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Analysis of early vigour in twenty modern cultivars of bread wheat (*Triticum aestivum* L.)

M. L. Maydup[A], *C. Graciano*[A], *J. J. Guiamet*[A], *and E. A. Tambussi*[A,B]

[A]Instituto de Fisiología Vegetal (INFIVE), Universidad Nacional de La Plata- CONICET, cc 327, 1900, La Plata, Argentina.

[B]Corresponding author. Email: tambussi35@yahoo.es

Abstract. Fast development of seedling leaf area is a relevant trait in order to increase early resource acquisition. The use of semi-dwarf genotypes of wheat has decreased early vigour of modern cultivars. We studied early vigour of 20 cultivars cropped in Argentina, and our main objectives were: (*i*) to analyse the genotypic variability in early vigour; (*ii*) to study morphological traits that can be good indicators of early vigour, such as seed mass, leaf width, and specific leaf area; and (*iii*) to determine whether increased dry mass allocation to roots impacts negatively on early vigour. Experiments with non-size-selected and size-selected seeds were carried out in a greenhouse. A field trial was also conducted in order to test the reliability of the greenhouse results. Seeds mass was the main parameter related to early vigour. However, results from the experiment with seeds selected by size (45–50 mg seed^{-1}) showed that seed mass *per se* only partially explains early vigour, since a significant coefficient of determination was observed between the seedling leaf area of each cultivar in both experiments (i.e. with randomly chosen or size-selected seeds).

We observed a high coefficient of determination between net assimilation rate and changes in the ranking of early vigour of the cultivars with time after transplant. Root biomass was positively correlated with leaf area, indicating that the traits were not mutually exclusive. We built simple models by multiple regression to predict early vigour, including some parameters that were easy to measure. Seed mass and leaf width taken together showed better fit than seed mass or leaf width alone. We found a significant coefficient of determination between early vigour in greenhouse and field experiments; thus, screening for early vigour under semi-controlled conditions may be feasible.

Additional keywords: early vigour, specific leaf area, *Triticum aestivum*, wheat.

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Introduction

Fast development of leaf area at early stages of development, i.e. early vigour, is a relevant trait in crops. First, in winter cereals, higher early vigour has been associated with higher water-use efficiency (Richards *et al*. 2001), since less water is evaporated directly from the soil underneath a canopy cover (Gregory *et al*. 2000) and most vegetative growth takes place under low evaporative demand early in the season. Second, faster development of leaf area can improve the crop's ability to compete with weeds, and thus, early vigour might help to reduce herbicide use (Coleman *et al*. 2001). Finally, early vigour has been also related to a higher capacity for nitrogen (N) uptake (Liao *et al*. 2004).

The wide use of semi-dwarf wheat genotypes after the Green Revolution increased the harvest index of modern cultivars, with shorter plants and higher grain yield than older varieties (e.g. Austin 1999; Annicchiarico *et al*. 2005). However, lower plant height is also associated with reduced early vigour, a pleiotropic and undesired effect of the high grain yield performance of modern semi-dwarf varieties (Richards *et al*. 2002).

Easy-to-measure parameters can estimate early vigour, and it can be a useful tool in wheat breeding programs, where large number of cultivars must be analysed. The width of seedling leaves has been proposed to predict early vigour in Australian germplasm of wheat (e.g. Rebetzke and Richards 1999).

From a mechanistic point of view, several characteristics have been associated with early vigour. On the one hand, seed mass showed a positive correlation with seedling vigour in several studies (Bremner *et al*. 1963; Lafond and Baker 1986; van Rijn *et al*. 2001; Aparicio *et al*. 2002). On the other hand, beyond the 'starting point' determined by seed mass, seedlings characteristics such as specific leaf area (SLA, the ratio between leaf area and leaf dry weight) have been reported as positively associated with early vigour in analysis of interspecific variation (e.g. van Rijn 2001). Finally, the daily increase of dry weight per unit dry weight (i.e. relative growth rate, RGR) may be implicated in differences in early vigour. Likewise, biomass allocation patterns (roots *v*. aboveground parts) can have an important impact on early vigour.

The aims of this work were: (*i*) to analyse the genotypic variability in early vigour in a set of 20 modern cultivars of bread wheat cropped in Argentina; (*ii*) to study morphological traits that may be reliable indicators of early vigour (such as seed mass, leaf width, and SLA); and (*iii*) to analyse the impact of biomass allocation patterns in the seedling on early vigour, in particular to see if an increase in root growth carries a penalty on early vigour or *vice versa*.

Material and methods

Plant material

Twenty modern bread wheat (*Triticum aestivum* L.) cultivars were used. In order to improve the potential range of variability, we choose cultivars from several different breeding programs. All cultivars are recommended for the same sowing date. The cultivars used in the study, the breeding company/institution, year of release, and country of origin are shown in Table 1. Seed material was obtained from trials conducted in 2005 in INTA Balcarce (Argentina), and the seeds were maintained in a cold chamber until sowing.

