J Indian bot Soc Vol 77 (1998) 87-90

INFLUENCE OF CALCIUM STRESS ON RED GRAM METABOLISM

P. SINHA, C.P. SHARMA AND C. CHATTERJEE
Botany Department, Lucknow University, Lucknow-226 007, India
(Accepted September, 1998)

Red gram (*Cajanus cajan* L.) var. upas was grown in refined sand at graded levels of calcium ranging from acute deficiency (0.025 mM l^{-1}) to excess (8 mM l^{-1}). Foliar symptoms of calcium deficiency appeared in plants grown at 0.025 mM Cal^{-1}. Calcium deficient leaves had decreased concentration of chlorphyll and sugars and increased concentration of starch. The activity of AT Pase and α-amylase was significantly reduced and that of acid phosphatase was markedly increased in young leaves of Ca stressed red gram. The quality of seed detriorated in Ca stress and which was reflected in lowered seed yield, protein, sugars and accumulation in phenols, starch and nitrate nitrogen concentrations.

In many plant species the cause of economic loss has been reported to be due to calcium stress (Brewbaker and Kwack, 1963) among many factors. Calcium is essential for both carbohydrate and protein metabolisms (Hewitt, 1983). In several plants, activity of certain enzymes is known to be altered by deficiency or excess of calcium eg. ATPase (Kuiper *et al.*, 1974) α- amylase (Deikman and Jones, 1985) and pyruvate kinase (Tomlinson and Turne, 1973), although calcium is not directly involved in several enzyme activity.

There is hardly any information available on the metabolic activity and seed quality of red gram influenced by Ca stress. Present investigation is aimed at studying the influence of calcium stress on seed quality, biomass, concentration of calcium, chlorophyll, sugars, starch, soluble protein and some enzyme activity in leaves of red gram by growing the plants at graded levels of calcium in refined sand.

MATERIALS AND METHODS

Red gram (*Cajanus cajan* L.) var. Upas was grown in purified sand (Hewitt, 1966) in clay pots, which can hold 12 kg sand, lined with alkathene. Each pot had a central drainage hole, whose rim was covered with clean watch glass lined with glass-wool. Calcium was supplied as $CaCl_2$ at seven graded levels i.e.-0.025,0.5, 1 2, 4, 6 and 8 mM. For each treatment 4 pots were taken. The concentration of the nutrient solution excluding Ca was: (in mM) KNO_3,4; $MgSO_4$2; NaH_2PO_4, 1.33; $NaNO_3$ 8; Fe-EDTA, 0.1; NaCl, 0.1; (in/µm) H_3BO_3, 30, $MnSO_4$, 10; $CuSO_4$,1; $ZnSO_4$, 1;Na_2MoO_4,$NiSO_4$/0.2/,1/;/ $CuSO_4$ No 0.1 and $CoSo_4$, 0.1. Total contribution of calcium from purified sand, water and nutrient solution was <0.004 mM.

Dry weight and calcium in the leaves and seed were determined in clear digests of oven dried leaf material and air dried seeds after wet digestion in nitric and perchloric acid (10:1) using a Systronics flame photometer model-121 at 80 and 168 days respectively. Pods and seed yield were recorded at 168 days. The concentration of chlorophyll, sugars, starch were estimated at 56 days and activity of some enzymes like acid-phosphatase, and α-amylase (Nautiyal *et al.*, 1993) were assayed at 68 days in young leaves. The activity of ATPase has been measured according to Fisher and Hodges (1969). In air dried seeds. the concentration of protein, sugars, starch, phenols and nitrate nitrogen were estimated as per the method described by Sinha and Chatterjee (1994).

All estimations were made in duplicate and analytical results have been tested statistically for significance at 5% level of probability.

RESULTS AND DISCUSSION

The visible calcium deficiency symptoms initiated on young leaves as wavyness of the margins and yellowing of emerging leaves initiating from edges and proceeding towards the apex. After 10 days, affected young leaves showed interveinal chlorosis and puckering of the leaf lamina. Owing to acute deficiency of calcium most of the flowers failed to mature and as a consequence of which the number of pods were reduced. These symptoms/ effects of low calcium were similar to those described for many other crop plants by Hewitt (1983) and for black gram (Nutiyal *et al.*, 1993). Both low (<4 mM/ l^{-1}) and excess (>4 mM l^{-1}) calcium decreased the dry weight of red gram (Fig. 1); calcium is known to play a direct role in stabilizing and maintaining the cell

