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BRIEF COMMUNICATION

Ribulose-1,5-bisphosphate carboxylase/oxygenase content and activity in wheat, rye and triticale

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Abstract

Photosynthetic parameters were measured in triticale and its parents wheat and rye. Soluble protein content in leaves, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content per fresh mass, total chlorophyll content, biomass yield, leaf area, leaf mass and specific leaf mass were higher but Rubisco content expressed as percentage of soluble protein, carboxylase activity, photosynthetic rate and stomatal conductance were significantly lower in rye than in wheat. Native-PAGE of Rubisco revealed that rye carboxylase was different from that of wheat. The difference was not related to either the small or large subunit of Rubisco but, may be, to the ionic and/or other properties of the Rubisco protein moiety. Triticale Rubisco was similar to wheat. For most of the studied physiological parameters, triticale showed much more similarity with wheat than with rye.

Additional key words: chlorophyll content, net photosynthetic rate, *Secale cereale*, stomatal conductance, *Triticum aestivum*, *Triticale octoploide*.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) is a bifunctional enzyme located in the chloroplast stroma. It catalyses photosynthetic CO_2 fixation to ribulose-1-5-bisphosphate (RuBP) and the first step of photorespiratory metabolism by oxygenation of the same substrate (Jensen and Bahr 1977). The enzyme constitutes up to 60 % of soluble leaf proteins (Ellis 1979). Genetic variability of Rubisco content and of carboxylase activity has been reported for barley and wheat (Nicco *et al.* 1993). In barley, variability has also been shown for net photosynthetic rate (P_N), leaf area and protein and chlorophyll contents (Sarrafi *et al.* 1987). A positive correlation between Rubisco activity and P_N was found by Ecochard *et al.* (1990). Triticale, a hybrid of wheat and rye, exhibits P_N significantly different from those of its parents (Uprety *et al.* 1987, Bobodzhanov *et al.* 1990). Triticale was shown to exceed wheat and rye in terms of leaf area, photosynthetic rate and other photosynthetic indices (Bobodzhanov *et al.* 1990), but total dry matter accumulation rate was consistently higher for both triticale and rye than wheat (Winzeler *et al.* 1989). However, the physiological and biochemical leading to such variations attributes have not been analysed. We report here, the differences in the amount of Rubisco, its carboxylase activity, its type and photosynthetic efficiency among wheat, rye and triticale.

Triticale (*Triticale octoploide* cv. DT-46) and its parents wheat (*Triticum aestivum* L. cv. PBW-34) and rye (*Secale cereale* L. cv. R-308), procured from the Division of Genetics, Indian Agricultural Research Institute, New Delhi, were raised in rectangular concrete pots (200 × 75 × 75 cm) in sandy loam soil under natural conditions. Ten plants were maintained per pot. Plants were fertilized with N, P and K corresponding to 100 kg(N) ha^{-1}, 60 kg(P) ha^{-1} and 40 kg(K) ha^{-1} for maintaining optimal growth. The newly formed fully expanded leaves (third or fourth from the base) were used for estimation of leaf area, P_N, Rubisco activity, and other biochemical attributes.

Soluble leaf protein content was determined by the method of Lowry *et al.* (1951). Carboxylase activity was determined by radiochemical method according to Raghavendra and Das (1977) as described by Nicco *et al.* (1993). A mixture of $NaH^{14}CO_3$ (specific activity 125.17 GBq mol^{-1}) and 20 μM $NaHCO_3$ along with 0.1 μM EDTA, 3.03 μM $MgCl_2$, 30 μM Tris HCl and 0.2 μM

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Abbreviations: LAR - leaf area ratio; P_N - net photosynthetic rate; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase.