Experimental set-up

Greenhouse experiments

In Experiment 1 (random seed size) a pool of seeds (representing the natural variability in seed size) was germinated on moistened filter paper in Petri dishes in a growth room at 28°C. After 72 h, seedlings were transplanted in 'multipots plastic trays' (8 × 5 rows, i.e. 40 pots per multipot) filled with soil (taken from the upper 20 cm of a Typic Argiudoll, Soil Taxonomy, Soil Survey Staff 2010). Each individual pot was 4 cm in diameter and 8.7 cm in depth (110 cm^3). Five seedlings of each cultivar were transplanted into each multipot, and each cultivar was planted in eight different multipots. On each sampling date, we harvested one seedling per cultivar from each multipot. Seedlings were grown in a greenhouse in La Plata, Argentina (34°54′24″S, 57°55′56″W). The seedlings were planted on 13 June, i.e. the experiment was carried out during the normal wheat growing season in Argentina.

Experiment 2 was carried out in a similar way to Expt 1, but before sowing, seeds were selected by size and only seeds in the range 45–50 mg $seed^{-1}$ were used.

Table 1. Names, breeding company/program, year of release, and country of origin of the cultivars used in the study

Cultivars marked with an asterisk were not included in the field experiment. The abbreviation for each cultivar is shown in parentheses

Cultivar	Breeding co./program	Year of release	Country of origin
ACA 303 (A303)	ACA Semillas	2001	Argentina
ACA 304 (A304)	ACA Semillas	2002	Argentina
Buck Arriero (ARR)	Buck Semillas	1997	Argentina
Buck Guapo* (GUA)	Buck Semillas	1999	Argentina
Buck Guatimozín (GTM)	Buck Semillas	2001	Argentina
ProInta. Molinero (MOL)	INTA	1999	Argentina
Buck Sureño (SUR)	Buck Semillas	1999	Argentina
Baguette 19 (BAG)	Nidera	2006	France
BioInta 3000 (B3000)	INTA	2003	Argentina
BioInta 3003* (B3003)	INTA	2004	Argentina
Klein Capricornio (CAP)	Criadero Klein	2004	Argentina
Klein Escorpion* (ESC)	Criadero Klein	1999	Argentina
Klein Escudo (ESDO)	Criadero Klein	2000	Argentina
Klein Gavilán* (GAV)	Criadero Klein	2004	Argentina
Klein Jabalí (JAB)	Criadero Klein	2002	Argentina
Klein Martillo (MAR)	Criadero Klein	–	Argentina
Klein Sagitario (SAG)	Criadero Klein	2000	Argentina
Premiun 11 (PR11)	Nidera	2005	France
ProINTA Puntal* (PUN)	INTA	1994	Argentina
INIA Torcaza* (TOR)	INIA	2004	Uruguay

Field experiment

In order to study whether cultivar differences in early vigour analysed in the greenhouse correlate with similar differences in the field, we carried out an experiment under realistic crop conditions. Fourteen of the 20 cultivars used in the greenhouse experiments were sowed on 29 June 2007 in La Plata (Experimental Field of Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina). Six of the 20 cultivars analysed in the greenhouse experiments were excluded because we had not enough seeds for a field experiment. The cultivars were planted in plots (plot size 1 by 3.4 m in five rows, with 20 cm spacing between rows). Sowing density was 260 plants m^{-2}. The experimental design was in randomised blocks with three replicates. The soil type was a Typic Argiudoll. Sowing depth was identical (2 cm) in all plots. Fertiliser was applied as 200 kg ha^{-1} of diammonium phosphate (i.e. 36 kg N ha^{-1} and 40 kg P ha^{-1}) immediately after sowing. Emergence took place on 17 July. Early vigour was measured on day 40 after sowing. Five seedlings per plot were sampled, i.e. 15 seedlings of each cultivar.

Morphological measurements

On days 10, 18, and 31 after transplanting (stages Z12, Z13, and Z14, respectively; Zadoks *et al*. 1974), we measured length and width of the leaf blades (all expanded leaves) and plant height in eight replicate plants of each cultivar, each replicate taken from a different multipot. Leaf length was determined as the distance between the ligule and the leaf blade apex. Plant (shoot) height was measured as the distance between the ground and the highest point of the shoot. Leaf width was measured in the middle of the blade. The area of each leaf blade was measured with a LI-COR LI-3000 area meter (Li-COR, Lincoln, NE, USA). On each sampling day (i.e. seedlings of 10, 18, and 31 days) roots were separated from the soil by washing with a gentle stream of water. Negligible amounts of organic debris (which could interfere with the identification of the roots (Manske *et al*. 2001) were present in the soil. Roots were washed several times over a sieve to eliminate soil particles. Dry weight of all plant parts was determined after drying at 70°C for several days until constant weight.

