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EFFECT OF SPLIT DOSES OF NITROGEN AND SEED RATE ON PROTEIN CONTENT, PROTEIN FRACTIONS AND YIELD OF WHEAT

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ABSTRACT

A semidwarf, high yielding with good quality traits Wheat variety viz. WH 711 was evaluated to study the effect of seed rate and nitrogen application (in split doses) on protein, protein fractions and yield of wheat. The results showed that splitting of recommended dose of nitrogen recorded higher total protein content (12.68%) as compared to control (10.23%). Similarly more true protein content was observed with split dose of N application (10.8%) as compared to control (7.8%). Among different fraction of proteins, the albumin fraction increased with increase in seed rate while prolamin and glutenin fractions remained unaffected. The albumin and the globulin fractions increased with splitting of nitrogen however, prolamin and glutenin fractions remained unaffected. Composition of high molecular weight glutenin subunits (HMW- GS) was determined by SDS-PAGE. The grain yield increased with increase in split doses of nitrogen and seed rate. Grain yield was significantly higher at 125 kg/ha and 112.5 kg/ha as compared to 100 kg/ha seed rate. Splitting of recommended dose of nitrogen increased the grain yield by 4.1%.

Keywords: wheat, yield, nitrogen, split dose, glutenin, protein, seed rate.

INTRODUCTION

Wheat is nature's unique gift to mankind as it produces excellent source of nutrition in terms of carbohydrates, minerals and proteins. Globally, wheat is being cultivated over an area of 227 million hectares with a production of 610 million tones. India occupies 2nd place in the world with a total production of 78.4 million tones which is 12% of the world food production from 28.5 million hectares of land (Mishra, 2008). Although wheat is grown in most of the states, the major production comes from the north western part of the country. The increase in domestic demand of baked and pasta products in the country and economic liberalization and global trade have offered opportunities for better utilization of wheat. Wheat quality needs uppermost attention to meet the trade requirements of the domestic and international markets. Quality of food grain is a complex phenomenon and may be influenced by several factors which may be genetic and/or environmental. Cultural practices considerably influence the grain quality. Adoption of suitable practices like seed rate and split nitrogen application play a key role on the quality of wheat. Nitrogen is the most important constituent of plant proteins and is required throughout the crop growth period from vegetative stage to subsequent harvesting. Application of nitrogen is known to mainly increase the gluten strength, protein content, sedimentation value, protein fractions, sedimentation value, pelshenke value etc. Many researchers have found that late season top dressed nitrogen addition as dry fertilizer material were most effective in attaining higher grain protein concentration, yield and increased fertilizer recovery and efficiency (Kumari *et al.*, 2000; Michael *et al.*, 2000; Anthony *et al.*, 2003). Therefore, availability of nitrogen to wheat during various phases of its growth and development is an important factor influencing the yield and quality of grain (Zende *et al.*, 2005).

Seed rate also plays a key role in the grain yield and quality of wheat. Seed rate governs the inter and intra plant competition, the numbers of tillers per plant, spikelet number per spike, grain size, grain shape etc. Likewise nitrogen nutrition, seed rate also influences the plant growth, development, seed size and other qualities of wheat. Although sufficient information is available on the effect of fertilizers on yield attributes but the information on the effect of time of nitrogen application and seed rate on biochemical parameters is meager. Keeping this aspect in mind, the present investigation is planned with the objectives to study the impact of time of nitrogen application and seed rate on protein, protein fractions and yield of wheat.

MATERIALS AND METHODS

The present investigation was carried out on Wheat cultivar WH 711 at Research Farm of Wheat Section, Department of Plant Breeding during the rabi season 2005-2006. The experiment was laid out in split plot design keeping three levels of seed rates i.e. 100, 112.5 and 125 kg/ha in sub-plots and nitrogen in main-plots @150 kg/ha in split doses, to study their effects on quality of wheat cultivar. Nitrogen was applied as per the following stages:

T_0 (no nitrogen), T_1 – ½ dose at sowing + ½ dose at CRI (first irrigation) , T_2 – 1/3rd dose at sowing + 2/3rd dose at first node (40-45 days), T_3 – 1/4th dose at sowing + ½ at first node + 1/4th dose at anthesis (85-90) and T_4 – 1/3rd dose at sowing + (2/3rd dose -6.9 kg N) at first node + 6.9 kg N as 3% Urea spray at post anthesis (95-100 days). Treatment without nitrogen (control) was included for comparison Crude protein eas estimated by micro-kjeldahl's method of AOAC (1990).True protein was separated from non- protein nitrogenous substances such as amides, amino acids, ammonium salts and nitrates by

