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II Effect of Applied Potassium in Increasing the Potential for Nitrogen Assimilation in Spinach (*Spinacia oleracea* L.)

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Abstract

Spinach (*Spinacia oleracea* L.) supplied with increasing levels of potassium (K) was grown in soil in a pot experiment under greenhouse conditions. The soil used, low in available potassium (40 mg/kg), was treated with a basal fertilizer application of nitrogen (100 mg N/kg soil) and phosphorus (30 mg P/kg soil) together with increasing amounts of potassium chloride (KCl; 0, 40, 80 and 120 mg K/kg of soil). Analysis of leaves from three-week old plants revealed that increasing amounts of applied potassium chloride (KCl) resulted in a progressive increase in the nitrate concentration (NO_3^-) as well as in the activities of the enzymes of the nitrogen metabolic pathway i.e. nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamine dehydrogenase (GDH). Highest accumulation of nitrate, as well as the activities of these nitrogen (N) assimilating enzymes, was observed in the K_{80} treatment. The results indicate the potential of potassium application for improving the nitrogen use efficiency (NUE) of spinach plants.

Introduction

In many parts of the world, there is a practice of imbalanced fertilization in which an excess supply of nitrogen is commonly accompanied by an inadequate supply of another nutrient. This may often be potassium (but it also applies to other nutrients e.g. phosphorus and sulphur), the lack of which restrict nitrogen uptake and utilization, and hence plant growth. When potassium is limiting, nitrogen fertilizers are used inefficiently by the crop. This nutrient imbalance is thus associated with low NUE of crop plants which is of detriment - not only to crop production directly, but also to the environment - because of the consequences of unused N by the crop. In addition to adverse environmental effects, a misuse of natural resources expended in producing the N fertilizers also has to be taken into account (Krauss, 2003). There is therefore a need to understand the importance of balanced fertilization in improving the NUE of plants. Identification and exploitation of positive interactions between nutrients provides a fundamental means of increasing returns in terms of yield, quality and NUE from applied N (Aulakh and Malhi, 2004).

Potassium plays a key role at various stages in N metabolism. At a basic level it is required in high concentrations in the synthesis of protein, which includes the binding of RNA to ribosomes. In green leaves chloroplasts account for about half the RNA and leaf protein. Since most chloroplast protein in green leaves is present as RuBP carboxylase, the CO_2 fixing enzyme in C3 plants, which requires potassium (as well as Mg) for activation, a lack of potassium inhibits photosynthesis and hence the ability for dry matter accumulation (Marschner, 1995). Engels and Kirkby (2001) envisage a cycling role for K^+ within the plant involving the transport and assimilation of nitrate (NO_3^-). During transport from root to shoot in the xylem, the negative charge of the nitrate is largely balanced by the positive K^+ charge as evident from chemical analysis of xylem sap. On assimilation of nitrate in the leaves, the negative charge is then transferred to organic compounds including carboxylic and amino acids which are transported together with K in the phloem from shoot to root. Nitrate uptake appears to be highly dependent on K. Without adequate K, nitrate accumulates in roots cells, which retards further nitrate uptake (Anjana *et al*., 2007a).

The significance of potassium in affecting uptake, translocation and reduction of NO_3^- is well documented (Ruiz and Romero, 2002). However, studies related to its effect on the enzymes of nitrogen assimilation and metabolism is meagre. Therefore, the present experiment considers the influence of potassium on foliar assimilation of nitrate as expressed by the nitrate concentration in the leaves and the activities of the most important nitrogen metabolizing enzymes involved namely nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH). The aim of this study was to help elucidate the mechanism for improving NUE with balanced potassium fertilization.

Crop plants absorb most nitrogen as nitrate, because ammonium is so readily oxidized to nitrate by nitrifying bacteria in soil. Reduction of nitrate to ammonium in plants occurs in two distinct reactions catalyzed by different enzymes, namely NR and NiR. Ammonium can be toxic to plant function and therefore must be rapidly assimilated into non-toxic organic compounds. In most plants, ammonium is assimilated into amino acids through the joint activity of two enzymes in the chloroplasts: GS and GOGAT. GS catalyzes the first step of ammonium incorporation into glutamate using ATP to yield glutamine. In the presence of reducing power from photosynthesis, GOGAT then catalyzes the transfer of the amide group of glutamine to 2-oxoglutarate resulting in the formation of two molecules of glutamate. One molecule of glutamate is thus produced from one molecule of

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2-oxoglutarate and one molecule of ammonia. The effectiveness of these enzymes in ammonia assimilation is highly dependent on photosynthetic activity and would thus be expected to be indirectly dependent on potassium nutritional status. An alternative, but now generally recognized as a less important pathway for the formation of glutamate, involves the reductive amination of 2-oxoglutarate by ammonium, catalyzed by mitochondrial GDH. In this paper we present findings of the effects of K plant status on the activities of these N assimilating enzymes in the leaves of spinach.

Materials and methods

A pot experiment was conducted at The Herbal Garden, Jamia Hamdard, New Delhi, during the winter season of 2005-2006 under greenhouse conditions. Prior to sowing, pots were lined with polythene bags and filled with 8 kg of soil low in available potassium (40mg K/kg soil). The soil (Lukhi soil series of Gurgaon) was loamy sand (83.6 per cent sand, 6.8 per cent silt and 9.6 per cent clay) with neutral reaction (pH 7.1). Its dry bulk density was 1.39 g/cm^3. The available nitrogen (30 mg/kg), phosphorus (4 mg/kg) and potassium (40 mg/kg) contents were low (Anjana *et al.*, 2007b). Spinach seeds (*Spinacia oleracea* L.) were sown and cultivated at three plants per pot, supplied at uniform levels of nitrogen and phosphorus i.e. 120 and 30 mg/kg soil, respectively but with increasing levels of potassium i.e. 0, 40, 80 and 120 mg/kg of soil. The sources of N, P and potassium were ammonium nitrate (NH_4NO_3), sodium phosphate (NaH_2PO_4) and KCl, respectively. Leaves were analyzed for nitrate concentration and nitrogen assimilating enzymes simultaneously at the 3-week stage of plant growth.

