STUDIES ON UPTAKE AND ASSIMILATION OF NITRATE NITROGEN IN COMMON BUCKWHEAT FAGOPYRUM ESCULENTUM MOENCH

ABSTRACT

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Nitrate uptake and utilization is presently considered as a major and early point of control of development of plant. Nevertheless, despite the extreme importance of nitrate in most agricultural ecosystems, a number of serious deficiencies remain in our understanding of the physiology and biochemistry of its uptake and assimilation. Therefore, an understanding of physiology and biochemistry of its uptake and assimilation is necessary to develop protocals for fertilizer regimes for improving the quantity as well as the quality of the harvest.

Even though quite a good amount of work has been done on the uptake and utilization of nitrate nitrogen in wheat, soyabeen, barley and maize, data on the utilization of nitrate nitrogen in common buckwheat (*Fagopyrum esculentum* Moench), a psuedocereal of extreme economic importance because of its short growth span, high nutritive value of its grains and its capacity to grow on poor soils, is scaty. A survey of the literature reveals that certain characteristics possessed by this crop give it an advantage over the conventional crops. The importance of the plant lies in the protein quality of its grains, short growth span and hardiness of the plant, Besidesthe foliage is used as a green vegetable and in an important commercial source of the glucoside "Rutin" which is used as a medicine. However, because of some problems associated with its growth like indeterminate growth habit, the crop has not being cultivated extensively and comes under the category of under utilized crops aa classified by International Bureau of Plant Genetic Resources (IBPGR). Although some studies have been made on the requirement of phosphate fertilization in buckwheat, not many reports are available on the nitrogen fertilization requirements in crop. The present study was therefore undertaken to:

(a) assess the various accessions of common buckwheat for the growth and yield attributes,

(b) characterize the uptake of nitrate in intact seedlings as well as excised roots of buckwheat seedlings under hydroponic culture, as a function of time, NO_3^- concentration, pH and accompanying ions,

(c) determine the relationships between photosynthetic activity and nitrate utilization in the plant during various phases of growth in the plant, so as to determine the nitrate nitrogen requirement of the crop at various stages of growth.

In order to assess the growth and yield attributes, the seeds of seven accessions of buckwheat which were procured from the NBPGR regional station at Shillong, were scanned by electon microscope for their seed coat characteristics. Based on the scanning electron microscopy of the seed coat, the seven accessions have been grouped into three categories. The data on the size and shape of the seeds of seven accessions further illustrated that the seeds of seven accessions were not similar to each other at least morphologically. However, the seven accessions did not differ from each other markedly in the chemical composition of their grains and growth behaviour. The conclusion has been corroborated by growth indices such as Leaf Area, LAR, NAR and RGR, calculated separately in each of the seven accessions.

Further an analysis of the polygonal diagram representing variables such as dry weight of stem, shoot, leaf, root and leaf area for the seven accessions at various stages of growth revealed that the seven accessions did not differ from each other, at least in their growth attribute.

The plants accumulated maximum dry matter in about three weeks after planting. However, the rate of dry matter production was maximum between 7 and 19 days after planting, in each of the seven accessions. A significantly positive relationship was observed between leaf area and dry matter accumulation in the crop.

The crop attained maturity in about six weeks time and completed its life cycle in about 9 to 10 weeks. However, because of the intermediate growth habit, the flowering extended from about 4 to 7 weeks after planting. However, among the seven accessions of buckwheat BDS-1354 distinguished itself by possessing determinate growth habit and synchroronization of seed maturity. Seedlings of the plant showed a linear and steady nitrate uptake during the initial 60 minutes upon exposure to the Hoagland's nutrient medium containing 5 mM nitrate as KNO_3^-. Significantly, there was no lag phase in the uptake of nitrate by the seedlings. After 60 minutes the uptake of nitrate gradually slowed down until it attained a plateau at t_{180} minutes. During the corresponding period the concentration of nitrate in the ambient nutrient

medium showed a gradual decrease with progressing time. When expressed as μmol nitrate taken up mg dry weight root^{-} min.$^{-1}$, the seedlings showed a maximum uptake rate during the initial 30 minutes of incubation in the nutrient medium. The rate of uptake showed a pregressive decrease with progressing time until no signficant uptake was observed after the 3rd hour of incubation. Decrease in the concentration of nitrate in the ambient nutrient medium had no apparent effect on the rates of nitrate uptake by the seedlings as a function of time. Seedlings in test solutions whose concentration of nitrate was kept constant, also showed a pattern of uptake similar to that shown by seedlings in test solutions in which the concentration of nitrate ions was allowed to deplete over the period. Thus, from an analysis of the cumulative uptake of nitrate by buchwheat seedlings and changes in the rate of uptake with progressing time, as determined in the present investigation, it can be assumed that the uptake of nitrate across the root plasma membrane in common buckwheat is mediated through a low capacity basic system. It seems reasonable to postulate that the carrier for nitrate ions in the seedlings is already present in the system because of an endogenous supply of nitrate. The observed decrease in the rate of uptake with time could be ascribed to a refilling of the available storage components in the seedlings and not to a decreasing nitrate concentration in the ambient nutrient medium, because the rate of uptake in the seedlings, which

were kept in test solution in which the level of nitrate was kept constant all through, showed a trend similar to that observed for seedlings which were kept in test solution in which no replenishment for the loss of nitrate as a result of the uptake were made.

When the concentration of nitrate in the nutrient medium was varied from 0.05 to 5.0 mM, the rate of nitrate absorption by buckwheat seedlings was a function of external nitrate concentration according to Michaelis-Menten Kinetics. The Michaelis-Menten constant (Km) and maximum velocity (V_{max}) for nitrate absorption by buckwheat seedlings were 200 µmol and 0.276 µmol mg dry weight $root^{-1}min.^{-1}$ respectively. In the presence of ammonium and chlorate ions, the uptake of nitrate by the seedlings was markedly suppressed; the magnitude of suppression increasing with the increasing concentration of either ammonium or chlorate ions. A Lineweaver-Burk plot for the uptake of nitrate ions as a function of substrate concentration, at various levels of either ammonium or chlorate clearly revealed that while the inhibition due to ammonium was non-competitive in nature, that due to the presence of chlorate ions was of competitive in nature. While the Km for nitrate uptake in the presence of ammonium remained 200 µmol, the uptake process in presence of 0.005 and 0.05 mM ammonium had a V_{max} of 0.083 and 0.064 µmol mg dry weight $root^{-1}min.^{-1}$. In the presence of 0.005 and 0.05 mM of chlorate, the Km for the uptake of nitrate was 307 and

500 μmol respectively. Chlorate ions had no effect on the V_{max} of the process. Our results indicate that the inhibition of the nitraate uptake by ammonium ions is not simply a case of ammonium providing a counter-ion for nitrate, the inhibition appears to be due the efect of ammonium ions on the net rate of nitrate influx into the seedlings. The inhibitory role of chlorate ions on nitrate uptake may be because the ion acts as an analogue for nitrate in the process of nitrate uptake by plants.

In the present investigation presence of nitrate ions in the nutrient solution had a stimulatory influence on the growth of the plants. The highest dry matter accumulation was observed in plants irrigated with Hoagland nutrient medium containing 20mM KNO_3. Similarly plants irrigated with Hoagland's nutrient medium containing 20 mM nitrate had the highest value for RGR, LAR and NAR. The presence of nitrate ions in the nutrient medium had a stimulatory effect on the net assimmilation rate of the plants. Thus plants irrigated with Hoagland's nutrient medium containing 20 mM nitrate showed a more than two-fold increase in NAR than those irrigated with nitrate free Hoagland's nutrient medium. Irrespective of the treatment, the highest value of RGR was recorded on 7th day after planting, after which it showed a consistent decrease with progressing time till it registered negative values on 67th day. There were a significant

difference in the number of grains produced per plant between those irigated with Hoagland's nutrient medium containing 5mM nitrate and those did not receive any nitrate. However, a marginal decline in the grain yield was observed in those plants supplied with Hoagland's nutrient medium containing 20 and 50mM nitrate ions. From the result it is clear that the increased concentration of NO_3^- in the nutrient medium beyond 5 mM did not play any positive role in increasing the grain yield for the crop.

The plants supplied with Hoagland nutrient solution containing 5, 20 and 50 mM nitrate ions, showed a nearly two-fold increase in leaf area as well as leaf dry matter accumulation than that of nitrate starved control plants. In contrast the total leaf area ratio was nearly independent of nitrate supply. However, the maximum leaf area ratio was observed in plants that were irrigated with Hoagland's nutrient medium supplemented with 5 mM nitrate. In *Fagopyrum esculentum*, the effect of nitrogen aplication on LAR could be assumed to be the major cause of the effects of the treatments on NAR and RGR. Further, the increased NAR and RGR with increase in the supply of external nitrate, augmented only vegetative growth and not the grain filling.

The results of study on partitioning of various nitrogenous components within the plants revealed that plant

supplied with Hoagland nutrient solution containing 5mM KNO_3^- had the maximum level of various nitrogenous constituents. Increasing the nitrate concentration beyond 5mM failed to bring about remarkable variations. When the whole plant was considered as a four interconnected units, namely, root, stem, petiole and leaf, the leaves were found to be externally self supporting in terms of nitrogen balance within the plant. The leaves had the highest level of NR activity in the plant as compared to root, stem and petiole. Probably, the petiole acquired reduced nitrogen for their growth from other tissues of the plant. In *Fagopyrum esculentum* leaves act as storage organs. This speculation is a reflection of significantly higher amounts of various N-components and NR activity in the levaes, specially in younger leaves. The expected highest rate of nitrate reduction were observed in laminae followed by root and then at a generally much lower level, the petiole and stem.

Fagopyrentum esculentum showed a significantly positive relationship linking nitrogen content, growth rate and plant mass. The percentage nitrogen in the plant declined as the plant mass increased. There was a linear relationship between percent N in the whole plant and $k_x(F)$, the growth rate coefficient. The linear relationship between percent N in the leaf and that in the whole plant showed that there is an interdependency between leaf and whole plant, in

respect of nitrogen, to support the growth and dry matter accumulation in the plant.

Our results indicate an optimum requirement of 5 mM nitrate in the irrigating solution for obtaining the maximum yields. Further, under conditions of sub-optimal nitrate supply, the dry matter production expressed as net assimmilation rate in the crop during the exponential phase of growth had a direct relationship with the nitrogen status of the plant with the equation

$$NAR = 0.308 + 0.0012\ x$$

where " x " is the nitrogen content of the plant expressed as mg/100 mg dry weight.

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CERTIFICATE

This is to certify that the thesis entitled " Studies on Uptake and Assimilation of Nitrate Nitrogen in common Buckwheat (*Fagopyrum esculentum* Moench) " submitted by Mr. S. Paulsamy for the degree of Doctor of Philosophy in Botany of the North-Eastern Hill University, Shillong embodies the record of the investigation carried out by him under my supervision in the Dept. of Botany, North Eastern Hill University, Shillong.

This work has not been submitted for this or any other degree of any other University.

(N. K. Chrungoo)
Supervisor

Dated: September 12, 1995
Shillong.

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CONTENTS

CHAPTER I

Introduction

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Among the physiologically well defined adaptations of plants to alterations in nutrient availability from the environment are changes in root nutrient acquisition and assimilation systems. Because the availability of nitrogenous nutrients is often rate limiting for plant growth, the effect of exogenously supplied nitrogenous compounds on plant growth has been of interest for many years (Clarkson, 1988). Nitrate is the major source of inorganic nitrogen utilized by plants and productivity is to a large extent determined by nitrogen nutrition. Because of the scarcity of nitrogen in a form ready for assimilation, the N cycle is of key importance to the structure and function of most agricultural ecosystems. plants, NO_3^- and NH_4^+ are the principal source of nitrogen.

Because of its importance in determining crop productivity, the relationship between uptake and utilization of NO_3^- ions and growth of the plants has been a subject of extensive investigations.

Among the best characterized responses of higher plant roots to exogenous nitrate are the induction of an enhanced level of nitrate uptake system and activity of enzymes responsible for the reduction of nitrate to ammonia (Neyra and Hageman, 1975). Ammonium ion enters the amino acid pool primarily by way of the action of the enzyme glutamine synthetase. The entire pathway can be written as:

$$NO_3^- \xrightarrow{\text{uptake}} NO_3^- \xrightarrow{\text{NR}} NO_2^- \xrightarrow{\text{NiR}} NH_4^+ \xrightarrow[+\text{Glu}]{\text{GS}} \text{Gln}$$

The absorption of nitrate by plant roots occurs with rate comparable to those for K^+, Rb^+, Cl^- and $H_2PO_4^-$ (Jackson *et al.*, 1973). The uptake efficiency of roots, however, plays a major role in regulating the ammount of nitrate supplied to the plant when soil nitrate is not a limiting factor. A great deal of progress has been made in defining aspects of the regulation of nitrate assimilation and an understanding is presently being reached of atleast the basic underlying mechanisms of some of the processes involved in the control of enzyme synthesis and activity. Other outstanding aspects of control where nitrogen and carbon metabolism are integrated and interact with each other

to balance the productivity of the plant, still remain unanswered. Nitrate uptake and utilization is presently considered as a major and early point of control of development of organisms. Nevertheless, despite the extreme importance of nitrate in most agricultural ecosystems, a number of serious deficiencies remain in our understanding of how this ion is absorbed, partitioned and assimilated by the plants. Therefore, an understanding of the physiology and biochemistry of its uptake and assimilation is necessary to develop protocols for fertilizer regimes for improving the quantity as well as the quality of the harvest.

Even though quite a good ammount of work has been done on the uptake and utilization of nitrate nitrogen in Wheat, Soyabean, Barley, Maize, no such work has been carried out on common buckwheat (*Fagopyrum esculentum* Moench) a pseudo cereal of extreme economic importance because of its short growth span, high nutritive value of its grains and its capacity to grow on poor soils. A survey of the literature reveals that certain characteristics possessed by this crop give it an advantage over the conventional crops. The importance of the plant lies in the protein quality of its grains, short growth span and hardiness of the plants; besides the foliage is used as a green vegetable and is an important commercial source of the glucoside Rutin which is used as a medicine. However, because of some problems associated with its growth like indeterminate growth habit, the crop has not been cultivated extensively and comes under

the category of underutilized crops as classified by National Bureau of Plant Genetic Resource (NBPGR). Although some studies have been made on the requirement of phosphate fertilization in buckwheat, there are no reports on nitrogen fertilization requirements in common buckwheat. The present study has therefore been undertaken to determine the kinetics of nitrate uptake in the plant and to work out the relationship between nitrate supply and growth so as to develop a nitrogen fertilizer programme for the crop for improved protein levels and grain yield.

QUALITIES AND USES OF COMMON BUCKWHEAT

1. Buckwheat proteins have better amino acid composition with high level of lysine which is generally deficient in proteins in most of cereals and millets. The nutritive value of proteins is comparable to that of Casein. Therefore the grains, unlike other cereals, can serve as a source of good quality protein even without supplementing with pulses or lysine.

2. Common buckwheat is a rich source of honey. One acre of land of common buckwheat can produce about 60 kg of honey

3. Buckwheat is a rich source of rutin, a glucoside. The leaves and flowers are richer in rutin which varies from 1.0 to 6.0 percent. The stems have little while seeds have no rutin.

4. The grain is used as an article of food in different countries. In the hills of India, Bhutan and Nepal, the flour is the staple food.

5. The plant is also used as a food for livestock

ESSENTIAL AMINO ACIDS OF BUCKWHEAT GRAINS AS COMPARED TO OTHER CEREALS

Food Grain	Lysine	Methionine	Tryptophan	Leucine
Buckwheat	5.9	3.7	1.4	5.8
Amaranth	5.0	4.4	1.4	4.7
Wheat	2.6	3.5	1.2	6.3
Rice	3.8	3.0	1.0	8.2
Maize	1.9	3.2	0.6	13.0
FAO/WHO Recomendation	5.5	3.5	1.0	7.0

*

Source: Proceedings of 2nd Amarant conference, 1979 (USA)

(As percentage of protein)

CHAPTER II

Review of Literature

The assimilation of inorganic nitrogen into organic compounds is, next to CO_2 assimilation in photosynthesis, the most important metabolic requirement of plants for growth and development. The principal source of inorganic nitrogen in higher plants is NO_3^-. Depending on the plant species and the concentration of nitrate in the ambient medium a portion of the nitrate is taken up by the plants where it is reduced. The uptake of nitrate is, therefore, the first step in the process of nitrate assimilation by plants. Nitrate uptake determines if and how much nitrate can be utilized by the plants. The reduction of nitrate to nitrite is usually the rate limiting step in the conversion of NO_3^- to organic nitrogen (Beevers and Hegeman,1969). Because the

availability of nitrogenous nutrients is often the rate limiting factor for plant growth, the effects of environmental nitrate on plant growth have been of interest for many years. Among the best characterized responses of higher plant roots to exogenous NO_3^- are the induction of an enhanced level of NO_3^- uptake system and the activity of enzymes responsible for the reduction of nitrate to ammonia. When availability of soil nitrate is not a limiting factor, the uptake efficiency of the roots play a major role in regulating the amount of nitrate supplied to the plant. Of the numerous reports available on the use of nitrogen by plants, only a few deal with the kinetic parameters that describe the mechanism of nitrate absorption by plants. The concentration dependent NO_3^- uptake in higher plants is currently believed to occur via one of the four ways: (a) two different uptake systems - one mediating uptake at lower concentrations and the other at higher concentrations (Doddema and Telkamp, 1979); (b) a single uptake system (Lycklama, 1963). (c) a single uptake system with distinct concentration dependent phases (Breteler and Nissen, 1982) and (d) a single saturable carrier-mediated uptake system plus a simple diffusion component (Ibarlucea *et al.*, 1981). Hole *et al.*, (1990) and Siddiqui *et al.*, (1990) have suggested the involvement of at least two mechanisms for uptake of nitrate ions by plant roots. At low external nitrate concentration ($[NO_3^-]_o$), net uptake of nitrate and nitrate influx are saturable and exhibit high affinities for nitrate; Km values for this high affinity transport system (HATS) fall in the range of 10-100 $mmol^{m-3}$. Based on a consi-

deration of normal cytoplasmic and external nitrate concentration, the measured negative electrical potential gradient across the plasma membrane (-70 to -250 mV) and the action of metabolic inhibitors it has been assumed that the induced high rate of nitrate uptake by plants is an active process (Clarkson, 1988; Glass *et al.*, (1990). Another characteristic feature of HATS for nitrate is its extremely low constitutive level of expression in plants deprived of nitrate (Siddique *et al.*, 1990). At higher concentration of nitrate, a second transport system has been reported to operate in the absorption of nitrate in barley (Rao and Rains, 1976), Corn (Pace and McClure, 1986), Tobacco (Guy *et al.*, 1988). This type of transport has been designated as low affinity transport system (LATS). Siddique *et al.*, (1990) and Glass *et al.*, (1990) probed the metabolic dependence of this low affinity transport system by Q_{10} determinations and metabolic inhibitors using $^{13}NO_3^-$. Their observations have lead them to conclude that LATS is a passive transport system with constitutive expression. Yet apart from noting the linear response to nitrate there has been virtually no characterization of this system. Yet another type of uptake system, referred to as BASIC system has been described in seedlings of sugarbeet (Mack and Tischner, 1990). This system by which seedlings, grown from seeds which germinated without an external N-supply, would absorb nitrate at low rates immediately upon exposure differs from the constitutive one because it was induced by the endogenous seed nitrate during germination. Paulsamy and Chrungoo (1994) have also reported

the operation of a similar type of system in seedlings of common buckwheat. Experiments with cereals have revealed that the rate of nitrate reduction is dependent on uptake rates (Ashley *et al.*, 1975). Jackson *et al.*, (1973) reported that roots of corn seedlings grown in an ammonium supplemented medium showed a biphasic pattern of nitrate uptake when transferred to a medium containing nitrate. Their results on experiments using RNA and protein synthesis inhibitors have lead them to suggest that the accelerated rate of nitrate uptake was dependent upon a continuous protein synthesis, implicating the involvement of NO_3^- transporter protein in the process. Apparent substrate inducibility of a root nitrate transporter in many higher plants has been known for over a decade. However, the reported induction periods required to achieve a steady state uptake rate differ; values ranging between 1-2 hours for corn roots (Neyra and Hageman, 1975), 3-4 hours for barley seedlings (Rao and Rains, 1976) and 6 hours for wheat seedlings have been reported (Jackson *et al.*, 1972). The lag period has been interpretted as the time required for the induction of the nitrate-specific transport system by nitrate.

The apparent inducible characteristics of the nitrate transport system in higher plants initiated a search for membrane proteins, which might be associated with the transport mechanism (McClure *et al.*, 1987). Clarkson, (1988) and Larsson and Ingemarsson (1989) have reported that uptake

of nitrate could be blocked with inhibitors of RNA and protein synthesis. In addition, certain amino acid modifying reagents, particularly phenylglyoxal, have been reported to inhibit nitrate uptake in induced systems (Ni and Beevers, 1990). Several newly synthesized plasmalemma and tonoplast proteins from 30 to 150 kD become labelled when NO_3^- starved maize roots are supplied with nitrate in the presence of ^{35}S-methionine (Dhugga *et al.*, 1988), indicating that nitrate uptake by roots is mediated by a plasma membrane localized protein system. Although no plasmalemma NO_3^- transport protein has been definitely identified from higher plants, genes encoding for NO_3^- transporter protein have been identified and cloned in prokaryotes and lower eukaryotes (Omata *et al.*, 1989; Scazzocchio and Arst, 1989; Unkless *et al.*, 1991). Jackson *et al.*, (1986) have observed that higher plants also have systems that translocate NO_3^- within and between cells. Although the effect of external nitrate on intra and inter - cellular translocation has not been defined, Jackson *et al.*, (986) have observed that these activities would also require a transporter. While Jackson *et al.*, (1986) have reported that the kinetic patterns for enhanced nitrate uptake into root cells and its translocation into xylem were similar. Redinburgh and Campbell (1991) have presented evidence to show that transport and translocation processes for NO_3^- are distinct. On the other hand ammonium uptake by plant roots during early exposure to NH_4^+ has been characterized as an initial brief phase of rapid uptake followed by a slower linear rate (Minoti *et al.*, 1969). Information on the

substrate inducibility of the NH_4^+ transporter has not been previously reported. The significance of NH_4^+ in the regulation of NO_3^- uptake and metabolism has been widely recognised and reported (Clarkson and Warner, 1979; Lewis *et al.*, 1987; Morgan and Jackson, 1988). However, most of the reports reveal that the interactions of ammonium and nitrate in higher plants depend not only on the composition of nutrient solution (Marcus-Wyner, 1983) but also on the genotypic and phenotypic responses of the plants to ammonium and/or nitrate in the soil (Bloom and Finazzo, 1986; Smart and Bloom, 1988).

After uptake the second step in the process of NO_3^- utilization by plants is the reduction of nitrate to nitrite by the enzyme, nitrate reductase (NR EC-1.6.6.1). Oaks *et al.*, (1972) and Jackson *et al.*, (1973) have concluded that continuous nitrate uptake was essential to maintain the activity of nitrate reductase enzyme in excised corn root. In an "Induced System" the activity of nitrate reductase in higher plants has been reported to be regulated by enzyme synthesis and/or degradation (Zilke and Filner, 1971; Somers *et al.*, 1983, Remmler and Campbell, 1986), rather than the activation and inactivation mechanisms as reported for algae. With the cloning of NR (Cheng *et al.*, 1986; Crawford, 1986) it has been possible to demonstrate that the substrate mediated induction of enzyme occurrs at the level of transcription. Beevers and Hegeman (1983) have demonstrated that nitrate reductase (NR) and nitrite reductase (NiR) depend on

the products of photosynthesis and photosynthetic electron transport for the supply of reducing power.

Although there is a strong correlation between increased rate of NO_3^- uptake and NR activity, the induction of nitrate uptake does not depend on functional NR (Jackson *et al.*, 1986; Larsson and Ingemarsson, 1989; Warner and Hussaker, 1989). Inactivating factors of NR, which may regulate level of NR activity in tissues have been found in extracts from a number of plant sources (Wallace and Oaks, 1986). Solomonson *et al.*,(1984) presented evidence describing the molecular basis of the action of corn root inactivating protein. They showed that, Cell Inactivating Protein (CIP) acted on Chlorella NR by cleavage of a 30 kD fragment from each of the NR sub unit. In addition, certain aminoacid modifying reagents, particularly phenylglyoxal, inhibit nitrate uptake in induced systems (Dhugga *et al.*, 1988; Ni and Beevers, 1990). Kumar and Abrol (1990) have shown that L-methionine sulphoximine, a potent inhibitor of GS, decreased NR activity by 50 percent at the end of 12 hours treatment while NiR was not affected. They inferred that the enzyme of nitrogen metabolism, except GS, were more or less resistent to MSO. Several newly synthesized plasmalemma and tonoplast proteins from 30 to 150 kD become labeled when NO_3^- starved maize roots are supplied with NO_3^- in the presence of ^{35}S-Methionine (Dhugga *et al.*, 1988; McClure *et al.*, 1987). These results suggest that NO_3^- uptake by roots is mediated by plasma membrane protein system.

Microscopic investigations of NR have presented a confusing picture of NR localization. In a histochemical investigation of etiolated barley leaves, Ekes (1981) demostrated ferricyanide reduction, a partial activity of NR, in the plastid envelope and suggested NR was present in this compartment. Vaughn and Duke (1981) have obtained both histochemical and immunochemical evidence to support a cytoplasmic localization for NR in soyabean cotyledons. Using immunocytochemical techniques Roldan *et al.*, (1987) have demonstrated the localization of NR in both cytoplasm as well as chloroplast of spinach leaves. However, a recent report of immunogold localization of NR in spinach leaves showed NR exclusively associated with the chloroplast (Kamachi *et al.*, 1987).

Since both NR and NO_3^- transport are simultaneously induced by NO_3^-, inhibited by protein and RNA synthesis inhibitors and increased in activity by supplying glucose to root (Butz and Jackson, 1977) a possible relationship between NO_3^- transport and NO_3^- reduction in the plasma membrane could be expected. Neyra *et al.*, (1975) favour the concept of "co-ordinated induction" of both NO_3^- transport system and nitrate reductase. It has further been suggested that the membrane associated nitrate reductase protein could function as carrier for NO_3^- transport. Although no plasmalemma NO_3^- transport protein has been definitely identified from higher plants, genes encoding for NO_3^- transporter protein have been identified and cloned in prokaryotes and lower eukaryotes

(Omata *et al.*, 1989; Scazzocchio and Arts, 1989; Unkless *et al.*, 1991).

Higher plants also have systems that translocates NO_3^- within and between cells (Jackson *et al.*, 1986). However, due to dependence of these processes on the uptake of external NO_3^-, it is difficult to separate the properties of translocation from transport. NO_3^- may be translocated intra cellularly to the vacuole, where it may get accumulated and be exchanged for cytoplasmic NO_3^- (Granstedt and Haffaker, 1982; Jackson *et al.*, 1986). This is particularly true in the leaf, where vacuole NO_3^- probably serves as a NO_3^- reservoir (Granstedt and Huffaker, 1982; Clarkson, 1988). Although the effect of environmental NO_3^- on intracellular translocation has not been defined, those activities would require a tonoplast NO_3^- translocator, which might be different from plasma membrane NO_3^- transporter. While the kinetic patterns for enhanced NO_3^- uptake into root cells and its translocation into the xylem are similar (Jackson *et al.*, 1986), there is evidence that indicates that transport and translocation process for NO_3^- are distinct (Redinbaugh and Campbell, 1991).

Belvins *et al.*, (1974) have studied the effects of cations on uptake of nitrate nitrogen in wheat seedlings. In some experiments uptake of nitrate supplied either in the form of KNO_3^- or $NaNO_3$, was nearly the same (Minotti *et al.*, 1969). In the presence of $CaSO_4$, nitrate uptake was much greater with K^+ than with Ca^{++} (Belvins *et al.*, 1978).

Minotti *et al.*, (1968) have shown that nitrate uptake and translocation were impaired in the absence of either K^{+} or Ca^{++}. Ammonium and nitrate ions interact in a characteristic way during their absorption by plants. In almost every case external ammonium has been found to strongly suppress net uptake of nitrate (Jackson, 1978; Haynes and Goh, 1978). Whether external ammonium affects nitrate influx in short term experiments has been a subject of much controversy. Based on the established interactions between nitrate and chlorate during their absorption by plants and the use of ^{36}Cl-Chlorate, Deane-Drummond and Glass (1983a) have observed that accumulation of radioactivity was unaffected by the composition of the ambient medium. They concluded that alterations in the rate of nitrate efflux, rather than influx, regulate net nitrate uptake. Base on the use of ^{13}N-nitrate Glass *et al.*, (1985) observed more than 40 percent inhibition of nitrate influx in barley and pea when 0.3-0.5 $mmol^{-3}$ ammonium was added to the ambient nutrient medium. Lee and Drew (1989) have reported similar results in their investigations in barley. According to these workers, inhibition of nitrate influx is in proportion to the log value of the concentration of ammonium ions in the ambient medium. This relationship has been found to extend over atleast three to four orders of magnitude. Glass *et al.*, (1985) have, however, suggested that ^{36}C-Chloride, a breakdown product of ^{36}C-Chlorate could introduce significant errors when labelled Chlorate is used as a tracer. Ullrich *et al.*, (1984) have attributed the decline in nitrate uptake in

Lemna, when ammonia was added to the ambient nutrient medium, to membrane depolarization.

Despite the extreme importance of NO_3^- in most agricultural ecosystem, a number of serious deficiencies remain concerning how this ion is absorbed, partitioned and assimilated, within plants. Central to such understanding is the development of methods for monitoring accurately the relative extents to which below and above ground parts of a plant contribute to nitrate reduction and the impact of such activities on the nutritional interdependence of plant parts for reduced and unreduced forms of nitrogen. The relationship between photosynthesis and nitrogen utilization in plants has been a subject of extensive investigation because of the importance of photosynthesis in plant productivity and the status of nitrogen as a limiting essential element. Dejong and Doyle (1985) and Olesinki *et al*., (1989) have suggested that nitrogen can affect photosynthesis by altering the concentration of photosynthetic pigments or the activity of enzymes involved in carbon fixation. Sinclair and Horie (1989) have, however, observed that the main effect of nitrogen nutrition on photosynthesis is due to changes in total leaf area and hence light absorption. Dale (1972) and Metivier and Dale (1977) have shown that nitrogen affects leaf extension rate, leaf length of the first leaf of a range of cultivars of *Hordeum vulgare* L. In general, application of nitrate at a dose equivalent to 22 kg ha^{-1} has been shown to result in a 20-30 percent increase in final length and area

of the first leaf. In *Triticum aestivum* L., Kemp and Blacklow (1982) found that addition of nitrogen as nitrate under field conditions lead to an almost doubling of the extension rate and a 50 per cent increase in the area of leaf 4. Similar responses to nitrogen nutrition have been reported in *Avena sativa* L. under controlled environmental as well as field conditions (Andrews *et al.*, 1989a; Dickson *et al.*, 1990). Radin (1983) has shown that reduction in leaf extension rate due to decreased availability of NO_3^- were small in case of maize and sorghum. On the basis of these observation he concluded that low levels of nitrogen nutrition inhibit leaf area growth more strongly in dicotyledonous species than in cereals. It was proposed that for dicotyledonous species, low nitogen nutrition levels resulted in reduced hydraulic conductivity. Further the transpiration generated water deficit in expanding leaves resulted in a reduced rate of cell expansion. In cereals, transpiration occurs from the exposed lamina but cell expansion occurs at the base of the leaf blade. Radin (1983) argued that this spatial separation of transpiration and cell expansion allowed turgor pressure to be maintained in expanding cells of nitrogen nutrition stressed plants despite water deficits in the leaf blade. On the basis of their observations Andrews *et al.*, (1991) have concluded that for temperate cereals, in general, increased external nitrate concentration resulted in a decrease in the duration of growth but increased maximum and mean growth rates and length of leaves.

Recording of plant growth as a tool to study the nitrogen requirements and to determine the optimum nitrogen fertilizer requirements of plants at various stages of growth, therefore, assumes much significance. The importance of growth analysis as a potential device to give an insight into the physiological basis of yield is well known (Watson, 1955). Several studies have related plant growth either as biomass or leaf area, to different levels of applied nitrogen (Nata, 1975), as most of the nitrogen in leaves is used for the synthesis of components of the photosynthetic apparatus (Epstein, 1972). Numerous investigations have related photosynthetic rate to the levels of various nitrogenous components in the whole plant or to leaf nitrogen concentration (Brown & Wilson, 1983; Novoa & Loomis, 1981). Differences in nitrogen nutrition cause physiological and morphological changes (Marschner, 1983). In addition, the demand for photosynthates for growth has also been reported to affect photosynthetic rates (Novoa & Loomis, 1981). The determination of the growth analysis coefficients may therefore show the effects of nitrogen nutrition on shoot growth (Hunt, 1978). The coefficients include RGR (the change in mass per unit mass per day), RLGR (the change in area per unit area per day), ULR (the change in mass per unit area per day, also called the net assimilation rate) and LAR (the ratio of leaf area to shoot mass). The ULR is the average gain in mass over 1 day by net photosynthesis and should be comparable to the instantaneous net photosynthetic rate (Osman, *et al.*, 1977). The RLGR and leaf area are probably

the characteristics most affected by nitrogen deficiency (Novoa & Loomis, 1981).

Nitrogen is often regarded as limiting to biomass production in agricultural ecosystem. Principles about the role of nitrogen have been incorporated into models that are being used to improve the efficiency of use of fertilizer (Aslyng and Hausen, 1985; Neetson, *et al.*, 1987) and organic manures (Bhat, *et al.*, 1980) and to minimize waste and environmental pollution. Fundamental to these models is knowledge about the dependence of plant growth on %N in the plants. Even when there is an ample supply of nitrogen and other nutrients, the concentration of nitrogen in plants declines as they grow. Numerous models (Greenwood and Barnes, 1978; Caloin and Yu, 1984; Agren, 1985a,b; Charles Edwards *et al.*, 1987; Hardwick, 1987) have been advanced to describe the phenomenon. Evidence has been obtained that the decline in the critical percent N in the plant (the minimum % N in the plant needed for maximum growth rate) is related to plant mass per unit area in much the same way for a variety of C_3 arable and herbage crops (Greenwood, 1982; Lemaire and Salette, 1984; Greenwood, *et al.*, 1986).

Leaf photosynthetic capacity of individual leaves from many species have been found to be highly correlated with leaf-N content (Field and Mooney, 1986; Hirose and Werger, 1987; Koch *et al.*, 1988). In fact, when the maximum leaf photosynthetic rate measured under standard conditions,

is plotted against % N in leaf dry matter for leaves of wide range of different C_3 wild species grown in different habitats, all the points fall closely about the same straight line (Field and Mooney, 1986). Relative growth rate in the early stages of growth has also been found to be linearly related to N concentration within the plant (Ingestad, 1979; Ericksson, 1981 and Agren, 1985b).

Incorporation of these relationships into simulation models of N-response is complicated by the fact that even when growing conditions are constant and supplies of nutrient and water meet crop demand, relative growth rate declines and absolute growth rate increases during growth. Both are affected by plant mass *per se*. A growth rate coefficient, however, has been devised that is independent of plant mass throughout the growing period. Moreover, this coefficient appears to be linearly related to the ratio : % N in the plant/critical % N during N-limited growth (Greenwood *et al.*, 1986). It therefore seems that a single model might be devised that relates % N in the plant dry matter to the rate of dry matter production as the fraction of the potential maximum and to plant mass per unit area. It would also be possible to determine how much N must be in the crop to permit maximum growth rate and how shortfall in that N restricts growth rate as the crop develops (Greenwood *et al.*, 1986).

Amongst the available biodiversity of crop plants, the International Bureau of Plant Genetic Resource (IBPGR), has identified common buckwheat (*Fagopyrum esculentum* Moench) as a potentially important crop species. This is because of the short growth span, capacity to grow in poor soil, high protein and lysine content of its grains and high nutritive value of honey produced as a result of pollination by bees of the crop. The plant thrives under cool temperatic conditions on rather poor well drained sandy soil (Gubbels, 1978). Flowering in the plant begins 5-6 weeks after the seed is sown and continues for at least a month owing to the indeterminate growth habit of the plant. The plant is generally grown as a rain fed crop in the hilly regions of the country.

Because of the critical importance of the crop in hill agriculture, specially around the Himalayan foothills, National Bureau of Plant Genetic Resource (NBPGR) has developed a germplasm bank for the crop and the regional station of NBPGR at Phagli (Simla) is devoted to the collection and maintenance of buckwheat germplasm from different regions. The centre maintains about 408 accessions of the plant in its repository. However, because of its low level of utilization the plant has not been a material of choice for scientists. Not much information is available on the agronomy, physiology and fertilizer requirements of the crop during differnt stages of its growth. Although some studies have been made on the phosphorus fertilizer requirement in buckwheat, with growth and yield as the reference

parameters (Ganyushima, 1972; Sokolor & Semihov, 1983; Potszyinski, 1984; Kalra, 1971; Strong and Soper, 1974; Gubbels, 1980), the reports on nitrogen fertilizer requirements of the crop are scanty. It has been reported that 200 kg of super phosphate and 50 kg N per hectare is beneficial for higher yields. In the hills of India a maximum dose of 15-20 kg P/ha has been recomended to raise a good crop when grown on soils of poor fertility. The crop has been estimated to remove 47 kg Nitrogen, 22 kg Phosphorus and 40 kg Potassium from the soil for each hectare planted and gives a yield of 1600 kg/ha (Campbell and Gubbels, 1978). Ganyushina (1972) has observed that application of nitrogen either as ammonium nitrate or urea increased the dry matter and chlorophyll content as well as the levels of proteins and soluble sugars. However, while ammonium nitrate increased the grain yield, urea had no effect on the same. Sokolov and Semikov (1983) have reported that local application of nitrogenous fertilizer in the form of a band at a depth of 30 cm, prior to sowing, considerably increased the grain yield of the crop. Singh and Atal (1982) have recomended applications of NPK combinations at 40, 60, 40; 50, and 40, 40 kg/ha, respectively, for high herbage yield and good grain quality in the crop. However, despite the extreme importance of NO_3^- as a nitrogen fertilizer in agricultural ecosystems, no data is available on the kinetics of nitrate ions, partitioning and assimilation in common buckwheat at various stages of growth. Information on these parameters would be of immense importance in devising adequate N fertilizer

programme for the crop for higher yields. With such a purpose in mind the study is aimed at :

(a) assessment of the various accessions of common buckwheat for the growth and yield attributes,

(b) characterizing the uptake of nitrate in intact as well as excised roots of buckwheat seedlings, under hydroponic culture, as a function of time, NO_3^- concentration, pH and accompanying ions,

(c) determination of the relationships between photosynthetic activity and nitrate utilization during various phases of growth in the plant,

(d) developmental of a mathematical model for the relationship between photosynthetic activity expressed in terms of relative growth rate and net assimilation rate, growth and the nitrate nitrogen requirement of the crop at various stages of growth.

CHAPTER III

Materials and Methods

Seven accessions of common buckwheat (Fagopyrum esculentum Moench), Viz. IC-18889, Kulugangri, PRB-8901, IC-13141, IC-13145, IC-13411 and BDS- were obtained from North Eastern Regional station of National Bureau of Plant Genetic resources, Shillong and maintained in the experimental fields of the Botany Department of North Eastern Hill University, Shillong. These accessions are native of different parts of North-Eastern States and Himalayan ranges. The geographical distribution of these accessions is shown in Fig. 1.

Regions of Buckwheat Cultivation in India.

1. Leh
2. Pahal gaon
3. Srinagar
4. Udhampur
5. Chamba
6. Kangra
7. Lahaul & Spiti
8. Kinnaur
9. Mandi
10. Kulu
11. Shima
12. Uttarkashi
13. Chamoli
14. Pauri
15. Almora
16. Pithoragarh
17. Darjeeling
18. Siliguri
19. Assam
20. Meghalaya
21. Arunachal Pradesh
22. Nagaland
23. Manipur

All the seven accessions were evaluated for their seed characters. Scanning electron microscopic photographs of the surface features of the seed coat of the accessions were taken using the method of Hayat (1974). Seeds were dehydrated by gradual and sequential passage through 30, 50, 70, 80, 90, 95 & 100 percent acetone. After dehydration the seeds were subjected to critical point drying, followed by fixing in a copper Stub for gold coating. Gold coating was done for about 5 minutes; soon after seed coat photograph was taken in SEM.

For the determination of weight per grain, 25 grains from each of the seven accessions were weighed in a monopan balance. The weight of each grain was calculated by dividing the total weight by the number of grains. For the determination of hull groat ratio, the hull fraction of the grains was separated from the grains manually and weighed. The weight of groat was calculated as the difference between total weight of the grain and the weight of the hull. The hull groat ratio has been calculated by dividing the weight of the hull by the groat weight.

