Grain Yield Potential Strategies in an Elite Wheat Double-Haploid Population Grown in Contrasting Environments

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ABSTRACT

The understanding of ecophysiological basis of wheat (*Triticum aestivum* L.) grain yield potential provides a useful framework to complement conventional breeding aimed at achieving genetic gains. This study analyzed the ecophysiological performance of an elite wheat mapping population (105 double-haploid lines derived from two modern cultivars, Bacanora and Weebil, with similar phenology but different and stable combinations of grain number per area unit (GN) and grain weight (GW) resulting in high grain yield) grown in four contrasting high-yielding environments, to determine the most successful strategies to increase grain yield potential. Main effect of environment on grain yield was significant ($p < 0.0001$) but the genotypic component was larger than genotype × environment interaction (30%). A robust and positive relationship between grain yield and biomass production was observed across all environments ($r^2 > 0.82$, $p < 0.0001$), and relatively high harvest indexes were expressed (0.39–0.51). While GN was clearly the dominant numerical component in terms of association with grain yield ($r^2 > 0.51$, $p < 0.0001$), a wide range in both components (i.e., GN and GW) was observed across all environments. This population represents a valuable resource for prebreeding studies, as the transgressive segregation in physiological and numerical yield components in combination with favorable expression of all agronomic traits could allow a fine phenotyping and mapping to identify key traits and quantitative trait loci linked with grain yield.

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Abbreviations: AGDM, above-ground dry matter; BA, Buenos Aires; BLUP, best linear unbiased predictor; CdO, Ciudad Obregón; °Cd, Celsius degree days; DH, double-haploid; GFP, grain filling phase; GFR, grain filling rate; GN, grain number per area unit; GNS, grain number per spike; GW, grain weight; GY, grain yield; HI, harvest index; ME, mega-environment; PAR, photosynthetically active radiation; PH, plant height; RUE, radiation use efficiency; SEP, stem elongation phase; SEPr, stem elongation phase relative to flowering time; SN, spike number per area unit; Val-1, Valdivia 1; Val-2, Valdivia 2; V_G, variance component for genotype; $V_{G\times E}$, variance component for genotype × environment; V_{res}, variance component for residual.

INCREASES IN WHEAT PRODUCTION will be necessary to underpin food security in the near future (Borlaug, 2007; Evans, 1998). The understanding of ecophysiological basis of grain yield (GY) potential provides a useful framework to complement

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conventional breeding aimed at maintaining or improving genetic gains in yield (Fischer, 2007; Foulkes et al., 2011). Global demand for wheat is growing at a faster rate than current genetic gains (Reynolds et al., 2009a), suggesting a need to increase conventional breeding efficiency, the main strategy of which is empirical selection for GY per se. Therefore, to complement this strategy, the use of simple ecophysiological traits as indirect selection criteria could accelerate breeding, especially in wheat, which has received intense selection pressure (Shorter et al., 1991). However, an important "bottleneck," not only to identify traits functionally linked to GY but also to understand genetics basis, is a detailed and accurate phenotyping of mapping populations. This information, combined with the available molecular tools, will provide a more comprehensive model for understanding the genotype–phenotype relationship and the genotype × environment interaction (Edmeades et al., 2004). Phenotyping should be performed in "elite" mapping populations (i.e., populations derived from crosses elite × elite) because such crosses do not introduce alleles long since discarded by selection for good agronomic performance (Rattey et al., 2009).

Grain yield is the product of the interaction between development and growth integrated over the whole crop cycle (Evans, 1993; Richards, 1996) and can be quantified through (i) growth analysis of biomass accumulation and partitioning and (ii) the main numerical components of GY (Slafer et al., 1994). Therefore, phenotyping can be based on two simple approaches to understand variation in GY potential: (i) above-ground dry matter (AGDM) and harvest index (HI) and/or (ii) grain number per area unit (GN) and grain weight (GW). Focusing on the first approach, genetic gains in wheat GY to date have been explained largely by increments in HI, but in the future must be linked to biomass improvement (Calderini et al., 1999; Miralles and Slafer, 2007). Different studies show that HI of high-yielding cultivars has progressed little since the 1990s (Reynolds et al., 1999; Sayre et al., 1997), suggesting that this trait has probably already been optimized by breeding although stability of HI remains an issue especially under stress (Reynolds et al., 2012). Considering the second approach focusing on numerical yield components, GN has historically shown a close positive association with GY (Calderini et al., 1999; Fischer, 2008; Peltonen-Sainio et al., 2007) while GN and GW are usually found to be negatively associated (Slafer and Andrade, 1989). However, considering the increase in GW of cultivars recently released by CIMMYT (Aisawi et al., 2010) and the lack of association between both numerical components found in an elite population such as Seri-Babax (Rattey et al., 2009), wheat breeding strategies could be more ambitious and try to improve both GN and GW.

The objective of the present study was to analyze the phenotypic variability in traits associated with GY potential in an elite wheat double-haploid (DH) population grown in contrasting yielding environments to assess (i) the most successful strategy to increase GY potential and (ii) their stability across the evaluated environments. The mapping population used is derived from the cross between two spring modern cultivars (Bacanora × Weebil) with similar phenology but different and stable combination of GN and GW resulting in high GY potential. Two approaches to analyze expression of GY were considered as described above.

