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SO_4^{2-} Uptake and Assimilation in Forage Rape (*Brassica napus*)

Ming Li, Yu Lan Jin*,**, Bok Rye Lee*, Lu Shen Li, Dae Hyun Kim and Tae Hwan Kim

유채의 SO_4^{2-} 흡수 및 동화에 관한 연구

이 명 · 김옥란*,** · 이복례 · 이노신 · 김대현 · 김태환

요 약

유채의 황 이용성에 대한 영향을 조사하기 위하여 유채 2 품종 (cv. Akela, Colosse)을 2.0 mM SO_4^{2-}와 0.2 mM SO_4^{2-}에서 SO_4^{2-} 흡수, ATP sulfurylase의 활성과 엽조직내의 glutathione (GSH) 함량을 측정하였다. 0.2 mM SO_4^{2-}에서 두 품종 모두 2.0 mM SO_4^{2-}에 비해 현저하게 낮은 SO_4^{2-} 흡수율을 나타냈다. 0.2 mM SO_4^{2-}에서 APT sulfurylase의 활성은 어린잎에서 두 품종 모두 증가하였으나 성숙된 잎에서의 활성은 큰 변화가 없었다. 0.2 mM SO_4^{2-}에서 glutathione의 함량은 어린잎에서 두 품종 모두 증가하였으나 성숙된 잎에서는 Akela에서만 현저하게 감소하였다. 이러한 결과들은 유채품종간의 SO_4^{2-} 흡수와 ATP sulfurylase의 특이성 뿐만 아니라, SO_4^{2-} 흡수가 glutathione과 같은 황을 포함하고 있는 화합물로의 동화와 밀접한 관련이 있음을 나타낸다.

(**Key words** : ATP sulfurylase, Glutathione, Rape, Sulfate uptake)

I. INTRODUCTION

Sulfur has been recognized as important nutrient for plants growth in agricultural productivity. Sulfur is considered as the fourth important major nutrient after nitrogen, potassium, and phosphorus, as it is essential for the formation of amino acid, proteins and fatty acids (Bloem et al., 2004; Zhao et al., 1997). Inorganic sulfate was major source of sulfur for the synthesis of sulfur containing amino acids, lipids, and protein. ATP sulfurylase is the first enzyme in the S-reduction pathway. The first step in the pathway of sulfate assimilation, involves activation of sulfate by ATP to form APS, catalyzed by ATP sulfurylase (Anderson, 1980). Most SO_4^{2-} reduction occurs in mesophyll cells in leaves, where the major part of activity is associated with chloroplasts and cytosol (Lunn et al., 1990).

It is well documented that sulfur deficiency decreased crop yields and quality, and even mild sulfur deficiency is known to influence overall plant quality (Hawkesford, 2000). Under condition of sulfur deficiency, firstly a decrease of S-

전남대학교 (Chonnam National University, Gwangju 500-757, Korea)
* BK21 Research Team for the Control of Animal Hazards using Biotechnology, College of Agriculture & Life Science, Chonnam National University, Gwangju 500-757, Korea
** Department of pharmaceutical analysis, College of Plant Protection, Qingdao Agricultural University, Qingdao, 266109, China
Corresponding author : Tae-Hwan Kim, Department of Animal Science & Institute of Agriculture Science and Technology, College of Agriculture & Life Science, Chonnam National University, Gwangju 500-757, Korea
Tel: +82-62-530-2126, Fax: +82-62-530-2129, E-mail: grassl@chonnam.ac.kr

containing amino acids in proteins is found (Bloem et al, 2004). Glutathione, one of major S-containing compounds has an important role in acting as a mobile pool of reduced sulfur in the regulation of plant growth and development. It is also an essential component of the cellular antioxidative defense system, which keeps reactive oxygen species under control (Noctor and Foyer, 1998). The higher glutathione concentrations in foliar tissues of plants were observed in various plant species exposed to environmental stress such as natural abiotic, biotic stresses (pathogens), or pollutant impacts (Noctor and Foyer, 1998; Payton et al., 2001; Herbinger et al., 2002).

The aims of this study were to estimated SO_4^{2-} uptake and its assimilation and to investigate S-deficiency effects on S utilization in two different genotypes of rape.

