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Wheat Response to Application Methods and Levels of Nitrogen Fertilizer: I. Phenology, Growth Indices and Protein Content

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Abstract: Fertilizer use efficiency of nitrogen is very low due to various types of losses. This two year field experiment was carried out to investigate the effect of various levels of nitrogen and its application methods on phenology, growth indices and quality of wheat. Fertilizer application methods (broadcast and side-dressing) were placed in the main plots, while nitrogen levels (60, 90, 120, 150, 180 and 210 kg/ha) applied in sub plots. Crop data showed that nitrogen methods had non-significant effect on phenology, physiology and quality parameters. Fertilizer application rates rendered significant effect on various parameters except days to emergence. Nitrogen at 210 kg/ha gave significant increase in the phenological and growth parameters. Leaf area index and leaf area duration were significantly affected by the rate of nitrogen fertilizers. Protein contents were also increased by the application of higher amount of nitrogen. Interaction among application methods and nitrogen levels showed significant variations in net assimilation rate, while crop growth rate remained significant in the second year and protein content had shown significant variation during the first year of study.

Key words: *Triticum aestivum*, physiology, growth rate, quality, assimilation rate, leaf area

INTRODUCTION

Wheat (*Triticum aestivum*) provides a major source of energy, protein and dietary fiber in human nutrition. It occupies the pivotal position in formulating agriculture based planning. In Pakistan, it adds 13.1% to the value addition in agriculture, 2.7% to gross domestic product and 39% of major crops share (Anonymous, 2011). Wheat is annually grown on an area of 9.045 million hectares with total production of 25 million tones and an average yield of 2750 kg/ha (Anonymous, 2011). Nonetheless, its average yield is far below in Pakistan than that in the developed countries (FAO, 2010) in spite of the fact that local varieties have great genetic potential of higher yields as in any country of the region.

Major yield limiting factors include late sowing, weeds infestation, water scarcity at critical growth stages and improper utilization of fertilizers. Among the fertilizers, use of N fertilizer normally affects production costs more than any other input (Ahmad *et al*., 2008; Aslam *et al*., 2011a). However, it enhances protein, protoplasm as well as chlorophyll formation. These cell components in return show positive influence on cell size, leaf area and ultimately photosynthetic activity (Rahman *et al*., 2000).

Nutrient management offers the opportunity for increasing wheat growth, yield and its nutrient components (Dilshad *et al*., 2010; Aslam *et al*., 2011b). It is a key factor to high yields and optimum economic returns by playing an important role in crop productivity (Dilshad *et al*., 2011). Nitrogen deficiency constitutes one of the major yield limiting factors for cereal production (Sarwar *et al*., 2012). A number of recent soil fertility surveys have revealed that among the essential nutrient elements (N, P, K, S, Mg, etc.) soils are mostly deficient in N (by 100%) and P (by 90%) in Pakistan (Shaheen *et al*., 2011).

Appropriate fertilizer application rates and methods enhance the nutrient use efficiency by reducing their losses. Significant effect of increased N levels applied as side-dressing in the form of urea on the number of wheat ears per unit area has been reported by Teixeira-Filho *et al*. (2007). However, Hassan *et al*. (2010) reported that nitrogen broadcast or side-dressing even at higher nitrogen rates (180 kg/ha) did not produce significantly higher values of crop growth parameters.Increase in net assimilation rate (NAR) is attributed to increased photosynthetic capacity of the leaves with improved nutrition of the plants (Ahmad *et al*., 1990). The NAR and Relative Growth Rate (RGR) improves with the increasing levels of N (Warraich *et al*., 2002). Similarly, Ayub *et al*. (1995) reported an increase

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by 20.3% in protein content of wheat grains with higher levels of nitrogen application.

Present research was initiated to determine the effect of various nitrogen levels and application methods on phenology, growth and physiology of wheat (cv. Zam-04) under agro-ecological conditions of Dera Ismail Khan.

