atmospheric sulfides as sulfur source.

**Keywords:** globe amaranth (*Gomphrena globosa* L.); atmospheric sulfur; growth condition; biomass; elements concentration

Sulfur (S) is one of the essential macro elements of plant and is regarded as the fourth key element next to N, P and K (Morris 1988). However, it is attributed rather catalytic and regulatory than structural functions are attributed to sulfur because it is much less abundant than other macro elements (Lewandowska and Sirko 2008). For example, there is on average about 30-fold more N, 8-fold more K and 2-fold more P than S in plant shoot dry matter (Marshner 2005). Its content strongly varies between species and may be dependent upon the developmental stage of the plant

(vegetative growth, seed production) and commonly ranges from 0.03 to 2 mmol/g dry weight (Tabatabai 1986, Pedersen et al. 1998, Hawkesford and De Kok 2006).

S is usually taken up as sulfate (Nikiforova et al. 2006). Generally, plants utilize sulfate taken up by the roots as an S source for growth and sulfur-deficient plants generate a lower yield and quality (Lunde et al. 2008). Plants are also able to take up sulfate from atmosphere and metabolize the sulfur dioxide (SO<sub>2</sub>) and/or hydrogen sulfur (H<sub>2</sub>S) as the sole source for growth upon sulfate deprivation of

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the root, as a result of  $\text{SO}_2/\text{H}_2\text{S}$  release from human technology (Hawkesford 2000, Hawkesford and De Kok 2006). The early studies believed that atmospheric S could not replace root sulfate, and it only takes up 10–20% S of the total S in plant (Cowling et al. 1973). However, several plants, such as *Brassica oleracea* and *Allium cepa* L., were found to absorb atmospheric S that completely replaced sulfate taken up by the roots as an S source for plant growth (Maas et al. 1987, De Kok et al. 1997, 2002a, Westerman et al. 2000a, b, Tausz et al. 2003, Durenkamp and De Kok 2004, Yang et al. 2006). However, the plants still need additional atmospheric S supplied for their growth. Atmospheric S is usually taken up via the stomata, turned into sulfate, metabolized with high affinity into cysteine, catalyzed by *O*-acetylserine (thiol)lyase, and then subsequently into other S metabolites (De Kok et al. 1997, De Kok and Tausz 2001, Hawkesford and De Kok 2007). Sulfate needs to be reduced to sulfide before it is metabolized into organic S compounds and the chloroplast appears to be the primary site for the reduction of sulfate to sulfide (Brunold 1990, 1993, Davidian et al. 2000).

In many developing countries, such as in China, S in soil has been decreasing without proper S fertilizer use.  $\text{SO}_2$  and  $\text{H}_2\text{S}$  pollution are of great significance as they are result of the rapid economic growth, industrialization and urbanization (Feng 2000). For example, volume of China  $\text{SO}_2$  emissions, totaled 24.68 million tons in 2007, was still the highest in the world, though they were decreased by 4.66% compared to 2006 (EPM 2008). It is well known that  $\text{SO}_2$  emissions and the resulting acid deposition have adverse impacts on forests, freshwaters and soils, killing insect and aquatic life-forms as well as causing damage to buildings and having impacts on human health. Thus, growing plants with high efficiency of absorbing atmospheric S would be very useful and important in China for sulfide pollution control and emission reduction.

Among S deprivation studies of many horticultural flower plants under soilless culture (Wang et al. 2008), a flowering plant, named globe amaranth (*Gomphrena globosa* L.), was found to have especially high ability to absorb atmospheric S. Globe amaranth, belonging to the family Amaranthaceae is an annual plant that can grow up to 60 cm in height. The true species has colorful scalelike perianths and may have white, red, purple, carmine and different shades of pink round, papery clover-like flowers. The flower heads are about 3–4 cm in length, and are borne on upright spikes from

summer until frost. The tiny, white true flowers within the flower heads are rather inconspicuous and insignificant. The narrow green leaves are opposite and oblong, 10–15 cm in length, and woolly-white when young, becoming sparsely white-hairy as they grow up.

This plant might fulfill its growth stage by absorbing atmospheric sulfides without root sulfate supply. However, information available in the literature on the primary nutrition status of the globe amaranth under root sulfate deprivation is little, especially on the S nutrition status. So the aim of the experiment was to investigate the plant growth and its primary nutrition conditions response to root sulfate deprivation including growth status, biomass production and elements concentration under hydroponic condition in order to further study its S metabolism physiology.

## MATERIALS AND METHODS

**Plant materials and pre-culture.** Globe amaranth variety used in the experiment was cv. Gnome Pink. The seeds were sterilized in 10% (v/v)  $\text{H}_2\text{O}_2$  for 5 min, rinsed thoroughly with deionized water, and germinated in vermiculite in a climate-controlled room for 30 days. Day and night temperatures were 29 and 22°C, respectively, with a relative humidity of 60–70%. The photoperiod was 14 h at a photon fluence rate of  $200 \pm 25 \mu\text{mol}/\text{m}^2 \text{ s}$  (PAR 400–700 nm). 30-day-old uniform seedlings were used for the further experiment.

