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 (Beavers 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\textsubscript{3} are the induction of an enhanced level of NO\textsubscript{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\textsubscript{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\textsubscript{3}]), 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\textsuperscript{-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 determinations and metabolic inhibitors using \(^{15}\text{NO}^-\). 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 Chrunghoo (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\textsuperscript{-2} 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 interpreted 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 NO₃⁻ starved maize roots are supplied with nitrate in the presence of ³⁵S-methionine (Dhugga et al., 1988), indicating that nitrate uptake by roots is mediated by a plasma membrane localized protein system. Although no plasmalemma NO₃⁻ transport protein has been definitely identified from higher plants, genes encoding for NO₃⁻ transporter protein have been identified and cloned in prokaryotes and lower eukaryotes (Omata et al., 1989; Scanzocchio and Arst, 1989; Unkless et al., 1991). Jackson et al., (1986) have observed that higher plants also have systems that translocate NO₃⁻ within and between cells. Although the effect of external nitrate on intra and intercellular translocation has not been defined, Jackson et al., (1986) 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 NO₃⁻ are distinct. On the other hand ammonium uptake by plant roots during early exposure to NH₄⁺ 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\textsuperscript{+} transporter has not been previously reported. The significance of NH\textsuperscript{+} in the regulation of NO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} 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 occurs 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\textsuperscript{−} uptake and NR activity, the induction of nitrate uptake does not depend on functional NR (Jackson \textit{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 \textit{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 \textit{et al.}, 1988; Ni and Beevers, 1990). Kumar and Abrol (1990) have shown that L-methionine sulphotximine, 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 resistant to MSO. Several newly synthesized plasmalemma and tonoplastic proteins from 30 to 150 kD become labeled when NO\textsuperscript{−}\textsubscript{3} starved maize roots are supplied with NO\textsuperscript{−}\textsubscript{3} in the presence of \textsuperscript{35}S-Methionine (Dhugga \textit{et al.}, 1988; McClure \textit{et al.}, 1987). These results suggest that NO\textsuperscript{−}\textsubscript{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) demonstrated 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 immuno-gold localization of NR in spinach leaves showed NR exclusively associated with the chloroplast (Kamachi et al., 1987).

Since both NR and NO\textsuperscript{-} transport are simultaneously induced by NO\textsuperscript{-}, 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\textsuperscript{-} transport and NO\textsuperscript{-} reduction in the plasma membrane could be expected. Neyra et al., (1975) favour the concept of "co-ordinated induction" of both NO\textsuperscript{-} transport system and nitrate reductase. It has further been suggested that the membrane associated nitrate reductase protein could function as carrier for NO\textsuperscript{-} transport. Although no plasmalemma NO\textsuperscript{-} transport protein has been definitely identified from higher plants, genes encoding for NO\textsuperscript{-} transporter protein have been identified and cloned in prokaryotes and lower eukaryotes.
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 intracellularly 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 $Ca^{2+}$ (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 \(^{36}\text{Cl}\)-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. Based on the use of \(^{15}\text{N}\)-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 \(^{36}\text{C}\)-Chloride, a breakdown product of \(^{36}\text{C}\)-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 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 Olesinski 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 nutritio 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 nitrogen 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 C₃ 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 C₃ 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 different 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 (Ganyushina, 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 recommended 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 recommended 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 NO₃ 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.