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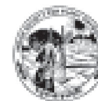

KINETICS OF NITRATE UPTAKE SYSTEM IN WHEAT GENOTYPES

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SUMMARY

The nitrate uptake kinetics (C_{min}, K_m, and V_{max}) were studied in two cultivars of wheat differing in the level of activity of enzyme nitrate reductase. The uptake kinetics especially K_m and V_{max} depend on the concentration of nitrate in rhizosphere. At low range of external nitrate concentration uptake system follows Michaelis-Menten saturation kinetics, while at high external nitrate concentration, the nitrate uptake system follows linear kinetics. The uptake system did not show any saturation at least up to 10mM external nitrate. Lineweaver-Burk plot transformation of the uptake data at low nitrate concentration, the K_m and V_{max} of HD 2285 (high nitrate reductase activity-HNR) and HD 1981 (low nitrate reductase activity-LNR) were found to be 0.186 and 0.725 mM, and 0.17 and 0.798 mmol g^{-1} fr. wt. h^{-1}, respectively. The C_{min} was calculated by estimating the accumulation of tissue nitrate and found to be 0.9 and 1.5 mM. At low range (0.05-0.5), the rate of nitrate uptake was higher in LNR genotype and at high external nitrate concentration, the uptake of nitrate was more in HNR genotype.

Key words: **C_{min}, K_m, nitrate reductase, nitrate uptake, V_{max}.**

INTRODUCTION

Plants take up nitrogen mostly in the form of nitrate (Siebrecht *et al.* 1995). The rate of uptake of nitrate by roots of higher plants is extremely sensitive to the availability of the nitrate ion in the surrounding medium. In a number of crop plants nitrate uptake has been shown to be biphasic viz. high affinity transport system (HATS) and low affinity transport system (LATS). The latter system operates at high concentration of nitrate in soil and also has high transport capacity. Whereas, HATS operates at comparatively low concentration of nitrate ion and has low transport capacity (Aslam *et al.* 1992, Siddiqi *et al.* 1989). HATS has both constitutive and inducible components as observed in many species (Glass and Siddiqi 1995, Kronzucker *et al.*1995). LATS has thus far shown only its constitutive nature (Siddiqi *et al.* 1989, Glass *et al.* 1992, Kronzucker *et al.* 1995) except the CHL1 transporter in *Arabidopsis* (Huang *et al.* 1996), which is a dual system. Glass *et al.* (1992) did electrophysiological study on nitrate uptake over a wide range of exogenous nitrate ion concentrations (from 100 μM to 20 mM) and reported that each of the nitrate transport system (HATS and LATS) is thermodynamically active and carrier mediated. There is no specific concentration of nitrate ion in various crop plants below which high affinity system may work. However, Siddiqi *et al.* (1989) reported that in barley the inducible and constitutive high affinity transport system typically operated below 0.2 mM nitrate whereas the LATS gave no evidence of saturation even at 50 mM concentration. For taking up nitrate plant needs a certain minimum concentration of nitrate ion in soil solution below which there is no net uptake. This minimum concentration (C_{min}) of nitrate again varies with the genotypes of plants. As low C_{min} value shows an efficient uptake system, the low C_{min} genotypes in a species can do well even in marginal soils (Murthy *et al.* 1998). Blumenthal *et al.* (1996) studied the C_{min} of nodulated alfalfa plants and reported that the non-nodulated

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varieties like Sarmac, has less C_{min} values (11 μM) as compared with the nodulated plants (up to 19 μM). Hence the C_{min} value varies with the type and the cultural conditions of the plant. In A*cer rubrum* (red maple) this value was 1 μM (Kelly *et al.* 2000), while Kalita and Nair (2001) reported 1.62 μM in HD 1593 and 5.5 μM in HD 1949. Hence, the objective of the present study was to study the differences in the kinetics of nitrate uptake in the wheat genotypes differing in the level of nitrate reductase activity.

