

Supplementation of Nitrogen and its Influence on Free Sugars, Amino Acid and Protein Metabolism in Roots and Internodes of Wheat

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Effect of different doses of nitrogen (N) (90, 120, 150 and 180 kg Nha⁻¹) on the activities of aminotransferases and alkaline inorganic pyrophosphatase (AIP) in relation to the accumulation of proteins, amino acids and sugars in roots and internodes at 15 and 40 days post anthesis (DPA) stages was studied in six wheat genotypes namely HD 2967, GLU 1101, PBW 343, BW 9022, PH-132-4840 and PBW 550. Supra-optimal N doses (150 kg Nha⁻¹ and 180 kg Nha⁻¹) accentuated glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline inorganic pyrophosphatase activities in correspondence with an increase in amino acid, protein and sugar content in both roots and internodes in all the six genotypes. Activities of analyzed enzymes were significantly high at 15 days post anthesis (DPA) stage and thereafter declined at maturity (40 DPA) in parallel with decrease in amino acid contents. Maximum activity of GOT, GPT and AIP was observed in HD 2967 and GLU 1101 genotypes along with higher build up of proteins and amino acids which resulted in higher grain yield. Activity of GPT was comparatively high over GOT, indicating its major role towards protein synthesis. Grain filling processes in terms of proteins and amino acids were positively correlated with GOT and GPT activities while sugars were correlated to AIP. Thus, nitrogen acquisition and assimilation resulted in favoured utilization of N in form of amino acid and proteins accumulation while sugar content was also stimulated. Due to immense activities of aminotransferases and higher contents of amino acids and proteins in GLU 1101 and HD 2967 genotypes at optimal dose and higher dose of N, these genotypes hold future potential for developing new cultivars with better grain quality characteristics.

Keywords: aminotransferases, amino acids, nitrogen doses, proteins, *Triticum aestivum*

Abbreviations: N, nitrogen; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; DPA, days post anthesis; TCA, trichloroacetic acid

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Introduction

Wheat is one of the most important widely grown crops in the world which serves as an important source of food and energy. It occupies central position in agriculture policies and farming (Shehzad et al. 2012). This crop is mainly grown for its high protein and starch content in grains.

Nitrogen is a critical input in agriculture and is a powerful tool for increasing grain yield in wheat. However, inadequate supply of N in plants frequently results with depressed protein levels, poor quality. Increase in the use of nitrogen fertilizer causes serious environmental concerns, such as nitrate leaching into ground water and run off into surface water. Efficient use of N by wheat is, therefore, needed to sustain or increase yield and quality, while reducing the negative impacts of fertilizer accumulation in the environment (Abedi et al. 2011; Li et al. 2016). Optimizing nitrogen use with adequate grain protein require the knowledge of expected nitrogen uptake and utilization efficiency within the plant based on the rate of nitrogen applied (Maqsood et al. 2012; Kaur et al. 2016a).

Nitrogen-efficient cultivars showed a higher potential of redox homeostasis, protein stability and regulation of nitrogen levels (Galani et al. 1991; Yousuf et al. 2017). Proteins involved in nitrogen sensing and assimilation exhibited diverse expression leading to the over expression of genes associated with stress tolerance (Dupont and Altenbach 2009; Li et al. 2016). Earlier study on the regulation of N uptake, assimilation and recycling during growth and development of wheat plants grown in the field was done at agronomic level by combining with whole plant molecular physiology (Kichey et al. 2006). Knowledge about the biochemical pathway in improving grain nitrogen content at sub-optimal dose of nitrogen in different wheat cultivars is still lacking. The enzymes glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are key enzymes involved in the assimilation of NH_4^+ in higher plants (Asthir and Tak 2017) and these enzymes are responsive to N supply (Wang et al. 2004). In the present study, six wheat cultivars showing differential response to supplied N were selected to study the effects of different doses of nitrogen on aminotransferases in relation with accumulation of proteins, amino acids and sugars in roots and internodes.

