# USING STOMATAL APERTURE-RELATED TRAITS TO SELECT FOR HIGH YIELD POTENTIAL IN BREAD WHEAT

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**Abstract:** More efficient wheat breeding methods are needed to meet demand for wheat from expected population growth in developing countries. This paper reports results from recent studies conducted at CIMMYT aimed at assessing the use of stomatal aperture-related traits (SATs) as indirect selection criteria for high yield-potential in bread wheat. Two classes of SATs were assessed: the instantaneous trait leaf porosity (POR), which is a close surrogate for stomatal conductance, and the integrative traits $\Delta^{13}C$ of leaf and grain and $\delta^{18}O$ of grain. For 3 out of the 4 populations of breeding lines tested in 2001–02, the results indicated strong prospects for using SATs in screening for high yield potential. Grain $\Delta^{13}C$, leaf $\Delta^{13}C$ and POR each showed promise, with moderate to high heritability, moderate to strong genetic correlations with yield, and yield gains from retrospective selection of about 40–50g $m^{-2}$ at trial mean yields of 520g $m^{-2}$. Grain $\delta^{18}O$ showed less promise. Heritability of this trait was moderately high, but grain $\delta^{18}O$ was not strongly correlated with yield and retrospective gains from selection were small. For one population, none of the SATs showed any convincing association with yield. The reasons for this need further investigation as SATs are evaluated over additional seasons

**Keywords:** stomatal aperture, yield potential

## INTRODUCTION

Since the initial impact of the Green Revolution, improvement in genetic yield potential of wheat stands at *ca*. 0.9% per year. This is about half what will be required to meet expected demand for wheat in developing countries, and indicates an urgent need to develop more efficient breeding methods for yield improvement.

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Traits related to stomatal conductance may prove useful for improving selection for yield potential. Research during the 1990's, aimed at understanding the physio logical basis of historic gains in yield potential at CIMMYT, showed a consistent correlation between the historic increase in yield potential among CIMMYT semi-dwarf bread wheats and changes in stomatal aperture-related traits (SATs). In summary, more-recent, higher yield-potential CIMMYT wheats had greater stomatal conductance and, therefore, cooler canopies than older, lower yield-potential releases, (Fischer et al. 1998). These differences in instantaneous physiological parameters were also reflected in the long-term stable-isotope composition of C and O measured in plant dry matter. More-recent CIMMYT releases showed greater discrimination against $\Delta^{13}C$ (higher $\Delta^{13}C$) and a lower $\delta^{18}O$ composition (lower $\delta^{18}O$) (Fischer et al. 1998, Barbour et al. 2000), both consistent with the observed historic changes in stomatal conductance. Earlier, Condon et al. (1987) had demonstrated positive associations between yield and $\Delta^{13}C$ under well-watered conditions in Australia and Reynolds et al. (1994) had shown cooler canopies to be associated with higher yield under warm, irrigated conditions in Mexico.

This paper reports results from recent studies conducted at CIMMYT aimed at assessing the utility of SATs as indirect selection criteria for high yield-potential in bread wheat. Two classes of SATs were assessed: the instantaneous trait leaf porosity (POR), which is a close surrogate for stomatal conductance, and the integrative traits $\Delta^{13}C$ of leaf and grain and $\delta^{18}O$ of grain.

## MATERIALS AND METHODS

### Germplasm

Large sets (n = 48–64) of random, $F_3$-derived $F_5$ lines from 4 crosses were grown. Two populations were from crosses already known to be varying for SATs among the progeny: Siete Cerros/Seri (Cross 1) and Quarrion/3*Genaro (Cross 2). Two populations were from crosses among elite parents selected from breeders' crossing blocks on the basis of measurements of SATs: Ures/Jun//Kaus/3/SW89.3243 (Cross 3) and SSeri1/SW89.3243 (Cross 4).

