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Calcium Chloride Stimulates Crop Yield, Photosynthetic Efficiency, Enzyme Activities and Nutraceuticals of Coffee Senna (*Senna occidentalis* L.) under Calcium-Deficient Soil

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ABSTRACT

Plant biological yield appears to be comparatively low in calcium-deficient soil of Aligarh, western Uttar Pradesh, India. Ca deficiency poses a serious yield and quality limitation for several crops, including medicinal herbs, in this region of India. In view of the importance of coffee senna as a medicinal legume, it was hypothesized that calcium application through soil could enhance crop productivity, photosynthetic efficiency, enzymatic activities and nutraceuticals. 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 (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) applied as calcium chloride ($CaCl_2$). The performance of the crop was assessed in terms of various growth, physiological, biochemical, yield and quality attributes at 120, 270, 300 and 330 days after sowing. Calcium application effectively increased most of the parameters studied. Of the five calcium levels, Ca_3 stimulated most of the attributes studied at the three growth stages (120, 270 and 300 DAS). In fact, Ca_3 increased seed-yield and seed-protein content by 27.6 and 10.6%, respectively, compared to control plants.

Keywords: carbonic anhydrase, $CaCl_2$, nitrate reductase, medicinal plant, tyrosinase

INTRODUCTION

Coffee senna (*Senna occidentalis* L.) of the family Fabaceae is also known as 'Negro-coffee' and locally known as 'Badi Kasondi'. It is an erect, foetid annual herb or under shrub, 60-150 cm in height. Leaves are 15-20 cm long, leaflets are ovate to ovate-lanceolate. The pods are brown, flat, slightly curved and 5-12 cm long. It contains 40 or more brown to dark-olive green, hard, shining, ovoid seeds about 4 mm long (The Wealth of India 1992). It is grown throughout tropical and subtropical countries of the world including India, for its roots, flowers and seeds, which have medicinal properties. It is a decongestant and used for the treatment of coughs, whooping cough, convulsions, and heart disease (The Wealth of India 1992). Furthermore, the seeds have been used as a substitute for coffee (The Wealth of India 1992; Morris 1999). The leaves and seeds of coffee senna contain anthraquinones (**Fig. 1**), chrysophenol, emodin, and their glycosides, physcion and rhein (The Wealth of India 1992). Leaf powder is used as an analgesic, antibacterial, anti-hepatotoxic, antifungal, anti-inflammatory, antiseptic, antiparasitic, antiviral, carminative, laxative, purgative and vermifuge (The Wealth of India 1992). The plant parts are used to cure sore eyes, haematuria, rheumatism, typhoid, asthma, leprosy, ringworm and disorders of hemoglobin. A decoction of the plant is used in hysteria, dysentery, itching, inflammation of the rectum and scorpion stings. The herb is reportedly used as a condiment and in perfumery (The Wealth of India 1992).

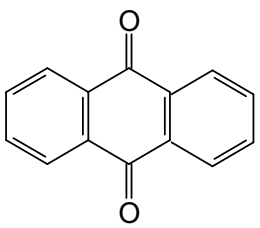

Fig. 1 Structural formula of anthraquinone.

However, plant biological yield appears to be comparatively low in Aligarh soils which are calcium-deficient (Khan and Mohammad 2006; Naeem and Khan 2006; Naeem *et al.* 2009a, 2009b). The optimal quantity of fertilizer application is one of the important factors for achieving high yield. In fact, mineral nutrients play a pivotal role in crop production especially for exploiting the yield potential of these plants. Moreover, the yield of most crop plants increases linearly within limits with the amount of fertilizer that they absorb (Franz 1983; Loomis and Connor 1992).

Thus, an experiment was designed to assess whether low calcium level of the soil in this region could be a possible reason for poor biological yield of coffee senna. However, no work has been done so far regarding the calcium requirement for this medicinal herb.

MATERIALS AND METHODS

Reagents

All reagents were purchased from E. Merck India Ltd., Mumbai, India.

Plant material and growth conditions

Healthy coffee senna seeds were received from the USDA-ARS, Plant Genetic Conservation Resources Unit, Griffin, GA, USA. Seeds of uniform size were selected and their viability was tested using 1% tetrazolium salt. The seeds were surface sterilized with 95% ethyl alcohol for 5 min and then washed thoroughly with distilled water before sowing.

Prior to sowing seeds, 5.0 kg of a homogenous mixture of soil and farmyard manure (4: 1) was filled into each pot. Physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2)

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– 8.0, E.C. (1:2) -0.48 m mhos/cm, available N, P and K – 97.46, 8.21 and 147.0 mg/kg soil, respectively and calcium carbonate was low. The soil samples were tested at the Government Soil Testing Laboratory, Quarsi Farm, Aligarh. A uniform basal level of P (10 mg/kg soil) was basally applied. Then seeds were sown at a depth of 2 cm in earthen pots (25 cm diameter × 25 cm height) containing sandy-loam soil.