**Experimental sites and design.** Pot experiments were conducted in a naturally-lit glasshouse from April 5 to October 31, 2007 at Zhejiang University, Hangzhou, China. The change trend of sulfides concentration in atmosphere in the experimental site in 2007 is shown in Figure 1 (EPM 2007).

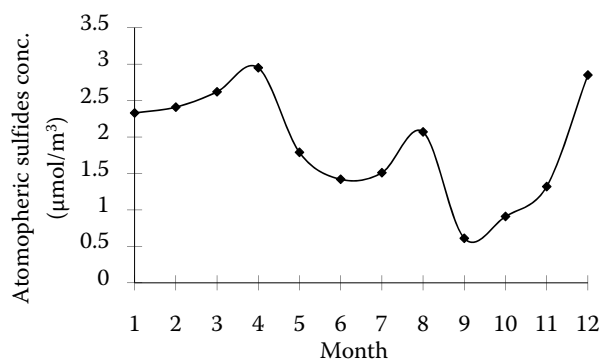


Figure 1. Atmospheric sulfides change trend in the experimental site in 2007

Uniform seedlings were transplanted to black plastic pots (30.0 cm in height and 25.5 cm in diameter, with a suitable plastic cover, 4 seedlings planted per pot). Two treatments, one was root sulfate deprivation (minus S) and the other was root sulfate supply (plus S), were set in the experiment. The plants were grown to maturity in hydroponic culture and laid out in a completely randomized design with three repetitions per treatment. In root sulfate deprivation treatment, 30-day-old seedlings were transferred to a 50% modified Hoagland nutrient solution with 2.50mM/l  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 0.26mM/l  $\text{KH}_2\text{PO}_4$ , 2.50mM/l  $\text{KNO}_3$ , 23.20 $\mu\text{M}$ /l  $\text{H}_3\text{BO}_3$ , 4.57 $\mu\text{M}$ /l  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.96 $\mu\text{M}$ /l  $\text{Zn}(\text{CH}_3\text{COO}) \cdot 2\text{H}_2\text{O}$ , 0.16 $\mu\text{M}$ /l  $\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$ , 0.26 $\mu\text{M}$ /l  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ .  $\text{MgSO}_4$  replaced  $\text{MgCl}_2$  in the modified Hoagland nutrient solution in root sulfate supply treatment. Nutrient solutions were refreshed once a week.

**Plant sampling and analysis.** One plant was removed from each pot for analyses on May 5, 31 days after transplanting. The second one was sampled on July 2 (the early flowering time), and the third one was taken on October 31 (the end of flowering). After being rinsed with 0.01M HCl (AR) and then with deionized water in order to clean thoroughly the samples, the plants were separated into three parts: the shoot, the flower and the root. All parts of the plant were dried at 70°C to constant weight. All samples were then ground to powder within a sample grinder (model Retsch MM301, Germany).

For N and P concentration determination, the powder of different tissues was digested firstly by boiling in concentrated  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}_2$ . Total N was determined by the Kjeldahl method, and total P concentration was determined by molybdenum antimony blue colorimetry (Lu 2000).

Total S in different parts of the plant was digested to sulfate by dissolving in 3 ml  $\text{HNO}_3$ , 2 ml  $\text{HClO}_4$  and 1 ml HCl, and the turbidity of the samples was measured on a spectrophotometer at 450 nm after addition of  $\text{BaCl}_2$  (Durenkamp and De Kok 2002b).

To determine the concentration of K, Ca, Mg and trace elements, the tissue powder was digested

first in 2.0 ml  $\text{HNO}_3$  and 0.5 ml  $\text{H}_2\text{O}_2$ , and digestion solutions were then allowed to cool to room temperature (25°C) and adjusted to a final volume of 25 ml with doubly deionized water (Zhang et al. 2008). All these elements in the solutions were determined by inductively coupled plasma mass spectrometry (ICP-MS, model Agilent 7500a, USA).

Leaf chlorophyll content was determined on May 20 and June 25 by Chlorophyll Meter (5M/HT4-SPAD-502, Japan).

All data statistical analyses were performed using Statistica (v. 5.5). Each value represented the average of three repetitions. Data were subjected to the analysis of variance (ANOVA) and significant differences in mean values were separated using the Duncan's Multiple Range Test ( $P \leq 0.05$ ).

## RESULTS

**Growth status.** After root sulfate deprivation treatment for one and a half month (on May 20), the SPAD value of the leaves of globe amaranth was 13.08, and was significantly different from the root sulfate-supplied plants (28.12). All leaves of sulfate-deprived plants were greenish yellow, whereas all leaves of sulfate-supplied plants were green. The symptoms of S deficiency were not very specific and just like other chlorosis in plants. However, when buds formed and were ready to bloom (on June 25), the SPAD value of leaves of plants under root sulfate deficiency treatment increased to 29.81, and there was no significant difference to the treatment of root sulfate supply (Table 1).