Moisture content of the seeds has been determined by "Low Constant Temperature Oven Method". A known fresh weight of the grains was placed in the preweighed weighing bottles and kept in a forced draught oven at 110 ± 2°C for 24 ± 1 hours. At the end of the prescribed period the bottles were placed in a desiccator for cooling for 1 hour. After

cooling, the final weight of the bottles was determined. The moisture content of the grains, expressed as percentage by weight, has been calculated by the following formula :

$$M_2 - M_3 \times \frac{100}{M_2 - M_1}$$

where M_1 = the weight in grams of the container and its cover

M_2 = the weight in grams of the container, its cover and its content before drying and

M_3 = the weight in grams of the containers, cover and contents after drying.

TISSUE CONSTITUENTS

For the estimation of grain tissue constituents, a known fresh weight (usually 1 gm) of the dehulled grains was fixed by plunging it into boiling 80 percent ethanol. After 24 hours the tissue was macerated in the alcohol in a tissue homogenizer. Separation into alcohol soluble and alcohol insoluble fractions was carried out by filtration under suction over Buchner funnel. The alcohol soluble fraction was made to volume and used for the estimation of soluble sugars, free amino acids, total phenolics and total lipids.

The alcohol insoluble residue was dried in a forced draught oven at 70°C for 72 hours over P_2O_5. The dried

material was weighed and used for the estimation of total starch and alcohol insoluble nitrogen.

Carbohydrates

Starch : The starch content of the grains has been determined by the method of McCready *et al*., (1950). A suitable quantity, generally 50 mg, of the alcohol insoluble material was transferred to a centrifuge tube containing a small volume of distilled water. The contents were heated over a water bath to gelatinize starch. After cooling, starch was extracted from the jelly by repeated trituration with 72 percent perchloric acid. The solution was filtered through a sintered funnel and the filtrate made to volume. To a suitable aliquot of the filtrate, 4ml of 0.1 percent anthrone in conc. H_2SO_4 was added; the solution was cooled for 10-15 minutes. Absorbance of the solution was measured at 700 nm in a spectronic 20 spectrophotometer.

Sugars : Reducing sugars were determined colorimetrically according to the method of Nelson (1944). From a suitable aliquot of the alcohol soluble fraction, alcohol was removed by keeping the tubes in a boiling water bath till the odour of alcohol disappeared completely. The sample was allowed to cool and then the sample was made to volume with

distilled water. To a suitable aliquot of the aquous extract, 1 ml of mixed copper reagent was added and the solution heated in a boiling water bath for 20 minutes. After cooling, 1 ml of arsenomolybdate reagent was added and the final volume made to 20 ml. Absorbance of the solution was measured at 490 nm in a spectronic 20 spectrophotometer. A calibration curve was prepared with glucose as the standard.

Total sugars were estimated as reducing sugars after hydrolysing enzymatically by 0.2 percent invertase (yeast). The solution was allowed to stand overnight, protected by layering a drop of toluene on top of the solution. Values for non reducing sugars have been obtained as the difference between total and reducing sugars.

Total Nitrogen

Nitrogen was estimated from the alcohol insoluble fraction by semi-micro Kjeldahl's method. A suitable amount of the alcohol insoluble powder was transferred to a Kjeldahl digestion flask and digested with concentrated H_2SO_4 using selenium-copper catalyst, according to the method of Chiball *et al*., (1943). Digestion was continued till the solution was faint blue in colour.

The digests were made to volume. Ammonia was estimated from the digests titrimatrically. Ammonia was steam distilled in a Markham's apparatus into boric acid buffer

and estimated by titration against N/140 H_2SO_4 containing phenol red-bromocresol green indicator (Conway and O'malley, 1942). Anhydrous ammonium sulphate was used as the standard. The percent of protein has been calculated by multiplying the content of nitrogen determined by Kjeldahl's-titrimetric method with a constant 6.25.

Total Free Amino Acids

Total free amino acid were estimated from the alcohol soluble fraction as α-amino nitrogen by the method of Rosen (1957). A suitable aliquot of the alcohol soluble fraction, from which alcohol had been completely removed by heating over a water bath, was made to 1 ml by distilled water. 0.5 ml of 0.002 M acetate-cyanide buffer, pH 5.4 and 0.5 ml of 3 percent ninhydrin were added to the solution in succession. The mixture was heated in a boiling water bath for 15 minutes followed by the addition of 4 ml of isopropyl alcohol-water diluent (1:1). After cooling absorbance of the coloured complex was recorded on a Spectronic-20 spectrophotometer at 550 nm with glycine as the standard.

Total Lipids

The lipids were extracted from the alcohol soluble fraction with Methanol-Chloroform mixture (1:1), after the alcohol was removed from the sample by gentle heating over a water bath. The solution was centrifuged at 1000 x g for 10

minutes and the supernatant transferred to preweighed petriplates. The solution was allowed to evaporate and the petriplates weighed again. The total lipid content was determined as the difference in the initial and final weight.

Total Phenolics

Total phenolics were estimated from the alcohol soluble fraction according to the method of Swain and Hills (1959). A suitable aliquot, usually 0.5ml of the ethanolic extract was diluted to about 7 ml with distilled water, followed by the addition of 0.5 ml Folin-Dennis reagent. After 3 minutes, 1 ml of a saturated solution of Na_2CO_3 was added and the mixture made to 10 ml with distilled water. Absorbance was measured after one hour at 700 nm in a spectronic-20 spectrophotometer with gallic acid as the standard.

GROWTH ANALYSIS

All the seven accessions of common buckwheat were assessed for the periodicity of their growth behaviour by conventional growth analysis under field conditions. To carryout the field experiment, a 7x4 metre field was selected in the campus of North-Eastern Hill University, Shillong. 14 raised beds, prepared as rows along the breadth of the field, were prepared; the distance between each row was approximately 50 cm and two rows were allotted for each accession.

Healthy seeds from each of the seven accessions were selected and washed thoroughly under running tap water. The seeds were germinated in petriplates in the laboratory in a incubator. The germinated seeds were transferred to a growth chamber maintained at 25°C and 65 percent R.H with constant illumination. The seeds were maintained in the growth chamber for 7 days till the cotyledonary leaves unfolded completely. The 7 day old seedlings from each of the seven accessions were transferred to the allotted raised beds in the field; 40 seedlings were sown in each row. The crop was watered periodically. Mild dressings of FYM were applied to the field twice, one at the time of sowing and the other after 30 days of transplanting.

All the seven accessions were sampled in a fixed order at random. The first harvest was made on 3rd day after planting and the subsequent harvests were made on 7, 19, 31, 43, 55 and 67 days after planting. 10 plants from each accession were harvested on a given sampling date. The harvested plants were washed in running tap water, blotted dry with the help of a filter paper and separated into stem, leaves and root portions. The length of the stem was measured by measuring tape. The leaf area was calculated by measuring the imprints of the leaves made on the ferrostate paper with the help of planimeter.

After recording their fresh weights, the samples of leaves, stem and root were allowed to dry for 48 hours in an oven at 80°C. The dried samples were cooled in a desiccator and then weighed for recording their dry weight. In the later part of the field experiment the observations were made regarding the time of flowering and number of grains.

The data recorded were used to derive the growth components or indices which were interpreted with reference to the differences amongst accessions. The growth components like Net Assimilation Rate (NAR), Relative Growth Rate (RGR) and Leaf Area Ratio (LAR) were calculated with the formula given by Watson (1950).

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \text{ mg/mg dry weight/day}$$

$$NAR = \frac{W_2 - W_1}{LA_2 - LA_1} \times \frac{\ln LA_2 - \ln LA_1}{t_2 - t_1} \text{ mg/cm}^2 \text{ leaf area/day}$$

$$LAR = \frac{(S_2 - S_1)(\ln W_2 - \ln W_1)}{(W_2 - W_1)(\ln S_2 - \ln S_1)} \text{ cm}^2\text{/mg dry weight}$$

where W_1 & W_2 are the initial and final dry weight of whole plant, LA_1 & LA_2 are initial and final leaf area of the harvest, t_1 & t_2 represents initial and final harvest dates, between two consecutive sampling intervals and S_1 & S_2 are

the initial and a final dry weight of leaf between two consecutive sampling intervals.

NITRATE UPTAKE

Seeds of common buckwheat (*Fagopyrum esculentum* Moench) were washed for one hour under running tap water followed by rinsing with deionized water. The washed grains were germinated for 48 hours in darkness at 27±2°C. The germinated seeds were transferred to a solution of 0.2 mM $CaSo_4$ for 24 hours. After 24 hours the seedlings were transferred to a modified full strength nitrate free Hoagland's nutrient solution (Arnon and Hoagland, 1940) having the following composition.

Salt	gram/liter
$Ca(SO_4)_2$	0.492
KH_2PO_4	0.23
$MgSO_4 \cdot 7\ H_2O$	0.49
H_3BO_3	0.00286
$MnCl_2 \cdot 4H_2O$	0.00181
$CuSO_4 \cdot 5H_2O$	0.00008
$ZnSO_4 \cdot 7H_2O$	0.00022
$H_2MOO_4 \cdot H_2O$	0.00009
$FeSO_4 \cdot 7H_2O$ 0.5% Tartaric acid 0.4%	0.6 ml

The germinated seedlings were suspended in the solution through a stainless steel mesh, so that the root portion dipped into the solution. The seedlings were maintained in a growth chamber under continuous white flourscent light 30 mmol cm^{-1}/ S^{-1} at 27±2°C and 65 percent R.H. The solution was aerated to provide ample Oxygen and solution mixing. For this purpose a peristaltic pump was used, in which continous air flow was maintained.

The assay of nitrate uptake was carried out on 8 day old seedlings from each of the seven accessions. The seedlings were transferred separately to beakers containing Hoagland nutrient solution supplemented with 5 mM KNO_3^-. The concentration of KNO_3^- in the solution was determined in an aliquot of the solution at t_o and one hour after the experiment was raised (t_{60} minutes). The difference between the nitrate level represented the amount of nitrate taken up by the seedlings.

Experiments were initiated by placing 8 day old seedlings (grown as described above) in 50 ml pyrex beakers containing 25 ml of Hoagland solution with 5 mM Nitrate given as KNO_3^-. For the determination of nitrate uptake as a function of time, samples in triplicate were drawn from each treatment solution at 15, 30, 45, 60, 120, 180, 240, and 300 minutes intervals and nitrate levels in 0.1 ml aliquot

of the test solution were determined using the Brucin reduction method, described by Nicholas and Nason (1957). According to this method, 0.1 ml of sample was taken in a test tube and the total volume made up to 5 ml with H_2O, 5 ml of H_2SO_4, 1 ml of sodium chloride solution and 0.25 ml of Brucine Sulphanalic acid mixture were added to the above solution and the test tube heated for 25 minutes in boiling water bath. The tubes were allowed to cool. The optical density of the cooled solution was read at 410 nM. Standard graph was prepared with KNO_3^- using the same procedure.

For the determination of nitrate uptake as a function of NO_3^- concentration in the nutrient medium, an experiment was raised in which the 8 day old seedlings were transfered to Hoagland nutrient solution containing varying levels of KNO_3^-. In each solution the amount of nitrate taken up was determined by drawing 0.1 ml aliquot from the test solution before the start of the experiment and 15 minutes after the seedlings were transfered to the solution. NO_3^- levels in each aliquots were determined by the Brucine reduction method described above, The difference in the nitrate levels in the ambient medium between t_o and t_{15} minutes represented the amount of nitrate taken up by the seedling. For the determination of effect of pH on the uptake of nitrate, the seedlings were transfered to Hoagland nutrient solution containing 5mM NO_3^-; each of the solution

having a diffrent pH. Uptake of nitrate under such conditions was determined in the same way as described above. For the determination of the effect of glucose, sucrose, DCMU, $KClO_3^-$ and NH_4^+ on the uptaeke of nitrate by the seedlings, the nutrient solution containing 5mM KNO_3^- was supplemented with arying amounts of the particular metabolite/compound. Uptake of the nitrate in the solution was determined in the same way as described above.

In order to determine the effect of depleting substrate concentration on the uptake of nitrate, an experiment was raised in which the level of nitrate in the ambient nutrient solution was kept constant throughout the duration of the experiment. This was done by constant replenishment of NO_3^- depleted from the nutrient solution. The amount of nitrate depleted from the solution was determined by measuring the nitrate content in suitable aliquots from the test solution at regular intervals. The drop in the nitrate content during two successive tests gave the amount of nitrate depleted during a particular time period. The uptake of nitrate from such solutions was determined by drawing suitable aliquot (0.1 ml) in triplicate at 15, 30, 45, 60, 120, 180, 240, and 300 minutes intervals and determining the level of nitrate in the test solution by Brucine reduction method (as described above).

The values for uptake rate expressed as μmol NO_3^- taken up mg dry weight $root^{-1}min^{-1}$, were computed from the values of nitrate at each sampling time, i.e., the difference between final and initial concentration of NO_3^- in the nutrient medium after a definite time interval has been expressed as amount of NO_3^- taken up by the plant. The cumulative uptake has been expressed as μmol nitrate taken up per 100 mg dry weight root during a particular time interval. The Km and Vmax values have been calculated from the data on uptake rate v/s NO_3^- concentration in the nutrient medium using Lineweaver and Burk plot (Lineweaver and Burk, 1934). The data of 1/v and 1/s were subjected to regression analysis by using the formula

$$Y = a + bX$$

The two seedlings which were used for nitrate uptake study were harvested from the nutrient medium, washed thoroughly in deionized water and separated into root and shoot portions. After measuring the fresh weight of the shoot and root one of the seedlings was used for NR activity study, as per the method of Joworski (1971). According to this method suitable quantity (usually 100 mg) of freshly harvested tissue was chopped into small pieces and incubated in a test tube in 4.5 ml of substrate solution containing 3.9 ml phosphate buffer (pH 7.5), 0.5 ml of 5 percent iso-propanol, and 0.1 ml of 0.02 M KNO_3^-. Incubation was done in

dark for one hour at 30° C in a water bath. After one hour from each sample, 0.8 ml aliquot was taken in sterilized test tubes to which, 0.6 ml of NEDH and 0.6 ml of sulphanilamide were added. Optical density of the solution was recorded at 450 nm in Spectronic 20 spectophotometer. Measurement of *in vivo* nitrate reductase activity was carried out separately in shoot and root portions of the harvested seedlings.

The root and shoot portion of the other seedling was dried in a forced draught oven at 80°C for 48 hours. The dried sample was weighed and used for the estimation of the tissue content of total and nitrate nitrogen. For the estimation of total nitrogen a suitable dry weight of the sample was digested as described earlier. The total nitrogen content was estimated titrimetrically by semi-micro Kjeldhal method as described earlier. The nitrate content of the dried sample was estimated after extraction with HCl. The solution was filtered through Whatman No. 1 filter paper. The nitrate content of the sample was estimated from the filtrate according to the method of Nicholsen and Nasson (1957).

From the titter value of total nitrogen, the content of total nitrogen per 100 mg dry weight root and per 100 mg dry weight shoot as well as per shoot and per root have been determined. Similarly the content of nitrate nitrogen per 100 mg dry weight shoot and per 100 mg dry weight root as well as per shoot and per root have been

determined from the data of absorbance for nitrate. The content of reduced nitrogen has been determined as the difference between total and nitrate nitrogen.

For the determination of nitrate uptake by excised roots the 8 day old seedlings, raised as described earlier, were washed in deionised water and dried on a filter paper. The root portion was excised from the seedlings by cutting with a sharp blade. A suitable fresh weight of the root tissue was transferred to Hoagland nutrient solution supplemented with 5 mM nitrate to determine the uptake, following the same protocol as that for intact seedlings.

PARTITIONING OF NITRATE IN WHOLE PLANT

For nitrate partitioning study the seedlings of common buckwheat were raised in the laboratory condition. Eight day old, seedlings were transplanted into pots, each containing 2.6 Kgs of fine sand washed with 0.3 per cent of H_2SO_4. The pots were arranged in 4 sets representing four treatments. The plants were maintained in the net house with four plants in each pot and 10 pots for each treatment. The pots were regularly supplied with full strength Hoagland solution, containing 5, 20 and 50 mM KNO_3^-, which represented the 3 levels of nitrate supplied to the plant. The untreated controls, representing one set on treatments, received Hoagland nutrient solution which did not contain any

nitrogen. The plant were harvested at random from each set of treatments, on 7, 19, 31, 43, 55 and 67 days after planting. At each interval 5 plants from each treatment were harvested. The harvested plants washed under running tap water and blotted dry on sheets of filter paper. The length of the shoot was measured and the plants were separated into stem, leaf, petiole and root segments. The stem was further divided into 2, 3 or 4 segments according to length. The fresh weight of each segment was determined and the segments dried in an over for 72 hours at 80°C. From the oven dried materials, the dry weight of each segment was determined. Based on dry weight data of different samples and leaf area, the growth indices, viz., RGR, NAR and LAR were calculated from the plants for each treatement and each samplings. In order to determine the partitioning of Nitrogen between various tissue segments of the plant, harvested plants were separated to shoot, leaves, petiole and stem. The leaves and petiole were numbered in the acropetal order. The stem was divided into 2, 3, or 4 segments from the base and each segment numbered in an acropetal order. In each segment the amount of various nitrogenous components viz. total nitogen, nitrate nitrogen and reduced nitrogen and the activity of nitrate reductase was determined. In figure the values for various nitrogenous constituents have been expressed with illustration in μg per total amount of dry weight of the particular unit. The units for NR activity have

been expressed as μmol nitrite reduced/100 mg fresh weight/hour.

Based on the information collected, an attempt has been made to determine the relationship between the nitrogen content of the plant and growth behaviour. The data thus obtained has been fitted to the models proposed by Greenwood *et al*, (1986; 1991). The theory, which backs the model attempts to relate the percentage nitrogen in plant dry matter to growth rate and to plant dry weight per unit area. It covers all stages of growth untill senscence, of crops grown at optimal and sub-optimal N-nutrition.

MODEL I

This model attempts to test the effects of applied nitrogen on the growth and photosynthtic activity with plants of common buckwheat (*Fagopyrum esculentu*) using the following equations;

$$\frac{dW(F,t)}{dt} = \frac{[Kx(F)] \times [W(F,t)]}{X + W(F,t)}$$

where W(F,t) is the dry weight of the plant (excluding root) per unit area

Kx (F), is the growth rate coefficient.

x is a constant

Integration of the above equation gives,

$$[Kx(F)]\,[T - T_o] = X \ln W(F,t) + W(F,t) - X \ln W(F,t_o) + W(F,t_o)$$

where, W (F,t) is the dry weight of the sample (excluding root) at time t and $W(F,t_o)$ is the dry weight of sample (excluding root) at time t_o. T_o represents the time of initial harvest and T represents the time of next harvest.

For specific conditions where there is just sufficient N-fertiliser to permit maximum growth, Greenwood *et al.*, (1991) have defined Kx(F) as Kc and W(F,t) as Wc(t), so that

$$\frac{d\,Wc(t)}{dt} = \frac{Kc \times Wc(t)}{X + Wc(t)}$$

where Kc is the critical growth constant and Wc(t) is critical dry weight at critical nitrogen level.

Greenwood *et al.*, (1986) have assumed that there is a relationship between critical percent N in the plant dry matter Nc(t) and plant dry weight Wc(t) and have defined the critical nitrogen constant in a plant by the equation

$$Nc(t) = 1.33 + \exp\,[1.4 - 0.26\,Wc(t)]$$

MODEL II

In this Model the relationship between percent N and photosynthesis has been derived by using the formula derived by Greenwood *et al.*, (1986). This has been done by regression of the rate of photosynthsis, P_L against the concentration of (N_L) nitrogen in the leaf. It is, therefore, written as

$$P_L = M_L N_L + C_L$$

where, P_L = Leaf Photosynthesis.

N_L = Nitrogen in the leaf

M_L and C_L are coefficients

There is a linear relationship between percent N in the leaf (N_L) and percent N in the whole plant (N_W). Therefore, it is written as,

$$N_L = M_W N_W + C_W$$

where, M_W and C_W are coefficients which have positive values. The integration of above equation gives

$$P_L = M_L M_W N_W + M_L C_W + C_L$$

For a given crop of a given size, $M_L C_W + C_L$ are constants so that P_L is linearly related to N_W.

The model based on the linear regression equation $Y = a + b X_{\#}$ can therefore used to relate photosynthetic efficiency of the leaf to the total nitrogen content within the plant. In the plant under study the leaf photosynthetic capacity defined as net assimilation rate has therefore been related to percent nitrogen in the whole plant at various stages of growth under varying levels of external nitrate supply.

The entire data presented in the present study represents experiments carried out over three consecutive years under laboratory and/or field conditions as applicable. The data represents the mean of at least three independent replicates for each study. The entire data has been subjected to statistical treatments and the LSD value depicting the level of significance at 5 percent probability determined using a computer programme for ANOVA.

CHAPTER IV

Grain Composition and Growth Analysis

i) Experimental

ii) Results

iii) Discussion

EXPERIMENTAL

Seven accession of common buckwheat (*Fagopyrum esculentum* Moench) viz. IC-18889, IC-13141, IC-13145, IC-13411, BDS-1345, Kulugangri and PRB-8901 were procurred from the North Eastern Regional Station of National Bureau of Plant Genetic Resources, Shillong. Mature grains from each accession were scaned by electron microscope for their seed code structure. The grains were also analyzed for moisture content, grain weight, hull groat ratio, germination percentage and the tissue level of total starch, total sugars, total proteins, free amino acids, phenolics, and total lipids. The concentration of the tissue constituents has been expressed as percent of grain dry weight.

Grains from each accession were germinated in the laboratory and 5 day old seedlings were transplanted in raised beds in the experimental field of the Botanical Garden of North Eastern Hill University, Shillong. The experimental design was a split plot with three replications for each accession. Plants from each of the accessions were harvested, at random, at periodic intervals till seed set. Shoot length, shoot dry weight, root dry weight, leaf dry weight and total leaf area was recorded for each harvested plant. From the data on dry weight and leaf area various indices of growth viz. Relative growth rate (RGR), Net assimilation rate (NAR), Leaf area ratio (LAR), were computed.

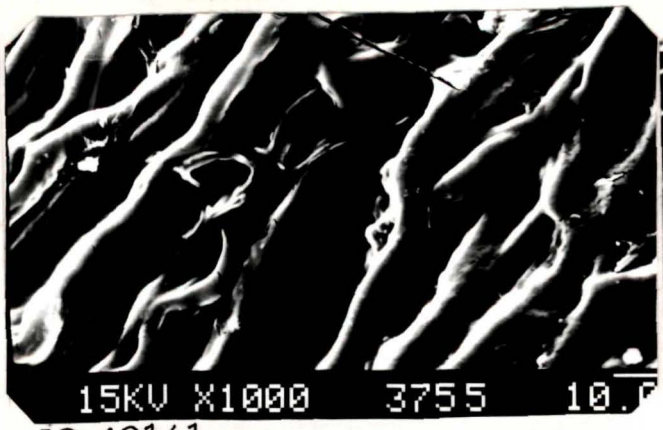
Results presented in this chapter are based on studies carried out in the year 1991. Each presented value represents the mean of at least five independent replicates. The data has also been subjected to ANNOVA and least significance calculated at P0.05.

RESULTS

The scanning electron microscopic photgraphs of seeds of seven accessions of buckwheat revealed marked variations in their pattern of surface waxy coating. The accession IC-13141 showed parallel waxy coating, while PRB-8901, IC-13411, IC-13145 and BDS-1345 showed thick waxy coating with high raticulation. However, Kulugangiri and

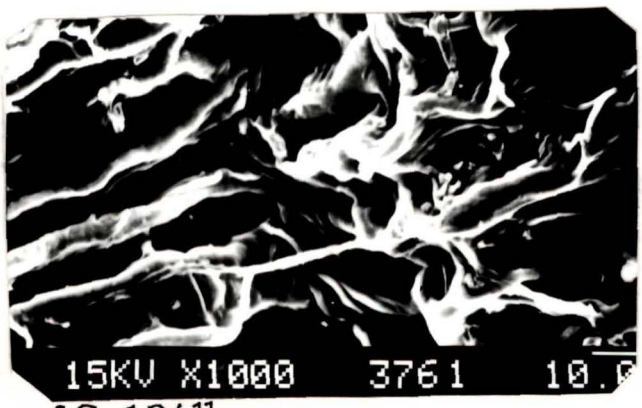
IC-18889 showed miled waxy coating with medium raticulation (Fig. 4.1). Marginal differences were also observed in the size of the grains from the seven accessions tested. Except for IC-13141 and IC-18889 which measured 0.27 and 0.318 mm respectively. The breadth of grains of PRB-8901, IC-13411, IC-13145, BDS-1354 and Kulugangri ranged between 0.409 to 0.45 mm. The grains of each of the seven accessions, analyzed, did not differ from each other in length. Thus the length of grains of all the accessions ranged between 0.60 to 0.65 mm (Fig. 4.2). The grains of common buckwheat showed an average grain weight ranging from 12 to 15 mg and a moisture content ranging from 10 to 13 percent. However, marked differences in the hull growth ratio between grains from different accessions were observed. While IC-13141 showed a lowest hull groat ratio of 0.31, IC-13145 had the highest hull groat ratio of 0.53 (Table 4.1). Expressed as percent of dry weight, the starch content of grains ranged between 53.1 to 55.0 percent and the content of total sugars ranged between 10.8 to 12.94 percent. The level of non-reducing was, however, more predominant than that of reducing sugars; reducing sugar constituted an average of 5 per cent of the total sugar content of the grains. Expressed as percent of dry weight, the total protein content of the grains was marginally more than 11.3 percent; no marked differences could be observed in the protein content of the grains between different accessions. Marked differences were, however observed in the level of total lipids and total phenolics in the grains of the seven accessions of common

Fig. 4.1 : The Scanning electron microscopic photographs of seeds of seven accessions of common buckwheat (*Fagopyrum esculentum* Moench).

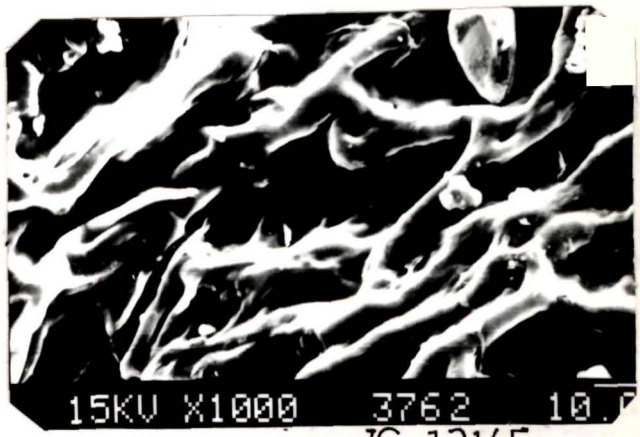


IC-13141

IC-8901

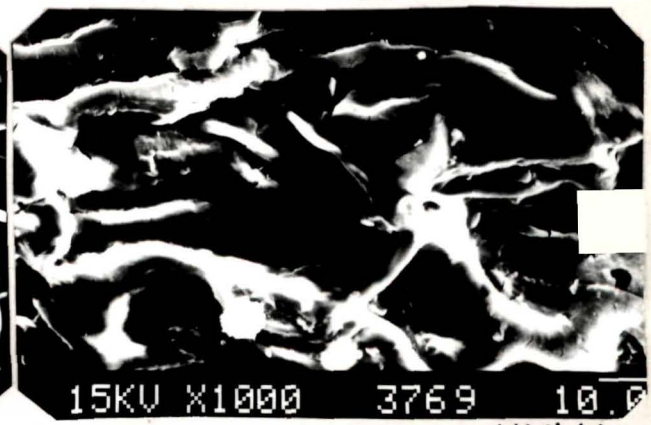


IC-13411

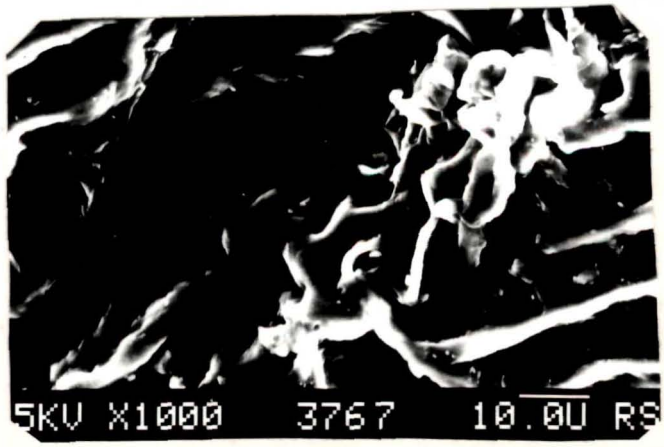


IC-13145

BDS-1354



KULU



IC-18889

Fig. 4.2 : Differences in the size, breadth and length of seeds of seven accessions of common buckwheat (Fagopyrum esculentum Moench)

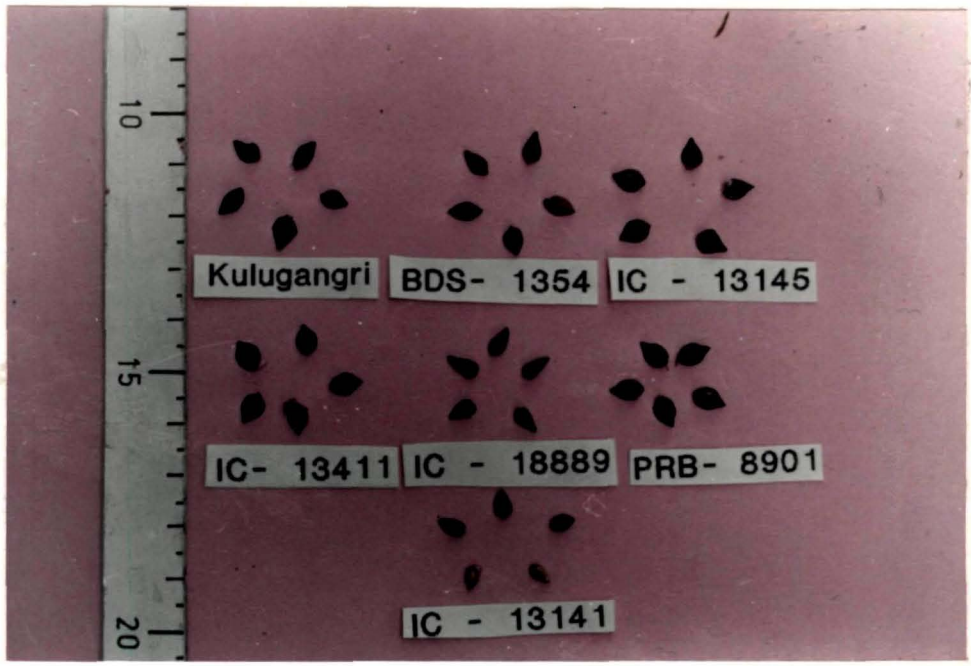

10
15
20
Kulugangri
BDS- 1354
IC - 13145
IC- 13411
IC - 18889
PRB- 8901
IC - 13141

Table 4.1: Changes in the Hull/groat ratio, percent moisture content and percent germination at 72 hours of the grains of seven accessions of common uckwheat (*Fagopyrum esculantum* Moench).

Accession	Grain weight (mg)	Hull/Groat Ratio	Moisture Content (%)	Percent Germination (72 hours)
IC-18889	13.7	0.34	13.0	88
Kulugangri	13.5	0.50	10.0	64
PRB-8901	14.4	0.42	12.0	92
IC-13141	15.5	0.31	10.0	80
IC-13145	13.5	0.53	13.0	84
BDS-1354	14.5	0.42	12.0	95
IC-13411	14.0	0.35	13.0	72

Table 4.2 : The content of total protein, total lipids, total amino acids, phenolics, reducing sugars, non-reducing sugars and starch in the grains of seven accessions of common buckwheat (*Fagopyrum esculentum* Moench).

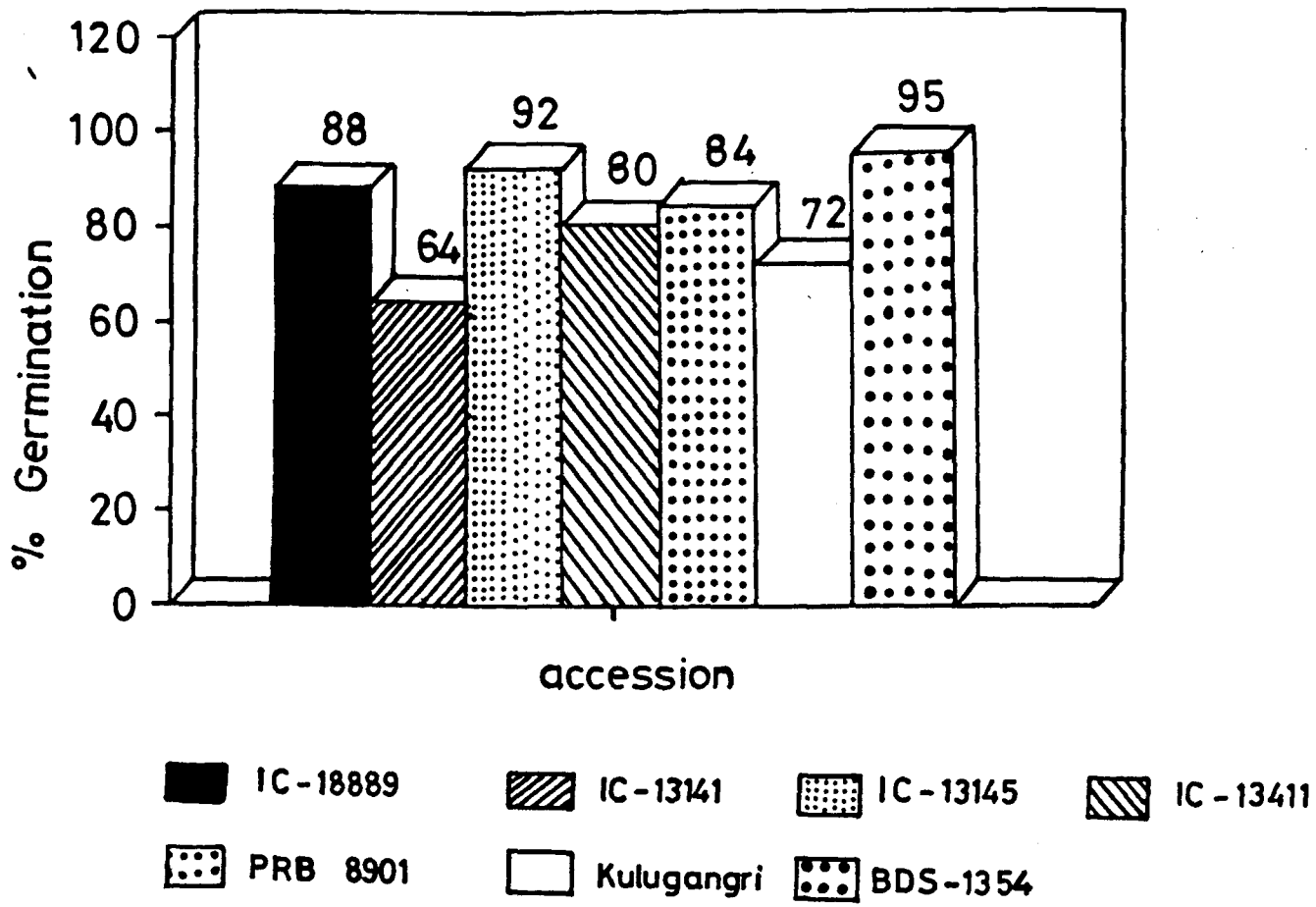
Component	Accessions						
	IC-18889	Kulu	PRB-8901	IC-13141	IC-13145	IC-13411	BDS-1354
Total Protein	11.20	11.30	11.50	11.2	11.30	11.20	11.50
Total lipids	5.90	7.00	3.00	6.00	4.00	4.00	6.00
Total amino acids	0.33	0.42	0.38	0.40	0.28	0.32	0.43
Phenolics	2.68	3.35	2.34	2.63	1.03	1.70	3.57
Reducing Sugars	4.77	3.88	3.65	4.77	3.71	5.10	4.73
Non-Reducing Sugars	6.03	8.02	6.88	6.12	7.66	7.84	7.26
Total Sugars	10.80	11.90	10.53	10.89	11.37	12.94	11.99
Starch	53.20	54.50	54.20	55.00	53.80	55.00	53.10

buckwheat. While PRB-8901 showed the lowest (3.0%) lipid content, Kulugangri had the highest content of total lipids representing about 7.0 percent of total grain dry weight. Amongst the accessions tested, IC-13145 had the lowest level of (1.03%) of total phenolics. The content of total phenolics was highest in grains of BDS-1354 which had nearly 3.6 mg total phenolics per 100 mg grain dry weight (Table 4.2).

All the seven accessions of buckwheat were analyzed for temporal changes in the pattern of growth. The seven accessions tested in our laboratory for their germination showed a germination percentage ranging between 60 to 90 per cent at 72 hours after imbibition. However, the grains could be separated into two groups based on their germination behaviour. At 72 hours of imbibition 70 to 90 percent grains of IC-18889, IC-13145, PRB-8901 and BDS-1354 had germinated. However, during the same time only 60 to 70 per cent grains of IC-13141, Kulugangri and IC-13411 showed visible signs of germination (Table 4.1, Fig. 4.3).

Being a short duration crop, the plants achieved a maximum dry weight in about 40 days after planting. Increase in the dry weight of the shoot followed a typical sigmoid curve with the lag phase lasting upto 7th day after planting. The logarithmic phase of dry matter accumulation in shoot lasted between 7th and 30th day when a more than 15 fold increase in dry weight of the shoot was observed. Beyond 40th day, however, no further increase in dry weight of the

Fig. 4.3 : Percentage of germination of seeds of common buckwheat (Fagopyrum esculentum Moench) under laboratory conditions at 72 hours of imbibition


120
100
80
60
40
20
0
% Germination
88
64
92
80
84
72
95
accession
IC-18889
IC-13141
IC-13145
IC-13411
PRB 8901
Kulugangri
BDS-1354

shoot could be observed (Table 4.3, Fig. 4.4). Increase in the dry weight of the roots in plants of common buckwheat followed a hyperbolic pattern without any lag phase; the maximum dry weight being recorded 40 days after planting (Table 4.3, Fig. 4.5). The changes in the pattern of dry matter accumalation in stem and leaf followed a trend similar to that shown by shoot. The logarithmic phase of dry weight accumulation in leaves, however, lasted from 7 to 20 days only beyond which the total leaf dry weight did not increase markedly (Table 4.4, Fig. 4.6 & 4.7). The total leaf area in each of the seven accessions increased linearly with time upto 30 days after planting recording a nearly 20 fold increase during the period. Beyond 30 days there was a slight decrease in the total leaf area of the plants (Table 4.5, Fig. 4.8). The leaf area ratio (cm^2 mg shoot $dryweight^{-1}$) of the plants showed a progressive increase with time during initial seven days after planting the seedlings. During this period a more than 6 fold increase in the LAR was observed. Difference was, however, observed on the 7th day in LAR between different accessions with BDS-1354 showing the least LAR, when compared with other accessions. After 7th day the LAR registered a marginal decrease upto 19th day. After 19th day there was no appreciable change with time in the LAR for each of the seven accessions (Table 4.6, Fig. 4.9).

In each of the seven accessions the rate of accumulation of dry matter (RGR) expressed as mg mg dry $weight^{-1}$ d^{-1} showed positive increments upto 43 days. The

Table 4.3 : Changes in the dry weight of shoot and dry weight of root with time in 7 accessions of buckwheat (*Fagopyrum esculantum* Moench) grown under field conditions.

Accession	Days after planting					
	7	19	31	43	55	67
	Dry Weight of Shoot (mg)					
IC-18889	7.1	50.8	89.9	91.4	85.1	85.0
Kulugangri	6.8	52.6	87.5	81.0	88.1	87.8
PRB-8901	7.2	54.2	87.5	93.0	91.6	87.3
IC-13141	7.3	55.5	83.1	90.5	88.3	86.0
IC-13145	6.9	57.3	82.6	87.3	85.5	83.0
IC-13411	7.3	58.0	82.6	86.1	84.3	83.3
BDS-1354	7.0	56.0	83.5	85.7	84.7	83.8
LSD P0.05 for Accessions = 4.27						
	Dry Weight of Root (mg)					
IC-18889	1.6	2.5	4.1	4.4	4.3	4.2
Kulugangri	1.6	2.9	4.3	5.6	4.2	4.1
PRB-8901	1.7	2.7	4.3	4.7	4.4	4.2
IC-13141	1.7	2.7	4.1	4.4	4.1	4.3
IC-13145	1.7	2.6	5.1	5.5	4.8	4.5
IC-13411	1.7	2.8	5.3	5.6	4.6	4.1
BDS-1354	1.6	2.7	5.6	5.8	4.8	4.4
LSD P0.05 for Accessions = 0.51						

Table 4.4 : Changes in the dry weight of stem and dry weight of leaf with time in 7 accessions of commonbuckwheat (*Fagopyrum esculantum* Moench) grown under field conditions.

