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Original Research

Effect of nitrogen on starch and protein content in grain influence of nitrogen doses on grain starch and protein accumulation in diversified wheat genotypes

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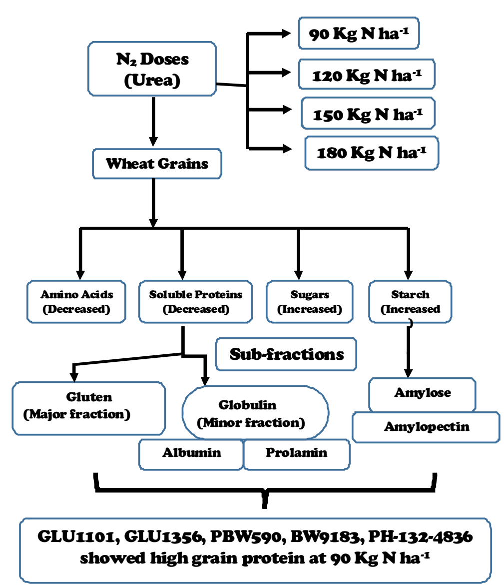
Abstract

Aim: Nitrogen is a critical input involved in plant metabolism growth and in different biochemical processes. Nitrogen participates directly in amino acid, protein and other cellular component syntheses, which are required for plant growth and development. Therefore, nitrogen application greatly influences starch and protein composition and, very little information is available on the effect of nitrogen fertilizer on protein sub fractions and starch components with little emphasis on quality characteristics. Therefore, the aim of the study was to evaluate the genotypic variation based on starch and protein accumulation under different doses of nitrogen.

Methodology: The present study was conducted to evaluate the effect of different doses of nitrogen in form of urea on grain quality parameters such as protein sub-fractions, starch sub-components from nine wheat genotypes (PBW 621, PBW 590, PBW 509, BW 9183, BW 8989, PBW 550, GLU 1101, GLU 1356, PH 132 4836) at four levels of nitrogen (N) optimal N dose i.e., recommended dose of N [RDN (120 Kg N ha^{-1})], suboptimal N dose [RDN-25% (90 Kg N ha^{-1})] and supra-optimal N doses [RDN+25% (150 Kg N ha^{-1}) and RDN+50% (180 Kg N ha-1)].

Results: PBW 550, BW 8989 and BW 9183 genotypes had higher sugars content (~20 mg g^{-1} d.wt.) and starch (~72.8%), whereas amino acids and protein content were low (~0.65 -100.3 mg g^{-1} d.wt., respectively). Sugar and starch content were inversely correlated with protein and amino acids indicating a compensatory effect. Higher build-up of grain protein in GLU 1101 (126.3 mg g^{-1} DW) and GLU 1356 (141.7 mg g^{-1} d.wt.) might be due to higher translocation of N from flag leaf to reproductive structures. Gluten constituted major seed storage proteins as its content was comparatively higher over other proteins in genotypes- PBW 590 and PH-132-4836 (~51.7 mg g^{-1} d.wt.) at RDN and RDN-25%. Whereas, amylose content was higher in BW 9183, GLU 1101, BW 8989 genotypes (~23.8 mg g^{-1} d.wt.) at RDN-25% while amylopectin content was more in BW 9183 and BW 8989 genotypes (~49.1 mg g^{-1} d.wt.) over other genotypes at RDN+25% and RDN+50% .

Interpretation: Due to consistent performance of GLU 1101, GLU 1356, PBW 590, BW 9183 and PH-132-4836 genotypes at sub-optimal nitrogen dose, these genotypes hold future potential for developing new cultivars with better grain quality parameters.



Introduction

Wheat (*Triticum aestivum* L.) is one of the three major cereals dominating agricultural world today. It accounts for majority of food products used for human diets. The quality of wheat based food products and the processing of wheat flour are strongly related to the composition of proteins and starch.

