

# Yield Response of Uniculm Wheat (*Triticum aestivum* L.) to Early and Late Application of Nitrogen: Flag Leaf Development and Senescence

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## Abstract

Nitrogen (N) supply increased flag leaf area by increasing cell number. N promoted initiation and development of floret primordia. N availability upto ear emergence affected fertile floret number more than spikelet number and increased the number of grains. Late application of nitrogen at 5 days after anthesis increased percentage nitrogen in grains, grain weight and grain number due to its effect on ability of floret to set grain. Larger number of florets and grains provided sink not only for carbon but also for nitrogen accumulation with high nitrogen availability. The accumulation of grain protein depended on the accumulation and partitioning of reduced N accumulated during the vegetative stage of growth and the relative contributions of nitrate assimilation and N redistribution during grain development in low as well as high nitrogen supply. Flag leaf of 'Gigas' wheat retained the ability to synthesize RuBPCO on induction of nitrate reductase by substrate supply at post anthesis stage. The reductions in proteolytic enzymes were coincident with loss of Chl, total soluble protein and nitrate reductase activity from the flag leaves.

**Keywords:** Flag leaf development, Senescence, Nitrogen supply, Yield, Uniculm wheat

## 1. Introduction

Nitrogen (N) is generally the most common limiting nutrient for growth and yield of crops worldwide. In cereals, N limits grain yield and quality via effects on plant biomass, and consequently on grain number, size, and protein concentration (Angus et al. 1993; Demotes-Mainard and Jeuffroy 2001a, b). Plant responses to N can be regulated directly by nitrate, or less directly via downstream metabolites (Stitt et al. 2002). Early season nitrogen application results in accumulation of dry matter by enhanced tiller number and larger photosynthetic surface (Morgan 1988). Late application of nitrogen at or after the emergence of flag leaf do not increase the leaf area but increase nitrogen contents of vegetative parts and prolongation of leaf area duration is the major cause for the increase in yield (Sprietz and Van de Haar 1978; Pearman et al. 1979). Strong relationship between leaf nitrogen concentration and single leaf photosynthetic rates occur which appeared to be associated with large fractions of leaf nitrogen composed in photosynthetic enzymes (Millard and Catt 1988; Shiraiwa and Sinclair 1993). Plants deficient in nitrogen will have lower photosynthetic rates, accumulate less dry matter, and produce lower yields (Delden 2001; Zhao et al. 2009). The photosynthetic rate per unit area of leaf depends on the development and maintenance of the photosynthetic system including energy-transducing components (e.g. thylakoid membranes), on enzymes of the photosynthetic carbon reduction (PCR) cycle (e.g. ribulose biphosphate carboxylase oxygenase RuBPCO) and enzymes of nitrogen assimilation (Farquhar et al. 1980). Longevity of leaf and regulation of breakdown of RuBPCO is an important factor in grain production. Increasing leaf photosynthesis will undoubtedly cause a higher nitrogen requirement for synthesis of more protein. The high leaf protein would increase the energy requirements of biomass and leaf area development, perhaps at a time when sink demand of the economic product would need to be enhanced to develop yield. Whether the extra investment in protein would later pay off in economic yield needs to be determined. In the present study, we analyzed the effects of nitrogen nutrition on leaf area, chlorophyll a and b, total soluble protein, amount of RuBPCO protein, rate of photosynthesis, activity of nitrate reductase, invertase (pH 5.0; 7.5), protease (pH 7.0) enzyme and grain yield in unculm wheat. The objective of the study was to assess the role of nitrogen supplied at vegetative stage in leaf area development and nitrogen availability at post anthesis stage to increase source size, assimilate supply and grain yield in unculm wheat.

## 2. Materials and Methods

Wheat (*Triticum aestivum* L. unicum genotype Gigas) plants (Atsmon and Jacob, 1977) were grown in cement pots (40cm x 40cm) containing 10 kg sandy loam soil. The experimental design was a completely randomized block with each treatment replicated four times. Fifty pots, each containing nine uniform plants, were maintained per treatment. Plants were watered regularly. Nitrogen treatments consists of low N (30 kg ha<sup>-1</sup> N1) and high N (90kg ha<sup>-1</sup> N2), low N + late application of N (30 kg ha<sup>-1</sup> +30 kg ha<sup>-1</sup> N3) and high N +late application of N (90 kg ha<sup>-1</sup> + 30kg ha<sup>-1</sup> N4) and 60 kg ha<sup>-1</sup> of P and K each in all treatments. Late N in both cases was given at five days after anthesis stage while low and high doses were split into two and given at seedling elongation and crown root initiation stages.