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stutzer's reagent and the nitrogen was estimated in the precipitates by kjeldahl's method. Proteins were fractionated into four classes (albumin, globulin, prolamin and glutenin) using a modified Osborne method of stepwise extraction based on protein solubility in different solvents (Lookhard and Bean, 1995). Albumins were extracted with water, globulins with 0.5 M NaCl, prolamins with 70 % ethanol and glutenins with 0.5 % SDS (sodium dodecyl sulphate) + 0.6 % 2-mercaptoethanol in 0.1 M borate buffer (pH 10). Flour to solvent ratio was 1:5. Nitrogen in each fraction was determined by micro- Kjeldhal method. Composition of high molecular weight glutenin subunits (HMW- GS) was determined by SDS-PAGE. Gliadins were analysed by the method of Metakovsky and Novoselskaya (1991).

STATISTICAL ANALYSIS

All the experimental data for various quality, biochemical, yield and chemical characteristics were subjected to statistical analysis by applying the technique of analysis of variance as described by Panse and Sukhatme (1978). The significance of treatments was judged with the help of F-test. Appropriate standard errors with critical differences at 5 per cent probability level of significance were worked out as described by Cochran and Cox (1963).

RESULTS AND DISCUSSIONS

Total protein content

Protein content data as affected by seed rate and stage of nitrogen application is shown in Table-1. Among seed rates, 112.5 kg seed rate/ha recorded significantly higher protein content in WH-711 as compared to 100 (1.34g/100 flour) and 125 kg/ha (11.35g/100g flour) seed rates which were at par. Under all seed rates, T_4 treatments (12.68 g/100g flour) showed higher values of protein content as compared to T_3. In WH-711, mean values of protein content were 10.23, 11.55 and 12.68 g/100g flour under T_0, T_2 and T_4 stage of nitrogen application treatments respectively. The interactive effect of seed rate and stage of nitrogen application on total protein content was not statistically sound. In the present investigation, the content of protein increased significantly with increasing dose of nitrogen application, though the seed rate did not produce any significant difference. This is supported by the study conducted by Weiser and Seilmeier (1998) who reported that protein content was strongly influenced by nitrogen. Sip *et al.*, (2000) reported that application of nitrogen in split doses increased grain protein content by 1.55%. Triboi *et al.* (2000) also reported that nitrogen supply is the most important environmental factor affecting protein content and composition.

Table-1. Effect of seed rate and stage of nitrogen application on total protein content (g/100 g flour) and soluble protein (g/100 g flour) of wheat cultivar WH-711.

Stage of Nitrogen application	Total protein content				True protein			
	Seed rates (Kg/ha)				Seed rates (Kg/ha)			
	100	112.5	125	Mean	100	112.5	125	Mean
T_0 : Control (No nitrogen)	10.13	10.30	10.26	10.23	7.98	7.98	7.48	
T_1 : ½ at sowing + ½ at CRI	11.43	11.43	11.16	11.34	9.08	8.64	9.08	
T_2 : 1/3 at sowing + 2/3 at 1st node	11.23	11.53	11.13	11.30	9.38	9.82	9.38	
T_3 : ¼ at sowing + ½ at 1st node + ¼ at anthesis	11.33	11.90	11.43	11.55	9.18	9.98	10.19	
T_4 :1/3 at sowing + (2/3rd- 7 Kg) N at 1st node + 3% Urea spray at post anthesis	12.56	12.73	12.76	12.68	10.97	10.97	10.57	
Mean	11.34	11.58	11.35		9.31	9.47	9.33	
LSD 0.05	Seed rate NS Nitrogen 0.506 Seed rate x Nitrogen NS				Seed rate NS Nitrogen 1.059 Seed rate x Nitrogen NS			

True protein

The data pertaining to the true protein content as influenced by seed rate and stage of N application are presented in Table-2. Seed rate did not influence true protein content significantly. Under varying stage of N application treatment, T_4 (10.83 g/100g flour) and T_0 (7.80g/100g flour) recorded highest and lowest true protein values. The interactive effect between seed rate and stage of N application was not found to be significant.