Accession	Days after planting					
	7	19	31	43	55	67
			Dry Weight Stem (mg)			
IC-18889	4.1	30.9	63.3	63.8	59.0	59.5
Kulugangri	4.1	29.7	62.5	51.3	60.0	61.5
PRB-8901	4.1	31.0	62.5	63.2	62.0	61.3
IC-13141	4.2	33.3	58.1	60.6	58.8	58.5
IC-13145	4.0	32.8	56.6	58.0	57.5	56.0
IC-13411	4.2	33.3	56.8	58.1	57.3	56.5
BDS-1354	4.1	33.0	57.1	58.1	57.6	57.0
LSD P0.05 for Accessions = 9.44						
			Dry Weight of Leaf (mg)			
IC-18889	3.1	19.9	26.6	27.6	26.1	25.5
Kulugangri	2.7	22.9	25.0	29.7	28.1	26.3
PRB-8901	3.1	23.2	25.0	29.8	29.6	26.0
IC-13141	3.1	22.2	25.0	29.9	29.5	27.5
IC-13145	2.9	24.5	26.0	29.3	28.0	27.0
IC-13411	3.1	24.8	25.8	28.1	27.0	26.8
BDS-1354	2.9	23.0	26.4	27.6	27.1	26.8
LSD P0.05 for Accessions = 1.60						

Table 4.5: Changes in the leaf area with time in 7 accessions of common buckwheat (*Fagopyrum esculantum* Moench) grown under field conditions.

Accession	Days after planting						
	3	7	19	31	43	55	67
	Leaf Area (cm^2)						
IC-18889	1.20	4.1	12.3	21.8	29.3	28.1	27.0
Kulugangri	1.25	4.5	13.1	21.6	26.0	23.6	22.8
PRB-8901	1.40	4.6	13.0	24.0	28.3	27.3	24.6
IC-13141	1.20	5.0	13.8	23.3	28.0	25.3	24.0
IC-13145	1.20	4.5	13.5	23.0	27.3	25.3	24.3
IC-13411	1.50	4.8	14.3	23.3	27.1	25.3	24.1
BDS-1354	1.30	4.8	13.3	19.6	27.0	26.8	25.0

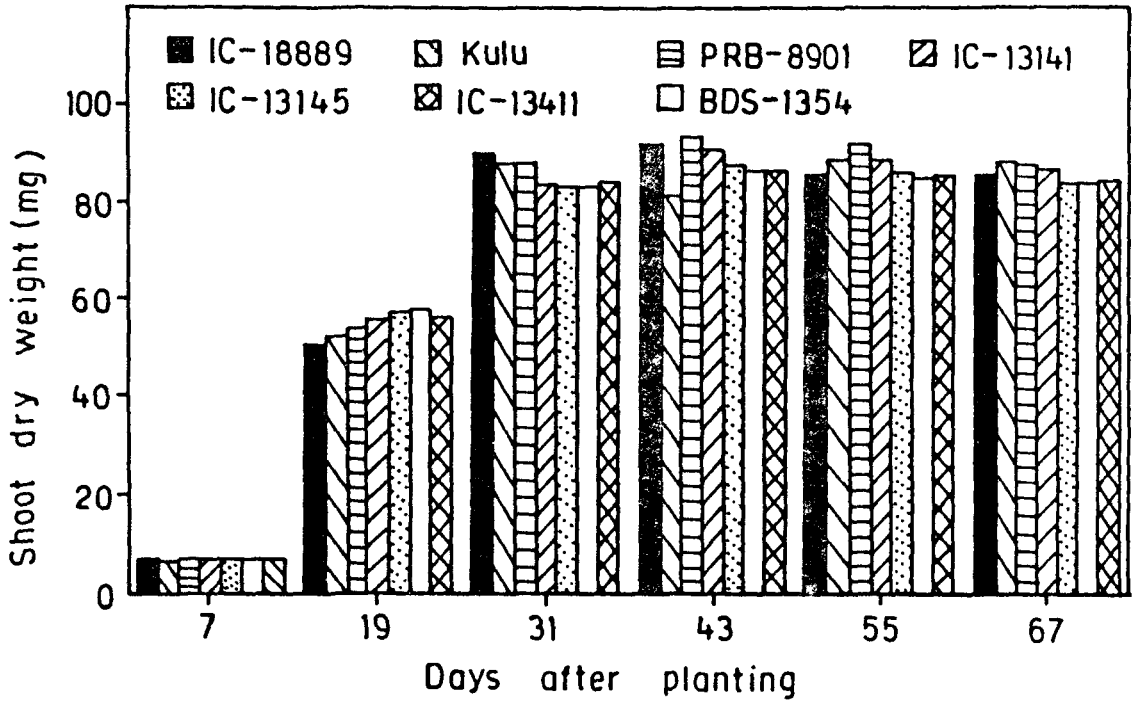
LSD P0.05 for Accessions = 1.72

Table 4.6 : Changes in the relative growth rate, net assimilation rate and leaf area ratio with time of 7 accessions of common buckwheat (*Fagopyrum esculenntum* Moench) grown under field conditions.

	Days After Planting						
Accessions	3	7	19	31	43	55	67
	RGR (mg mg dry weight^{-1}d^{-1})						
IC-18889	0.007	0.04	0.159	0.047	0.002	-0.006	-
Kalu	0.029	0.028	0.159	0.042	0.004	-0.004	-0.0070
PRB-8901	0.014	0.043	0.154	0.039	0.005	-0.001	-0.0040
IC-13141	0.01	0.046	0.156	0.033	0.007	-0.002	-0.0020
IC-13145	0.007	0.035	0.16	0.003	0.005	-0.002	-0.0020
BDS-1354	0.03	0.034	0.16	0.034	0.002	-0.002	-0.0020
IC-13411	0.017	0.045	0.158	0.031	0.003	-0.002	-0.0014
LSD P0.05 for accession = 0.028							
	NAR (mg/Cm^{-2} leaf area d^{-1})						
IC-18889	0.021	0.43	0.487	0.212	0.008	-0.018	-0.002
Kalu	0.068	0.398	0.488	0.200	0.018	-0.006	-0.002
PRB-8901	0.037	0.400	0.490	0.174	0.019	-0.005	-0.014
IC-13141	0.028	0.398	0.483	0.134	0.024	-0.005	-0.008
IC-13145	0.020	0.401	0.518	0.128	0.015	-0.007	-0.002
BDS-1354	0.074	0.223	0.600	0.187	0.099	-0.008	-0.004
IC-13411	0.043	0.410	0.490	0.120	0.114	-0.007	-0.006
LSD P0.05 for accession = 0.042							
	LAR (cm^{-2} leaf area/ dry weight)						
IC-18889	0.085	0.50	0.230	0.240	0.320	0.330	0.310
Kalu	0.100	0.66	0.249	0.246	0.280	0.267	0.259
PRB-8901	0.102	0.63	0.239	0.274	0.300	0.290	0.280
IC-13141	0.085	0.68	0.240	0.280	0.300	0.286	0.279
IC-13145	0.082	0.65	0.230	0.270	0.312	0.290	0.292
BDS-1354	0.104	0.65	0.246	0.282	0.314	0.300	0.280
IC-13411	0.110	0.68	0.230	0.230	0.310	0.316	0.298
LSD P0.05 for accession = 0.284							

Fig. 4.4 : Changes in the shoot dry weight with time in 7 accessions of common buckwheat (Fagopyrum esculentum Moench)during growth under natural field condition.

Fig. 4.5 : Changes in the root dry weight with time in common buckwheat (Fagopyrum esculentum Moench) during growth under natural field conditions.


IC-18889
Kulu
PRB-8901
IC-13141
IC-13145
IC-13411
BDS-1354
Shoot dry weight (mg)
0
20
40
60
80
100
7
19
31
43
55
67
Days after planting

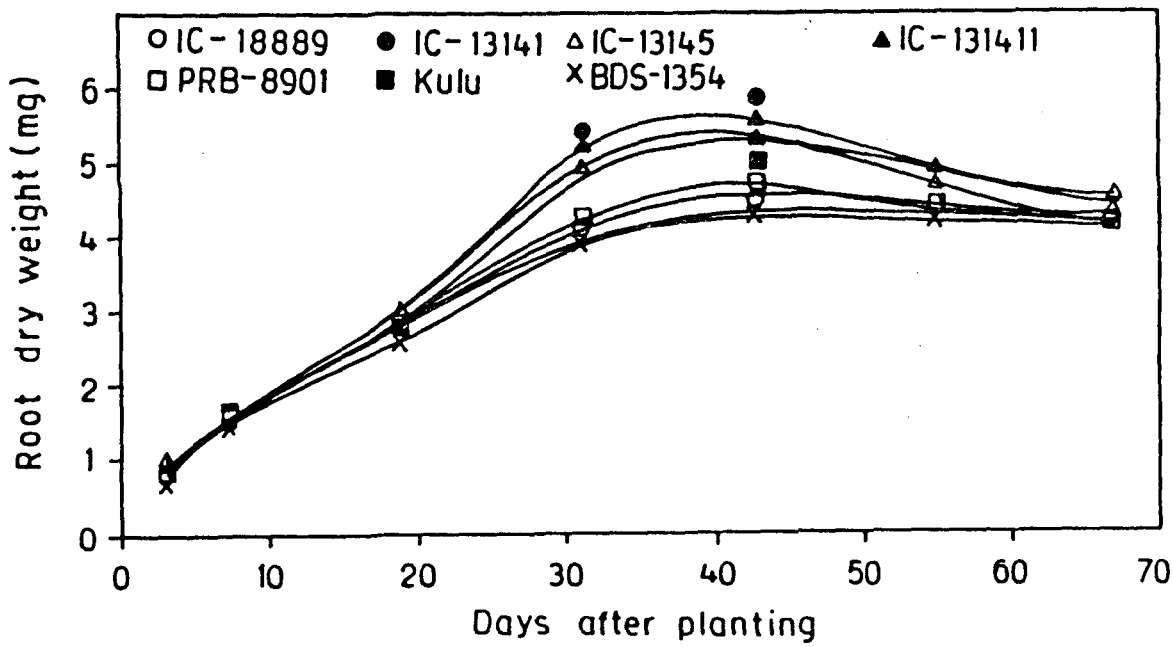
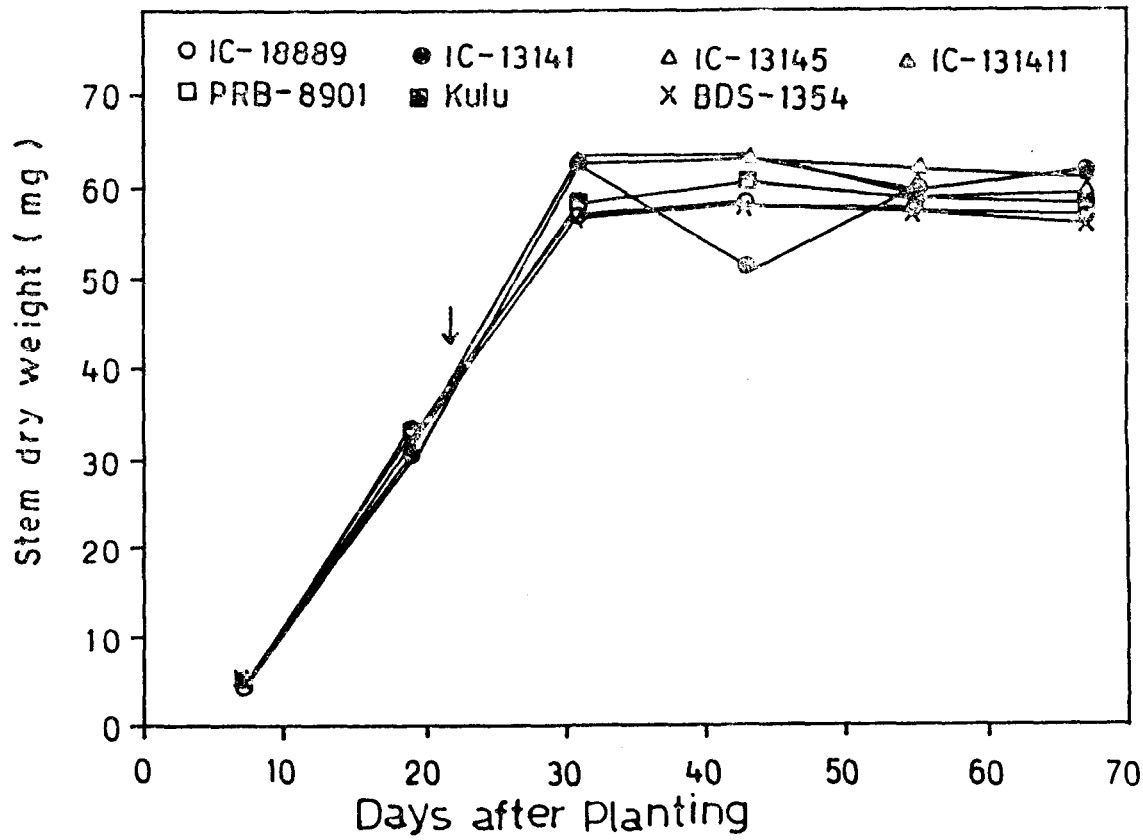

IC-18889
IC-13141
IC-13145
IC-131411
PRB-8901
Kulu
BDS-1354
Root dry weight (mg)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
Days after planting

Fig. 4.6 : Changes in the stem dry weight with time in 7 accessions of common buckwheat (Fagopyrum esculentum Moench) during growth under natural field condition.

Fig. 4.7 : Changes in the leaf dry weight with time in 7 accessions of common buclwheat (Fagopyrum esculentum Moench) during growth under natural field condition.


IC-18889
IC-13141
IC-13145
IC-131411
PRB-8901
Kulu
BDS-1354
Stem dry weight (mg)
Days after Planting

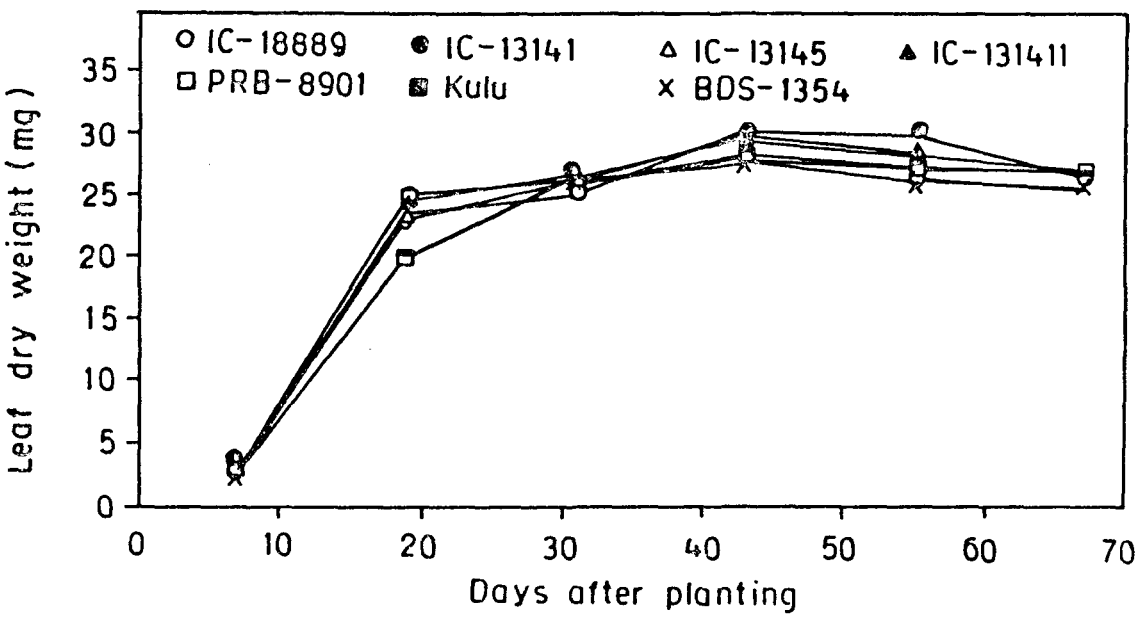
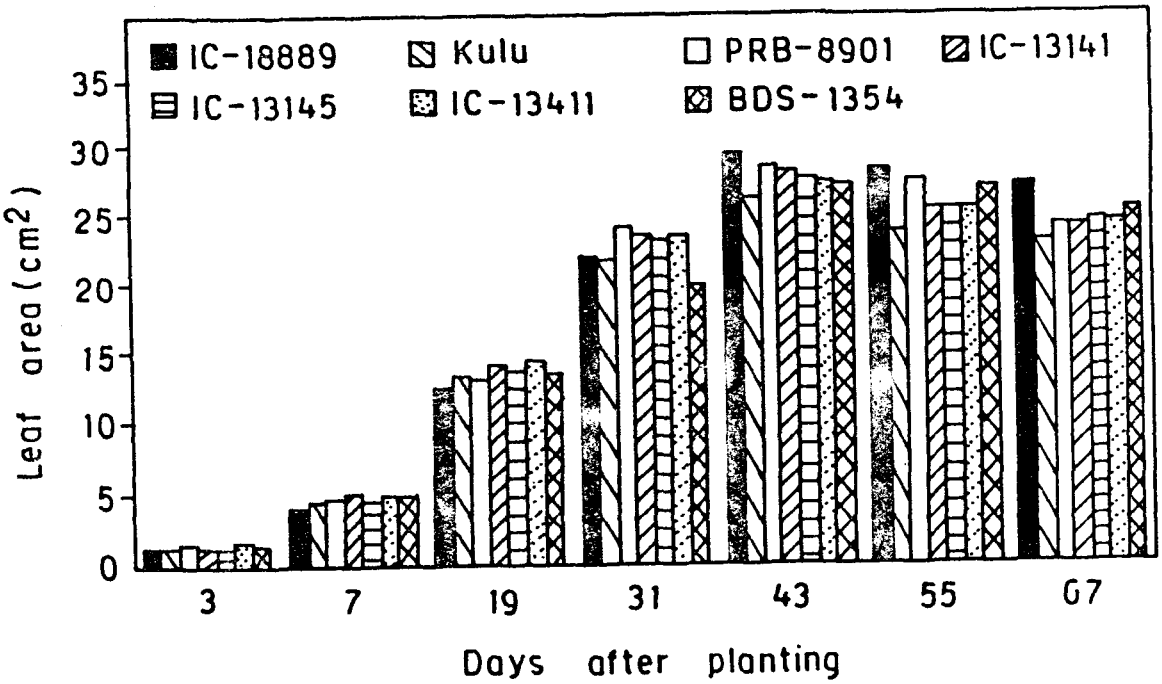
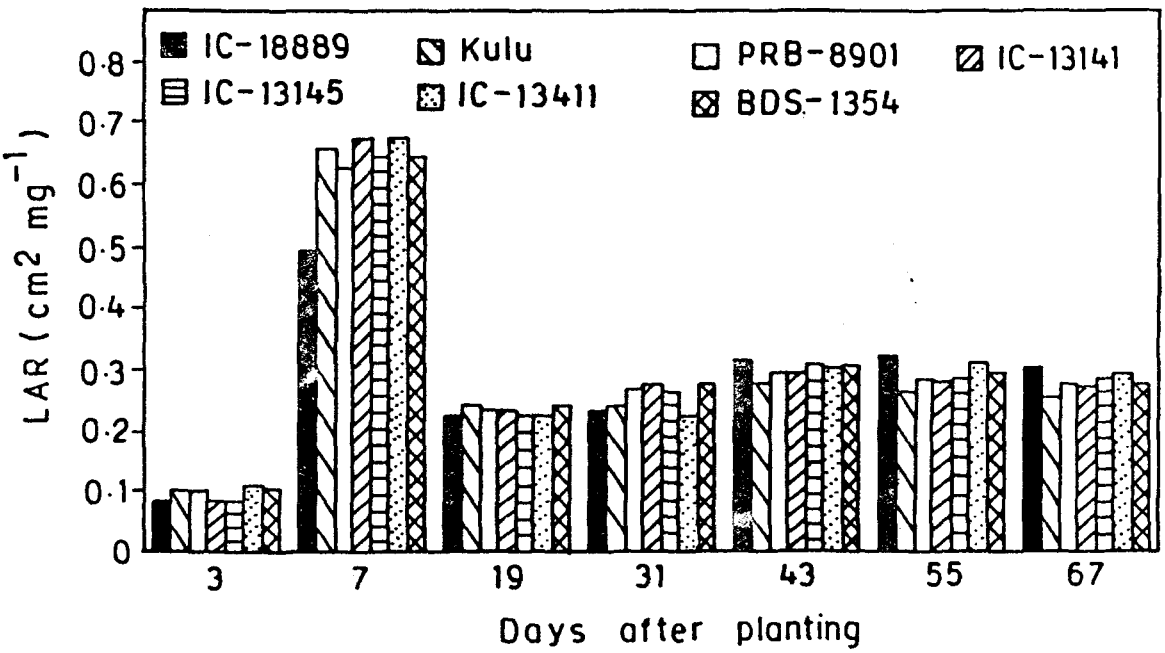

IC-18889
IC-13141
IC-13145
IC-131411
PRB-8901
Kulu
BDS-1354
Leaf dry weight (mg)
Days after planting

Fig 4.8 : Changes in the leaf area with time in 7 accessions of common buckwheat (Fagopyrum esculentum moench) during growth under natural field condition.

Fig 4. 9 : Changes in the leaf area ratio with time in 7 accessions of common buckwheat (Fagopyrum esculentum MOench) during growth under natural field condition.

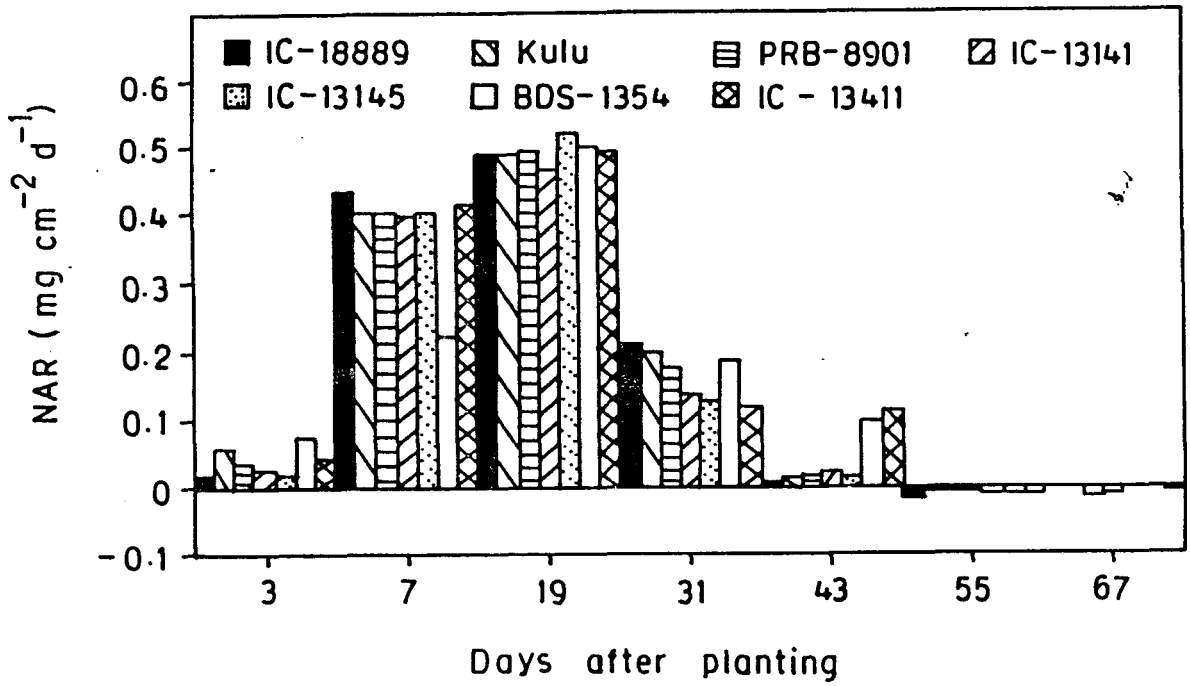

IC-18889
Kulu
PRB-8901
IC-13141
IC-13145
IC-13411
BDS-1354
Leaf area (cm²)
0
5
10
15
20
25
30
35
3
7
19
31
43
55
67
Days after planting

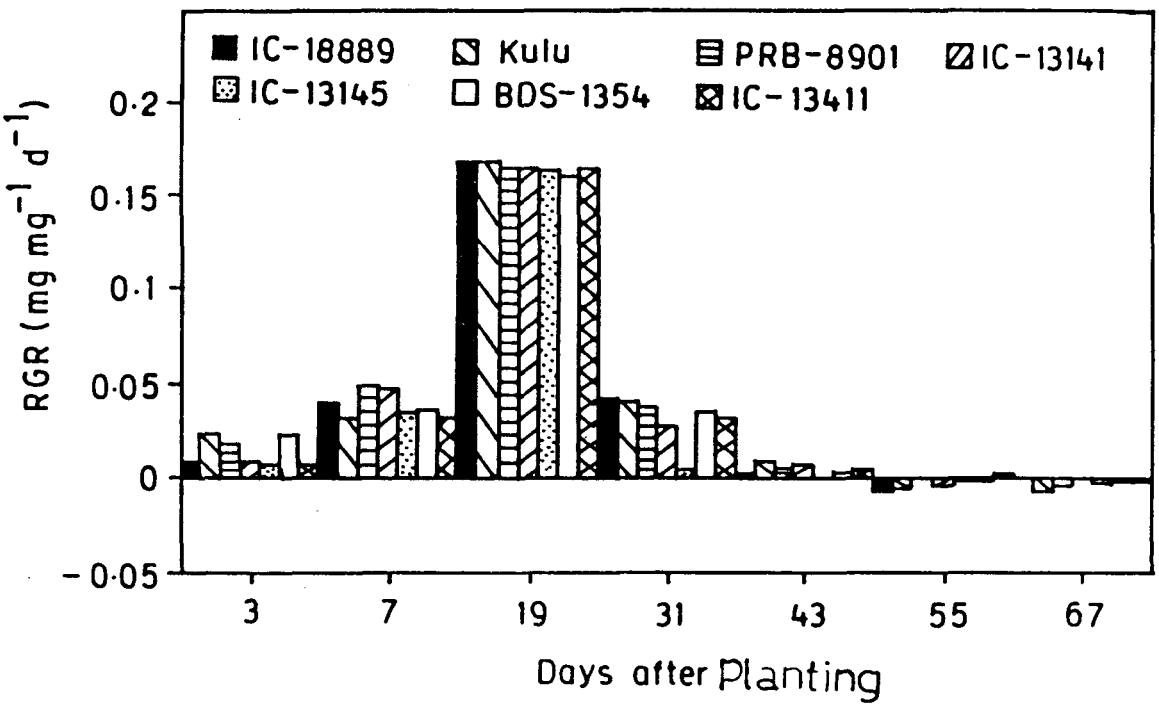

IC-18889
Kulu
PRB-8901
IC-13141
IC-13145
IC-13411
BDS-1354
LAR (cm² mg⁻¹)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
3
7
19
31
43
55
67
Days after planting

values for RGR showed a progressive increase till 19 days, recording a more than 3 fold increase during the period. After 20th day, however, the relative growth rate of plants started to decline with progressing time. While, the values showed a positive sign between 20 and 43 days, a negative deviation for the relative growth rate was observed between 43 and 67 days. The highest values for RGR were recorded on 19th day. Even though Kulugangri and BDS-1354 showed slightly higher values for RGR during the first week of growth, there were no significant differences in RGR between the accessions at any stage of growth (Table 4.6, Fig. 4.10). The net assimilation rate (NAR) expressed as mg cm^2 leaf area d^{-1} for each of the seven accessions increased between 3rd and 19th day of growth after which it started to decline with time. However, the magnitude of increase was more marked between 3rd and 7th day than during the time interval between 7 and 19 days. Positive values for the parameter were, however observed till 43 days after planting. Beyond 43rd day of growth values for NAR showed a negative deviation. While IC-18889, Kulugangri, PRB-8901, IC-13141, IC-13145 and IC-13411 showed a similar pattern and rates of net assimilation of dry matter, the values for NAR for BDS-1354 showed marked differences from those recorded for other accessions. In case of BDS-1354 the NAR showed a longer duration for higher values (Table 4.6, Fig. 4.11).

Fig 4. 10. : Changes in the relative growth rate with time in 7 accessions of common buckwheat (Fagopyrum esculentum Moench) during growth under natural field conditions.

Fig 4. 11 : Changes in the net assimilation rate with time in 7 accessions of common buckwheat (Fagopyrum esculentum Moench during growth under natural field condition.


IC-18889
Kulu
PRB-8901
IC-13141
IC-13145
BDS-1354
IC - 13411
NAR (mg cm^{-2} d^{-1})
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
3
7
19
31
43
55
67
Days after planting


IC-18889
Kulu
PRB-8901
IC-13141
IC-13145
BDS-1354
IC-13411
RGR (mg mg^{-1} d^{-1})
0.2
0.15
0.1
0.05
0
-0.05
3
7
19
31
43
55
67
Days after Planting

Discussion

Our results on the chemical composition of buckwheat grains reveals that the various nutritive components of the crop are comparable to those reported for cereals like rice and wheat. Joshi and Paroda in their monograph "buckwheat in India" reported that while the grain is variously used as an article of food in different countries the forage of the crop is extensively used as fodder for cattle Proteins being one of the determinants of nutritive value of any food grain, numerous workers have analysed the protein content in different cultivators of buckwheat. While Pomeranz and Robbins (1972) have found 13.7 percent protein in ten Buckwheat cultivars, Kraft and Javornik (1979) have reported a maximum of 11.5 percent protein in thirteen cultivars of buckwheat. Kirilenko (1981) has determined the grain protein content in some varieties of common buckwheat derived from various breeding techniques. He could not find significant differences in protein content in the same variety grown in different years. The range of protein content was from 15 percent to 18 percent. In the present investigation the seven accessions of common buckwheat *Fagopyrum esculentum* Moench showed an average of 11.5 percent of protein content on grain dry weight basis. There were no significant differences in the protein content in the grain from the seven accessions of buckwheat analysed in our laboratory.

Various investigators in their studies with buckwheat grains, have reported that besides containing a high level of protein the grains also a good source of carbohydrate and fats. Faroog and Tahir (1988) reported that the leaves of *F. esculentum*, *F. Sagittatum*, *F. tataricum* and *F. kashmirianum* had higher levels of sugars and starch and relatively lower levels of total phenolics than the other species indicating that it is more suitable as a green vegetable. The total carbohydrates content of 70 percent in grains of common buckwheat as observed in the present study was comparable to the amount of 72.9 percent as reported by Joshi and Paroda in their monograph "Buckwheat in India". The lipid and phenolics content of 6 percent and 2.5 percent respectively makes the plant suitable for human consumption.

Even though the buckwheat seeds constitute an important source of dietary proteins and carbohydrates and high biological value (Pomeranz 1973, Pomeranz *et al.*, 1975) it carries an inherent property of less digestibility (Food Policy and food science service FAO 1970, Farrell 1978, Eggum *et al.*, 1981, Thacker *et al.*, 1983). Ikeda and Kusuno (1978) have presented evidence for the occurrence of a trypsin inhibitor in buckwheat seed. There are also some reports indicating the presence of a proteinase inhibitor in buckwheat seed (Laporate and Tremolieres 1962, Pokrouski *et al.*, 1978 and 1980). The low digestibility has been ascribed to the presence of the trypsin inhibitors in buckwheat grains, by these workers.

Joshi and Paroda (1991) have reported that buckwheat is a short duration crop (3-4 months) and requires moist and cool temperate climate to grow. They have further reported that it is a suitable crop for summer season at higher altitudes. In the present investigation, seeds of *Fagopyrum esculentum* Moench were sown in the month of July. The crop attained maturity in about 6 weeks time and completed its life cycle in about 9 to 10 weeks. However, because of the indeterminate growth habit the time period of flowering extended from about 4 to 7 weeks after planting (Table 4.7). Among the severn accessions of buchwheat BDS-1354 distinguished itself by possessing determinate growth habit and synchronization of seed maturity. Therefore BDS-1354 can be introduced among the farmers for wider cultivation. The percentage of germination observed with *Fagopyrum esculentum* Moench was comparable with the 90 percent germination observed by Gohil and Rathar (1981) with different accessions of buckwheat from western Himalayas. Common buckwheat (*F. esculentum* Moench) is a herbacious erect annual attaining a height of 60 to 180 cm. (Joshi and Paroda 1991). The stem is hallow, angular with swollen nodes with red, pink and green colour. The leaves are alternate triangular with the blades being hesitate or cordate. The upper leaves were almost sessile, but lower leaves with petiole of considerable length. The in florescence was auxiliary and terminal Cyme with more or less densely clustered flowers (Fig. 4.12).

Table 4.7 : Changes in the harvest index, days of apprearance of flower, days of appearance of grain and grain weight in common buchwheat (*Fagopyrum esculantum* Moench).

Accession	Harvest index (%)	Days of appearance of flower	Days to appearance of first grains	Total grain weight (grams) per plant
IC-18889	2.006	21	30	2.506
Kulugangri	2.120	24	30	2.730
PRB-8901	2.176	22	32	2.800
IC-13141	2.170	22	29	2.744
IC-13145	2.220	24	29	2.730
BDS-1354	2.600	22	30	3.220
IC-13411	2.080	24	30	2.548

Fig 4. 12 : The plants of 7 accessions of common buckwheat (Fagopyrum esculentum Moench) grown in the botonical garden of Botany Department of North-Eastern Hill University Shillong : A - 7 th day after planting ; B- 19th day after planting ; C- 43rd day after planting.

A

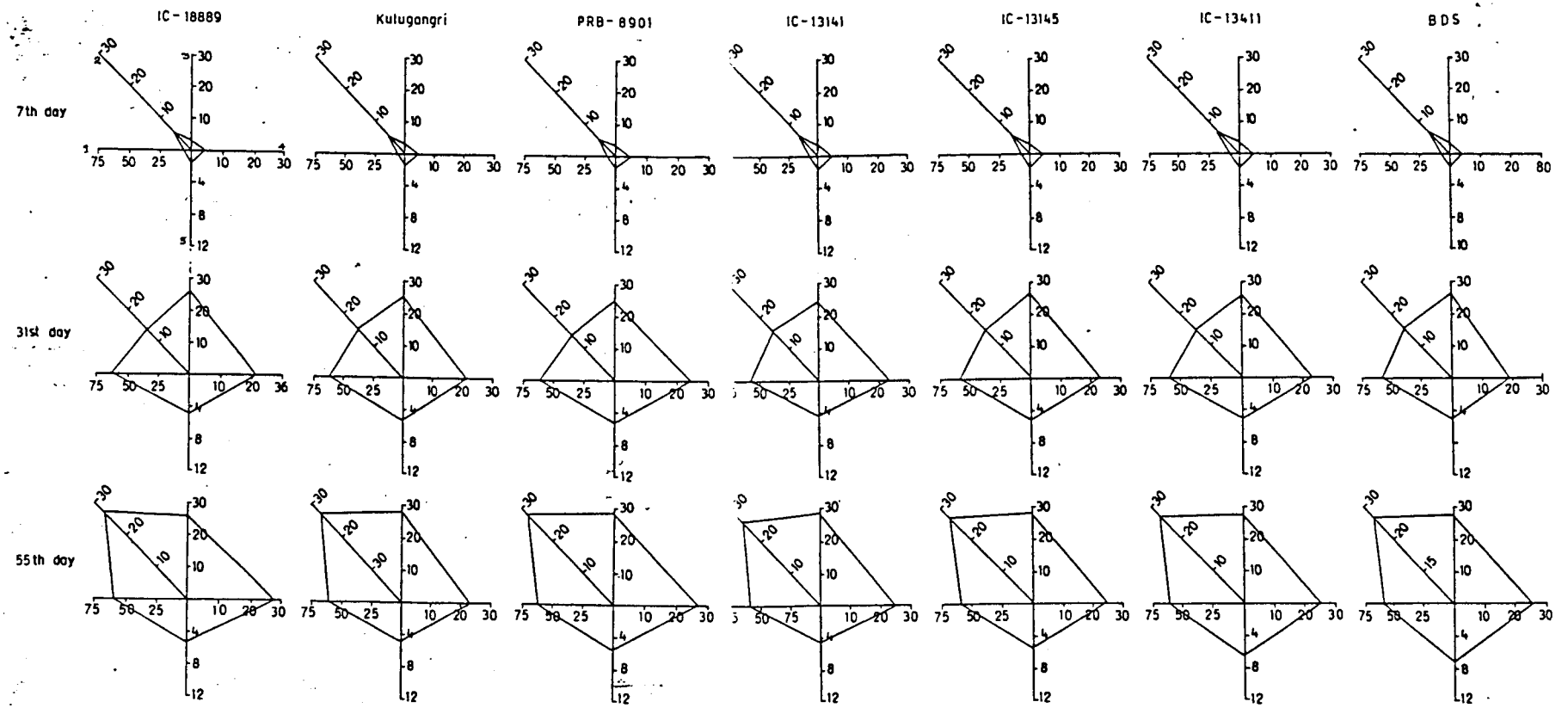
B

C

Joshi and Paroda (1991) have reported 408 accessions of buckwheat in their monograph. Buckwheat Catalogue reported by Joshi and Paroda was prepared by using; plant height, number of branches, number of internodes, colour of stem, number of leaves, petiole length, colour of petiole, length of the leaf, leaf breadth, blade shape, leaf colour at maturity, leaf margin colour, flowering, flower colour, maturity, length of cyme, seeds per cluster, seed colour and seed shape as the descriptors. Taking the above mentioned descriptors as the reference the seven accessions of buckwheat as analysed by us fall under a single category except minor fluctuations in their growth pattern. An analysis of the polygonal diagram in which variables such as dry weight of stem, shoot, leaf, root and leaf area, for the seven accessions at various stages of growth, have been combined into one figure (Fig. 4.13), reveals that the seven accessions did not differ from each other markedly in their growth characters. However, scanning electronic microscopic observation of seed coat of the seven accessions revealed differences in their seed coat pattern and thickness. Based on the pattern and thickness, the seven accessions could be classified into three groups.

In so far as the kinetics of growth is concerned all the seven accessions showed the same behaviour except minor differences in their growth curves. When tested for significance at 5 percent probability, the differences however, proved to be insignificant. The results of dry

Fig 4. 13 : Polygonal diagram representing variations with time in the Stem dry weight (1) Total shoot dry weight (2) Total leaf dry weight (3) Total leaf area (4) and Total root dry weight (5) in 7 accessions of common buckwheat (Fagopyrum esculentum MOench)


IC - 18889
Kulugangri
PRB - 8901
IC - 13141
IC - 13145
IC - 13411
BDS
7th day
31st day
55th day

matter accumulation, revealed by dry weight data of shoot, stem and root, separately, showed that the plants achieved a maximum rate of dry matter accumulation in about three weeks after planting. However, a declining trend was observable after 40th day after planting. This declining trend could be attributed to dry matter loss from these region of the plant during later stage of growth. While people differ in their views on the fate of the carbohydrate and dry weight lost from the stems, it is generally accepted that during later stage of growth there is a shift in the sink from stem/leaves to the grains. The loss in dry weight has also been reproted to be due to actual transportation of dry matter from shoot to the grains during later stage of development. The estimate varying from 2.7 percent of grain yield in wheat (Rawson and Evans 1971) to 70 percent in barley (Gallagher, *et al.*, 1975). It seems probable that there are differences between species, and between genotypes of a species, in the amounts of stem losses which can occur and in the extent to which substances lost are translocated to the grains. Austin *et al.* (1977) have reported that the dry weight loss from internodes of the stems of six genotypes of wheat as 172 gm cm^{-2}. They have viewed the loss of materials from stem as a reflection of the balance between the demands exerted by the grains and the supply from the assimilatory organs.

In all the seven accessions of *Fagopyrum esculentum* Moench the leaf area (cm^2) reached its maximum on 43rd day. However, LAR in all the seven accessions reached its peak in

between 7th and 19th day after planting. In the present study direct relationship was observed between leaf area and dry matter accumulation. The increase in dry weight of the shoot corresponded with the changes in the total leaf area of the plant. Leaves being the source of photosynthates, their contribution towards the dry matter accumulation naturally has a significant role. However, opinions on the significance of LA as well as LAR as a growth index in the conventional growth analysis vary.

Watson (1952, 1963) has laid much emphasis on leaf area as an index of growth. According to him, leaf area is a better determinant of crop growth, mainly because, the photosynthetic capacity of the crops depends on leaf area which in turn responsible for dry matter production. Intervarital difference in CGR have been found almost invariably to be positively related to leaf area of the various varieties of pea (Mahon, 1982), wheat (Rewson *et al*., 1983) and perenial rye (Rhoden, 1972). Correlation between leaf growth and tuber growth in potato have been described by Humphries and Dyson (1967). They found that connection between leaf growth and tuber growth in potato. They found that a growth inhibitor which slowed leaf growth hastened tuber growth for a few weeks. This result suggest that supply of photosynthate controls dry matter accumulation in the potato tubers. However, in the view of Jalliffe *et al*., (1990) leaf area ratio was the least interesting factor in growth analysis. They have been tempted to make such a suggestion because leaf

area ratio was not strongly affected by population density treatments. Therefore, they turned their attention towards soil resources and suggested that the competition for soil nutrients and/or oxygen may have been the main source of interference during crop growth. In the present investigation though attention was not paid on the interference of soil nutrients and oxygen on growth, all the seven accessions tested were cultured in the same field with similar environmental and edaphic factors.

