

Proxy-based approach to wheat selection for photosynthetic efficiency

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Abstract

Approaches based on physiological understanding of yield are necessary for developing genotypes combining high yielding potential and agronomic traits of superior adaptation, and for understanding yield limiting factors. Yet, direct measurement of a particular physiological variable is often difficult. This study was set to develop a proxy-based approach to wheat selection for photosynthetic efficiency. After conceptualizing a theoretical framework of links between the traits of photosynthesis, water relations, leaf morphology and anatomy, and their likely proxies; a comparative screening of 23 *Eps* wheat cultivars was conducted in field at NIAB by means of photosynthetic gas exchange measurement, followed by isotopic measurements (of $\Delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) in the leaf matter, and morphological and anatomical measurement. Data were explored for parametric assumptions of normal distribution, and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests respectively, and then were subjected to both partial Pearson correlation analysis and mixed analysis of variance (*Mixed ANOVA*) at $p < .01$; and Bonferroni test. Having ranked a number of traits according to their likely association with particular proxy, the results showed that photosynthetic rate and WUE (Water Use Efficiency) were highly and statistically significant ($p < .01$) associated with *SLA* (*Specific Leaf Area*). Based on these results, the study concluded that *SLA* would be potential proxy of both A_{max} and WUE in wheat.

Key words: Proxy-based selection, photosynthesis, WUE, wheat, SLA
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Introduction

Proxy-based crop selection: A conceptual framework

Given the complexity of yield trait, it is apparent that a comprehensive approach to crop improvement has the greatest probability of achieving increased productivity. Based on the understanding that any improvement in grain yield results from underlying physiological processes; approaches based on physiological understanding of yield are necessary for identification of traits putatively related to yield and adaptation, and selection criteria that could be exploited (Slafer *et al.*, 2005; Reynolds & Borlaug, 2006; Foulkes *et al.*, 2011). Yet, direct measurement of a particular physiological variable is often difficult.

Therefore, this work theorized an idealized conceptual framework for a "proxy-based approach" in terms of physiological research in wheat (*fig. 1*).

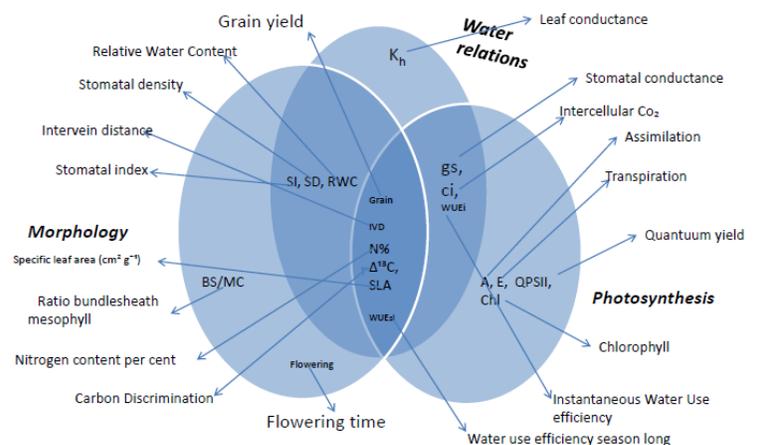


Figure.1 Conceptual framework of interaction between physiological variables at leaf level

Photosynthetic gas exchange

It has been suggested that the maximal rate of CO_2 assimilation (A_{max}), under natural conditions, at atmosphere CO_2 supply under optimal environmental conditions, is characteristic constitutional feature of

specific crop cultivar (Larcher, 2003). In a steady state, the rate of CO_2 diffusion can be ascribed by Fick's first law. Hence:

$$A_n = g_c(C_a - C_c) = (C_a - C_c)/r_c \quad (1.1)$$

where, g_c is the leaf conductance for CO_2 , C_a and C_c are the mole fractions of CO_2 in the air and at the site of carboxylation, respectively; r_c is the inverse of g_c (leaf resistance to CO_2 diffusion).

The ratio of CO_2 assimilation to transpiration which is termed instantaneous water use efficiency (WUE_i) of photosynthesis can be calculated as follow:

$$WUE_i = A_n/E = g_c(C_a - C_i)/g_w(W_i - W_a) \quad (1.2)$$

$$= C_a(1 - C_i/C_a)/1.6(W_i - W_a)$$

Where g_w is the leaf conductance for water vapor; W_i and W_a are the mole of water vapor in the intercellular spaces and in air, respectively; r_w is the inverse of g_w , and E is the rate of leaf transpiration.

Leaf morphology and anatomy

The influence of leaf morphology and anatomy on photosynthetic activity has long been recognized (Jellings & Leech, 1984; Garnier & Laurent, 1994). According to Nobel (1983), and Sharkey (1985), differences in photosynthetic rate among plant cultivars

may be attributed to differences in biochemical, morphological and anatomical features of their leaves. Similarly, Taiz & Zeiger (2010) suggested that morphological aspects of the leaf such as leaf size can be a determinant in influencing the thickness of the boundary layer.