MATERIALS AND METHODS

Site and weather: This field experiment was performed at Agronomic Research Area, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan for two years (2009-10 and 2010-11). Physico-chemical analysis of the soil showed that it was slightly alkaline [pH 8.22, ECe 2.09 dS/m, SAR 6.15 $(meq/L)^{1/2}$], low in organic matter (0.29%) and nitrogen (0.014 %) and the texture silty clay. Monthly weather data revealed that in both years (2009-10 and 2010-11), the respective maximum/minimum temperatures were highest at the time of sowing in October (33/16 and 34/19°C) decreasing towards January being the lowest (16/5 and 17/3°C) and again increased being highest at harvesting in April (37/19 and 34/16°C). Total precipitation during this period (wheat season) was only 23.3 and 48.5 mm, respectively.

Experimental: Field was laid out in randomized complete block design with split plot arrangement having four replications. Nitrogen fertilizer application methods (broadcasting and side-dressing) were assigned to main plots, while nitrogen levels (60, 90, 120, 150, 180 and 210 kg N/ha) were maintained in sub-plots. Fertilizers of phosphorus (120 kg P_2O_5/ha) and potassium (90 kg K_2O/ha) were applied uniformly in the whole field under these treatments. Sources of fertilizer were urea for N, single super phosphate for P and sulfate of potash for K. Whole amounts of phosphorus and potassium were applied as basal fertilizers during land preparation just before wheat sowing. Nitrogen was applied in two split doses; initially before seed sowing and then at first irrigation through broadcast and side-dressing methods as per treatments plan. Wheat variety Zam-04 was sown with recommended seed rate of 120 kg/ha. Size of sub-plots (for N levels treatments) was 5 x 1.8 m. Chemical herbicides were used to control the weeds in the experimental field.

Crop parameters: During this study, most of the attributes related to phenology, growth and physiology of wheat crop were recorded. Among them, days to heading, days to maturity, leaf area index were noted as stated by Watson (1958). Leaf area was recorded at 42 and 84 Days of Sowing (DAS). Net Assimilation Rate (NAR) and crop growth rate (CGR) were determined through the procedures given by Gardner *et al.* (1985):

$$\text{Crop growth rate} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{GA}$$

Where:

W_2	:	Final dry weight
W_1	:	Previous dry weight of plants
T_2	:	Weight recording time
T_1	:	Previous weight recording time
GA	:	Ground area

Protein determination: Grain protein contents were measured thorough Kjeldahl's procedure. For this, 600 seeds were taken from each treatment and grinded. Then by Gunning and Hibherds method, 0.5 g sample was digested in 10 mL conc. H_2SO_4 at 400°C for 2-3 hours until solution became transparent. Distillation was performed in MicroKjeldahl's apparatus (Jackson, 1973) to assess the N contents in grains. Protein contents were calculated by multiplying the nitrogen contents of seed with a constant factor of 5.71 for wheat (Peter and Young, 1980).

Statistical analysis: Data on crop parameters influenced by various treatment variables were analyzed statistically through MSTAT-C software by Fisher's analysis of variance technique and subsequently least significance difference test was applied for comparing the treatment means (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Phenological attributes of wheat: Data on periodic biological/growth phenomena of wheat, such as days taken to emergence, heading and maturity, in relation to nitrogen fertilizer application methods and graded doses (Table 1). In both the years (2009-10 and 2010-11) there was statistically non-significant difference between two N fertilizer application methods (broadcast and side-dressing) and among six N application rates (60 to 210 kg/ha) for days taken to seed emergence. However, during the 2nd year (2010-11) the increasing rate of N fertilizers application slightly reduced the time for seed germination (11.1 days at N60 vs. 10.1 days at N210). Interaction of both factors (N fertilizer application methods x N application rates) was also non-significant during both the years.

