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VARIATION IN THE INDUCTION POTENTIAL OF LEAF NITRATE REDUCTASE IN WHEAT GENOTYPES

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SUMMARY

A study was conducted with 15 day old sand culture grown seedlings of 21 genotypes of wheat to assess the induction potential of Nitrate Reductase (NR) in the leaves by nitrate and to assess whether induction of the enzyme is limited by uptake of nitrate by root. Feeding various concentrations of nitrate, rangning from 0 to 100 mM, to excised leaves as against seedling with intact roots showed that in 11 out of 21 genotypes, uptake of NO_3^- by roots was not a constraint in the induction of the enzyme. In the remaining 10 genotypes root uptake limited the expression of induction potential significantly, but only at their maximal induction concentration. Maximal induction concentration showed genotype dependent variation, with a majority of the genotypes showing maximal induction with a concentration of 30 mM NO_3^- in the media. However, in HD-2172 (G_3) and WH-542 (G_7) maximal induction occurred with 15 mM, in HD-2329 (G_2) and WJ-134 (G_8) with 7.5 mM and in E-340 (G_6) with 3.75 mM nitrate. A concentration of 100 mM NO_3^- in the medium significantly inhibited the induced enzyme activity of all the genotypes with the exception of HD-2380 (G_{10}) and Kalyansona (G_{17}). In the former no significant effect above 15 mM was discernible while in the latter maximal induced activity was observed with 100 mM NO_3^-. E-340 (G_6) which showed the least maximal induction concentration of 3.75 mM in combination with high induced enzyme activity, was a high biomass producer at low soil N level in field experiment. Such a trait could possibly be utilized in developing genotypes with high nitrogen use efficiency, especially under low soil nitrogen level.

Key words: **Induction potential, nitrate reductase, wheat genotypes.**

INTRODUCTION

Among the nutrients usually applied to crop plants, nitrogen elicits more yield response than any other nutrient. However, its utilization rarely exceeds 50 per cent (Prasad *et al.*, 1971). Unutilized nitrogen which is lost to the environment, is not only difficult to recycle but is also a potential health hazard. For sustainable agriculture, therefore, there is a need for improving the efficiency of nitrogen usage by crop plants. To achieve this, apart from modifying cultural practices, a more desirable approach is to identify/develop genotypes, which are efficient in extracting and utilizing both soil native and fertilizer nitrogen. Existence of genotypic variation for nutrient use efficiency (NUE) is well known (Karrow and Maranville 1994, Nair and Chatterjee 1990, Papakosta 1994). Such variations could be related to differences in the efficiency of acquisition by root or in utilization by plant. Identification of specific physiological and biochemical traits underlying such variations is therefore of paramount importance in improving NUE of the present day high yielding cultivars. The present study was undertaken to identify and locate such traits in wheat. In India, wheat, which occupies nearly 24.5 million hectares, accounts for a major share of N-fertilizer

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consumption. Improving the N-utilization of the high yielding wheat cultivars is of primary concern in sustainable agriculture. Improvement of nitrogen utilization efficiency of wheat crop could be theoretically brought out : (i) by improving the uptake of nitrate, the form in which nitrogen is usually present in arable soils and (ii) by improving the efficiency of its assimilation. The two processes are, however, interlinked so that the limiting step in assimilation, which is the conversion of nitrate to nitrite catalyzed by the enzyme nitrate reductase (NR) is substrate dependent for its induction (Hageman *et al.* 1967, Reilly 1990). Tischner *et al.* (1993) also reported the role of NO_3^- as a trigger for induction of nitrate uptake and nitrate reductase activity. Here again, the induction is controlled by the flux concentration of nitrate rather than by the tissue nitrate concentration (Shaner and Boyer 1976) and hence, the induction is controlled by current uptake of nitrate. Hannachi *et al.* (1998) reported the dependency of leaf nitrate reductase activity on NO_3^- flux into shoot in wheat. The question, therefore, arises whether genotypes differ in induction potential and if so, whether the expression of such differences is substrate concentration dependent. Whether the uptake of nitrate by roots could be limiting the expression? The present study attempted to analyse these aspects in wheat genotypes.

