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EVALUATION OF ADVANCED WHEAT (*TRITICUM AESTIVUM* L.) LINES REVEALED GENETIC VARIATION FOR CARBON ISOTOPE DISCRIMINATION UNDER DROUGHT STRESS

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ABSTRACT

Experiments were carried out in field and controlled conditions at Plant Breeding Institute-Cobbitty, the University of Sydney, Australia. The objective of the study was to determine the relationship of carbon isotope discrimination (CID) with wheat biomass and morphological traits. Results showed significant ($P \leq 0.05$) variation between evaluated wheat advanced lines for flag leaf CID and morphological traits. Correlation and principal component analysis revealed close relationships between the traits such as CID and wheat whole plant biomass obtained under green house. In field conditions CID was showed correlations with grain yield per plant and morphological traits such as plant height and harvest index. Correlation between the CID, grain yield and harvest index was not consistent across environment, it was significantly negative under green house and positive under field conditions. In short, development of high yielding genotypes for stress environment is possible through selection of higher CID. The results further showed that total biomass (TB) and yield (Y) were significantly affected by the environment while plant height and vegetative biomass showed medium heritability. It was concluded that CID was promising criteria for the discrimination of production, wheat germplasm under stress condictions.

KEYWORDS: harvest index; carbon isotope discrimination; yield components; moisture stress; biomass; Pakistan.

INTRODUCTION

Globally water stress is a major production constraint of wheat (*Triticum aestivum* L.). It is important to enhance the germplasm resistance against this abiotic stress. The presence of adequate amount of genetic variation is prerequisite to develop stress resistant cultivars. Genetic variation has been shown to exist within wheat germplasm for drought resistance (Rauf *et al*., 2016; Christy *et al*., 2018; Tricker *et al*., 2018). Therefore, plant breeders evaluate diverse germplasm for estimating the magnitude of genetic variation within germplasm and identifying the source of resistance which can be utilized as parents for the establishment of transgressive segregation or may be directly released as cultivar for targeted condition. Germplasm has been screened on diverse selection criteria related to drought tolerance in wheat such as canopy temperature discrimination (Pinto and Reynolds, 2015), seedling survivability (Tomar *et al.,* 2016), yield (Sukumaran *et al.,* 2018), cuticular waxes and leaf hairiness (Huussain *et al.*, 2019) to select resistant or susceptible genotypes (Rauf *et al*., 2016). However, most of these criteria were associated with high labor, significant impact of environment, related with survival of plant and only measured at crop maturity (Richards, 1996; Rauf *et al.,* 2016). The usefulness of selection criterion was judged on the basis of presence of adequate amount of genetic variation, heritability, positive relationship with yield and could screen the germplasm before the onset reproductive growth phase (Richards, 1996).

In recent years, carbon isotope discrimination (CID) has been emerged as an important selection criterion of drought resistance germplasm and indicates transpiration efficiency of a genotype (Yasir *et al*., 2013; Lobos *et al.,* 2014; Zhang *et al*., 2015; Rauf *et al.,* 2016). The CID has been utilized in various studies which results showed that germplasm was selected through CID and genetic variation for CID existed within diverse germplasm (Yasir *et al*., 2013; Lobos *et al*., 2014; Zhang *et al.,* 2015). However, CID is polygenic traits and loci located at chromosome no 1A, 3A, 4A and 5A (Mora *et al.,* 2015). The trait has shown moderate heritability over range of environment and also showed positive correlation with yield and total biomass under normal environment (Zhang *et al.,* 2015; Munjonji *et al.,* 2018). Moreover, it showed negative relationship with transpiration efficiency thus selection for CID could increase the drought resistance in field (Rebetzke *et al.,* 2008; Foulkes *et al.,* 2016). However, contrasting reports are also present showing that genetic variation for CID was masked by the environmental (Araus *et al.*, 2003). Furthermore, heritability and its relationship with yield under stress were unclear.

On the basis of these findings, wheat germplasm was evaluated for various morphological and carbon isotope discrimination under both green house and field conditions to estimate the magnitude of genetic variation, heritability and correlation coefficient among the traits. The results would help to reveal the usefulness of CID as selection criteria for drought tolerance and its relationship with grain yield under drought stress.

