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Photosynthetic characteristics in two wheat genotypes as affected by nitrogen nutrition

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Abstract

Chlorophyll (Chl) *a* and *b* content, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content and activity, and electron transport rate were measured in flag leaves of wheat genotypes Uniculm and Kalyansona, grown at suboptimal and optimal supply of nitrogen. The Chl content, RuBPCO activity, and electron transport rate were decreased due to suboptimal nitrogen supply only in Kalyansona. There was no change in the ratio of RuBPCO and photosystem 2 (PS2) activity at various stages which suggests that there was no alteration in distribution of N due to additional N supply.

Additional key words: chlorophyll, photochemical activity, photosystem 2, ribulose-1,5-bisphosphate carboxylase/oxygenase, *Triticum aestivum*.

Introduction

Nitrogen nutrition plays a crucial role in determining photosynthetic capacity of plants as 75 % of the N is contained in chloroplast proteins. This fraction of N is directly related to components of photosynthesis, *i.e.* pigment protein complexes, photosystem reaction centres, components of electron transport chain, and coupling factors (Evans 1989). N nutrition affects lamina growth as well (*e.g.* Morita and Kono 1975, Makino *et al.* 1984, Sivasankar *et al.* 1993). Evans and Terashima (1987) observed a 60 % reduction in chlorophyll (Chl) content per leaf area unit, accompanied by equivalent reduction in the contents of PS1 and PS2, plastoquinone pool, cytochrome *f* and coupling factor activity due to N stress. According to Ramalho *et al.* (1997) N supply was the key factor in photosynthetic acclimation to high irradiance in *Coffea*.

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Abbreviations: Chl - chlorophyll; MV - methyl viologen; PD - phenylenediamine; PS2 - photosystem 2; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase.

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Wheat genotypes respond differentially to supply of N (Sivasankar *et al.* 1998a,b) In one genotype there was a drastic reduction of leaf area at inadequate N, while the net photosynthetic rate (P_N) rate was maintained. In the other genotype, there was a decrease in P_N as well as in biochemical activities but the leaf area was maintained. Significant differences in the flag leaf area and other laminae characteristics related to availability of soil N were found at different stages of growth and development (Abrol *et al.* 1976, Abrol 1990). In the present study, the photosynthetic characteristics as affected by suboptimal and optimal N supply of two wheat genotypes were investigated.

Materials and methods

Wheat (*Triticum aestivum* L.) genotype Uniculm 'Gigas' (V1) and cultivar Kalyansona (V2) were grown in earthen pots (35 × 40 cm) with soil and nitrogen was applied at two different concentrations corresponding to 3 (N1) and 12 g(N) m^{-2} (N2). Half of the N was applied as basal nutrition and rest in two equal splits at 30 and 45 d after sowing. Entire phosphorus and potassium were applied as basal dose of 8 g m^{-2} in the form of single superphosphate and muriate of potash. Flag leaves were sampled at full expansion (68 d), maturity (87 d) and early senescence (108 d). At each sampling, middle portion of lamina was cut with a sharp blade, leaving off a 2 cm portion both at the base and apex.

Pigments were extracted with 80 % acetone and Chl (*a*+*b*) content was determined according to Arnon (1949). Thylakoids were isolated according to Sabat *et al.* (1991). Whole chain electron transport [H_2O→methyl viologen (MV)] and photosystem 2 (PS2) activity [H_2O→phenylenediamine (PD)→$Fe_3(CN)_6$] were monitored as O_2 evolution or consumption in a polarographic assembly at 25 ± 1 °C under an irradiance of 480 W m^{-2}. The basal reaction medium contained 100 mM sucrose, 3 mM $MgCl_2$, 10 mM NaCl, and 50 mM Hepes-KOH (pH 7.5). The concentrations of electron acceptors $Fe_3(CN)_6$, PD, and MV were 2, 0.5 and 0.5 mM, respectively. In the H_2O→MV assay, reaction mixture also contained 1 mM sodium azide to inhibit peroxidase activity. For all the assays, a similar Chl concentration (28 μg) was used.