Wheat grain proteins is an important trait and plays a crucial role in forming a strong, cohesive dough that will retain gas and produce light baked products (Abedi *et al.,* 2011). These properties make wheat suitable for the preparation of a great diversity of food products: breads, noodles, pasta, cookies, cakes, pastries and many other foods. The mature wheat grains comprises of 18-20% proteins which are further classified into four categories on the basis of their solubility- albumin (water), globulin (salt), gluten (alkali) and prolamin (propanol) (Osborne 1907). Gluten is large complex proteins composed of glutenin and gliadins, which is important for baking quality because of their impact on water absorption capacity of the dough. Its elasticity and extensibility can affect wheat flour quality extensively (Torbica *et al.,* 2007). Albumin and globulin probably have a critical role in flour quality, while they also have dual role as nutrient reserves for the germinating embryo (Singh and Skerritt, 2001).

Starches with varying pasting characteristics are of major concern for food processing because of their potential to modify the texture and quality of the end use based products. It also serves as a source of carbon during yeast fermentation in bread making, in setting of the bread loaf and in retrogradation during storage (Singh *et al.,* 2010). Starch is divided into two broad categories: amylose and amylopectin. Amylose consist of glucose units having linear chain linked by α-1,4 linkage, while amylopectin has an additional α-1,6 linkages. The content of amylose varies from 20 to 30%, whereas amylopectin constitutes about 75% of cereal starches. High amylose content (> 40%) in starch is used as thickeners and as strong gelling agents while amylopectin content in starch improves homogeneity, stability, texture of gelled starch that enhances the stability of starch gel at frosting and defrosting of frozen foods (Massaux *et al.,* 2008).

Wheat is preferably grown for bread and other flour products because of its supreme baking performance (Dewettinck *et al.,* 2008). The wheat quality characteristics are usually influenced by genotype, environmental factors and the interaction between genotype and environment. Nitrogen nutrition, an indispensible factor for wheat production during growth especially through the grain filling period is identified as a major constraint to wheat grain quality world wide (Dupont and Altenbach., 2003).

Albumin and globulin are controlled merely by genotypes than nitrogen treatment whereas prolamin and glutenin are largely determined by nitrogen (Fuertes-Mendizabal *et al.,* 2010). Reports indicate that different nitrogen rates can cause changes in the total amount of different grain proteins. Infact, the composition of proteins fractions has found to be much more affected by nitrogen management than temperature (Hurkman *et al.,* 2013). In other words, high nitrogen supply increases grain proteins concentration linearly while grain yield response to added nitrogen had a diminishing return relationship. Apparently, when nitrogen very limiting, small nitrogen addition resulted in greater grain yield with decreased proteins concentration caused by dilution of the plant nitrogen. Thus, nitrogen nutrition is the main factor affecting storage proteins as well as the technological quality of the grain. Nitrogen nutrition is also considered as the third most environmental factor influencing starch composition and properties (Xiong *et al.,* 2014). Zadeh *et al.* (2013) showed that a moderate reduction in N lead to small increases in starch content in wheat. Increased N fertilization improves amylopectin content while content of amylose in rice varieties decreases. Thus, nitrogen influences both proteins and starch composition (Kindred *et al.,* 2008).

Although nitrogen application greatly influences starch and proteins composition, very little information is available on the effect of nitrogen fertilizer on protein sub fractions and starch components with little emphasis on quality characteristics. However, none of the earlier studies have reported the effects of different doses of nitrogen application on grain quality parameters such as starch (amylose and amylopectin) and proteins (albumin, globulin, gluten and prolamin) sub-components of wheat. Therefore, the aim of the study was to evaluate the genotypic variation based on starch and protein accumulation under different doses of nitrogen.

Materials and Methods

Plants materials and nitrogen treatment : Seeds of nine wheat (*Triticum aestivum* L.) genotypes viz: PBW 621, PBW 590, PBW 509, PBW 550, BW 9183, BW 8989, GLU 1101, GLU 1356 and PH 132-4836 were raised in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The crop was raised under different doses of nitrogen - optimal N dose i.e. recommended dose of N (RDN, 120 Kg N ha^{-1}), suboptimal N dose (RDN-25%, 90 Kg N ha^{-1}) and supra-optimal N doses RDN+25% (150 kg N ha^{-1}) and RDN+50% (180 kg N ha^{-1}) in plots consisting of 4 rows of 1 m each. Row to row spacing was maintained at 9 inches while spacing between the plots was 40 cm. Nitrogen was applied as urea. Basal dose of di-ammonium phosphate and NPK was added at the time of sowing. Mature grains were used for analysis of grain quality parameters analysis. There were three replications for each parameter.