Calculations

The SLA was calculated as the ratio between leaf area and leaf weight. The RGR (based on dry weight) and net assimilation rate (NAR) were calculated as in Poorter (1989). In order to obtain the respective pairs to calculate RGR and NAR, seedlings of two consecutive harvests were ordered and paired as described in Causton and Venus (1981). Seedling leaf area approximately doubled between subsequent harvests, allowing for the estimation of growth parameters (RGR and NAR) without a large error (Poorter 1989; Villar *et al*. 2004).

Statistical analyses

Statistical analyses were carried out using the Statistica 5.1 software (StatSoft Inc., Tulsa, OK, USA). Differences in parameters were analysed by ANOVA. Means were compared by Tukey's test ($P<0.05$).

Principal components analysis

Mean values from each cultivar were used to analyse principal components based on a correlation matrix, in order to explore in a descriptive way the parameters most related to early vigour. Parameters of the first sampling date (day 10) and seedling leaf area of the third sampling date (day 31) of Expt 1 (random seed size) were included in the analysis. A multiple regression analysis was performed with the backward stepwise method with F to enter equal to 2 and F to remove equal to 1.

Regressions were fitted between total leaf area and seed weight of each cultivar, and between leaf areas in Expt 1s and 2. In the latter regression, a slope equal to unity means that there was no change in leaf area between plants from Expt 1 (non-selected seeds) and Expt 2 (size-selected seeds). In order to determine whether the slope of the regressions differed from unity, a *t*-analysis was performed with the output data of the regression for each sampling date (10 and 18 days after transplanting). The *t*-value was calculated as: $t_{calculated}=(1-B_{slope})/StErr_{slope}$. The results were compared with t_{table} for $n-2$ degrees of freedom (d.f. = 18). To confirm the results of this analysis, the Spearman correlation was calculated. The same non-parametric correlation was used to analyse whether the ranking of cultivars in Expt 1 changed for the different sampling dates (10, 18, and 31 days after transplanting).

Results

There was significant variation in early vigour in the set of 20 cultivars of bread wheat analysed in the greenhouse. The least vigorous cultivars had a seedling leaf area representing only 59, 55, and 60% of the most vigorous cultivar at 10, 18, and 31 days after transplanting, respectively (Table 2).

Seedling leaf area was closely (and positively) related to the average weight of kernels (seed mass) ($r^2=0.46$, 0.66, and 0.76 at 10, 18, and 31 days after transplanting, respectively; Fig. 1*a–c*). The coefficient of determination (i.e. r^2) increased from 10 to 31 days after transplanting (see Fig. 1*a–c*). To further analyse early vigour independently of the effects of seed size, we carried out Expt 2 with only seeds weighing 45–50 mg. The variability in early vigour between the 20 cultivars observed in Expt 1 (i.e. where the seeds were not selected by mass) was partially maintained in Expt 2 (i.e. where seed sizes were similar). When we compared the leaf area per seedling of each cultivar in Expt 1 *v.* Expt 2, we found a significant coefficient of determination ($r^2=0.47$, 10 days after planting; Fig. 1*d*, open circles) between experiments. At this sampling date, the slope of the relationship did not differ from 1 (*t*-test with $P=0.14$) and the intercept of the regression did not differ from 0 ($P=0.16$). Consistently, the non-parametric Spearman correlation was significant (Spearman $r=0.68$, $P<0.001$). At the second sampling date (i.e. 18 days after planting), the coefficient of determination between early vigour in both experiments decreased, although it was still statistically significant ($r^2=0.30$; Fig. 1*d*, filled circles). At this sampling date, the intercept of the regression was >0 ($P<0.001$) and the slope was <1 ($P=0.001$). Therefore, at 18 days after transplanting,

Table 2. Expt 1. Early vigour of 20 cultivars of bread wheat (*Triticum aestivum* L.) grown in a glasshouse

Measurements were made at 10, 18, and 31 days after planting. Cultivars were ranked according to their seedling leaf area, and the ranking position at each harvest date is indicated. In parentheses, the seedling leaf area is expressed as a percentage of the cultivar with the highest early vigour at each harvest time (i.e. the cultivar positioned 1 in the ranking). Cultivars with the abbreviation B., K., and PI correspond to Buck, Klein (breeding companies), and ProInta (a public breeding program)

