Effect of water stress on carbon isotope discrimination and Rubisco activity in bread and durum wheat genotypes

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ABSTRACT

Carbon isotope discrimination (CID) has been suggested as an indirect tool for selection of water use efficiency in wheat. This study evaluates the role of Rubisco activity in regulating carbon isotope discrimination (CID) and discriminate the response of durum (PDW-233) and bread (C-306, HD-2329) wheat genotypes for carbon isotope discrimination and Rubisco activity under induced water stress hydroponically created by using different concentrations of PEG. In C-306, a well known drought tolerant genotype, CID was positively correlated with Rubisco activity. In C-306 CID was positively correlated with both Rubisco activity ($r = 0.588^*$ at 5). A positive but not a strong correlation was observed between Rubisco activity and CID ($r = 0.418$) in durum type PDW-233. Although in HD-2329 the Rubisco activity ($r = 0.303$) was positively correlated with CID, the correlations were not significant. We show that carbon isotope discrimination technique is a potential tool for selection of lines with high Rubisco activity in the wheat breeding program. [*Physiol. Mol. Biol. Plants* 2009; 15(3) : 281-286] E-mail : shailesh_agri@yahoo.com; shailesh.physiology@gmail.com

Key words : Carbon isotope discrimination, Rubisco activity, water stress, bread wheat, durum wheat

Abbreviations : CID, Δ , = carbon isotope discrimination, ‰ = parts per thousand PEG = polyethylene glycol, * = 5% significance level RUBP = Ribulose- 1, 5- bis phosphate, Rubisco = Ribulose -1, 5-bis-phosphate carboxylase/oxygenase, WUE = Water Use Efficiency.

INTRODUCTION

Carbon in atmosphere, is present in more than one isotopic form, with lighter isotope (^{12}C) existing in far greater natural abundance (98.9 %) than heavy isotopes (^{13}C , 1.1 %). The molecules with heavier isotope ($^{13}\text{CO}_2$) diffuse slowly because of mass difference and are also slow in reacting during any biochemical process. Discrimination against the heavy isotope of carbon ($\Delta^{13}\text{C}$) during the diffusion of CO_2 through the stomata and later during the carboxylation reaction is catalyzed by Rubisco. Farquhar, 1989 hypothesized that discrimination (D) is mainly caused in C_3 species by (i) fractionation due to CO_2 diffusion, (ii) changes in stomatal resistance or assimilation rate, which affect the ratio of internal to ambient concentration of CO_2 , and

(iii) fractionation by enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). A strong relationship between carbon-13 discrimination and WUE is established (Shailesh, 2004). Plants fractionate carbon isotope during photosynthesis however, the magnitude of fractionation varies with plant type, environment, genotype and other factors.

The principal components of photosynthesis that influence discrimination are diffusion of CO_2 through stomata and the carboxylation process mediated by Rubisco (O'Leary, 1988, 1993). Drought decreased the initial and total Rubisco activity but did not reduce the amount of Rubisco protein per unit leaf area (Medrano *et al.*, 1997). WUE as well as yield levels can be enhanced only when mesophyll types (g_m) selected are, used in breeding program (Von Caemmerer and Farquhar, 1981) or by determining certain mesophyll component such as Rubisco content and its activity (Hudson *et al.*,

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1994; Mate *et al.*, 1998). Marques *et al.* (1995) showed that Rubisco activity decreased slightly at moderate water deficit and sharply at severe drought. A reduction in rate of reaction catalyzed by Rubisco was caused by change in availability of CO₂ at chloroplast level or the availability of RUBP or the loss of Rubisco capacity. They further concluded that the primary mechanism for inhibition of photosynthesis under both mild and severe water deficit is a reduced CO₂ supply to Rubisco, as the intercellular CO₂ decrease under stress condition (Marques *et al.*, 1995).

Hence, the present investigation was conducted with two objectives: 1) to evaluate the contribution of Rubisco activity in regulating foliar carbon isotope discrimination and 2) to discriminate the response of durum and bread wheat genotypes for carbon isotope discrimination and Rubisco activity under induced water stress.

