Indian J. Plant Physiol., Vol. XXIX, No. 4, pp. 363-367 (December, 1986)

ASSESSMENT OF POTENTIAL FOR NITRATE REDUCTION IN CHICKPEA LEAVES (*CICER ARIETINUM* L.)

P. B. VARADE, K. G. WASNIK[1] AND T. C. POKHRIYAL[2]

Division of Biochemistry,
Indian Agriculture Research Institute, New Delhi-110 012

SUMMARY

Leaf nitrate reductase activity (NRA) was measured in fertilizer treated and non fertilizer treated chickpea plants. Fertilizer treated plants showed higher nitrate reductase activity. When leaf+petiole +stem and leaf+petiole were incubated in Hoagland solution separately, it was observed that NRA was more in the former part. Leaf NRA started declining when nitrate was excluded from the incubation medium. There was an enhancement of NRA in chickpea leaves when sucrose was included along with nitrate in the incubation medium. The possible role of nitrate flux, and photosynthates on nitrate reduction in chickpea leaves is discussed.

INTRODUCTION

It has been reported that nitrate reductase activity (NRA) could be correlated with actual accumulation of reduced nitrogen (Brunetti and Hageman, 1976; Dalling *et al.*, 1975). These studies show that nitrate flux, availability of reductant as well as NRA affect the assimilation of nitrate and all these parameters are responsible for the potential of leaf NRA (Hageman, 1979, Reed, *et al.*, 1980; Shaner and Boyer, 1970). Such studies which have been reported for wheat and triticale only needs to be extended to other crops. In the present communication NRA of chickpea from leaf+petiole+stem and leaf+petiole parts incubated in Hoagland solution, is reported.

MATERIALS AND METHODS

The seeds of chickpea variety BG 203 were sown in the field during rabi season after mixing the seeds with rhizobium inoculum obtained from the

1. Division of Plants Physiology, I.A.R.I., New Delhi-110 012.
2. Forest Research Institute, Dehradun, U.P.

Division of Microbiology, IARI, New Delhi-110 012. There were two fertilizer treatment is *i.e.* (i) no nitrogen (ii) nitrogen 120 kg/ha. Plants from both fertilizer treated and untreated groups were selected for the experiments. These plants were brought to the laboratory in moist muslin cloth. Plants from both the groups were separated in to leaf+petiole+stem and leaf +petiole sub groups. They were incubated in Hoagland solution containing (i) 15mM NO_3 (ii) 15mM NO_3 with 1% sucrose (iii) without nitrate but containing. 1% sucrose. After the incubation for 24 hours in sufficient light, leaflets were separated out into pieces weighed and assayed for *in vivo* NRA according to the method described by Klepper *et al.*, 1971; and Hageman & Hucklesby 1971 with some modifications (Nair and Abrol, 1977).

RESULTS AND DISCUSSION

Leaf NRA after incubation in Hoagland solution with 15 mM KNO_3

It was observed that leaf NRA was more in leaf+petiole+stem as compared to leaf+petiole incubated under same condition (Table I). However, NRA was more in fertilizer treated plants as compared to untreated plants. The leaf NR activity could further be enhanced slightly by including 1% sucrose along with nitrate in the incubation mixture.

Table I : Activity of leaf nitrate reductase in chickpea plants.

Treatment	Time and contents of incubation medium	Leaf & Petiole	Leaf+Petiole+Stem
		mμmoles of NO_3 gm fr. wt^{-1}. hr^{-1}.	
Nitrogen O	O hr.	853	853
Nitrogen 120 kg/h	O hr.	914	914
Nitrogen O	24 hr. Incubation (15 mM KNO_3 in hoagland solution)	1022	2233
Nitrogen 120 kg/hectare	24 hr. incubation (15 mM KNO_3 in hoagland solution)	1059	2582
Nitrogen O	24 Inc ubation (15mM KNO_3+1% sucrose in hoagland solution)	1490	2335
Nitrogen 120kg/h.	24 hr. incubation (15 mM KNO_3+1% sucrose in hoagland solution)	1425	2655

Leaf NRA from leaf+petiole+stem of fertilizer treated plants

It was observed that leaf NRA was more in fertilizer treated plants (Table I). When 15mM KNO_3 was excluded from the incubation medium, there was decaying of leaf NR activity (Table II). However, when 1% sucrose was included along with 15mM KNO_3 there was slight increase in leaf NRA. When stem is attached the effect of added sucrose is not exhibited. This means that a flux from stem is already taking place.