10 Days after transplanting			18 Days after transplanting			31 Days after transplanting		
Ranking position	Cultivar	Leaf area (cm^2 $seedling^{-1}$)	Ranking position	Cultivar	Leaf area (cm^2 $seedling^{-1}$)	Ranking position	Cultivar	Leaf area (cm^2 $seedling^{-1}$)
1	B. Guapo	5.79 (100)	1	B. Guapo	11.85 (100)	1	K. Sagitario	20.27 (100)
2	B. Arriero	5.26 (90.8)	2	K. Sagitario	11.76 (99.2)	2	B. Guapo	19.92 (98.3)
3	ACA 304	5.06 (87.3)	3	B. Arriero	11.60 (97.9)	3	B. Sureño	19.88 (98.1)
4	K. Jabalí	5.00 (86.3)	4	ACA 303	11.40 (96.2)	4	K. Escudo	19.61 (96.7)
5	K. Sagitario	4.89 (84.5)	5	K. Escudo	11.30 (95.4)	5	BioInta 3000	19.56 (96.5)
6	ACA 303	4.87 (84.1)	6	B. Sureño	11.12 (93.8)	6	K. Jabalí	18.86 (93.0)
6	K. Escudo	4.87 (84.1)	7	K. Martillo	11.08 (95.4)	7	B. Arriero	17.60 (86.8)
6	K. Escorpión	4.87 (84.1)	8	BioInta 3000	11.05 (93.3)	8	ACA 303	17.50 (86.4)
9	B. Sureño	4.81 (83.0)	9	ACA 304	10.87 (91.7)	9	PI Molinero	17.15 (84.6)
10	K. Capricornio	4.80 (82.8)	10	K. Jabalí	10.17 (85.8)	10	K. Escorpión	17.10 (84.4)
11	BioInta 3000	4.77 (82.3)	11	K. Gavilán	9.93 (83.8)	11	B.Guatimozín	16.91 (83.4)
12	K. Gavilán	4.43 (76.4)	12	K.Capricornio	9.80 (82.7)	12	ACA 304	16.88 (83.3)
13	K.Martillo	4.36 (75.3)	13	K. Escorpión	9.69 (81.7)	13	K Martillo	16.25 (80.2)
14	PI Molinero	4.05 (69.9)	14	PI Molinero	9.49 (80.0)	14	K. Gavilán	16.07 (79.3)
15	Baguette 19	3.95 (68.2)	15	B. Guatimozín	8.96 (75.6)	15	Capricornio	15.28 (75.4)
16	B. Guatimozín	3.87 (66.9)	16	Baguette 19	8.64 (72.9)	16	PI Puntal	14.81 (73.1)
17	Premium 11	3.65 (63.0)	17	PI Puntal	8.50 (71.7)	17	Premium 11	13.72 (67.7)
18	PI Puntal	3.47 (59.9)	18	Premium 11	8.21 (69.3)	18	BioInta 3003	12.92 (63.8
19	INIA Torcaza	3.43 (59.2)	19	BioInta 3003	7.61 (64.2)	19	INIA Torcaza	12.75 (62.9)
20	BioInta 3003	3.40 (58.6)	20	INIA Torcaza	6.47 (54.6)	20	Baguette 19	12.18 (60.1)

