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Full Length Article

Nitrogen Nutrition, its Assimilation and Remobilization in Diverse Wheat Genotypes

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Abstract

Nitrogen is an essential element required for plant growth and development. However, over-use of nitrogen fertilizer is not only costly but also environmentally unsafe. Therefore, identification of genotypes possessing higher nitrogen use efficiency (NUE) at sub-optimal dose of nitrogen is the key challenge. In this study, effect of nitrogen on the activities of nitrogen assimilatory enzymes, total soluble proteins and amino acids was studied in wheat genotypes of diverse physiology (*viz.*, GLU 1101, GLU 1356, GLU 2001, GLU 700, PH132-4836, PH132- 4840). Nitrogen was applied at recommended dose (RDN), RDN-50%, RDN-25% and RDN+25% using urea as a source. With increase of nitrogen rate, significant increase in the activities of nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase was noted, which caused an increase in protein and amino acid contents in all the genotypes. Activity pattern of studied enzymes revealed an increasing trend from tillering to anthesis stage and thereafter declined in parallel with decrease in protein and amino acid contents. Conversely, nitrogen and chlorophyll content showed a consistent decline with leaf growth. Genotypes GLU 1356 and GLU 2001 maintained higher activities of nitrogen assimilatory enzymes in parallel with higher NUE at RDN-50% and RDN-25%, while genotypes PH132-4836 and PH132-4840 were found to be promising at RDN+25%. A positive correlation of nitrogen assimilatory enzymes (nitrate reductase and glutamine synthetase) with NUE and nitrogen content was also observed indicating that these might be the rate limiting enzymes in nitrogen metabolism. In crux, 25% less than the recommended dose of nitrogen had significant influence on nitrogen metabolism especially in GLU 1356 and GLU 2001 due to high activities of nitrate reductase and glutamine synthetase. These nitrogen efficient genotypes may be exploited in enhancing wheat crop productivity under lower dose of nitrogen to save environment and input cost. © 2015 Friends Science Publishers

Keywords: NUE; Nitrogen assimilating enzymes; Protein; Nitrogen amino acid; Chlorophyll content
Abbreviations: N: Nitrogen, NR: Nitrate reductase, NIR: Nitrite reductase, GS: Glutamine synthetase, GOGAT: Glutamate synthase, GDH: Glutamate dehydrogenase, NUE: Nitrogen use efficiency

Introduction

Wheat (*Triticum aestivum* L.) is one of the three main cereals cultivated worldwide and is mainly used for human consumption. The crop is grown mainly for grain starch and protein content and therefore requires huge amount of nitrogen (N) fertilizer for good grain productivity. In plants, N is important for growth and survival and is a necessary component of proteins, enzymes and metabolic products, and is involved in their synthesis as well as transfer of energy (Kichey *et al*., 2006; Horchani *et al*., 2011). Indeed, N fertilization constitutes one of the most important agronomic practices in cereals due to elimination of crop rotation (Crews and Peoples, 2004). However, overexploitation of N-fertilizer is harmful for environment and economy (Giambalvo *et al*., 2010). Therefore, identification of genotypes showing higher productivity and nitrogen use efficiency (NUE) at sub-optimal doses of N is imperative.

Nitrogen use efficiency is the product of two physiological factors: (i) N uptake efficiency, defined as the amount of N uptake by the crop per unit of N available and (ii) N utilization efficiency, defined as the grain yield per unit of N uptake (Giambalvo *et al*., 2010). The mechanisms regulating these processes are complex, but it is vital that they should be well understood to improve NUE in plants (Kant *et al*., 2011). Nitrogen uptake and utilization efficiency is closely related to nitrate (NO_3^-) and ammonium (NH_4^+) assimilatory pathways. The first step involved in acquiring N is the uptake system which is mediated by

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transporters (Orsel *et al*., 2002) followed by its reduction to NH_4^+ that constitutes first step of amino acid biosynthesis by enzymes nitrate reductase (NR) and nitrite reductase (NIR), respectively (Meyer and Stitt, 2001). The efficiency of NH_4^+ assimilation could contribute to the overall plant NUE (Foulkes *et al*., 2009). The assimilation of NH_4^+ into amino acids occurs via action of glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes (Esposito *et al*., 2005). Glutamate dehydrogenase (GDH) constitute an alternative pathway of NH_4^+ assimilation (Hirel *et al*., 2007; Shrawat and Good, 2008). Accumulative evidence reveals that organic N supplied can have profound effect on plant cell metabolism. Glutamine synthetase and GOGAT are key enzymes involved in the assimilation of NH_4^+ in higher plants (Zhou *et al*., 2004); these enzymes are responsive to N supply (Wang *et al*., 2004).

