THE EFFECT OF SULPHUR DEFICIENCY ON ION ACCUMULATION WITH SPECIAL REFERENCE TO ^{15}N AND ^{35}S TRANSPORT AND METABOLISM IN CHICKPEA (*CICER ARIETINUM* L.)

BY

MD. BADRUDDIN
M. Sc. (Rajshahi)

A THESIS
SUBMITTED TO DHAKA UNIVERSITY

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

OCTOBER 1999

THE EFFECT OF SULPHUR DEFICIENCY ON ION ACCUMULATION WITH SPECIAL REFERENCE TO ^{15}N AND ^{35}S TRANSPORT AND METABOLISM IN CHICKPEA (*CICER ARIETINUM* L.)

By

Md. Badruddin
M.Sc. (Rajshahi)

A Thesis
Submitted to Dhaka University

for the degree of

DOCTOR OF PHILOSOPHY

October 1999

To

my

parents

This is to certify that the research work embodying this thesis has been carried out under my supervision in the Plant Physiology laboratory, Department of Botany, University of Dhaka. The research work presented herein is original. This thesis contains no material published elsewhere or extracted from a thesis presented by the candidate for another degree or diploma. No other person's work has been used without due acknowledgment. This thesis has not been published for the award of any other degree or diploma in any other University.

Jadu Lal Karmoker

Professor Jadu Lal Karmoker
Supervisor

Table of contents

Chapter 8

Chapter 9

Abstract

Sulphur deficiency resulted in a decrease in accumulation of ^{15}N in the root and shoot of nonnodulated and nodulated chickpea seedlings ranging from 6% to 50% within 24 h of ^{15}N treatment.

One-day sulphur deficiency caused a 44- and 48-fold increase in ^{35}S accumulation in the root and the shoot of the nodulated chickpea seedling respectively. However, sulphur deficiency increased the accumulation of ^{35}S by a 4.5- and 3- fold in the root and the shoot of the nonnodulated chickpea seedling respectively. Five-day sulphur deficiency also caused an increase in ^{35}S accumulation of similar magnitude in seedlings of both nodulated and nonnodulated genotypes.

Sulphur deficiency decreased the accumulation of K^+ and Na^+ in roots and shoots of both nonnodulated and nodulated chickpea seedlings in solution culture as well as in sand culture except an increased accumulation of these ions in the nonnodulated chickpea seedling at long term deficiency in solution culture.

Ca^{2+} accumulation was increased in the root and shoot of the seedling of the nonnodulated genotype but that was decreased in the shoot of the seedling of

the nodulated genotype following sulphur deficiency treatment. Sulphur deficiency treatment resulted in an increase in Mg^{2+} accumulation in roots and shoots of 15-d and 21-d-old seedlings of both the genotypes in both solution and sand culture conditions.

The accumulation of iron and Zn^{2+} increased in the shoot of 7-d-old seedling but that was decreased in roots of seedlings of both the genotypes. Sulphur deficiency resulted in a decrease in the accumulation of Zn^{2+} and Fe^{2+} in roots and shoots of 15-d-old and 21-d-old seedlings of nonnodulated and nodulated genotypes.

Phosphate accumulation was decreased in roots and shoots of chickpea seedlings of both nonnodulated and nodulated genotypes except an increase in that in the root of the 21-d-old nonnodulated seedling following sulphur deficiency treatment.

The accumulation of nitrate was increased in the seedling of the nonnodulated genotype following sulphur deficiency treatment probably due to decrease in NO_3^- assimilation. In the nodulated genotype, NO_3^- accumulation was decreased in the early stage of growth of the chickpea seedling. But that was increased in the root in subsequent growth periods.

The decreased accumulation of nitrate in the leaf and higher accumulation of that in the root indicated less dependence of chickpea seedlings on nitrate.

Sulphur deficiency decreased nitrate reductase activity (NRA) in both the genotypes.

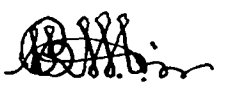
Sulphur deficiency increased the accumulation of reducing and total sugars in the root but that was decreased in the leaf.

Sulphur deficiency caused an increase in total amino acid content in both roots and leaves of seedlings of both the genotypes.

Sulphur deficiency caused a decrease in the dry matter contents of the root and shoot.

The relationship between sulphur deficiency-induced effects on ^{15}N and ion transport with that on metabolism, and the relationship of these factors with the growth of chickpea seedlings will be discussed

This thesis contains no material published elsewhere or extracted from a thesis presented by the candidate for another degree or diploma. No other person's work has been used without acknowledgment. This thesis has not been submitted for the award of any other degree or diploma in any other University.



Md. Badruddin

Acknowledgement

I express my sincere gratitude to my supervisor Professor Jadu Lal Karmoker B.Sc (Hons.), M.Sc. (Dhaka), Ph.D. (La Trobe, Australia) who introduced me to this research problem and guided me during the course of this Ph.D. work and helped me in the preparation of the manuscript. I also thank Mrs. Nilima Karmoker for her inspiration during this research work.

I am grateful to Professor M. M. Haque, Chairman, Department of Botany, University of Dhaka for providing me the research facilities in the Department of Botany. I also thank all the teachers of the Department of Botany, especially Dr. Muniruzzaman Khondker, Dr. Parveen Rashid for their encouragement and help during this research work. I also thank the officers and other members of the staff of the Department of Botany for their help.

I thank the BINA authorities for permitting me to work on the Ph. D. programme. In this respect, I deeply acknowledge the Ex-Director General, Dr. A. J. Miah and the present Director General of BINA and the Ex-Head of Soil Science Division, BINA, Dr. M. Idris Ali for their encouragement and unconditional permission to work in the laboratories of Soil Science Division. I also acknowledge Dr. S. U. Patwary and Dr. S. M. Rahman, Director (Research), BINA for their co-operation. I thank the staff members of Soil Science Division for their help during chemical analysis. I am indebted to Mr. R. K. Dutta, CSO of crop Physiology Division, BINA for his encouragement and coperation. I acknowledge the help of Dr. Ataur Rahman, Director (Adm. and Supp. Serv.), Dr. M.A. Satter , PSO, Mr. Safiqullah, PSO, Mr. Maheb Hossain, Training Officer, BINA for their help during my Ph.D. work. I acknowledge the help of the authorities of IFRB, Atomic Energy Establishment, Savar, Dhaka. I thank Dr. Lokman Hakim, SSO, IFRB, for helping me during ^{35}S analysis.

I thank Professor D. T. Clarkson, Long Ashton Research Station, Bristol University, U.K. for providing ^{15}N rich KNO_3.

I thank my second brother Md. Belal Uddin for his encourgement during my higher studies. I also remember my teacher Late Dr. Sultanul Alam, Professor of Botany, University of Rajshahi who would be very much pleased to see my Ph.D. thesis.

I owe my wife, Mrs. Tahera Begum and my sons, Md. Badrul Hasan and Md. Badrul Hossain for their continuous inspiration during the whole course of my Ph.D. work.

Abbreviations

ae%	atom excess %
AOAC	Association of official analytical chemists. 1970. Methods of analysis: Micro-kjheldahl method. 11th ed. ASS of Offi. Anal. Chem.. Washington, DC, 11th edition P 858.
ASI	Agro Services International Inc. 1983. Agro Services International By Arvel H. Hunter, Vice-president and director of analytical services
DAG	Days after germination
Fe-EDTA	Fe-ethylenediaminetetraacetic acid
gfw	Gram fresh weight
LSC	Liquid scintillation counter
NEDD	Napthyl-ethyldiaminedihydrochloride
NR	Nitrate reductase
NRA	Nitrate reductase activity
POPOP	1,4-bis-2-(4-Methyl-5-phenyloxazolyl)-Benzene
PPO	2,5-diphenyloxazole
PVC	Polyvinyl chloride
S	Sulphur
S-deficient	Sulphur-deficient

Chapter 1

General introduction

Sulphur is considered as the fourth major nutrient element for crops (Platou and Jones 1982) . But sulphur requirement is different for different crops and in the order oilseed > pulses > cereals (Kanwar and Mudahar 1983). Nitrogen and sulphur are linked to each other with respect to plant metabolism. As for example, one part of sulphur is required for every 15 parts of nitrogen during protein synthesis (Dijkshoorn *et al.* 1960; Adams and Sheard 1966; Dijkshoorn and van Wijk 1967; Baily 1986). The largest portion of nitrogen in the plant is in the protein. Ratio of protein-N and protein-S was shown to be 15 to 1 in plant parts (Legget *et al.* 1966). When sulphur is deficient the ratio of total N to total S may exceed the 15:1 ratio resulting in a decrease in the formation of protein. Sulphur deficiency caused an increase in accumulation of inorganic-N (NO_3^- or NH_4^+) and organic nonprotein-N (amino -N or amide-N) in the tissue (Pasricha and Randhawa 1975; Bordeleau *et al.* 1981; Shewry *et al.* 1983). Sulphur deficiency in crops resulted in a reduction of leaf area, seed number, seed weight, delayed floral initiation and anthesis (Janzen 1986; Nybe and Nair 1987; Onkersingh and Nandlal 1987; Ruhal and Paliwal 1987; Naren and Virupaksha 1990; Tiwari 1994). It reduced growth rate and root shoot ratio (Clarkson *et al.*

1989), plant protein, chlorophyll content and photosynthetic CO_2 fixation (Qi B.Z. 1989; Tiwari *et al.* 1994) Sulphur deficiency caused a reduction in the synthesis of nitrate reductase (NR) enzyme which resulted in the minimal supply of reduced nitrogen to the developing organs (Onkersingh and Nandlal 1987). When sulphur was lacking or inadequate, nitrogen assimilation was hampered due to the inadequate supply of sulphur containing amino acids and thus nitrogen uptake and translocation was hampered (Stewart and Porter 1969). All these conditions ultimately reduced yield. Again application of sulphur to the soil reduced molybdenum and selenium toxicity in the soil resulting in an improved plant growth and yield (Bowa *et al.* 1990; Mikkilesen and Wan 1990).

Though sulphur is considered as one of the major nutrients but for many years this received little attention because fertilizer and atmospheric inputs supplied the soil with adequate amounts of sulphur (Scott 1985; Sinclair 1986; Syers *et al.* 1987). Now-a-days, sulphur deficiencies in soil and sulphur deficiency symptoms in the crops is known throughout the world (Eppendorfer 1977; Randall and Wirgley 1986; Richards 1990; Eppendorfer and Eggortn 1992; Knudsen and Pedersen 1993; Schnug and Haneklaus 1994a; 1994b). Sulphur deficiencies had been reported in soil of 35 states of USA (Metson 1973; Tabatabai 1986; Rechcigl 1989). In Latin America, sulphur deficiency was recorded in at least nine countries. Soil of eighteen

African countries was found to be deficient in sulphur (Morris 1986). Sulphur deficiency is prevailing in tropical Asian countries including China, India, Thailand, Indonesia, Bangladesh (Morris 1986; Blair *et al.* 1979). Sulphur deficiency is an important problem in Indian agriculture. About 15 to 20% crop lands of India are facing sulphur deficiency and field responses to sulphur application had been reported for about 30 crops. Sulphur deficiency in Indonesian agriculture was promoted by intensive cropping. Sulphur responses was found in rice as well as in other food crops in Indonesia (Leijder and Aldjabri 1972; Ismunadji *et al.* 1975).

In Bangladesh, many Rabi and Kharif crops like rice, wheat, sugarcane, pulses, tobacco had shown an increase in yield in response to applied sulphur in comparison to no sulphur plots (Islam *et al.* 1986). Bhuiyan and Islam (1986) from their fertilizer trial experiment reported sulphur as the 2nd most limiting nutritional factor after nitrogen for higher crop yield under extensive rice cultivation. Hossain (1978) analysed a large number of soil samples from the farmers field and found sulphur content below 10 ppm suggesting sulphur deficiency. Portch and Islam (1986) reported that the sulphur deficiency condition in Bangladesh was severe than that reported earlier. FAO (Zinc and sulphur deficiency project report, 1983. TCP/BGD/2203, UNDP, Bangladesh) report and scientists from BARI (Bangladesh Agricultural Research Institute) and BRRI (Bangladesh Rice

Research Institute) also reported sulphur deficiency throughout Bangladesh and considered sulphur as a major constraint for higher yield (Short *et al.* 1982; Talukder and Islam 1982a; 1982b; Talukder *et al.* 1984; Portch and Islam 1986).

Since 1960 attention was given to the significance of sulphur in crop production. During the past quarter century world sulphur consumption in all forms was increased. Furthermore, removal of sulphur by crops in the world nearly reached upto 9 million tons (Morris 1986). As a result, sulphur deficiency are occurring at greater frequency throughout the world and particularly in the country like Bangladesh where crop removal of sulphur are fast exceeding the fertilizer application and other inputs. The removal of sulphur in excess of its addition might lead to depletion of this nutrient element from the soil (Morris 1986). The sulphur deficiency problem is likely to become acute in the future as the level of native sulphur is being depleted below the critical level.

The main causes of sulphur deficiency have been identified by many workers (Morris 1986). These may be summarized as:

1) the use of high analysis nitrogenous and phosphatic fertilizers containing little or no sulphur. It is acute in Bangladesh;

2) use of high yielding crop varieties which remove more sulphur from the soil resulting in a depletion of sulphur from the soil reserve;

3) increased use of farm residues as a source of fuel for cooking in many developing countries ;

4) decreased use of sulphur in pesticides and fungicides;

5) increased leaching of sulphur due to increased use of lime and phosphorus on acid soils;

6) control of industrial emission of sulphur.

Major portion of the cultivated land of Bangladesh remain inundated for a long period of the year. Prolonged submergence of soil decreases the availability of native sulphur due to excessive leaching of sulphur as sulphate. Sulphur deficiency in Bangladesh soils are becoming more acute in high textured and irrigated areas where high yielding varieties of crop have been introduced.

1.1 Effects of sulphur deficiency on ^{15}N and nitrate transport

Nitrogen is the key element for plant growth. Both nitrate and ammonium forms of nitrogen are taken up and utilized by plants. The form in which nitrogen translocation occurs is dependent upon nitrogen sources and root

metabolism. Nearly all ammonium-N is assimilated in the root whereas nitrate is transported within the plant through N-rich molecules like glutamine and asparagine. Many workers reported the synergistic relationship of nitrogen and sulphur in plant metabolism and the maximum yield response to nitrogen and sulphur was achieved when the supply of nitrogen and sulphur was balanced (Rehm and Caldwell 1970; Nyborg *et al.* 1974; Jaggi *et al.* 1977; Pasricha *et al.* 1977; Janzen and Bettany 1984; Murphy 1986; Booth *et al.* 1991a, 1991b). Stewart and Porter (1969) reported that the lack of sulphur in the plant environment limits the efficiency of the applied nitrogen, and sulphur nutrition was necessary to achieve the maximum utilization of applied nitrogen. Sulphur and nitrogen fertilization increased the concentration of each other along with increased protein level (Adams and Sheard 1966; Walker and Booth 1992). Protein content was adversely affected under low sulphur level with the concomitant increase in the nonprotein-N in the plant (Pasricha and Randhawa 1975; Bordeleau *et al.* 1981; Shewry *et al.* 1983; Bapat *et al.* 1984). Generally, during grain filling, nitrogen was delivered from the post anthesis nitrogen uptake and from vegetative reserves (Cox *et al.* 1985; 1986). Rao and co-workers (1977) suggested that good varieties were those which would translocate high percentages of leaf and stem nitrogen to developing grains. Thus, nitrogen remobilization from the stem and root is an important additional reserve pool during grain filling. Sulphur in plant tissue might

reflect the relative availability of nitrogen and sulphur for protein synthesis (Metson 1973).

Many researchers used isotopically lebelled ^{15}N-fertilizer to determine the uptake and distribution pattern of nitrogen and its utilization efficiency within the plant (Hill Cotingham and Jones 1975; Kato *et al.* 1984; Broadbent *et al.* 1987; Clarkson *et al.* 1989; Karmoker *et al.* 1991). From the study of the effect of sulphur deficiency on nitrogen transport in barley it was shown that the deprivation of sulphur reduced the uptake and transport of ^{15}N and total nitrogen to the shoot without any measurable change on the growth rate of plants ((Karmoker *et al.* 1991).They also reported that the accumulation of amides in sulphur-deprived roots was probably due to a failure to export either the product of root nitrate assimilates or phloem derived amino-N. Similarly, Burney and Bush (1985) suggested that there appeared to be mutual regulation of both nitrate and sulphate assimilation pathway.

There are reports that sulphur deficiency in many crops resulted in an accumulation of amino compounds like glutamine and asparagine (Adams and Sheard 1966; Eppendorfer 1968; Millard *et al.* 1985; Karmoker *et al.* 1991). In S-deficient plants, the accumulation of glutamine acted to down regulate the nitrogen uptake (Clarkson 1986). Both ammonium and sulphate

influxes in barley roots were found to be depressed in the earliest stage of sulphur deprivation (Clarkson *et al.* 1989). They also reported that due to lack of sulphur, nitrogen uptake was also deminished.

Sulphur deficiency caused an accumulation of soluble nitrogen and a decrease in protein synthesis (Grant 1991). Sulphur and nitrogen fertilizers significantly increased the uptake and concentration of each other along with increase in protein content (Rendig and McComb 1961; Adams and Sheard 1966; Walker and Booth 1992).

Generally, the percent of total nitrogen in the plant is influenced by both the level of sulphur in the culture medium and the nitrogen sources. When inorganic nitrogen was provided either the uptake of nitrogen or utilization of nitrogen in the plant was influenced by the level of sulphur in the nutrient (Stewart and Porter 1969).

1.2 Effects of sulphur deficiency on accumulation of ^{35}S

Smith (1975) working with tobacco found that the rate of SO_4^{2-} transport was controlled primarily by the size of intracellular SO_4^{2-} pool. Accumulation of sulphate was high in cells with small SO_4^{2-} pool and low in cells with large SO_4^{2-}pool. The rate of sulphate accumulation was low in cells grown in sulphur containing medium but it was high in cells grown in S-deficient

condition (Smith 1980; Smith and Lung 1988). Uptake of SO_4^{2-} in S-starved plants was higher than that in plants supplied with sulphur in the beginning (Holobrada 1977). But Dreyfuss (1964) suggested genetic control of SO_4^{2-} uptake. Saccomani and co-workers (1984) reported that ^{35}S uptake at 30 minutes following sulphur deficiency was higher in roots than that in leaves.

1.3.1 **Effects of sulphur deficiency on accumulation of K^+ and Na^+**

Concentrations of potassium and sulphur in plants were found to be interrelated. Higher concentration of potassium was recorded with increasing level of sulphur in plants (Hariram and Dwivedi 1992, Sairam and co-workers 1995). Ligero and co-workers (1981) reported that potassium uptake was depressed when sulphur supply in the medium was low. Andonova (1992) reported in maize that the harmful effect of potassium was enhanced when sulphur supply in the medium was less.

Rennenberg and Kaiser (1989) reported that Na^+ accumulation remained unaffected due to sulphur deficiency in photoheterotrophic tobacco cell culture.

1.3.2 Effects of sulphur deficiency on accumulation of Ca^{2+} and Mg^{2+}

Sairam and co-workers (1995) found enhanced calcium uptake in presence of sulphur in *Phalaris aquatica*. Accumulation of calcium was positively correlated with the supply of sulphur (Ligero *et al.* 1981). Bugakova and co-workers (1981) found that calcium content was depressed when sulphur supply was reduced in the growth medium.

Matocha (1971) reported that magnesium in large quantity influenced sulphur uptake in bermuda grass due to magnesium -sulphur imbalance in the plant.

1.3.3 Effects of sulphur deficiency on accumulation of Fe^{2+} and Zn^{2+}

Hariram and Dwivedi (1992) found synergistic relationship of zinc with sulphur. Sairam and co-workers (1995) reported increased uptake of zinc due to sulphur application in *P. aquatica* in comparison to no sulphur treatment. Zinc transport in upper plant parts was influenced by application of sulphur (Aulakh and Dev 1978, Singh and Ram 1992). Singh and Ram (1992) reported that sulphur application upto 120 kg ha^{-1} progressively increased zinc content in straw and grain of chickpea.

Synergistic relationship of sulphur and iron transport was also reported (Bugakova *et al.* 1981; Singh and Ram 1992).

1.3.4 Effects of sulphur deficiency on accumulation of phosphate and nitrate

Sulphur and phosphorus interact with each other with respect to their uptake and assimilation. At high levels of phosphorus, sulphur deposition in the soil was hampered (Lancaster *et al.* 1971; Aulakh and Pasricha 1977). This might be due to the fact that high phosphorus level increased the availability of the native sulphur in the soil creating the imbalance of sulphur and phosphorus in the soil. Synergistic relationship of sulphur and phosphorus was reported in plant growth and development in soybean (Kumar and Singh 1980) and in chickpea (Hariram and Dwivedi 1992). Phosphorus uptake was increased when sulphur was applied in lower amount (Singh 1970; Gaur *et al.* 1971). Sairam and co-workers (1995) also reported that both sulphur and phosphorus uptake was increased when sulphur was applied along with phosphorus.

Nitrate is an important source of plant nitrogen in upland crops. Whatever form of nitrogen is applied to the soil it is readily converted to nitrate. Synergistic relationship of nitrate and sulphur was reported by many researchers. For example, maximum yield was achieved when the supply of

nitrogen and sulphur in the medium was balanced because of their involvement in protein synthesis (Rehm and Caldwell 1970 ; Nyborg *et al.* 1974; Jaggi *et al.* 1977; Booth *et al.* 1991b). When sulphur was lacking in the growth medium, utilization of reduced nitrogen for the synthesis of amino acids was decreased (Hocking *et al.* 1987; Karmoker *et al.* 1991) and protein synthesis was affected. This resulted in the accumulation of nitrate in the plants (Stewart and Porter 1969). Baker and co-workers (1971) reported that amide-N and nitrate-N were two compounds related to the quality of the cattle food. The higher the quantities of these elements the lower the quality of fodder. High concentration of nitrate due to short supply of sulphur reduced the quality of forage due to accumulation of these two compounds. Higher concentration of nitrate in the external growth medium increased nitrate concentration in the plant parts. But when sulphur supply was increased the nitrate accumulation was reduced.

1.4 **Effects of sulphur deficiency on nitrate reductase**

In plants, nitrate is converted to ammonium before assimilation into organic compounds. In course of reduction of nitrate to ammonium, nitrite is the intermediate compound. Nitrate reductase enzyme catalyses the reduction of nitrate to nitrite (Guerrero *et al.* 1981). The synthesis of nitrate reductase enzyme was interrupted by boron and sulphur stresses (Tucker *et al.* 1961;

Wright and Davison 1964). The synthesis of nitrate reductase enzyme was decreased when sulphur supply in the medium was limited (Onkersingh and Nandlal 1987). Pal and co-workers (1976) recorded an increase in nitrate reductase activity (NRA) in tobacco with increasing levels of sulphur fertilizer. On the other hand, Oputa (1971) reported that NRA in sulphur deficient maize seedlings was only 17% of that found in + S control plants. Hence, it is apparent that sulphur deficiency may decrease nitrate reductase activity.

1.5 Effects of sulphur deficiency on sugar and amino acid level

Rendig and co-workers (1976) reported that accumulation of amide-N in sulphur deficient maize plants was associated with low level of sugars. The low sugar content resulted from the poor photosynthetic ability of chlorotic sulphur deficient plants. Cowling and co-workers (1977) reported a 24- and 8-fold increase in arginine and glutamine accumulation respectively due to sulphur deficiency. Karmoker and co-workers (1991) reported that sulphur deficiency resulted in a decline in uptake and transport of ^{15}N which was associated with a 6- to 8- fold increase in arginine and 2- to 4- fold increase in glutamine accumulation.

1.6 Effects of sulphur deficiency on soluble protein accumulation

The synthesis of soluble protein by plants depends upon their capacity to utilize N and production of reduced N needed for protein synthesis. The level of protein in the plant indicates its efficiency to produce other structural material necessary for the plant. There are reports that sulphur deficiency affects the normal course of soluble protein synthesis (Mertz and Matsumoto 1956; Thibodeau and Jaworski 1975; Passera and Ghisi 1982).

Reports on the effect of sulphur deficiency on ^{15}N transport and distribution, the accumulation and distribution of cations and anions, and nitrate reductase activity in chickpea are rare. Therefore, chickpea was selected as plant material for this research work.

The objectives of this research work are to study the effect of sulphur deficiency on

a) ^{15}N transport at different growth periods of chickpea;

b) accumulation and transport of ^{35}S in chickpea;

c) accumulation and distribution of monovalent and divalent cations and anions at different growth stages of chickpea;

d) nitrate reductase activity and soluble protein content;

e) reducing and total sugars and total free amino acid contents;

f) correlation of all the informations obtained in an attempt to establish a relationship between the biochemical changes like disturbances in reducing and total sugars, total free amino acid level, nitrate reductase activity and ^{15}N and ion transport, and the overall effects of these factors on growth.

Chapter 2

Material and Methods

2.1 Plant material

Chickpea (*Cicer arietinum* L) cv. Nabin (nodulated) and nonnodulated chickpea genotype nod$^-$ 4993 were used as plant material. Seeds were collected through the courtesy of Bangladesh Agricultural Research Institute, Gazipur.

2.2 *Surface sterilization of seeds*: Seeds were surface sterilized to avoid fungal infection by soaking the seeds with 1% sodium hypochloride for one minute followed by washing 7 to 8 times in tap water and three times in distilled water.

2.3 *Nutrient solution*: Letcombe solution was used in all the experiments. The composition of Letcombe solution was as follows:

+ S solution contained 5 mM KNO_3; 2 mM $Ca(NO_3)_2$; 4 mM $NaNO_3$; 1 mM KH_2PO_4 ; 1.5 mM $MgSO_4$; 0.092 mM H_3BO_3; 0.0967 mM Fe-EDTA;

0.0025 mM $CuSO_4$; 0.00016 mM $NH_4Mo_7O_2$; 0.0328 mM $MnSO_4$; 0.00765 mM $ZnSO_4$.

–S solution contained 5 mM KNO_3; 2 mM $Ca(NO_3)_2$; 4 mM $NaNO_3$; 1 mM KH_2PO_4 ; 2 mM $MgCl_2$; 0.092 mM H_3BO_3; 0.0967 mM Fe-EDTA; 0.0016 mM $CuCl_2$; 0.00016 mM $NH_4Mo_7O_2$; 0.036 mM $MnCl_2$; 0.0077 mM $ZnCl_2$.

For N-free solution instead of $NaNO_3$, $Ca(NO_3)_2$ and KNO_3, 1 mM NaCl, 2 mM $CaCl_2$ and 5 mM KCl were used as Na^+, Ca^{2+} and K^+ source respectively.

2.4 Methods of growing plants:

2.4.1 Sand culture technique

2.4.1.1 *Purification of sand*: Quartz sand was soaked in 3% HCl for 24 hours. The sand was then washed thoroughly with running tap water to remove the acid. The sand was finally washed with distilled water until the pH was neutral (Hewitt 1966). The sand was dried in sunlight .

2.4.1.2 *Sand culture of plants*: For growing plants in sand, PVC pots were filled with 5 kg purified and dried quartz sand. Twenty surface sterilized chickpea seeds were sown in sand and moistened with deionized water for

germination. After germination of seeds, the sand was flashed with 0.1 strength Letcombe solution. Seven days after germination 15 uniform seedlings were kept in each pot and the rest was uprooted and then the nutrient solution was applied twice in a week.

2.4.2 *Solution culture technique*: Surface sterilized seeds were soaked in distilled water and aerated for half an hour with the help of an air compressor. Then seeds were blotted and spread over a stainless steel net supported over a plastic tub (7 l capacity) containing approximately 5 l of 0.1 strength Letcombe solution. Seeds were covered by a black polyethylene sheet to avoid light for 48 hours to facilitate their germination. The black cover was then removed and the plants were allowed to grow. The net was placed on the solution in such a way that the solution remain just in touch of the net. The solution was aerated continuously by an air pump (Gallenkamp). After germination of seeds, plastic tubs containing the seedlings were transferred to a growth cabinet (model EA-7BH, Environ. AIR, Sydney, Australia). Plants were grown under 25 ± 1^0C /16^0 ± 1^0C day/night temperature and 10 h/14 h day/night length, relative humidity was 75% by day and 65% at night. The light intensity was 40 thousand lux. The nutrient solution was replenished every three days.

2.5 Methods of studying the accumulation and transport of ^{15}N

Collection of plant material: Plants were grown in solution culture following the method described in section 2.4.2. 500 ml of the freshly prepared 0.1 strength Letcombe solution containing enriched ^{15}N (12.2% atom excess $K^{15}NO_3$) was taken in a 600 ml beaker. In the experimental 0.1 strength Letcombe solution containing ^{15}N, $Ca(NO_3)_2$ was replaced with equivalent amount of $CaCl_2$. + S and –S solutions were prepared using 0.1 strength Letcombe solution with 5 mM $^{15}NO_3$ (12.2% atom excess $K^{15}NO_3$). Experiments were done with 7-d and 15-d-old seedlings. Sulphur deficiency was applied for 1 day and 5 days. Each sulphur deficient plant (1-d or 5-d) was transferred to ^{15}N solution. Root and shoot samples were collected after 6, 12 and 24 hours of ^{15}N application. After collection of samples, roots were washed with 0.1M calcium sulphate for removing free space ions (Karmoker and Van Steveninck 1978). Samples were then dried in an oven at 70^0C for 72 h. Dry weight of each sample was recorded. Three replicates were taken for each treatment.

Digestion of plant material: Plant samples were digested in tecator tubes on a tecator heater (model tecator digestion system 40, 1016 digestor). Samples were taken in tubes. To each of the tubes 5 ml of 1:1 water: sulphuric acid mixture was added and left for 6 hours. Then to each tube, 0.2 g of reduced

iron was added and the tubes were heated initially at 100^0 C for 45 minutes. When water froths disappeared the tubes were removed from the heater and cooled to room temperature. Then to each tube 3 ml of concentrated sulphuric acid and 0.3 g of Na_2SO_4 : $CuSO_4$: selenium powder mixture in the ratio of 25:1:1 were added and the tubes were heated at 370^0C until the digestion was completed with white fumes. Each sample was then made upto final volume of 20 ml.

Distillation and concentration of distillate: Nitrogen was estimated from the digested aliquot by distillation in a micro kjheldhal distillation unit (model Buchi 315, USA, methodology as per AOAC 1970). Released ammonium due to distillation was collected in 0.1N HCl. The collected aliquot was titrated with 0.1N NaOH. After final titration, the aliquot was acidified with 0.005N HCl. This titrated aliquot was then concentrated upto necessary mark (mg N in the distillate x 10µl, volume of capillary tube / 2.2 µg N in the 10 cm Pyrex tube to get a approximate pressure between 1.8 and 2.6 torr in the tube) and was ready for infiltration in the capillary tube.

Sample preparation for ^{15}N measurement : 10 µl of concentrated sample was infiltrated in 1.0 cm capillary tube. Each of the capillary tubes containing the sample was infiltrated in a 10 cm pyrex tube. To each of these pyrex tubes, CuO (15 mg) and CaO (15 mg) were also infiltrated and the

tubes were sealed at high vacuum (3.10^{-5} milli bar) through a sample preparation unit (model No. LH Combitron CM330, Germany). The sample tubes were then kept in a muffle furnace for four hours at 500^0C to complete the reaction with CuO (Dumas reaction). Peak heights of ^{15}N and ^{14}N were taken by ^{15}N analyser (model Jasco N-150 ^{15}N analyser, Japan spectroscopic Co. Ltd, Japan).

Calculation: Respective peak heights were measured and the ratio of ^{15}N and ^{14}N were recorded. Total ^{15}N abundance was calculated according to the formula 100/2R+1, where R = ratio of the peak heights of $^{14}N/^{15}N$. ^{15}N atom excess was obtained by subtracting the ratio with natural abundance of 0.366%. The ^{15}N was expressed as ae% (atom excess %). Total amounts of nitrogen absorbed by the plant parts were calculated following the method of Munoz and co-workers (1993). The calculation was done as follows: total N absorbed (mg N = %N x dry weight x ^{15}N x 10).

2.6 Methods of studying the accumulation and transport of ^{35}S

Collection of plant material: Plants were grown in solution culture following the method described in sec. 2.4.2. Three plants were transferred to each dunking frame (Clarkson *et al.* 1989). ^{35}S was dispensed in + S and –S Letcombe solution to obtain a activity of 30 μCi. 500 ml of freshly

prepared +S and – S 0.1 strength Letcombe solution containing ^{35}S was taken in two different beakers. Dunking frames with plants were transferred to + S and – S solutions. Samples were collected after 10 min., 20 min., 30 min., 1 h, 3 h and 6 h of ^{35}S application. Roots were washed with 0.01 strength Letcombe nutrient solution for half a minute and blotted with tissue paper. The root and shoot were separated and dried in an oven at 70^0C for 72 h.

Preparation of scintillant: 8 g of POPOP and 0.5 g of PPO were dissolved in 1 litre of toluene. 500 ml triton-x-100 was added to this mixture.

2.6.1 Extraction and scintillation counting of ^{35}S

Extraction of ^{35}S: Dried root and shoot samples were transferred to test tubes and each sample was boiled with 10 ml of distilled water for 20 minutes in a water bath. The extraction was repeated with two further changes of 5 ml of distilled water. The extract was then diluted to 20 ml.

Counting of ^{35}S: One ml of sample was taken in a scintillation vial and 9 ml of scintillant was added to each of the vials. Radioactive decay was counted for 1 minute in a scintillation counter (model LSC 2 , NE Technology, UK.)

2.7 Methods of application of sulphur deficiency treatment for ion transport study

Three uniform seedlings (grown as described in sec. 2.4.2.) were attached to a dunking frame (Clarkson *et al.* 1989) and the dunking frames with the seedlings were placed in freshly prepared 0.1 strength Letcombe solution for 24 hours. Then the seedlings were transferred to 5 l of 0.1 strength + S Letcombe solution and 5 l of 0.1 strength – S Letcombe solution. Samples were collected after 1-d, 3-d and 5-d of S-deficiency treatment. Root and shoot samples were separated. Roots were washed in two 1 minute changes of 0.1 mM $CaSO_4$ (50 ml each) to remove free space ions (Karmoker and Van Stevenenick 1978; 1979a; 1979b). Three replicates were taken in each treatment. Samples were then dried in an oven at 70^0C to a constant weight. Dry weight of each sample was recorded.

2.7.1 Digestion of plant material for the measurement of cations and anions

Samples were digested in a tecator heater (model tecator digestion system 40, 1016 digestor). Samples were taken in the tecator tubes separately. To each tube, 2 ml of concentrated sulphuric acid was added and the tube was left for two hours. Two glass balls were placed in each tube to avoid bumping. After heating initially at low temperature the sample tubes were taken out and cooled to room temperature. Then 1 ml of 1:1

perchloric:sulphuric acid (v/v) mixture was added to each tube and the tubes were put on the tecator heater at 370^{0}C and heating was continued until the solution was clear. After cooling , the samples were finally made upto a final volume of 50 ml.

2.7.2 Measurement of K^+, Na^+, Ca^{2+}, Mg^{2+} , Fe^{2+} and Zn^{2+}

The amount of sodium and potassium were measured by flame photometer (model 866150, Corning Ltd., Essex, England). Calcium , magnesium, iron and zinc were measured by atomic absorption spectrophotometer (model 2348, Perkin Elmer, USA) following ASI method.

2.7.3 Digestion of plant material for measurement of phosphate

Phosphate was determined from the aliquot obtained by digesting the plant material according to sec. 2.7.1. Phosphate was determined by vanado-molybdate-nitric yellow colour method (Jackson 1967).

2.7.3.1 Preparation of the vanado-molybdate-nitric acid reagent :

Solution A was prepared just before use by dissolving 25 gram of ammonium molybdate in 400 ml warm distilled water . Solution B was prepared by dissolving 1.25 gram of ammonium metavanadate in 300 ml of

boiling distilled water. 250 ml of HNO_3 was added to the vanadate solution. After cooling the mixture, ammonium molybdate was poured into it and finally it was made upto a final volume of 1000 ml. Solution A and solution B were mixed just before use.

2.7.3.2 Measurement of phosphate

10 ml of each extract was taken in a 50 ml volumetric flask. To each volumetric flask 10 ml of nitric acid-vanadate-molybdate mixture was added and the final volume was made to 50 ml with distilled water. A permanent yellow colour appeared. The optical density of the aliquot was measured at 470 nm with a spectrophotometer (model systronic uv vis spectrophotometer 118, India). A set of standards was also prepared and optical densities of these were also recorded.

2.8 Extraction and measurement of nitrate

Extraction of nitrate: Fresh root and shoot samples were weighed separately and taken in 20 ml test tubes. 10 ml of distilled water was added to each test tube and allowed to stand for half an hour.. The test tubes with the soaked tissue were boiled for half an hour in a boiling water bath. Extracts were

decanted off in separate test tubes and the procedure was repeated twice. Each extract was made upto a final volume of 25 ml with distilled water.

Measurement of nitrate: Nitrate was determined following the method of Cataldo and co-workers (1975). The method was as follows: 0.8 ml of 5% salicylic acid (dissolved in concentrated sulphuric acid) was added to 0.2 ml extract. After 20 minutes 19 ml of 2N NaOH was added and shaken well. A yellow colour appeared. The optical density was measured with a spectrophotometer at 410 nm after cooling the aliquot at room temperature.

2.9 Nitrate reductase assay and measurement of nitrate reductase activity

The NRA was assayed and measured following the method of Stewart and Orebamjo (1979).

The incubation medium was 0.1M K-buffer (pH 7.5), 1.5% KNO_3, 0.02% (v/v) neutronix 600 and 1.5% (v/v) propanol. Root and leaf samples were cut into small sections (nearly 2 mm). For each sample, 50 mg of fresh tissue was taken in 5 ml incubation medium and vacuum infiltrated (10 minutes) as described by Klepper and co-workers (1971). The samples were then transferred to shaking water bath and incubated in darkness at 25^0C for one hour. Then 1 ml of the medium was taken in a test tube for determination of

nitrite. To each of the 1 ml sample, 1 ml of 1% sulphanylic acid and 1 ml of 0.02% NEDD (Napthyl-ethyldiaminedihydrochloride) was added for colour development. The optical density was measured at 540 nm using a spectrophotometer. The difference in nitrite production between zero time and 1 hour of incubation was used to calculate the activity of nitrate reductase and expressed as μ mol NO_2^- gfw^{-1} h^{-1}.

2.10 Extraction and measurement of total free amino acid

Extraction of amino acid: Dried samples of roots and leaves were taken into separate test tubes . To each test tube, 3 ml of methanol (conc.) was added. After 48 hours of addition, methanol was decanted and collected in a separate test tube. The procedure was repeated. The combined extract was evaporated in a water bath to ca. 1 ml and the final volume was made to 10 ml with distilled water.

Measurement of amino acid: Citric acid buffer was prepared by dissolving 160 g of citric acid and 64 g of NaOH in 1000 ml of distilled water.

Ninhydrin reagent was prepared by dissolving 1g of ninhydrin in 100 ml of methoxy ethanol. 40 mg of ascorbic acid was dissolved in 25 ml of distilled water. These solutions were mixed together and the final volume was made upto 140 ml with distilled water.

For reaction, 1 ml of extract was taken in a test tube. 1.4 ml of citric acid buffer and 1.2 ml of ninhydrin reagent were added to the extract. The aliquot was then heated for 20 minutes. After heating, the samples were cooled immediately in ice cold water. The optical density was measured at 570 nm using a spectrophotometer (Dutta, R.K., personal communication).

2.11 Extraction and measurement of total and reducing sugar

Extraction of reducing and total sugar : Sugar was extracted by boiling fresh leaf and root tissue in 5 ml of 80% ethanol for 5 minutes. The extraction was repeated with two further changes of 5 ml of 80% ethanol. To remove chloroplast, leaf extracts were partitioned three times with a mixture of 4:1 petroleum spirit (bp. 40-60) and 80% ethanol (v/v). The ethanol was evaporated to approximately 3 ml. The extract was then diluted to 20 ml with distilled water (Karmoker 1981).

Measurement of reducing and total sugar : Reducing sugar was determined by Somogyi-Nelson method (Nelson 1944; Somogyi 1952). Total sugar was determined by the phenol-H_2SO_4 method of Dubois and co-workers (1956).

2.12 Extraction and measurement of soluble protein

Extraction of soluble protein: Fresh root and leaf samples were weighed and homogenised in 3 ml of 2 mM KH_2PO_4 buffer with a mortar and pestle. The homogenised sample was then centrifused at 3000 rpm for 5 minutes. The supernatant was collected and the procedure was repeated with 2 ml of buffer. The supernatant was collected in a respective test tube. The combined extract containing soluble protein was made upto a final volume of 5 ml with 2 mM phosphate buffer. One ml of supernatant was placed in a separate test tube and 5 ml of 5% trichloroacetic acid was added to the test tube. The protein was allowed to precipitate for 5 minutes and then centrifused for 5 minutes at 3000 rpm. The supernatant was discarded. The pellet was suspended in 1 ml of distilled water.

Measurement of soluble protein: 0.5 ml of extract was taken in a test tube. 2.5 ml of freshly prepared alkaline copper solution (Lowry solution) was added to the extract. After 20 minutes, 0.25 ml 1N folin reagent was added to the test tube. The mixture was shaken well. Just after 30 minutes the optical density was measured at 660 nm with a spectrophotometer.

Chapter 3

Effects of sulphur deficiency on ^{15}N accumulation and transport in nonnodulated and nodulated chickpea seedlings at different developmental stages

3.1 Introduction

Nitrogen and sulphur assimilation are closely related (Friedrich and Schrader 1978). Nitrogen and sulphur fertilization significantly increased the uptake and concentration of each other along with an increase in protein content (Walker and Booth 1992; Adams and Sheared 1966). The intensity of nitrogen metabolism and the rate of protein synthesis controls the import of nitrogen by the plant parts. The study of the effect of sulphur deficiency on nitrogen transport in barley showed that deprivation of sulphur reduced the uptake and transport of nitrogen to the shoot of barley (Karmoker *et al.* 1991; Clarkson *et al.* 1989). Many researchers used isotopically labeled ^{15}N fertilizer to determine the uptake and distribution pattern of nitrogen within plant organs (Kato *et al.* 1984, Hill Cotingham and Jones, 1975).

This experiment was undertaken to study the effect of sulphur deficiency on nitrogen uptake and distribution in chickpea using ^{15}N as a tracer in solution culture.

3.2 Material and Methods

Plants were grown in 0.1 strength complete Letcombe nutrient solution for 7 and 15 days in the growth cabinet according to section 2.4.2. Sulphur deprivation was applied in each set of plants for 1-d and 5-d. Control and sulphur deficient plants were then transferred to 0.1 strength Letcombe nutrient solution having 5 mM $K^{15}NO_3$ (12.2% atom excess). Samples were collected after 6, 12 and 24 h of $K^{15}NO_3$ application. The root and shoot were separated. Root samples were washed with 0.01 Letcombe nutrient solution to remove free space ions. Samples were dried in the oven at 70^0C upto 72 h. Samples were then digested with sulphuric acid, ^{15}N was determined following the method as in section 2.5.

3.3 Results

3.3.1 Effects of sulphur deficiency on ^{15}N accumulation and distribution in 7- d-old nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of 7-d-old nonnodulated chickpea seedlings, 1-d sulphur deficiency increased the accumulation of ^{15}N by 12% at 12 h of ^{15}N application (Fig. 1a) whereas 1-d sulphur deficiency applied to nodulated chickpea seedlings caused a 6.2% to 46% increase in ^{15}N accumulation in the root from 6 to 24 h of ^{15}N treatment (Fig. 2a).

In the shoot of the 1-d S-deficient nonnodulated chickpea seedling, ^{15}N accumulation was decreased by 10% to 33% from 6 to 24 h of ^{15}N treatment (Fig. 1b). On the other hand, ^{15}N accumulation in the shoot of the 1-d S-deficient nodulated seedling was inhibited by 18.2% at 24 h of addition of ^{15}N (Fig. 2b).

Five-day sulphur deficiency applied to nonnodulated chickpea seedlings depressed the accumulation of ^{15}N in the root by 10% to 17% at 6 to 24 h of ^{15}N application (Fig. 3a). Similarly, five-day sulphur deficiency treatment applied to nodulated chickpea seedlings resulted in a 5% and 10 % decrease in ^{15}N accumulation in the root at 12 and 24 h of addition of ^{15}N respectively (Fig. 4a).

In the shoot of the nonnodulated chickpea seedling, 5-d sulphur deficiency resulted in a decrease in accumulation of ^{15}N by 3.5% to 20% from 6 to 24 h of ^{15}N treatment (Fig. 3b) whereas in 5-d S-deficient nodulated chickpea seedling ^{15}N accumulation was decreased in the shoot by 36% and 55% at 12 and 24 h of ^{15}N treatment respectively (Fig. 4b).

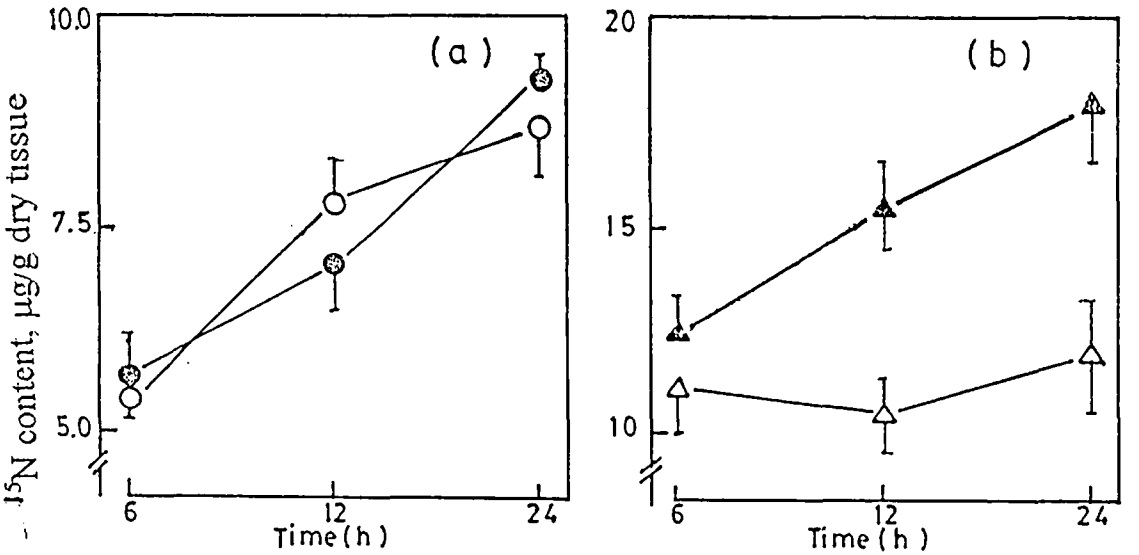


Fig. 1. The effect of 1-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Solid symbols------ + S; open symbols----- – S. O root; Δ shoot. Each value is the mean of three replicates ± standard error.

