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Association of source/sink traits with yield, biomass and radiation use efficiency among random sister lines from three wheat crosses in a high-yield environment

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SUMMARY

For many years yield improvement reported in wheat was associated with increased dry matter partitioning to grain, but more recently increases in above-ground biomass have indicated a different mechanism for achieving yield potential. The most likely way of increasing crop biomass is by improving radiation use efficiency (RUE); however there is evidence that sink strength is still a critical yield limiting factor in wheat, suggesting that improving the balance between source and sink (source/sink (SS)) is currently the most promising approach for increasing yield, biomass, and RUE. Experiments were designed to establish a more definitive link of SS traits with yield, biomass and RUE in high-yield environments using progeny deriving from parents contrasting in some of those traits. The SS traits formed three main groups relating to (i) phenological pattern of the crop, (ii) assimilation capacity up until shortly after anthesis, and (iii) partitioning of assimilates to reproductive structures shortly after anthesis. The largest genetic gains in performance traits were associated with the second group; however, traits from the other groups were also identified as being genetically linked to improvement in performance parameters. Because many of these traits are interrelated, principal component analysis (PCA) multiple regression and path analysis were used to expose these relationships more clearly. The trait most consistently associated with performance traits was biomass at anthesis (BMA). The PCA indicated a fairly close association among traits within this group (i.e. assimilation-related traits) while those from the other two groups of SS traits (i.e. phenological and partitioning) appeared to have secondary but independent effects. These conclusions were partially born out by stepwise multiple regression for individual crosses where BMA was often complemented by traits from the two other groups. Taken together, the data suggest that the assimilation traits biomass in vegetative stage (BMV) and BMA have partially independent genetic effects in this germplasm and were complementary to achieving improved performance. The identification of a number of SS traits associated with yield and biomass, which both PCA and multiple regression suggest as being at least partially independent of one another, support the idea that additive gene action could be achieved by adopting a physiological trait based breeding approach where traits from different groups are combined in a single background. A second breeding intervention based on these results would be in selecting progeny for BMA and BMV using spectral reflectance approaches since those traits that lend themselves to large-scale screening. Path analysis confirmed the importance of the spike primordial stage in the genotype by environment interaction for these traits.

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INTRODUCTION

Increasing the genetic yield potential of crops remains an important research objective for applied scientists for a number of reasons. Year to year variation in yield due to unpredictable weather as well as biotic stresses can have major economic impacts; food security is still not guaranteed for millions of resource-poor people in both urban and rural areas; there is good evidence – in wheat at least – that improved genetic yield potential of cultivars have impact in both favourable as well as marginal agro-ecosystems (Calderini & Slafer 1999; Reynolds & Borlaug 2006). The physiological basis of yield improvement in wheat has been reviewed by different workers (Loss & Siddique 1994; Slafer *et al.* 1994; Calderini *et al.* 1999; Reynolds *et al.* 1999; Fischer, 2007). For many years, most of the yield improvement reported was associated with increased dry matter partitioning to grain while above-ground biomass was not modified (Austin *et al.* 1980; Kulshrestha & Jain 1982; Calderini *et al.* 1995; Sayre *et al.* 1997). In addition, physiological determinants of biomass, especially radiation use efficiency (RUE) of the crop, was apparently unchanged (Calderini *et al.* 1997; Fischer *et al.* 1998). However, more recently, increases in above-ground biomass have been reported (Singh *et al.* 1998; Reynolds *et al.* 1999, 2001; Donmez *et al.* 2001; Shearman *et al.* 2005), indicating a different mechanism for achieving yield potential. Furthermore, despite the theoretical upper limit of harvest index (HI) estimated at 0·60 (Austin *et al.* 1980), there has been no great improvement in partitioning since it reached *c.*0·50 in the mid 1980s (Fischer & Quail 1990). Therefore, the conclusions reached previously by experts that investments in raising wheat yield potential should simultaneously focus on improving source and sink (Richards 1996; Slafer *et al.* 1996) seem to be still valid.

The most likely way of increasing crop biomass is by improving RUE (Slafer *et al.* 1999). Various approaches for raising RUE of wheat have been the subject of review (Reynolds *et al.* 2000), with genetic modification of Rubisco probably being the most recent (Parry *et al.* 2003, 2007). The theoretical limits to RUE were revised by Loomis & Amthor (1996) and when applied to the irrigated wheat environment of the current study suggest that significant increases in RUE are attainable (Reynolds *et al.* 2000). Furthermore, there is an ever increasing body of evidence that suggests sink strength is still a critical yield limiting factor in wheat (Fischer 1985; Slafer & Savin 1994; Abbate *et al.* 1995; Miralles *et al.* 2000; Borras *et al.* 2004; Miralles & Slafer, 2007) and that improving the balance between source and sink is currently the most promising approach for raising yield, biomass, and RUE (Reynolds *et al.* 2001, 2005; Shearman *et al.* 2005; Foulkes *et al.* 2007).

Candidate traits for improving the source/sink (SS) balance come from a number of studies. Bingham (1969) suggested that increasing the relative partitioning of assimilates to the developing spike by anthesis, i.e. spike index, might increase grain set. This was later confirmed by Austin *et al.* (1980) when they analysed the physiological bases of wheat breeding improvement in the UK. In addition, work looking at the association between resources available during spike growth stage and the spike index have supported the idea (Fischer 1985; Slafer *et al.* 1990; Abbate *et al.* 1995). Based on examination of the relationship between photoperiod and changes in relative duration of phenological phases, Slafer *et al.* (1996) proposed increasing the relative duration of spike growth (RSG) through manipulation of genetic sensitivity to photoperiod as a means to reach higher spike mass. Subsequent work by Miralles *et al.* (2000), in which duration of spike growth phase was increased through manipulation of photoperiod, showed that grain set could be increased in this way. Another way to increase investment in spike growth would be to increase pre-anthesis RUE and therefore biomass at anthesis (BMA), making more assimilates available to increase spike mass. Higher dry matter partitioning to the spikes could also be a complementary alternative. Gonzalez *et al.* (2005) showed that photoperiod manipulation increased spike index. In addition, 7Ag.7DL translocation lines that showed improved agronomic performance over their recurrent parents and the following SS traits showed superior expression in tandem with yield (12%) and final biomass (9%): BMA (5%), spike mass (15%), spike index (9%), while RSG was not affected (Reynolds *et al.* 2005). In a subsequent study, BMA was increased artificially with a brief light treatment that increased the rate of biomass accumulation during spike-growth or booting stage (dBMs). The treatment was inevitably associated with increased BMA (21%) but there was a larger increase in spike mass (27%) and substantially increased RUE (10%) during grain filling (Reynolds *et al.* 2005). Work looking at winter wheat cultivars has also shown that pre-anthesis RUE was positively associated with yield gains (Shearman *et al.* 2005).