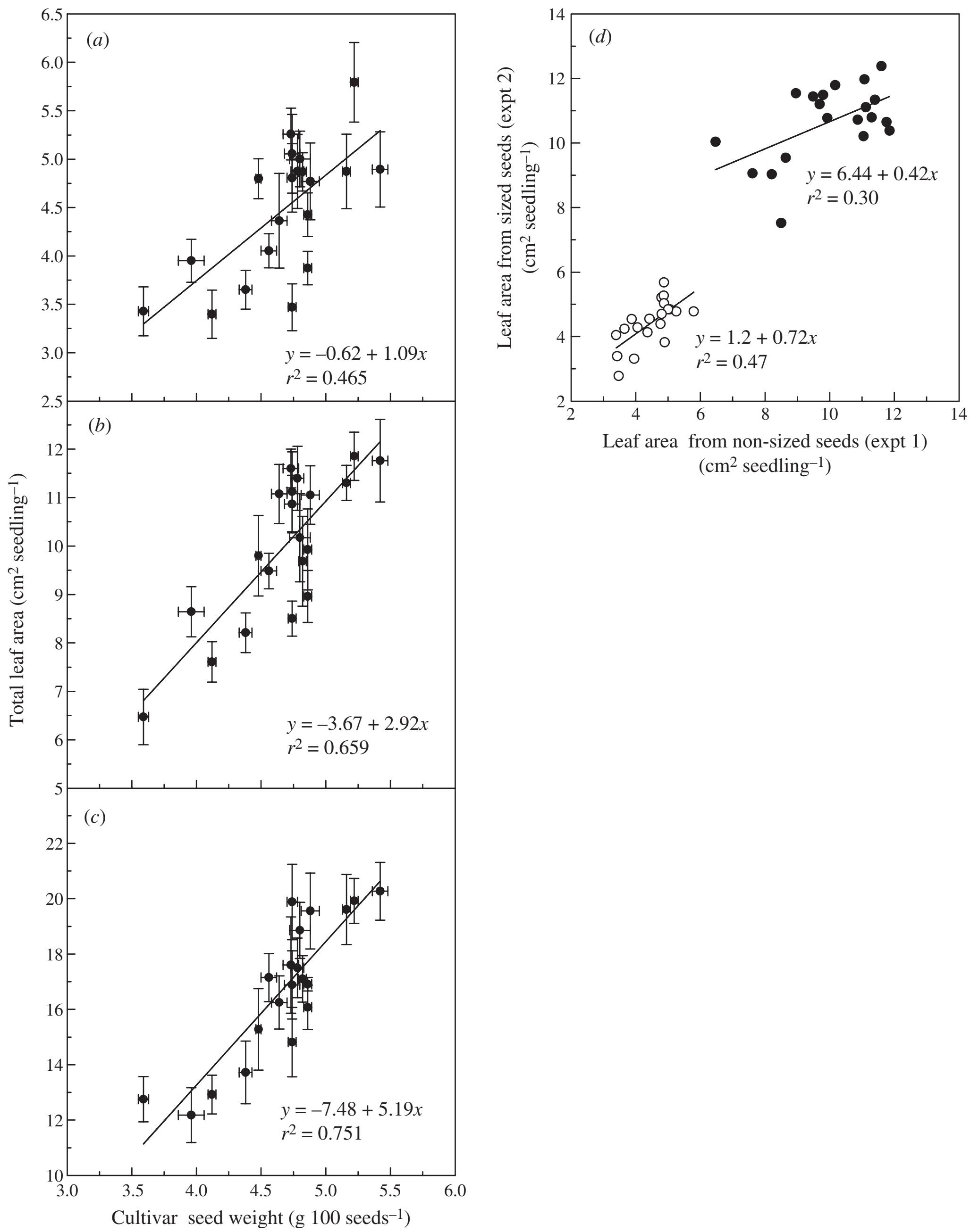

Fig. 1. (*a–c*) Relationship between seed mass and seedling leaf area in 20 cultivars of bread wheat grown in a greenhouse in multipots, at 10, 18, and 31 days after transplanting, respectively. Each point represents the mean of each cultivar ± s.e. Equations and coefficients of determination (r^2) are shown. (*d*) Relationship between early vigour of the cultivars in Expt 1 (i.e. non-sized seeds) *v.* early vigour of the same cultivars in Expt 2 (seeds sized 45–50 mg for each cultivar). Data are from harvests at 10 days (open) and 18 days (closed symbols) after transplanting. Linear regression and coefficients of determination (r^2) are shown for both sampling times.

cultivars with lower early vigour had higher leaf area if the seeds were selected by size than if the seed were non-selected. This result was confirmed by a non-significant Spearman correlation (Spearman $r = 0.43$, $P = 0.06$).

The coefficients of determination for the relationship between SLA and early vigour were small ($r^2 = 0.31$ and 0.17 for days 18 and 31, respectively; Fig. 2*a*, *b*). Root dry weight showed a positive correlation ($r^2 = 0.54$) with seedling leaf area (Fig. 2*d*).

Leaf width was the morphological parameter most closely correlated with early vigour (in Expt 1, $r^2 = 0.764$, 0.821, and 0.769, for leaves 1, 2, and 3, respectively, at 31 days after planting, calculated from *r* values in Table 3). On other hand, leaf length was a poorer indicator of early vigour than leaf width ($r^2 = 0.58$