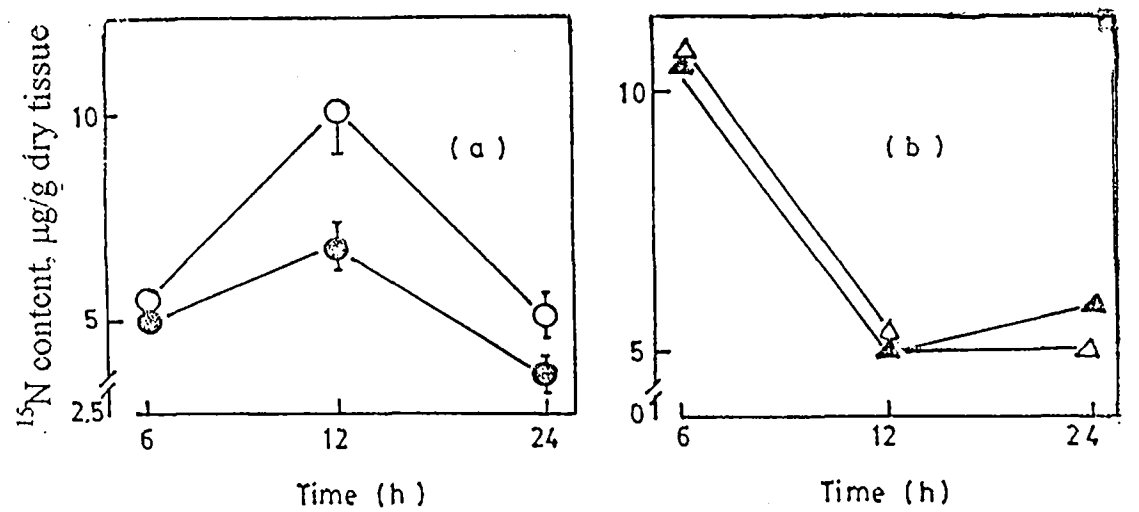


Fig. 2. The effect of 1-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

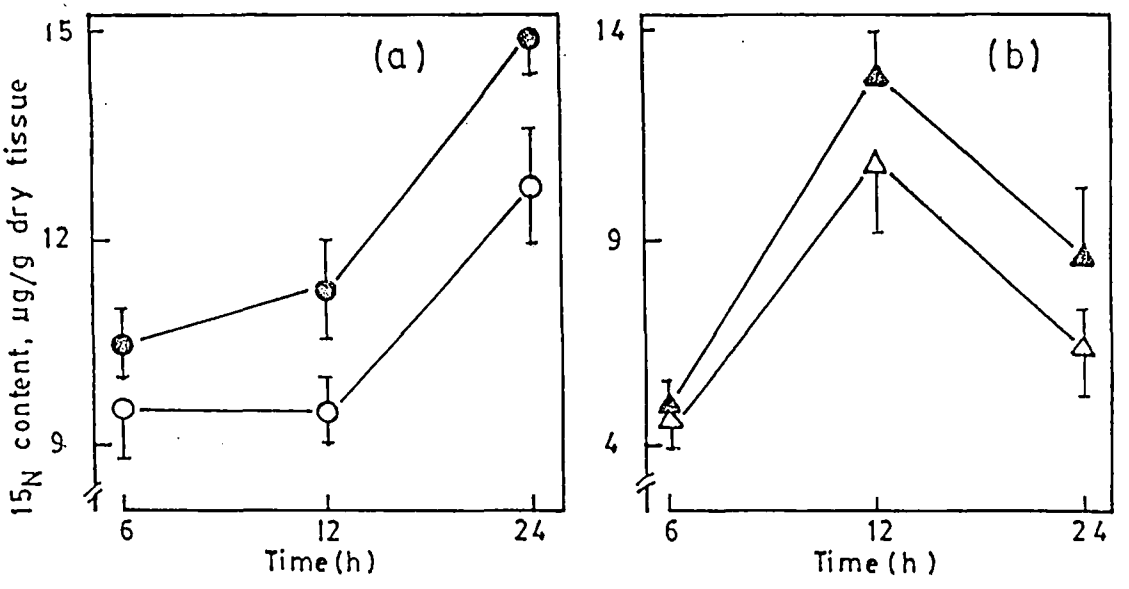


Fig. 3. The effect of 5-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

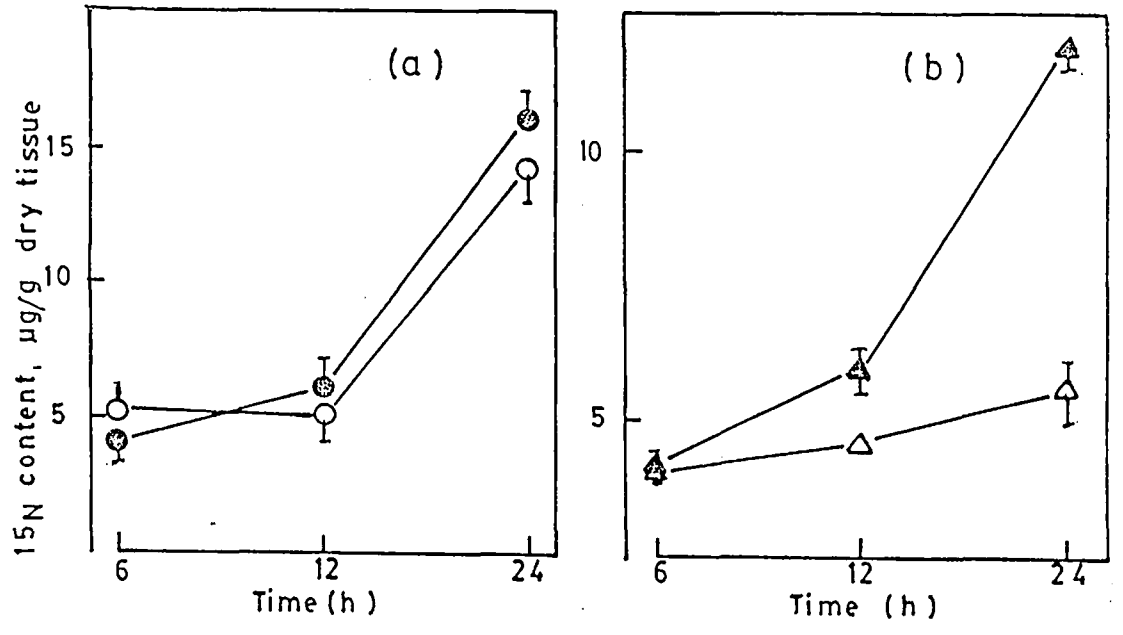


Fig. 4. The effect of 5-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

3.3.2. Effects of sulphur deficiency on ^{15}N accumulation and distribution in 15-d-old nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of 15-d-old nonnodulated genotype, 1-d sulphur deficiency inhibited the accumulation of ^{15}N by 27.5% and 41.1% at 6 and 24 h of ^{15}N application respectively (Fig. 5a). On the other hand, 1-d sulphur deficiency applied to nodulated chickpea seedlings caused a 20.4% and 3.8% increase in ^{15}N accumulation in the root at 6 and 24 h of addition of ^{15}N respectively (Fig. 6a).

In the shoot of the seedling of nonnodulated chickpea genotype, 1-d sulphur deficiency inhibited the accumulation of ^{15}N by 24% to 28% from 6 to 24 h of ^{15}N treatment over that of the + S control (Fig. 5b) whereas ^{15}N accumulation was inhibited by 31% and 6% in the shoot of 1-d S-deficient nodulated seedling at 6 and 24 h after addition of ^{15}N respectively (Fig. 6b).

In the root of 5-d S-deficient seedling of nonnodulated genotype, ^{15}N accumulation was inhibited by 22% and 21% at 6 and 24 h respectively except an increase in accumulation by 13% at 12 h of ^{15}N application (Fig. 7a) whereas 5-d sulphur deficiency treatment applied to nodulated seedlings had no effect on ^{15}N accumulation in the root (Fig. 8a).

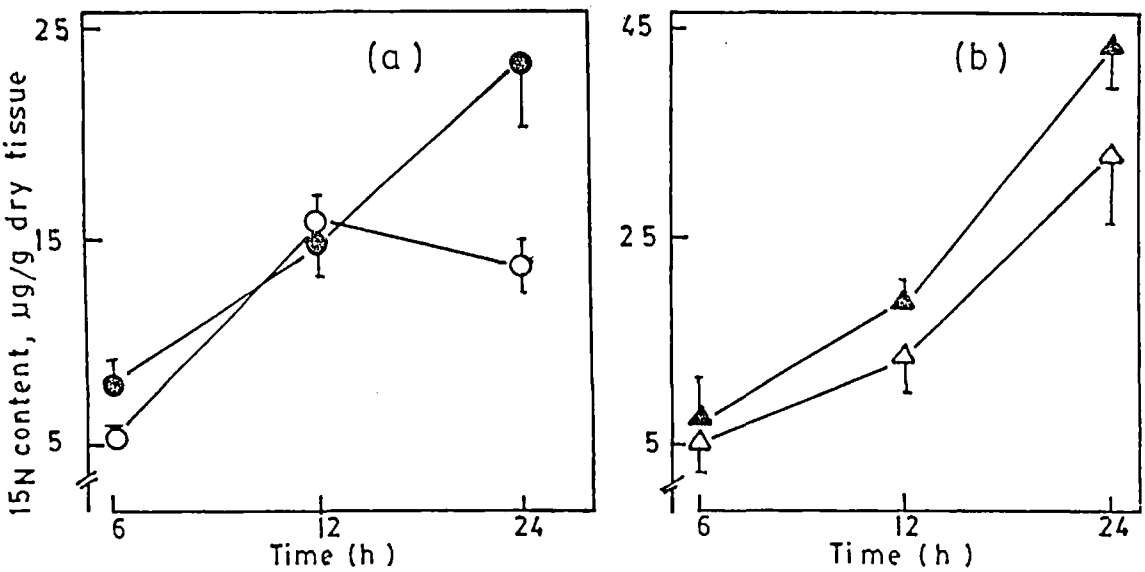


Fig. 5. The effect of 1-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

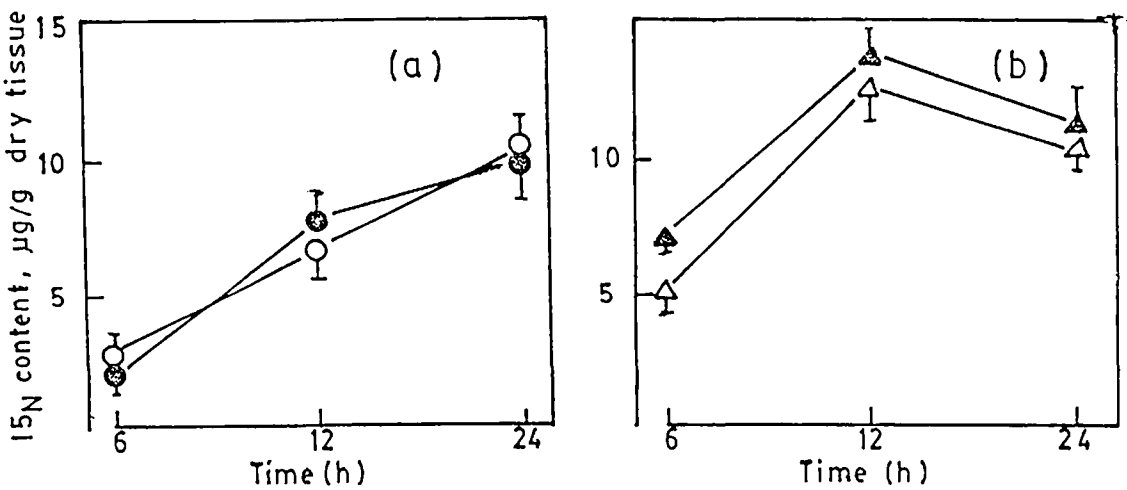


Fig. 6. The effect of 1-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

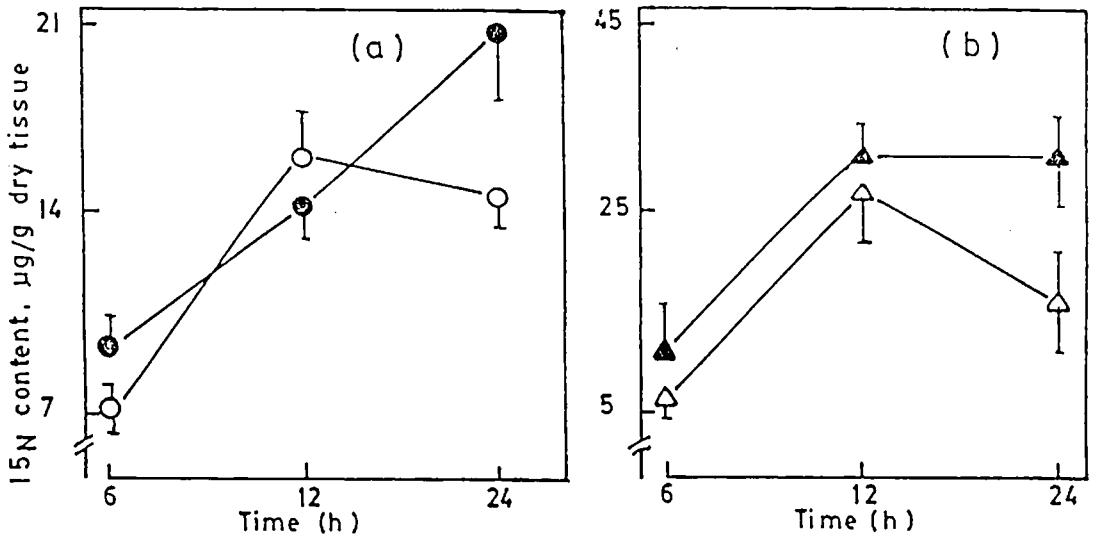


Fig. 7. The effect of 5-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

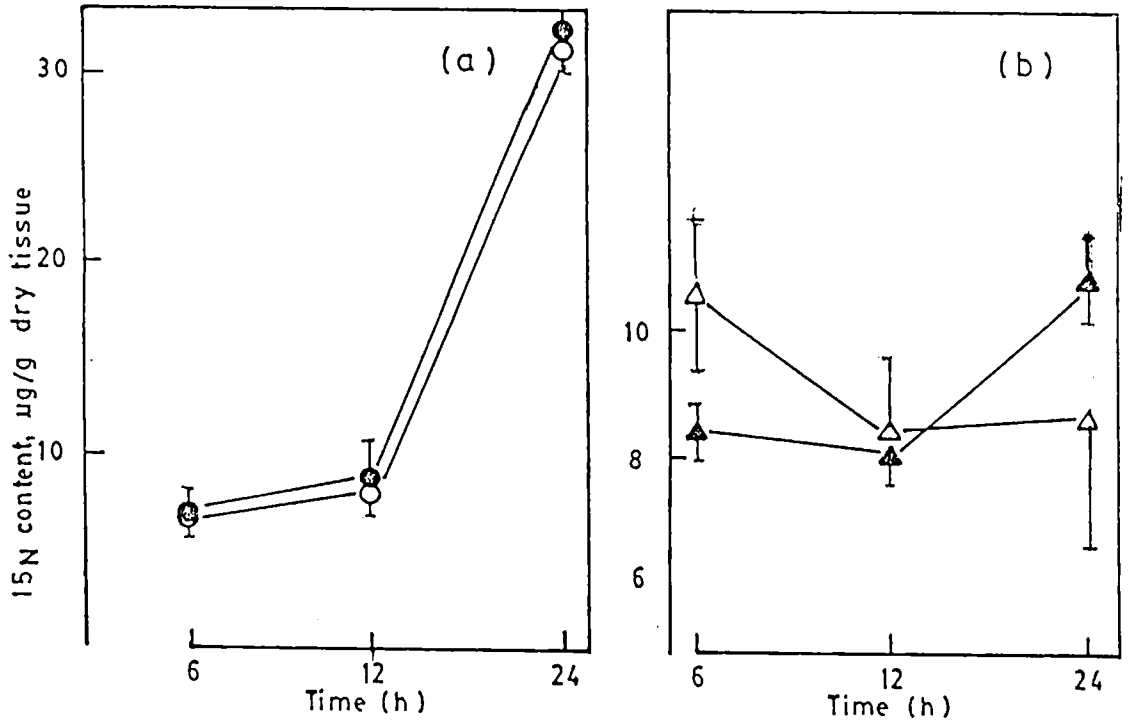


Fig. 8. The effect of 5-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

In the shoot of the 5-d S-deficient nonnodulated chickpea seedling, ^{15}N accumulation was inhibited by 32.5%, 13% and 29.5% at 6, 12 and 24 h of ^{15}N application respectively (Fig. 7b). Similarly, accumulation of ^{15}N was inhibited by 27% in the shoot of the 5-d sulphur deficiency-treated nodulated seedling at 24 h of addition of ^{15}N except an initial increase in ^{15}N accumulation at 6 h of tracer treatment (Fig. 8b).

3.3.3 Effects of sulphur deficiency on growth of 7-d-old nonnodulated and nodulated chickpea seedlings grown in solution culture

One-day sulphur deficiency applied to 7-d-old nonnodulated seedlings had no effect on the accumulation of dry matter in the root and shoot (Fig. 9a & 9b). On the other hand, 5-d sulphur deficiency applied to nonnodulated chickpea seedlings decreased the root dry matter accumulation by 12.5 % to 10.3% over a period of 24 h (Fig. 11a) whereas shoot dry matter accumulation decreased in these 5-d S-deficient seedlings by 7% (Fig. 11b).

One-day sulphur deficiency decreased the accumulation of the root dry matter by 9.7% to 12% in nodulated seedlings over a period of 24 h (Fig. 10a). One-day sulphur deficiency had no effect on the accumulation of the dry matter in the shoot of the nodulated seedling (Fig. 10b).

Five-day sulphur starvation applied to nodulated seedlings caused a 3% to 13% decrease in the root dry matter content over a period of 24 h (Fig. 12a).

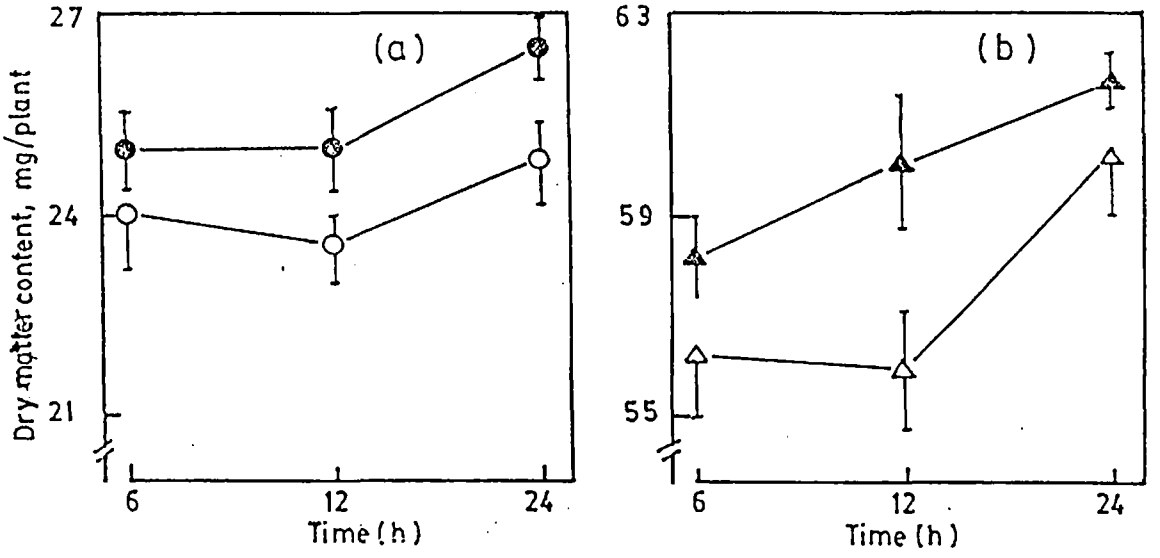


Fig. 9. The effect of 1-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

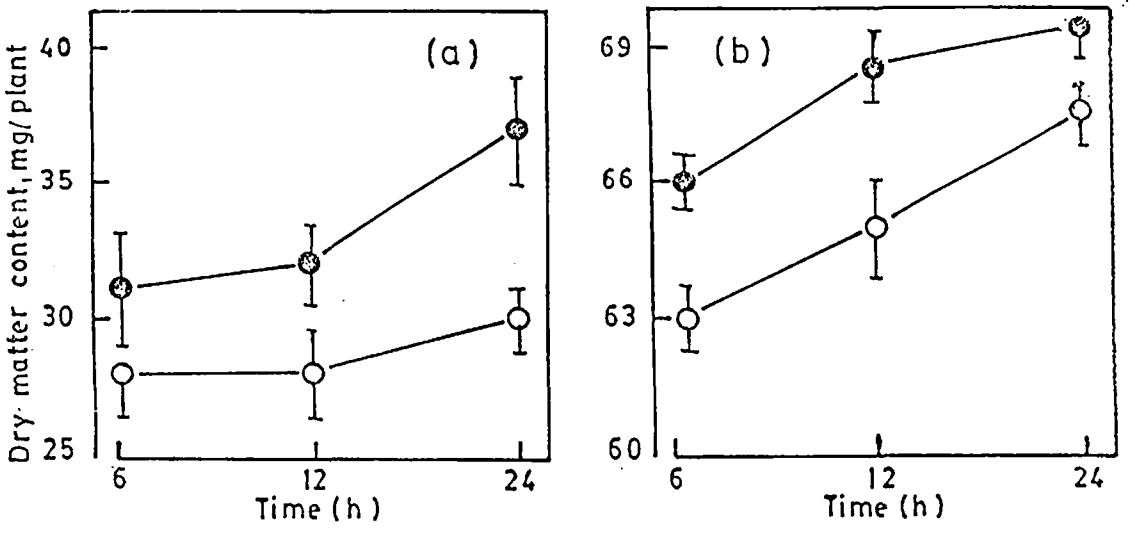


Fig. 10. The effect of 1-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

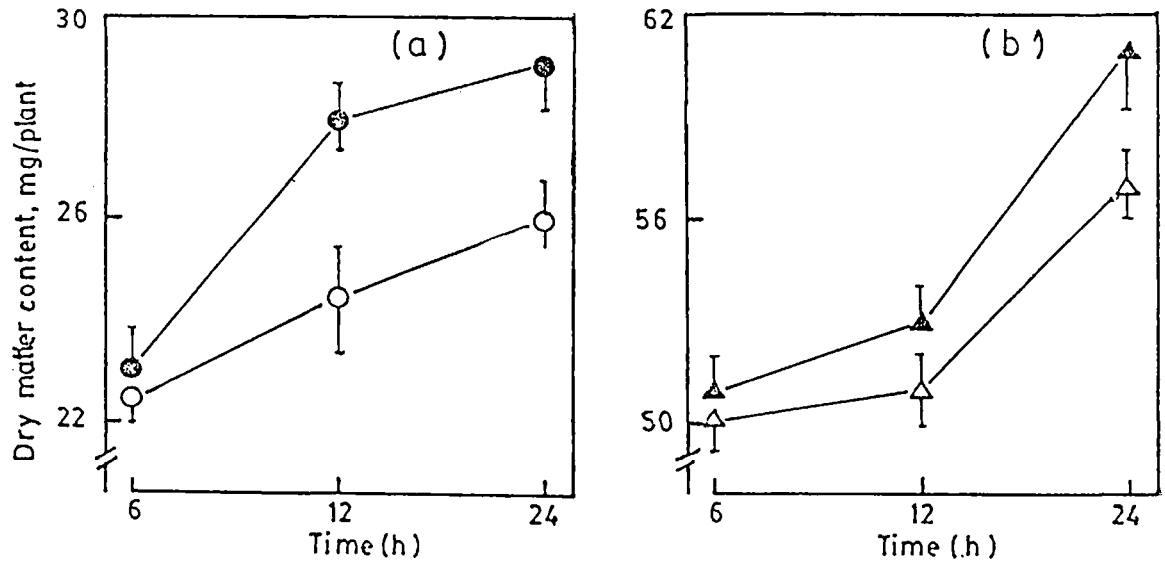


Fig. 11. The effect of 5-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

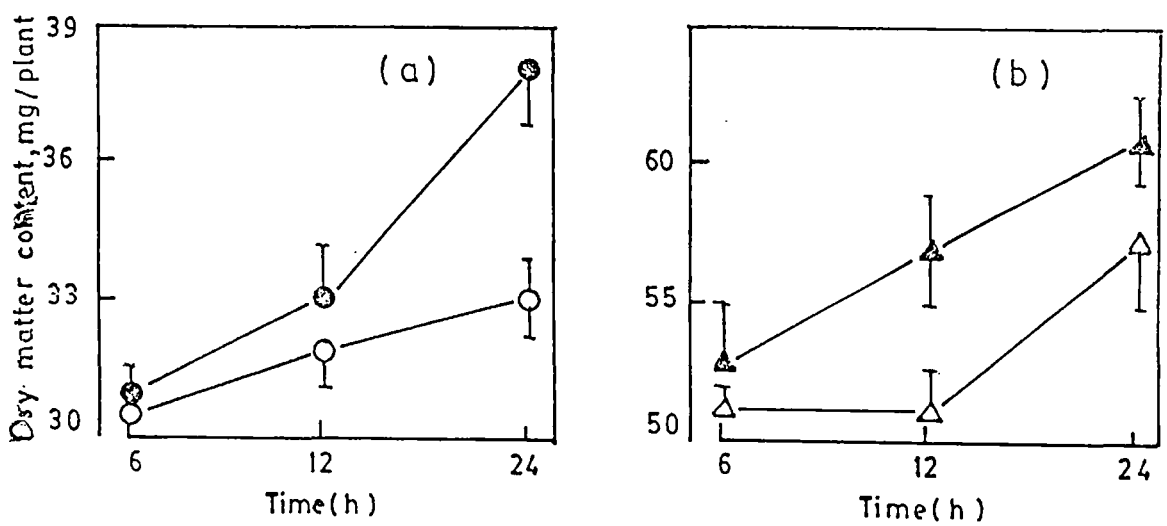


Fig. 12. The effect of 5-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

Similarly, dry matter content of the shoot in the nodulated seedling was decreased by 3% to 9.4% over a period of 24 h following 5-d sulphur deficiency treatment (Fig. 12b).

3.3.4 Effects of sulphur deficiency on growth of 15-d-old nonnodulated and nodulated chickpea seedlings grown in solution culture

1-d sulphur deficiency had no effect on the accumulation of dry matter in the root and shoot of the nonnodulated chickpea seedling (Fig. 13a & 13b).

Five-day sulphur deficiency decreased the accumulation of dry matter in the root and shoot of the nonnodulated chickpea seedling by 24% to 32% and 6% to 24% respectively over a period of 24 h (Fig. 15a & 15b).

One-day sulphur deficiency resulted in a 3.3% to 12% decrease in the dry matter content of the root of the nodulated seedling (Fig. 14a). Similarly, 1-d sulphur deficiency caused a 3.6% to 15% decrease in the dry matter content of the shoot over a period of 24 h (Fig. 14b).

Five-day sulphur deficiency caused a 8.8% to 17% decrease in the dry matter content of the root of the nodulated chickpea seedling over a period of 24 h (Fig. 16a). Similarly, 5-d sulphur deprivation resulted in a 3% to 9%

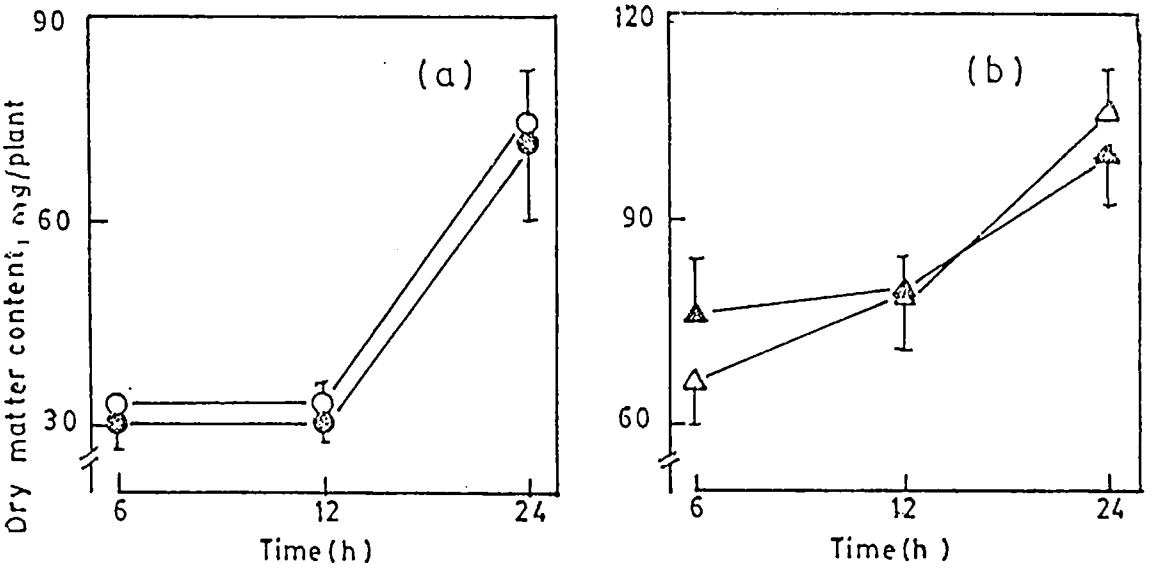


Fig. 13. The effect of 1-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

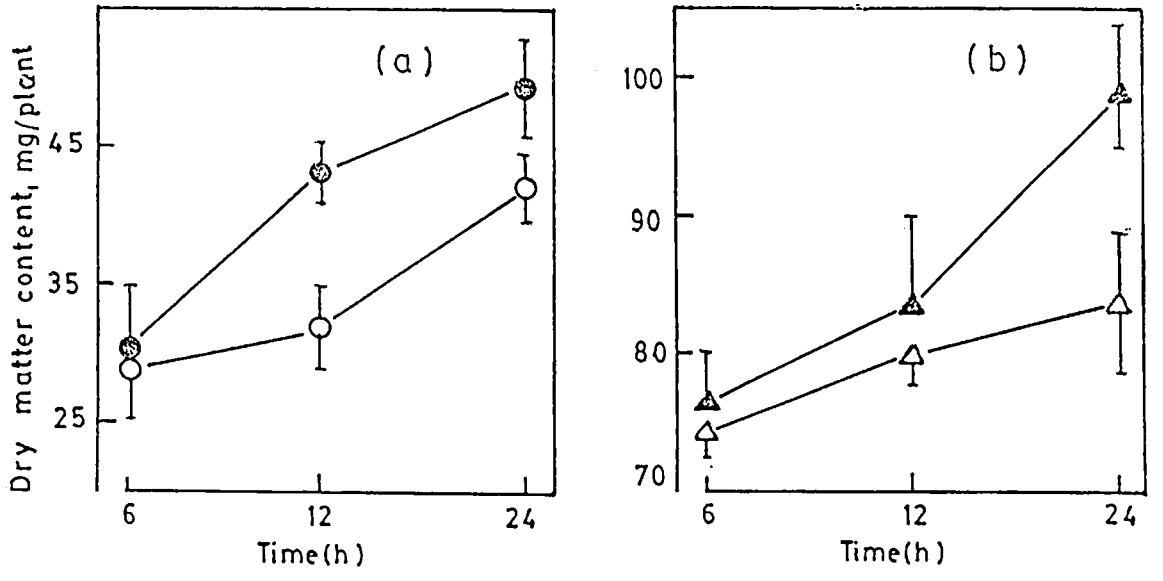


Fig. 14. The effect of 1-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

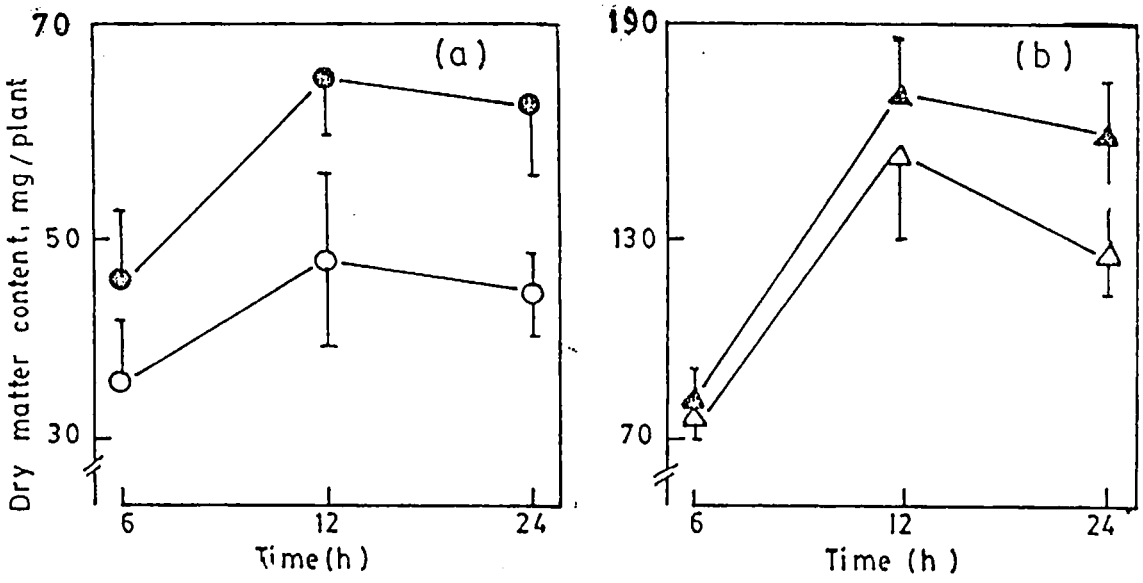


Fig. 15. The effect of 5-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

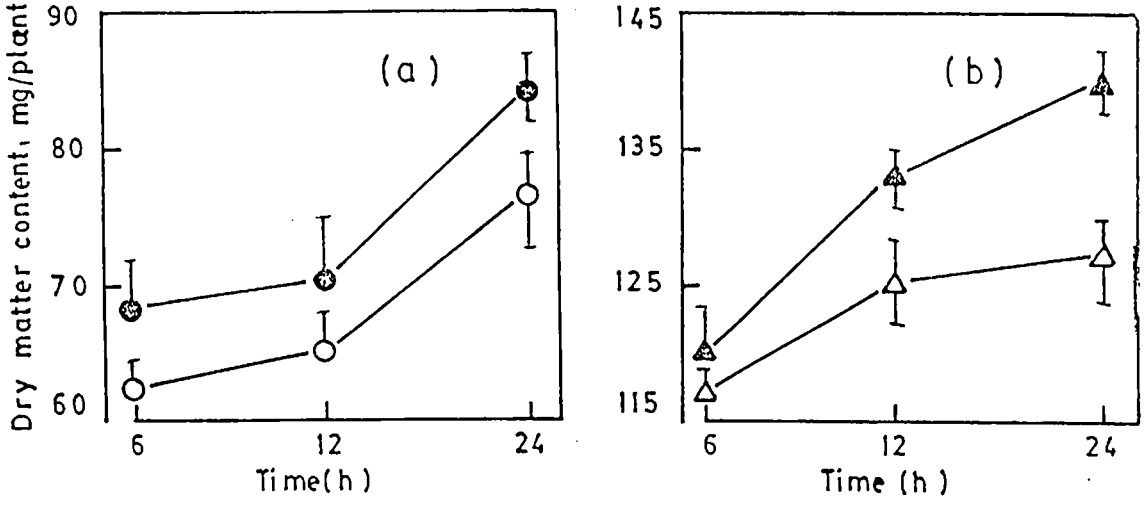


Fig. 16. The effect of 5-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

decrease in the dry matter content of the shoot of these seedlings over a period of 24 h (Fig. 16b).

3.4 Discussion

Sulphur deficiency caused an inhibition of ^{15}N accumulation in the root and shoot of 15-d-old chickpea seedlings of nonnodulated and nodulated genotypes (Fig. 5 to 8). Similar sulphur deficiency-induced inhibition of uptake and transport of ^{15}N was observed in barley seedlings (Karmoker *et al.* 1991; Clarkson *et al.* 1989).

However, ^{15}N accumulation in the root of 7-d-old seedlings of both the genotypes was increased at 1-d of sulphur deficiency while that in shoots of seedlings of both the genotypes was decreased (Fig. 1 to 4). The decrease in ^{15}N in the shoot with a concomitant increase of that in the root might be due to decreased translocation of ^{15}N from the root to the shoot. This view is supported by the work of Copper and Clarkson (1986) who found that recycling of ^{15}N occurs between the root and the shoot in cereal.

According to Horms and Nowik (1990a; 1990b; 1990c) ^{15}N remobilization was observed at time course of ^{15}N assimilation.

Sulphur deprivation applied to 7-d-old and 15-d-old chickpea seedlings caused a decrease in the dry weight of the root and the shoot after 5-d of sulphur deficiency treatment. While no effect of sulphur deficiency was observed in case of the accumulation of dry matter in the root and the shoot of 7-d-old and 15-d-old chickpea seedlings after 1-d of sulphur deficiency treatment (Fig. 9 to 16). On the contrary, Clarkson and co-workers (1989) observed that sulphur deficiency caused a slight increase in the accumulation of dry matter. Present results are supported by the work of Tiwari (1994) who found that sulphur deficiency resulted in a decrease in leaf area (Tiwari 1994; Tiwari *et al.* 1994). Sulphur deficiency-induced inhibition of dry matter accumulation (Fig. 9 to 16) may be due to sulphur deficiency-induced decrease in carbohydrate synthesis (Tso and McMurtrey 1960). This view is supported by the findings of Hall and co-workers (1972) who reported that S-deficient leaves fixed 25% less CO_2 than the normal plants which might result in a decrease in the accumulation of dry matter.

Chapter 4

Effects of sulphur deficiency on ^{35}S accumulation and distribution in 15-d-old nonnodulated and nodulated chickpea seedlings

4.1 Introduction

Sulphate or a variety of reduced form of SO_4^{2-} can satisfy the requirement of sulphur for higher plants (Thomas 1958). Sulphur assimilation in the higher plant mainly occurred in the leaves and was enhanced by light (Kylin 1960; Schwenn and Trebst 1976; Anderson 1980; 1990). Therefore, sulphur must be translocated from the root to the leaf (Willenbrink 1967).

The rate of sulphate transport was low in cells grown in sulphur containing medium but it was high in cells grown in S-deficient condition (Smith 1980; Smith and Lung 1988). Accumulation of SO_4^{2-} in S-starved plants was higher than that in plants which was supplied with sulphur in the beginning (Holobrada 1977). Dreyfuss (1964) reported genetic control of SO_4^{2-} uptake and transport. At 5-d of S-starvation, the rate of SO_4^{2-} uptake was enhanced by 46% and 30% in higher and lower yield group of maize genotypes respectively (Saccomoni *et al.* 1984). They reported that ^{35}S uptake at 30 minutes was higher in roots than that in leaves of S-starved plants.

respectively (Saccomoni *et al.* 1984). They reported that ^{35}S uptake at 30 minutes was higher in roots than that in leaves of S-starved plants.

The present experiment was conducted to study the effect of sulphur deficiency on ^{35}S accumulation and transport in chickpea.

4.2 Material and Methods

Chickpea plants were grown in nutrient culture for 15 days according to sec. 2.4.2. Sulphur deficiency was then applied to each set of plants for 1-d and 5-d periods. For isotopic chase experiment, 3 ml of 10 μCi ^{35}S was added to 500 ml of 0.1 strength Letcombe solution (Sec. 2.3) in each set of seedlings. Samples were collected after 10 min., 20 min., 30 min., 1 h, 3 h and 6 h of ^{35}S application. The root and the shoot were separated. Roots were rinsed in three 1 minute changes of 0.01 mM $CaSO_4$. Root and shoot samples were dried in an oven at 70^0C for 48 h. ^{35}S was extracted by boiling the samples for 15 minutes with distilled water (sec. 2.6.1.).

For radioassay of ^{35}S, 1 ml of sample was taken in a scintillation vial and 9 ml of scintillant (Sec. 2.6.1.) was added to each vial. Radioactive decay was counted for one minute for each sample using a scintillation counter (model LSC 2, SE Technology, UK).

4.3 Results

4.3.1 Effects of 1-d sulphur deficiency on ^{35}S uptake and translocation in nonnodulated and nodulated chickpea seedlings

In the root of the nonnodulated chickpea seedling, 1-d sulphur deficiency caused a 4.7- to 9.5-fold increase in ^{35}S accumulation from 10 to 30 minutes of chase period. This stimulatory effect was maintained upto 6 h of treatment (Fig. 17a). Similarly, one-day sulphur deficiency caused a 17- to 44-fold increase in ^{35}S accumulation in the root of the nodulated chickpea seedling from 10 to 60 minutes and the stimulatory effect was maintained upto 6 h of treatment (Fig. 18a).

^{35}S accumulation was increased by a 2.1- to 2.8-fold in the shoot of the nonnodulated S-deficient seedling from 10 to 60 minutes. This increasing tendency of ^{35}S accumulation was sustained upto 6 h of isotopic chase (Fig. 17b). In the shoot of the nodulated seedling, ^{35}S accumulation was increased by 4- to 48- fold from 10 to 60 minutes following sulphur deficiency treatment and this stimulatory effect was maintained upto 6 h of isotope treatment (Fig. 18b).

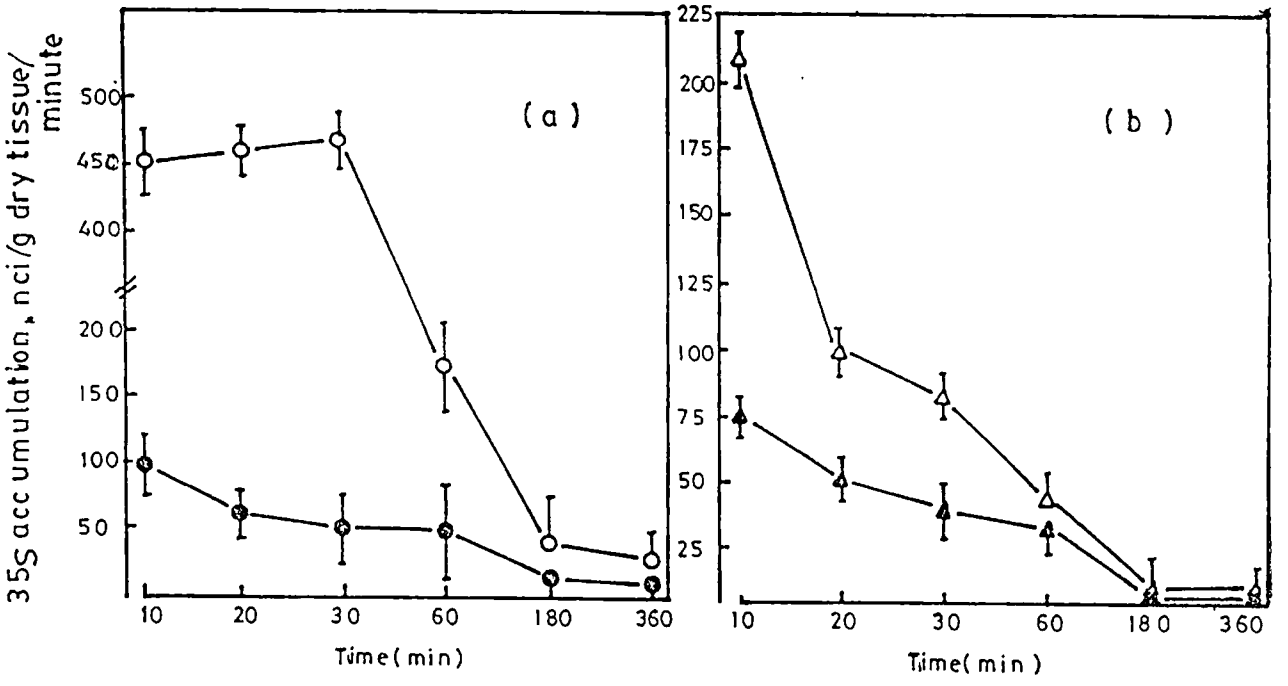


Fig. 17. The effect of 1-d sulphur deficiency on ^{35}S accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

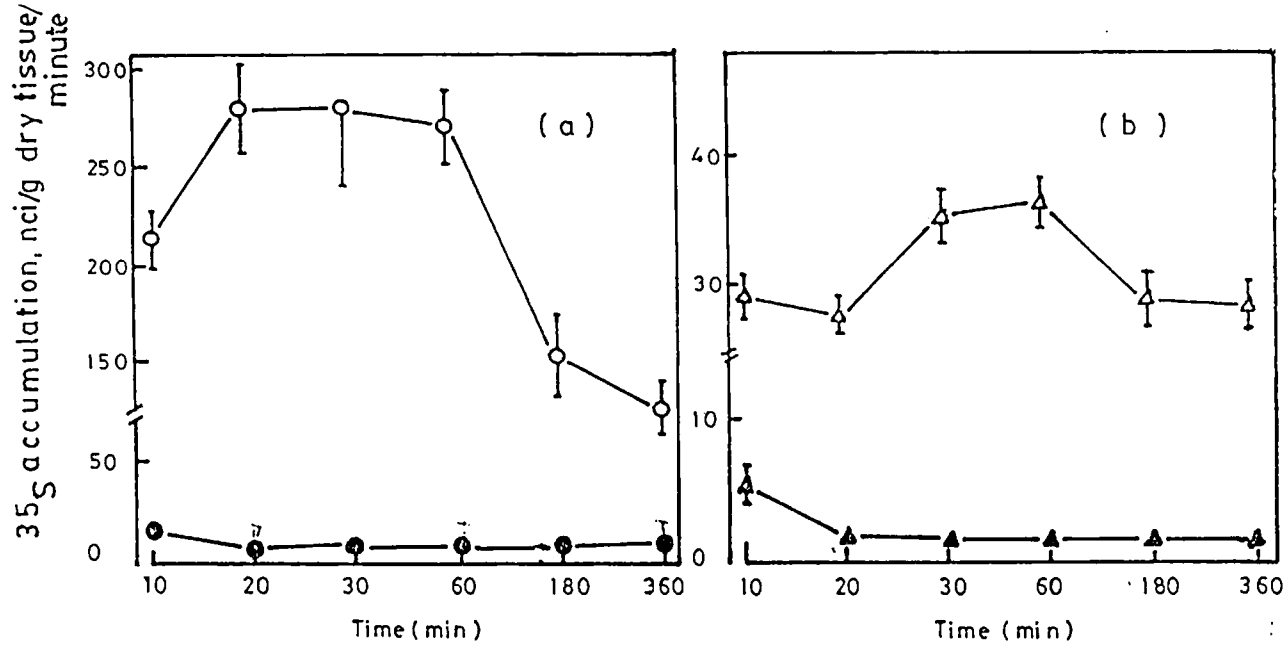


Fig. 18. The effect of 1-d sulphur deficiency on ^{35}S accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

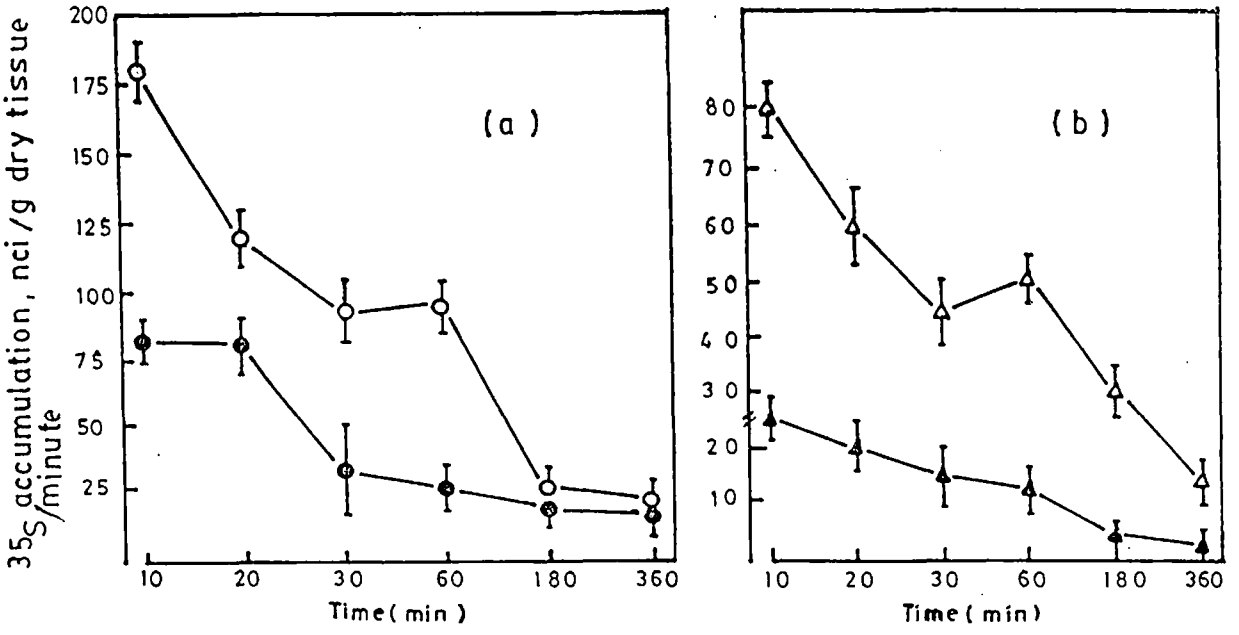


Fig. 19. The effect of 5-d sulphur deficiency on ^{35}S accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

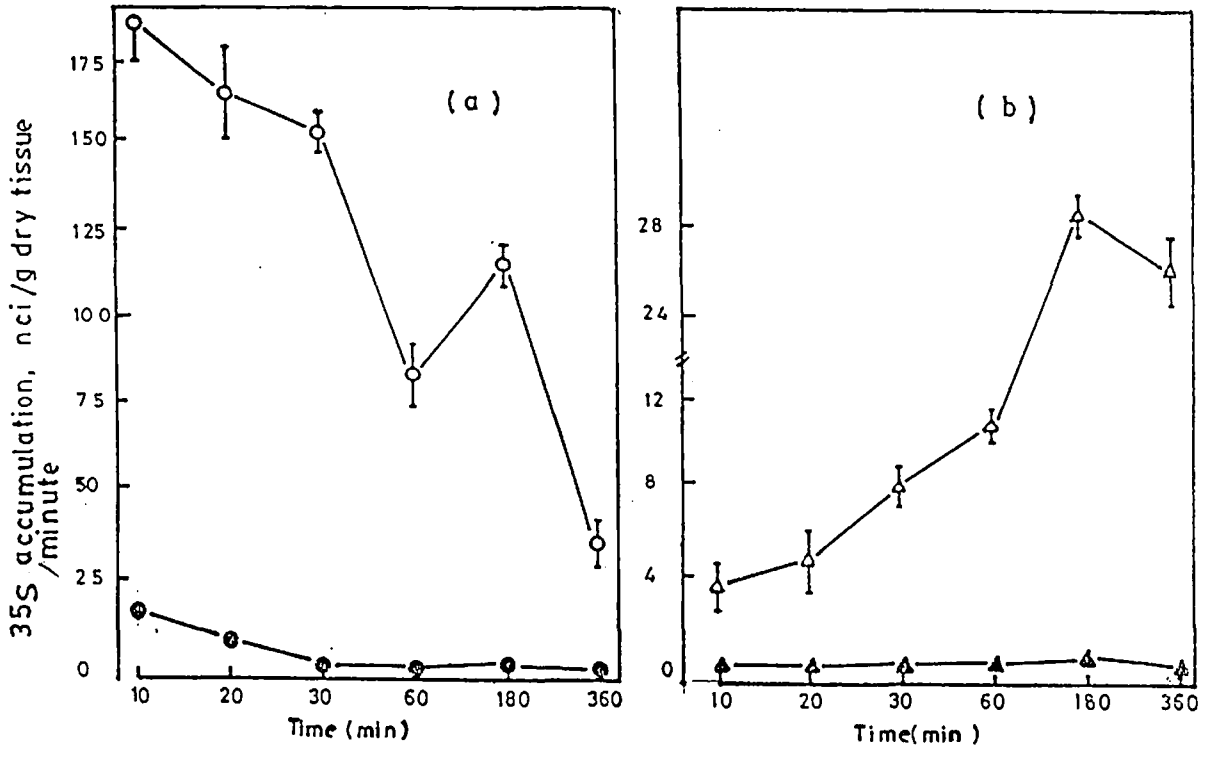


Fig. 20. The effect of 5-d sulphur deficiency on ^{35}S accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

4.3.2 Effects of 5-d sulphur deficiency on ^{35}S uptake and translocation in nonnodulated and nodulated chickpea seedlings

^{35}S accumulation in the root of 5-d S-deficient nonnodulated chickpea seedling was increased from a 2.2- to 3.7-fold from 10 to 60 minutes of ^{35}S application. This stimulatory effect was sustained upto 6 h of isotope treatment (Fig. 19a). Five-day of S-starvation resulted in a 19- to 24-fold increase in ^{35}S accumulation from 10 to 60 minutes in the root of the nodulated chickpea seedling and the stimulatory effect was maintained upto 6 h of isotope treatment (Fig. 20a).