### Trial Management

The 4 populations were sown in 2-replicate trials at CIMMYT's field station at Obregon, NW Mexico, using lattice designs with repeated checks. Populations were sown in $6m^2$ yield plots and in small plots ($1.5m \times 2$ rows) simulating breeders' early-generation observation plots. Sowing was in mid-late October 2001, anthesis occurred towards the end of February and plots were harvested after grain maturity in late April. Yield plots were also sown in 2002 and 2003 to obtain a more robust estimate of yield potential, i.e. yield averaged over 3 seasons. All sowings received 5–6 irrigations, each of *ca.* 100mm. Weeds were controlled by early herbicide application and then by hand. Pests and diseases were controlled with foliar sprays when necessary.

### Measurements

Grain yield was measured by machine-harvesting yield plots grown in all 3 seasons and the 2-row plots grown in 2001–02. Data on stomatal aperture traits (SATs) was collected on 2-row plots in 2001–02 to simulate the use of SATs in breeders' observation plots.

Leaf porosity (POR) of six sun-lit flag-leaves per plot was measured using a Thermoline viscous-flow porometer. Raw data from the porometer (counts) was inverted (1/counts) to generate POR data linearly related to stomatal conductance over the range of counts measured on irrigated wheat plants (Rebetzke et al. 2001). Single sets of data were collected from each plot once in the 2 weeks before anthesis and once in the 2 weeks after anthesis. POR data was collected 3–12d after irrigation and on cloud-free days without high wind between 1000h and 1500h.

Recently-expanded leaf material was sampled in early January 2002, near the time of full ground cover, for stable isotope analysis. Subsamples of grain for isotope analysis were taken after machine-harvest in April 2002. Analysis of carbon isotope composition ($\Delta^{13}C$) of ground, dried (70 °C) leaf and grain samples was done using a Europa 'ANCA' sample preparation system and a Europa '20–20' ratio mass spectrometer. Values of carbon isotope discrimination ($\Delta^{13}C$) were calculated assuming a $\Delta^{13}C$ of air of $-8‰$. Grain samples only were analysed for oxygen isotope composition $\delta^{18}O$) using the same mass spectrometer after pyrolysis of dry matter at very high temperature and separation of CO from $N_2$ using gas chromatography (Barbour et al. 2000).

### Statistical Analysis of Data

Data were analysed using mixed models (REML) after checking for normality and error variance homogeneity. Data transformation was not required. Estimates of heritability ($h^2$), and of phenotypic ($r_p$) and genetic ($r_g$) correlations of yield and SATs, were calculated from components of variance and co-variance.

## RESULTS

### Variation in Yield and SATs

The mean grain yield in these trials of 520g $m^{-2}$ was very similar for all 3 yield estimates, i.e. 3-year-mean plot yield, 2001–02 plot yield and 2001–02 two-row yield (Table 1). Average yields were similar for the 4 populations and all 3 yield estimates showed large, highly significant genotypic variation. Depending on cross and yield estimate, yield varied over a range of *ca.* 120–300g $m^{-2}$ among lines. Average values for SATs measured on 2-row plots in 2001–02 were similar for the 4 populations (Table 1). Within each population there was substantial genotypic variation for the stable isotopes and for POR. The stable isotopes showed the highest heritability ($h^2$), similar to or higher than $h^2$ of yield estimates (Table 1). Heritability of POR was similar to $h^2$ of yield.

*Table 1.* Summary of variation in grain yield and SATs among $F_{3:5}$ lines from four crosses