Materials and Methods

Plant material and N treatment

Seeds of six wheat (*Triticum aestivum* L.) genotypes, viz. HD 2967, GLU 1101, PBW 343, BW 9022, PH-132-4840 and PBW 550 (cross and pedigree of the genotypes represented in Table 1) were grown in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The crop was grown under four doses of nitrogen – optimal N dose recommended dose of N (RDN, 120 kg Nha^{-1}), suboptimal N dose (RDN-25%, 90 kg Nha^{-1}) and supra-optimal N doses (RDN + 25%, 150 kg Nha^{-1}) and (RDN + 50%, 180 kg Nha^{-1}) in plots consisting of 4 rows of 1 m each. Row to row spacing was maintained at 22.86 cm while spacing between the plots was 40 cm. Nitrogen was applied in form of urea. Phosphorous and potassium

Table 1. Important features of six wheat genotypes

Genotype	Pedigrees	Important features
HD 2967	ALD/COC//URES/3/ HD2160M/HD2278	Released variety for timely sown irrigated conditions
GLU 1101	GLUPRO/3*C518 (BWL 0992)	High grain protein contents conferred by <i>Gpc-B1</i> gene originally introgressed from <i>Triticum dicoccoides</i> (Tetraploid wild wheat)
PBW 343	D/VG 9144//KAL/BB/3/YACO'S'/4/ Vec#5	Widely sown released variety for timely sown irrigated conditions
BW 9022	C 591/3*PBW343	Introgression lines with genetic input from tall traditional cultivars
PH-132-4840	PH132/WL711//PBW343	High grain protein content (control mechanism not known)
PBW 550	WH594/RAJ3856//W485	Good grain released variety for timely sown irrigated conditions

were applied at 65 and 30 kg ha⁻¹ using diammonium phosphate and sulphate of potash as sources, respectively. Roots and internodes at 15 and 40 days post anthesis (DPA) stage were collected for various physiological and biochemical parameters. Fresh tissue was used for enzyme assays whereas dry tissue (80 °C for 48 ~ 72 h in an oven) was used for protein, amino acid and sugar analysis. There were four plots for each treatment and every treatment was analyzed in triplicate for each determination.

Determination of free sugars, amino acid and proteins

Free sugars were extracted sequentially with 80% and 70% ethanol from ethanol-preserved samples. The text extracts were clarified with basic lead acetate and from these extracts and concentrations of total sugars were determined (Asthir and Bhatia 2012). Total free amino acids were extracted and determined as described earlier (Asthir and Bhatia 2012). Soluble proteins extracted in 0.1 M NaOH and precipitated with trichloroacetic acid and were estimated (Bala et al. 2010).

Extraction and assay of enzymes

For extraction of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase (GOT/GPT, EC 2.6.1.1//2.6.1.2, L-aspartate/L-alanine: 2-oxaloglutarate aminotransferases) from roots and internodes, 50 mM Tris HCl buffer (pH 7.5) containing 100 mM β-mercaptoethanol, 2 mM MgCl₂, 2 mM EDTA, 10 mM cysteine were used. For extracting alkaline inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1), roots and internodes were homogenized at 0–4 °C in 50 mM Hepes buffer (pH 7.5) containing 5 mM MgCl₂, 1 mM disodium EDTA, 2.5 mM DTT, 0.5 mg cm⁻³ BSA, 0.05% (v/v) Triton-X 100. Homogenates were centrifuged at 10,000 g for 15 min and the extraction procedure was repeated with the pellet obtained. The pooled supernatants were

passed through SephadexG-25 column equilibrated with the above buffer without EDTA and Triton X-100. The activities of GOT and GPT were determined according to Tonhazy et al. (1960a,b). Alkaline inorganic pyrophosphatase (AIP, EC 3.6.1.1) was assayed by the method of Heppel (1955). In all enzyme assays, the conditions for linear rates with respect to substrate concentration, time, optimum temperature and pH were determined in preliminary assays.