Observations were recorded in flag leaf at anthesis five days before in six replicates and ten, twenty and thirty days after late application of equal dose of nitrogen in low and high nitrogen plants using three replicates. The rate of photosynthesis was measured by Infra Red Gas Analyzer (ADC-225MK3). Diffusive resistance and transpiration was measured using Li-1600 Steady State Porometer (LiCor Inc.USA). Leaf area was recorded by leaf area meter (Li-3100). Total N in grains at harvest was determined by Nitrogen Auto analyzer (*Technicon*, Dublin, Ireland). Total soluble protein (Bradford, 1976) and RuBP carboxylase protein were estimated following Lawlor et al (1989). Chlorophyll extraction was done in 80% ethanol and chlorophyll a and b were calculated as described by Arnon (1949). Leaf characteristics were analysed under light microscope in fresh tissue using impressions of leaf adaxial surface. Protease enzyme activity was estimated according to Frith et al (1975), invertase activity using Sumner and Howell (1935) and *in vivo* nitrate reductase (NR) was assayed following Klepper et al. (1971). Total starch and soluble carbohydrates content were estimated following Hassid and Neufeld (1964) and Yemm and Wills (1954), respectively. Photosynthetic efficiency was also measured by photosystem I signal using ESR (Bruker ER 200 ESR spectrometer - Germany) in isolated chloroplast suspension in the division of plant physiology, IARI, New Delhi, India.

Statistical analysis of data was done by analysis of variance (ANOVA) of single factor using MStat software.

## 3. Results

### 3.1 Characteristics of flag leaves and plant growth

The characteristics of flag leaves were strongly affected by nitrogen supply (Table 1). Number of leaves remained same; density and number of veins on the lamina increased from 24 with low nitrogen to 39 with increased supply of nitrogen. Total number of stomata per square millimeter of flag leaf increased from 140 to 153 with increased availability of nitrogen. Nitrogen deficiency had a reducing effect on flag leaf length and expansion. Flag leaf width was 1.8 cm and 2.42 cm with low and high nitrogen supply, respectively. Flag leaf area increased from 36.21 cm<sup>2</sup> to 64.37 cm<sup>2</sup> in high nitrogen plants as compared to low nitrogen plants at ear emergence stage. Fresh weight per unit leaf area was higher and fresh weight per flag leaf was approximately double with higher nitrogen. At pre anthesis stage, rate of photosynthesis in flag leaf of nitrogen deficient plants was higher as compared to flag leaf of plants grown on high nitrogen (Table 1). Photosynthetic efficiency was higher in nitrogen deficient flag leaf (Fig1). Total plant dry matter production was strongly affected by nitrogen nutrition. At the time of sample harvest (about 90 days after sowing i.e. ear emergence), plant dry weight increased from 2.963 g in low nitrogen to 4.619 g in high nitrogen supply at vegetative stage. The increase in dry weight per plant was closely paralleled by changes in plant height and flag leaf area. Thus, increased growth of wheat plants at high-nitrogen was associated with greater allocation of dry matter to the shoots.

### 3.2 Chlorophyll contents

Total chlorophyll content and chlorophyll b (mg g<sup>-1</sup> fresh weight) were increased in flag leaves with high dose as well as late nitrogen application (Table 2). Higher ratio of Chl a/b at 30 days after nitrogen application was due to the faster depletion of Chl b in both low and high N plants. Chlorophyll a content was higher in flag leaves supplied with low dose and low plus late dose of nitrogen. Total chlorophyll content was related with rate of photosynthesis in plants growing with high soil nitrogen at grain fill stage. Reduction in total chlorophyll content at 30 days after late application of nitrogen reflected progress of senescence. The basal part of larger leaves with higher supply of nitrogen stayed green longer period (a week) while smaller leaves with low nitrogen supply were yellow uniformly. Senescence started at anthesis from tip of flag leaf towards base of lamina in both treatments.

### 3.3 Nitrate reductase activity

Under high and low nitrogen levels, nitrate reductase activity was highest in flag leaf followed by second or penultimate leaf and then the third leaf of unicum wheat (Table 3). Highest NR activity was also observed in larger flag leaf of unicum wheat with late application of nitrogen.