Nitrogen use efficiency is a genotype-dependent response (Le Gouis *et al*., 2000). Furthermore, its effect on N uptake and/or high N utilization was highlighted in different crops like durum wheat (Giambalvo *et al*., 2010) and bread wheat (Alizadeh and Ghaderi, 2006; Dawson *et al*., 2008). The integration of various agro-physiology and biochemical parameters to screen wheat genotypes is quite useful to optimize grain yield (Vinod, 2007). Our knowledge on the aspects of N acquisition and its subsequent assimilation is still incomplete due to genotypic variation at biochemical and physiological levels. Moreover, agricultural practices towards extensive use of N fertilizers are negotiating towards a better knowledge of various pathways employing NUE in economically important crops particularly wheat. Identification of key metabolic steps in genotypes optimizing N in low-N conditions is essential for the sustainability of agriculture (Lian *et al*., 2006). This entire process requires a deep understanding of various biochemical responses of wheat with respect to different N levels. Thus, characterizing genotypes for N-use is a vital step for improving crop productivity at suboptimal dose of N.

In this study, we analyzed six genotypes, with differential inherent genetic background for N metabolism (N assimilation and remobilization) at four N levels. These genotypes were selected on the basis of differential inherent genetic background with respect to N assimilation and remobilization.

Materials and Methods

Six wheat genotypes namely GLU 1101, GLU 1356, GLU 2001, GLU 700, PH132-4836 and PH132-4840 (Table 1) were raised in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.91°N, 75.85°E and 252 m asl), India. The crop was sown on October 29, 2010 in plots consisting of 4 rows of 1 m each in 22.5 cm spaced rows and with 40 cm between the plots. The experiment was conducted in randomized complete block design in factorisal arrangement with three replicates.

Nitrogen was applied, in the form of urea (46% N), at 120 kg N ha^{-1} (recommended N dose, RDN), 90 kg N ha^{-1} (RDN-25%), 60 kg N ha^{-1} (RDN-50%) and 150 kg N ha^{-1} (RDN+25%). Phosphorus and potassium were applied at 65 and 30 kg ha^{-1} using diammonium phosphate (DAP) and sulphate of potash as sources, respectively. Whole phosphorus and potash and half of N were applied as basal dose, rest half of N was applied with 1st irrigation. Analysis of various biochemical parameters was done at tillering (30-40 days after sowing; DAS), anthesis (about 100-140 DAS depending upon genotype) and post-anthesis (15 days post anthesis) stages from the flag leaf. Days taken for phenological events in tested wheat genotypes are given in Table 2.

Nitrate reductase activity was determined following the procedure of Jaworski (1971), NIR by the method of Verner and Ferari (1971) and GS by the method of Kanamori and Matsumoto (1974), GOGAT and GDH by Bulen (1956). Content of soluble protein was determined employing the method of Lowry *et al*. (1951) and amino acid by Lee and Takahashi (1966). Nitrogen content was estimated by the method of McKenzie and Wallace (1954). Chlorophyll content was determined as described by Arnon (1949).

Grain yield of each plot was recorded after threshing. Biomass was determined by taking weight of above ground dried plants along with the spikes and was expressed as kg/plant. Thousand grain weight was calculated by randomly selecting sample from harvested seeds of each plot. Length of three randomly selected plants from each plot was measured from base to tip and mean values computed. NUE was calculated as the grain yield per N supply (fertilizer N + soil mineral N at planting kg grain yield kg^{-1} N) (López-Bellido and López-Bellido, 2001).

All the values were mean of three replicates. Data obtained were subjected to statistical analysis at 5% level of CD using CPCS1 software developed by Department of Statistics, PAU, Ludhiana, India.

Results

In this study, NR, NIR, GS, GOGAT, GDH and content of metabolites increased significantly in response to high N fertilization during plant development. A significant increase in the activities of NR, NIR, GS, GOGAT and GDH was observed till anthesis and thereafter it decreased towards plant maturity at all N doses. However, N content showed consistent decrease from initial stages till maturity. With plant development, there was little change in soluble protein and chlorophyll content while amino acid content gradually increased from tillering to anthesis and decreased thereafter (Table 3).

At anthesis and post-anthesis stages, genotype GLU 1356 showed highest NR activity at all four N doses. At tillering stage, GLU 1356 showed higher activity of NR at RDN-25% and RDN and GLU 2001 at RDN-50% and RDN+25% over other genotypes (Table 3).

Table 1: Parentage of wheat genotypes used in the study

Genotypes	Parentage	Important trait
GLU 1101	GLUPRO/3*C518(BWL 0992)	High grain protein contents conferred by *Gpc-B1* gene introgressed from *Triticum dicoccoides*
GLU 1356	GLUPRO/3*PBW554(BWL 0975)	
GLU 2001	GLUPRO/3*PBW568 (BWL 0977)	
GLU 700	GLUPRO/3*PBW568 (BWL 0985)	
PH132-4836	PH132/WL711//PBW343	High grain protein content (control mechanism not known)
PH132-4840	PH132/WL711//PBW343	

Table 2: Days taken for phenological events in tested wheat genotypes

Genotypes	Days to tillering	Days to anthesis
GLU 1101	38	128
GLU 1356	41	131
GLU 2001	34	124
GLU 700	34	124
PH132-4836	38	128
PH132-4840	32	122

Table 3: Effect of nitrogen application on leaf nitrate reductase and nitrite reductase activities at three developmental stages of different wheat genotypes