It appears that leaf thickness plays an important role in leaf functioning and relates plant strategy of resource acquisition and use. Wide variation in leaf thickness among plant cultivars had been observed (Evans, 1999; Poorter *et al.*, 2009). Roderick *et al.* (1999), showed that leaf thickness is closely related to SLA (the ratio of leaf area to leaf dry matter); as such SLA can be used as surrogate of leaf thickness. It was also suggested that SLA is a trait that may be up to 60 to 90% heritable (Rebetzke *et al.*, 2004; Songrui *et al.*, 2008).

This research examined the possibilities of potential use of SLA for screening photosynthetic efficiency of wheat, and was set to address the specific questions of;

- i) To what extent variation in SLA accounts for variations in photosynthetic capacity and water use in wheat?
- ii) What links leaf venation to photosynthetic rate in wheat?

Materials and Methods

The 23 *Eps* cultivars (table 1) were characterized in this research. Varietal differences in timing to anthesis independent of sensitivity to photoperiod and vernalization had been found (Ford *et al.*, 1981), and referred to as "earliness per se (*Eps*)" (Hoogendoorn, 1985), or "intrinsic earliness". Slafer (1996) reported that *Eps* genes not only regulate anthesis time but also effect in the transition from vegetative to reproductive apices, early and late spike development, and stem elongation. As grain yield components are determined during these phases (Slafer & Whitechurch, 2001), it is therefore relevant to examine how *Eps* relate to physiological processes of performance.

Photosynthetic gas exchange measurements

Snapshot measurements of photosynthetic gas exchange were performed on wheat leaves of 23 *Eps* cultivars in the field located at 52° 13' N, 04° 59' E at National Institute of Agricultural Botany (NIAB, Cambridge, the UK), using a portable LICOR LI-6400XT (LI-COR Inc., Lincoln, Nebraska).

Measurements were taken at anthesis (GS65), on fully expanded flag leaf of the main tiller (two leaves per plot, & 4 replications per cultivar) randomly chosen in the center of each plot (46 plots in total). Parameters were set in the LI-COR as; Relative humidity to 60-80%, the block temperature at 20°C, the CO_2 reference to 400 ppm, flow rate at 400 $\mu\text{mol s}^{-1}$, the photosynthetic active radiation (PAR) of 1000 $\mu\text{mol quanta s}^{-1}$. After the setting of the parameters, a leaf was placed in the sensor head, and enclosed the chamber, and then measurement was recorded on the LI-COR. The measurement was repeated on the second leaf in the same plot before moving to another plot, and the same leaves were marked for further sampling and analysis (*SPAD* measurements; SLA ; $\Delta^{13}C$; leaf N%; & $\Delta^{18}O$). The portable LI-COR 6400XT provides real time measurement of CO_2 uptake (A), transpiration (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i).

Chlorophyll content measurement

The chlorophyll measurement was taken non-destructively at anthesis (*GS65*) on a fully expanded flag leaf (two leaves per plot & four replicates per cultivar), using a hand held portable chlorophyll meter *SPAD-502* (Konica Minolta Sensing Inc., Osaka, Japan). The values measured by the chlorophyll meter *SPAD-502* correspond to the amount of chlorophyll present in the plant leaf. The values are calculated based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll is different (Minolta, 1989).

Specific leaf area ($cm^2 g^{-1}$) measurement

The same flag leaf samples on which gas exchange and chlorophyll content measurements were taken in the field at *NLAB* were collected for specific leaf area (*SLA*) measurement. At anthesis (*GS65*), two flag leaves were sampled per plot (four replicates per cultivar): leaf was cut from plant, rapidly wrapped in moist paper, placed in plastic bag, put in cool box, and taken to the laboratory of physiological ecology at department of plant sciences (University of Cambridge) for further measurements.

In the laboratory, each leaf was recut under distilled water to remove the petiole, and placed immediately into a tube filled with distilled water, and stored in refrigerator at $4^{\circ}C$ for 6 hours to ensure fully rehydration of the leaves (Garnier *et al.*, 2001). After this period, the leaf blade was taken out of the tube, and blotted dry with tissue paper to remove any surface water. The leaf area (*LA*), one side of the leaf, was measured with *ImageJ* (version 1.42g, National Institute of Health, USA). Then, the sample was oven dried in a paper envelope at $75^{\circ}C$ for 24 hours. The leaf dry weight (*DW*) was obtained by reweighing the sample on micro-balance after oven drying.

The *SLA* was calculated as the ratio of leaf area (*LA*) to dry weight (*DW*):

$$SLA (cm^2 g^{-1}) = \frac{LA (cm^2)}{Dw (g)} \quad (2.1)$$

Carbon discrimination ($\Delta^{13}C$) and leaf *N* measurement

At the Godwin laboratory (Cambridge University, UK), the dried ground leaf samples weighed ($1mg$) into a tin capsule were analyzed for both $\delta^{13}C$ and percentage of nitrogen using Costech elemental analyzer attached to a Thermo Delta *V* mass spectrometer in continuous flow mode. The mass spectrometer software measures the

$^{12}C/^{13}C$ and the $^{14}N/^{15}N$ ratio, and *N* percentage in the sample. Reference standards from IAEA (International Atomic Energy Agency) in Vienna are also run at intervals throughout the sequence and these values are used to calibrate to the international standards for $\delta^{13}C$ *PDB* and $\delta^{15}N$ in air.