Determination of free sugars, amino acid, starch and proteins : Free sugars were extracted sequentially from mature grains with 80% and 70% EtOH from ethanol-preserved samples. The test extracts were clarified with basic lead acetate and from these extracts and concentration of total sugars were determined (Dubois *et al.,* 1956). From sugars free grain samples, starch was determined by perchloric acid method as described by (Dubois *et al.,* 1956). Total free amino acids were extracted and determined as described earlier by Lee and Takahashi (1966). Soluble proteins extracted in 0.1 NaOH and precipitated with trichloroacetic acid and estimated by the method of Lowry *et al.* (1951).

Determination of proteins sub-fractions : The sequential extraction of different protein fractions in the whole grain flour was carried out by extracting wheat flour (100 mg) sequentially with 5 ml of 1 N NaOH for albumin, 5 ml of 10 % NaCl for globulin, 5 ml of 55% propanol for prolamin and 5 ml of distilled water for gluten. The extractions were carried out on magnetic stirrer for 15 min at room temperature and the suspensions were centrifuged for 20 min at 15,000 x g. To a 2 ml of supernatant, 2 ml chilled 20% TCA was added and mixed thoroughly. After aging for 1 hr at 4ºC, the contents were again centrifuged at 14,000 x g for 15 min and the precipitates were dissolved in 0.5 N NaOH for proteins sub-fractions estimation (Lowry *et al.,* 1951).

Determination of amylose and amylopectin content : The sequential extraction of starch granules *i.e.,* small (amylose) and large (amylopectin) was done by the method of Takeda *et al.* (1989). Further, separation of amylose and amylopectin from mature grain samples was carried out by the method of Peng *et al* (1999). Amylose and amylopectin was estimated colorimetrically according to Chrastil (1987).

Statistical analysis : Values reported are average of triplicate observations and was subjected to analysis of variance (ANOVA). Correlation was also carried out for determining the relationship between different variables.

Results and Discussion

The level of soluble protein content not only reflects the level of plant nitrogen metabolism, but is also regarded as an important indicator of degree of leaf senescence, especially in wheat grain filling stage (Dupont and Altenbach, 2003). The major effect of N fertilization on wheat grain quality characteristics is exerted through changes in the grain final N content (Fuertes-Mendizabal *et al.,* 2010). Protein concentration in flour is the main quality determinant for wheat. In the present study, the effect of different doses of nitrogen on starch and protein accumulation was analysed in different wheat genotypes.

Metabolites (amino acids, proteins, sugars and starch) in nine wheat genotypes seeds as influenced by different doses of nitrogen are given in Table 1. Application of increasing levels of N *i.e.,* RDN+25% (150 kg N ha^{-1}) and RDN+50% (180 kg N ha^{-1}) led to an increased content of amino acids and proteins whereas RDN-25% (90 kg N ha^{-1}) and RDN, (120 kg N ha^{-1}) doses resulted in decreased content in wheat seeds. Amino acids content varied with respect to genotypes but were comparatively higher in genotypes GLU 1101 (2.51 mg g^{-1} d.wt.) and GLU 1356 (2.49 mg g^{-1} d.wt.) due to *Gpc-B1* gene indicating higher translocation of N from the vegetative organs to sink tissue, and thus influencing grain proteins and amino-acids accumulation over other genotypes. In rice varieties, free amino N-content increased with increasing application of N (Farooq *et al.,* 2012). Maximum protein content was observed in GLU 1356 at RDN+25% (136.7 mg g^{-1} d.wt.) and RDN+50% (141.7 mg g^{-1} d.wt.) as compared to RDN-25% (135.8 mg g^{-1}) (Table 1). In maize, application of highest 300 Kg N ha^{-1} rather than 75Kg N ha^{-1} led to increased proteins content (Khan *et al.,* 2008). Thus, under low N conditions, the amino acids content decreased significantly and continually during leaf development. Slight decrease in amino acids content under low N supply could be interpreted as dilution of a stable organic N pool by increasing leaf volume. Higher N supply caused an overall increase in amino acids content and the large part of which is stored in vacuole (Masclaux *et al.,* 2010). Thus, N fertilization can be used for nutritional improvement of cereals by increasing and maintaining proteins and essential amino acids content.