MATERIAL AND METHODS

Plant Material and Environments

A wheat DH population used in this study consisted of 105 lines derived from a cross between Weebil and Bacanora, performed in CIMMYT (Mexico) and developed by the John Innes Centre (the United Kingdom). The main advantage of using this DH population is its application in a realistic breeding program, as both parents are modern cultivars within the elite CIMMYT germplasm, in contrast to other studies that use populations derived from highly contrasting parents without commercial relevance and with phenological patterns that are sometimes extremely different (Borràs et al., 2009). Four field trials in contrasting environments were performed (Table 1). Experiments were conducted in the Norman E. Borlaug experimental station near Ciudad Obregón (CdO), Mexico, in the experimental field of Facultad de Agronomía, Universidad de Buenos Aires, in Buenos Aires (BA), Argentina , and in the experimental field of Universidad Austral de Chile, in Valdivia, Chile, where two growing seasons were performed (Valdivia 1 [Val-1] and Valdivia 2 [Val-2]). In CdO, the soil was a coarse sandy clay mixed with montmorillonitic clay, classified as Typic Caliciorthid, 0.76% of organic matter content, and pH 7.7. In BA, the soil corresponded to a silty clay loam, classified as Vertic Argiudoll, 2% of organic matter content, and pH 5.4. In Valdivia, the soil was a volcanic ash, classified as Typic Hapludand, 14% of organic matter content, and pH 5.8. In this last location 7 Mg $CaCO_3$ ha^{-1} was applied on plots to avoid Al toxicity, which is usual in the acidic soils of southern Chile. For each field trial, the crop agronomic practices applied were the ones commonly used by farmers in the production system for each location (i.e., Yaqui Valley for CdO, Rolling Pampas Region for BA, and Araucania and Los Rios Regions for Valdivia). In CdO each replicate was sown in a 2.5 m long and 0.8 m wide plot consisting of one raised bed with two rows per bed (0.25 m between rows). In BA, each replicate was sown in flat plots, 2.1 m long and 0.9 m wide with five rows (0.175 m between rows). In Valdivia, each block consisted of a continuous plot where each genotype was sown in three rows 1.5 m long (0.15 m between rows), flanked by a row (0.15 m apart and 1.5 m long) of the local spring wheat 'Pandora INIA' at each side. All trials were irrigated (furrow irrigation in CdO and irrigation by sprinklers in the other sites) and adequate nutrients applied (i.e., fertilization was adequate to obtain more than 20 mg kg^{-1} of P per hectare at sowing in all environment and 180 or 250 kg of N per hectare at tillering in BA and CdO or Valdivia, respectively). Plant pathogens, pests, and weeds were prevented or controlled by recommended chemical treatments in all trials.

Table 1. Description of environments, including location and general characteristics of each field trial, where the double-haploid population was evaluated.

Environment	CdO†	BA†	Val-1†	Val-2†
Location	Ciudad Obregón, Mexico	Buenos Aires, Argentina	Valdivia, Chile	Valdivia, Chile
Latitude	27°25′ N	34°35′ S	39°47′ S	39°47′ S
Longitude	109°54′ W	58°29′ W	73°14′ W	73°14′ W
Altitude, m asl	38	26	19	19
Growing season	2009/2010	2009	2008/2009	2009/2010
Crop system	Raised bed	Flat	Flat	Flat
Experimental design (repetitions)	Alpha lattice (2)	Completely randomized (3)	Randomized complete block (3)	Randomized complete block (3)
Sowing date	2 Dec.	5 July	23 Aug.	4 Sept.
Seed rate, seeds m^{-2}	170–190	250	350	350

†CdO, Ciudad Obregón; BA, Buenos Aires; Val-1, Valdivia 1; Val-2, Valdivia 2.

Phenotyping

In all experiments phenological stages of first node detectable (GS31) (Zadoks et al., 1974) and anthesis (GS65) (Zadoks et al., 1974) in main stems were determined when 50% of the plot reached those stages. Nondestructive measurement of the first node detectable was used as a phenotypic marker to determine the onset of the stem elongation phase (SEP), which coincides with the terminal spikelet differentiation in wheat under most agronomic conditions (Slafer and Rawson, 1994). In BA and CdO, physiological maturity was determined directly through the dynamic of dry matter accumulation (twice per week starting at 10 d postanthesis), considering this stage when dry matter accumulation in those grains ended. In Valdivia, physiological maturity was assessed indirectly by the color of peduncle as a morphological marker, assuming that this stage coincides with the complete loss of green color from the peduncle or the flag leaf (Hanft and Wych, 1982). Crop cycle was divided in three phases: (i) the leaves and spikelets initiation phase, from emergence to first node detectable, (ii) the SEP, from first node detectable to anthesis, and (iii) the grain filling phase (GFP) from anthesis to physiological maturity. Durations of phases were expressed in thermal time units, using daily mean temperature recorded in a weather station localized at each experimental field and assuming a base temperature of 0°C for preanthesis phases (Kirby et al., 1985) and of 8.2°C for GFP (Slafer and Savin, 1991). Plant height from the soil to the base of the spike was recorded in five plants per plot around mid grain filling.

Biomass accumulation, its partitioning, GY, and numerical components were determined in the four field trials. Aboveground dry matter accumulated during the whole cycle was estimated indirectly (see below), through GY and HI, in CdO (Hobbs and Sayre, 2001) and directly in the rest of field trials. Approximately a week after physiological maturity, 0.3 m of two central rows and 0.5 m in the central one was harvested in BA and Valdivia trials, respectively. The samples were oven dried at 60°C until constant weight (approximately 72 h), which was then recorded. These samples were used to estimate AGDM and GY per square meter, which were obtained after threshing the spikes and weighing the grains. Then HI was calculated as the quotient between sample AGDM and sample GY. In CdO, HI was calculated in a similar way with a sample of 25 stems harvested a week after physiological maturity. In this trial the remaining plot (approximately 1.6 m^2) was mechanically harvested approximately 2 wk after physiological maturity. A subsample of these grains was used to estimate the relative humidity and to obtain dry GY and AGDM (used to estimate it the HI calculated previously) per area unit. Grain weight was measured in two subsamples of 100 grains and GN was calculated as the quotient between GY and GW in all trials. Subsequently, the grain filling rate (GFR), which is one of the traits used to describe GW together with the GFP (Egli, 1981), was estimated as the quotient between GW and GFD. Spike number per area unit (SN) was measured directly in BA, Val-1, and Val-2 from the harvested sample and indirectly in CdO, through biomass partition to spike obtained by weighing spikes before threshing in sample used for HI (Hobbs and Sayre, 2001). Finally, another subcomponent of GN, grain number per spike (GNS), was estimated as the quotient between GN and SN in all trials.