The NAR for the seven accessions, increased upto 19 days after which it showed a rapid decrease between 19th day and 31st day. Net assimilation rate is the net difference between the amount of dry matter assimilated and respired. According to Shivakumar and Shaw (1978) important in the calculation of NAR by classical methods is the assumption that dry weight is linearly related to leaf area. In their observation with soyabeans, they found that there was a linear relationship between leaf area and dry matter accumulation. They further suggest that NAR, like RGR has photosynthetic and respiratory components and the relative importance of respiration increases with plant age. Kollar *et al.*, (1970) has interpreted the increase in the NAR with progressing time as a response of the photosynthetic apparatus to an increased demand for assimilates. The decline in the NAR after 19 days, as observed in the present study could be attributed to either/or (a) diversion of dry matter towards grains; (b) a lower photosynthetic activity in lower

leaves due to mutual shading and (c) increase in the respiratory activity during later stage of growth.

The plants of common buckwheat showed a maximum RGR value of 0.16 mg mg^{-1} dry weight^{-1} d^{-1} and a maximum NAR value of 0.6 mg cm^{2} leaf area^{-1} d^{-1}. These values compare well with the rates of 0.167 and 0.445 for RGR and NAR, respectively, reported by Blackman and Wilson (1950) in case of *Fagopyrum esculentum* Moench. A large number of workers have reported that relative growth rate change with time (e.g. Blackman, 1961; Eagles, 1969; Evans, 1972). Hunt and Burnett (1973) showed that it only changed slightly over a period of eight weeks in plants of *Lolium perenne*. Unit shoot root resembles unit leaf rate expect that the increase in the total weight of the plant is calculated relative to the weight of the shoot rather than leaf area. Unit leaf rate has been reported by a number of workers to show no significant trends with time during the vegetative phase of growth (Heath, 1937; Hammond and Kirkham, 1949; Blackman and Wilson, 1951). Other workers have reported a decline in unit leaf rate with age (Ballard and Petrie, 1936; Eagles, 1971).

Blackman and Wilson (1950) in their comparative studies with *H. annus* and *F. esculentum*, have correlated relative growth rate with light intensity. They found that the maximum relative growth rate was attained by *Fagopyrum esculentum* at higher intensity of light and the magnitude of relative growth rate at any light intensity is dependent on

the net assimilation rate and the leaf area ratio. However, the result obtained from their data showed that higher leaf area ratio of buckwheat more than offset any difference in the assimilation rates in determining the greater relative growth rate of *F. esculentum*.

The low yield of *F. esculentum* Moench grown in the Botany Department field of NEHU could be attributed to climatic and adaphic factors prevailed on the particular period of plant growth of the region. Veremichik (1972) and Gubbels (1978) found that the yield of common buckwheat increased with high soil moisture. Ruszkowski and Zebrowski (1982) have reported that, in the same climatic conditions the productivity of buckwheat is higher on heavier soil than on lighter soil. Krotov (1963) reported that flowering at temperatures above 30°C is accompanied by desiccation of fruit and lowering of yield. Since the temperature in Shillong is on an average lower than 30°C, the possibility of desiccation of fruits due to high temperature can be ruled out. However, lighter soil, less moisture content, late spring and early fall frosts and shading of the field could be the causative factors for low yield.

The present investigation deals with the importance prospects and limitations which are associated with the classification and cultivation of seven accessions of *Fagopyrum esculentum* Moench in the North-Eastern regions. The results of the present investigation clearly reveals that

the criteria used for the classification of accessions are arbitrary and some more physiological features should be taken into consideration for the same purpose. Even though, the seven accessions differ from each other morphologically as supported by SEM photographs of seed coat morphology, they show similar values in their nutritive content and growth pattern.

CHAPTER V

Kinetics of Nitrate by Seedlings and Isolated Roots

i) Experimental

ii) Results

iii) Discussion

EXPERIMENTAL

Seeds of common buckwheat (*Fagopyrum esculentum* Moench) were washed for 1 hour under running tap water followed by rinsing with deionized water. The rinsed grains were germinated for 48 hours in darkness at 27±+2°C. The 48 hours old germinated seeds were transferred to a solution of 0.2 mM $CaSO_4$ and maintained there for 24 hours. The 72 hour old seedlings were transferred to a full strength nitrate free Hoagland's nutrient medium and maintained in a growth chamber under continuous white light (30 mol $Cm^{-2}S^{-1}$) at 27±2°C and 65 percent RH. The solution was aerated to provide ample oxygen and solution mixing.

Experiments were initiated by placing two eight-day old seedlings (grown as above) in 50 ml Pyrex beakers containing 25 ml of full strength Hoagland's nutrient solution supplemented with 5mM nitrate given as KNO_3^-. The seedlings were held in the centre of the beaker by a stainless steel wire mesh. For the determination of nitrate uptake as a function of time, samples, in triplicate, were drawn from each treatment solution at periodic intervals viz. 15, 30, 45, 60, 120, 180, 240 and 300 minutes : nitrate levels in 0.1 ml aliquots of the test solution were determined using the Brucine reduction method. To determine the effect of depletion of substrate concentration on the uptake of nitrate, an experiment was raised in which the level of nitrate in the ambient nutrient solution was kept constant throughout the duration of the experiment by periodic replenishments. The uptake of nitrate from such solution was determined by drawing suitable aliquots, in triplicate, at periodic intervals and estimating the content of nitrate in the aliquot. For studying the effect of substrate concentration, pH and metabolic inhibitors/activators on nitrate uptake by buckwheat seedlings, samples, in triplicate, were drawn from each treatment solution at t_{15} and t_{90} minutes; the content of nitrate in the sample was determined as described above. The values for uptake, expressed as μmol NO_3^- taken up mg dry weight root^{-1} min^{-1}. were computed from the concentration of nitrate and the volume of the solution at

each sampling. The Km and Vmax values were calculated from the data of uptake vs. NO_3^- concentration in the nutrient medium using the Lineweaver-Burk plot. After the termination of the experiment, the two seedlings were harvested. One of the seedlings was dried and used for the determination of tissue level of total and nitrate nitrogen. The other seedling was used for the assay of NR activity. *In vivo* nitrate reductase activity was measured in the shoot as well as the root tissues of the harvested seedlings as described in Materials and Methods.

RESULTS

Expressed as cumulative uptake, the seedlings of common buckwheat showed a linear and steady uptake of NO_3^- with time during the first hour without any lag phase; the uptake of nitrate gradually slowed down beyond 60 minutes until it attained a plateau at t_{180} min. (Table 5.1; Fig. 5.1). Between 0-180 minutes, nearly 25 µmol of NO_3^- were taken by each mg dry weight of the root. Correspondingly, the concentration of nitrate in the ambient nutrient medium showed a gradual decrease with progressing time up to t_{180} min. While the nutrient solution had a concentration of 5 mM NO_3^- at t_0, the level of nitrate ions in the medium showed a progressive decrease with time till t_{180} minutes when it was 1.5 mM, thereby recording a more than 3 fold increase (Table

5.1; Fig. 5.2). An analysis of the uptake of nitrate from the ambient medium by the seedlings as a function of time revealed that the rate of uptake was maximum during the first 30 minutes. During this period the seedlings showed an uptake rate of 0.37 μmol NO_3^- mg dry weight $root^{-1}$ $minute^{-1}$. The rate of uptake, however, decreased gradually with a progressing time till no significant uptake was observed at 240 minutes. The decline in the rate of uptake was significantly marked between 30 and 120 minutes of incubation. During this period a nearly five fold decrease in the rate of uptake was observed. After 120 minutes, however, the rate of uptake remained by and large stationery (Table 5.1; Fig. 5.2). In order to determine the effect of decrease in the concentration of nitrate in the ambient nutrient medium on the uptake of nitrate by seedlings experiments were raised in which the concentration of nitrate in the ambient nutrient medium was maintained at a constant level by periodic replenishments. The uptake of nitrate by the seedlings under such conditions showed a pattern similar to that observed for seedlings maintained under conditions of depleting external nitrate concentration. The seedlings showed a maximum uptake rate of 0.42 μmol/mg dry weight root/ minutes which was maintained upto 45 minutes of incubation. After 45 minutes, the uptake rate showed a study decline with progressing decline upto 240 minutes; the decline in the uptake rate was, however, more marked between 45 and 120

Table 5.1 : Changes in the uptake of nitrate by seedlings of common buckwheat (*F. esculentum* Moench) growing under hydroponic culture in Hoagland's nutrient solution supplemented with 5 mM KNO_3^-, and the concentration of nitrate in the nutrient medium, with progressing time.

Time (min.)	Cumulative uptake (μmol NO_3^- taken up/ mg dry weight root)	Uptake rate (μmol NO_3^- taken up/ mg dry weight/min.)	NO_3 Con. (mM) in the nutrient medium
0	0.00	0.00	0.00
15	5.50	0.36	8.00
30	11.15	0.37	4.00
45	14.23	0.20	3.30
60	16.15	0.12	3.10
120	20.38	0.07	2.90
180	24.23	0.06	2.00
240	24.23	0.00	1.50
300	24.23	0.00	1.50

Table 5.2 : Changes in the rate of nitrate uptake with time by seedlings of common buckwheat (*F. esculentum* Moench) cultured in Hydroponic system with Hoagland's nutrient solution supplemented with 5 mM KNO_3^- in which nitrate ions were maintained by periodic replenishment.

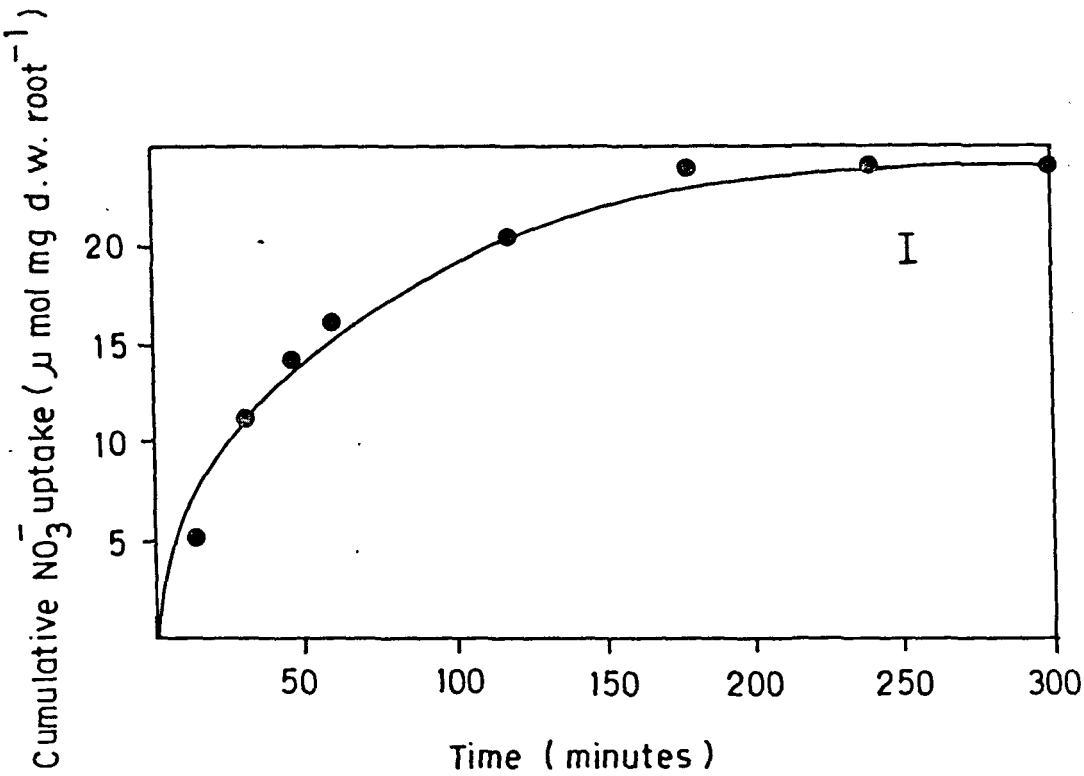
Time (Minutes)	Uptake rate (µmol NO_3^- taken up/mg dry weight root /minute
0	0.00
15	0.42
30	0.42
45	0.42
60	0.30
75	0.25
90	0.21
105	0.12
120	0.11
135	0.10
150	0.10
165	0.08
180	0.06
195	0.05
210	0.03
225	0.01
240	0.01

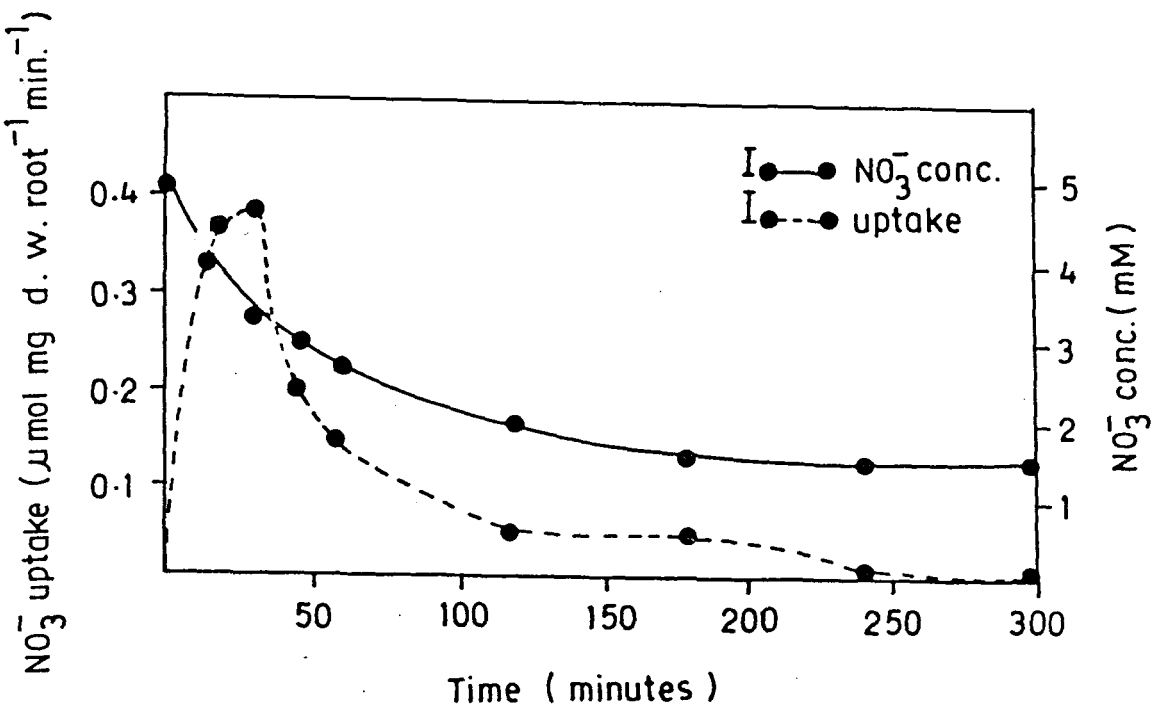
Table 5.3 : The effect of H^+ ion concentration of the nutrient medium on the uptake of nitrate by seedlings of common buckwheat (*F. esculentum* Moench) growing under hydroponic culture in Hoagland nutrient medium supplemented with 5 mM KNO_3^- .

pH	Uptake rate (µmol NO_3^- taken up/mg dry weight root /minute
2.0	0.00
3.0	0.02
4.5	0.12
5.0	0.16
5.5	0.32
6.0	0.34
6.5	0.34
7.0	0.16
8.0	0.16
9.0	0.06

Fig.5.1; Cumulative NO_3^- uptake as a function of time by seedlings of common buckwheat (*Fagopyrum esculentum*), from Hoagland's nutrient solution containing 5 mM NO_3^-. Vertical line in the figure represents LSD at $P_{0.05}$.

Fig.5.2; Nitrate uptake by seedlings of common buckwheat as a function of time. The solid curve depicts thechanges in the concentration of nitrate in the nutrient medium. The broken curve represents the change in the rate of uptake of nitrate by the seedlings with time. Vertical lines in the figure represent LSD at $P_{0.05}$.


Cumulative NO_3^- uptake (μ mol mg d.w. root^{-1})
20
15
10
5
I
50
100
150
200
250
300
Time (minutes)

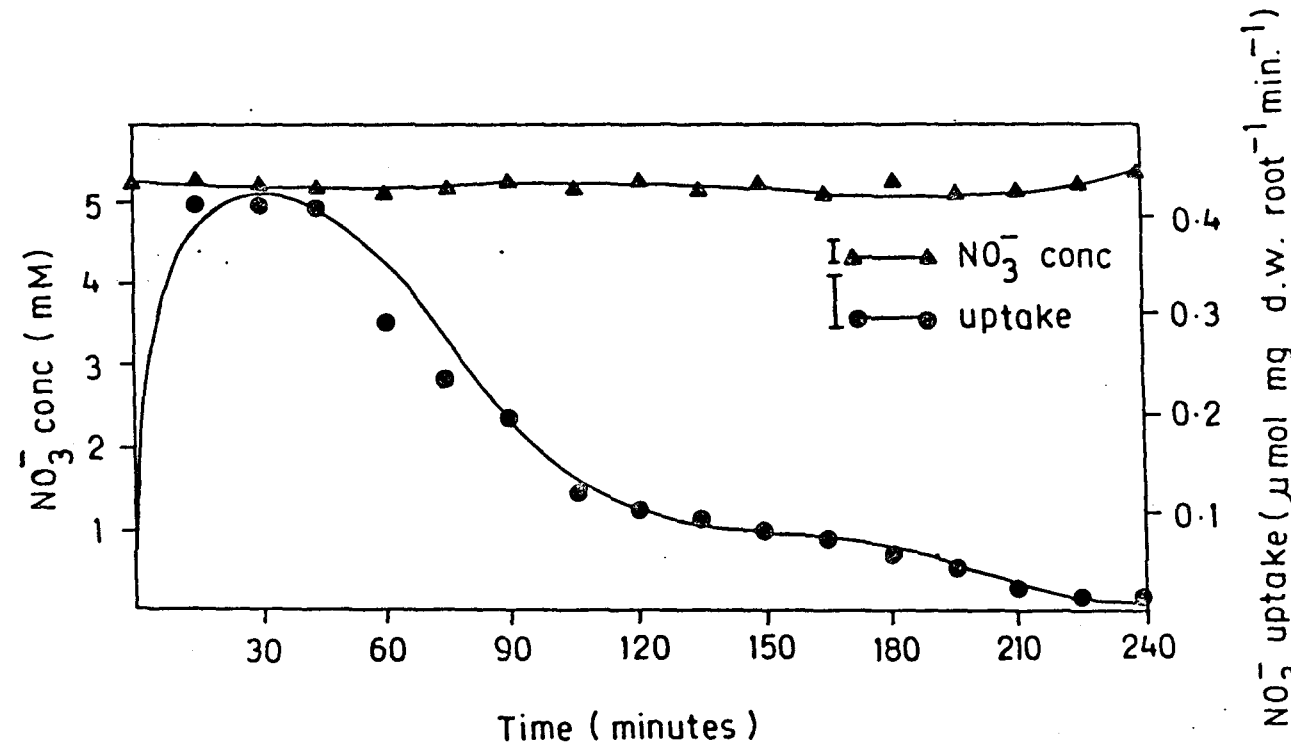

NO_3^- uptake (μ mol mg d. w. root^{-1} min.$^{-1}$)
0·4
0·3
0·2
0·1
I NO_3^- conc.
I uptake
5
4
3
2
1
NO_3^- conc. (mM)
50
100
150
200
250
300
Time (minutes)

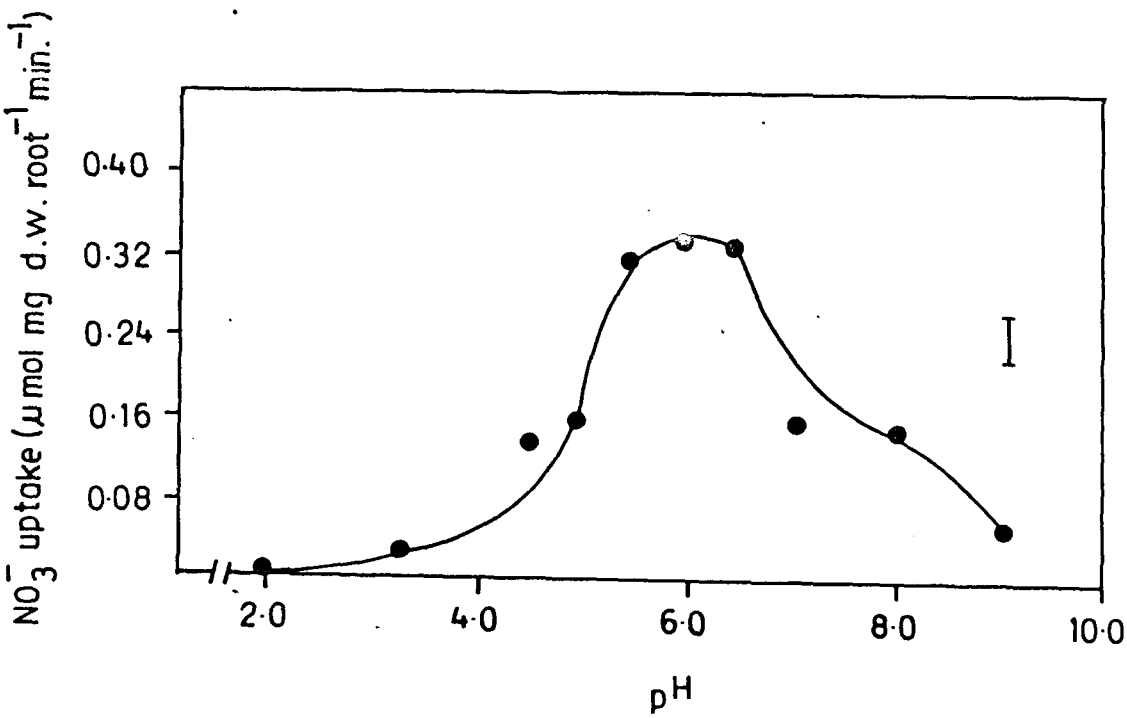
minutes (Table 5.2; Fig. 5.3). The change in the concentration of nitrate in the ambient nutrient medium, did not appear to have any significant influence on the rate of nitrate uptake. The H^+ concentration of the nutrient solution had a marked influence on the uptake of nitrate by the seedlings. Thus, in relation to pH, optimum uptake of nitrate was observed between pH 5.5 and 6.5 in 0.01 M Tris-HCl buffer. The uptake rate dropped significantly even a change of 0.5 units of pH, beyond the optimum range, towards the acidic or alkaline scale (Table 5.3; Fig. 5.4).

The uptake of nitrate by the seedlings was dependent on the concentration of nitrate in the ambient nutrient medium. Thus, when the concentration of NO_3 in the nutrient medium was varied from 0.05 to 5 mM, the rate of nitrate uptake by buckwheat seedlings was a function of external nitrate concentration, according to Michaelis-Menten Kinetics. Changes in the concentration of nitrate in the ambient nutrient medium resulted in a linear increase in the uptake of nitrate by the seedlings up to 1 mM external NO_3^- concentration. Thus, there was a four-fold increase in the uptake rate with change in the concentration of nitrate from 0.05 to 1 mM. Beyond 1 mM concentration of nitrate, the uptake of nitrate slowed down and tended to form a plateau. Thus a typical hyperbolic relationship could be observed for the relationship between the concentration of nitrate in the

Fig. 5.3 : Nitrate uptake by seedlings of common buckwheat (Fagopyrum esculentum MOench) as a function of time. The concentration of nitrate was adjusted by adding suitable amounts of NO_3^- at periodic intervals. The curve with data points (▲) represents concentration of nitrate in the nutrient medium. The curve with data points (●) depicts the changes in the rate of uptake with time. Vertical line in the figure represents LSD at p0.05

Fig. 5.4 : Effect of pH of the ambient nutrient medium on the uptake of nitrate by seedlings of common buckwheat (Fagopyrum esculentum MOench) Vertical line in the figure represents LSD at p 0.05


NO_3^- conc (mM)
NO_3^- uptake (µmol mg d.w. root^{-1} min.$^{-1}$)
NO_3^- conc
uptake
Time (minutes)
1
2
3
4
5
0·1
0·2
0·3
0·4
30
60
90
120
150
180
210
240


NO_3^- uptake (µmol mg d.w. root^{-1} min.$^{-1}$)
0·08
0·16
0·24
0·32
0·40
2·0
4·0
6·0
8·0
10·0
pH

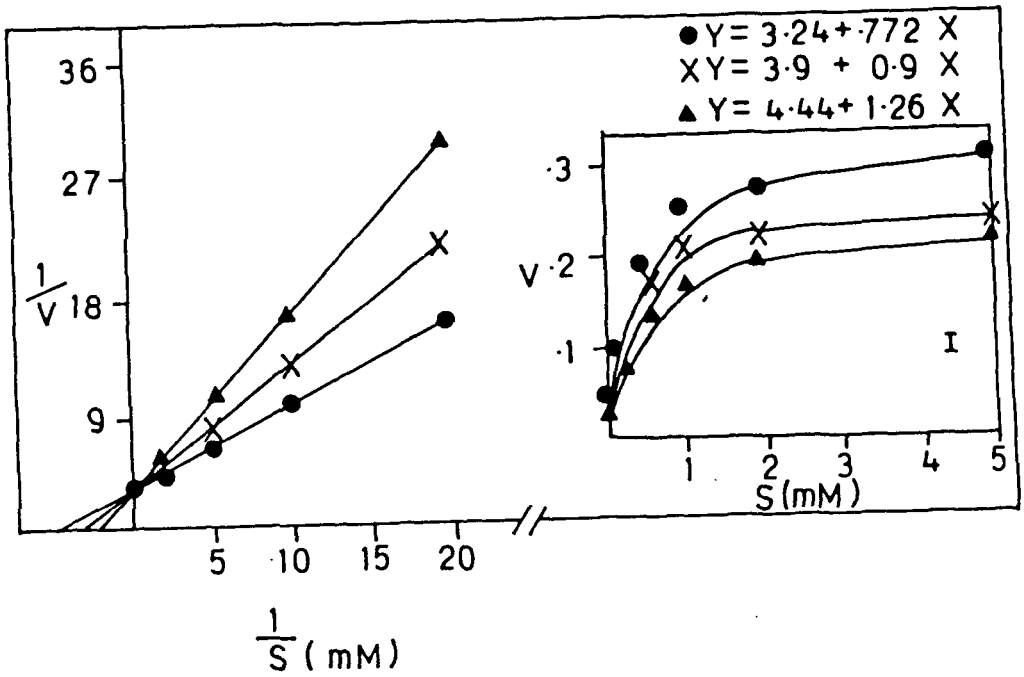
ambient nutrient medium and uptake of the ions in seedlings of common buckwheat. A plot of the values of 1/V against 1/S gave a linear relationship with a slope value of 0.772 and intercept value of 3.24. The Lineweaver and Burk plot for the relationship between uptake of nitrate by the seedlings and the concentration of nitrate in the ambient nutrient medium revealed a maximum velocity (V_{max}) of 0.276 μmol/mg dry weight root/minute and a Michaelis-Menten (Km) constant of 200 μ mol in the presence of 0.01 M Tris-HCl buffer at pH 6.5 (Table 5.4; Fig 5.5). Chlorate and ammonium ions markedly suppressed the uptake of nitrate by buckwheat seedlings; the magnitude of suppression increasing with increased concentrations of either NH_4^+ or ClO_3^- ions. When compared with the untreated controls, superimposition of 0.05 mM $KClO_3^-$ at 5 mM external nitrate concentration lead to a nearly two-fold decrease in the uptake of nitrate by the seedlings. At the same external nitrate concentration, however, 0.05 mM of NH_4^+ lead to a five-fold decrease in the amount of nitrate taken up by the seedlings (Table 5.4; Figs. 5.5, 5.6). A Lineweaver-Burk plot for uptake of nitrate as a function of external nitrate concentration, at different levels of either NH_4^+ or ClO_3^-, clearly revealed that while the inhibition due to NH_4^+ was non-competitive in nature, that due to the presence of ClO_3^- in the nutrient medium was of competitive in nature. Analysis of the regression of 1/V against 1/S in the presence of ClO_3^- ions in the ambient nutrient medium revealed

Table 5.4 : Effect of varying concentration of nitrate (substrate) on the rate of nitrate uptake (V) by seedlings of common buckwheat (*Fagopyrum esculentum* Moench) cultured under hydroponic culture in Hoagland nutrient solution supplemented with varying doses of either potasium chlorate or ammonium sulphate.

NO_3^- (mM)	Conc	Control		0.005 (mM) KCl O_3^-		0.05 (mM) KCl O_3^-		0.005 (mM) $(NH_4)_2SO_4$		0.05(mM) $(NH_4)_2SO_4$	
(S)	(1/S)	V	1/V	V	1/V	V	1/V	V	1/V	V	1/V
		Uptake rate (μmol NO_3^- taken up/mg dry weight root /minute									
0.05	20	0.05	20.00	0.045	22.20	0.033	30.30	0.016	80.40	0.012	83.3
0.1	10	0.05	20.00	0.066	15.15	0.055	18.18	0.027	37.03	0.020	50.0
0.2	5	0.09	10.98	0.108	9.25	0.083	12.00	0.039	25.60	0.033	30.3
0.5	2	0.20	5.00	0.166	6.02	0.141	7.09	0.055	18.18	0.041	24.4
1.0	1	0.25	4.00	0.208	4.80	0.166	6.02	0.075	13.33	0.049	20.4
2.0	0.5	0.26	3.84	0.225	4.44	0.208	4.80	0.083	12.04	0.052	19.2
5.0	0.2	0.30	3.24	0.241	4.14	0.235	4.29	0.116	8.62	0.058	17.2

Fig.5.5; Lineweaver and Burk plot for the relationship between substrate concentration and nitrate uptake in seedlings of common buckwheat in the presence of varying concentrations of ClO_3^- ions. ●, control; X, 0.005mM ClO_3^-; ▲, 0.01mM ClO_3^-. Inset: V/S relationship for the same. Vertical line in the inset figure depict LSD for any two observations at $P_{0.05}$.

Fig.5.6; Lineweaver and Burk plot for the relationship between substrate concentration and nitrate uptake in seedlings of common buckwheat in the presence of varying concentrations of NH_4^+ ions. ●, control; X, 0.005mM NH_4^+; ▲, 0.01mM NH_4^+. Inset: V/S relationship for the same. Vertical lines in the inset figure depict LSD for any two observations at $P_{0.05}$.


● Y = 3·24 + ·772 X
X Y = 3·9 + 0·9 X
▲ Y = 4·44 + 1·26 X
36
27
1/V 18
9
5 10 15 20
1/S (mM)
·3
V ·2
·1
I
1 2 3 4 5
S (mM)

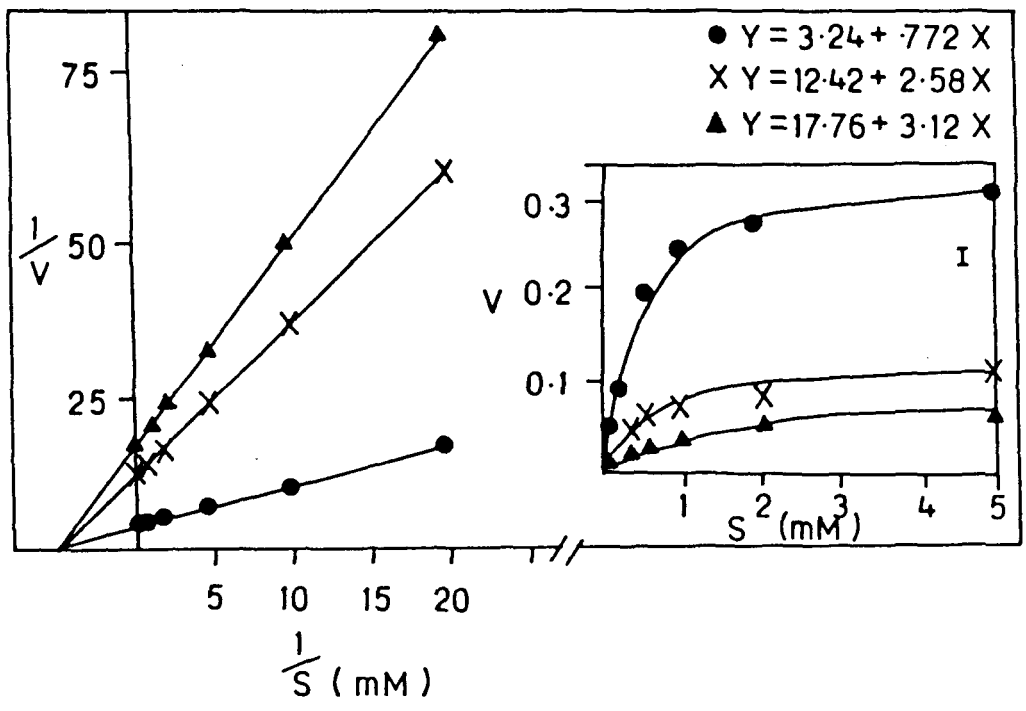

● Y = 3·24 + ·772 X
X Y = 12·42 + 2·58 X
▲ Y = 17·76 + 3·12 X
75
1/V 50
25
5 10 15 20
1/S (mM)
0·3
V 0·2
0·1
I
1 2 3 4 5
S (mM)

Table 5.5 : Valuews of a, b, r^2, Km and V_{max} for the relationship of 1/V against 1/S for nitrate uptake by seedlings of common buckwheat (*F. esculentum* Moench) growing under hydroponic culture in Hoagland's nutrient solution supplemented with varying level of NO_3^-.

	a	b	r^2	Km (µmol/mg dry weight root/ min.)	V_{max} (µmol/mg dry weight root/ min.)
Control	3.24	0.772	0.98	200	0.276
+					
0.005 (mM) $KClO_3^-$	3.90	0.900	0.94	307	0.276
+					
0.05 (mM) $KClO_3^-$	4.44	1.260	0.95	500	0.276
+					
0.005 (mM) NH_4^+	12.42	2.580	0.91	200	0.083
+					
0.05 (mM) NH_4^+	17.76	3.120	0.96	200	0.064

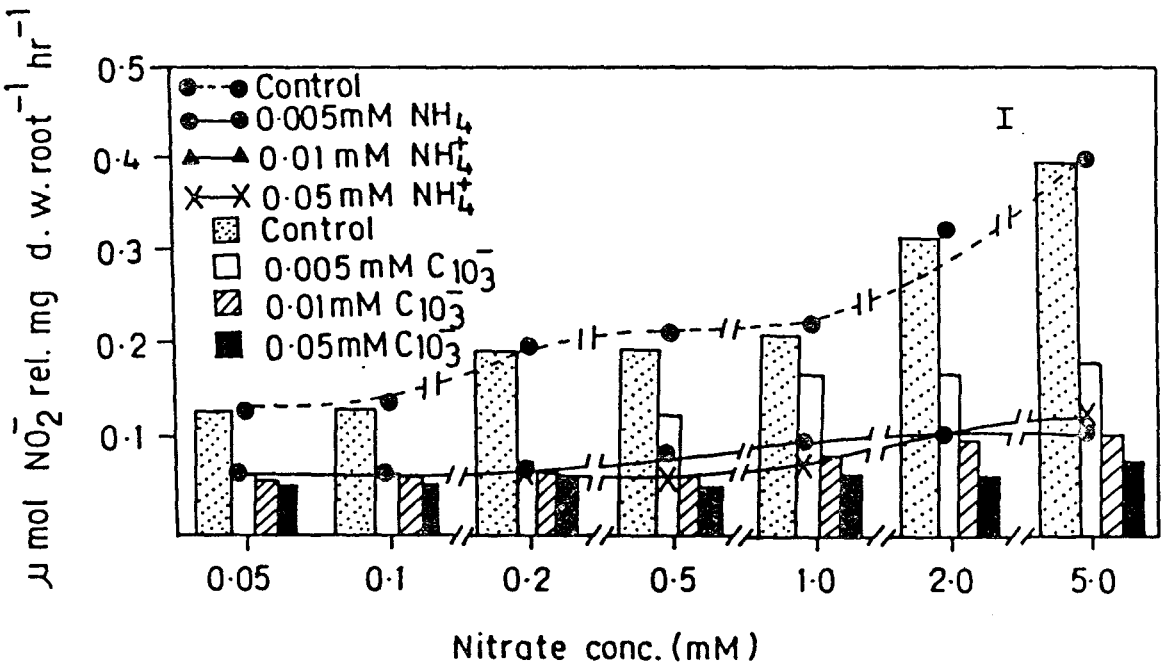
that presence of ClO_3^- ions in the nutrient medium affected both the intercept as well as the slope of the regression that described the relationship between the concentration of nitrate in the ambient medium and the uptake of nitrate by the seedlings. Ammonium ions did not affect the intercept but altered the slope of the regression. The values for V_{max}, Km, the intercept of the regression equation and slope for the relationship of 1/V against 1/S for uptake of nitrate by buckwheat seedlings in the control experiment as well as in presence of varying concentrations of ammonium and chlorate ions are presented in Table 5.5. Thus, the Km and V_{max} for the uptake of nitrate by seedlings of buchwheat under ydroponic culture in Hoagland's nutient medium, pH 6.5, was 200 μmol and 0.276 mM/mg dry weight root/minute respectively. In the presence of chlorate ions in the nutrient medium, the Km for the process was 307 and 500 μmol at chlorate ion concentration of 0.005 and 0.05 mM respectively. Chlorate ions had no effect on the V_{max} for nitrate uptake by buckwheat seedlings. While ammonium ions had no effect on the Km for the uptake of nitrate by buckwheat seedlings, the V_{max} for the uptake of nitrate was markedly affected. The uptake process showed a V_{max} of 0.083 and 0.064 μmol/mg dry weight root/minute in the presence of 0.005 and 0.05 mM NH_4^+ respectively (Table 5.5; Figs. 5.5, 5.6).

The activity of Nitrate reductase in the root as well as the in leaf tissues showed a progressive increase with increase in concentration of nitrate in the ambient nutrient medium. The magnitude of increase was, however, more marked in the root tissues than in the leaf. Thus, while there was a four-fold increase in the activity of nitrate reductase in root with increase in the concentration of nitrate in the ambient nutrient medium from 0.05 mM to 5 mM, the activity of the enzyme in the leaf tissues registered less than two-fold increase with increase in external nitrate ion concentration from 0.05 to 5 mM (Table 5.6; Figs. 5.7, 5.8). Ammonium and chlorate ions had an inhibitory effect on the activity of nitrate reductase in the root tissues; the inhibition being to the level of more than 50 per cent of the untreated control. While the effect of chlorate ions showed a dose dependency, the magnitude of inhibition did not show any increase with increase in the concentration of ammonium ions in the nutrient medium (Table 5.6; Fig. 5.7). The activity of the enzyme in leaves from seedlings growing in Hoagland's nutrient medium containing choriate ions, was marginally lesser as compared to those growing in the medium which did not have any chlorate ions. The magnitude of decrease in the activity of the enzyme was, however, more marked at higher concentration of nitrate and chlorate ions. Ammonium ions on the other hand markedly suppressed the activity of nitrate reductase in the leaf tissues; the

Table 5.6 : Effect of varying concentrations of $(NH_4)_2SO_4$ and $KCLO_3$ on root NR activity of common buckwheat (*Fagopurum esculentum* Moench) maintained for one hour under hydroponic culture in Hoagland nutrient solution containing varying amounts of KNO_3 and supplemented with varying doses of potassium chlorate or ammonium sulphate.

KNO_3^- Conc (mM)	Control	0.005 (mM) NH_4^+	0.05 (mM) NH_4^+	0.005 (mM) CLO_3^-	0.05 (mM) CLO_3^-
	μmol NO_2 released mg dry weight $root^{-1}hr^{-1}$				
0.05	0.12	0.066	0.080	0.074	0.066
0.10	0.12	0.068	0.080	0.072	0.066
0.20	0.20	0.068	0.025	0.092	0.069
0.50	0.20	0.093	0.102	0.130	0.064
1.00	0.21	0.115	0.124	0.175	0.077
2.00	0.31	0.107	0.124	0.197	0.078
5.00	0.41	0.122	0.124	0.214	0.095

Fig. 5.7 : Changes in the nitrate reductase activity in root tissues from seedlings of common buckwheat (*Fagopyrum esculentum* Moench) kept in varying concentrations of NO_3^- either separately or in combination with different levels of NH_4^+ (curve)/ClO_3^- (histogram). vertical lines in the figure represents LSD for any two observations at P0.05.


µ mol NO_2^- rel. mg d. w. root^{-1} hr^{-1}
0·5
0·4
0·3
0·2
0·1
Control
0·005mM NH_4
0·01 mM NH_4^+
0·05 mM NH_4^+
Control
0·005 mM $C_{10_3}^-$
0·01mM $C_{10_3}^-$
0·05mM $C_{10_3}^-$
I
0·05
0·1
0·2
0·5
1·0
2·0
5·0
Nitrate conc. (mM)

magnitude of inhibition increasing with increase in the concentration of ammonium ions in the ambient nutrient medium (Table 5.7; Fig. 5.8).

