Calcium ameliorates photosynthetic capacity, nitrate reductase, carbonic anhydrase, nitrogen assimilation, yield and quality of *Cassia sophera* L. – a medicinal legume

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ABSTRACT

Plant biological yield appears to be comparatively low in calcium deficient soils of Aligarh, Western Uttar Pradesh, India. Here, Ca deficiency poses a serious yield and quality limitation for several crops, including various medicinal legumes in this region of India. *Cassia sophera* L. is an important medicinal herb of great therapeutic value and is used in the modern as well as traditional systems of medicine. In view of the medicinal importance of *Cassia sophera* L., a hypothesis was designed to determine whether calcium application through soil could enhance the photosynthetic efficiency, enzymatic activities, nitrogen assimilation, yield and quality attributes. The plants were grown in pots containing soil supplied with five levels of calcium, viz. 0, 40, 80, 120 and 160 mg Ca kg⁻¹ soil (Ca₀, Ca₁, Ca₂, Ca₃ and Ca₄, respectively) applied as calcium chloride (CaCl₂). The performance of the crop was assessed in terms of various growth, physiological, biochemical, yield and quality attributes at 120, 150, 180 and 210 days after sowing. Calcium application proved significantly effective on most of the attributes studied. Of the five calcium levels, Ca₃ showed the best results that significantly stimulated most of the attributes studied at the three growth stages. [Physiol. Mol. Biol. Plants 2009; 15(3) : 237-247] E-mail : naeem_phd@yahoo.co.in; naeemgaur@gmail.com

Key words : Photosynthesis, nitrate reductase, carbonic anhydrase, anthraquinone, medicinal legume, *Cassia sophera* L.

INTRODUCTION

Senna sophera (*Cassia sophera* L.) is commonly known as 'Kasunda' or 'Banar' and belongs to family Fabaceae. It is diffuse, sub-glabrous, shrubby herb or undershrub, 0.7-3.0 m in height. Plant parts contain anthraquinones, chrysophenol, physcion and emodin. The plant leaves possess purgative properties. A paste of leaves and root-bark is a useful application in skin diseases like ringworm and ulcers (The Wealth of India, 1992). The seeds are cathartic, used as a febrifuge and administered in diabetes and in acute bronchitis (Dastur, 1977; Kirtikar and Basu, 1987). A paste of the roots is sometimes substituted for the leaf-juice in ringworm. Its infusion is considered useful in diabetes and its juice in asthma and with honey in diabetes (The Wealth of India, 1992). Anthraquinones are an important group of natural products occurring in higher plants as glycosides. They are found in a large number of plant families including family Fabaceae.

The optimal quantity of fertilizer application is one of the important factors for achieving high yields. In fact, mineral nutrients play a pivotal role in crop production especially for exploiting the yield potential of these plants. In fact, yields of most crop plants increase linearly within limits with the amount of fertilizer that they absorb (Franz, 1983; Loomis and Conner, 1992). To meet the increasing demand, the scientific cultivation of these medicinal plants could improve their yield and quality by improving soils physically, chemically and biologically.

However, plant biological yield appears to be comparatively low in Aligarh soils which are considerably calcium-deficient (Khan and Mohammad, 2006; Naeem and Khan, 2006). With this in mind, a hypothesis was framed that the low calcium level of the soil in this region (Aligarh) of Western Uttar Pradesh, India could be a possible reason for poor biological yield of *Cassia sophera* L. Moreover, there are no reports, so far regarding the requirement of calcium for this important medicinal herb. Thus, a pot culture experiment was

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performed on *Cassia sophera* L. to test the above hypothesis.

MATERIALS AND METHODS

Plant material and growth conditions

The seeds of *Cassia sophera* L. were obtained from Regional Research Institute of Unani Medicine, Aligarh. Healthy seeds of uniform size were selected and their viability was tested using 1 % tetrazolium salt solution. The seeds were surface sterilized with 95 % ethyl alcohol for five minutes and then washed thoroughly with distilled water before sowing. Prior to seed sowing, 5.0 kg homogenous mixture of soil and farmyard manure (4:1) was filled in each pot (25 cm diameter × 25 cm height). Physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2) - 7.5, E.C. (1:2) -0.52 m mhos/cm, available N, P, K and Ca -92.68, 8.13, 140.3 and 36.0 mg kg⁻¹ soil, respectively. The soil samples were tested at the Government Soil Testing Laboratory, Quarsi Farm, Aligarh.