	Tillering stage				Anthesis stage				Post-anthesis stage			
N doses/ Genotypes	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha
	Nitrate reductase (μmol NO_2 formed/h/g FW)											
GLU 1101	0.63± 0.10ab	1.07± 0.11a	1.16± 0.06a	1.01± 0.16a	2.36± 0.06b	2.39± 0.12a	2.26± 0.00a	4.84± 0.32b	1.43± 0.02b	1.49± 0.07a	2.46± 0.14b	3.34± 0.17b
GLU 1356	1.07± 0.15cd	1.39 ± 0.02b	1.65± 0.07c	1.74± 0.06bc	3.34± 0.03c	3.81± 0.14c	4.69± 0.17d	4.97± 0.08b	3.48± 0.10d	3.42± 0.05c	3.40± 0.01c	4.42± 0.01c
GLU 2001	1.29± 0.00d	1.34± 0.10a	1.57± 0.01bc	1.81± 0.07c	2.40± 0.03b	3.26± 0.00b	3.60± 0.01c	3.51± 0.33a	0.56± 0.24a	1.52± 0.11a	1.55± 0.01a	3.37± 0.09b
GLU 700	0.33± 0.00a	0.96± 0.20ab	1.10± 0.12a	1.47± 0.08b	1.42± 0.03a	2.34± 0.10a	3.62± 0.01c	3.61± 0.03a	1.40± 0.11b	2.47± 0.03b	2.48± 0.12b	2.60± 0.01a
PH132-4836	0.82± 0.13bc	1.28± 0.19ab	1.32± 0.04ab	1.66± 0.02bc	2.24± 0.09b	2.35± 0.04a	2.81± 0.13b	3.28± 0.03a	2.14± 0.02c	2.36± 0.06b	2.45± 0.10b	3.42± 0.22b
PH132-4840	0.77± 0.12bc	1.16± 0.10a	1.19± 0.01a	1.54± 0.05bc	1.51± 0.14a	2.17± 0.04a	3.26± 0.14c	4.48± 0.04b	1.33± 0.05b	1.43± 0.06a	2.48± 0.16b	2.48± 0.04a
CD (5%)	N-0.086, Genotypes(G)-0.105, N×G0.209				N-0.104, G-0.127, N×G-0.255				N-0.090, G-0.110, N×G-0.220			
	Nitrite reductase (μmol NO_2^- released/h/g FW)											
GLU 1101	0.13± 0.04a	0.14± 0.01a	0.23± 0.03a	0.34± 0.03ab	0.44± 0.02ab	0.46± 0.04b	0.64± 0.02a	0.64± 0.01a	0.37± 0.03ab	0.45± 0.03ab	0.44± 0.03ab	0.54± 0.01a
GLU 1356	0.36± 0.03c	0.45± 0.02b	0.45± 0.01bc	0.48± 0.02c	0.65± 0.03c	0.65± 0.03c	0.85± 0.03b	0.86± 0.02b	0.52± 0.03c	0.67± 0.03c	0.79± 0.01e	0.84± 0.01b
GLU 2001	0.23± 0.03ab	0.35± 0.03b	0.36± 0.03b	0.46± 0.05bc	0.54± 0.03bc	0.57± 0.01c	0.63± 0.07a	0.80± 0.02b	0.35± 0.05ab	0.54± 0.04b	0.55± 0.02d	0.88± 0.00b
GLU 700	0.15± 0.02ab	0.16± 0.03a	0.23± 0.02a	0.25± 0.01a	0.36± 0.05a	0.34± 0.02a	0.57± 0.02a	0.58± 0.02a	0.26± 0.04a	0.34± 0.03a	0.36± 0.01a	0.49± 0.01a
PH132-4836	0.39± 0.01c	0.40± 0.06b	0.42± 0.02bc	0.48± 0.03c	0.65± 0.01c	0.65± 0.01c	0.65± 0.01a	0.87± 0.04b	0.41± 0.02bc	0.43± 0.00ab	0.54± 0.02cd	0.56± 0.04a
PH132-4840	0.25± 0.01b	0.38± 0.01b	0.51± 0.01c	0.54± 0.01c	0.52± 0.01b	0.57± 0.02c	0.63± 0.02a	0.63± 0.04a	0.42± 0.03bc	0.47± 0.04b	0.48± 0.02bc	0.51± 0.01a
CD (5%)	N-0.023, G-0.029, N×G-0.057				N-0.025, G-0.030, N×G-0.061				N-0.022, G-0.027, N×G-0.054			

Means sharing the same letters, for a parameter at a phonological stage, do not differ significantly at p 0.05

Highest NIR activity was observed in genotypes GLU 1356 and PH132-4836 at sub-optimal doses while at optimum and higher dose, PH132-4840 showed higher activity compared to other genotypes at tillering stage. At anthesis, both GLU 1356 and PH132-4836 predominated at sub-optimal doses while at RDN and RDN+25%, GLU 1356 and PH132-4836 respectively showed high activity and at post-anthesis, GLU 1356 showed highest activity at all levels of N nutrition (Table 3). Although GS activity increased with increase in N dose but activity at RDN was statistically at par with RDN-25% depicting that RDN-25% gave comparable results to optimum dose. GLU 1356 was found to be highly efficient for GS activity at all N doses and at all three stages (Table 4). At tillering and post-anthesis stages, GLU 1356 showed appreciable activities of GOGAT and GDH at all N doses however, at anthesis, GLU 1356 showed highest activity at RDN-50% only and PH132-4840 at RDN-25%, RDN and higher dose (Table 4).