The $\delta^{13}C$ value was used to compute the $\Delta^{13}C$ following Farquhar *et al.* (1982);

$$\Delta^{13}C = \left(\frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p} \right) / 1000 \quad (2.2)$$

Where the $\delta^{13}C_a$ is the delta value of *C* in the air and the $\delta^{13}C_p$ is the delta value of *C* in the sample.

Inter-vein distance (*IVD*) measurement

Leaf tissue of $5mm \times 15mm$ to be used for vein measurement was cut in the middle area between the central vein and the leaf edge, on the same each leaf sample. Then, the tissues samples were fixed in a solution of 4% para-formaldehyde overnight at $4^{\circ}C$; and washed into 100% ethanol and dehydrated passing into ethanol concentration series of 30 %, 40%, 50%, 60%, 70%, 80%, 90% and staying in each concentration for 45 minutes at $4^{\circ}C$. Thereafter, samples were transferred into ethanol 95% with eosin 0.1%, and cooled overnight at $4^{\circ}C$; and then moved into three series of 100% ethanol at room temperature for 45 minutes each.

The embedding followed through the sequences of steps; first under the mixture of 50% ethanol and 50% technovit, then under 100% technovit, and third, into mixture of 100% technovit and hardener *I* for 45 minutes per step. Thereafter, the samples were polymerized overnight in made up of technovit plus hardener *I* and *II* into mould covered with parafilm. Finally, samples were mounted with araldite on wooden block and kept cool and dry to harden fully over night at room temperature. Four μm thick cross sections were cut using a glass knife on microm *HM340E*; and stained with 0.1% toluidine blue. Sections were photographed on microscope at a magnification of $\times 40$ using a digital camera (Nikon Coolpix *P5100*). The inter-veins distance was measured by means of *ImageJ* (version 1.42g, National institute of health, USA), and measured as the distance between the half of a major vein and half of minor vein (Dengler *et al.*, 1994) (*fig.2.1*).



Figure 2.1 Veins and mesophyll area viewed under microscope 100 μm

Leaf relative water content (RWC) measurement

The two fully expanded intact flag leaf samples were collected at GS65 from two randomly chosen plants in each plot (4 replicates per cultivar) for RWC measurement. The samples were immediately placed into pre-weighed plastic tubes and sealed the lid, placed in cooled container, and taken to the laboratory of physiological ecology (University of Cambridge, the UK) for measurement.

In the laboratory, the leaf fresh weight was measured as the weight of the tube containing the sample minus the weight of the tube. Thereafter, 1cl of distilled water was added to each tube containing the sample and was placed

Results and discussion

Proxy-based ranking and magnitude of variability in performance of *Eps* cultivars

Genotypic ranking for $\Delta^{13}\text{C}$ values based upon leaf samples revealed consistency in rankings for both SLA and WUE_i . The $\Delta^{13}\text{C}$ in leaf organic matter was lowest in seven cultivars: *BBCB-91-232-4-16-1-4*; *BBCB-90-231-8-17-6-6*; *Badger*; *BBCB-91-232-4-1-5-5-1*; *SR96-1-412-1-*

in refrigerator at 4°C for 24 hours for leaf to reach full turgor; then, samples were taken out the tubes and blotted dry with paper towel, and the turgid leaf weight of the sample was measured; thereafter they were oven dried at 75°C for 24 hours, and reweighed for dry weight afterwards.

The leaf RWC was computed following Barrs and Weatherley (1962);

$$\text{Leaf RWC (\%)} = ((FW - DW) / (TW - DW)) * 100 \quad (2.3)$$

where; FW = fresh weight; TW = turgid weight; and DW = dry weight

Statistical analysis

The statistical analysis of the data was performed using *SPSS 16.0* for windows (*SPSS Inc., Chicago, IL, USA*). Data were explored for parametric assumptions of normal distribution, and homogeneity of variance: The test for normal distribution was performed using both histograms, and Kolmogorov-Smirnov test to produce $K-S$ test and normal $Q-Q$ plots. The Levene's test was used to test for homogeneity of variance. Then, graphing of means, ranking for variations in performance among cultivars, and the analysis for the linear relationships were performed by means of bar charts and scatter-plots respectively. Thereafter, the partial Pearson correlation analysis was conducted. Finally, data were subjected to the mixed analysis of variance (*Mixed ANOVA*) at $p < .01$; and Bonferroni test.