Experimental design

The experiment was conducted in the pots in the net house at the Botany Department, A.M.U., Aligarh (27° 52' N latitude, 78° 51' E longitude, and 187.45 m altitude). The experiment was conducted according to simple randomized complete block design using five levels of calcium, viz. viz. 0, 40, 80, 120 and 160 mg Ca kg⁻¹ soil (Ca₀, Ca₁, Ca₂, Ca₃ and Ca₄, respectively) applied as CaCl₂ at the time of sowing. Each treatment was replicated three times and each replicate had three plants. Thus, each treatment consisted of 9 pots, each of the pots having a single plant. The pots were watered regularly. Plants were grown under naturally illuminated environmental conditions. A uniform basal dose of P (10 mg kg⁻¹ soil) was given basally in the form of KH₂PO₄. The seeds were sown in pots at a depth of 2 cm in the soil.

Growth analysis

Three plants from each treatment were removed with roots intact at three growth stages and analyzed. The plants were sampled at vegetative (120 days after sowing i.e. DAS), flowering (150 DAS) and pod-filling (180 DAS) stages. Three plants from each treatment were harvested with the roots and washed with tap water to remove adhering foreign particles. Water adhering to the roots was removed with blotting paper and fresh weights of plants were taken and dried at 80 °C for 24 h for recording the dry weights.

Yield analysis

At harvest (210 DAS), nine plants from each treatment were uprooted randomly and were used for computing yield attributes including number of pods per plant, number of seeds per pod, 100-seed weight and seed-yield per plant. The pods were threshed and cleaned. The seeds cleaned and counted. Further, seeds were sun dried to maintain constant moisture content for recording a more accurate 100-seed weight. Seed-yield was calculated accordingly.

Physiological and biochemical analysis

The fresh leaves were used for the analysis of various physiological and biochemical attributes except leaf - N, P, K and Ca contents.

Net photosynthetic rate, stomatal conductance and transpiration rate

Net photosynthetic rate, stomatal conductance and transpiration rate were measured on cloud-less clear days at 1100 hours at ambient temperature on fully expanded *Cassia sophera* L. leaves using IRGA (Infra Red Gas Analyzer), Li-Cor 6200 Portable Photosynthesis System (Lincoln, Nebraska, USA). Photosynthesis was measured at 150 DAS.

Total chlorophyll and carotenoid content

Total chlorophyll and carotenoid content were estimated in fresh leaves according to the methods of Mac Kinney (1941) and MacLachlan and Zalik (1963). Fresh tissue from interveinal leaf-areas was ground using a mortar and pestle containing 80 % acetone. The optical density (OD) of the solution was recorded at 645 and 663 nm for chlorophyll content and at 480 and 510 nm for carotenoids content estimation using the spectrophotometer (Spectronic 20D, Milton Roy, USA) and the values were expressed as mg g⁻¹ FW.

Nitrate reductase activity

Nitrate reductase (E.C. 1.6.6.1) activity was estimated by the intact tissue assay method developed by Jaworski (1971), which is based on the reduction of nitrate to nitrite according to the following biochemical reaction:

The amount of nitrite formed was determined spectrophotometrically. Two hundred mg of fresh chopped leaves were weighed and transferred to a

plastic vial. Each vial contained 2.5 mL phosphate buffer (pH 7.5), 0.5 mL potassium nitrate solution and 5 % isopropanol. After incubation, 1 % sulphanilamide and 0.02 % N-(1-Naphthyl) ethylenediamine dihydrochloride (NED-HCL) were added. The test tubes were kept for 20 minutes at room temperature for colour development. The OD of colour was recorded at 540 nm using a spectrophotometer. Nitrate reductase activity was expressed as $\text{n mol NO}_2^- \text{ g}^{-1} \text{ FW h}^{-1}$.