Proteins fractionations

The data presented in Table-3 demonstrated that prolamin and and glutelin are the principal proteins of wheat endosperm accounted for about 60-70 % of the total protein. Albumin and globulin together accounted for about 25-28 % of the total endosperm proteins. Slight variations were, however, noticed in the relative proportions of these fractions at different treatments and seed rates.

Albumin and globulin increased with increase in stage of nitrogen application while the prolamin and glutelin remained unaffected. Regarding the seed rate treatments, albumin and globulin content showed slight increase while the prolamin and glutelin remained unchanged. Ottman *et al.*, (2000) also reported that nitrogen application at near anthesis stage increases protein content, grain yield and grain weight. Similar results were earlier reported by Patel (2004) and Lestache *et al.* (2004). This increase in protein content was due to consistent increase in different fractions of proteins. The protein is formed by different amino-acids which are constituted in higher amount due to higher availability of nitrogen in more splits. Secondly, the foliar application of 3% urea spray at reproductive phase directly contributed to the grain protein formation and different fractions. Nitrogen applied at heading stage markedly increased the content of prolamin while that applied at booting stage significantly increase the content of globulin and glutenin.

Table-2. Effect of seed rate and stage of nitrogen application on protein fraction (g/100 g flour) of wheat cultivar WH-711.

Seed rate (kg/ha)	**Protein fractions**				
	Protein content	**Albumin**	**Globulin**	**Prolamin**	**Glutelin**
100	11.34	1.02 (8.90)	1.74 (15.30)	3.70 (32.60)	3.51 (30.90)
112.5	11.58	0.98 (8.60)	1.84 (16.20)	3.92 33.80)	3.59 (31.00)
125	11.35	1.22 (10.70)	1.84 (15.80)	3.68 (32.40)	3.43 (30.20)
Stage of Nitrogen application					
T_0 : Control (No nitrogen)	10.23	1.08 (10.55)	1.28 (12.51)	3.52 (34.40)	3.28 (32.06)
T_1 : ½ at sowing + ½ at CRI	11.34	1.24 (10.93)	1.53 (13.48)	3.91 (34.47)	3.72 (32.80)
T_2 :1/3 at sowing + 2/3 at 1st node	11.55	1.29 (11.16)	1.64 (14.19)	4.03 (34.89)	3.72 (32.20)
T_3 : ¼ at sowing + ½ at 1st node + ¼ at anthesis	11.30	1.36 (12.03)	1.64 (14.51)	3.85 (34.07)	3.64 (32.21)
T_4 : 1/3 at sowing + (2/3rd – 7Kg) N at 1st node + 3% Urea spray at post anthesis	12.68	1.73 (13.43)	1.79 (15.22)	4.12 (32.49)	3.92 (30.91)

The values in parenthesis represent per cent of total proteins.

Table-3. Effect of seed rate and stage of nitrogen application on grain yield (q/ha) of wheat cultivar WH-711.

Stage of Nitrogen application	**Grain yield** (q/ha)			
	Seed rate (kg/ha)			
	100	**112.5**	**125**	**Mean**
T_0 : Control (No nitrogen)	20.4	22.4	21.9	21.5
T_1 : ½ at sowing + ½ at CRI	40.4	41.0	43.6	41.7
T_2 :1/3 at sowing + 2/3 at 1st node	38.1	42.9	43.1	41.4
T_3 : ¼ at sowing + ½ at 1st node + ¼ at anthesis	40.3	41.4	43.8	41.8
T_4 :1/3 at sowing + (2/3rd –7 Kg) N at 1st node + 3% Urea spray at post anthesis	40.2	43.7	46.6	43.5
Mean	35.9	38.3	39.8	
LSD 0.05	Seed rate 1.9 Nitrogen 3.7 Seed rate x Nitrogen NS			

Electophoretic pattern of gliadins and glutenins

To further investigate variation in the different protein fractions electrophoresis was performed. High molecular weight (HMW) glutenin subunit composition of WH-711 was determined by SDS-PAGE. The HMW glutenin subunits are coded by genes at three genetically unlinked loci Glu-A1, Glu-B1 and Glu-D1 which occurs on chromosomes 1A, 1B and 1D, respectively and allelic variation at locus has produced extensive variability in wheat varieties (Payne and Lawerence, 1983). The role of

HMW glutenin subunits has been studied in bread making (Kasarda, 1989; Shewry *et al.*, 1992; Sewa Ram, 2003, Horvat *et al.*, 2006) quality. In the present investigation, one allele for the Glu-A1 locus (1), one allele (7 + 9) at Glu-B1 locus and one allele (2+12) at Glu-D1 locus were identified. The results in Figure-1 indicated that glutenin banding pattern did not vary with stage of nitrogen application and seed rate. Similarly the gliadin banding pattern (Figure-2) did not change in the present investigation. Chakraborty and Khan (1988) also obtained similar pattern with gliadins. This is probably due to the fact that gliadin mRNA's in certain varieties of bread wheats are very short lived (Jackson, 1983).

