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Sulfur Deficiency Effects on Sulfate Uptake and Assimilatory Enzymes Activity in Rape Plants

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유채에서 황 결핍이 황산염 흡수 및 동화관련 효소활력에 미치는 영향

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요 약

유채 (*Brassica napus* L.)에서 외생적 황 공급이 SO_4^{2-} 흡수와 동화에 대한 영향을 알아보고자 SO_4^{2-} 농도를 세가지 수준 (1 mM SO_4^{2-}, 대조구; 0.1 mM SO_4^{2-}, 결핍; 0 mM SO_4^{2-}, 무공급)으로 25시간 처리한 후 식물조직내에서의 SO_4^{2-} 농도, ATP sulfurylase와 APS reductase 활성을 측정하였다. SO_4^{2-}의 흡수와 식물조직내에서의 SO_4^{2-}의 농도는 결핍과 무공급 조건하에서 현저하게 감소하였다. ATP sulfurylase 활성은 외부 황 공급의 감소에 따라 증가한 반면, APS reductase 활성은 감소하였다. 황 무공급에 따른 이 두 효소 활력의 유의적인 차이는 어린잎과 중간잎에서만 고찰되었다. 이러한 결과는 한정된 황 조건하에서 특히 어린잎에서 SO_4^{2-} 동화는 더욱 민감하게 반응한다는 것을 제시한다.
(**Key words** : SO_4^{2-} uptake, APS reductase, ATP sulfurylase, S deficiency, Rape)

I . INTRODUCTION

Sulfur, as one of the most versatile elements in living organisms, is an essential mineral nutrient required for plant growth in agricultural productivity (Hell, 1997). Because it is essential for the formation of amino acids, lipids, proteins and fatty acids, Sulfur is considered as the fourth important major nutrient following nitrogen, potassium, and phosphorus (Bloem et al., 2004; Zhao et al., 1997). Besides, disulfide bridges in proteins play structural and regulatory roles. Sulfur is also required for the synthesis of various other compounds, such as thiols, sulpholipids and secondary sulfur compounds, which play an important role in the nutritional physiology and in the protection and adaptation of plants against stress and pests (Matsubayashi et al., 2002).

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In the environment, Sulfur is primarily available as sulfate (SO_4^{2-}), which is taken up by root into plants and then reduced to sulfide through a series of enzymatic reactions (Schmidt and Jager, 1992; Leustek and Saito, 1997; Bick and Leustek, 1998; Leustek et al., 2000; Hopkins et al., 2004). After sulfate is taken up via the root from the soil solution and transported by the xylem to the leaves. It is then reduced to Adenosine-5'-phosphosulfate (APS), and either converted to methionine or incorporated into cysteine, GSH (glutathione), and proteins. The first metabolic transformation in the sulfur assimilation pathway is to generate Adenosine-5'-phosphosulfate and pyrophosphate (PPi), catalyzed by ATP sulfurylase (ATPS). The major one of the two forms of ATPS is found in plastids and the other one is found in the cytoplasm (Leustek et al. 2000). The products, APS and PPi, must be converted to other compounds immediately so as to drive the former reaction. APS is reduced to sulfite by APS reductase (Suter et al. 2000; Kopriva et al. 2002). The additional metabolic pathway of APS is that it can be sulfated to generate 3'-phosphoadenosine-5'-phosphosulfate (PAPS). The former is the dominant pathway.

For some years, sulfur is to become the major element limiting for the plant growth. Certain estimations predict that sulfur deficiency in agriculture eco-system shall be at the world-wide symptom in a near future. This S-oligotrophisation is bound to several phenomena; 1) strong restriction of SO_2 emissions to avoid atmospheric pollution (considerable decrease in S deposition from atmospheric S) during the last two decades, 2) shirking the intensive culture system with S demand plants for high productivity, 3) decline of the employment of S-based antifungal products.