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; Bala *et al.,* 2016). Sugars content decreased at lower N doses i.e. RDN-25% and RDN+25% over RDN except in cv BW 8989. Maximum content of sugars and starch was observed in genotypes PBW 550 (20.3 mg g^{-1} d.wt., 73.31%) and BW 9183 (19.7 mg g^{-1} d.wt., 73.31%) at RDN in contrast with amino acid and proteins content which was comparatively less thereby showing negative correlation (Table 1). These results were in accordance with studies done in sweet sorghum indicating an increase in sugars content with increasing level of N (Almodares *et al.,* 2008). Infact, N fertilizer affects distribution of A-type and B-type starch granules which affects the content and proportion of starch in wheat grains (Xiong *et al.,* 2014). Correspondingly, distribution of starch granules is regulated by timing and amount of N fertilizer applied.

Accumualtion of protein subfractions in mature grains of nine wheat genotypes as influenced by different doses of nitrogen are given in Table 2. Amongst different proteins, the concentration of gluten was highest over prolamin, globulin and albumin. A significant increase in gluten, globulin, albumin and prolamin content was observed with RDN+50% and RDN+25% as compared to RDN. Gluten, confers properties of elasticity and extensibility that are essential for functionally of wheat flours (Torbica *et al.,* 2007). The range of gluten content in different genotypes were in the following order: PBW 590 (53.2 mg g^{-1} d.wt.) > PH-132-4836 (52.7 mg g^{-1} d.wt.) > GLU 1101 (52.5 mg g^{-1} d.wt.) and PBW 509 (52.3 mg g^{-1} d.wt.) at RDN+50%. Globulin is

Table 1 : Effect of different doses of nitrogen on wheat grains metabolites

Genotypes	Nitrogen application (kg N ha^{-1})	Amino acids (mg g^{-1} d.wt.)	Soluble proteins (mg g^{-1}d.wt.)	Sugars (mg g^{-1} d.wt.)	Starch (%)
PBW 621	T_1	0.71	121.9	14.7	63.97
	T_2	1.11	139.3	15.1	71.06
	T_3	2.17	141.6	14.7	69.17
	T_4	2.45	142.7	14.8	66.50
PBW 590	T_1	0.60	110.5	15.3	64.61
	T_2	1.03	122.3	16.5	69.82
	T_3	2.23	122.7	15.8	69.75
	T_4	2.27	126.5	15.9	66.48
PBW 509	T_1	0.71	105.5	15.7	65.19
	T_2	0.75	114.0	16.9	71.81
	T_3	2.40	114.3	16.4	71.69
	T_4	2.50	136.7	16.1	68.07
BW 9183	T_1	0.67	103.9	17.9	66.39
	T_2	0.69	106.7	19.7	73.31
	T_3	1.89	106.6	18.9	73.29
	T_4	2.11	116.9	18.2	68.30
BW 8989	T_1	0.63	96.7	18.7	65.63
	T_2	0.72	116.1	12.3	72.27
	T_3	2.14	106.3	19.8	72.13
	T_4	1.91	121.5	19.5	68.34
PBW 550	T_1	0.67	101.7	18.1	64.61
	T_2	0.63	101.5	20.3	73.31
	T_3	2.15	101.9	19.5	71.43
	T_4	2.05	101.7	18.3	68.28
GLU 1101	T_1	0.60	109.7	15.2	64.09
	T_2	0.20	126.1	16.1	69.41
	T_3	2.14	125.8	15.6	69.23
	T_4	2.51	126.3	5.50	66.80
GLU 1356	T_1	0.99	135.8	14.1	64.21
	T_2	0.98	136.3	15.1	69.81
	T_3	2.41	136.7	14.6	69.79
	T_4	2.49	141.7	14.5	66.24
PH132-4836	T_1	0.64	113.7	14.7	65.13
	T_2	0.86	116.5	16.1	71.27
	T_3	2.03	116.9	15.4	71.23
	T_4	2.06	136.3	15.2	67.23
CD (5%)	**A-N Doses**	**A - 0.124,**	**A - 0.500,**	**A - 0.362,**	**A - 0.237,**
	B- Genotypes	**B - 0.264,**	**B - 1.061,**	**B - 0.767,**	**B - 0.504,**
		AB - 0.524	**AB – 2.127**	**AB - 1.135**	**AB – 1.008**