The plants with sulfate supply started flowering on June 21, while sulfate-deprived plants bloomed on July 2. Due to the fadelessness of the plant, flowers of all the plants weighed much more than their shoots in the late flowering time. The sulfate-deprived plants had even more flowers than the Plus S ones (Figure 2).

**Biomass production.** Without S supply for 1 month (on May 5), above- and underground dry

Table 1. The third leaf SPAD values of globe amaranth

| Treatments    | May 20             | June 25            |
|---------------|--------------------|--------------------|
| Plus sulfate  | 28.12 <sup>a</sup> | 31.25 <sup>a</sup> |
| Minus sulfate | 13.08 <sup>b</sup> | 29.81 <sup>a</sup> |

Means with the same letter within a column are not significantly different by the Duncan's Multiple Range Test at  $P \leq 0.05$  level ( $r = 3$ ); plus sulfate (plus S), the treatment of sulfate supply in hydroponic culture; minus sulfate (minus S), the treatment of sulfate deprivation in hydroponic culture

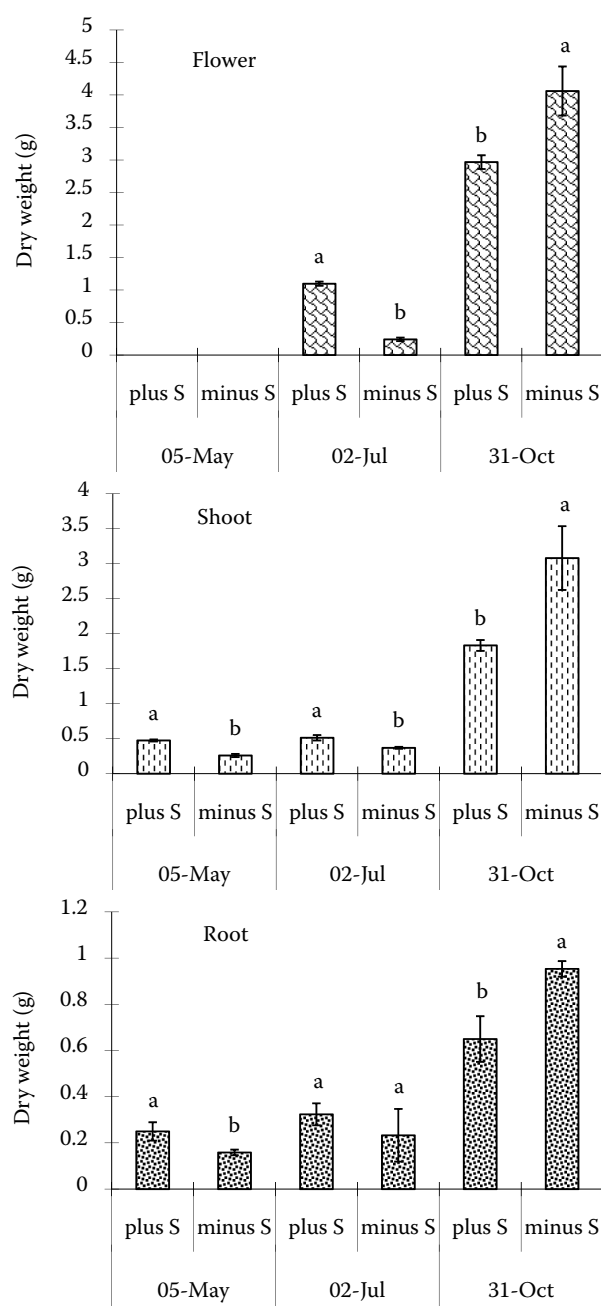


Figure 2. Biomass of globe amaranth with and without root sulfate added

weight of the plants were decreased significantly by 45.3% and 36.4%, respectively, as compared with the root sulfate supply treatment (Figure 2). At early flowering time (on July 2), shoot with the sulfate deprivation treatment was 0.37 g and flower was 0.24 g, whereas the sulfate supply treatment resulted in the values of 0.51 g (shoot) and 1.10 g (flower). The aboveground dry matter under sulfate deprivation treatment was decreased significantly by 62.1%, while  $\text{SO}_4^{2-}$  deprivation did not reduce

root weight (Figure 2). Globe amaranth shoot growth was affected more than root growth upon  $\text{SO}_4^{2-}$  deprivation; more prolonged sulfate deprivation generally results in changes of shoot/root biomass ratio in favor of root production (Stuiver et al. 1997, Yang et al. 2003, 2006, Buchner et al. 2004) and root morphology by increasing the total absorptive surface of the root system (Kutz et al. 2002, López-Bucio et al. 2003). However, the sulfate-deprived plants seemed to have a rapid growth when they were in bloom; their above- and underground dry weights were much higher than those of sulfate-supplied plants at the end of flowering sampling on October 31. The average dry weights of flower, shoot and root with sulfate deprivation treatment were increased significantly by 36.9, 68.2 and 46.7% respectively, as compared with the respective parts of the sulfate-supplied plants (Figure 2).