### 3.4 Total soluble protein and RuBPCO contents

RuBPCO protein content (Fig2) and total soluble protein content (Fig3) was higher in flag leaves of plants those received higher dose of nitrogen at vegetative stage and late application of nitrogen further increased the content.

### 3.5 Rate of photosynthesis, diffusive resistance and transpiration

The rate of photosynthesis was maintained high during grain fill and later period of senescence as a consequence of increase in nitrogen content of leaves in terms of RuBPCO protein contents with late application of nitrogen (Table 4). Diffusive resistance was low and transpiration was more with availability of nitrogen irrespective of higher or late supply of nitrogen in the soil.

### 3.6 Invertase activity [neutral (pH 7.5) and acidic (5.0)], total starch content and total soluble carbohydrates

Activity of neutral invertase was low and that of acidic invertase was high significantly at ear emergence in fully expanded flag leaf of nitrogen deficient plants compared to those supplied with higher nitrogen; at vegetative and anthesis stages (Table 5). Activity of acid and neutral invertase was lowest at post anthesis stage (10 days after nitrogen application). Invertase activities increased at late stage of senescence and nitrogen availability was related with decreased hydrolytic activities.. Total starch and total soluble carbohydrates content were low in flag leaves those received higher nitrogen from soil as compared to low nitrogen at vegetative stage (Table 5) and the values were significant.

### 3.7 Protease (pH 7.0) activity

The enzyme protease (pH 7.0) had higher activity at high soil nitrogen levels and at onset of senescence (Fig4). Senescence started at the same time in all treatments but the process was faster in low nitrogen plants. The complete loss of total soluble protein occurred about one week later in plants grown in the high soil nitrogen than in the nitrogen deficient plants, therefore, senescence was delayed significantly. Late application of nitrogen did not change the time of senescence in low nitrogen plants. The flag leaves were completely yellow with low nitrogen supply while those received high nitrogen were half green and half yellow. Protease activity increased with increase in nitrate reductase activity, N influx, and RuBPCO content with high and late application of nitrogen.

### 3.8 Yield components

The first basal grain that was missing in spikes of low nitrogen plants became visible in spikes of high nitrogen plants and the grain was filled with late application of nitrogen. Number of spikelets was unchanged and grains number was increased in plants supplied with 90 kg ha<sup>-1</sup> nitrogen at vegetative stage compared to nitrogen limited plants (Table 6). Total grain weight, grain number per ear, 100-seed weight were significantly increased by soil nitrogen fertilization while percentage of nitrogen in grains was unchanged indicating unchanged carbon to nitrogen ratio in grains of unicultm wheat under all treatments of nitrogen.

## 4. Discussion

### 4.1 Nitrogen application at vegetative stage and leaf characteristics

High nitrogen availability prior to double ridge when the final leaf primordia were being initiated (Longnecker et al. 1993) was important in determining size of flag leaf and total leaf number was unchanged as compared to low nitrogen supply in unicultm. Nitrogen availability had a systematic and large effect on leaf length and width displaying increased number of veins and stomata, indicating increased cell division and production of more cells. Nitrogen deficiency decreased the size of leaves, mainly by reducing cell number. Nitrogen deficiency had been associated with asymmetrical cell division, stopping of cell division at different positions from the base of the leaf, reduced cell flux, small mature cell size, reduced cell elongation and duration of cell elongation (Jovanovic et al. 2004; Fricke et al. 1997; MacAdam et al. 1989; Volenec and Nelson 1983; Nova and Loomis 1981). Leaf growth induction resulting in longer leaves and higher cell wall contents had been reported in grasses (Casey et al. 1999; Skinner and Simmons 1993; Lawlor et al. 1989). Higher fresh weight per unit leaf area indicated larger total volume and area of multiple layers of mesophyll cells with ample nitrogen (observed under light microscope and our unpublished data on sunflower), therefore, large number of mesophyll cell utilized photosynthets in structural tissue. The reduction in leaf expansion and peduncle length or stem height was accompanied by increased invertase activity contrary to sucrose and fructans utilization (Ruuska et al., 2008; Huber et al. 1989; Volenec and Nelson, 1984) and starch accumulation (Paul and Stitt 1993) in dicots due to a low nitrogen supply. The accumulation of total soluble carbohydrates and non – structural carbohydrates in nitrogen deficient plants suggested that carbohydrate supply is not the cause of the leaf growth reduction under low nitrogen supply. Nitrogen deficiency could, for example, lead to sugar accumulation by decreasing demand

for carbon skeletons for amino acid and protein synthesis. Higher invertase activities were related with senescence process at later stages of grain filling. An early start of senescence can be expected to be favourable when the supply of nitrogen is low.