In the other hand, rape (*Brassica napus* L.) plants have a characteristic of high demand for S during vegetative growth period. The seeds contain high level of proteins with relatively large quantity of S-containing amino acids (Zhao et al., 1997). In this context, rape plants, including oil seeds and forage type, are particularly sensitive to S-utilization efficiency (Holmes, 1980) under stressed condition. In addition, the plants require S for the synthesis of glucosinolates, a group of thioglucoside compounds which has been reported to be associated with plant defense mechanism against fungi and insects (Chew, 1988). The study on the physiological significance of S deficiency in rape plants will facilitate the prediction of responses to decreased S inputs and may provide useful diagnostic indicators of S status. Our objectives of this study were to investigate changes in SO_4^{2-} uptake and two key enzymes related to sulfur assimilation pathway in response to S-deficiency in rape plants.

II. MATERIALS AND METHODS

1. Plant culture

Seeds of rape (*Brassica napus* L. cv. mosa) were germinated in bed soil (containing peatmot, perlite, cocopeat, and zeolite; 15:15:60:10, v/v). When plants were grown to the 3 leaves stage, they were transferred to 2.3 L pots filled with 2 L of complete nutrient solution (Kim et al., 1991). The nutrient solution was continuously aerated and renewed every 5 days. Natural light was supplemented by metal halide lamps that provided a light of 200 μmol $m^{-2}s^{-1}$ at the canopy height for 16 h day^{-1}. Eight-week-old

plants were harvested at 3 days after the supplies of newly nutrient solution for experiment.

Control plants were fed with the complete nutrient solution (1.0 mM SO_4^{2-}) as shown at Table 1. For S-deficient (0.1 mM SO_4^{2-}) and S-deprivation (0 mM SO_4^{2-}) treatments, SO_4^{2-} concentration in the nutrient solution was decreased to 0.1 mM or withdrawn SO_4^{2-} source from the control solution with controlling cation/anion balance. Three plants per treatment were harvested at 25 hour after treatment. Each plant was separated into old leaves, middle leaves, young leaves, and roots. The tissue samples of each plant were immediately frozen in liquid N and stored in deep-freezer for further analysis.

2. Determination of sulfate uptake

SO_4^{2-} uptake was determined by subtracting the current concentration at the sampling time from the initial concentration, and expressed as μmol SO_4^{2-} g^{-1} FW. The SO_4^{2-} conentration was determined using an ion chromatography (Dionnex, DX 120, USA) equipped with an Ionpac AS14A column and AG4A-SC guard column. The eluent solution consisted of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ and the regenerant of 0.025 N H_2SO_4. The flow rate was 2.3 ml min^{-1}.

3. Analysis of ATP-sulfurylase

About 0.2 g fresh tissues were quickly ground with liquid N with, then use 1.0 ml extract buffer which contains 10 mM Na_2EDTA, 20 mM Tris-HCl (pH 8.0), 2 mM DTT and 1% PVP, extract at 4℃ with rotation about 1 hour followed by centrifugation at 13,000 rpm for 10 minutes at the same temperature. The supernatant was collected to analyze the ATP sulfurylase activity. Molybdate-dependent formation of pyrophosphate was measured to represent the activity of ATP-sulfurylase (Lappartient and Touraine 1996).

4. Analysis of APS reductase

Approximately 0.2 g tissues were ground with liquid nitrogen before extracted by 0.6 ml KPO_4 Buffer (pH 8.5) containing 1 mM EDTA, 1 mM DTT and 1 mM Cysteine at 4℃. After centrifugation, the supernatant was used to analyze the APS reductase. The activity of APS reductase is easily measured with ferricyanide as the electron acceptor and by following the decrease in absorbance at 420 nm.

Table 1. Composition of nutrient solution used for the hydroponic culture

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	0.5	$ZnSO_4 \cdot 7H_2O$	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		

Ⅲ. RESULTS

1. SO_4^{2-} concentration in leaf tissues

The changes in SO_4^{2-} concentration in leaf tissues grown in nutrient solution containing 0, 0.1 and 1.0 mM SO_4^{2-} during 25 hours are presented at Table 2.