**Macro- and medium elements concentration.** Compared to the sulfate-supplied plant, where 30.2% of N was accumulated in the root, it was 55.1% of N in sulfate-deprived plant (Table 2). There was the highest Mg concentration in the shoot of sulfate-deprived plant and the lowest concentration in the root of sulfate-supplied plant. Deprivation of S caused a significant decrease in the concentration of total P, K, Ca, S both in shoot and root (Table 2). In addition, shoots had higher concentration of those four elements than roots in both sulfate supply and deprivation treatments. At this sampling time, S concentration in the shoot and root of sulfate-deprived decreased by 71.8% and 78.9%, respectively, as compared to the sulfate-supplied plant. When plants starved for sulfate, the decreased sulfate uptake led to reduced assimilation activity and affected many different metabolic processes (Hirai and Saito 2004). Eventually, the limited supplies of S in plants resulted in decreased plant tissue S content. Decreases in S content resulted in the inhibition of sulfate assimilation, reduced amounts of chlorophyll and imbalance of nitrogen as well as of other elements (Nikiforova et al. 2003, 2005, Schachtman and Shin 2007). Overall, these changes led to a reduced rate of metabolism and growth of plant.

At early flowering time (on July 2), N concentration did not differ largely among the three plant parts in sulfate-supplied plant, whereas the value was significantly much higher in the flower of sulfate-deprived plant than in its shoot and root (Table 2). S concentration in the corresponding three parts of the plant was increased markedly whether it was the deprived of sulfate or not when

Table 2. Macro- and medium elements concentration and N/S value in different organs in globe amaranth with and without root sulfate supply

| Treatments    | N                  | P                  | K                 | Ca                | Mg                 | S                  | N/S                |
|---------------|--------------------|--------------------|-------------------|-------------------|--------------------|--------------------|--------------------|
| (%)           |                    |                    |                   |                   |                    |                    |                    |
| May 5         |                    |                    |                   |                   |                    |                    |                    |
| Plus sulfate  |                    |                    |                   |                   |                    |                    |                    |
| Shoot         | 1.39 <sup>b</sup>  | 0.39 <sup>a</sup>  | 1.00 <sup>a</sup> | 0.95 <sup>a</sup> | 0.67 <sup>b</sup>  | 0.39 <sup>a</sup>  | 3.56 <sup>c</sup>  |
| Root          | 1.16 <sup>c</sup>  | 0.12 <sup>b</sup>  | 0.56 <sup>b</sup> | 0.13 <sup>c</sup> | 0.14 <sup>d</sup>  | 0.19 <sup>b</sup>  | 6.11 <sup>b</sup>  |
| Minus sulfate |                    |                    |                   |                   |                    |                    |                    |
| Shoot         | 0.85 <sup>d</sup>  | 0.08 <sup>c</sup>  | 0.56 <sup>b</sup> | 0.39 <sup>b</sup> | 0.73 <sup>a</sup>  | 0.11 <sup>c</sup>  | 7.73 <sup>b</sup>  |
| Root          | 1.70 <sup>a</sup>  | 0.07 <sup>c</sup>  | 0.28 <sup>c</sup> | 0.10 <sup>c</sup> | 0.21 <sup>c</sup>  | 0.04 <sup>d</sup>  | 42.50 <sup>a</sup> |
| July 2        |                    |                    |                   |                   |                    |                    |                    |
| Plus sulfate  |                    |                    |                   |                   |                    |                    |                    |
| Flower        | 1.28 <sup>b</sup>  | 0.27 <sup>b</sup>  | 0.96 <sup>a</sup> | 0.58 <sup>c</sup> | 0.31 <sup>b</sup>  | 0.26 <sup>cd</sup> | 4.92 <sup>c</sup>  |
| Shoot         | 1.07 <sup>b</sup>  | 0.08 <sup>c</sup>  | 1.05 <sup>a</sup> | 1.63 <sup>a</sup> | 0.78 <sup>a</sup>  | 0.54 <sup>a</sup>  | 1.98 <sup>d</sup>  |
| Root          | 0.83 <sup>b</sup>  | 0.06 <sup>c</sup>  | 0.52 <sup>b</sup> | 0.22 <sup>d</sup> | 0.16 <sup>b</sup>  | 0.39 <sup>b</sup>  | 2.13 <sup>d</sup>  |
| Minus sulfate |                    |                    |                   |                   |                    |                    |                    |
| Flower        | 2.62 <sup>a</sup>  | 0.33 <sup>a</sup>  | 0.94 <sup>a</sup> | 0.63 <sup>c</sup> | 0.33 <sup>b</sup>  | 0.18 <sup>de</sup> | 14.56 <sup>a</sup> |
| Shoot         | 0.86 <sup>b</sup>  | 0.08 <sup>c</sup>  | 0.97 <sup>a</sup> | 1.35 <sup>b</sup> | 0.77 <sup>a</sup>  | 0.32 <sup>bc</sup> | 2.69 <sup>cd</sup> |
| Root          | 1.15 <sup>b</sup>  | 0.05 <sup>c</sup>  | 0.46 <sup>b</sup> | 0.35 <sup>d</sup> | 0.22 <sup>b</sup>  | 0.10 <sup>e</sup>  | 11.50 <sup>b</sup> |
| October 31    |                    |                    |                   |                   |                    |                    |                    |
| Plus sulfate  |                    |                    |                   |                   |                    |                    |                    |
| Flower        | 0.95 <sup>ab</sup> | 0.27 <sup>a</sup>  | 0.84 <sup>b</sup> | 0.82 <sup>c</sup> | 0.25 <sup>d</sup>  | 0.23 <sup>ab</sup> | 4.13 <sup>b</sup>  |
| Shoot         | 1.01 <sup>ab</sup> | 0.10 <sup>bc</sup> | 1.12 <sup>a</sup> | 2.01 <sup>a</sup> | 0.84 <sup>a</sup>  | 0.34 <sup>a</sup>  | 2.97 <sup>c</sup>  |
| Root          | 1.01 <sup>ab</sup> | 0.07 <sup>c</sup>  | 0.49 <sup>c</sup> | 0.44 <sup>d</sup> | 0.34 <sup>cd</sup> | 0.25 <sup>ab</sup> | 4.04 <sup>b</sup>  |
| Minus sulfate |                    |                    |                   |                   |                    |                    |                    |
| Flower        | 1.18 <sup>a</sup>  | 0.24 <sup>a</sup>  | 0.78 <sup>b</sup> | 0.69 <sup>c</sup> | 0.22 <sup>d</sup>  | 0.20 <sup>b</sup>  | 5.90 <sup>a</sup>  |
| Shoot         | 0.78 <sup>b</sup>  | 0.10 <sup>bc</sup> | 0.76 <sup>b</sup> | 1.67 <sup>b</sup> | 0.62 <sup>b</sup>  | 0.28 <sup>ab</sup> | 2.79 <sup>c</sup>  |
| Root          | 0.80 <sup>b</sup>  | 0.06 <sup>c</sup>  | 0.45 <sup>c</sup> | 0.83 <sup>c</sup> | 0.49 <sup>bc</sup> | 0.23 <sup>ab</sup> | 3.48 <sup>bc</sup> |