Days taken to heading showed non-significant effect of N fertilizer application methods in both the years. While the rates of N application showed significant influence on the days to heading during both the years (Table 1). The highest number of days to heading was recorded with N210 (120) and (88) during 2009-10 and 2010-11, respectively. The least days to heading was recorded in N60 for both the years. Interaction between the application methods and rates of N fertilizer was non-significant in both the years. More days taken to heading recorded with 210 kg N/ ha could be due to continuous cell division and multiplication with sufficient amount of nitrogen which prolonged vegetative stage. These

Table 1: Effect of various nitrogen fertilizer application methods and rates on phenological attributes of wheat

Crop attributes	Year	Application method	Fertilizer nitrogen application rate (kg ha^{-1}) N60	N90	N120	N150	N180	N210	Mean
Days to emergence	2009-10	Broadcast	8.25	8.75	8.25	8.00	8.50	8.25	8.33^{NS}
		Side-dressing	8.25	8.50	8.75	8.25	8.50	8.00	8.38
		Mean	8.25	8.63	8.50	8.13	8.50	8.13	
	2010-11	Broadcast	11.0	11.5	10.8	10.8	10.3	9.5	10.6^{NS}
		Side-dressing	11.3	11.0	11.0	10.8	10.3	10.8	10.8
		Mean	11.1	11.1	10.9	10.8	10.3	10.1	
Days to heading	2009-10	Broadcast	106.3	107.0	108.5	110.0	111.5	120.0	110.7^{NS}
		Side-dressing	106.8	108.0	108.8	109.0	110.5	120.0	110.3
		Mean	106.5 E	107.5 DE	108.6 CD	109.5 C	111.0 B	120.0 A	
		$LSD_{0.05}$ for nitrogen levels = 1.205							
	2010-11	Broadcast	76.0	78.3	78.0	78.5	79.3	87.5	79.8^{NS}
		Side-dressing	76.8	78.0	77.5	78.5	79.3	88.5	79.6
		Mean	76.4 D	78.5 C	77.8 C	78.5 BC	79.3 B	88.0 A	
		$LSD_{0.05}$ for nitrogen levels = 0. 986							
Days to maturity	2009-10	Broadcast	142.0	143.3	144.0	145.5	147.5	153.3	146.0^{NS}
		Side-dressing	142.3	143.8	144.5	146.0	147.0	152.3	146.0
		Mean	142.1 E	143.5 DE	144.3 D	145.8 C	147.3 B	152.8 A	
		$LSD_{0.05}$ for nitrogen levels = 1.400							
	2010-11	Broadcast	138.0	139.0	140.0	141.0	142.5	147.0	141.3^{NS}
		Side-dressing	138.3	139.8	140.8	141.5	144.3	151.0	142.6
		Mean	138.1 E	139.4 DE	140.4 CD	141.3 C	143.4 B	149.0 A	
		$LSD_{0.05}$ for nitrogen levels = 1.695							

Mean values within a row followed by different letters (upper case) exhibit statistically significant difference among them at $P \leq 0.05$. NS = Non-significant difference.

results are in line with that reported by Hameed *et al.* (2003) who found delayed heading by each increment in nitrogen doses.

Total number of days for maturity of wheat crop was also significantly influenced by the application of different rates of nitrogen fertilizers (Table 1). Maximum days to maturity were 152.8 and 149.0 recorded during the first and second year, respectively by the application of nitrogen at 210 kg/ha. Minimum days to maturity were noted in N60 (60 kg N/ha). Possible reason for such results could be that use of nitrogen dose at higher rates caused enormous vegetative growth that postponed the crop maturity. These findings are supported by Ling and Silberbush (2002) who reported that foliar and soil application of N enhanced the growth characteristics. Methods of fertilizer application, viz., broadcast and side-dressing did not show significant effect on the days to maturity. The interaction between methods and rates of N fertilizer application was also found non-significant for days to maturity.

Leaf area index: Leaf Area Index (LAI) is the main physiological determinant of crop yield. It describes the surface growth and light use during crop period. Leaf area index at 42 and 84 Days after Sowing (DAS) was determined in both the years 2009-10 and 2010-11 (Table 2). Results showed significant difference among various nitrogen levels. The highest leaf area index after 42 days in the 1st year was 0.39 found in the treatment receiving 210 kg N/ha. Similarly, 0.15 was the LAI recorded in the next year. The lowest LAI in both the cases was found with the N60 dose. Increasing nitrogen rates gradually increased the leaf area index having the highest value at 210 kg/N ha. Similarly, leaf area index at 84 DAS showed significant difference among various nitrogen levels in both the years (Table 2). The highest leaf area index (4.36) was obtained in 1st year and 2.67 in the second year under the treatment where 210 kg N/ha was applied. However, N application methods had non-significant effect on leaf area index at both stages (42 and 84 DAS) in two years. Interaction of fertilizer application methods and nitrogen levels also remained non-significant. The increase in leaf area index with higher nitrogen levels might be due more leaf area on account of more accumulation of assimilates. Heinemann *et al.* (2006) reported enhanced leaf area index by applying higher levels of nitrogen fertilizer.

