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EFFECTS OF SULPHUR DEFICIENCY ON GROWTH, SUGARS, PROLINE AND CHLOROPHYLL CONTENT IN MUNGBEAN (*VIGNA RADIATA* L. VAR. BARI MUNG-6)

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Abstract

Sulphur deficiency caused an increase in accumulation of reducing sugar and proline in the root, stem and leaf of mungbean (*Vigna radiata* L. var. BARI MUNG-6) seedlings grown in solution culture. Sulphur deficiency caused a 2.2-fold increase of reducing sugar level in the root at 24 hrs of treatment but caused a decrease in accumulation of total sugar in the root and leaf but increased that of stem of mungbean. It caused a 5.4-fold increase in proline level in the root at 24 hrs of treatment. Sulphur deficiency decreased root and shoot length, leaf number, dry weight of the root and shoot of mungbean plants grown in sand culture. It also decreased chlorophyll a content by 40.1% and chlorophyll b content by 77.3% at 21 days of treatment in leaves of mungbean plants under the same condition.

Introduction

Mungbean (*Vigna radiata* L.) is an important legume of Asia origin, and is widely cultivated in the countries of Asia, Australia and Africa (Yang *et al.* 2008). It is an important summer pulse crop of many South Asian countries including India, Pakistan, Bangladesh, Thailand and Korea (Hussain *et al.* 2006). Sulphur is indispensable for the growth and metabolism of all plants (Vidyalakshmi *et al.* 2009). It plays various critical roles in catalytic or electrochemical function of biomolecules in the cells (Saito 2004).

Sugar level was increased in sulphur stressed *Allium cepa* (Chandra and Pandey 2014a). Somal and Yapa (2008) reported that sulphur deficiency increased proline content in the leaf of cowpea (*Vigna unguiculata* L.). The addition of sulphur increased free proline content in the leaf of *Pisum sativum* L. (Osman and Rady 2012).

Sulphur deficiency affects the photosynthetic apparatus severely and the chlorophyll content was reduced by 49% because of a general reduction of PS I and PS II (Lunde *et al*. 2008). It decreased chlorophyll content in Indian mustard (Bashir *et al*. 2015), *Brassica napus* L. (Lee *et al*. 2014), leaves of *Vigna mungo* L. (Chandra and Pandeya 2014b) and mulberry plants (Tewari *et al*. 2010). It decreased root and shoot biomass in *Brassica napus* L. (Lee *et al.* 2014) and root and shoot length in *Medicago sativa* L. (Wang *et al.* 2003).

There is no report on the effect of sulphur deficiency on the sugar, proline and pigment content of mungbean (*Vigna radiata* L.). In this paper, the effect of sulphur deficiency on the accumulation of reducing and total sugars, proline, pigment content and growth is reported.

Materials and Methods

Mungbean (*Vigna radiata* L. var. BARI MUNG-6) was used as plant material. The seeds were obtained from Bangladesh Agricultural Research Institute (BARI), Gazipur. Plants were grown in solution culture (Long Ashton Solution) (Professor D.T. Clarkson, Long Ashton Research Station, Bristol, U.K. personal communication) to study the accumulation of reducing

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and total sugar, proline content and also in sand culture (Hewitt 1966) to study growth parameters and pigment contents. Seeds were surface sterilized by 5.25% sodium hypochlorite solution according to Samad and Karmoker (2013). Then the seeds were spread over a cotton gauge placed in a lid having holes (3 cm diameter) and the lid with seeds was placed on a beaker containing 500 ml of half-strength Long Ashton solution. The beaker was painted black to avoid the exposure of roots to the light. The beakers were kept in dark for 48 hrs to facilitate the germination of seeds. After germination, the beakers with the seedlings were placed in light bank at 22°C/18°C day/night temperature, 13 hrs/11 hrs day/night length and light intensity was 160 $\mu Em^{-2}s^{-1}$. The relative humidity was 65 - 80%. Sulphur-containing half-strength Long Ashton solution (+S-solution) was used as control. Sulphur-free half-strength Long Ashton solution (–S-solution) was used as sulphur-deficiency treatment. Solutions of control and treatments were aerated continuously by an air compressor. In case of sand culture, sterilized seeds were sown in pots filled with purified sand and kept at natural environmental conditions. In the sand culture experiment, +S-solution was used as control and –S-solution was used as sulphur-deficiency treatment. Reducing and total sugars, proline were measured in the root, stem and leaves of the seedlings after 24, 48, 72 and 96 hrs of sulphur deficiency treatment. Determination of pigment content and growth parameters were measured after 7, 14 and 21 days of treatment in plants grown in sand culture.