Therefore, experiments were designed to establish a more definitive link of SS traits with yield, biomass and RUE in high-yield environments using progeny deriving from parents contrasting in some of those traits. The SS traits formed three main groups relating to (i) phenological pattern of the crop (RSG and relative duration of grain filling (RGF)); (ii) realized assimilation capacity up until shortly after anthesis (biomass at flag leaf emergence, biomass in vegetative stage (BMV), dBMs, and BMA); and (iii) partitioning of assimilates to reproductive structures shortly after anthesis (spike mass, spike index and absolute spike size). The specific objectives of the experiments

Table 1. *Weather data averaged for different growth stages in three wheat cycles, NW Mexico, 2002–04*

	Air temperature (°C)		Radiation (MJ/m²/d)	Duration (days)	°C days
Growth stage and year	Max	Min			
2002 Cycle					
(*Growth stage*)					
1 Canopy establishment	24·9	6·4	14·3	26	407
2 Spike primordia	26·3	5·7	14·4	26	416
3 Rapid spike-growth	24·6	7·5	15·6	26	417
4 Grainfill: first half	27·5	7·6	21·7	22	386
5 Grainfill: second half	27·9	8·3	23·4	21	380
2003 Cycle					
Stage 1	25·2	7·8	14·8	26	430
Stage 2	27·7	7·9	15·3	25	445
Stage 3	27·2	11·1	15·3	23	440
Stage 4	25·3	9·0	20·4	22	378
Stage 5	29·1	9·2	24·5	20	383
2004 Cycle					
Stage 1	26·8	6·0	13·0	26	426
Stage 2	23·0	8·6	14·6	26	411
Stage 3	24·0	6·4	18·6	28	425
Stage 4	27·0	8·2	22·4	22	388
Stage 5	30·6	11·7	24·7	19	401

were to study in three sets of random sister lines: (1) which SS traits were best associated with yield, biomass and RUE; (2) the association among SS traits to indicate which trait combinations may result in additive gene action for agronomic performance; (3) how environmental factors at different growth stages impact on growth parameters to determine year to year variation in agronomic performance.

MATERIALS AND METHODS

Crop environment

All experiments were conducted at the CIMMYT experimental station near Cd. Obregon, NW Mexico (20° 27′ N, 54° 109′ W, 38 m asl) during the spring wheat season (late November sowing and April harvest). The site is a temperate, high-radiation environment and appropriate irrigation, weed, disease and pest control were implemented to avoid any biotic or abiotic stresses. However, nitrogen fertilizer was applied at a rate (150 kg N/ha), which in combination with residual soil N estimates was designed to achieve approximately 0·80–0·90 of maximum yield potential (normally 7–8 t/ha; Sayre *et al.* 1997) in order to avoid yield losses associated with lodging. Phosphate fertilizer was applied at a rate of 25 kg P/ha. Plants were sown as plots 5 m long and 1·6 m wide consisting of two raised beds with 3 rows/bed (0·20 m between rows) at seed rates of 100 kg/ha. Plots were sown in randomized lattice designs with two replications on three consecutive wheat cycles.

Emergence dates were 5 December 2001, 2 December 2002 and 6 December 2003. These three cycles will be referred to subsequently as the 2002, 2003 and 2004 growth cycles, respectively. A summary of weather data averaged for five different growth stages in each year is presented in Table 1. The growth stages consisted of three periods up until anthesis (average date) of approximately equal day-degree length, and two periods during grain filling of approximately equal day-degree length. The growth stages corresponded approximately as follows: (1) canopy establishment, (2) spike primordia, (3) rapid spike-growth, (4) first half of grain filling and (5) second half of grain filling.

Agronomic and physiological measurements

Dry vegetative biomass (BMV) was estimated a few days after the last plots achieved full canopy closure (approximately Zadoks stage 35) and BMA was measured shortly (5 days) after anthesis (Zadoks stage 70) of each individual plot. These cuts consisted of the above-ground tissue from three rows of a 0·50 m length of the bed, starting at least 0·50 m from the end of the plot to avoid border effects. Fresh biomass was oven dried at 70 °C for 48 h for dry weight measurement. The trait dBMs was calculated as $BMA-BMV$ divided by the number of days between their respective harvests. The trait spike index was estimated by selecting at random 12 normal spike-bearing culms from biomass cuts shortly after anthesis and measuring the dry weight of the spikes

and culms separately, spike index being the coefficient of the dry weights, respectively. The trait spike size was the average dry weight of the 12 spikes. The trait spike mass was calculated by multiplying BMA by spike index. Dates of subsequent phenological stages were estimated visually: 0·50 terminal spikelet stage (using a binocular microscope), 0·50 anthesis and 0·50 physiological maturity by the colour of spikes. These values were used to derive two additional phenological parameters: (i) relative duration of rapid spike growth (RSG), i.e. the number of days between terminal spikelet and anthesis stages as a percentage of the number of days between crop emergence and physiological maturity; (ii) the RGF, i.e. days between anthesis and maturity divided by days between emergence and maturity. After physiological maturity was reached, yield was measured by machine-harvesting a bordered area of 4·8 m². Prior to that, a random sub-sample of 100 spike-bearing culms were removed from each plot, dried, weighed and threshed, so that HI could be estimated. Using these data and an estimate of mean kernel weight (MKW), yield components were calculated: spikes/m², grains/spike (GSP), grains/m² (GM2) and final aboveground biomass. RUE was estimated for biomass shortly after anthesis and for biomass at maturity using the sum of incident photosynthetically active radiation from emergence to the day of the anthesis cut and until date of maturity, respectively, after correcting for predictable losses in light interception using the model presented by Reynolds *et al.* (2000). However, the correction assumes no genetic effects in early light interception or staying green at the end of grain-filling. Visual estimates could not detect differences in staying green (not shown); however, observed differences in BMV could have been the result of differences in early light interception. Nonetheless, the fact that growth rate between Zadoks 35 and 70 (dBMs) was highly correlated with estimated RUE shortly after anthesis ($r = 0.79$) suggested that the effects of early light interception were relatively minor and that the estimated RUE values were a reasonable approximation with respect to genetic effects. Canopy temperature was measured on sunny days with an infra-red thermometer on all genotypes on three or four different occasions during boot stage and again during grain filling.

