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BRIEF COMMUNICATION

ATP-sulfurylase activity, photosynthesis, and shoot dry mass of mustard (*Brassica juncea* L.) cultivars differing in sulfur accumulation capacity

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Abstract

Sulfur (S) is an essential nutrient element required in a large quantity by mustard. S regulates photosynthesis and plant growth through improving nitrogen (N) acquisition. Mustard cultivars Alankar, Varuna, Pusa Jai Kisan, and SS2 differing in S accumulation capacity calculated as sulfate transport index (STI) were tested for ATP-sulfurylase activity, S and N accumulation, photosynthesis, and shoot dry mass (DM) at 30 and 60 d after sowing (DAS). The activity of ATP-sulfurylase, shoot N content, net photosynthetic rate (P_N), leaf area, and shoot DM of the cultivars were in the order: Pusa Jai Kisan>Alankar>Varuna>SS2. ATP-sulfurylase activity was strongly and positively correlated with P_N and shoot DM in all the cultivars. Hence ATP-sulfurylase activity may be used as a physiological trait for augmenting photosynthesis and shoot DM.

Additional key words: dry mass; leaf area; nitrogen content; sulfate transport index.

Sulfur (S) is an essential element required in higher quantity by mustard (Zhao *et al.* 1993, Lakkinani and Abrol 1994, McGrath and Zhao 1996). It is a precursor of cysteine and methionine, amino acids involved in the synthesis of compounds containing reduced S (Marschner 1995, Scherer 2001). A sufficient S supply improves photosynthesis and growth (Ahmad *et al.* 2005b, Khan *et al.* 2005) through regulating N assimilation (Reuveny *et al.* 1980, Kopriva *et al.* 2002, Scherer 2008). A larger accumulation of N maintains high chlorophyll content and high activity of enzymes of Calvin cycle (Lawlor *et al*. 1989), and enhances growth (Schnug *et al.* 1993, Khan *et al.* 2005) because of the established role of S and N in cell differentiation and overall growth of plants (Marschner 1995). The assimilatory pathways of S and N have been considered functionally convergent and well coordinated as the availability of one element regulates the other (Reuveny *et al*. 1980, Schnug *et al*. 1993). The availability of S regulates the activity of nitrate reductase and the accumulation of N (Pal *et al*. 1976).

The assimilation of S in plants is initiated by its activation and the reaction is catalyzed by the enzyme, ATP-sulfurylase. It is, therefore, assumed that the cultivars differing in ATP-sulfurylase activity may show different photosynthetic potential and shoot dry mass (DM). To test this assumption four cultivars of mustard differing in S accumulation capacity were tested for ATP-sulfurylase activity, sulfate content, net photosynthetic rate (P_N), and shoot DM. The relationship of ATP-sulfurylase activity with P_N and shoot DM was also established.

Cultivars Alankar, Varuna, Pusa Jai Kisan, and SS2 of mustard (*Brassica juncea* L. Czern & Coss.) were raised from seeds in 23-cm diameter plastic pots filled with acid-washed sand purified according to Hewitt (1966). The experiment was carried out under natural day/night [photosynthetically active radiation (PAR) >900 μmol (photon) m^{-2} s^{-1}; temperature 22±3 ºC; relative humidity (RH) 62–72 %] in the Department of Botany, Aligarh Muslim University, Aligarh, India. Two plants per pot were maintained and fed with 250 cm^3 of half-strength Hoagland nutrient solution (Hewitt 1966) every alternate day and 200 cm^3 of de-ionized water daily. The composition of the nutrient solution was 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM $NH_4H_3PO_4$, 50 μM KCl, 25 μM H_3BO_3,

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Abbreviations: DAS – days after sowing; DM – dry mass; DTT – dithiothreitol; PAR – photosynthetically active radiation; P_N – net photosynthetic rate; PVP – polyvinylpyrrolidone; RH – relative humidity; STI – sulfate transport index.
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2 μM $MnCl_2$, 2 μM $ZnCl_2$, 0.5 μM $CuCl_2$, 0.5 μM $(NH_4)_6Mo_7O_{24}$, 20 μM $Na_2FeEDTA$, and 1 mM SO_4^{2-} as $MgSO_4.Mg^{2+}$ was maintained at 1 mM by the addition of $MgCl_2$. The pH of the solution was adjusted to 6.5±0.1 with NaOH. The nutrient solution was replaced weekly. The pots were arranged in a randomized block design and replicated three times.

The activity of leaf ATP-sulfurylase, contents of SO_4^{2-} and N in leaf, and P_N were measured in one plant from each cultivar and its replicates at 30 and 60 d after sowing (DAS). At these sampling times, leaf area of these plants was measured and plant DM was determined. Root SO_4^{2-} content was also determined at 30 DAS. S accumulation capacity determined as sulfate transport index (STI) was calculated as the ratio of sulfate content in root and leaf and expressed as percentage. The relationship of ATP-sulfurylase activity with P_N and shoot DM of the four cultivars was worked out.