Reducing and total sugars were extracted by boiling fresh root, stem and leaf tissue in two changes of 5 ml of 80% ethanol for five minutes in a hot water bath according to Karmoker and Van Steveninck (1979). Reducing sugar was measured following Somogyi-Nelson method (Nelson 1944, Somogyi 1952). Total sugar was measured following Dubois *et al.* (1956). Proline was measured following Bates *et al.* (1973). Chlorophyll a and b contents were determined by using specific absorption co-efficient of Mckinney (1940) and the formulae of Maclachalan and Zalik (1963). The amounts of carotenoid were determined by the equation of Von Wettstein (1957).

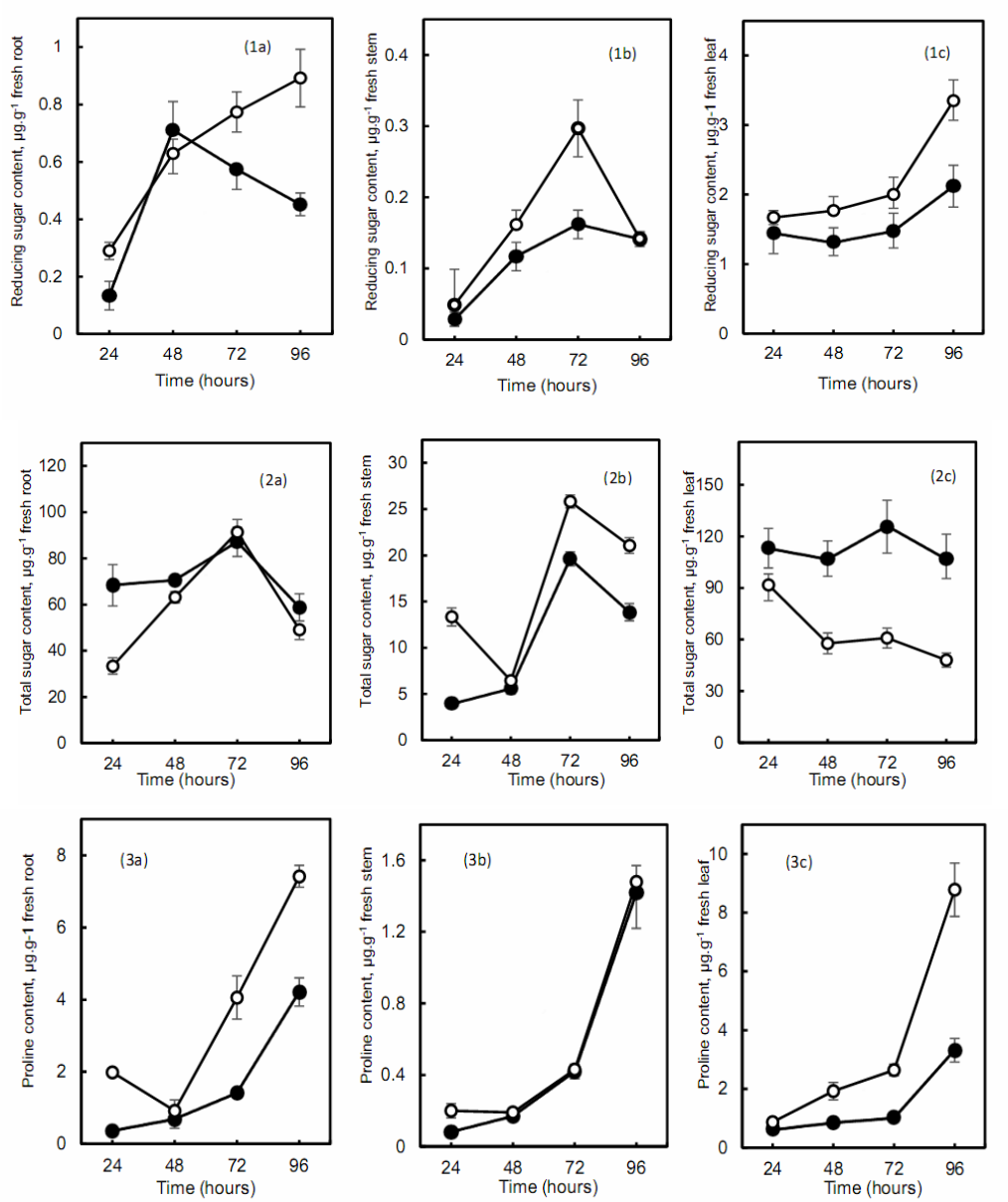
Results and Discussion

Sulphur deficiency increased accumulation of reducing sugar in root by 2.2-fold at 24 hrs and the stimulatory effect was maintained up to 96 hrs of treatment (Fig. 1a). It stimulated accumulation of reducing sugar in the stem by 69 to 83% from 24 to 72 hrs of treatment (Fig. 1b). It increased accumulation of reducing sugar in the leaf by 15 to 58% from 24 to 96 hrs of treatment (Fig. 1c). This result was supported by Chandra and Pandey (2014a) who found that sulphur deficiency increased reducing sugar in *Allium cepa*.

In the root, sulphur deficiency caused a decrease in accumulation of total sugar by 51% at 24 hrs of treatment and the inhibitory effects was nullified at 72 to 96 hrs of treatment (Fig. 2a). But it increased the accumulation of total sugar in the stem (Fig. 2b). The accumulation of total sugar in the leaves gradually declined from 19 to 55% from 24 to 96 hrs of treatment (Fig. 2c). On the contrary, sulphur deficiency increased the accumulation of total sugar in *Allium cepa* (Chandra and Pandey 2014a).