Leaf area duration: Data regarding the leaf area duration at 42 and 84 days after sowing showed a significant effect of fertilizers rates in both the years (Table 3). The highest leaf area duration recorded at 42 DAS in the first year was 2.36, while it was 0.93 in the second year in the treatments receiving 210 kg N/ha. In second year, it was statistically at par with the treatment receiving 180 kg N/ha. Similarly, at 84 DAS the highest leaf area duration was found in the treatments receiving nitrogen at 210 kg/ha in the years 2009-10 and 2010-11

Table 2: Effect of various nitrogen fertilizer application methods and rates on leaf area index of wheat at 42 and 84 days after sowing

Crop attributes	Year	Application method	Fertilizer nitrogen application rate (kg ha^{-1}) N60	N90	N120	N150	N180	N210	Means
Leaf area index (42 DAS)	2009-10	Broadcast	0.26	0.27	0.29	0.31	0.33	0.35	0.30^{NS}
		Side-dressing	0.29	0.31	0.34	0.36	0.40	0.42	0.35
		Mean	0.27 F	0.29 E	0.31 D	0.33 C	0.37 B	0.39 A	
		$LSD_{0.05}$ for nitrogen levels = 0.016							
	2010-11	Broadcast	0.08	0.09	0.10	0.11	0.13	0.13	0.11^{NS}
		Side-dressing	0.11	0.13	0.14	0.15	0.17	0.17	0.14
		Mean	0.10 E	0.11 D	0.12 C	0.13 B	0.15 A	0.15 A	
		$LSD_{0.05}$ for nitrogen levels = 0.003							
Leaf area index (84 DAS)	2009-10	Broadcast	3.61	3.79	4.05	4.24	4.53	4.58	4.13^{NS}
		Side-dressing	2.99	3.22	3.48	3.72	3.95	4.15	3.58
		Mean	3.30 C	3.50 C	3.76 B	3.98 B	4.24 A	4.36 A	
		$LSD_{0.05}$ for nitrogen levels = 0.022							
	2010-11	Broadcast	1.99	2.17	2.38	2.43	2.72	2.75	2.41^{NS}
		Side-dressing	1.57	1.75	2.00	2.15	2.51	2.59	2.10
		Mean	1.78 D	1.96 C	2.19 B	2.29 B	2.62 A	2.67 A	
		$LSD_{0.05}$ for nitrogen levels = 0.122							

Mean values within a row followed by different letters (upper case) exhibit statistically significant difference among them at $P \leq 0.05$. NS = Non-significant difference. DAS = Days after sowing

Table 3: Effect of various nitrogen fertilizer application methods and rates on leaf area duration of wheat at 42 and 84 days after sowing