Means within a column on the same sampling time with the same letter are not significantly different by Duncan's Multiple Range Test at  $P \leq 0.05$  level ( $r = 3$ ). Plus sulfate, the treatment of sulfate supply in hydroponic culture; minus sulfate, the treatment of sulfate deprivation in hydroponic culture

it was in the bloom (Table 2). However, compared to the sulfate-supplied plant, S concentrations in the flower, shoot and root of the sulfate-deprived plant were decreased significantly by 30.8%, 40.7% and 74.4%, respectively. The flower of sulfate-deprived plant tended to have the highest N, P and K concentration, and the highest Ca, Mg and S appeared in the shoot of sulfate-supplied plant (Table 2). At this sampling time, Mg concentration in the organs of sulfate-deprived plants showed no significant difference compared to the cor-

responding parts of sulfate-supplied plant (Table 2), that was in coincidence with the SPAD valued on June 25 (Table 1).

At the end of flowering (on October 31), there was still the highest Ca, Mg and S in the shoot of sulfate-supplied plant and the highest N and P concentration in the flower of sulfate-deprived plant. The root accumulated extra percentages of Ca and Mg after flowering, and their values were almost twice as high as that at the early flowering time. Concentration of other elements had simi-



lar values to those from July 2. Strange enough, the S concentration declined in the tissues of the sulfate-supplied plant since flowering, but kept unchanged in flower and shoot of sulfate-deprived plant and this accumulation in its root just went on. S concentration in the organs of sulfate-deprived plant showed no significant difference to the corresponding parts of sulfate-supplied plant at this time (Table 2, Figure 3). The results at whole plant level showed that the demand of sulfate and its distribution in the plant were driven presumably by the S demand for growth at any specific developmental stage.

**N/S as affected by sulfate supply.** On the first sampling (May 5), the highest N/S was obtained in the root of sulfate-deprived plant, i.e. 42.50, and was about 7 times higher than in the root of sulfate-supplied plant. However, once the plant was in the bloom, the highest N/S appeared in the flower of the sulfate-deprived plant. In the early flowering time, the ration of N to S was 14.56 in the flower of sulfate-deprived plant; it was about 3 times higher than in the flower of the sulfate-supplied plant; however, it declined to 5.90 at the end of flowering time sampling (Table 2).