In the shoot of 5-d S-deficient nonnodulated seedling, accumulation of ^{35}S was increased from a 3- to 18-fold at 10 to 360 minutes of ^{35}S application (Fig. 19b). Similarly, in the shoot of the nodulated seedling exposed to 5-d S-starvation, the accumulation of ^{35}S was gradually increased by a 12- to 46-fold from 10 to 360 minutes of ^{35}S treatment (Fig. 20b).

4.4 Discussion

^{35}S accumulation was dramatically increased in roots and shoots of intact chickpea seedlings of both the genotypes following 1-d and 5-d of sulphur deficiency treatment (Fig. 17 to 20). This dramatic increase in ^{35}S accumulation in S- deficient plants may be due to the demand of sulphur

created in the plant as a result of prolonged sulphur starvation. This view is supported by the fact that the rate of stimulation of ^{35}S accumulation was gradually decreased from 1 h to 6 h of isotope treatment (Fig. 17 to 20).

Holobrada (1977, 1978) reported in maize that when sulphur was supplied to S-starved plants, the S-deficient plants absorbed more sulphur than that of + S controls. The rate of the uptake of sulphur was also higher in S-starved plants. The fact is that S-deficient plants incorporate a large quantity of the absorbed sulphur to protein within 1 h of treatment. The low level of sulphur in S-deficient plant, large number of free exchange sites and active sulphate uptake, quick depletion of these sites due to extensive exhaustion of absorbed sulphate into the metabolic reduction processes had brought about a quick uptake of sulphate (Holobrada 1978).

Clarkson and co-workers (1989) reported that when sulphate supply was restored in S-starved barley plants, sulphate influx was quickly increased over the subsequent 24 h.

The accumulation of ^{35}S in the root was higher than that in the shoot. (Fig. 17-20). This result is supported by Srivastava and co-workers (1989) who reported in sugarcane that uptake of ^{35}S in 30 minutes was higher in the root.

The decreased transport of ^{35}S in the shoot of the S-deficient plant with concomitant increase in accumulation in the root may be due to increased rate of accumulation of ^{35}S in the root vacuole.

Results presented herein indicated that initial accumulation of ^{35}S in the seedling of the nonnodulated genotype was higher than that of the seedling of the nodulated genotype. Furthermore, it reflected that transport of ^{35}S from the root to the shoot was higher in the seedling of the nonnodulated genotype than that of the seedling of the nodulated genotype. Thus, these two genotypes differ in their efficiencies of accumulation and translocation of sulphur. Saccomoni and Ferrari (1989) reported genotypic variation of sulphur uptake efficiency. They found that S-deprivation for 5 days enhanced the rate of SO_4^{2-} uptake by 46% and 30% in high and low yield genotypes of maize respectively.

Chapter 5

Effects of sulphur deficiency on accumulation and distribution of monovalent cations, anions and divalent cations and anions in intact chickpea seedlings at different developmental stages

5.1 Introduction

Effects of sulphur deficiency on monovalent cations: Sulphur affects K^+ uptake and distribution in plants. For example, Ligero and coworkers (1981) found a decreased K^+ uptake in pea when sulphur supply was low. Greenway and Pitman (1965) reported that sulphur deficiency depressed K^+ transport in *Hordeum vulgare.*

Effects of sulphur deficiency on Ca^{2+} and Mg^{2+} accumulation and distribution: Bugakova and co-workers (1981) showed that Ca^{2+} content in pea was decreased when sulphur supply in the growth medium was limited.

Rennenberg and Kaiser (1989) reported that accumulation of Mg^{2+} was increased due to sulphur deficiency in tobacco cell culture. On the contrary, Bugakova and co-workers (1981) reported that sulphur deficiency decreased the accumulation of Mg^{2+} in pea.

Effects of sulphur deficiency on Fe^{2+} *and* Zn^{2+} *accumulation and distribution*: Sulphur deficiency resulted in a decrease in accumulation of Fe^{2+} in pea (Bugakova *et al.* 1981) and in wheat (Wankhade *et al.* 1989).

Sulphur deficiency caused a decrease in Zn^{2+} uptake in *Phalaris aquatica* (Sairam *et al.* 1995).

Effects of sulphur deficiency on accumulation and distribution of phosphate and nitrate : Phosphate accumulation was increased in pea (Ligero *et al.* 1981) and in tomato (Martinez *et al.* 1983) following less supply of sulphur.

Stewart and Porter (1969) reported that the lack of sulphur in the plant environment limited the efficiency of applied nitrogen. Protein synthesis was adversely affected under low sulphur level with the concomitant increase in the nonprotein-N accumulation in the plants (Pasricha and Randhawa 1975; Bordeleou *et al.* 1981; Shewry *et al.* 1983; Bapat *et al.* 1984). Clarkson and co-workers (1989) reported that influx of nitrate and ammonium was depressed in barley in the earliest stage of sulphur deprivation.

In this section, the effect of sulphur deficiency on the accumulation of K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, PO_4^{3-} and NO_3^- in chickpea seedlings will be reported.

5.2 Material and Methods

Plants were grown in solution culture in controlled environmental condition as described in sec. 2.4.2. Seven, 15 and 21-d-old plants were used in the experiment. Root and shoot samples were collected for analysis of ions according to sec. 2.7. at 1-d, 3-d and 5-d of sulphur deficiency treatment. Free space ions were removed and samples were dried according to 2.7. Root and shoot samples were digested following the method described in sec 2.7.1. K^+ and Na^+ were measured by flame photometer (model 866150, Corning Ltd., Halstead, Essex, England). Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} were extracted and determined according to sec. 2.7.1. and sec. 2.7.2. Phosphate was extracted as described in sec. 2.7.1. Phosphate was measured by vanadomolybdate method (Jackson 1967) according to sec. 2.7.3.1. and sec. 2.7.3.2. Nitrate was determined using fresh root and shoot tissue according to sec. 2.8.

5.3 Results

5.3.1 Effects of sulphur deficiency on accumulation and distribution of K^+ and Na^+ in 7-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of nonnodulated genotype, sulphur deficiency inhibited the accumulation of K^+ by 11%, 5% and 12.5% at 1-d, 3-d and 5-d

of treatment respectively (Fig. 21a). Similarly, sulphur deficiency inhibited the accumulation of K^+ by 3.8% to 14.5% in the root of the seedling of the nodulated genotype from 1-d to 5-d of treatment (Fig. 22a).

Accumulation of K^+ in the shoot of the seedling of nonnodulated genotype was increased by 8% to 14% from 1-d to 5-d of sulphur deficiency whereas the same was decreased in the shoot of the seedling of nodulated genotype by 1.8% to 31.4% from 1-d to 5-d of treatment (Fig. 21b & 22b).

In roots of seedlings of nonnodulated chickpea sulphur deficiency inhibited the accumulation of Na^+ by 50% at 5-d of treatment. Similarly, in the root of nodulated genotypes, sulphur deficiency inhibited the accumulation of Na^+ by 14% and 18% at 3-d and 5-d of treatment respectively except an initial promotion in the nodulated genotype at 1-d of sulphur deficiency treatment (Fig. 23a & 24a).

Sulphur deficiency increased the accumulation of Na^+ in the shoot of the seedling of the nonnodulated genotype by 13% to 40% from 1-d to 5-d of treatment. On the other hand, in the shoot of the seedling of the nodulated genotype, sulphur deficiency inhibited the accumulation of Na^+ by 9% at 5-d of treatment and this inhibitory trend was maintained upto 1-d to 3-d of treatment (Fig. 23b & 24b).

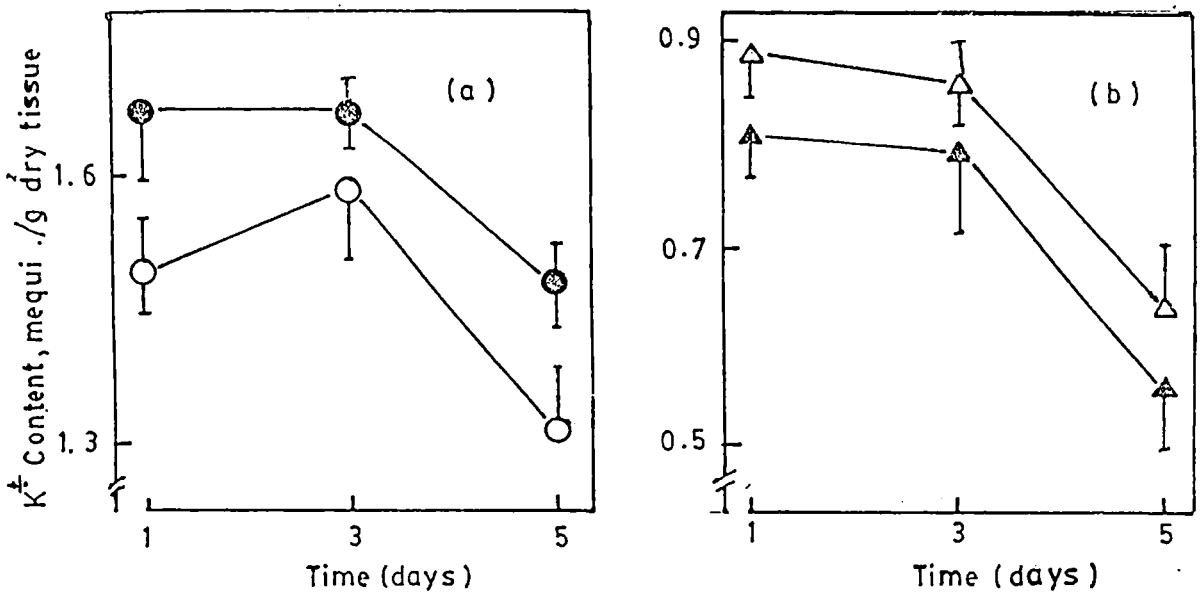


Fig. 21. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Solid symbols------ + S , open symbols-------- – S. O root, Δ shoot. Each value is the mean of three replicates ± standard error.

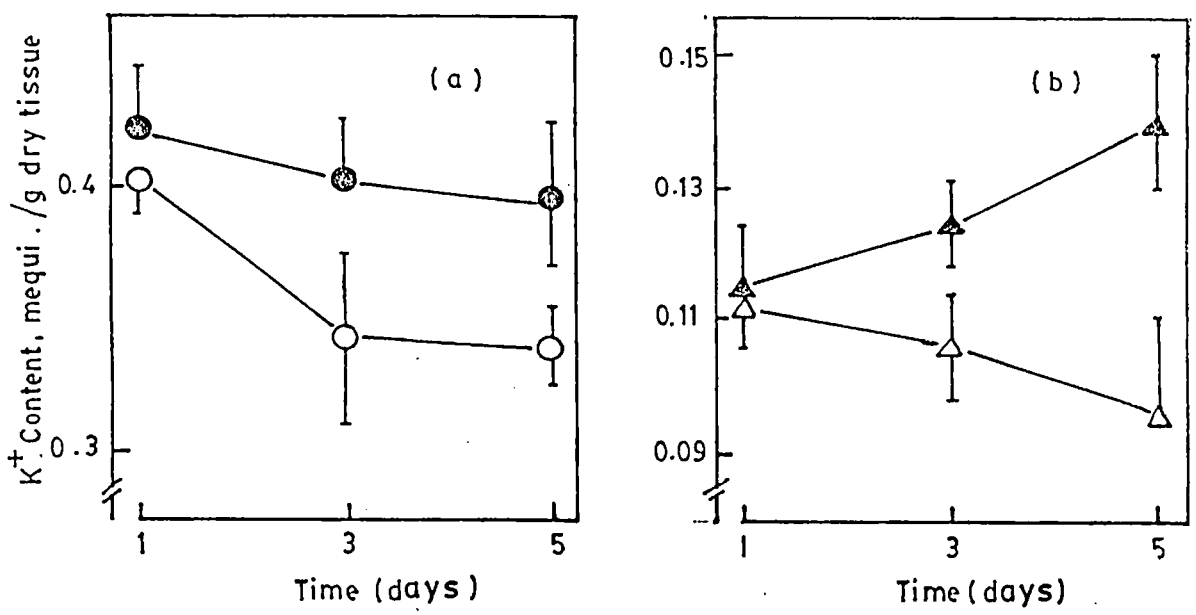


Fig. 22. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

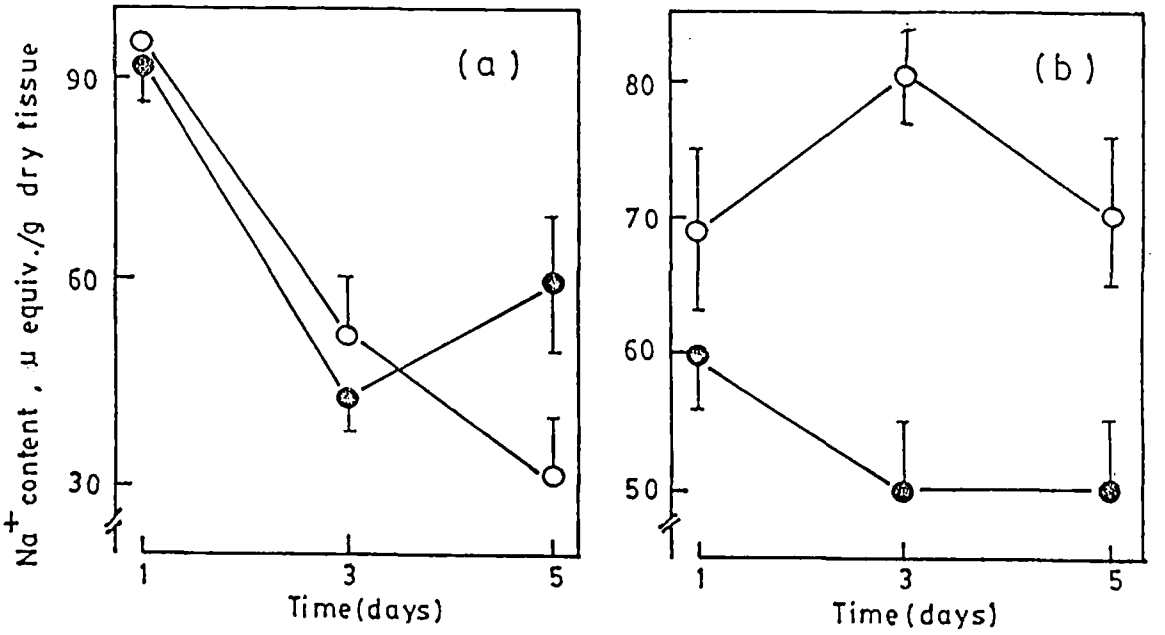


Fig. 23. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

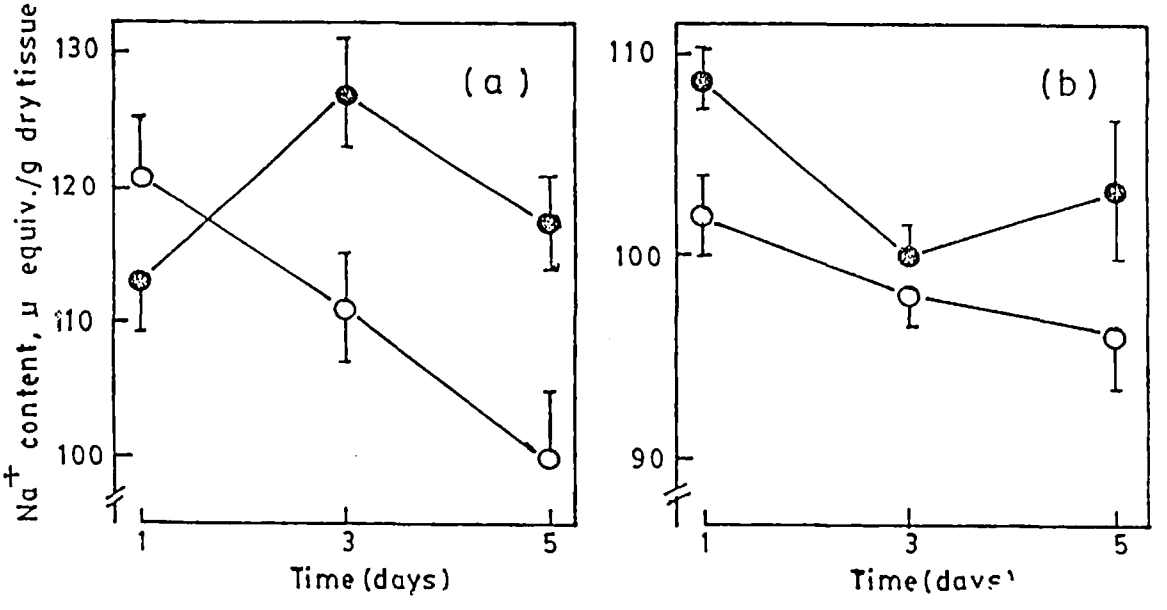


Fig. 24. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

5.3.2 Effects of sulphur deficiency on accumulation and distribution of K^+ and Na^+ in 15-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of the nonnodulated genotype, sulphur deficiency increased the accumulation of K^+ by 10% and 12% at 3-d and 5-d of treatment respectively. On the contrary, Sulphur deficiency showed an inhibitory trend in K^+ accumulation in the root of the seedling of nodulated genotype (Fig. 25a & 26a).

Sulphur deficiency showed inhibitory effect in K^+ accumulation in the shoot of 15-d-old nonnodulated chickpea seedling at 3-d and 5-d of treatment. In the shoot of the seedling of the nodulated genotype, sulphur deficiency decreased K^+ accumulation by 9.5% and 16% at 3-d and 5-d of treatment respectively (Fig. 25b & 26b).

In the root of the nonnodulated chickpea seedling, sulphur deficiency increased the accumulation of Na^+ by 17% at 3-d of treatment. On the contrary, in the seedling of the nodulated genotype, Na^+ accumulation was inhibited in the root by 7.1%, 16.4% and 12.9% following 1-d, 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 27a & 28a).

In the shoot of the seedling of the nonnodulated genotype, sulphur deficiency decreased the accumulation of Na^+ by 11% at 1-d of treatment.

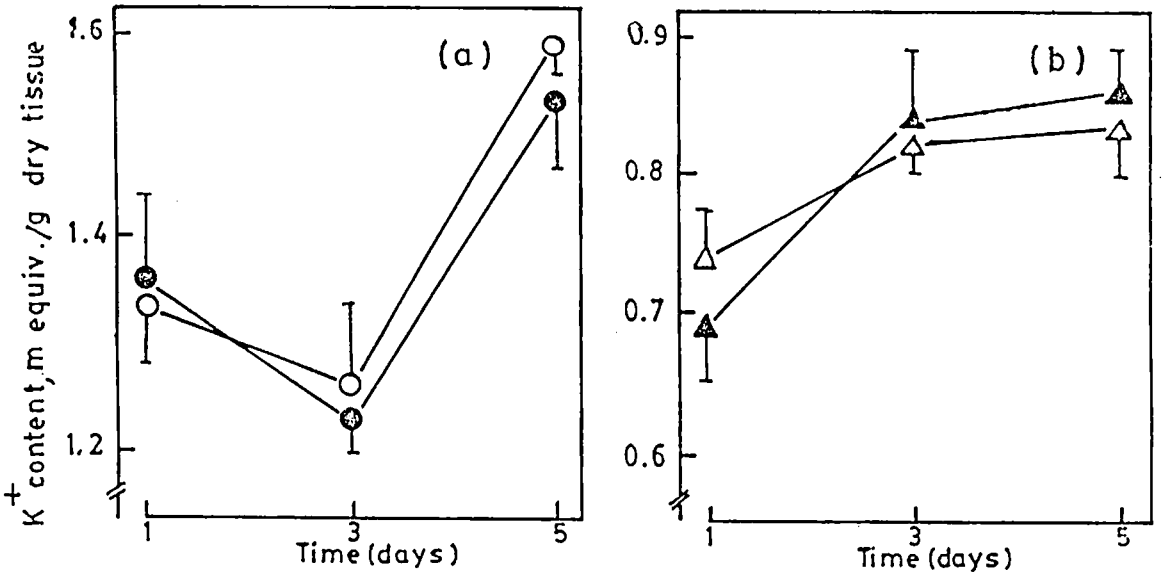


Fig. 25. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

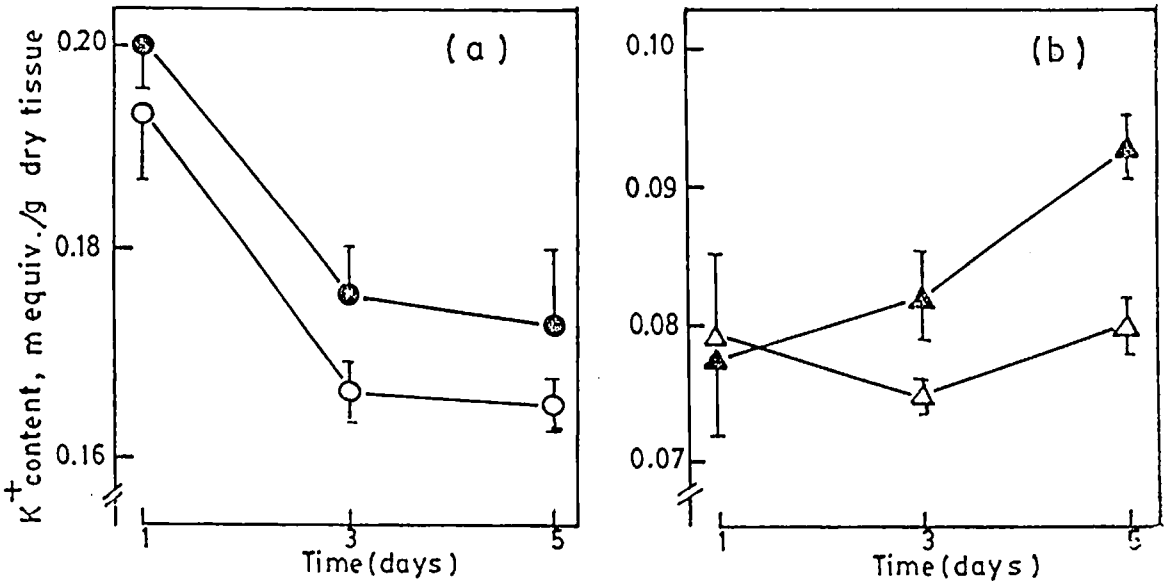


Fig. 26. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

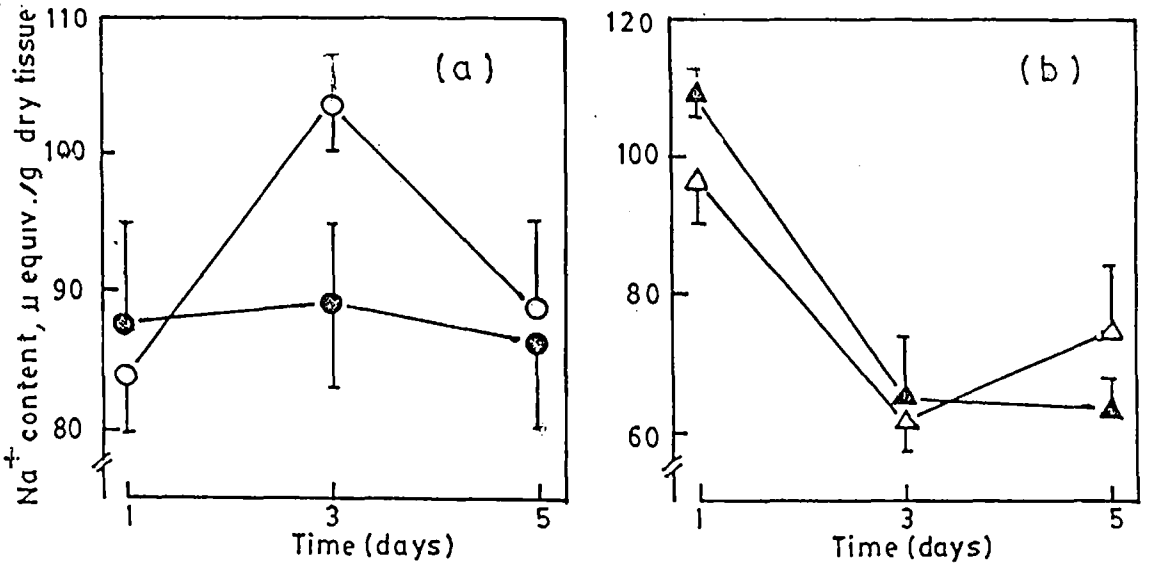


Fig. 27. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

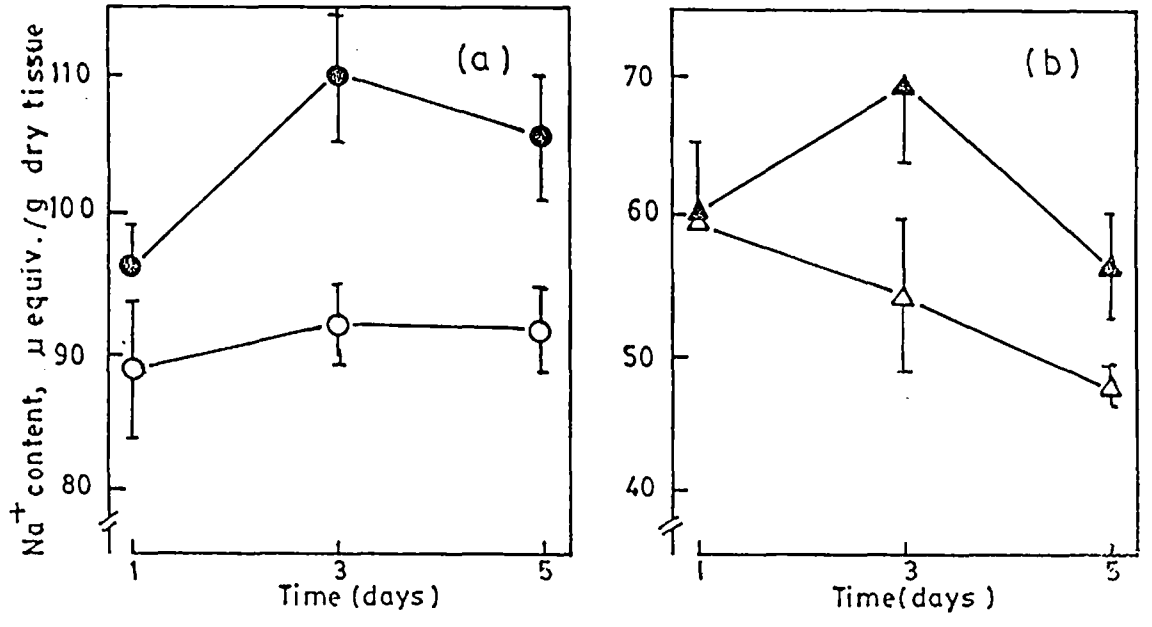


Fig. 28. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

On the other hand, 5-d of sulphur deficiency increased Na^+ accumulation by 16% (Fig. 27b). Accumulation of Na^+ in the shoot of the nodulated chickpea seedling was inhibited by 21% and 15.5% following 3-d and 5-d of sulphur deficiency respectively but had no effect at 1-d of sulphur deficiency treatment (Fig. 28b).

5.3.3 Effects of sulphur deficiency on accumulation and distribution of K^+ and Na^+ in 21-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

Potassium accumulation in the root of the 21-d-old nonnodulated chickpea seedling was depressed by 7.2% at 1-d of sulphur deficiency treatment. This inhibitory effect was sustained upto 5-d of sulphur deficiency treatment (Fig. 29a). In the 21-d-old nodulated chickpea seedling, sulphur deficiency inhibited the accumulation of K^+ in the root by 25.1% to 30% from 1-d to 3-d of treatment and the inhibitory effect was sustained upto 5-d of treatment (Fig. 30a).

In the shoot of the nonnodulated seedling, sulphur deficiency inhibited the accumulation of K^+ by 10% at 5-d of treatment (Fig. 29b). Similarly, accumulation of K^+ was decreased in the shoot of the nodulated chickpea seedling by 6.1% to 14.3% from 1-d to 5-d of treatment (Fig. 30b).

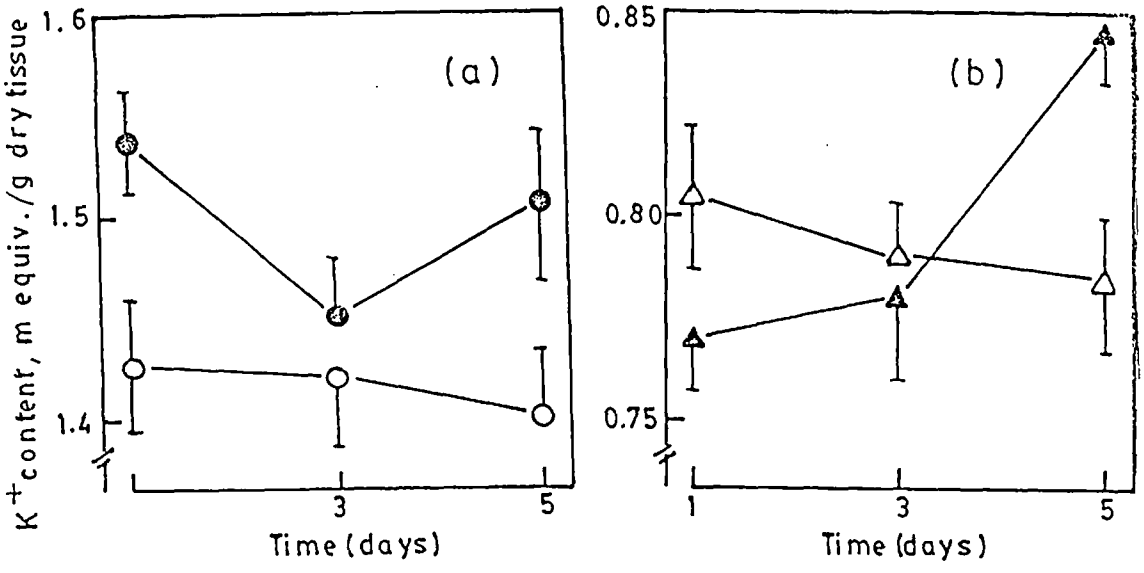


Fig. 29. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 21-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

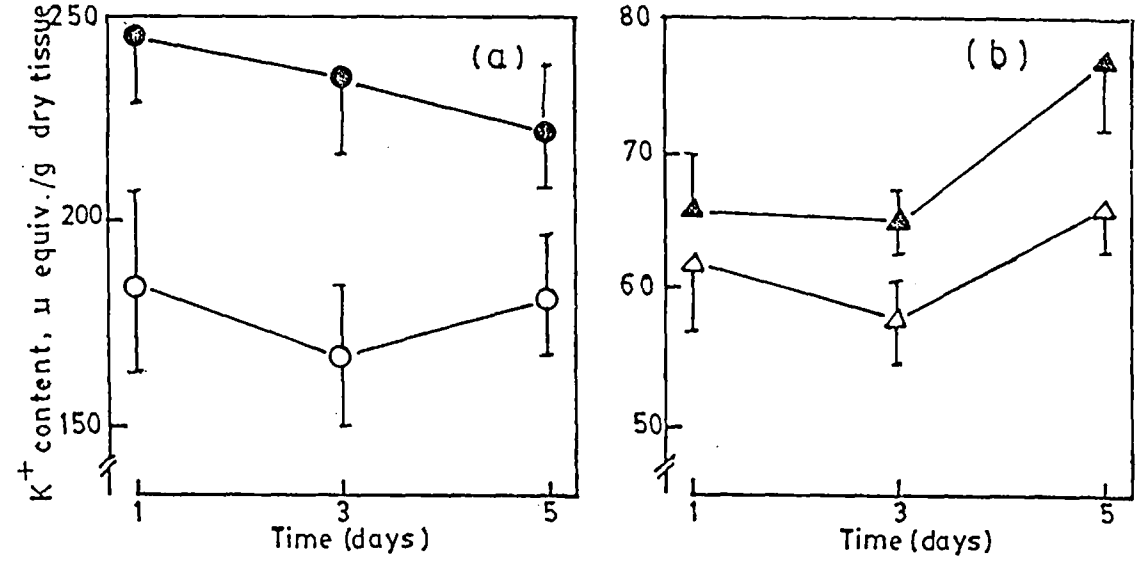


Fig. 30. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 21-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

No effect of sulphur deficiency was observed on Na^+ accumulation in the root and the shoot of the nonnodulated chickpea seedling (Fig. 31a & 31b). Sulphur deficiency caused an inhibition of Na^+ accumulation in the root of the nodulated seedling by 20% and 15% at 3-d and 5-d of treatment respectively (Fig. 32a). In the shoot, Na^+ accumulation was decreased by 6.2% to 25.8% from 1-d to 5-d of sulphur deficiency treatment (Fig. 32b).

5.3.4 Effects of sulphur deficiency on Ca^{2+} and Mg^{2+} accumulation in 7-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the nonnodulated chickpea seedling, sulphur deficiency inhibited the accumulation of Ca^{2+} by 11% at 1-d of treatment. The inhibitory effect was sustained upto 3-d of treatment (Fig. 33a). On the contrary, accumulation of Ca^{2+} in the root of the nodulated chickpea seedling was increased under sulphur deficiency by 15.7% and 9.1% at 1-d and 5-d of treatment respectively (Fig. 34a).

Sulphur deficiency caused an increase in Ca^{2+} accumulation in the shoot of the seedling of the nonnodulated genotype by 13% at 1-d of treatment and this was followed by an inhibition of Ca^{2+} accumulation upto 5-d of treatment (Fig. 33b). In the shoot of the seedling of the nodulated genotype,

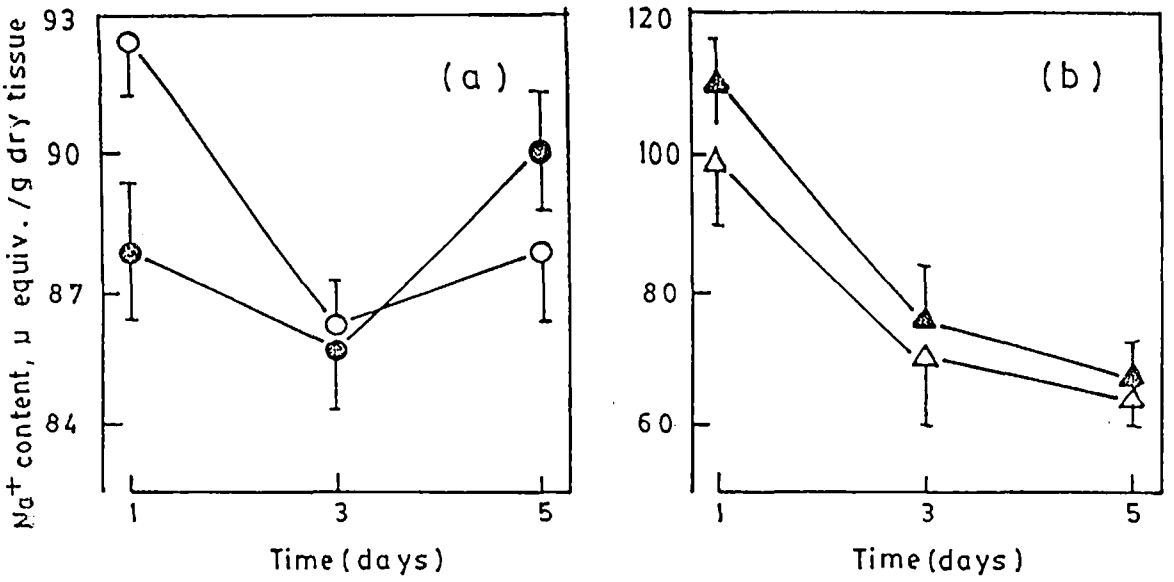


Fig. 31. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 21-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

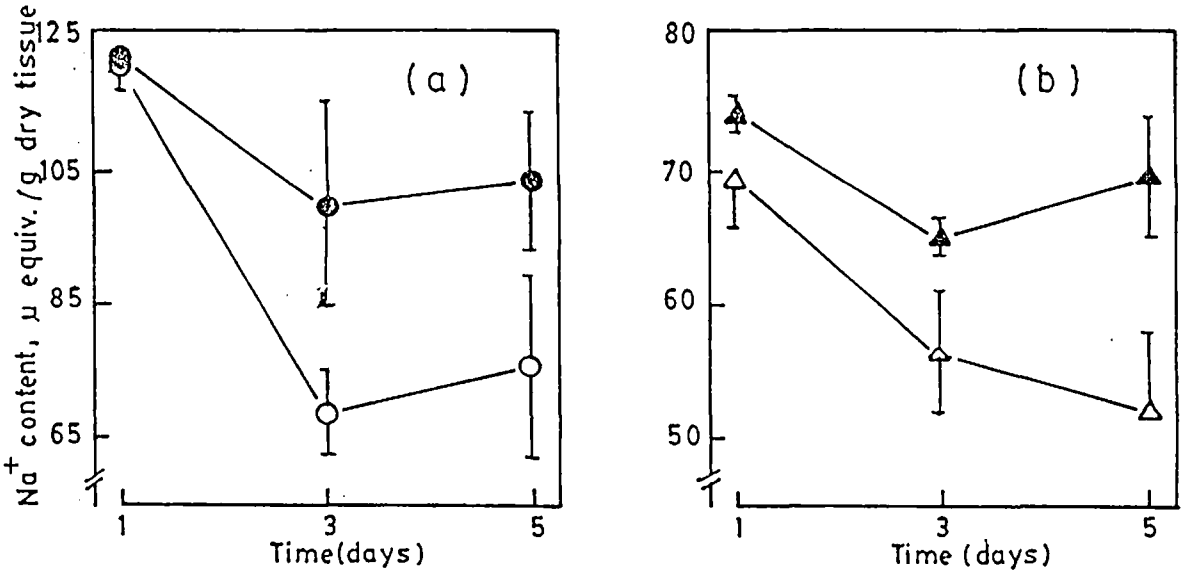


Fig. 32. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 21-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

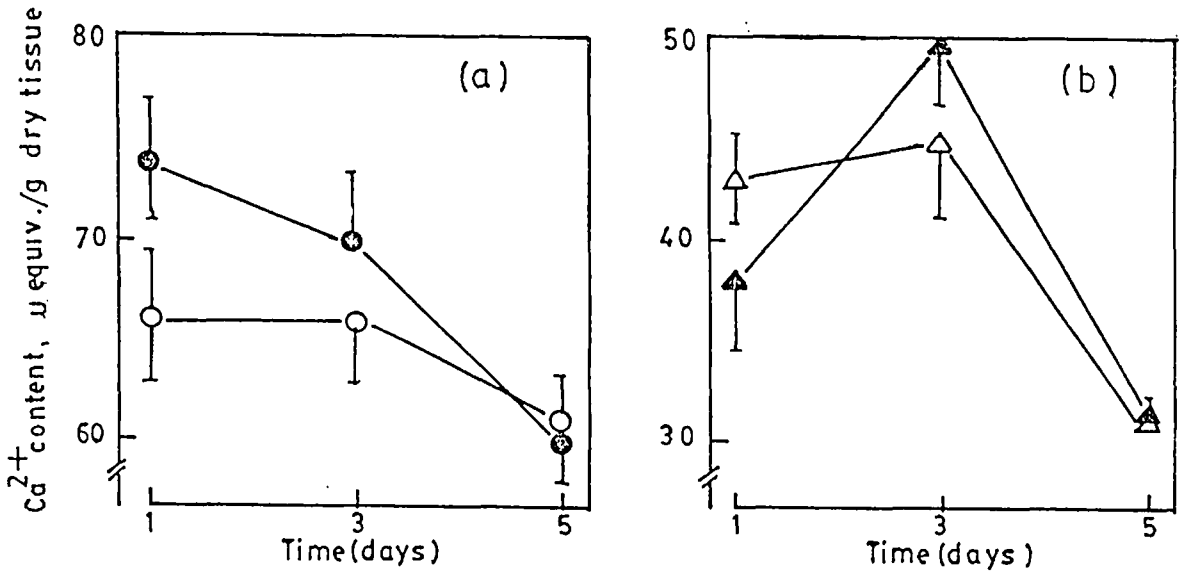


Fig. 33. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

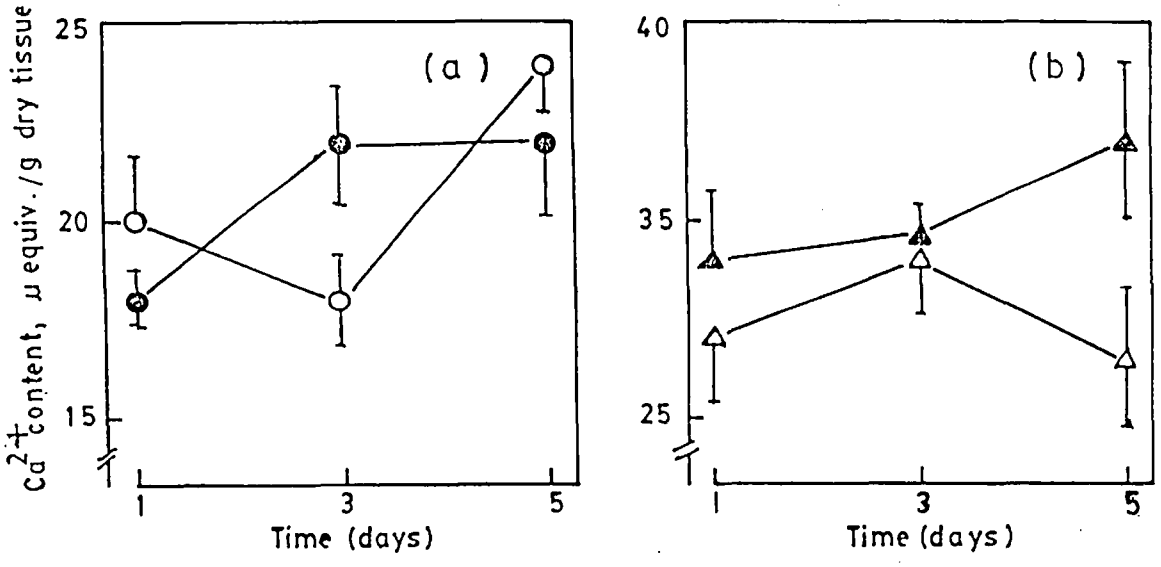


Fig. 34. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

Ca^{2+} accumulation was depressed by 24.2% and 28.8% at 1-d and 5-d respectively following sulphur deficiency treatment (Fig. 34b).

Mg^{2+} accumulation in the root of the nonnodulated chickpea seedling was increased by 26% to 41% at 1-d to 5-d of deficiency treatment. On the contrary, in the root of the seedling of the nodulated genotype, sulphur deficiency caused 22% and 24.5% inhibition of Mg^{2+} accumulation at 3-d and 5-d of treatment respectively (Fig. 35a & 36a).

In the shoot of the seedling of the nonnodulated genotype, sulphur deficiency resulted in an increase in the accumulation of Mg^{2+} by 23.6%, 80% and 15% at 1-d, 3-d and 5-d of treatment respectively. However, sulphur deficiency had no effect on the accumulation of Mg^{2+} in the shoot of the nodulated chickpea seedling (Fig. 35b & 36b).