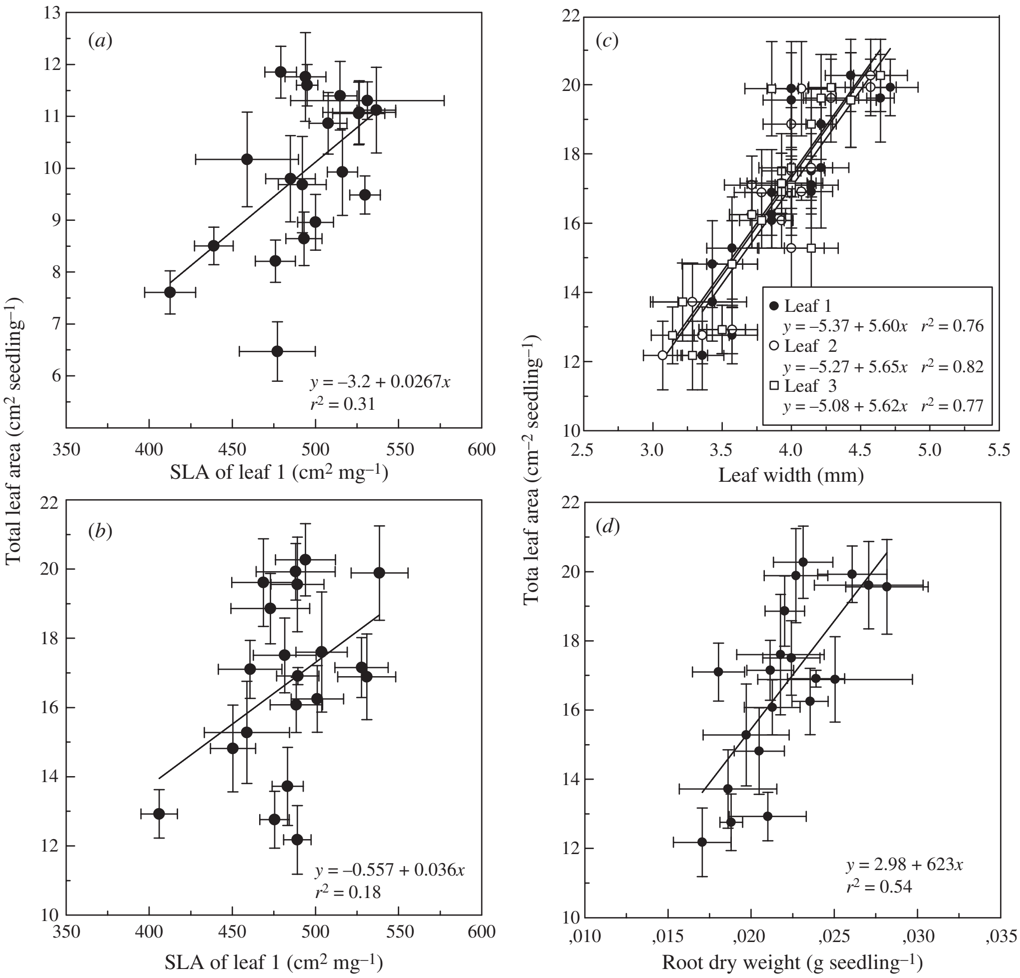

Fig. 2. Relationships between specific leaf area (SLA) at (*a*) 18 and (*b*) 31 days after transplanting, (*c*) leaf width, or (*d*) root dry weight *v.* early vigour at day 31 (leaf area per plant) in 20 cultivars of bread wheat grown in a greenhouse. Each point represents the mean of each cultivar ± s.e.

and 0.28 for leaves 1 and 2, respectively, calculated from *r* values in Table 3).

In Table 2, we compare the ranking of the 20 cultivars at the three sampling dates (10, 18, and 31 days after planting) in Expt 1. Although the correlations between early vigour at the different sampling dates were significant (Spearman correlation = 0.85, $P < 0.001$ between 10 and 18 days and between 18 and 31 days after transplanting), the relative position of some cultivars in this ranking changed substantially (see also Fig. 3*a*). For instance, the positions of cvv. Sagitario, Sureño, and BioInta 3000 improved throughout the experiment (Table 2). By contrast, other cultivars decreased in their relative position (e.g. ACA 304). In other words, some cultivars may show higher or lower early vigour depending on time after germination. In order to explain this, we calculated the NAR between successive harvest dates. Changes in the ranking of early vigour of the cultivars between two successive harvests were associated with differences in NAR. There was a positive correlation ($r^2 = 0.59$) between NAR and the change of position in the ranking of early vigour of the cultivars between 18 and 31 days after planting (Fig. 3*b*), i.e. a higher net assimilation per area of leaf allowed some cultivars to increase their early vigour with respect to the initial conditions and thereby partially compensate for seed-size effects.

Principal component (PC) analysis (Fig. 4) showed that early vigour at the third sampling date (day 31) was highly variable between cultivars, and it varied in the same direction as 1000-grain weight and the morphological parameters of the first leaf at the first sampling date (day 10). The PC axis 2 grouped parameters of the second leaf, so traits of the second leaf provided variability related to early vigour (see direction of the arrows in Fig. 4).

