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EVALUATION OF DIVERSE WHEAT GENOTYPES FOR POTENTIAL BIOMASS PRODUCTION THROUGH PHYSIOLOGICAL PARAMETERS AT SEEDLING STAGE UNDER CONTROLLED ENVIRONMENT

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Abstract

Thirty wheat genotypes from UK, CIMMYT and Pakistan were evaluated under controlled environment conditions for their potential biomass production by measuring stomatal conductance (g_s; porometry), leaf photosynthesis (IRGA), carbon isotope discrimination and carbon content (isotope ratio and mass analysis) at Rothamsted Research, Harpenden, UK during 2011. Amongst the dwarf genotypes, Rht2 and Rht3 showed lower stomatal conductance than Seri 32B, Seri 87B and Bathoor-07. For these 5 genotypes and another genotype 'Inqalab' photosynthetic performance was determined by means of IRGA measurements. Of these genotypes Inqalab had the highest photosynthetic activity (A), stomatal conductance (g_s), transpiration (E) and leaf intercellular CO_2 but it also had the lowest water use efficiency (A/g_s) and intrinsic water use efficiency (A/E). Seri-87B had the greatest water use efficiency (A/g_s) and intrinsic water use efficiency (A/E). All the Pakistani genotypes had large stomatal conductances and high ^{13}C delta (Δ) and thus may be expected to produce high biomass under irrigations and optimum inputs.

Introduction

Crop performance is the result of the actions of thousands of genes and their interactions with both environment and agronomic practices. Conventional breeding has been very successful in enhancing the yield potential of crops through improvements in harvest index, by allocating more biomass to seed production (Borlaug & Dowswell, 2005; Duvick, 2005). This was mainly achieved with little or no knowledge of the factors governing the genetic variability that breeders exploited for crop improvement (Borlaug, 2007). However, the potential of this approach may now have been fully realised.

To increase future harvests, more biomass will have to be produced per unit area of land; this will require improvements in light use efficiency, light conversion efficiency, or both (Zhu *et al.*, 2010). For improvements in photosynthetic capacity to result in additional wheat yield, extra assimilates must be produced and partitioned to developing spikes and grains and/or potential grain weight increased to accommodate the extra assimilates (Foulkes *et al*., 2011).The carbon assimilation is integrated over the entire growing season and crop canopy, therefore, the small increases in the rate of net photosynthesis can translate into large increases in biomass and hence yield. This in turn will lead to increases in biomass and harvestable grain in the absence of further increases in harvest index (Parry *et al*., 2011). Increasing photosynthesis will increase crop biomass and ultimately the crop will produce higher yield potential, provided that other constraints do not become limiting (Ainsworth & Long, 2005).

Crop yield potential is the yield of a cultivar when grown in environments to which it is adapted (solar radiation, temperature, day length) with nutrients and water non-limiting and with pests, diseases, weeds, and other stresses effectively controlled (Evans & Fischer, 1999). Yield potential is close to attainable yield (the best yield achieved through skilful use of the best available technology). The on-farm yields normally realize from 60-80% of attainable yield (Foulkes *et al*., 2009). Therefore, yield potential in crops is principal focus and target for plant breeders as it is closely and directly linked to both attainable and on-farm yields (Fischer & Edmeades, 2010). Selection for higher yield potential has frequently resulted increase in productive biomass under stressed and unstressed environments (Foulkes *et al.*, 2007). Thus the higher attainable yields under relatively favourable conditions, as well as under moderate abiotic stresses are an important outcome of the breeding for yield potential. To date, improvement of yield potential is mainly because of yield *per se*, but there is strong evidence that understanding traits at the physiological level will help to identify trait interactions and indirect selection criteria that may accelerate breeding progress (Reynolds *et al.*, 2009). Evaluation of genetic variability of potential biomass production in wheat may be the initial and important step to understand physiological basis of potential biomass production and its utilization as indirect selection criteria for improvement of yield potential in wheat.

Potential biomass production evaluated through physiological parameters in diverse wheat genotypes at optimum inputs under controlled environment has been studied and findings are reported in this manuscript.

Materials and Methods

Thirty wheat genotypes from UK, CIMMYT and Pakistan (Table 3) were planted in plastic pots with 4 replicates in a completely randomized design and then grown under controlled environmental conditions (Table 1) at Rothamsted Research, Harpenden, UK during 2011. Each replicate consisted of a pot containing 5 seeds of the same genotype. Soil composition was a composite of Rothamsted Nematode Mix, Rothamsted Weed Mix and Rothamsted Prescription mix (compost) with some added nutrients (Table 2). Stomatal conductance was measured using a Delta-T type Ap4 Porometer, whilst the plants were at seedling stage, three weeks after planting. Porometer measurements were recorded at the mid-leaf position of a fully elongated and opened, younger leaf on four plants per replication. This leaf position was found to yield the most consistent data (not shown).

Table 1. Detail of controlled environment.