Germplasm

Lines consisted of random F4 derived bulks from three crosses. Cross 1 was Condor/R143// Ente/Mexicali_2/3/A. Squarrosa (TAUS)/4/Weaver /5/Bacanora and 34 sister lines were studied with the parents. Cross 2 was Sonalika/Attila, and Cross 3 was Sonalika/Borlaug, for which 23 sister lines and the parents were studied in each cross. Between F1 and F4 generations the populations were managed as low-density bulks without selection pressure being applied. The parents were chosen for high yield potential and for contrasts in RSG, spike index and BMA (unpublished data).

Statistical analyses

In order to obtain the proportion of the total sums of squares accounted for the genotype by year inter-action for each trait, a combined analysis of variance (ANOVA) was conducted with the PROC GLM procedure from SAS (SAS version 9.1.3; SAS Institute Inc., 2004), with all the effects, environments (years), reps within years, blocks within years and reps, genotypes and genotype by environment inter-action (GEI) being considered as fixed effects.

Since the traits were measured in different units, the principal component analysis (PCA) was performed based on the correlation matrix using the PRINCOMP procedure from SAS, and then graphing the first two eigenvectors associated with the first two largest eigenvalues which accounted for 0·71 of the total variance.

The multiple regression was realized with the PROC REG procedure from SAS using the stepwise selection procedure.

Broad-sense heritabilities (h^2) for each trait were estimated over the 3 years as follows:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_{ge}^2/e) + \sigma^2/re}$$

where r = number of repetitions, e = number of environments (years), σ^2 = error variance, σ_g^2 = genotypic variance and σ_{ge}^2 = GEI variance. Similarly the genetic correlation can be estimated as

$$\rho_g = \frac{\sigma_{gxy}}{\sqrt{\sigma_{gx}^2 \sigma_{gy}^2}}$$

where ρ_g = genetic correlation between the traits X and Y, σ_{gxy} = genetic covariance between the traits, and σ_{gx}^2 and σ_{gy}^2 are the genetic variances for traits X and Y, respectively. The genetic covariance was estimated using the statistical property of the sum of two random variables, which states: $\sigma_{(x+y)}^2 = \sigma_x^2 + \sigma_y^2 + 2\sigma_{xy}$, which can be rearranged and written as

$$\sigma_{xy} = \frac{\sigma_{(x+y)}^2 - \sigma_x^2 - \sigma_y^2}{2}$$

For both broad-sense heritability and genetic correlations, all the variance components were estimated using the PROC MIXED procedure from SAS considering all the terms in the model (environments, replications within environments, blocks within replications and environments, genotypes and GEI) as random effects.

The structural equation modelling (SEM) is a statistical methodology used to quantitatively analyse

the causal structure among a number of variables where each may function as a dependent variable in some equations and as an independent variable in others. To use SEM, prior knowledge of the direction of the causal relationships is assumed and specified through a path diagram and the model is then algebraically specified by a system of regression-type equations. A final model is then developed by fitting successive models and retaining significant variables. The final model yields path coefficients (PCs) and a path diagram that contains only significant paths thus giving insight into important relationships between the traits. For more details see Vargas *et al.* (2007).

In the present study, two alternative path analyses (SEM) were realized, the first based on all the main effects (environment and genotype main effects) and the GEI and the second one based only on the GEI. Both were conducted using the CALIS procedure from SAS following the steps laid out by Hatcher (1998) using the maximum likelihood (ML) estimation method. For the SEM based on all the effects, the analysis was performed directly on the observed variables and without including external environmental covariates. A final model was developed by fitting successive models and retaining significant predictor variables, which resulted in a better-fitting model.

For the SEM based on GEI for characterizing the complex relationships of GEI, where many traits function as both dependent variables to be predicted by environmental and genetic factors (exogenous variables) and also as independent predictor variables of other traits further downstream, the exogenous variables were chosen by using a forward stepwise selection procedure. Again the final model was developed by fitting successive models and retaining significant predictor variables, which resulted in a better-fitting model.

RESULTS

Agronomic response of germplasm

A summary of the mean agronomic response of sister lines from each of the three crosses, averaged over three cycles, is presented in Table 2. Crosses 1 and 3 had a better yield performance on average but agronomic parameters for progeny from all crosses were within the ranges to be expected from elite/elite crosses. The maximum and minimum values of any genotype (averaged over 3 years) considering all three crosses are presented and support this conclusion, for example when considering the range for HI, height, phenology, etc. As mentioned earlier, these trials were N managed to achieve approximately 0·80–0·90 of maximum yield potential to avoid the confounding effects of lodging, which can be considerable and

certainly greater than the generally insignificant genotype × N interaction effects among contrasting but relatively high N levels in this environment (K. D. Sayre, personal communication). Genotype by year interaction was significant for all parameters and the proportion of the total sums of squares from ANOVA that were associated with GEI averaged 0·13 for yield and 0·18 for final biomass. SS traits showed values similar to yield or lower, except for dBMs, which averaged 0·30 and was the highest of any trait. Both yield and final biomass were subject to path analysis to determine the basis of this GEI in terms of the interaction of environmental variables with phenological stage and growth parameters, and results will be presented subsequently. For reference, the actual range in duration from emergence to anthesis and relative duration between terminal spikelet and anthesis (as a proportion of the period from emergence to physiological maturity) respectively were 78–87 days and 0·28–0·34 for Cross 1; 68–84 days and 0·27–0·41 for Cross 2, and 68–88 days and 0·27–0·42 for Cross 3 (average values across all three cycles).