Carbonic anhydrase activity

The carbonic anhydrase (E.C. 4.2.1.1) activity in fresh leaves was analyzed using the method described by Dwivedi and Randhawa (1974). Two hundred mg of fresh leaf pieces were weighed and transferred to petri plates, followed by incubation of the leaf tissue in 10 mL of 0.2 M cystein hydrochloride solution for 20 minutes at 4 °C. Thereafter, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022 % bromothymol blue was added to the homogenate. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. Carbonic anhydrase (CA) activity was expressed as $\mu \text{M CO}_2 \text{ kg}^{-1} \text{ leaf FW s}^{-1}$.

Nutrient analysis

Leaf samples from each treatment were used for the estimation of leaf -N, P, K and Ca contents. The leaves were dried in a hot air oven at 80 °C for 24 h. Dried leaves were powdered and the powder was passed through a 72 mesh. The sieved powder was used for N, P, K and Ca content. One hundred mg of oven-dried leaf powder was carefully transferred to a digestion tube and 2 mL of AR (analytical reagent) grade concentrated sulphuric acid was also added. This solution was heated on a temperature controlled assembly at 80 °C temperature for about two hours and then cooled for about 15 minutes at room temperature. Afterwards 0.5 mL of 30 % hydrogen peroxide (H_2O_2) was added to the solution. The addition of H_2O_2 followed by heating was repeated until the contents of the tube became colourless. The prepared aliquot (peroxide-digested material) was used to estimate per cent N, P, K and Ca in the leaves on a dry weight basis.

Nitrogen content

Leaf-nitrogen content was estimated using the method of Lindner (1944). A 10 mL aliquot (peroxide-digested material) was poured into a 50 mL volumetric flask where 2 mL of a 2.5 N sodium hydroxide and 1 mL of 10 % sodium silicate solutions were added to neutralize the acid excess and prevent turbidity. A 5 mL aliquot of this

solution was poured into a 10 mL graduated test tube and a 0.5 mL Nessler's reagent was added. The contents of the test tubes were allowed to stand for 5 minutes for maximum colour development. The OD of the solution was recorded at 525 nm using the spectrophotometer. The reading of each sample was compared with the standard calibration curve prepared by using graded solutions of ammonium sulphate in order to estimate the percent nitrogen content.

Phosphorus content

The method of Fiske and Subba Row (1925) was used to estimate the leaf-phosphorus content in the digested material. The same aliquot was used to determine the leaf-P content. A 5 mL aliquot was poured into a 10 mL graduated test tube where 1 mL of molybdic acid (2.5 %) was added, followed by addition of 0.4 mL 1-amino-2-naphthol-4-sulphonic acid. When the colour became blue, the volume was increased to 10 mL with the addition of double distilled water. The OD of the solution was recorded at 620 nm using the spectrophotometer.

Potassium and calcium contents

Potassium and calcium contents in the leaves were analyzed flame-photometrically. Both contents were estimated in the same aliquot with the help of emission spectra using specific filters. In the flame-photometer, the solution (peroxide-digested material) was discharged through an atomizer in the form of a fine mist into a chamber, where it is drawn into a flame. Combustion of the elements produces light of a particular wavelength [$(\lambda_{\text{max}}$ for K= 767 nm (violet)]. The light produced was passed through the appropriate filters to impinge upon a photoelectric cell that activates a galvanometer. Both leaf potassium and calcium content were estimated in the same aliquot and the readings were recorded with the help of emission spectra using specific filters in a flame-photometer (Model, C150, AIMIL, India).

Seed-protein content

The protein content of seed was estimated using the method of Lowry *et al.* (1951). Seeds of *Cassia sophera* L. were ground to powder using a mortar and pestle. The protein of seed powder was extracted with 5 % cold trichloroacetic acid (TCA). In addition, 0.5 mL of Folin phenol reagent was added rapidly with immediate mixing. The seed powder containing solution was maintained at room temperature for thirty minutes. Extracted protein was measured at 660 nm using a spectrophotometer. The reading was compared with a calibration curve obtained by using known dilutions of standard egg

albumin solution and the percent seed protein content was calculated on dry a weight basis.