MATERIALS AND METHODS

Twenty-one genotypes of wheat, selected from an earlier field evaluation of 230 genotypes for biomass production in response to varying levels of fertilizer, were taken as experimental material. They were E-574(G_1), HD-2329 (G_2), HD-2172 (G_3), WJ-146 (G_4), HD-1949 (G_5), E-340 (G_6), WH-542 (G_7), WJ-134 (G_8), E-3899 (G_9), HD-2380 (G_{10}), HD-1106 (G_{11}), HD-2651 (G_{12}), Narmada (G_{13}), C-306 (G_{14}), Sonalika (G_{15}), P-159-3 (G_{16}), Kalyansona (G_{17}), RAJ-3805 (G_{18}), MACS-9 (G_{19}), IP-1 (G_{20}) and NP-824 (G_{21}). Seeds of the genotypes G_1, G_4, G_6, G_8, G_9 and G_{18} were obtained from Directorate of Wheat Research, Karnal, while the seeds of the rest of the genotypes were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi.

The seeds were surface sterilized and germinated overnight on moist filter paper in petridishes. Germinating seeds (around 10 in numbers) were transferred to bottom perforated long glass tubes containing acid washed sand for further growth under natural environment. These tubes with seedlings were kept in a net house. From third day after transfer, the seedlings were irrigated with Hoagland solution (one fourth strenght) containing 0.5 mM nitrate on alternate days until sampling. The experiment was conducted with 15-day-old seedling. The study was carried out during normal crop growing season. Genotypic potential for substrate induction of the enzyme was assessed through nitrate feeding of the excised leaves. The leaves were excised under water and incubated on the solution of required strenght (direct induction). To assess whether root uptake is limiting the expression of induction potential, the seedlings with the intact roots were fed with the required concentration of nitrate through the root (indirect induction).

Excised leaves/intact seedlings were thoroughly washed, first with tap water and than with double distilled water and transferred to Erlenmeyer flask containing 100 ml of Hoagland solution (one fourth strenght) with the required concentration of nitrate. Nitrate concentrations were 0, 3.75, 7.5, 15.0, 30.0 and 100.0 mM NO_3^-. The seeldlings/excised leaves were secured at the neck of the flask, with the help of non-absorbent cotton in such a way that only the root portion/cut end of the leaves remained dipped in the solution. The solution also contained 0.5 mM calcium as calcium sulphate. Nitrate reductase activity of the leaf tissue was assayed *in-vivo* 24 hours after induction, following the method of Hageman and Hucklesby (1971) modified by Nair and Abrol (1973). The experiment was replicated thrice and the data obtained were analyzed as per the procedure prescribed for Factorial Randomised Block Design of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Induction potential of the excised leaves

The induction potential of the excised leaves increased with increasing concentration of nitrate in the medium, though the concentration at which induction maxima was observed depended upon genotypes (Table 1 and Fig. 1). In all the genotypes, however, 100 mM nitrate significantly inhibited the induction compared to the next lower concentration used (30 mM) except in G_{10} and G_{17}. Thirteen out of 21 genotypes showed maximum induction

Table 1. *In vivo* nitrate reductase activity (μmol NO_3^- g^{-1} dry w h^{-1}) in 15 day old seedlings of 21 wheat gentoypes after induction with six different concentrations of nitrate for 24h.