Table 4: Effect of nitrogen application on leaf glutamine synthetase, glutamate synthase and glutamate dehydrogenase activities at three developmental stages of different wheat genotypes

	Glutamine synthetase (µmol γ-glutamylhydroxamate formed/min/g FW)											
	Tillering stage				Anthesis stage				Post-anthesis stage			
N doses/ Genotypes	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 Kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 Kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 Kg N/ha
GLU 1101	2.57b	2.70ab	3.30b	2.97a	6.13b	6.77ab	8.67b	9.57a	5.40ab	6.93bc	7.80b	8.50bc
GLU 1356	5.04d	5.27c	5.40c	6.13c	8.23c	10.30c	16.57d	16.47e	9.00c	8.60d	10.20c	11.63d
GLU 2001	3.50c	4.93c	4.58c	4.71b	6.20b	7.84b	8.60b	14.13d	6.57b	7.73cd	7.80b	8.67bc
GLU 700	1.30a	1.63a	1.73a	2.67a	5.10a	5.57a	6.70a	9.93ab	4.37a	5.17a	5.43a	7.37ab
PH132-4836	1.37a	3.03b	3.27b	3.23a	5.27a	6.30a	7.17a	10.93bc	5.20a	5.73ab	6.13a	6.40a
PH132-4840	3.13bc	5.20c	5.27c	5.77c	9.03c	9.63c	10.10c	11.97c	8.30c	8.67d	9.07c	9.47c
CD (5%)	N- 0.024, G- 0.256, N×G- 0.511				N- 0.250, G- 0.306, N×G- 0.611				N- 0.309, G- 0.378, N×G- 0.756			
	Glutamate synthase (µmol NADH oxidized/min/g FW)											
GLU 1101	0.79a	1.50b	1.63b	2.24a	2.41a	3.70a	4.29b	4.55b	1.27a	3.75b	4.41bc	5.30c
GLU 1356	1.34b	2.75c	3.33d	4.75c	3.56c	5.58c	5.57d	5.70c	3.75d	4.70c	5.66d	6.56e
GLU 2001	1.30b	1.36b	1.63b	3.37b	3.13b	3.69a	4.73bc	5.36c	2.23b	3.79b	4.49c	5.68d
GLU 700	0.41a	0.62a	1.22a	2.25a	3.36bc	3.51a	3.56a	3.67a	1.19a	1.80a	2.36a	3.24a
PH132-4836	0.63a	1.52b	2.71c	3.46b	3.51c	4.38b	5.10cd	5.70c	2.86c	3.69b	4.01b	5.53cd
PH132-4840	0.67a	1.45b	2.80c	3.50b	3.25bc	5.82c	5.22d	6.55d	2.10b	3.67b	4.12bc	4.54b
CD (5%)	N- 0.083, G- 0.102, N×G- 0.204				N- 0.090, G- 0.111, N×G- 0.221				N- 0.086, G- 0.106, N×G- 0.211			
	Glutamate dehydrogenase (µmol NADH oxidized/min/g FW)											
GLU 1101	0.69a	1.02a	1.40a	1.80a	1.11a	2.65a	4.60b	5.20bc	2.81d	3.28b	3.58b	4.70c
GLU 1356	2.13d	3.28e	3.46d	3.40d	4.81e	4.78c	5.37c	5.65c	2.24c	5.09d	5.56d	5.78e
GLU 2001	0.58a	1.59c	2.14b	2.22bc	3.80d	4.60c	5.42c	5.49bc	3.51e	4.66d	4.54c	4.86cd
GLU 700	0.79ab	1.25ab	1.45a	2.12b	2.85bc	2.73a	3.19a	3.63a	1.31a	1.85a	2.10a	2.52a
PH132-4836	1.63c	2.26d	2.28b	3.23d	2.48b	4.06b	4.23b	5.07b	1.48ab	3.10b	3.43b	4.09b
PH132-4840	1.11b	1.55bc	2.88c	2.42c	3.12c	4.81c	5.49c	5.60bc	1.86bc	3.78c	3.81b	5.13d
CD (5%)	N- 0.071, G- 0.087, N×G- 0.175				N- 0.096, G- 0.117, N×G- 0.235				N- 0.084, G- 0.103, N×G- 0.207			

Means sharing the same letters for a parameter at a phonological stage don't differ significantly at p 0.05

At all three stages of plant development, GLU 1356 genotype revealed higher N content at RDN-50%, RDN-25% and RDN over other genotypes, although with higher dose of N PH132-4836 showed maximum N content at anthesis and post-anthesis stages (Table 5). Genotype GLU 1356 showed highest protein content at sub-optimal and recommended dose while at higher dose there was variation with respect to genotypic behavior at all three stages studied (Table 5). A higher build-up of protein content in this genotype was probably due to presence of *Gpc-B1* gene. In fact, this gene is responsible for accumulation, assimilation and translocation of N from flag leaf to grain in the form of amino acid and protein. A highest amino acid content was recorded at RDN+25%, which was significantly higher over RDN. At RDN-50% and RDN-25%, consistent genotypic response as observed for proteins at all three stages was noted while at RDN+25% GLU 2001 and PH132-4836 showed higher accumulation of amino acids (Table 5). Genotypes with *Gpc-B1* gene showed lower chlorophyll content compared to PH genotypes at all stages studied irrespective of N dose indicating that these genotypes senescence early compared to other genotypes (Table 3).