MATERIALS AND METHODS

Seeds of wheat variety HD 2285 (high nitrate reductase activity-HNR) and HD 1981 (low nitrate reductase activity-LNR) were washed with double distilled water and surface sterilized with 0.1% $HgCl_2$ for 3 min. The seeds were then rinsed 5 to 6 times with double distilled water to remove the traces of mercuric chloride. Five seeds were inserted in each tooth of the template stand. The stands were kept in rectangular tub and the entire set up was aerated and kept in growth chamber for 15 days in ¼th strength nitrogen free (-N) Hoagland solution. The pH of the growing medium was 6.5. The temperature, day night cycle and relative humidity inside the growth chamber were 25^0C 16h/8h and 80 %, respectively. The 15 days old seedlings of both the varieties were harvested and time course study (from 0-48 h) of nitrate reductase (NR) activity was done by incubating the seedlings in aerated 10 mM KNO_3 solutions (50 ml) at a photon flux intensity of 600μmol m^{-2} s^{-1}. The activity of enzyme in the leaf tissue was estimated as described by Klepper *et.al.* (1971) and modified by Nair and Abrol (1973).

To study the kinetics of nitrate uptake system low (0.05–0.5 mM) and high concentration of nitrate solutions (1-10 mM) were used in the incubation medium and uptake of nitrate was studied by depletion method. The seedlings of the two cultivars of wheat were harvested from nitrogen free (-N) Hoagland solution and incubated in various concentrations of KNO_3 solution for 12 h at a photon flux density of 600 μmol m^{-2} s^{-1}). The nitrate left in the incubation solution was estimated by the method of Downes (1978). The seedlings of both the wheat cultivars were also incubated in low concentration of nitrate (1.1 μM-150 μM) to calculate the C_{min} of both the genotypes. For tissue nitrate content estimation the treated seedlings were dried to the constant weight and nitrate content was estimated by the method of Downes (1978).

RESULTS AND DISCUSSION

The activity of nitrate reductase was significantly lower in LNR genotype as compared to the activity in the leaves of HNR genotype at all stages of measurement (Fig 1). Earlier studies have indicated the presence of high (HNR) and low NR (LNR) cultivars (Abrol 1990, Abdin *et al*. 1992, Jain *et al*. 1997), and under similar conditions of growth NR transcript was higher in the HNR genotypes as compared to the LNR genotypes (Jain and Abrol 2005). Un-induced seedlings of both the genotypes showed similar pattern of NRA despite the differences in the level of activity (Fig 1). The NR activity in the leaves of the un-induced seedlings increased slightly but not significantly in both HNR and LNR genotypes upon incubation in the 10mM nitrate solution after 24h.

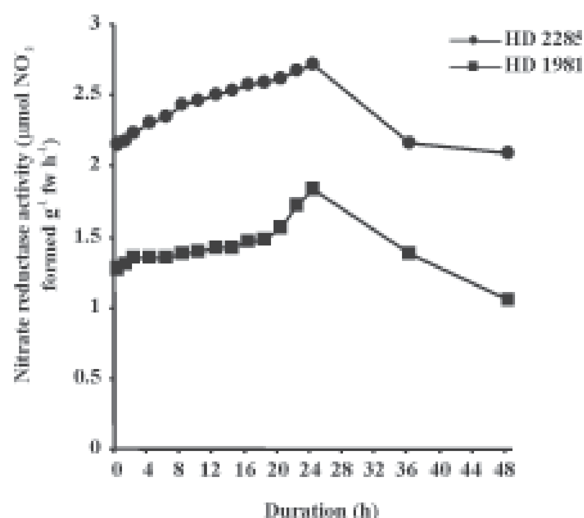

Fig. 1. Activity of the enzyme nitrate reductase (NR) in the leaves of HD 2285 and HD 1981 genotypes grown in nitrogen free (-N) Hoagland solution. The seedlings were incubated in the various concentrations of KNO_3 solutions for various time intervals.