We explored whether a multiple regression analysis with several traits could predict early vigour better than regressions with individual parameters. When seed mass and leaf width (see Eqns 3 and 4 in Table 4) were included in this multiple regression, the statistical fit was improved, i.e. the sum of residual squares was significantly lower than for leaf width alone. For example, r^2 was 0.76 for the regression of early vigour and width of the first leaf, but it increased to 0.85 when kernel size was included in a multiple regression equation (compare Eqn 3 and Eqn 1 in Table 4). For leaf 2, the trend was similar, although not as marked ($r^2 = 0.86$ *v.* 0.82, respectively). The SLA, a parameter

Table 3. Expt 1. Correlation matrix between several seed and seedling parameters of 20 cultivars of *T. aestivum* grown in a greenhouse

SLA, Specific leaf area. Note that in order to show the sign of each correlation, values are the r coefficient (and not the coefficient of determination, r^2). The results of this matrix correspond to 31 days after planting of pre-germinated seeds. Seeds were not selected by size

	Seedling height	Length leaf 1	Width leaf 1	Length leaf 2	Width leaf 2	Length leaf 3	Width leaf 3	Length leaf 4	Leaf 1 area	Leaf 2 area	Leaf 3 area	Leaf 4 area	Root dry wt	SLA leaf 1	SLA leaf 2	SLA leaf 3	SLA leaf 4	Seedling leaf area	Shoot : root ratio
1000-grain wt	0.434	0.749	0.795	0.587	0.834	0.373	0.847	0.216	0.835	0.856	0.832	0206	0.655	0.228	0.223	0.006	0.227	0.868	0.381
Seedling height	–	0.354	0.300	0.677	0.394	0.971	0.358	–0.100	0.382	0.626	0.637	–0039	0.260	0.518	0.245	–0.124	–0.187	0.532	0.431
Length leaf 1		–	0.716	0.705	0.742	0.323	0.743	0.091	0.928	0.780	0.631	0070	0.596	–0.001	0.019	–0.152	0.073	0.764	0.306
Width leaf 1			–	0.291	0.843	0.228	0.776	0.357	0.888	0.741	0.819	0344	0.672	0.243	0.413	0.288	0.415	0.874	0.252
Length leaf 2				–	0.504	0.683	0.496	–0.269	0.588	0.744	0.482	–0219	0.350	0.095	–0.113	–0.451	–0.389	0.532	0.395
Width leaf 2					–	0.295	0.924	0.261	0.851	0.894	0.852	0259	0.771	0.227	0.290	0.115	0.286	0.906	0.278
Length leaf 3						–	0.291	–0.246	0.325	0.562	0.548	–0183	0.170	0.462	0.201	–0.239	–0.328	0.426	0.369
Width leaf 3							–	0.287	0.823	0.849	0.832	0266	0.674	0.198	0.202	0.049	0.327	0.877	0.397
Length leaf 4								–	0.251	0.030	0.343	0977	0.330	0.373	0.229	0.665	0.938	0.448	0.202
Leaf 1 area									–	0.849	0.775	0235	0.731	0.201	0.251	0.050	0.253	0.900	0.256
Leaf 2 area										–	0.843	0060	0.724	0.287	0.321	–0.092	0.037	0.888	0.289
Leaf 3 area											–	0347	0.616	0.421	0.351	0.290	0.307	0.940	0.459
Leaf 4 area												–	0.291	0.468	0.318	0.683	0.904	0.461	0.229
Root dry wt													–	0.273	0.377	0.080	0.324	0.741	–0.217
SLA Leaf 1														–	0.777	0.470	0.301	0.420	0.010
SLA Leaf 2															–	0.518	0.308	0.383	–0.260
SLA Leaf 3																–	0.610	0.256	0.013
SLA Leaf 4																	–	0.420	0.145
Seedling leaf area																		–	0.388