| Cross | Trait value[1] | Grain yield (g $m^{-2}$) | | | Stable isotopes (‰) | | | POR | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3-year yield 2001–04 | Plot yield 2001–02 | 2-row yield 2001–02 | Leaf $\Delta^{13}C$ | Grain $\Delta^{13}C$ | Grain $\delta^{18}O$ | Pre-anthesis | Post-anthesis |
| Cross 1 | mean | 500 | 539 | 493 | 18.7 | 17.9 | 31.6 | 8.7 | 6.2 |
| (n = 64) | max | 584 | 642 | 610 | 19.6 | 18.9 | 33.1 | 10.1 | 8.7 |
| | min | 342 | 342 | 343 | 17.7 | 16.3 | 30.6 | 6.5 | 1.2 |
| | lsd | 53 | 91 | 84 | 0.6 | 0.5 | 0.9 | 1.2 | 1.8 |
| | $h^2$ | 0.69 | 0.62 | 0.61 | 0.50 | 0.78 | 0.52 | 0.54 | 0.80 |
| Cross 2 | mean | 542 | 532 | 557 | 18.5 | 18.0 | 31.0 | 8.4 | 3.9 |
| (n = 48) | max | 595 | 612 | 645 | 19.2 | 18.6 | 31.9 | 10.2 | 6.8 |
| | min | 472 | 392 | 462 | 17.7 | 17.1 | 29.8 | 5.4 | 1.4 |
| | lsd | 30 | 57 | 84 | 0.5 | 0.4 | 0.7 | 1.8 | 2.4 |
| | $h^2$ | 0.78 | 0.63 | 0.39 | 0.56 | 0.80 | 0.60 | 0.33 | 0.43 |
| Cross 3 | mean | 523 | 513 | 517 | 18.3 | 17.9 | 31.9 | 7.1 | 3.9 |
| (n = 48) | max | 583 | 589 | 598 | 19.1 | 18.7 | 32.9 | 8.5 | 6.8 |
| | min | 435 | 335 | 386 | 17.6 | 17.0 | 30.6 | 5.6 | 2.2 |
| | lsd | 30 | 66 | 87 | 0.5 | 0.4 | 0.7 | 1.3 | 2.1 |
| | $h^2$ | 0.77 | 0.59 | 0.41 | 0.44 | 0.69 | 0.56 | 0.27 | 0.31 |
| Cross 4 | mean | 511 | 499 | 507 | 18.1 | 17.8 | 32.3 | 8.0 | 4.2 |
| (n = 48) | max | 634 | 581 | 597 | 19.2 | 18.4 | 33.7 | 9.1 | 7.0 |
| | min | 453 | 390 | 369 | 17.4 | 16.4 | 30.8 | 6.9 | 1.2 |
| | lsd | 43 | 74 | 89 | 0.8 | 0.4 | 0.9 | 1.1 | 1.6 |
| | $h^2$ | 0.64 | 0.34 | 0.43 | 0.36 | 0.71 | 0.63 | 0.37 | 0.59 |
| All | mean | 519 | 521 | 519 | 18.4 | 17.9 | 31.7 | 8.1 | 4.6 |
| | $h^2$ | 0.72 | 0.55 | 0.46 | 0.47 | 0.75 | 0.58 | 0.38 | 0.53 |

[1] For each cross, the mean, maximum and minimum values are shown for each yield estimate and SAT, as is the lsd ($P < 0.05$) and heritability ($h^2$).

## Associations of SATs with Yield

Phenotypic correlations ($r_p$) of plot yield with SATs are shown in Table 2 in comparison with $r_p$ of plot yield with 2-row yield. Correlations tended to be greater with 3-year plot yield than with plot yield measured in 2001–02. The strength of correlations of plot yield with SATs and with 2-row yield varied with population. Correlations were strongest for Cross 1 and weakest for Cross 4.

For Crosses 1, 2 and 3, correlations were always in the direction expected, i.e. positive for $\Delta^{13}C$ and POR, negative for $\delta^{18}O$. The results for Cross 4 were inconsistent with the other 3 crosses. For Cross 4, phenotypic correlations of SATs with plot yield were small and variable in direction. Even the phenotypic correlations of 2-row yield with plot yield were small (0.0 with plot yield in 2001–02; 0.2 with 3-year plot yield). The very poor correlation of 2-row yield with 3-year plot yield indicates that there may have been unobserved problems with the 2-row plots of Cross 4 that caused more 'random' variation in 2-row yield and perhaps more 'random' variation in SATs.