Statistical Analyses

The first step of the analysis consisted of the estimation of best linear unbiased predictors (BLUPs) for each DH line (or parental line) and trait, fitting a linear mixed model. As experimental designs were different among environments (Table 1), it was decided to use a postblocking design assigning a number of rows and columns to each plot in each field trial. Best linear unbiased predictors of genotypic effects were estimated using a mixed model where genotype, genotype × environment, row within environment, and column within environment were the random effects and environment was the fixed one. Variance components were estimated for each random term while a standard test of fixed effects was performed for environment. These analyses were performed with the MIXED procedure from SAS (SAS, 1999).

The second step of the analysis, using BLUPs, consisted on a descriptive statistic (i.e., mean, SD, quartiles, and variability range) to evaluate the DH population behavior at each trial and linear and nonlinear regression analysis to determine relationships between GY and its main components. Besides, BLUPs across all trials and in each mega-environment (ME) established (see Results) were used in a principal component analysis and biplots to analyze trait correlations. As the traits had different units, columns were standardized. The genetic correlation between any two traits can be approximated by the cosine of the angle formed by their two vectors, provided that the two principal components of the biplot explain a sufficient amount (>50%) of the total variability (Kroonenberg, 1995). In this case, two traits are positively correlated if the angle

Table 2. Climatic factors in each evaluation environment. Detail of photoperiod, mean temperature, and incident photosynthetically active radiation (PAR) explored, in average, by the population (standard deviation is in brackets) during different crop phases in which wheat in sensitive to them: leaves and spikelets initiation phase (LSP), stem elongation phase (SEP), and grain filling phase (GFP).

Climatic factor	Crop phase	CdO†	BA†	Val-1†	Val-2†
Photoperiod, h	LSP	11.4 (0.02)	11.7 (0.04)	13.4 (0.04)	13.8 (0.08)
	SEP	12.1 (0.10)	13.2 (0.10)	15.1 (0.08)	15.5 (0.08)
Mean temp., °C	LSP	15.2 (0.02)	12.7 (0.03)	10.5 (0.04)	9.4 (0.08)
	SEP	15.6 (0.08)	13.8 (0.26)	13.1 (0.21)	11.1 (0.23)
	GFP	17.9 (0.45)	18.9 (0.46)	16.5 (0.39)	14.3 (0.16)
Incident PAR, MJ m^{-2} d^{-1}	LSP	7.1 (0.06)	5.0 (0.05)	9.6 (0.07)	7.8 (0.09)
	SEP	9.9 (0.38)	8.2 (0.26)	12.4 (0.18)	9.6 (0.40)
	GFP	12.4 (0.23)	9.5 (0.13)	14.1 (0.36)	10.6 (0.31)

†CdO, Ciudad Obregón; BA, Buenos Aires; Val-1, Valdivia 1; Val-2, Valdivia 2.

between their vectors is <90°, negatively correlated if the angle is >90°, or independent if the angle is 90°, and the length of the vectors indicates how much variability is explained for that specific trait. These analyses were performed with InfoStat (Di Rienzo et al., 2010).

RESULTS

Environmental Characterization and Population Behavior

Climatic factors in the four different environments (Table 2) had clear impacts on flowering time and plant height (Fig. 1). The test of fixed effects (meaning environment in this study) was significant ($p < 0.0001$) for these trait while the estimated variance component for genotype (V_G) had a higher relative magnitude with respect to the variance component for genotype × environment ($V_{G \times E}$) and variance component for residual (V_{res}) (Table 3). The V_G was around the triple that of $V_{G \times E}$ for both traits while it was twice V_{res} for plant height and eightfolds greater than V_{res} for flowering time. Across the environments, flowering time was more variable than plant height in the DH population (range in flowering time and plant height was 63 and 38% of general mean, respectively) (Fig. 1). According to population means, the higher the latitude the shorter the flowering time (1335, 1177, 913, and 885 Celsius degree days [°Cd] in CdO, BA, Val-1, and Val-2, respectively). As flowering time was calculated in thermal time units, that result is explained by the different photoperiods among environments (11.7, 12.3, 14.1, and 14.6 h in CdO, BA, Val-1, and Val-2, respectively). In Valdivia, the delay in the sowing date (Val-2) (Table 1) exposed the population to a longer and therefore more inductive photoperiod (Table 2). The range in flowering time decreased as the photoperiod increased (295, 264, 191, and 181°Cd in CdO, BA, Val-1, and Val-2, respectively) from lower to higher latitude (Table 1). Those values, measured in thermal time units, accounted for 19, 16, 13, and 12 d in CdO, BA, Val-1, and Val-2, respectively. Considering plant height, the population range was similar among environments (0.21, 0.25, 0.22, and 0.21 m in CdO, BA, Val-1, and Val-2, respectively). On average, the DH population achieved taller plants in CdO and Val-1 (average plant height of 0.84 and 0.82 m, respectively) than in BA and Val-2 (0.76 m in both environments).

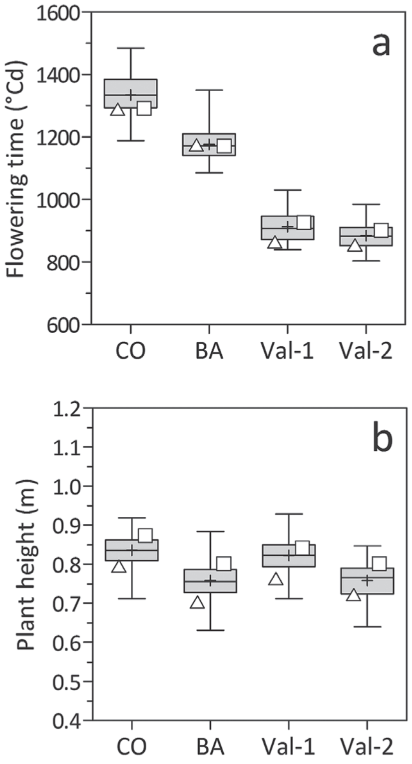

Figure 1. (a) Flowering time and (b) plant height values registered in the double-haploid population in different environments. Box and whiskers of best linear unbiased predictors are presented for each environment, indicating the population minimum, first percentile, mean (cross), median, third percentile, and maximum. Parental lines are indicated by triangles (Bacanora) and squares (Weebil). °Cd, Celsius degree days; CdO, Ciudad Obregón; BA, Buenos Aires; Val-1, Valdivia 1; Val-2, Valdivia 2.