Total anthraquinone glycosides content

Total anthraquinone glycosides content in seeds were analyzed using a spectrophotometer as described in Standard of ASEAN Herbal Medicine (ASEAN Countries, 1993). Seed powder (1.2 g) was refluxed with 30 ml H₂O, centrifuged for 10 min, 0.1 ml of 2M HCL added and then extracted three times with 15 ml chloroform. The chloroform layer was discarded, and the aqueous layer was collected. After that 0.10 g of sodium carbonate was added and the content was shaken thoroughly for 3 min. Aglycone fraction was extracted with ether resulting in the formation of two layers (aqueous layer and ether layer). The ether layer was evaporated and the residue was taken, dissolved in 10 ml of 0.5% (w/v) magnesium acetate in MeOH. Consequently, a pink colour developed. The OD of this solution developed and was recorded at 515 nm using a spectrophotometer and expressed as percent dry weight.

Statistical Analysis

The data were analyzed statistically by one-way ANOVA. LSD was calculated at 5 % according to Gomez and Gomez (1984) for determining significance of the data. Duncan's Multiple Range Test (DMRT) was also employed to separate means using different letters in the Tables and Figures.

RESULTS

Growth attributes

The effect of Ca on all the attributes was found significant at all the stages except number of seeds per pod, 100-seed weight and total anthraquinone glycosides content which were found non-significant. The Ca₃ level proved optimum for most of the attributes studied. However, the effect of Ca₄ was equal to that of Ca₃ at all the growth stages (Tables 1 & 2).

The effect of Ca was significant on fresh weight per plant and treatment Ca₃ proved superior to the control at 120, 150 and 180 DAS. Ca₃ significantly enhanced the fresh weight by 22.3, 29.3 and 23.2 % over Ca₀ at 120, 150 and 180 DAS, respectively (Table 1). The effect of Ca₃ on dry weight per plant was similar to that noted for fresh weight i.e. Ca₃ produced maximum dry weight by 37.6, 46.8 and 50.1 % at 120, 150 and 180 DAS, respectively over Ca₀. The control (Ca₀) gave minimum value at 120, 150 and 180 DAS (Table 1).

Biochemical attributes

Application of Ca was found significant on biochemical attributes studied at all the stages. Calcium treatments, Ca₁, Ca₂, and Ca₃ accelerated the net photosynthesis, transpiration and stomatal conductance by 20.8 %, 18.4 % and 14.3 % respectively over the non-treated plants (Fig. 2). The application of calcium to *Cassia sophora* L. plants favourably influenced the concentrations of the photosynthetic pigments (chlorophyll and carotenoids content) at 120, 150 and 180 DAS (Fig. 1). The chlorophyll and carotenoid contents increased with the increase in calcium levels. The maximum total chlorophyll content was given by Ca₃ and the percent increase was 10.4, 15.2 and 12.6 % at 120, 150 and 180 DAS, respectively than that of control (Fig. 2). The total carotenoid content were also increased by Ca₃ level which proved optimum and recorded 15.3, 15.8 and 13.4 % higher value at 120, 150 and 180 DAS, respectively than the Ca₀ (Fig. 1).

Exogenous application of calcium generated maximum nitrate reductase activity at all stages over the respective control. A progressive increase was observed in Ca₃ by 40.6, 38.2 and 30.5 %, at the respective stages. Ca₀ recorded minimum value at all stages over the control

Table 1. Effect of five basal levels of calcium (Ca₀, Ca₁, Ca₂, Ca₃ and Ca₄, respectively) on fresh and dry weights per plant of *Senna sophora* (*Cassia sophora* L.) at 120, 150 and 180 DAS. LSD ($p \leq 0.05$) was employed to separate the means in the Table 1. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of three replicates.

