PHOTOSYNTHETICA 45 (1): 147-152, 2007

BRIEF COMMUNICATION

Photosynthetic variation and photosynthetic nitrogen use efficiency in *Brassica* species with different genetic constitution of ribulose-1,5-bisphosphate carboxylase

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Abstract

Net photosynthetic rate (P_N) was high in genotypes with 'C' genome both in the nucleus and cytoplasm. This may be attributed to the co-ordinated manner of acting of both genome sources. Leaf mass per area (LMA) and chlorophyll content increased with leaf nitrogen (N) content but did not show any correlation with P_N. The factors which affected P_N had the same effect on photosynthetic nitrogen use efficiency (pNUE). Thus, differential allocation of N to the various components influences plant pNUE which is not significantly affected by genome constitution.

Additional key words: chlorophyll; leaf mass per area; net photosynthetic rate.

In the past, close correlations were found between crop yields and nitrogen (N). N is an essential component of the biochemical constituents that drive the yield-producing processes. Increase of total production requires sufficient N to reach asymptote, but to achieve the greatest production with the least N requires optimization, which occurs at less than maximum production (Lawlor *et al*. 2001). Crop species differ in their response to N in terms of their capacity to produce biomass. Photosynthetic nitrogen use efficiency (pNUE), the ratio of net photosynthetic rate (P_N) to leaf N content, is an important parameter in determining the response of leaf N to carbon exchange rate.

The differences in pNUE observed between species may arise from environmental conditions, morphological, genetic, physiological, or biochemical features of plants. An evidence for the relationship between leaf N content and leaf characteristics which influence P_N exists. Leaf area (Kemp 1980, Gastal and Lemaire 2002) and CO_2 exchange rate (Bolton and Brown 1980, Caemmerer and Farquhar 1981, Sage and Pearcy 1987b, Evans and Terashima 1988, Evans 1989, Press *et al*. 1993) increase with increased levels of N. N nutrition may affect P_N through leaf and mesophyll conductance. Other factors such as leaf mass per area (LMA) or partitioning of dry matter to stem may also affect pNUE (Brown and Wilson 1983). At the biochemical level, high N supply increased leaf protein and chlorophyll (Chl) contents and content of several enzymes involved in CO_2 fixation (Wong 1979, Hesketh *et al*. 1981, Girardin *et al.* 1985, Evans 1989, Ogunella *et al*. 1989).

The basis of variation in pNUE is therefore not well established and hence we tried to gain an insight into the relationship between leaf N content, P_N, and related leaf characteristics. This relationship was assessed with respect to different genome constitutions of the genotype resulting in diverse varied types of photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase (RuBPC). *Brassica* species, which have well-characterized cytoplasmic and nuclear substitution lines and ploidy differences, offered an ideal experimental material.

Seeds were obtained from the collections maintained

Received 24 February 2006, *accepted* 18 September 2006.
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Abbreviations: DAS – Days After Sowing; DM – dry mass; FM – fresh mass; LMA – leaf mass per area; LN – leaf nitrogen; N – nitrogen; P_N – net photosynthetic rate; pNUE – photosynthetic nitrogen use efficiency; RuBPC – ribulose-1,5-bisphosphate carboxylase.
Acknowledgements: We thank Dr Shyam Prakash for providing the seed material, and Professor and Head of the Division of Plant Physiology and Project Director NRL for providing the necessary facilities. The senior author is thankful to Director IARI for financial assistance to carry out the work.

Table 1. Euploid, amphiploid, and alloplasmic lines of *Brassica* used in this study: their nuclear and cytoplasmic genome constitution, net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}], leaf mass per area (LMA) [g m^{-2}], and total chlorophyll (Chl) [mg m^{-2}] content. Means of three replicates at 50 and 60 d after sowing (DAS). C.G. = cytoplasmic genome; N.G. = nuclear genome; S.C. = somatic chromosome; *capital letter denotes the origin of large subunit from the cytoplasmic genome and small letter explains the origin of small subunit from nuclear genome. CD at 5 % for P_N (G = genotype, S = sampling date): G = 2.54, S = 1.04, G×S = 3.59; for LMA: G = 0.44; S = 0.18; G×S = 0.62; for Chl: G = 5.47; S = 2.23; G×S = 7.74.