Interestingly, the DH population showed transgressive segregation in flowering time and plant height at each environment (Fig. 1). The variability was similar across environments and between both traits (approximately 5 SD). Flowering time was similar between Bacanora and Weebil in CdO and BA, with a difference lower than 5°Cd (i.e., a difference lower than 0.1 SD of population) and close to the population mean while in Valdivia, Weebil was slightly later than Bacanora with approximately 55°Cd of difference between them (approximately 1.2 SD). In terms of plant height, Weebil was taller than Bacanora across environments with a difference of approximately 0.1 m (approximately 2 SD). In spite of the differences described above, DH lines were within the range considered optimal for GY (approximately between 0.7 and 1 m) (Miralles and Slafer, 1995; Richards, 1992).

The DH population showed differences in GY and its physiological and numerical components across

Table 3. Variance components (standard errors are indicated within parenthesis) for genotype (V_G), genotype × environment ($V_{G\times E}$), and residual (V_{res}) for different traits evaluated in the double-haploid population: grain yield (GY), flowering time (FT), plant height (PH), above-ground dry matter (AGDM), harvest index (HI), grain number per area unit (GN), and grain weight (GW).

Trait	V_G	$V_{G\times E}$	V_{res}
FT, °Cd†	2,066 (313)	685 (66)	242 (15)
PH, m	0.0015 (0.0002)	0.0006 (0.0001)	0.0007 (0.00001)
GY, g m^{-2}	6,832 (1,496)	5,193 (1,096)	17,363 (1,109)
AGDM, g m^{-2}	13,002 (3,452)	11,675 (3,403)	65,124 (3,982)
HI	0.00029 (0.00007)	0.00027 (0.00005)	0.00076 (0.00005)
GN, grains m^{-2}	7,315,291 (1,261,375)	1,509,494 (557,952)	11,055,036 (674,446)
GW, g	9 (1.4)	5 (0.5)	3 (0.2)

† °Cd, Celsius degree days.

environments (Fig. 2). The test of fixed effects was significant ($p < 0.0001$) for GY and also for both its physiological and numerical components while the estimated V_G had a variable relative magnitude with respect to the $V_{G\times E}$ and V_{res} (Table 3). The V_G was greater than $V_{G\times E}$ for all traits, varying from approximately 10% in AGDM and HI to fivefold in GN while it was relatively lower than V_{res} for all traits (V_G was between 20 and 50% of V_{res}), except for GW where it was substantially greater than V_{res} (V_G was the triple that of V_{res}). Grain yield was clearly higher in Valdivia trials (population means of 1193 and 1214 g m^{-2} in Val-1 and Val-2, respectively) than in CdO (583 g m^{-2}) and BA (679 g m^{-2}) but with wider ranges across the population (317, 443, 634, and 669 g m^{-2} in CdO, BA, Val-1, and Val-2, respectively). This behavior across environments was similar in AGDM but less apparent in GN. In this sense, it was possible to divide the field trials into two MEs according to marked differences in GY ($p < 0.0001$) as well as main components ($p < 0.0001$). Thus, a high-yielding ME (i.e., more than 800 g m^{-2}) was determined for Valdivia trials and a low-yielding ME (i.e., less than 800 g m^{-2}) included CdO and BA.

Similar to phenology and plant height, the DH population showed transgressive segregation in GY and its components at each environment (Fig. 2). Both GY and AGDM were similar between parental lines in BA (difference lower than 0.1 SD), but they tended to be greater in Weebil than Bacanora in the other trials (i.e., differences of approximately 0.5 SD for GY and of approximately 0.7 SD for AGDM). These elite cultivars showed similar values of HI at each trial. As expected, Bacanora established a greater GN in all environments (differences greater than 1 SD) while Weebil had heavier grains (differences of approximately 2 SD) in agreement with the basis for the initial choice as parents. Although the range in these traits of the DH population was different across field trials, it was greater than 5 SD in most of them (approximately it was greater than 3000 grains m^{-2} and 7 mg for GN and GW, respectively).