MATERIAL AND METHODS

Plant materials

Bread (C-306, HD-2329) and durum (PDW 233) wheat genotypes procured from the Division of Genetics & Plant Breeding, IARI, New Delhi, India, were used for the present investigations.

Experimental conditions

All the plants were grown in nutrient sufficient solution culture under controlled conditions in the National Phytotron facility of the Institute. The plants were exposed to water stress after a stable plant growth were achieved (i.e. at 35 days after transplanting) using different concentration (10, 20 and 30 %) of PEG-6000 and physiological and biochemical observations were taken at 24, 48 and 72 hours after PEG treatment. PEG was used to induce water stress because it is an inert polymer, with a non-ionic and virtually impermeable long chain (Couper and Eley 1984) which helps to maintain uniform water potential during the whole experimental period.

Carbon isotope discrimination (CID)

For determination of carbon isotope discrimination, leaves of different genotypes at different PEG treatments were randomly detached, oven dried at 80 °C till constant weights were achieved. Carbon-13 discrimination (Δ) was measured in ground samples with the help of Isotope Ratio Mass Spectrometer (IRMS) at the National facility

for stable isotope studies in biological science at the Department of Crop Physiology, UAS, Bangalore.

Rubisco activity

Estimation of ribulose-1, 5-bisphosphate carboxylase / oxygenase (Rubisco; EC 4.1.1.39) activity was done using radiochemical method (Raghavendra and Das, 1977) as described by Nicco *et al.*, 1993. A reaction mixture consisting of enzyme extract, NaH¹⁴CO₃ (specific activity 125.17 GBq mol⁻¹) and 20 μ M NaHCO₃ along with 0.1 μ M EDTA, 3.03 μ M MgCl₂, 30 mM Tris HCl and 0.2 μ M RuBP (Fair *et al.*, 1973) were used in the assay medium and the incorporated radioactivity was measured by scintillation spectroscopy (Packard TRICARB- Model 1600 TR, Meriden, USA) at the Nuclear Research Laboratory, IARI, New Delhi.

Statistical analysis

The data collected were analyzed statistically by using factorial completely randomized design (CRD). The values of observation have been represented as means of three replications in all the experiments. The CD was calculated at 5 % and expressed for all the possible interactions between stage, genotypes and treatments.

RESULTS

Carbon isotope discrimination

Mean carbon isotope discrimination (Δ ¹³C) averaged over PEG treatment and time was significantly higher for HD-2329 (24.1 %) followed by C-306 (23.1 %). A clear distinction in carbon isotope discrimination between bread and durum wheat type was evident with PDW-233 recording lowest relative discrimination value of 22.3 % as compared to bread wheat types. The difference in carbon isotope discrimination (between control and PEG-treated samples) was insignificant at 10 per cent PEG but significant at 20 and 30 per cent PEG. Carbon isotope discrimination, in general decreased with progressive water stress from 0 to 30 per cent PEG. C-306 showed appreciably lower discrimination even under 10 per cent upto 30 per cent PEG treatment, when compared with carbon isotope discrimination in control (0 % PEG) while for the other two genotypes the difference between control and different PEG treatment were of much smaller magnitude (Table 1). There is an applicable reduction in carbon isotope discrimination in C-306 under water stress condition, over control, while for other two genotypes the discrimination under water stress was not much different from those of control. The CID difference in C-

Table 1. ^{13}C isotope discrimination (CID, Δ , ‰) of three wheat genotypes (Bread, C-306, HD-2329; and durum, PDW-233) at 0, 24, 48 and 72 hours after PEG (0, 10, 20 and 30 ‰) application to the nutrient solution (Values in parenthesis are genotypic means; Mean H: 0h, 23.63; 24h, 23.11; 48h, 23.02; 72h, 22.95).