Calcium (mg Ca kg ⁻¹ soil)	Fresh weight per plant (g)			Dry weight per plant (g)		
	DAS			DAS		
	120	150	180	120	150	180
Ca ₀	13.20 ^c	64.08 ^c	121.90 ^d	2.45 ^c	11.84 ^d	21.78 ^d
Ca ₁	14.10 ^{bc}	67.19 ^c	128.02 ^c	2.71 ^{bc}	13.64 ^c	25.87 ^c
Ca ₂	14.75 ^b	71.95 ^b	135.99 ^b	2.96 ^b	15.24 ^b	28.60 ^b
Ca ₃	16.15 ^a	82.84 ^a	150.14 ^a	3.37 ^a	17.38 ^a	32.70 ^a
Ca ₄	15.85 ^a	82.13 ^a	149.60 ^a	3.30 ^a	16.95 ^a	32.36 ^a
LSD at 5%	1.07	3.58	4.85	0.315	1.55	2.43

Table 2. Effect of five basal levels of calcium (Ca_0 , Ca_1 , Ca_2 , Ca_3 and Ca_4 , respectively) on yield and quality attributes of *Senna sophera* (*Cassia sophera* L.) at harvest (210 DAS). LSD ($p \leq 0.05$) was employed to separate the means in the Table 2. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of three replicates.

Calcium (mg Ca kg^{-1} soil)	Number of pods per plant	Number of seeds per pod	100-seed weight (g)	Seed-yield per plant (g)	Seed-protein content (%)	Total anthraquinone glycosides content (%)
Ca_0	57.0 ^d	33.0 ^a	1.85 ^a	31.54 ^d	15.43 ^d	0.013 ^a
Ca_1	60.3 ^c	33.0 ^a	1.83 ^a	33.46 ^c	15.87 ^c	0.014 ^a
Ca_2	64.2 ^b	33.3 ^a	1.87 ^a	35.47 ^b	16.32 ^b	0.016 ^a
Ca_3	70.6 ^a	33.5 ^a	1.88 ^a	39.03 ^a	17.40 ^a	0.016 ^a
Ca_4	69.2 ^a	33.4 ^a	1.85 ^a	38.80 ^a	17.45 ^a	0.015 ^a
LSD at 5 %	3.19	0.502	0.05	1.50	0.434	0.004

(Fig. 2). Carbonic anhydrase activity was significantly enhanced by the calcium application at 120, 150 and 180 DAS (Fig. 2). The CA activity was found highest in Ca_3 treated plants over Ca_0 at all three growth stages. This treatment increased CA activity exceeding the control by 17.2, 15.1 and 13.2 % at 120, 150 and 180 DAS, respectively in comparison to the control plants (Fig.2).

The effect of calcium levels on nitrogen content was found maximum at all the stages. Treatment Ca_3 proved optimum and registered 21.9, 16.9 and 20.6 % higher

value at 120, 150 and 180 DAS, respectively over the control (Fig. 3). Data presented in Figure 3 indicates that Ca_3 proved optimum and increased the phosphorus content by 26.3, 19.6 and 14.3 % at 120, 150 and 180 DAS, respectively over the control (Fig. 3). Leaf-potassium content was also affected significantly by the calcium levels. Ca_3 treatment proved superior to the rest of the treatments and recorded highest value for potassium content by 16.8, 22.6 and 18.2 % at 120, 150 and 180 DAS, respectively over Ca_0 (Fig. 3). The calcium application also contributed to the increase in total

Fig. 1. Effect of five calcium levels (Ca_0 , Ca_1 , Ca_2 , Ca_3 and Ca_4 , respectively) on net photosynthetic rate (A), transpiration rate and stomatal conductance (B) of *Senna sophera* (*Cassia sophera* L.) studied at 150 DAS. LSD ($p \leq 0.05$) was employed to separate the means at the top of the bars. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of three replicates.

Fig. 2. Effect of five calcium levels (Ca₀, Ca₁, Ca₂, Ca₃ and Ca₄, respectively) on total chlorophyll (A) and carotenoid contents (B), nitrate reductase activity (C) and carbonic anhydrase activity (D) of *Senna sophora* (*Cassia sophora* L.) studied at 120, 150 and 180 DAS. LSD ($p \leq 0.05$) was employed to separate the means at the top of the bars. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of three replicates.

calcium content at 120, 150 and 180 DAS. Treatment Ca₃ significantly enhanced calcium content by 24.7, 26.4 and 20.8 % at 120, 150 and 180 DAS, respectively over the control. Ca₀ showed poorest effect (Fig. 3).

