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Comparative sulfur use efficiency and water stress tolerance in two *Brassica napus* cultivars

Bok-Rye Lee, Tae-Hwan Kim*, Sang-Hyun Park, Qian Zhang and Rashed Zaman
Chonnam National University, Gwangju, Republic of Korea
*Corresponding author e-mail: grassl@jnu.ac.kr

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Introduction

S-deficiency and/or S-deprivation decreases the cell sap osmotic potential resulting from a net increase of intracellular solutes rather than from a loss of cell water and chlorophyll content resulting in a restriction of Rubisco synthesis and provoked the chlorosis of young leaves (Lee *et al.*, 2014; Muneer *et al.*, 2014). These imply that S-deficiency results in a general inhibition of photosynthesis and protein synthesis. On the other hand, several studies have indicated that S nutrition has a potential role in stress tolerance and defense mechanism. Sulfur is an essential element in the formation of sulfhydryl (S-H) and disulphide bond (S-S). These bonds are important for the stabilization of protein structures. In this context, the roles of S nutrition in alleviating negative responses to salinity stress (Fatma *et al.*, 2014) and iron deficiency (Muneer *et al.*, 2014) have been widely reported. In this study, we hypothesized that cultivar variation in sulfur use efficiency (SUE) under Polyethylene glycol (PEG)-induced water stress may be attributed to S-uptake efficiency (SUpE; S uptake per S supplied), and that the genotype having higher SUE is more tolerant to PEG-induced water stress. To test this hypothesis, direct quantifications of S uptake was done by a ^{34}S tracing method. The responses of photosynthetic activity-related parameters to PEG-induced water stress were also assessed in relation to SUE in two *B. napus* cultivars.

Materials and Methods

PEG-induced water stress and isotope labeling: Eight-week-old plants were divided in two groups for the application of Polyethylene glycol (PEG-6000). One group of experimental plants was supplied normal nutrient solution for control, whereas the other group was supplied 8% PEG-6000 with normal nutrient solution for 72 h. For the ^{34}S feeding, S sources in the above mentioned hydroponic solution were replaced by ^{34}S labeling solution containing 1.5 mM $K_2{}^{34}SO_4$ with 1.0% ^{34}S atom excess throughout the experiment period. Plants samples were separated into leaves and roots.

Measurements: Leaf water potential was immediately determined as the petiole xylem-pressure potential using a pressure chamber. Photosynthesis, transpiration and stomatal conductance were measured using a portable photosynthesis measurement system (LI-6400). Freeze-dried powder samples (1-5 mg) were weighed (± 10 μg) into tin capsules for the determination of total S. The S content and ^{34}S atom % of all fractions were determined by a continuous flow isotope mass spectrometer (IsoPrime, GV Instrument, Manchester, UK). Sulfur use efficiency (SUE) was calculated by dividing total amount of newly absorbed S by S amount supplied during treatment as expressed by mg S taken up g^{-1} S fed. Rubisco content was measured by method of Makino *et al.* (1985).

Results and Discussion

PEG-induced water stress decreased the leaf water potential (LWP) gradually in both cultivars for 72 h, showing higher decrease in *cv*. Mosa (-66.7%) than *cv*. Saturnin (-56.9%) (Fig. 1A). The LWP has been often used for the criterion of degree of stress exposed in many stress physiological works because the decrease in LWP with development of hydraulic or osmotic stress. A significant decrease in biomass, caused by water-stress, was observed only in only in *cv*. Mosa (Fig. 1B). S uptake [sum of newly absorbed S (NAS) in leaves and roots)] for 72 h of treatment was 24.1 mg S $plant^{-1}$ in cv. Mosa and 28.7 mg S $plant^{-1}$ in *cv*. Saturnin for the non-stressed control plants (Fig. 1C). Reduction of NAS caused by water stress was largely less in *cv*. Saturnin (-40.9%) than in *cv*. Mosa (-63.9%), indicating that *cv*. Saturnin is more efficiently absorbed S under water-stressed condition (Fig. 1C). Similarly, cv. Saturnin was estimated to have the highest capacity of N acquisition over eight *B. napus* cultivar water deficit stressed condition (Lee *et al.* 2015). For assessing the genotypic variation in tolerance to nutrient deficiency, the studies mainly focused on nitrogen-use efficiency, but very less on SUE. In this study, sulfur use efficiency (SUE) based on S uptake was calculated by dividing total newly absorbed S by the amount of S supplied for 72 h of treatment. PEG-induced water stress resulted in a reduction of SUE with varietal difference, showing higher reduction in *cv*. Mosa (- 66.7%) than in *cv*. Saturnin (-40.4%) (Fig. 1D). These results indicate that *cv*. Saturnin is higher S-efficient genotype compared to *cv*. Mosa.

