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Responses of penultimate and flag leaves of wheat to different nitrogen supply

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Abstract

Uniculm wheat (*Triticum aestivum* L.) was grown to maturity at four concentrations of nitrogen corresponding to 3 (N1), 6 (N2), 9 (N3) and 12 (N4) g m^{-2}. Penultimate and flag leaves were examined throughout the ontogeny. Sub-optimal concentrations of N resulted in sharp decline in both area and dry mass of the leaves. Decline in leaf area was due to fewer mesophyll cells. Net photosynthetic rate (P_N) increased up to full expansion, remained constant for about a week and then declined. P_N, nitrogen, ribulose-1,5-bis-phosphate carboxylase/oxygenase (RuBPCO) amount and activity, chlorophyll and soluble protein contents were similar at all the N concentrations. Both amount and activity of RuBPCO in the flag leaf were about two fold higher as compared to penultimate leaf, but P_N was similar. This indicates the presence of an excess amount of RuBPCO in the flag leaf.

Additional key words: chlorophyll, photosynthesis, ribulose-1,5-bisphosphate carboxylase/oxygenase, soluble proteins.

Introduction

Nitrogen nutrition plays a crucial rôle in determining the photosynthetic capacity of the plants in both natural and agricultural environments (Abrol *et al.* 1999). Photosynthetic functions of leaves require a large content of reduced nitrogen compared to other tissues of the plants. Nitrogen in photosynthetic apparatus is found predominantly in the pigment-protein complexes of the light reactions as well as in the proteins associated with photosynthesis carbon reduction cycle especially RuBPCO (Evans and Seeman 1984). Of the total leaf nitrogen, about 25 - 30 % is in RuBPCO, and 25 % in light harvesting and electron transport components (Sivasankar *et al.* 1993). Studies have indicated that the net photosynthetic rate (P_N) is correlated with nitrogen content of the leaf (Evans 1989, Ahmad and Abdin 2000) and the components of photosynthetic system may change over the range of nitrogen contents.

The amount of nitrogen per unit leaf area is highly variable and depends on both environmental and internal factors (Laurer *et al.* 1993). Much of the variation in photosynthetic nitrogen use efficiency in species differing in specific leaf area can be accounted for by differences in investment of nitrogen in RuBPCO (Millard and Catt, 1988) and other photosynthetic components. Leaf nitrogen also affects the size and number of chloroplasts per mesophyll cell and also the cross sectional area of the cells (Lawlor *et al.* 1989, Sivasankar *et al.* 1998).

In the previous experiments, the different response of uniculm genotype Gigas to applied N in comparison with other wheat cultivars was studied (Sivasankar *et al.* 1998). In present communication detailed analysis of upper two leaves which are photosynthetically active during grain filling period is discussed. We have also examined the relationship between leaf N, P_N and RuBPCO protein in the penultimate and flag leaf of uniculm genotype, as they differ markedly in their response to N stress in terms of leaf area.

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Abbreviations: chl - chlorophyll; c_i - internal CO_2 concentration; DAS - days after sowing; DMSO - dimethyl sulphoxide; FL - flag leaf; g_s - stomatal conductance; P_N - net photosynthetic rate; PL - penultimate leaf; RuBPCO - ribulose-1,5-bis-phosphate carboxylase/oxygenase; TCA - tricloroacetic acid.

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Materials and methods

Plants: Wheat (*Triticum aestivum* L. uniculm genotype Gigas) plants (Atsmon and Jacob 1977) were grown in earthen pots (35 × 40 cm) containing 10 kg sandy loam soil at four nitrogen levels corresponding to 3 (N1), 6 (N2), 9 (N3) and 12 (N4) g m^{-2}. Half of the N was applied as basal dose and the rest in two equal amounts at 30 and 45 days after sowing (DAS). P and K were also applied. The experimental design was a completely randomized block with each treatment replicated four times. Fifty pots, each containing eight plants, were maintained per treatment.