Experimental design

A pot culture experiment was conducted in a net house at the Botany Department, A.M.U., Aligarh (27° 52' N latitude, 78° 51' E longitude, and 187.45 m altitude). Growth and biochemical attributes of coffee senna were determined at 120 (vegetative stage), 270 (flowering stage) and 300 (pod-filling stage) days after sowing (DAS). The plants were dried at 80°C for 24 h. The experiment was conducted according to a simple randomized complete block design using five levels of calcium, viz. 0, 40, 80, 120 and 160 mg Ca kg^{-1} soil (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) applied as calcium chloride ($CaCl_2$) at the time of sowing. Each treatment was replicated three times and each replicate had three plants. One healthy plant was maintained in each pot. The pots were watered thoroughly. Plants were grown under naturally illuminated environmental conditions.

Growth analysis

A simple pot experiment was designed in terms of various growth, physiological, biochemical, yield and quality attributes to find out the response of coffee senna at different growth stages by applying basal levels of calcium. The plants were sampled at 120, 270 and 300 DAS. At these three growth stages, three plants of each treatment were carefully 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 the fresh weight of plants was recorded. The plants were dried at 80°C for 24 h, and dry weights were then recorded.

Yield analysis

At harvest (330 DAS), nine plants of each treatment were randomly uprooted and were used for computing yield attributes including the number of pods $plant^{-1}$, the number of seeds pod^{-1}, 100-seed weight and seed-yield $plant^{-1}$. The pods were threshed, cleaned and then counted. Afterwards, the number of seeds pod^{-1} and 100-seed weight were recorded. Seed-yield was calculated accordingly.

Physiological and biochemical analysis

The youngest fully expanded fresh leaves were used for the analysis of various physiological and biochemical attributes, including total chlorophyll and carotenoid content, nitrate reductase activity and carbonic anhydrase activity except for leaf -N, -P, -K and -Ca contents.

1. Measurement of net photosynthetic rate, stomatal conductance and transpiration rate

Net photosynthetic rate (P_N), stomatal conductance (gs) and transpiration rate were measured on cloud-less clear days at 11:00 am on the youngest fully expanded leaves of coffee senna placed in a 1-l leaf chamber of Li-6200 portable photosynthesis system (Lincoln, Nebraska, USA) at light saturation intensity between 11 and 12 h. The IRGA (Infra Red Gas Analyzer) was calibrated and zero was adjusted approximately every half hour during the measurement period. These measurements were recorded three times in each treatment. Photosynthesis was measured only at 270 DAS.

2. Estimation of total chlorophyll and carotenoids content

Total chlorophyll and carotenoids content from fresh leaves were estimated using the method of Mackinney (1941) and MacLachlan and Zalik (1963), respectively. Fresh tissue from interveinal leaf areas was ground using a mortar and pestle in 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 caroteniod content and estimated using a spectrophotometer (Spectronic 20D, Milton Roy, USA). These contents were expressed as mg/g FW.

3. Determination of nitrate reductase activity

NR (NR; E.C. 1.6.6.1) activity was estimated by the intact tissue method developed by Jaworski (1971), which is based on the reduction of nitrate to nitrite as per the following biochemical reaction:

$$NO_3^- + NADH + H^+ \xrightarrow{NR} NO_2^- + NAD^+ + H_2O$$

Nitrite formed was determined spectrophotometrically. 200 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 min at room temperature for colour development. The OD of colour was recorded at 540 nm using a spectrophotometer. NR activity was expressed as nM NO_2^-/g FW/h.

4. Determination of carbonic anhydrase activity

Carbonic anhydrase (CA; E.C. 4.2.1.1) activity in fresh leaves was analyzed using the method described by Dwivedi and Randhawa (1974). 200 mg of fresh leaf pieces were weighed and transferred to Petri dishes. The leaf pieces were dipped in 10 mL of 0.2 M cystein hydrochloride for 20 min at 4°C. 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. CA activity was expressed as μM CO_2/kg leaf FW/s.

5. Nutrient analysis

Leaf samples of 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 hrs. Dried leaves were powdered and the powder was passed through a 72 mesh sieve. The sieved powder was used for N, P, K and Ca contents. 100 mg of oven-dried leaf powder was carefully transferred to a digestion tube and 2 mL of AR grade concentrated sulphuric acid was added to it. It was heated on a temperature controlled assembly for about 2 hrs. After heating, the contents of the tube turned black. It was cooled for about 15 min at room temperature and then 0.5 mL 30% hydrogen peroxide (H_2O_2) was added drop by drop. The addition of H_2O_2 followed by heating was repeated until the contents of the tube turned colourless. The prepared aliquot (H_2O_2-digested material) was used to estimate N, P, K and Ca contents.