Yield and quality attributes

The effect of calcium on number of pods per plant was found significant over the Control. Treatment Ca₃ significantly enhanced 23.9 % more number of pods over the Ca₀, which gave the least value (Table 2). However, the effect of Ca₄ showed parity with that of

Ca₃. The effect of calcium application on number of seeds per pod and 100-seed weight was found non-significant (Table 2). As expected, Ca₃ gave highest value for seed-yield (23.7 % higher than Control) as shown in Table 2.

The basal calcium increased seed-protein content significantly in comparison to control (Table 2). Of the five calcium levels, treatment Ca₃ proved superior to other treatments and gave maximum seed-protein content by 12.8 % over the control (Table 2). However, the effect of Ca₄ was equaled with that of Ca₃. Seed-protein

Fig. 3. Effect of five calcium levels (Ca₀, Ca₁, Ca₂, Ca₃ and Ca₄, respectively) on leaf –nitrogen (A), phosphorus (B), potassium (C) and calcium (D) contents of *Senna sophera* (*Cassia sophera* L.) studied at 120, 150 and 180 DAS. LSD ($p \leq 0.05$) was employed to separate the means at the top of the bars. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of three replicates.

content was found minimum in non treated calcium plants (control). The effect of calcium application on total anthraquinone glycosides content in seeds was found non-significant (Table 2).

DISCUSSION

The present study clearly indicates that application of calcium significantly enhanced growth attributes (plant fresh and dry weights) at all the growth stages of *Cassia sophera* L. Ca₃ level of calcium (120 mg Ca kg⁻¹ soil)

proved the best and significantly increased fresh and dry weights per plant over the respective controls at the respective growth stages (Table 1).

The growth of a plant depends on the availability of calcium in the soils and that controls a wide variety of physiological and cellular processes (Nayyar, 2003). Calcium regulates many physiological functions such as, cell elongation, cell division and differentiation, stabilization of cell wall and plasma membrane, membrane stability and cell integrity, polymerization of proteins, regulation of enzymes and fruit development (Marschner,

1974; Hanson, 1983; Kirkby and Pilbeam, 1984; Hepler and Wayne, 1985; Mengal and Kirkby, 1987; Berridge *et al.*, 1998; Marschner, 2002; Ng and McAnish, 2003; Nayyar, 2003; White and Broadley, 2003; Hirschi, 2004). Furthermore, calcium plays a crucial role in plant growth and development by regulating cell division and modulating the enzyme activities of the cells (Rasmussen and Means, 1989). The enhancing effect of calcium on growth attributes of the medicinal plant corroborates the findings of Savithramma (2002, 2004), Naeem *et al.* (2005), Khan and Naeem (2006) and Naeem and Khan (2006).

It has been observed that the leaves of calcium treated plants trap more sunlight to increase the rate of photosynthesis as compared to the control plants (Khan *et al.*, 2001). Furthermore, calcium is very important for plant metabolism in general and for photosynthetic process in particular (Ramalho *et al.*, 1995). Calcium plays a dual function in photosynthesis including Ca^{2+} ions, bounding to photosystem II (PS II) involved in oxygen evolution and, provide a structural role in the peripheral antenna assembly (Ramalho *et al.*, 1995).

The photosynthetic pigments (chlorophyll and carotenoids content) were maximally exhibited by plant leaves in response to calcium application at the respective growth stages (Fig. 2). These content were enhanced in plants treated with Ca_3 at all the growth stages of *Cassia sophera* L. (Fig. 2). These results are in line with the findings of Savithramma (2004), Naeem *et al.* (2005) and Khan and Naeem (2006). As far as total chlorophyll and carotenoids content in leaves of *Cassia sophera* L. are concerned, the maximum content was found at flowering stage (150 DAS) and minimum at pod-filling stage (180 DAS), respectively (Fig. 2).