Sampling and measurements: The penultimate and flag leaves were sampled weekly from emergence until senescence. Green area of the leaves was determined using leaf area meter (*Delta T Devices*, Burwell, England). The leaves were dried in hot air oven at 80 °C to a constant mass. Chlorophyll content was determined by extracting the pigments using dimethyl sulfoxide (DMSO) according to Hiscox and Israelstom (1978). Net photosynthetic rate (P_N), stomatal conductance (g_s) and internal CO_2 concentration (ci) were measured using photosynthetic system *LI 6200*, (*Li-Cor*, Lincoln, USA). Reduced nitrogen in the dried samples was measured with N-autoanalyzer (*Technicon*, Dublin, Ireland). Protein content was estimated following the method of Lowry *et al.* (1951).

The mesophyll cell size, number, surface area and volume of the flag leaf at full expansion were determined by the method of Lawlor *et al.* (1989). Leaf tissue was digested with 5 % chromic acid in 1M HCL for 3 - 4 d in dark and the digest was filtered through 106 µm mesh to remove large debris. The mesophyll cells were counted by haemocytometer. The mesophyll cell area was calculated from length and middle width from about 50 - 60 mesophyll cell.

The penultimate and flag leaves were sampled weekly from emergence until senescence for estimating the amount of RuBPCO and its activity by following the protocol as described Lawlor *et al.* (1989).

Statistical analysis: The data obtained was analysed statistically by following the methods of Gomez and Gomez (1984).

Results

Leaf dry mass and area: Maximum dry mass was recorded at N4 and the minimum at N1 (Fig. 1*A*). In the penultimate leaf (PL), the maximum dry mass was recorded at 87 DAS at all the four levels of N followed by a sharp decline at later stages of growth whereas in the flag leaf (FL), the maximum dry mass was at about 80 DAS followed by a steady decline.

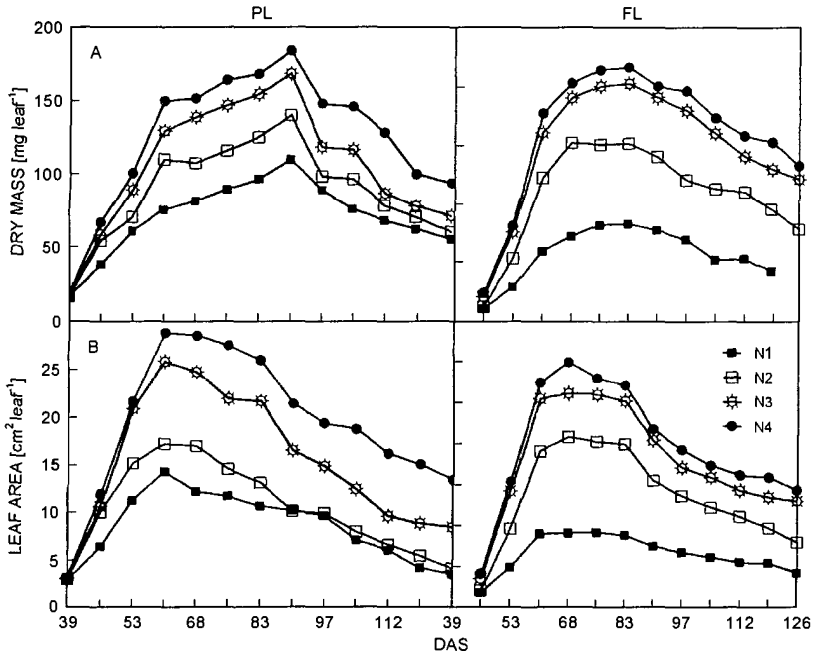

Fig. 1. Ontogenetic changes in dry mass (*A*) and leaf area (*B*) of penultimate (PL) and flag (FL) leaves of uniculm wheat grown at four N concentrations viz. 3 (N1), 6 (N2), 9 (N3), and 12 (N4) g m^{-2}. CD at 5 %: dry mass PL and FL, 12.09 and 16.47, respectively; leaf area PL and FL, 1.613 and 1.854, respectively.

Maximum area in the PL was around 61 DAS followed by a gradual decline whereas in the FL, it was around 68 to 73 DAS (Fig. 1*B*). At N4, the area of the PL and the FL were two to three fold higher as compared to the values at N1. There was greater reduction in the area of FL at sub-optimal nitrogen level than that of the PL area (Fig. 1*B*).