Table II : Activity of nitrate reductase in leaf+petiole+stem in fertilizer treated plants.

Time	Contents of incubation medium	Leaf NRA mμ moles NN_3 reduced gm fr wt^{-1} hr^{-1}
0 hr	Nil	915
24 hr	Hoagland solution without KNO_3	120
24 hr	Hoagland solution with 15 mM KNO_3	936
24 hr	Hoagland solution with 15 mM KNO_3 +1% sucrose	1072

Leaf NRA in leaf +petiole+stem from fertilizer traetd and untreated plants

When 15mM KNO_3 was excluded from the incubation medium, in fertilizer treated as well as untreated plants, there was considerable decrease in leaf RNA (Table III). The results further confirmed that this decline was two fold lower in non fertilizer treated plants. Addition of both sucrose and KNO_3 resulted in more than three fold increase in leaf NRA.

It appears observed that there was possibility of increasing leaf NRA. This potential enhancement was dependant on initial level of NRA which was induced by application of fertilizer. Further leaf NRA was dependant on the flux of nitrate which was continuously maintained by the stem. The flux of reductants also appeared to be partially met by stem. After achieving

Table III : Induction of leaf NRA in leaf+petiole+stem from fertilizer treated and untreated plants.

Time	Contents of incubation medium	Leaf NRA mμ moles gm fr. wt^{-1} hr^{-1}	
		Nitrogen 0	Nitrogen 120 kg/h
0	Nil	638	1268
24 hrs	Hoagland solution without KNO_3+sucrose 1%	76	155
24 hrs	Hoagland solution with 15 mM KNO_3+1% sucrose	2670	3334

enhancement of leaf NRA by maintaining optimal nitrate flux by applying fertilizer, it could further be enhanced if sucrose was provided along with the nitrate. The possible reason may be that this exogenous supply of sucrose provides necessary carbon skeleton for reduced nitrogen to convert it in to amino acids. In the present study leaf NRA was more in fertilizer treated plants. Addition of nitrate and sucrose increased the leaf NRA activity. These results suggests that there is considerable potential in the leaf which remains unrealized for the nitrate assimilation. This could be realized with proper supply of nitrate flux and photosynthates, which may improve the yield in chickpea.

REFERENCES

Brunetti, N. and Hageman, R. H. (1976). Comparison of *in vivo* and *in vitro* assays of nitrate reductase in Wheat (*Triticum aestivum* L.). Seedlings. *Plant Physiol.*, 58: 583-587.

Dalling, M. S., Halloram, G. M. and Wilson, J. H. (1975). The relation between nitrate reductase activity and grain nitrogen productivity in wheat. *Aust. J. Agri. Res.*, 26: 1-10.

Hageman, R. H. and Hucklesby, D. P. (1971). Nitrate reductase-In : Methods in Enzymol. (Ed. San Pietro), Vol. 23: Part A, PP 491-503.

Hageman, R. H. (1979). Integration of nitrogen assimilation in relation to yield. In Herwitt. E. J. and Cutting, C. V. Eds. Nitrogen Assimilation of Plants. Academic Press, New York, 591.

Klepper, L., Flesher, D. and Hageman, R. H. (1971). Generation of reduced nicotinamide adenine dinucleotide for nitratrate reduction in green leaves. *Plant Physiol.*, 48: 580-90.

Nair, T. V. R. and Abrol, Y. P. (1977). Studies on nitrate reducing system in developing wheat ears: *Crop. Sci.*, 17: 438-42.

Reed, A. S., Below, F. E. and Hageman, R. H. (1980). Grain protein accumulation and the relationship between leaf nitrate reductase and protease activities during grain development in maize (Zea mays L.) I. Variation between genotypes, *Pl. Physiol.*, 66: 164-70.

Shaner, D. L. and Boyer, J. S. (1976). Nitrate reductase activity in maize (Zea mays L.) leaves. I. Regulation by nitrate flux. *Plant Physiol.*, 58: 499-504.