MATERIALS AND METHODS

All studies were conducted in the Plant breeding Institute, University of Sydney.

Green house experiment

Sixty wheat advanced lines were grown under green house conditions. Equal volume of soil was added to pot (30 × 10 cm) and determined soil field capacity by gravimetric method. Fertility of the soil was raised by adding 2% farm yard manure. Two seed of each line was sown in each pot. The germinated seedlings were thin to single plant for geting uniform plant population. Temperature and high intensity was maintained at 27 ± 2°C 700 μmol $m^{-2} s^{-1}$, respectively during growth period. Experiment was carried out in completely randomized design with three replications.

A weighed amount of soil sample was used to make saturated paste. The saturated paste was made by adding given volume of water. The soil paste was weighed after incubation at 70°C to constant weight. The loss in weight was used to calculate the saturation% of the soil. Field capacity was considered 50% of the saturation% of the soil. In non-stress regime, moisture contents were maintained to field capacity throughout the growth period. However, drought stress was induced by maintaining 60% of total field capacity throughout the growth period.

Field experiment

Same set of wheat germplasm lines (sixty wheat advanced lines) were planted in rainfed condition under randomized complete block design (RCBD) arrangements having three blocks. Drought stress was induced by growing the trial without supplemental irrigation and received only rainfall which was below the optimum level. Each line was sown as single row with sixty plants row^{-1}. Agronomic practices were carried out according to commercial production package. Weeds were controlled manually and diseases were scored as absent.

Carbon isotope discrimination

Carbon isotope composition was measured on flag leaves of wheat genotypes collected from green house and field experiments. Carbon isotope discrimination (CID) was calculated from following equations:

$$\Delta = (\delta_a - \delta_p)/(I + \delta_p)$$

Where, δ_a and δ_p are the carbon isotope composition of air and plant material, respectively. Measurement of δ_a was obtained from the greenhouse and field. Carbon isotope analysis of air and plant material was measured according to the method described by Brugnoli & Lauteri (1991). Samples were collected from drought stressed plants of green house and field. For measurement, samples were oven dried and heated to remove CO_2 from the samples. The CO_2 was purified by cryogenic traps. Isotope ratio was determined on mass spectrometer using purified CO_2.

Morphological trait measurement

Morphological traits includes plant height, place length, harvesting index and no. of spkikelets were measured at different growth stages through standard procedures.

Biometrical and statistical analyses

The data was subjected to the analysis of variance in completely randomized design with three replications for green house and RCBD with three blocks for field conditions. Data was subjected to factor analysis to determine the relative importance of traits. Means were used to calculate correlations. All data was analyzed using statistical software package Mini Tab 15. Broad sense heritability was estimated according to the method given by Allard (1960).

RESULTS AND DISCUSSION

Overall mean, ranges and heritability estimates of the evaluated germplasm under drought stress condition of field and green house has been given in Table 1. These estimates showed that traits such as total biomass (TB) and yield (Y) were significantly affected by the environment while traits such as plant height (PH), vegetative biomass (BM) showed medium heritability. Analyses of variance showed significant variation for varieties under both green house and field conditions (Table 1). Data regarding carbon isotope discrimination (CID) obtained from green house and field condition has been separately presented in Table 2 which showed that the magnitude of heritability has been highest for CID in both green house and field conditions. Heritability is an index of usefulness of the plant trait as selection criteria, therefore, CID may be to discriminate germplasm for drought tolerance (Table 2).

Correlation analysis was carried out which showed that yield under drought condition was positively ($P \leq 0.01$) correlated with PH, BM, GW, TB and CID of field conditions. These results suggest the exploitation of these traits for the enhancement of yield under drought conditions (Table 3). Among these traits, CID could potentially be exploited because it is related with drought resistance, with high heritability and have positive relationship with yield.

Table 1. Analysis of variance for carbon isotope discrimination of wheat advanced lines in green house (drought regime) and field condition.