The kinetics of the nitrate uptake system in both the cultivars was studied in 15-days old seedlings grown in nitrogen free (-N) Hoagland solution. In HNR genotype after 12 h incubation the rate of nitrate uptake was proportional to the concentration of the nitrate in the external medium and increased by 1.81 times at 0.2 mM as compared to the rate of uptake at 0.05 mM (Table 1). No significant change in nitrate uptake rate could be observed beyond 0.2 mM nitrate concentration and uptake of nitrate followed Michaelis-Menten saturation kinetics (Fig. 2 a). The Lineweaver-Burk plot transformation in HD 2285 at low external nitrate concentration showed a straight line, with an equation of:

$$y= 0.2556x + 1.3799$$

The V_{max} or I_{max} of the system was 0.725 µmol g^{-1} fr. wt. h^{-1} and K_m of the uptake system in HD 2285 was 0.186 mM. Michaelis-Menten saturation kinetics for nitrate uptake at low external nitrate concentrations was also observed in HD 1981 (LNR) genotype (Fig. 2b). The uptake system after 12h incubation revealed V_{max} and K_m value of 0.798 µmol g^{-1} fr. wt. h^{-1} and 0.17 mM, respectively. The high affinity component of the nitrate uptake system in LNR genotype increased by about 1.81 fold at 0.5 mM external nitrate concentration compared to uptake at 0.05 mM external nitrate concentration (Table 1). Similar type of uptake kinetics have also been reported by Glass *et al.* (1990) and Siddiqi *et al.* (1989). For both the cultivars the saturation level in the uptake

Table 1. Nitrate uptake rate by the nitrogen starved seedlings of HD 2285 and HD 1981 incubated in range of external concentrations of KNO_3 for 12 h.

Nitrate conc.	Nitrate uptake rate (µmol g^{-1} root fw h^{-1})	
(mM)	HD 2285	HD 1981
0.05	0.135	0.154
0.075	0.245	0.289
0.1	0.298	0.337
0.125	0.34	0.367
0.15	0.36	0.395
0.2	0.38	0.431
0.3	0.378	0.431
0.5	0.376	0.432
1	3.116	3.156
1.5	3.76	4.388
2	5.663	4.831
2.5	6.372	6.978
3	6.796	8
4	7.257	9.102
5	9.952	10.412
7	12.023	12.1
10	17.259	14.03
	SE (m): 0.24 CD (P= 0.05): 0.874	SE (m): 0.163 CD (P= .05): 0.592

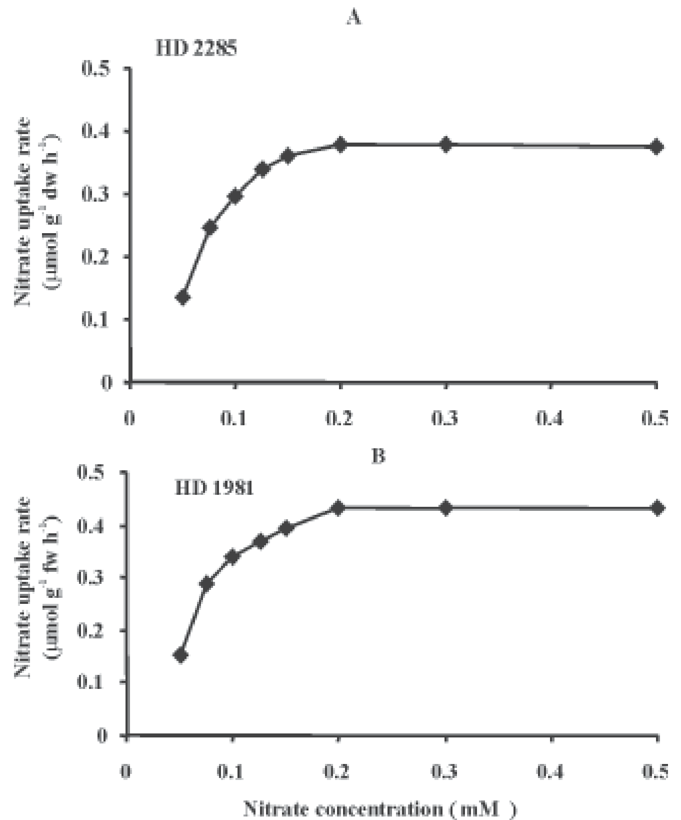

Fig. 2 (a&b). Michelis-Menten saturation kinetins of the high affinity component of nitrate uptake system in uninduced seedlings of HD 2285 and HD 1981 incubated in the various concentrations for 12 h

rate was between the external concentrations of 0.2-0.3 mM nitrate.