5.3.5 Effects of sulphur deficiency on Ca^{2+} and Mg^{2+} accumulation in 15-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the nonnodulated chickpea seedling, sulphur deficiency increased the accumulation of Ca^{2+} at 3-d and 5-d of treatment by 18.9% and 53.8% respectively (Fig. 37a) whereas accumulation of Ca^{2+} was

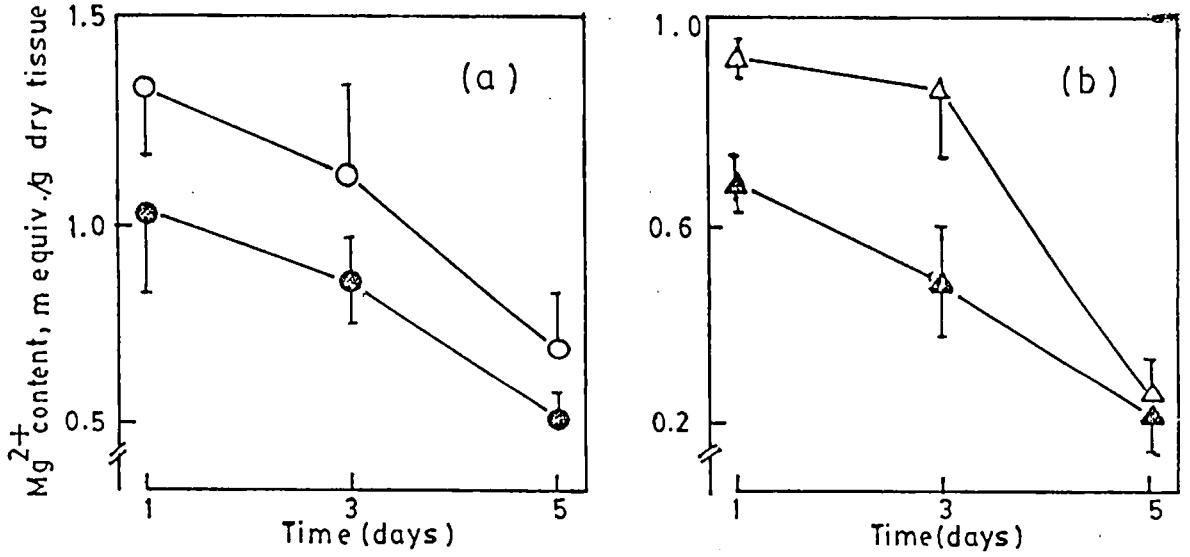


Fig. 35. The effect of sulphur deficiency on Mg^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

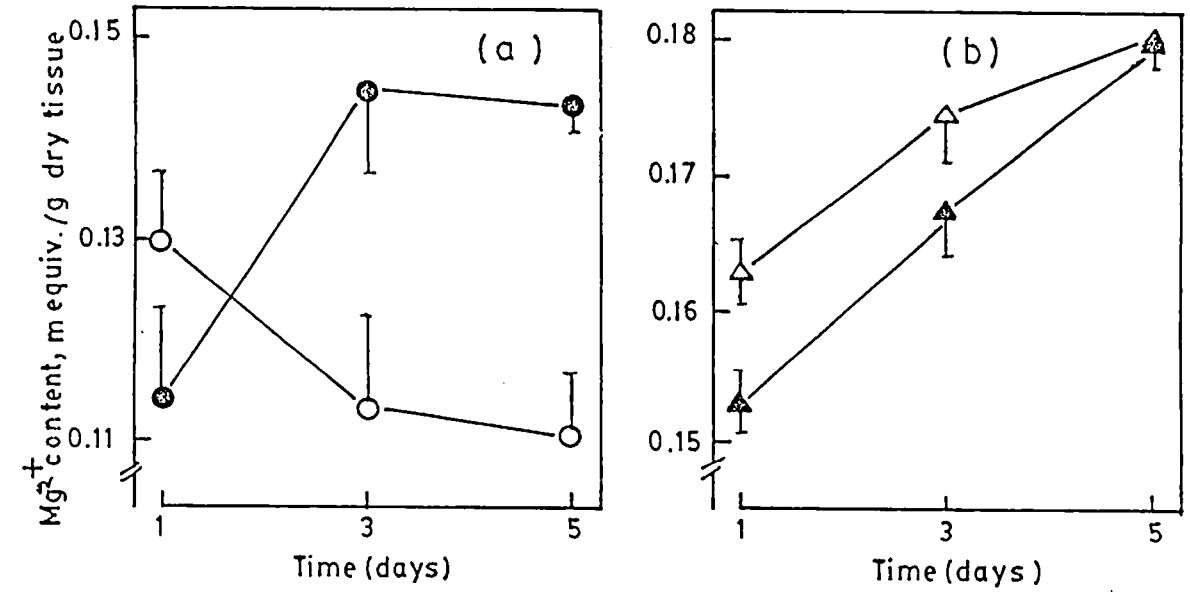


Fig. 36. The effect of sulphur deficiency on Mg^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

decreased in the root of the nodulated sulphur-deficient seedling by 7.5% and 7.9% at 3-d and 5-d of S-treatment respectively (Fig. 38a).

In the shoot of the nonnodulated chickpea seedling, sulphur deficiency resulted in an increase in accumulation of Ca^{2+} by 15%, 25% and 15% at 1-d to 5-d of treatment respectively (Fig. 37b). On the other hand, Ca^{2+} accumulation was increased in the shoot of the seedling of the nodulated genotype by 16% at 1-d following a decrease in accumulation of that by 14.5% and 9% at 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 38b).

In the root of the nonnodulated chickpea seedling, sulphur deficiency increased the accumulation of Mg^{2+} by 18.5% and 53% at 3-d and 5-d of treatment respectively. Similarly, Mg^{2+} accumulation was increased in the shoot of the seedling of the nonnodulated genotype by 22% and 33% at 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 39a.& 39b). On the contrary, sulphur deficiency had very little effect on Mg^{2+} accumulation in the root and the shoot of 15-d-old nodulated seedlings (Fig. 40a & 40b).

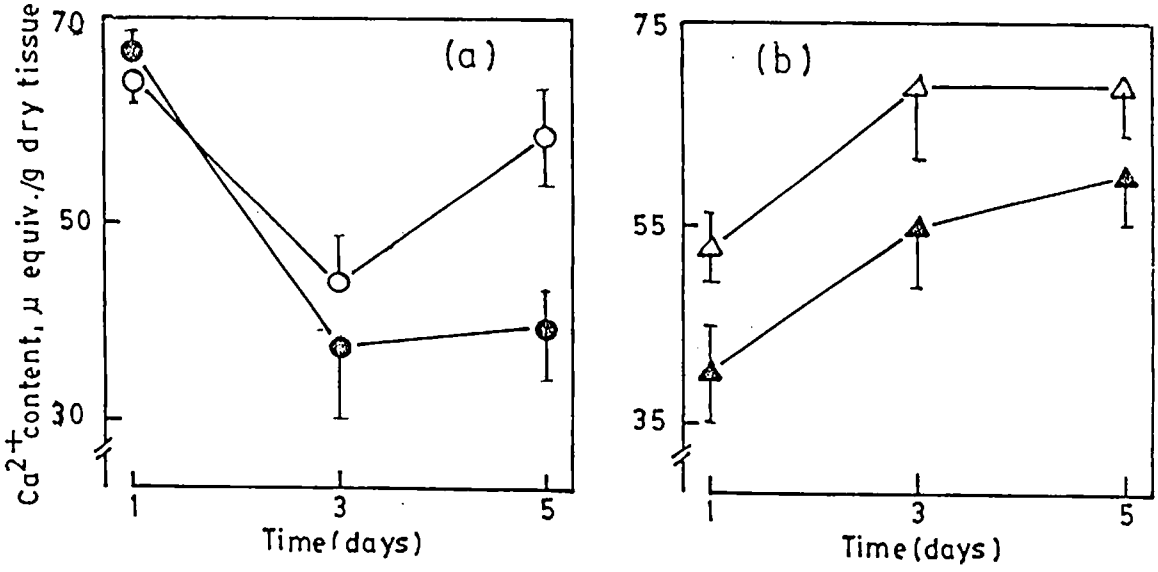


Fig. 37. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

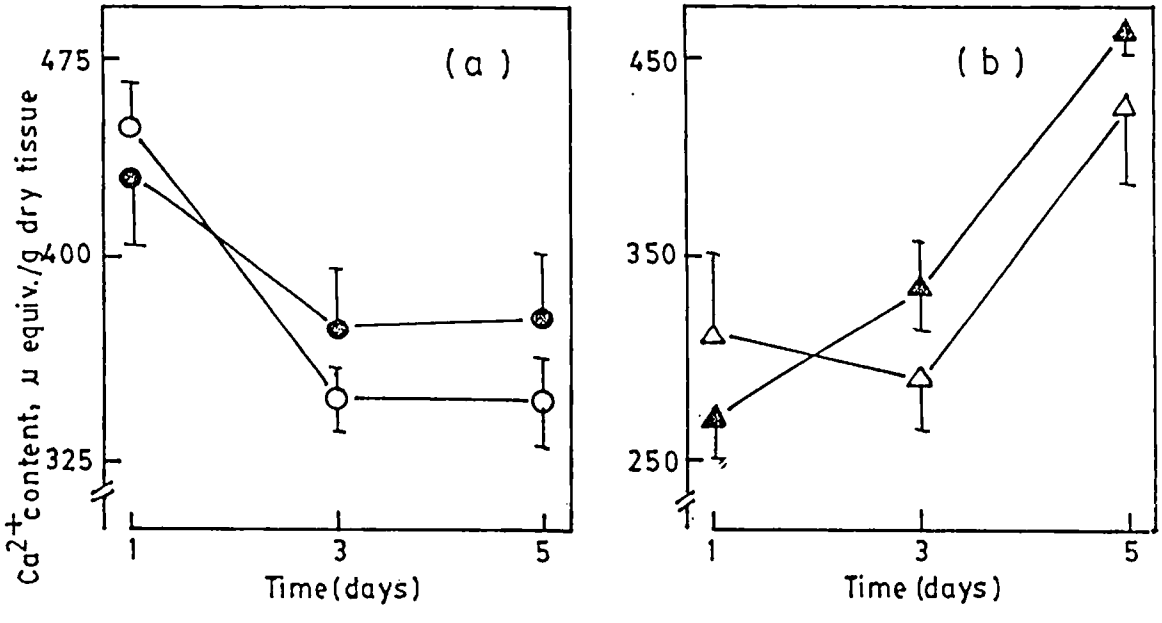


Fig. 38. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

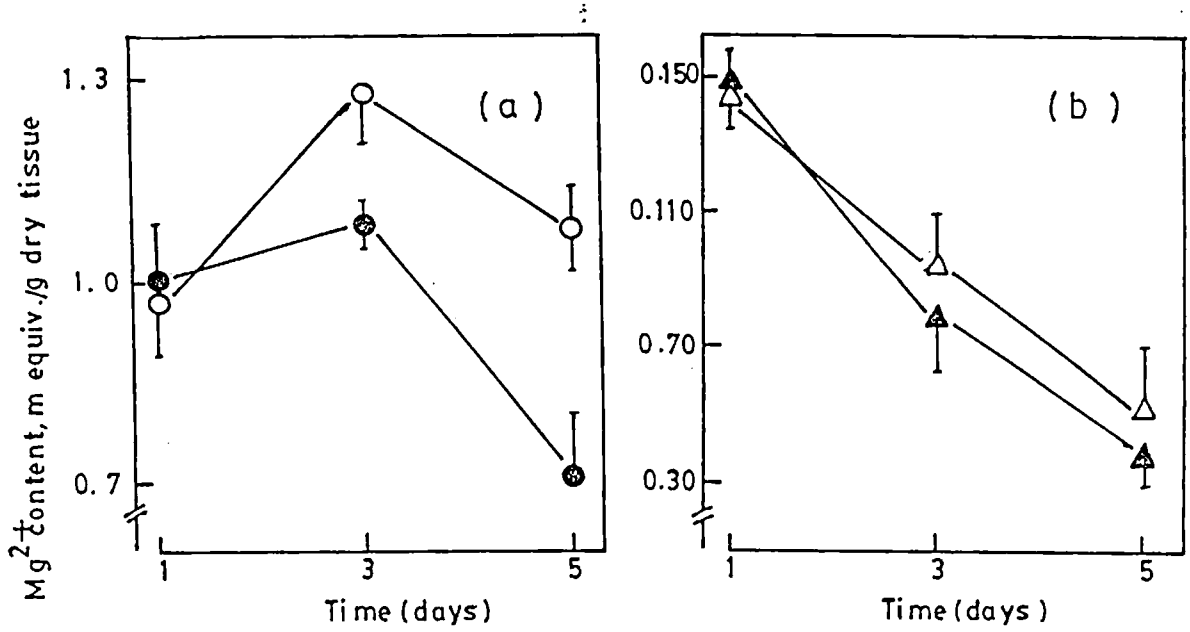


Fig. 39. The effect of sulphur deficiency on Mg^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

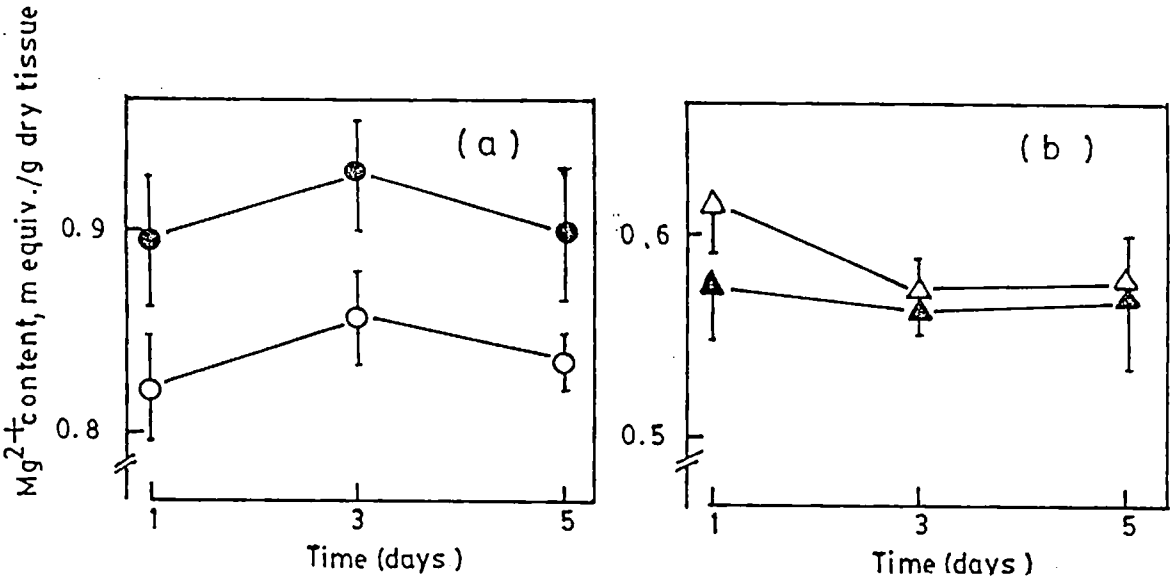


Fig. 40. The effect of sulphur deficiency on Mg^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

5.3.6 Effects of sulphur deficiency on Ca^{2+} and Mg^{2+} accumulation in 21- d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

Calcium accumulation in the root of the nonnodulated chickpea seedling was increased by 7% and 12% at 3-d and 5-d of treatment respectively (Fig. 41a). On the contrary, accumulation of Ca^{2+} was inhibited in the root of the nodulated seedling upto 5-d of sulphur deficiency treatment (Fig. 42a). Accumulation of Ca^{2+} in the shoot of the nonnodulated seedling was increased by 10% and 7.5% at 1-d and 3-d of sulphur deficiency treatment respectively (Fig. 41b). On the other hand, Ca^{2+} accumulation was depressed in the shoot of the nodulated seedling by 13.9% to 13.2% at 1-d to 5-d following deficiency treatment (Fig. 42b).

Sulphur deficiency caused an increase in accumulation of Mg^{2+} in the root of the nonnodulated seedlings by 13.4%, 9.35% and 17% at 1-d, 3-d and 5-d of treatment respectively (Fig. 43a). Similarly, sulphur deficiency slightly increased the accumulation of Mg^{2+} in the root of the nodulated seedling (Fig. 44a).

In the shoot of the nonnodulated chickpea seedling, sulphur deficiency caused an increase in accumulation of Mg^{2+} by 17.6% and 13.3% at 3-d and 5-d of treatment respectively (43b). Similarly, sulphur deficiency

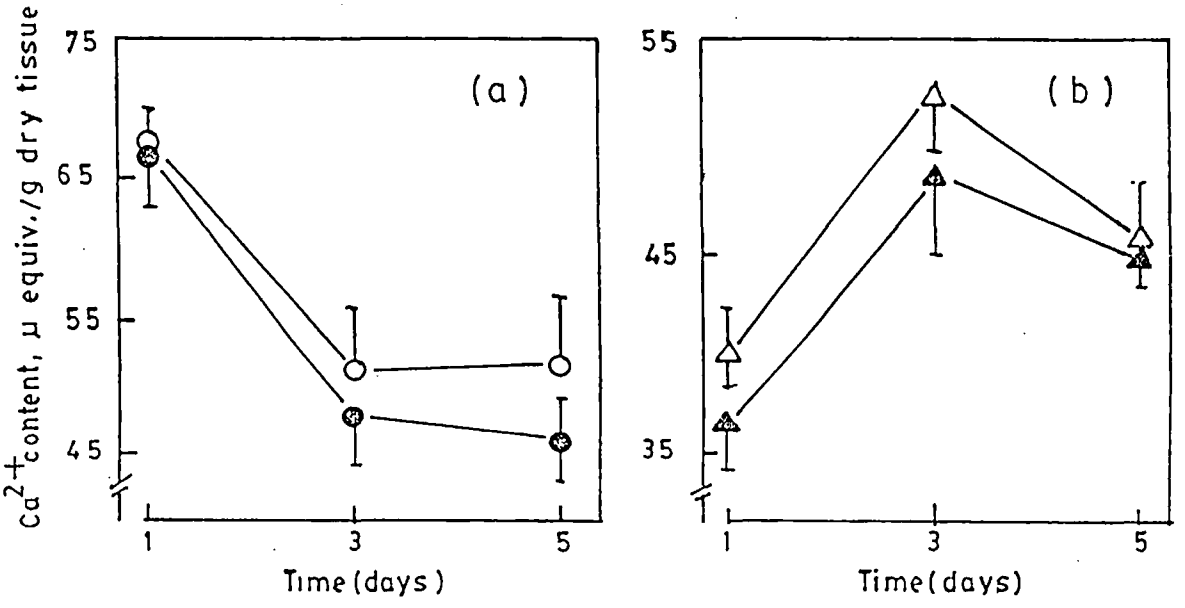


Fig. 41. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 21-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

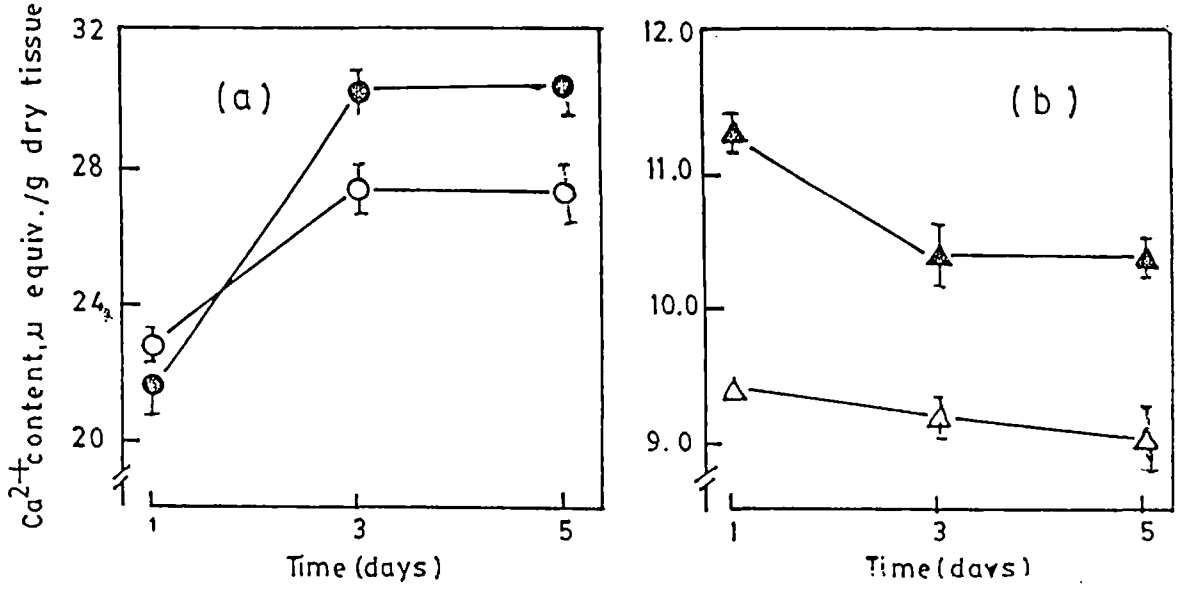


Fig. 42. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 21-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

increased Mg^{2+} accumulation in the shoot of the nodulated seedling by 13.1% at 5-d of treatment (Fig. 44b).

5.3.7 Effects of sulphur deficiency on Fe^{2+} and Zn^{2+} accumulation in 7-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of the nonnodulated genotype, sulphur deficiency increased the accumulation of Fe^{2+} by 7.3% and 30.5% at 3-d and 5-d of treatment respectively (Fig. 45a). Accumulation of Fe^{2+} was depressed in the root of the S-deficient nodulated chickpea seedling by 10.4% at 3-d of treatment except an initial 22% increase in the accumulation of that at 1-d of treatment (Fig. 46a).

In the shoot of the seedling of the nonnodulated genotype, accumulation of Fe^{2+} was inhibited by 39.1% at 5-d of sulphur deficiency treatment. Similarly, accumulation of Fe^{2+} in the shoot of the seedling of the nodulated genotype was inhibited by 26.7 % to 39.3% than that of the control from 1-d to 5-d of sulphur deficiency treatment (Fig. 45b & 46b).

In the root of the nonnodulated chickpea seedling, sulphur deficiency inhibited the accumulation of Zn^{2+} by 29.5% to 38% from 1-d to 5-d of treatment (Fig. 47a). Similarly, accumulation of Zn^{2+} in the root of S-

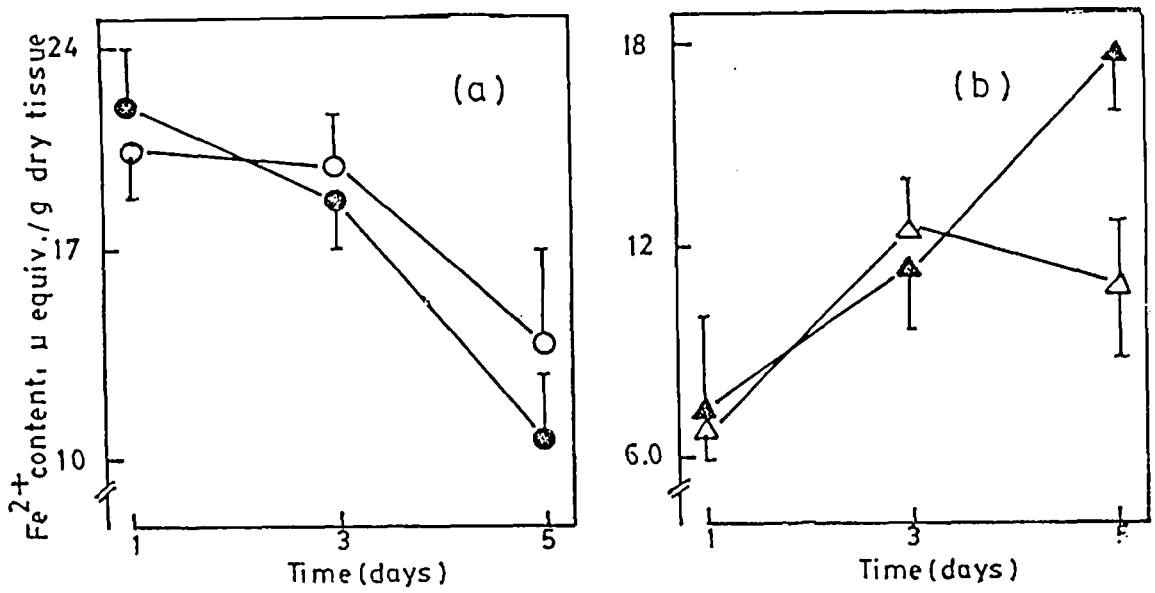


Fig. 45. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

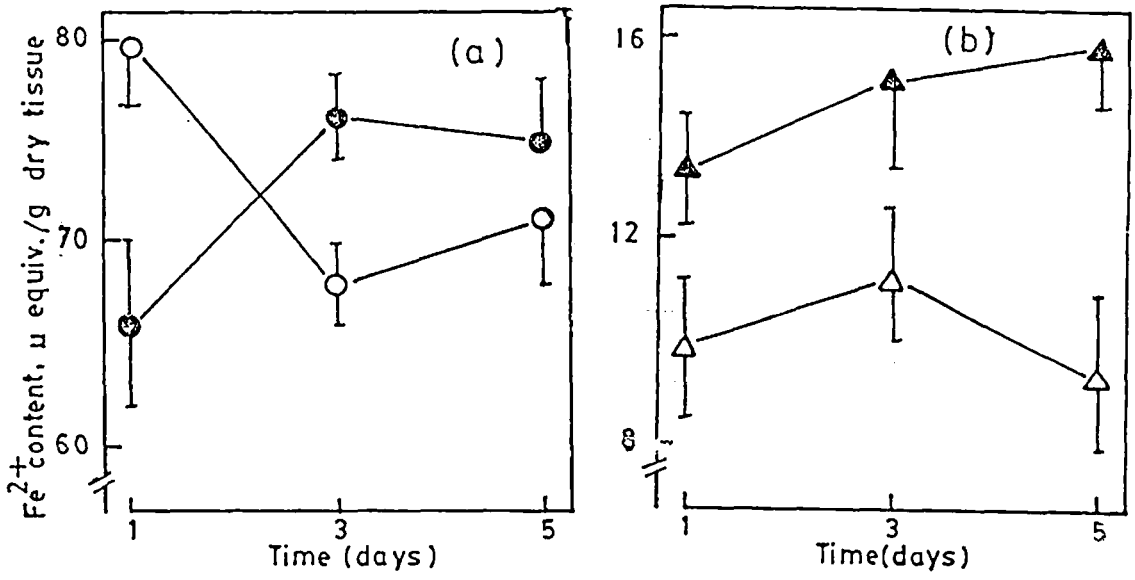


Fig. 46. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

deficient nodulated chickpea seedling was decreased by 22% and 13.5% at 3-d and 5-d of S-treatment respectively (Fig. 48a).

In the shoot of the seedling of the nonnodulated genotype, sulphur deficiency inhibited the accumulation of Zn^{2+} by 17% and 33% at 3-d and 5-d of treatment respectively except an initial increase in accumulation of that at 1-d of treatment. Similarly, Zn^{2+} accumulation in the shoot of the nodulated chickpea seedling was inhibited by 10.5% and 19% at 1-d and 5-d of sulphur deficiency treatment respectively (Fig. 47b & 48b).

5.3.8 Effects of sulphur deficiency on Fe^{2+} and Zn^{2+} accumulation in 15-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of the nonnodulated genotype, inhibitory effect of sulphur deficiency on Fe^{2+} accumulation was observed from 1-d to 5-d of treatment (Fig. 49a). Similarly, Fe^{2+} accumulation was inhibited in the root of the nodulated chickpea seedling by 8.2% and 12.6% following 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 50a).

Accumulation of Fe^{2+} in the shoot of the seedling of the nonnodulated genotype was decreased by 9.7% and 13.7% at 3-d and 5-d of sulphur deficiency treatment respectively except an initial increase in accumulation

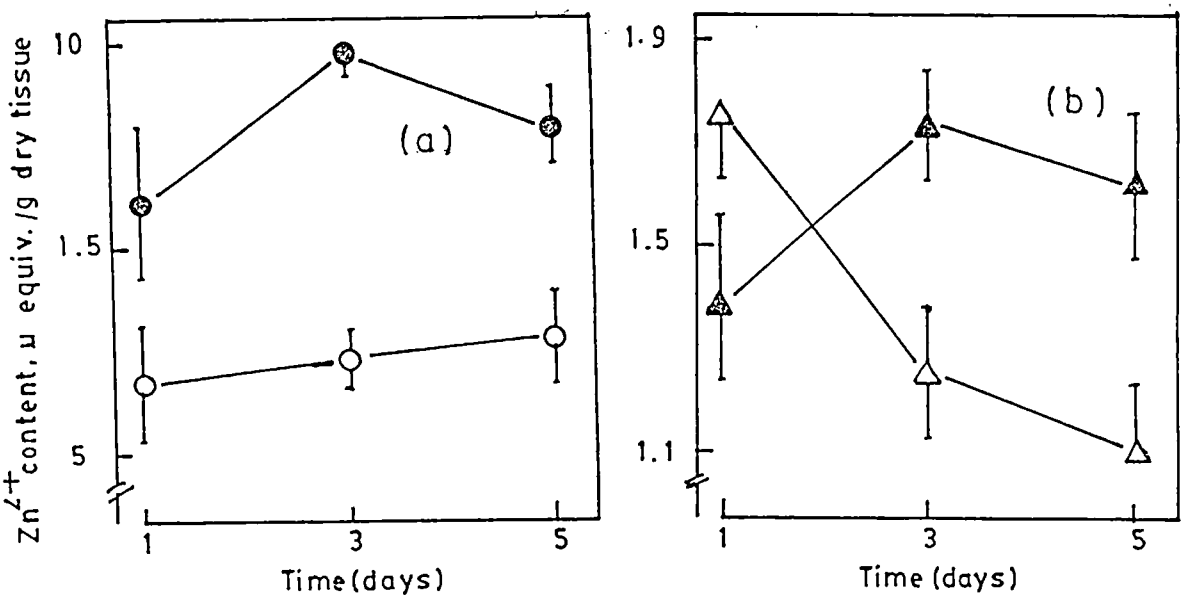


Fig. 47. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

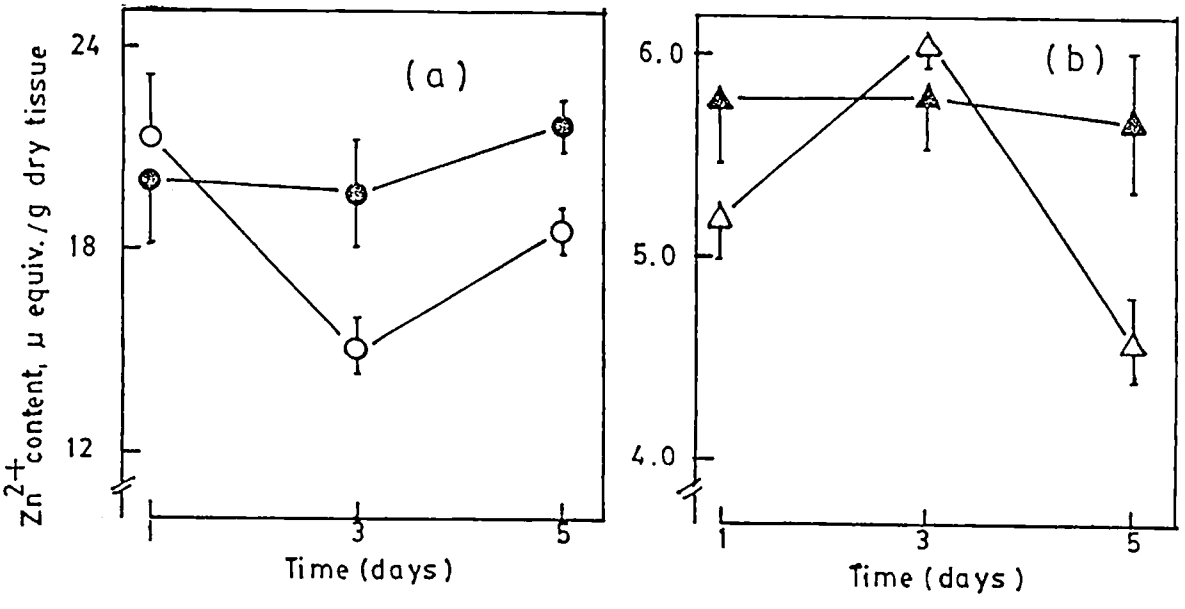


Fig. 48. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

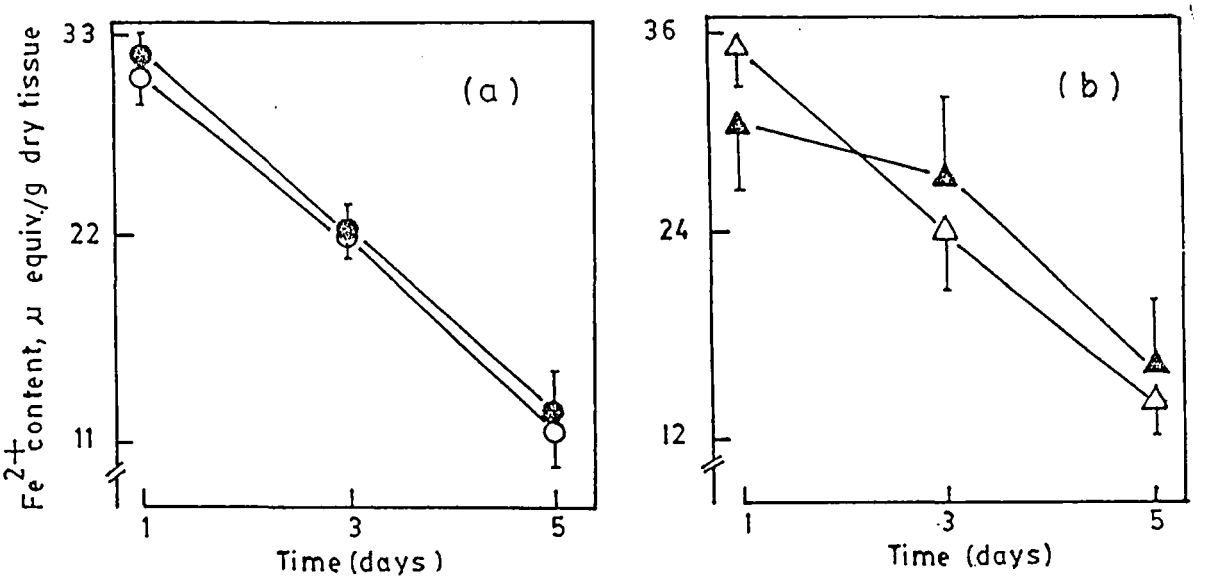


Fig. 49. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

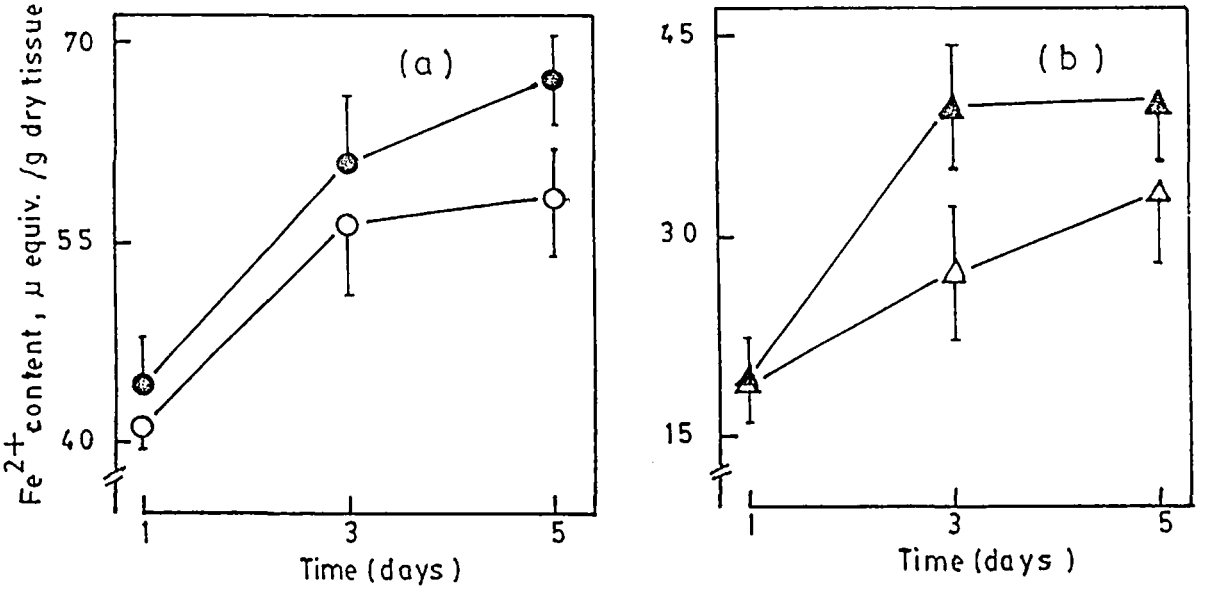


Fig. 50. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

of that at 1-d of treatment (Fig. 49b). In the shoot of the nodulated chickpea seedling, Fe^{2+} accumulation was inhibited by 14.7% to 25.7% at 3-d to 5-d of treatment respectively (Fig. 50b).

Sulphur deficiency inhibited the accumulation of Zn^{2+} in the root of the nonnodulated chickpea seedling by 22% to 24.5% from 1-d to 5-d of treatment (Fig. 51a). Similarly, zinc accumulation in the root of the nodulated genotype was inhibited by 10.2%, 19.3% and 13.4% at 1-d, 3-d and 5-d respectively following sulphur deficiency treatment (Fig. 52a).

Accumulation of Zn^{2+} in the shoot of the seedling of the nonnodulated genotype was increased by 12% and 15% under sulphur-deficient condition at 1-d and 5-d respectively (Fig. 51b). On the contrary, in the shoot of the nodulated chickpea seedling, sulphur deprivation has an inhibitory effect on Zn^{2+} accumulation in all growth stages (Fig. 52b).

5.3.9 Effects of sulphur deficiency on Fe^{2+} and Zn^{2+} accumulation in 21-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

Iron accumulation in the root of the nonnodulated chickpea seedling was decreased by 3.6% to 17.4% at 1-d to 5-d of sulphur deficiency treatment (Fig. 53a). Similarly, accumulation of Fe^{2+} in the root of the nodulated

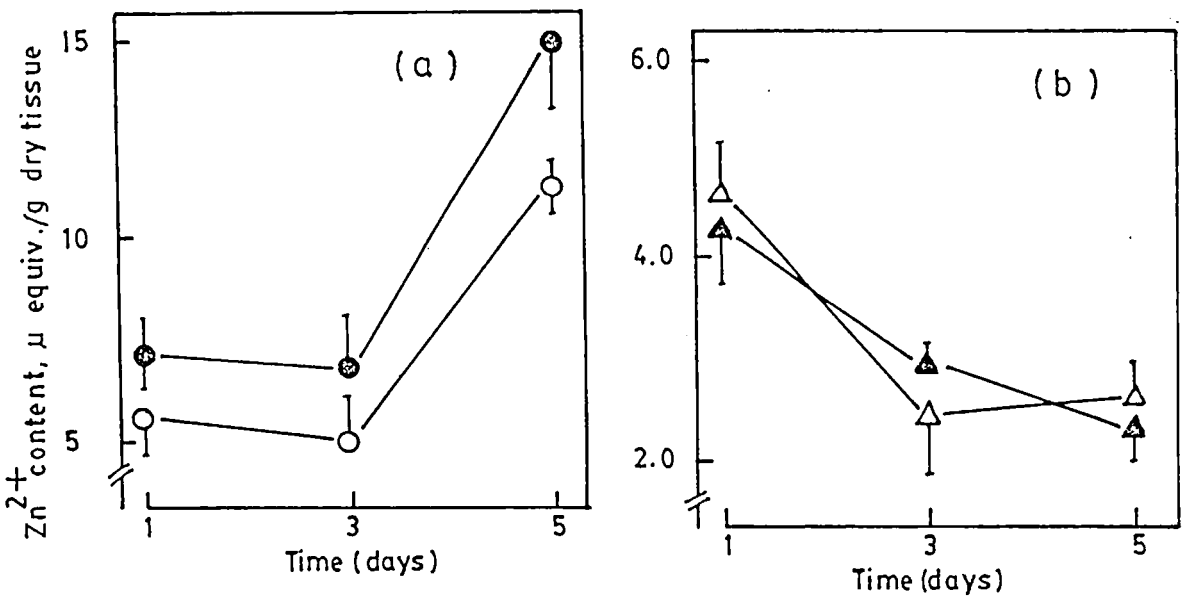


Fig. 51. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

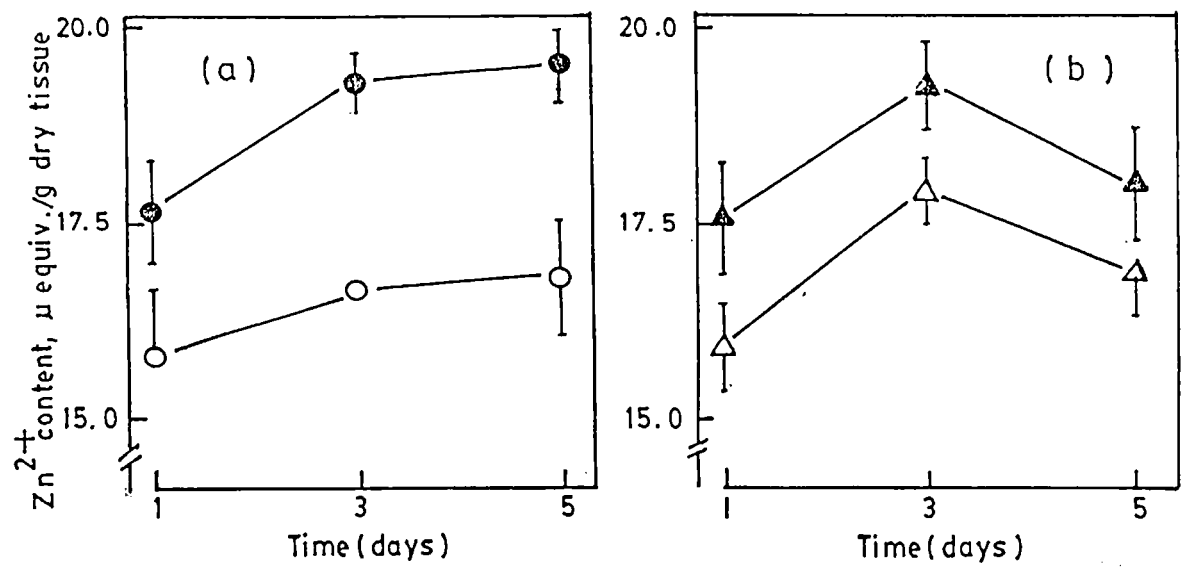


Fig. 52. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

seedling was inhibited by 10.5% and 8.1% under sulphur-deficient condition at 3-d and 5-d of treatment respectively (Fig. 54a).

Accumulation of Fe^{2+} in the shoot of the nonnodulated chickpea seedling was inhibited by 14% and 12% at 3-d and 5-d of sulphur deficiency treatment (Fig. 53b). In the shoot of the nodulated chickpea seedling, Fe^{2+} accumulation was decreased by 9.5% and 30.6% at 3-d and 5-d of sulphur deficiency respectively (Fig. 54b).

Zinc accumulation in the root of the nonnodulated chickpea seedling was depressed by 14.2%, 18.4% and 10% at 1-d, 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 55a). In the root of the nodulated chickpea seedling, Zn^{2+} accumulation was inhibited by 20.4%, 12.4% and 12.6% at 1-d, 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 56a).

In the nonnodulated chickpea seedling, Zn^{2+} accumulation was inhibited in the shoot by 6% and 12.5% at 3-d and 5-d of sulphur deficiency treatment (Fig. 55b). Similarly, in the shoot of the nodulated chickpea seedling, Zn^{2+} content was inhibited by 12.5% and 13.9% at 1-d and 3-d of sulphur deficiency treatment respectively (Fig. 56b).