Genotype	Feeding treatment	NO_3^- -N levels (mM) (N)						Mean
(G)	(F)	0	3.75	7.5	15	30	100	
G1 (18.73)	D	11.63	16.92	19.5	21.46	32.35	16.41	19.71
	I	14.7	15.44	18.4	19.84	24.78	13.18	17.72
G2 (14.85)	D	12.23	20.89	23.62	15.14	13.47	7.9	15.54
	I	10.55	16.3	23.68	14.96	12.16	7.23	14.14
G3 (20.63)	D	10.18	23.12	22.52	26.24	18.76	21.59	20.41
	I	10.06	18.52	20.76	28.82	25.69	21.31	20.86
G4 (13.62)	D	3.18	7.92	16.74	20.62	24.10	18.66	15.20
	I	2.97	5.61	10.02	14.43	19.66	19.49	12.03
G5 (15.03)	D	13.04	16.49	17.63	16.79	20.87	12.27	16.19
	I	8.82	11.34	14.56	17.07	17.31	14.12	13.87
G6 (21.72)	D	5.97	29.49	27.60	26.79	23.37	19.35	22.04
	I	5.64	28.58	26.75	25.51	23.32	18.64	21.41
G7 (13.17)	D	11.61	11.63	14.72	19.78	17.70	15.78	15.21
	I	5.84	10.00	14.13	15.71	11.41	9.67	11.13
G8 (16.13)	D	5.42	20.34	25.08	18.74	16.57	13.41	16.59
	I	5.68	19.63	24.33	17.82	15.68	10.88	15.67
G9 (17.69)	D	8.44	18.27	17.57	21.13	27.88	12.24	17.58
	I	8.65	16.51	17.43	23.13	25.94	15.11	17.79
G10 (16.92)	D	4.92	15.19	18.04	22.88	21.89	22.46	17.57
	I	4.17	13.89	17.09	20.01	20.26	22.72	16.28
G11 (14.51)	D	6.69	11.54	12.06	20.06	26.50	10.45	14.61
	I	6.62	10.63	11.64	20.27	25.68	11.58	14.41
G12 (14.31)	D	7.49	12.77	15.42	17.70	17.53	15.87	14.47
	I	7.75	12.27	15.72	17.43	18.26	13.48	14.15
G13 (16.96)	D	4.39	15.13	19.08	19.94	27.21	20.4	17.69
	I	4.84	15.06	17.58	19.09	22.40	18.34	16.22
G14 (17.60)	D	6.85	16.22	19.25	25.99	31.06	13.16	18.76
	I	6.74	13.87	19.16	26.16	22.33	10.42	16.45
G15 (10.74)	D	3.83	4.95	11.49	18.06	19.71	9.49	11.26
	I	3.79	6.00	11.42	14.28	14.26	11.61	10.23
G16 (9.16)	D	4.55	7.77	8.29	9.51	17.2	9.72	9.51
	I	3.44	6.76	9.03	10.4	12.33	11.32	8.82
G17 (16.02)	D	8.92	10.98	10.71	13.44	21.81	38.39	17.34
	I	8.57	10.63	11.72	12.72	20.79	23.71	14.69
G18 (12.94)	D	4.14	8.71	13.02	16.49	22.83	18.89	14.02
	I	2.98	8.02	12.78	16.13	16.69	14.54	11.86
G19 (19.58)	D	9.72	14.54	16.11	20.43	29.89	27.49	16.69
	I	9.83	16.04	15.71	20.85	28.97	25.42	19.47
G20 (13.10)	D	4.04	9.84	11.49	14.02	22.09	18.46	13.32
	I	4.15	8.88	10.01	14.07	22.31	18.03	12.88
G21 (16.44)	D	5.21	14.19	16.10	19.86	28.56	23.58	17.92
	I	4.06	11.98	15.39	18.82	22.95	16.62	14.97
Mean		(6.95)	(13.87)	(16.51)	(18.86)	(21.55)	(16.51)	
CD at 5% P	F = 0.216; N = 0.374; G = 0.700; F X N = 0.529; F X G = 0.990; N X G = 1.715; F X N X G= 2.41							

D = Direct feeding through cut end of excised leaves; I = Indirect feeding through intact root system; Values in parentheses are mean of main effect.

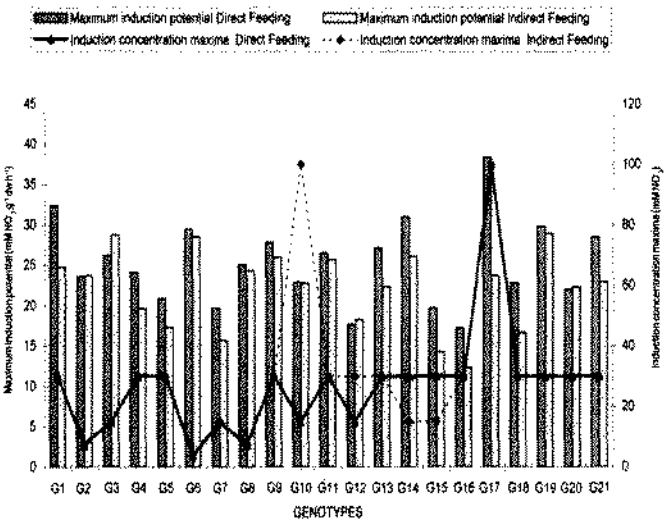

Fig. 1. Genotypic variation in maximum induction potential and induction concentration maximum in 21 wheat genotypes

