Sulfur Use Efficiency Is a Significant Determinant of Drought Stress Tolerance in Relation to Photosynthetic Activity in Brassica napus Cultivars

Bok-Rye Lee, Rashed Zaman, Jean-Christophe Avice, Alain Ourry, Tae-Hwan Kim

To cite this version:
Bok-Rye Lee, Rashed Zaman, Jean-Christophe Avice, Alain Ourry, Tae-Hwan Kim. Sulfur Use Efficiency Is a Significant Determinant of Drought Stress Tolerance in Relation to Photosynthetic Activity in Brassica napus Cultivars. Frontiers in Plant Science, Frontiers, 2016, 7, pp.1-11. 10.3389/fpls.2016.00459. hal-02183573

HAL Id: hal-02183573
https://hal-normandie-univ.archives-ouvertes.fr/hal-02183573
Submitted on 27 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Sulfur Use Efficiency Is a Significant Determinant of Drought Stress Tolerance in Relation to Photosynthetic Activity in *Brassica napus* Cultivars

Bok-Rye Lee1,2†, Rashed Zaman1†, Jean-Christophe Avice3,4, Alain Ourry3,4 and Tae-Hwan Kim1*†

1 Department of Animal Science, Institute of Agricultural Science and Technology, College of Agriculture and Life Science, Chonnam National University, Gwangju, South Korea, 2 Biotechnology Research Institute, Chonnam National University, Gwangju, South Korea, 3 Université de Caen Basse-Normandie, Caen, France, 4 UMR INRA-UCBN 950 Ecophysiologie Végétale, Agronomie et Nutritions N, Université de Caen Basse Normandie, Caen, France

To investigate the varietal difference in sulfur use efficiency (SUE) and drought stress tolerance, *Brassica napus* ‘Mosa’ and ‘Saturnin’ were exposed to polyethylene glycol (PEG)-induced drought stress for 72 h. Direct quantification of S uptake, *de novo* synthesis of amino acids and proteins was performed by tracing $^{34}$S. The responses of photosynthetic activity in relation to SUE were also examined. The total amount of newly absorbed S decreased with drought stress in both cultivars but the decrease rate was significantly higher in Mosa (−64%) than in Saturnin (−41%). Drought stress also decreased the amount of S assimilated into amino acids ($^{34}$S-amino acids) and proteins ($^{34}$S-proteins). The total amount of S incorporated into amino acids and proteins was generally higher in Saturnin (663.7 µg S per plant) than in Mosa (337.3 µg S per plant). The estimation of SUE based on S uptake (SUpE) and S assimilation (SUaE) showed that SUE was much higher in Saturnin than in Mosa. The inhibition of photosynthetic activity including Rubisco protein degradation caused by drought stress was much lower in the cultivar with higher SUE (Saturnin). The present study clearly indicates that the genotype with higher SUE is more tolerant to PEG-induced drought stress.

**Keywords:** *Brassica napus*, PEG-induced drought stress, photosynthetic activity, $^{34}$S tracing, sulfur use efficiency

**INTRODUCTION**

*Brassica napus* L.) is increasingly grown throughout the world. It has a wide range of uses including vegetable oil, animal feed, and alternative fuel (Abdallah et al., 2010). Oilseed rape is also considered to be an excellent rotation crop which enhances suppression of soil-borne pathogens and a catch crop species that limits leaching of mineral nutrients into the aquifer (Kirkegaard et al., 1997). World production of oilseed rape is growing rapidly, with FAO reporting...
that 47 million tons of oilseed rape was produced in 2007, and 58.4 million tons estimated to have been produced in the 2010–2011 (Saedindia and Gohari, 2012).

In general, oilseed rape, and Brassica species have a characteristically high sulfur (S) demand during vegetative growth for the synthesis of proteins. For example, the production of 1 ton of rape seeds requires about 16 kg S (Blake-Kaliff et al., 2001), compared with 2–3 kg of S per ton of wheat grain (Zhao et al., 1999). Moreover, these plants contain relatively high amounts of S-containing secondary metabolites, viz. glucosinolate, which accounts for up to 20% of the organic sulfur content (Castro et al., 2004; Aghajanzadeh et al., 2014). However, S-deficiency is a common phenomenon in many agro-ecosystems, caused by a massive decrease of S inputs from atmospheric deposition and reduced application of S-containing fertilizer in the last three decades (Zhao et al., 1997). S-deficiency and/or S-deprivation decrease the cell sap osmotic potential due to a net increase of intracellular solutes, rather than from a loss of cell water (Kusaka et al., 2005). In addition, S-deficiency reduces chlorophyll and Rubisco content, and provokes chlorosis of young leaves (Gilbert et al., 1997; Lee et al., 2014; Muneer et al., 2014). These results imply that S-deficiency results in a general inhibition of photosynthesis and protein synthesis. In B. napus, studies carried out under controlled greenhouse (Balint and Rengel, 2008; Dubouset et al., 2010) or field conditions (Schnug et al., 1993; Fismes et al., 2000) have shown that S-deficiency reduces N-use efficiency, and that N-deficiency can also affect et al., 1993; Fismes et al., 2000) have shown that S-deficiency reduces N-use efficiency, and that N-deficiency can also affect (Rengel, 2008; Dubousset et al., 2010) or field conditions (Schnug et al., 2009; Astolfi and Zuchi, 2013) or field conditions (Schnug et al., 2009; Astolfi and Zuchi, 2013). Sulfur is an essential element in the formation of sulfhydryl (S-H) and disulfide bonds (S-S). These bonds are important for the stabilization of protein structures (Saito, 2000). In this context, the role of S nutrition in alleviating negative responses to salinity stress (Khan et al., 2009; Astolfi and Zuchi, 2013; Fatma et al., 2014) and iron deficiency (Zuchi et al., 2009; Muneer et al., 2014) have been widely reported.