Estimation of nitrate and *in vitro* assays of nitrate NR, nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase activity (GOGAT) and glutamate dehydrogenase (GDH) activities were carried out using the standard protocols followed in our laboratory.

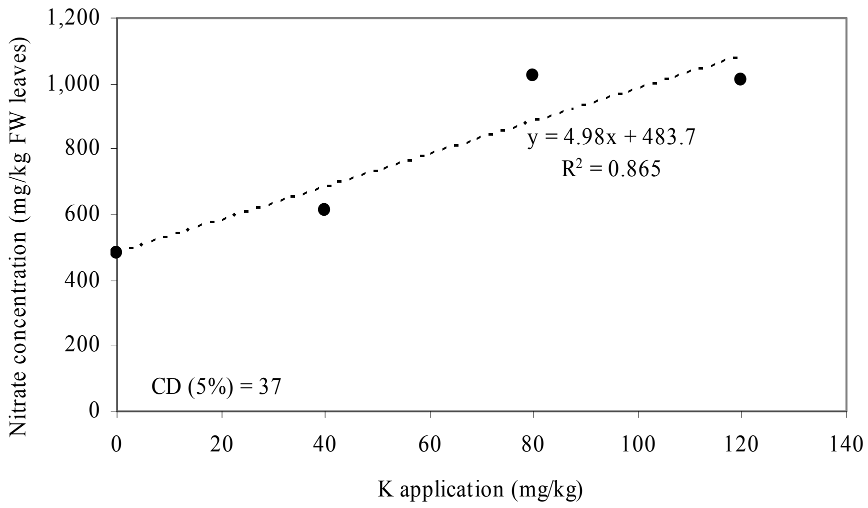

Fig. 1. Nitrate concentration in leaves with increasing application of K.

Results and discussion

All the parameters of nitrogen metabolism analyzed in spinach leaves were directly influenced by potassium application.

Nitrate levels showed a linear response to potassium fertilization with maximum nitrate concentration at K_{80} (Fig. 1), representing 112 per cent increase with respect to the lowest concentration found at K_0 application rate. These results were expected since a close relationship exists between K^+/NO_3^- uptake and because K^+ has been demonstrated to be the major accompanying cation of NO_3^- during transport in the xylem (Blevins, 1985).

Nitrate reductase activity (NRA) also showed a linear and positive response with increasing potassium application with the highest activity at K_{80} (Table 1), an increase over the control of 101 per cent. This finding is in accordance with the increasing nitrate concentrations because NR is a substrate inducible enzyme.

Table 1. Effect of applied potassium on the activities of nitrogen metabolizing enzymes in spinach leaves at 3-week stage of plant growth.

Enzyme activity	Applied K (mg/kg)				CD (5%)
	K_0	*K_{40}*	*K_{80}*	*K_{120}*	
Nitrate reductase activity (NRA; µmoles nitrite h^{-1} mg^{-1} protein)	0.296	0.376	0.597	0.589	0.009
Nitrite reductase (NiR; µmoles nitrite h^{-1} mg^{-1} protein)	3.210	4.914	5.251	5.217	0.023
Glutamine synthetase (GS; µmoles GHA min^{-1} mg^{-1} protein)	0.1691	0.2015	0.2905	0.2893	0.004
Glutamate synthase (GOGAT; µmoles NADH min^{-1} mg^{-1} protein)	0.0332	0.0496	0.0534	0.0522	0.001
Glutamine dehydrogenase (GDH ; µmoles NADH min^{-1} mg^{-1} protein)	0.1928	0.2293	0.2719	0.2695	0.003

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Similarly, activities of all other enzymes of the pathway namely, NiR, GS, GOGAT and GDH continued to increase with increasing supply of potassium (K_{80}), leveling off thereafter. The increases in these enzymes at K_{80} in comparison to the control were 64 per cent, 72 per cent, 61 per cent and 41 per cent for NiR, GS, GOGAT and GDH, (Table 1) respectively.

The results indicate that increasing potassium application rates facilitates the uptake and transport of nitrate towards the aerial part of the plants, which in turn enhances the activities of the nitrogen assimilating enzymes. The highest levels of nitrate, as well as the nitrogen metabolizing enzymes at K_{80} indicate this to be the most efficient rate of application in promoting nitrate utilization in this soil. The parallel increase in activities of nitrogen assimilating enzymes with nitrate concentration in relation to the progressive increase in potassium application shows, moreover, that these enzymes act in a coordinated manner in order to assimilate nitrogen in the plants. Potassium thus appears to play a significant role in improving NUE of plants by means of enhancement of nitrate uptake with concomitant increase in nitrogen metabolizing capacity of the plants.

Conclusions

Under conditions of low availability of soil K, together with imbalanced N/K fertilization in spinach production, application of potassium enhanced nitrate uptake stimulated the activities of the nitrogen assimilating enzymes thus improving the NUE of the plants. Further studies on balanced fertilization need to be carried out using different crops and soils to prevent environmental degradation and wastage of natural resources.

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