Association of SS traits with performance traits

A number of different analytical approaches were taken to establish which of the SS traits were best associated with performance traits. PCA was made across the three cycles of the experiment for individual crosses as well as all genotypes from the three crosses together; PCA was also run for individual years using all genotypes. Genetic correlation was made of yield and final above-ground biomass with SS traits considering crosses separately and together across years. Phenotypic correlations were made of yield, final biomass, and RUE with SS traits. Stepwise multiple regression was performed for SS traits on yield, final biomass and RUE.

Considering the PCA across genotypes of all crosses and years, yield, biomass and RUE can be seen to be associated most strongly with traits associated with assimilation, namely BMV and dBMs and to a lesser extent with BMA (Fig. 1). There was no apparent association with any of the phenological traits RSG, RGF, days from emergence to anthesis (ANT) and days from emergence to physiological maturity (MAT). Of the partitioning traits only spike mass showed association with performance, spike size (g) and HI showed no association and spike index showed a negative association. When considering PCA for individual years (combining crosses) the SS traits relating to in-season biomass estimates were consistently associated with performance traits, but when considering individual crosses BMA showed the most consistent association. For RSG there was a consistent weak association with performance traits in all years but its relationship with yield varied

Table 2. *Growth parameters of random sister lines from three crosses averaged across genotypes and three growing cycles, and genotype range represented by max and min 3 year average values considering genotypes from all crosses, NW Mexico, 2002–04*

Phenology	Terminal spikelet (days)	Anthesis (days)	Maturity (days)	RSG* (propn)	RGF† (propn)
Cross 1 ($n=36$)	39	81	120	0·35	0·32
Cross 2 ($n=25$)	35	75	118	0·33	0·36
Cross 3 ($n=25$)	37	75	120	0·32	0·37
S.E.	1·6	2	1	0·014	0·014
Minimum	30·0	68	115	0·28	0·27
Maximum	44·8	88	124	0·44	0·42

Pre-harvest	Biomass vegetative (g/m²)	Biomass anthesis (g/m²)	Spike growth rate (g/m²/d)	RUE pre-grainfill (g/MJ)	Spike Index	Spike mass (g/m²)
Cross 1 ($n=36$)	328	818	19·4	1·79	0·317	256
Cross 2 ($n=25$)	244	681	17·8	1·64	0·341	228
Cross 3 ($n=25$)	256	704	18·4	1·70	0·356	247
S.E.	28	47	1·9	‡	0·017	16·8
Minimum	185	577	15·8	1·42	0·275	196
Maximum	395	917	23·8	2·09	0·397	304

Final harvest	Yield dry weight (g/m²)	Kernel dry weight (g)	Grains/m²	Harvest Index	Biomass dry weight (g/m²)	RUE (g/MJ)	Spikes/m²	Grains/spike	Height (m)
Cross 1 ($n=36$)	585	37·9	15 500	0·462	1270	1·53	293	54·7	0·96
Cross 2 ($n=25$)	508	42·1	12 200	0·466	1100	1·36	254	49·6	0·94
Cross 3 ($n=25$)	562	44·6	12 800	0·481	1180	1·43	273	48·3	0·91
S.E.	26	1·9	931	0·013	62	‡	17	3·2	0·020
Minimum	422	32·9	9500	0·422	910	1·15	200	38·5	0·82
Maximum	685	50·5	19 500	0·517	1450	1·78	345	65·5	1·01

* RSG = relative duration of spike growth period.
† RGF = relative duration of grain filling period.
‡ Calculated from means.

considerably when considering different crosses. The trait RGF showed a more or less reciprocal relationship to RSG. For spike index, the overall tendency considering years and crosses was for a weak negative association with yield.

Genetic correlations of SS traits with the performance traits yield and final biomass are presented in Table 3. The SS traits that were most consistently and strongly associated with performance traits were BMA, BMV and spike mass. Interestingly, although a positive relationship was found between BMA and the length of the emergence–anthesis period a strong association between BMA and RUE was also found (Fig. 1). The trait spike index had a tendency to be negatively associated with yield but was more strongly and consistently negatively associated with final biomass. One of the phenological traits RGF

was consistently and negatively associated with both yield and biomass, while RSG showed more inconsistent results, being negative for Cross 1 and positive for the other two. The trait dBMs showed inconsistent genetic correlations with yield and final biomass. Phenotypic correlations were run between SS traits and three performance related traits – yield, final biomass, and RUE – and generally results were quite consistent with genetic correlations. Spearman correlation coefficients were significant but generally weaker than genetic correlations for BMV, BMA and spike mass, except that BMV did not show significance for Cross 2. For spike index, while the general trend was also towards a negative association with biomass and RUE, results were only significant for Cross 3. The phenological trait RGF also showed negative association with performance traits but

Table 3. *Broad-sense heritability and genetic correlations with yield and with final biomass of SS traits for random sister lines of three crosses averaged over three growth cycles, NW Mexico, 2002–04*

	RSG	RGF	BMV	BMA	dBMs	SPM	SPI
Broad-sense heritability							
All genotypes	0·58	0·95	0·60	0·83	0·04	0·69	0·54
Avg. three crosses	0·37	0·92	0·49	0·77	0·18	0·70	0·44
Cross 1	0·12	0·87	0·58	0·63	0·21	0·56	0·40
Cross 2	0·50	0·97	0·40	0·87	0·12	0·81	0·45
Cross 3	0·49	0·91	0·48	0·80	0·21	0·72	0·48
Genetic correlation with yield							
All genotypes	0·44	−0·42	0·85	0·75	0·00	0·76	−0·26
Avg. three crosses	0·22	−0·30	0·67	0·67	0·40	0·53	−0·10
Cross 1	−0·16	−0·16	0·83	0·95	0·90	0·55	0·12
Cross 2	0·44	−0·47	0·33	0·57	−0·25	0·54	−0·34
Cross 3	0·37	−0·26	0·84	0·50	0·55	0·49	−0·08
Genetic correlation with final biomass							
All genotypes	0·55	−0·63	0·84	0·89	−0·07	0·73	−0·59
Avg. three crosses	0·25	−0·49	0·54	0·72	−0·18	0·67	−0·43
Cross 1	−0·44	−0·30	0·60	0·72	−0·18	0·87	−0·30
Cross 2	0·83	−0·66	0·28	0·76	−0·53	0·59	−0·62
Cross 3	0·35	−0·52	0·73	0·68	0·16	0·56	−0·37