Day temperature	18°C	Night temperature	15°C
Day length	16 h	Light intensity	700 µmol m^{-2} s^{-1}
Day humidity	70%	Night humidity	80%

Five genotypes were selected: Rht2 and Rht3 for their low and Seri 32B, Seri 87B and Bathoor-07 for their high stomatal conductance, as determined using the Porometer, which had the advantage (over the IRGA) of speed, facilitating a rapid pre-screen. Further in-depth analyses of these 5 genotypes were carried out by means of an IRGA (Li-Cor 6400 XT). In each case, data was recorded for the youngest fully expanded leaf, maintaining a sample relative humidity at 60%, a sample (leaf) CO_2 concentration of 390 (µmol CO_2 mol^{-1} of the air) and a leaf temperature of 22°C. The Photosynthetically Active Radiation (*PAR*) was kept constant at 750µmol photons m^{-2} s^{-1}, similar to the light intensity of the controlled environment chamber.

Two shoots of each genotype from separate plants in a single pot were collected and combined six weeks after planting, into 50 ml centrifuge tubes and rapidly frozen, in situ, in liquid nitrogen. After collection, all samples were stored at -80°C. The samples were freeze dried and crushed into fine powder by means of a Retsch centrifugal mill. Two mg of each sample was used for determination of the carbon isotope ratio (^{13}C delta). Data were analysed for ANOVA by Genstat (2011)

Table 2. Composition of nutrients added to compost.

Nutient	Osmocote plus 3/4month (%)	Osmocote exact 3/4month (%)	PGmix (%)
N	15	16	14
P_2O_5	11	11	16
K_2O	13	11	18
MgO	2	3	0.7
Bo	0.02	0.02	0.03
Mo	0.02	0.02	0.2
Cu	0.05	0.05	0.12
Mn	0.06	0.06	0.16
Zn	0.015	0.015	0.04
Fe (chelated)	0.15	0.2	0.09

Results

Mean data for stomatal conductance and carbon isotope discrimination of 30 wheat genotypes at seedling stage is depicted in Table 3. Analysis of variance of stomatal conductance and carbon isotope ratio of evaluated (Pakistan, Rht, CIMMYT and UK types) wheat genotypes is presented in Table 4. Types were significantly different (p<0.01) for stomatal conductance and highly significantly different (p<0.001) for ^{13}C delta. Type x line interaction was not significant (p>0.05) for neither stomatal conductance nor ^{13}C delta (Table 5). Pakistani genotypes possessed highest stomatal conductance (205.50 mmol H_2O m^{-2} s^{-1}) followed by the UK genotypes (146.66 mmol H_2O m^{-2} s^{-1}). Pakistani genotypes possessed the highest ^{13}C delta (-25.26‰) followed by CIMMYT (-25.29‰) although this difference was not significant. However, they were significantly different from the Rht and UK types (p<0.05). The UK genotypes possessed lowest ^{13}C delta, which was significantly different (p<0.05) from the Pakistan, Rht and CIMMYT types. Inqalab showed highest photosynthetic activity, stomatal conductance, transpiration and leaf intercellular CO_2 but it possessed lowest water use efficiency (A/g_s) and intrinsic water use efficiency (A/E). Seri-87B showed the highest water use efficiency (A/g_s) and intrinsic water use efficiency (A/E) amongst genotypes studied (Tables 6 and 7).

Discussion

Significant differences for stomatal conductance among four types (Pakistan, Rht, CIMMYT and UK) of wheat genotypes at optimum management at seedling stage under controlled environment revealed existence of variations in photosynthetic activity in studied germplasm. Higher stomatal conductance of Pakistani genotypes found during current study indicates that they may tend to diffuse more CO_2 to chloroplast and thus have greater photosynthetic activity and produce more biomass. Positive relationship between stomatal conductance and photosynthetic activity in wheat has already been reported by earlier researchers (Muller & Whitsitt, 1996; Ahmed *et al.*, 2010). High photosynthetic activity of Pakistani genotypes was also revealed by the highest ^{13}C delta value. Pakistani genotypes evaluated under current project are direct/indirect selections from CIMMYT breeding material and thus have the similar genetic background, therefore, non-significant differences were observed between Pakistan and CIMMYT genotypes for carbon isotope discrimination. Moreover, highest ^{13}C delta value of Pakistani genotypes is one of the important indicators for high biomass production under irrigation and optimal management. The existence of genetic variation as regards Δ was also reported for various cereals (Impa *et al*., 2005; Misra *et al*., 2006). Stomatal conductance and leaf photosynthetic capacity in grain crops have positive and negative effects on Δ, respectively (Richards, 2000). Positive relationship between Δ and yield in bread and durum wheat under well-watered or moderate drought conditions has earlier been reported by Merah *et al.,* (2001). Present and earlier findings revealed that highest yielding genotypes will have high Δ and consequently the lowest *WUE* under irrigation and optimal inputs.

Table 3. Stomatal conductance (mmol H_2O m^{-2} s^{-1}), $\delta^{13}C$ (‰) and Zadocks scale of 30 wheat genotypes at seedling stage grown under controlled environment.