T_1 - RDN-25% (90 kg N ha^{-1}), T_2 - RDN (120 kg N ha^{-1})]; T_3 - RDN+25% (150 kg N ha^{-1}); T_4 - RDN+50% (180 kg N ha^{-1})

another important proteins fraction from nutritional point of view as it determines the bread making quality of wheat. Globulin content was found to be maximum in genotypes GLU 1101 (34.8 mg g^{-1} d.wt.), PH-132-4836 (33.8 mg g^{-1} d.wt.) and BW 8989 (33.5 mg g^{-1} d.wt.) at RDN+50%. This increase was almost significant at all N-levels. Maximum content of albumin (31.2 mg g^{-1} d.wt.) and prolamin (32.0 mg g^{-1} d.wt.) was observed in PBW 550 genotype at RDN+50% (Table 2). Proteins sub-fractions decreased with decrease in nitrogen application indicating, thereby that maximum albumin and prolamin content was found in wheat seeds treated with RDN+50% followed by RDN+25% and RDN dose, whereas minimum albumin and prolamin content was found in mature seeds treated with RDN-25% and RDN, respectively. Results of the study indicated that increase in nitrogen doses led to consistent increase in different fractions of proteins. Similarily, among various treatments of nitrogen doses in spring triticale grain, highest protein concentration sub-fractions was recorded with higher doses of nitrogen (Wojtkowaiki *et al.,* 2013).

Starch sub-fractions (amylose and amylopectin) are other important characters of wheat grain quality evaluation for

Table 2 : Effect of different doses of nitrogen on proteins sub-fractions and starch sub-components in wheat grains

Genotypes	Nitrogen applicaton (kg N ha^{-1})	Gluten (mg g^{-1} d.wt.)	Albumin (mg g^{-1} d.wt.)	Globulin (mg g^{-1} d.wt.)	Prolamin (mg g^{-1} d.wt.)	Amylose (mg g^{-1} d.wt.)	Amylopectin (mg g^{-1} d.wt.)
PBW 621	T_1	50.3	27.5	27.3	27.4	22.97	46.35
	T_2	51.4	27.6	28.1	27.8	22.53	48.75
	T_3	51.9	28.8	31.1	28.0	22.17	47.93
	T_4	52.5	28.9	33.7	28.3	22.06	45.73
PBW 590	T_1	51.3	26.5	26.2	27.2	23.61	47.25
	T_2	51.5	27.0	26.3	28.0	23.52	48.28
	T_3	52.4	28.2	29.0	28.4	22.57	47.75
	T_4	53.2	28.8	31.1	29.2	21.82	46.61
PBW 509	T_1	50.9	26.8	25.7	27.2	24.19	48.18
	T_2	51.0	28.0	26.7	27.4	23.89	49.07
	T_3	51.9	28.4	28.7	27.5	23.69	48.89
	T_4	52.3	29.0	31.8	28.4	23.33	46.95
BW 9183	T_1	51.2	28.1	26.5	27.0	25.39	48.78
	T_2	51.3	28.3	28.1	28.0	25.37	50.09
	T_3	51.4	28.5	28.2	28.2	25.24	49.81
	T_4	51.9	29.2	32.6	29.5	24.97	48.39
BW 8989	T_1	51.0	27.9	27.1	27.6	24.63	49.36
	T_2	51.1	28.5	27.9	28.1	24.35	49.81
	T_3	51.4	28.6	28.6	28.3	24.13	49.53
	T_4	51.8	29.0	33.5	28.6	23.87	47.25
PBW 550	T_1	50.4	30.5	28.4	29.6	22.83	47.74
	T_2	51.3	30.8	28.8	30.5	22.61	48.93
	T_3	52.1	31.0	29.6	31.4	22.43	48.57
	T_4	52.2	31.2	31.1	32.0	22.14	46.63
GLU 1101	T_1	51.5	26.8	27.1	28.0	22.14	47.23
	T_2	51.5	28.0	28.2	28.4	21.87	48.14
	T_3	52.0	28.4	29.4	30.7	21.63	47.74
	T_4	52.5	31.2	34.8	30.9	21.41	45.93
GLU 1356	T_1	51.1	27.1	26.5	26.6	22.32	47.52
	T_2	51.5	27.2	27.1	26.9	22.16	48.78
	T_3	52.0	27.7	29.3	27.8	21.97	48.17
	T_4	52.4	28.9	31.6	28.0	21.61	46.21
PH132-4836	T_1	51.8	28.3	26.9	28.3	24.27	47.18
	T_2	52.1	29.0	27.2	28.7	24.13	48.25
	T_3	52.5	30.2	28.7	31.3	24.07	48.23
	T_4	52.7	31.0	33.8	31.9	23.86	46.97
CD (5%)	**A- N Doses**	**A - 0.117,**	**A- 0.612,**	**A - 0.691,**	**A - 0.652,**	**A - 0.148,**	**A – 0.386,**
	B- Genotypes	**B - 0.236,**	**B - 0.129,**	**B – 0.146,**	**B - 0.139,**	**B - 0.315,**	**B – 0.819,**
		AB -0.473	**AB -1.259**	**AB –1.293**	**AB -1.278**	**AB -0.631**	**AB – 1.164**