Crop attributes	Year	Application method	Fertilizer nitrogen application rate (kg ha$^{?1}$) N60	N90	N120	N150	N180	N210	Means
Leaf Area duration (42 DAS)	2009-10	Broadcast	1.56	1.65	1.77	1.88	2.01	2.14	1.84^{NS}
		Side-dressing	1.73	1.86	2.05	2.17	2.43	2.57	2.14
		Mean	1.65 F	1.76 E	1.91 D	2.03 C	2.22 B	2.36 A	
		$LSD_{0.05}$ for nitrogen levels = 0.099							
	2010-11	Broadcast	0.52	0.60	0.64	0.69	0.77	0.80	0.67^{NS}
		Side-dressing	0.68	0.77	0.84	0.89	1.02	1.06	0.88
		Mean	0.60 E	0.68 D	0.74 C	0.79 B	0.90 A	0.93 A	
		$LSD_{0.05}$ for nitrogen levels = 0.049							
Leaf Area duration (84 DAS)	2009-10	Broadcast	21.68	22.75	24.30	25.61	27.22	27.48	24.82^{NS}
		Side-dressing	17.95	19.34	20.90	22.35	23.75	24.91	21.53
		Mean	19.82 C	21.05 C	22.60 B	23.90 B	25.49 A	26.20 A	
		$LSD_{0.05}$ for nitrogen levels = 1.402							
	2010-11	Broadcast	11.98	13.04	14.29	14.58	16.33	16.53	14.46^{NS}
		Side-dressing	9.43	10.55	12.04	12.91	15.10	15.57	12.60
		Mean	10.70 D	11.80 C	13.16 B	13.75 B	15.72 A	16.05 A	
		$LSD_{0.05}$ for nitrogen levels = 0.733							

Mean values within a row followed by different letters (upper case) exhibit statistically significant difference among them at $P \leq 0.05$. NS = Non-significant difference. DAS = Days after sowing

and it was statistically at par with the treatments receiving nitrogen at 180 kg/ha. The methods of fertilizers application and interaction were non-significant in both the years.

Crop growth rate: Crop Growth Rate (CGR) of wheat showed non-significant effect of fertilizer application methods during both years (2009-10 and 2010-11), however, the rates of N fertilizer significantly enhanced the crop growth rate (Table 4). The highest crop growth rate of 4.81 and 4.77 g/m/d was recorded in N210 (210 kg N/ha) and N180 (180 kg/ha) during the first year and it was lower in the second year. In 2010-11, the CGR was 3.03 in N210 and it was statistically at par with that of N180 having the value of 2.95. The interaction between the methods and rates of N fertilizers application was found significant at $P \leq 0.05$ during the second year of the experiment. The increase in CGR ultimately increases total dry matter at the end of growing season. Moreover, increase in CGR at higher N levels was mainly due to larger LAI, since CGR is a product of the LAI and Net Assimilation Rate (NAR). These results are also endorsed by that of Rahman *et al*. (2000) who reported that nitrogen application created a significant impact on leaf photosynthesis, leaf area index, crop growth rate and biomass production of wheat.

Table 4: Effect of various nitrogen fertilizer application methods and rates on crop growth rate and net assimilation rate of wheat

Crop attributes	Year	Application method	Fertilizer nitrogen application rate (kg ha^{-1}) N60	N90	N120	N150	N180	N210	Means
Crop growth rate (g m^{-2} day^{-1})	2009-10	Broadcast	3.35	3.67	4.08	4.40	5.07	5.10	4.28^{NS}
		Side-dressing	3.15	3.40	3.63	3.93	4.47	4.52	3.85
		Mean	3.25 A	3.53 D	3.85 C	4.17 B	4.77 A	4.81 A	
		$LSD_{0.05}$ for nitrogen levels = 0.504							
	2010-11	Broadcast	1.82 I	2.14 g	2.39 f	2.48 e	2.82 c	2.87 c	2.42^{NS}
		Side-dressing	1.65 j	2.04 h	2.37 f	2.59 d	3.08 b	3.19 a	2.49
		Mean	1.74 E	2.09 D	2.38 C	2.54 B	2.95 A	3.03 A	
		$LSD_{0.05}$ for nitrogen levels = 0.145; $LSD_{0.05}$ for interaction = 0.083							
Net assimilation rate (g m^{-2} day^{-1})	2009-10	Broadcast	11.15 cd	11.82 bc	12.15 ab	12.12 ab	12.27 ab	12.25 ab	11.96^{NS}
		Side-dressing	10.72 d	11.75 bc	12.14 ab	12.44 ab	12.72 a	12.76 a	12.09
		Mean	10.94C	11.78 B	12.15 AB	12.28 AB	12.50 A	12.51 A	
		$LSD_{0.05}$ for nitrogen levels = 0.597; $LSD_{0.05}$ for interaction = 0.845							
	2010-11	Broadcast	9.44 f	9.83 e	10.18 d	10.49 c	11.22 a	10.59 b	10.35^{NS}
		Side-dressing	9.34 fg	9.33 fg	9.16 g	9.34 fg	9.79 e	9.39 fg	9.39
		Mean	9.38 D	9.58 CD	9.67 CD	9.91 BC	10.51 A	10.17 AB	
		$LSD_{0.05}$ for nitrogen levels = 0.400; $LSD_{0.05}$ for interaction = 0.231							