A completely different mechanism of nitrate uptake was observed at high external concentration of nitrate (1- 10 mM), a 7.2 fold increase in the rate of nitrate uptake at 1 mM compared to that of at 0.5 mM concentration (Table 1) in HNR genotype. The rate of nitrate uptake increased gradually with the increase in the concentration of external nitrate *i.e.* 1mM to 10mM and a linear relationship was observed between rate of nitrate uptake and nitrate concentration in both the genotypes (Table 1 and Fig. 3). The regression equation of the linear kinetics of the nitrate uptake system in HD 2285 was y=1.5058x +1.9988 and in HD 1981 y= 1.2018x + 3.3034. Hence, under high external nitrate concentration uptake mechanism followed linear kinetics. No saturation in the uptake rate was observed at least up to 10 mM concentration of external nitrate. Glass *et al.* (1992) and Siddiqi *et al.* (1989) have concluded that the K_m value of the high affinity system lies in between 5-300 mM. High K_m value indicates that the transporter protein has low affinity for its substrate. HD 2285 has comparatively low affinity for nitrate as compared to HD 1981 at low concentrations of nitrate (for high affinity system) in the rhizosphere. Barber (1984) reported that V_{max} was a critical parameter for nitrate uptake and variation in K_m had no effect. The plants having higher V_{max} can use nitrogen more efficiently (Laine *et al.* 1993). From the data it appears that at low range of external nitrate concentration HD 1981 seems to be more efficient. Whereas, cv. HD 2285 took up more nitrate at high external nitrate concentration. In a well fertilized soil HD 2285 has the ability to take up nitrate more efficiently than HD 1981. The higher uptake of nitrate ions by the HNR genotype could be responsible for the higher activity of the enzyme nitrate reductase in HNR genotype (Jain and Abrol 2005) apart from the adequate amount of NADH present in HNR genotypes as compared to LNR genotypes (Abdin *et al.* 1992).

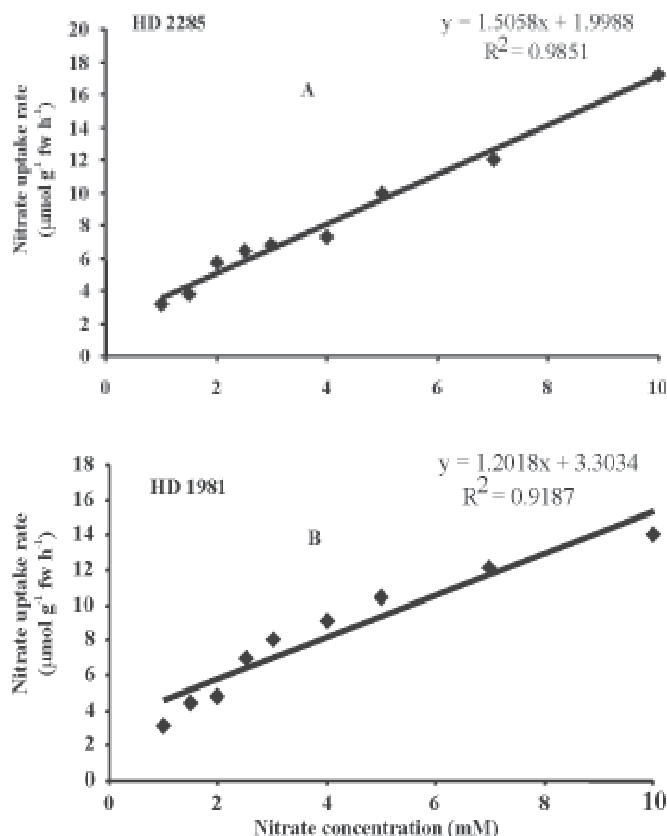

Fig. 3 (a&b). Linear kinetics of the low affinity component of the nitrate uptake system in uninduced 15 d old seedlings of cv. HD 2285 and HD 1981 incubated in the various concentrations for 12h