Genotype (G)	(H) Hours after PEG Treatment	PEG Concentration (%) (T)				Mean
		0	10	20	30	
C-306 (23.10)	0	23.77	–	–	–	23.80
	24	23.54	23.62	22.30	22.82	23.10
	48	23.89	22.38	22.20	22.10	22.60
	72	24.07	22.95	22.39	22.50	23.00
	Mean	23.80	23.00	22.30	22.50	–
HD-2329 (24.10)	0	24.38	–	–	–	24.40
	24	24.46	24.16	24.40	24.44	24.30
	48	24.42	23.85	24.43	24.22	24.20
	72	24.50	24.13	24.36	22.37	23.80
	Mean	24.40	24.00	23.90	23.70	–
PDW233 (22.30)	0	22.75	–	–	–	22.80
	24	22.13	22.60	22.07	22.45	22.30
	48	22.58	22.03	22.01	22.17	22.20
	72	22.44	21.92	21.77	22.09	22.10
	Mean	22.50	22.20	22.00	22.20	–
	Mean T	23.56	23.08	22.73	22.80	–
CD at 0.05	G	0.045	GXT	0.091	GXTXH	0.182
	T	0.052	GXH	0.091	–	–
	H	0.052	TXH	0.105	–	–

306 is in conformity with known drought tolerance nature of this genotype. The only problem with C-306 is that the low discrimination in this genotype is accompanied by a low photosynthesis under water stress. PDW-233, under normal conditions, has lowest discrimination but higher photosynthetic capacity. The mean values across time shows a uniform pattern of the variation in carbon isotope discrimination, but in case of C-306, there is marginal increase in the CID value after 72 hours of water stress. This may be attributed to the fact that C-306 is a drought tolerant genotype and after 72 hours it shows recovery from the water stress by increasing the root biomass, and so it is able to discriminate more between the normal and isotopic forms of carbon to be utilized for CO_2 fixation.

Rubisco activity

Differences in Rubisco activity of bread and durum wheat genotypes under normal and water stress condition has been presented in (Table 2). PDW-233 showed highest ($4.0 \mu\text{mol CO}_2/\text{g/hr}$) while C-306 showed least ($2.3 \mu\text{mol CO}_2/\text{g/hr}$) Rubisco activity. The activity when averaged over time of sampling under control (0 ‰ PEG) was highest for PDW-233 ($5.4 \mu\text{mol CO}_2/\text{g/hr}$) as compared to HD-2329 ($3.9 \mu\text{mol CO}_2/\text{g/hr}$) and C-306 ($2.7 \mu\text{mol CO}_2/\text{g/hr}$). The Rubisco activity decreased by 25 and 50 per cent under 10 per cent and 20 per cent PEG treatment respectively when compared to control. When compared at different time after PEG application the Rubisco activity declined appreciably over 0 h upto 48 h but

Table 2. Rubisco activity (μ mol CO₂ fixed/g/hr) of three wheat genotypes (Bread-C- 306, HD-2329; and durum, PDW-233) at 0, 24, 48 and 72 hours after PEG (0, 10, 20 and 30 %) application to the nutrient solution (Values in parenthesis are genotypic means; Mean H: 0h, 4.8; 24h, 2.9; 48h, 2.8; 72h, 2.9).

Genotype (G)	(H) Hours after PEG Treatment	PEG Concentration (%) (T)				Mean
		0	10	20	30	
C-306 (2.3)	0	2.3	–	–	–	2.3
	24	2.2	2.7	2.3	1.9	2.3
	48	3.1	2.9	1.8	2.6	2.6
	72	3.2	1.9	1.8	1.8	2.2
	Mean	2.7	2.5	2.0	2.1	
HD-2329 (2.9)	0	4.0	–	–	–	4.0
	24	2.8	1.8	1.8	3.1	2.4
	48	3.1	2.5	2.8	2.8	2.8
	72	2.9	2.7	2.3	2.8	2.7
	Mean	3.2	2.3	2.3	2.9	–
PDW-233 (4.0)	0	5.1	–	–	–	5.1
	24	6.0	3.6	4.0	2.8	4.1
	48	5.5	4.1	0.5	1.8	3.0
	72	5.1	5.6	0.4	4.1	3.8
	Mean	5.4	4.4	1.7	2.9	–
	Mean T	4.0	3.0	2.0	2.6	–
CD at 0.05	G	0.02	GXT	0.14	GXTXH	0.29
	T	0.08	GXH	0.14	–	–
	H	0.08	TXH	0.17	–	–