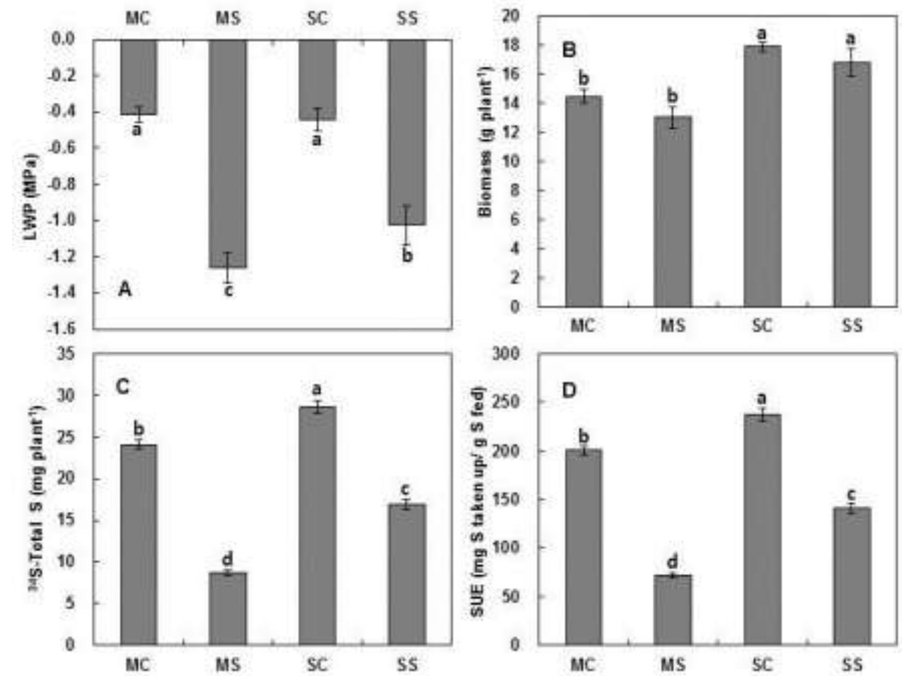

Fig 1: Leaf water potential (LWP, A), biomass (B), S-uptake (C) and sulfur use efficiency (SUE, D) in cv. Mosa or cv. Saturnin under control or PEG-induced water stress conditions for 72 h. MC, Mosa control; MS, Mosa stress; SC, Saturnin control; SS, Saturnin stress. Data are presented as mean ± SE for n = 3. Means denoted by the different letter are significantly different at $P < 0.05$ according to the Duncan's multiple range test.

PEG-induced water stress decreased net photosynthesis rate significantly with varietal difference, representing 74.6% and 58.5% decrease in *cv*. Mosa and cv. Saturnin, respectively at 72 h after treatment when compared to controls (Fig. 2A). Varietal difference and water stress effects on stomatal conductance and transpiration were similar with that of net photosynthesis rate (Fig. 2B, C). The reduction in photosynthesis rate results from stomatal closure, which is associated with the increased concentrations of ions and other solutes in the cells are increased and thus decreasing the osmotic potential. Stomatal closure decreases available internal CO_2 and restricts water loss through transpiration. In this study, PEG-induced water stress degraded Rubisco protein in cv. Mosa (-75.6%) and *cv*. Saturnin (-51.1%) (Fig. 2D). The inhibition of phtosynthetic activity and Rubisco degradation was much less in *cv*. Saturnin, which is higher S-efficient for uptake and assimilation (Fig. 2). It thus suggests that SUE has significant roles in alleviating negative responses of photosynthetic activity caused by water stress. It has been reported that surplus or sufficient S-supply favours the formation of Fe-S clusters in the photosynthetic apparatus and electron transport system, thereby alleviating the photosynthetic activity inhibited by salt stress (Fatma *et a*l., 2014) and Fe deficiency (Muneer *et al*., 2014).

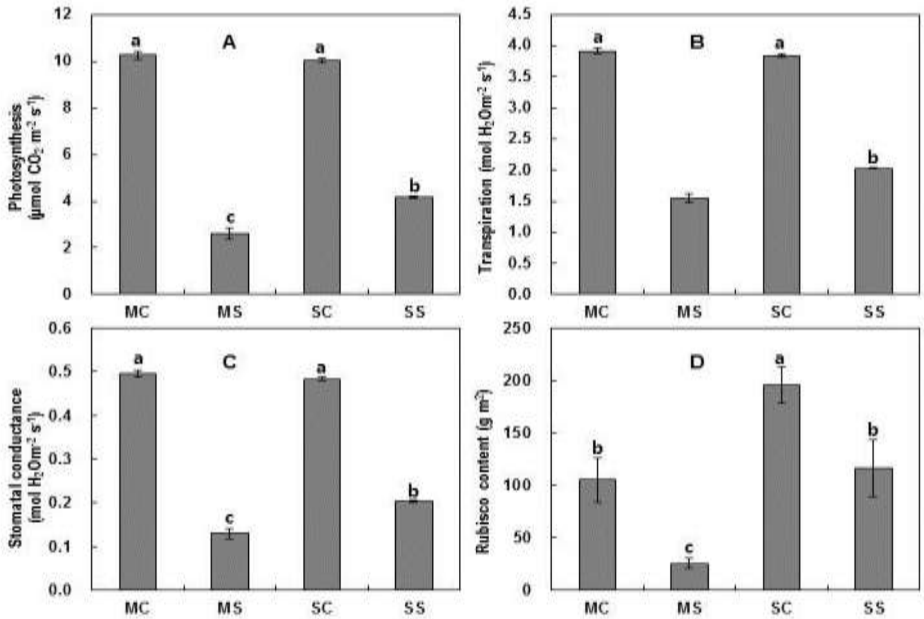

Fig 2: Photosynthesis rate (A), stomatal conductance (B), transpiration (C) and Rubisco content (D) in cv. Mosa or cv. Saturnin under control or PEG-induced water stress conditions for 72 h. MC, Mosa control; MS, Mosa stress; SC, Saturnin control; SS, Saturnin stress. Data are presented as mean ± SE for n = 3. Means denoted by the different letter are significantly different at $P < 0.05$ according to the Duncan's multiple range test.

Conclusion

This study suggests that SUE based on S uptake has significant roles in alleviating negative responses of photosynthetic activity to water stress. Thus, SUE is certainly a desired feature for the management of crops against water stress and/or in breeding program aimed at improving stress tolerance.

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