Nitrogen and soluble protein contents: Nitrogen content of the leaves was high immediately after emergence and declined thereafter (Fig. 2*A*). During the initial growth stages, the N content was higher at N3 and N4 than at N1 and N2. At later stages, there were no differences among the different N treatments. In both the leaves, the soluble protein content increased till the full expansion and then declined (Fig. 2*B*). There were no significant differences among the different nitrogen treatments. In FL, the values were higher than in PL.

Net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (c_i) and total chlorophyll (Chl) content: Despite large reduction in the leaf area, P_N, g_s, c_i and Chl content were similar at all the nitrogen levels in both the leaves (Figs. 2*C*, 3*A*,*B*). Maximum P_N was at 61 and 73 DAS in the PL and FL, respectively, and declined thereafter during the ear emergence.

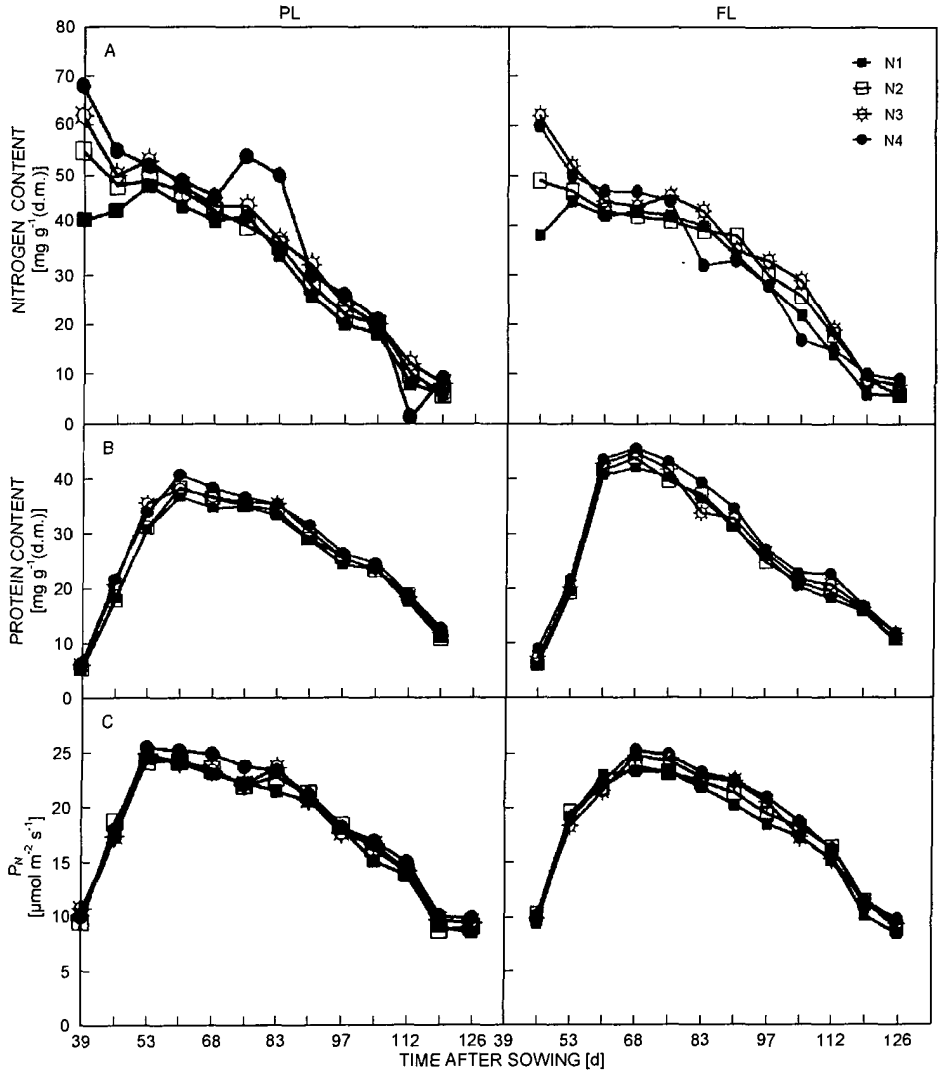

Fig. 2. Ontogenetic changes in nitrogen content (*A*), soluble protein content (*B*), and net photosynthetic rate, P_N (*C*) of penultimate (PL) and flag (FL) leaves of uniculm wheat grown at four N concentrations. CD 5 % for nitrogen content in PL and FL is 0.518 and 0.480, respectively. No significant difference was there between protein content and P_N in both the leaves.

