

# 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 protocols 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, soyabean, 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 scanty. 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,

and to determine the chemical composition of their grains and foliage.

(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,  $\text{NO}_3^-$  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 NBFGR regional station at Shillong, were scanned by electron 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 synchronization 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  $\text{KNO}_3^-$ . 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  $\mu\text{mol nitrate taken up mg dry weight root}^{-1} \text{ min}^{-1}$ , the seedlings showed a maximum uptake rate during the initial 30 minutes of incubation in the nutrient medium. The rate of uptake showed a progressive decrease with progressing time until no significant 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 buckwheat 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 ( $K_m$ ) and maximum velocity ( $V_{max}$ ) for nitrate absorption by buckwheat seedlings were 200  $\mu\text{mol}$  and 0.276  $\mu\text{mol mg dry weight root}^{-1}\text{min}^{-1}$  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  $K_m$  for nitrate uptake in the presence of ammonium remained 200  $\mu\text{mol}$ , the uptake process in presence of 0.005 and 0.05 mM ammonium had a  $V_{max}$  of 0.083 and 0.064  $\mu\text{mol mg dry weight root}^{-1}\text{min}^{-1}$ . In the presence of 0.005 and 0.05 mM of chlorate, the  $K_m$  for the uptake of nitrate was 307 and

500  $\mu\text{mol}$  respectively. Chlorate ions had no effect on the  $V_{\text{max}}$  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 effect 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  $\text{KNO}_3$ . 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 assimilation 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 irrigated 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  $\text{NO}_3^-$  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 application 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  $\text{KNO}_3^-$  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 leaves, 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 ml 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 assimilation rate in the crop during the exponential phase of growth had a direct relationship with the nitrogen status of the plant with the equation

$$\text{NAR} = 0.308 + 0.0012 x$$

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