The rapid decline in cytokinin levels in xylem sap and leaf tissue had been associated with inhibition of leaf expansion in response to nitrogen deprivation (Palmer et al. 1996; Wagner and Michael 1971). A large effect of nitrogen on cell division was observed in a dicot *Ricinus communis* provided that nitrogen starvation was initiated early in leaf development, in the period of intense cell division (Roggatz et al. 1999). Our data showed that nitrogen allocated to the uppermost internode (peduncle) and flag leaf contributed in their increased size with higher supply of nitrogen. An increasing proportion of shoot carbon and nitrogen is allocated to non – photosynthetic tissues irrespective of whether this is achieved through alteration in the leaf: stem ratio (Belanger and Richards 2000) or through changes in the proportion of photosynthetic and structural tissue within laminae. The increase in leaf area during crop growth, achieved by building and positioning new leaves in the light, necessitates proportionally more structural tissues of low nitrogen content. Therefore, trade-off between allocation of nitrogen to maintain photosynthetic activity of existing leaves, and allocation of nitrogen to build new leaf tissue in order to increase leaf area, is complicated by the additional cost in structural carbon and nitrogen (Gastal and Lemarie 2002). The present study clearly showed that high nitrogen at early stages stimulated flag leaf growth, synthesis of RuBPCO enzyme and reduced stomatal conductance. Research aimed at understanding the basis for yield potential improvement (Fischer et al. 1998) has shown a consistent correlation between increases in yield potential achieved for CIMMYT semidwarf varieties and flag-leaf photosynthetic rate and stomatal conductance. The chlorophyll a/b ratio that reflects the relative abundance of light harvesting proteins to reaction center complexes (Evans and Terashima 1987; Evans 1989; Makino et al. 1985) was decreased with nitrogen supply. The higher amplitude signal of photosystem I of isolated chloroplasts from unculm seedlings from pots suggested the increased substrate (RuBP) for CO<sub>2</sub> fixation that could be related to increased activation of RuBP carboxylase in nitrogen deficient plants (Machler et al 1988) compared to high nitrogen plants. Higher nitrogen use efficiency of nitrogen deficient flag leaf could be explained by higher availability of light to single layer of palisade parenchyma and rate of photosynthesis. A better transmittance of epidermal cells in the blue than in the UV and thus the chlorophylls in the mesophyll below the epidermis receive a higher irradiance exciting chlorophyll fluorescence (Barnes et al. 2000) under low nitrogen supply. We might assume that 30 kg ha<sup>-1</sup> N was enough to build up same amount of leaf area unto sixth leaves and same number of spikelet as in 90 kg ha<sup>-1</sup> nitrogen on maturity. Therefore, RuBPCO in leaves beneath flag leaf served as the storage protein, contributed in buildup of new leaves with higher nitrogen contents and increased number of cells in flag leaf, increased number of fertile florets and high the flag leaf protein in turn enhanced endosperm cell storage capacity.

#### 4.2 Nitrogen application at post anthesis stage and yield components

Our data suggested that assimilates availability from flag leaf developed under high nitrogen supply, at the pre-anthesis late reproductive phase (Fischer 1975; 1985) determined the number of fertile florets at anthesis and in turn final grain number and that would be key trait to improve wheat yield without changing the anthesis date. The relationship between the spike dry weight and the number of fertile florets at anthesis (Fischer and Stockman 1980; Brooking and Kirby 1981; Stockman et al. 1983; Slafer and Andrade 1993), and the coincidence of spike and stem growth with floret degeneration (Kirby 1988), led to the suggestion that wheat yield is restricted by the availability of assimilates for spike growth. Flag leaves in wheat are strongly involved in source–sink relations and are important source of nutrients during grain filling through photo assimilate partitioning and nutrient remobilization. Amino acid remobilization was closely linked to protein degradation and occurred relatively early in senescence, when the cell integrity was still well maintained, whereas strong oxidative events occurred at later senescence stage (Hortensteiner and Feller 2002; Prochazkova and Wilhelmova 2009).