SSRS-67-147-5-1-12-2-2; *SSSR-67-147-6-2-4-19-B-7*, with the means values ranging between $21.4 \pm 0.1\%$ and $22.0 \pm 0.0\%$ (*tab.3.1*). On the other range, the two cultivars: *RRSR-15-159-2-7-4-A-4*; *SR99-1-413-2-15*, showed the highest $\Delta^{13}\text{C}$ value with the mean in the range of $23.1 \pm 0.1\%$ and $23.0 \pm 0.0\%$. The mixed *ANOVA* depicted statistically significant difference at $p < 0.01$ between cultivars (*fig. 3.1*).

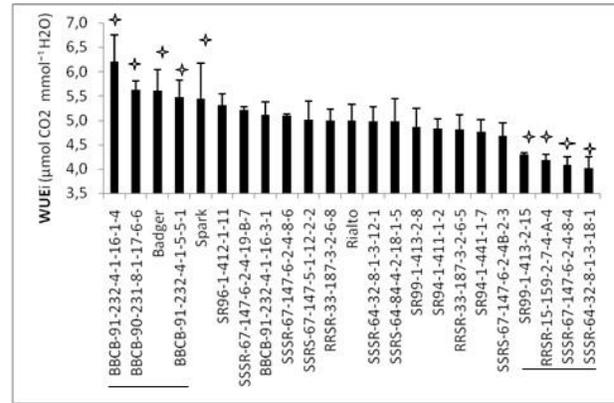
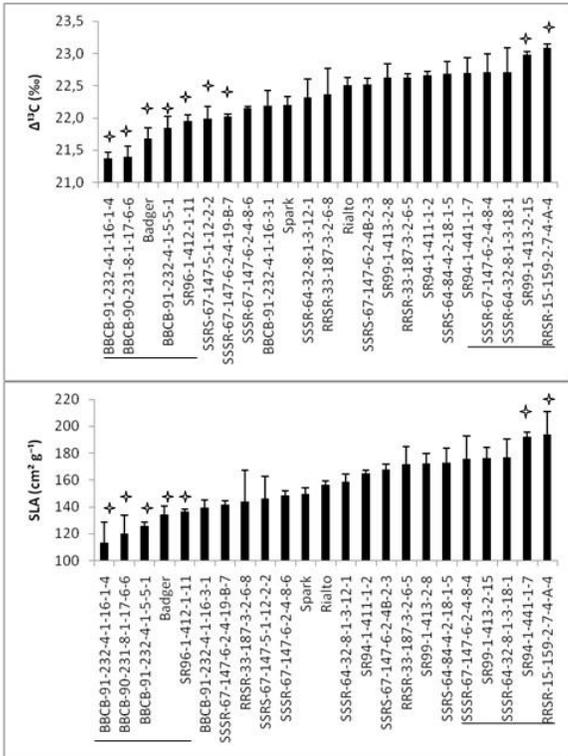


Figure 3.1 Ranking for variation in $\Delta^{13}C$, SLA , and WUE_i . The star (✱) on the top of the bar indicates statistical significance of difference depicted by the mixed ANOVA between the means of cultivars at $p < 0.01$. The bars represent the mean value \pm SE. and IVD : cultivars with low SLA tended to have both higher leaf RWC and short IVD , while low leaf RWC and larger IVD were generally observed in cultivars with higher SLA . The photosynthetic net assimilation rate varied between $31.9 \pm 1.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $22.4 \pm 1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ (tab. 3.1).

Although the pattern of variation in leaf organic $\Delta^{13}C$ appeared like had been driven by SLA , the trend was also observed in variation with RWC .