---

Received December, 1997

Table 1. Effect of variable Ca supply on pod and seed yield and quality in red gram seeds, concentration of Ca, protein, sugars, starch, phenol and nitrate nitrogen (d 168).

	mM Ca supply							L.S.D P=0.05
	0.025	0.5	1	2	4	6	8	
Wt. of plant^{-1}: g	19.1	36.8	43.3	46.5	56.8	43.9	39.4	8.66
Wt. of seeds plant^{-1}: g	9.2	19.7	24.9	27.5	31.5	26.5	24.1	3.10
In seed Calcium: % in fresh wt.	0.13	0.30	0.30	0.41	0.81	0.92	1.52	0.21
Proteins: % in fresh wt.	14.8	18.2	22.0	22.5	28.5	21.8	19.0	5.26
Sugars: % in fresh wt.	1.15	1.31	1.43	1.59	1.35	1.58	1.11	0.03
Starch: % in fresh wt.	44.3	40.2	39.6	36.8	41.8	39.9	33.9	0.89
Phenols: % in fresh wt.	0.020	0.016	0.016	0.015	0.013	0.021	0.015	0.001
Nitrate Nitrogen: % in fresh wt.	0.066	0.053	0.052	0.050	0.060	0.053	0.066	0.001

Table 2. Effect of variable Ca supply on specffic activities of some enzymes in young leaves of red gram (d 60)

	mM Ca supply							L.S.D P=0.05
	0.025	0.5	1	2	4	6	8	
Acid phosphatase:/ug Pi liberated	149	124	125	115	38	68	70	10
Adenosine triphosphatase: /ug Pi liberated	163	253	268	284	268	159	153	30
α-amylase: mg starch hydrolysed	0.018	0.024	0.033	0.027	0.027	0.031	0.065	0.006

membranes by bridging the phosphate and carboxylate group of phospholipids and proteins at membrane surface, this is involved in maintenance of cell organization as a result yield is depressed in low Ca. In calcium stress, marked reduction in seed yield was observed (Table 1), might be due to either failure of pollen germination (Reiss and Herth, 1979) or poor growth of pollen tube at low Ca supply. The growth of pollen tube is maintained and controlled by presence of extra cellular Ca^{2+} (Mascarenhas and Machlis, 1964). This might explain the reduced seed formation at low calcium supply, an observation similar to that of reduced ball yield of calcium deficient cotton (Combrink, 1984). At low Ca the quality and yield of red gram seeds deteriorated because the concentration of protein and sugars decreased and that of phenols, starch and nitrate nitrogen which accumulated in Ca deficiency, which might suggest the essentiality of Ca not only in pollen germination but most probably in the maturation of ovule for the seed formation (Tabel 1).