Drought stress is one limiting factor to plant growth. In Korea, drought occurs predominantly from March to the early June, when the growth and development of most varieties of oilseed rape (winter type) actively progress. Oilseed rape is, therefore, often exposed to drought stress during the early stages of growth. Selection for physiologically efficient S-use cultivars may have value in breeding programs aimed at improving stress tolerance, grain yield, and quality. In this study, we hypothesized that cultivar variation in SUE under PEG-induced drought stress may be attributed to two components: (i) S-uptake efficiency (SUaE; S uptake per S supplied) and (ii) S-assimilation efficiency (SUAe; S assimilated to amino acids and proteins per S supplied), and that the genotype producing higher SUE is more tolerant to PEG-induced drought stress. To test this hypothesis, S uptake and the amount of S incorporated into amino acids and proteins were directly measured by a $^{34}$S tracing method. The responses to PEG-induced drought stress of parameters related to photosynthetic activity were also assessed in relation to SUE in two B. napus cultivars.

# MATERIALS AND METHODS

## Plant Culture

The surface-sterilized seeds of B. napus ‘Mosa’ and ‘Saturnin’ were sown into bed soil in a tray. At the three-leaf stage, seedlings were transferred to 2.5 L pots filled with hydroponic nutrient solution containing (mM for the macro elements): 1.0 NH$_4$NO$_3$; 0.4 KH$_2$PO$_4$; 1.0 K$_2$SO$_4$; 0.5 K$_2$HPO$_4$; 3.0 CaCl$_2$; 0.5 MgSO$_4$; 0.15 K$_2$HPO$_4$; 0.2 Fe- Na EDTA; and (µM for the micro elements): 14 H$_2$BO$_3$; 5.0 MnSO$_4$·H$_2$O; 3.0 ZnSO$_4$·7H$_2$O; 0.7 CuSO$_4$·5H$_2$O; 0.7 (NH$_4$)$_6$MoO$_7$·4H$_2$O; 0.1 CoCl$_2$. Seedlings were then grown in a greenhouse. The nutrient solution was continuously aerated and renewed every 5 days. Natural light was supplemented by metal halide lamps, which generated c. 400 µmol photons m$^{-2}$ s$^{-1}$ at the canopy height for 16 h per day.

## PEG-Induced Drought Stress and Isotope Labeling

Eight-week-old plants were divided in two groups for the application of (PEG-6000). One group of experimental plants was supplied normal nutrient solution as a control. The other group was supplied 8% PEG-6000 with normal nutrient solution for 72 h. For the $^{34}$S feeding, S sources in hydroponic solution were replaced by $^{34}$S labeling solution containing 1.5 mM K$_2$SO$_4$ with 1.0% $^{34}$S atom excess.

## Measurements and Sampling

Leaf water potential was measured as the petiole xylem-pressure potential using a pressure chamber (PMS Instrument Co. Corvallis, OR, USA). Photosynthesis rate, stomatal conductance and transpiration were measured in a greenhouse using a portable photosynthesis measurement system (LI-6400. LI-COR, Inc. Lincoln, NE, USA). One fully expanded mature leaf per plant was tagged with a small wire, and measurements were taken over 72 h of treatment. Measurement was done every day, 4 h after the beginning of the photoperiod on the same tagged leaf. The first sampling (0 h) was conducted just before the drought stress treatment (the beginning of $^{34}$SO$_4^{2-}$ feeding) was applied at 10:00 h. Additional measurements were taken at 24, 48, and 72 h after treatment. The harvested plants were separated into leaves and roots. All plant samples were immediately frozen in liquid
Sulfur Use Efficiency Effects on Photosynthesis

Chemical Fractionation and Isotope Analysis

About 200 mg of finely ground freeze-dried samples were extracted twice with 100 mM sodium-phosphate buffer (pH 7.5) at 4°C. Proteins in the combined supernatant were precipitated with 80% (v/v) acetone and centrifuged at 10,000×g at 4°C for 10 min. The resulting pellets, which corresponded to NAS incorporated into protein fractions (34S-protein), were re-suspended in 0.5 mL of ultra-pure water. To measure NAS in sulfate (34S-sulfate), the aliquot obtained after protein precipitation was evaporated under vacuum at 4°C and centrifuged. The resulting supernatant was passed through an H+ column (Dowex 50W × 8). The pH of the solution collected from the H+ column was adjusted to neutral pH, and this solution was concentrated to a final volume of 0.5 mL. The NAS incorporated into amino acids (34S-amino acids) was eluted from the Dowex 50W × 8 column by using 25 mL of 0.5 M HCl and concentrated to 1 mL. For the fractionated liquid samples, an appropriate sample volume (usually 0.1 mL) was dropped into a tin capsule and freeze-dried before S and 34S quantification. The NAS (34S-total S) was obtained from 5 mg of freeze-dried powder samples. The S content and 34S atom % of all fractions was determined by a continuous flow isotope mass spectrometer (IsoPrime, GV Instrument, Manchester, UK). The 34S abundances measured were converted to (RSA, i.e., % of recently incorporated atoms relative to the total numbers of atoms in the sample) via the following equation (1):

\[
RSA = \frac{(34\text{S atom } \% \text{ measured} - \text{natural } 34\text{S atom } \%)}{(34\text{S atom } \% \text{ of fed } 34\text{SO}_4^{2-} \text{ - natural } 34\text{S atom } \%)} \times 100
\]  

in which the natural 34S atom % was adopted from the 34S atom % of non-34S-fed plants.