Mean values within a row followed by different letters (upper case) and interaction values of N application methods x rates (bearing lower case letters) exhibit statistically significant difference among them at $P \leq 0.05$. NS = Non-significant difference

Table 5: Effect of various nitrogen fertilizer application methods and rates on protein contents (%) in wheat grains

Year	Application method	Fertilizer nitrogen application rate (kg ha^{-1}) N60	N90	N120	N150	N180	N210	Means
2009-10	Broadcast	8.73 g	9.51 f	11.07 d	11.39 cd	12.12 b	13.06 a	10.97^{NS}
	Side-dressing	8.60 g	10.50 e	11.11 d	11.60 c	12.12 b	12.81 a	11.12
	Mean	8.66 F	10.00 E	11.09 D	11.47 C	12.13 B	12.94 A	
	$LSD_{0.05}$ for nitrogen levels = 0.257; $LSD_{0.05}$ for interaction = 0.364							
2010-11	Broadcast	8.83	10.19	11.11	11.31	12.04	12.68	11.15^{NS}
	Side-dressing	8.90	10.56	10.95	11.34	12.21	12.94	11.02
	Mean	8.86 E	10.37 D	11.03 C	11.32 C	12.13 B	12.81 A	
	$LSD_{0.05}$ for nitrogen levels = 0.345							

Mean values within a row followed by different letters (upper case) exhibit statistically significant difference among them at $P \leq 0.05$. NS = Non-significant difference.

Net assimilation rate: The NAR of wheat was significantly increased by the application nitrogen fertilizers during the two experimental years (Table 4). In the first year, the recorded NAR was 12.51 and 12.50 in the treatments receiving nitrogen at 210 and 180 kg/ha, respectively. During the second year of the experiment, the NAR was lower as compared to that in first year but was found greater in the treatment N180 (10.51 g/m/day) which was statistically at par with N210 (10.17 g/m/day). Methods of fertilizer application showed non-significant difference on the NAR of wheat. The interaction between N fertilizer application methods and rates showed significant effect in both the experimental years. Warraich *et al.* (2002) found improved NAR and RGR with the increasing levels of N fertilizer. Increase in net assimilation rate may be attributed to enhanced photosynthetic capacity of leaves with improved nutrition of the plants (Ahmad *et al.*, 1990).

Protein contents: The protein contents in wheat grains exhibited significant increase with the higher levels of nitrogen application (Table 5). During the first year of experiment, the highest protein contents (12.94%) were recorded under the treatment receiving the highest dosage of N fertilizer (210 kg N/ha). Similar results were obtained during the second year. Methods of N fertilizer application had non-significant difference during both the years. Interaction between the methods and rates of fertilizer application was significant in the first year but was non-significantly different in the second year of the experiment. These results are in line with the findings of Maqsood *et al.* (2000) who reported increase in protein contents with higher levels of nitrogen. Ayub *et al.* (1995) also reported that nitrogen fertilizer application increased the wheat grain protein content by 20.3% compared to control without nitrogen.

Conclusion: This study compared different methods and rates of N fertilizer application and their interactions on the phenological and growth attributes of wheat crop and protein contents in wheat grains. Most of these crop characteristics were improved with increased nitrogen levels bearing maximum values at the highest rate of 210 kg N/ha. However, the two methods of N fertilizer application (broadcast and side-dressing) did not show significant difference for any of the crop parameters studied in this experiment. Study concludes that dose of N fertilizer needs to be increased as the current recommended dose is 120-150 kg N/ha. However, N fertilizer can be applied with a more convenient and economical broadcast method without any risk of reduction in crop growth and grain quality.

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