The uptake of nitrate was markedly higher in seedlings in the presence of glucose. Thus, presence of 2 mM glucose in the nutrient medium lead to a nearly 30 percent increase in the amount of NO_3^- taken up by the seedlings as compared to the control. Sucrose, however, had no effect on uptake of nitrate by the seedlings. Neither glucose nor sucrose had, however, any effect on the activity of NR in roots of the seedlings. A marked suppression in the uptake of nitrate by the seedlings was observed in the presence of the photosynthetic electron transport inhibitor DCMU. The uptake of nitrate by seedling in the presence of 1.0 mM DCMU (3, 3, 4-dichlirohenyl, 1-dimethylurea) was only about 8 percent of that observed in the control, thus registering a 92 percent decrease in the uptake. DCMU had a marked inhibitory effect on the activity of NR in roots of the seedlings. The activity of the enzyme in presence of DCMU was only 4 percent of that observed in the roots of control seedlings (Table 5.8). The wavelength of the incident light under which the seedlings were maintained also had an effect on the uptake rates. However, while ther was no difference in the rate of nitrate uptake between seedlings maintained under white fluorescent light or red light, in seedlings maintained under blue light,

Table 5.7 : The activity of nitrate reductase in the first leaves from seedlings of common buckwheat (*Fagopurum esculentum* Moench) maintained for one hour under hydroponic culture in Hoagland nutrient solution containing varying amounts of KNO_3^- and supplemented with varying doses of potassium chlorate or ammonium sulphate.

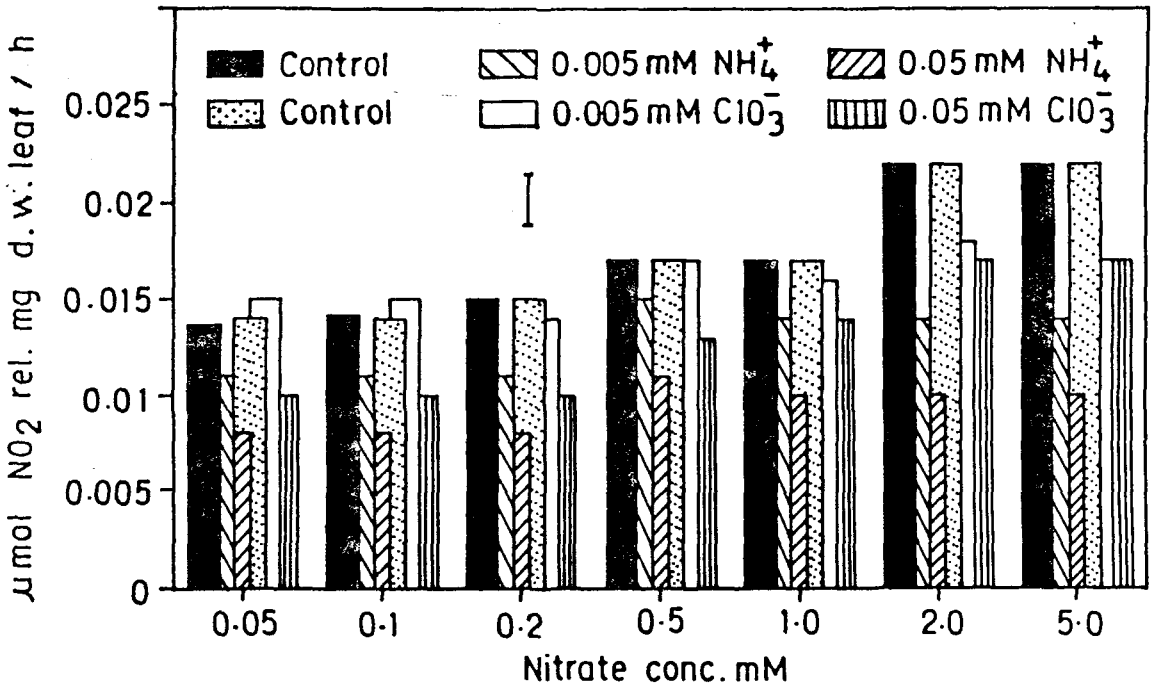
KNO_3^- Conc (mM)	Control	0.005 (mM) NH_4^+	0.05 (mM) NH_4^+	0.005 (mM) CLO_3^-	0.05 (mM) CLO_3^-
	μmol NO_2^- released mg dry weight $root^{-1}hr^{-1}$				
0.05	0.014	0.012	0.008	0.015	0.01
0.10	0.014	0.012	0.008	0.015	0.01
0.20	0.015	0.012	0.008	0.015	0.01
0.50	0.017	0.016	0.013	0.015	0.014
1.00	0.017	0.016	0.013	0.016	0.014
2.00	0.023	0.016	0.013	0.016	0.014

Table 5.8 : Effect of glucose, sucrose and DCMU on the uptake of nitrate by intact seedlings and the activity of nitrate reductase in the roots of common buckwheat (*Fagopyrum esculentum* Moench).

Treatment	Conc.	Nitrate uptake (μmol NO_3^- taken mg dry wt $root^{-1}min^{-1}$	NR activity (μmol NO_3^- released mg dry wt $root^{-1}min^{-1}$
Control		0.41 ± 0.1	0.4 ± 0.12
Glucose	2.0 mM	0.54 ± 0.12 (131)*	0.42 ± 0.14 (105)*
Sucrose	2.0 mM	0.41 ± 0.12 (100)	0.41 ± 0.14 (100)
DCMU	1.1 μM	0.032 + 0.02 (7.8)	0.016 + 0.04 (4.0)

* Figures in parentheses represent activity as per cent on the untreated control.

Fig. 5.8 : Changes in the nitrate reductase activity in leaf tissues from seedlings of common buckwheat (*Fagopyrum esculentum* Moench) kept in varying concentrations of NO_3^- either separately or in combination with different levels of NH_4^+ or ClO_3^- . vertical lines in the figure represents LSD for any two observations at P0.05.


Control
Control
0·005 mM NH_4^+
0·005 mM ClO_3^-
0·05 mM NH_4^+
0·05 mM ClO_3^-
µmol NO_2 rel. mg d.w. leaf / h
0·025
0·02
0·015
0·01
0·005
0
0·05
0·1
0·2
0·5
1·0
2·0
5·0
Nitrate conc. mM

the uptake of nitrate was only 40 per cent of that observed in control seedling maintained under white fluorescent light. Uptake of nitrate in seedlings exposed to far red light was, however, only 10 percent of that observed for seedlings kept continuously in white light (Table 5.9; Fig. 5.9).

For studying the role of shoot on the uptake of nitrate by the seedlings experiment were carried out on determination of nitrate uptake rates by isolated roots from seedlings of common buckwheat. For studying the uptake of nitrate by isolated roots, eight day old seedlings (grown as described above) were removed from the nutrient solution, washed with deionized water and blotted dry on a filter paper. The root portion was detached from the seedlings and a suitable fresh weight transferred to Hoagland's nutrient medium containing 5 mM KNO_3^-.

Expressed as cumulative uptake, the isolated roots from seedlings of common buckwheat showed a linear and steady uptake of NO_3^- during the first 30 minutes without any lag phase. Thereafter, the uptake gradually slowed down till it reached a plateau at t_{60} minutes. During this period a total of 14.5 µmol of nitrate was taken up by each mg dry weight of the root. When expressed as µmol nitrate taken up/mg dry weight root/minute, the uptake was maximum at 15 minutes of incubation after which it showed a gradual decrease with

Table 5.9 : Influence white, red, blue and far red lights on the uptake by seedlings of common buckwheat (*F. esculentum* Moench) growing in hydroponic culture in Hoagland nutrient solution supplemented with 5 mM KNO_3^-.

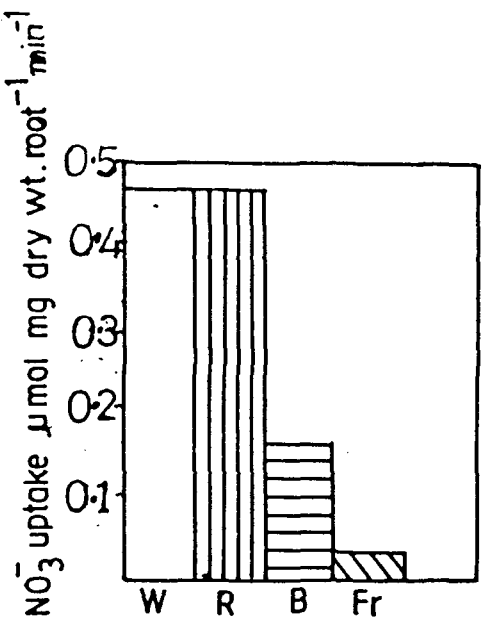
Light quality	Uptake rate (μmol NO_3^- taken up/mg dry weight root /minute
Whilte light	0.45
Red	0.45
Blue	0.16
Far red	0.03

Table 5.10 : Changes in the cumulative nitrate uptake with time by isolated roots of common buckwheat (*F.esculentum* Moench) supplied with 5 mM KNO_3^-.

Time minutes	Cumulative uptake (μmol NO_3^- taken up /mg dry weight root)	uptake rate (μmol NO_3^- taken up/ mg dry weight root/min.
15	7	0.55
30	10	0.40
45	14	0.39
60	14	0.29
120	14	0.15
180	14	0.00

Fig. 5.9 : Nitrate uptake as a function of light quality in seedlings of common buckwheat (*Fagopyrum esculentum* Moench) from Hoagland nutrient solution containing 5 mM NO_3^-.

W - White light
R - Red light
B - Blue light
Fr - Far red light


NO_3^- uptake µmol mg dry wt. root^{-1} min^{-1}
0·5
0·4
0·3
0·2
0·1
W
R
B
Fr

progressing time till no significant uptake could be observed at t_{180} minutes. A maximum uptake rate of 0.55 μmol/mg dry weight root/minute was recorded at 15 minutes incubation time (Table 5.10; Fig. 5.10). The H^+ ions concentration of the nutrient medium had a pronounced effect on the rate of uptake of nitrate by the excised roots of buckwheat seedlings. In the highly acidic range of pH 2.0 and 3.0, root did not take up any nitrate from the nutrient medium. The rate of uptake increased gradually with decrease in the H^+ ion concentration when the maximum uptake was observed between pH 4.5 and 6.0. With further increase in the H^+ ion concentration the rate of uptake showed a gradual decline. At pH 8.0, the uptake was 1/3rd of that observed at pH 6.0 of 0.01 M Tris-HCl buffer (Table 5.11; Fig. 5.11).

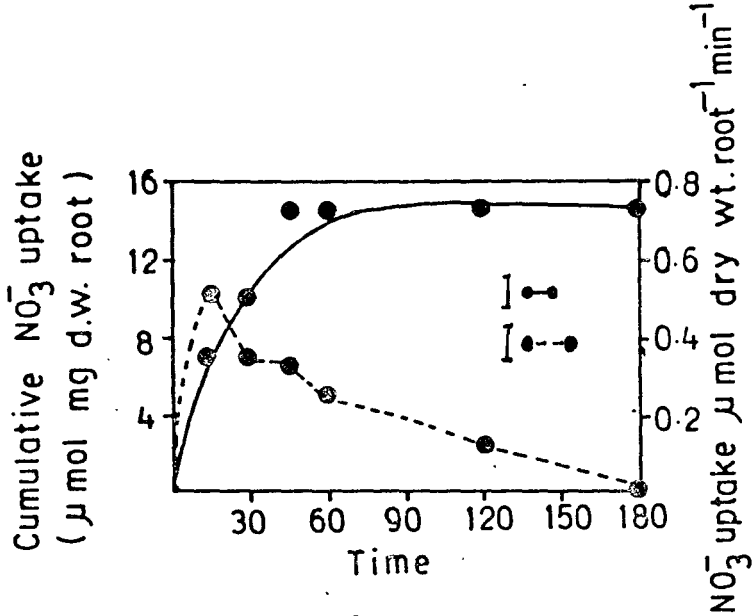
The uptake of nitrate by excised roots showed a hyperbolic relationship with the concentration of nitrate ions in the nutrient medium. Thus when the concentration of nitrate ion in the ambient medium was varied from 0.05 to 5 mM, the rate of nitrate uptake by excised roots was a function of external nitrate concentration, according to Michael's-Menten Kinetics. Increase in the concentration of nitrate in the ambient nutrient medium lead to a linear increase in the uptake of nitrate by the excised roots up to 1 mM external NO_3^-. Beyond the concentration of 1 mM the rate of uptake tended to form a plateau with the uptake rate

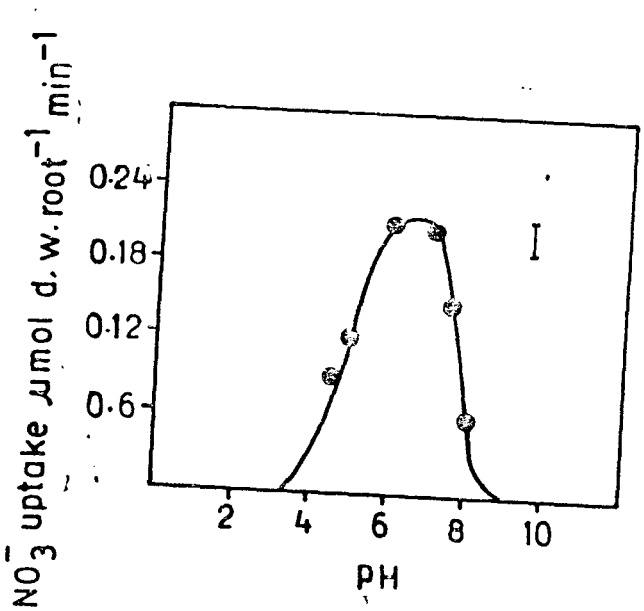
Table 5.11 : The effect of H^+ ion concentration of the nutrient medium on the uptake of nitrate by isolated roots common buckwheat (*F. esculentum* Moench) growing under hydroponic culture in Hoagland nutrient medium supplemented with 5 mM KNO_3^- .

Time (minutes)	μmol NO_3^- taken up /mg dry weight root/minute
15	0.55
30	0.40
45	0.39
60	0.29
120	0.15
180	-

Fig. 5.10 : Nitrate uptake by isolated roots of common buck-wheat (*Fagopyrum esculentum* Moench) as a function of time. The solid curve depicts the changes in the concentration of nitrate in the nutrient medium. The broken curve represents the change in the rate of uptake of nitrate by isolated roots with time. Vertical lines in the figure represents LSD at P0.05.

Fig. 5.11 : Effect of pH of the ambient nutrient medium on the uptake of nitrate by isolated roots of common buckwheat (*Fagopyrum esculentum* Moench). Vertical lines represents LSD at Po.05.


Cumulative NO_3^- uptake
(µ mol mg d.w. root)
16
12
8
4
30
60
90
120
150
180
Time
0·8
0·6
0·4
0·2
NO_3^- uptake µ mol dry wt. $root^{-1} min^{-1}$


NO_3^- uptake µmol d. w. $root^{-1} min^{-1}$
0·24
0·18
0·12
0·6
2
4
6
8
10
PH

remaining nearly stationery beyond 2 mM external nitrate concentration. Thus a typical hyperbolic relationship could be observed for the relationship between external nitrate concentration and nitrate uptake in excised roots of common buckwheat. The values for inverse of "V" and "S" gave a linear relationship with a slope of 2.586 and intercept value of 3.147. The Lineweaver and Burk plot for the uptake of nitrate with varying external nitrate concentration revealed a maximum velocity (V_{max}) of 0.208 μmol/mg dry weight root/ minute with a Km value of 227 μmol in the presence of 0.01 M Tris buffer at pH 6.5. Superimposition of either chlorate or ammonium ions in the nutrient medium lead to a marked suppression in the uptake of nitrate by the excised roots, the magnitude of suppression increasing with increasing concentrations of either NH_4^+ or ClO_3^-. The dose dependency of suppression was, however, more marked in case of chlorate ion induced suppression than that for ammoniumion induced suppression (Table 5.12; Figs. 5.12, 5.13). A Lineweaver-Burk plot for uptake of nitrate as a function of external nitrate concentration at varying concentrations of either ClO_3^- or NH_4^+ clearly revealed that while the inhibition due to the presence of ClO_3^- was of competitive nature. That due to the presence of NH_4^+ was non-competitive in nature. The presence of the chlorate ions affected both the intercept as well as the slope of the regression equation describing the relation-ship of 1/V vs. 1/S. While ammonium ions in the ambient

Table 5.12 : Effect of varying concentration of NO_3^-(S) on the rate of nitrate uptake (V) by isolated root system of common buckwheat (*Fagopyrum esculentum* Moench) maintained under hydroponic culture in Hoagland nutrient solution supplemented with varying doses of either KCl O_3^- or $(NH_4)_2SO_4$.

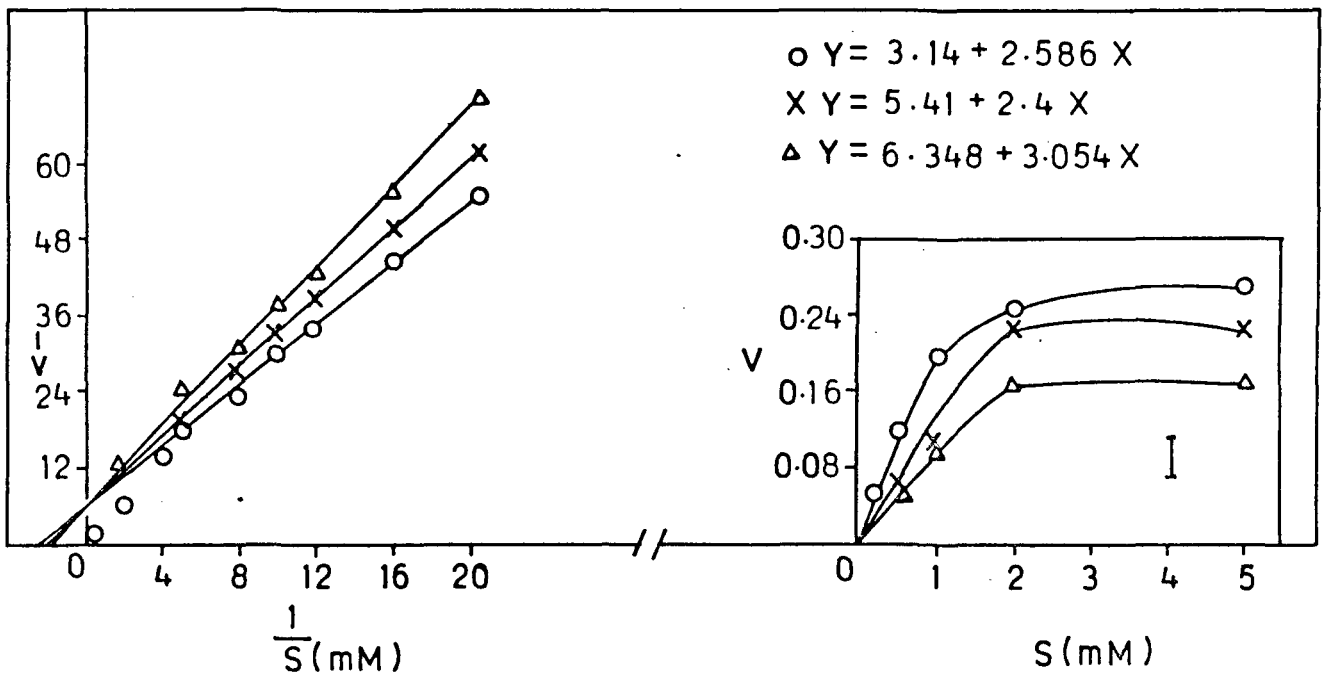
NO_3^- (mM)	Conc	Control		0.005 (mM) KCl O_3^-		0.05 (mM) KCl O_3^-		0.005 (mM) $(NH_4)_2SO_4$		0.05(mM) $(NH_4)_2SO_4$	
(S)	(1/S)	V	1/V	V	1/V	V	1/V	V	1/V	V	1/V
				Uptake rate (μmol NO_3^- taken up/mg dry weight root /minute							
0.05	20	1.11	0.75	1.00	1.00	9.90	1.10	0.625	1.60	0.62	1.60
0.1	10	2.00	0.50	1.81	0.55	1.61	0.62	0.780	1.28	0.62	1.60
0.2	5	3.33	0.30	2.85	0.35	2.50	0.40	0.780	1.28	0.62	1.60
0.5	2	7.50	0.13	4.54	0.22	4.16	0.24	3.900	0.25	3.12	0.32
1.0	1	12.50	0.08	6.66	0.15	6.25	0.16	6.250	0.16	5.46	0.18
2.0	0.5	15.62	0.06	14.00	0.07	10.93	0.09	6.250	0.16	6.25	0.16
5.0	0.2	17.18	0.05	14.00	0.07	10.93	0.09	6.250	0.15	6.25	0.16

nutrient medium did not affect the intercept they altered the slope of the regression that described the relationship of 1/V against 1/S. The values for V_{max}, Km, the intercept and slope for the relationship of 1/V with 1/S in the control as also in the presence of ammonium and chlorate ions are presented in Table 5.13. While the Km for the uptake nitrate by excised roots in presence of 0.005 and 0.05 mM chlorate ions was 357 and 416 μmol respectively, the V_{max} for the process of uptake was 0.138 μmol/mg dry weight root/minute. Ammonium ions had no effect on the Km for the uptake of nitrate by excised roots. However, the V_{max} for the process was markedly affected by the presence of ammonium ions in the nutrient medium. Thus the uptake of nitrate in presence of 0.005 and 0.05 mM ammonium had Km of 0.227 μmol. The V_{max} for the process in the presence of 0.005 and 0.05 mM ammonium was 0.069 and 0.052 μmol/mg dry weight root/minute respectively (Table 5.13; Figs. 5.12, 5.13).

When expressed on per seedling basis the uptake of nitrate by each seedling showed a dependency on the concentration of the substrate in the ambient nutrient medium. Thus with increase in the concentration of nitrate from 0.05 to 1.0 mM, a linear increase in the uptake by the seedlings was observed. Beyond 1.0 mM nitrate concentration, however, there was no change in the amount of nitrate taken up by the seedling. Correspondingly, the amount of nitrate

Fig. 5.12 ; Lineweaver and Burk plot for the relationship between substrate concentration and nitrate uptake in isolated roots of common buckwheat (*Fagopyrum esculentum* Moench) in the presence of varying concentrations of NH_4^+ ions, ●, control; x 0.005 mM $KClO_3^-$; ▲, 0.01 mM $KClO_3^-$. Inset : V/S relationship for the same. vertical line in the inset of figure depicts LSD for any two observations at P0.05.

Fig. 5.13 ; Lineweaver and Burk plot for the relationship between substrate concentration and nitrate uptake in isolated roots of common buckwheat (*Fagopyrum esculentum* Moench) in the presence of varying concentrations of NH_4^+ ions, ●, control; x 0.005 mM NH_4^+; ▲, 0.01 mM NH_4^+. Inset : V/S relationship for the same. vertical line in the inset of figure depicts LSD for any two observations at P0.05.


o Y= 3·14 + 2·586 X
X Y= 5·41 + 2·4 X
Δ Y= 6·348 + 3·054 X
60
48
36
1/V
24
12
0
4
8
12
16
20
1/S(mM)
0·30
0·24
V
0·16
0·08
0
1
2
3
4
5
S(mM)

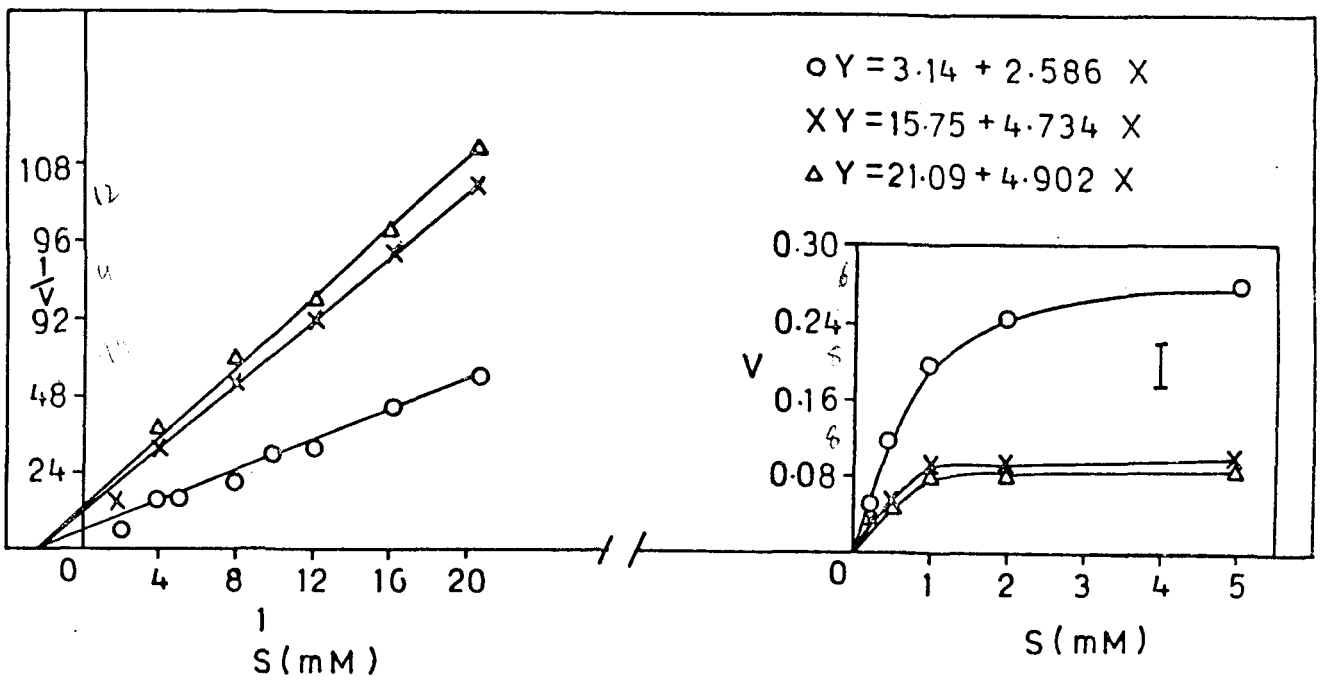

o Y=3·14 + 2·586 X
X Y=15·75 + 4·734 X
Δ Y=21·09 + 4·902 X
108
96
1/V
92
48
24
0
4
8
12
16
20
1/S(mM)
0·30
0·24
V
0·16
0·08
0
1
2
3
4
5
S(mM)

Table 5.13 : Values of a, b, r^2, Km and V_{max} for the relationship of 1/V against 1/S for nitrate uptake by seedlings of common buckwheat (*F. esculentum* Moench) maintained under hydroponic culture in Hoagland's nutrient solution supplemented with varying level of NO_3^-.

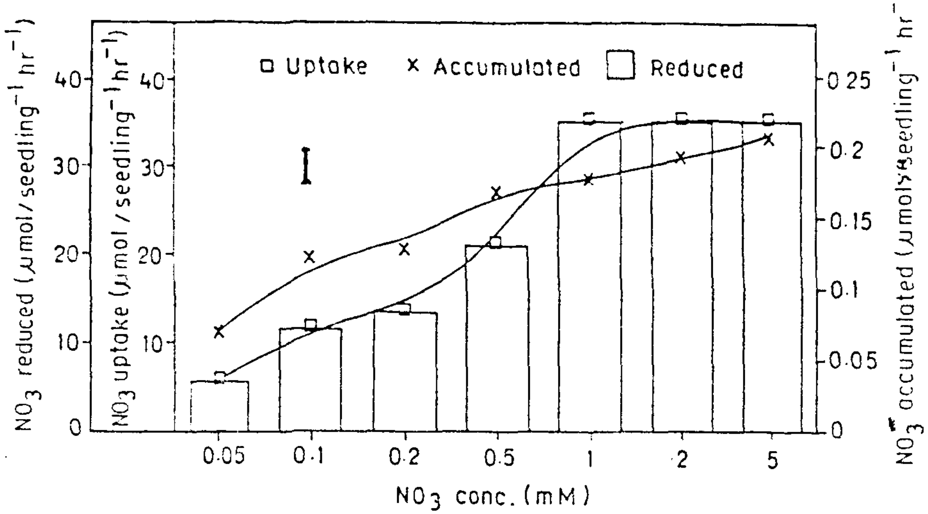
	a	b	r^2	Km (µmol/mg dry weight root/ min.)	V_{max} (µmol/mg dry weight root/ min.)
Control	3.15	2.586	0.89	227	0.208
+					
0.005 (mM) $KClO_3^-$	5.41	2.400	0.79	357	0.208
+					
0.05 (mM) $KClO_3^-$	6.35	3.054	0.81	416	0.208
+					
0.005 (mM) NH_4^+	15.75	4.734	0.82	227	0.069
+					
0.05 (mM) NH_4^+	21.09	4.902	0.89	227	0.052

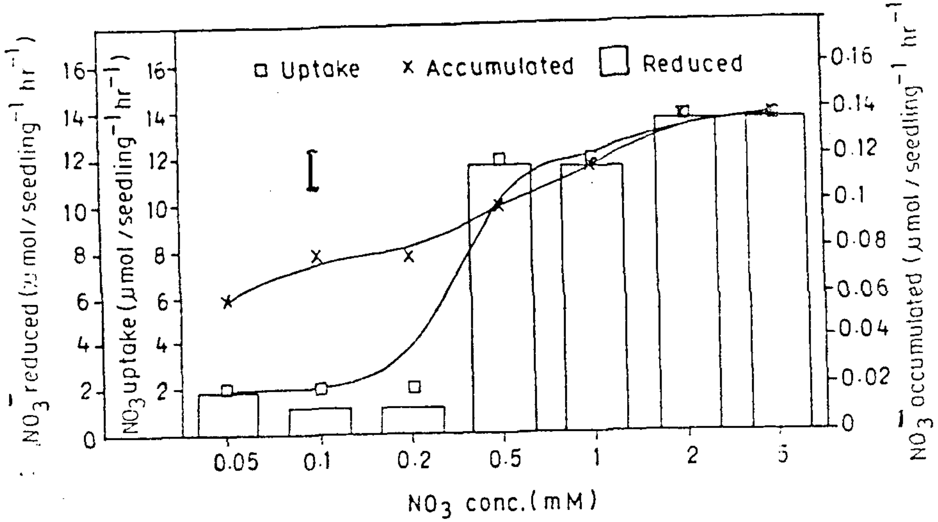
accumulated by seedling showed a linear increase with increase in the concentration of nitrate in the medium. Changes in the amount of nitrogen reduced by the seedlings in the corresponding period followed a pattern in similar to that observed for uptake rate (Table 5.14; Fig. 5.14). Superimposition of ammonium ions in the nutrient medium, had a markedly inhibitory effect on the uptake, accumulation and reduction of nitrate in the seedlings; the magnitude of inhibition increasing with increase in the concentration of ammonium ions (Table 5.14; Fig. 5.14). Chlorate ions had raltively less marked effect in the uptake and accumulation of nitrate by the seedlings. Chlorate ions had, however, no significant effect on the process of reduction of nitrate in the seedlings (Table 5.15; Fig. 5.15). Thus at external nitrate concentration of 5 mM, superimposition of 0.05 mM ammonium lead to a 77 percent decrease in the uptake and nearly 80 percent decrease in the reduction of nitrate in the seedlings, the treatment resulted in a 55 percent decrease in the accumulation of nitrate in the seedlings. At the same external nitrate concentration, however, superimposition of chlorate ions lead to only 11 percent decrease in the uptake and reduction of nitrate by the seedlings; the accumulation of nitrate being to the level of 50 percent of that observed in the control seedlings.

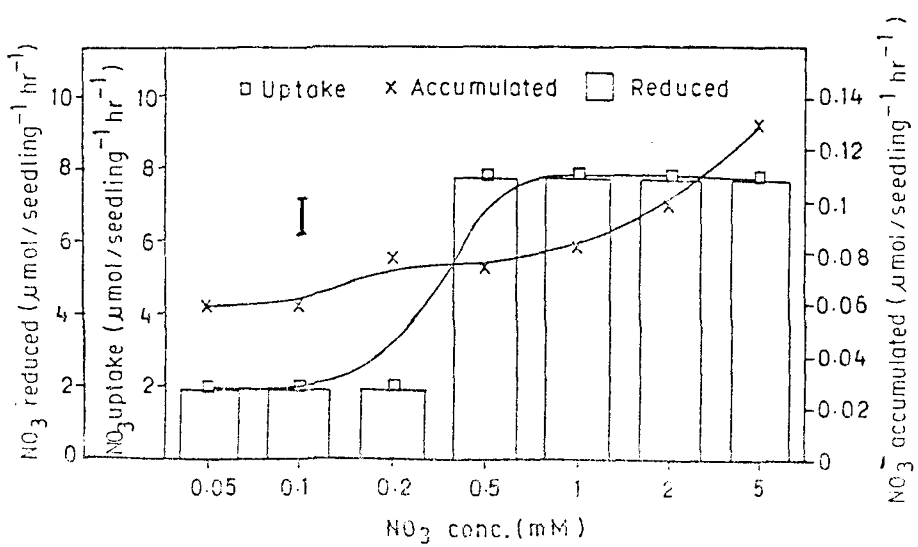
Table 5.14 : Effect of different concentrations of $(NH_4)_2SO_4$ on uptake, accumulation and reduction of nitrate by seedlings of common buckwheat (*F. esculentum* Moench) cultured in hydroponic system with Hoagland nutrient solution supplemented with 5 mM KNO_3^-.

Concentration of Substrate	NO_3^- uptake (µmol/seedling)	NO_3^- accumulated (µmol/seedling)	NO_3^- reduced (µmol/seedling)
		Control	
0.005	5.80	0.06	5.73
0.100	11.71	0.12	11.58
0.200	13.67	0.12	13.54
0.500	21.09	0.16	20.93
1.000	35.15	0.17	34.98
2.000	35.15	0.19	34.96
5.000	35.15	0.20	34.94
		0.005 $(NH_4)_2SO_4$	
0.005	1.95	0.059	1.89
0.100	1.95	0.076	1.18
0.200	1.95	0.076	1.18
0.500	11.71	0.090	11.61
1.000	11.71	0.110	11.59
2.000	13.67	0.130	13.53
5.000	13.67	0.130	13.53
		0.01 $(NH_4)_2SO_4$	
0.005	1.95	0.05	1.89
0.100	1.95	0.05	1.89
0.200	1.95	0.07	1.87
0.500	7.81	0.07	7.73
1.000	7.81	0.08	7.72
2.000	7.81	0.09	7.71
5.000	7.81	0.12	7.68
		0.05 $(NH_4)_2SO_4$	
0.005	1.95	0.03	1.91
0.100	1.95	0.03	1.91
0.200	1.95	0.05	1.89
0.500	7.81	0.06	7.74
1.000	7.81	0.09	7.71
2.000	7.81	0.09	7.71
5.000	7.81	0.09	7.71

Fig. 5.14; Changes in the uptake, accumulation and reduction of NO_3^- by seedlings of common buckwheat (*Fagopyrum esculentum* Moench) kept in varying concentrations of NO_3^- superimposed with varying concentration of NH_4^+ ions. A, control; B, 0.005 mM NH_4^+; C, 0.01 mM NH_4^+; D, 0.05 mM NH_4^+. Vertical line in the figure represents LSD at P0.05.


Uptake
Accumulated
Reduced
NO_3 reduced (µmol / seedling^{-1} hr^{-1})
NO_3 uptake (µmol / seedling^{-1} hr^{-1})
NO_3^- accumulated (µmol / seedling^{-1} hr^{-1})
0
10
20
30
40
0.05
0.1
0.15
0.2
0.25
0.05
0.1
0.2
0.5
1
2
5
NO_3 conc. (mM)


Uptake
Accumulated
Reduced
NO_3^- reduced (µmol / seedling^{-1} hr^{-1})
NO_3 uptake (µmol / seedling^{-1} hr^{-1})
NO_3^- accumulated (µmol / seedling^{-1} hr^{-1})
0
2
4
6
8
10
12
14
16
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.05
0.1
0.2
0.5
1
2
5
NO_3 conc. (mM)


Uptake
Accumulated
Reduced
NO_3 reduced (µmol / seedling^{-1} hr^{-1})
NO_3 uptake (µmol / seedling^{-1} hr^{-1})
NO_3^- accumulated (µmol / seedling^{-1} hr^{-1})
0
2
4
6
8
10
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.05
0.1
0.2
0.5
1
2
5
NO_3 conc. (mM)

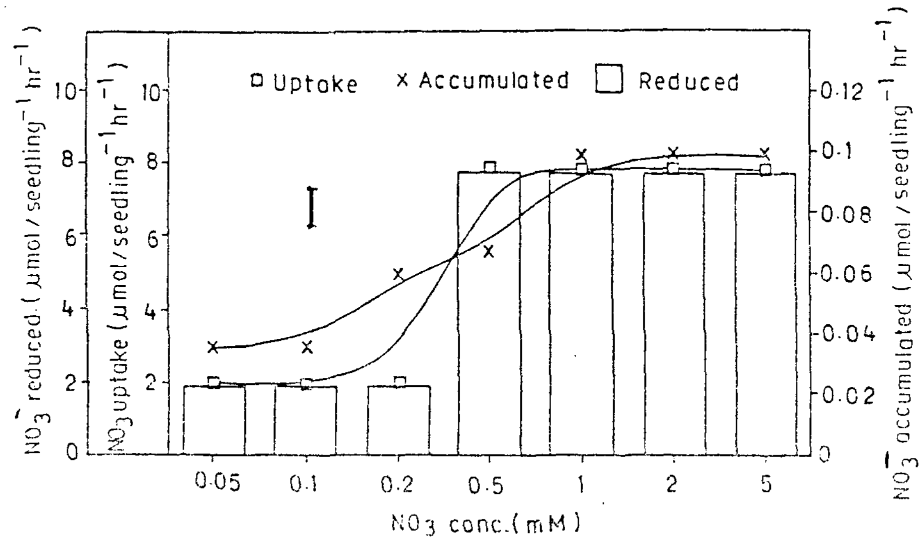
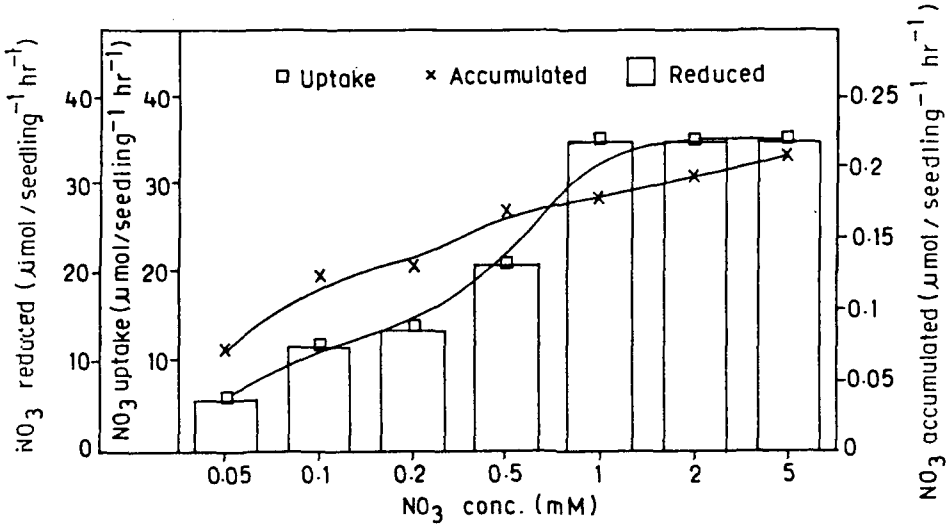
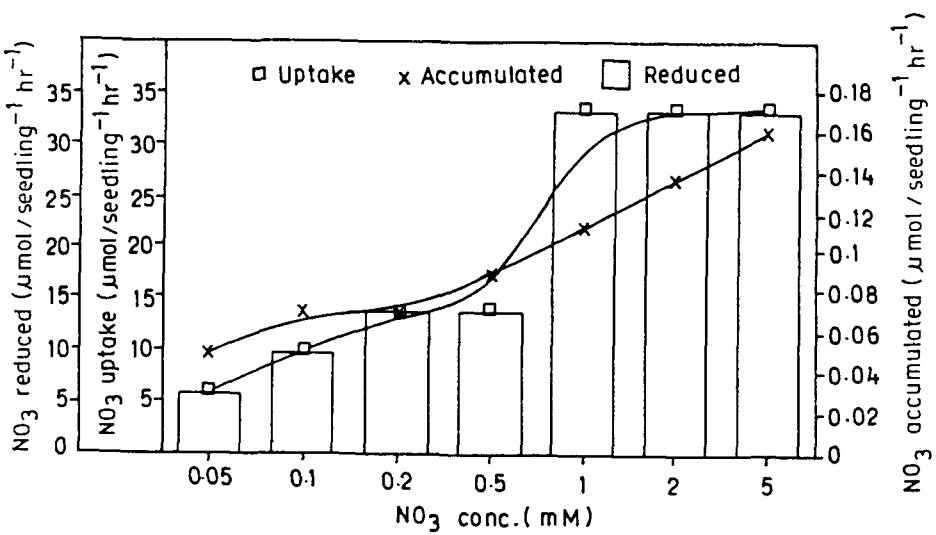

Uptake
Accumulated
Reduced
NO_3^- reduced (µmol / seedling^{-1} hr^{-1})
NO_3 uptake (µmol / seedling^{-1} hr^{-1})
NO_3^- accumulated (µmol / seedling^{-1} hr^{-1})
0
2
4
6
8
10
0.02
0.04
0.06
0.08
0.1
0.12
0.05
0.1
0.2
0.5
1
2
5
NO_3 conc. (mM)

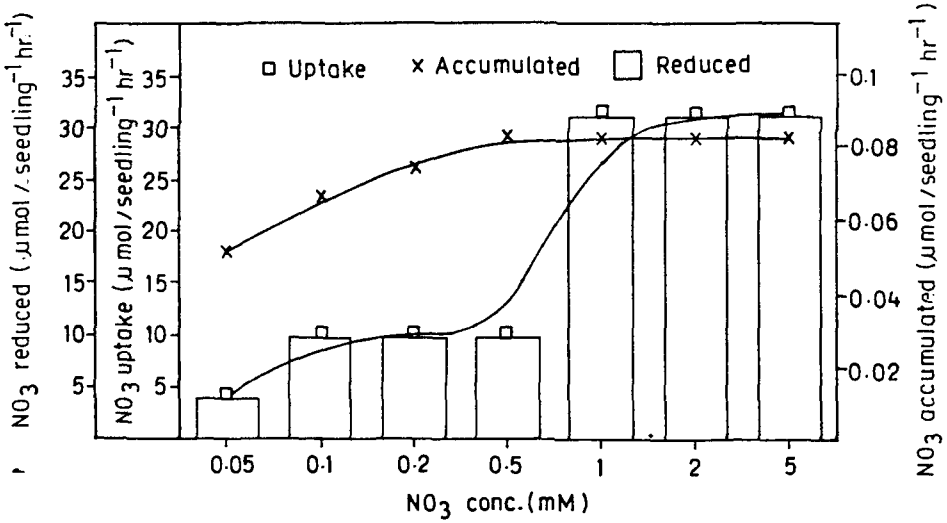
Table 5.15 : Effect of varying concentrations of $KClO_3^-$ on uptake, accumulation and reduction of nitrate by the seedlings of common buckwheat (*F. esculentum* Moench) cultured in hydroponic system with Hoagland nutrient solution supplemented with 5 mM KNO_3^-.