In our study, nitrate reductase activity and nitrogen flux are most active in young leaf, but are limited in the senescent leaf as evidenced in penultimate and leaf next to it. RuBPCO synthesis, *rbcS* and *rbcL* mRNAs decrease with leaf senescence (Imai et al. 2008); low nitrogen and high nitrogen leaves at post anthesis stage and five days after late application of nitrogen had potential actively to synthesize RuBPCO with an increase in nitrogen flux (nitrate reductase activity) correlated well with increased contents of the enzyme protein and delayed senescence of flag leaf of high nitrogen contents in our study. Flag leaf CO<sub>2</sub> exchange rates during grain fill are usually positively correlated with nitrogen concentration (Hunt and Poorten 1985) except when drought-induced stomatal closure occurs (Rawson and Hackett 1974). Late application of nitrogen maintained total soluble proteins, increased protease activity at 10, 20 and 30 days after anthesis in both low and high

nitrogen flag leaves of unicultm wheat. The enzyme activity was highest in all nitrogen treatments at 10 days after anthesis. Higher activity of protease was related with protein degradation and mobilization of reduced nitrogen at later stages when leaf lamina was turning yellow. Protease activity was significantly higher in high nitrogen flag leaf at 10 after nitrogen application at post anthesis stage and improvement in translocation efficiency reflected difference made in the contents of RuBPCO protein with late nitrogen supply. Our results confirmed the assumption made by several authors (Thornley 2004; Lawlor et al. 1989 Hageman 1986; Makino et al. 1985) that soluble proteins in vegetative organs form a unique pool of nitrogen available for growing vegetative and storage organs and the size of this pool reflects the nitrogen status of the plant. Semenov et al. (2007) assumed specific leaf nitrogen had no affect on the radiation use efficiency. This implied an increase in the efficiency of RuBPCO to fix carbon, so a lower amount of protein nitrogen will be required per leaf area to fix the same amount of carbon (Parry et al. 2003). Considering that high yield with high grain quality can be achieved with high applied nitrogen, the potential improvement in nitrogen use efficiency due to alteration of leaf nitrogen was significant. High protease activities were related with the high N contents of grains in different treatments.

During grain filling, weights of grains are determined from seven to ten days after flowering, therefore, the difference of current photosynthets and reserved assimilates between low and high nitrogen supply at vegetative stage was expressed in bolder seeds and appearance of basal spikelet with high nitrogen supply while nitrogen supply at post anthesis stage filled the grain in basal spikelet and improved grain nitrogen. Grain yield depended on the rate and duration of grain filling (Langer and Liew 1973; Nass and Reiser 1975) while the number and duration of spikelet initiation was not affected with high nitrogen application. Some other experiments showed that high N regimes before floral initiation increased spikelet number, but generally did not affect the time of spike development (Whingwiri and Kemp 1980). Nitrogen deficiency decreased spikelet number and delayed time of double ridge and terminal spikelet (Longnecker et al. 1993). Changing nitrogen supply levels at the end of floret initiation and at anthesis showed (Steer et al. 1984) that seed number was determined by nitrogen supply before floret initiation (Whingwiri and Stern 1982). In agreement with the result of Whingwiri and stern (1982), the present results showed that high N slightly increased spike dry weight, N contents though not significantly, but did not affect the duration of spikelet initiation and time of spike development. However, the effect of N supply on rate of florets initiation and maximum floret number varied from report to report. Our data showed that nitrogen influx at post anthesis stage might help in the development of florets formed at or after terminal spikelet formation and contribute in increased grain number and grain N content. The contribution of flag leaf nitrogen to grain was 20% in high nitrogen and 14% in nitrogen deficient crops (Lawlor et al. 1989) and high percentage of nitrogen contributed to maintaining photosynthetic integrity. Therefore, high nitrate reductase activity contributed in nitrogen assimilation, RuBPCO synthesis, delayed leaf senescence; sustain leaf photosynthesis, improved the efficiency of redistribution, of carbon and nitrogen assimilated prior to ear emergence, during grain formation as suggested previously (Dwyer *et al.* 1995; Frederick and Camberato 1995; Deckard *et al.* 1973; Eilrich and Hageman 1973; Croy and Hageman 1970). We conclude that single seed weight was influenced by nitrogen supply throughout plant development but mainly between floret initiation and anthesis. The grain size at low potential weight spikelet positions was dependent on grain number. Grain nitrogen depend on flag leaves having a high level of RuBPCO, nitrate reductase activity and a high translocation efficiency i.e. protease activity at later stages.