RuBPCO protein content and RuBPCO activity: RuBPCO protein and its activity increased to a maximum value at full expansion and then declined (Figs. 4*A*,*B*). No significant changes were observed among the different N levels. FL recorded the higher amount and activity of RuBPCO per unit area compared with PL. The amount of

RuBPCO was almost two fold higher in FL than that in the PL. The same trend was also observed in its activity. In spite of the very high activity and amount of RuBPCO per unit area in the flag leaf no increase in P_N was observed in the flag leaf.

Mesophyll cell characteristics: The number of mesophyll cells per unit leaf area remained similar in both the leaves at different nitrogen concentrations (Table 1). There were large reductions in the total number of cells per leaf (around 45 % in the PL and more than 50 % in the FL) at sub-optimal N. No such differences were recorded in cell volume and surface area (Table 1).

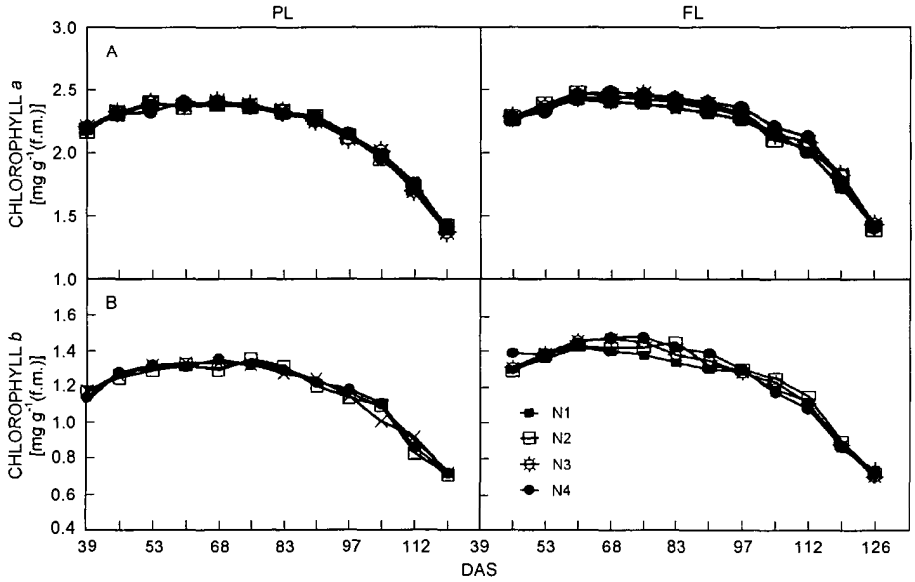

Fig 3. Ontogenetic changes in chlorophyll *a* (*A*) and chlorophyll *b* (*B*) contents in penultimate (PL) and flag (FL) leaves of uniculm wheat grown at four N concentrations. No significant difference in both the leaves were found.