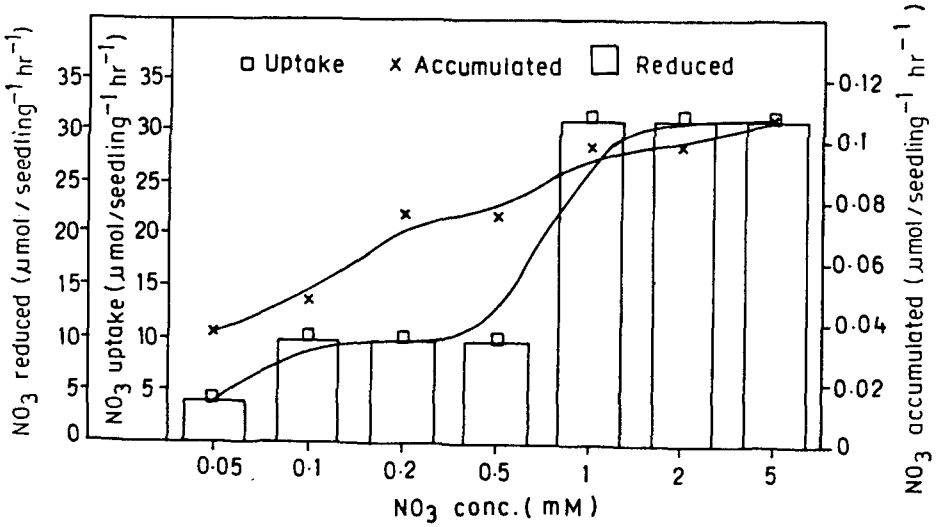
NO_3^- Concentration (mM)	NO_3^- uptake (µmol/seedling)	NO_3^- accumulated (µmol/seedling)	NO_3^- reduced (µmol/seedling)
	Control		
0.005	5.80	0.06	5.73
0.100	11.71	0.12	11.58
0.200	13.67	0.12	13.54
0.500	21.09	0.16	20.93
1.000	35.15	0.17	34.98
2.000	35.15	0.19	34.96
5.000	35.15	0.20	34.94
	0.005 mM $KClO_3^-$		
0.005	5.80	0.04	5.89
0.100	9.70	0.06	9.63
0.200	13.67	0.06	13.60
0.500	13.67	0.08	13.58
1.000	33.20	0.11	33.08
2.000	33.20	0.13	33.06
5.000	33.20	0.16	33.04
	0.01 mM $KClO_3^-$		
0.005	3.90	0.05	3.84
0.100	9.76	0.06	9.69
0.200	9.76	0.07	9.68
0.500	9.76	0.08	9.67
1.000	31.25	0.08	31.16
2.000	31.25	0.08	31.16
5.000	31.25	0.08	31.16
	0.05 mM $KClO_3^-$		
0.005	3.90	0.03	3.86
0.100	9.76	0.04	9.71
0.200	9.76	0.07	9.68
0.500	9.76	0.07	9.68
1.000	31.25	0.09	31.15
2.000	31.25	0.09	31.15
5.000	31.25	0.10	31.15

Fig. 5.15 ; Changes in the uptake, accumulation and reduction by the seedlings of common buckwheat (*Fagopyrum esculentum* Moench) kept in varying concentrations of NO_3^- superimposed with varying concentration of $KClO_3^-$ ions. A, control; B, 0.005 mM $KClO_3^-$; C, 0.01 mM $KClO_3^-$; D, 0.05 mM $KClO_3^-$.


Uptake
Accumulated
Reduced
NO3 reduced (µmol / seedling-1 hr-1)
NO3 uptake (µmol/seedling-1 hr-1)
NO3 accumulated (µmol / seedling-1 hr-1)
NO3 conc. (mM)
0.05
0.1
0.2
0.5
1
2
5


Uptake
Accumulated
Reduced
NO3 reduced (µmol / seedling-1 hr-1)
NO3 uptake (µmol/seedling-1 hr-1)
NO3 accumulated (µmol / seedling-1 hr-1)
NO3 conc.(mM)
0.05
0.1
0.2
0.5
1
2
5


Uptake
Accumulated
Reduced
NO3 reduced (µmol / seedling-1 hr-1)
NO3 uptake (µmol/seedling-1 hr-1)
NO3 accumulated (µmol/seedling-1 hr-1)
NO3 conc.(mM)
0.05
0.1
0.2
0.5
1
2
5


Uptake
Accumulated
Reduced
NO3 reduced (µmol / seedling-1 hr-1)
NO3 uptake (µmol/seedling-1 hr-1)
NO3 accumulated (µmol/seedling-1 hr-1)
NO3 conc.(mM)
0.05
0.1
0.2
0.5
1
2
5

DISCUSSION

Plant roots show selectivity in absorption of ions from the soil. The uptake of ion is dependent on free energy of H^+ across the plasmalemma of the cells of the root cortex and carriers possessing specific affinities for particular ions (Spanswick, 1981; Sze, 1985). In higher plants the anion NO_3^- is taken up uniquely, in that it is subjected to both positive and negative feed back regulation. The absorption of nitrate by plant roots has been reported to occured with rates comparable to those for K^+, Rb^+, Cl^- and $H_2Po_4^-$ (Jackson *et al.*, 1973). A low level of tissue nitrate and low rates of net NO_3^- uptake have been observed with the plants which have not been pretreated with NO_3^- before the determination of nitrate (Clarkson, 1986). Following exposure to nitrate, root $[NO_3^-]$ and NO_3^- uptake have been reported to increase several fold with time (Glass, 1988). Glass (1988) has observed that this increase in the uptake was a specific response of the root to the presence of NO_3^- in the ambient environment. The net uptake rate of NO_3^- by plants can be modelled as a summation of two factors: (i) induction of transportor - this would increase the uptake rate with time until a steady state is achieved, (ii) ambient nitrate concentration - a progressive depletion of nitrate would result in lower uptake rates. In the present investigation the uptake of nitrate by buckwheat seedlings with progressing time follows a typical hyperbolic curve without any lag

phase. In sugarbeet, Mack and Tischner (1990), have observed that seedlings grown from seeds which germinated without an external nitrogen supply absorbed nitrate at low rates immediately upon exposure. Such a low capacity uptake mechanism has been referred to as a 'basic' system instead of a 'constitutive' one because it was assumed that the system was induced by the endogenous seed nitrate during germination. Rudolf *et al.*, (1988) in their experiment with barely roots have observed that the low capacity uptake system was always present even in the absence of nitrate in the ambient environment of the root. In the present case, the seedlings of common buckwheat started to absorp nitrate immediately upon exposure to the ions, without any lag phase. Further the uptake of nitrate increased with time up to 180 minutes after which it remained by and large stationary. The seedlings showed a maximum of uptake rate during the initial 30 minutes of incubation after which the rate of uptake decreased with the progressing time when no significant uptake was observed after t_{240} minutes. From an analysis of the cumulative uptake of the ion and the changes in the rate of uptake with time, as observed in the present investigation it is quite clear that the uptake of nitrate conforms to a 'low capacity basic system'. Tahir (1985) has reported that the hull was a source of nitrate for buckwheat seeds during early stages of germination. It seems reasonable to postulate that the carrier for nitrate ions in the seedlings is already present

in the system because of an endogenous supply of nitrate during their early stages of growth. There was a significant decrease in the rate of uptake of nitrate by the seedlings after 30 minutes of incubation in the nutrient solution containing 5 mM nitrate. Correspondingly, however, the level of nitrate in the ambient nutrient medium showed a marked decrease with progressing time up to 240 minutes. Could, the decrease in the uptake rate therefore ascribed to the decrease in the concentration of nitrate in the ambient nutrient medium ? To answer this question, an experiment was raised in which the concentration of nitrate in the ambient medium was maintained at 5 mM by periodic replenishment. The rate of nitrate uptake in seedlings, from Hoagland nutrient solution in which the level of nitrate was maintained at 5 mM by periodic replenishment, showed a trend similar to that observed for seedlings which were kept in a test solution in which no replenishments for loss of nitrate as a result of uptake were made. Thus, the observed decrease in the rate of nitrate uptake with time could be ascribed to (i) refilling of the available storage components in the seedlings and/or (ii) any factor which influences the turnover of proteins involved in the translocation of nitrate in the plant, and not to a decreasing nitrate concentration in the nutrient medium. Behl *et al.*, (1988) have reported that p-flurophenylamine induced supression of nitrate transport into xylem effect on the uptake of nitrate ions by nitrate starved

barley roots. They have emphasized about the secretion of ions into the xylem vessels as a mechanism indirectly modifying the uptake of ions.

Changes in the pH of the ambient nutrient medium had a marked effect on uptake of nitrate by buckwheat seedlings, with almost complete suppression of uptake at pH values below 4.0 and above 9.0. Bassioni (1971) has attributed such an effect of pH on nitrate uptake to the deleterious effect of high concentration of H^+ ions (at low pH value) on the root system and a direct competition between OH^- and NO_3^- ions for transport across membrane at high pH values. Since at pH values between 6.0 and 6.5, the nitrate ion concentration (5 mM) would exceed that of the OH^- ions by a factor of 5×10^4, a possible competition for nitrate transporter by OH^- ions would be ruled out. This would result in higher uptake rates as has been observed in the present study.

In the present study, nitrate uptake as a function of substrate concentration followed a saturation curve. This is a common feature to almost all carrier mediated transport systems (Annera *et al.*, 1990, Siddigui *et al.*, 1990, Aslam *et al.*, 1992). However the kinetics of NO_3^- uptake are clearly more complex than the simple Michelis-Menten relationship, at least in a great majority of cases (Nissen, 1974, 1991; Nandi *et al.*, 1987). In the present investigation the uptake of

nitrate by buckwheat seedlings from Hoagland's nutrient solution (pH, 6.5) had a V_{max} of 0.276 µmol/mg dry weight root/minute and a Km value of 200 µmol.

There is no consensus in the literature as to whether the complexity of the kinetics of ion uptake (including NO_3^-) arises from the simultaneous functioning, in the plasmalemma, of two or more independent mechanisms, one of which may be diffusion (Doddema and Telkamp, 1979 Lee and Drew, 1986, Glass *et al.*, 1990 Aslam *et al.*, 1982) or whether there is only single mechanism either cooperative (Hodges, 1973) or multiphasic (Nissen, 1971, 1977, 1991). From our observation with *Fagopyrum esculentum* it is clear that the mechanism of nitrate uptake in the plant is mediated through the low concentration high affinity system. The Km for nitrate absorption by buckwheat seedlings and isolated roots from Hoagland's nutrient solution (pH, 6.5) was 200 and 227 µmol, respectively. A range of Km values have been reported for the uptake of nitrate by the low concentration, high affinity system. The reported values range from 4 µmol in *Arabidopsis thaliana* (Doddema and Telkamo, 1979) to 250 µmol in *Hordium vulgare* (Chatrotwong *et al.*, 1979). Similarly there has been great variation in the reported affinity for NO_3^- of the high concentration mechanism with a Km of 100 µmol for *Hordium vulgare* (Siddiqui *et al.*, 1990) and 25 µmol for *Arabidopsis thaliana* (Doddema and Telkamp, 1979). This

variation in the affinity of the two systems for NO_3^- has been attributed to (i) differences in endogenous nitrate levels and, hence cell/tissue nitrate status (Butz and Jackson, 1977, Lecand Drew, 1986, Siddiqui *et al.*, 1990) and/or (ii) inherent inter and intraspecific differences in NO_3^- uptake capacity (Rao and Rains, 1976). There can be no doubt that the latter factor may explain at least the variability observed between species.

The presence of either NH_4^+ or ClO_3^- ions in the nutrient medium had an inhibitory effect on the uptake of nitrate by buckwheat seedlings. While NH_4^+ ions are known to repress uptake and assimilation nitrate in cyanobacteria, algae and fungi (Marznef, 1981; Andrews *et al.*, 1989; Fernandez and Cardenes, 1989). Their effects in higher plants are not clearly established. Bloom and Finazzo (1986) have reported the inhibition of net nitrate uptake in two cultivars of barley by $(NH_4)_2So_4$. A Lineweaver-Burk plot of the uptake v/s nitrate concentration at various levels of ammonium, as worked out in the present incestigation reveals the non-competitive nature of inhibition. Several explanation have been forwarded for NH_4^+ ion induced inhibition of NO_3^- absorption. (i) The inhibition may be a direct result of NH_4^+ accumulation in the cells or due tot he accumulation of some product of NH_4^+ assimilation pathway (ii) slowing of the activation or synthesis of the NO_3^-

absorption system, (iii) inhibition of nitrate reduction pathway in the cells (Deane-Drummond and Glass, 1983). It may, therefore, be that the inhibition is at some level other than one involved in uptake of nitrate. While NH_4^+ ions had no effect on the Km for nitrate uptake by buckwheat seedlings, the ions markedly reduced the V_{max} for the process. While the process had a V_{max} of 0.276 μmol/mg dry weight root/min. in the presence of 0.005 and 0.05 mM ammonium, the uptake of nitrate by buckwheat seedlings had a V_{max} of 0.083 and 0.064 μmol/mg dry weight root/min. Lee and Drew (1989) have reported a 38-43 % inhibition of nitrate uptake by 0.5 μmol m^{-3} ammonium ions in barley; the V_{max} for nitrate influx being lowered by 20 - 42 % in the presence of 0.5 μmol m^{-3} ammonium, as compared to the ammonium free control. It appears that the inhibition of nitrate uptake by $(NH_4)_2SO_4$ as observed in the present study is not simply a case of NH_4^+ providing a counter - ion for NO_3^-, rather the inhibition may be due to the effect of the ammonium ions on the net rate of nitrate influx into the root tissues.

Although ClO_3^- ions also suppressed uptake of nitrate by buckwheat seedlings, derivation of the Lineweaver-Burk plot for V as a function of S at various levels of chlorate, clearly reveals the competitive nature of the inhibition. In the presence of 0.005 and 0.05 mM of ClO_3^-, the Km for uptake of nitrate by buckwheat seedlings was 307

and 500 μmol respectively. Chlorate ions had no effect on the V_{max} of the process. Chlorate has been reported to behave as a analogue for nitrate during NO_3^- uptake by *Arabidopsis thaliana* (Doddema and Otten, 1979) barley plants (Deane-Drummondand and Glass, 1989) and during NO_3^- uptake and assimilation by Chlorella (Tromballa and Broda, 1971). In chera Deane-Drummond (1984) has observed that chlorate also appeared to act as a substitute for NO_3^- at the induction site, although the concentration of chlorate required to elicit a response was lower than nitrate.

Ammonium and chlorate ions had an inhibitory effect on the reduction of nitrate in the root tissues. However, while chlorate ions showed a dose response for the inhibition, ammonium ions did not show any such response. In the present study, however, the ammonium induced repression of NR activity is quite clear. In mosses, Mallapadidam *et al.* (1991) have reported that ammonia repressed NRA even in the presence of nitrate. Hageman and Flesher (1960) have clearly established the requirement of both light and NO_3^- for the appearance of NRA in leaves. The requirement has been related to either the activation of a pre-existing protein or to the *de novo* synthesis of the enzyme protein. In *Chlorella vulgaris*, Losada *et al.*, (1970) have demostrated an immediate disappearance of NRA when NH_4^+ was added to the medium. The repression of NRA could thus ascribed to one or more of the

following : (i) an inhibition of NO_3^- uptake, (ii) an inactivation or enhanced degradation of the enzyme, (iii) an inhibition of transcription or translation of mRNA coding for the nitrate reductase protein. The dose dependency of the effect of ClO_3^- ions on the *in vivo* reduction of nitrate in root tissues as observed in the present study reveals the competitive nature of the effect of ClO_3^- ion on nitrate reduction. Aberg (1947) has emphasized the toxic effect of ClO_3^- ions on plants. He has suggested that some derivatives of chlorate produced within the plant cell, and not the chlorate ion *per se*, were the actual toxins. The inhibitory effect of chlorate ions on nitrate reduction, as observed in the present study, could also be ascribed to the toxic nature of the products of chlorite, (ClO_2^-) and hypochlorite (ClO^-) ions, the reduction product of chlorate. Hofstra (1977) and Labrie (1991) have also reported a decrease in NR activity in plants treated with chlorate.

The stimulation of nitrate uptake by glucose as observed in the present study indicates a requirement of metabolic energy for uptake of nitrate by roots. It is true that glucose could have many direct or indirect effects on protein synthesis, in general, and on the appearance of nitrate reductase in particular. Travi's and co-workers (1970, 1971) have shown that glucose is responsible for maintenance of polyribosomes. Their results suggest that glucose could

enhance the synthesis of nitrate reductase by increasing the protein synthesizing machinery in the system.

However, the inhibition (more than 90 %) of ion absorption in the presence of DCMU as observed in the present study suggests that the factors which affect photosynthetic electron transport do have a marked influence on nitrate uptake. Gray and Cresswell (1984) have reported that infiltration of excised maize leaves with DCMU, an electron transport blocker of photosystem II, resulted in diminished rates of nitrate uptake and utilization. Inhibition of photosynthetic electron transport will result in a decline in both ATP levels and flow of reducing equivalents to the cytoplasm. In the present investigation, DCMU has been observed to suppress nitrate reduction in buckwheat seedlings. The inhibitory effect of DCMU on nitrate uptake by buckwheat seedlings thus could be attributed to its inhibitory role in nitrate reduction rather than an effect on the mechanism of transport. In the present investigation, although, no marked difference could be observed between uptake rates in seedlings, subjected to continuous illumination with either fluorescent light or with red light, a more than 60 percent suppression of nitrate uptake was observed in seedlings maintained continuously under blue light. The results demonstrate the role of light in nitrate uptake. According to Beevers *et al.*, (1965), light increases

nitrate uptake by leaves, leading to higher intracellular concentration of NO_3^- for induction.

Our investigations on the uptake of nitrate by buckwheat seedlings thus clearly indicate that the absorption and reduction of nitrate by seedlings of common buckwheat (*Fagopyrum esculentum* Moench) are energy dependent process linked to the operation of the photosynthetic electron transport chain in the plant. The results obtained in the present investigation also indicate that the process of uptake of nitrate by the seedlings is mediated via the low capacity " BASIC SYSTEM " of uptake.

The kinetic properties of the uptake process by excised roots were much similar to that observed for the intact seedlings. In both, the cases pH optima for uptake ranged between 5.5 and 6.5 with a Km value ranging between 200-220 μmol. These results indicate the existence of a species specific nitrate transport protein through which the nitrate ions are transported across the plasma membrane of the roots.

CHAPTER VI

Uptake and Partitioning of Nitrate by Plants Under Sand Culture

i) Experimental

ii) Results

iii) Discussion

EXPERIMENTAL

Seedlings of common buckwheat were raised in the laboratory as described earlier. Eight day old seedlings were transplanted into pots, each containing 2.6 kgs. of sand washed with 0.3 percent H_2SO_4. The pots were arranged in 4 sets representing four treatments viz. control, 5, 20 and 50 mM of nitrate given us KNO_3^- in Hoagland's nutrient medium. The plants were maintained in a net house with four plants in each pot and 10 pots for each treatment. Harvest were made on 7, 19, 31, 43, 55 and 67 days after planting. Five plants were harvested at random from each set of treatments. The

harvested plants were washed under the running tap water and blotted dry betwen sheets of filter paper. The length of the shoot was measured and the plants were separated into stem, leaf, petiole and root segments. Leaf area was measured with the help of planimeter. The fresh weight of each segment was determined on a balance followed by drying in an oven at 80°C for 72 hours. From the oven dried materials, the dry weight of each segment was determined. Based on dry weight data of the samples and leaf area, the growth indices, viz., RGR, NAR and LAR have been calculated for the plants for each treatment and each sampling. In order to determine the partitioning of nitrogen between various tissue segments of the plant, the harvested plants were separated into shoot, leaves, petiole, and stem portions; the leaves and petiole being numbered in an acropetal order. The stem was divided into segments from the base and each segment was numbered in an acropetal order. In each segment the amount of various nitrogenous components and the level of NR activity was determined. In figure the values for nitrogenous constituents have been expressed both on percent dry weight basis as well as μg per total amount of dry weight of the particular unit. the activity of NR has been expressed as μmol nitrate released per 100 mg dry weight of the tissue segment per hour. Based on the model of Greenwood *et al.*, (1991) an attempt has been made to express the relationship between growth rate and percentage nitrogen in the crop at

various stages of development. An investigation has been carried out to understand the relationship between photosynthesis and the nitrogen status in the leaves and the whole plant by using the model of Greenwood *et al.*, (1986).

RESULTS

Irrespective of the treatment the dry weight of the shoot increased linearly with time between 7 and 43rd days after planting; the dry weight of the shoot also increased with increase in to amount of nitrate supplied, with plant receiving a dose of 20 or 50 mM KNO_3^- showing the maximum dry weight. On 7^{th} day there was no marked difference in the dry weight of the shoots between treatments. The magnitude of difference however increased with progressing time till 43rd day after planting. Further, the differences was more marked between plants irrigated with 0, 5 and 20 mM nitrate. Plant that were irrigated with Hoagland nutrient solution containing 50 mM nitrate showed the same level of dry matter accumulation as that observed in plants that received 20 mM nitrate (Table 6.1; Fig. 6.1).

Changes in the total leaf dry weight of the plants followed the same trend as that observed for the shoot. Irrespective of the level of applied nitrate a linear increase in the total leaf dry weight was observed between day 7 and 31. The total leaf dry weight in plants receiving an external supply of nitrate, continued to increase with

progressing time till it attained a plateau on 55th day. In the case of controls, however, the total leaf dry weight attained stationary phase after day 43 of planting only. Marked differences were observed in the total dry weight of the leaves between the untreated control and those that received either 5 or 20 or 50 of nitrate with the irrigating solution. Thus, while the control attained a maximum dry weight of 18 mg, plants receiving 50 mM of nitrate attained a maximum of 35 mg of dry weight. There were no significant differences in the leaf dry weight between plants receiving either 20 or 50 mM nitrate externally (Table 6.1; Fig. 6.2).

The changes in the total leaf area of the plants with time followed the same pattern as that observed for leaf dry weight. However, the value for the parameter did not differ significantly in plants receiving either 5, 20 or 50 mM of KNO_3^-. The leaf area of the plants receiving external nitrate was marginally higher than that of control plants, on the 7th day. However, the magnitude of difference in the leaf area between plants which did not receive any external supply of nitrate and those irrigated with nitrate supplemented solution increased with progressing time, till it was nearly two-fold on 43rd day after planting (Table 6.2; Fig. 6.3).

There was no marked differences in the leaf area ratio of the plants, with progressing time. However, when

Table 6.1 : Effect of varying doses of externally supplied nitrate on shoot dry weight and leaf dry weight of common buchwheat (*Fagopyrum esculentum*) grown under sand culture.

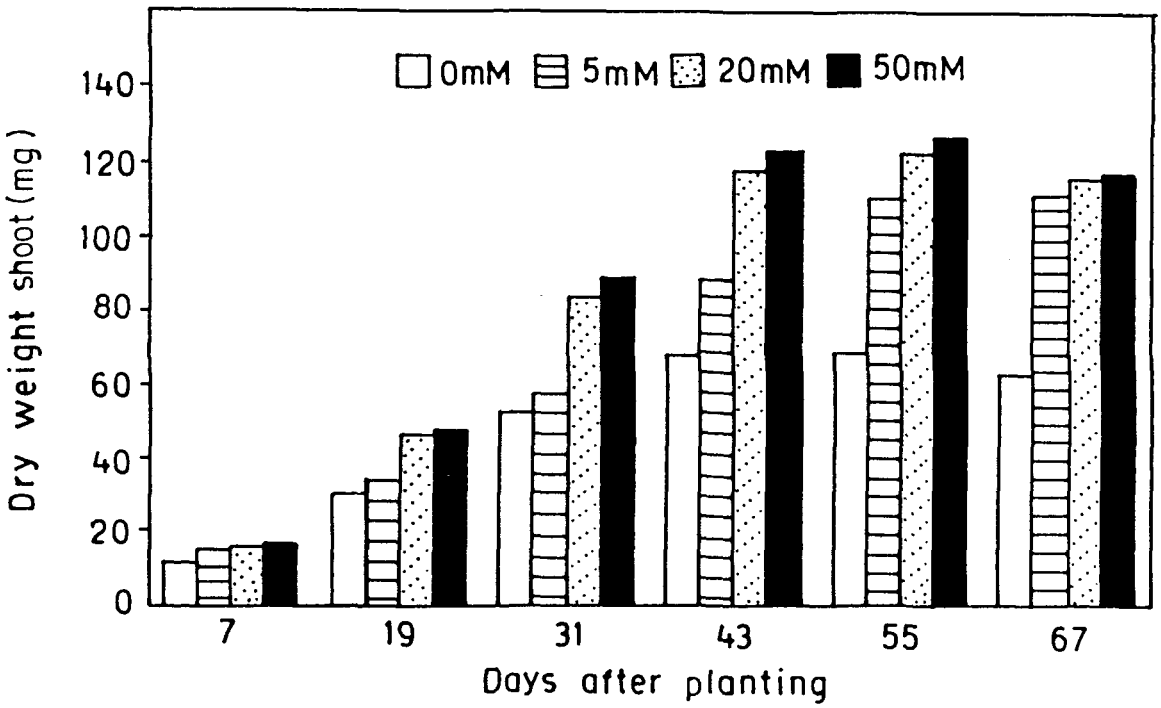
Days after Planting	Nitrate Concentration (μmol)			
	Control	5	20	50
	Dry weight Shoot (mg)			
7	11.2	15.05	16.05	16.55
19	30.5	33.88	46.25	48.20
31	52.4	57.60	84.00	89.00
43	67.5	88.30	117.50	122.50
55	68.5	110.50	121.50	126.50
67	62.5	111.00	116.50	116.50
	Dry weight Leaf (mg)			
7	4.0	5.00	6.0	6.0
19	11.0	10.68	16.0	17.0
31	16.0	16.00	28.0	30.0
43	19.0	23.00	34.0	33.5
55	19.5	30.50	35.0	35.0
67	18.0	30.00	34.5	35.0

Table 6.2 : Effect of varying doses of externally supplied nitrate on changes with time in the total leaf area, leaf area ratio relative growth rate and net assimilation rate in plants of common buckwheat grown under sand culture.

Nitrate conc. (mM)	Days after Planting					
	7	19	31	43	55	67
			LA (cm^2)			
0	3.5	9.5	15.5	17.5	16.5	16.0
5	5.5	14.5	24.5	32.5	31.5	30.5
20	6.0	15.5	25.5	32.5	32.5	31.5
50	6.0	14.5	26.0	32.0	31.5	30.5
			LAR cm^2 leaf dry weight per day			
0	0.31	0.31	0.29	0.25	0.24	0.25
5	0.36	0.42	0.42	0.36	0.28	0.27
20	0.37	0.33	0.30	0.27	0.26	0.27
50	0.36	0.30	0.29	0.26	0.24	0.26
			RGR mg dry weight/day			
0	0.1025	0.083	0.0307	0.0211	0.00122	-0.0076
5	0.175	0.0676	0.0442	0.0356	0.0186	0.0003
20	0.19	0.088	0.0497	0.0279	0.002	-0.003
50	0.199	0.089	0.051	0.026	0.0026	-0.0068
			NAR mg dry weight cm^2/day			
0	0.330	0.351	0.106	0.0755	-0.005	-0.03
5	0.583	0.402	0.171	0.0851	0.074	0.001
20	0.637	0.408	0.181	0.095	0.013	0.0016
50	0.675	0.45	0.19	0.122	0.02	0.0025

Fig.6.1; Effect of varying doses of NO_3^- on the changes in shoot dry weight with time in plants of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.

Fig.6.2; Effect of varying doses of NO_3^- on the changes in leaf dry weight with time in plants of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.


0mM
5mM
20mM
50mM
Dry weight shoot (mg)
0
20
40
60
80
100
120
140
7
19
31
43
55
67
Days after planting

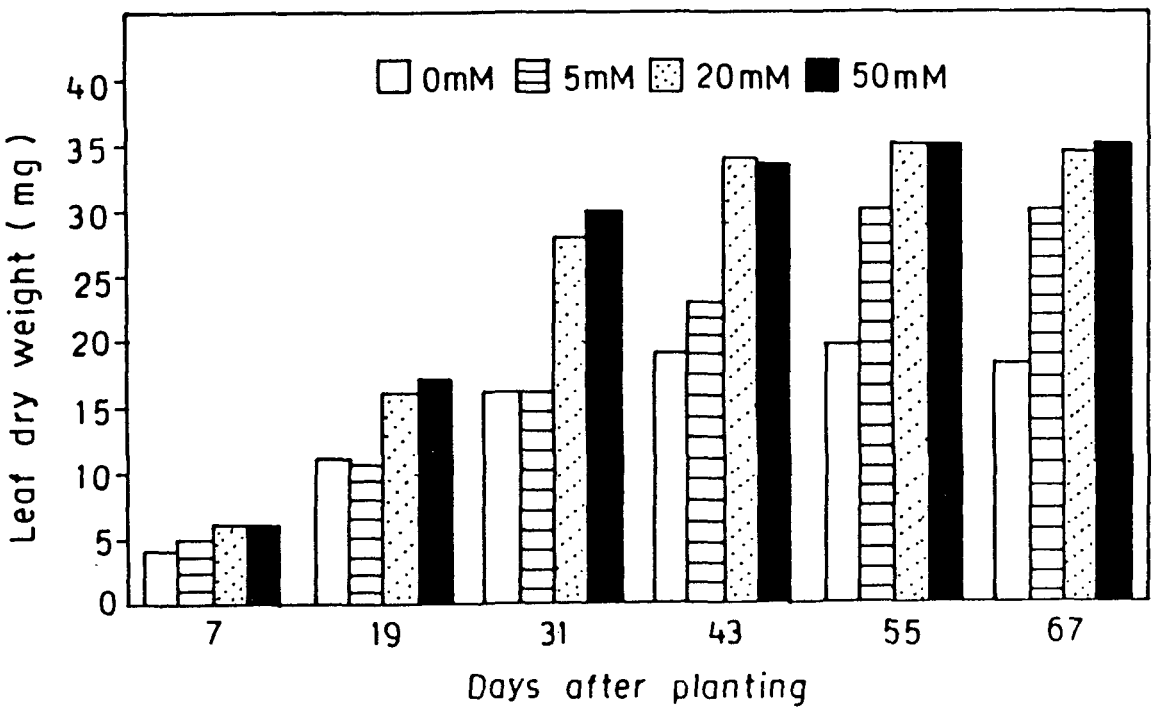
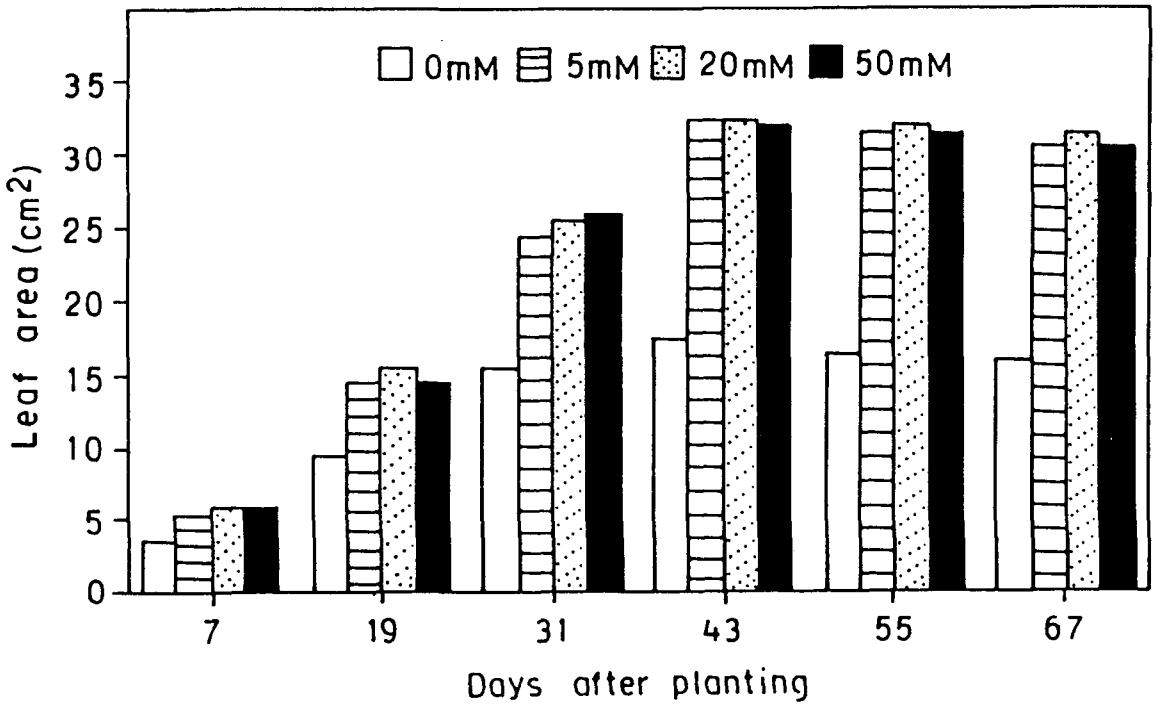
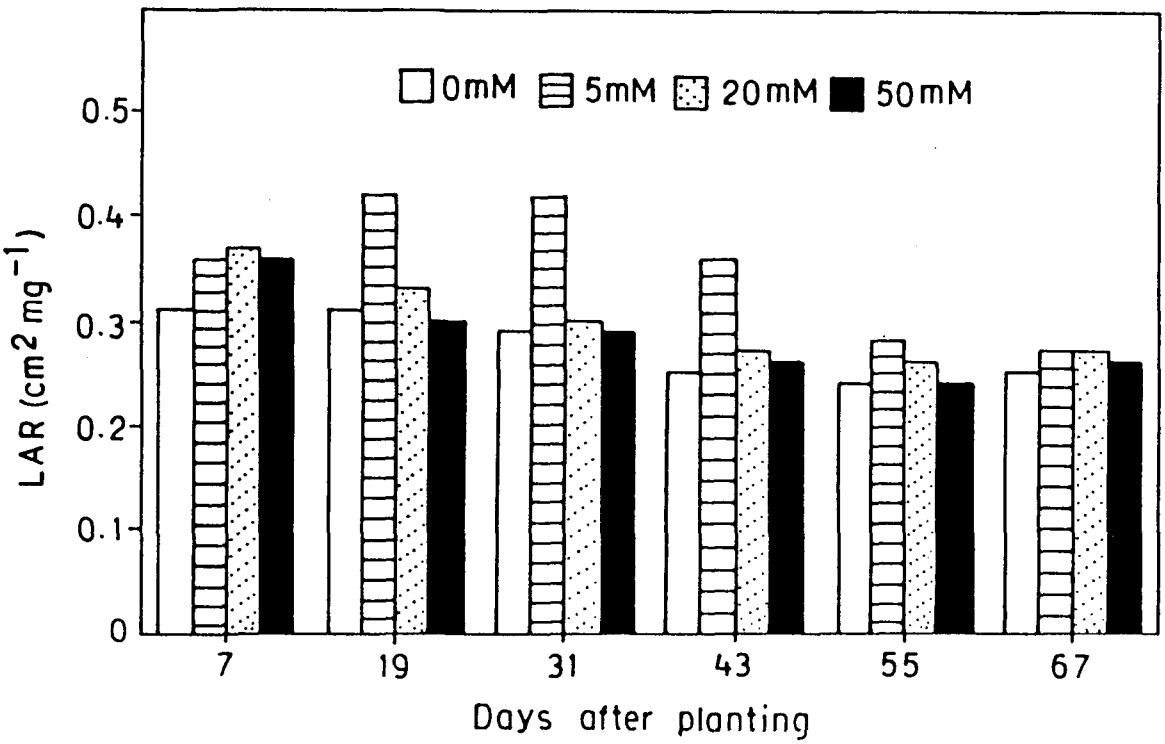

0mM
5mM
20mM
50mM
Leaf dry weight (mg)
0
5
10
15
20
25
30
35
40
7
19
31
43
55
67
Days after planting

Fig. 6.3; Effect of varying doses of NO_3^- on the changes in Leaf area ratio with time in plants of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.

Fig. 6.4; Effect of varying doses of NO_3^- on the changes in leaf area ratio with time in plants of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.