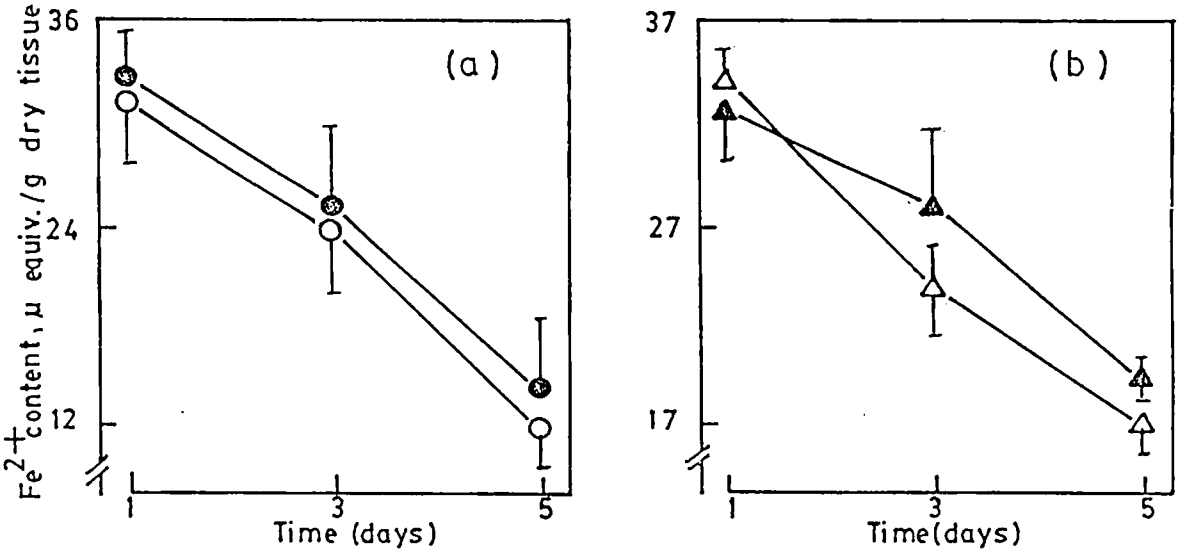


Fig. 53. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 21-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

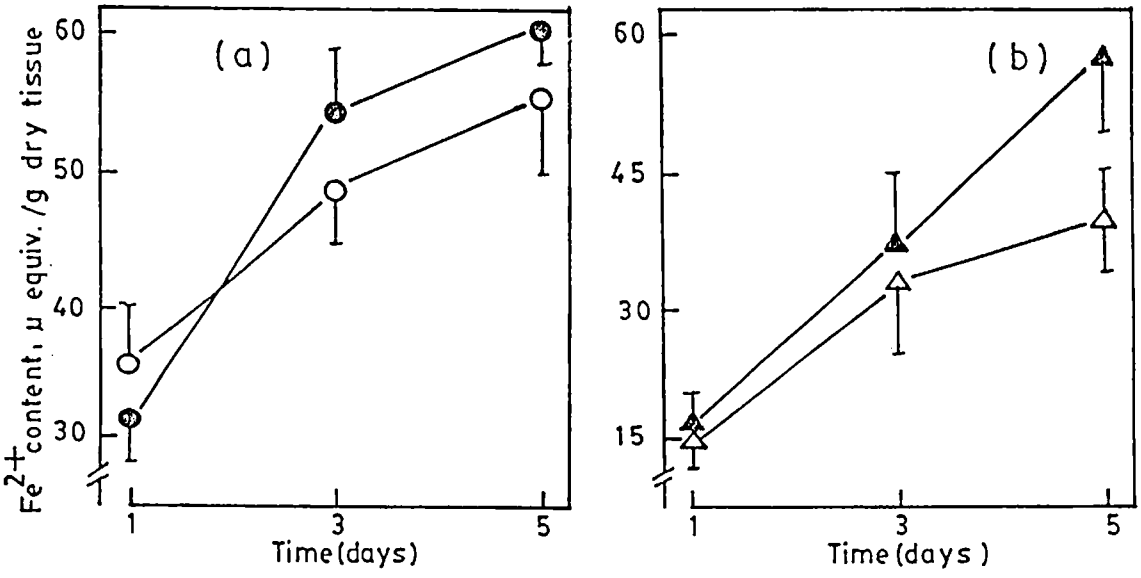


Fig. 54. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 21-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

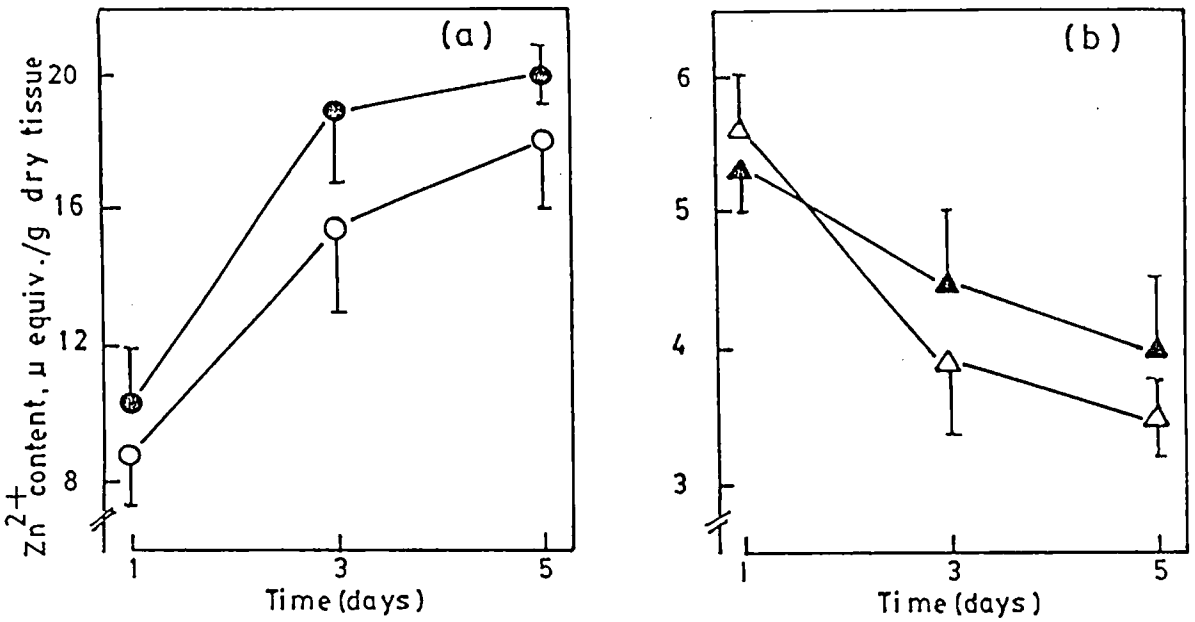


Fig. 55. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 21-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

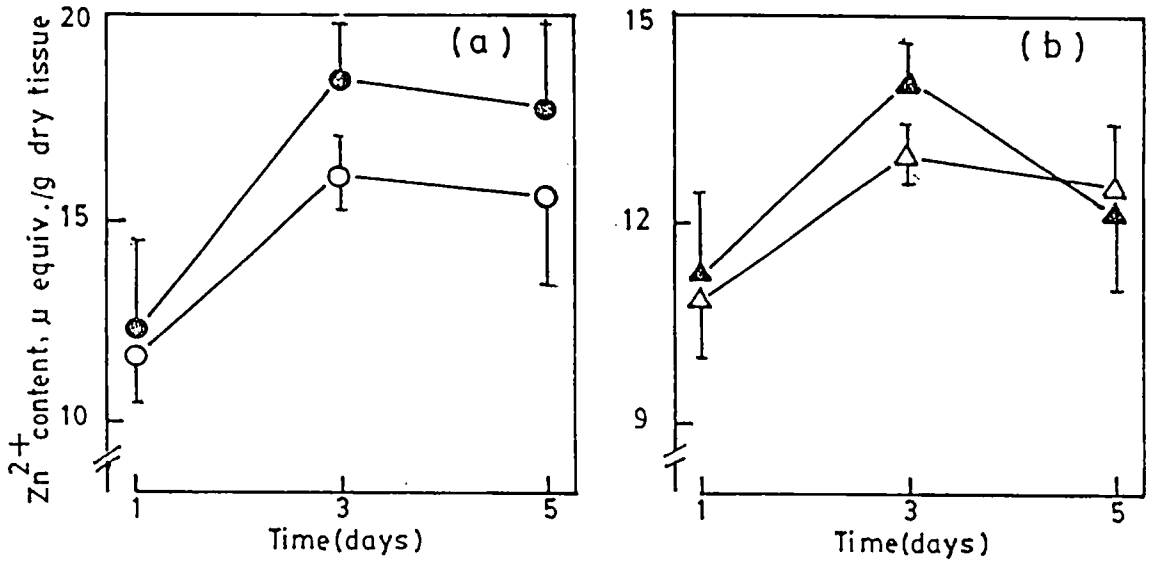


Fig. 56. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 21-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

5.3.10 Effects of sulphur deficiency on accumulation and distribution of phosphate in 7-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

Phosphate accumulation in the root of the seedling of the nonnodulated genotype was inhibited by 9% at 3-d of sulphur deficiency treatment whereas in the root of the nodulated chickpea seedling, sulphur deficiency inhibited phosphate accumulation by 15.9% at 5-d of treatment (Fig. 57a & 58a).

In the shoot of the seedling of the nonnodulated genotype, sulphur deficiency inhibited the accumulation of phosphate by 5.5% and 21.6% at 1-d and 3-d of treatment respectively. Similarly, phosphate accumulation was inhibited in the shoot of the seedling of the nodulated genotype by 17.2% and 22.1% at 1-d and 5-d of treatment respectively following sulphur deficiency treatment (Fig. 57b & 58b).

5.3.11 Effects of sulphur deficiency on accumulation and distribution of phosphate in 15-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of nonnodulated chickpea seedling, phosphate accumulation was inhibited by 7%, 9% and 10.2% following 1-d, 3-d and 5-d of sulphur

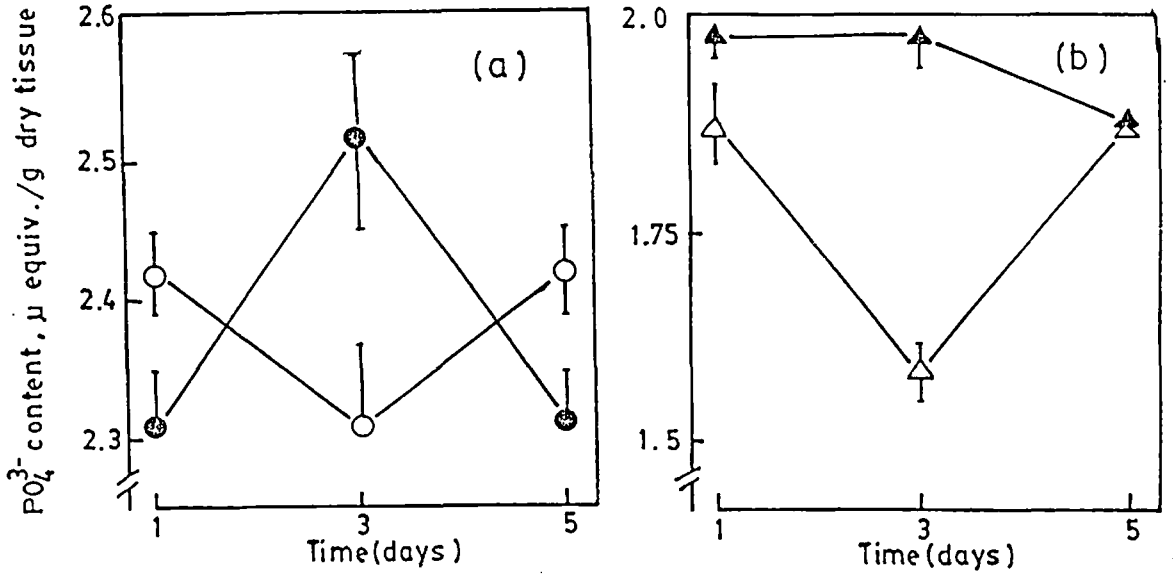


Fig. 57. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

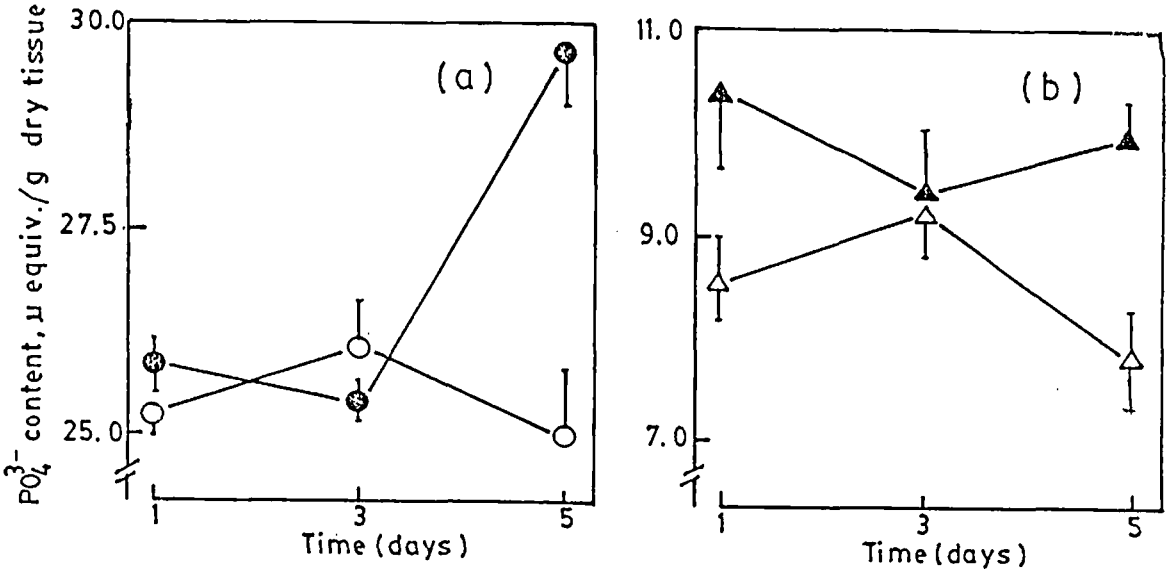


Fig. 58. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

deprivation respectively. Similarly, accumulation of phosphate in the root of the seedling of the nodulated genotype was decreased by 4% to 16% from 1-d to 5-d of sulphur deprivation (Fig. 59a & 60a).

Accumulation of phosphate in the shoot of the seedling of the nonnodulated genotype was increased by 19% at both 1-d and 3-d of sulphur deficiency treatment. On the contrary, in the shoot of the nodulated chickpea seedling, sulphur deficiency had inhibitory effects on phosphate accumulation at 1-d and 5-d of treatment (Fig. 59b & 60b).

5.3.12 Effects of sulphur deficiency on accumulation and distribution of phosphate in 21-d-old intact nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the nonnodulated chickpea seedling, phosphate accumulation was increased by 5% to 13% from 1-d to 5-d of sulphur deficiency treatment (Fig. 61a). On the other hand, phosphate accumulation in the root of the seedling of nodulated genotype was depressed by 3.8% to 15.6% at 1-d to 5-d of sulphur deficiency treatment (Fig. 62a).

Phosphate accumulation in the shoot of the nonnodulated chickpea seedling was decreased by 8% and 25% at 1-d and 5-d of sulphur deficiency treatment (Fig. 61b). Similarly, sulphur deficiency had an inhibitory effect

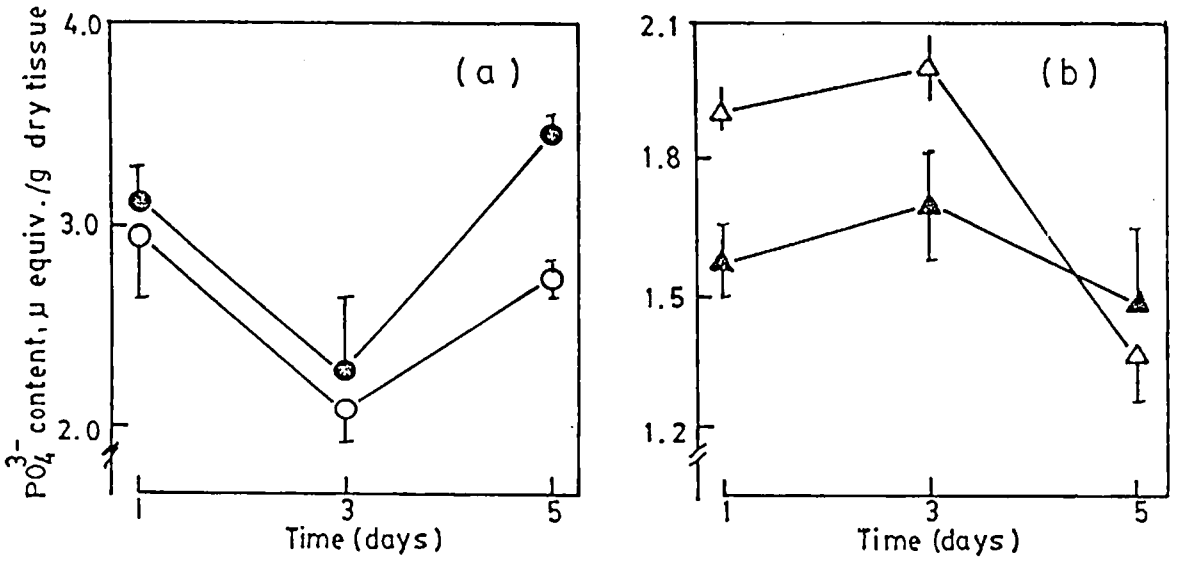


Fig. 59. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

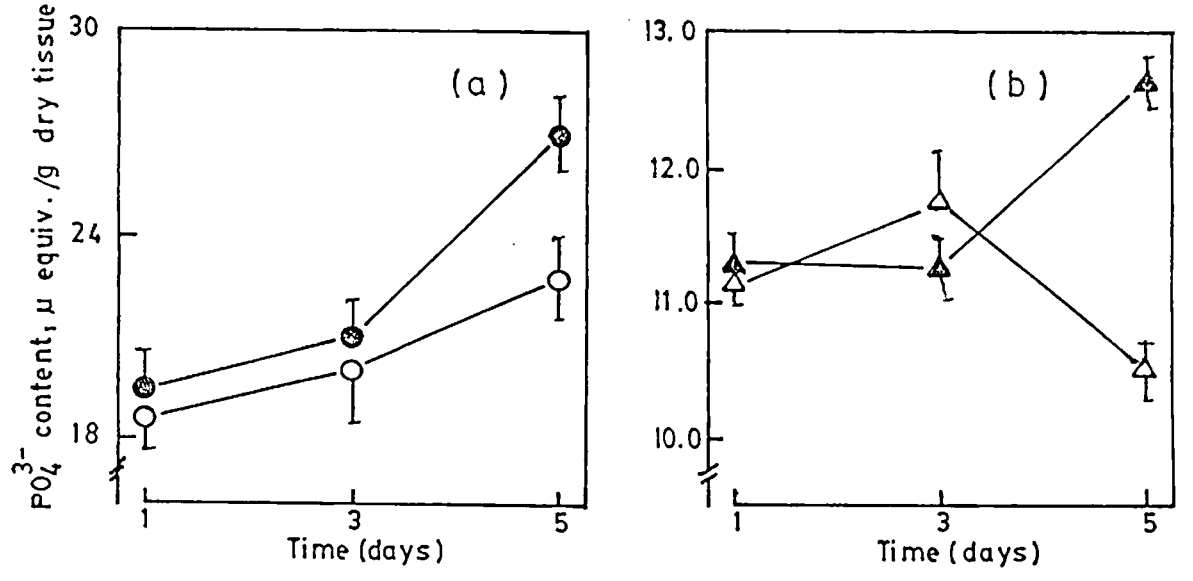


Fig. 60. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

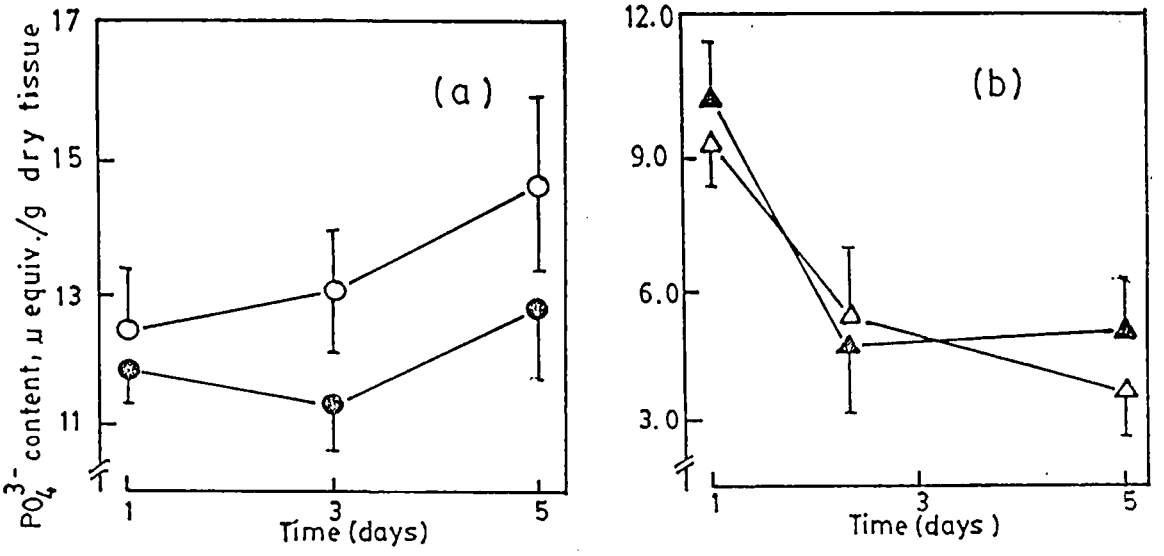


Fig. 61. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 21-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

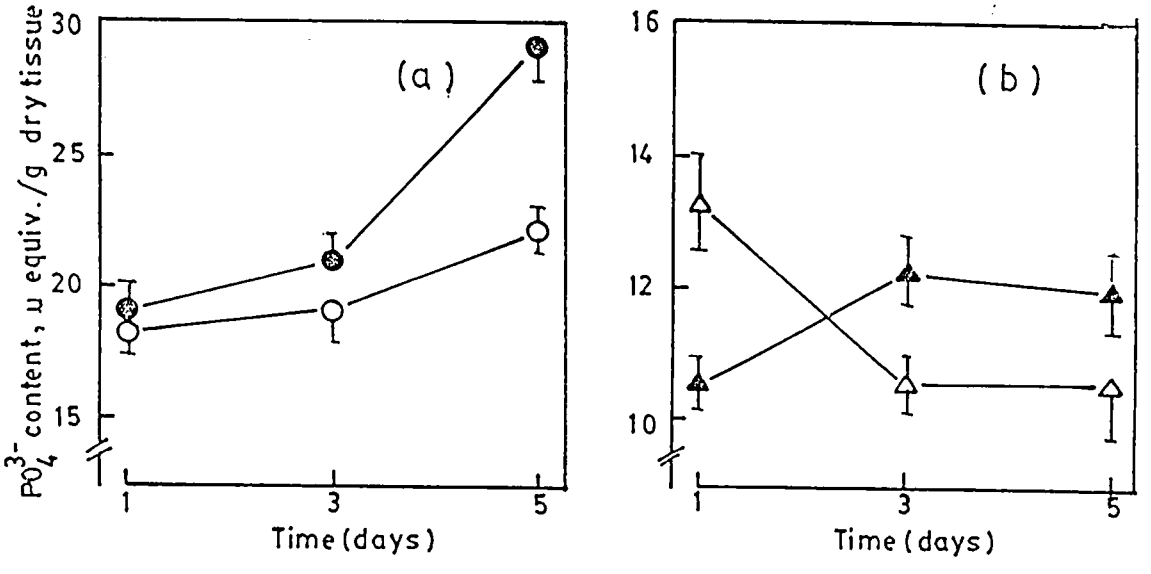


Fig. 62. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 21-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

on the accumulation of PO_4^{3-} in the shoot of the nodulated chickpea seedling (Fig. 62b).

5.3.13 Effects of sulphur deficiency on nitrate accumulation in 7-d-old nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of the nonnodulated genotype, sulphur deficiency increased the accumulation of NO_3^- by 15.2% to 68.4% from 1-d to 5-d of sulphur deficiency treatment. Similarly, NO_3^- accumulation was increased in the root of the seedling of the nodulated genotype by 51.4% and 34.4% at 1-d and 5-d of sulphur deficiency treatment respectively (Fig. 63a & 64a).

Accumulation of NO_3^- in the shoot of the seedling of the nonnodulated genotype was increased by 29% and 35.3% at 1-d and 5-d of sulphur deficiency treatment respectively (Fig. 63b). In the shoot of the seedling of the nodulated genotype, sulphur deficiency increased the accumulation of NO_3^- by 25% at 5-d of treatment (Fig. 64b).

5.3.14 Effects of sulphur deficiency on nitrate accumulation in 15-d-old nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the seedling of the nonnodulated genotype, sulphur deficiency increased the accumulation of NO_3^- by 36.1% at 5-d with an initial decrease

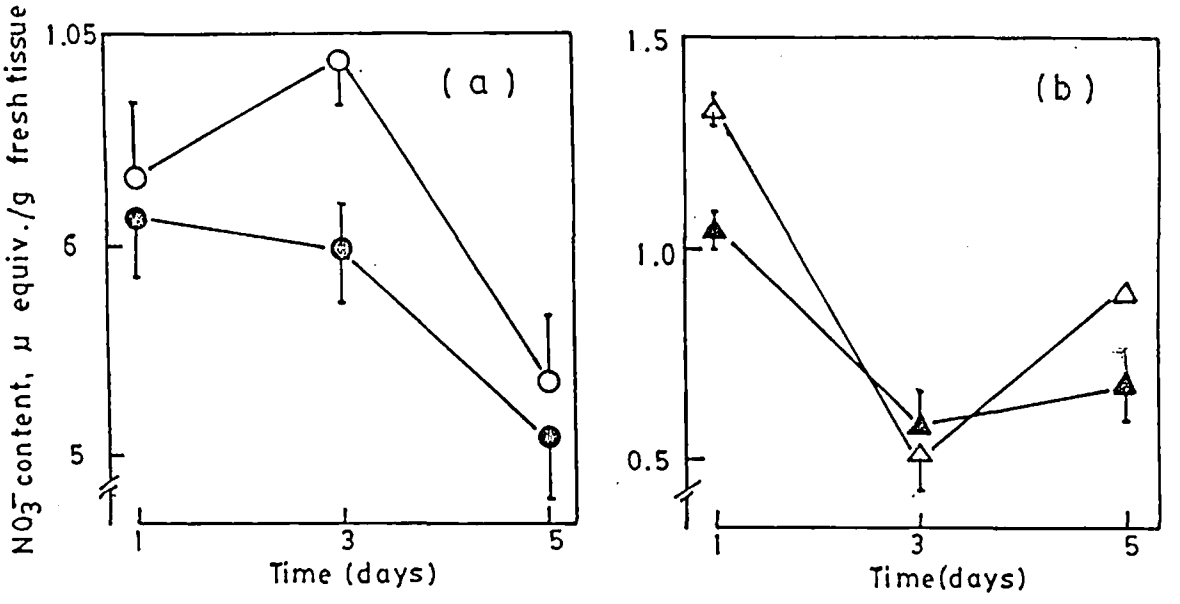


Fig. 63. The effect of sulphur deficiency on NO_3^- accumulation in the (a) root and (b) shoot of 7-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

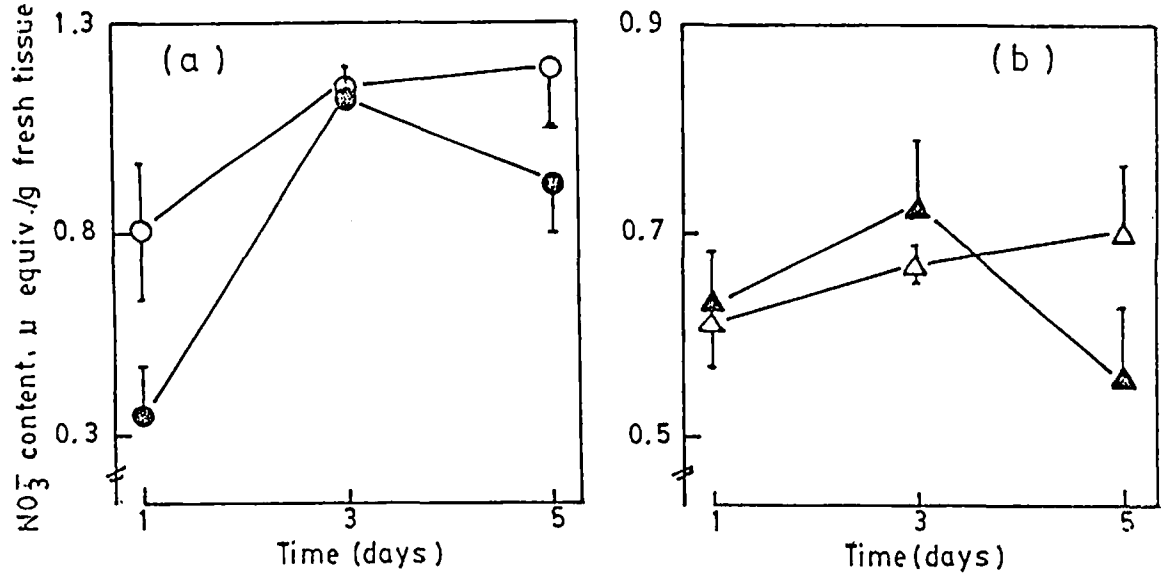


Fig. 64. The effect of sulphur deficiency on NO_3^- accumulation in the (a) root and (b) shoot of 7-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

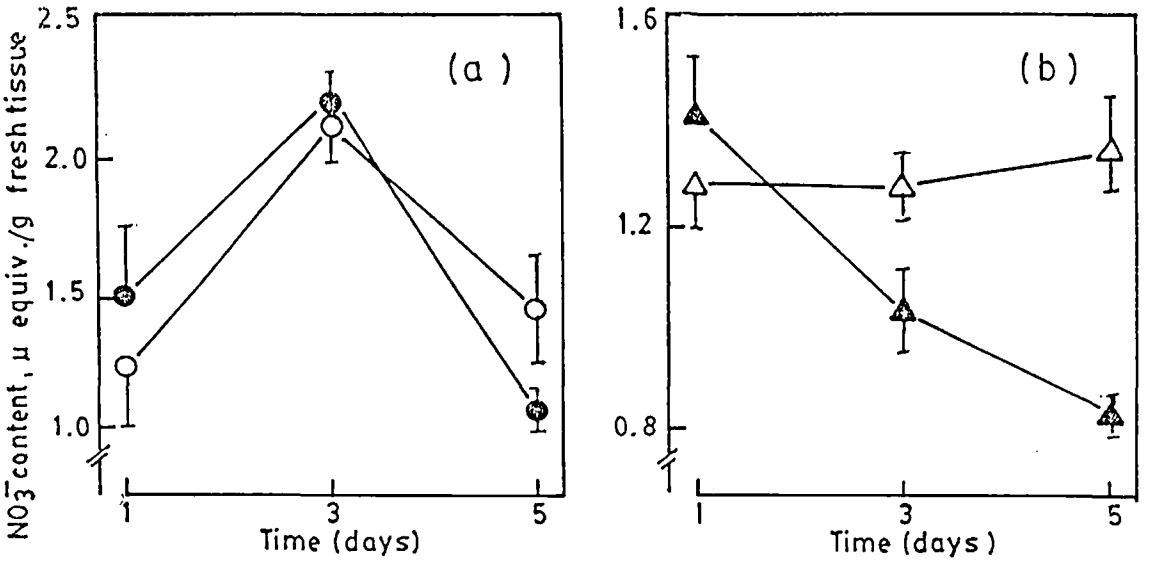


Fig. 65. The effect of sulphur deficiency on NO_3^- accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

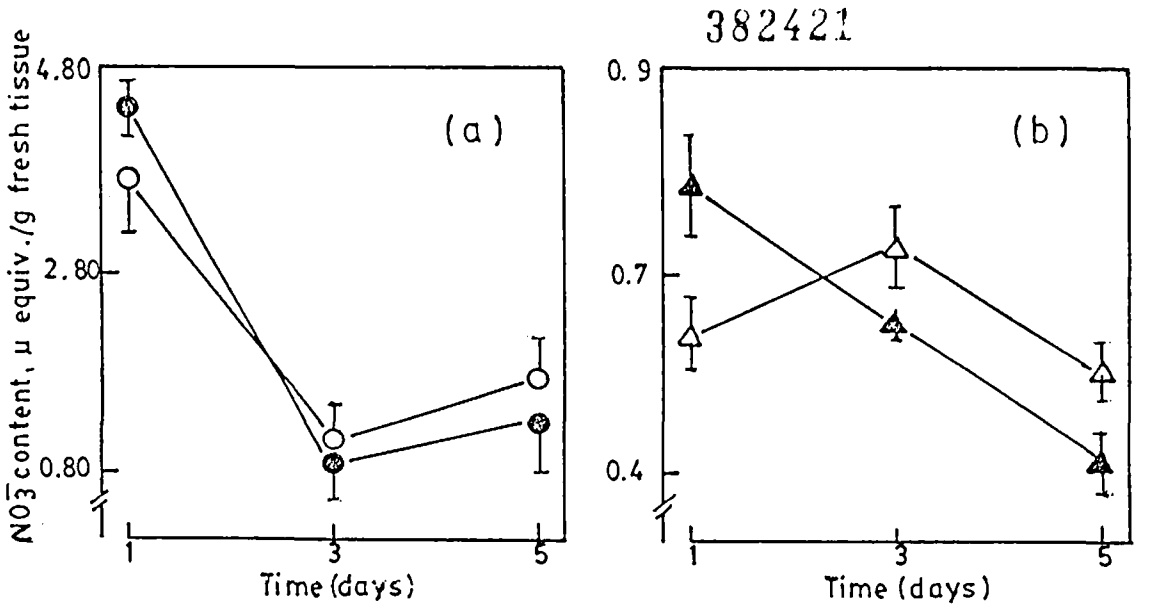


Fig. 66. The effect of sulphur deficiency on NO_3^- accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 21.

in that by 18.7% at 1-d of treatment (Fig. 65a). Similarly, in nodulated seedlings, sulphur deficiency increased the accumulation of NO_3^- by 20% and 28.8% at 3-d and 5-d of treatment respectively except an initial decrease in that by 19.9% at 1-d of treatment (Fig. 66a).

In the shoot of the seedling of the nonnodulated genotype, sulphur deficiency increased the accumulation of NO_3^- by 22% and 68% over that of the control at 3-d and 5-d of treatment respectively (Fig. 65b). Similarly, in the shoot of the nodulated chickpea seedling, sulphur deficiency increased the accumulation of NO_3^- by 16.% and 35.2% at 3-d and 5-d of treatment respectively except an initial inhibition of that by 29% at 1-d of treatment (Fig. 66b).

5.4 **Discussion**

The effect of sulphur deficiency on the accumulation of K^+ and Na^+ in chickpea seedlings at different developmental stages: Potassium accumulation was stimulated in the shoot of 7-d-old nonnodulated chickpea seedlings followed by a gradual decrease of that in 15-d and 21-d-old plants (Fig. 21, 25, 29). But its accumulation was decreased in the root. Sulphur deficiency inhibited K^+ accumulation in all the developmental stages of the plants of nodulated genotype (Fig. 22, 26, 30). These results are supported

by Singh and Singh (1982) who reported decreased K^+ accumulation in *Mentha arvensis* var. piperances due to sulphur deficiency. Similarly, suboptimal concentration of sulphur was found to decrease K^+ accumulation in rice (Pillai and Singh 1975), wheat (Ivanic 1978) and in *Phalaris acquatica* (Sairam *et al.* 1995). On the contrary, sulphur deficiency increased K^+ content in tomato (Martinez *et al.* 1983). On the other hand, Rennenberg and Kaiser (1989) found that sulphur deficiency behaved differently in different genotypes of the same species of tobacco with respect to K^+ uptake and accumulation. Sulphur deficiency resulted in an increase in accumulation of K^+ in heterotrophic tobacco culture but had little effect on photoheterotrophic tobacco cells.

The effect of sulphur deficiency on the accumulation of Na^+ was different in different genotypes of chickpea at different developmental stages (Fig. 23, 24, 27, 28, 31 & 32). Accumulation of Na^+ increased in the root of the seedling of the nonnodulated genotype, while in that of the nodulated genotype, sulphur deficiency resulted in a decrease in accumulation of Na^+ throughout all the developmental stages (Fig. 24, 28 & 32). Similarly, Rennenberg and Kaiser (1989) reported that sulphur deficiency resulted in an increase in accumulation of Na^+ in the heterotrophic tobacco cell culture while it showed no effect on Na^+ uptake in the photoheterotrophic cell culture.

The variability of K^+ and Na^+ accumulation in different genotypes may be the result of genotypic variation in K^+/Na^+ uptake efficiency of the genotypes. This result shows that sulphur deficiency eliminates the K^+/Na^+ selectivity (Van Stevenenick and Steveninck 1972).

The effect of sulphur deficiency on the accumulation of Ca^{2+} and Mg^{2+} in chickpea seedlings at different developmental stages: Under sulphur deficiency, accumulation of Ca^{2+} was increased in the root and the shoot of the seedling of the nonnodulated genotype while it was decreased in the root and the shoot of the chickpea seedling of the nodulated genotype in almost all developmental stages (Fig. 33, 34, 37, 38, 41 & 42) except an initial increase in the root of the 7-d-old nodulated genotype. This variability of Ca^{2+} accumulation in different genotypes may be the result of genotypic variation of Ca^{2+} uptake efficiency of the genotypes. Bugakova and co-workers (1981) found that in pea, lowering of sulphur concentration in the growth medium resulted in a decrease in accumulation of Ca^{2+}.

Magnesium accumulation was depressed in the root of 7-d-old nodulated chickpea seedlings but it was increased in that of 21-d old plant. On the other hand, Mg^{2+} accumulation was increased in almost all developmental stages of the root and the shoot of the seedling of the nonnodulated genotype (Fig. 35, 36, 39, 40, 43 & 44). This variation in Mg^{2+} accumulation between

the genotypes may also be due to the genotypic variation in ion uptake efficiency and differences in root functions of the nodulated and nonnodulated chickpea genotypes. Similarly, Rennenberg and Kaiser (1989) found differential effects of Mg^{2+} accumulation due to sulphur deficiency in different genotypes of tobacco. Furthermore, this genotypic variation in uptake kinetics may be another factor for the differential response of the varieties to a particular element. Matocha (1971) reported that when Mg^{2+} is available in S-deficient condition sulphur-magnesium imbalance may develop. Martinez and co-workers (1983) reported that in tomato, sulphur deficiency increased leaf Mg^{2+} content. On the other hand, Bugakova and co-workers (1981) reported decreased accumulation of Mg^{2+} in the root and the shoot of pea due to sulphur deficiency.

Sulphur deficiency decreased K^+ accumulation and increased Mg^{2+} accumulation (Fig. 21, 22, 25, 26, 29, 30, 35, 36, 39, 40, 43 & 44). Claassen and Barber (1977) found that when shoot K^+ content was higher Mg^{2+} uptake was lower and when K^+ content was lower Mg^{2+} influx was higher. This indicates competitive nature of K^+ and Mg^{2+} absorption.

The effect of sulphur deficiency on the accumulation of Fe^{2+} and Zn^{2+} in chickpea seedlings at different developmental stages: Sulphur deficiency decreased Fe^{2+} accumulation in chickpea seedlings at all the developmental

stages (Fig. 45, 46, 49, 50, 53 & 54). Similar results were reported by Bugakova and co-workers (1981) in pea who showed that reduced rate of sulphur supply in the growth medium resulted in a decrease in Fe^{2+} accumulation. Iron takes part in the synthesis of nitrate reductase (NR) enzyme (Garret and Nason 1967). Earlier, it was reported that sulphur deficiency affects enzyme activity (Oputa 1971; Onkersingh and Nandlal 1987). Therefore, any change of NR activity by sulphur deficiency may be related to the changes in accumulation of Fe^{2+}.

Sulphur deficiency decreased accumulation of Zn^{2+} in roots and shoots of seedlings of both the genotypes (Fig. 47, 48, 51, 52, 55 & 56). On the other hand, Wankhade and co-workers (1989) reported that sulphur deficiency increased uptake of Zn^{2+} in wheat.

The effect of sulphur deficiency on the accumulation of PO_4^{3-} in chickpea seedlings at different developmental stages: Phosphate accumulation was decreased in the sulphur deficient nonnodulated and nodulated chickpea seedlings (Fig. 57, 58, 59, 60, 61 & 62). On the other hand, Bugakova and co-workers (1981) reported an increase in PO_4^{3-} accumulation in the root and upper plant parts of pea under sulphur deficiency.

The effect of sulphur deficiency on the accumulation of NO_3^- in nonnodulated and nodulated chickpea seedlings at different developmental stages: NO_3^- accumulation was increased in both the root and the shoot of the seedling of the nonnodulated genotype at almost all developmental stages (Fig. 63 & 65). On the other hand, the accumulation of NO_3^- was decreased at the early stages of plant growth in S-deficient seedlings of the nodulated genotype. But in the subsequent periods of development, when NO_3^- supply was continued, sulphur deficiency resulted in an increase in accumulation of NO_3^- in the root and the shoot than that of the + S control (Fig. 64 & 66). Similarly, Bush and co-workers (1981) reported increased accumulation of NO_3^- in tall fescue grown in S-deficient condition. In tomato, sulphur deficiency resulted in an increase in accumulation of NO_3^- (Martinez *et al.* 1983). The accumulation of NO_3^- in various crops was increased under sulphur deficiency (Friederich and Schrader 1978; Stewart and Porter 1969; Coleman 1957, Mertz and co-workers 1952, Vickery and Pucher 1939).

Chapter 6

Effects of sulphur deficiency on nitrate reductase activity in nonnodulated and nodulated chickpea seedlings grown in (a) solution and (b) sand culture conditions

6.1 Introduction

Chickpea meets up its nitrogen demand mostly (about 70%) by nitrogen fixation. The rest of the nitrogen for plant growth comes from soil as well as from applied sources. Whatever form of nitrogen fertilizer is applied in the soil it is readily converted to either nitrate or ammonium ions. In the process of nitrogen assimilation nitrate reductase enzymes play a vital role (Guerrero *et al.* 1981). Nitrate reductase (NR) is induced in the presence of nitrate which functions as a substrate (Beevars *et al.* 1965; Nelson *et al.* 1984). NR may also be induced , in some crops, when NH_4^+ is used as N source (Haba 1990; Langelean and Troelstro 1992). Sulphur is considered as one of the limiting elements for the synthesis of nitrate reductase enzyme. Onkersingh and Nandlal (1987) reported decreased NR activity in sunflower due to sulphur deficiency. Oputa (1971) reported that NR in the S-deficient maize seedlings was only 17% of that found in seedlings supplied with sulphur. As NR provides reduced N for plant growth so NR activity is considered as the indicator of N utilization capacity of the plants.

In this experiment the effect of sulphur deficiency on nitrate reductase activity was studied.

6a.1 Effects of sulphur deficiency on nitrate reductase activity in nonnodulated and nodulated chickpea seedlings grown in solution culture

In this section, the effect of sulphur deficiency on nitrate reductase activity in plants grown in solution culture is reported.

6a.2 Material and Methods

Seedlings were grown in solution culture according to sec. 2.4.2. for 14 days. Surface sterilized seeds of each genotype were sown. Seven days after germination (DAG) extra seedlings were removed keeping twelve healthy seedlings in each pot. 0.1 strength Letcombe nutrient (Sec. 2.3) solutions without N was changed twice in a week upto 14-d of germination. Nitrogen was not provided so that NR activity could be stopped. Sulphur deficiency was applied at 15-d-old seedlings. Nitrate reductase activity (NRA) was determined in S-deficient and + S control seedlings after 1-d, 2-d, 3-d, 4-d, 5-d and 6-d of nitrate addition in order to compare the effect of sulphur deficiency on the induction of NR activity at daily interval in S-deficient and + S control conditions. 5 mM NO_3^- was added to 0.1 strength Letcombe

nutrient solution in both + S and -S solutions before the first analysis of NR activity. The analysis of NR activity was done following the method of Stewart and Orebamjo (1979) (Sec. 2.9).

6.3. Results

6a.3 Effects of sulphur deficiency on nitrate reductase activity in nonnodulated and nodulated chickpea seedlings grown in solution culture

In the root of the 1-d S-deficient nonnodulated chickpea seedling, no NR was detected after 24 h of NO_3^- application. In the later period, sulphur deficiency resulted in an inhibition of NR activity by 11% to 15% from 2- to 6-d of NO_3^--N addition (Fig. 67a).

In the root of the nodulated chickpea seedling, sulphur deficiency inhibited the activity of NR by 20.9%, 25%, 4.4% and 22.9% at 2-d, 4-d, 5-d and 6-d of NO_3^--N addition respectively except a slight increase in NR at 1-d of nitrate addition (Fig. 68a).

In leaves of 1-d S-deficient nonnodulated chickpea seedlings, NR activity was not detected after 1-d of NO_3^- -N application. But sulphur deficiency inhibited the activity of NR by 11% and 6% at 3-d and 6-d of NO_3^--N application respectively (Fig. 67b).

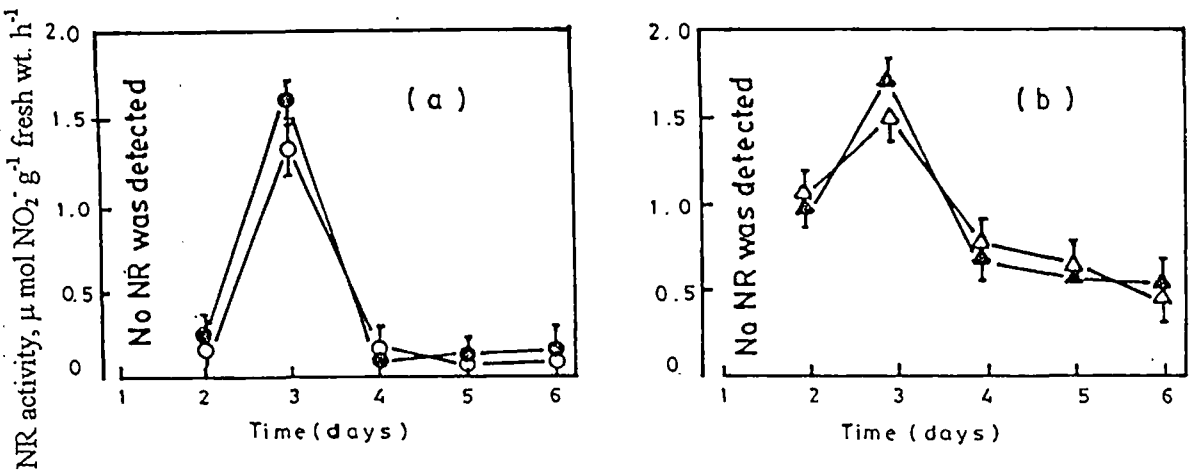


Fig. 67. The effect of sulphur deficiency on nitrate reductase activity in the (a) root and (b) leaf of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Solid symbols------ + S , open symbols------- – S. O root, Δ shoot. Each value is the mean of three replicates ± standard error.

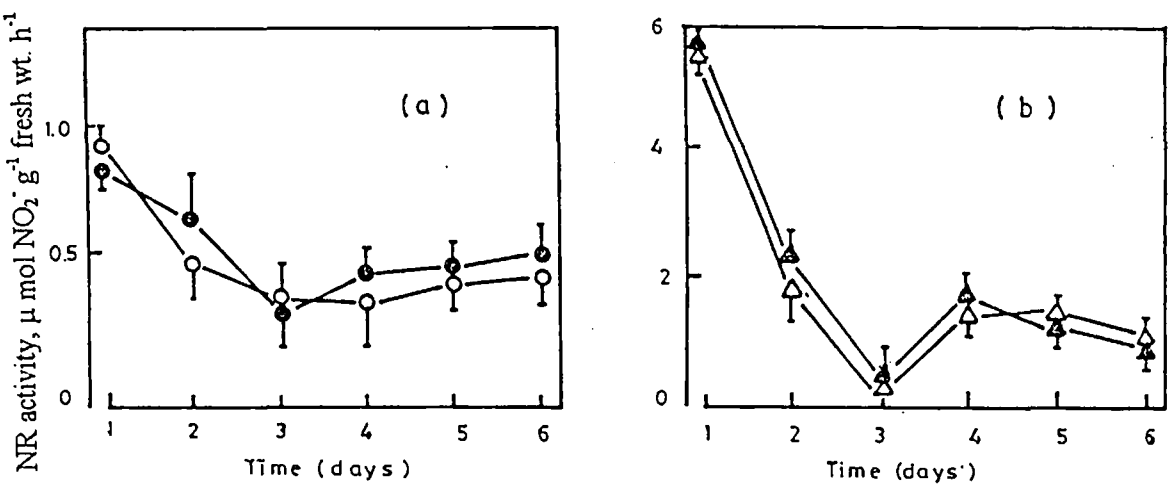


Fig. 68 . The effect of sulphur deficiency on nitrate reductase activity in the (a) root and (b) leaf of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

In the leaf of the nodulated chickpea seedling, sulphur deficiency inhibited the activity of NR by 21%, 14% and 11% at 2-d, 3-d and 4-d of NO_3^- -N addition respectively (Fig. 68b).

6b Effects of sulphur deficiency on nitrate reductase activity in 7-d S-deficient nonnodulated and nodulated chickpea seedlings grown in sand culture

6b.1 Introduction

In this section, the effect of sulphur deficiency on nitrate reductase activity in seedlings grown in sand culture is reported. This was done in order to compare the results on nitrate reductase activity obtained in seedlings grown in sand culture in natural condition with that in seedlings grown in solution culture in controlled environmental condition.

6b.2 Material and Methods

Two genotypes of chickpea, nonnodulated and nodulated were grown in the PVC pots containing 5 kg purified quartz sand. (Sec. 2.4.1.2). Surface sterilized seeds of each genotype were sown. Seven days after germination (DAG) extra seedlings were removed keeping 6 seedlings in each pot. 0.1 strength Letcombe nutrient (Sec. 2.3) solution without N was changed twice in a week upto 14-d of germination. Nitrogen was not added so that NR

activity could be stopped. Sulphur deficiency was applied to 15-d-old seedlings for 7 days. 5 mM NO_3^- was added to both + S and - S solution before 48 h of the analysis of NR activity. Nitrate reductase activity was determined following the method of Stewart and Orebamjo (1979) (Sec. 2.9).

6.3 Results

6b.3 Effects of sulphur deficiency on nitrate reductase activity in 7-d S-deficient nonnodulated and nodulated chickpea seedlings grown in sand culture

In the root of the seedling of the nonnodulated genotype sulphur deficiency did not affect the activity of NR (Table 1). But sulphur deficiency inhibited the activity of nitrate reductase (NR) in the root of the seedling of the nodulated genotype by 59.5% (Table 1)

Sulphur deficiency inhibited the activity of NR in leaves of nonnodulated seedlings by 19% (Table 1). Similarly, sulphur deficiency inhibited the activity of NR by 27.9% in leaves of seedlings of the nodulated genotypes (Table 1).