At the time of treatment (time 0), SO_4^{2-} concentration was 37.7, 90.5 and 170.6 μM g^{-1} DW (in average of 3 treatments) in young, middle and old leaves, respectively. After 25 hours after treatment, SO_4^{2-} concentration significantly decreased in S-deficient (0.1 mM SO_4^{2-}) and S-deprived (0 mM SO_4^{2-}) condition, while it maintained at same level it slightly increased in the control (1.0 mM SO_4^{2-}).

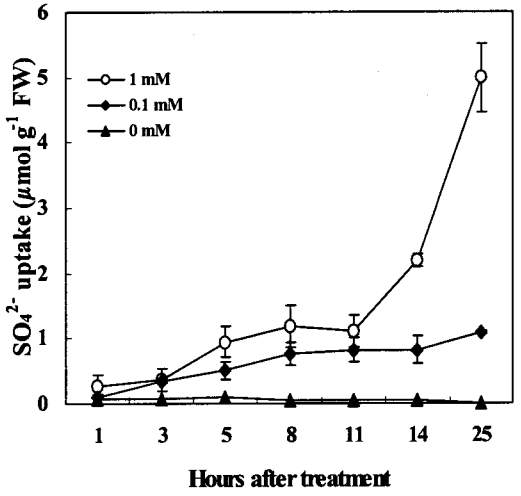

Fig. 1. Changes of SO_4^{2-} uptake (μmol g^{-1} FW) measured at the complete S supply (control, 1.0 mM SO_4^{2-}), S deficiency (0.1 mM SO_4^{2-}) and S deprivation (0 mM SO_4^{2-}) during 25 hours after treatment. The values are means ± SE of three replicates.

2. SO_4^{2-} uptake by root

The SO_4^{2-} uptake during 25 hours was presented at Fig. 1. For the first 3 hours, there was no significant difference among 3 sulfur levels applied. At the control (1.0 mM SO_4^{2-}), SO_4^{2-} uptake slightly increased to 1.10 μmol g^{-1} FW until 11 h after treatment, and then rapidly increased to 4.98 μmol g^{-1} FW at 25 h. A slight increase was observed at S-deficiency treatment (0.1 mM SO_4^{2-}), but the rate of increase was much less than that of the control, presenting 1.08 μmol g^{-1} FW of SO_4^{2-} uptake at 25 h. No SO_4^{2-} absorption was observed in the S-deprivation treatment throughout the sampling times.

Table 2. Sulfate concentration (μM g^{-1} DW) in different leaf tissues grown in nutrient solutions containing 0, 0.1 and 1.0 mM SO_4^{2-} throughout the experimental period. The values indicate mean±SE for 3 replicates

Treatment	Time (hours)	Young	Middle	Old
		μM g^{-1} DW		
1.0 mM SO_4^{2-}	0	37.2±4.0	85.4±3.7	167.4±12.1
	25	45.4±3.6	91.3±6.2	175.4±16.5
0.1 mM SO_4^{2-}	0	35.3±3.5	94.6±6.5	170.2±14.5
	25	19.6±3.0	76.2±4.5	141.4±12.6
0 mM SO_4^{2-}	0	40.5±2.9	91.4±4.7	174.3±17.2
	25	8.9±1.1	62.5±3.3	116.1±10.4

3. ATP sulfurylase activity

The changes in the activity of ATP sulfurylase measured at 25 hours after treatment are given at Fig. 2. The activity of ATP Sulfurylase was significantly increased under S-deficient condition (S-deficiency, 0.1 mM and S-deprivation, 0 mM) in the young and middle leaves (Fig. 2). S-deprivation resulted in the activation of ATP sulfurylase by 30% and 60% respectively. The increase in ATP sulfurylase under S-deficient condition was much less, accounting to only 11% and 19% in the young and middle leaves. In the old leaves, comparing with young and middle leaves, 20% increase in the activity was estimated in S-deficient condition, representing a similar increasing rate. However, the increasing rate largely attenuated in S-deprivation condition.