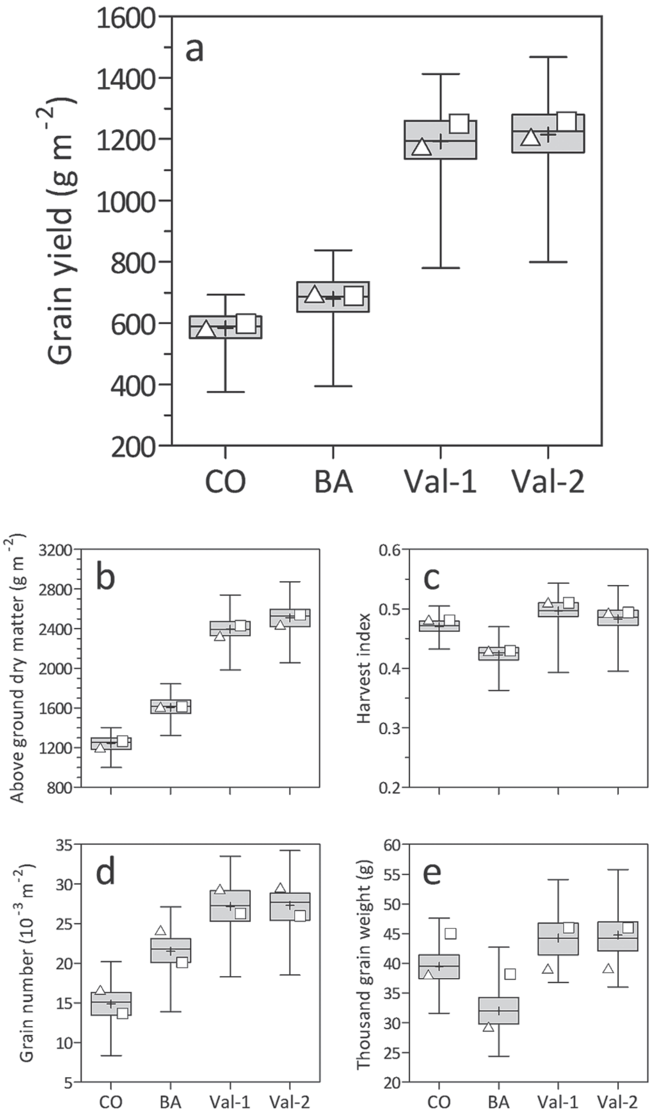

Figure 2. (a) Grain yield and its physiological (b) above-ground dry matter and (c) harvest index and its numerical components, (d) grain number per area unit and (e) grain weight values, registered in the double-haploid population in different environments. Box and whiskers of best linear unbiased predictors are presented for each environment, indicating the population minimum, first percentile, mean (cross), median, third percentile, and maximum. Parental lines are indicated by triangles (Bacanora) and squares (Weebil). CdO, Ciudad Obregón; BA, Buenos Aires; Val-1, Valdivia 1; Val-2, Valdivia 2.

Grain Yield Potential Strategies

Across environments, GY variation was mainly explained by AGDM and GN and to a lesser extent by HI and GW, respectively (Fig. 3). Although both physiological

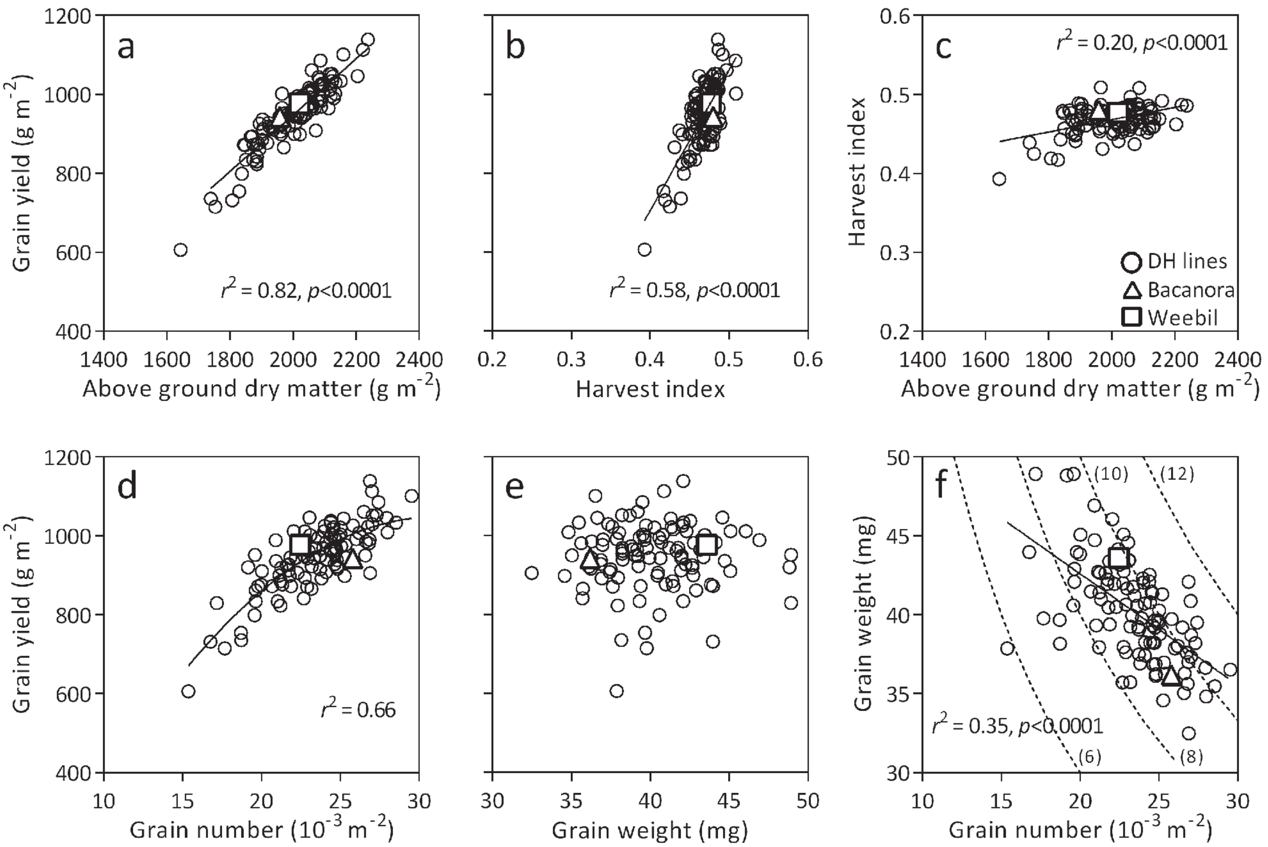

Figure 3. Relationship between grain yield and its physiological (b) above-ground dry matter and (c) harvest index and its numerical components, (d) grain number per area unit and (e) grain weight, for best linear unbiased predictors of double-haploid (DH) population across all environments. Relationship between (c and f) both components of each approximation are also presented. Lines or curves indicate the linear or nonlinear regression, respectively, with the corresponding regression coefficient, and dotted curves (f) indicate iso-grain yield.