A significant increase in grain yield and its components was observed in wheat supplied with higher dose of N over optimum. However, yielding ability of genotypes at RDN-25% was statistically at par with recommended dose. Genotypic variation existed for yield component at all four doses of N. Yield was maximum for GLU 1356 at RDN-50%, while GLU 2001 was superior at RDN-25% (Table 6). Influence on 1000 grain weight corresponded with yield at RDN-50% and RDN+25% (Table 6). Maximum biomass was observed for genotype GLU 1356 at sub-optimal and optimum doses (Table 6). Plant height is an important index of growth and is also an important parameter for yield. GLU 700 was the tallest among all genotypes (Table 6), while maximum tiller number and spikelet number was recorded in GLU 1356 at all N doses (Table 6).

Nitrogen use efficiency is based on yield performance i.e. grain yield per N input, NUE decreased with increasing dose of N. A decrease in NUE with increasing fertilizer rate is due to less increase in grain yield in comparison to N supply. Genotype GLU 1356 showed highest NUE at RDN-50% (43.3 kg kg^{-1}) and GLU 2001 at RDN-25% (39.4 kg kg^{-1}) treatments (Table 7). As observed in this study, N dose can be decreased up to RDN-25% without marked loss of grain yield. A pooled correlation analysis was performed for biochemical and physiological parameters studied. Positive correlation of all N-assimilating enzymes (NR, NIR, GS, GOGAT and GDH) with N content, NUE and yield at 1 and 5% level of significance was observed (Table 8). Correlation of NR and GS with N content (r= 0.993, r=0.980, respectively) and NUE (r=0.963, r=0.829, respectively) was stronger than other enzymes. This depicted that these enzymes are important for acquiring sufficient amount of N required to the plant reflecting the overall physiological status of the plant (Kichey *et al*. 2006). NUE correlated positively with yield indicating that NUE depends on the grain yield of the crop.

Table 5: Effect of nitrogen application on nitrogen content, total soluble proteins, total free amino acids and chlorophyll content at three developmental stages of different wheat genotypes

	Nitrogen content (%)											
	Tillering stage				Anthesis stage				Post-anthesis stage			
N doses/ Genotypes	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha
GLU 1101	3.09b	3.13ab	3.21a	3.28a	2.04a	2.31a	2.42a	2.50a	1.40b	1.75b	1.60a	1.76ab
GLU 1356	5.44d	5.78d	6.81e	6.72d	4.87d	5.26b	5.23c	4.20d	2.49d	2.67c	2.55c	2.46d
GLU 2001	4.25c	5.42d	5.52d	6.89c	2.28ab	3.06b	3.11b	3.11bc	1.88c	1.98b	2.22b	2.44cd
GLU 700	2.65a	2.66a	3.01a	3.53a	2.01a	2.21a	2.24a	2.70ab	0.33a	1.11a	1.61a	1.61a
PH132-4836	3.82c	4.03c	4.44b	4.66b	2.52bc	3.05b	3.32b	5.65c	1.76bc	1.95b	2.14b	2.79cd
PH132-4840	3.35b	3.47b	5.06c	5.34c	2.87cd	3.11b	3.22b	3.46c	2.09c	2.09b	2.25b	2.22bc
CD (5%)	N- 0.101, G- 0.123, N×G- 0.247				N- 0.104, G- 0.127, N×G- 0.254				N- 0.085, G- 0.104, N×G- 0.207			
	Total soluble proteins (mg/g)											
GLU 1101	140.7a	148.8a	150.5a	155.5a	145.0a	144.2a	144.3ab	155.0a	143.0a	149.0a	155.0ab	160.0a
GLU 1356	141.0c	147.2c	161.0b	152.4d	164.5d	170.2b	172.0c	168.0b	148.5a	160.0a	162.0c	163.0c
GLU 2001	133.0ab	145.1ab	147.0c	161.0cd	160.4c	168.0b	170.2c	171.0ab	142.0b	145.0b	156.0b	166.7c
GLU 700	133.4a	143.7b	152.0a	158.0ab	162.0bc	168.1a	169.7a	172.0ab	141.5a	149.0a	163.5a	166.0a
PH132-4836	131.4ab	143.1ab	158.0b	159.2bc	151.0d	158.0b	163.0bc	174.2ab	137.0a	146.0a	158.3b	162.0b
PH132-4840	132.0b	142.4a	145.3ab	153.5cd	158.0ab	150.2b	154.0c	158.1b	144.5a	147.0b	153.0c	155.4c
CD (5%)	N- 1.831, G- 2.243, N×G- 4.487				N- 1.548, G- 1.896, N×G- 3.793				N- 1.732, G- 2.121, N×G- 4.242			
	Total free amino acids (mg/g)											
GLU 1101	1.84b	1.95a	1.74bc	2.08a	2.05b	2.81a	2.57a	2.68a	1.96b	2.04a	2.18b	2.19a
GLU 1356	1.64e	2.44e	2.67e	2.68e	2.38d	2.95d	3.08e	2.76f	2.16d	2.68d	2.63e	2.40f
GLU 2001	1.28c	1.61d	1.67ab	2.76e	1.95c	2.14b	2.57c	3.05e	1.46a	1.78b	1.96c	2.86c
GLU 700	1.59a	1.54b	1.62c	2.48c	1.39a	2.04a	2.48b	2.66b	1.61a	1.98a	2.18a	2.46b
PH132-4836	1.38d	1.54c	1.63a	2.56d	2.03b	2.36c	2.72d	3.21c	1.58b	1.59a	2.66e	2.54e
PH132-4840	1.39d	1.60d	2.37d	1.91b	1.85b	2.15b	2.72d	2.86d	1.79c	1.92c	2.19d	2.50d
CD (5%)	N- 0.136, G- 0.167, N×G- 0.334				N- 0.139, G- 0.171, N×G- 0.341				N- 0.120, G- 0.147, N×G- 0.294			
	Chlorophyll content (mg/g)											
GLU 1101	2.62c	2.84ab	2.75bc	2.84ab	2.46c	2.50bc	2.50b	2.52ab	2.32d	2.39d	2.45bc	2.57b
GLU 1356	2.58a	2.60cd	2.79ab	2.73a	2.45ab	2.51b	2.60a	2.62a	2.36c	2.41a	2.36a	2.38ab
GLU 2001	2.60ab	2.64a	2.67a	2.70a	2.52b	2.57c	2.59b	2.63bc	2.40ab	2.43b	2.48c	2.59b
GLU 700	2.61bc	2.77d	2.80c	2.88ab	2.48b	2.55d	2.57a	2.64c	2.19ad	2.36e	2.45b	2.41a
PH132-4836	2.69a	2.78b	2.88a	2.90b	2.60a	2.62a	2.83ab	2.87b	2.42b	2.50c	2.55ab	2.60ab
PH132-4840	2.53b	2.82c	2.90b	2.99b	2.61bc	2.66ab	2.72a	2.81c	2.48d	2.49ab	2.51a	2.57b
CD (5%)	N- 0.088, G- 0.108, N×G- 0.186				N- 0.112, G- 0.128, N×G- 0.204				N- 0.102, G- 0.125, N×G- 0.198			