Source of Variation	SS	df	MS	F	P-value	F crit
Green house						
Varieties	453.55	59	7.69	1.56	0.02	1.43
Error	590.39	120	4.92			
Total	1043.94	179				
Field conditions						
Varieties	325.34	59	5.514189	1.61	0.01	1.43
Blocks	3.49	2	1.743327	0.51	0.60	3.07
Error	404.84	118	3.430836			
Total	733.66	179				

Table 2. Mean, minimum, maximum, and heritability (h^2) estimates for various traits i.e. plant height (PH), vegetative biomass (BM), spikelet per spike (SLS^{-1}), grain weight (GW), Yield (Y), harvest index (HI), total biomass (TB), ΔCID (F) under field condition and ΔCID (GH) C13/C12 in green house.

Component	Field condition								ΔCID (GH)
	PH	BM (g)	SLS^{-1}	GWS^{-1}(g)	Y(g $plant^{-1}$)	HI	TB(g)	ΔCID (F)	
Mean	84.07	38.38	17.43	2.69	19.56	0.34	57.93	28.76	32.22
Max	97.00	47.29	21.00	3.30	24.10	0.38	71.39	29.86	32.72
Min	75.00	24.90	14.00	2.25	14.30	0.28	39.20	27.79	31.77
GCV%	42.19	29.14	43.28	34.15	29.74	39.37	26.14	41.39	15.93
PCV%	88.51	77.34	73.37	67.35	78.68	76.35	64.34	79.33	26.37
h^2	0.54	0.58	0.64	0.59	0.39	0.61	0.41	0.79	0.81

* GCV= genotypic coefficient of variation; PCV= genotypic coefficient of variation , h^2= heritability

Table 3. Correlation coefficients among various traits under drought stress i.e. plant height (PH), biomass (BM), spikelet per spike (SLS^{-1}), grain weight (GW), Yield (Y), harvest index (HI), total biomass (TB), ΔCID (GH) C13/C12 in green house and C13/C12 in field.

Traits	PH	BM (g)	SLS^{-1}	GW(g)	Y(g)	HI	TB(g)	ΔCID (GH)
Biomass(gm)	0.69*							
No.of spikelets/spike	0.06^{NS}	-0.15^{NS}						
Grain weight/spike	0.77**	0.51*	0.17^{NS}					
Yield(gm)	0.71**	0.64**	-0.03^{NS}	0.79*				
Harvest index	0.05^{NS}	-0.39^{NS}	0.16^{NS}	0.34^{NS}	0.45^{NS}			
Total Biomass	0.76**	0.96	-0.12^{NS}	0.66*	0.84*	-0.10^{NS}		
C13/12 G.H	0.05^{NS}	0.51*	-0.48*	-0.15^{NS}	-0.14^{NS}	-0.54*	0.29^{NS}	
C13/12 Field	0.56**	0.26^{NS}	-0.36^{NS}	0.31^{NS}	0.54*	0.37*	0.43*	0.20^{NS}

Principal component analysis was carried to determine the genetic diversity within germplasm with respect to traits related to the drought stress. Analysis showed that at least three factors showed eigenvalue greater than unity, these factors exhibited more than 67.75% of the genetic variability within germplasm. First factor represented 48% of the total genetic variability in the germplasm (Fig 1). Factor 1 showed that traits such as plant height, biomass, grain weight, yield, total biomass and CID contributed positively to the genetic variation present in the germplasm (Fig. 1, Table 4). In factor 2, traits such as Harvest index, SLS^{-1} and CID of field experiment showed positive contribution to the genetic variability while other traits showed negative correlation with variability (Fig. 1, Table 4). In Factor 3, only CIDs showed positive contribution to the genetic variability. The directional change in the contribution of a trait to the genetic variation may be due to presence of correlation among traits (Table 2). However, CIDs did not show directional change over these factors. Therefore, CIDs could reliably be used to access the genetic diversity of the germplasm to drought stress tolerance. Moreover, principal component analysis also placed CIDs and yield on the same axis showing that CID could also show genetic variation within germplasm for yield.

Results of the present studies showed that there was significant variation among the wheat accessions related to biomass and drought resistance. Genotypic (GCV) and phenotypic coefficient of variation (PCV) was used to determine variation within germplasm. Traits such as plant height, spikelet spike[-1] and CID under field conditions showed high GCV and PCV estimates. Heritability of the traits were also estimated that CID had the highest heritability estimates. High heritability ensure good selection efficiency and genetic advancement, therefore traits with high heritability could be exploited as selection criterion (Rauf *et al.,* 2016). Previous results have also shown that board sense heritability estimates for CID were high across range of environments (Rebetzke *et al*., 2008; Zhang *et al.,* 2015). However, it was also mentioned that CID had negative relationship with grain protein contents, and increase in the yield could occur at the expense of baking quality under rainfed conditions (Zhang *et al*., 2015).