Our data confirmed the physiological potential for increasing kernel weight at distal spikelet positions strongly endorsing the objective of breeders to raise yields through increasing grain weight potential. Our work suggest optimization of fertility of florets/spikelet and endosperm cell division regulating the availability of nitrogen; could achieve assimilate diversion to grain position showing lower grain weight and longer duration of grain fill at distal positions.

## 5. Conclusion

Nitrogen supply affected plant growth and productivity by altering leaf area, photosynthetic capacity and nitrogen status of flag leaf that improved number and ability of florets to set grain. Nitrogen was involved in the functioning of meristematic tissues and in the determination of the protein content of harvested organs. During the reproductive stage, nitrogen is translocated to growing grains, which decreases the photosynthetic capacity, nitrate reductase activity and indirectly hampers the ability of roots to further take up nitrogen.

The present study assigns an important role to flag leaf blades in the supply of reduced nitrogen at early and late reproductive stage of unicultm wheat. Maintenance of nitrate reductase activity during the reproductive phase increased grain weight and prevent the depression of percent grain protein frequently observed when grain yields are high. Gigas inclusion in breeding programs could be advantageous under high input, high density crop

conditions, because they clearly permit larger ears, with more and larger grains, than can be obtained with normal cultivars even under favourable conditions or when tillering is restricted. Although the harvest index of the gigas lines is not higher than that of modern cultivars, the gigas characteristics may make further increase in harvest index possible. Some modern varieties with high yield potential are characterized by heavy ears and large thick leaves (Austin et al. 1980) approaching those of the gigas type, and further selection in this direction, aided by the gigas genes, could be productive.

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Table 1. Characteristics of flag leaf, plant height and green leaf area of unculm wheat at full expansion of flag leaf in relation to nitrogen supply at vegetative stage

Plant character	Treatments		C. D. 5%
	N1	N2	
Length of flag leaf (cm)	19.62	26.64	00.82
Width of flag leaf (cm)	01.80	02.42	00.14
Leaf Area (cm <sup>2</sup> )	36.21	64.37	04.31
Fresh weight of flag leaf (g)	00.48	00.97	00.02
Vein number	24.00	39.00	01.16
Number of Stomata mm <sup>-2</sup>	140	153	01.85
Total Chlorophyll contents (mg g <sup>-1</sup> f. wt.)	3.43	3.70	00.12
Photosynthetic rate (mg CO <sub>2</sub> dm <sup>-2</sup> hr <sup>-1</sup> )	27.33	22.00	01.62
Plant height (cm)	60.90	64.62	01.43
Total green leaf area (cm <sup>2</sup> )	57.44	107.12	07.97

N1 = Low Nitrogen

N2 = High Nitrogen

Table 2. Chlorophyll a, Chlorophyll b and total chlorophyll contents of unicum wheat in relation to nitrogen supply

Treatments	Chlorophyll a	Chlorophyll b	Chl a: Chl b	Total Chlorophyll
N+10 Days				
N1	4.420	1.877	2.354	6.297
N2	3.590	3.950	0.908	7.540
N3	4.777	2.533	1.885	7.310
N4	3.660	3.960	0.924	7.620
C. D. 5%	0.022	0.031	0.003	0.010
N+20 Days				
N1	4.330	1.390	3.115	5.720
N2	3.240	3.360	0.964	6.600
N3	4.560	1.797	2.537	6.357
N4	3.000	3.270	0.917	6.270
C.D. 5%	0.019	0.017	0.002	0.330
N+30 Days				
N1	2.200	0.730	3.013	2.930
N2	2.020	2.070	0.975	4.090
N3	2.240	0.840	2.666	3.080
N4	2.070	2.100	0.985	4.170
C. D. 5%	0.019	0.019	0.003	0.014

N1 = Low Nitrogen

N2 = High Nitrogen

N3 = Low + Late Nitrogen

N4 = High + Late Nitrogen

Table 3. Nitrate reductase activity in flag leaf of unicum wheat in relation to nitrogen supply

Treatments	Nitrate reductase Activity (n mole nitrite produced /g f. wt/hr).			
	Position of leaf			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Total NR activity
Anthesis + 10 days (5 days after late supply of nitrogen)				
N1	232.50	187.50	116.70	536.20
N2	375.00	312.50	232.50	919.50
C. D. 5%	0.899	0.233	0.233	
Anthesis +20 days (15 days after late supply of nitrogen)				
N1	108.33	70.66	60.66	239.59
N2	141.67	120.66	66.67	329.00
C. D. 5%	0.023	0.023	0.023	