Ⅱ. MATERIALS AND METHODS

1. Plant culture

Seeds of rape (*Brassica napus* L.) species (cv. Akela and Colosse) were germinated and grown in a controlled environment on a nutrient solution (Kim et al., 2003) in a 2 L polyvinyl chloride pots. The nutrient solution was renewed every 6 days. The treatment of sulfate deficiency was exposed to 8 week old plants. Control plants were fed with the complete nutrient solution containing 2 mM SO_4^{2-}. The composition of nutrient solution is presented at Table 1. For S-deficient treatment SO_4^{2-} concentration in the nutrient solution was decreased to 0.2 mM by depriving K_2SO_4 and $MgSO_4$ from the control solution. Plants were harvested at 3 d after treatment. Shoot material was divided into young and old leaves. Samples were immediately frozen in liquid nitrogen for further analysis.

2. Determination of sulfate uptake

Sulfate uptake was determined by substracting the current concentration at sampling time from the initial concentration and expressed as μmol SO_4^{2-} g^{-1} FW taken up from nutrient solution. During the time of treatment, the SO_4^{2-} concentration in sample solution was determined by ion chromatography (Dionex, DX-120, USA) with an IonPac AS14A column and AG4A-SC guard column. The eluent containing 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ was used at a flow rate of 2.3 ml min^{-1}.

3. Analysis of ATP sulfurylase

About 0.2 g of fresh leaves were rapidly

Table 1. Composition of nutrient solution

Macro element	Concentration (mM)	Micro element	Concentration (μM)
$CaCl_2 \cdot 2H_2O$	1.5	H_3BO_3	14
NH_4NO_3	1.0	$MnSO_4 \cdot 4H_2O$	5.0
K_2SO_4	1.5	$ZnSO_4 \cdot 7H_2O$	3.0
$MgSO_4$	0.5	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.7
KH_2PO_4	0.5	$CuSO_4 \cdot 5H_2O$	0.7
Fe·Na · EDTA	0.2	$CoCl_2 \cdot 6H_2O$	0.1
K_2HPO_4	0.5		

ground at 4℃ in a buffer consisting of 10 mM Na_2EDTA, 20 mM Tris-HCl (pH 8.0), 2 mM DTT and 1% PVP, using a 1:4 (w/v) tissue-to-buffer ratio. The homogenate was centrifuged at 13,000 rpm for 10 min at 4℃. The supernatant was used for ATP sulfurylase assays. ATP sulfurylase activity was measured using molybdate-dependent formation of pyrophosphate, as described by Lappartient and Touraine (1996).

4. Determination of total glutathione (GSH)

Approximately 0.2 g sample were extracted with 1.5 ml of 5% 5-sulfosalicylic acid. After centrifugation at 12,000 rpm for 10 min, 100 µl of supernatant was mixed with 700 µl daily buffer containing 143 mM sodium phosphate, 6.3 mM sodium EDTA (pH 7.5) and 0.3 mM NADPH, 100 µl of 6 mM 5,5'-dithiobis-2-nitro-benzoic acid and 100 µl water. Then, 5 µl of GSSG reductase (50 U/ml) was added with mixing to initiate the assay. The amount of GSH is determined from a standard curve in which the GSH equivalents present is plotted against the rate of change of absorbance at 412 nm (the absorbance value only can be read above 0.5).

III. RESULTS AND DISCUSSIN

1. SO_4^{2-} uptake

SO_4^{2-} uptake in two rape cultivars under complete S supply (2.0 mM SO_4^{2-}) or S-deficient (0.2 mM SO_4^{2-}) condition was presented at Fig 1. During the first 24 h, under complete solution (2.0 mM SO_4^{2-}) condition, Akela has 2.5-fold higher SO_4^{2-} uptake than Colosse. After 72 h of treatment, SO_4^{2-} uptake of complete solution was 2.4 and 2.0-fold higher in Akela and Colosse, respectively, compared to S-deficient condition. These results suggest that the cultivars having high SO_4^{2-} uptake under complete nutrient solution also showed high SO_4^{2-} uptake under S-deficient condition. Schonhof et al., (2007) suggest that SO_4^{2-} uptake is depressed under S limiting condition in broccoli plants. Depression of SO_4^{2-} uptake was observed in maize hybrid (Quggiotti et al., 2003), sugar beet (Thomas et al., 2000), and spinach (Prosser et al., 2001). However, a increase of SO_4^{2-} uptake capacity in response to decreased SO_4^{2-} availability has been observed in tobacco and maize single cells (Hatzfeld et al., 1998) as well as in whole plants (Clarkson et al., 1993). Such contradictory results may be attributed to different plant tissues, leaf age, internal S level and metabolic interaction with other nutrient especially C and N. These changes in SO_4^{2-} uptake capacity is correlated with a modification in the relative abundance of mRNA encoding putative root high affinity sulfate transporters (Vidmar et al., 1999; Takahashi et al., 2000).