The amounts of NAS in the S containing compounds were calculated using equation (2):

\[
NAS = (RSA \times S \text{ content measured in a compound})/100.
\]

Determination of Sulfur Use Efficiency

Sulfur use efficiency was assessed based on S uptake and S assimilation, respectively, in controls and PEG-induced drought stressed plants. SUE based on S uptake (SUpe) was calculated as the total amount of NAS (34S) per gram of S provided in the nutrient solution during 72 h of the experiment, and expressed by mg S taken up g−1 S fed. SUE based on S assimilation (SUaE) was calculated as the total amount of S assimilated into amino acids and proteins (34S-amino acid + 34S-protein) per gram of S provided in the nutrient solution during 72 h of the experiment, and expressed by mg S assimilated g−1 S fed.

SDS-PAGE and Quantification of Rubisco Protein

Leaf samples were homogenized in chilled mortar with ice-cold extraction buffer containing 25 mM Tris-HCl (pH 7.8), 50 mM MgCl2, 2.5 mM EDTA, and 1 mM DTT. The homogenate was centrifuged at 10,000×g at 4°C for 10 min and the supernatant was applied to SDS polyacrylamide gel electrophoresis (SDS-PAGE) for determination of Rubisco content. Twenty microgram proteins were separated in 1.0 mm thick gels containing 12.5% acrylamide (propenamide). The large subunit of Rubisco was detected by staining with Coomassie brilliant blue R-250. The individual band was extracted with formamide, and quantified by the Bradford dye binding assay using BSA as a standard (Makino et al., 1985).

Statistical Analysis

Analysis of variance (ANOVA) and Duncan’s multiple range test were employed to compare the means of each treatment. Statistical significance was postulated at P < 0.05. Statistical analysis of physiological and biochemical measurements was carried out using the software SAS 9.1.3.

RESULTS

PEG-Induced Drought Stress Effects on Leaf Water Potential and Biomass in B. napus Cultivars

Variatel differences and PEG-induced drought stress effects on Ψw were significant (Table 1). Drought stress decreased the Ψw gradually in both cultivars throughout the experiment, showing higher decrease in Mosa (−66.7% at 72 h after treatment) than in Saturnin (−56.9%) (Table 2). The Ψw decreased in drought-stressed leaves, accompanied by the decrease in chlorophyll content in both cultivars. Varietal differences in biomass of drought-stressed plants was significance only in leaves (Table 1), and was overall higher in Saturnin in both controls and drought-stressed plants. A significant decrease in leaf biomass, caused by drought-stress, was observed only in Mosa at 72 h after treatment (Table 2).

Amounts of Newly Absorbed S in Total S and in Sulfate Fraction

 Cultivar variation in NAS in total S (34S-total S) was significant in both leaves and roots (Table 1). Sum of 34S-total S in leaves and roots (34S-total S) at 72 h of treatment was 24.1 mg S plant−1 in Mosa and 28.7 mg S plant−1 in Saturnin for the non-stressed control plants (Table 3). PEG-induced drought stress decreased the amount of 34S-total S in leaves and showed a significant cultivar difference, with a higher decrease in Mosa (−80.3%) than in Saturnin (−49.8%). A significant decrease in the amount of 34S-total S in roots (−40.6%) was apparent only in Mosa (Table 3).

 The amount of NAS in sulfate fraction (34S-sulfate) in leaves showed cultivar difference in both controls and drought stressed plants (Table 3). PEG-induced drought stress decreased the
amount of $^{34}$S-sulfate in leaves by 28.9 and 17.5%, respectively, in Mosa and Saturnin at 72 h after treatment, when compared to controls. However, PEG-induced drought stress overall increased the amount of $^{34}$S-sulfate in roots, while cultivar variation was not significant (Tables 1 and 3).

**De novo Synthesis of Amino Acids and Proteins**

To investigate the assimilation of SO$_4^{2-}$ taken up during 72 h of PEG-induced drought stress, the amount of NAS incorporated into amino acids ($^{34}$S-amino acids) or proteins ($^{34}$S-proteins) were quantified. Cultivar variation and drought stress effects on the $^{34}$S-amino acids were significant ($P < 0.001$) (Table 1). Overall, the amount of $^{34}$S-amino acids was higher in Saturnin than in Mosa in both controls and drought-stressed plants for the entire course of the experiment (Figures 1A,B). PEG-induced drought stress decreased $^{34}$S-amino acids significantly in both leaves and roots with varietal difference, showing 47.8 and 39.1% decreases in the leaves and roots of Mosa versus 37.0 and 35.7% in Saturnin at 72 h after treatment, when compared to controls (Figures 1A,B).