Table 3.1: The magnitude of variability in traits among Eps cultivars

Variety	$\Delta^{13}C$	WUE_i	An	SLA	RWC	Chlor	IVD
BBCB-91-232-4-1-16-1-4	21.4 \pm 0.1**	6.2 \pm 0.5**	31.9 \pm 1.1**	114 \pm 15**	83 \pm 2**	52.8 \pm 1.5**	0.36 \pm 0.01**
BBCB-90-231-8-1-17-6-6	21.4 \pm 0.2**	5.6 \pm 0.2**	30.1 \pm 1.8**	120 \pm 13**	77 \pm 4*	50.4 \pm 0.7*	0.30 \pm 0.02**
Badger	21.7 \pm 0.2**	5.6 \pm 0.4**	31.8 \pm 1.2**	134 \pm 6**	83 \pm 2**	51.8 \pm 1.7**	0.37 \pm 0.00*
BBCB-91-232-4-1-5-5-1	21.8 \pm 0.2**	5.5 \pm 0.3**	28.1 \pm 0.8**	126 \pm 3**	77 \pm 1*	50.1 \pm 1.5*	0.32 \pm 0.01*
SR96-1-412-1-11	22.0 \pm 0.1**	5.3 \pm 0.2**	27.6 \pm 1.5**	137 \pm 2**	78 \pm 2*	49.8 \pm 0.2*	0.37 \pm 0.00**
SSSR-67-147-5-1-12-2-2	22.0 \pm 0.2**	5.0 \pm 0.4*	26.5 \pm 2.2*	146 \pm 16*	76 \pm 1	50.2 \pm 0.1*	0.37 \pm 0.00*
SSSR-67-147-6-2-4-19-B	22.0 \pm 0.0**	5.2 \pm 0.1*	27.0 \pm 2.5*	142 \pm 3*	76 \pm 1	49.5 \pm 0.3*	0.38 \pm 0.01*
SSSR-67-147-6-2-4-8-6	22.1 \pm 0.0	5.1 \pm 0.0	26.8 \pm 0.7*	149 \pm 4	75 \pm 3	48.4 \pm 0.2	0.40 \pm 0.01
BBCB-91-232-4-1-16-3-1	22.2 \pm 0.2	5.1 \pm 0.3	27.0 \pm 0.9*	140 \pm 6*	74 \pm 1	48.4 \pm 0.3	0.42 \pm 0.01
Spark	22.2 \pm 0.1	5.4 \pm 0.7**	29.8 \pm 1.9*	150 \pm 5	77 \pm 0*	48.2 \pm 0.6	0.39 \pm 0.00
SSSR-64-32-8-1-3-12-1	22.3 \pm 0.3	5.0 \pm 0.3	23.8 \pm 1.3	159 \pm 6	76 \pm 3	47.4 \pm 1.7	0.43 \pm 0.00
RRSR-33-187-3-2-6-8	22.4 \pm 0.4	5.0 \pm 0.2	25.1 \pm 1.0	144 \pm 23*	76 \pm 1	48.0 \pm 0.4	0.38 \pm 0.00*
Rialto	22.5 \pm 0.1	5.0 \pm 0.3	24.1 \pm 1.4	157 \pm 3	74 \pm 1	47.6 \pm 0.7	0.42 \pm 0.00
SSRS-67-147-6-2-4B-2-3	22.5 \pm 0.1	4.7 \pm 0.3*	24.4 \pm 0.1	168 \pm 4*	71 \pm 2	47.9 \pm 0.7	0.43 \pm 0.01
SR99-1-413-2-8	22.6 \pm 0.2	4.9 \pm 0.4	23.2 \pm 0.8	172 \pm 8*	70 \pm 1*	45.8 \pm 0.9*	0.43 \pm 0.02
RRSR-33-187-3-2-6-5	22.6 \pm 0.1	4.8 \pm 0.3*	23.6 \pm 2.1	172 \pm 13	70 \pm 4*	47.0 \pm 0.3	0.44 \pm 0.00
SR94-1-411-1-2	22.7 \pm 0.1	4.8 \pm 0.2	24.6 \pm 1.5	165 \pm 2*	70 \pm 3*	47.9 \pm 1.0	0.42 \pm 0.01
SSRS-64-84-4-2-18-1-5	22.7 \pm 0.2	5.0 \pm 0.5**	23.5 \pm 1.3	173 \pm 11**	68 \pm 5*	46.7 \pm 1.3	0.46 \pm 0.00*
SR94-1-441-1-7	22.7 \pm 0.2	4.8 \pm 0.3*	23.5 \pm 1.2	192 \pm 4*	70 \pm 4*	46.9 \pm 0.5	0.43 \pm 0.00
SSSR-67-147-6-2-4-8-4	22.7 \pm 0.3	4.1 \pm 0.2**	23.4 \pm 1.3	176 \pm 17*	66 \pm 2*	46.4 \pm 0.7	0.44 \pm 0.01
SSSR-64-32-8-1-3-18-1	22.7 \pm 0.4	4.0 \pm 0.2**	21.1 \pm 0.8	177 \pm 14*	67 \pm 1*	44.1 \pm 1.2*	0.46 \pm 0.00*
SR99-1-413-2-15	23.0 \pm 0.1**	4.3 \pm 0.0**	22.7 \pm 0.6*	176 \pm 8**	65 \pm 3**	45.7 \pm 0.8*	0.44 \pm 0.01
RRSR-15-159-2-7-4-A-4	23.1 \pm 0.1**	4.2 \pm 0.1**	22.4 \pm 1*	194 \pm 17**	65 \pm 4**	45.5 \pm 0.6*	0.47 \pm 0.01**

The values are means \pm SE. Mean value that is statistically significant different compared to the control “Rialto” is marked: * designates a significant difference at $p < 0.05$, and the ** indicates the significance of difference at $p < 0.01$. The values for $\Delta^{13}C$ are expressed in ‰, for WUE_i in $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$, in $\text{cm}^2 \text{ g}^{-1}$ for SLA , in $\mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$ for A_n , in ‰ for RWC, SPAD units for chlorophyll content, and in mm for inter-vein distance (IVD).

Relationships and Correlations between SLA and traits of photosynthetic rate

The extent to which a trait is useful as a selection criterion depends upon with its relationships with others traits of performance. The regression analysis and partial Pearson correlation depicted consistently the relationships between SLA and the traits of photosynthetic efficiency (fig.3.2).