There was no change in nitrate content of shoot and root tissues below 0.9 mM KNO_3 concentration as compared to the tissue nitrate content of control plants in HNR genotype (Fig 4 a & b). For LNR genotype the C_{min} value was calculated to be at 1.5 mM nitrate concentration. There was no significant change in root nitrate content of both HD 2285 and HD 1981 below 2.5 mM KNO_3 concentration. Below a certain threshold value no net uptake is possible. The minimum concentration of nitrate ion or C_{min} in present investigation in HNR genotype was 0.9 mM and in LNR genotype 1.5 mM. The earlier reports indicate that the C_{min} concentrations for nitrate vary from more than 1.0 to 50 mM depending upon the plant species as well as the environmental conditions (Deane-Drummond and Chaffey 1985 and Marschner *et al.* 1991). The low value of C_{min} indicates an efficient uptake system in varieties/species, which perform well in marginal land (Murthy *et al.* 1998).

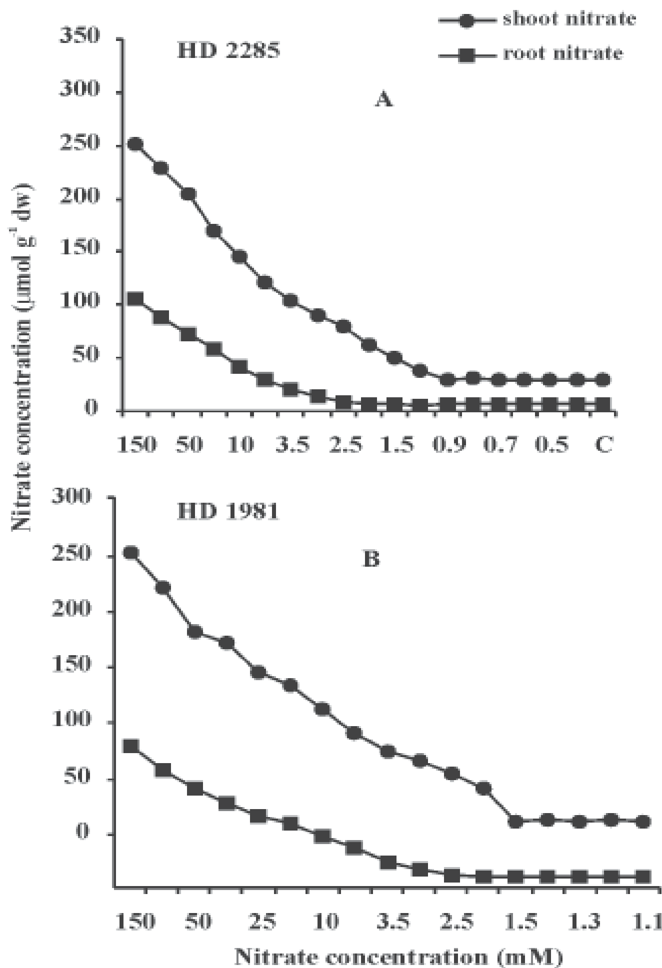

Fig. 4 (a&b). Changes in the tissue nitrate concentration in the seedlings of HD 2285 and HD 1981 grown in +N Hoagland solution and incubated in the low range of KNO_3 solution for 24h to determine the C_{min} (CD at 5%: 4.51 and 2.56, and 4.37 and 2.87 for shoot and root nitrate concentration in HD 2285 and HD 1981, respectively)

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