Plant genotype	N.G.	S.C.	C.G.	RuBPC*	P_N DAS			LMA			Chl		
		No.			50	60	mean	50	60	mean	50	60	mean
B. campestris (Pusa Kalyani)	AA	20	[A]	Aa	16.9	12.9	14.9	27	32	29	263	343	303
B. nigra	BB	16	[B]	Bb	8.9	13.7	11.3	30	21	26	217	346	281
B. oleracea (Knol khol)	CC	18	[C]	Cc	17.2	16.9	17.1	42	44	43	284	421	352
B. juncea (Pusa Bold)	AABB	36	[A]	Aab	15.7	9.4	12.6	31	36	33	303	334	318
B. napus – excel	AACC	38	[C]	Cac	15.6	13.9	14.7	32	36	34	384	352	368
B. carinata-1	BBCC	34	[B]	Bbc	15.1	10.6	12.8	33	32	33	336	332	334
B. carinata-241	BBCC	34	[C]	Cbc	14.2	13.1	13.6	30	36	33	317	399	359
B. napus-706	AACC	38	[A]	Aac	9.4	9.4	9.4	27	30	28	304	297	301
B. oxyrrhina	OO	18	[O]	Oo	8.7	5.6	7.2	34	46	40	263	466	365
B. oxycampestris	OOAA	38	[O]	Oao	15.8	8.4	12.1	39	32	36	399	361	379
CMS (oxy) *B. campestris*	AA	20	[O]	Oa	16.2	11.6	13.9	27	31	29	245	259	252
CMS (oxy) *B. juncea*	AABB	36	[O]	Oab	15.5	9.1	12.3	26	33	29	228	278	253

by Dr. Shyam Prakash, NRCPB, New Delhi, India. The genotypes included a number of synthetic amphiploids derived from interspecific hybridisation, their euploid parents, and the alloplasmic lines (Table 1). The alloplasmic lines differed in the large subunit of RuBPC as the nuclear genome has been substituted into various cytoplasms. Plants were raised in pots kept outdoors at Indian Agricultural Research Institute, New Delhi, India (28º4′, 77º9′E, 228 m a.s.l.). Five plants were grown in each pot filled with 10 kg of 3 : 1 mixture of sandy loam soil to farm yard manure under identical cultivation conditions. There were three replicate pots for each genotype. NPK equivalent to 60 : 30 : 30 kg per ha, respectively, was supplied in four equal split doses. The plants were watered at weekly interval. Sampling was done at 50 and 60 DAS. At each sampling date, measurement was made on the youngest fully expanded leaf of three randomly selected plants from three pots. P_N was measured using portable IRGA (model *6000*, *LiCor*, USA). After the gas exchange measurements the leaf was excised and its area measured; fresh mass (FM) of the same leaf was also recorded. The leaves were then finally sliced and a representative sample was used for measurement of Chl content following non-maceration technique of Hiscox and Israelstam (1979). The remaining leaf slices were dried to a constant mass to estimate dry mass (DM) and N content. Leaf N content was estimated following a modified Kjeldahl procedure with the help of N-autoanalyser (Technicon Monograph 1 1971). RuBPC protein content in the crude extracts was estimated by polyacrylamide gel electrophoresis following the procedure of Rintamäki *et al*. (1988) and quantified using purified RuBPC run along with the extracts. Fresh leaf samples were ground in Tris-HCl buffer, pH 8.0, and the soluble protein present in the crude extract was determined by filter paper binding assay of Minamide and Bamburg (1990). Calibration curve was prepared with bovine serum albumin treated similarly as the crude extracts. The mean values of the different parameters of all the three replicates were subjected to statistical analysis using *MSTAT-C* package. In the following text, the nuclear genome of the genotypes is indicated in double letters whereas cytoplasmic genome as letters in parentheses.