ATP-sulfurylase activity was assayed by the method of Lappartient and Touraine (1996). One g fresh leaf tissue was ground at 4 ºC in a buffer consisting of 10 mM Na_2EDTA, 20 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol (DTT), and 0.01 g per cm^3 polyvinylpyrrolidone (PVP), using 1 : 4 (m/v) tissue to buffer ratio. The homogenate was centrifuged at 20 000×*g* for 10 min at 4 ºC. The supernatant was used for *in vitro* ATP-sulfurylase assay. The enzyme activity was measured using molybdate-dependent formation of pyrophosphate. The reaction was initiated by adding 0.1 cm^3 of extract to 0.5 cm^3 of the reaction mixture, which contained 7 mM $MgCl_2$, 5 mM of Na_2MoO_4, 2 mM of Na_2ATP, and 0.032 units per cm^3 of sulfate-free inorganic pyrophosphate in 80 mM Tris-HCl buffer (pH 8.0). Another aliquot from the same extract was added to the same reaction mixture except that Na_2MoO_4 was absent. Incubations were carried out at 37 ºC for 15 min, after which phosphate was determined spectrophotometrically.

One hundred mg of dried fine powder of root and leaf was digested in a mixture of concentrated HNO_3 and 60 % $HClO_4$ (85 : 1, v/v) and the content of sulfate was estimated using the turbidimetric method of Chesnin and Yien (1950). The content of N in leaf was determined in acid-peroxide digested dried material by the method of Lindner (1944).

P_N was measured using infrared gas analyzer (*LiCOR 6200, Nebraska*, NE, USA) in fully expanded uppermost leaves on plants used for ATP-sulfurylase assay at saturating irradiance between 11:00–12:00 h. The atmospheric conditions during the measurements were: PAR 990±20 μmol(photon) m^{-2} s^{-1}; temperature 22±1 ºC, and RH 62±3 %. Leaf area was measured with a leaf area meter (*LA 211*, *Systronics*, New Delhi, India). Shoot DM was determined after drying the plant at 80 ºC to a constant mass.

Data were analyzed statistically and standard error was calculated. Least significant difference (LSD) was calculated using ANOVA to identify significant difference with the help of *SPSS* version *10* (USA).

All the cultivars differed in STI: Pusa Jai Kisan showed maximum STI followed by Alankar, Varuna, and SS2 (Table 1). A similar pattern of ATP-sulfurylase activity was found; the activity increased by 16.67 to 25.84 % from 30 to 60 DAS (Table 1). Also the contents of leaf sulfate and N increased from 30 to 60 DAS (Table 1). Similar cultivar and time differences existed in P_N, leaf N content, plant growth. P_N, leaf area, and shoot DM (Table 1). Leaf sulfate content was greatest in Alankar followed by Pusa Jai Kisan, Varuna, and SS2 at both the sampling times.

A strong positive correlation (p<0.01) between ATP-sulfurylase activity and P_N and shoot dry mass was found (Fig. 1).

Table 1. Contents of root and leaf sulfate (SO_4^{2-}) and leaf nitrogen (N) [mg kg^{-1}(DM)], sulfate transport index (STI) [%], activity of ATP-sulfurylase [mmol kg^{-1}(protein) s^{-1}], net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}], leaf area [cm^2 per plant], and shoot dry mass (DM) [g per plant] of four cultivars of mustard (*Brassica juncea* L.) at 30 or 60 d after sowing (DAS). Means±SE (n = 3). *Values are significantly different at p<0.05. STI was calculated as the ratio of root to leaf sulfate content.

	DAS	Alankar	Varuna	Pusa Jai Kisan	SS2
Root SO_4^{2-} content	30	2.20±0.06	1.22±0.03	2.65±0.07*	1.02±0.02
STI	30	42.23±2.66	35.26±2.15	61.34±3.81*	33.77±1.56
ATP-sulfurylase activity	30	2.77±0.11*	2.55±0.08	3.61±0.12	1.67±0.09
	60	3.39±0.10*	3.00±0.09	4.55±0.14	4.55±0.11
Leaf SO_4^{2-} content	30	5.21±0.40*	3.46±0.30	4.32±0.40	3.02±0.20
	60	9.11±0.50*	5.36±0.20	7.97±0.30	4.53±0.30
Leaf N content	30	58.38±2.40*	56.98±1.80	64.54±1.80	54.60±2.10
	60	60.55±2.10*	58.36±2.00	67.48±2.20	55.70±1.80
P_N	30	13.25±0.56*	12.85±0.42	14.56±0.50	10.00±0.42
	60	24.82±7.40*	23.23±7.80	28.22±8.20	18.07±7.50
Leaf area	30	158.0±9.4*	150.0±9.2	190.0±7.3	140.0±7.4
	60	188.0±10.5*	175.0±6.6	230.0±12.5	152.0±6.1
Shoot DM	30	7.33±0.32*	5.43±0.18	9.17±0.32	4.20±0.22
	60	12.10±0.72*	8.21±0.62	16.88±0.82	6.80±0.32