6. Estimation of nitrogen

Leaf-nitrogen content was estimated using the method of Lindner (1944). A 10 mL aliquot (H_2O_2-digested material) was placed in a 50-mL volumetric flask. To this, 2 mL of 2.5 N sodium hydroxide and 1 mL of 10% sodium silicate solutions were added to neutralize excess acid and to prevent turbidity, respectively. In a 10 mL graduated test tube, 5 mL aliquot of this solution was removed and 0.5 mL Nessler's reagent was added. The contents of the test tubes were allowed to stand for 5 min for maximum colour development. The OD of the solution was recorded at 525 nm using a spectrophotometer. The reading of each sample was compared with the standard calibration curve of ammonium sulphate to estimate the percent nitrogen content.

7. Estimation of phosphorus

The method of Fiske and Subba Row (1925) was used to estimate the leaf-phosphorus (P) content in the digested material. The same aliquot was used to determine the leaf-P content. A 5 mL aliquot was taken in a 10 mL graduated test tube. To it, 1 mL molybdic

Table 1 Effect of five levels of calcium (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) on plant fresh and dry weights (g) of coffee senna (*Senna occidentalis* L.) at 120, 270 and 300 DAS (means of three replicates). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). The data shown are means of three replicates ± SE.

Attributes	DAS	Calcium (mg Ca kg^{-1} soil)				
		Ca_0	Ca_1	Ca_2	Ca_3	Ca_4
Fresh weight $plant^{-1}$	120	16.93 ± 0.73 c	19.62 ± 0.59 b	19.87 ± 0.73 b	22.04 ± 0.61 a	22.01 ± 0.57 a
	270	102.64 ± 1.02 d	113.75 ± 1.76 c	121.78 ± 1.41 b	136.75 ± 1.67 a	136.32 ± 1.22 a
	300	187.45 ± 1.50 d	200.29 ± 1.34 c	214.06 ± 1.37 b	235.47 ± 1.26 a	234.42 ± 1.37 a
Dry weight $plant^{-1}$	120	3.21 ± 0.16 d	3.48 ± 0.14 c	3.79 ± 0.28 b	4.61 ± 0.16 a	4.56 ± 0.09 a
	270	18.90 ± 0.40 d	21.61 ± 0.34 c	23.12 ± 0.47 b	27.35 ± 0.31 a	26.9 ± 0.37 a
	300	34.12 ± 0.47 d	39.35 ± 0.37 c	43.19 ± 0.34 b	47.68 ± 0.24 a	47.35 ± 0.29 a

Table 2 Effect of five levels of calcium (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) on number of pods $plant^{-1}$, number of seeds pod^{-1}, 100-seed weight (g) and seed-yield (g) $plant^{-1}$ of coffee senna (*Senna occidentalis* L.) at 330 DAS (means of three replicates). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). The data shown are means of three replicates ± SE.

Attributes	Calcium (mg Ca kg^{-1} soil)				
	Ca_0	Ca_1	Ca_2	Ca_3	Ca_4
Number of pods $plant^{-1}$	54.5 ± 0.29 c	56.8 ± 0.87 c	61.3 ± 1.15 b	69.2 ± 1.04 a	67.3 ± 0.81 a
Number of seeds pod^{-1}	44.6 ± 0.17 a	44.7 ± 0.23 a	44.8 ± 0.12 a	45.3 ± 0.23 a	45.0 ± 0.35 a
100-seed weight	1.82 ± 0.02 a	1.81 ± 0.01 a	1.84 ± 0.02 a	1.85 ± 0.02 a	1.82 ± 0.01 a
Seed-yield $plant^{-1}$	39.16 ± 0.14 d	43.41 ± 0.19 c	45.72 ± 0.15 b	49.98 ± 0.20 a	49.65 ± 0.18 a

acid (2.5%) was added carefully, followed by the addition of 0.4 ml 1-amino-2-naphthol-4-sulphonic acid. When the solution turned blue, the volume was made up to 10 mL with the addition of double distilled water. The solution was shaken for 5 min. The OD of the solution was read at 620 nm using a spectrophotometer.

8. Estimation of potassium and calcium

Potassium (K) and calcium (Ca) contents were analyzed using flame-photometrics. Both leaf-K and -Ca contents in the same aliquot were estimated and recorded with the help of emission spectra using specific filters in a flame-photometer (Model, C150, AIMIL, India). Leaf-N, -P, -K and -Ca contents were expressed in terms of percent dry weight.

9. Estimation of seed-protein content

The seed-protein content was estimated using the method of Lowry *et al.* (1951). Coffee senna seed was ground to a powder using a mortar and pestle. The seed powder was transferred to a mortar to which 5% cold trichloroacetic acid (TCA) was added. Extracted protein was measured at 660 nm using a spectrophotometer. The reading was compared with a calibration curve obtained by using known dilution of standard egg albumin solution and the percent seed protein content was calculated on a dry weight basis.