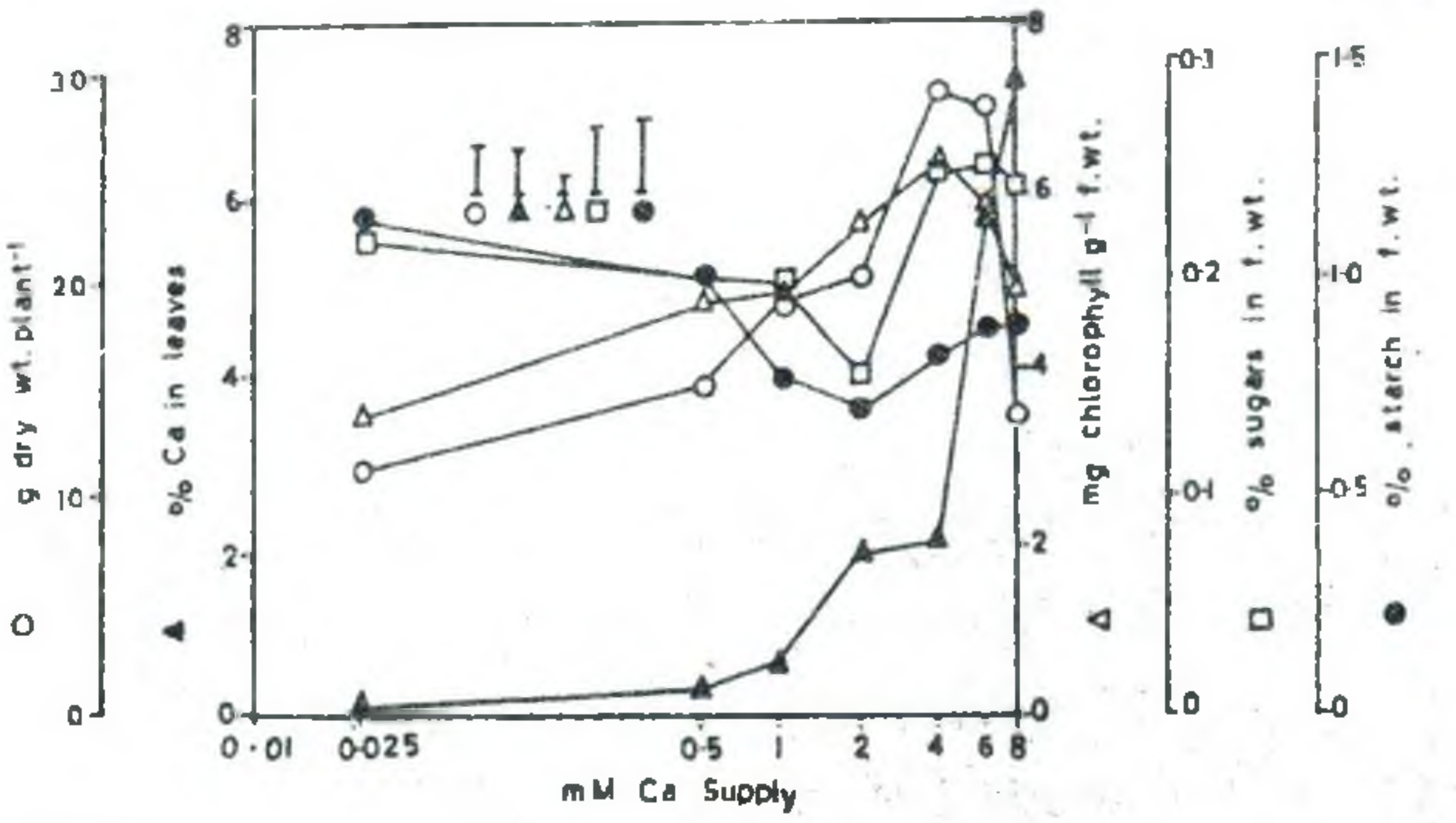

Figure 1 : Effect of variable calcium on the dry weight (o), tissue calcium in young leaves (▲), chlorophyll (Δ), Sugars (□) and starch (•) in red gram leaves. Vertical bars represent LSD (P=0.05).

In red gram, increase in calcium supply from low to excess, increased the calcium concentration in leaves (Fig. 1) and seeds (Table 1). The lower concentration of calcium in young leaves might be due to calcium being immobile, an observation in consonance with the reports on sugarcane (Murthada *et al.*, 1988) and that of Needham (1983) might explain the appearance of visible deficiency symptoms on young leaves only.

The chlorophyll concentration in red gram leaves was decreased significantly by low calcium and not so markedly by excess calcium (Fig.1). In Ca deficient leaves the lowering of chlorophyll concentration is due to the involvement of Ca in stability and maintenance of chloroplast (Barr *et al.*, 1980; Hewitt,1983) and in excess Ca the decrease in chlorophyll is due to induced iron deficiency in such conditions (Mengel and Kirkby, 1983; Hewitt, 1983).

The depression in growth and yield of redgram at excess Ca supply might be due to its inhibitory role in such high amount to the plant (Pooviah and Leopold, 1973).

Deficiency of calcium decreased the sugars in leaves (Fig. 1) and seeds (Table 1) of red gram because low Ca is known to reduce markedly the photosynthetic activity of plants by inhibiting many steps of electron transport sequences (Hartt and Burr, 1967; Barr *et al.*, 1980).

The increase in nitrate nitrogen and phenol concentration in Ca deficient red gram seeds is in agreement with the findings of Paulsen and Harper (1968) in wheat might be the result of Ca playing an important role in influencing nitrogen assimilation and metabolism of pulses (Mehrotra, 1990).

The lowered activity of ATPase and a-amylae in Ca deficient red gram leaves (Table 2) might be due to the role of Ca in activating the synthesis and translocation of the enzymes in plants (Wynjones and Lunt, 1967; Marschner, 1986) or might be due to the direct involvement of calcium in an ion pump to transport ions across the membrane (Dodds and Ellis, 1968; Bush *et al.*, 1986). The increased activity of acid phosphatase in leaves at low and excess Ca supply might be due to high accumulation in organic phosphorus in such conditions as has been reported elsewhere (Nautiyal *et al.*, 1993).