Genotype	Type	Stomatal conductance	$\delta^{13}C$ (‰)	Zadocks scale
Consort	UK	146.66	-26.89	2-3
Battalion	-do-	126.94	-26.63	2-2
Gladiator	-do-	146.65	-25.97	2-2
Robigus	-do-	122.55	-26.69	2-2
Xi19	-do-	66.50	-26.00	2-2
Istabraq	-do-	150.41	-26.71	2-3
Rht	Rht	76.08	-25.74	2-3
Rht1	-do-	123.71	-25.93	2-2
Rht2	-do-	56.44	-25.61	2-4
Rht3	-do-	45.84	-26.85	2-2
Bavia Cora	CIMMYT	111.08	-25.01	2-4
Hist 10	-do-	88.98	-25.03	2-3
Hist 13	-do-	78.25	-25.00	2-4
Mex 19	-do-	108.64	-24.61	2-4
Mex 20	-do-	141.40	-25.01	2-4
Oax 93	-do-	89.39	-25.19	2-4
Pub 94	-do-	79.87	-25.62	2-3
Seri 11B	-do-	81.66	-24.98	2-4
Seri 20B	-do-	156.54	-25.89	2-4
Seri 29B	-do-	133.82	-25.55	2-4
Seri 31B	-do-	162.77	-25.78	2-4
Seri 32B	-do-	188.68	-25.18	2-4
Seri 87B	-do-	178.75	-25.53	2-4
Seri 143B	-do-	112.60	-25.81	2-3
W 15	-do-	165.56	-25.14	2-3
Bathoor-07	Pakistan	205.50	-24.98	2-3
Tatara	-do-	97.15	-25.53	2-4
Takbeer	-do-	97.59	-25.13	2-3
NIFA Barsat 09	-do-	100.66	-25.50	2-3
Inqalab	-do-	161.31	-25.10	2-3

Table 4. Analysis of variance of stomatal conductance (g_s) and delta ^{13}C ($\delta^{13}C$) of 30 wheat genotypes recorded at seedling stage grown under controlled environment.

SOV	df	g_s		$\delta^{13}C$	
		Mean sq.	F	Mean sq.	F
Type	3	1.27	0.01^{*}	10.17	0.001^{***}
Type x line	26	0.40	0.23^{ns}	0.55	0.16^{ns}
Error	87	0.32	-	0.41	-
Total	119	-	-	-	-

Table 5. Types' mean values for stomatal conductance and $\delta^{13}C$ and LSD values of Type x line interaction of stomatal conductance and ^{13}C delta of 30 wheat genotypes recorded at seedling stage grown under controlled environment.

Type	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	$\delta^{13}C$ (‰)
Pakistan	205.50	-25.26
Rht	76.08	-26.03
CIMMYT	111.08	-25.29
UK	146.66	-26.48
	LSD (5%)	
Comparison	**Stomatal conductance**	**$\delta^{13}C$**
Pakistan vs Rht	38	0.40
Pakistan vs CIMMYT	29	0.29
Pakistan vs UK	34	0.36
Rht vs CIMMYT	32	0.36
Rht vs UK	36	0.41
CIMMYT vs UK	27	0.31

Table 6. Photosynthetic activity (*A*), stomatal conductance (g_s) and transpiration (*E*), of 6 wheat genotypes under controlled environment.

Genotypes	Photosynthetic activity (*A*) (μmol $m^{-2}s^{-1}$)	Stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$)	Transpiration (*E*) (mmol H_2O $m^{-2}s^{-1}$)
Rht2	20.54 ± 1.01	0.27 ± 0.03	2.62 ± 0.24
Rht3	18.86 ± 3.65	0.24 ± 0.06	2.28 ± 0.53
Seri-32B	17.04 ± 7.00	0.18 ± 0.10	1.85 ± 0.90
Seri-87B	22.95 ± 1.95	0.28 ± 0.06	2.65 ± 0.51
Bathoor-07	19.06 ± 1.14	0.23 ± 0.02	2.29 ± 0.19
Inqalab	**25.25 ± 1.31**	**0.51 ± 0.11**	**4.18 ± 0.67**

Table 7. Leaf intercellular CO_2 (*Ci*), water use efficiency (*A*/*E*) and intrinsic water use efficiency (A/g_s) of 6 wheat genotypes under controlled environmental conditions.

Genotypes	Parameters		
	Leaf intercellular CO_2 (μmol CO_2 mol air^{-1})	Water use efficiency	Intrinsic water use efficiency
Rht2	259.37 ± 11.66	7.89 ± 0.63	76.48 ± 7.27
Rht3	250.25 ± 20.38	8.40 ± 1.00	82.64 ± 13.55
Seri-32B	217.41 ± 47.49	**9.49 ± 1.92**	**98.47 ± 26.12**
Seri-87B	244.38 ± 24.38	8.86 ± 1.26	86.02 ± 15.55
Bathoor-07	252.60 ± 5.15	8.34 ± 0.47	83.05 ± 5.38
Inqalab	299.42 ± 16.19	6.16 ± 1.05	51.91 ± 13.25

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