1<sup>st</sup> = Flag2<sup>nd</sup> = Penultimate3<sup>rd</sup> = Next to Penultimate

Table 4. Photosynthesis, diffusive resistance and transpiration of unicultm wheat in relation to nitrogen supply

Treatments	Photosynthesis (mg CO <sub>2</sub> /dm <sup>2</sup> /hr)			Diffusive resistance (S cm <sup>-1</sup> )			Transpiration (μg H <sub>2</sub> O cm <sup>-2</sup> S <sup>-1</sup> )		
	N+10	N+20	N+30	N+10	N+20	N+30	N+10	A+20	N+30
N1	16.65	8.16	4.08	0.18	0.67	0.89	40.01	17.88	15.85
N2	16.63	13.47	4.49	0.18	0.52	0.84	39.32	20.00	16.58
N3	18.62	10.88	4.08	0.18	0.44	0.67	39.00	21.03	18.40
N4	18.68	15.83	5.35	0.19	0.50	0.72	38.88	19.91	16.50
C. D. 5%	1.01	0.73	0.29	N.S.	0.03	0.04	N.S.	1.33	1.06

N1 = Low Nitrogen

N2 = High Nitrogen

N3 = Low + Late Nitrogen

N4 = High + Late Nitrogen

Table 5. Activity of invertase, total starch and total soluble carbohydrates of unicultm wheat flag leaf in relation to nitrogen supply

Treatment	Invertase (μg reducing sugars mg <sup>-1</sup> protein 30 min <sup>-1</sup> )		Starch (mg g <sup>-1</sup> f.wt.)	Total soluble carbohydrates (mg g <sup>-1</sup> f.wt.)
	pH(5.0)	pH (7.5)		
Ear emergence				
N1	95.20	72.80	4.45	-
N2	86.80	78.40	6.69	-
C.D.	00.50	00.99	0.07	
Anthesis				
N1	114.8	84.00	2.95	2.16
N2	92.40	81.20	2.35	1.35
C.D.	00.42	00.77	0.08	0.03
N+ 10 day				
N1	40.70	30.55	1.70	1.08
N2	61.00	30.55	1.45	1.26
N3	63.62	38.16	1.45	0.95
N4	63.62	35.65	1.45	1.00
C. D. 5%	00.95	00.04	0.02	0.02
N +20				
N1	176.40	109.20	2.90	1.22
N2	176.40	100.80	2.75	1.26
N3	165.20	112.00	2.60	2.13
N4	151.20	100.80	2.45	1.12
C. D. 5%	00.55	00.96	0.02	0.01

N1 = Low Nitrogen

N2 = High Nitrogen

N3 = Low + Late Nitrogen

N4 = High + Late Nitrogen

Table 6. Yield components of unicum wheat in relation to nitrogen supply

Treatments	Grain Yield/Ear (g)	Grain Number/Ear	100 Seed Weight (g)	Nitrogen in grain (%)
N1	2.734	96	3.262	2.86
N2	4.992	121	3.859	3.00
N3	2.846	99	3.298	2.78
N4	5.035	129	3.910	3.17
CD at 5%	0.43	12.35	0.10	NS