Cultivar variation and drought stress effects for the $^{34}$S-proteins were also highly significant ($P < 0.001$) (Table 1). Overall, the amount of $^{34}$S-proteins was higher than those assimilated into other fractions, particularly in the leaves (Figures 1C,D). Varietal difference in $^{34}$S-protein was also observed in non-stressed controls (331.4 and 109.1 µg S plant$^{-1}$ in the leaves and roots of Mosa versus 526.0 and 153.9 µg S plant$^{-1}$, respectively, in Saturnin at 72 h after treatment). Drought stress decreased $^{34}$S-proteins significantly in both leaves and roots. The decreases in $^{34}$S-proteins caused by drought stress was higher in leaves than in roots with cultivar variation, showing 42.3 and 37.7% decrease in the leaves and roots of Mosa versus 26.8 and 21.2%, respectively, in Saturnin at 72 h after treatment when compared to controls (Figures 1C,D).

**Sulfur Use Efficiency Based on S Uptake and Its Assimilation Into Amino Acids and Proteins**

Cultivar variation in SUE based on S uptake (SUPE), calculated by dividing total NAS by the amount of S supplied for 72 h of treatment, was significant (Table 1). SUPE in control plants was 200.4 and 238.0 mg S uptake g$^{-1}$ S fed, respectively, in Mosa and Saturnin (Figure 2A). PEG-induced drought stress resulted in a reduction of SUPE with varietal difference, showing higher reduction in Mosa (−66.7%) than in Saturnin (−40.4%).

Sulfur use efficiency based on S assimilation (SUaE), calculated by dividing total amount of S assimilated into amino acids and proteins by the amount of S supplied for a given time, are presented in Figure 2B. Cultivar variation was significant ($P < 0.001$) for SUaE, with a range from 4.86 mg S assimilated g$^{-1}$ S taken up (Mosa) to 7.71 mg (Saturnin) in controls and from 2.80 mg S assimilated g$^{-1}$ S taken up (Mosa) to 5.51 mg (Saturnin) in PEG-induced drought stressed plants. The decrease in SUaE due to drought stress was largely higher in Mosa (−42.3%) than in Saturnin (−28.5%).
TABLE 2 | Changes in $\psi_w$, total chlorophyll and biomass of *B. napus* 'Mosa' and 'Saturnin' under control or PEG-induced drought stress condition for 72 h.

| Physiological parameters/Treatment       | Hours after treatment |
|-----------------------------------------|----------------------|
|                                         | 0        | 24     | 48     | 72     |

**Leaf water potential ($\psi_w$, MPa)**

| Treatment  | control       | PEG-stressed | control       | PEG-stressed |
|------------|---------------|--------------|---------------|--------------|
| Mosa       | $-0.41 \pm 0.04a$ | $-0.42 \pm 0.05a$ | $-0.42 \pm 0.05a$ | $-0.42 \pm 0.04a$ |
| Saturnin   | $-0.45 \pm 0.07a$ | $-0.44 \pm 0.06a$ | $-0.42 \pm 0.03a$ | $-0.44 \pm 0.06a$ |

**Total chlorophyll content (mg g$^{-1}$ FW)**

| Treatment  | control       | PEG-stressed | control       | PEG-stressed |
|------------|---------------|--------------|---------------|--------------|
| Mosa       | $2.85 \pm 0.07b$ | $3.15 \pm 0.28ab$ | $3.17 \pm 0.61a$ | $2.99 \pm 0.39a$ |
| Saturnin   | $3.28 \pm 0.17a$ | $3.28 \pm 0.12a$ | $3.47 \pm 0.04a$ | $3.38 \pm 0.31a$ |

**Biomass (g plant$^{-1}$)**

| Treatment  | Leaves | Roots |
|------------|--------|-------|
| Mosa       | control       | control       |
|            | $9.78 \pm 0.65b$ | $4.79 \pm 0.25b$ |
|            | PEG-stressed | PEG-stressed |
|            | $9.21 \pm 0.56b$ | $4.75 \pm 0.08a$ |
| Saturnin   | control       | control       |
|            | $11.87 \pm 0.33a$ | $5.63 \pm 0.36a$ |
|            | PEG-stressed | PEG-stressed |
|            | $11.68 \pm 0.96a$ | $5.65 \pm 0.48a$ |

Values are means ± SD of three replicates. Values in a vertical column followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

TABLE 3 | Changes in amount of NAS ($^{34}$S-total S) and non-assimilated sulfate ($^{34}$S-sulfate) in leaves and roots of *B. napus* 'Mosa' and 'Saturnin' under control or PEG-induced drought stress condition for 72 h.

| Physiological parameters/Treatment       | Hours after treatment |
|-----------------------------------------|----------------------|
|                                         | 0        | 24     | 48     | 72     |

**Newly absorbed S ($^{34}$S-total S, mg plant$^{-1}$)**

| Treatment  | Leaves | Roots |
|------------|--------|-------|
| Mosa       | control       | control       |
|            | -        | $4.74 \pm 0.65b$ | $2.94 \pm 0.23a$ |
|            | PEG-stressed | PEG-stressed |
|            | $0.98 \pm 0.14c$ | $1.88 \pm 0.15b$ |
| Saturnin   | control       | control       |
|            | $7.73 \pm 0.81a$ | $2.43 \pm 0.40ab$ |
|            | PEG-stressed | PEG-stressed |
|            | $3.69 \pm 0.44b$ | $1.92 \pm 0.15b$ |