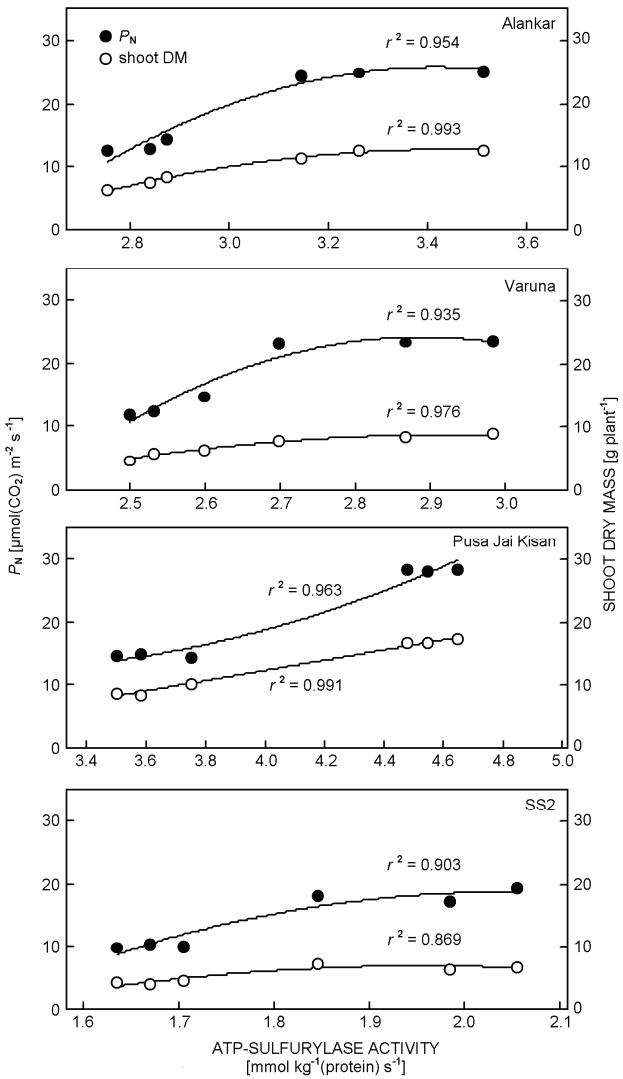

Fig. 1. Correlations (p<0.01) between ATP-sulfurylase activity and shoot dry mass (DM) or net photosynthetic rate (P_N) in four cultivars of *Brassica juncea*.

ATP-sulfurylase catalyzes the first step of S assimilation pathway and therefore may act as a point of regulation in S uptake and its subsequent reduction (Hawkesford and Wray 2000). The cultivar Pusa Jai Kisan exhibited maximum ATP-sulfurylase activity and also P_N and plant growth (Table 1). A higher ATP-sulfurylase activity and sulfate-transport index in Pusa Jai Kisan indicates its higher sulfate accumulation capacity. An increased accumulation of sulfate, therefore, helped increase in N content (Table 1). As ATP-sulfurylase activity was maximum in Pusa Jai Kisan, the sulfate accumulated was assimilated efficiently into reduced S resulting in leaf sulfate content lesser than in Alankar. A higher accumulation of sulfate resulted in higher N content and P_N in Pusa Jai Kisan as N is necessary for high contents of chlorophyll and ribulose-1,5-bisphosphate carboxylase protein (Lawlor *et al.* 1989). Ahmad and Abdin (2000) and Ahmad *et al.* (2005b) have also found increased P_N with adequate S supply, and inadequacy of S caused the accumulation of non-protein N in the vegetative tissue at the expense of protein-N.

The higher activity of ATP-sulfurylase and N accumulation in Pusa Jai Kisan also resulted in the development of larger leaf area. Larger leaf area in Pusa Jai Kisan intercepted more photons and efficiently utilized solar radiation resulting in higher P_N. P_N integrated over time and leaf area resulted in the increased DM accumulation. This suggests that the cultivar Pusa Jai Kisan possesses greater S accumulation and assimilation capacity which was possibly associated with the higher sulfate transporter system. The difference in sulfate uptake and S transporter system in mustard genotypes has been shown by Ahmad *et al.* (2005a). However, an association of ATP-sulfurylase activity with photosynthesis and shoot DM has not been reported. A strong positive correlation observed between the activity of ATP-sulfurylase and P_N and shoot DM in all four cultivars (Fig. 1) suggests their concomitant relation. Hence the activity of ATP-sulfurylase may be used as a physiological trait for augmenting photosynthesis and shoot DM accumulation in mustard.

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