Abbreviations used: RSG and RGF = relative duration of spike growth and grain filling periods, respectively. BMV and BMA = biomass at full canopy cover and anthesis, respectively. dBMs = growth rate during spike growth period. SPM and SPI = spike mass (g/m²) and spike index shortly after anthesis.
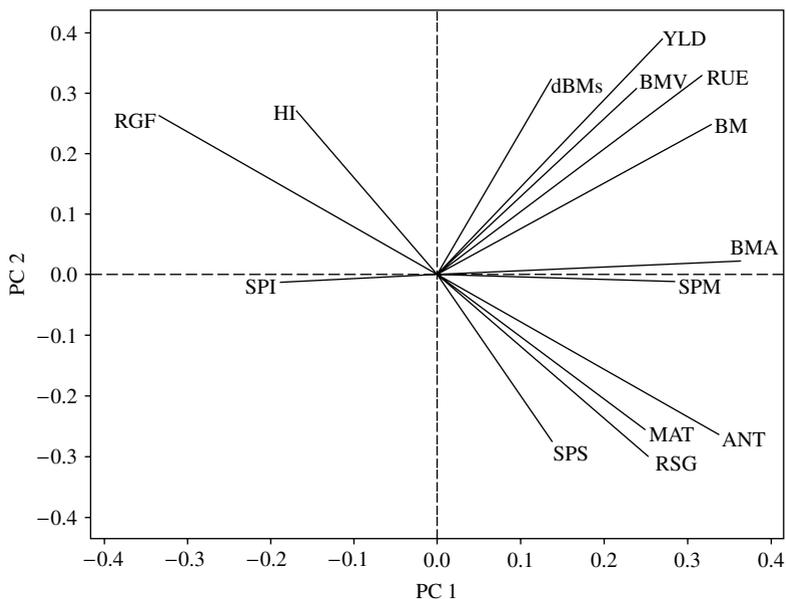

Fig. 1. PCA of SS traits with yield and biomass considering 86 genotypes from three crosses grown over three crop cycles, NW Mexico, 2002–04. SPM and SPI = spike mass (g/m²) and spike index shortly after anthesis.

coefficients were not significant in Cross 1, while RSG again showed positive association but it was not significant for any performance traits in Cross 1 either. The trait dBMs showed significant positive association with performance related traits in Cross 1 only. Phenotypic correlations for spike size (g) were positively associated with all three performance traits for Cross 1 only. Phenotypic correlations between

Table 4. *Stepwise multiple regression of SS traits on yield, final biomass, and RUE for random sister lines of three crosses averaged over three growth cycles, NW Mexico, 2002–04*

	Step	Variables	Model R^2	F	Prob $> F$
Yield					
Cross 1	1	BMA	0·528	38·060	0·001
(n = 36)	2	SPI	0·616	7·570	0·010
	3	RGF	0·650	3·040	0·091
	4	dBMs	0·675	2·450	0·128
Cross 2	1	RSG	0·226	6·700	0·016
(n = 25)	2	SPS	0·373	5·150	0·034
	3	BMA	0·524	6·690	0·017
Cross 3	1	BMA	0·161	4·410	0·047
(n = 25)	2	RGF	0·228	1·910	0·180
Biomass					
Cross 1	1	BMA	0·619	55·140	0·001
(n = 36)	2	RSG	0·643	2·280	0·140
Cross 2	1	BMA	0·389	14·640	0·001
(n = 25)	2	SPS	0·553	8·070	0·010
	3	dBMs	0·706	10·900	0·003
Cross 3	1	BMA	0·324	11·000	0·003
(n = 25)	2	SPI	0·370	1·610	0·218
	3	SPM	0·407	1·310	0·265
Rue					
Cross 1	1	BMA	0·597	50·370	0·001
(n = 36)	2	RGF	0·661	6·200	0·018
Cross 2	1	BMA	0·230	6·860	0·015
(n = 25)	2	SPS	0·508	12·460	0·002
	3	RGF	0·645	8·110	0·010
Cross 3	1	BMA	0·150	4·040	0·056
(n = 25)	2	SPI	0·275	3·810	0·064

Abbreviations of variables: RSG and RGF = relative duration of spike growth and grain filling periods, respectively. BMA = biomass shortly after anthesis. dBMs = crop growth rate during spike growth period. SPM, SPI and SPS = spike mass (g/m²), spike index and spike size (g) shortly after anthesis.

performance traits and canopy temperature measured both during the boot stage and during grain filling were very highly significant.

Stepwise multiple regression was run on performance traits – yield, biomass and RUE – using all SS traits for the three crosses and results are presented in Table 4. The SS trait BMA was involved with eight out of the nine regression models and was the first step in five cases, while BMV was included in five models, in conjunction with BMA in three cases. The SS traits related to phenological pattern RGF and RSG were included in four and three models respectively. The trait spike size (g) was included in all three models for Cross 2 but not in the other crosses. The traits spike mass, dBMs and spike index were

included in one model each. When comparing the SS traits that were adopted among the nine models, no strong pattern emerged except for the fact that more variation was explained for all three performance traits in Crosses 1 and 2 than for Cross 3, and BMA appeared to be more important in explaining variation in final biomass and RUE than it was for grain yield.

An SS trait that has been reported previously as being associated with GM2 in a set of Argentinean cultivars released after 1984 (Abbate *et al.* 1998) is the grain number to spike dry matter ratio at anthesis. In the current study, the trait showed a similar range of genetic variation (60–100 grains/g) as observed previously and showed a 0·6 correlation with GM2 in Crosses 1 and 2 while the association was reciprocal with MKW. However, the trait was not associated significantly with yield, biomass, RUE or SS traits for any of the crosses.