10. Estimation of total anthraquinone glycosides content

Total anthraquinone glycosides content in seeds were analyzed using the spectrophotometer as described in Standard of ASEAN Herbal Medicine (ASEAN Countries 1993). Seed powder (1.2 g) was refluxed with 30 mL H_2O, centrifuged for 10 minutes and 0.1 mL of 2 M HCL was added and then extracted three times with 15 mL chloroform. The chloroform layer was discarded, and the aqueous layer was collected. Afterward 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 and expressed as per cent on the dry weight basis.

Statistical analysis

The data of all parameters measured were statistically analysed using analysis of variance (ANOVA) by SPSS (ver. 12; SPSS Inc., Chicago, IL, USA). Data were presented as mean ± SE.

RESULTS

Growth and yield attributes

Effect of Ca application on plant fresh and dry weights was found to be statistically significant. Of the five Ca levels, Ca_3 produced maximum plant fresh weight (30.2, 33.2, and 25.6% more than the control) at 120, 270 and 300 DAS, respectively (**Table 1**). Treatment Ca_3 increased plant dry weight by 43.6, 44.7 and 39.7% more than the control at 120, 270 and 300 DAS, respectively (**Table 1**).

The influence of basal calcium on the number of pods/plant was significant compared to the control. Ca_3 produced 27.0% more pods than the control. However, Ca_4 was at par with Ca_3 (**Table 2**). Different Ca levels did not significantly affect the number of seeds pod^{-1} and 100-seed weight (**Table 2**). The data presented in **Table 2** indicates that seed-yield was significant affected by applied calcium levels. Ca_3 generated maximum seed-yield (27.6% more than Ca_0) (**Table 2**).

Physiological and biochemical attributes

According to the data presented in **Fig. 2**, the influence of calcium on photosynthesis was significant. Calcium at the rate in Ca_3 accelerated net photosynthetic rate, transpiration rate and stomatal conductance by 24.5, 13.5 and 14.3% more than the control plants (**Fig. 1**). Ca_3 resulted in maximum total chlorophyll content (12.5, 17.4 and 13.3%, respectively) at 120, 270 and 300 DAS. This treatment also gave maximum carotenoid content, i.e. 12.3, 15.4 and 10.6% higher at 120, 270 and 300 DAS, respectively than Ca_0 (**Fig. 2**).

All levels of calcium generated maximum NR activity over the control at all three growth stages. The highest NR activity (40.4, 31.7 and 29.7%) was observed in treatment Ca_3 at 120, 270 and 300 DAS, respectively (**Fig. 3**).

On the other hand, CA activity was significantly enhanced by the application of Ca at all the three growth stages (**Fig. 3**). Treatment Ca_3 increased CA activity by 17.2, 15.1 and 13.0% at 120, 270 and 300 DAS, respectively compared to the control plants (**Fig. 3**).

Ca_3 enhanced leaf-N content by 20.2, 25.2 and 20.8% at 120, 270 and 300 DAS, respectively (**Fig. 4**). The effect of basal application of calcium significantly affected leaf-P content at all stages except for 300 DAS. Ca_3 gave 21.0 and 16.2% higher leaf-P content than Ca_0 at 120 and 270 DAS (**Fig. 4**). The maximum K content recorded in Ca_3-treated plants was 23.8, 23.4 and 13.6% at 120, 270 and 300 DAS, respectively more than the control (**Fig. 4**). As expected, Ca_3 significantly enhanced the Ca content by 27.3, 22.3 and 19.4%, at 120, 270, and 300 DAS, respectively more than