0mM
5mM
20mM
50mM
Leaf area (cm2)
0
5
10
15
20
25
30
35
7
19
31
43
55
67
Days after planting

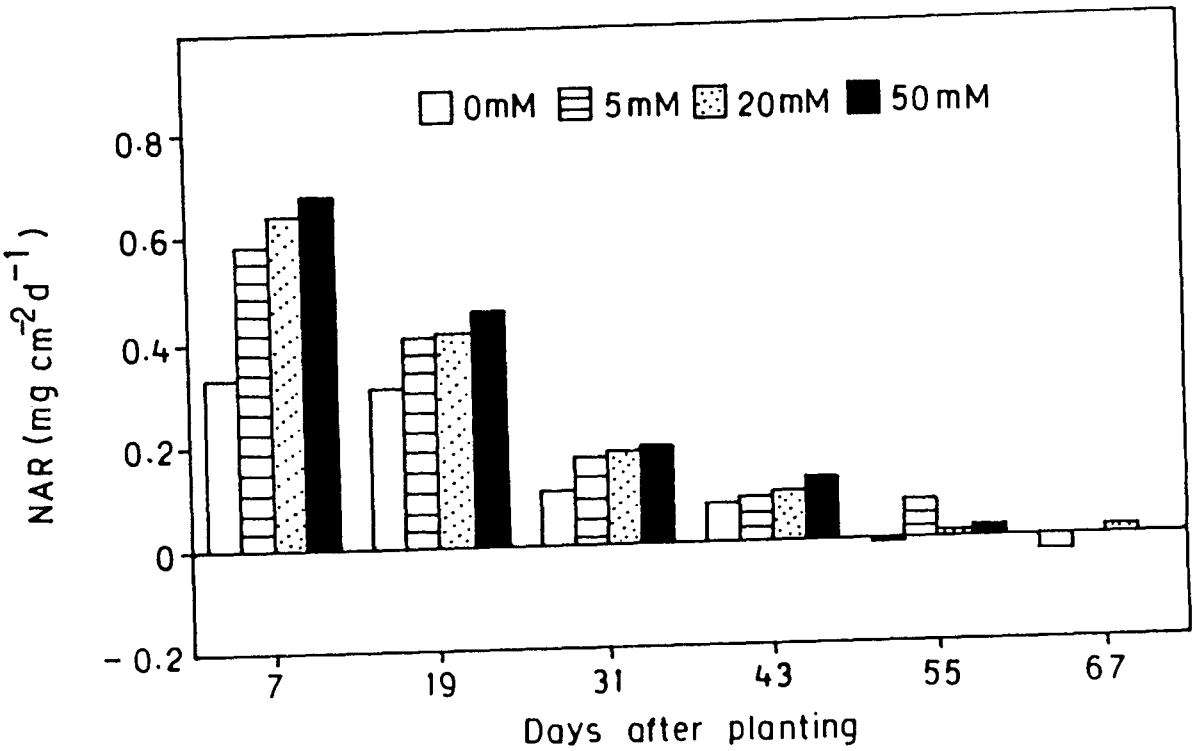

0mM
5mM
20mM
50mM
LAR (cm2 mg-1)
0
0.1
0.2
0.3
0.4
0.5
7
19
31
43
55
67
Days after planting

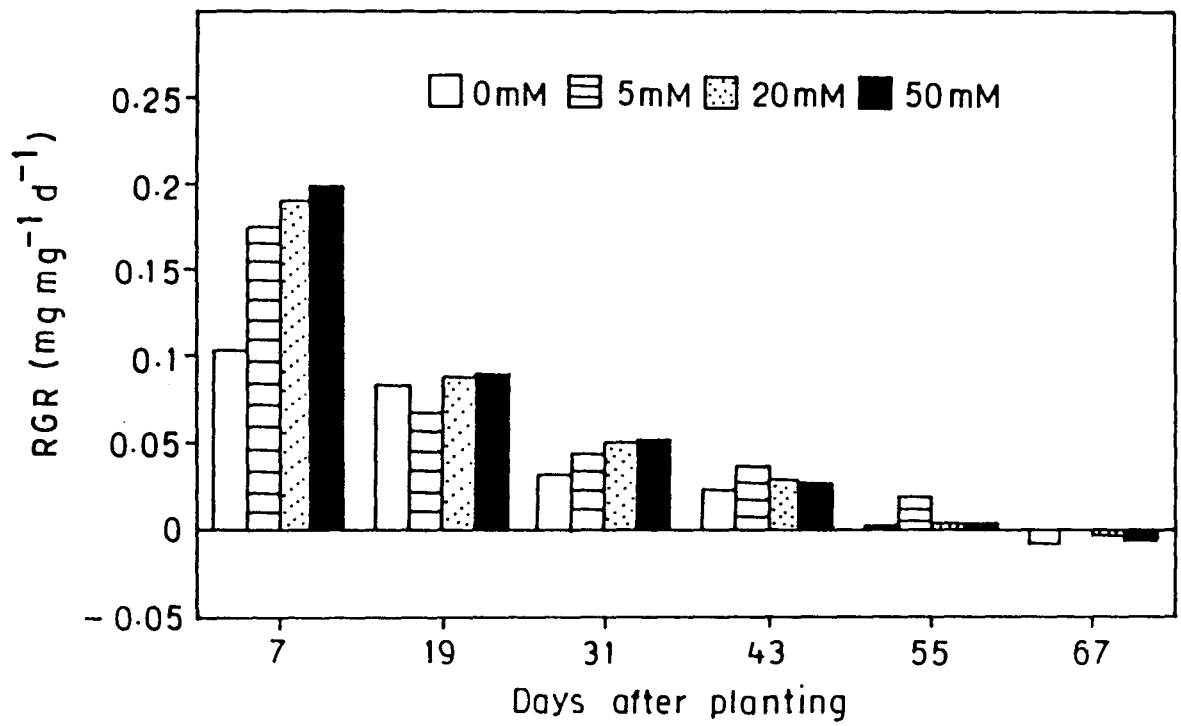
compared with untreated controls, plants irrigated with nitrate containing nutrient solution showed marginally higher leaf area ratio at any stage of growth. Within the treatments, however, the maximum leaf area ratio was shown by plants that were irrigated with Hoagland's nutrient medium containing 5 mM nitrate (Table 6.2; Fig. 6.4). The plants showed the highest net assimilation rate between 7th and 19th day after planting. The rate of assimilation decreased with progressing time till it showed negative values after 55th day of planting. Nitrate ions had a stimulatory effect on the net assimilation rate of the plants. Thus, plants irrigated with Hoagland's nutrient solution containing 50 mM KNO_3^- showed a nearly two-fold increase in the net assimilation rate than that recorded for untreated controls. While the untreated control plants recorded positive increments for NAR upto 43 days of planting, those receiving an external supply of nitrate showed positive increment for the net assimilation rate up to 55 days of planting (Table 6.2; Fig. 6.5).

Irrespective of the treatment, relative growth rate for the crop, expressed as mg dry matter accumulated mg. dry weight^{-1} day^{-1}, showed the highest value on 7th day after planting. With progressing time, however, the growth rate showed a consistent decline till it registered negative values on day 67. Except on 7th day when the plant treated with either 5, 20 or 50 mM nitrate showed higher values for

Fig.6.5: Effect of varying doses of NO_3^- on the changes in net assimilation rate with time in plants of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.

Fig.6.6; Effect of varying doses of NO_3^- on the changes in relative growth rate with time in plants of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.


0mM
5mM
20mM
50mM
NAR (mg cm^{-2} d^{-1})
0·8
0·6
0·4
0·2
0
- 0·2
7
19
31
43
55
67
Days after planting

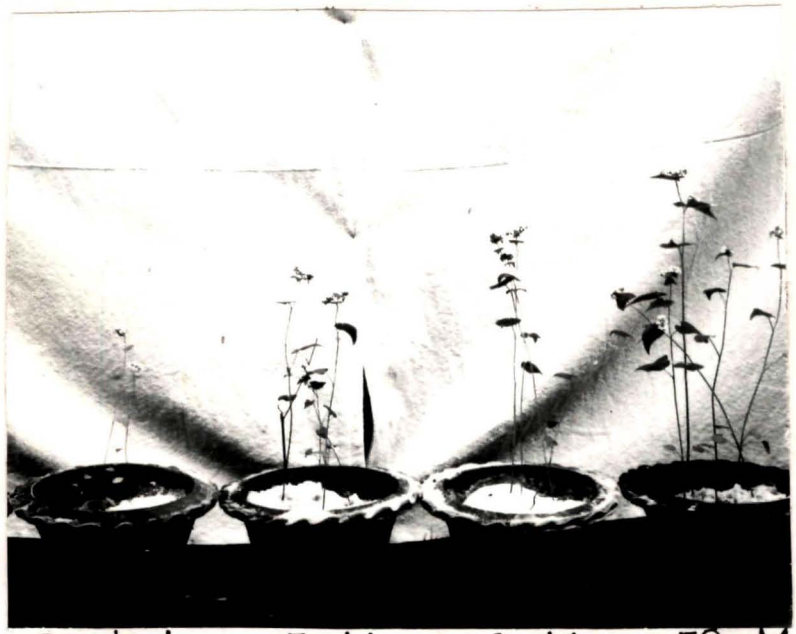

0mM
5mM
20mM
50mM
RGR (mg mg^{-1} d^{-1})
0·25
0·2
0·15
0·1
0·05
0
- 0·05
7
19
31
43
55
67
Days after planting

RGR, there was no significant differences in the raltive growth rates of the plants irrigated with Hoagland's nutrient medium supplemented with varying concentration of nitrate (Table 6.2; Fig. 6.6). However, plants irrigated with nitrate containing Hoagland nutrient medium showed positive increments for relative growth rate for a much higher duration than those which were irrigated with nitrate free nutrient solution (Table 6.2; Figs. 6.6, 6.7).

Irrespective of the treatments the content of total nitrogen in the plants, expressed as mg/100 mg dry weight of the plant, showed a progressive decrease with progressing time between 7^{th} and 67^{th} day after planting. The magnitude of decrease was, however, more marked, between 7^{th} and 19^{th} day after planting. Further, marked difference were observed in the content of total nitrogen between plants irrigated with nitrate supplemented nutrient solution and those which were irrigated with nitrate free nutrient solution. Thus, while the untreated control had a 0.7 percent total nitrogen content on 7th day, plant receiving 5 mM nitrate had a total nitrogen content of 1.2 percent on dry weight basis. The magnitude of difference, in the content of total nitrogen in plants receiving either 0, 5, 20 or 50 mM nitrate nitrogen, however, narrowed down with progressing growth of the plant. When expressed as µg total nitrogen/plant, the content of total nitrogen in the plants

Fig. 6.7 A: Plants of common buckwheat growing under sand culture and irrigated with Nitrate free Hoagland nutrient solution (a), Hoagland nutrient solution supplemented with 5 mM NO_3^- (b), Hoagland nutrient solution supplemented with 20 mm NO_3^- (c), Hoagland nutrient solution supplemented with 50 mM NO_3^- (d). Photograph taken 31 days after planting.

Fig. 6.7 B: A close up photograph of plants of common buckwheat growing under sand culture with Hoagland nutrient solution supplemented with 50 mM NO_3^- . Photograph taken 31 days after planting.



Control 5mM 20mM 50mM

50mM

showed a consistent increase with a progressing time between 7 and 43rd days. During this period the plants in each of the 4 treatment sets recorded a two-fold increase in the content of total nitrogen. After 43rd days, there was no significant increase in the total nitrogen content of the plants. Plants irrigated with nitrate containing nutrient solution consistently maintained higher levels of total nitrogen than those which were irrigated with nitrate free nutrient solution (Table 6.3; Fig. 6.8).

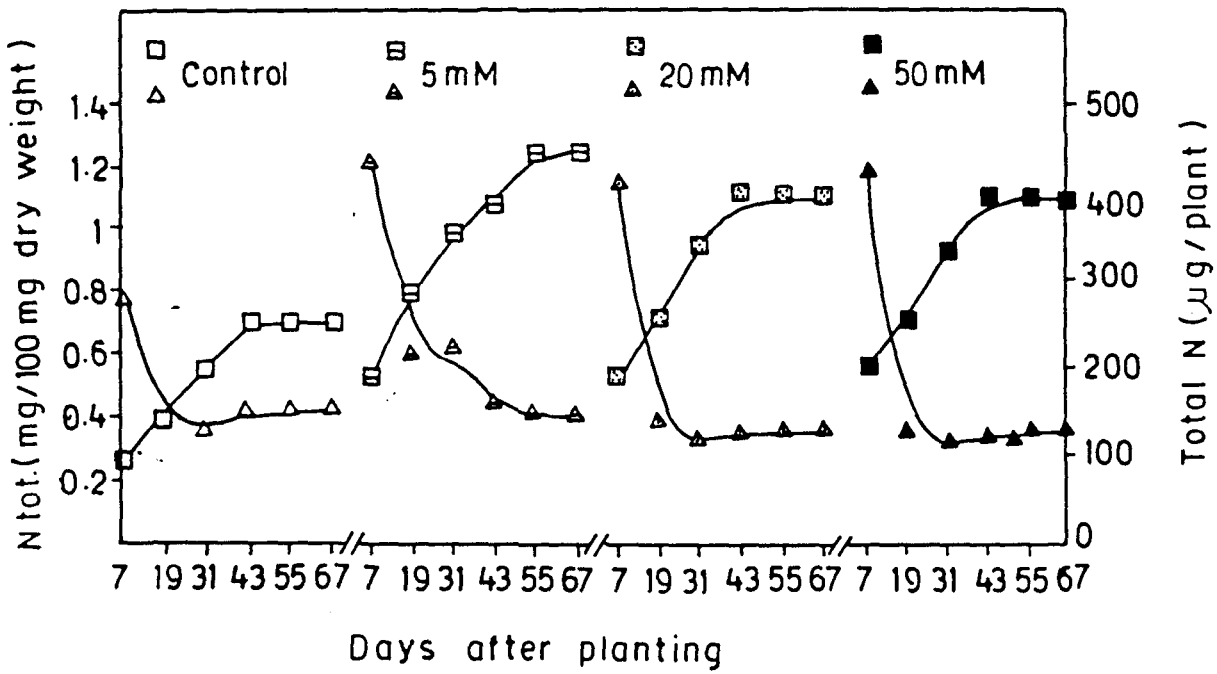
In order to determine the partioning of nitrogen between various tissue segments of the plant, the harvested plants were separated into shoot, leaves, petiole and stem. The dry weight of each segment was determined, after drying in an oven at 80°C for 72 hours. The dried samples were digested and the digests used for the estimation of total nitrogen content of the segment. The nitrate content of each segment was determined after extraction with HCl. In each segment the activity of nitrate reductase was also determined. The content of reduced nitrogen has been determined as the difference between total nitrogen and nitrate nitrogen.

When expressed as amount present per plant, the content of total nitrogen in the plants showed a consistent increase with progressing time between 7 and 43 day after

Table 6.3 : Effect of varying doses of externally supplied nitrate on the leaf nitrogen content and total nitrogen content in plants of common buckwheat grown under sand culture.

Days of Planting	Nitrate Concentration (mM)			
	Cont.	5	20	50
Total Nitrogen (mg/100 mg dry weight leaf)				
7	0.62	0.70	0.91	0.91
19	0.50	0.66	0.75	0.79
31	0.46	0.62	0.66	0.72
43	0.44	0.60	0.64	0.65
55	0.41	0.60	0.62	0.64
67	0.41	0.53	0.57	0.60
Total Nitrogen (mg/100 mg dry weight soot)				
7	0.71	0.81	1.13	1.17
19	0.38	0.59	0.60	0.62
31	0.31	0.50	0.52	0.53
43	0.29	0.40	0.42	0.43
55	0.29	0.38	0.41	0.43
67	0.30	0.37	0.42	0.42

Fig.6.8; Changes in the concentration of total nitrogen expressed as mg/100mg plant dry weight (△, ▲, ▲, ▲) with time and the content of total nitrogen per plant (□, ▤, ▩, ■) in plants of common buckwheat (Fagopyrum esculentum Moench) grown in sand culture and net house conditions under varying levels of externally supplied nitrate.


Control
5 mM
20 mM
50 mM
N tot.(mg/100 mg dry weight)
Total N (µg/plant)
1.4
1.2
1
0.8
0.6
0.4
0.2
500
400
300
200
100
0
7 19 31 43 55 67 7 19 31 43 55 67 7 19 31 43 55 67 7 19 31 43 55 67
Days after planting

planting. After 43rd day the level of total nitrogen in the plant remained by and large stationary. Plants that received an external supply of nitrate accumulated markedly higher level of total nitrogen than those which did not receive any nitrate externally. Thus, plant receiving 5 mM of nitrate showed a nearly two fold increase in the content of accumulated nitrogen. There were no marked difference in the amount of nitrogen accumulated by plants receiving either 5, 20 or 50 mM of KNO_3^-. However, differences were observed in the pattern of accumulation of nitrate nitrogen in the plants. Thus with increase in the supply of external nitrate, the plants accumulated more of nitrate nitrogen than reduced nitrogen. Irrespective of the treatment, the content of total, nitrate and reduced nitrogen, expressed on per plant basis, showed a consistent increase with progressing time. Thus a more than three fold increase in the level of each of the constituents was recorded with progressing time between 7 and 55 days. When compared with the untreated control plants, marked differences were observed in the level of total and nitrate nitrogen in plants receiving nitrate supplemented irrigating solution. Thus there was a more than two-fold increase in the content of total nitrate and nitrate nitrogen respectively, in all the nitrate treated plants. However, no marked differences were observed in total and nitrate nitrogen levels in plants receiving either 5, 20 and 50 mM nitrate in the irrigating nutrient solution. The

highest accumulation of each total, nitrate and reduced nitrogen was observed in the leaf tissue of the plants; the petiole accumulated the least amount of each of these constituents. During the early stages of growth, the shoot accumulated more of total and nitrate nitrogen than the stem tissues of the plants in all the treatments.

However, the pattern of accumulation reversed with progressing growth, with the stem showing more accumulation of total and nitrate nitrogen than the root (Figs. 6.9, 6.10, 6.11, 6.12). Irrespective of treatments the plants showed higher activity of nitrate reductase during the initial stages of growth. With progressing time, however, the activity of NR declined markedly. Higher level of NR activity was observed in plants that were irrigated with nitrate supplemented Hoagland's nutrient medium (Figs. 6.9, 6.10, 6.11, 6.12).

Nitrogen fertilizer has a decisive influence on the growth and yields of most stable crops throughout the world. Principles about the role of nitrogen have been incorporated into models that are being used to improve the efficiency of the use of fertilizers. Fundamental to these models is the knowledge about the dependence of plant growth on the content of total nitrogen in the plants. Evidence has been obtained that the decline in critical % N (the minimum % N in the

Fig.6.9; The content of total nitrogen ●; nitrate nitrogen □; reduced nitrogen ○ ; and the level of activity of nitrate reductase expressed as μmol NO_2^- released/100 mg fresh weight ○ ; in root o; stem ▶ ; petiole x; and leaf ●; of common buckwheat (*Fagopyrum esculentum* Moench) cultivated in sand with Hoagland nutrient solution deprived of nitrate supply at 7th day adfter planting (Fig. A), 19th day after planting (Fig. B), 43rd day after planting (Fig. C) and 55th day after planting (Fig. D).

Fig. 6.10: The content of total nitrogen ○: nitrate nitrogen □: reduced nitrogen ○: and the level of activity of nitrate reductase expressed as umol NO_2^- released/100 mg fresh weight ○: in the root ○: stem ►: petiole ×: and the leaf ●; of common buckwheat (Fagopyrum esculentum Moench) cultivated in sand with Hoagland nutrient solution supplemented with 5 mM nitrate, on 7th day after planting (Fig. A), 19th day after planting (Fig. B), 43rd day after planting (Fig. C) and 55th ady after planting (Fig. D).

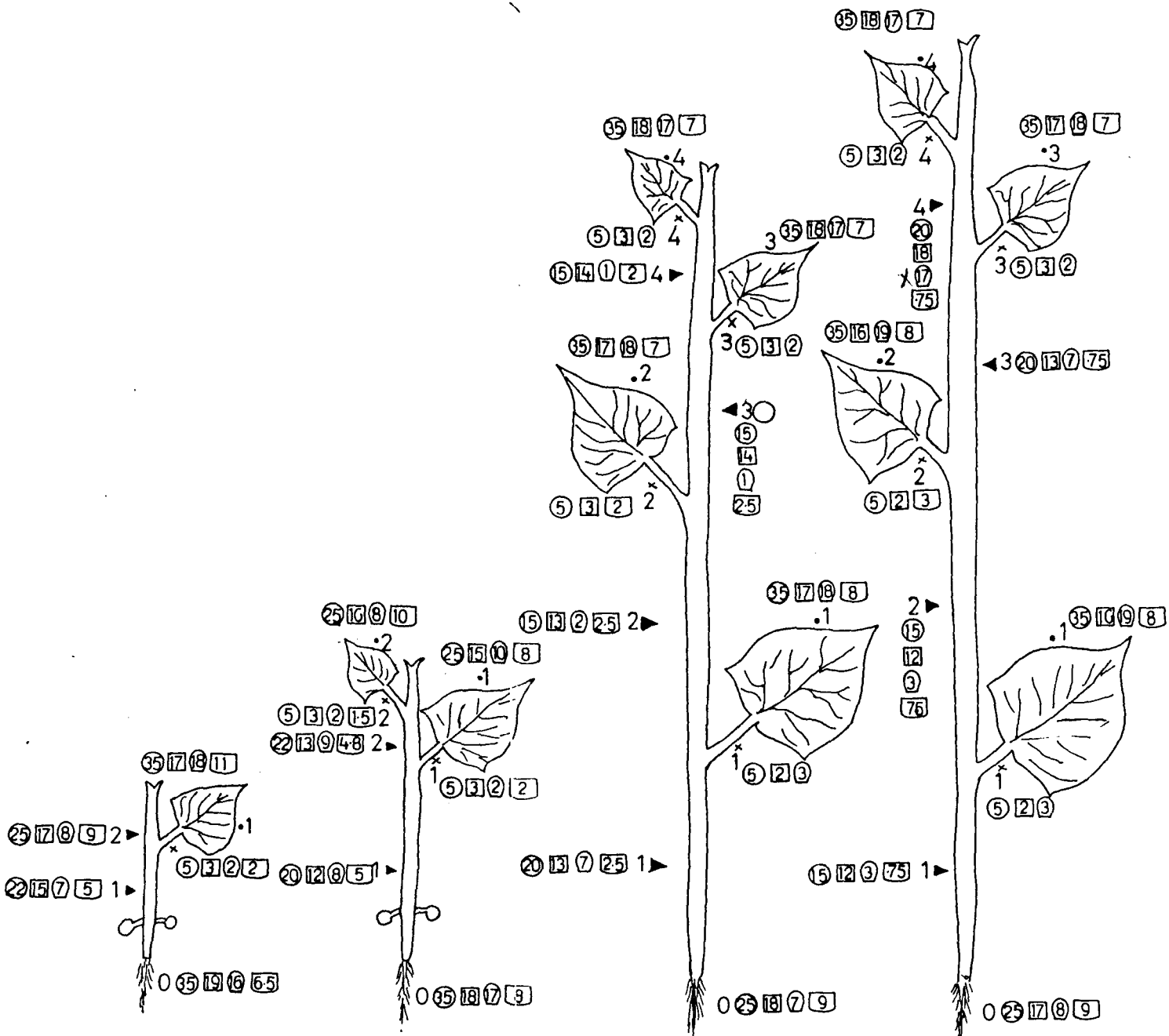


Fig. 6.11: The content of total nitrogen ○; nitrate nitrogen □; reduced nitrogen ⬭; and the level of activity of nitrate reductase expressed as umol NO_2^- released/100 mg fresh weight ⬭; in the root ○; stem ▶; petiole ×; and the leaf ●; of common buckwheat (Fagopyrum esculentum Moench) cultivated in sand with Hoagland nutrient solution supplemented with 20 mM nitrate, on 7th day after planting (Fig. A), 19th day after planting (Fig. B), 43rd day after planting (Fig. C) and 55th ady after planting (Fig. D).

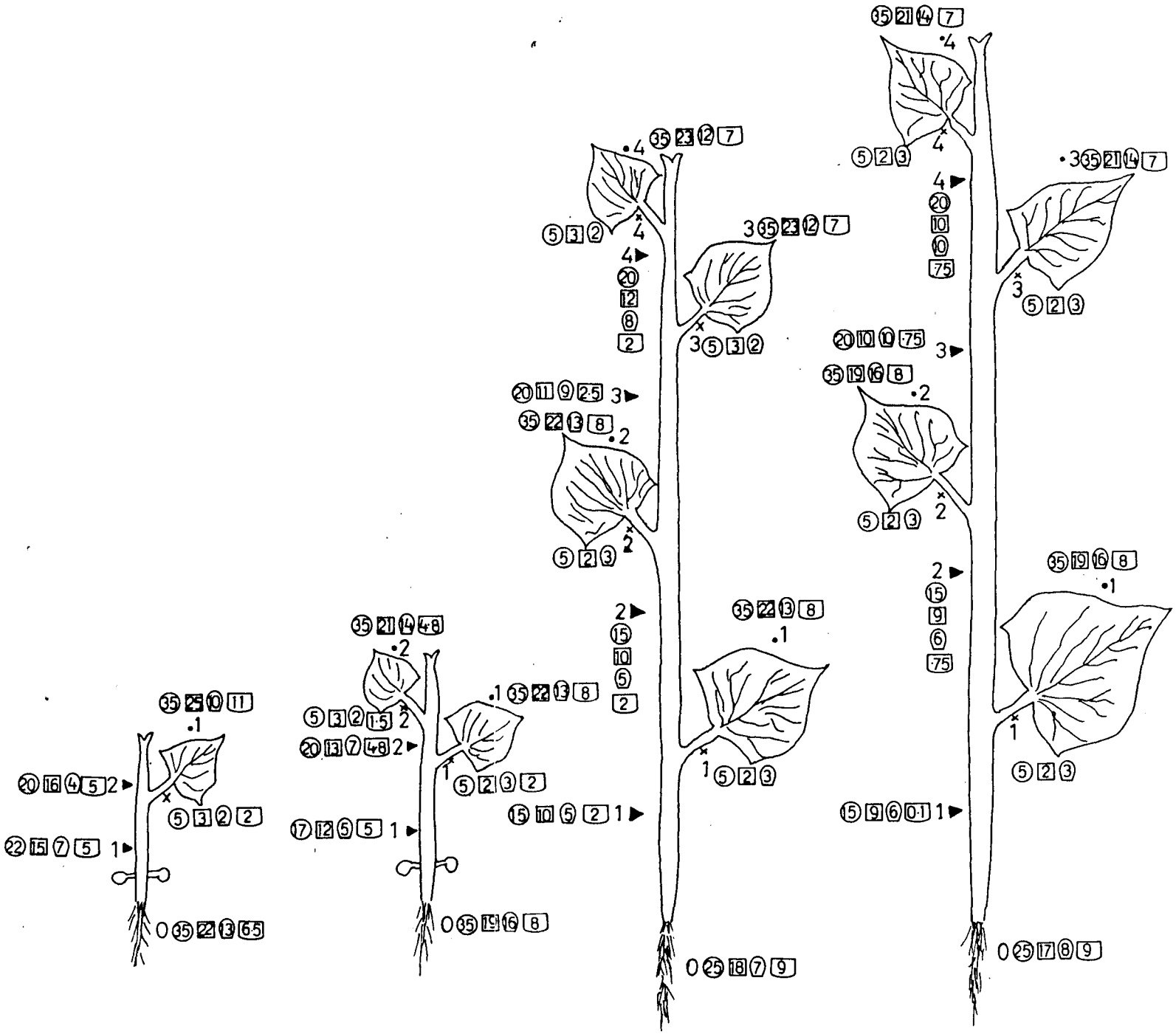
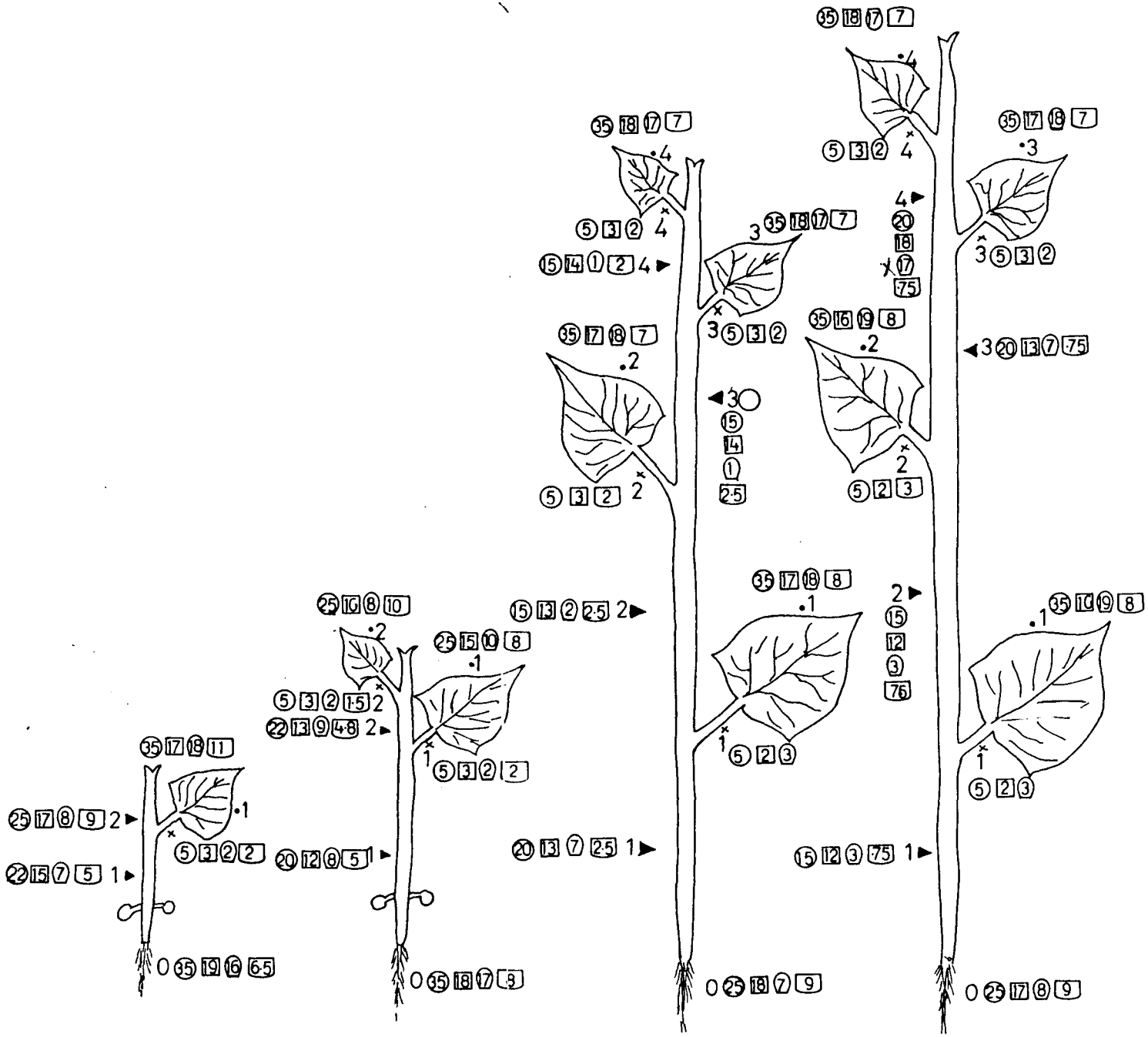


Fig. 6.12: The content of total nitrogen○; nitrate nitrogen □; reduced nitrogen◯; and the level of activity of nitrate reductase expressed as umol NO_2^- released/100 mg fresh weight ◯; in the root○; stem▶; petiole✕; and the leaf●; of common buckwheat (Fagopyrum esculentum Moench) cultivated in sand with Hoagland nutrient solution supplemented with 50 mM nitrate, on 7th day after planting (Fig. A), 19th day after planting (Fig. B), 43rd day after planting (Fig. C) and 55th ady after planting (Fig. D).



plant needed for maximum growth rate) in the plant is related to plant mass per unit area in much the same manner for a variety of C_3 arable and herbage crops. Leaf photosynthetic capacity has also been found to be highly correlated with the nitrogen status of the plant. Incorporation of these relationships into simulation models of N- response is. however. complicated by the fact that even when growing conditions are constant and supplies of nutrients and water meet the crop demand. RGR declines and absolute growth rate increases during growth. Greenwood *et al.*, (1991) have devised a growth rate coefficient that is independent of plant mass throughout the growing period. This coefficient has been devised on the earlier observations of Greenwood *et al.*, (1986) that the coefficient is linearly related to the ratio of % N in plants to the critical % N during the N limited growth. In the foregoing chapters, therefore an attempt has been made to describe the content of nitrogen in the plant in relation to the levels of the applied nitrogen fertilizer and its relationships with photosynthetic activity. The experimental data has been subjected to treatment with mathematical models developed by Greenwood *et al.*, (1986, 1991). Attempts have been made to test the effects of applied nitrate nitrogen on the growth and photosynthetic activity with plants of common buckwheat using the equations;

$$\frac{dW(F,t)}{dt} = \frac{[Kx\ (F)]\ [W(F,t)]}{x + W(t)} \qquad (1)$$

Where W(F,t) is the dry weight of the plant (excluding root) per unit area

Kx(F) is the growth rate coefficient

x is a constant

Integration of above equation would give

$$[Kx\ (F)][T-T_o] = x\ln w(F,t) + w(F,t) - x\ln w(F,t_o) - w(F,t_o) \qquad \ldots(2)$$

Where W(F,t) is the dry weight of the sample (excluding root) at time t, $W(F,t_o)$ is the dry weight of the sample (excluding root) at time t_o and t_o represents the time of initial harvest and t represents the time of next harvest. Greenwood *et al.*, (1991) have defined Kx(F) as Kc and W(F,t) as Wc(t), so that

$$\frac{dWc(t)}{dt} = \frac{KcxWc(t)}{x + Wc(t)} \qquad (3)$$

where Kc is a critical growth constant and Wc(t) is the critical dry weight at critical nitrogen level.

Greenwood *et al.*, (1986) have assumed that there is a positive relationship between critical % N in the plant dry matter Nc(t) and plant dry weight Wc(t) and have defined the critical nitrogen content in a plant by the equation

$$Nc\ (t) = 1.33 + \exp\ [1.4 - 0.26\ wc(t)] \qquad (4)$$

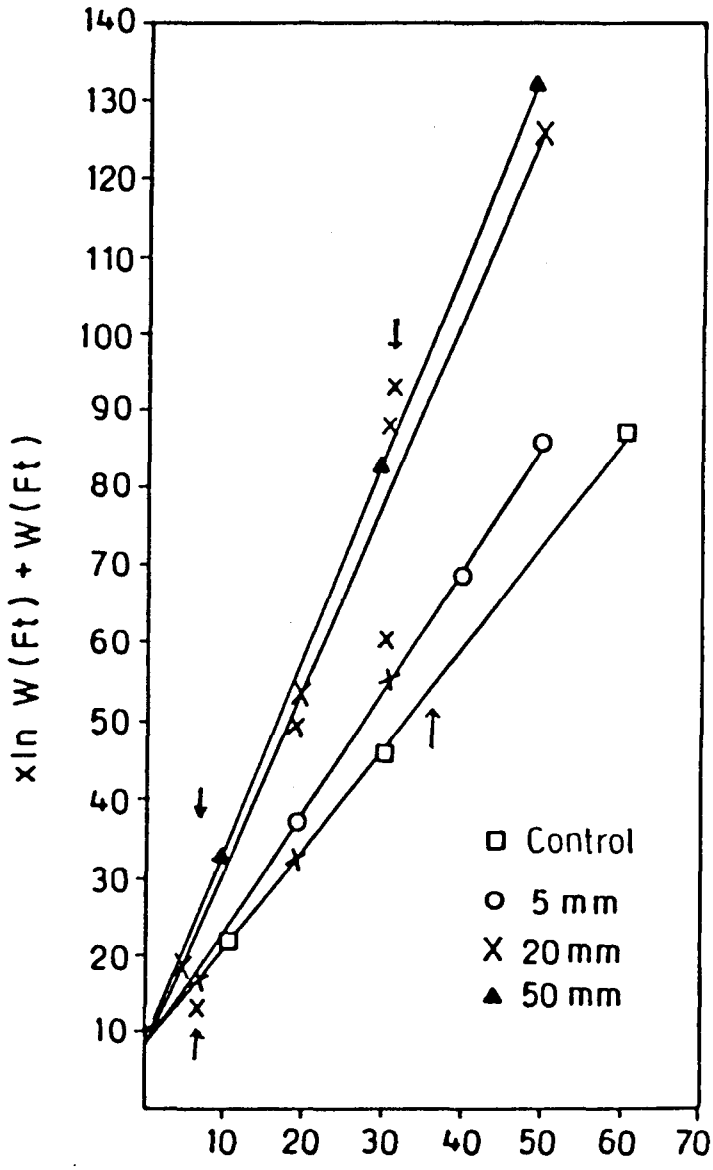
In the present study a growth rate coefficient has been extrapolated which is independent of plant mass throughout during the exponential phase of growth. Attempts have also been made to relate the growth rate coefficient with % N in the plant.

When derived from the model of Greenwood *et al.*, (1991) the RGR defined as xln W(Ft) + W(Ft) for the crop showed a linear and positive relationship with progressing time for each of the four levels of nitrate under which the plants were growing. The treatment had a marked effect on the slope of the regression equation describing the relationship between RGR and time. Plants showed higher values for RGR than untreated controls. When compared with the controls the relative growth rate increased with increase in the concentration of nitrate upto 20 mM. With further increase in the concentration of nitrate beyond 20 mM there was no further increase in the RGR for the crop (Table 6.4; Figs. 6.13, 6.14). Corresponding with the increase in RGR, Kx(F), the growth rate coefficient calculated from equation also showed a positive relationship with the level of applied nitrate in the ambient medium. Thus, the value for the coefficient increased linearly with increase in the level of nitrate from 0 to 20 mM. Beyond 20 MM nitrate

Table 6.4 : Changes in the relative growth rate, expressed as $x \ln W(F,t) + W(F,t)$, with changing time in common buckwheat (*Fagopyrum esculentum*) supplied with varying doses of nitrate under sand culture.

Nitrate conc. (mM)	Days after planting				
	7	19	31	43	55
	$x \ln W(F,t) + W(F,t)$				
Control	13.60	33.92	56.35	71.71	72.72
5 mM	17.76	37.40	61.65	92.78	115.20
20 mM	18.82	50.08	88.43	122.26	126.29
50 mM	19.35	52.07	93.49	127.30	131.34

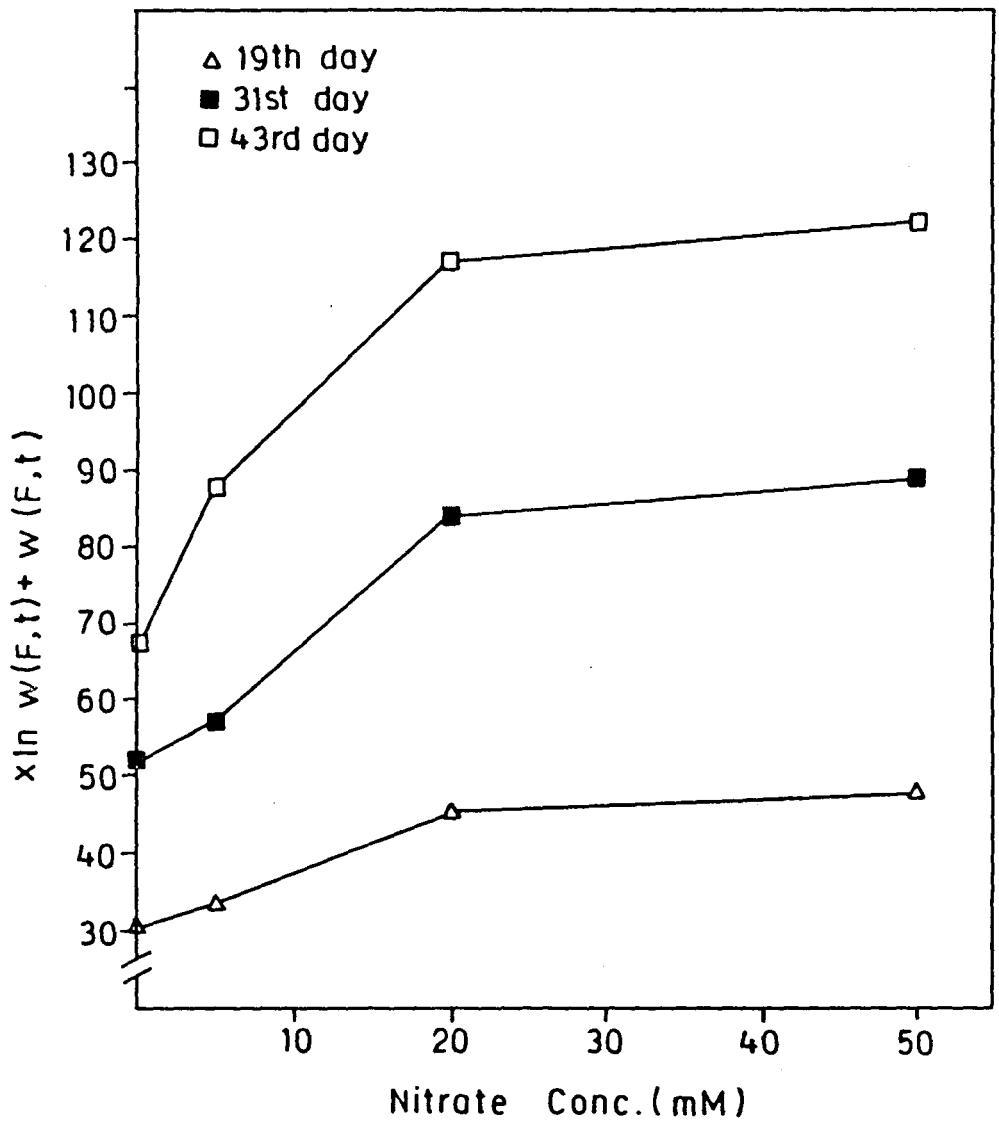
Fig. 6.13; Changes in relative growth rate, expressed as x lnw (F,t) + W(F,t) with time in common buckwheat (Fagopyrum esculentum Moench) supplied with varying doses of nitrate in sand culture under net house conditions.



Values of a, b, and r^2 for relationship between RGR expressed as Xln w(F,t) + w(F,t) with time for plants of common buckwheat growing in sand culture under varying levels of nitrate concentration.

Nitrate Conc. (mμ)	a	b	r^2
Control	9.350	1.300X	0.965
5 mμ	4.829	1.692X	0.932
20 mμ	7.000	2.392X	0.977
50 mμ	7.419	2.493X	0.975

Fig.6.14; Changes in the relative growth rate expresed as Xln W(F,t) + W(F,t) in common buckwheat (*Fagopyrum esculentum* Moench) on 19th day, 31st day and 43rd day after growing in sand culture under net house conditions.


△ 19th day
■ 31st day
□ 43rd day
130
120
110
100
90
80
70
60
50
40
30
x ln w(F,t)+ w (F,t)
10
20
30
40
50
Nitrate Conc.(mM)

concentration there was no marked increase in the value for the coefficient (Table 6.5; Fig. 6.15). On the basis of this data the minimal level of applied nitrate required to achieve the maximum growth has been determined. Thus under conditions of the present study a concentration of 20mM nitrate in the external medium could support maximum growth of the crop. The growth rate coefficient (Kx (F)) at this level of applied nitrate has been described as critical growth coefficient (kc(F)). Our results thus reveal a critical growth rate coefficient of 2.803 for the crop under conditions of the present study. The growth rate coefficient had a significantly positive correlation with the concentration of total nitrogen in the plant (Table 6.3; Fig. 6.16). However the slope of the equation describing the relationship between Kx(F) and concentration of total nitrogen in the plant increased with increase in the age of the plant. The relative growth rate described as a Kx(F)/x + W(F,t) also had a significantly positive relationship with % nitrogen, expressed on dry weight basis, in the plant (Table 6.6; Fig. 6.17). It is clear from the equation that a great deal of the effects of growth dilution and deficiency of nitrogen can be accounted for by this relationship and thus by differences in relative growth rate. The effects of N deficiency on relative growth rate can, therefore, be treated by assuming a linear relationship between the growth rate coefficient (Kx(F) and % N in the plant. Our results reveal a significant

Table 6.5 : Changes in the Kx(F) with changing concentration of nitrate supplied to common buckwheat (*Fagopyrum esculentum*) grown under sand culture.