Average P_N ranged from 7.2 to 17.1 μmol(CO_2) m^{-2} s^{-1} in the 12 genotypes examined. *B. oleracea* genotypes with CC[C] genome showed the highest P_N, followed by *B. campestris* with AA[A] genome and *B. napus* genotypes having AC[C] genome. *B. oxyrrhina*, the only wild species included in this study, showed extremely low P_N (Table 1). Sampling time also had a significant influence on P_N in most of the genotypes. Seasonal mean LMA also showed variation from 26 to 43 g m^{-2} (Table 1). Low and high values of LMA were recorded in the diploid, *B. nigra* with BB[B] genome had the lowest LMA, whereas *B. oleracea* with CC [C] genome was characterized by the highest LMA. CMS (oxy) *B. juncea* and CMS (oxy) *B. campestris* had low Chl content, and *B. oxycampestris* followed by *B. napus* and *B. oxyrrhina* high Chl content (Table 1).

Large variability in leaf nitrogen (LN) content ranging from 137.2 to 252.4 mmol m^{-2} was observed (Table 2). The lowest content was observed in CMS (oxy) *B. campestris* and *B. nigra* and the highest one in the wild species *B. oxyrrhina* and *B. oleracea*. Both the *carinata* genotypes had similar LN content even though they possessed different cytoplasmic genome. Out of the two *napus* genotypes, cv. 706 having [A] cytoplasmic genome had significantly lower LN content than cv. excel

Table 2. Leaf N content [mmol m^{-2}] and photosynthetic nitrogen use efficiency (pNUE) [nmol(CO_2) $mmol^{-1}$(N) s^{-1}] of *Brassica* genotypes varying in genome constitution. Means of three replicates at 50 and 60 DAS. CD at 5 % for leaf N (G = genotype, S = sampling date): G = 27.06, S = 11.04, G×S = 38.26; for LMA: G = 11.88; S = 4.85; G×S = 16.80.

Plant genotype	RuBPC	Leaf N DAS			pNUE		
		50	60	mean	50	60	mean
B. campestris (Pusa Kalyani)	Aa	168.3	202.2	185.2	100.4	63.7	82.1
B. nigra	Bb	153.4	125.3	139.4	58.0	110.5	84.2
B. oleracea (Knol khol)	Cc	223.4	256.1	239.8	77.1	66.8	71.9
B. juncea (Pusa Bold)	Aab	157.3	152.5	154.9	99.1	63.1	81.1
B. napus – excel	Cac	198.5	205.7	202.1	78.8	67.2	72.9
B. carinata-1	Bbc	179.0	173.5	176.2	84.6	60.8	72.7
B. carinata-241	Cbc	155.2	201.3	178.2	91.3	65.1	78.2
B. napus-706	Aac	174.9	164.1	169.5	55.0	57.8	56.4
B. oxyrrhina	Oo	224.5	280.3	252.4	39.8	20.4	30.1
B. oxycampestris	Oao	256.0	206.8	231.4	62.8	41.1	51.9
CMS (oxy) *B. campestris*	Oa	129.8	144.7	137.2	128.9	80.7	104.8
CMS (oxy) *B. juncea*	Oab	143.8	146.0	144.9	107.7	62.5	85.1

with [C] cytoplasmic genome. No ploidy effect was observed for leaf N content; on the contrary, both the highest and lowest values were observed in diploids (Table 2).

Contents of soluble and RuBPC proteins increased with increasing LN and LMA. The former parameter showed a correlation of $r = 0.578^{*}$ with LN and $r = 0.615^{*}$ with LMA. On the other hand, the RuBPC protein showed a stronger correlation with LN ($r = 0.752^{**}$), LMA ($r = 0.776^{**}$), and soluble protein content ($r = 0.868^{**}$). Similarly, increasing content of LN also enhanced Chl content with the result of a fairly good correlation between total Chl and LN contents ($r = 0.875^{**}$) or LN content and LMA ($r = 0.849^{**}$).

Calculation of pNUE from P_N and LN content showed variability from 30.1 to 104.8 nmol(CO_2) $mmol^{-1}$(N) s^{-1} among the species. CMS (oxy) *B. campestris* showed the highest pNUE, while *B. oxyrrhina* was characterized by the lowest values of this parameter. Altogether there was no effect of ploidy or genome constitution on pNUE (Table 2).