**Non-assimilated sulfate ($^{34}$S-sulfate, $\mu$g plant$^{-1}$)**

| Treatment  | Leaves | Roots |
|------------|--------|-------|
| Mosa       | control       | control       |
|            | $63.8 \pm 2.5ab$ | $58.2 \pm 4.3a$ |
|            | PEG-stressed | PEG-stressed |
|            | $57.3 \pm 9.8b$ | $67.1 \pm 10.9a$ |
| Saturnin   | control       | control       |
|            | $83.2 \pm 3.5a$ | $54.6 \pm 6.3a$ |
|            | PEG-stressed | PEG-stressed |
|            | $76.4 \pm 14.8ab$ | $62.3 \pm 12.5a$ |

Values are means ± SD of three replicates. Values in a vertical column followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.
Rubisco Protein Content and Photosynthetic Activity

Rubisco protein patterns showed considerable varietal difference in PEG-induced drought stressed plants (Figure 3A). PEG-induced drought stress degraded the prominently large subunits of Rubisco in the low intensity band and slightly degraded the smaller subunits in Mosa (Figure 3A Lane 2–3), while much less change was observed in Saturnin (Figure 3A Lane 4–5). The decrease in Rubisco protein due to drought stress was estimated as 75.6% in Mosa and 51.1% in Saturnin at 72 h after treatment (Figure 3B).

Polyethylene glycol-induced drought stress decreased the net photosynthesis rate significantly with varietal difference, representing 74.6% and 58.5% decrease in Mosa and Saturnin, respectively at 72 h after treatment when compared to controls (Figure 4A). Varietal difference and drought stress effects on stomatal conductance and transpiration were similar to that of the net photosynthesis rate (Figures 4B,C).

DISCUSSION

The present data allow us to interpret firstly the physiological basis of genetic variation in SUE under PEG-induced drought stress. Secondly, the physiological significance of SUE in drought stress tolerance, with a particular focus on photosynthetic activity, will be discussed for two B. napus cultivars with different SUE. Following PEG application for 72 h, drought-stress occurred in both B. napus cultivars as shown by $\Psi_w$ values (Table 2). The final $\Psi_w$ in PEG-induced drought stressed plants reached a minimum value of $-1.26$ and $-1.02$ MPa, respectively, in Mosa and Saturnin. These values were slightly lower than that ($-0.87$ MPa) recorded over eight B. napus cultivars exposed to water-deficit stress for 7 days (Lee et al., 2015), indicating that PEG provokes drought stress more quickly than water deficiency treatment. These values were, however, higher than that ($-1.41$ MPa) recorded for four annual clover species exposed to water-stress (Iannucci et al., 2002). The $\Psi_w$ has often been used as the criterion for the degree of stress in many studies of stress physiology because the decrease in $\Psi_w$, caused by increase in hydraulic or osmotic stress, is responsible for decrease in photosynthetic CO$_2$ assimilation rates (Costa Franca et al., 2000), nitrogen assimilation (Kim et al., 2004; Lee et al., 2009), and cell extension processes (Singh et al., 2000).

As expected, PEG-induced drought stress resulted in a reduction of S uptake, estimated by newly absorbed $^{34}$S amount accumulated during treatment, with varietal difference (Tables 1 and 3). The rate of decrease in total amount of S uptake caused by drought stress was generally less in Saturnin ($-40.9\%$) than in Mosa ($-63.9\%$), indicating that Saturnin is able to more efficiently absorb S under drought-stressed conditions.

FIGURE 1 | Amount of S assimilated into amino acids and proteins in leaves (A,C) and roots (B,D) of cultivars Mosa (white bar) or Saturnin (dark gray bar) under control (non-dotted bar) or PEG-induced drought stress (dotted bar) conditions for 72 h. Data are presented as mean ± SE for $n = 3$. Means denoted by the different letter are significantly different at $P < 0.05$ according to the Duncan’s multiple range test.
Sulfur Use Efficiency Effects on Photosynthesis

FIGURE 2 | Sulfur use efficiency based on S uptake (SUe) (A) and S assimilation (SUE) (B) of cultivars Mosa (white bar) or Saturnin (dark gray bar) under control (non-dotted bar) or PEG-induced drought stress (dotted bar) conditions at 72 h. Data are presented as mean ± SE for n = 3. Means denoted by the different letter are significantly different at P < 0.05 according to the Duncan’s multiple range test.

Similarly, Saturnin was estimated to have the highest capacity of N acquisition of eight *B. napus* cultivars exposed to a water deficit stressed condition (Lee et al., 2015). The proportion of NAS distributed to leaves under drought-stressed conditions was also generally higher in Saturnin (76.7% of total S newly absorbed) than in Mosa (58.0%). These results suggest that PEG-induced drought stress restricts the translocation of newly absorbed SO$_4^{2-}$ to leaves, as a consequence of low SO$_4^{2-}$ absorption. Low absorption of NO$_3^-$ and decreased translocation into leaves were observed in white clover (Lee et al., 2009) and *B. napus* species with low N use efficiency (Lee et al., 2015) under water deficiency.