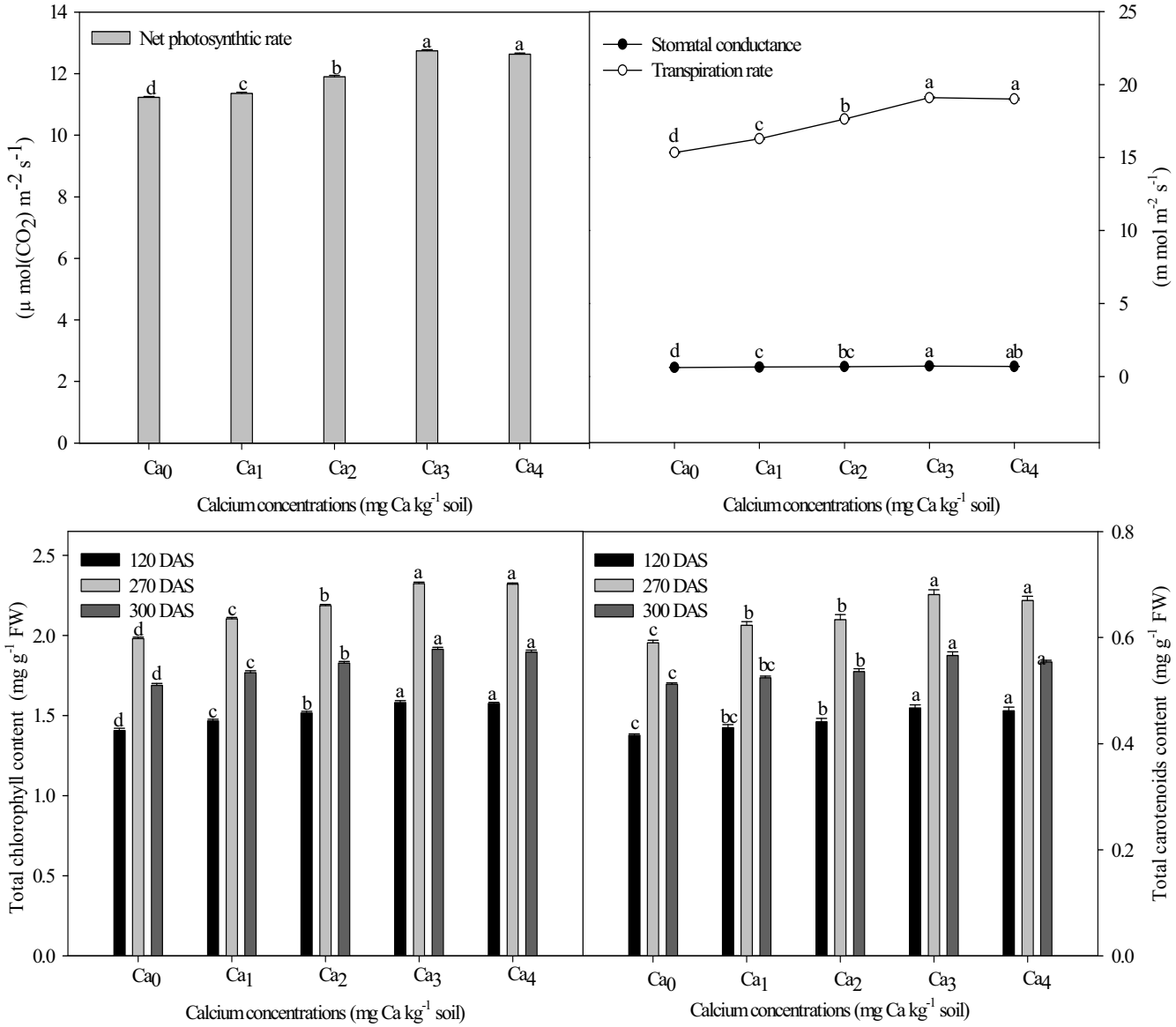

Fig. 2 Effect of five calcium levels (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) on net photosynthetic rate, transpiration rate and stomatal conductance (270 DAS), total chlorophyll and carotenoid content of coffee senna studied at 120, 270 and 300 DAS (means of three replicates). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Error bars (-) show SE.