Path analysis of trait relationships: main effects of genotypes and interaction of genotypes with years

Path analysis is a means of investigating the chronological or mechanistic order in which variables impact on each other. However, since the path analysis is recommended for a great number of genotypes, it was conducted for genotypes of all crosses together (n = 86) and the results are not necessarily expected to coincide with those seen when considering individual crosses. Furthermore, because almost all the variables are highly correlated (calculated as linear combinations of other variables) using the methods described could generate singularities in the model, therefore only ten variables were included in the model at any one time and different combinations of SS traits were tested in order to achieve a model that explained the largest degree of variation. As a consequence the traits spike index, spike size (g) and RGF were not adopted in the models presented (Figs 2 and 3). Despite the fact that yield components are known to show compensation among each other, both these numerical components as well as physiological variables (SS traits) were included in path analysis to determine the influence of the latter on the former. In fact, several analyses with different combinations of variables were run and those presented represent the models that explained the largest proportion of variation. For example, an analysis with biomass as the final product instead of yield is presented elsewhere in this issue (Vargas *et al.* 2007). Since the result of path analysis is quite complex with this many variables, the path diagram representing a single year has been chosen to illustrate main effects (Fig. 2). The PCs will now be considered in approximate chronological order of effect during the cycle, and the average PC across the three crop cycles will be presented in parentheses.
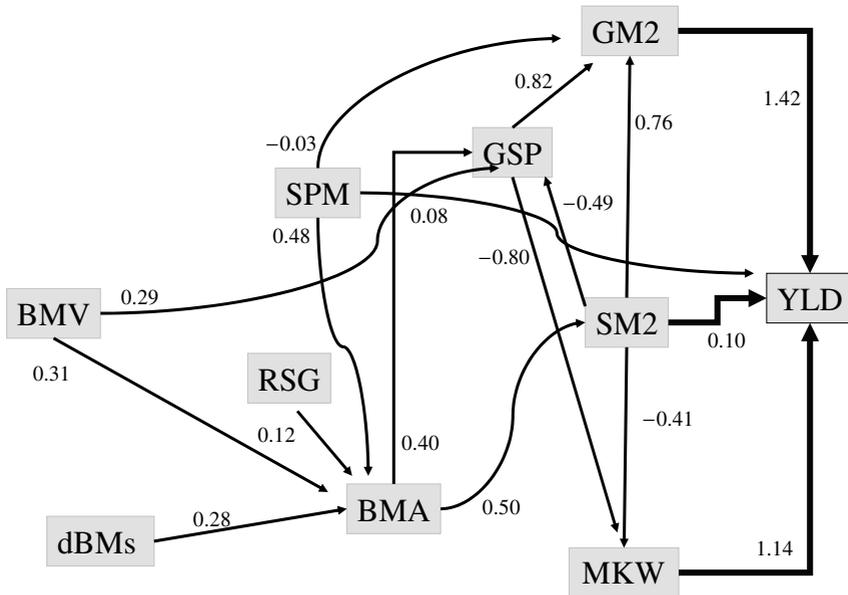
Fig. 2. Path analysis of main effects of SS traits on YCs and of YCs on yield for 86 genotypes from three crosses, NW Mexico, 2004 (PCs shown). SPM = spike mass (g/m²).
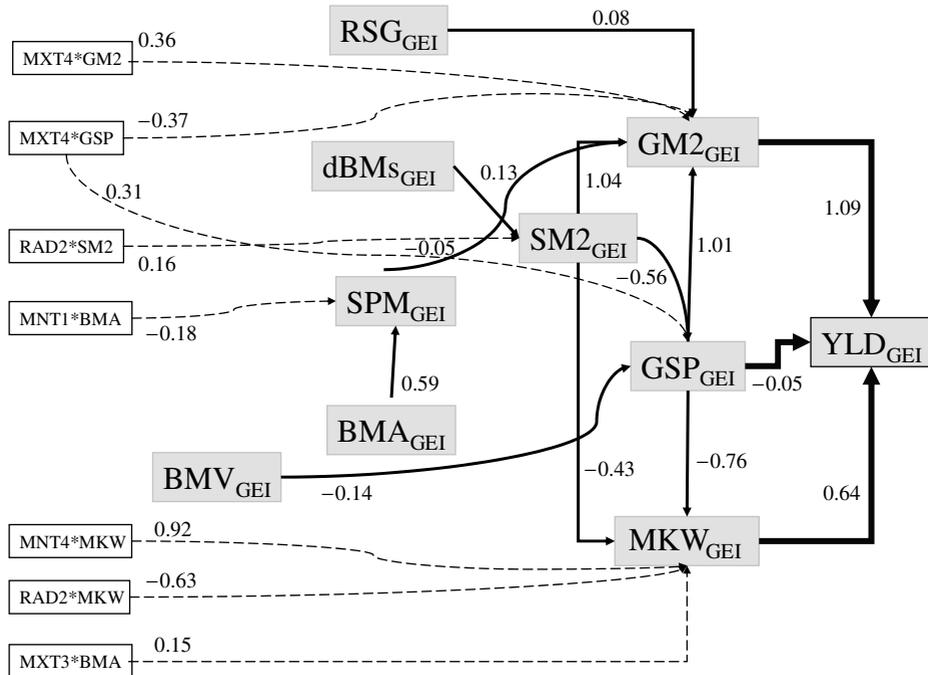

Fig. 3. Path analysis of GEI effects among environmental variables, SS traits, YCs and yield, for 86 genotypes from three crosses, over three crop cycles, NW Mexico, 2002–04 (PCs shown). SPM = spike mass (g/m²).