The experiment revealed an intimate positive association between SLA and $\Delta^{13}C$ (fig.3.2). The linear regression accurately described the significant dependence of $\Delta^{13}C$ upon SLA ($R^2=0.89$; $p<0.01$). The cultivars with low SLA exhibited lower values of $\Delta^{13}C$ in their leaf matter than cultivars with higher SLA (fig.3.2).

Table 3.2 Pearson correlation between SLA, an, N, and Chlorophyll content

Parameters	SLA($\text{cm}^2 \text{ g}^{-1}$)	A_n ($\mu\text{mol m}^2 \text{ s}^{-1}$)	Flag leaf N%	Chlorophyll (SPAD units)
SLA($\text{cm}^2 \text{ g}^{-1}$)				
A_n ($\mu\text{mol m}^2 \text{ s}^{-1}$)				
Flag leaf N%				
Chlorophyll (SPAD units)				

** .Correlation is significant at the 0.01 level (1-tailed).

Significant statistical Pearson correlation ($p < 0.01$) was observed between SLA and the flag leaf nitrogen content at anthesis ($r=-0.92$). Similarly, the photosynthetic assimilation and chlorophyll content at anthesis were strongly related to SLA ($R^2=0.77$, ψ $R^2=0.78$ respectively).

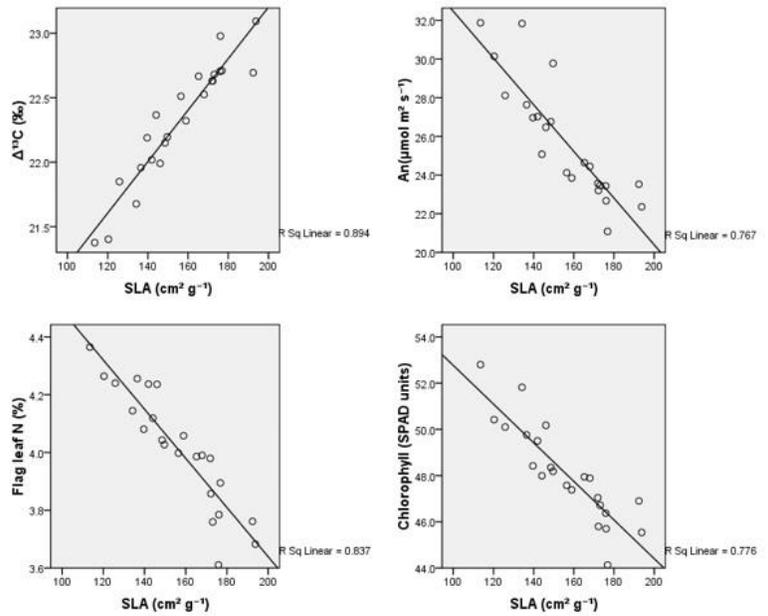


Figure 3.2 Relationship between SLA and traits of photosynthetic efficiency

Relationships and correlations between SLA and traits of leaf water

Based on the overall physiology of plant, the link between SLA , leaf RWC, WUE_i , and IVD have been investigated, and the regression analysis indicated that SLA was significantly ($p < 0.01$) related both to the leaf RWC and IVD ($R^2 = 0.78$; $R^2 = 0.72$ respectively).

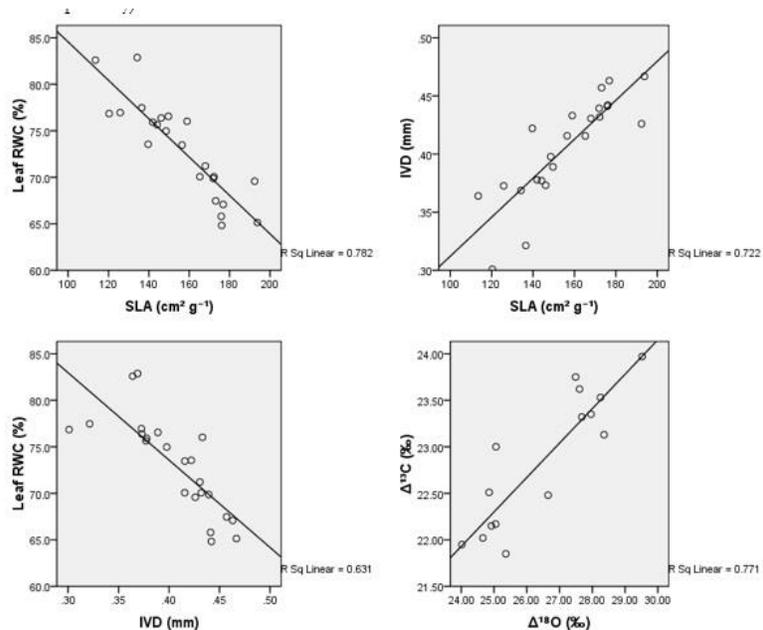


Figure 3.3 Relationships between SLA, RWC, and IVD

The variation of SLA among cultivars was associated with differences in leaf RWC, with dense leaves exhibiting higher water content than thin leaves. The values of SLA were statistically significant ($p < 0.01$) related

to the WUE_i ($R^2=0.75$). Additionally, closer veins were associated and statistically significant correlated with higher leaf water content, and lower SLA (fig. 3.3, & tab.3.3).