As expected, genetic correlations ($r_g$) of SATs with yield were larger than values of $r_p$, (Table 3). Values of $r_g$ were in most cases greater for 3-year plot yield than for 2001–02 plot yield. Genetic correlations of 2-row yield with plot yield were consistently high (0.7–1.0), except for Cross 4 (*ca.* 0.5). Values of $r_g$ for plot yield with leaf $\Delta^{13}C$, grain $\Delta^{13}C$ and POR were moderate to high in most cases, except for Cross 4. Value of $r_g$ for $\delta^{18}O$ were lower. For Crosses 1, 2 and 3, genetic correlations of plot yield with $\Delta^{13}C$, $\delta^{18}O$ and POR were usually in the direction anticipated. For Cross 4, the direction of $r_g$ was more variable and the values were low.

Calculations from retrospective selection of the top and bottom 25% of lines based on SATs generated average gains in plot yield of up to 40g $m^{-2}$ (Table 4). Gains in plot yield in 2001–02 were similar to gains in 3-year yield. Average yield

*Table 2.* Phenotypic correlations ($r_p$) of SATs measured on 2-row plots with grain yield measured on large plots. Correlations are shown with grain yield in the year SATs were measured and with 3-year average grain yield

| Yield estimate | Cross | 2-row yield | Leaf $\Delta^{13}C$ | Grain $\Delta^{13}C$ | Grain $\delta^{18}O$ | POR Pre-anth | POR Post-anth |
|---|---|---|---|---|---|---|---|
| **2001–02** | Cross 1 | 0.63 | 0.42 | 0.56 | −0.16 | 0.38 | 0.48 |
| | Cross 2 | 0.42 | 0.19 | 0.20 | −0.16 | 0.07 | 0.04 |
| | Cross 3 | 0.39 | 0.03 | 0.16 | −0.02 | 0.19 | 0.17 |
| | Cross 4 | 0.00 | −0.12 | −0.05 | 0.02 | −0.19 | −0.13 |
| | Average | 0.36 | 0.13 | 0.22 | −0.08 | 0.11 | 0.14 |
| **2001–04** | Cross 1 | 0.62 | 0.40 | 0.60 | −0.19 | 0.45 | 0.50 |
| | Cross 2 | 0.59 | 0.38 | 0.48 | −0.20 | 0.13 | 0.21 |
| | Cross 3 | 0.52 | 0.19 | 0.40 | −0.11 | 0.26 | 0.23 |
| | Cross 4 | 0.20 | −0.06 | 0.07 | −0.09 | −0.21 | −0.14 |
| | Average | 0.48 | 0.23 | 0.39 | −0.15 | 0.15 | 0.27 |

*Table 3.* Genetic correlations (rg) of SATs measured on 2-row plots with grain yield measured on large plots. Correlations are shown with grain yield in the year SATs were measured and with 3-year average grain yield

| Yield estimate | Cross | 2-row yield | Leaf $\Delta^{13}C$ | Grain $\Delta^{13}C$ | Grain $\delta^{18}O$ | POR Pre-anthesis | POR Post-anthesis |
|---|---|---|---|---|---|---|---|
| **2001–02** | Cross 1 | 0.90 | 0.80 | 0.84 | −0.22 | 0.53 | 0.73 |
| | Cross 2 | 1.00 | 0.35 | 0.33 | −0.12 | 0.48 | 0.32 |
| | Cross 3 | 0.86 | 0.34 | 0.44 | 0.11 | −0.07 | 0.46 |
| | Cross 4 | 0.48 | −0.07 | −0.01 | 0.22 | 0.21 | 0.14 |
| | Average | 0.81 | 0.36 | 0.40 | 0.00 | 0.29 | 0.41 |
| **2001–04** | Cross 1 | 0.71 | 0.69 | 0.76 | −0.15 | 0.68 | 0.71 |
| | Cross 2 | 0.85 | 0.54 | 0.62 | −0.26 | 0.41 | 0.40 |
| | Cross 3 | 0.93 | 0.44 | 0.54 | −0.12 | 0.16 | 0.36 |
| | Cross 4 | 0.55 | 0.11 | 0.00 | 0.07 | 0.01 | 0.01 |
| | Average | 0.76 | 0.45 | 0.48 | −0.12 | 0.32 | 0.37 |