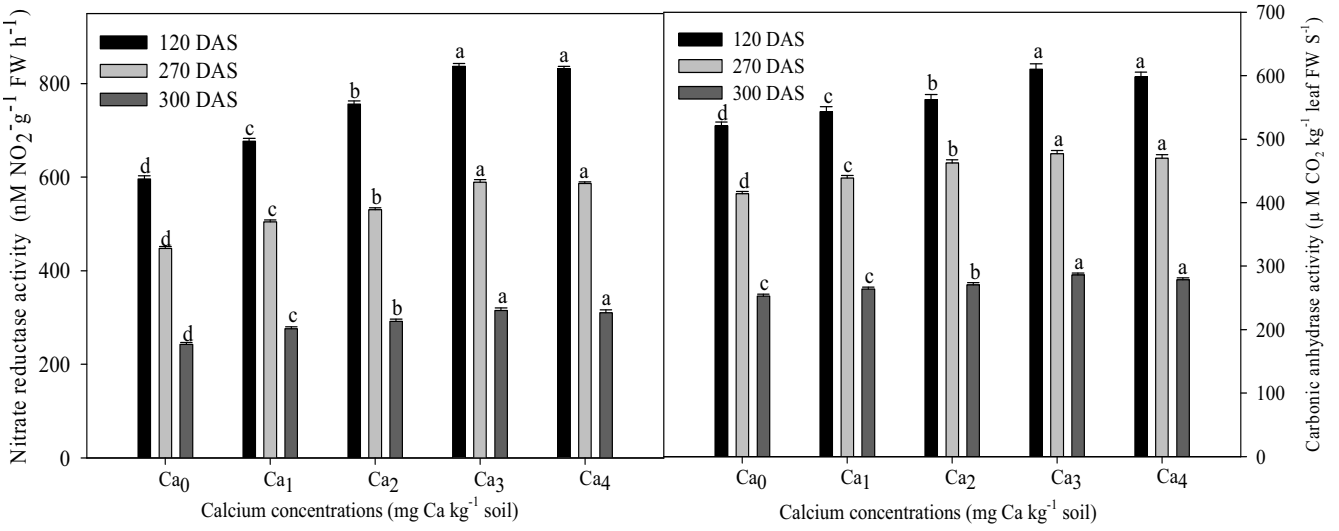

Fig. 3 Effect of five calcium levels (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) on nitrate reductase activity, carbonic anhydrase activity of coffee senna studied at 120, 270 and 300 DAS (means of three replicates). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Error bars (-) show SE.

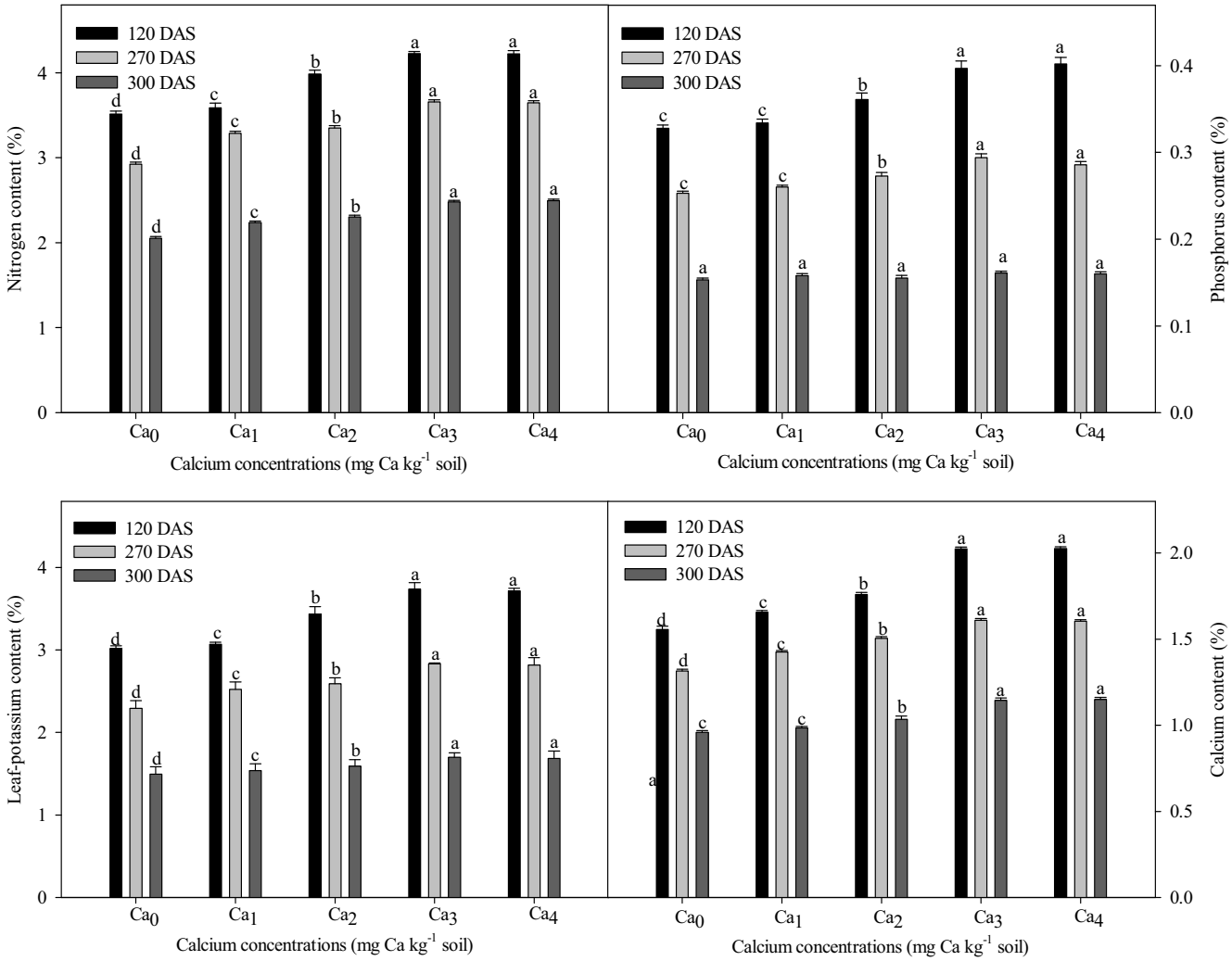

Fig. 4 Effect of five calcium levels (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) on leaf -N, -P, -K and -Ca contents of coffee senna studied at 120, 270 and 300 DAS (means of three replicates). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Error bars (-) show SE.