The effect of exogenous application of calcium on nitrate reductase (NR) activity of *Cassia sophera* L. was significant at all the growth stages as shown in Figure 2. NR activity was significantly enhanced as a result of basal supply of calcium (Ca_3) compared to the control. The reduction of nitrate in the leaves depends upon the presence of calcium ions and a metabolic connection exists between nitrate assimilation and Ca content of leaves (Dekock *et al.*, 1979). However, the role of calcium in nitrogen metabolism depends upon the nitrogen source. In case, the N source is NH_4^+ , the application of calcium increases NH_4^+ uptake and improves N utilization in the plant, resulting in increased production of dry matter (Fenn *et al.*, 1994). The role of Ca as an activator for nitrate reductase activity in leaves has been reported by Sane *et al.* (1987), Fenn *et al.*

(1994) and Ruiz *et al.* (1999). The above results are further supported by a strong positive correlation between NR activity and leaf-Ca content in the present study (Table 3). In addition, NR activity decreased with increasing age of the plants, comparatively slowly from vegetative to flowering stage and it decreases rapidly from flowering to fruiting stage of *Cassia sophera* L. (Naeem and Khan, 2009).

Carbonic anhydrase enzyme has an active role in photosynthesis, which is established by its presence in all photosynthesizing tissues. This enzyme catalyzes the reversible hydration of CO_2 , thereby increasing its availability for ribulose 1,5- biphosphate carboxylase-oxygenase (RuBisCO) (Badger and Price, 1994; Khan *et al.*, 2004). The enhancement in CA activity due to external calcium application could be the result of readily availability of this nutrient at the site of its metabolism. Furthermore, the increase in CA activity would enhance the rate of CO_2 assimilation. Consequently this was reflected in production of enhanced dry matter (Fig. 2) presumably owing to the improvement in net photosynthetic rate. A probable cause for the enhancement of CA activity might be the influence of calcium on the *de novo* synthesis of CA, which involves translation/transcription machinery of the plant cells (Okabe *et al.*, 1980).

The effect of calcium on leaf -N, P, K and Ca contents was positively significant at all the three growth stages (Fig. 3). The control plants which were not supplied with nutrients, had to totally depend on the nutrients present in low concentration in the soil. On the other hand, calcium supplied plants got an adequate supply, ensuring continuous absorption by roots followed by smooth translocation to the foliage. The increased accumulation of N, P, K and Ca nutrients due to calcium supply may be because of enhanced dry matter accumulation, synthesis of higher amounts of chlorophyll and carotenoids and increased activity of NR (Table 1 & Fig. 2-3). In fact, increasing supply of calcium concentrations has been reported to markedly augment the absorption of N, P, K and Ca contents in legumes and other crops as reported by Bell *et al.* (1989), Ruiz *et al.* (1999), Savithramma (2002, 2004) and Naeem and Khan (2006). Leaf -N, P, K and Ca contents decreased with increasing age of the plants, as observed in Fig. 3. Such a decrease in N, P, K and Ca contents of leaf may be due to continuous utilization of these nutrients by the developing pods and their translocation from vegetative (sink) to reproductive parts (source).

The yield attributes (number of pods and seed-yield

Table 3. Correlation coefficient values (r) of various attributes with leaf-phosphorus and calcium contents (120 DAS), seed-yield and seed-protein content (210 DAS) of *Senna sophora* (*Cassia sophora* L.)

Attributes	DAS	Leaf-phosphorus content	Leaf-calcium content	Seed-yield	Seed-protein content
Fresh weight per plant	120	0.991**	0.985**	0.997**	0.987**
	150	0.996**	0.982**	0.994**	0.998**
	180	0.999**	0.992**	0.999**	0.998**
Dry weight per plant	120	0.995**	0.992**	0.998**	0.988**
	150	0.988**	0.990**	0.993**	0.977**
	180	0.984**	0.986**	0.991**	0.977**
Total chlorophyll content	120	0.994**	0.987**	0.999**	0.995**
	150	0.978**	0.978**	0.987**	0.974**
	180	0.984**	0.995**	0.979**	0.963**
Total carotenoid content	120	0.996**	0.994**	0.990**	0.989**
	150	0.994**	0.979**	0.993**	0.997**
	180	0.985**	0.971**	0.992**	0.990**
Nitrate reductase activity	120	0.975**	0.976**	0.985**	0.970**
	150	0.987**	0.978**	0.995**	0.991**
	180	0.991**	0.990**	0.996**	0.987**
Leaf-nitrogen content	120	0.926*	0.932*	0.944*	0.923*
	150	0.981**	0.970**	0.990**	0.986**
	180	0.979**	0.970**	0.989**	0.984**
Leaf-phosphorus content	120	—	0.995**	0.998**	0.995**
	150	0.993**	0.981**	0.995**	0.991**
	180	0.958*	0.926*	0.957*	0.971**
Leaf-potassium content	120	0.999**	0.998**	0.996**	0.991**
	150	0.990**	0.985**	0.997**	0.990**
	180	0.989**	0.987**	0.995**	0.986**
Leaf-calcium content	120	0.995**	—	0.992**	0.983**
	150	0.981**	0.979**	0.990**	0.980**
	180	0.959**	0.967**	0.971**	0.949*
Number of pods per plant	210	0.996**	0.992**	0.998**	0.989**
Number of seeds per pod	210	0.966**	0.973**	0.954*	0.938*
100-seed weight	210	0.581 ^{NS}	0.604 ^{NS}	0.552 ^{NS}	0.512 ^{NS}
Seed-yield per plant	210	0.998**	0.992**	—	0.996**
Seed-protein content	210	0.995**	0.983**	0.996**	—
Total anthraquinone glycosides content	210	0.794 ^{NS}	0.836 ^{NS}	0.796 ^{NS}	0.737 ^{NS}