Thousand-grain weight (g)

Thousand-grain weight was calculated by randomly selecting twelve samples from harvested seeds of each plot and the mean value was computed.

Statistical analysis

All the values reported are the means of three replicates. Data obtained was subjected to statistical analysis of variance for factorial experiments in randomized block design at 5% level of CD using CPCS1 software developed by Department of Statistics, PAU, Ludhiana, India.

Results

Activities of aminotransferases, viz. glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline inorganic pyrophosphatase (AIP) in relation to accumulation of protein, amino acid and sugars in six wheat genotypes viz. HD 2967, GLU 1101, PBW 343, BW 9022, PH-132-4840 and PBW 550 under four doses of N – i.e. optimal N dose i.e recommended dose of N (RDN, 120 kg Nha⁻¹), suboptimal N dose (RDN-25%, 90 kg Nha⁻¹) and supra-optimal N doses (RDN + 25%, 150 kg Nha⁻¹), (RDN + 50%, 180 kg Nha⁻¹) in form of urea were studied. Higher doses of nitrogen (supra-optimal N doses, 150 kg Nha⁻¹ and 180 kg Nha⁻¹) increased activities of aminotransferases which resulted in increased content of protein and amino acids. For instance, internodes of GLU 1101 and roots of HD 2967 possess comparatively higher proteins, amino acids and sugars contents over other studied genotypes. Maximum activity of aminotransferases and contents of proteins, amino acids was found at 15 DPA (actively metabolizing stage) as compared to 40 DPA stage (Figs 1 and 2). Glutamate pyruvate transaminase activity was higher over GOT, indicating a major role of this enzyme during proteins biosynthesis. Amino acid and protein accumulation were also high at supra-optimal N dose (RDN + 50%, 180 kg Nha⁻¹) while sugar content was maximum at recommended dose of N (RDN, 120 kg Nha⁻¹) at 40 DPA stage in roots and internodes (Fig. 2). As in case of proteins and amino acids, accumulation of sugars is also affected by different doses of nitrogen as reflected in increased activity of alkaline inorganic pyrophosphatase. However, maximum soluble sugar content was observed at RDN as compared to other sub and supra-optimal doses of nitrogen. Moreover, soluble sugar content showed an increasing trend at maturity (40 DPA). Maximum activity of this enzyme was observed in genotype GLU 1101 (flag leaf) and HD 2967 (grains) at both stages. Consequently,