Table 1. The effect of 7-d sulphur deficiency on NR activity in roots and leaves of 15-d-old nonnodulated and nodulated chickpea plants grown in sand culture. Each value is the mean of three replicates ± standard error

		NR activity, μ mol NO_2^- g^{-1} fresh weight h^{-1}	
	Treatment	Root	Leaf
nonnodulated genotype	+ S	0.40 ± 0.01	4.4 ± 0.18
	- S	0.41 ± 0.02	3.5 ± 0.03
Nodulated genotype	+ S	0.37 ± 0.04	1.9 ±. 0.2
	- S	0.15 ± 0.02	1.4 ± 0.2

6.4 **Discussion**

Nitrate reductase (NR) was decreased in the root and leaf in most cases (Fig. 67, 68 and Table 1). This might be due to the fact that sulphur deficiency might result in less availability of sulphur containing amino acids (Klepper *et al.* 1971) which may cause inhibition of regeneration of NADH acting as the energy donor for NR enzyme (Oaks *et al.* 1972). Similar results of reduced NR activity due to sulphur deficiency was reported in sunflower (Onkersingh and Nandlal 1987) and in maize (Oputa 1971; Schrader *et al.* 1968). Sulphur deficiency-induced inhibition of NR activity (Fig. 67, 68 and Table 1) might be due to the fact that NR is a sulphydryl dependent enzyme (Evan and Nason 1953, Schrader *et al.* 1968).

It is apparent from the result of daily change of NR activity that induction of NR activity was inhibited in roots of both genotypes (Fig. 67, 68 and Table 1). But in leaves, its induction was inhibited initially in the nodulated genotype upto 4-d of NO_3^- addition and then the inhibiting effect was nullified (Fig. 68b). But in the nonnodulated chickpea seedlings, NR induction in leaves was depressed at 3-d and 6-d of NO_3^- addition (Fig. 67b). This might be due to the variation in N-utilization efficiency of the genotypes. It was reported that NR has short half-life of about 4 h (Schrader *et al.* 1968). Privalle *et al.* (1989) reported that maximum NR induction in maize occurred within 2 h of nitrate addition. It decreased after that period

despite the continuous presence of nitrate in the medium. Oji and Izawa (1968) reported increased NR activity upto 72 h of nitrate addition. Thereafter, the tendency of NR induction was found to be decreased at 7-d of nitrate addition. In the present experiment, it might so happen that NR induction was increased in S-supplied seedlings upto certain period and thereafter the enhanced induction of NR was stopped resulting in the lowered NR activity. Whereas in S-deficient seedlings, the NR induction was reduced initially. But in the later stages, due to the continuous presence of NO_3^-, the constitutive NR of leaves might be more functional and thus NR showed higher activity in S-deficient seedling than that of + S controls. On the other hand, in the dark, the constitutive NR of the root was not effectively functional and the NR activity was always lower.

Nitrate reductase activity was decreased following sulphur deficiency in seedlings grown in solution culture under controlled environmental condition and also in seedlings grown in sand culture under natural condition. Therefore, the effect of sulphur deficiency on nitrate reductase activity under these two growth conditions may be reconciled.

Chapter 7

Effects of sulphur deficiency on the accumulation of reducing and total sugar, total free amino acid in nonnodulated and nodulated chickpea seedlings

7.1 Introduction

Effects of sulphur deficiency on sugar level: Carbohydrate provides the reductant energy and carbon skeleton required for the reduction of nitrate as well as synthesis of amino acids and proteins. Sulphur availability influences the synthesis of reducing and total sugar in plant. Rendig and McComb (1961) reported that plants grown in solution with low levels of sulphur contained relatively larger amounts of amide-N but lesser amounts of sugar than that of plants grown in solution containing adequate sulphur. Mertz and Matsumoto (1956) reported that a severe deficiency of sulphur in the plant may disrupt the normal course of protein synthesis which would ultimately be reflected in the changes in kinds and amounts of various carbohydrate. Rendig and co-workers (1976) reported that sulphur deficiency resulted in an increase in the concentration of amide-N and a decrease in the concentration of soluble sugar in corn.

This study was undertaken to investigate the effect of sulphur deficiency on sugar accumulation in chickpea. An attempt was made to correlate the effect of sulphur deficiency on sugar level with the effect of that on amino acid accumulation.

Effects of sulphur deficiency on total free amino acid accumulation: Sulphur-containing amino acids play an important role in the protein synthesis which is essential for plant structure and function. Hocking and co-workers (1987) reported that in sunflower sulphur deficiency resulted in a decrease in accumulation of S-containing amino acids like cystein and methionine but caused an increase in that of arginine. Cowling and co-workers (1977) reported that sulphur deficiency caused a 24-fold increase in accumulation of asparagine and a 8-fold increase in the accumulation of glutamine in forage plants. Karmoker and co-workers (1991) reported that S-deprivation resulted in a 6- to 8-fold increase in asparagine and 2- to 4-fold increase in glutamine accumulation in the root tissue of barley. Sulphur deficiency caused an increased accumulation of glutamine and asparagine (Adams and Sheared 1966; Millard *et al.* 1985).

In this section, the effect of sulphur deficiency on total amino acid accumulation in chickpea seedlings is reported.

7.2 Material and Methods

Extraction and measurement of reducing and total sugars: Chickpea seedlings were grown in Letcombe solution according to sec. 2.3. Sulphur deficiency was applied to 15-d-old seedlings. Samples were collected after 1-d, 3-d and 5-d of sulphur deficiency treatment. Fresh samples were separated into the root and leaf. Samples were analysed for reducing sugar following the Nelson (1944) and Somogyi (1952) methods. Total sugar was measured following the method of Dubois and co-workers (1956). Reducing sugar and total sugar were expressed as milligram $gram^{-1}$ fresh tissue.

Extraction and measurement of total free amino acid: Chickpea seedlings were grown in 0.1 strength Letcombe solution according to sec. 2.3. Sulphur deficiency was applied to 15-d-old seedlings. Samples were collected after 1-d, 3-d and 5-d of sulphur deficiency treatment. The root and leaf samples were dried in an oven for 72 h at 70^0C. Dried samples were soaked in 70% alcohol in test tubes for 1 h and those were then boiled in a water bath for half an hour and the extracts were decanted in separate test tubes. The process was repeated twice. Total free amino acid was determined using methoxyethanol and citrate buffer (R.K. Dutta, BINA, personal communication). Total free amino acid was expressed as milligram $gram^{-1}$ dry tissue.

7.3 Results

7.3.1 Effects of sulphur deficiency on total sugar accumulation in nonnodulated and nodulated chickpea seedlings

In the root of the nonnodulated chickpea seedling, total sugar accumulation was inhibited by 31% and 29% at 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 69a). On the contrary, sulphur deficiency resulted in a 9.9%, 15.4% and 15.2% increase in accumulation of total sugar in the root of the nodulated chickpea seedling at 1-d, 3-d and 5-d of treatment respectively (Fig. 70a).

Total sugar accumulation in the leaf of the nonnodulated chickpea seedling was inhibited by 33% and 8% at 3-d and 5-d of S-starvation respectively (Fig. 69b).

In the leaf of the seedling of the nodulated genotype, sulphur deficiency inhibited the accumulation of total sugar by 13.6% and 13.4% at 3-d and 5-d of treatment respectively except an initial increase at 1-d of deficiency treatment (Fig. 70b).

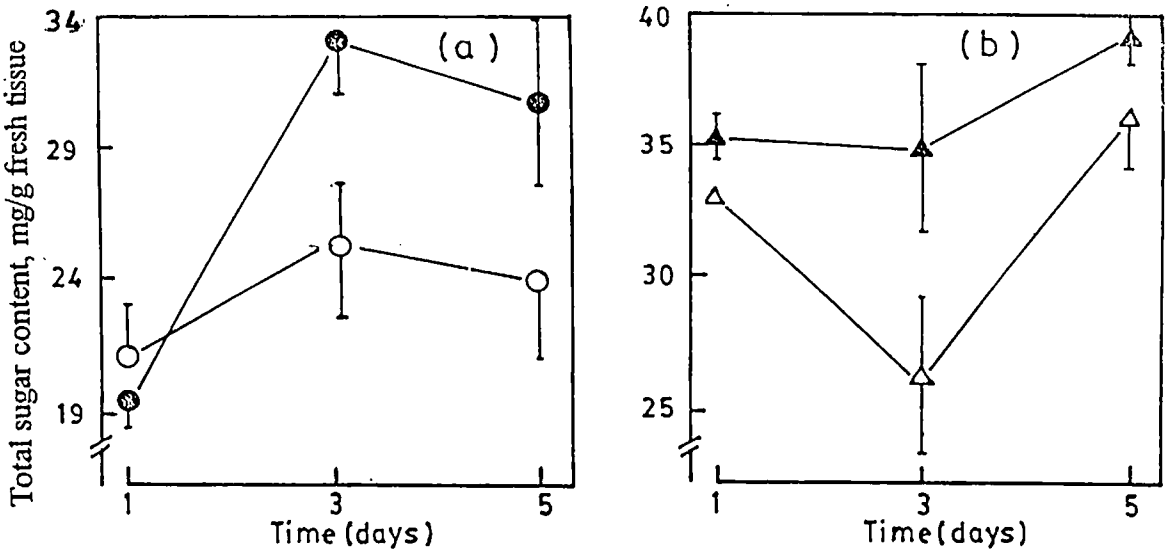


Fig. 69. The effect of sulphur deficiency on total sugar accumulation in the (a) root and (b) leaf of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

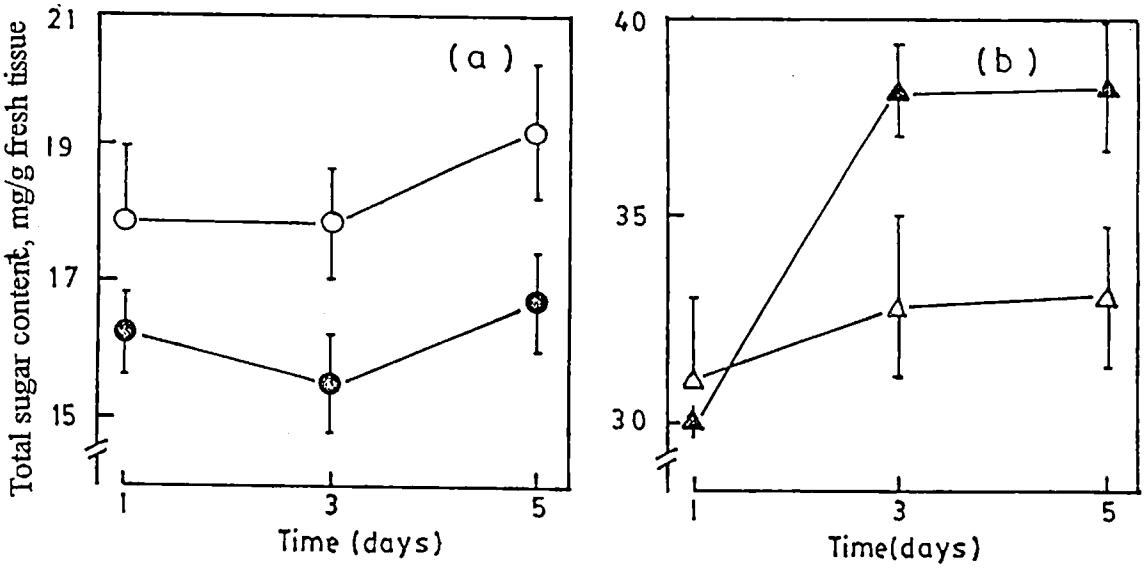


Fig. 70. The effect of sulphur deficiency on total sugar accumulation in the (a) root and (b) leaf of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

7.3.2 Effects of sulphur deficiency on reducing sugar accumulation in nonnodulated and nodulated chickpea seedlings

In the root of the nonnodulated chickpea seedling, sulphur deficiency increased the accumulation of reducing sugar by 4% to 31% from 1-d to 5-d of treatment (Fig. 71a). Similarly, Sulphur deficiency caused a 18.6% and 16.7% increase in the accumulation of reducing sugar in the root of the nodulated chickpea seedling at 3-d and 5-d of treatment respectively (Fig. 72a).

In leaves of nonnodulated chickpea seedlings, sulphur deficiency decreased reducing sugar accumulation by 18% and 16% at 3-d and 5-d of treatment respectively (Fig. 71b). Similarly, sulphur deficiency inhibited the accumulation of reducing sugar by 27.7% and 24.2% in the leaf of the nodulated chickpea seedling at 3-d and 5-d of treatment respectively except an initial increase in reducing sugar accumulation at 1-d of treatment (Fig. 72b).

7.3.3 Effects of sulphur deficiency on total amino acid accumulation in nonnodulated and nodulated chickpea seedlings

Sulphur deficiency caused an increase in the accumulation of total amino acid in the root of the nonnodulating chickpea seedling by 19%, 14.5% and 47.4% at 1-d, 3-d and 5-d of treatment respectively (Fig. 73a). Similarly, in

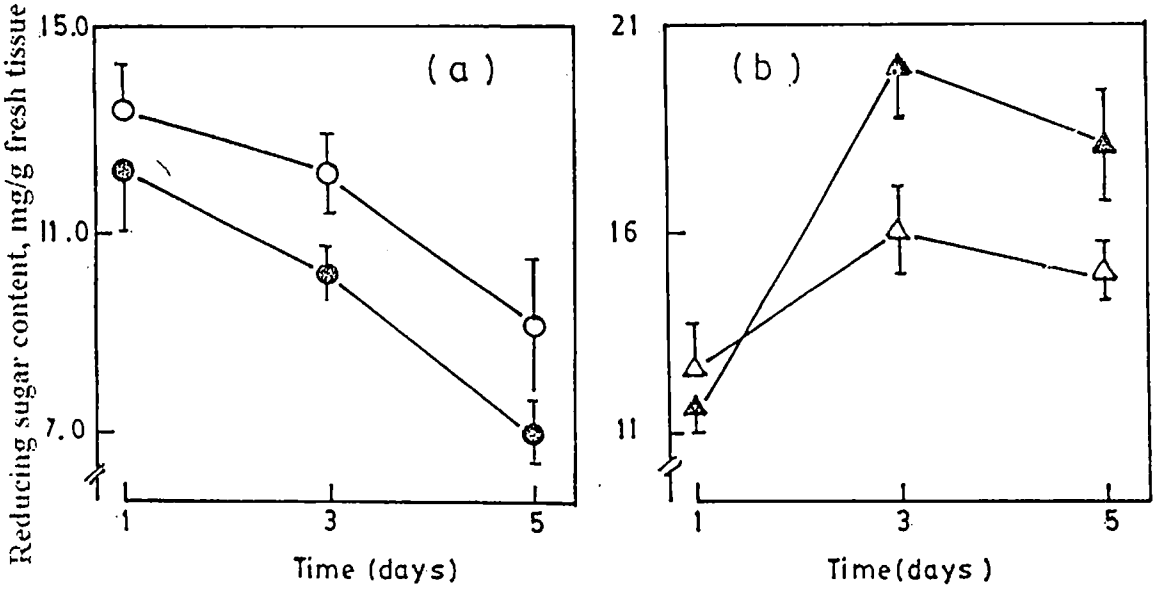


Fig. 71. The effect of sulphur deficiency on reducing sugar accumulation in the (a) root and (b) leaf of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

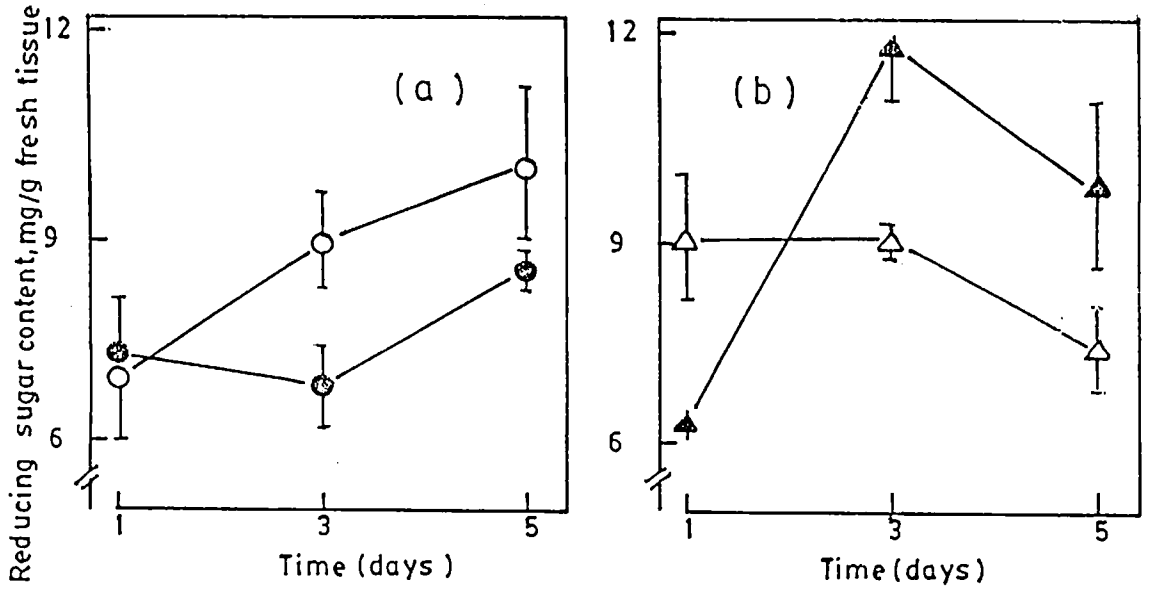


Fig. 72. The effect of sulphur deficiency on reducing sugar accumulation in the (a) root and (b) leaf of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

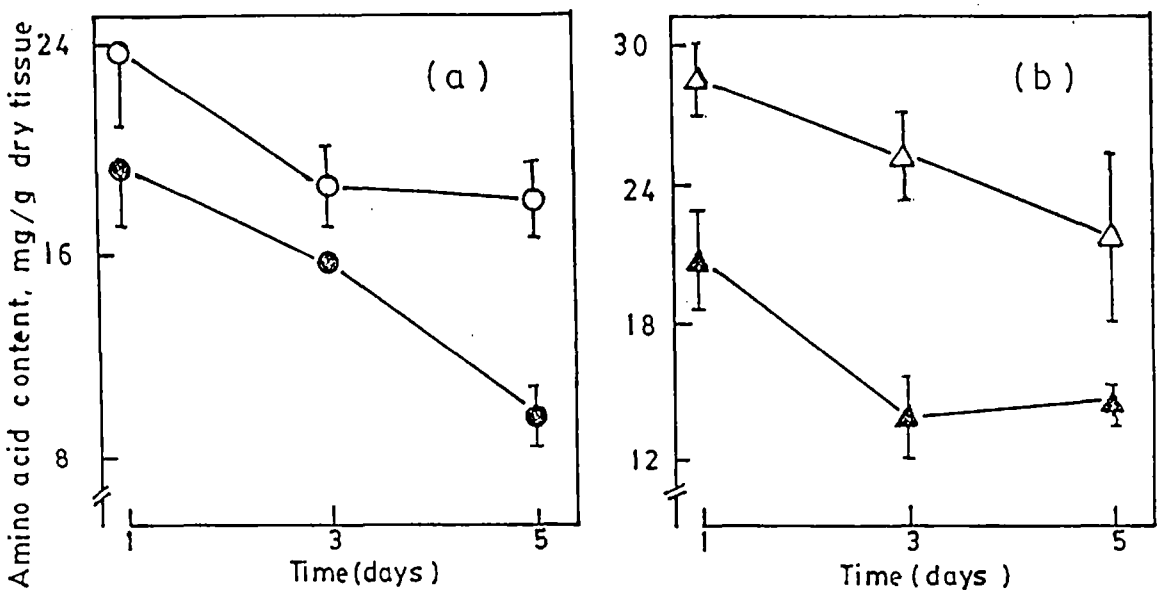


Fig. 73. The effect of sulphur deficiency on total amino acid accumulation in the (a) root and (b) leaf of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

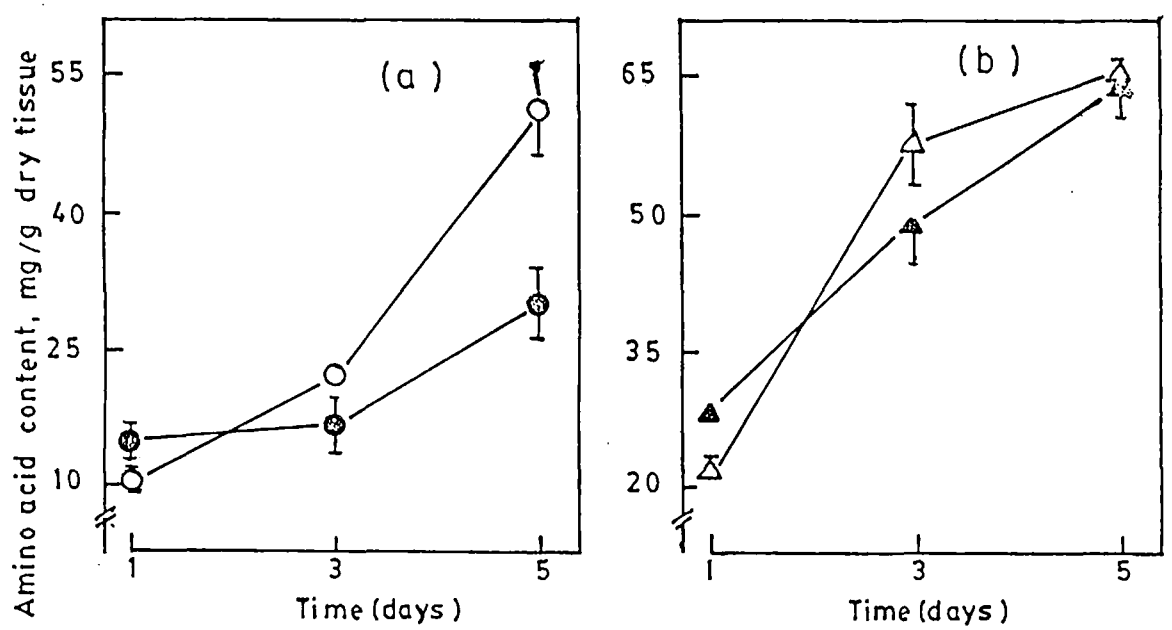


Fig. 74. The effect of sulphur deficiency on total amino acid accumulation in the (a) root and (b) leaf of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

the root of the nodulated chickpea seedling, sulphur deficiency increased the accumulation of total amino acid by 65.9% and 44.9% respectively at 3-d and 5-d of treatment except an initial inhibition of that by 25.5% at 1-d of treatment (Fig. 74a).

In the leaf of the nonnodulated chickpea seedling, sulphur deficiency resulted in an increase in accumulation of total amino acid by 28%, 45% and 34% at 1-d, 3-d and 5-d of treatment respectively (Fig. 73b).

Similarly, accumulation of total amino acid in the leaf of the nodulated chickpea seedling, was increased by 19.7% and 9.2% at 3-d and 5-d of treatment respectively except an initial decrease at 1-d of sulphur deficiency (Fig. 74b).

7.4 Discussion

The effect of sulphur deficiency on sugar content: In the root, sulphur deficiency increased the accumulation of total and reducing sugars. On the other hand, it inhibited the accumulation of total and reducing sugar in leaves of seedlings of both genotypes of chickpea (Fig. 69 to 72). Earlier, Srivastava and Ranjan (1970) reported increased sucrose accumulation in all organs of flax following sulphur deficiency. Increase in the total and reducing sugars in the root (Fig. 70a, 71a and 72a) with a concomitant

decrease in those in the leaf (Fig. 69b to 72b) might be due to increased transport of sugar from the leaf to the root. Furthermore, the increased accumulation of sugar in the root due to sulphur deficiency might be the result of less utilization of the carbohydrate in the subsequent synthesis of the plant structural material as a result of unavailability of sulphur containing amino acids. Sulphur deficiency-induced inhibition of sugar in leaves (Fig. 69b to 72b) might be due to the decrease in the fixation of CO_2 than that of the + S control plants (Hall *et al.* 1972). Bussye and co-workers. (1993) reported that free amino acid and free sugar levels were weakly correlated with growth and biomass production. Chlorophyll synthesis was also inhibited due to sulphur deficiency (Tiwari 1994) which might be related to decrease in sugar concentration in the leaf of S-deficient plant (Fig. 69b to 72b).

Effects of sulphur deficiency on free amino acid accumulation: In chickpea seedlings, total free amino acid was increased in roots and leaves of seedlings of both the genotypes of chickpea at 3-d and 5-d of sulphur deficiency treatment (Fig. 73 & 74). This result was supported by Dietz (1989) who found that sulphur deficiency increased the accumulation of amino acids in spinach leaves. Similarly, sulphur deficiency increased accumulation of amino acids in tomato, soybean, mustard, sunflower and flax (Nightingle 1932; Eaton 1935; 1941; 1942; Anderson and Spencer

1950). Karmoker and co-workers (1991) reported that sulphur deprivation resulted in a 6- to 8-fold increase in asparagine and 2- to 4-fold increase in glutamine accumulation in the root tissue of barley. Cowling and co-workers (1977) found that in sulphur-deficient plants, there is a 24- fold increase in accumulation of asparagine and a 8-fold increase in accumulation of glutamine. Mertz and co-workers (1952) found that when sulphur deficiency started, N-assimilation was disturbed but the conversion of NO_3^- and NH_4^+ into organic-N might continue, and a cessation of protein synthesis made free amino acids to be accumulated in the tissue. On the contrary, Hocking and co-workers (1987) reported that sulphur stress, applied during seed filling of sunflower, showed low levels of essential amino acids in seeds. They found that sulphur deficiency resulted in a decrease in accumulation of cystein and methionine and a increase in that of arginine. Zhao and co-workers (1991) reported that application of sulphur increased the methionine and cystein contents which might be at the expense of aspartic acid/asparagine. It is believed that sulphur containing amino acids are derived from aspartic acid (Zhao *et al.* 1991).

There is a reciprocal correlation between amino acid accumulation and sugar accumulation. In the present experiment, sugar accumulation was decreased and amino acid accumulation was increased. Similarly, Singh and Singh (1971) reported that phosphorus deficiency disturbed N-metabolism at the

stage of amide and amino acid formation which resulted in an accumulation of carbohydrate. Rendig and McComb (1959) showed that in alfalfa, at low level of sulphur, when the amide-N content was high, the sugar content was low. As the sulphur level was increased, the amide-N content was decreased and the sugar content was increased. The reciprocal correlation between elevated amide-N and lowered sugar content in plants grown under sulphur deficiency might be due to the asparagine synthetase activity in roots which is closely related to their glucose content (Rendig *et al.* 1976, Oaks 1967).

Chapter 8

Effects of sulphur deficiency on the accumulation of soluble protein in nonnodulated and nodulated chickpea seedlings grown in sand and solution culture conditions

8.1 Introduction

Soluble proteins are active as enzymes or antigens (Doby 1965). So the quantity of soluble protein is one of the important factors for synthesis of other plant material. Qi, B.Z. (1989) reported that reduced dose of sulphur affected NRA and soluble protein content in plants. Grant (1991) reported that sulphur containing amino acids e.g. cystein and methionine combined with other amino acids to form plant protein. Sulphur deficiency in canola depressed protein synthesis (Grant 1991). Sulphur deficiency decreased soluble protein in maize (Friederich and Schrader 1978); in timothy and switchgrass (Friederich *et al.* 1977).

This study was undertaken to find out the effect of sulphur deficiency on soluble protein accumulation in two genotypes of chickpea.

8.2 Material and Methods

Seedlings were grown in sand culture in the glasshouse according to sec. 2.4.1.2. Sand was soaked with 0.1 strength Letcombe nutrient solution with no nitrogen (-N) (Sec. 2.3) for 14 DAG (days after germination). Sulphur deficiency was applied on 15-d-old seedling. Soluble protein of the root and leaf samples in 7-d S-deficient seedlings were determined. 48 h before soluble protein analysis, nitrate nitrogen was applied to the S-deficient and + S control seedlings. Soluble protein was extracted in 0.1 M of K-buffer and determined following Lowry and co-workers (1951) as described in Sec. 2.12.

Another experiment was also conducted in solution culture condition (Sec. 2.4.2). This was done in order to compare the results on soluble protein accumulation obtained in seedlings grown in sand culture in natural condition with that in seedlings grown in solution culture. Seedlings were grown in 0.1 strength Letcombe nutrient (Sec. 2.3) with no nitrogen (-N) upto 14 DAG. Sulphur deficiency was applied on 15-d-old seedling. 48 h before soluble protein analysis, nitrate nitrogen was applied to the S-deficient and + S control seedlings. The root and the leaf samples were analysed for soluble protein following the method of Lowry and co-workers (1951) (Sec. 2.12) after 1-d, 3-d and 5-d of sulphur deficiency treatment.

8.3 Results

8.3.1 Effects of 7-d sulphur deficiency on the accumulation of soluble protein in 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture.

In the root of the chickpea seedling of nonnodulated genotype, sulphur deficiency decreased the accumulation of soluble protein by 16.4% (Table 2). On the other hand, sulphur deficiency caused an increase in soluble protein accumulation in the root of the seedling of nodulated chickpea genotype by 11.4% (Table 2).

Sulphur deficiency inhibited the accumulation of soluble protein in leaves of nonnodulated genotypes by 28.4% whereas in leaves of the nodulated genotype sulphur deficiency had no effect on soluble accumulation (Table 2).

8.3.2 Effects of sulphur deficiency on the accumulation of soluble protein in 15-d-old nonnodulated and nodulated chickpea seedlings grown in solution culture.

Sulphur deficiency inhibited the accumulation of soluble protein in the root of the seedling of the nonnodulated genotype by 34%, 32% and 12.5% at 1-d, 3-d and 5-d of treatment respectively (Fig. 75a).

Table 2. The effect of 7-d sulphur deficiency on soluble protein accumulation in roots and leaves of 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture. Each value is the mean of three replicates ± standard error

		Soluble protein content, mg gram^{-1} fresh tissue	
	Treatment	Root	Leaf
nonnodulated genotype	+ S	0.61 ± 0.07	3.6 ± 0.17
	- S	0.51 ± 0.06	2.9 ± 0.17
Nodulated genotype	+ S	1.32 ± 0.17	4.9 ±. 0.05
	- S	1.47 ± 0.17	5.0 ± 0.40

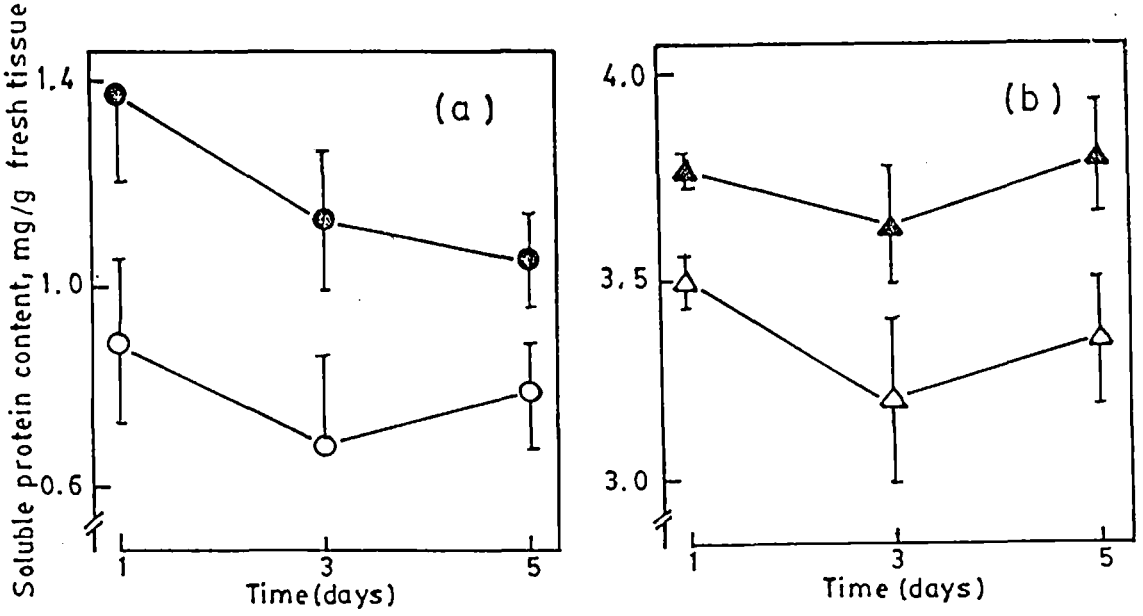


Fig. 75. The effect of sulphur deficiency on soluble protein accumulation in the (a) root and (b) leaf of 15-d-old nonnodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

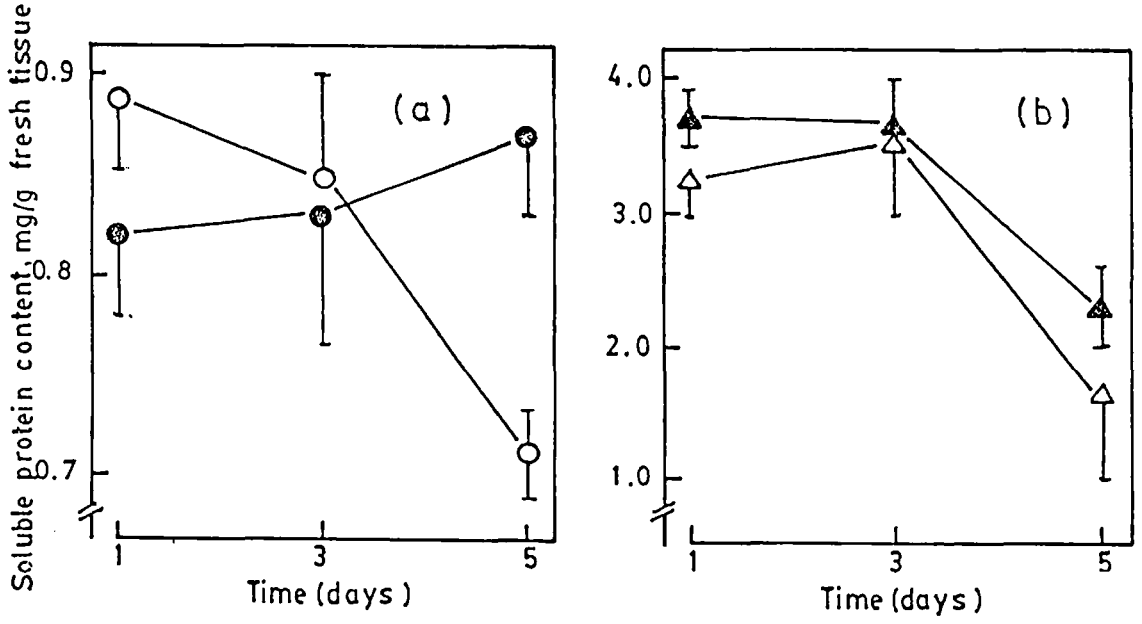


Fig. 76. The effect of sulphur deficiency on soluble protein accumulation in the (a) root and (b) leaf of 15-d-old nodulated chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

In the root of chickpea seedling of the nodulated genotype, sulphur deficiency caused an increase in soluble protein content by 7.3% and 2.4% at 1-d and 3-d of treatment respectively. But 5-d sulphur deficiency inhibited its accumulation by 23% (Fig. 76a).

In leaves of the seedlings of the nonnodulated genotype, sulphur deficiency inhibited the accumulation of soluble protein by 7%, 20% and 11% at 1-d, 3-d and 5-d of treatment respectively (Fig. 75b).

In leaves of the nodulated chickpea seedlings, sulphur deficiency also inhibited the accumulation of soluble protein by 12% and 26.5% at 1-d and 5-d of treatment respectively (Fig. 76b).

8.4 **Discussion**

Sulphur deficiency inhibited the accumulation of soluble protein in the root and the leaf of the chickpea seedling of the nonnodulated genotype (Fig. 75 and Table 2). But in the nodulated genotype, sulphur deficiency increased the accumulation of soluble protein in the root (Fig. 76a and Table 2). Whereas in leaves of chickpea seedlings of the nodulated genotype, sulphur deficiency inhibited the accumulation of soluble protein in most of the deficiency periods (Fig. 76b). Sulphur deficiency-induced increase in the accumulation of soluble protein in the root of the nodulated seedling of

chickpea with a concomitant decrease of that in the leaf (Fig. 76) may be due to the decrease in the translocation of protein to the leaf. These opposing effects of sulphur deficiency on soluble protein content in nodulated and nonnodulated varieties (Fig. 75 & 76 and Table 2) might be due to the genotypic variation. It appears that nodulated genotype might have a more effective mechanism for assimilation of nitrogen to form soluble protein. A decrease in the soluble protein synthesis following sulphur deficiency was also reported in wheat, maize and blackgram (Qi. B.Z. 1989; Bapat *et al.* 1984; Passera and Ghisi 1982; Friedrich and Schrader 1978; Rendig *et al.* 1976; Mertz and Matsumoto 1956; Mertz *et al.* 1952). Friedrich and Schrader (1978) reported in maize that both NRA and soluble protein synthesis diminished due to sulphur deficiency. Zhao and co-workers (1991) found that when sulphur supply was inadequate for seed production, protein synthesis in the seed was also suspended in oilseed rape. Thibodeau and Jaworski (1975) reported an increase in soluble protein in leaves of the field grown soybean during pod filling whereas Harper and Paulsen (1967) reported differential proportion of the accumulation of soluble protein.

Chapter 9

Effects of sulphur deficiency on transport of ^{15}N and other ions in nonnodulated and nodulated chickpea seedlings grown in sand culture.

9.1 Introduction

This experiment was conducted in order to compare the effect of sulphur deficiency on transport of ^{15}N and other ions in plants grown in solution culture under controlled environmental conditions with that of plants grown in sand culture in field conditions.

9.2 Material and Methods

Seedlings were grown in sand culture according to sec. 2.4.1.2. under natural environmental conditions. Fifteen-d-old seedlings grown in sand culture were subjected to sulphur stress and accumulation of ^{15}N and other ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}, PO_4^{3-}, NO_3^-) were studied. ^{15}N was measured according to sec. 2.5. Ions were extracted and measured according to sec. 2.7. and 2.8.

9.3 Results

9.3.1 Effects of sulphur deficiency on ^{15}N accumulation in 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture

^{15}N accumulation in the root of the 1-d S-deficient nonnodulated chickpea seedling was inhibited by 8% to 12.5% at 6 to 24 h of tracer application. Similarly, one-day sulphur deficiency treatment applied to the 15-d-old nodulated chickpea seedling caused a decrease in ^{15}N accumulation by 6%, 24% and 11% in the root at 6, 12 and 24 h of addition of ^{15}N respectively (Fig. 77a & 78a).

One-day sulphur deficiency resulted in a decrease in ^{15}N accumulation in the shoot of the 15-d-old nonnodulated chickpea seedling by 14.6% at 24 h of ^{15}N treatment (Fig. 77b). In the shoot of the 15-d-old nodulated chickpea seedling, 1-d sulphur deficiency inhibited ^{15}N accumulation by 9% at 24 h with an initial increase by 6% at 6 h of ^{15}N application (Fig. 78b).

Five-day sulphur deficiency, applied to the 15-d-old nonnodulated chickpea seedling, inhibited the accumulation of ^{15}N in the root by 28.8% and 36.8% at 12 and 24 h of ^{15}N treatment respectively except an 20% initial increase of that at 6 h of treatment (Fig. 79a). Similarly, 5-day sulphur deprivation,

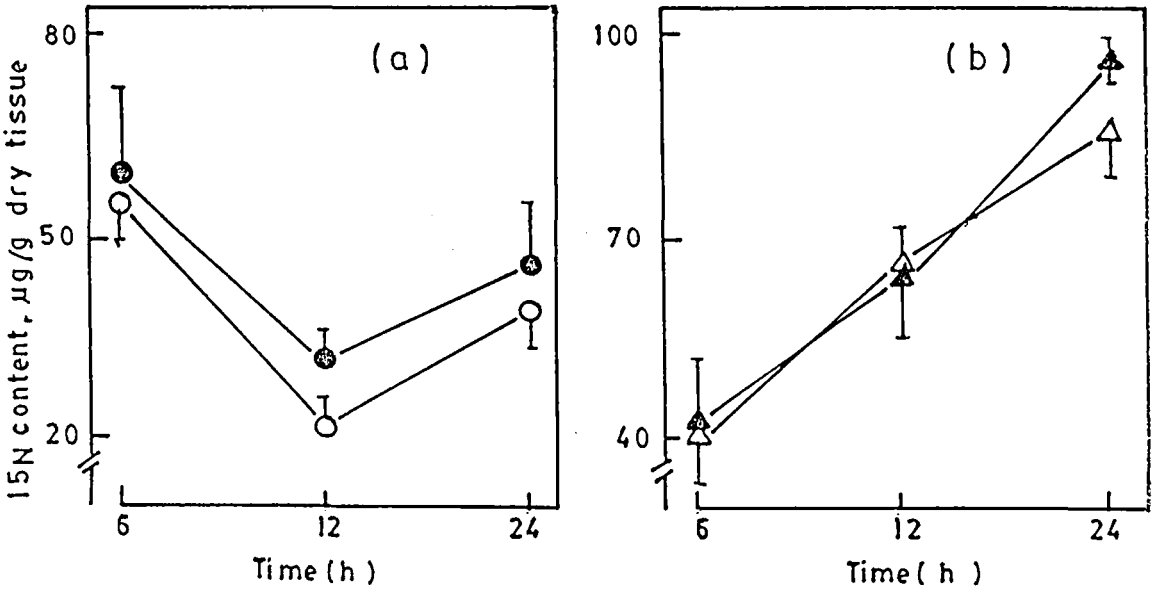


Fig. 77. The effect of 1-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Solid symbols------ + S; open symbols----- – S. ○ root; △ shoot. Each value is the mean of three replicates ± standard error.

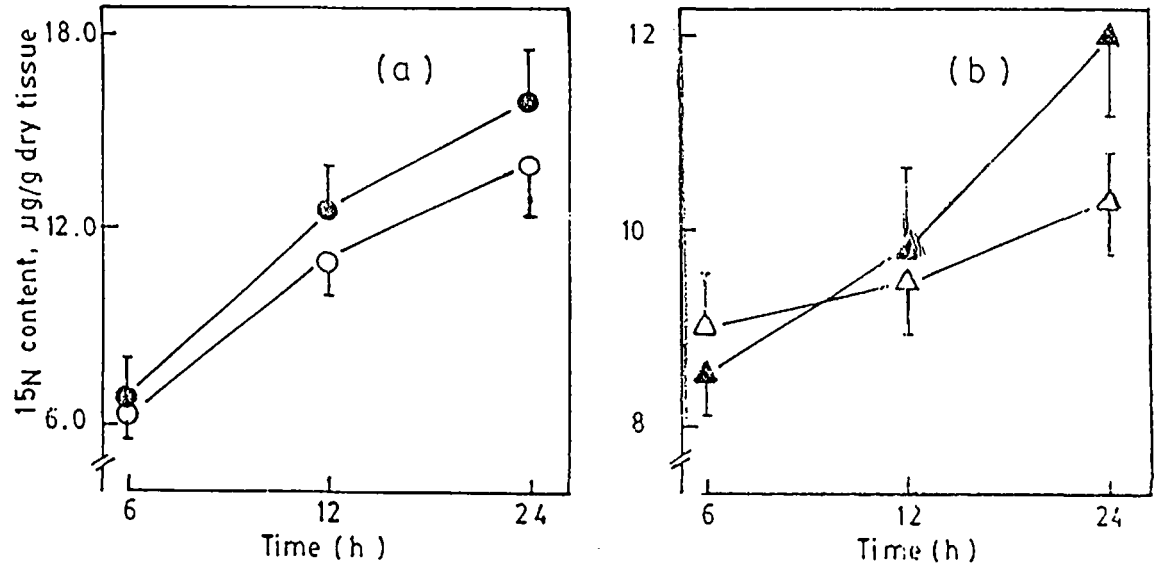


Fig. 78. The effect of 1-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

applied to the 15-d-old nodulated chickpea seedling, caused a 11.5% and 21.4% decrease in ^{15}N accumulation in the root at 12 and 24 h of addition of ^{15}N respectively (Fig. 80a).

In shoot of these 5-d S-deficient nonnodulated chickpea seedling, ^{15}N accumulation was decreased by 24% at 24 of ^{15}N application with an initial increase in accumulation of that by 29.5% at 6 h of treatment (Fig. 79b). On the other hand, ^{15}N accumulation was inhibited by 53% in the shoot of the 5-d sulphur deficiency-treated nodulated chickpea seedlings at 12 h of application of ^{15}N (Fig. 80b).

9.3.2 Effects of sulphur deficiency on growth of 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture

One-day sulphur deficiency applied to 15-d-old nonnodulated chickpea seedlings had no effect on the dry matter content of the root and shoot (Fig. 81a & 81b). On the other hand, 1-d sulphur deprivation decreased dry matter content of the root of the nodulated seedling by 8% and 15% at 6 and 12 h of addition of ^{15}N respectively (Fig. 82a). Similarly, the dry matter content of the shoot of the S-deficient nodulated seedling was decreased by 9% at 12 h of addition of ^{15}N (Fig. 82b).