Table 3.3 Pearson correlations between SLA , $\Delta^{13}C$, RWC , A_n , and IVD

Parameters	A_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	RWC (%)	SLA ($\text{cm}^2 \text{g}^{-1}$)	$\Delta^{13}C$
IVD (mm)	-0.811**	-0.794**	0.849**	0.870**
A_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		0.887**	-0.876**	-0.916**
RWC (%)			-0.884**	-0.916**
SLA ($\text{cm}^2 \text{g}^{-1}$)				0.945**

Moreover, the Pearson correlation analysis indicated statistically significant correlation ($p < .01$) between IVD and leaf $\Delta^{13}C$ ($r = -0.87$). The combined statistical significant correlation ($p < .01$) observed between SLA and IVD , A_n , leaf RWC , and $\Delta^{13}C$ values in leaf matter (tab. 3.3), showed SLA would be a proxy of both A_n and leaf water status.

Discussion

This research dealt with the proxy-based approach to crop screening for physiological variables. The focus was particularly given to the proxies of photosynthesis and water use at the leaf level. In contrast to the previous works (Ehleringer *et al.*, 1993; Rebetzke *et al.*, 2006; Bindumadhava *et al.*, 2006) that tended to focus on single tool such $\Delta^{13}C$ for selection, this study built on comprehensive examination of the interactions between physiological processes, to develop an integrated proxy of a particular physiological variable.

Emphasis in discussion will be led on the following three aspects; Ranking for proxies and implications for cultivars selection.

- ii) SLA as an integrated proxy of photosynthesis and water use.
- iii) The links of leaf venation to photosynthesis.

Ranking for proxies and implication for crop selection

Consistency of genotypic ranking is essential for breeding to be effective in selecting for a particular quantitative trait (Ehleringer *et al.*, 1993). Ranking of the 23 *Eps* cultivars for low $\Delta^{13}C$ based on leaf material, did show consistent pattern of variation, and were largely maintained in the ranking for low SLA (fig.3.2). In agreement with photosynthetic gas exchange results, the ranking for WUE_i were mostly similar to the ranks for

both low SLA and $\Delta^{13}C$ in leaf organic matter; thus suggesting it is possible to relate WUE_i in wheat genotype to both SLA and carbon discrimination values in leaf.

The consistent pattern of ranking for SLA and $\Delta^{13}C$ among cultivars is of particular interest in the way SLA is cheap and easy to measure than $\Delta^{13}C$: it provides indication that SLA could be used in place of $\Delta^{13}C$ for selecting wheat cultivars. The results are in agreement with Farquhar *et al.* (1982) who first proposed that $\Delta^{13}C$ is a promising parameter for assessing and eventually selecting plant genotypes for water use efficiency in C_3 plants. The results are also consistent with Vogel (1993) who suggested the $\delta^{13}C$ value in leaf matter of terrestrial C_3 plants growing under natural conditions range from -22 to -34‰; in close agreement, the $\delta^{13}C$ of leaf dry matter among the 23 *Eps* cultivars tested in this study, varied between -28 and -31‰.

Many authors have exploited the ranking for selection of crop cultivars (Ehleringer *et al.*, 1993; Acquah, 2012), and their results showed ranking could be a powerful tool to make inference in crop selection: for example, Hall *et al.* (1993) used the consistency of ranking for $\Delta^{13}C$ and grain yield for selection of cowpeas, and they observed the ranking of accession for $\Delta^{13}C$ was remarkably consistent when the same genotypes were grown over different drought conditions, years, and date of sampling, but at the same conditions. Similarly, Garnier *et al.* (2001) based on ranking of species for functional traits, found the species ranking for a given trait remained mostly consistent in space and time. Further, Condon *et al.* (2004) used the genotypic ranking for selecting broad sense heritability of $\Delta^{13}C$ in wheat. Similarly, Rajabi (2006) exploited ranking of leaf organic $\Delta^{13}C$ for selecting sugar beet cultivars for drought tolerance, and observed the ranking was closely maintained over time.

Overall, considering the consistent of the ranking for the proxies of photosynthetic rate and water use among the 23 *Eps* cultivars screened in this research, and the statistical significant difference between each particular cultivar and the control 'Rialto', five cultivars were proposed as selected for photosynthetic and water use efficiency: BBC-91-232-4-1-16-1-4; BBCB-90-231-8-1-17-6-6; Badger; BBCB-91-232-4-1-5-5-1; SR96-1-412-1-11.