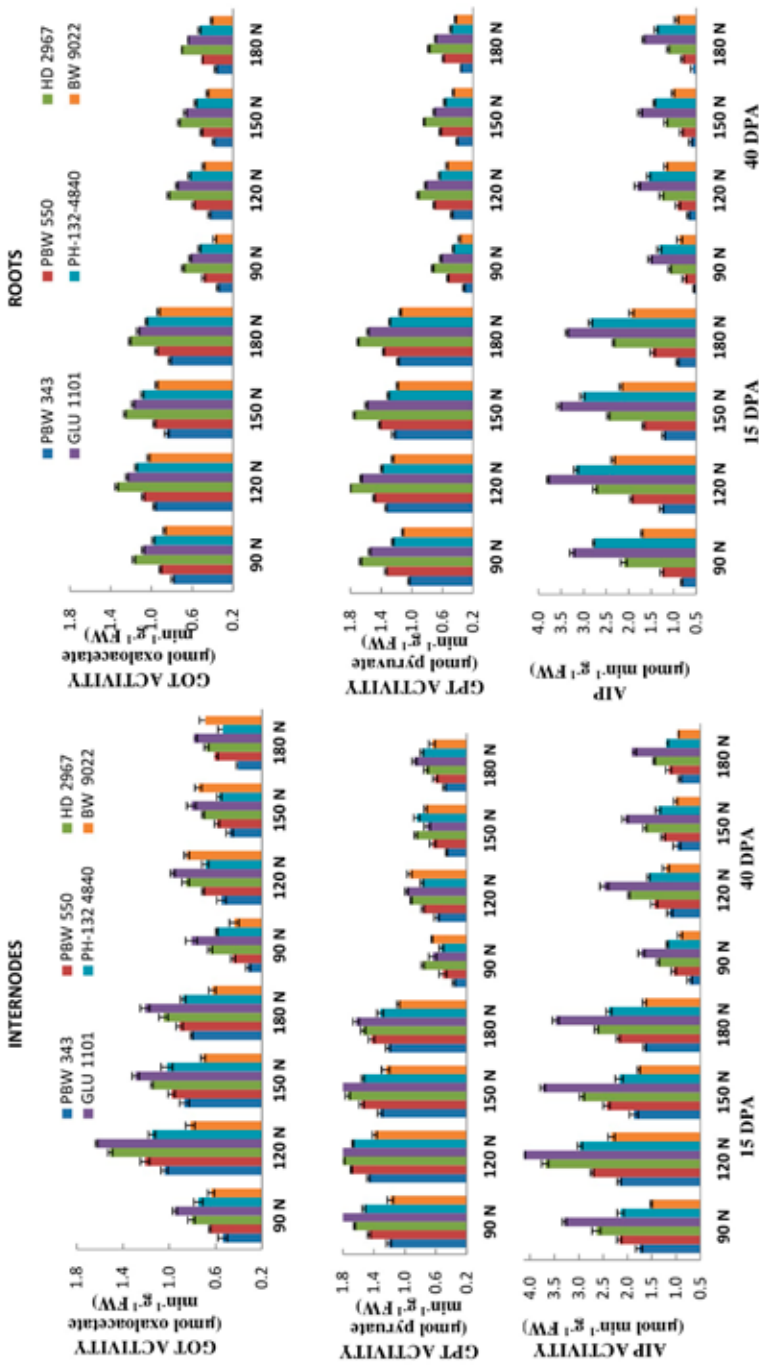


Figure 1. Effect of different doses of nitrogen (N) on glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline inorganic pyrophosphatase (AIP) activities in roots and internodes of six wheat cultivars