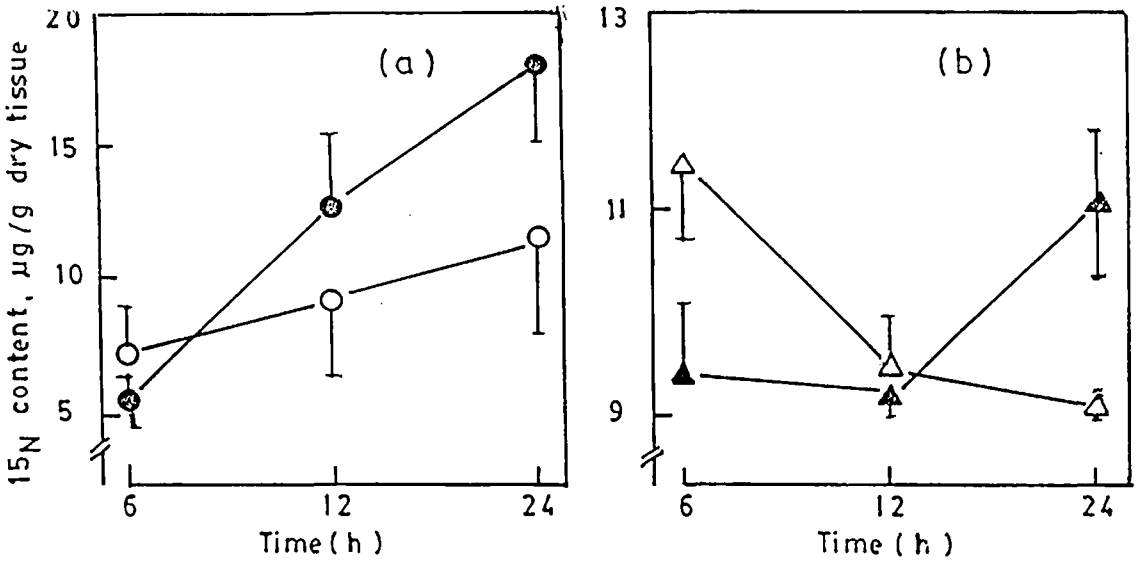


Fig. 79. The effect of 5-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

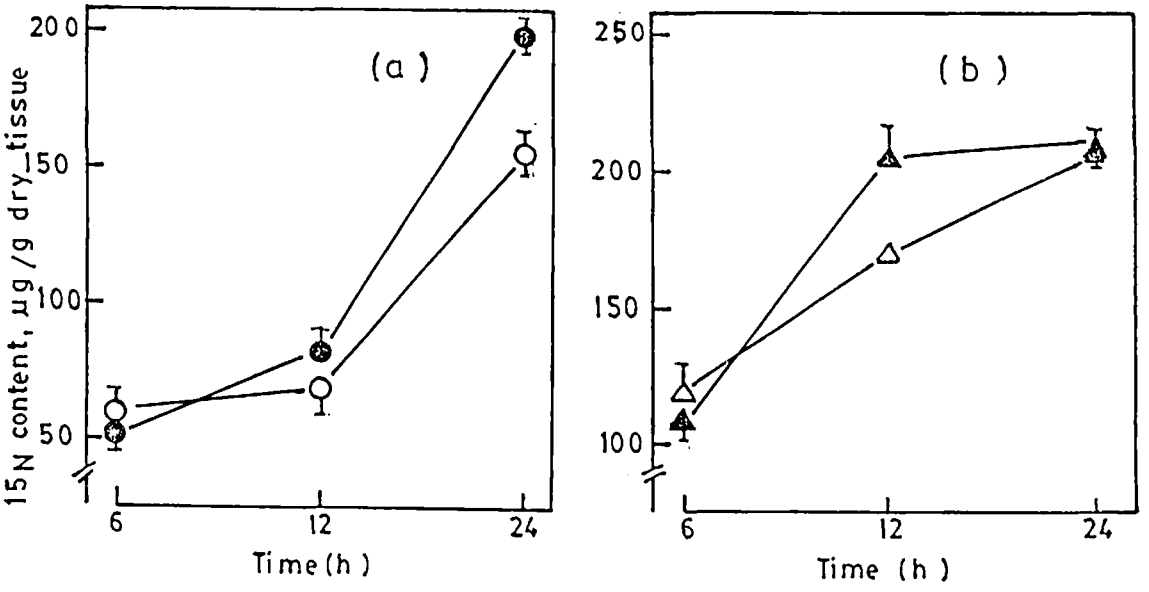


Fig. 80. The effect of 5-d sulphur deficiency on ^{15}N accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

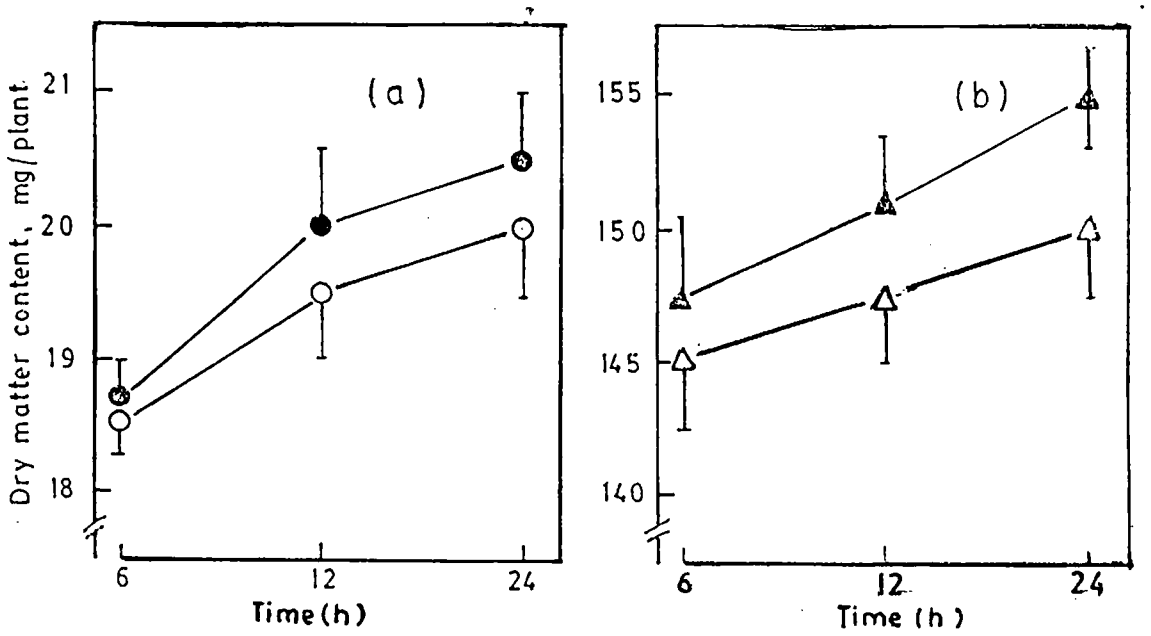


Fig. 81. The effect of 1-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

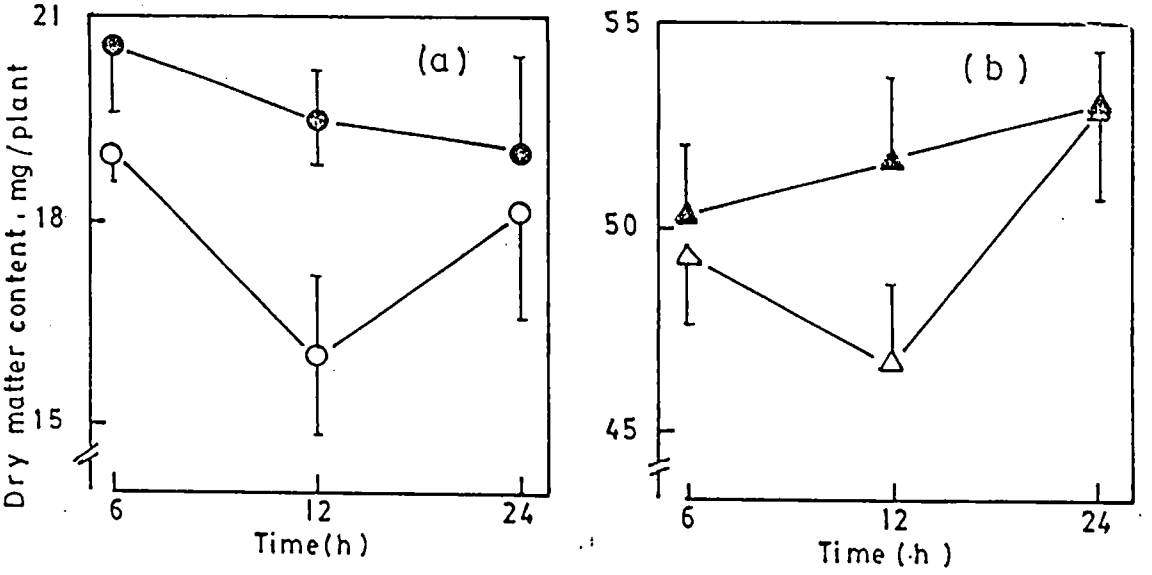


Fig. 82. The effect of 1-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

Five-day sulphur deficiency had no effect on the dry matter content of the root of the nonnodulated chickpea seedling (Fig. 83a). But dry matter contents of shoots of these seedlings were inhibited by 3.5% to 11% at 6 to 24 h of ^{15}N application (Fig. 83b).

Five-day sulphur deficiency caused a 6.2% to 9% increase in the dry matter of the root at 12 and 24 h of ^{15}N application (Fig. 84a). Five-day sulphur deprivation decreased the dry matter of the shoot of the nodulated seedling by 4% to 31% from 6 to 24 h of addition of ^{15}N (Fig. 84b).

9.3.3 Effects of sulphur deficiency on accumulation and distribution of K^+ and Na^+ in 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture

K^+ accumulation in the root was inhibited by 8.5% to 13.7% in the nonnodulated and 33% to 50% in the nodulated chickpea seedling respectively at 1-d to 5-d of sulphur deficiency treatment (Fig. 85a & 86a).

In the shoot of the nonnodulated chickpea seedling, K^+ accumulation was decreased by 5% following sulphur deficiency treatment.(Fig. 85b). Accumulation of K^+ in the shoot of the seedling of nodulated genotype was decreased by 13%, 24% and 10% at 1-d, 3-d and 5-d of treatment respectively (Fig. 86b).

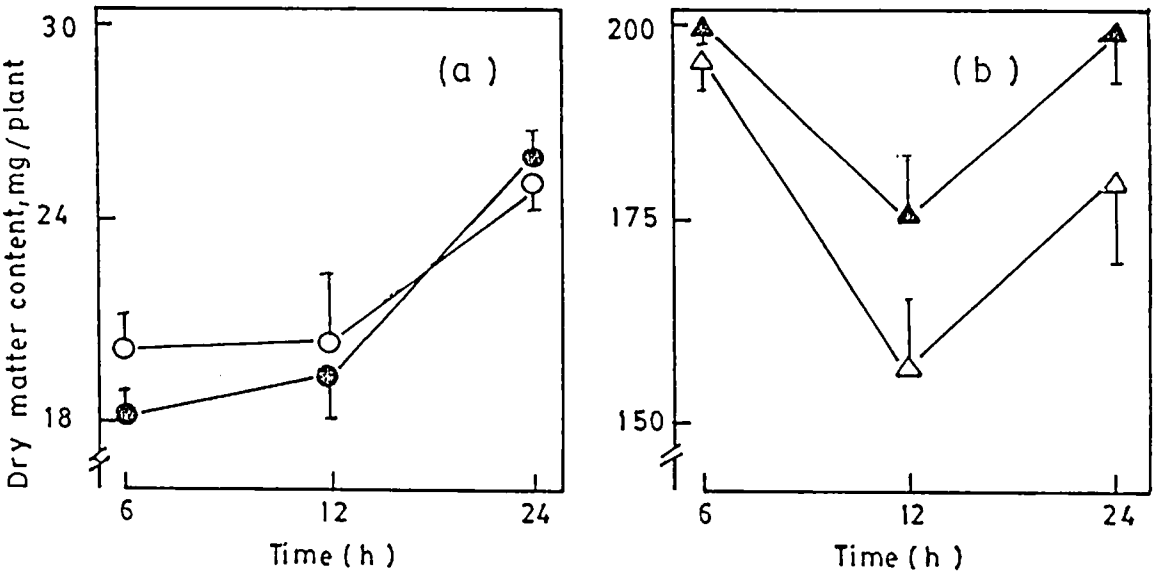


Fig. 83. The effect of 5-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

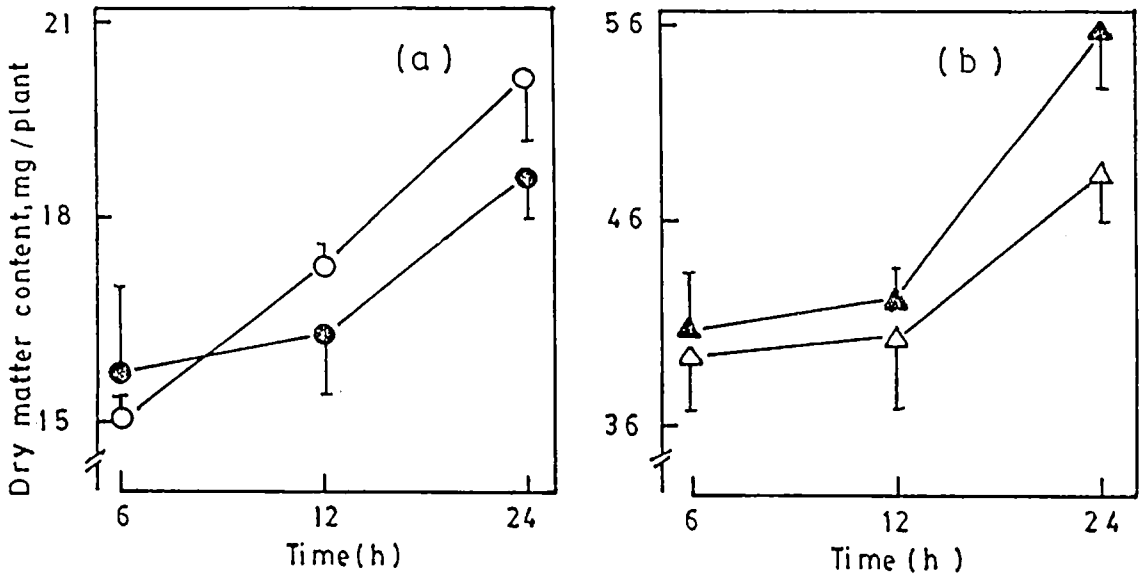


Fig. 84. The effect of 5-d sulphur deficiency on dry matter accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 77.

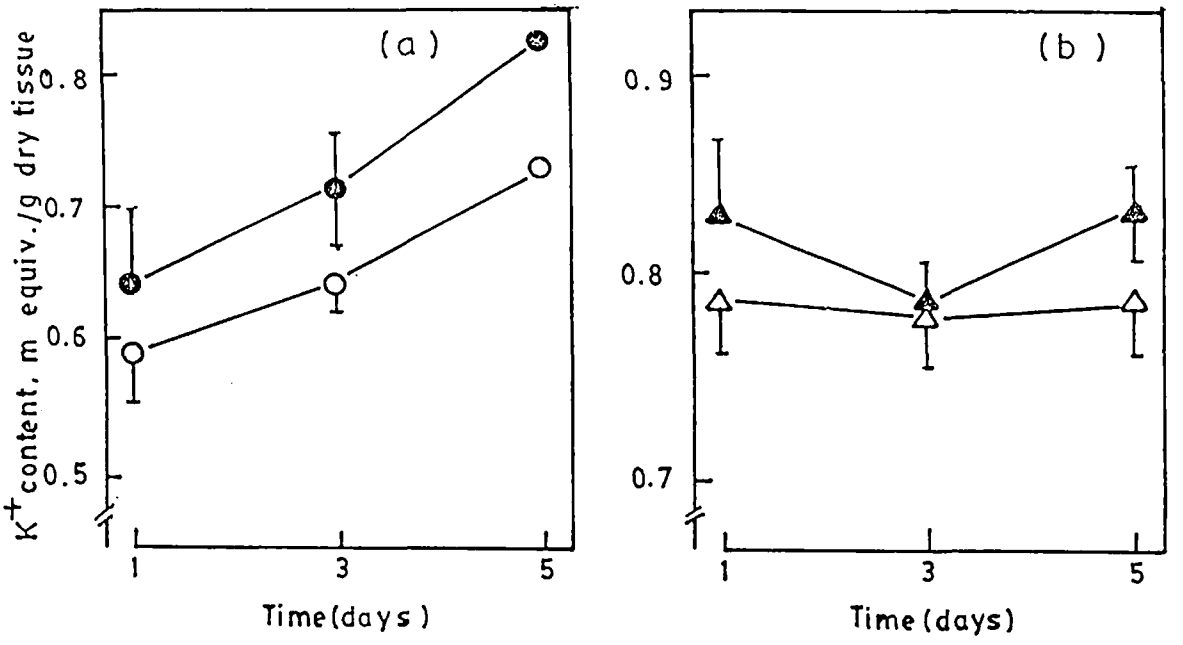


Fig. 85. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Solid symbols------ + S; open symbols----- – S. ○ root; △ shoot. Each value is the mean of three replicates ± standard error.

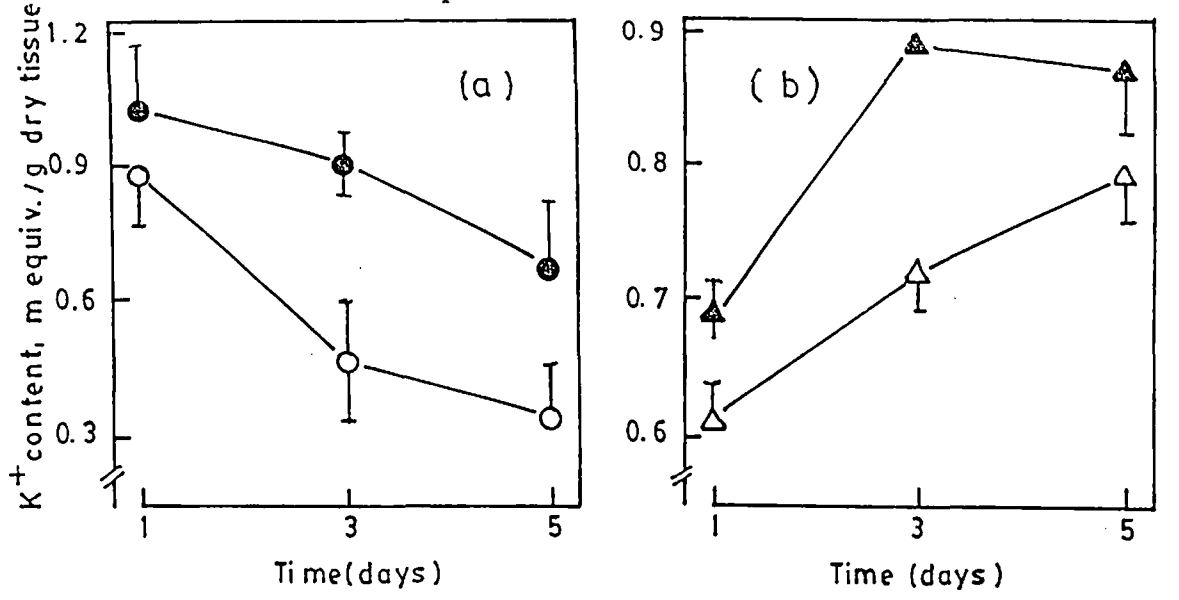


Fig. 86. The effect of sulphur deficiency on K^+ accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

In the root of the nonnodulated chickpea seedling, sulphur deficiency increased Na^+ accumulation by 6%, 22% and 5% at 1-d, 3-d and 5-d of treatment respectively (Fig. 87a). On the other hand, in the nodulated genotype, Na^+ accumulation in the root was inhibited by 19%, 16% and 26% at 1-d, 3-d and 5-d of treatment respectively (Fig. 88a).

Na^+ accumulation in the shoot of the nonnodulated genotype was increased by 25% and 26% at 3-d and 5-d of sulphur deficiency treatment respectively. On the contrary, Na^+ accumulation in the shoot of the nodulated seedling was decreased by 14% and 15% at 1-d and 3-d of sulphur deficiency treatment respectively (Fig. 87b & 88b).

9.3.4 Effects of sulphur deficiency on Ca^{2+} and Mg^{2+} accumulation and distribution in 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture

Calcium accumulation in the root of the nonnodulated chickpea seedling was increased by 12% and 18.4% at 1-d and 3-d of sulphur deficiency treatment. On the contrary, Ca^{2+} accumulation was decreased in the root of the nodulated seedling by 30.6% and 16% at 3-d and 5-d of treatment respectively (Fig. 89a & 90a).

In the shoot of the nonnodulated seedling, Ca^{2+} accumulation was decreased by 13.6% at 5-d of sulphur deficiency treatment. Similarly, sulphur

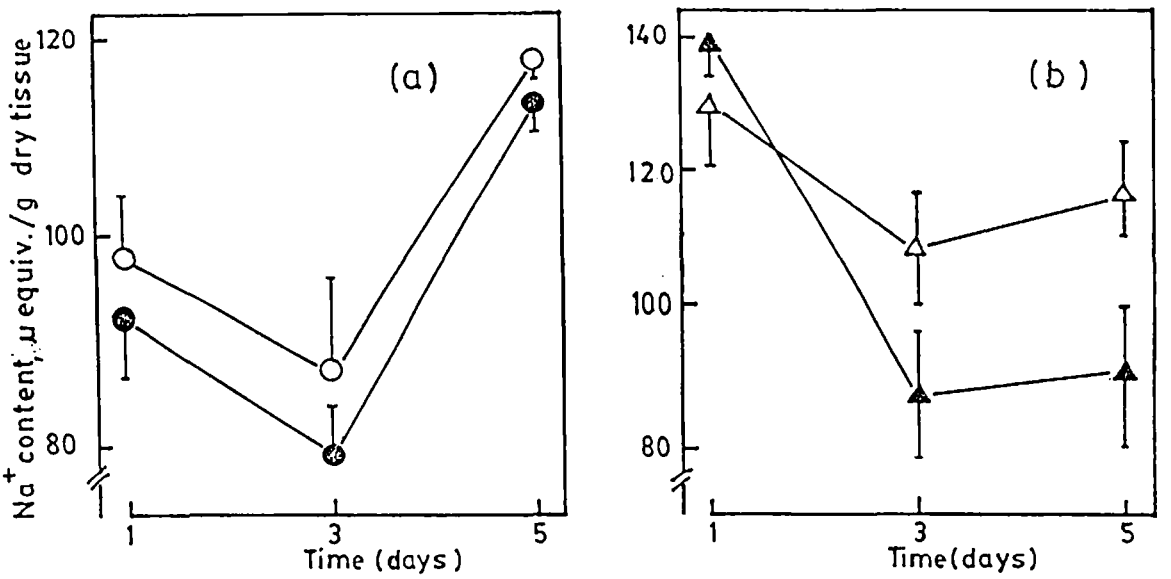


Fig. 87. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

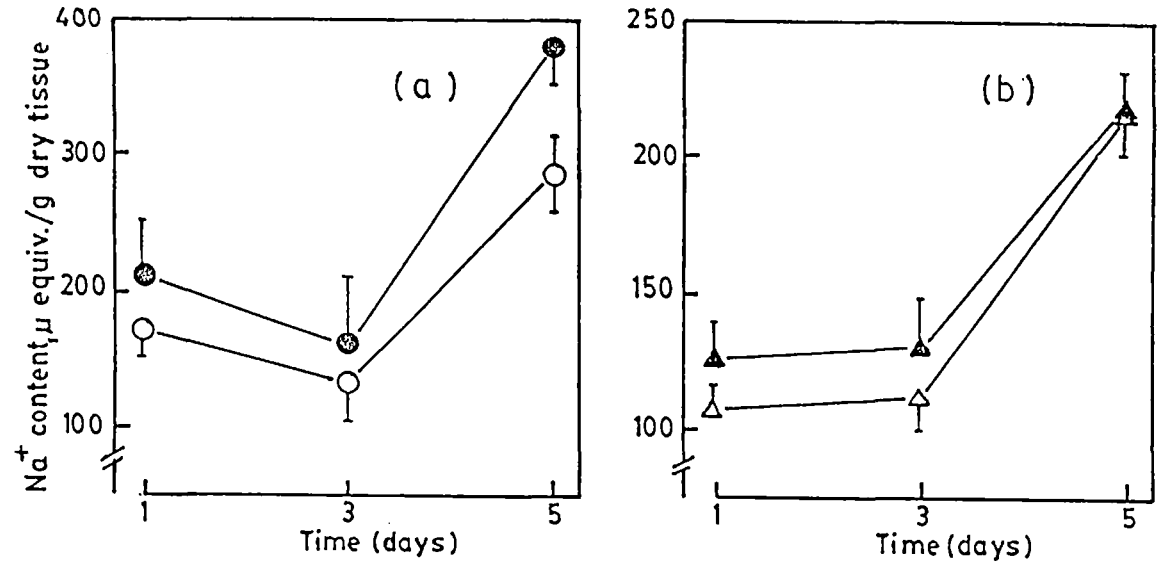


Fig. 88. The effect of sulphur deficiency on Na^+ accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

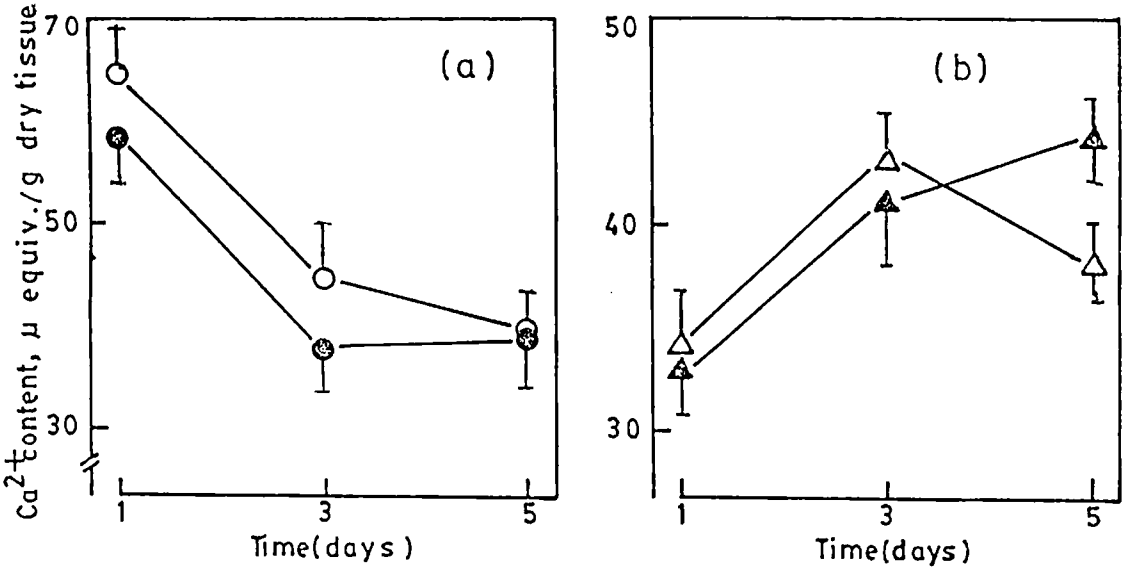


Fig. 89. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

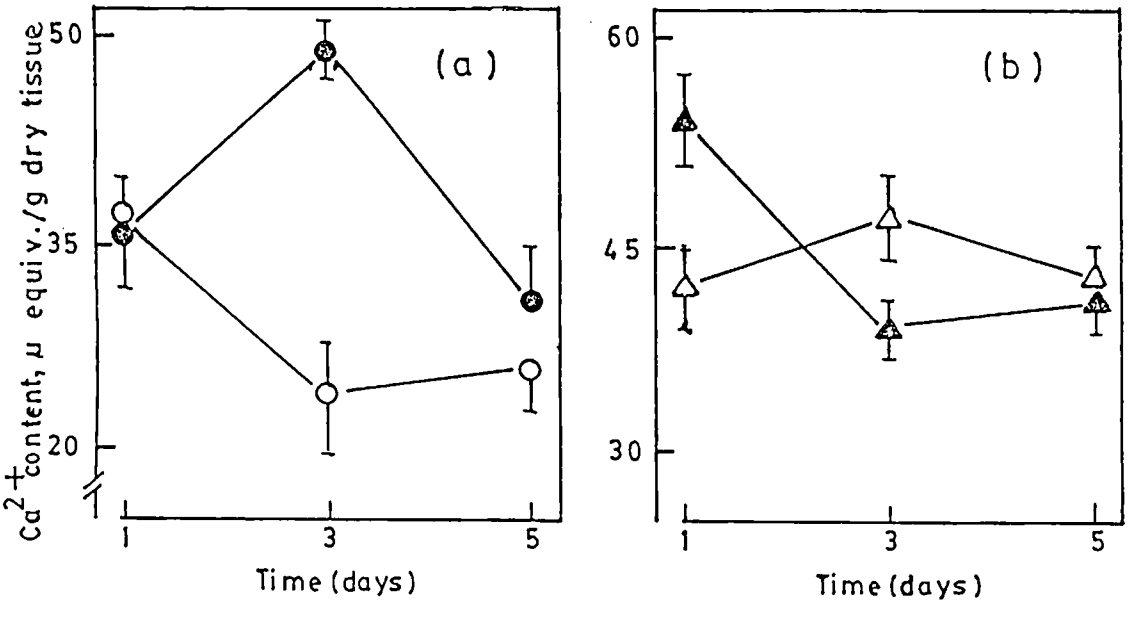


Fig. 90. The effect of sulphur deficiency on Ca^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

deficiency decreased the accumulation of Ca^{2+} in the shoot of the seedling of nodulated genotype by 22% at 1-d of treatment. But accumulation of that was increased by 20% and 5% at 3-d and 5-d of treatment respectively (Fig. 89b & 90b).

Accumulation of Mg^{2+} was increased in the root of the nonnodulated chickpea seedling by 51% and 20.6% at 1-d and 3-d of sulphur deficiency treatment respectively and this stimulatory effect was sustained upto 5-d of treatment. Similarly, in the root of the nodulated seedling, Mg^{2+} accumulation was increased by 7% to 34% from 3-d to 5-d of treatment (Fig. 91a & 92a). In the shoot of the nonnodulated chickpea seedling, sulphur deficiency increased the accumulation of Mg^{2+} by 66%, 25% and 10% at 1-d, 3-d and 5-d of treatment respectively. Similarly, accumulation of Mg^{2+} in the shoot of the nodulated seedling was increased by 37% to 60% from 3-d to 5-d of deficiency treatment (Fig. 91b & 92b).

9.3.5 Effects of sulphur deficiency on Fe^{2+} and Zn^{2+} accumulation and distribution in 15-d-old intact nonnodulated and nodulated chickpea seedlings grown in sand culture

In the root of the nonnodulated seedling, sulphur deficiency resulted in a decrease in accumulation of Fe^{2+} at 3-d and 5-d of treatment (Fig. 93a). On the contrary, Fe^{2+} accumulation was increased in the root of the seedling of

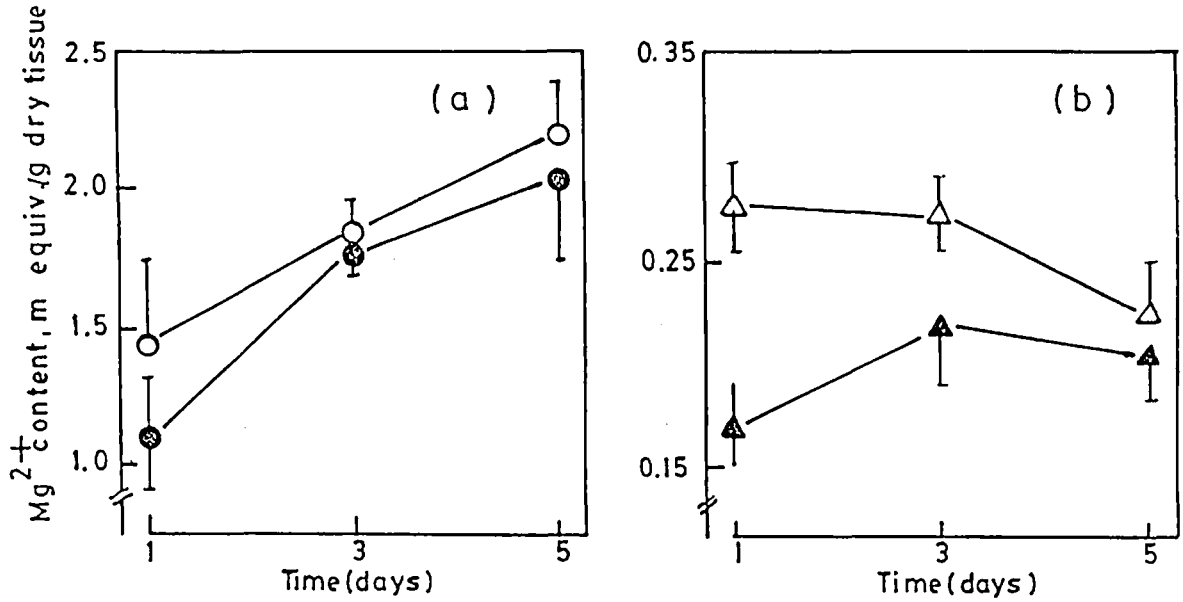


Fig. 91. The effect of sulphur deficiency on Mg^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

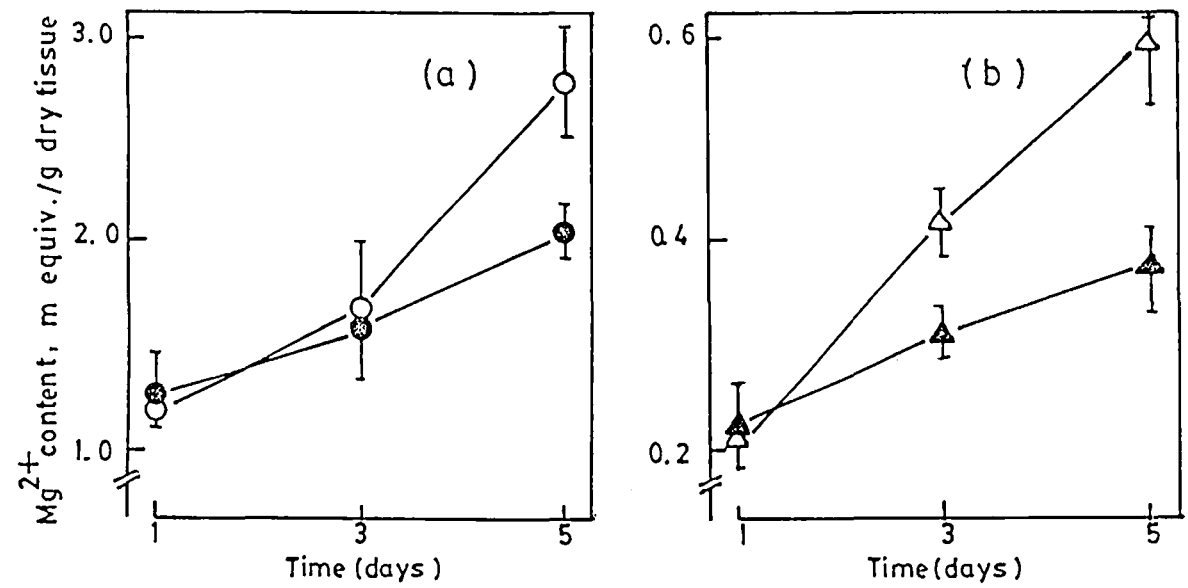


Fig. 92. The effect of sulphur deficiency on Mg^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

the nodulated genotype by 10.5% and 14.5% at 3-d and 5-d of sulphur deficiency treatment respectively (Fig. 94a).

In the shoot of the nonnodulated seedling, sulphur deficiency inhibited the accumulation of Fe^{2+} by 6% and 27% at 3-d and 5-d of treatment respectively (Fig. 93b). Similarly, in the shoot of the nodulated seedling, sulphur deficiency inhibited the accumulation of Fe^{2+} by 26% and 46% at 3-d and 5-d of treatment respectively (Fig. 94b).

In the root of the S-deficient nonnodulated chickpea seedling, Zn^{2+} accumulation was inhibited by 3%, 13% and 10% at 1-d, 3-d and 5-d of treatment respectively (Fig. 95a).The accumulation of that was also inhibited in the nodulated S-deficient seedling by 26%, 15% and 5% at 1-d, 3-d and 5-d of treatment respectively (Fig. 96a).

Zn^{2+} accumulation in the shoot of the seedling of nonnodulated genotype was increased by 14% and 18% at 1-d and 3-d of sulphur deficiency followed by a 9% decrease in accumulation of that at 5-d of treatment. On the other hand, in the nodulated seedling, sulphur deficiency inhibited the accumulation of Zn^{2+} by 17% and 4% at 1-d and 3-d of treatment respectively (Fig. 95b & 96b).

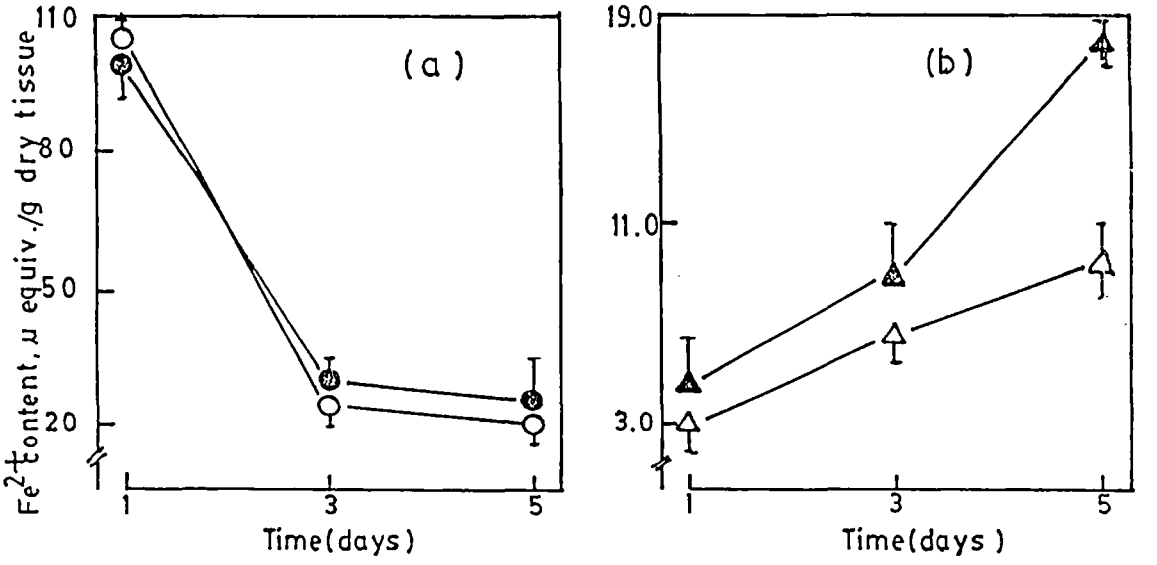


Fig. 93. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85

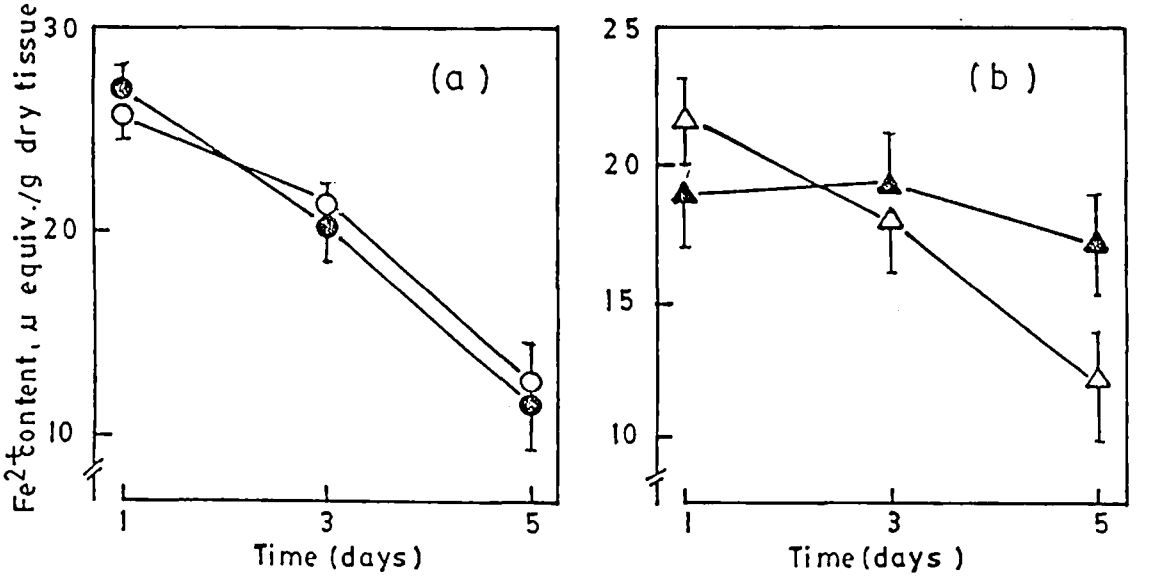


Fig. 94. The effect of sulphur deficiency on Fe^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

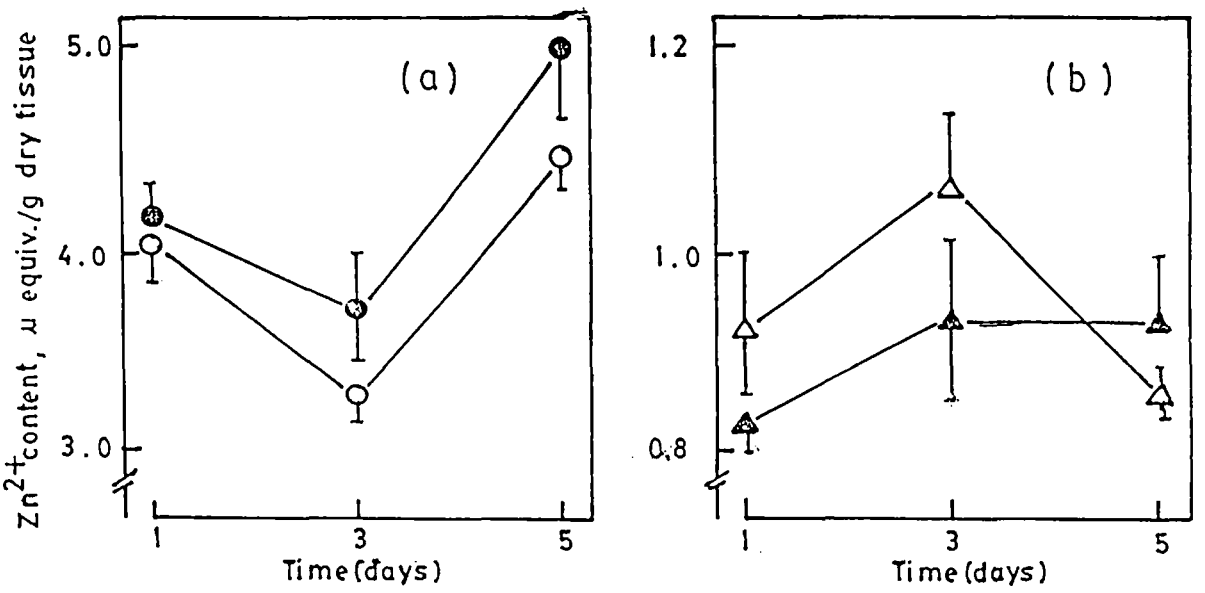


Fig. 95. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

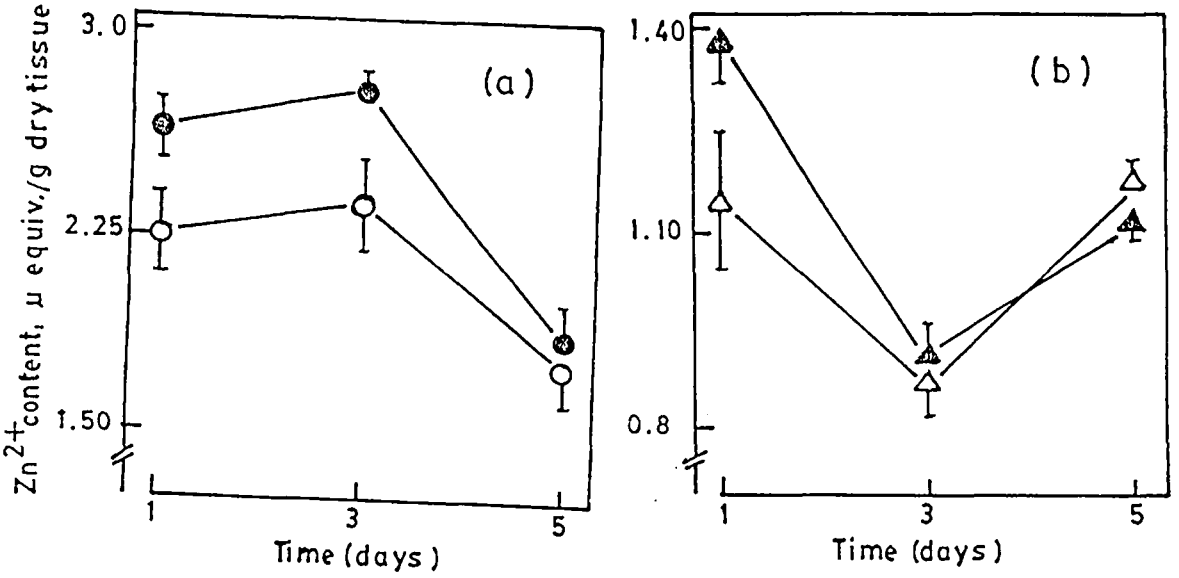


Fig. 96. The effect of sulphur deficiency on Zn^{2+} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

9.3.6 Effects of sulphur deficiency on accumulation and distribution of phosphate in 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture

In the root of the nonnodulated chickpea seedling, sulphur deficiency resulted in an increase in accumulation of PO_4^{3-} by 53% at 1-d of treatment followed by a decrease in that by 11% and 7.5% at 3-d and 5-d of treatment respectively (Fig. 97a). On the contrary, PO_4^{3-} accumulation in the root of the nodulated seedling was decreased by 21%, 9% and 21% at 1-d, 3-d and 5-d of treatment respectively (Fig. 98a).

In the shoot of the seedling of the nonnodulated genotype, sulphur deficiency decreased the accumulation of PO_4^{3-} by 6% to 27% from 1-d to 5-d of treatment. On the other hand, sulphur deficiency caused an 8.5% increase in PO_4^{3-} accumulation in the shoot of the seedling of the nodulated genotype at 5-d of treatment (Fig. 97b & 98b).

9.3.7. Effects of sulphur deficiency on accumulation and distribution of nitrate in 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture

Sulphur deficiency caused an increase in the accumulation of NO_3^- by 36.3% in the root of nonnodulated seedlings whereas the same was inhibited by 19.4% in the nodulated seedlings (Table 3).

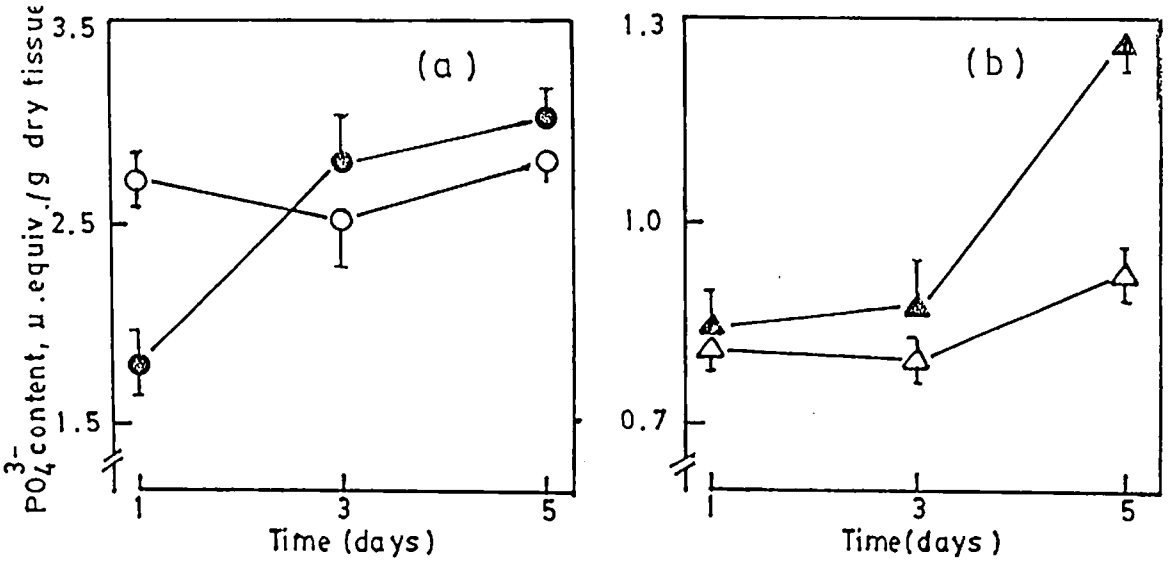


Fig. 97. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 15-d-old nonnodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85

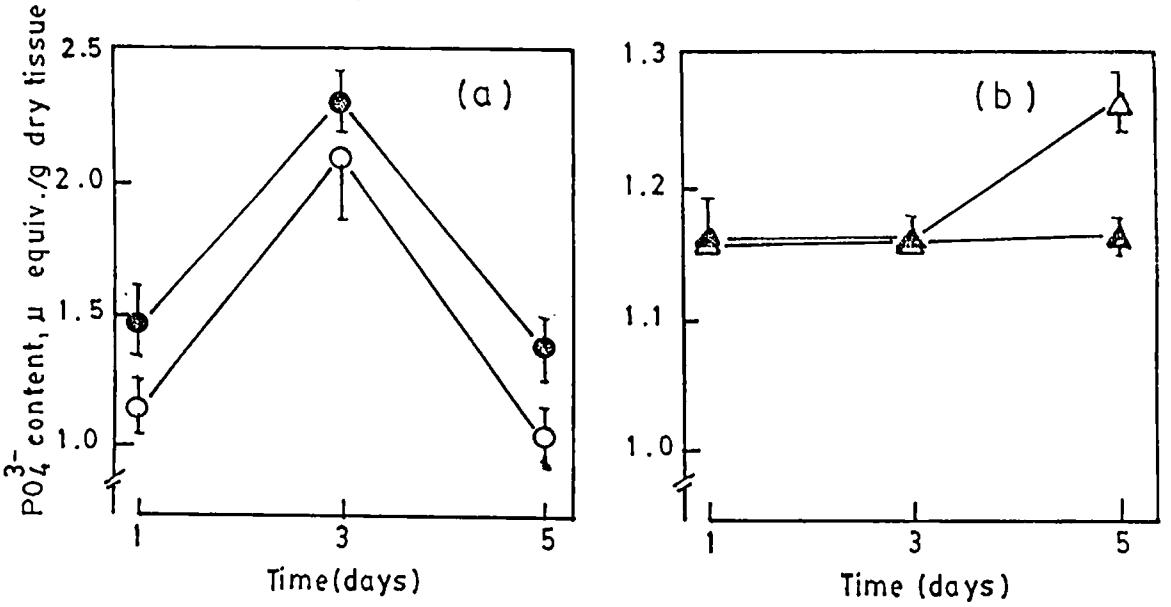


Fig. 98. The effect of sulphur deficiency on PO_4^{3-} accumulation in the (a) root and (b) shoot of 15-d-old nodulated chickpea seedlings grown in sand culture. Otherwise as Fig. 85.