As to the whole plant, N/S of the sulfate-deprived plant was 5.2 times higher than that of the sulfate-supplied plant on May 5. In bloom, N/S of the sulfate-deprived plant declined to be 3.2-fold higher than in the sulfate-supplied plant; towards the end of flowering, no significant difference of the N to S ratio between the two treatments of plants was observed (Table 2). However, in a study on wheat by Gilbert et al. (1997), the optimal ratio of N/S was 15.00; similarly, in corn, Wang et al. (2003) found the ratio of total N/S in root of 10.70 with the sulfate supply, and 48.70 without the sulfate supply.

Whether sulfate was supplied in root or not, more than 80% S was accumulated mainly in the above-

ground part of globe amaranth (Figure 3). After flowering, the ratio of S in flower to the whole plant was generally increasing; however, S ratio in the flower of sulfate-deprived plant was lower than that of the sulfate-supplied plant (Figure 3).

**Trace elements concentrations.** Generally, deprivation of  $\text{SO}_4^{2-}$  for one month depressed the trace elements concentration in plant (Table 3). But we could also find that Fe concentration in the sulfate-deprived root was similar to that in the sulfate-supplied root, and Mn concentration in the root and Mo concentration in the shoot were even increased significantly by 72.7% and 167.0%, respectively, compared to their counterparts in sulfate-supplied plant. Higher Fe and Cu concentration was found in root than in shoot, and higher Mn, Zn and B concentration in shoot. Fe concentration was much higher than other elements, the values being from ten to hundred times higher.

At early flowering time (Table 3), however, Fe concentration decreased markedly in all parts of the plant whether sulfate was added or not. Similarly Mo concentration decreased markedly in the shoot and root of sulfate-supplied plant. Sulfate deprivation did not cause a decrease in the proportion of trace elements allocated to the plant tissues at this time, but even raised Zn, B and especially Mo concentration (Table 3).

At the end of flowering time, Fe concentration in shoot continued to decrease, whereas the values in flower and root began to increase whether the sulfate was supplied or not in hydroponic solutions. Zn and Mo concentration decreased a lot in flower of the sulfate-deprived plant compared to sampling at the early flowering time (Table 3).

S occurs in the environment in a variety of oxidative states that range from  $-2$  in its most reduced form (sulfide  $\text{-S}^{2-}$ ) to  $+6$  in its most oxidized form (sulfate  $\text{-SO}_4^{2+}$ ). Though inorganic sulfate is the primary

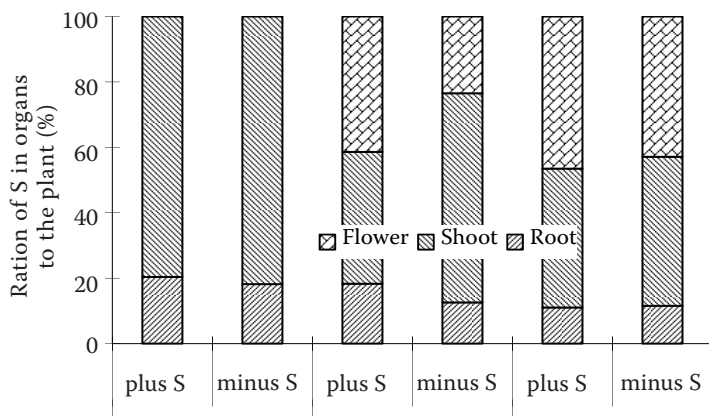


Figure 3. Ratio of S in organs in globe amaranth with and without root sulfate added

Table 3. Trace elements concentration in different organs in globe amaranth with and without root sulfate supply