Polyethylene glycol-induced drought stress significantly decreased the amount of S assimilated into amino acids ($^{34}$S-amino acids) and proteins ($^{34}$S-proteins). The rate of decrease in $^{34}$S-proteins was much higher in leaves (−32.8% on average for the two cultivars) than in roots (−28.1%). Similarly, we recently reported that the inhibition of N assimilation into amino acids and proteins due to water-deficit stress occurred more severely in leaves than in roots (Lee et al., 2015). These results indicate that protein synthesis in leaves may be a major sink for S and N assimilation and is likely to be more inhibited by water stress (Kim et al., 2004) and nutrient deficiency (Lee et al., 2013, 2014). Decreases in sulfate uptake, translocation and further assimilation in drought stressed plants (Table 1; Figure 1) are likely to be driven by low sulfur demand for protein synthesis (De Kok et al., 2002; Anderson and Fitzgerald, 2003). Significant cultivar variation in the impacts of PEG-induced drought stress on de novo protein synthesis in leaves was found, with higher rates of decrease of $^{34}$S-proteins in Mosa (−42.3%) than in Saturnin (−26.8%). A similar finding was made for roots. These results indicate that the inhibition of protein incorporation derived from newly absorbed SO$_4^{2-}$, caused by drought stress, occurs much less often in cultivars with high S uptake (e.g., Saturnin). This suggests that newly absorbed SO$_4^{2-}$ is preferentially assimilated into proteins, rather than being stored in vacuoles, so that the capacity of S acquisition is an essential determinant for SUE under drought stress.

For assessing the genotypic variation in tolerance to nutrient deficiency, most studies have focused on nitrogen-use efficiency...
Sulfur Use Efficiency Effects on Photosynthesis

Figure 2B

Figure 4

Figure 5A,D

Figure 5B,C,E,F

FIGURE 4 | Changes in photosynthesis rate (A), stomatal conductance (B), and transpiration (C) in cultivars Mosa (triangles) or Saturnin (circles) under control (open symbols) or PEG-induced drought stress (closed symbols) conditions for 72 h. Data are presented as mean ± SE for n = 3. Means denoted by the different letter are significantly different at P < 0.05 according to the Duncan’s multiple range test.

(Ahmad et al., 2005, 2008; Berry et al., 2010), rather than on SUE. The physiological basis of varietal differences has been mostly interpreted by the relationships between growth and yield characteristics. In this study, cultivar variation in SUE, based on S uptake (SUpE) and S assimilation (SUaE), was assessed using $^{34}$S tracing. This data, to the best of our knowledge, is the first to directly quantify NAS and its assimilation into amino acids and proteins in response to PEG-induced drought stress. Cultivar variation was significant for SUpE, expressed as mg S taken up per g S, supplied under PEG-induced drought stress treatment (Figure 2A). The SUpE of Saturnin was 1.2 times higher in the control and 1.9 times higher in drought stressed plants than those of Mosa. Similarly, SUE based on S assimilation (SUaE), expressed as mg S assimilated per g S supplied, was also significantly higher in Saturnin in both controls and drought stressed plants (Figure 2B). In addition, the decreases in SUpE and SUaE caused by PEG-induced drought stress were smaller in Saturnin that those in Mosa. These results indicate that Saturnin is a genotype with a higher SUE compared to Mosa. In a recent study on nitrogen use efficiency based on N uptake (NUpE) and N assimilation (NUaE), Saturnin was also identified as one of the most N-use efficient genotypes of eight B. napus cultivars (Lee et al., 2015). This indicates that cultivar variation in SUE is likely to be similar to that of NUE. For B. napus, studies carried out under controlled greenhouse (Balint and Rengel, 2008; Dubousset et al., 2010) or field conditions (Schnug et al., 1993; Fismes et al., 2000) have shown that S nutrition affects the N-use efficiency and vice versa.

In this study, PEG-induced drought stress resulted in a gradual decrease in photosynthetic rate, along with a reduction in stomatal conductance and transpiration (Figure 4). These results are in accordance with previous results received in experiments with white clover cultivated under water deficiency (Lee et al., 2009). The reduction in the photosynthesis rate results from stomatal closure, which is associated with increased concentrations of ions and other solutes in the cells and the associated decrease in the osmotic potential (Cornic, 1994). Stomatal closure decreases available internal CO$_2$ and restricts water loss through transpiration (Chaves, 1991; Tezara et al., 1999). In this study, PEG-induced drought stress degraded Rubisco proteins in both the B. napus cultivars examined (Figure 3). The inhibition of photosynthetic activity and Rubisco degradation was less significant in Saturnin, which showed a higher efficiency in S uptake and S assimilation (Figures 3 and 4). This suggests that a good SUE has the potential to alleviate negative responses caused by drought stress on photosynthetic activity. Indeed, significant correlation of SUpE or SUaE with $\Psi_w$ affected by PEG-induced drought stress was found (Figures 5A,D). Positive relationships between SUpE or SUaE with net photosynthesis rate and Rubisco content, recorded at 72 h after treatment for the two B. napus cultivars examined in this study, were also significant (Figures 5B,C,E,F). Reduced photosynthesis under S-deficient conditions has been widely observed in various plant species (Astolfi et al., 2012; Fatma et al., 2014; Muneeer et al., 2014). S-deficiency has an early effect on CO$_2$ assimilation and on Rubisco activity and protein abundance, thereby inducing chlorosis (Gilbert et al., 1997). The lack of Rubisco synthesis and chlorosis reflects a general inhibition of de novo synthesis of photosynthetic apparatus (Hawkesford, 2000). However, in B. juncea and B. campestris, high S-fertilization increases chlorophyll, Rubisco, and protein contents, and leads to better photosynthetic capacity, compared to the plants grown without S (Ahmad and Abdin, 2000; Fatma et al., 2014). It has also been reported that surplus or sufficient S-supply favors the formation of Fe-S clusters in the photosynthetic apparatus and electron transport system, alleviating the photosynthetic activity inhibited by salt stress (Resurreccion et al., 2002; Nazar et al., 2011) or Fe deficiency (Astolfi et al., 2012; Muneeer et al., 2014). Application of exogenous ally isothiocyanate, which is one of the products of hydrolysis of glucosinolates, induced stomatal closure leading to the elevation of guard cell cytosolic Ca$^{2+}$ to avoid water loss (Khokon et al., 2011). Recently it was...
shown that an excess S supply improved photosynthesis under salt stress condition (Fatma et al., 2014). These positive effects of surplus S-supplementation may be explained by increased S-availability for the formation of S-containing compounds such as amino acids (cysteine and methionine for assembly of new proteins), antioxidant peptides (GSH for protection against oxidative damage), and sulfhydryl (S-H) and disulfide (S-S) bonds that are important for the stabilization of protein structure (Saito, 2000), and S-Fe clusters that function in vital processes such as respiration, photosynthesis, and sulfur and nitrogen metabolism (Balk and Pilon, 2011). These results suggest that SUpE is a determinant for total S-availability, which has significant roles in alleviating negative responses of photosynthetic activity to drought stress, and is a preliminary factor for SUaE. Indeed, the data presented here shows that the inhibition of photosynthetic activity (including Rubisco degradation) is much less apparent in the cultivar with higher SUpE and SUaE (Saturnin), and that this cultivar can be identified as a genotype more tolerant to PEG-induced drought stress, compared to the inefficient SUE cultivar (Mosa).