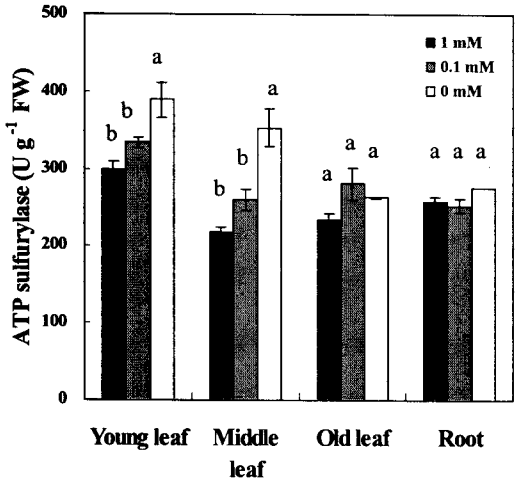

Fig. 2. ATP sulfurylase activity (U g^{-1} FW) in different leaf tissues and root after 25 hours of treatment (control, 1.0 mM SO_4^{2-}; S deficiency, 0.1 mM SO_4^{2-}; S deprivation, 0 mM SO_4^{2-}). The values are means ± SE of three replicates. Bars labeled with the same letters are not significantly different ($P>0.05$) according to Duncan's multiple range test.

4. APS reductase

The activity of APS reductase tended to decrease with the decrease of SO_4^{2-} supply level, in all leaf tissues (Fig. 3). The rate of decrease was the highest in 0 mM SO_4^{2-} treatment, especially in young leaves, which presented a decrease of more than 25% under S-deprivated condition. The activity was the highest in the young leaves, and followed by the middle and old leaves in 3 all treatments. The lowest activity was observed in roots, the increase was significant only at S-deficient condition.

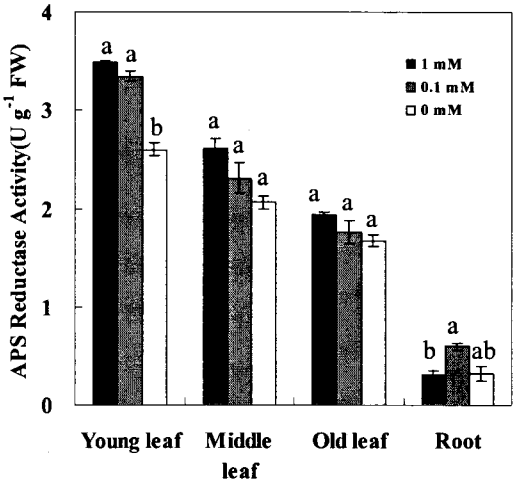

Fig. 3. APS reductase activity (U*g^{-1} FW) in different leaf tissues and root after 25 hours of treatment (control, 1.0 mM SO_4^{2-}; S deficiency, 0.1 mM SO_4^{2-}; S deprivation, 0 mM SO_4^{2-}). The values are means ± SE of three replicates. Bars labeled with the same letters are not significantly different ($P>0.05$) according to Duncan's multiple range test.

IV. DISCUSSION

Sulfate (SO_4^{2-}) concentration in rape plant was leaf tissue specific. Before treatment of