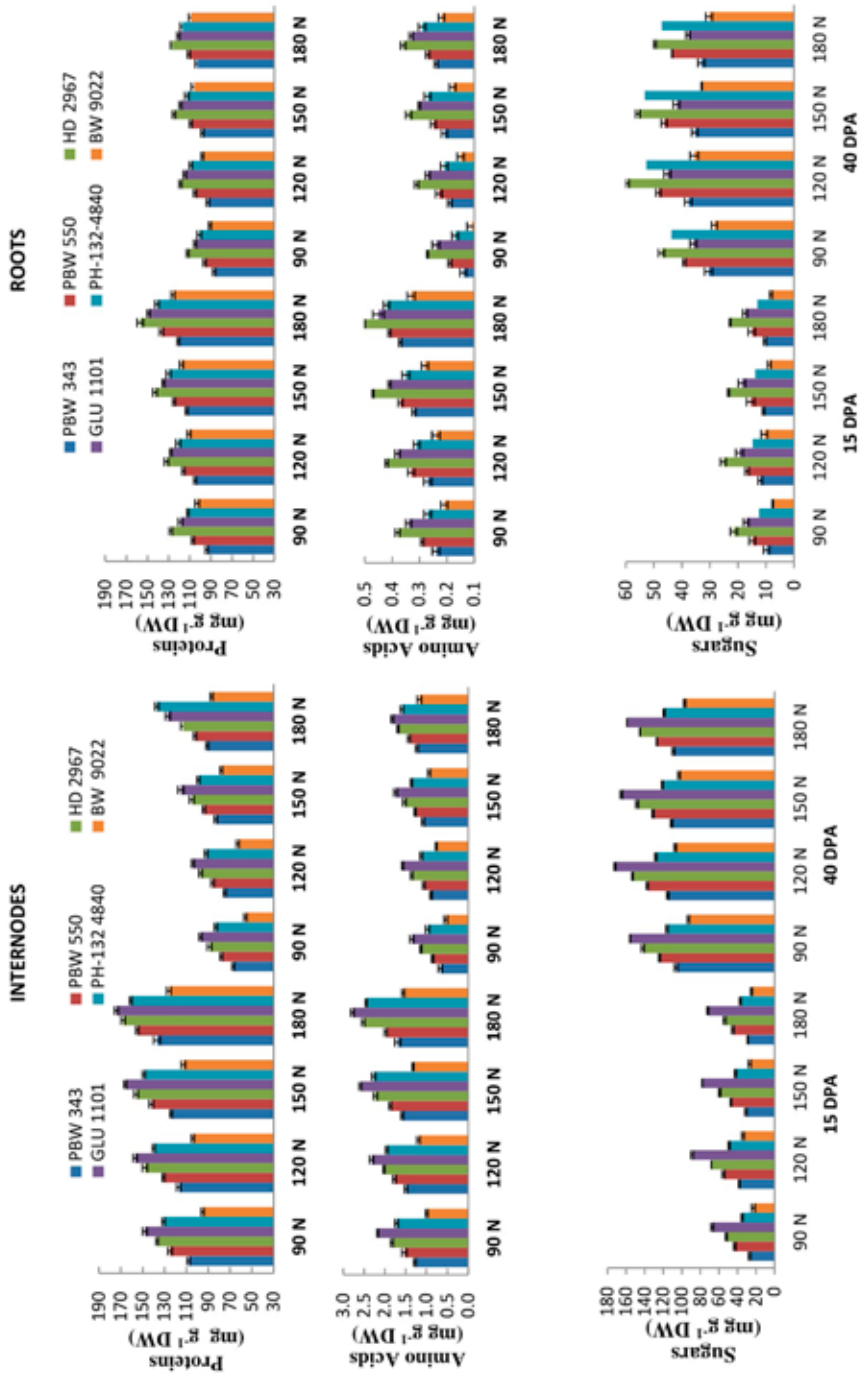


Figure 2. Effect of different doses of nitrogen (N) on accumulation of proteins, amino acids and sugars content in roots and internodes of six wheat cultivars

accumulation of soluble sugar was observed in cvs GLU 1101 (internodes) and HD 2967 (roots) at both stages.

Thousand grain weight was maximum at higher dose of nitrogen i.e. RDN + 50% as compared to other doses of nitrogen and grain weight was most affected in cultivar HD 2967 at higher doses of nitrogen. Decrease in aminotransferases and alkaline inorganic pyrophosphatase activities was more apparent in PBW 550 in relation to accumulation of proteins, amino acids and sugars in roots and internodes (Table S1*). Analysis of variance (ANOVA) indicated significant variation among cultivars, stages and treatments for all the characters.

Correlation among proteins and GOT were stronger than GPT during development stages (Table S1). Total sugars was significantly but negatively correlated with thousand grain weight while protein content was positively correlated.