Absorption spectra of acetone buffer-suspended chloroplasts were recorded on the *Hitachi 557* spectrophotometer. The amount of chloroplast preparation used for recording absorption spectra was adjusted at 750 nm to give the same absorbance. The base line was corrected carefully and spectra were scanned at least three times. The ratio of absorbances of blue to red peak (A_{436}/A_{678}) was taken as an index of the change in radiation scattering by the chloroplasts (Grover *et al.* 1986).

On the same samples, N content, soluble proteins (Lowry *et al.* 1951) and RuBPCO activity and its content (Lawlor *et al.* 1989) were determined.

Results

Chl (*a+b*) content, RuBPCO protein content and activity in the flag leaves of V2 were lower at suboptimal (N1) than at adequate (N2) nitrogen supply. In contrast, V1 did not show a similar reduction in response to low N at all growth stages (Table 1). The Chl *a/b* ratio did not change in both the genotypes as a result of varied N supply. The whole chain ($H_2O \rightarrow MV$) and PS2 [$H_2O \rightarrow PD \rightarrow Fe_3(CN)_6$] electron transport capacities were reduced at N1 compared to N2 in V2 and the reduction progressed towards senescence (Table 1). In contrast, V1 exhibited similar rates of electron transport (whole chain and PS2 activity) at all the three stages of sampling at both N levels. In both genotypes, the ratio of RuBPCO to PS2 activity remained similar at both N treatments and all stages (Table 2).

Table 1. Nitrogen concentration [%(d.m.)], chlorophyll *a+b* content [g kg^{-1}(f.m.)], RuBPCO protein content [g m^{-2}] and activity [μmol $m^{-2}s^{-1}$], and photochemical activities [μmol(O_2) g^{-1}(Chl) s^{-1}] in flag leaves of two wheat genotypes (V1, V2) in response to suboptimal and optimal nitrogen supply (N1, N2) at three growth stages 68 d (full expansion), 87 d (maturity), and 108 d (early senescence).

Age	Genotype + treatment	Nitrogen	Chl *a+b*	Chl *a/b*	RuBPCO protein	RuBPCO activity	Whole chain	PS2
68 d	V1N1	4.38	3.09	1.81	2.24	62.6	1.56	9.94
	V1N2	4.88	4.08	1.65	2.31	65.0	1.61	10.00
	V2N1	3.73	2.77	1.80	1.43	34.4	1.53	6.83
	V2N2	4.85	3.88	1.77	2.21	53.5	1.50	8.75
CD at 5 %		0.26	0.25	0.17	0.01	2.6	0.11	0.66
87 d	V1N1	3.89	3.80	1.77	2.09	56..8	1.67	9.78
	V1N2	4.15	4.00	1.67	2.15	59.4	1.67	9.78
	V2N1	2.28	2.70	1.88	1.21	25.9	1.94	6.50
	V2N2	3.82	3.76	1.64	2.08	44.6	2.58	9.06
CD at 5 %		0.26	0.20	0.13	0.01	3.2	0.01	0.59
108 d	V1N1	2.98	3.35	2.00	1.22	36.6	1.22	5.97
	V1N2	3.14	3.57	1.91	1.50	39.8	1.31	6.00
	V2N1	1.82	2.00	1.91	0.83	19.1	0.97	3.50
	V2N2	3.00	3.25	1.97	1.48	32.4	1.36	5.17
CD at 5 %		0.15	0.20	0.21	0.01	1.9	0.08	0.29

Absorption spectra of buffer-suspended chloroplasts were recorded and the A_{436}/A_{678} ratio was taken as an index of change in radiation scattering by the chloroplasts. At all stages, there was no change in the absorption spectra and ratio of different peaks induced by N nutrition except in V1N2. Absorption spectra at full expansion stage (Fig. 1) showed a slight increase in the ratio of blue to red absorbance at low N at early senescent stage of V2 (Table 2). No shifts in the red or blue absorption maxima was observed. The changes at 478 nm region are possibly associated with the carotenoid content (Fig. 1).