Biomass estimated at full canopy cover (BMV) showed relatively high PCs with spikes/m² (0·20) and BMA (0·21). In one year only it was associated with GSP (Fig. 2) and therefore there was a significant PC between these variables for GEI (Fig. 3). These results suggest that while early establishment strongly influences final spike density, more subtle indirect effects at this stage may influence compensation among yield components. Growth rate during spike growth stage (dBMs) showed relatively high PCs with BMA (0·48) in all years as to be expected and generally negative PCs with GSP (−0·20). The trait dBMs showed a negative PC with spikes/m² (−0·29) in year 1 only and there was a significant PC between these variables for GEI (Fig. 3). While it is predictable that dBMs would have a strong influence on BMA it is interesting that there was a negative effect on GSP and suggests that higher growth rate in this period may be achieved through increased tiller survival with the inevitable compensation in yield components. However, this is contrary to what has been found in other studies showing that higher crop growth rate during this period increases the number of viable flowers and grains per spike (Miralles & Slafer, 2007). Relative duration of rapid spike growth (RSG) showed a positive PC with BMA (0·35) while BMA showed strong PC with GSP (0·50) and with spikes/m² (0·34). In 2002, only BMA showed a strong PC with spike mass and consequently there was a strong PC for the GEI of BMA with spike mass. This strong GEI is not easy to explain. In subsequent years spike mass showed a PC in the direction of BMA implying that genetically determined high spike mass was a driving force for high BMA. The only PC associated with GEI for BMA is with minimum temperature in growth stage 1; the 2002 cycle had the coolest mean daily temperature (though minimum temperature itself was not the coolest of the three cycles) and cycle was associated with relatively low number of spikes/m² so it is unclear what cause and effect may have been, if any. When the variable spike index was included in the path analysis model of main effects (instead of spike mass) it showed no consistent effects while in the GEI model it showed no connection with any yield component and weaker connections with other variables compared with spike mass. The PCs for main effects among yield components were predictable (Fig. 2) and the GEI effects (Fig. 3) will be discussed subsequently.

Path analysis of trait relationships: interaction of genotypes with weather data

Since yield and biomass showed significant interactions with year, path analyses of interaction effects (Fig. 3) were modelled with weather data considering temperature and radiation parameters for five phenological stages (Table 1). The intention was to determine whether GEI effects could be traced to the influence of weather on traits during specific growth stages. Most of the PCs for indirect (weather) effects related directly to yield components (YCs). Of the SS traits the only strong PC was between BMA and spike mass and was associated with a reversal in the direction of the PC between these variables which is not easily explained by environmental data as already stated. There were relatively weak PCs associated with BMA, in one case the interaction with minimum temperature in stage 1 (canopy establishment) seemed to impact on spike mass (Fig. 3), suggesting that even the earliest environmental events can have subtle effects on SS traits. The other example was the interaction of BMA and maximum temperature in stage 3 (rapid spike growth) which impacted on MKW; at that stage temperature influenced grain weight potential (Calderini & Reynolds 2000; Ugarte et al. 2007) or else it was related to compensation among YCs.

When considering YCs, the largest PCs were associated with MKW. Radiation in stage 2 (the spike primordia) and minimum temperature in stage 4 (first half of grain filling) were clearly associated with significant GEI effects. However, when considering MKW across genotypes of all crosses broad-sense heritability was 0·90, so these effects must have been quite subtle; scatter plots of MKW among years show fairly tight and linear grouping along lines that show close to a 1:1 relationship. The other YC with relatively large PCs associated with weather effects was the other major YC GM2. In this case, maximum temperature in the first half of grain filling interacted both directly on GM2 and indirectly through GSP to influence GM2. It is not unexpected that differences in temperature in the period between anthesis and mid grain filling would impact on GM2 directly and via GSP, as kernel abortion is likely to be occurring in distal florets (Calderini & Reynolds 2000). Again, these effects are likely to be very subtle as broad-sense heritability for GM2 was 0·91; however, while scatter plots among years for GM2 showed generally tight and linear relationships there were occasional outliers that were most likely accounting for the GEI effects. An interaction showing a relatively weak PC was that of radiation in stage 2 with spikes/m², suggesting differential sensitivity of genotypes in their assimilation capacity that affected tiller growth or survival. The magnitude of PCs for direct effects among YCs and with yield suggest that variation in spikes/m² was more likely to cause GEI effects on GM2 than on MKW while variation in GM2 is more likely than MKW to cause GEI in yield. This is to be expected given the relatively conservative nature of MKW relative to GM2 (Sadras, 2007). In the present study, GEI for the latter was associated with 12 % of the total sums of squares from ANOVA versus 9·3 % for MKW.

The GEI effects were also modelled using path analysis for final biomass (see Fig. 3; Vargas *et al.* 2007). Despite the fact that biomass was associated with a larger proportion of sums of squares for GEI than yield, the results were almost identical as those outlined for yield.

DISCUSSION

Two of the main objectives of the present study were to determine which SS traits were best associated with performance parameters – yield, biomass and RUE – and to analyse the association among SS traits to ascertain which traits in combination may result in additive gene action. Considering the three main groups of SS traits (i) phenological pattern, (ii) assimilation capacity up until shortly after anthesis and (iii) partitioning of assimilates to reproductive structures, it is clear from the results that the largest genetic gain in performance traits were associated with the second group. However, traits from the other groups were also identified as being genetically linked to improvement in performance parameters. Since many of these traits are interrelated either physiologically (and numerically in some cases), analytical procedures including PCA, multiple regression and path analysis were adopted with the view to discern these relationships more clearly.

Interrelationships among SS traits

The SS trait most consistently associated with performance traits was BMA. The PCA indicated a fairly close association among traits within this group (i.e. assimilation related traits) while those from the other two groups of SS traits (i.e. phenological and partitioning) appeared to have secondary but independent effects (Fig. 1). These conclusions were partially borne out by stepwise multiple regression for individual crosses, where BMA was often complemented by traits from the two other groups and especially the traits spike size (g) and RGF (Table 4). However, BMV was in fact the trait that most often complemented BMA in multiple regression and the PCA (Fig. 1) also suggested a degree of independence. Taken together, the data suggest that the assimilation traits BMV and BMA have partially independent genetic effects in this germplasm and were complementary to achieving improved performance.

Path analysis of main effects was an attempt to test assumptions about causal relationships among the variables (Vargas *et al.* 2007). It did not produce any surprises with respect to relationships among YCs, and generally confirmed the predictable relationships among SS traits. For example, BMV showed a relatively high association with BMA and spikes/m². Early vigour has been proposed as

an important trait for improving yield in wheat (López-Castañeda *et al.* 1995). The trait dBMs showed high association with BMA and generally negative PCs with GSP, presumably reflecting compensation among YCs. Nevertheless, some results were more surprising. The trait RSG also showed a positive association with BMA, confirming the PCA result (Fig. 1) and this may have been associated with the fact that larger RSG was strongly associated with days to the anthesis which in turn was somewhat associated with larger BMA (see subsequent discussion). The trait BMA showed strong association with both GSP and spikes/m², indicating the importance of BMA in forming two major YCs. In the light of results found in the present study, RUE was one of the main causes of higher BMV and BMA. Then, BMV favoured spikes/m² and spike mass (associated with BMA) improved GSP. Fertile florets/spike and GSP have been shown to be positively associated with spike mass (Miralles *et al.* 2000; Gonzalez *et al.* 2005). In addition, spike mass was a factor in determining BMA.