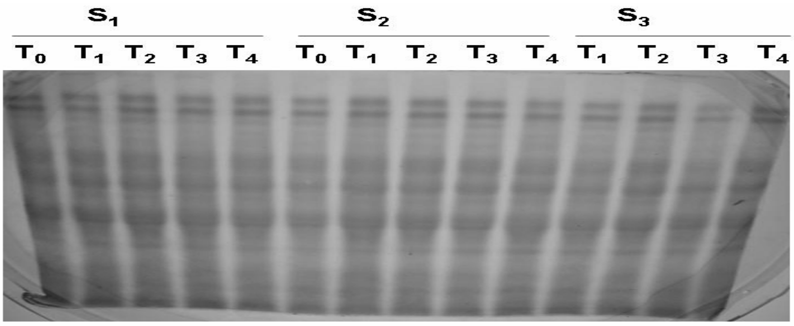

Figure-1. Effect of split doses of nitrogen application and different seed rates on glutenin banding pattern.

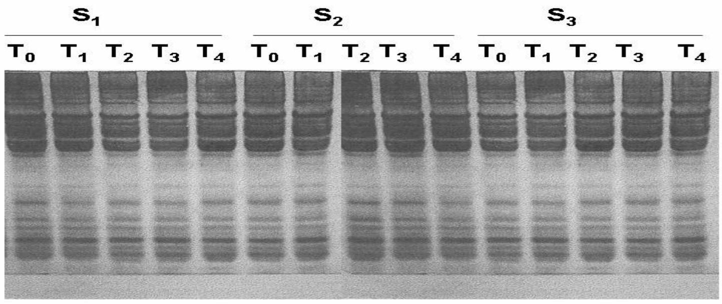

Figure-2. Effect of split doses of nitrogen application and different seed rates on gliadin banding pattern.

T_0 – No nitrogen
T_1 – ½ at sowing + ½ at crown root initiation (CRI)
T_2 – 1/3 at sowing + 2/3 at first node (42 days)
T_3 – ¼ at sowing + ½ at first node ¼ at anthesis
T_4 – 1/3 at sowing + (2/3- 6.9 Kg N) at first node + 6.9 Kg N as urea spray (3%) at post anthesis.
S_1: 100 (Recommended seed rate) $S_{2:}$ 112.5
$S_{3:}$ 125

Grain yield

The grain yield increased with increase in split doses of nitrogen and seed rate. Grain yield was significantly higher at 125 kg/ha and 112.5 kg/ha as compared to 100 kg/ha seed rate. Splitting of recommended dose of nitrogen at T_4 increased the grain yield by 4.1% (43.5 q/ha) than the grain yield obtained (41.7 q/ha) at T_1. Increase was due to higher number of plants and more number of tillers per meter row length. The similar response of decrease in yield with decreasing seed rates have been reported by Naik *et al.*, (1991); Parihar and Singh (1995); Samra and Dhillon (2002) and Anureet (2006).

CONCLUSIONS

In present studies grain yield and protein content increased with increase in seed rates and split dose of nitrogen application. T_4 treatment (1/3 at sowing + (2/3rd – 7 Kg) N at 1st node + 7 Kg N as 3% Urea spray at post anthesis) is the best treatment for getting higher yield and protein content Although grain yield and protein content have a negative correlation, but this belief is not true in nutritional studies as the higher grain yield and better grain quality in terms of protein content and hectoliter weight can be increased side by side through proper management and timely application of nitrogen. This fact is also imperative from the study made by Bushuk (1985) and Zende *et al*., (2005) that the protein content of wheat strongly depends on agronomic and environmental factors, such as soil nitrogen, soil moisture etc. during the growing season. When the nitrogen is applied in splits, crop do not suffer at any stage of development due to nitrogen deficiency rather increased supply at the time of higher demand give better results.

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