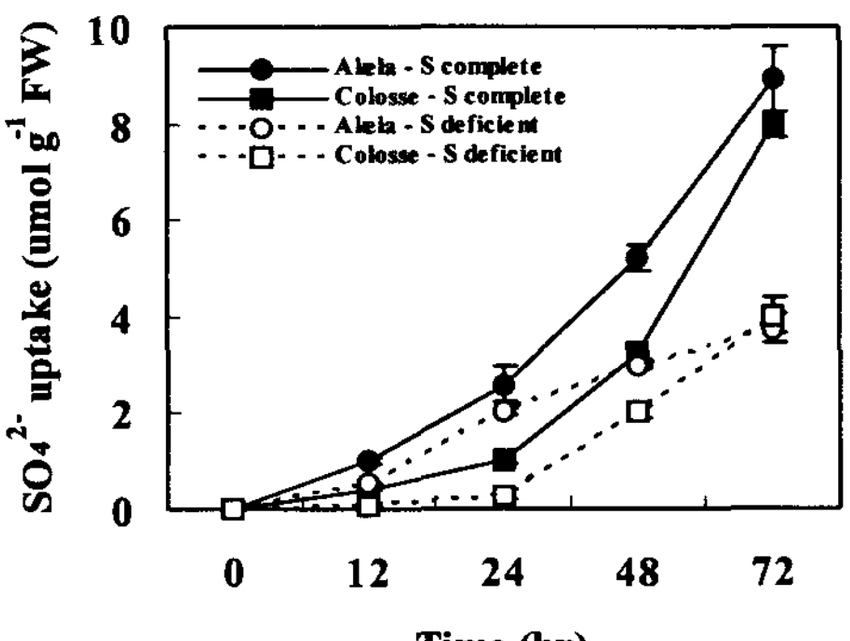

Fig. 1. Changes of SO_4^{2-} uptake measured at complete S supply (2.0 mM) and S- deficient condition (0.2 mM) in rapes. The values are means ± SD of three replicates.

2. ATP Sulfurylase

To examine S-deficiency effects on the assimilation of SO_4^{2-}, ATP sulfurylase activity was measured in young and old leaves grown at complete S supply and S-deficient condition (Fig. 2). ATP sulfurylase activity increased after S-deficient treatment in young leaves. The rate of increase were 14.2 and 60.7% in Akela and Colesse, respectively. In old leaves, no significant changes were observed in Akela by decreasing SO_4^{2-} supply. However, ATP sulfurylase activity decreased 20.1% in the old leave of Colesse, which have a low SO_4^{2-} uptake under complete S supply condition. It has been widely reported that the activity of ATP sulfurylase extracted from roots of intact canola (*Brassica napus* L. cv Drakkar) increased after withdraw of the S source from the nutrient solution (Lappartient & Touraine, 1996). The increase in activity of ATP sulfurylase by S starvation were also observed in tobacco and intack canola (Hatzfeld et al., 1998; Lappartient and Touraine, 1996). In soybean, cold treatment induced mRNA accumulation and enhanced the specific activity of ATP sulfurylase activity (Phartiyal et al., 2006). Since ATP sulfurylase is the first enzyme in the sulfur assimilation pathway of plants, it is reasonable to expect that S deficiency or starvation would enhance the expression of this enzyme.