N1 = Low Nitrogen

N2 = High Nitrogen

N3 = Low + Late Nitrogen

N4 = High + Late Nitrogen

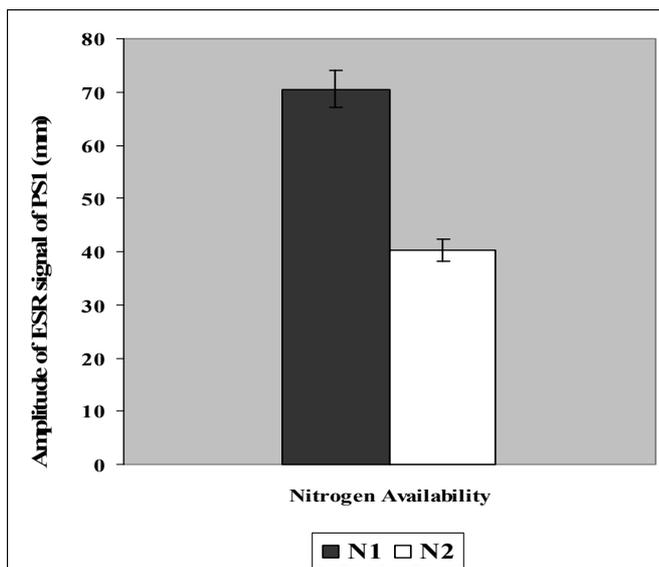
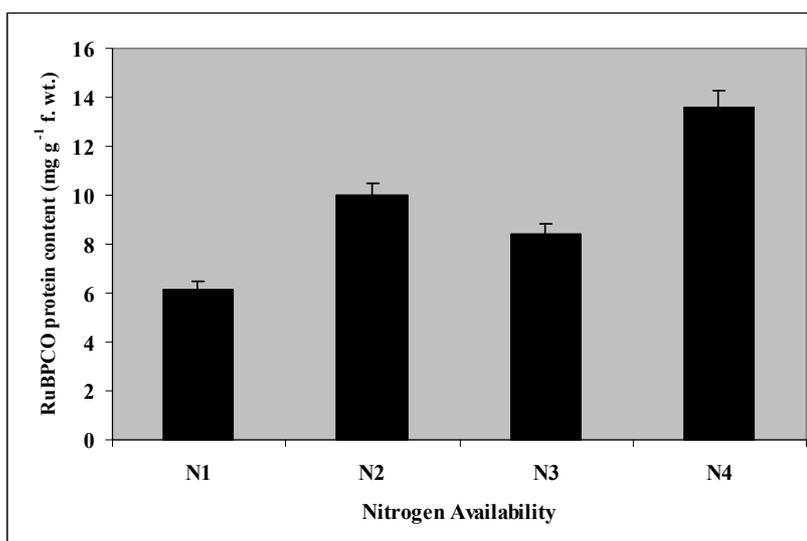


Figure 1. Photosynthetic efficiency (PS 1 - ESR signal) in flag leaf of unicum wheat in relation to nitrogen supply

Figure 2. RuBPCO protein content (mg g<sup>-1</sup> fresh weight) in flag leaf of unicum wheat in relation to nitrogen supply

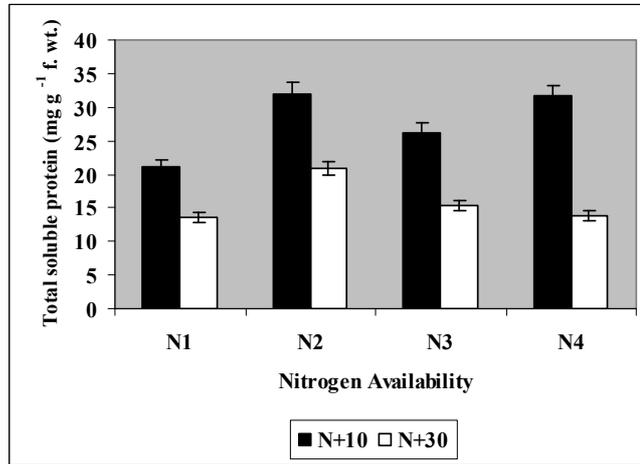


Figure 3. Total soluble protein content (mg g<sup>-1</sup> fresh weight) in flag leaf of unicult wheat in relation to nitrogen supply

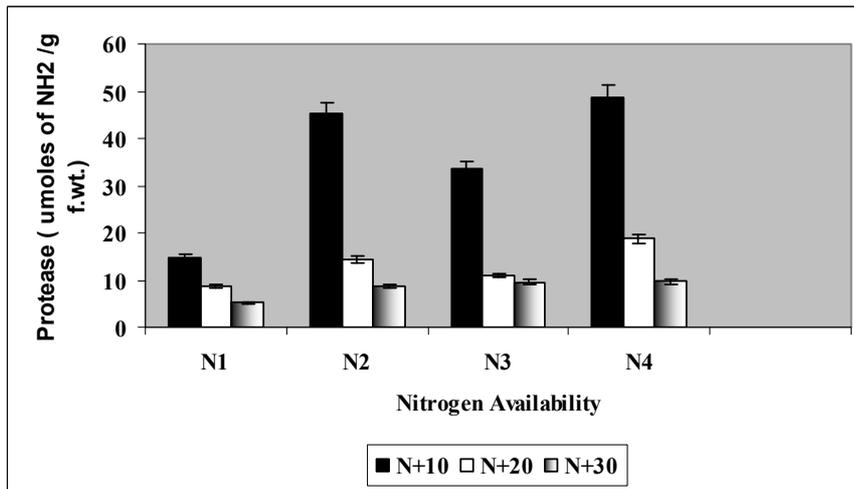


Figure 4. Protease activity in flag leaf of unicult wheat in relation to nitrogen supply