with 30 mM nitrate. In another 4, viz., G_3, G_7, G_{10} and G_{12} maximum induction was observed with 15 mM concentration with no significant difference between 30 mM and 15 mM in the latter two. In yet another 2, G_2 and G_8, the maximum activity was observed with 7.5 mM nitrate. In G_6, maximum induction occurred with the lowest concentration of nitrate (3.75 mM) used. In the remaining genotype G_{17}, maximum induction occurred by 100 mM nitrate. The genotype G_6, showed a maximum induced activity value of 29.5 µmol NO_3^- reduced g^{-1} dry w h^{-1} with the lowest concentration of 3.75 mM and was one of the three genotypes which showed high induced enzyme activity. The other two did so with higher media concentrations. In G_{10} no significant effect was observed with concentrations higher than 15 mM.

The range of genotypic variations in induced activity values were 6, 3.3, 2.4 and 4.9 fold respectively with 3.75, 7.5, 30 and 100 mM media nitrate concentrations. Though interactions were observed among all the three factors, G_{15} showed consistently low activity while G_3 and G_6 showed consistently high activity across the concentrations indicating that the differences observed could be true variations. Thus, the expression of the induction potential though was influenced by the substrate concentration; genotypic dependence of the expression was clearly discernible. As mentioned above, the maximum differences in the expression were observed with 3.75 mM NO_3^-, indicating that the induction potential among the genotypes vary more at low available nitrogen. At this concentration, G_6 showed maximum induction and G_3 had 88 per cent of its maximal activity observed at 15 mM. Since, it is the flux of nitrate that influences the induction of the enzyme, these two genotypes have the ability to take up and maintain the required substrate NO_3^- concentration at the induction site, even under very low ambient nitrate concentration. It may be possible to utilize this trait to develop varieties that are efficient in N utilization under low applied nitrogen levels.

Expression of the enzyme induction potential through root feeding

The efficiency differences among the genotypes to assimilate either native soil or added nitrate may not only depend upon the enzyme potential but also on the ability of the roots to extract nitrate and maintain its flux at the site of enzyme induction (Table 1 and Fig. 1). Genotypes showed no consistent differences in the induction potential between direct and indirect (root) feeding. However, a concentration dependent limitation by root untake was observed, mainly at maximal induction concentration. Out of the 13 genotypes, which had their induction maxima at 30 mM based on direct feeding, root uptake limited the expression in 10 genotypes. Out of the 4 genotypes with 15 mM as a maximal induction concentration, root uptake limited the induction in two, and in G_{17} which showed maximum induction with 100 mM, root uptake limited the induction. In the two genotypes G_2 and G_8 with 7.5 mM and in G_6 with 3.75 mM as maximal induction concentration, the expression of induction potential was not limited by root uptake.

As in the case of direct feeding, in most of the genotypes, induction concentration maxima for root feeding also were found to be 30 mM. Soil nitrate concentration usually is in the range of 0.5 to 5 mM and may reach 30 mM or more only transiently immediately after fertilizer application (Pitman *et al.* 1976). However, NR induction is causally related to the nitrate flux concentration rather than ambient or tissue concentrations (Shaner and Boyer 1976). As in the case of direct feeding, 100 mM of nitrate inhibited the induced enzyme activity in all the genotpyes with notable exceptions of two viz., G_{10} and G_{17}. In field grown wheat and barely seedlings Nair and Chatterjee (1990) have reported inhibition of enzyme induction with

high dose of applied fertilizer. Maximal induction concentration in a few genotypes, however, was less than 30 mM. In G_3 and G_7 maximal induction with root feeding occurred with 15.0 mM, in G_8 with 7.5 mM and in G_6 with 3.75 mM. In G_{17} highest induced activity was observed with 100 mM, the concentration that inhibited the induction in all the other genotypes, except G_{10}, however the induced activity here was only 62 per cent of the direct feeding. The significance of this observation in terms of fertilizer responsiveness of the genotypes in the field needs further investigation. Nevertheless, G_6 which showed the least maximal induction concentration was one of the high biomass producers at low nitrogen level (P. Kalita and T.V.R. Nair, unpublished observation).

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