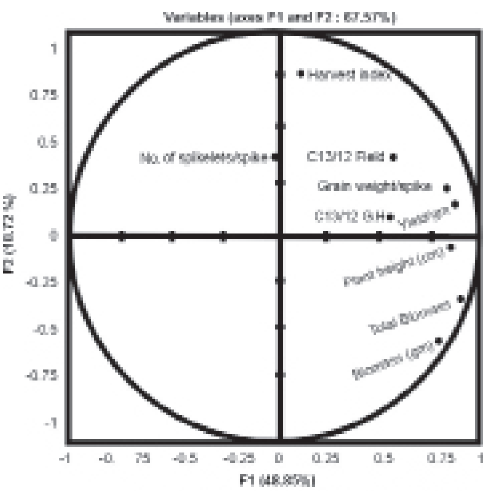

Fig. 1. Correlation between variable and factors

Table 4. Contribution of various plant traits to the factors (F1, F2 and F3).

Plant Trait	F1	F2	F3
Plant Height(cm)	16.89	0.14	2.40
Biomass(g)	14.83	18.72	0.19
Spike spike^{-1}	0.01	10.50	3.98
Grain weight/spike	16.21	4.15	3.52
Yield (g)	17.73	1.93	7.02
Harvest index	0.38	46.50	5.17
Total Biomass	18.88	6.50	1.73
C13/12 G.H	7.28	0.60	44.72
C13/12 Field	7.80	10.96	31.26

Results of the present studies showed that there was significant variation among the wheat accessions related to biomass and drought resistance. Genotypic (GCV) and phenotypic coefficient of variation (PCV) was used to determine variation within germplasm . Traits such as plant height, spikelet spike^{-1} and CID under field conditions showed high GCV and PCV estimates. Heritability of the traits were also estimated that CID had the highest heritability estimates. High heritability ensure good selection efficiency and genetic advancement, therefore traits with high heritability could be exploited as selection criterion (Rauf *et al.,* 2016). Previous results have also shown that board sense heritability estimates for CID were high across range of environments (Rebetzke *et al*., 2008; Zhang *et al.,* 2015). However, it was also mentioned that CID had negative relationship with grain protein contents, and increase in the yield could occur at the expense of baking quality under rainfed conditions (Zhang *et al*., 2015).

The correlation estimates showed that CID under field conditions had positive relationship with grain yield and other morphological traits. Thus selection for high CID could also help in improving the biomass and yield. Relationship of CID with grain yield of drought environment has been documented earlier (Akhater *et al*., 2008; Rebetzke *et al*., 2008; Zhang *et al.,* 2015; Munjonji *et al.,* 2018). Traits that are related with abiotic stresses and have positive relationship with yield may be selected to minimize yield losses under specific conditions. Such types of ideotypes may able to take advantage of non-droughted years and also minimize yield losses under stress condition (Kalyar *et al*., 2014).

Principal component analysis has been extensively used to study the genetic diversity within germplasm and to determine relatively important trait that depict the highest genetic variation of the germplasm (Royo *et al.,* 2002). Results showed that variability within germplasm was explained by at least two factors. These factors explained 67.75% of variation (as per principal component analysis) and showed relative importance of various traits. Studies showed that CID contributed positively to the genetic variation present in the germplasm in all factors (Fig. 1; Table 4). Previous studies have also shown high genetic variation for CID within germplasm of wheat species (Zhang *et al*., 2015; Munjonji *et al*., 2018).

CONCLUSION

On the basis of results, it was concluded that CID was promising criteria for the discrimination of productive wheat germplasm under stress conditions. This conclusion was based on the wheat germplasm variation for CID under field conditions, correlation with grain yield or biomass and heritability estimates.

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2.	Muhammad Akram Khan	Designed research and contributed as supervisor	 _(on behalf)