Table 2. Ratios of RuBPCO content or activity and PS2 activity, and ratio of absorbances (A_{436}/A_{678}) by isolated chloroplasts in two wheat (V1,V2) genotypes at three growth stages.

Genotype/ treatment	RuBPCO content/PS2			RuBPCO activity/PS2			A_{436}/A_{678}		
	68 d	87 d	108 d	68 d	87 d	108 d	68 d	87 d	108 d
V1N1	0.62	0.59	0.56	0.17	0.16	0.17	1.32	1.38	1.48
V1N2	0.64	0.61	0.69	0.18	0.17	0.18	1.35	1.37	1.49
V2N1	0.58	0.51	0.65	0.14	0.12	0.15	1.33	1.40	1.48
V2N2	0.70	0.63	0.75	0.17	0.14	0.17	1.35	1.38	1.40
CD at 5 %							NS	0.02	0.03

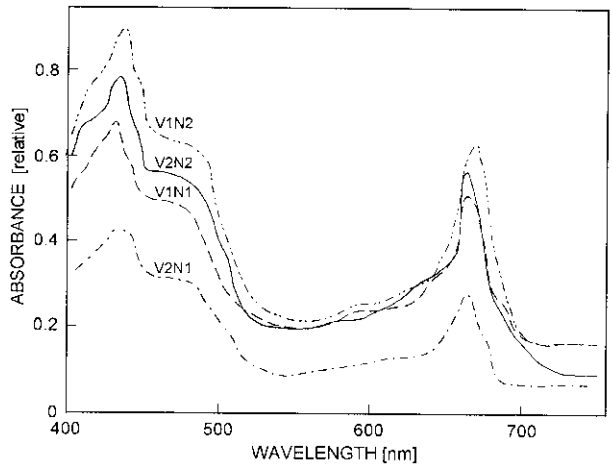

Fig. 1. Absorption spectra at different wavelengths by isolated chloroplasts of fully expanded flag leaf of two wheat genotypes (V1, V2), grown at suboptimal and optimal N supply (N1, N2).

Discussion

For this study, flag laminae on the main shoot of the two genotypes were selected for detailed investigation because of their different response in terms of P_N and lamina area to suboptimal N supply (Sivasankar *et al.* 1998a). The photosynthetic capacity of the laminae is related to their N content primarily because the proteins of Calvin cycle and thylakoids represent the major part of the N compounds. In the genotype V1, where the P_N was similar at both N levels, there was no change in the concentrations of N, Chl, and RuBPCO protein, the RuBPCO activity and photochemical activities. Significant differences in Chl content at stage I may be related to differences in development. In V2, the decline in P_N at low N is reflected in reduction of Chl and RuBPCO content and activity, and activities of whole electron transport chain and PS2. However, the Chl *a/b* ratio did not change thereby indicating that N deficiency probably did not cause a change in partitioning of N between the light harvesting complexes and PS2 reaction centres.

Evans (1983) in wheat and Uchida *et al.* (1980) in rice, reported that the ratio of RuBPCO/Chl was independent of N nutrition. Similar response to N in terms of electron transport and RuBPCO activity was reported in spinach (Evans and Terashima 1987) and *Atriplex* (Medina 1971). A similar RuBPCO/PS2 activities ratio at suboptimal and optimal N nutrition in both the genotypes showed that the pattern of distribution of N within the chloroplast was not affected. But in V2 the reduction in PS2 activity due to low N was more prominent than reduction in whole electron transport chain. This can be due to closure of fraction of PS2 centres under stress (Giardi *et al.* 1996). In our earlier study, the cell number per unit leaf area was not changed with the leaf N content (Sivasankar *et al.* 1998b). Probably, under optimal N supply, with increasing N content per unit leaf area, higher proportion of N goes towards thylakoid and Calvin cycle enzymes.

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