components (i.e., AGDM and HI) tended to be positively and significantly associated with GY, the relationship with AGDM was more robust and consistent than with HI in all environments ($r^2 = 0.82$–0.85 and $p < 0.0001$ and $r^2 = 0.28$–0.63 and $p < 0.0001$ for AGDM and HI, respectively). Few DH lines reached HI values higher than 0.50 (Fig. 2c) and a weak relationship was observed between AGDM and HI ($r^2 = 0.20$, $p < 0.0001$) across (Fig. 3c) or even at each (data not shown) environment. Interestingly, GY was asymptotically related with GN suggesting that GY tended to be stabilized at a very high GN (Fig. 3d). This behavior was more evident in BA than in the other environments, where the regression coefficient of linear and nonlinear regression between GY and GN were similar (data not shown). A negative association between GN and GW was found (Fig. 3f) in BA ($r^2 = 0.32$, $p < 0.0001$), CdO ($r^2 = 0.34$, $p < 0.0001$), Val-1 ($r^2 = 0.26$, $p < 0.0001$), and Val-2 ($r^2 = 0.25$, $p < 0.0001$). In spite of the negative relationship between GN and GW, GY was not significantly associated ($p > 0.1$) with GW (Fig. 3e).

Genetic correlations between GY and traits related to biomass accumulation and partitioning and to the numerical components in the DH population across all environments are illustrated in Fig. 4. Principal components analysis explained around 60% of total variability among genotypes using the first two axes; the cosine of the angle between two trait vector approximates the magnitude of the correlation between them (Kroonenberg, 1995). As stated above, GY was positively associated with AGDM ($r = 0.92$, $p < 0.0001$), GN ($r = 0.80$, $p < 0.0001$), and HI ($r = 0.77$, $p < 0.0001$). Other traits evaluated including GW, flowering time, and plant height did not show association with GY. Grain number per area unit was more associated to GNS ($r = 0.88$, $p < 0.0001$) than SN ($r = 0.56$, $p < 0.0001$) while GW, which tended to be negatively associated with GN ($r = -0.59$, $p < 0.0001$) as stated above, was positively correlated with GFR ($r = 0.68$, $p < 0.0001$).

To analyze the population's GY strategy in each ME, principal components analyses were performed (Fig. 5). More than 50% of the variability at each ME was explained with the two first principal components (Fig. 5) and more than 65% when the third component was considered (data not shown). According to these biplots, AGDM was the strongest trait correlated with GY in both ME ($r > 0.85$, $p < 0.0001$ in both cases) while HI was associated with GY only in the high-yielding ME ($r = 0.82$, $p < 0.001$). Variations in GY were better associated with GN in the high- ($r = 0.82$, $p < 0.0001$) than in the low-yielding ME ($r = 0.55$, $p < 0.0001$). In addition, both numerical components were more negatively correlated in low- ($r = -0.70$, $p < 0.0001$) than in high-yielding ME ($r = -0.53$, $p < 0.0001$).

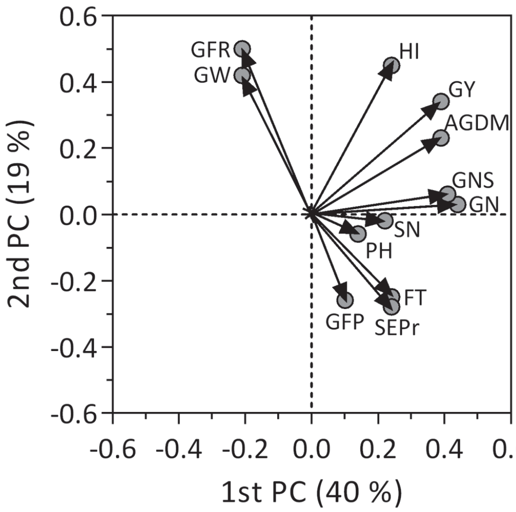

Figure 4. Principal components analysis of grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), grain number per area unit (GN), grain weight (GW), spike number per area unit (SN), grain number per spike (GNS), flowering time (FT), plant height (PH), stem elongation phase relative to FT (SEPr), grain filling phase (GFP), and grain filling rate (GFR) for best linear unbiased predictors of double-haploid population across all environments. Percentage of variability explained by each principal component (PC) is indicated in brackets in each axis of the biplot.

Variations in GN were strongly associated with GNS in high-yielding ME ($r = 0.94$, $p < 0.0001$) although in low-yielding ME both traits were poorly correlated ($r = 0.35$, $p = 0.002$) and GW was positively associated to GFR in both ME ($r > 0.60$, $p < 0.0001$ in both cases). Similar to the general analysis, flowering time and plant height did not explain the differences observed in GY in any ME.

Above-ground dry matter and GN were also the main traits explaining the performance of 25% top yielding DH lines selected across both ME (Fig. 6). Grain yield across all environments had a stronger correlation with that in the high-yielding ($r = 0.96$, $p < 0.0001$) than in the low-yielding ME ($r = 0.49$, $p < 0.0001$). In fact, a low correlation was found when GY of low- and high-yielding ME was plotted, showing genotype × environment interaction. The analysis of the performance of the best (six) yielding DH lines in relation with their parents in both MEs reinforced the previous results: transgressive segregation was observed in GY, AGDM, and GN. The 25% top yielding DH lines (11 and 7% than the parental mean in high- and low-yielding MEs, respectively) were those that produced more biomass (7 and 5% than the parental mean in high- and low-yielding MEs, respectively) and established more grains (7 and 11% than the parental mean in high- and low-yielding MEs, respectively). Differences in GN tended to be explained by changes in GNS (7 and 6% than the parental mean in high- and low-yielding MEs, respectively).