Discussion

Genotypic variation among crop plants provides a valuable tool in selection of cultivars with desirable traits as it respond differentially to various doses of nitrogen. Supra-optimal N doses (150 kg Nha⁻¹ and 180 kg Nha⁻¹) during grain filling stage affects several key metabolic steps which are involved in determining grain yield and quality of wheat. Our research findings depicted the role of aminotransferases enzymes in developing grains leading to biosynthesis of proteins and amino acids (Asthir and Tak 2017). However, further studies related to alteration in carbon-nitrogen metabolism under different doses of nitrogen during grain filling processes is still lacking. Kikuchi et al. (1999) also reported that with increase of nitrogen concentration, an increase in GOT and GPT activity was observed in correspondence with an increase in amino-acids and proteins content of both roots and internodes. Apparently, GOT and GPT activities coincided well with proteins deposition in roots and internodes. Our findings on increased proteins, amino acids and sugars content under higher nitrogen doses are consistent with the ones reported in maize (Khan et al. 2008), rice (Farooq et al. 2012) and sweet sorghum (Almodares et al. 2008). Similarly, internodes of GLU 1101 and roots of HD 2967 revealed higher activities of aminotransferases and contents of proteins and amino acids suggesting a possible relationship with NH₄⁺-assimilation and remobilisation. GPT play a major role in transfer of C3 units to maintain a nitrogen-carbon balance through the interaction between alanine aminotransferase (AlaAT) and pyruvate; while GOT might be responsible in N-metabolism and participate in the consumption of glutamic acid for further metabolism as reported by Liu et al. (2014). It is also probable that the resulting amino acids were substrates for GOT and GPT, thus inducing these enzyme activities. Since the activity of transaminases is stimulated under conditions, i.e. supra-optimal doses of N that are otherwise deleterious to protein synthesis, we speculate that aminotransferase might be acting in the direction of deamination to provide amino-acids, especially glutamic acid for common N-pool as also reported by Ge et al. (2008). Likewise, both aminotransferase activities were higher in roots than internodes indicating that amino acids are first transformed

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

into other amino acids and proteins in roots prior to their transport into shoots (Ma 2004). However, increased protein content leads to deterioration in dough quality which could partly be attributed to the decline in glutenin-gliadin ratio (Bencze et al. 2004).

Carbohydrate distribution within plant is also affected by N supply which strongly influences the processes of carbon assimilation, allocation and partitioning (Mehta et al. 2011; Xiong et al. 2014). For example, alkaline inorganic pyrophosphatase activity was enhanced by higher doses of nitrogen, leading to increased content of soluble sugars, by other authors (Bybordi and Ebrahimian 2011) in *Brassica napus* L. Similarly, Kaur et al. (2016b) reported that nitrogen fertilization increased sugars content in rice. The greater biochemical activity (sugar and protein) and higher N-use efficiency at low nitrogen rates could be used in selection for N-efficient rice genotypes. This relationship between nitrogen fertilizer and consumption of soluble sugars may be due to metabolism nitrogen fixation. Since some of the intermediate metabolic in TCA cycle is used for amino acids and protein synthesis, so the amount of carbohydrate (sugars) is reduced specially in wheat crop. This progressive increase in sugar content with grain development may be due to their enhanced mobilization for starch biosynthesis as also reported by Balla et al. (2011). Alternatively, it also suggests that pathways of sugars and proteins were correlated under different doses of nitrogen. Therefore, a complementary effect in the balance of carbon and nitrogen metabolism reflects as content of sugars increased there was parallel influence on amino acids and proteins. Moreover, inhibition of starch biosynthesis and funneling the accumulating sugars into respiratory metabolism generating carbon skeletons for amino acid biosynthesis in wheat grains as reported by Almodares et al. (2009). An appreciable activity of alkaline inorganic pyrophosphatase was significantly high at 15 DPA and was low at 40 DPA indicating a significant role in starch biosynthesis. The major role of alkaline inorganic pyrophosphatase might be in protein degradation at 40 DPA due to its hydrolytic nature.

Our study reveals that, GLU 1101 and HD 2967 genotypes seemed to respond better to lower and higher doses of nitrogen in terms of protein and amino acids accumulation. Individual grain weight which considered as one of the major yield contributor was also significantly affected by higher doses of nitrogen. Higher N supply increases grain weight due to higher assimilates in form of carbohydrates and amino compounds during the lag phase when the number of storage cells and starch granules are being formed. Thus, it appears that the application of nitrogen increased the proteins percentage which in turn increased the thousand grain weight.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <https://akademai.com/loi/0806>

Electronic Supplementary *Table S1*. Correlation coefficients between ammonia assimilating enzymes with proteins, amino acids and sugars content and thousand grain weight in roots and internodes