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RuBP (Fair *et al.* 1973) were used in the assay medium and the incorporated radioactivity in the acid-stable fraction was determined by scintillation spectroscopy (*Packard TRICARB*-Model 1600TR, Meriden, USA) at the Nuclear Research Laboratory of Indian Agricultural Research Institute, New Delhi. The amount of Rubisco in the crude extract was estimated by polyacrylamide gel electrophoresis following the procedure of Rintamaki *et al.* (1988). Partially purified ribulose-1-5-bisphosphate carboxylase (*Sigma*, St. Louis, USA) was run along with the crude extract. Protein in the crude extract was separated on a 6 % native PAGE with discontinuous electrophoresis buffer system of Laemmli (1970). 4 % stacking gel was used. An electrophoresis current of 10 mA was applied initially and then increased to 30 mA. After 4 h, the gel was removed and stained overnight with 0.1 % (m/v) Coomassie brilliant blue (R-250). The gels were destained in water:methanol:glacial acetic acid mixture (45:45:10 v/v) and relative mobility of Rubisco was observed. Depending on the mobility, the Rubisco was designated as fast or slow. The amount of Rubisco protein was estimated by cutting the stained bands of Rubisco holo-protein from the non denaturing gels and extracting in 4 cm^3 of 1 % (m/v) aqueous sodium dodecyl sulfate (SDS) for 24 h at 4 °C in a shaking water bath. The absorbance was measured at 585 nm and the amount of Rubisco was determined. Rubisco subunits, large and small, were seperated according to the method of Mummenhoff and Hurka (1986) using SDS-PAGE on 12 % (m/v) resolving gel with 4 % (m/v) polyacrylamide gel stacker. The buffer system of Laemmli (1970) was used. Rest of the procedure was similar to that used for native-PAGE.

Leaf P_N and stomatal conductance were measured using portable infrared gas analyzer (*LICOR-6200*, Lincoln, USA). The observations were taken three hours after sunrise on two consecutive days for five plants selected at random from each of the three replicates. After determining P_N, the same leaves were sampled for estimation of leaf area, which was measured using a leaf area meter (*Hayashi Denkoh Co. Ltd.*, Tokyo, Japan). Specific leaf mass (SLM) was calculated by dividing leaf dry mass by its area. Whole plant biomass and leaf area ratio were also estimated.

Among the three species, rye showed the highest leaf protein content while it was more or less similar for wheat and triticale (Table 1). Rubisco content of those also showed similar pattern when expressed on a fresh mass basis but when expressed as percentage of total soluble proteins the Rubisco content of rye was significantly lower than that of wheat and triticale due to low amount of leaf protein and high enzyme content. The observed values are comparable with those reported by Martinez-Bustamante (1987) for different wheat cultivars. In rye, higher Rubisco content was not connected with higher carboxylase activity. Wheat and triticale, despite their lower Rubisco content, showed carboxylase activities similar to those of rye. Specific carboxylase activity of rye was appreciably lower than that of the other two species. High enzyme content does, therefore, not always correspond to high carboxylase activity (Nicco *et al.* 1993).

Table 1. Soluble leaf protein content and Rubisco content and activity in wheat, rye and triticale. Means ± SE, $n = 10$ (CD - critical difference at $P = 0.05$, n.s. - non significant).

	Protein content $[\mathrm{mg\ g^{-1}(d.m.)}]$	Rubisco content $[\mathrm{mg\ g^{-1}(d.m.)}]$	[% of soluble protein]	Rubisco activity $[\mathrm{\mu mol(^{14}CO_2)\ kg^{-1}(d.m.)\ s^{-1}}]$	$[\mathrm{\mu mol(^{14}CO_2)\ g^{-1}(Rubisco)\ s^{-1}}]$	Mobility
Wheat	94.9 ± 0.08	59.2 ± 0.8	62.4 ± 0.5	17.06 ± 0.16	0.288 ± 0.0033	slow
Rye	176.9 ± 0.12	102.5 ± 1.3	57.9 ± 0.7	16.56 ± 0.17	0.161 ± 0.0050	fast
Triticale	92.0 ± 0.08	56.5 ± 0.8	61.4 ± 0.8	16.77 ± 0.19	0.297 ± 0.0031	slow
CD	12.7	5.4	2.7	n.s.	0.008	

Furthermore, native-PAGE revealed clear cut variation of Rubisco properties among the three genotypes (Table 1, Fig. 1). Rubisco, of rye, showed faster mobility than that of wheat and triticale. This variation in the mobility of Rubisco may be due to difference in either of the small (nuclear DNA encoded) or large (chloroplast DNA encoded) subunit of Rubisco. SDS-PAGE of the enzyme (Fig. 2), however, revealed no difference for the small or the large subunit of Rubisco among the studied genotypes. This suggests that the difference in the mobility of Rubisco, observed among rye and wheat or triticale (Fig. 1) may be related to ionic or other properties of the Rubisco protein moiety rather than its molecular mass. For all the above physiological parameters, triticale followed wheat more than rye.