Means sharing the same letters, for a parameter at a phonological stage, do not differ significantly at p 0.05

Table 6: Effect nitrogen application on plant height, grain yield, yield-related traits and total biomass of different wheat genotypes

	Plant height (cm)				Tiller number (per m row length)				Number of spikelet/spike			
N doses/ Genotypes	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha
GLU 1101	85a	82a	91c	90a	57ab	83cd	86b	102a	15ab	15ab	17b	18b
GLU 1356	84ab	87c	83ab	88a	80b	80ab	100a	109ab	17bc	18b	18ab	18ab
GLU 2001	80b	84bc	80a	87ab	50a	93d	94a	105ab	16c	17ab	17ab	18a
GLU 700	86a	87a	96ab	98a	62a	71bc	97ab	102ab	15abc	15a	16ab	19ab
PH132-4836	81ab	85b	88a	89ab	69ab	84abc	94ab	99b	15abc	16ab	17ab	17a
PH132-4840	85b	81ab	86ab	88b	55a	76a	77a	82a	16a	16ab	16a	17ab
CD (5%)	N- 15.64, G- 18.31, N×G- 21.52				N- 13.45, G- 15.89, N×G- 19.83				N- 1.314, G- 1.589, N×G- 1.967			
	Yield (kg/m^2)				1000-grain wt (g)				Total biomass (kg/m^2)			
GLU 1101	0.210a	0.330a	0.380ab	0.420a	35.56b	37.11a	37.22bc	39.00a	1.07a	1.27b	1.40b	1.67a
GLU 1356	0.260bc	0.310b	0.360b	0.390b	37.89a	38.67b	38.78c	41.00b	1.67b	1.70ab	1.77a	1.83c
GLU 2001	0.230ab	0.350bc	0.320ab	0.370ab	35.00ab	37.77b	38.22bc	39.11ab	1.20a	1.50bc	1.67b	1.87ab
GLU 700	0.200ab	0.270c	0.310a	0.390a	33.78b	35.89ab	37.30a	38.11a	1.30ab	1.37c	1.50ab	1.50ab
PH132-4836	0.250c	0.340ab	0.370a	0.410ab	36.56a	36.93ab	37.89ab	38.00ab	1.03b	1.43a	1.57ab	1.60a
PH132-4840	0.230ab	0.310bc	0.360ab	0.430a	37.00ab	37.33ab	38.92bc	41.00b	1.13ab	1.17ab	1.27a	1.43a
CD (5%)	N- 0.102, G- 0.124, N×G- 0.153				N- 1.624, G- 1.837, N×G- 2.869				N- 0.081, G- 0.136, N×G- 0.296			