Specific leaf area (SLA , $\text{cm}^2 \text{g}^{-1}$): An integrated proxy of photosynthetic rate and water use in wheat
The results in this work provided considerable evidences that SLA is a potential indicator of plant photosynthetic

rate and water use. The comparison of cultivars varying in SLA revealed a greater pattern of association between SLA and A_n (fig.3.2). Some sets of hypothesis could help explain the negative association between SLA and photosynthetic assimilation rate; the first explanation is by the mechanistic that links SLA to the leaf biochemical characteristics (Chlorophyll content, leaf N , Rubisco); According to Lambers *et al.*(2008) one mechanism which sun leaves on a plant achieves a high A_{max} is by producing thicker (low SLA) leaves and which provide spaces for more chloroplasts per unit leaf area. Similarly, Evans and Poorter (2001) indicated that thicker leaves (thus low SLA) are associated with increases in number of chloroplasts and the amount of photosynthetic enzymes; thereby may enhance the photosynthetic capacity per unit leaf area. In similar fashion, von Caemmerer and Farquhar (1981) argued that the capacity of the leaf tissue for photosynthetic CO_2 assimilation depends to a large extent on its Rubisco content.

Over the past years, a number of correlations have been uncovered relating photosynthetic capacity of the leaf to leaf N content (Evans, 1989; Schulze *et al.*, 1994; Reich *et al.*, 1994): That is the higher leaf N content was found to be associated with higher rate of photosynthesis. The mechanistic causes of these relationships were attributed to the large amount organic N present in the chloroplasts, most of it in the photosynthetic machinery (Evans and Seemann, 1989). It had also been found that RuBP carboxylase and chlorophyll content both tend to increase with leaf N content (Evans and Terashima, 1988), They argued that with increased leaf N , the chlorophyll content and electron transport capacity increase. It was also observed that the amount of light absorbed by a leaf, and the diffusion pathway of CO_2 through its tissue depend, at least partially, on its thickness (Agusti *et al.*, 1994; Syvertsen *et al.*, 1995).

Therefore, the strong relationships obtained between SLA and both leaf chlorophyll and nitrogen contents (fig.3.2); provide indication that SLA would possibly be a good proxy to distinguish variation in photosynthetic capacity in wheat.

In addition, the SLA appeared to be a potential indicator of plant water use and related to $\Delta^{13}C$ in this study (tab.2.2). In agreement with photosynthetic gas exchange results, the $\Delta^{13}C$ measured in the leaf dry matter were also negatively correlated with WUE_i as expected for C_3 plants (Farquhar *et al.*, 1982). The relationships between $\Delta^{13}C$ and WUE_i indicated it is possible to relate instantaneous photosynthetic gas exchange to estimate

water use efficiency in wheat genotypes with $\Delta^{13}C$ values of leaf matter.

Moreover, the finding of consistent positive relationships between SLA and $\Delta^{13}C$ is of particular interest (fig. 3.2): it linked indirectly the WUE_i and SLA , and hence indicating the possibility of using SLA as surrogate measure of $\Delta^{13}C$ in selection for WUE in wheat. The proposition of using SLA as surrogate for $\Delta^{13}C$ and WUE is promising in the way it is easiest and cheap to measure while measurement of $\Delta^{13}C$ requires expensive analytical device makes it more expensive proxy to obtain.

Closer association between SLA and leaf RWC (fig. 3.3) was observed in this research. Leaf water content is known to be related to several leaf physiological variables (Kramer & Boyer, 1995). For instance, Farquhar *et al.* (1989) argued that the leaf RWC closely reflects the balance between water supply and transpiration rate. The argument was supported by Yamasaki & Dillenburg (1999) who suggested that leaf RWC is a useful indicator of plant water balance. Additional insights were provided by many authors that observed that decreasing of the leaf RWC of both C_3 and C_4 plants progressively decreased the photosynthetic CO_2 assimilation which eventually stopped below a threshold of leaf RWC (< 70%)(Chaves, 1991; Cornic, 1994; Lawlor & Cornic, 2002). Taken together, the strong relationships between SLA and leaf RWC , and $\Delta^{13}C$ observed in this research, further confirmed the proposition that SLA constitutes a good estimate of plant water status.

Linking leaf venation to photosynthesis

Leaf veins form the transport network for water, nutrients, and carbon for nearly all plants (Brodribb *et al.*, 2005). Leaf venation is highly diverse within and across species (Cochard *et al.*, 2004; Sack & Frolle, 2006; Ellis *et al.*, 2009; Brodribb *et al.*, 2005). The aim of this research was to investigate the link of leaf venation to the photosynthetic efficiency, and how it relates to leaf morphology.

Leaf venation in this study of 23 *Eps* cultivars fell into distinct groups: the group of closer veins which demonstrated a strong association with leaves of low SLA , and another group of larger IVD which was associated with leaves of higher SLA (fig.3.3). Additionally, our data indicated the leaves with low SLA (with also closer veins) to be associated with higher leaf RWC , higher A_n , and higher WUE_i than leave with

These results corroborate with Scoffoni *et al.* (2012) who found that xylem cavitation which is often observed during dehydration, was better tolerated in their study by leaf with higher vein density. Another explanation would be that vein density would relate to photosynthesis because the vein surface area would probably to limit photosynthate transport away from the leaf. In conclusion, our data suggested the link of *IVD* to the photosynthetic rate would probably be rooted in the effect of *IVD* on leaf RWC.

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