*Significant at $P>0.05$ (0.878); **Significant at $P>0.01$ (0.959); NS = Non-significant DAS = Days after sowing

per plant) were significantly increased after various levels of Ca application (Table 2). A greater seed-yield of calcium treated plants was mainly due to increased number of pods (Table 2). The role of calcium in increasing seed-yield can possibly be ascribed to its involvement in the processes of photosynthesis and translocation of carbohydrates to young pods (Sawan *et al.*, 2001). The results are in accordance with the findings of others in this regard (Khan *et al.*, 2001; Naeem *et al.*, 2005; Naeem and Khan, 2006). The results of the present study are also supported by correlation studies where seed-yield was found to be significantly and positively related to most of the attributes studied at various stages of growth of *Cassia sophera* L. Additionally seed-yield was significantly correlated with fresh and dry weights, total chlorophyll and carotenoids contents, leaf-N, P, K and Ca contents, NR activity and yield attributes (Table 3).

External application of calcium increased seed-protein content significantly as compared to control. Of the five levels, Ca₃ proved the best and enhanced seed-protein content over no calcium application (Table 2). It is an established fact that calcium binds certain proteins known as calmodulins (CaM) that are directly stimulated by calcium. These proteins phosphorylate several enzymes to activate them (Marschner, 2002). Moreover, calcium increased accumulation of N, P, K and Ca contents that might have been responsible for the enhanced synthesis of proteins during seed development. A favourable effect of application of calcium on seed-protein content has also been reported by Savithramma (2004), Naeem *et al.* (2005) and Naeem and Khan (2006). The strong positive correlation of leaf-N, P, K and Ca contents with seed-protein content at various growth stages found in the present study further supports this view (Table 3).

The effect of calcium on anthraquinone glycosides content in the seeds of *Cassia sophera* L. was found non-significant (Table 2). Anthraquinones compound in plants, occurring as glycosides with one or more sugar molecules. They possess astringent, purgative, anti-inflammatory, antitumour and bactericide effects. Anthraquinone glycosides act as stimulant cathartics and increase the tone of the smooth muscle in the wall of large intestine. They also participate in the processes of metabolism, respiration, division of cells, oxidative phosphorylation, complexation with DNA and RNA, and perhaps, in other physiological processes of vital importance. In higher plants, anthraquinones are present in oxidized, reduced, glycosides and condensed forms. Furthermore, anthraquinones are used as dyes,

pigments, analytical reagents and chemical means for plant protection (Muzychkina, 1998; Thomson, 1987, 1996).

CONCLUSION

Thus on the basis of the present study, it was concluded that a basal dose of calcium (120 mg Ca kg⁻¹ soil) improved the physiological, biochemical and yield performance of the crop. The low level of calcium in the soil of Aligarh, Western Uttar Pradesh, India may be one of the main causes of poor biological yield of *Cassia sophera* L. Thus, the economic dose of calcium might be recommended for maximizing the productivity and quality of *Cassia sophera* L. used as a drug in the modern as well as traditional systems of medicine.

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