Means sharing the same letters, for a parameter, do not differ significantly at p 0.05

Discussion

Genotypic variation among crop plants provides a valuable tool in the selection of genotypes with desirable traits as they respond differentially to various conditions (Dawson *et al*., 2008). In wheat, assimilate supply in the form of N is never a limiting factor rather its controlled utilization is a major phenomenon linked to grain activity as sink (Asthir and Bhatia, 2014). Enzyme activities were assayed in the flag leaves as they contribute towards grain filling and

Table 7: Effect of nitrogen application on nitrogen use efficiency (kg kg^{-1}) in various wheat genotypes

N Doses Genotypes	60 kg N/ha	90 kg N/ha	120 kg N/ha	150 kg N/ha
GLU 1101	35.0	36.7	31.7	28.0
GLU 1356	43.3	39.4	30.0	26.0
GLU 2001	39.3	38.9	26.7	24.7
GLU 700	33.3	30.0	25.8	26.0
PH132-4836	31.7	37.8	30.8	27.3
PH132-4840	38.3	34.4	31.0	28.7
Mean	36.8	36.2	29.3	26.8

Table 8: Correlation coefficients between biochemical and physiological traits at recommended dose of nitrogen (120 kg N/ha)

	Grain yield	NR activity	NIR activity	GS activity	GOGAT activity	GDH activity	Soluble proteins	Amino acids	Chlorophyll content	Grain weight	Biomass	Plant height	Tiller number	Spikelet number	NUE
N content	0.850*	0.993**	0.975**	0.980**	0.948**	0.963**	0.991 **	0.894 *	0.848 *	0.843*	0.714 ns	-0.664 ns	0.513 ns	0.840*	0.907*
Grain yield		0.899 *	0.818 *	0.932 **	0.865 *	0.485 ns	0.852 **	0.423 ns	0.500 ns	0.556 ns	-0.098 ns	-0.619 ns	0.000 ns	0.033 ns	0.972**
NR activity			0.762 ns	0.716 ns	0.768 ns	0.756 ns	0.881 *	0.956**	-0.434 ns	0.677 ns	0.872 ns	-0.243 ns	0.746 ns	0.909*	0.963 **
NIR activity				0.756 ns	0.970**	0.936**	0.350 ns	0.752 ns	0.128 ns	0.829*	0.596 ns	-0.724 ns	0.463 ns	0.718 ns	0.856 *
GS activity					0.848*	0.919**	0.834 *	0.852 *	-0.194 ns	0.954**	0.512 ns	-0.594 ns	0.149 ns	0.773 ns	0.829 *
GOGAT activity						0.952**	0.220 ns	0.814*	0.108 ns	0.928**	0.517 ns	-0.706 ns	0.346 ns	0.697 ns	0.804 *
GDH activity							0.813 *	0.760 ns	-0.061 ns	0.915*	0.615 ns	-0.776 ns	0.349 ns	0.811 ns	0.633 ns
Soluble proteins								0.445 ns	-0.547 ns	0.086 ns	0.868*	0.128 ns	0.789 ns	0.729 ns	0.859 *
Amino acids									-0.362 ns	0.757 ns	0.729 ns	-0.293 ns	0.624 ns	0.816*	0.514 ns
Chlorophyll content										0.062 ns	-0.647 ns	-0.228 ns	-0.529 ns	-0.578 ns	0.452 ns
Grain weight											0.369 ns	-0.628 ns	0.081 ns	0.638 ns	0.631 ns
Biomass												-0.225 ns	0.887*	0.939**	0.092 ns
Plant height													-0.053 ns	-0.406 ns	-0.719 ns
Tiller number														0.701 ns	0.157 ns
Spikelet number															0.211 ns

** - Significant at p 0.01, * - Significant at p 0.05

grain N status throughout maturity of the plants (Jain *et al*., 2011). Earlier reports using maize as a model plant, revealed a decreasing trend of N uptake and assimilation at leaf ageing and that the decrease was enhanced when plants were N starved (Hirel *et al*., 2005). However, reverse trend was observed in crops raised under higher levels of N_2 which revealed coordinated increase in the activities of all N assimilating enzymes (Anjana *et al*., 2011).

In this study, we elaborated N assimilation in diverse genotypes raised under different N levels to understand biochemical basis of source sink transition of wheat leaves in relation to their variability. Previous studies have shown that plants take up a range of organic N substrates (urea, amino acids) directly from soil using a range of passive and active transport systems (Jones *et al*., 2005). Tissue type and concentration of N can significantly affect the activities of N assimilatory enzymes in plants (Zhou *et al*., 2004). NR catalyses the first step of NO_3^- assimilation in plants, leading to reduction of NO_3^- to NO_2^- in the cytosol. Nitrite accumulating in cytosol is transported into chloroplast for its reduction to NH_4^+ ions via NIR (Lea, 1993). Ammonia is assimilated into organic form as glutamine and glutamate, which serves as the N donors in the biosynthesis of amino acids, nucleic acids and other N containing compounds such as chlorophyll.