|               | Fe                   | Mn                 | Cu                  | Zn                  | B                   | Mo                 |
|---------------|----------------------|--------------------|---------------------|---------------------|---------------------|--------------------|
|               | (mg/kg)              |                    |                     |                     |                     |                    |
| May 5         |                      |                    |                     |                     |                     |                    |
| Plus sulfate  |                      |                    |                     |                     |                     |                    |
| Shoot         | 650.07 <sup>b</sup>  | 7.84 <sup>a</sup>  | 24.01 <sup>b</sup>  | 28.81 <sup>a</sup>  | 27.60 <sup>a</sup>  | 11.88 <sup>d</sup> |
| Root          | 1040.11 <sup>a</sup> | 4.90 <sup>b</sup>  | 31.21 <sup>a</sup>  | 19.21 <sup>b</sup>  | 13.80 <sup>c</sup>  | 19.26 <sup>b</sup> |
| Minus sulfate |                      |                    |                     |                     |                     |                    |
| Shoot         | 390.07 <sup>c</sup>  | 8.46 <sup>a</sup>  | 12.90 <sup>d</sup>  | 15.08 <sup>c</sup>  | 16.11 <sup>b</sup>  | 31.72 <sup>a</sup> |
| Root          | 975.17 <sup>a</sup>  | 8.46 <sup>a</sup>  | 15.49 <sup>c</sup>  | 6.56 <sup>d</sup>   | 9.47 <sup>d</sup>   | 13.88 <sup>c</sup> |
| July 2        |                      |                    |                     |                     |                     |                    |
| Plus sulfate  |                      |                    |                     |                     |                     |                    |
| Flower        | 107.2 <sup>d</sup>   | 9.17 <sup>b</sup>  | 34.93 <sup>b</sup>  | 52.27 <sup>e</sup>  | 38.45 <sup>cd</sup> | 2.35 <sup>f</sup>  |
| Shoot         | 287.01 <sup>b</sup>  | 14.62 <sup>a</sup> | 33.21 <sup>b</sup>  | 113.57 <sup>c</sup> | 76.04 <sup>a</sup>  | 8.73 <sup>e</sup>  |
| Root          | 467.56 <sup>a</sup>  | 8.89 <sup>b</sup>  | 45.34 <sup>a</sup>  | 71.07 <sup>d</sup>  | 34.35 <sup>cd</sup> | 13.48 <sup>d</sup> |
| Minus sulfate |                      |                    |                     |                     |                     |                    |
| Flower        | 105.99 <sup>d</sup>  | 4.63 <sup>c</sup>  | 35.13 <sup>b</sup>  | 168.46 <sup>b</sup> | 41.32 <sup>c</sup>  | 17.73 <sup>c</sup> |
| Shoot         | 209.34 <sup>c</sup>  | 10.69 <sup>b</sup> | 37.9 <sup>b</sup>   | 189.45 <sup>a</sup> | 53.97 <sup>b</sup>  | 56.99 <sup>a</sup> |
| Root          | 504.96 <sup>a</sup>  | 9.55 <sup>b</sup>  | 44.5 <sup>a</sup>   | 81.62 <sup>d</sup>  | 31.05 <sup>d</sup>  | 24.52 <sup>b</sup> |
| October 31    |                      |                    |                     |                     |                     |                    |
| Plus sulfate  |                      |                    |                     |                     |                     |                    |
| Flower        | 175.53 <sup>c</sup>  | 8.81 <sup>c</sup>  | 41.37 <sup>ab</sup> | 68.82 <sup>cd</sup> | 32.66 <sup>bc</sup> | 3.04 <sup>c</sup>  |
| Shoot         | 141.84 <sup>d</sup>  | 26.53 <sup>b</sup> | 34.34 <sup>b</sup>  | 165.53 <sup>a</sup> | 51.31 <sup>a</sup>  | 10.9 <sup>b</sup>  |
| Root          | 724.81 <sup>a</sup>  | 24.2 <sup>b</sup>  | 43.26 <sup>ab</sup> | 72.23 <sup>c</sup>  | 27.9 <sup>c</sup>   | 12.48 <sup>b</sup> |
| Minus sulfate |                      |                    |                     |                     |                     |                    |
| Flower        | 122.34 <sup>d</sup>  | 7.97 <sup>c</sup>  | 38.91 <sup>b</sup>  | 59.75 <sup>d</sup>  | 32.07 <sup>c</sup>  | 3.81 <sup>c</sup>  |
| Shoot         | 172.3 <sup>c</sup>   | 39.63 <sup>a</sup> | 43.26 <sup>ab</sup> | 171.34 <sup>a</sup> | 47.33 <sup>ab</sup> | 20.73 <sup>a</sup> |
| Root          | 641.18 <sup>b</sup>  | 41.34 <sup>a</sup> | 54.12 <sup>a</sup>  | 112.86 <sup>b</sup> | 32.34 <sup>bc</sup> | 12.39 <sup>b</sup> |

Means with the same letter within a column on the same sampling day are not significantly different by the Duncan's Multiple Range Test at  $P \leq 0.05$  level ( $r = 3$ ); plus sulfate (plus S), the treatment of sulfate supply in hydroponic culture; minus sulfate (minus S), the treatment of sulfate deprivation in hydroponic culture

source of S used by plant, S may be taken up from the atmosphere as well, as  $H_2S$  and mainly  $SO_2$  are emitted to the atmosphere as a result of volcanic activity, decomposition of biological tissues and anthropogenic activities (Hawkesford 2000, Maathuis 2009). Atmospheric  $SO_4^{2-}$  derived from industry and coal burning frequently reaches levels of over  $100 \mu g/m^3$  (Maathuis 2009). It has been proved that atmospheric levels of  $\geq 0.1 \mu l/l$  of these S gases should be sufficient to cover the organic S need for growth of most plant species (Durenkamp and De Kok 2004).

Due to prolonged  $SO_4^{2-}$  deprivation from root, S deficiency symptoms of globe amaranth gradually disappeared (Table 1). Why did this phenomenon appear? It might be caused by a capacity of the plant to capture atmospheric sulfides.