CONCLUSION

This study suggests that SUE based on S uptake (SUpE) and S assimilation (SUaE) displays significant role in alleviating negative responses to drought stress on photosynthetic activity. Thus, an improved SUE is certainly a desired feature for the management of crops against drought stress and for breeding programs with the target to improve the stress tolerance of plants.

AUTHOR CONTRIBUTIONS

B-RL and T-HK designed the experiment and wrote the manuscript. B-RL carried out the experiment. T-HK, J-CA, and AO participated in data interpretation and critical reading of the manuscript. RZ has been involved in revising the article for important intellectual content.

FUNDING

This work was supported by the National Research Foundation of Korea (NRF) grant (NRF-2013R1A2A2A01014202).

ACKNOWLEDGMENT

We gratefully thank Marie-Paule Bataillé for conducting isotopic analysis.
REFERENCES

Abdallah, M., Dubouset, L., Meriot, F., Etienne, P., Avic, J. C., and Oury, A. (2010). Effect of mineral sulphur availability on nitrogen and sulphur uptake and remobilization during the vegetative growth of Brassica napus L. J. Exp. Bot. 61, 2635–2646. doi: 10.1093/jxb/erq096

Aghajanzadeh, T., Hawkesford, M. J., and De Kok, L. J. (2014). The significance of glucosinolates for sulfur storage in Brassicaceae seedlings. Front. Plant Sci. 5:704. doi: 10.3389/fpls.2014.00704

Ahmad, A., and Abdin, M. Z. (2000). Effect of sulphur application on lipid, RNA and fatty acid content in developing seeds of rapeseed (Brassica lipoida L.). Plant Sci. 150, 71–76. doi: 10.1016/S0168-9452(99)00167-3

Ahmad, A., Hussain, A., Ibqal, S., and Husnain, Z. (2005). "Sowing, growth, radiation use efficiency and yield of rice (Super basmati) as affected by sowing date and split nitrogen application," in Proceedings of the International Seminar on Rice Crop, (Lahore Paks: RRI), 315–321.

Ahmad, A., Khan, I., Abrol, Y. P., and Ibqal, M. (2008). Genotypic variation of sulfur-use efficiency in Indian mustard. Environ. Pollut. 154, 462–466. doi: 10.1016/j.envpol.2007.10.007

Anderson, J. W., and Fitzgerald, M. A. (2003). "Sulfur distribution and redistribution," in Sulphur in Plants, eds Y. P. Abrol and A. Ahmad (Dordrecht: Kluwer Academic), 113–134.

Astolfi, S., and Zuchi, S. (2013). Adequate S supplies protects barley plants from adverse effects of salinity stress by increasing thiold contents. Acta Physiol. Plant. 35, 175–181. doi: 10.1007/s11738-012-1060-5

Astolfi, S., Zuchi, S., Neumann, G., Cesco, S., di Toppi, L., and Pinton, R. (2012). Response of barley plants to Fe deficiency and Cd contamination as affected by S starvation. J. Exp. Bot. 63, 1241–1250. doi: 10.1093/jxb/err344

Balint, T., and Rengel, Z. (2008). Nitrogen efficiency of canola genotypes varies between vegetative stage and grain maturity. Euphytica 164, 421–432. doi: 10.1007/s10681-008-9693-6

Balk, J., and Pilon, M. (2011). Ancient and essential, the assembly of iron–sulfur clusters in plants. Trends Plant. Sci. 16, 218–226. doi: 10.1016/j.tplants.2010.12.006

Berry, P. M., Spink, J., Foulkes, M. J., and White, P. J. (2010). The physiological basis of genotypic differences in nitrogen use efficiency in oilseed rape (Brassica napus L.). Field Crops Res. 119, 365–373. doi: 10.1016/j.fcr.2010.08.004

Blake-Kalff, M., Zhao, J., Hawkesford, M., and McGrath, S. (2001). Using plant inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. Physiol. Plants 125, 474–489. doi: 10.1046/j.1399-3054.2001.00578.x