external SO_4^{2-} application, the concentration of sulfate in old leaf was already the highest, and followed by middle and young leaf (Table 2). This indicates that sulfate distribution and accumulation in old and mature leaf might be the first magnitude. After 25 hours of treatment, SO_4^{2-} concentration was significantly affected by the level of external SO_4^{2-} application. The percentage of decrease in SO_4^{2-} concentration caused by S-deficiency (0.1 mM SO_4^{2-}) or S-deprivation (0 mM SO_4^{2-}) was the highest in young leaves, even though the absolute concentration was lowest among leaf tissues. This suggests that sulfate metabolism in young leaf tissue would be much more restricted by S-limited nutrition compared with old and mature tissues. The kinetics of SO_4^{2-} uptake was well reflected to the external SO_4^{2-} application. After 25 hours of treatment, the highest SO_4^{2-} uptake was found in the control (1.0 mM SO_4^{2-}), and followed by S-deficiency (0.1 mM SO_4^{2-}) treatment. The difference of SO_4^{2-} uptake responding to 3 treatments became distinct with passing the time of treatment. Similar result was reported that SO_4^{2-} uptake is reduced with S-limiting treatment (Schonhof et al., 2007); in all treatment of sulfur deficiency. Total S concentration in the leaves is closely paralleled with SO_4^{2-} concentration in the nutrient solution (Mechteld et al., 1998). These results suggest that uptake and subsequent distribution of SO_4^{2-} to the leaves are closely associated with S-demand and S-status in plant tissues.

To determine how does endogenous SO_4^{2-} assimilate in leaves with different age and root tissues in response to the exogenous SO_4^{2-} level, the activity of ATP sulfurylase and APS reductase was measured after 25 hours of treatment. A significant increase in ATP sulfurylase activity responding to S-deprivation treatment was observed only in young and middle leaves. It could be suggested that the active site of SO_4^{2-} assimilation might be young tissue rather than old or mature leaf tissues. Furthermore, a significant increase in 0 mM SO_4^{2-} clearly indicates that the SO_4^{2-} reserved in cytoplasm, especially vacuole become to be a primary source of S-assimilation under S-limited condition. In fact, the activities of ATP sulfurylase the highest in the youngest leaves, and followed by the middle and old leaves in all three treatments (Fig. 2). In addition, the rate of decrease of APS reductase also was the highest in S-deprivation treatment, especially in young leaves (Fig. 3). Young shoot tissues are usually the most susceptible to the disruption of normal growth and metabolism when the external supply of SO_4^{2-} becomes limited (Gilbert et al., 1997). The chlorophyll content rapidly decreased in the middle and particularly the young leaves of plants grown in S-deficient (0.1 mM SO_4^{2-}) and S-deprived (0 mM SO_4^{2-}) condition, whereas no significant changes was observed in the control (1.0 mM SO_4^{2-})(data not shown). For instance, developing leaves are strong S sinks, but show a net loss of S after full expansion (Sunarpi and Anderson, 1996). At sufficient S supply, approximately 50% of total S in the young leaves was incorporated into soluble S, and 42% accumulate as SO_4^{2-}(mechteld et al., 1998). It has been thus suggested that the assimilation of S in the middle and old leaves is much less than that in the young leaves, which are currently developing. It has been suggest that SO_4^{2-} stored in the vacuoles of mesophyll cells is only released under S stress and that this release is too slow to support new growth (Clakson et al.,

1983; Bell et al., 1995).

In conclusion, S uptake and their distribution to the plant tissues are closely related with the availability of this nutrient element in the soil medium. SO_4^{2-} assimilation in young leaf tissue would be much more sensitively responded to S-limited nutrition compared with old and mature tissues.

V. ABSTRACT

To determine SO_4^{2-} uptake and its assimilation in response to the exogenous SO_4^{2-} supply level in forage rape (*Brassica napus* L.), the concentration of this element in plant tissues and the activity of ATP sulfurylase and APS reductase was measured after 25 hours of treatment (1.0 mM SO_4^{2-}, control; 0.1 mM SO_4^{2-}, S deficiency; 0 mM SO_4^{2-}, S deprivation). SO_4^{2-} uptake and the concentration in the plant tissues significantly decreased in S-deficient and S-deprived condition, while it maintained at nearly same level in the control. The activity of ATP sulfurylase tended to increase with decreasing the exogenous SO_4^{2-} supply, while that of APS reductase to decrease. A significant change in both enzymes responding to S-deprivation treatment was observed only young and middle leaves. The results indicated that SO_4^{2-} assimilation in young leaf tissues would be much more sensitively responded to S-limited nutrition.

VI. ACKNOWLEDGEMENT

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