The authors are thankful to ICAR for providing financial help during the course of study.

REFERENCES

Barr R , K S Troxel & F L Crane 1980 EGTA a calcium chelator inhibits electron transport in photosystem II of spinach chloroplasts at two different sites. *Biochem Biophys Res Commun* **92** 106-212.

Brewbaker J L & B H Kwack 1963 The essential role of calcium ion in pollen germination and pollen tube growth; *Am J Bot* **50** 859-865.

Bush D S, M Cornnejo, C Huang & R L Jones 1986 Calcium stimulated secretion of α-amylase during development in barley (*Hordeum vulgara* cultivar. Himalaya) aleurone protoplasts; *Plant Physiol.* **82** 566-574.

Combrink N J J 1984 Physiological leaf abnormalities and ball yield of cotton *(Gossypium hirsutum)* as affected by potassium, calcium and boron nutrition; *SAFR J Plant Soil* **1** 106-110.

Deikman J & R L Jones 1985 Control of α-amylase RNA accumulation by gibberellic acid and calcium in barley aleurone layers; *Pl. Physiol.* **78** 192-198.

Dodds J J A & R J Ellis 1966 Cation stimulated adenosine triphosphate activity in plant cell walls; *Biochem J* **101** 31 P.

Fisher J & T K Hodges 1969 Monovalenion stimulated adenosine triphosphatase from oat root. *Plant Physiol.* **44** 385-395.

Hartt C E & G O Burr 1967 Factors affecting photosythesis in sugarcane; *Proc. Int. Soc. Sugarcane Technol* **12** 590-609.

Hewitt E J 1966 *Sand and Water Culture Methods Used in the Study of Plant Nutrition;* 2nd edn (England; Commonw. Agric. Bureau).

Hewitt E J 1983 The effect of mineral deficiencies and excess on growth and composition. In *"Diagnosis of Mineral Disorders in Plants"*. Vol I Principles (C Bould, E J Hewitt, P Needham & J B D Robinson eds) H M S O London p 54-110.

Kuiper P J C, M Kahrs C E E Stuiver & A Kylin 1974 Lipid composition of whole roots and of Ca^{2+} and Mg^{2+} activated adenosine tirphosphatases from wheat and oat as related to mineral nutrition *Physiol Plant* **32** 33-36.

Marschner H 1986 *Mineral Nutrition of Higher Plants;* Academic Press, London.

Mascarenhas J P & L G Machlis 1964 Chemotropic response of the pollen of *Antirrhinum majus* to calcium, *Pl Physiol* **39** 70-77.

Mehrotra MN K 1990 Secondary Nutrients Ca Mg and S and plant productivity *JPAS* Jaunpur **1** 207-231.

Mengel K & E A Kirkby 1982 *"Principles of Plant Nutrition"* 3rd ed Int Potash Inst Bern Switzerland.

Murtadha H M, J W Maranville & R B Clark 1988 Calcium deficiency in sorghum grown in controlled environmets in relation to nitrate/ammonium ratio and nitrogen source *Agron J* **80** 125-130.

Nautiyal N, L Cahudhary, R Jain & C Chatterjee 1993 Influence of variable calcium on chlorophyll concentration and some enzyme activities in black gram *Proc Indian Natn Sci Acad B 56* p 511-516.

Needham P 1983 The occurrence and treatmennt of Mineral Disorders in Plants In *"Diagnosis of Mineral Disorders in Plants"* Vol I Principles (C Bould E J Hewitt J B D Robinson eds) H M S O London p 137.

Paulsen G M & J E Harper 1968 Evidence for a role of calcium in wheat seedlings. *Pl Physiol* **43** 775-780.

Poovaiah B W & A C Leopold 1973 Deferral of leaf senescence with calcium, *Pl Physiol* **52** 236-239.

Reiss H D & W Herth 1979 Calcium ionophore A 23187 affects localized wall secretion in tip region of pollen tubers of *Lilium longiflorum, Planta* **145** 225-232.

Sinha P & C Chatterjee 1994 Influence of boron on yield and grain quality of pearl millet, *Indian J Agric Sci* **64** 836-840.

Tomlinson J D & J F Turner 1973 Pyruvate kinnase of higher plants, *Biochem Biophys Acta* **329** 128-139.

Wynjones R G & O R Lunt 1967 The function of calcium in plants, *Bot Rev* **33** 407-426.