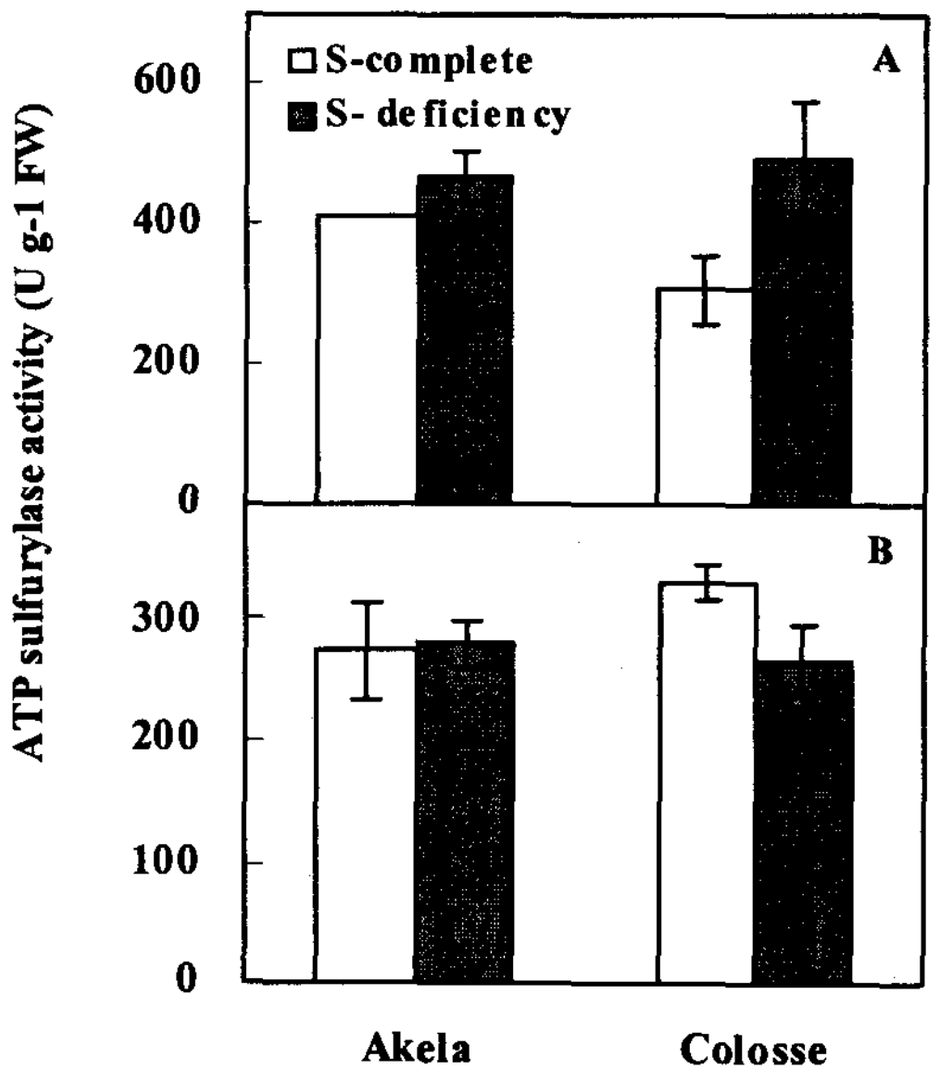

Fig. 2. Changes in ATP sulfurylase measured at complete S and S-deficient condition in young leave (A) and old leave (B). The values are means ± SD of three replicates.

3. Glutathione concentration

The changes in GSH concentration, which are major metabolite of sulfur, measured at young and old leaves grown complete S supply level and S-deficiency treatment, are presented at Fig. 3. S deficiency treatment significantly increased GSH concentration in all cultivar examined in young leaves. The rate of increase caused by S-deficiency was higher in Akela (+166.9%), which have a high SO_4^{2-} uptake under complete S supply condition. In old leaves, GSH concentration was less affected by S-deficiency treatment in Colosse. However, a remarkable decrease was observed in Akela (−74.1%). These suggest that GSH synthesis in leaves is species specific in relation to the internal demand for S leading to corresponding changes in the SO_4^{2-} uptake significant higher than in old leaves after S-deficient treatment. In addition, the results clearly indicate that S deficiency increased glutathione synthesis in the active site of S assimilation, as like young leaves. Hartmann (2000) observed that GSH concentration is higher in young leaves than old ones. Similarly, total and reduced GSH were