The lowest P_N was recorded in rye, while it did not vary significantly between wheat and triticale (Table 2). The low carboxylase activity of rye may be one of the reasons for its low photosynthetic rate. These results find support of Winzeler *et al.* (1989) and of Bobodzhanov *et al.* (1990). Further, stomatal conductance was also significantly lower in rye than in triticale and wheat which might limit CO_2 transport (Table 2). Observations on stomatal conductance of rye leaves was in contrast to that reported by Uprety and Sirohi (1987). The chlorophyll content was maximum in rye followed by triticale and wheat.

The three species also varied significantly in the growth parameters (Table 3). Rye produced highest biomass followed by wheat and triticale. The observation find support in Winzeler *et al.* (1989) who showed a consistently greater dry matter accumulation in rye and triticale than in wheat. Rye also recorded higher leaf area and leaf mass as compared to other two species. However, differences for these attributes were significant even between wheat and triticale. Triticale showed the least leaf area. Bobodzhanov *et al.* (1990), on the contrary, have reported that triticale exceeds wheat and rye in leaf area. Leaf area ratio was maximum in wheat followed by rye and triticale. Specific leaf mass did not vary significantly between rye and triticale. A higher SLM for rye and triticale, compared to wheat, was due to higher leaf mass and lower leaf area.

Table 2. Photosynthetic rate, stomatal conductance, and chlorophyll content in wheat, rye and triticale. Means ± SE, $n = 10$; CD - critical difference at $P = 0.05$.

	Photosynthetic rate [μmol(CO_2) $m^{-2} s^{-1}$]	Stomatal conductance [cm s^{-1}]	Chlorophyll content [mg g^{-1}(d.m.)]
Wheat	20.9 ± 0.50	0.755 ± 0.023	63.8 ± 0.8
Rye	14.1 ± 0.29	0.192 ± 0.060	89.0 ± 0.8
Triticale	18.2 ± 0.72	0.770 ± 0.036	78.4 ± 0.6
CD	3.0	0.181	5.2

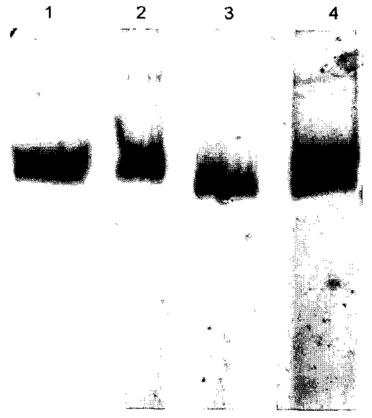

Fig. 1. Native-PAGE of Rubisco from wheat (*1*), triticale (*2*), rye (*3*), and combination of wheat, triticale, rye (*4*).

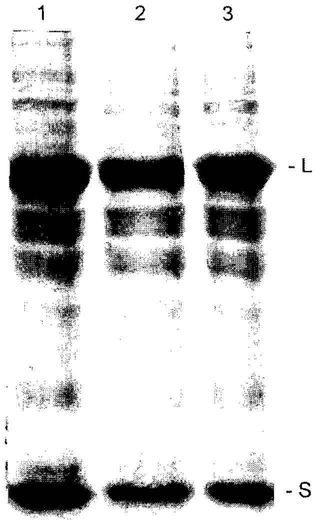

Fig. 2. SDS–PAGE of Rubisco showing large (*L*) and small (*S*) sub-unit of Rubisco in wheat (*1*), triticale (*2*) and rye (*3*).

Table 3. Growth parameters in wheat, rye and triticale. Means ± SE, $n = 10$; CD - critical difference at $P = 0.05$.

	Biomass [g(d.m.) $plant^{-1}$]	Leaf area [cm^2 $plant^{-1}$]	Leaf mass [mg(d.m.) $plant^{-1}$]	Leaf area ratio [cm^2 g^{-1}(plant d.m.)]	Specific leaf mass [mg(d.m.) cm^{-2}]
Wheat	2.565 ± 0.25	141.0 ± 4.8	269.1 ± 3.7	55.0 ± 0.87	1.90 ± 0.12
Rye	5.298 ± 0.37	246.4 ± 5.2	548.1 ± 5.2	46.5 ± 0.89	2.37 ± 0.18
Triticale	1.971 ± 0.32	79.9 ± 4.3	189.4 ± 3.5	40.5 ± 0.72	2.22 ± 0.13
CD	1.84	25.4	28.2	3.2	0.17

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