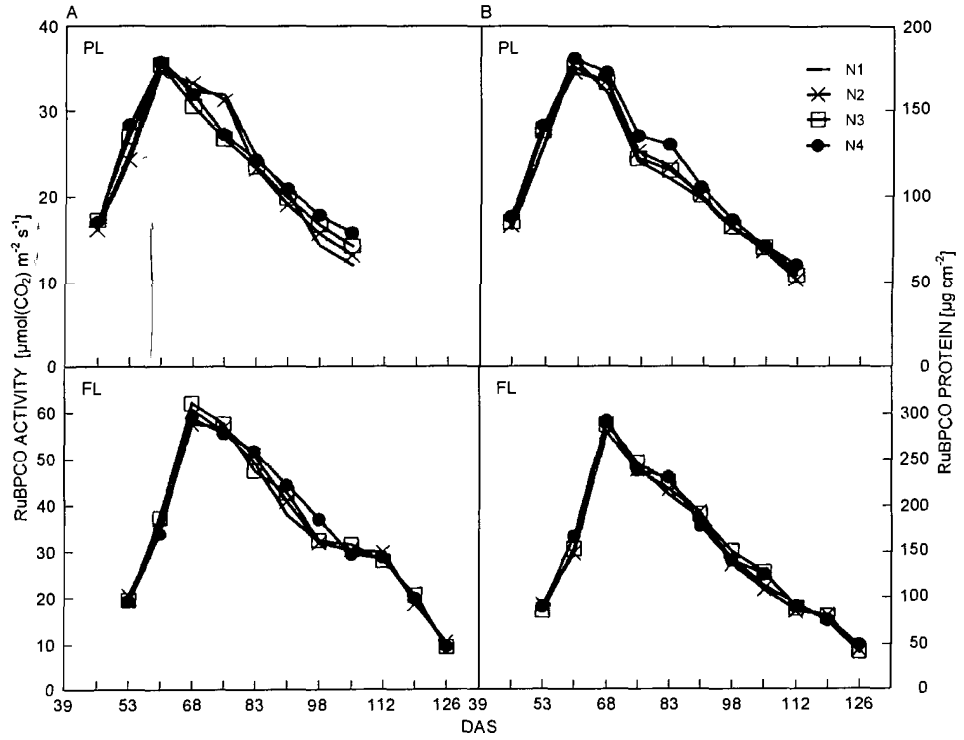

Fig 4. Ontogenetic changes in the RuBPCO activity (*A*) and amount (*B*) in the penultimate (PL) and flag (FL) leaves of uniculm wheat grown at four N concentrations. No significant difference in both the leaves were found.

Table 1. Mesophyll cell characteristics of PL and FL of uniculm wheat at full expansion at two nitrogen concentrations N1 and N4 (A_{leaf} - leaf surface area, V_{mes} - mesophyll cell volume).

	Cell number [$\times 10^{-5}$ cm^{-2}]		Cell number [$\times 10^{-5}$ leaf^{-1}]		V_{mes}/A_{leaf} [μm^3 cm^{-2}]		Cell surface area [μm^2]		A_{mes}/A_{lcaf}	
	PL	FL	PL	FL	PL	FL	PL	FL	PL	FL
N1	2.92	3.28	38.69	29.68	1.02	0.91	13705	10428	40.90	34.20
N4	2.71	2.99	67.75	84.08	1.04	0.93	15350	12210	41.60	38.10
$LSD_{0.05}$	ns	ns	2.18	2.18	ns	ns	ns	ns	ns	ns

Discussion

The increased dry matter in uniculm wheat as a result of increased N supply is caused by increase in the photosynthetic area. P_N per unit area of the individual leaves did not change. Morgan (1988) stated that early season N application enhances photosynthetic area but hardly influences P_N of the individual leaves. In our study there was no effect on the area as well as P_N of the lower four leaves (data not shown). Significant differences in leaf size at the various N supply started from fifth leaf onwards and PL and FL showed maximum differences in the size. PL and FL, respond differently in terms of their growth and chlorophyll content at sub-optimal N supply as compared to other leaves (Sivasankar *et al.* 1998, Jain *et al.* 1999). Relationship between cell number and leaf nitrogen content has been reported by Gastal and Nelson (1994). Large differences in leaf area were mainly due to differences in mesophyll cell number as variation in cell size was insignificant. Hence, it is possible that N stress induced variations in laminae size are controlled by the rate of cell production and not by variation in final size of these cells.

Various studies have indicated that the fraction of leaf N represented by RuBPCO increases as leaf N content increases and that the amount of RuBPCO protein in FL is in excess for P_N (Lawlor *et al.* 1989, Theobald *et al.* 1998). Comparison of amount and activity of RuBPCO between PL and FL revealed that the RuBPCO content of FL was more than two fold higher than that of PL and their P_N were similar. This observation clearly indicates that high enzyme content of FL is not fully utilized for photosynthesis. Even under low N supply FL invests much more nitrogen in RuBPCO than is required for carboxylation. The function of RuBPCO has often been discussed as a storage protein. As this enzyme is degraded faster than the other soluble proteins (Makino *et al.* 1984a,b, Sage *et al.* 1990).

There is a parallel decline in the P_N and nitrogen concentration. Carbon dioxide fixed by the PL and FL contribute significantly to the total CO_2 fixed by the shoot and particularly the grain yield. In the present study, because of similar P_N at all the N levels, the increase in leaf area is reflected in the total CO_2 fixed by the leaves during their entire duration.

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