Up to the early flowering stage, the growth of the plant aboveground part was affected by  $SO_4^{2-}$  deprivation more than root growth, which resulted in a decrease in the shoot/root ratio (Figure 2). That was in coincidence with the previous studies which reported that the prolonged sulfate deprivation

would generally result in changes of shoot/root biomass ratio in favor of root production (Stuiver et al. 1997, Yang et al. 2003, 2006, Buchner et al. 2004) and root morphology by increasing the total absorptive surface of the root system (Kutz et al. 2002, López-Bucio et al. 2003). However, at the end of flowering period, all of the three organs weighted significantly more than the corresponding parts of the sulfate-supplied plant when root sulfate deprivation was prolonged (Figure 2). It seems that the activated root production and root morphology at prolonged sulfate deprivation could activate the growth of the aboveground parts of globe amaranth. The results confirmed that this species had a rather low root sulfate need for growth in normal atmospheric sulfides condition (Figure 1) and showed that the effect of S was primarily on the number of grains per ear, indicating that S deficiency either reduces the initiation of spikelet and/or floret, or increased the mortality of floret. It was in compliance with the conclusions of Archer (1974) on wheat and Scott et al. (1984) on barley. Recent data also suggested that when *Brassica* was grown at a maintained 5  $\mu$ M sulfate concentration (the sulfate concentration in Hoagland nutrient solution is 2000  $\mu$ M), plant growth was quite normal although the sulfate content of the shoots was somewhat lower compared to plants grown at 100 or 500  $\mu$ M (Hawkesford and De Kok 2006).

Yang (2006) proved that  $\text{SO}_4^{2-}$  deprivation did not affect total N content of shoot and root of cv. Kasumi, whereas David et al. (1951) reported that the percentage of total N was always greater when the  $\text{SO}_4^{2-}$  supply was limited in cotton. The findings of our experiment were similar to Yang (2006). S concentration of plants under sulfate deprivation was less than 30% of that in sulfate-supplied plants on May 5, and it led to decreases of P, K, Ca concentration excluding Mg. S concentration in sulfate-deprived plants increased with the plant's growth, and P, K, Ca, and Mg concentration turned to be similar to those in the sulfate-supplied plant. A similar trend occurred in the trace elements concentration as well. The S demand might be dependent upon the developmental stage of the plant. In the perspective of whole plant S metabolism, the requirement was the provision of adequate S to optimize vegetative plant growth, and hence reproductive potential, and ultimately to provide S for seed tissues to maximize fecundity. It was unclear to what extent the external or internal sulfate concentration in the root itself was the sensing factor in the modulation of the sulfate efficiency in general.

N/S in plants of the sulfate deprivation treatment was higher than in the sulfate-supplied plants. Whether the sulfate was supplied to the plant or not, the highest root N/S was before the plant was in bloom; during the blooming period, the flower N/S was higher than that in roots and shoots (Tables 2 and 4). It was suggested that for S deficiency diagnosis of globe amaranth, the root could be an optional organ to estimate plant S deficiency symptom before flowering; in bloom, the flower would be a better organ. That was not always in accordance with the findings of Wang et al. (2003) who reported that the root N/S of corn could be a diagnosis index to determine whether S supply was deficient.

To globe amaranth, S content per seed was generally only about 5  $\mu$ g, and it could be recognized that no sulfate was imported into the aqueous culture solutions for the deionized water and analyzed reagents used during the hydroponic experiment. However, towards the end of flowering time, S content in the sulfate-deprived plants was 18.95 mg per plant on average. Where did S come from in the sulfate-deprived plant? S deficiency symptoms of globe amaranth gradually disappeared with prolonged S deprivation from root and the plant did fulfill its whole growth stage without root sulfate supply. A rational hypothesis was that globe amaranth (*Gomphrena globosa* L.) could efficiently utilize the atmospheric sulfides as its S source for plant growth. But up to now, there was no direct evidence that could prove the phenomena shown in the case of globe amaranth (*Gomphrena globosa* L.); to explore the plant atmospheric sulfides utilization characteristics directly with the physiological and biological approaches was thus certainly what should be done next, for sulfate starvation of plants led to a series of metabolic and physiological responses aiming at adopting plant metabolism to the available nutrient supply and to acquire a new homeostatic balance (Nikiforova et al. 2005). After all, the primary target site for effects of sulfate deprivation were the S containing metabolites, the amino acids cysteine and methionine and their immediate derived metabolites such as GSH and SAM (Nikiforova et al. 2006). Amino acid content in plants was usually balanced in a delicate way (Hofgen et al. 1995). Yet, environmental conditions were affecting plant amino acid compositions.

Nevertheless, the plant having high effective assimilation of atmospheric sulfides may be of great use in ameliorating the environment and for farming as a green organic S fertilizer.



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