Table 3. The effect of 7-d sulphur deficiency on nitrate accumulation in roots and shoots of 15-d-old nonnodulated and nodulated chickpea seedlings grown in sand culture. Each value is the mean of three replicates ± standard error

		Nitrate content, μ equivalent g^{-1} fresh tissue	
	Treatment	Root	shoot
nonnodulated genotype	+ S	58.7 ± 5.8	80.4 ± 9.0
	- S	80.0 ± 3.5	86.1 ± 4.7
Nodulated genotype	+ S	60.3 ± 6.5	187.6 ± 5.6
	- S	48.6 ± 7.8	157.3 ± 7.8

Accumulation of NO_3^- in the shoot of the nonnodulated seedlings was increased by 7% whereas that in the nodulated seedlings was inhibited by 15.9% following sulphur deficiency treatment (Table 3).

9.4 **Discussion**

In plants grown in sand culture under natural condition, sulphur deficiency inhibited K^+, Na^+, Fe^{2+}, Zn^{2+} and PO_4^{3-} accumulation and distribution in chickpea seedlings of both nonnodulated and nodulated genotypes (Fig. 85 to 88, 93 to 98). Sulphur deficiency was reported to decrease accumulation of K^+ in wheat (Ivanic 1978) and in rice (Pillai and Singh 1975). Accumulation of Fe^{2+} and Zn^{2+} were also decreased in wheat (Wankhade *et al.* 1989) and in *P. aquatica* (Sairam *et al.* 1995). Under S-deficient condition phosphate accumulation was also reported to be decreased in pea (Ligero *et al.* 1981). On the contrary, accumulation of Ca^{2+}, and Mg^{2+} were increased in seedlings of both genotypes (Fig. 89 to 92). Accumulation of NO_3^- was increased in the root and shoot of the seedling of nonnodulated chickpea (Table 3). Sulphur deficiency-induced increase in the accumulation of nitrate is also supported by Bush and co-workers (1981) who found a similar increase in that in tall fescue. These results are supported by the work of Mertinez and co-workers (1983) who found a sulphur deficiency-induced increase in accumulation of Mg^{2+} and NO_3^- in tomato. On the other

hand, in the seedling of the nodulated genotype, sulphur deficiency decreased the accumulation of nitrate in both the root and shoot (Table 3). Hence, the nonnodulated and nodulated genotype of chickpea showed varietal differences with respect to their nitrate uptake capacities.

Sulphur deficiency decreased ^{15}N uptake and transport in roots and leaves of the nonnodulated and nodulated chickpea seedlings (Fig. 77 to 80). Similarly, sulphur deficiency decreased ^{15}N uptake and transport in barley (Karmoker *et al.* 1991).

It is apparent from the results that sulphur deficiency decreased ^{15}N accumulation in roots and shoots of nonnodulated and nodulated chickpea seedlings grown in both solution culture in controlled conditions (Fig. 1-8 , chapter 3) and sand culture under field conditions (Fig. 77 to 80, chapter 9).

Conclusion

In this concluding chapter all the results are coordinated in an attempt to correlate the effect of sulphur deficiency on ^{15}N and ion transport with biochemical changes and overall effects of these factors in growth of chickpea. A comparison of the effect of sulphur deficiency on nonnodulated and nodulated genotypes of chickpea was also made.

^{15}N uptake and translocation in S-deficient chickpea seedlings was reduced in both the genotypes (Fig. 1 to 8 chapter, 3 and Fig. 79 to 82, chapter 9). The reduction of ^{15}N uptake and distribution in nonnodulated and nodulated chickpea might be the result of a decrease in soluble protein accumulation (Fig. 75 & 76, chapter 8), the synthesis of which was disturbed by sulphur deficiency. Sulphur deficiency also affected NR activity as well as ion transport. Both the genotypes were equally affected with respect to ^{15}N uptake under sulphur deficiency.

Sulphur deficiency caused a dramatic increase in accumulation of ^{35}S in roots and shoots of seedlings of both nonnodulated and nodulated genotypes (Fig. 17 to 20, chapter 4). However, high rate of ^{35}S accumulation in the S-

sulphur starved seedlings is a common phenomenon to meet the demand of this particular ion created due to prolonged sulphur deficiency.

K^+ and Na^+ accumulation was decreased in roots and shoots of seedlings of both the genotypes with the exception that in long-term sulphur deficiency K^+ and Na^+ accumulation was increased in the root and shoot of the nonnodulated genotype (Fig. 21 to 32, chapter 5 and Fig. 87 to 90, chapter 9). Similarly, Fe^{2+} and Zn^{2+} accumulation was decreased in the root and shoot of both the genotypes except an increase in accumulation of Fe^{2+} and Zn^{2+} in 7-d-old seedlings of nonnodulated genotype (Fig. 45 to 56, chapter 5 and Fig. 93 to 96, chapter 9). The reduced uptake of K^+, Na^+, Fe^{2+} and Zn^{2+} might be associated with disturbed metabolic function which was the result of biochemical changes like reduced synthesis of NR enzyme (Fig. 67 & 68, chapter 6 and Table 1) and carbohydrate (Fig. 69 to 72, chapter 7).

In the seedling of the nonnodulated genotype, accumulation of Ca^{2+} and Mg^{2+} was increased under sulphur deficiency (Fig. 33, 35, 37, 39, 41, 43, chapter 5). On the other hand, Mg^{2+} accumulation was decreased in the root but long term deficiency resulted in an increase in accumulation of that in the shoot of the seedling of nodulated genotype whereas Ca^{2+} accumulation was decreased in both the root and shoot of this genotype (Fig. 34, 36, 38, 40, 42, 44, chapter 5). The initial increase in the accumulation of Ca^{2+} and

Mg^{2+} in the root and shoot of the nonnodulated chickpea seedling whereas initial decrease in the accumulation of those ions in the root and shoot of nodulated chickpea seedling show that nonnodulated genotype is more efficient in accumulation of Ca^{2+} and Mg^{2+} than the nodulated genotype under S-deficient condition. Matocha (1971) reported that when Mg^{2+} is available in S-deficient condition, there might be a sulphur-magnesium imbalance.

It was found that phosphate accumulation was depressed due to sulphur deficiency in both nonnodulated and nodulated genotypes (Fig. 57 to 62, chapter 5 and Fig. 97 & 98, chapter 9) except an increase in accumulation in the root of 21-d-old nonnodulated chickpea seedling. The decreased accumulation of phosphate might be due to decreased root growth (Fig. 9 to 16, chapter 3) in S-deficient condition as was reported in alfalfa by Martel and Zizka (1977).

Sulphur deficiency resulted in an increase in the accumulation of nitrate in seedlings of both the genotypes (Fig 63 to 66, chapter 5). It was recorded that sulphur deficiency reduced the synthesis of NR (Fig. 67 & 68, chapter 6 and Table 1) and soluble protein (Fig. 75 & 76, chapter 8 and Table 2). This increase in the accumulation of nitrate in chickpea might be correlated with lower synthesis of soluble protein due to less availability of substrate which

resulted in the disruption of utilization of NO_3^- (Radin *et al.* 1975; Radin 1978).

Sulphur deficiency resulted in a decrease in the accumulation of sugars in the leaf but its accumulation in the root was increased (Fig. 69 to 72, chapter 7). Total accumulation of sugar was decreased in both the genotypes of chickpea (Fig. 69 to 72, chapter 7). This indicates the decrease in the synthesis of carbohydrate in sulphur limiting condition. Similarly, sulphur deficiency resulted in a decrease in sugar content in alfalfa (Rendig and McComb 1961).

Total amino acid increased in all plant parts of both the genotypes of chickpea (Fig. 73 & 74, chapter 7). Generally, the assimilated N is incorporated to C-skeleton to form amino acids. In sulphur-deficient condition, the sulphur containing amino acid synthesis might be reduced (Bordeleau *et al.* 1981; Martinez *et al.* 1983; Shewry *et al.* 1983) due to less availability and slower transport of sulphur from the root to the leaf (Fig. 17 to 20, chapter 4) and hence might increase the synthesis of sulphur free amino acids (Holobrada 1977; Coleman 1957; Mertz *et al.* 1952). This view supports the observed increase in accumulation of total amino acids in chickpea.

Nitrate reductase activity was reduced and soluble protein accumulation was depressed in chickpea seedlings (Fig. 67 & 68, chapter 6 and Table 1; Fig 75 & 76, chapter 8 and Table 2). Furthermore, total NR activity in seedlings of nodulated genotype was higher than that in seedlings of the nonnodulated genotype (Fig. 67 & 68, chapter 6). This result proved the existence of genetic variability of NR activity. Earlier, it was reported that NR was genetically controlled (Oakes *et al.* 1972; Zieserl and Hageman 1969). According to Worner and co-workers (1969) NR activity was influenced by different rates of enzyme synthesis.

It is apparent from the above discussion that sulphur deficiency has more or less similar inhibitory effects on ^{15}N transport, stimulatory effects on ^{35}S transport, inhibitory effects on protein synthesis and ion transport in both nonnodulated and nodulated genotypes of chickpea except that of nitrate where sulphur deficiency-induced stimulatory effects on the accumulation of nitrate in both the genotypes of chickpea was observed (Fig. 63 to 66, chapter 5 and Table 3, chapter 9).

^{15}N transport decreased in chickpea seedlings in both solution culture in the controlled environmental conditions and sand culture in the field condition (Fig. 1 to 8, chapter 3 and 79 to 82, chapter 9). Accumulation of Ca^{2+} and Mg^{2+} increased in seedlings grown in both solution culture in the controlled

environment and sand culture in the field condition. Similarly, phosphate accumulation decreased in seedlings grown in both solution culture in the control environment and sand culture in the field condition (Fig. 57 to 62 chapter 5 and 97 & 98. chapter 9). Therefore, the result obtained with respect to the effect of sulphur deficiency on ^{15}N, Ca^{2+}, Mg^{2+}, PO_4^{3-} transport in seedlings grown in solution culture in the controlled environmental condition may be reconciled with that in seedlings grown in sand culture in the field condition.

Overall effects of sulphur deficiency on ion and ^{15}N transport, on the accumulation of protein, carbohydrate and amino acid was coordinated in Fig. 99. This figure shows that sulphur deficiency resulted in a decrease in ^{15}N transport (Fig. 1 to 8, chapter 3; Fig. 79 to 82, chapter 9); it reduced soluble protein accumulation (Fig. 75 & 76, chapter 8 and Table 2). The decrease in ^{15}N transport and accumulation might lead to a decrease in soluble protein synthesis. Sulphur deficiency might result in a decrease in sulphur containing amino acid synthesis and increase in total free amino acid accumulation (Fig. 73 & 74, chapter 7) which might affect the synthesis of soluble protein. Sulphur deficiency decreased the accumulation of total carbohydrate (Fig. 69 to 72, chapter 7). The decrease in carbohydrate accumulation as well as the decrease in soluble protein synthesis might lead to the shortage of energy supply which may affect ion accumulation and

translocation (Fig. 21 to 32, and 45 to 62, chapter 5; Fig. 85 to 88 & 93 to 98, chapter 9). These adverse effects on inorganic nutrition and biochemical processes might lead to the decrease in growth (Fig. 9 to 16, chapter 3 and 81 to 84, chapter 9) and finally may result in a decrease in the yield of chickpea.

Finally, the fundamental information obtained during the present investigation on the effect of sulphur deficiency on ^{15}N and ion transport and metabolic processes in chickpea may be useful in solving the sulphur deficiency problem prevailing in Bangladesh.

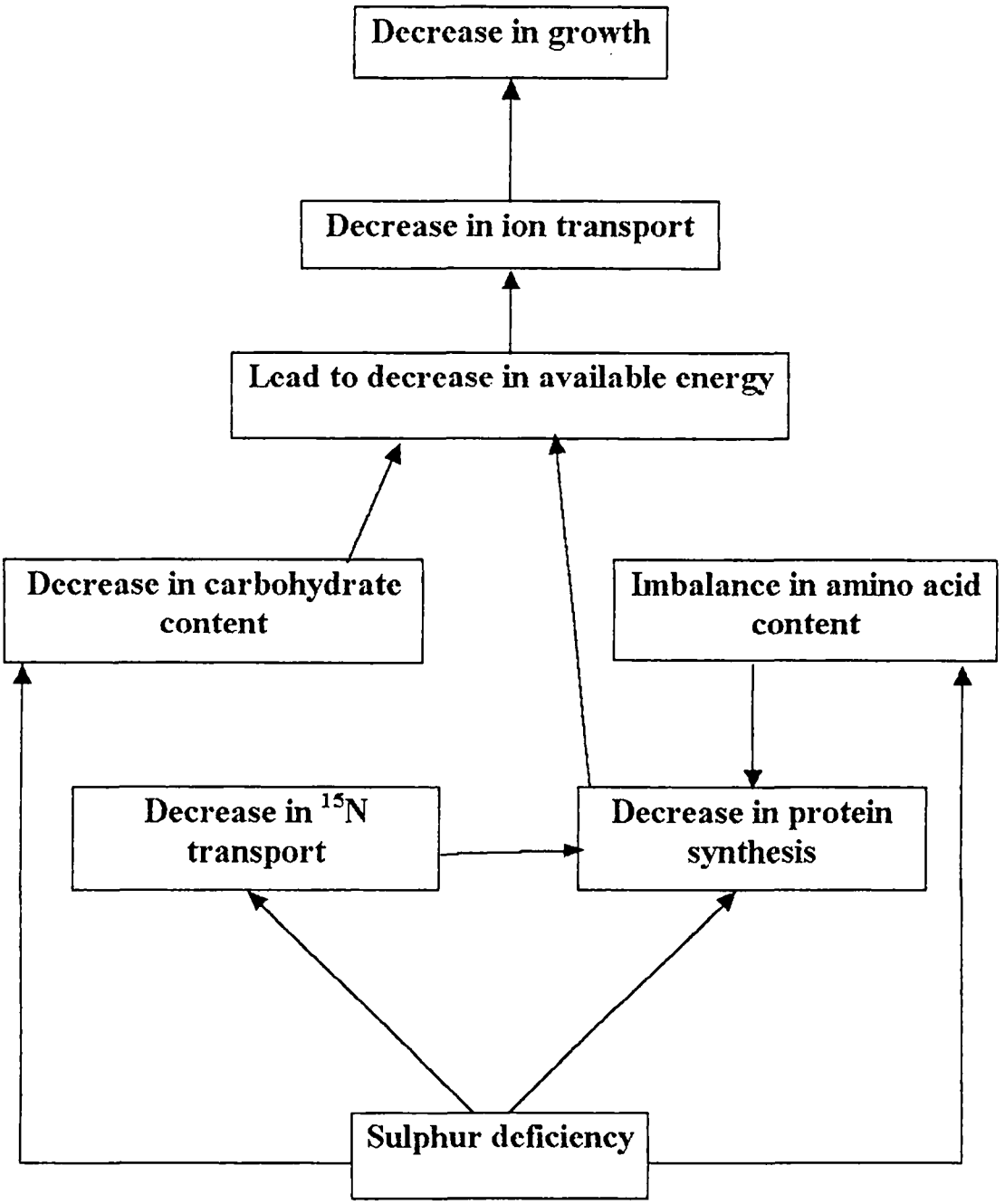


Fig. 99. Overall effects of sulphur deficiency on metabolism and ion and ^{15}N transport and its relation with growth

References

Adam, C.A. and R.W. Sheard. 1966. Alteration in the nitrogen metabolism of *Medicago sativa* and *Dactylis glomerata* as influenced by potassium and sulfur nutrition. Can. J. Plant Sci. **46**: 671-680.

Anderson, A.J. and D. Spencer. 1950. Sulphur and nitrogen metabolism in legumes and nonlegumes. Aust. J. Sci. Res. **B3**: 431-449.

Anderson, J.W. 1980. Assimilation of inorganic sulfate into cystein. *In* The Biochemistry of plants. B.J. Miflin ed. Vol. **5**: 203-223. Academic press, New York..

Anderson, J.W. 1990. Sulfur metabolism in plants. *In*: The Biochemistry of plants. Miflin, B.J. and P.J. Lea eds., Vol. **5**: 327-375. Academic press, San Diego, CA.

Andonova, P. 1992. Uptake and release of potassium and nitrogen by mineral deficient maize plants treated with polyethylene glycol. Fiziologya Rasteniyata **18**: 55-61

Aulakh, M.S. and G. Dev. 1978. Interaction effect of calcium and sulphur on the growth and nutrient composition of alfalfa (*M. sativa* L. Pers.), using ^{35}S. Plant and Soil **50**: 125-134.

Aulakh, M.S. and N.S. Pasricha. 1977. Interaction effect of sulphur and phosphorus on growth and nutrient content of moong (*Phaseolus aureus* L.). Plant and Soil **47**: 341-351.

Bailey, L.D. 1986. The sulphur status of Eastern Canadian prairie soils. Sulphur response and requirements of alfalfa (*Medicago sativa* L.), rape (*Brassica napus* L.) and barley (*Hordeum vulgare* L.). Canadian J. Soil Sci. **66**: 209-216.

Baker, A.V., N.H. Peck and G.E. MacDonald. 1971. Nitrate accumulation in vegetables. I. Spinach grown in upland soils. Agron. J. **63**: 126-130.

Bapat, P.N., S.B. Sinha and J.P. Mahajan. 1984. Protein and non-protein synthesis in blackgram as influenced by sulphur and phosphorus fertilizer. J. Nucl. Agric. Biol. **13**: 112-114.

Beevers, L., L.E. Schrader, D. Flesher and R.H. Hageman. 1965. The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings. Plant Physiol. **40**: 691-698.

Bhuiyan, N.I. and M. Islam. 1986. Sulphur deficiency problems in wetland rice soils in Bangladesh agriculture. *In*: Sulphur in Agricultural Soils. Proc. International symposium. BARC, Dhaka, Bangladesh.

Blair, G.J., C.P. Mamaril, P. Umar, A.E.O. Momuat and C. Momuat. 1979. Sulfur nutrition of rice. I. A survey of soils of South Sulawesi, Indonesia. Agron. J. **71** : 473-477.

Booth, E.J., K.C. Walker and E. Schnug. 1991a. A comparison of the response to oxidized and reduced sulphur in oilseed rape. *In*: Proc. 2nd European Society of Agron. Warwick, England.

Booth, E.J., K.C. Walker and E. Schnug. 1991b. The effect of site, foliar sulphur and nitrogen application on glucosinolate content and yield of oilseed rape (*Brassica napus* L.) *In*: Proc. GCIRC Eighth International rapeseed congress, Saskatoon, Canada. pp. 567-572.

Bordeleau, L. M., M. Giroux, R. Ouellet and H. Antoun. 1981. Effects of sulphur and nitrogen on symbiotic nitrogen fixation in seedling of lucerne (*Medicago sativa* L.). Canadian J. Plant Science **61**: 639-645.

Bowa, S.S. , S.K. Dhillon and K.S. Dhillon. 1990. Effect of sulphur on the absorption of selenium by different fodder crops . Indian J. Dairy Sci. **43** : 564-570.

Broadbent, F.E., S.K. De Datta and E.V. Laureles. 1987. Measurement of nitrogen utilization efficiency in rice genotypes. Agron. J. **79**: 786-791.

Bugakova, A.N., A.N. Knoppe and T.N. Lepesheva. 1981. Effects of sulphur nutrition conditions on the content of certain mineral elements in pea plants. Fiziologia Biolkhimiya Kulturnykh Rastenii. **13:** 46-46.

Burney, P.E. and L.P. Bush. 1985. Interaction of nitrate and sulphate reduction in tobacco. I. Influence of availability of nitrate and sulfate. J. Plant Nutr. **8**: 505-515.

Bush, L.P., J.E. Legget, M. King and E. Vercent. 1981. Sulphur molybdenum nutrition effects in tall fescue. Canadian J. Bot. **59**: 536-541.

Buysse, J., F. Smolders and R. Merckx. 1993. The role of free sugars and amino acids in the regulation of biomass partitioning and plant growth. Plant and Soil **193**: 191-194.

Cataldo, D.A., M. Haroon, L.E. Schrader and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. **6**: 71-80.

Claassen, N and S.A. Barber. 1977. Potassium influx characteristics of corn root and interaction with N, P, Ca and Mg influx. Agron. J. **69**: 860-864.

Clarkson , D.T. 1986. "Regulation of the absorption and release of nitrate by plant cells" a review of current ideas and methodology. Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants. *In*: Lanbers *et al.* eds., pp. 3-27. Murtirus Nijhoff publ. Dordrecht. The Netherlands.

Clarkson, D.T., L.R. Saker, J.V. Parvez. 1989. Depression of nitrate and ammonium transport in barley plants with diminished sulphate status: Evidence of co-regulation of nitrogen and sulphate intake. J. Exp. Bot. **40**: 953-964.

Coleman, R.G. 1957. The effect of sulphur deficiency on the free amino acids of some plants. Austr. J. Biol. Sci. **10**: 50-56.

Copper, H.D. and D.T. Clarkson. 1986. Cycling of amino nitrogen and other nutrients between shoot-root in cereal- a possible mechanism integrating shoot and root in the regulation of nutrient uptake. J. Exp. Bot. **40**: 753-762.

Cowling, D.W., D.R. Lockyer and A.W. Bristow. 1977. Uptake and utilization of sulphur and nitrogen by forage plants. Grassland Res. Inst., UK, Annual Report, pp. 28-29.

Cox, M.C., C.O. Qualoet and D.W. Rains. 1985. Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein. Crop Sci. **25**: 435-440.

Cox, M.C., C.O. Qualset and D.W. Rains. 1986. Genotypic variation for nitrogen assimilation and translocation in wheat: III. nitrogen translocation in relation to grain yield and protein. Crop Sci. **26:** 737-740.

Deitz, K.J. 1989. Recovery of spinach leaves from sulfate and phosphate deficiency. J. Plant Physiol. **134**: 551-557.

Dijkshoorn, W. and A.L. Van Wijk. 1967. The sulfur requirements of plants as evidenced by N:S ratio in the organic matter. A review of published data. Plant and Soil **26**: 129-157.

Dijkshoorn, W., J.E.M. Lampe and P.F.J. Van Burg. 1960. A method of diagnosing the sulfur nutrition status of herbage. Plant and Soil **13**: 227-241.

Doby, G. 1965. Plant Biochemistry. Interscience Publishers, London, New York, Sydney. pp. 309.

Dreyfus, J. 1964. Characterization of a sulfate and thiosulfate transporting system in *Salmonella typhimurium*. J. Biol. Chem. **239**: 2292-2297.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Robers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**: 350-356.

Eaton, S.V. 1935. Influence of sulphur deficiency on the metabolism of soybean. Bot. Gaz. **97**: 68-100.

Eaton, S.V. 1941. Influence on sulphur deficiency on the metabolism of sulfur. Bot. Gaz. **102**: 536-556.

Eaton, S.V. 1942. Volatile content of black mustard plants. Bot. Gaz. **104**: 82-89.

Eppendorfer, W. 1968. The effect of nitrogen and sulphur on changes in nitrogen fractions of barley plants at various early stages of growth and on yield and amino acid composition of grain. Plant and Soil **29**: 424-438.

Eppendorfer, W.H. 1977. Effects of varying amounts of sulphur and nitrogen on yield, N/S ratio and amino acid composition of successive cuts of Italian ryegrass. *In*: yearbook 1977. pp. 42-57. Royal Veterinary and Agricultural University, Copenhagen,

Eppendorfer, W.H. and B.O. Eggortn. 1992. Dietary fibre, sugar, starch and amino acid content of kale, ryegrass and seed of rape and field beans as influenced by S and N fertilization. Plant Food Hum. Nutr. **42**: 359-371.

Evans, H.J. and A. Nason. 1953. Pyridine nucleotide-nitrate reductase from extracts of higher plants. Plant Physiol. **28**: 233-254.

Friedrich, J.W. and L.E. Schrader. 1978. Sulfur deprivation and nitrogen metabolism in maize seedlings. Plant Physiol. **61**: 900-903.

Friedrich, J.W., D. Smith and L.E. Schrader. 1977. Nitrate reductase activity and nitrogen fractions in timothy and switchgrass as influenced by nitrogen and sulfur fertilization. Can. J. Plant Sci. **57**: 1151-1157.

Garret, R.H. and A. Nason. 1967. Proc. Natl. Acad. Sci., USA. **58**: 1603-1610.

Gaur, B.L., H.G. Singh and G.A. Cahoon. 1971. Effect of sulphur in the prevention of incipient iron chlorosis in corn. Soil Sci. Plant Analysis **2**: 259-266.

Grant, C.A. 1991. Sulphur requirements of Canola. Sulphur in Agric. **15**: 3-6.

Greenway, H. and H.G. Pitman. 1965. Potassium retranslocation in seedlings of *Hordeum vulgare*. Aust. J. Biol. Sci. **18**: 235-247.

Guerrero, M.G., J.M. Vega and M. Losada. 1981. The assimilatory nitrate reducing system and its regulation. Ann. Rev. Plant Physiol. **32**: 169-204.

Haba, D.L. Purificaceion, E. Aguera and J. Maldonado. 1990. Differential effects of ammonium and tungsten on nitrate and nitrite uptake and reduction by sunflower plants. Plant Sci. (Limerick) **70**: 21-26.

Hall, J.D., R. Barr, A.H. Al-Abbas and F.L. Crane. 1972. The ultrastructure of chloroplasts in mineral deficient maize leaves. Plant Physiol. **50**: 404-409.

Hariram and K.N. Dwivedi. 1992. Effects of sulphur sources on yield and uptake of some major nutrients by chickpea (*Cicer arietinum* L.). J. Indian Soc. Soil Sci. **40**: 388-389.

Harper, J.E. and G.M. Paulsen. 1967. Changes in reduction and assimilation of nitrate during the growth cycle of winter wheat. Crop Sci. 7: 205-209.

Hewitt, E.J. 1966. *In*: sand and water culture methods used in the study of plant nutrition. Commn. Agric. Bureaux, Farnham Royal, England., G.R.

Hill-Cottingham, D.G. and C.P. Lloyd-Jones. 1975. Nitrogen-15 in apple nutrition investigations. J. Sci. Food Agric. **26**:165-173.

Hocking, P.J., P.J. Randall and A. Pinkerton. 1987. Sulphur nutrition of sunflower (*Helianthus anuus* L) as affected by nitrogen supply: Effects on vegetative growth, the development of yield components and seed yield and quality. Field Crop Res. **16**: 157-175.

Holobrada, M. 1977. The influence of S-deficient pretreatment on sulphur uptake and assimilation by intact maize plants. I. Uptake and transport. Agrochimica **21**: 235-242.

Holobrada, M. 1978. The influence of S-deficient pretreatment on sulphur uptake and assimilation by intact maize plants. III. Further study of uptake and incorporation. Agrochimica **22**: 42-47.

Horms, H. and G. Nowak. 1990a. Effects of foliar applied nitrogen and kinetin on nitrogen redistribution during grain growth in wheat: II.

Protein pattern and proteolytic enzyme activity. Angewandte Botanic **64**: 261-269.

Horms, H. and G. Nowak. 1990b. Effects of foliar applied nitrogen and kinetin on nitrogen redistribution during grain growth in wheat. II. Protein pattern and proteolytic enzyme activity. Angewandte Botanic **64**: 261-269.

Horms, H. and G. Nowak. 1990c. Effects of foliar applied nitrogen and kinetin on nitrogen redistribution during grain growth in wheat: III. Mobilization of nitrogen from vegetative plant parts. Angewandte Botanic **64**: 435-444.

Hossain, A. 1978. Proceedings of the Workshop on sulphur nutrition on rice, BRRI, Joydebpur, Bangladesh. pp. 29-38.

Islam, M.S., K.M. Hossain, N.I. Sarker, S. Altamash and J.U. Sarker. 1986. Experimental methodology and responses of principal crops to sulphur fertilization in Bangladesh. *In*: Sulphur in agricultural soils. Proceedings, International symposium, Dhaka, Bangladesh. BARC pp. 184-211.

Ismunadji, M., I. Zulkarnaini and M. Mikake. 1975. Sulphur deficiency in lowland rice in Java. Contr. Centr. Res. Inst. Agric. Bogor **14**: 1-17.

Ivanic, J. 1978. Effect of various sulphur fertilizers on the nutrient uptake and yield of spring barley. Pol'hohspodarstvo **24**: 593-604.

Jackson, M.L. 1967. Phosphorus determination for soils. *In*: Soil Chemical Analysis, pp. 151-156. Prentice-Hall of India (Pvt.) Ltd. New Delhi.

Jaggi, R.C., M.S. Aulakh and G. Dev. 1977. Interaction effect of nitrogen and sulphur on growth and nutrient uptake by maize. J. Nucl. Agric. Biol. **6**: 18-20.

Janzen, H.H. 1986. S-nutrition of rapeseed. Dissertation Abstracts International, B (Science and Engineering) **46**: 2507B.

Janzen, H.H. and J.R. Bettany. 1984. Sulphur nutrition of rapeseed: I. Influence of fertilizer nitrogen and sulphur rates. Soil Sci. Soc. Am. J. **48**: 100-107.

Kanwar, J.S. and M. Modahar. 1983. Fertilizer sulphur and food production-research policy implications for tropical countries. IFDC, Muscles Shoala, Alabama.

Karmoker, J.L., D.T. Clarkson, L.R. Saker, J.M. Rooney and J.V. Parves. 1991. Sulphate deprivation depresses the transport of nitrogen to the xylem and the hydraulic conductivity of barley (*Hordeum vulgare* L.) roots. Planta **185**: 269-278.

Karmoker, J.L. 1981. Ph. D. Thesis, School of Agriculture, La Trobe University, Australia.

Karmoker, J.L. and R.F.M. Van Steveninck. 1978. Stimulation of volume flow and ion flux by abscisic acid in excised root systems of *Phaseolus vulgaris* L. cv. Redland pioneer. Planta **141**: 37-43.

Karmoker, J.L. and R.F.M. Van Steveninck. 1979a. The effect of abscisic acid on the uptake and distribution of ions in intact seedlings of *Phaseolus vulgaris* L. cv. Redland pioneer. Physiol. Plant. **45**: 453-459.

Karmoker, J.L. and R.F.M. Van Steveninck. 1979b. The effect of abscisic acid on sugar in seedlings of *Phaseolus vulgaris* L. cv. Redland pioneer. Planta **146**: 25-30.

Kato, T., H. Yamagata and S. Tsukahara. 1984. Storage forms and reservoirs of nitrogen used for new shoot development on *Satsuma mandarin* trees. Japan Soc. Hortic. Sci. **52**: 393-398.

Klepper, L., D. Flesher and R.H. Hageman. 1971. Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiol. **48**: 580-590.

Knudsen, L. and C.A. Pedersen. 1993. Sulphur fertilization in Danish Agriculture. Sulphur in Agric. **17**: 29-31.

Kumar, V. and M. Singh. 1980. Interaction of sulfur, phosphorus and molybdenum in relation to uptake and utilization of phosphorus by soybean. Soil Science **130**: 26-31.

Kylin, A. 1960. The incorporation of radio sulphur from external sulphate into different sulphur fractions of isolated leaves. Physiol. Plant. **13**: 366-379.

Lancaster, D.L., B.M. Jones, J.H. Oh and J.E. Ruckman. 1971. Effect of sulphur fertilization of forage species on yield, chemical composition and *in vitro* rumen microbial activity of sheep. Agron. J. **63**: 621-623.

Langlean, J.G. and S.R. Troelestra. 1992. Growth, Chemical composition and nitrate reductase activity of Rumex Species in relation to form and level of nitrogen supply. Plant Soil **145**: 215-229.

Leggett, G.E., B.A. Stewart and D.W. Jones. 1966. Sulfur deficiency of sugar beats. Proc. 17th Ann. Pacific North-West Fertilizer Conf. pp. 157-164.

Leidger, R.A. and M. Aldjabri. 1972. Sulphur deficiency under conditions of wet rice cultivation with specific reference to a vertisol near Ngawi, East Java. Newsletter Soil Study Group, Bogor, **1/2**: 21-29.

Ligero, F., C. Lluch and L. Recalde. 1981. Effect of nitrogen and sulphur fertilizer application on growth, leaf area and content of N, P, Ca and Na in the leaf of bean plants (*Phaseolus vulgaris* L.). Anales Edaphologia Agrobiologia **40**: 1279-1280.

Lowry, O.H., N.J. Roserrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. **193**: 265-275.

Martel, Y.A. and J. Zizka. 1977. Yield and quality of alfalfa as influenced by additions of S to P and K fertilizer under greenhouse conditions. Agron. J. **69**: 531-535.

Martinez, V., A. Cerda, M. Caro and F.G. Fernandez. 1983. Development and mineral composition of tomato plants (*L. Esculentus* Mill.) in relation to sulphate concentration in the nutrient medium. Anales de Edafologia Adrobiologia **42**: 1252-1268.

Matocha, J.E. 1971. Influence of sulfur sources and magnesium on forage yields of coastal Bermudagrass (*Cynodon dactylon* (L) Pers.). Agron. J. **63**: 493-496

Mertz, E.T. and H. Matsumoto. 1956. Further studies on the amino acids and proteins of sulfur deficient alfalfa. Arch. Biochem. Biophys. **63**: 50-63.

Mertz, E.T., V.L. Singleton and C.L. Garey. 1952. The effect of sulphur deficieny on the amino acids of alfalfa. Arch. Biochem. Biophys. **38**: 139-145.

Metson, A.J. 1973. Sulphur in forage crops. Sulphur Institutes, Washington D.C. Technical bulletin 20.

Mikkelsen, R.L. and H.F. Wan. 1990. The effect of selenium on sulphur uptake by barley and rice. Plant and Soil **121**: 151-153.

Millard, P., G. S. Sharp and N.M. Scott. 1985. The effect of sulphur deficiency on the uptake and incorporation of nitrogen in ryegrass. J. Agric. Sci. **105**: 501-504.

Morris, R.J. 1986. The importance of sulphur in agriculture-An Overview. *In*: Proceedings of the International Symposium on Sulphur in Agricultural Soil, Dhaka, 1986. BARC, Dhaka, Bangladesh.

Munoz, N., J. Guerri, F. Legaz and E. Primo-Millo. 1993. Seasonal uptake ^{15}nitrogen-nitrate and distribution of absorbed nitrogen in peach trees. Plant and Soil **150**: 263-269.

Murphy, M.D. 1986. Sulphur deficiency in Irish soils and its effect on herbage production. Ph.D. Thesis, Dublin City University, Dublin, Ireland.

Naren, A.P. and T.K. Virupaksha. 1990. Effects of sulphur deficiency on the synthesis of -setarin, a methionine rich protein of Italian millet. Cereal Chemistry. **67**: 136-168.

Nelson, D.R., R.J. Bellville and C.A. Porter. 1984. Role of nitrogen assimilation in seed development of soybean. Plant Physiol. **74**: 128-133.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for determination of glucose. J. Biol. Chem. **153**: 375-380.

Nightingale, G.T., L.G. Schermerhorn and R.M. Addoms. 1932. Effects of sulphur deficiency on metabolism in tomato plant. Plant Physiol. **7**: 565-595.

Nybe, E.V., and P.C.S. Nair. 1987. Nutrient deficiency in black pepper (*Piper nigram.*) calcium, magnesium and sulphur. Agric. Res. J. Kerala **25**: 52-65.

Nyborg, M., C.F. Bentley and P.B. Hoyt. 1974. Effect of sulphur deficiency on seed yield of turnip rape. Sulphur Institute J. **10**: 14-15.

Oaks, A. 1967. Asparagine synthesis in *Zea mays.* Biochem. Biophysics Acta **141**: 436-439.

Oaks, A., W. Wallace and D. Stevens. 1972. Synthesis and turnover of nitrate reductase in corn roots. Plant Physiol. **50**: 649-654.

Oji, Y. and G. Izawa. 1968. Utilization of nitrate nitrogen in higher plants. 7. Functions of NADH; Nitrate oxidoreductase with special reference to its inducibility and enzyme activities varying among leaf positions in the rice plants. J. Sci. Soil Manure, Japan **39**: 380-386.

Onkersingh and J.K. Nandlal. 1987. Effects of sulphur deficiency on leaf area ratio and dry-matter production in sunflower (*Helianthus annus*

L. var. Mordein) related with their nitrate reductase activity in leaves. Indian J. Plant Physiol. **30**: 368-371.

Oputa, C.O. 1971. Amide nitrogen and soluble sugar interrelationships in *Zea mays* L. as influenced by sulfur nutrition. Ph. D. Thesis, University of California, Davis.

Pal, U.R., D.R. Gossett, J.L. Shim and J.E. Leggett. 1976. Molybdenum and sulfur nutrition effects on nitrate reduction in barley and tobacco. Can. J. Bot. **54**: 2014-2022.

Pasricha, N.S. and N.S. Randhawa. 1975. Effects of sulphur fertilization on the nitrogen metabolism in barseem. Indian J. Agric. Sci. **45**: 213-218.

Pasricha, N.S., V.K. Nayyar, N.S. Randhawa and M.K. Sinha. 1977. Influence of sulphur fertilization on suppression of molybdenum by barseem grown on molybdenum toxic soil. Plant and Soil **46**: 245-250.

Passera C. and R. Ghisi. 1982. Sulfulyrase and o-acetyleserine sulfhydrylase in isolated mesophyll protoplasts and bundle sheath strands of sulphur deprived maize leaves. J. Exp. Bot. **33**: 432-438.

Pillai, P.B. and H.G. Singh. 1975. Effect of different sources of sulphur and iron on flag-leaf composition and grain yield of rice on calcareous soils. Indian J. Agric. Sci. **45**: 340-343.

Platou, J. and M.B. Jones. 1982. Sulphur the fourth major nutrient. *In*: sulphur in Agriculture Bulletin, 1982, pp. 1-33..

Portch, S. and M.S. Islam. 1986. Sulphur: A major constraint to high yields in Bangladesh. Sulphur in Agric. **10**: 14-15.

Privalle, L.S., K.N. Lahners, M.A. Mullins and S. Rothstein. 1989. Nitrate effect on nitrate reductase activity and nitrate reductase *m*RNA levels in maize suspension culture. Plant Physiol. **90**: 962-967.

Qi, B.Z. 1989. The effects of sulphur nutrition of some physiological parameters in relation to carbon and nitrogen metabolism in wheat and maize. Acta Agronomica Sinica **15**: 31-35.

Radin, J.W. 1978. A physiological basis for the division of nitrate assimilation between roots and leaves. Plant Sci. Lett. **13**: 21-25.

Radin, J.W., C.R. Sell and W.R. Jordan. 1975. Physiological significance of the *in vivo* assay for nitrate reductase in cotton seedlings. Crop Sci. **15**: 710-713.

Randall, P.J. and C.W. Wrigley. 1986. Effects of sulphur supply on the yield, composition and quality of grain from cereals, oilseeds and legumes. *In*: Pomaranz,Y. (ed.) advances in Cereal Sciences and Technology, vol. VIII, pp. 171-205.

Rao, K.P., D.W. Rains, C.O. Quelset and R.S. Huffaker. 1977. Nitrogen nutrition and grain protein in two spring wheat genotypes differing nitrate reductase activity. Crop Sci. **17**: 283-256.

Rechcigl, J.E. 1989. Importance of sulphur fertilization of forages. The Florida Cattle men, April.

Rehm, G.W. and A.C. Caldwell. 1970. Sulfur uptake by corn as influenced by ammonium and nitrate-N. Soil Sci. Soc. Am. Proc. **34**: 327-329.

Rendig, V.V. and E.A. McComb. 1959. Effects of nutritional stress on plant composition. I. The interaction of added nitrogen with varying sulfur supply. Soil Sci. Soc. Am. Proc. **23**: 377-380.

Rendig, V.V. and E.A. McComb. 1961. Effects of nutritional stress on plant composition. II. Changes in sugar and amide nitrogen content of normal and sulfur deficient alfalfa during growth. Plant and Soil **14**: 176-186.

Rendig, V.V., C. Oputa and A.E. McComb. 1976. Effects of sulfur deficiency on nonprotein nitrogen, soluble sugars and N/S ratios in young corn (*Zea mays* L.) plants. Plant and Soil **44**: 423-437.

Rennenberg, H. and W.M. Kaiser. 1989. Ion composition of tobacco cells cultured under sulphur deficiency. Plant and Cell Physiol. **30**: 325-333.

Richards, I.R. 1990. Sulphur as a crop nutrient in the United Kingdom. Sulphur Agric. **14**: 8-10.

Ruhal, D.S. and K.V. Paliwal. 1987. Sulphur requirement of maize and sesamum in nutrient solution. Indian J. Plant Physiol. **30**: 71-77.

Saccomani, M. and G. Ferrari. 1989. Sulfate influx and efflux in seedlings of maize genotypes of different productivity. Maydica **34**: 171-177.

Saccomani, M., G. Cacco and G. Ferrari. 1984. Effects of nitrogen and/or sulfur deprivation on the uptake and assimilation steps of nitrate and sulfate in maize seedlings. J. Plant Nutr. **7**:1043-1057.

Sairam, R.K., A.R. Till and G.J. Blair. 1995. Effect of sulfur and molybdenum levels on growth, nitrate assimilation and nutrient content of *Phalaris aquatica.* J. Plant Nutr. **18**: 2093-2103.

Schnug, E. and S. Haneklaus. 1994a. Sulphur deficiency in *Brassica napus*. *In*: Biochemistry, Symptomatology, Morphogenesis, Sonderheft 144, Landbauforschung Volkenrods.

Schnug, E. and S. Haneklaus. 1994b. Ecological aspects of plant sulphur supply. *In*: Proceedings of XV International Soil Science Conference, Acapulco.

Schrader, L.E., G.L. Ritenour, G.L. Elrich and R.H. Hageman. 1968. Some characteristics of nitrate reductase from higher plants. Plant Physiol. **43**: 930-940.

Singh, D.P. and J.N. Singh. 1971. Nitrogen and carbohydrate metabolism in Japanese mint as affected by phosphorus deficiency and seasonal variation. Physiol. Plant **24**: 126-129

Singh, H.G. 1970. Wheat response to sulphur in Udaipur. Sulphur Inst. J. Autumn, 1970.

Smith, I.K. 1975. Sulfate transport in cultured tobacco cells. Plant Physiol. **53**: 303-307.

Smith, I.K. 1980. Sulfate transport in cultured tobacco cells. Effect of nitrogen and sulfur nutrition on sulfate permease and o-acetyleserine sulfuhydrylase. Plant Physiol. **66**: 877-883.

Smith, I.K. and A. L. Lung. 1988. Translocation of sulfate in soybean (*Glycine max* L. Merr.). Plant Physiol. **86**: 798-802.

Somogyi, H. 1952. Notes on sugar determination. J. Biol. Chem. **195**: 19-23.

Srivastava, R.K. and S. Rajan. 1970. Effects of calcium and sulfur deficiencies on sugar metabolism in *Linum usitatimum* L.. plant. Acta Agron. J. Hung. **19**: 161-163.

Srivastava, A.K., K. Singh, A.K. Ghosh, R. Danash and Y.R. Saxena. 1989. Phosphorus and sulphur uptake by excised roots of plant cane and ratoon in sugarcane (*Saccharum spp.*). J. Nuclear Agric. and Biol. **17**: 179-181.

Stewart, B.A. and L.K. Porter. 1969. Nitrogen:sulfur relationship in wheat (*Triticum aestivum* L.), Corn (*Zea mays* L.) and Bean (*Phaseolus vulgaris* L.). Agron J. **61**: 267-271.

Stewart, G.R. and T.O. Orebamjo. 1979. Some unusual characteristics of nitrate reduction in *Erythrana senegalensis.* DC. New Phytol. **83**: 311-319.

Syers, J.K., D. Curtin and R.J. Skinner. 1987. Soil and fertilizer sulphur in U.K. agriculture. Proc. of the fertilizer Society, London.

Tabatabai, M.A. 1986. Sulphur in Agriculture. American Society of Agronomy, Madison, WI.

Talukder, K.H. and M.S. Islam. 1982a. Yield responses of maize to different rates of phosphorus and sulphur during the kharif season of Bangladesh. Bangladesh J. Agric. Res. **7**: 108-113.

Talukder, K.H. and M.S. Islam. 1982b. The yield responses of groundnut to sulphur and zinc fertilization in Grey Terrace soils of Joydevpur. Bangladesh J. Agric. Res. **7**: 49-53.

Talukder, K.H., M.S. Islam, N.I. Sarker and O.J. Majumder. 1984. The yield response of chickpea to sulphur and zinc fertilization in calcareous brown floodplain soils of Jessore. Bangladesh J. Soil Sci. **20**: 31-36.

Thibodeau, P.S. and E.J. Jaworski. 1975. Pattern of nitrogen utilization in the soybean. Planta **127**: 133-137.

Thomas, M.D. 1958. Assimilation of sulfur and physiology of essential sulphur compounds. *In*: Encyclopedia of Plant Physiol. W. Ruhland, ed. **9**: 37-63, Springer, Berlin.

Tiwari, R.J 1994. Response of gypsum on morpho-physiochemical properties of cotton cultivars under salt affected vertisols of Madhya Pradesh. Crop Res. 7: 197-200.

Tiwari, R.J., K. Dwivedi and S.K. Verma. 1994. Effect of gypsum application on nutrient content in cotton leaves grown on a sodic vertisol. Crop Research 7: 193-196.

Tso, T.C. and J.E. McMutrey Jr. 1960. Mineral deficiency and organic constituents in tobacco plants. II. Amino acids. Plant Physiol. **35**: 865-870.

Tucker, J.M., D.R. Cordy, L.J. Berry, W.A. Harvey and T.C. Fuller. 1961. Nitrogen poisoning in livestock. Calif. Agric. Exp. Sta. and Ext. Ser. Cir. 506.

Van Steveninck, R.F.M. and M.E. Steveninck. 1972. Effects of inhibitors of protein and nucleic acid synthesis on the development of ion uptake mechanism in beet root slices (*Beta vulgaris*). Physiol. Plant. **27**: 407-411.

Vickery, H.B. and G.W. Pucher. 1939. The metabolism of amides in green plants. III. The mechanism of amide synthesis. J. Biol. Chem. **128**: 703-713.

Walker, K.C. and E.J. Booth. 1992. Sulphur research on oilseed rape in Scotland. Sulphur in Agric. **16**: 15-19.

Wankhade, S.G., B.D. Patil, P. Ratnakar and P.S. Naphade. 1989. DTPA extractable Zn, Cu, Fe and Mn and their uptake by wheat as influenced by varying levels of elemental sulphur. PKV Research J. **13**: 96-99.

Worner, R.W., R.H. Hageman, J.W. Dudley and R.J. Lambert. 1969. Influence of nitrate activity in *Zea mays* (L.). Proc. Natl. Acad. Sci. US **62:** 785-792.

Willenbrink, J. 1967. Der transport der phosphor-und schewefelhaltigen verbindungen. *In*: Handbuch der pflanzenphysiologie,, Bd.XIII, Ruhland. W., ed. pp. 178-199, Berlin, Heidelberg, New York, Springer.

Wright M.J. and K.I. Davison. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. Advances in Agron. **16**: 197-217.

Zhao, F.J. , J. K. Syers, E.J. Evans and P.E. Bilsborrow. 1991. Sulphur and oilseed rape production in the United kingdom . Sulphur in Agric. **15**: 13-16.

Zieserl, J.F. and R.H. Hageman. 1962. Effect of genetic composition on nitrate reductase activity in maize. Crop Sci. 2: 512-515.