DISCUSSION

As more genetic backgrounds are genotyped and phenotyped across different environments, genetic gains could be improved as breeding decisions are based on better conceptual models (Lopes et al., 2012; Reynolds et al., 2009b). Before discussing the strategies for GY potential observed in this elite population, it is important to consider the variations in flowering time and plant height among individuals within the population, which should be reduced to obtain significant results for breeding (Reynolds et al., 2009a). Although parental lines were elite cultivars with similar flowering time and adaption, transgressive segregation was expressed, in line with evaluations observed of elite populations not only in spring wheat (Borràs-Gelonch et al., 2012; García et al., 2011) but also in barley (*Hordeum vulgare* L.) (Borràs et al., 2009) and rice (*Oryza*

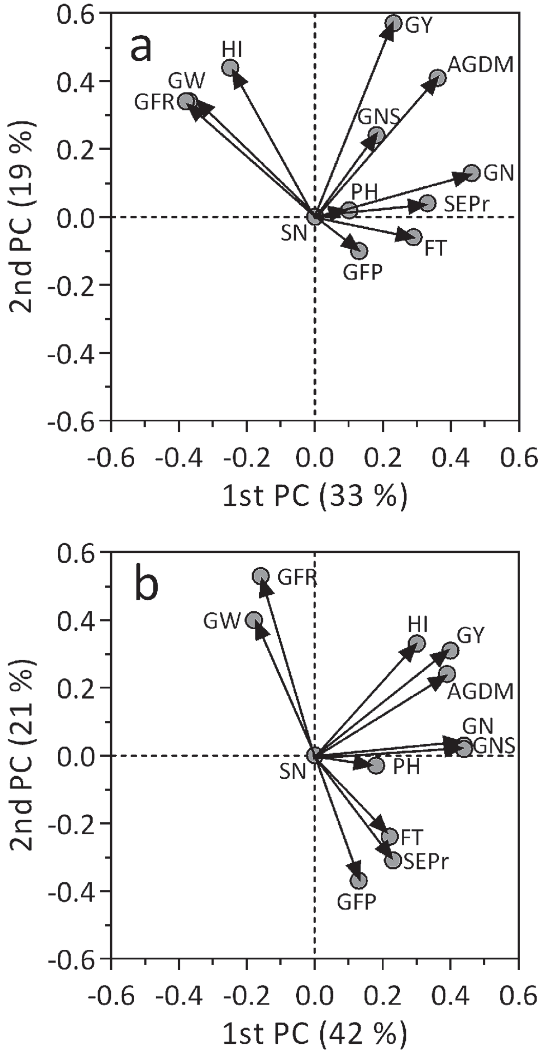

Figure 5. Principal components analysis of grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), grain number per area unit (GN), grain weight (GW), spike number per area unit (SN), grain number per spike (GNS), flowering time (FT), plant height (PH), stem elongation phase relative to FT (SEPr), grain filling phase (GFP), and grain filling rate (GFR) for best linear unbiased predictors of double-haploid population across (a) low- and (b) high-yielding mega-environments. Percentage of variability explained by each principal component (PC) is indicated in brackets in each axis of the biplot.

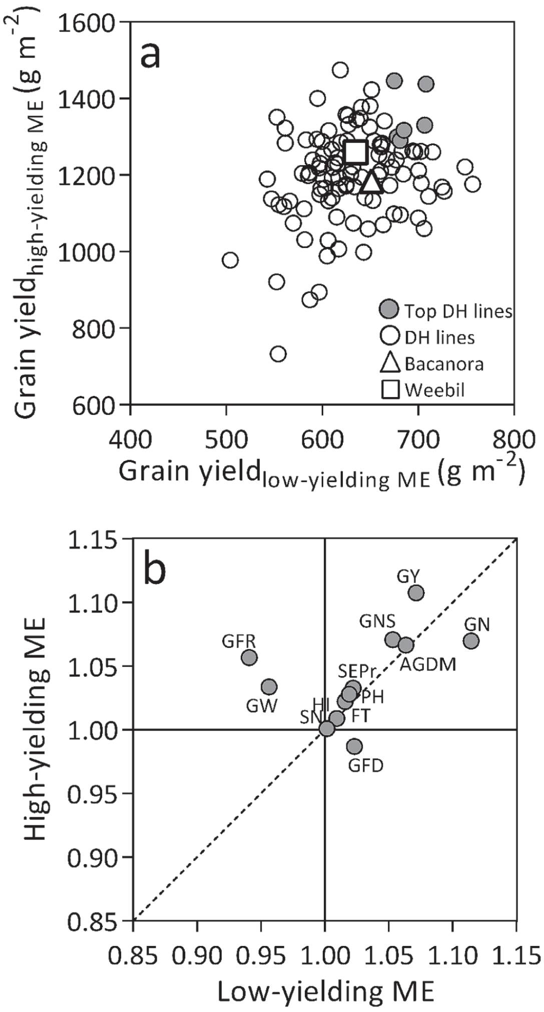

Figure 6. Contrast between best linear unbiased predictors of double-haploid (DH) population across both mega-environments (MEs). (a) Relationship between grain yield in high- and low-yielding ME, identifying DH lines selected in the 25% top ranking (closed symbols) across both ME. (b) Relationship between traits means of top DH lines relative to the parental mean in low- and high-yielding ME. Traits evaluated are grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), grain number per area unit (GN), grain weight (GW), spike number per area unit (SN), grain number per spike (GNS), flowering time (FT), plant height (PH), stem elongation phase relative to FT (SEPr), grain filling phase (GFP), and grain filling rate (GFR). Vertical and horizontal lines indicate parental mean in each ME and dotted line represent the 1:1 relationship.

sativa L.) (Zhou et al., 2001). The reduction in the range of flowering time as photoperiod was lengthened suggests that individuals within the population have different photoperiod sensitivity. Thus, the more inductive the photoperiod the narrower the range of variability among DH lines within the population. However, considering the phenotypic distribution, the range in flowering time of 50% of DH lines (i.e., DH lines included between the first and third quartile) was lower than 5 d in all environments. Besides, the main effect of this trait was higher than the genotype × environment interaction. Therefore, considering (i) the relatively narrow and consistent variability in flowering time and (ii) that plant height of most of the DH lines was within the optimal range for GY (approximately between 0.7 and 1 m) (Miralles and Slafer, 1995; Richards, 1992), the elite wheat DH population evaluated in this study could be a useful mapping population to increase the precision in quantitative trait loci analysis, avoiding confounded results as a consequence of the simple variation in traits already fixed by breeding (Reynolds and Tuberosa, 2008; Reynolds et al., 2009b).