However, the trait spike index that partially defines relative spike mass was not indicated as being involved in this relationship and in fact showed a surprisingly low and erratic association with other traits in general. This result was not consistent with other work indicating the importance of spike index in determination of grain number (Gonzalez *et al.* 2005). Although work conducted on a set of Argentinean cultivars released after 1984, while showing significant genetic variation in spike index (from 28 to 34%), found that the trait was not associated with yield or grain number (Abbate *et al.* 1998). On the other hand, associations between number of grains/spike and post-anthesis assimilation rate have been found in SS treatments initiated before or at the beginning of the grain filling period but not after it (Savin & Slafer 1991; Calderini & Reynolds 2000), and in other experiments (Reynolds *et al.* 2001, 2005; Shearman *et al.* 2005). One reason why different SS traits appear to have variable influence in determining performance traits in different studies may be related to variations in phenological patterns of the lines being studied; this factor will be discussed in the section on comparing genotypes in experimental breeding populations.

Interpretation of genotype by year (weather) interaction

The attempt to find association of the GEI effect with specific traits and phenological stages was not especially fruitful in the current study, at least compared with other statistical approaches such as partial least squares (PLS) analysis that have been undertaken in the same environment (Reynolds *et al.* 2004), although involving very different types of germplasm.

Theoretically, path analysis using GEI effects is more powerful than PLS as it encompasses a number of response variables simultaneously when partitioning significance to interactions with specific weather parameters throughout the growth cycle. However, in the current analysis PCs for indirect (weather) effects were related mostly to YCs and only a few weak associations were identified with in-season traits and their interpretation was either unclear or not especially revealing. The largest indirect (weather) effects were associated with the two main YCs MKW and GM2. One thing that was consistent with the PLS analysis of GEI was the importance of weather conditions in the spike primoridia and early grain filling stages in influencing GEI (Reynolds et al. 2004). In summary, the path analysis for GEI effects reaffirmed the predictable phenomenon of compensation among YCs but also hinted that the relationship between BMA and partitioning to spike mass may be dynamically influenced by weather, although unfortunately no weather variables were identified by path analysis to follow up on.

Comparing genotypes in experimental breeding populations

While the timing of phenological stages such as anthesis can be controlled by choice of genotypes in studies with unrelated fixed lines, experiments aimed at estimating genetic effects of traits employ the random progeny of experimental crosses. In this kind of population, data can be confounded by two major factors. The first is that some genotypes may have generally poor agronomic adaptation; however, this can be relatively easily overcome by selecting suitable populations from a range of crosses among contrasting but agronomically elite parents, as was the case in the present study. The second confounding factor is genetic variation in flowering date. This is not generally considered to be problematic if the population's overall maturity class fits the target environment. However, this is almost certainly a false assumption and the most likely reason why, for example, QTL studies frequently identify Ppd loci as those most strongly associated with adaptation to stress environments, as has been the case for drought adaptation studies in rice (Lafitte et al. 2007). It is well established in wheat that key developmental processes such as kernel set are determined within relatively narrow developmental windows and can be especially sensitive to environmental conditions (Fischer 1980, 1985; Abbate et al. 1998). Therefore, genotypes growing side by side but which pass through key developmental stages on different dates are likely to trigger different physiological responses at the whole plant level. In summary, the potentially confounding effects of uncontrolled variation in phenology have yet to be fully overcome in studies

with experimental populations aimed at identifying candidate traits and genes for crop improvement, though some progress has been reported recently (Olivares-Villegas et al., in press). The germplasm in the current study showed a 10–20 day range in days to flowering (depending on the cross) and is likely to have influenced some results. For example, while BMA was strongly correlated with RUE (Fig. 1) there was also an association with the duration of the emergence–anthesis period which varied from cross to cross. There was also an association of RSG with BMA, and it appeared that larger RSG was also strongly associated with the duration of the emergence to anthesis period. Nevertheless, this is the first study reported which looks comprehensively at the association of SS and performance traits in sister lines and in spite of variation in phenology some very clear patterns emerge which have the potential to be applied in breeding as will be discussed in the following section.

Implications for breeding

Assuming that the genetic backgrounds chosen for this work are representative of other germplasm sources used in breeding for yield potential, the present results provide a set of traits that can be applied in a number of ways. The first intervention would be in crossing where potential parents can be screened for the SS traits and crosses made between parents with complementary characteristics. The identification of a number of SS traits associated with yield and biomass, which both PCA and multiple regression suggest as being at least partially independent of one another, support the idea that additive gene action could be achieved by adopting a physiological trait-based breeding approach where traits from different groups are combined in a single background (Reynolds & Trethowan, 2007). The traits identified in the present study fit into two main groups: pre-anthesis source traits (including BMV, BMA and dBMs), pre-anthesis sink traits (including spike mass and spike index) and phenology traits as RSG and RGF. The importance of post-anthesis assimilation rate probably in response to sink size was also indicated in the present study by the strong association of performance traits with canopy temperature (CT) during grain filling.

A second breeding intervention based on these results would be in selecting progeny for those traits that lend themselves to large-scale screening. It is fortuitous that BMA was identified in the present study as the trait best associated with yield and biomass since a rapid screening protocol for distinguishing genetic differences in BMA and other crop stages such as BMV has recently been tested and validated in the same environment (Babar et al. 2006a). The methodology involves measurement of

spectral reflectance indices with a hand-held probe and which also distinguishes between yield (Babar *et al.* 2006 *b*). The high heritability of both BMA and BMV (Table 3) supports their value as early generation screening traits. The third way in which the information generated by the current study can be applied to crop improvement would be to identify new and better sources of the SS traits in germplasm collections where the same rapid screening methodologies could be applied at least to screen large collections of accessions for BMV and BMA.

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