Sulphur deficiency increased proline accumulation in root by 5.4- to 2.9-fold from 24 to 72 hrs of treatment (Fig. 3a). In the stem, sulphur deficiency increased proline accumulation by 2.4-fold at 24 hrs and the stimulatory effect was nullified from 48 to 96 hrs of treatment (Fig. 3b). In the leaf, it increased accumulation of proline by 2.2- to 2.7-fold from 48 to 96 hrs of treatment (Fig. 3c). Similarly, Somal and Yapa (2008) reported that sulphur deficiency enhanced proline level significantly in the leaves of cowpea (*Vigna unguiculata* L.). Sulphur deficiency-induced increase in proline level is an indicator of stress. Besides the increase in proline level may help to

maintain osmotic potential of cytoplasm of cells which is important for survival of plants under stress (Hayat *et al.* 2012).



Figs 1 - 3: 1. Effects of sulphur deficiency on the accumulation of reducing sugar in the (a) root, (b) stem and (c) leaf of intact mungbean seedlings grown in solution culture. Solid symbols (●) represent +S and open symbols (O) represent –S. Each value is the mean of three replicates; Bars represent ± standard error of the mean value. 2. Effects of sulphur deficiency on the accumulation of total sugar in the (a) root, (b) stem and (c) leaf of intact mungbean seedlings grown in solution culture. Otherwise as Fig.1. 3. Effects of sulphur deficiency on the accumulation of proline in the (a) root, (b) stem and (c) leaf of intact mungbean seedlings grown in solution culture. Otherwise as Fig.1.

Sulphur deficiency caused a decrease in the content of chlorophyll a by 13.0 to 40.1%, chlorophyll b by 15.7 to 77.3% from 7 to 21 days of treatment in mungbean plants grown in sand culture (Fig. 4a,b,c). Similarly, sulphur deficiency decreased chlorophyll content in Indian mustard (Bashir *et al*. 2015) and *Brassica napus* L. (Lee *et al*. 2014). This result is in agreement with the work of Chandra and Pandey (2014b) who found that the photosynthetic pigments of leaves in black gram (*Vigna mungo* L.) was significantly decreased by sulphur stress. Sulphur deficiency-induced decrease in chlorophyll content of leaves may slow down photosynthesis.