increased marginally at 72 h with few exceptions. Bread wheat types C-306 and HD-2329 were able to maintain appreciably higher Rubisco activity even under stress condition than the durum type PDW-233 where reduction in Rubisco activity was almost upto 50 per cent under stress (average of 10, 20 and 30 % PEG) when compared with the activity under control. The low Rubisco activity in C-306 as compared to HD-2329 and PDW-233 may be responsible for lower rates of photosynthesis observed in this genotype. As C-306 is a drought tolerant genotype, it develops profuse rooting (increase in root biomass) under high PEG concentrations; this improves the water use efficiency and thus recovery of the stomatal tissue which got affected under water stress. The recovery of stomatal tissue results in the increased

supply of CO₂ to Rubisco protein and thus, the genotype shows marginal increase in Rubisco activity at 72 hours of water stress.

In C-306 CID was positively correlated with both Rubisco activity ($r = 0.588^*$). A positive but not a strong correlation was observed between Rubisco activity and CID ($r = 0.418$) in durum type PDW-233. Although in HD-2329 the Rubisco activity ($r = 0.303$) was positively correlated with D the correlations were not significant.

DISCUSSION

Carbon isotope discrimination (CID) has been suggested as an indirect tool for selection of water use efficiency

in wheat. Effect of different physiological attributes on carbon isotope discrimination on WUE has also been investigated (Shailesh, 2004). In this present investigation an attempt has been made to evaluate the contribution of Rubisco activity in regulating foliar carbon isotope discrimination and to discriminate the response of durum and bread wheat genotypes for carbon isotope discrimination and Rubisco activity under induced water stress.

Discrimination, in general, declined under water stress for all genotypes. This may be attributed to partial or complete stomatal closure under water stress condition, which cause, diffusion limitation for both CO₂ and H₂O and result in smaller CID. Qingwu Xue *et al.*, 2002 observed that wheat plants under less water stress posses higher CID value. C-306 which is regarded as drought tolerant type showed significant decrease in discrimination under stress compared to control, indicating that this genotype experiences more osmotic stress as compared to other genotypes. Richards *et al.*, 2002 suggested a necessity of selecting low D types under water stress. Our results on variation in carbon discrimination under normal water stress condition showed an applicable reduction in D in C-306 under water stress over control, while for the other two genotypes, the discrimination under water stress was not much different from those of normally grown plants (control). The carbon isotope discrimination difference for C-306 is in conformity with known drought tolerance nature of this genotype.

Our results also found support from the work of Cornic and Briantais *et al.*, 1991 who observed that under severe dehydration stress, photosynthesis may be controlled by chloroplast capacity to fix CO₂ rather than diffusion characteristic. It has been shown in sunflower that non-stomatal limitation of photosynthesis may be related to reduced carboxylation efficiency, reduced RUBP regeneration or reduced amount of functional Rubisco. We show that maximum Rubisco activity decreased slightly at mild dehydration stress and sharply at dehydration stress. The decrease in photosynthesis under severe drought may be due to decrease in Rubisco specific activity. The supply of CO₂ to Rubisco may be limited under dehydration stress either by stomatal closure or by stomatal tissue shrinkage, diminishing the intercellular air space. Drought reduces the initial and total Rubisco activity per unit area but does not reduce the amount of Rubisco protein per unit leaf area. Therefore, the drought tolerant genotypes, like C-306, may be able to increase their photosynthesis as they try to recover from the

dehydration stress by increasing the root biomass. This recovery may allow gradual recovery in stomatal tissue and hence, increased supply of CO₂ to Rubisco, thus, increasing the Rubisco activity.

CONCLUSION AND FUTURE PROSPECTS

In conclusion the present study with three wheat genotype in nutrient solution under moderate to severe dehydration stress revealed that CID was positively related to Rubisco activity. The low D values were recorded for C-306 and PDW-233 both of which showed tolerance to water stress. The present study suggests that we should select for low CID types that are likely to perform better under water stress. Rubisco activity is the major physiological parameter for carbon isotope discrimination of durum and bread wheat genotypes under water stress. This should be tested in other crops also.

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