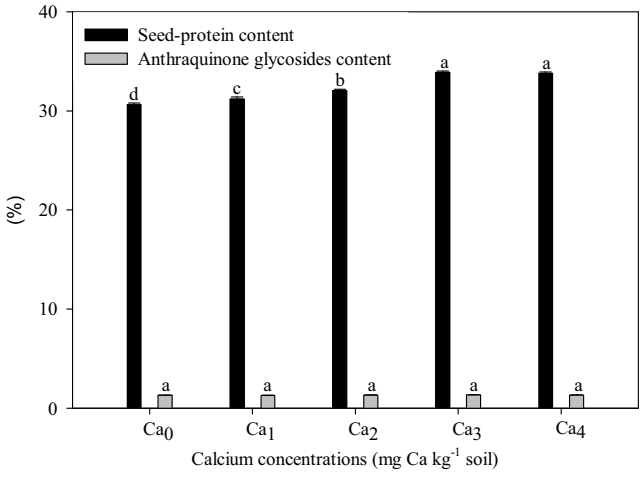

Fig. 5 Effect of five calcium levels (Ca_0, Ca_1, Ca_2, Ca_3 and Ca_4, respectively) on seed-protein and total anthraquinone glycosides content of coffee senna studied at 150 DAS (means of three replicates). Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). Error bars (-) show SE.

Ca_0 (**Fig. 4**).

Ca_3 significantly increased seed-protein content by 10.6% more than the control (**Fig. 5**). The effect of different calcium levels on total anthraquinone glycosides content in the seeds was found to be non-significant (**Fig. 5**).

DISCUSSION

The present study clearly indicates that application of calcium significantly enhanced growth attributes (plant fresh and dry weights) of coffee senna at all growth stages with 120 mg Ca /kg soil proving to be best (**Table 1**).

The growth of a plant depends on the availability of calcium in the soil and that controls a wide variety of physiological and cellular processes (Nayyar 2003). Calcium facilitates rapid translocation of photosynthates from plant parts thus leading to an increase in yield (Mengel and Kirkby 1987). Further, calcium regulates many physiological functions such as cell elongation, cell division and differentiation, stabilization of the cell wall and plasma membrane, membrane stability and cell integrity, polymerization of proteins, regulation of enzymes and fruit development (Marschner 1974; Kirkby and Pilbeam 1984; Hepler and Wayne 1985; Mengel 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 cells (Rasmussen and Means 1989). The enhancing effect of calcium on growth attributes of this medicinal plant corroborates the findings of Savithramma (2002, 2004) for *Boswellia ovalifoliolata* Bal. & Henry, *Pterocarpus santalinus* L. and *Syzygium alternifolium*, Naeem *et al*. (2005) for mungbean (*Vigna radiata* L.), Arshi *et al*. (2005, 2006) for senna (*Cassia angustifolia* Vahl.) and chicory (*Cichorium intybus* L.), Khan and Naeem (2006) for mungbean (*Vigna radiata* L.), Naeem

and Khan (2006) for *Cassia tora* L., Murillo-Amador *et al*. (2007) for cowpea (*Vigna unguiculata* L.), kidney bean (*Phaseolus vulgaris* L.), Dordas (2009) for oregano (*Origanum vulgare* sp. *hirtum*) and Naeem *et al*. (2009a) for senna sophera (*Cassia sophera* L.).

The yield attributes (number of pods and seed-yield/ 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). It clearly indicates that availability of calcium at an early stage of growth helps in active growth and metabolism, which ultimately leads to an increase in yield. Our results are similar to results of other researchers in this regard (Khan *et al*. 2001; Arshi *et al*. 2006; Khan and Naeem 2006; Murillo-Amador *et al*. 2007; Dordas 2009; Naeem *et al*. 2009a). The above researchers found a significant effect of Ca treatment on yield attributes in mustard (*Brassica juncea* L. Czern & Coss), wheat (*Triticum aestivum* L.), senna (*Senna aungustifolia* L.), mungbean (*Vigna radiata* L.), cowpea (*Vigna unguiculata* (L.), kidney bean (*Phaseolus vulgaris* L.) and senna sophera (*Cassia sophera* L.), respectively.

It has been observed that the leaves of calcium-treated plants trap more sunlight to increase the rate of photosynthesis compared to the control plants (Khan *et al*. 2001). Furthermore, calcium is very important for plant metabolism in general and for photosynthesis in particular (Ramalho *et al.* 1995). Calcium plays a dual function in photosynthesis. On the one hand, Ca^{2+} ions, bound to photosystem II (PS II), are involved in oxygen evolution and, on the other, play a structural role in the peripheral antenna assembly (Ramalho *et al.* 1995). Moreover, calcium is effective in the protection of chlorophylls and proteins, as well as in the functional ability of PS-II (Swamy *et al*. 1995; Savithramma 2004).