In flag leaf of diverse genotypes, variability in the activities of NR, NIR, GS, GOGAT was observed at various levels of N implying that different wheat genotypes had distinct N use thresholds and, therefore, they had diverse adaptive mechanism to regulate N assimilation. GLU 1356 was found to be efficient for NR and NIR activity at sub-optimal doses as has been previously reported (Yang *et al*., 2005; Purcino *et al*., 2008). Activities of NR, NIR, GS, GOGAT and GDH were highest at anthesis stage, indicating an extensive NH_4^+ assimilation and remobilization, which are tightly interrelated processes at plant growth and development (Miflin and Habash, 2002). The transition of source-sink relation of flag leaf from vegetative stage to anthesis and post-anthesis stages might be responsible in inducing N-remobilization in senescing leaves at post-anthesis stage. The study provided evidence that high NR activity is associated with the enhanced activity of the enzymes of pathway. Chandna *et al*. (2012) reported that N efficient genotypes differed in all aspects of N metabolism viz. amount and rate of NO_3^- taken up, accumulation and assimilation by the tissue and also N harvest. As observed for NR activity, GS and GOGAT activities were also significantly high in GLU 1356 and GLU 2001 than other

genotypes suggesting that N efficient genotypes exhibited higher activities of all the enzymes at all stages of plant analysis leading to higher yield and N harvest. Overall there has been limited reports indicating that increased NR, NIR, GS or GOGAT alone could be useful strategy to increase yield or NUE because all work in conjunction.

The decreasing content of N in leaves may be linked to low N uptake and utilization at later stages of plant development. However, chlorophyll content showed minor variation with plant development. An increase in chlorophyll content with increasing level of N could be attributed to increased synthesis of molecules under higher N availability (Mehta *et al*., 2011). In senescing leaves, the activities of all N assimilating enzymes were reduced indicating chloroplast breakdown involving *de facto* NIR, GS and GOGAT proteolysis. Furthermore, at post-anthesis stages, the photosynthesis virtually declines because of the hydrolysis of flag leaf cellular components into transport compounds with low C/N ratio to develop seed for their accumulation. Total N content in leaves also reflects the overall physiological status of the plant in relation to N fertilization, which is one of the main parameters used to predict N requirement (Kichey *et al*., 2006). Major sinks of the N taken from the soil are proteins (Landry and Delhaye, 2007). High activities of NR, NIR and GS in GLU 1356 and GLU 2001 corresponded well with high contents of protein and amino acid in these genotypes. As depicted from our results, low N supply resulted in significant decline in enzyme activities as well as amino acid and protein content.

In this study, N fertilization had a significant influence on grain yield for all the tested genotypes and is in agreement with many previous reports (Hussain, 2007; Arnall *et al*., 2009). Under low N levels, the highest grain yields were achieved for GLU 1356 and GLU 2001. Shah (2008) reported that under optimal N nutrition, CO_2 assimilation is improved resulting in increased spikelet number and seed yield/plant. Low N supply decreases grain weight due to less supply of the grain with carbohydrates and amino compounds (Paponov *et al*., 2005). A highest accumulation of dry matter in terms of amino acids in GLU 1356 and GLU 2001 could be due to high supply of assimilates to the sink leading to high grain yield. A similar positive response of increased N dose to growth and yield attributes in sweet sorghum was observed by Miri *et al*. (2012). In this regard, Warraich *et al*. (2002) reported that an increase in the number of fertile tillers with the increasing levels of N owing to reduction in mortality of tillers. Biochemical and physiological differences in these genotypes will facilitate breeders as a benchmark for increasing NUE and crop productivity by crossing these genotypes.

Under higher N supply, NUE decreased due to inconsistent increase between grain yield and N supply as also observed in this study. Dawson *et al*. (2008) suggested that under these conditions, plants are unable to assimilate enough N and losses rise resulting in low NUE. In our study, highest NUE and grain yield was recorded for genotypes GLU 1356 and GLU 2001 under sub-optimal doses of N. Field studies on barley have clearly shown differences in the NUE of various genotypes (Abeledo *et al*., 2008; Anbessa *et al*., 2009), which can be improved by increasing N uptake and NUE under low N supply (Moose and Below, 2009; Beatty *et al*., 2010). Very low NUE of even 6% was registered for wheat genotypes under 180 kg N ha^{-1} of urea ammonium nitrate which could be attributed to N loss to environment (Kanampiu *et al*., 1997).

Data showed s strong relationship among all N-assimilating enzymes and N content and NUE. Among all the studied enzymes, NR and GS showed highest positive correlation with N content and NUE. This indicated that NR and GS could serve as marker enzymes associated with NUE. In this study, grain yield was also correlated positively with most of the parameters and this has also been reported by previous researchers (Hefny, 2007).

Conclusion

A higher NUE and grain yield in GLU 1356 and GLU 2001 at sub optimal dose of N is probably linked to higher activities of NR and GS enzymes. A strong positive correlation of NR and GS with NUE indicated key metabolic steps influencing N metabolism. Apparently these two enzymes can be exploited for producing superior cultivars having high NUE at suboptimal dose of N. Furthermore, RDN-25% dose is sufficient to ensure maximum yield potential for most of the genotypes. Due to stable performance of GLU 1356 and GLU 2001 at RDN-25%, these genotypes hold potential for developing new cultivars with improved NUE.

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