*Table 4.* Yield gains in large plots from retrospective divergent selection based on SATs measured on 2-row plots. Yield gains (g $m^{-2}$) were calculated for a selection intensity of 25%, i.e [average yield of top 25% of lines based on indirect traits] minus [average yield of bottom 25% of lines based on indirect traits]. Yield responses to retrospective selection for SATs

| Yield estimate | Cross | 2-row yield | Leaf $\Delta^{13}C$ | Grain $\Delta^{13}C$ | Grain $\delta^{18}O$ | POR Pre-anthesis | POR Post-anthesis |
|---|---|---|---|---|---|---|---|
| **2001–02** | Cross 1 | 95 | 93 | 93 | 20 | 54 | 78 |
| | Cross 2 | 77 | 28 | 33 | 19 | 12 | 29 |
| | Cross 3 | 44 | 14 | 28 | −6 | 13 | 25 |
| | Cross 4 | 19 | −1 | −12 | −25 | 16 | 7 |
| | Average | 59 | 34 | 36 | 2 | 24 | 35 |
| **2001–04** | Cross 1 | 74 | 61 | 81 | 14 | 56 | 62 |
| | Cross 2 | 58 | 40 | 39 | 13 | 10 | 26 |
| | Cross 3 | 39 | 19 | 34 | 10 | 21 | 17 |
| | Cross 4 | 31 | 9 | 8 | −13 | 4 | −6 |
| | Average | 51 | 32 | 41 | 6 | 23 | 25 |

gains from retrospective selection were positive based on all SATs. They were highest for grain $\Delta^{13}C$ and leaf $\Delta^{13}C$, a little lower for POR (post-anthesis then pre-anthesis) and lowest for grain $\delta^{18}O$. Yield gains from retrospective selection were poorest, sometimes negative, for all SATs applied to Cross 4. Averaged over the other 3 crosses, retrospective selection based on grain $\Delta^{13}C$ gave yield gains of 50g $m^{-2}$. This is about 10% of the average trial yield. Selection based on leaf $\Delta^{13}C$ and POR gave yield gains of *ca.* 40g $m^{-2}$, averaged over Crosses 1, 2 and 3.

## DISCUSSION

For 3 out of the 4 populations, the results of this study indicated strong prospects for using SATs in screening for high yield potential. Grain $\Delta^{13}C$, leaf $\Delta^{13}C$ and POR showed the most promise, with moderate to high heritability, moderate to strong genetic correlations with yield, and yield gains from retrospective selection of about 40–50g $m^{-2}$. Grain $\delta^{18}O$, which reflects the effect of changes in stomatal conductance on canopy temperature, showed less promise. Heritability of this trait was moderately high, but grain $\delta^{18}O$ was not strongly correlated with yield and retrospective gains from selection were small. It is possible that a more direct measure of canopy temperature may prove more effective.

For one population (Cross 4), none of the SATs showed any convincing association with yield. The reasons for this need further investigation. It may reflect poor growth of plants in the 2-row plots relative to growth in yield plots, since for this population the correlations between 2-row yield and plot yield were much weaker than for the other 3 populations. Factors resulting in poor growth in the 2-row plots may have also resulted in variation in stomatal conductance and related traits that was not reflected in yield measured in large plots, either in 2001–02 or subsequent years.

The results of this study indicate that SATs may have potential to cut costs associated with yield testing by, for instance, being used to cull the number of lines taken to the yield-plot stages of a breeding program or reducing the number of years of testing in yield plots. Freeing up resources from the yield-testing activity may permit early-generation testing of a greater number of progeny from more crosses. Measuring SATs is not without cost, of course. Each involves provision of labour, some equipment and there are substantial analysis costs for $\Delta^{13}C$. While this additional cost is a disadvantage for $\Delta^{13}C$, the collection of stable isotope samples is not dependent on the sunny, stable weather conditions that are required for collection of POR data. Nor is there a strong requirement for operator training. Samples are dried, ground and sent to a lab for analysis.

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