Total chlorophyll and carotenoids content were maximally exhibited by plant leaves in response to exogenous applications of calcium at all growth stages (**Fig. 2**). Total chlorophyll and carotenoids content were enhanced in plants treated with Ca_3 at all three growth stages (**Fig. 2**). These results are in line with the findings of Savithramma (2004), for *Boswellia ovalifoliolata* Bal. & Henry., *Pterocarpus santalinus* L. and *Syzygium alternifolium*, Naeem *et al*. (2005) for mungbean (*Vigna radiata* L.), Arshi *et al*. (2005, 2006) for senna (*Cassia angustifolia* Vahl.) and chicory (*Cichorium intybus* L.), Khan and Naeem (2006) for mungbean (*Vigna radiata* L.), Naeem and Khan (2006) for *Cassia tora* L., Murillo-Amador *et al*. (2007) for cowpea (*Vigna unguiculata* L.) and kidney bean (*Phaseolus vulgaris* L.) and Naeem *et al*. (2009a) for senna sophera (*Cassia sophera* L.). They observed a positive effect of calcium treatment in their studies for the above-mentioned plants.

As far as total chlorophyll and carotenoids content in leaves of coffee senna are concerned, the maximum content was found at the flowering stage (270 DAS) and minimum at the pod-filling stage (300 DAS), respectively (**Fig. 2**). These results are similar to the results of Naeem *et al*. (2009a) for *Cassia sophera* L. and Naeem *et al.* (2009b) for hyacinth bean. Generally, degradation of chlorophyll and carotenoid contents presumably began with leaf senescence as a result of aging effect, however the highest concentrations of photosynthetic pigments occurred when the leaf blade was fully mature and remained maximum with slight fluctuations for photosynthesis (Lopez-Cantarero *et al*. 1994).

NR activity was significantly enhanced as a result of the basal supply of calcium (Ca_3) compared to the control (**Fig. 3**). 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. If 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 NR activity in leaves has been reported by Sane *et al*. (1987), Fenn *et al.* (1994) and Ruiz *et al.* (1999). In addition, NR activity decreased with increasing age of the plants, comparatively slowly from the vegetative to the flowering stage and it decreases rapidly from the flowering to the fruiting stage of coffee senna (Naeem and Khan 2009; Naeem *et al.* 2009a).

CA 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 RuBisCO (Badger and Price 1994; Khan *et al.* 2004). The enhancement of 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 the enhanced production of dry matter (**Table 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 three growth stages (**Fig. 4**). 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 of it, ensuring continuous absorption by roots followed by smooth translocation to 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**). In fact, increasing the supply of calcium has been reported to markedly augment the absorption of N, P, K and Ca contents in legumes (Bell *et al*. 1989). The beneficial effect of calcium application on N, P, K and Ca contents has also been reported by Bell *et al*. (1989), Ruiz *et al.* (1999), Savithramma (2002, 2004) and Naeem *et al*. (2009a) in various plants. 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 the leaf may be due to continuous utilization of these nutrients by the developing pods and their translocation from vegetative (sink) to reproductive parts (source). These results are similar to the results of Naeem and Khan (2006) for *Cassia tora* L. and Naeem *et al.* (2009) for *Cassia sophera* L.

Like leaf-nutrients and yield attributes, seed-protein content was also significantly enhanced by the application of calcium. Ca_3 proved the best and enhanced seed-protein content over no calcium application (**Fig. 5**). 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 the 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), Naeem and Khan (2006), Bahmaniar and Ghajar (2008) and Naeem *et al*. (2009a) for *Boswellia ovalifoliolata* Bal. & Henry, *Pterocarpus santalinus* L. and *Syzygium alternifolium*, *Cassia tora* L., soybean (*Glycine max* L.) and senna sophera (*Cassia sophera* L.), respectively.

Fig. 4 indicates that the response of calcium levels on anthraquinone glycosides content in the seeds of coffee senna was non-significant. Anthraquinone compounds in plants are found as glycosides with one or more sugar molecules (**Fig. 1**). 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 Muzychkina (1998). 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 (Thomson 1987, 1996; Muzychkina 1998). In higher plants, anthraquinones are present in oxidized, reduced, glycosides and condensed forms. Furthermore, anthraquinones used as dyes, pigments, analytical reagents and chemical means for plant protection (Thomson 1987, 1996; Muzychkina 1998).

CONCLUSION

On the basis of our results it may be postulated that a basal dose of calcium (120 mg kg^{-1} soil) improved the overall performance of the crop and was best. The low level of calcium in this region's soil may be one of the main causes of poor biological yield of coffee senna. Thus, the economic dose of calcium might presumably be recommended for maximizing the productivity and quality of coffee senna used as a drug in modern as well as traditional systems of medicine.

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