The wild species *B. oxyrrhina* showed the lowest P_N. Contrary to our results, a tendency of the wild species to show markedly higher P_N compared to the cultivated ones was found for wheat, sorghum, pearl millet, and cotton (Khan and Tsunoda 1970, Gifford and Evans 1981). An effect of ploidy on P_N was observed in *Festuca* (Byrne *et al.* 1981), *Solanum phureja* (Pehu *et al.* 1988), and *Triticum* (Austin *et al.* 1982). However, no visible effect of ploidy on P_N was found in the present investigation. Actually, both the highest and lowest values were noticed among the diploids. However, in another study with 19 genotypes of *Brassica* no species-dependent differences have been observed (Hobbs 1988).

The chloroplast genome plays a significant role in the genetic control of photosynthesis. Uprety *et al.* (1990) attributed the high photosynthetic efficiency of *B. carinata* to the effect of chloroplast genome from the maternal parent. However, variability in P_N found among *Brassica* genotypes used in our study could not be attributed to chloroplast genome. Genotypes possessing the same chloroplast genomes but differing in nuclear genome showed variation in P_N. The control of P_N also did not reside entirely with nuclear genome, though Johnson *et al.* (1988) had attributed high P_N in wheat to the effect of nuclear genome. In the present study, an observation of significance was that the presence of 'C' genome both in the nucleus and cytoplasm resulted in high P_N. For instance, *B. oleracea*, *B. carinata* cv. 241, and *B. napus* cv. excel (having CC[C], BC[C], and AC[C] genomes, respectively) had high P_N. It is therefore likely that P_N in *Brassica*, similarly to other species, is under the joint control of both nuclear and chloroplast genomes acting in coordination.

An important leaf characteristic that very often (Hesketh *et al.* 1981, Hobbs 1988), but not always (Brinkman and Frey 1978), influences P_N is LMA or leaf thickness. However, we did not find correlation between LMA and P_N, which is similar to the findings of Heichel and Musgrave (1969) in maize. On the other hand, Hobbs (1988) and Suresh *et al.* (1996) in *Brassica* species showed the existence of significant positive relationship between the two parameters, and El-Sharkawy *et al.* (1965) found a negative relationship in cotton.

To capture the energy used in photosynthesis and convert it into chemical energy efficiently, a large amount of Chl per unit area is needed. These Chls exist in light-harvesting protein complexes (LHPC) (Sage and Pearcy 1987a) and reaction centre proteins in the thylakoid membrane (Okamura *et al.* 1982). Ellison *et al.* (1983) found a positive relationship between flag leaf P_N and Chl contents in wheat. Other studies also showed association between genetic variability in P_N and Chl content (Buttery and Buzzell 1977, Hobbs and Mahon 1982,

Suresh *et al.* 1996). However, in our investigation of *Brassica* species no relationship was found between P_N and Chl.

All the bichemical and photobiological processes of the photosynthesis require several nitrogenous compounds and hence as much as 30-50 % of the leaf N is concentrated in chloroplast (Field and Mooney 1986, Evans 1989). Increase in P_N is generally a linear function of leaf N content (Yoshida and Coronell 1976, Bolton and Brown 1980, Field and Mooney 1986, Evans 1989, Press *et al*. 1993, Lynch and Rodriguez 1994, Reddy *et al*. 1996, Suresh *et al.* 1996, Grindlay 1997). Correlation coefficients from 0.51 to 0.97 have been reported when both parameters were expressed per leaf surface area. A corollary of this relationship is that if P_N is to be enhanced, there is a need for increasing the N content per unit area. This can be brought about by two ways: (*1*) through increasing the concentration of N per unit mass, and (*2*) by increasing leaf thickness or LMA. In the 12 *Brassica* genotypes we examined, both factors contributed to the increase of LN content per unit leaf area, though the actual N content contributed to a lesser extent compared to LMA. The contribution of LMA to the LN increase ranged from 3.6 % [CMS (oxy) *B. campestris* and *B. napus*] to 48.3 % [*B. oleracea* (knol khol)] while increase in N content ranged from 23.5 to 74.8 %. In four genotypes, viz. CMS (oxy) *B.campestris, B. juncea* (cv. Pusa Bold), *B. carinata* (cv. 1), and *B. oleracea* (Knol khol), the increase in LN came entirely from the increase in LMA, while in two other genotypes, *B. juncea* (alloplasmic) and *B. campestris,* the contribution from the actual increase in N content was marginally higher than increases in LMA. The overriding role of LMA in increasing LN led to a strong correlation between the two ($r = 0.887^{**}$). The increase in leaf thickness usually results from the presence of additional cell layers, which means that there would be greater requirement of N to synthesise soluble and structural proteins and other N requiring compounds like Chl and nucleic acids. In a study with herbaceous community, Hirose and Werger (1994) attributed changes in LN within a species to the differences in N content, while the differences between species were due to the differences in their LMA.