Lee, B. R., Jin, Y. L., Avic, J. C., Clignet, J. B., Oury, A., and Kim, T. H. (2009). Increased proline loading to phloem and its effect on nitrogen uptake and assimilation in water stressed white clover (Trifolium repens). New Phytol. 182, 654–663. doi: 10.1111/j.1469-8137.2009.02795.x

Lee, B. R., Jin, Y. L., Park, S. H., Zaman, R., Zhang, Q., Avic, J. C., et al. (2015). Genotypic variation in N uptake and assimilation estimated by 15N tracing water deficit-stressed Brassica napus. Environ. Exp. Bot. 109, 73–79. doi: 10.1016/j.envexpbot.2014.08.004

Lee, B. R., Muneer, S., Jung, W. J., Avic, J. C., Oury, A., and Kim, T. H. (2014). Partitioning of newly absorbed and previously stored nitrogen and sulfur under sulphate deficient nutrition. J. Plant Nutr. 37, 1702–1716. doi: 10.1080/01904167.2014.889148

Lee, B. R., Muneer, S., Kim, K. Y., Avic, J. C., Oury, A., and Kim, T. H. (2013). S-deficiency responsive accumulation of amino acids is mainly due to hydrolysis of the previously synthesized proteins – not to de novo synthesis in Brassica napus. Physiol. Plants. 147, 369–380. doi: 10.1111/j.1399-3045.2012.01669.x

Makino, A., Mae, T., and Ohira, K. (1985). Enzymic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase purified from rice leaves. Plant Physiol. 79, 57–61. doi: 10.1104/pp.79.1.57

Muneer, S., Lee, B. R., Park, S. H., Zhang, Q., and Kim, T. H. (2014). Involvement of sulphur nutrition in modulating iron deficiency responses in photosynthetic organelles of oilseed rape (Brassica napus L.). Photosynth. Res. 119, 319–329. doi: 10.1007/s11120-013-9953-8

Nazar, R., Ibqal, N., Masood, A., Syed, S., and Khan, N. A. (2011). Understanding the significance of sulphur in improving salinity tolerance in plants. Environ. Exp. Bot. 70, 80–87. doi: 10.1016/j.envexpbot.2010.09.011

Prosser, I. M., Schneider, A., Hawkesford, M. J., and Clakson, D. T. (1997). "Changes in nutrients compensation, metabolite concentrations and enzyme activities in spinach in the early stages of S-deprivation," in Sulphur Metabolism in Higher Plants, eds W. J. Cram, L. J. De Kok, I. Stulen, C. Brunold, and H. Rennenberg (Leiden: Backhuys Publishers), 339–342.

Rausch, T., Gromes, R., Liedschille, V., Muller, I., Bogs, J., Galovic, V., et al. (2007). Novel insight into the regulation of GSH biosynthesis in higher plants. Plant Biol. 9, 565–572. doi: 10.1055/s-2007-965580

Resurreccion, A. P., Makino, A., Bennett, J., and Mae, T. (2002). Effect of light intensity on the growth and photosynthesis of rice under different sulfur concentrations. Soil Sci. Plant Nutr. 48, 71–77. doi: 10.1080/0380768.2002.1049173
Saeidnia, S., and Gohari, A. R. (2012). Importance of Brassica napus as a medicinal food plant. J. Med. Plant Res. 6, 2700–2703.

Saito, K. (2000). Regulation of sulfate transport and synthesis of sulfur-containing amino acids. Curr. Opin. Plant Biol. 3, 188–195. doi: 10.1016/S1369-5266(00)00063-7

Schnug, E., Haneklaus, S., and Murphy, D. P. L. (1993). Impact of sulphur fertilization on fertilizer nitrogen efficiency. Sulphur. Agric. 17, 8–12. doi: 10.1021/jf071710s

Singh, S., Sharma, H., Goswami, A., Datta, S., and Singh, S. (2000). In vitro growth and leaf composition of grapevine cultivars as affected by sodium chloride. Biol. Plantarum 43, 283–286. doi: 10.1023/A:1002720714781

Szalai, G., Kellos, T., Galiba, G., and Kocsy, G. (2009). Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. J. Plant Growth Regul. 28, 66–80. doi: 10.1007/s11033-012-2402-5

Tezara, W., Mitchell, V. J., Driscoll, S. D., and Lawlor, D. W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401, 914–917. doi: 10.1038/44842

Warrilow, A. G. S., and Hawkesford, M. J. (1998). Separation, subcellular location and influence of sulphur nutrition on isoforms of cysteine synthase in spinach. J. Exp. Bot. 49, 1625–1636. doi: 10.1093/jxb/49.327.1625

Zhao, F. J., Bilsborrow, P. E., Evans, E. J., and McGrath, S. P. (1997). Nitrogen to sulfur ratio in rapeseed and in rapeseed protein and its use in diagnosing sulfur deficiency. J. Plant Nutr. 20, 549–558. doi: 10.1080/01904169709365273

Zhao, F. J., Hawkesford, M. J., and McGrath, S. P. (1999). Sulphur assimilation and effects on yield and quality of wheat. J. Cereal Sci. 1, 1–17. doi: 10.1006/jcrs.1998.0241

Zuchi, S., Cesco, S., Varanini, Z., Pinton, R., and Astolfi, S. (2009). Sulphur deprivation limits Fe-deficiency responses in tomato plants. Planta 230, 85–94. doi: 10.1007/s00425-009-0919-1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Lee, Zaman, Avice, Ourry and Kim. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.