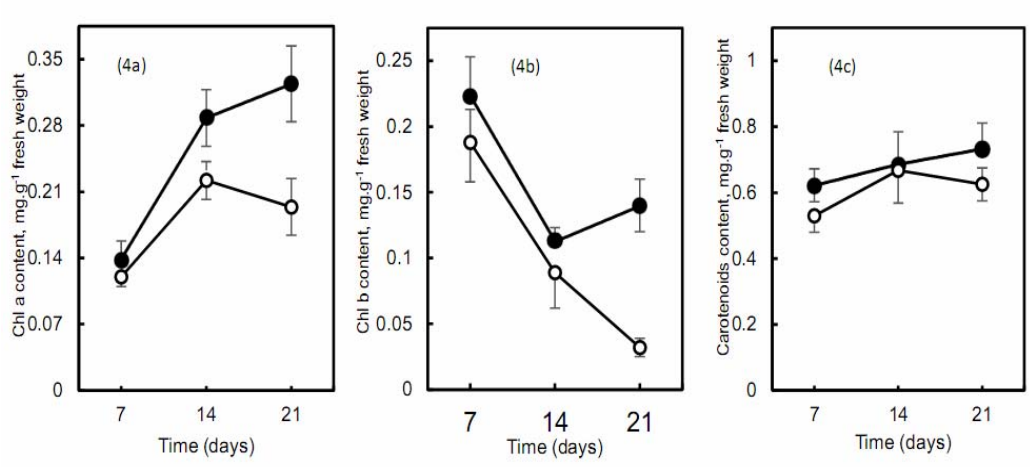


Fig. 4. Effects of sulphur deficiency on (a) chlorophyll a, (b) chlorophyll b and (c) carotenoids content of leaf of mungbean grown in sand culture. Otherwise as Fig. 1.

Table 1. The effect of sulphur deficiency on growth parameters of mungbean plants grown in sand culture.

Duration of treatment (days)	Root length (cm)		Shoot length (cm)		Leaf no./ plant		Dry wt. of root (g)		Dry wt. of shoot (g)	
	+S	–S	+S	–S	+S	–S	+S	–S	+S	–S
0 - 7	3.65 ± 0.15	2.75 ± 0.25	16.05 ± 1.30	12.40 ± 0.60	5.00 ± 0.00	5.00 ± 0.00	0.005 ± 0.003	0.004 ± 0.005	0.049 ± 0.005	0.038 ± 0.04
0 - 14	6.0 ± 0.35	4.600 ± 0.50	23.00 ± 1.00	20.25 ± 1.50	8.00 ± 0.33	6.00 ± 0.33	0.010 ± 0.001	0.080 ± 0.001	0.135 ± 0.007	0.106 ± 0.008
0 - 21	6.75 ± 0.25	5.25 ± 0.25	31.85 ± 0.55	28.00 ± 1.00	11.00 ± 0.57	9.00 ± 0.33	0.020 ± 0.003	0.015 ± 0.001	0.266 ± 0.028	0.233 ± 0.011

Each value is the mean of three replicates; ± standard error.

Sulphur deficiency decreased the length of root and shoot from 7 to 21 days of treatment in mungbean plants grown in sand culture. It also decreased leaf number. It also decreased dry weight of root by 20 to 25% and shoot by 22.4 to 12.4% at 7 to 21 days of treatment (Table 1). Similarly, Wang *et al.* (2003) reported that sulphur deficiency reduced root and shoot length in alfalfa plant (*Medicago sativa* L.). Sulphur deficiency decreased root and shoot biomass in *Brassica napus* L. (Lee *et al.* 2014).

Sulphur deficiency-induced increase in proline level may play a role in maintaining osmotic potential of cytoplasm of cells which is important for survival of plants under stress. Decrease in chlorophyll contents of leaves by sulphur deficiency may slow down the rate of photosynthesis.

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