The strong correlations between LN, LMA, and soluble protein indicate preferential allocation of soluble N for the synthesis of proteins. Thus, increase in LMA may lead to increment in Chl content, soluble proteins, and the major component of soluble protein in C_3 leaves, *i.e*. RuBPC or *vice versa*. This should ultimately lead to linear increase in P_N with increasing LN. However, no correlation was found between P_N and LN content when data obtained from twelve *Brassica* genotypes were considered together. Similarly, no significant relationship existed between P_N and LMA or Chl content, but a positive relationship (though not very strong) was observed between P_N and soluble protein ($r = 0.364$) or RuBPC ($r = 0.322$) contents indicating that P_N is more related to the partitioning of N into soluble components, especially the RuBPC protein, than to total N content or structural components.

B. oxyrrhina which had the highest LN and LMA showed the lowest P_N. When this genotype was excluded from the analysis, strong correlations between P_N and LN ($r = 0.495$) or LMA ($r = 0.655^{*}$) indicated that photosynthesis/N relationship can be drastically altered by other factors not requiring nitrogenous constituents.

pNUE declined with increase in leaf N content among genotypes, resulting in a negative relationship between the two ($r = –0.777^{**}$) while it was positively related to P_N ($r = 0.623^{*}$). The negative relationship between LN and pNUE may result from the inefficient partitioning of N into compounds involved in photosynthetic reactions with increasing leaf N. Factors that affect P_N negatively had the same effect on pNUE as P_N and pNUE were positively related. This implies that the carbon economy of the plant is closely connected to the N economy. The allocation of N to different compounds thus affects plant efficiency with respect to the use of N. Field and Mooney (1986) also reported that pNUE is positively related to P_N but negatively to LN. Inverse relationship between LN and pNUE has also been reported by Hirose and Werger (1994). Based on a model, Hikosaka and Terashima (1995) predicted that at low N availabilities low leaf N contents are advantageous in terms of NUE.

Biochemical basis for the genotypic variation in pNUE has been attributed to the differences in the partitioning of N between structural and soluble components and to the differences in the amount, specific activity, and kinetic properties of RuBPC (Seeman *et al*. 1984, Evans and Austin 1986, Makino *et al*. 1987). RuBPC has low catalytic rate per mass of protein, so P_N commonly found in leaves of C_3 plants require a large amount of RuBPC. The amount of RuBPC may reach 8 g m^{-2} and constitute up to 30 % of N in wheat leaves and up to 50 % of the soluble protein (Lawlor *et al.* 1989). Actively metabolizing leaves contain correspondingly large amount of N. If N supply during leaf growth is lower than that required to sustain the potential rate of protein synthesis, the amount of components formed is inadequate for maximum P_N (Lawlor *et al.* 1989). In *Brassica* genotypes used in our study, considerable variation was found in the contents of soluble protein, RuBPC, and Chl per unit N, the latter being indicative of the differences in Chl-binding proteins or enzymes participating in Chl synthesis and/or degradation. Nevertheless, no significant relationship was found between pNUE and RuBPC and Chl when expressed on N basis. In *B. nigra* a positive correlation between pNUE and soluble protein per unit N or RuBPC per unit N was observed, though this did not apply to the relationship with Chl per unit N. The only attribute which contributed to high pNUE of *B. nigra* was that it contained much Chl per unit N, indicating a better photon capturing capacity of this genotype. The maximum pNUE may be limited under natural conditions by

reductions in P_{max} due to low irradiance or temperature. For this reason, Field and Mooney (1986) described pNUE as the potential photosynthetic NUE as it may not reflect the resource efficiency under varying natural conditions and because leaf P_N is generally measured under optimum conditions.

We conclude that pNUE is not affected by ploidy level or genome constitution of the *Brassica* species. Increase in leaf N resulted in genotypes less efficient with respect to pNUE which shows that N is not partitioned to the photosynthesis reactions.

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