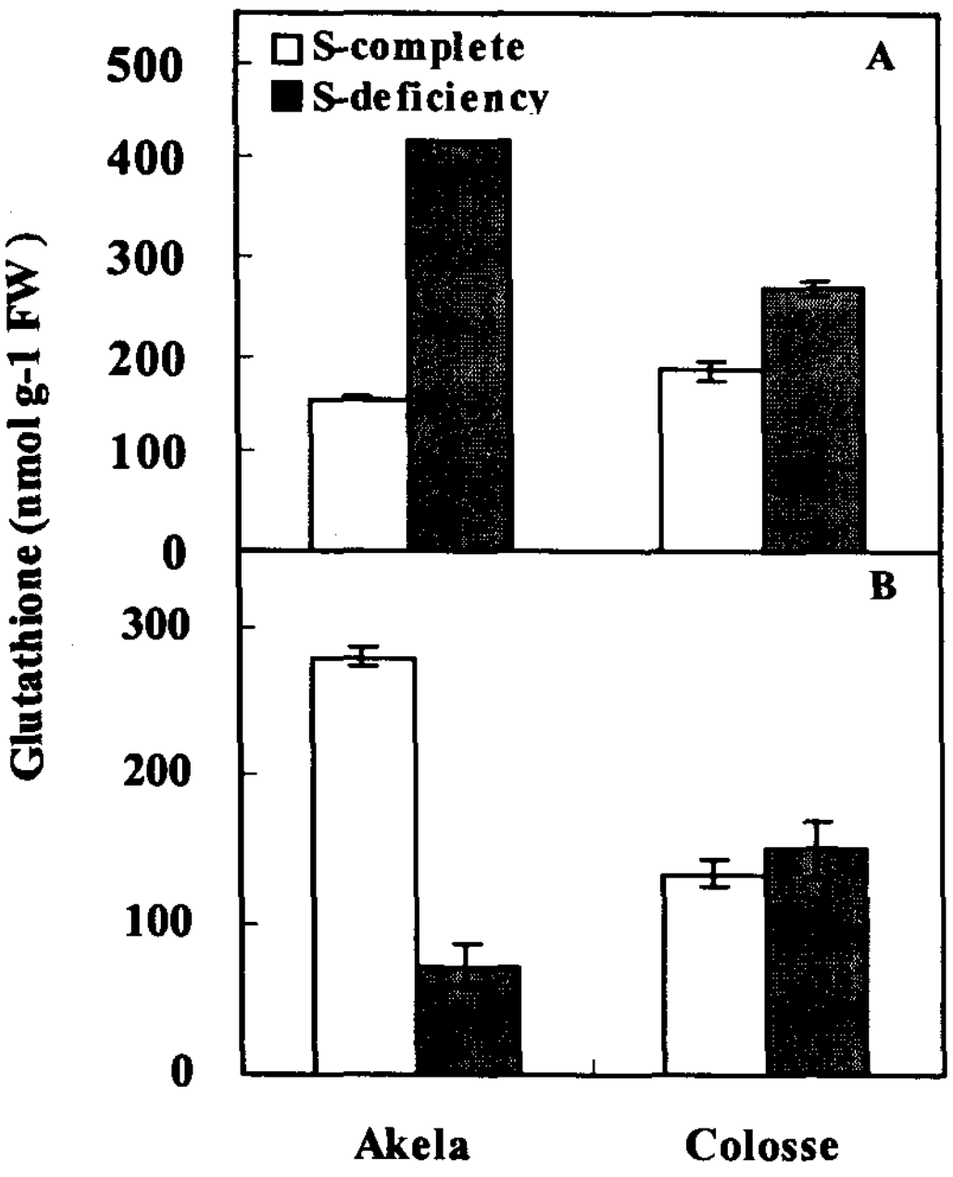

Fig. 3. Changes of glutathione concentration in young and old leaves after 3 days of S-deficiency treatment. The values are means ± SD of three replicates.

decreased, the ratio of GSH/glutathione disulfide (GSSG) was markedly increased under drought-stressed spring wheat (Chen et al., 2004). This author suggested that the higher ratio of GSH/ GSSG, the rate of GSH synthesis might be essential for stress resistance of plants. The different responses of leaves of different ages to S deficiency have to be taken into account for the development of field diagnostic tests to determine whether plants are S deficient.

Ⅳ. ABSTRACT

To investigate the sulfate utilization efficiency that has been examined in rape (*Brassica napus* L.) cultivars (cv. Akela and Colosse). During 72 h of treatment, in two cultivars, SO_4^{2-} uptake was significantly higher in complete S condition (2.0 mM SO_4^{2-}) than that of the S-deficient condition (0.2 mM SO_4^{2-}). In young leaves, ATP sulfurylase activity increased after S-deficient treatment. However, in old leaves, ATP sulfurylase activity was not significantly changed in Akela. Glutathione concentration in young leaves significantly increased in all cultivars examined under S-deficient condition. The rate of increase in glutathione concentration caused by S-deficiency treatment was higher in Akela. However, in old leaves, the glutathione concentration in Akela significantly decreased. The results suggest that SO_4^{2-} uptake and ATP sulfurylase in rape plants were species specific, and that SO_4^{2-} uptake was highly related to its assimilation into S containing compound such as glutathione.

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