The DH population expressed higher GY in Valdivia than in BA and CdO. Lower temperatures and higher (or similar) incident photosynthetically active radiation (PAR) was experienced in Val-1 and Val-2 than in BA and CdO. In general, the photothermal quotient (i.e., the ratio between the amount of PAR intercepted by the crop and mean temperature above a base temperature) during the critical period has been shown to be positively related to GN (Fischer, 1985; Magrin et al., 1993) while mean temperature during grain filling is negatively associated with GW (Stone, 2001; Wardlaw et al., 1980). In this sense, considering the ratio between accumulated incident PAR and mean temperature during SEP, the environmental ranking was Val-2 > Val-1 > BA > CdO, similar to the final rank of both GN and GY. Regarding GW, the environmental rank was as expected considering the mean temperature during grain filling, Val-2 > Val-1 > CdO > BA.

The robust and positive relationship between GY and AGDM observed across all contrasting environments was consistent with the suggestion that genetic gains in biomass production is a way to improve wheat GY potential (Calderini et al., 1999; Reynolds et al., 2009a; Slafer and Andrade, 1991). However, a necessary condition is to maintain the current levels of partitioning to grain (Austin et al., 1980; Miralles and Slafer, 2007). Average HI estimated for the DH population was close to the maximum values reported in modern spring wheat cultivars (approximately 0.45) (Fischer, 2007) in BA and CdO and tended to be higher in Valdivia. These results reinforce the idea that enhancing biomass production within optimal plant height range while avoiding penalties in expression of HI is a viable way to genetically increase GY (Fischer, 2011; Miralles and Slafer, 2007). Evidence in the literature suggest that improvements in biomass production in current wheat germplasm will most likely be achieved by focusing on radiation use efficiency (RUE) constraints rather than on radiation interception (Loomis and Amthor, 1996; Parry et al., 2011; Reynolds et al., 2005). In fact, there is evidence for improvement in RUE both during the whole cycle (Shearman et al., 2005) as well as postanthesis (Calderini et al., 1997) in modern wheat cultivars.

As expected, GY was mainly explained by changes in GN rather than in GW, in agreement with the literature (e.g., Calderini et al., 1999; Fischer, 2008; Peltonen-Sainio et al., 2007). This result highlights the importance of GN for GY potential improvements. However, in the present study, GW seemed to be more plastic than generally reported (Sadras, 2007), with similar relative variability as for GN. As stated, one of the contrasting characteristics of the parental lines (i.e., Bacanora and Weebil) was their stable contrast between GN and GW at similar GYs. Across the environments, GN and GW tended to be negatively associated, suggesting a trade-off in GN due to reductions in GW (Slafer and Andrade, 1989). Grain weight reductions could suggest a possible source limitation during grain filling in high-yielding DH lines. However, given that within each environment (i) variability in GW was observed for a given GN and vice versa and (ii) the trade-off between GW and GN was less than 1:1, larger GN could contribute to improved GY potential although partial compensation is expected. Therefore, overall, in this population GY was co-limited by source and sink as reported for modern wheat cultivars (Borrás et al., 2004, and references therein; Slafer and Savin, 1994).

Differences in GN were positively associated with changes in GNS more than SN, mostly in high-yielding environments. The low variability found in SN could be associated with the fact that DH lines fully exploited the growing conditions, reaching adequate SN for each particular environment where the DH population was evaluated. In line with the ecophysiological approach proposed by Fischer (1983, 2008) where GN can be analyzed as the product of spike dry weight at anthesis and spike fertility coefficient (i.e., grains per gram of spike), a longer SEP relative to anthesis has been proposed as a way to increase the spike dry weight and, in turn, GN and GY potential (Miralles and Slafer, 2007; Slafer et al., 2001). However, no close relationship was observed between GN and SEP relative to flowering time (SEPr) in the population evaluated here, suggesting that a longer SEPr is a subtle way to increase spike dry weight, resulting in unclear effects (García et al., 2011).

CONCLUSION

Taking into account the two main avenues to increase wheat yield (Reynolds et al., 2012), (i) increasing photosynthetic capacity and efficiency to produce more assimilates (Parry et al., 2011) and (ii) optimizing partitioning to grain of extra assimilates while maintaining lodging resistance (Foulkes et al., 2011), this elite mapping population could be an important source of germplasm to carry out both precision phenotyping and gene discovery. First, most of the DH lines showed relatively narrow variability in flowering time and plant height, traits already optimized for most wheat target environments in elite cultivars and that otherwise confound analysis when other more complex trait are evaluated (Reynolds and Tuberosa, 2008; Reynolds et al., 2009b). Second, an important transgressive segregation in GY was observed. The 25% top yielding DH lines yielded 11 and 7% more than the parental mean in high- and low-yielding MEs, respectively. The current study demonstrated the importance of enhancing biomass production as a strategy to increase GY potential and suggests likely variation in RUE among DH lines. To maintain or even improve current levels of expression for HI, extra assimilates produced must be partitioned to more grains and/or higher potential GW (Calderini and Reynolds, 2000; Foulkes et al., 2011). To achieve this aim, the establishment of more fertile flowers (Miralles and Slafer, 2007) with bigger carpels (Calderini and Reynolds, 2000), that is, more grain with greater potential weight, could be necessary, requiring breaking the evolutionary trade-off (Sadras and Denison, 2009). Although GN clearly dominated GW as the main GY component, important variability in both numerical components was found in the elite population across the environments explored, suggesting its value in more detailed physiological and genetic studies to understand the relationship between GN and potential GW to optimize both in new genotypes.

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