T_1 - RDN-25% (90 kg N ha^{-1}); T_2 - RDN (120 kg N ha^{-1})]; T_3 - RDN+25% (150 kg N ha^{-1}); T_4 - RDN+50% (180 kg N ha^{-1})

pasta products and these components increased in BW 9183, BW 8989 and PH-132-4836 genotypes (Table 2), indicates preferential utilization of these genotypes for nutritional and technological properties (including adhesion, foam strengthening, gelling, glazing, moisture retention, stabilizing and texturizing) of wheat. The application of N fertilizer altered the starch characteristics in wheat grains. Highest amylose content was found in the genotype BW 9183 (25.39%) followed by BW 8989 (24.63%) and PH-132-4836 (24.27%) at RDN-25%, whereas lowest amylose content was observed in genotype GLU 1101 (21.41 %) at RDN+50% thus representing that with increase in nitrogen doses, amylose content decreases, respectively.

Maximum amylopectin content was observed in genotype BW 9183 (50.09%) followed by BW 8989 (49.81%) and PBW 509 (49.07%) at optimal dose of N over other genotypes. Starch sub-fractions depicting that amylopectin was significantly affected by nitrogen doses, whereas amylose content was in significantly affected by nitrogen doses. Thus, it was inferred that with increase in the amount of nitrogen fertilizer, activation of starch branching enzymes might have resulted in increase in

Table 3 : Correlation coefficients between amnio acids, proteins, sugars, starch, proteins sub-fractions and starch sub-components in mature grains

	Amino acids	Proteins	Sugars	Starch	Gluten	Albumin	Globulin	Prolamin	Amylose
Protein	0.787								
Sugars	-0.44	-0.813							
Starch	-0.559	-0.798	0.881						
Gluten	0.055	0.281	-0.493	-0.497					
Albumin	-0.600	-0.608	0.471	0.409	0.008				
Globulin	-0.344	0.063	-0.163	-0.135	-0.206	0.444			
Prolamin	-0.658	-0.530	0.215	0.151	0.309	0.912	0.431		
Amylose	-0.434	-0.513	0.611	0.834	-0.188	0.045	-0.306	-0.115	
Amylopectin	-0.439	-0.642	0.753	0.901	-0.585	-0.058	-0.268	-0.177	0.827

amylopectin percentage decreasing amylose content. Similar results were reported in rice varieties by Li *et al.* (2010) indicating a negative correlation between nitrogen rate and amylose content.

A pooled correlation analysis was done for various biochemical parameters studied at different doses of nitrogen. Total sugars were significantly but negatively correlated with amino acids and proteins, while positively correlated with sugars and starch content (Table 3). All protein subfractions were positively correlated with amino acids and proteins, while negatively correlated with sugars and starch. Amylose and amylopectin was negatively correlated with amino acids and proteins while positively correlated with sugars and starch content indicating, thereby all the metabolites of mature grains were significantly correlated with each other.

In conclusion, distribution of proteins, starch and their sub fractions in wheat genotypes are significantly affected by different doses of nitrogen, and genotypic response play a vital role in enhancing proteins content thereby improving grain quality traits.

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