Nitrate conc. (mM)	Kx(F)
Control	1.794
5 mM	2.196
20 mM	2.803
50 mM	2.920

Fig.6.15; Effect of varying doses of NO_3^- on the growth rate co-efficient Kx (F) in plants of common buckwheat (*Fagopyrum exculentum* Moench) grown in sand culture under net house conditions.

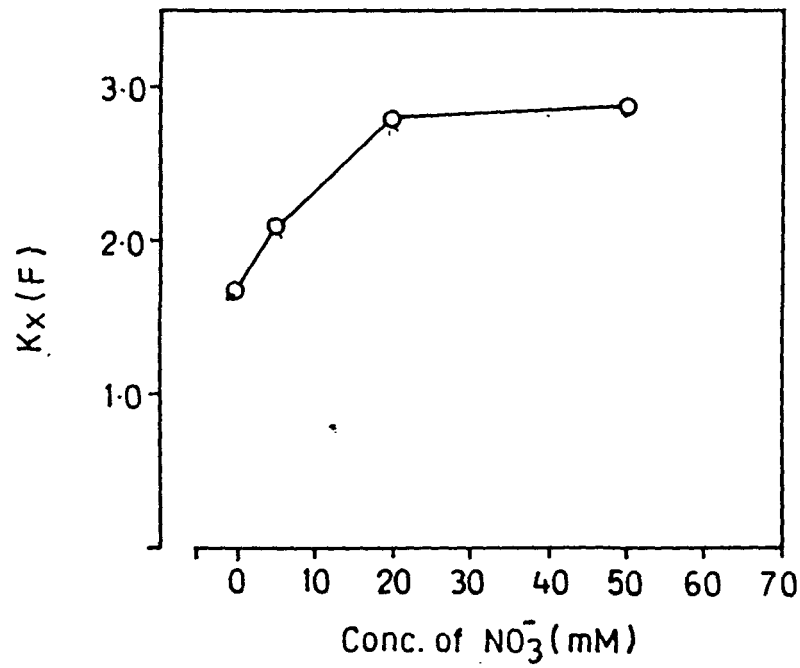

3·0
2·0
1·0
Kx (F)
0
10
20
30
40
50
60
70
Conc. of NO_3^- (mM)

Table 6.6 : Relationship between Kx(F)/x + W(F,t) and time in common buckwheat (*Fagopyrum esculentum*) supplied with varying doses of nitrate under sand culture.

Nitrate conc. (mM)	Days after planting			
	7	19	31	43
	Kx(F)/x + W(F,t)			
Control	0.147	0.0560	0.0330	0.0260
5 mM	0.136	0.0374	0.0374	0.0246
20 mM	0.164	0.0329	0.0329	0.0230
50 mM	0.166	0.0593	0.0324	0.0236

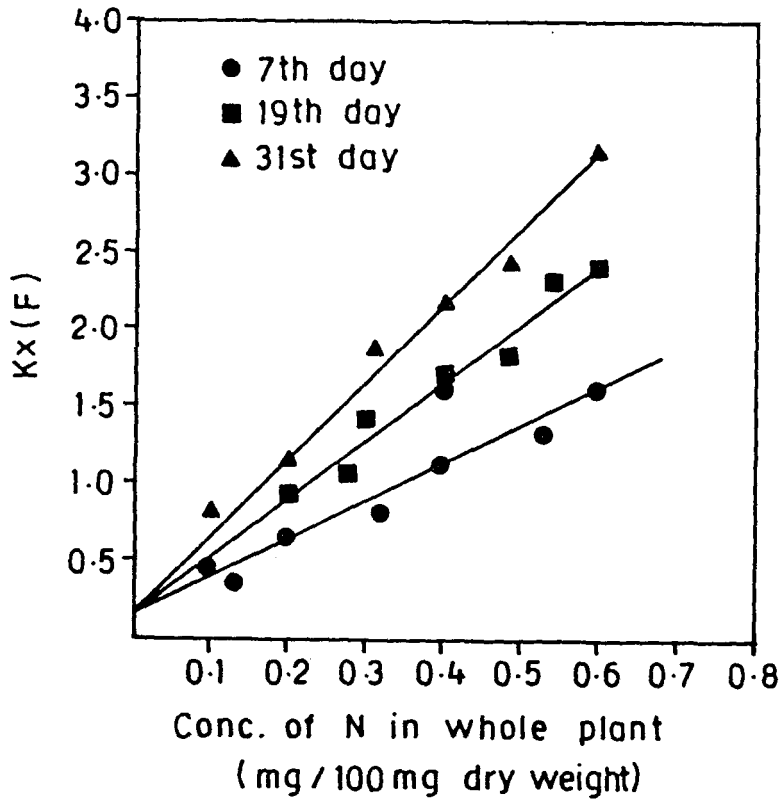
Fig.6.16; The relationship between growth rate coefficient (Kxf) and the concentration of total nitrogen in plant of common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions on 7th day, 19the day and 31st day after planting.

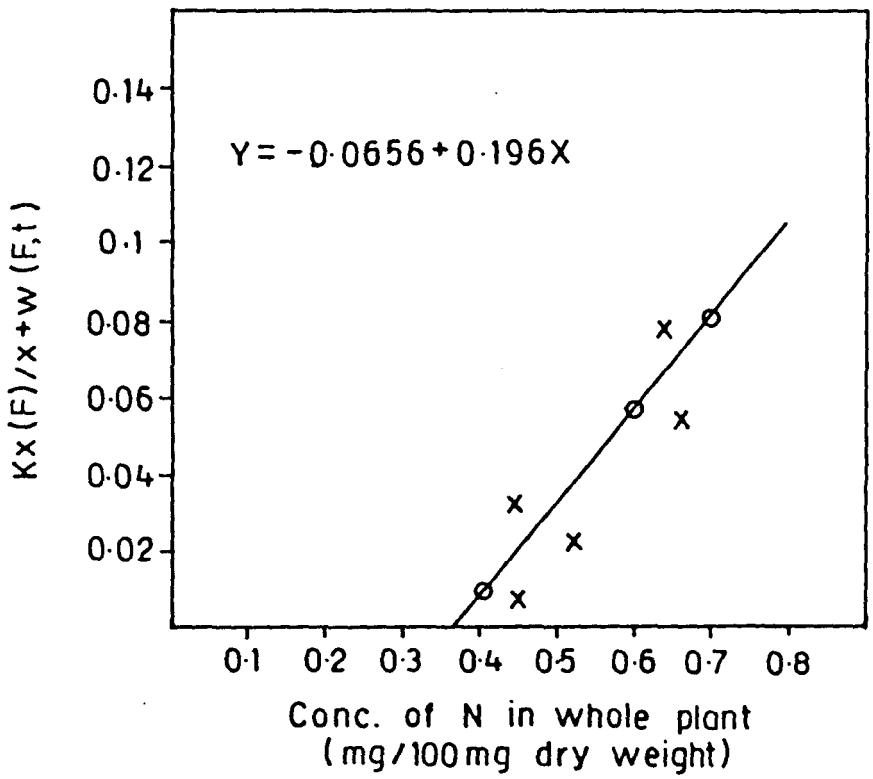
when Y = 0.267 + 2.251 x for 7th day

Y = 0.201 + 4.049 X for 19th day

Y = 0.365 + 4.379 x for 31st day

Fig.6.17; The Relationship between relative growth rate expressed as Kx(F)/x + W(F,t) and % N in whole plant in common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.


4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
Kx (F)
● 7th day
■ 19th day
▲ 31st day
0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8
Conc. of N in whole plant
(mg / 100 mg dry weight)


0·14
0·12
0·1
0·08
0·06
0·04
0·02
Kx (F)/x+W (F,t)
Y = -0·0656 + 0·196X
0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8
Conc. of N in whole plant
(mg/100 mg dry weight)

and positive correlation between the two. Kx(F) was linearly relate to % N at each harvest date: the gradient increasing with the increase in the concentration of nitrate in the irrigating solution. However, the value of NO_3^- ,corresponding to Kx(F) = 0.

Increase in the level of nitrate in the ambient nutrient medium had a positive effect on the accumulation of total nitrogen in the leaves as well as whole plant. However, the maximum accumulation of total nitrogen was observed at an ambient nitrate supply of 20 mM. Beyond 20 mM ambient nitrate supply there was no further increase in the content of total nitrogen in the plants (Figs. 6.18, 6.19). The photosynthetic activity in the plant, described as net assimilation rate, showed a positive relationship with the content of nitrogen in the leaf. Thus NAR showed a consistent increase with increase in the concentration of nitrogen in the leaf, at any stage of growth (Fig. 6.20). Greenwood *et al.*, (1986) have described the relationship between photosyntheic activity in the leaf with percent nitrogen in the leaf by the equation

$$P_L = M_L N_L + C_L \qquad (5)$$

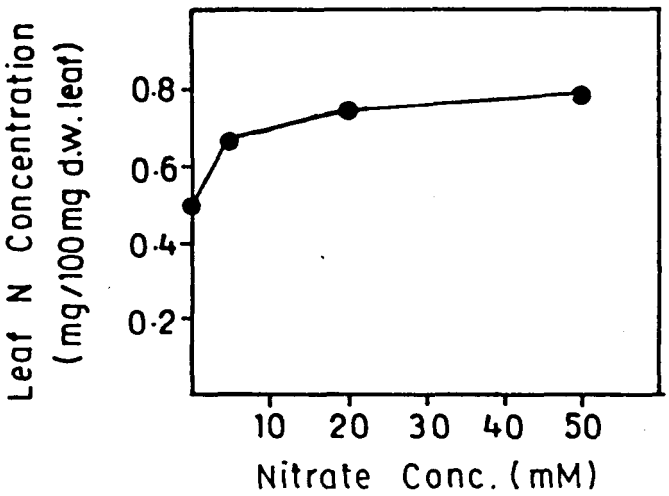
where P_L = leaf photosynthesis

N_L = content of percent nitrogen in the leaf

C_L and M_L are constants a and b respectively.

Fig.6.18; Changes in the nitrogen content of leaf expressed as mg/100 mg dry weight leaf in common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions supplied with varying levels of external nitrate.

Fig.6.19; Changes in the nitrogen content of whole plant expressed as mg/100 mg dry weight in common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions supplied with varying levels of external nitrate.


Leaf N Concentration
(mg/100mg d.w. leaf)
0·8
0·6
0·4
0·2
10
20
30
40
50
Nitrate Conc. (mM)

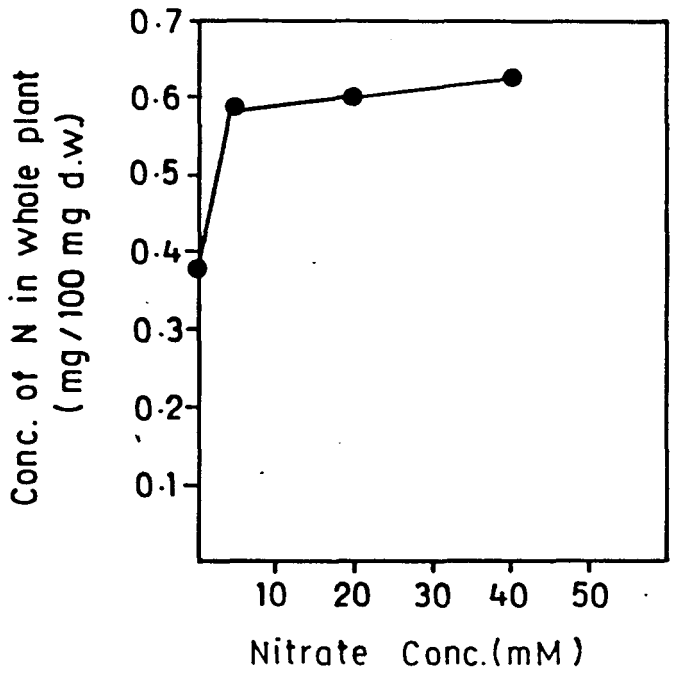
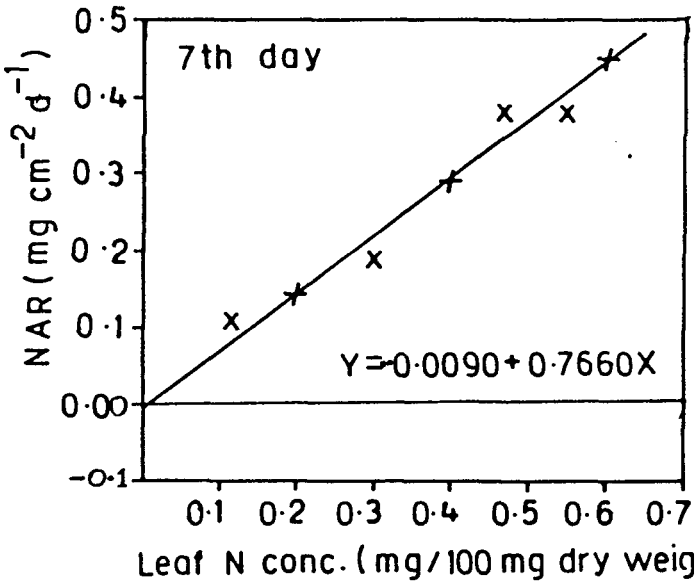
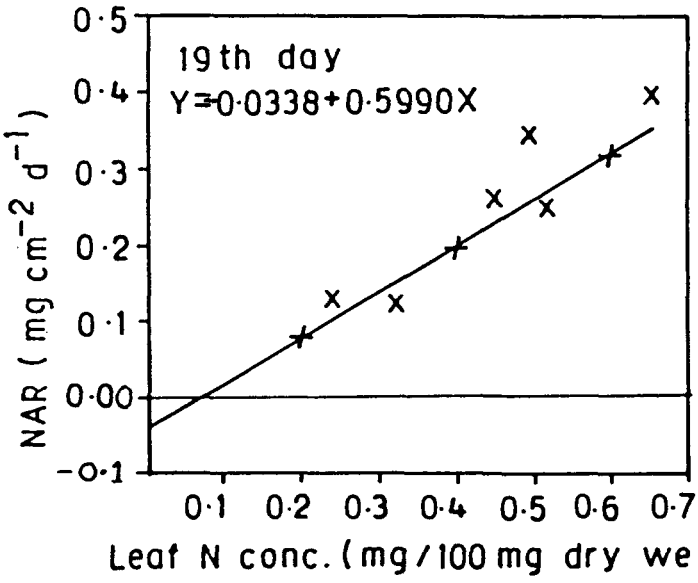
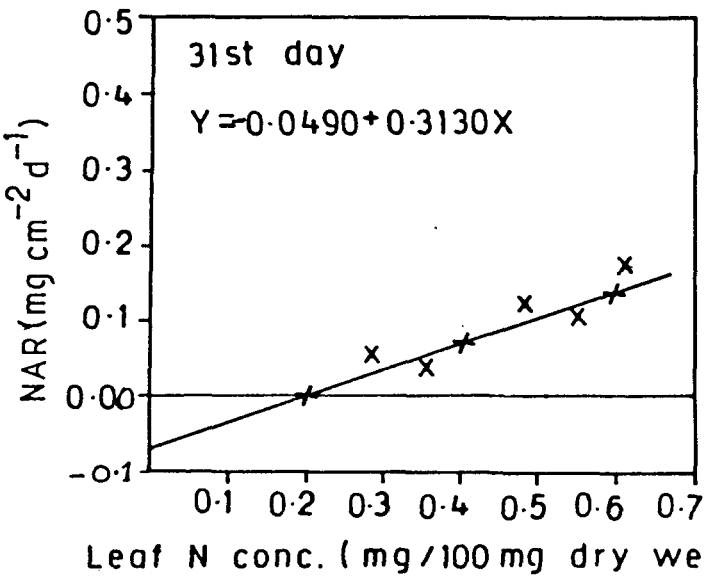

Conc. of N in whole plant
(mg/100 mg d.w.)
0·7
0·6
0·5
0·4
0·3
0·2
0·1
10
20
30
40
50
Nitrate Conc.(mM)

Fig. 6.20; Relationship between net assimilation rate and leaf nitrogen concentration expressed as (mg/100 mg dry weight) during different harvest period in common buckwheat (*Fagopyrum esculentum* Moench) grown in sand culture under net house conditions.


7th day
Y=-0·0090+0·7660X
NAR (mg cm^{-2} d^{-1})
0·5
0·4
0·3
0·2
0·1
0·00
-0·1
0·1 0·2 0·3 0·4 0·5 0·6 0·7
Leaf N conc. (mg/100 mg dry weight)


19th day
Y=-0·0338+0·5990X
NAR (mg cm^{-2} d^{-1})
0·5
0·4
0·3
0·2
0·1
0·00
-0·1
0·1 0·2 0·3 0·4 0·5 0·6 0·7
Leaf N conc. (mg/100 mg dry weight)


31st day
Y=-0·0490+0·3130X
NAR(mg cm^{-2} d^{-1})
0·5
0·4
0·3
0·2
0·1
0·00
-0·1
0·1 0·2 0·3 0·4 0·5 0·6 0·7
Leaf N conc. (mg/100 mg dry weight)

The values for a, b and r^2 for the equation $P_L = M_L N_L + C_L$ obtained for plants of common buckwheat, growing under varying levels of nitrate fertilization, at various dates of harvest are presented in Table 6.8. An analysis of the data on net assimilation rate as a function of leaf nitrogen content reveals that a general decline, with progressing time, in the value of the slope of the regression equation that described the relationship between NAR and leaf nitrogen content. It is thus clear that with progressing growth there was an increase in the nitrogen requirement of the plant to sustain minimal photosynthetic activity.

The relationship between the content of total nitrogen in the leaf with the content of total nitrogen in the whole plant has been defined by Greenwood *et al.*, (1986) by the equation

$$N_L = M_W N_W + C_W \qquad (6)$$

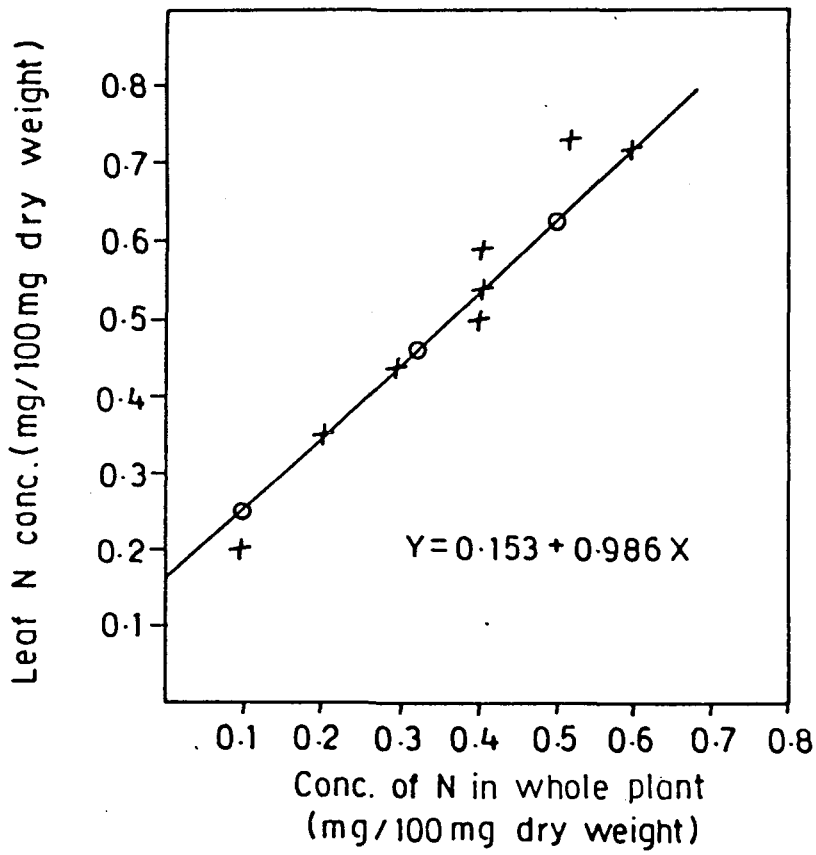
where N_L = content of percent nitrogen in the leaf

N_W = content of percent nitrogen in the whole plant

M_W and C_W are constant a and b respectively.

In the present study the concentration of N in the leaf showed a linear relationship with the average nitrogen concentration in the whole plant over a substantial range (average r^2 = 0.9) (Fig. 6.21). Integration of equations 5

Fig. 6.21; Relationship between total leaf nitrogen content expressed as mg/100mg dry weight and total nitrogen content in the plant expressed as mg/100mg plant dry weight in common buckwheat (Fagopyrum esculentum MOench) grown under varying levels of externally supplied nitrate, under net house conditions.


Leaf N conc.(mg/100mg dry weight)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
Y=0·153+0·986X
0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8
Conc. of N in whole plant
(mg/100mg dry weight)

and 6 would give the relationship between the photosynthetic activity in the leaf described as NAR with the content of total nitrogen in the whole plant by the equation

$$P_L = M_L M_W N_W + M_L C_W + C_L$$

where P_L = leaf photosynthetic activity

N_W = content of percent nitrogen in the whole plant

$M_L C_W + C_L$ and $M_L M_W$ are the constants a and b respectively.

To provide an estimate of the importance of the constant, average value of M_L, C_L, Mw and C_W were obtained. Substitution of these values in equation 6 would give $P_L = 0.0099 N_W + 0.656$ - on 19th day after planting. $P_L = 0.0012 N_W + 0.308$ on 31st day after planting and $P_L = -0.0554 N_W + 0.372$ on 43rd day after planting for plant of common buckwheat growing under sand culture under varying levels of nitrate supply.

DISCUSSIONS

The presence of nitrate ions in the nutrient solution had a stimulatory influence on the growth of the plants. Thus when compared with those plants which were grown in a nitrate free medium, plants growing in a medium containing nitrate ions had significantly higher levels of

dry matter. A nearly two-fold increase in the amount of dry matter accumulated was observed in plants irrigated with Hoagland's nutrient medium containing 20 mM KNO_3^-. There were no marked differences in the dry matter content of plants receiving either 20 or 50 mM of nitrate. This observation is in accordance with the results of Huett and Dettmann (1991). They have reported that the optimum predicted N-level for maximum dry matter production was 14.2 mM^{-1} for Lettuce, 18.1 mML^{-1} for Zucchini squash and 11.6 mML^{-1} for potato. In their study on the efficiency of leaves in dry matter production, Pearman, *et al.*, (1977) have reported that nitrogen fertilization had a stimulatory effect on the accumulation of dry weight of the vegetative part of the plants. They have stated that, when nitrate is given in higher amounts, more photosynthat was directed to the stem, which in turn lead to higher dry matter accumulation.

The total number of grains produced by plants receiving 5 mM nitrate was 180 per plant. This was nearly 50 percent more than the yield obtained for plants which did not receive any nitrate. However, a marginal decline in the grain yield was observed in those plants irrigated with Hoagland's nutrient medium containing 20 or 50 mM nitrate. From the results, it is obvious that the optimum N-requirement for maximum grain yield in common buckwheat was lower than that for total dry matter production. Thus while the plants

showed a maximum grain yield under 5 mM nitrate supply, the maximum dry matter production by vegetative parts of the plant was achieved in plants irrigated with a nutrient solution containing 20 mM KNO_3^-. The results further illustrate that there is a competition for assimilates between vegetative and reproductive parts of the plant. Huett and Dettman (1991) have reported that, the optimum N-levels for maximum yield in lettuce were 5 to 11 mML^{-1} and for potato was 7 mML^{-1}. In their analysis of the growth of spring wheat, supplied with different amounts of nitrogen fertilizer, Thomas and Thorn (1971) have observed higher amounts of nitrogen produced bigger plants with large leaves but with decreased grain yield. They have attributed the decreased grain yield to a significant change in the rate of photosynthesis of the leaves. This change makes the leaves inefficient in contribution towards grain production. Pearman *et al.*, (1977) have attributed, this inefficiency of the leaves of crops, given higher doses of nitrogen, in producing grain, to the effect of nitrogen on respiration.

As compared to the untreated control plants irrigated with Hoagland nutrient solution containing 5, 20 or 50mM nitrate, showed a nearly 2 fold increase in leaf area, upto 43rd day after planting. This was also reflected in the dry matter accumulation of leaves. Irrespective of the treatments, dry weight of the leaf increased with progressing

time upto 43rd day. Beyond 43rd day there was no marked change in the dry weight of the leaf with progressing time. In the present experiment, the dry matter accumulation in the leaf was dose dependent; thus leaf dryweight increased with increased nitrate content in the Hoagland's nutrient solution. In contrast, the total leaf area ratio was by and large independent of nitrate supply; however, plants receiving 5 mM nitrate had a marginally higher leaf area ratio than the nitrate starved control plants or plants treated with 20 or 50 mM nitrate. It is clear from the results that, in *Fagopyram esculentum*, 5 mM nitrate was sufficient to maintain optimum photosynthetic activity in the crop during the experimental phase of growth. Differences in the nitrogen treatments affect leaf expansion (Bottrice *et al.*, 1980) by influencing photosynthetic quantum yields and dark respiration or by making changes in the demand for photosynthetic rates (Nova and Loomis, 1981). Further, cellular expansion and leaf water relation ship has been reported to be affected by nitrogen deficiency (Radin and Bryer, 1982; Radin and Parker, 1979). Nitrogen deficiency may also cause secondary deficiencies of other essential nutrients (smith, 1962). Huett *et al.*, (1985) observed that in *Amaranthus powelli* wats. Variations in the nitrogen content brought about changes in the leaf area of the plants. In the present investigation, when the nitrate concentration in the irrigating solution was increased, the leaf area of

the plant also showed a corresponding increase. Therefore it is probable to make an assumption that, in *Fagopyrum esculentum*, increased nitrate level resulted in increased cell expansion and cell division. The increased cell expansion and cell division helped only to increase the leaf area and leaf dry weight but did not play any role in the ratio of leaf area to whole plant dry weight. The presence of nitrate ion had a stimulatory effect on net assimilation rate in common buckwheat; thus a more than 2.5 fold increase in net assimilation rate was observed in plants supplied with Hoagland's nutrient solution containing 5, 20 or 50 mM KNO_3^-, than that observed in nitrate starved control plants. However, there was no marked difference in the NAR, between plants receiving 5, 20 or 50 mM KNO_3^-. Leaf being a major contributor for dry matter accumulation in the plant, the factors influencing its photosynthetic capacity, affected the NAR. When compared with nitrate starved control plants, a nearly 2 fold increase in RGR was observed in plants irrigated with 5, 20 or 50 mM KNO_3^-. Like NAR, the RGR too did not show any marked variation between plants receiving either 5, 20 or 50 mM KNO_3^-. Blackman (1961) has summarized the effect of nitrogen upon plant growth by stating that increases in relative growth rate due to augmentations in nitrogen supply could be attributed to increases in leaf dry weight ratio rather than to changes in leaf area. In

Fagopyrum esculentum, the effect of nitrogen application on LAR appears to be the major cause of increase in NAR and RGR.

In order to determine the partitioning of variuos nitrogenous components within the plant, plants from each of the four treatments were harvested at different intervals and harvested plants were separated into different units. From each unit nitrate nitrogen, total nitrogen, reduced nitrogen and NR activity have been calculated. The results, obtained during the course of the study reveal a maximum level of various nitrogenous components in plants irrigated with 5 mM KNO_3^-. Increase in the nitrate concentration beyond 5 mM failed to bring about any significant variations, in the levels of various nitrogenous components in the plants. Leaves were extremely self supporting in terms of accumulating total nitrogen, and nitrate nitrogen.Petioles, by contrast were inactive in nitrate assimilation. Probably the petioles must acquired reduced nitrogen for their growth from other tissues of the plants. These observations are in accordance with the findings of Jeschke and Pate (1991), who while describing the nutritional interactions of shoot parts of different age and type, have suggested that in caster bean, the laminae of the three lowest mature leaves were more than self supporting in terms of generating various nitrogenous components.

In the present study, levels of various nitrogenous components were maintained, in root as well as basal parts of the stem, throughout the period of study. This observation is in accord with the theory of downward translocation of nitrogenous compounts, proposed by Jeschke and Pate (1991). While the leaves possessed, relatively higher amounts of various nitrogenous constituents, the stem tissues accumulated relatively lower levels of various nitogenous constituents. This is in consonance with the observation of Robinson and Millar (1987) in potato. There observation clearly revealed that the young leaves possessed significantly higher levels of various nitrogenous constituents than the stem tissues.

In *Fagopyrum esculentum* leaves probably act as storage organs. This speculation is a reflection of the significantly higher levels of various nitrogenous compounts in the leaves, especially in younger leaves. From the leaves the nitrogen reserves are presumably diverted towards grains during their development.

Fagopyrum esculentum showed a positive relationship linking nitrogen content, growth rate and plant mass. The percentage nitrogen in the plant declined as the plant grows. For this phenomena, Greenwood, *et al.*, (1991) have proposed a hypothesis. According to this hypothesis the photosynthetic

activties in the plant, described as rate of dry matter production are strongly influenced by the content of nitrogen in photosynthesis tissues of the plants. The relationship between the nitrogen status of the leaves and the activity of RuBP carboxylase in the leaves has been emphasized by Evans (1983). Results obtained in the present investigation clearly revealed a linear and positive relationship between percentage nitrogen in the whole plant and the growth rate coefficient $K_x(F)$, as derived according to the model proposed by Greenwood *et al.*, (1991). The linear and positive relationship between percent N in the leaf and that in the whole plant as observed in the present study reveals the interdependency between leaf and whole plant, in respect of nitrogen status, to support the growth and dry matter accumulation in the plants.

In the present study, the plants receiving 20 mM nitrate showed the maximum rate of dry matter accumulation. Besides, the plants also accumulated the maximum amount of total nitrogen at this level of nitrate fertilization. However, the yield response of *Fagopyrum esculentum* to nitrogen seems to be optimum at low (5 mM) than at high levels of applied nitrate. Under conditions of suboptimal nitrate fertilization the rate of dry matter increase appears to be limited by the photosynthetic and associated factors. This supports the concept in common buckwheat that

there is a positive relationship between nitrate fertilization and nitrogen accumulation in the plant. Our results also clearly establish the positive relationship between the nitrogen status of the plant and the rate of dry matter production by the plant. During the experimental phase of growt, the relationship between nitrogen status of the plant and photosynthetic activity in common buckwheat can be described by the equation $P_L = 0.308 + 0.0012\ N_W$ where P_L = leaf photosynthesis. Further reduction of N - supply has also reduced (% N) in the leaves, stems and roots in proportion to one another. Since the rate of photosynthesis varied at different phases of growth, *Fagopyrum esculentum* needs to be given N - fertilizer at least three different stages of its young age.

CHAPTER VII

General Summary and Conclusion

Nitrate uptake and utilization is presently considered as a major and early point of control of development of plant. Nevertheless, despite the extreme importance of nitrate in most agricultural ecosystems, a number of serious deficiencies remain in our understanding of the physiology and biochemistry of its uptake and assimilation. Therefore, an understanding of physiology and biochemistry of its uptake and assimilation is necessary to develop protocals for fertilizer regimes for improving the quantity as well as the quality of the harvest.

Even though quite a good amount of work has been done on the uptake and utilization of nitrate nitrogen in wheat, soyabeen, barley and maize, data on the utilization of nitrate nitrogen in common buckwheat (*Fagopyrum esculentum* Moench), a psuedocereal of extreme economic importance because of its short growth span, high nutritive value of its grains and its capacity to grow on poor soils, is scaty. A survey of the literature reveals that certain characteristics possessed by this crop give it an advantage over the conventional crops. The importance of the plant lies in the protein quality of its grains, short growth span and hardiness of the plant, Besidesthe foliage is used as a green vegetable and in an important commercial source of the glucoside "Rutin" which is used as a medicine. However, because of some problems associated with its growth like indeterminate growth habit, the crop has not being cultivated extensively and comes under the category of under utilized crops aa classified by International Bureau of Plant Genetic Resources (IBPGR). Although some studies have been made on the requirement of phosphate fertilization in buckwheat, not many reports are available on the nitrogen fertilization requirements in crop. The present study was therefore undertaken to:

(a) assess the various accessions of common buckwheat for the growth and yield attributes,

(b) characterize the uptake of nitrate in intact seedlings as well as excised roots of buckwheat seedlings under hydroponic culture, as a function of time, NO_3^- concentration, pH and accompanying ions,

(c) determine the relationships between photosynthetic activity and nitrate utilization in the plant during various phases of growth in the plant, so as to determine the nitrate nitrogen requirement of the crop at various stages of growth.

In order to assess the growth and yield attributes, the seeds of seven accessions of buckwheat which were procured from the NBPGR regional station at Shillong, were scanned by electon microscope for their seed coat characteristics. Based on the scanning electron microscopy of the seed coat, the seven accessions have been grouped into three categories. The data on the size and shape of the seeds of seven accessions further illustrated that the seeds of seven accessions were not similar to each other at least morphologically. However, the seven accessions did not differ from each other markedly in the chemical composition of their grains and growth behaviour. The conclusion has been corroborated by growth indices such as Leaf Area, LAR, NAR and RGR, calculated separately in each of the seven accessions.

Further an analysis of the polygonal diagram representing variables such as dry weight of stem, shoot, leaf, root and leaf area for the seven accessions at various stages of growth revealed that the seven accessions did not differ from each other, at least in their growth attribute.

The plants accumulated maximum dry matter in about three weeks after planting. However, the rate of dry matter production was maximum between 7 and 19 days after planting, in each of the seven accessions. A significantly positive relationship was observed between leaf area and dry matter accumulation in the crop.

The crop attained maturity in about six weeks time and completed its life cycle in about 9 to 10 weeks. However, because of the intermediate growth habit, the flowering extended from about 4 to 7 weeks after planting. However, among the seven accessions of buckwheat BDS-1354 distinguished itself by possessing determinate growth habit and synchroronization of seed maturity. Seedlings of the plant showed a linear and steady nitrate uptake during the initial 60 minutes upon exposure to the Hoagland's nutrient medium containing 5 mM nitrate as KNO_3^-. Significantly, there was no lag phase in the uptake of nitrate by the seedlings. After 60 minutes the uptake of nitrate gradually slowed down until it attained a plateau at t_{180} minutes. During the corresponding period the concentration of nitrate in the ambient nutrient

medium showed a gradual decrease with progressing time. When expressed as μmol nitrate taken up mg dry weight root^{-} min^{-1}, the seedlings showed a maximum uptake rate during the initial 30 minutes of incubation in the nutrient medium. The rate of uptake showed a pregressive decrease with progressing time until no signficant uptake was observed after the 3rd hour of incubation. Decrease in the concentration of nitrate in the ambient nutrient medium had no apparent effect on the rates of nitrate uptake by the seedlings as a function of time. Seedlings in test solutions whose concentration of nitrate was kept constant, also showed a pattern of uptake similar to that shown by seedlings in test solutions in which the concentration of nitrate ions was allowed to deplete over the period. Thus, from an analysis of the cumulative uptake of nitrate by buchwheat seedlings and changes in the rate of uptake with progressing time, as determined in the present investigation, it can be assumed that the uptake of nitrate across the root plasma membrane in common buckwheat is mediated through a low capacity basic system. It seems reasonable to postulate that the carrier for nitrate ions in the seedlings is already present in the system because of an endogenous supply of nitrate. The observed decrease in the rate of uptake with time could be ascribed to a refilling of the available storage components in the seedlings and not to a decreasing nitrate concentration in the ambient nutrient medium, because the rate of uptake in the seedlings, which

were kept in test solution in which the level of nitrate was kept constant all through, showed a trend similar to that observed for seedlings which were kept in test solution in which no replenishment for the loss of nitrate as a result of the uptake were made.

When the concentration of nitrate in the nutrient medium was varied from 0.05 to 5.0 mM, the rate of nitrate absorption by buckwheat seedlings was a function of external nitrate concentration according to Michaelis-Menten Kinetics. The Michaelis-Menten constant (Km) and maximum velocity (V_{max}) for nitrate absorption by buckwheat seedlings were 200 μmol and 0.276 μmol mg dry weight $root^{-1}min.^{-1}$ respectively. In the presence of ammonium and chlorate ions, the uptake of nitrate by the seedlings was markedly suppressed; the magnitude of suppression increasing with the increasing concentration of either ammonium or chlorate ions. A Lineweaver-Burk plot for the uptake of nitrate ions as a function of substrate concentration, at various levels of either ammonium or chlorate clearly revealed that while the inhibition due to ammonium was non-competitive in nature, that due to the presence of chlorate ions was of competitive in nature. While the Km for nitrate uptake in the presence of ammonium remained 200 μmol, the uptake process in presence of 0.005 and 0.05 mM ammonium had a V_{max} of 0.083 and 0.064 μmol mg dry weight $root^{-1}min.^{-1}$. In the presence of 0.005 and 0.05 mM of chlorate, the Km for the uptake of nitrate was 307 and

500 μmol respectively. Chlorate ions had no effect on the V_{max} of the process. Our results indicate that the inhibition of the nitrate uptake by ammonium ions is not simply a case of ammonium providing a counter-ion for nitrate, the inhibition appears to be due the efect of ammonium ions on the net rate of nitrate influx into the seedlings. The inhibitory role of chlorate ions on nitrate uptake may be because the ion acts as an analogue for nitrate in the process of nitrate uptake by plants.

In the present investigation presence of nitrate ions in the nutrient solution had a stimulatory influence on the growth of the plants. The highest dry matter accumulation was observed in plants irrigated with Hoagland nutrient medium containing 20mM KNO_3^-. Similarly plants irrigated with Hoagland's nutrient medium containing 20 mM nitrate had the highest value for RGR, LAR and NAR. The presence of nitrate ions in the nutrient medium had a stimulatory effect on the net assimmilation rate of the plants. Thus plants irrigated with Hoagland's nutrient medium containing 20 mM nitrate showed a more than two-fold increase in NAR than those irrigated with nitrate free Hoagland's nutrient medium. Irrespective of the treatment, the highest value of RGR was recorded on 7th day after planting, after which it showed a consistent decrease with progressing time till it registered negative values on 67th day. There were a significant

difference in the number of grains produced per plant between those irigated with Hoagland's nutrient medium containing 5mM nitrate and those did not receive any nitrate. However, a marginal decline in the grain yield was observed in those plants supplied with Hoagland's nutrient medium containing 20 and 50mM nitrate ions. From the result it is clear that the increased concentration of NO_3^- in the nutrient medium beyond 5 mM did not play any positive role in increasing the grain yield for the crop.

The plants supplied with Hoagland nutrient solution containing 5, 20 and 50 mM nitrate ions, showed a nearly two-fold increase in leaf area as well as leaf dry matter accumulation than that of nitrate starved control plants. In contrast the total leaf area ratio was nearly independent of nitrate supply. However, the maximum leaf area ratio was observed in plants that were irrigated with Hoagland's nutrient medium supplemented with 5 mM nitrate. In *Fagopyrum esculentum*, the effect of nitrogen aplication on LAR could be assumed to be the major cause of the effects of the treatments on NAR and RGR. Further, the increased NAR and RGR with increase in the supply of external nitrate, augmented only vegetative growth and not the grain filling.

The results of study on partitioning of various nitrogenous components within the plants revealed that plant

supplied with Hoagland nutrient solution containing 5mM KNO_3^- had the maximum level of various nitrogenous constituents. Increasing the nitrate concentration beyond 5mM failed to bring about remarkable variations. When the whole plant was considered as a four interconnected units, namely, root, stem, petiole and leaf, the leaves were found to be externally self supporting in terms of nitrogen balance within the plant. The leaves had the highest level of NR activity in the plant as compared to root, stem and petiole. Probably, the petiole acquired reduced nitrogen for their growth from other tissues of the plant. In *Fagopyrum esculentum* leaves act as storage organs. This speculation is a reflection of significantly higher amounts of various N-components and NR activity in the levaes, specially in younger leaves. The expected highest rate of nitrate reduction were observed in laminae followed by root and then at a generally much lower level, the petiole and stem.

Fagopyrentum esculentum showed a significantly positive relationship linking nitrogen content, growth rate and plant mass. The percentage nitrogen in the plant declined as the plant mass increased. There was a linear relationship between percent N in the whole plant and $k_x(F)$, the growth rate coefficient. The linear relationship between percent N in the leaf and that in the whole plant showed that there is an interdependency between leaf and whole plant, in

respect of nitrogen, to support the growth and dry matter accumulation in the plant.

Our results indicate an optimum requirement of 5 mM nitrate in the irrigating solution for obtaining the maximum yields. Further, under conditions of sub-optimal nitrate supply, the dry matter production expressed as net assimmilation rate in the crop during the exponential phase of growth had a direct relationship with the nitrogen status of the plant with the equation

$$NAR = 0.308 + 0.0012\ x$$

where " x " is the nitrogen content of the plant expressed as mg/100 mg dry weight.

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