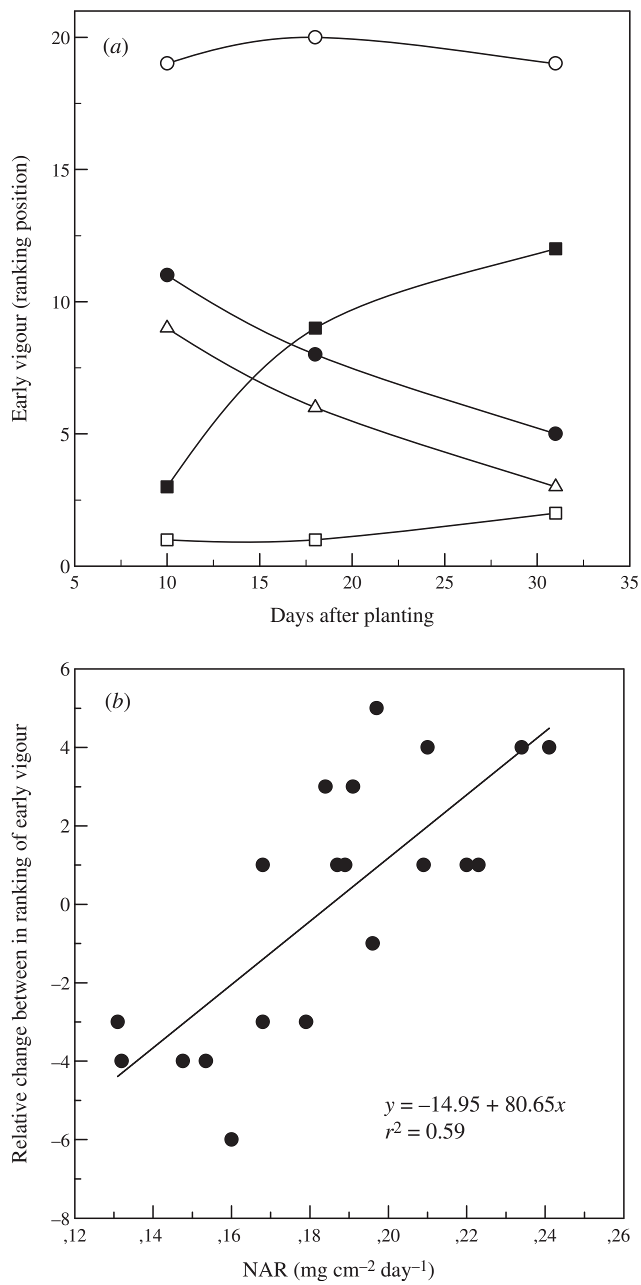

Fig. 3. (*a*) Changes with time (10, 18, and 31 day after transplanting) in the relative position in the ranking of early vigour of selected cultivars grown in a greenhouse: ○, cv. INIA Torcaza; ●, cv. BioINTA 3000; □, cv. Buck Guapo; ■, cv. ACA 304; △, cv. Buck Sureño. (*b*) Relationship between net assimilation rate (NAR) and the change in the ranking position of early vigour, for the set of 20 cultivars used in this study. Each point represents the mean of each cultivar.

poorly related to early vigour when it was considered alone (Table 2), did not show a significant statistical contribution in a multiple regression (data not shown). Following on from PC analysis, a similar multiple regression analysis was used to predict seedling leaf area on day 31 from leaf width on day 10 after planting. These equations are shown in Table 4 (Eqns 5 and 6). A multiple correlation including 1000-grain weight and width of leaf 1 (day 10) *v.* early vigour (day 31) showed a better fit than a regression against only width of leaf 1 (sum of squares was statistically different between both equations).

In Fig. 5 we compare early vigour in Expt 1 (greenhouse, non-sized seeds, 31 days after planting) *v.* early vigour in the field 40 days after sowing. There was a close and significant correlation between early vigour in both experiments ($r^2 = 0.66$). By contrast, early vigour in the field did not correlate closely with seedling leaf area at 10 and 18 days after planting in the greenhouse experiment (data not shown).

Discussion

Characteristics associated with early vigour: seed mass and biomass allocation

The influence of seed mass on early vigour is typically high (e.g. Lafond and Baker 1986; López-Castañeda *et al*. 1996; Aparicio *et al*. 2002). However, when we compared the results of Expt 1 (i.e. where the seeds were not selected by mass) with Expt 2 (i.e. where seed sizes were similar for all cultivars), we found a significant relationship between both experiments, i.e. differences in early vigour cannot be accounted for by seed mass alone. For instance, recently it has been reported that seed density (beyond seed mass) could be a relevant factor related to early vigour and seedling establishment (Ball *et al*. 2011). We did not analyse seed density in this study, but we are aware that this trait could also be involved in differences in early vigour in our set of cultivars.

Beyond the ‘starting point’ determined by seed mass, differences in biomass allocation (e.g. leaves *v.* roots) could also affect the growth performance of the seedling (Poorter 1989). Therefore, we explored whether root dry weight and seedling leaf area were negatively related in this set of cultivars, that is, if root growth might represent a penalty on early vigour by diverting assimilates from the production of new leaves. Our results suggest the opposite—early vigour was not penalised by the presence of a larger seedling root system (Liao *et al*. 2004). Similar results were reported by van den Boogaard *et al*. (1997). Root : weight ratio (the dry mass of roots per unit of plant mass) and leaf area ratio (the dry mass of leaves per unit of plant mass) were positively correlated in the set of 10 cultivars of *T. aestivum* analysed in that study ($r^2 = 0.63$, calculated from table 3 in van den Boogaard *et al*. 1997). Greater root growth is a relevant trait to improve shoot growth, especially in marginal environments (Manske *et al*. 2001, and references cited therein). Seminal roots are the main roots in the seedlings and penetrate the soil more deeply than secondary roots (Manske *et al*. 2001; Richards *et al*. 2002). In short, although results derived from our experimental set-up (seedlings growing in small containers under good irrigation) do not allow a direct extrapolation to field conditions *a priori*, in the set of cultivars analysed we did not observe negative effects on early vigour of higher root mass.

Seedling growth parameters

The RGR can be divided into two components according to the following equation (Poorter 1989):

$$\mathrm{RGR} = \mathrm{NAR} * \mathrm{LAR}$$

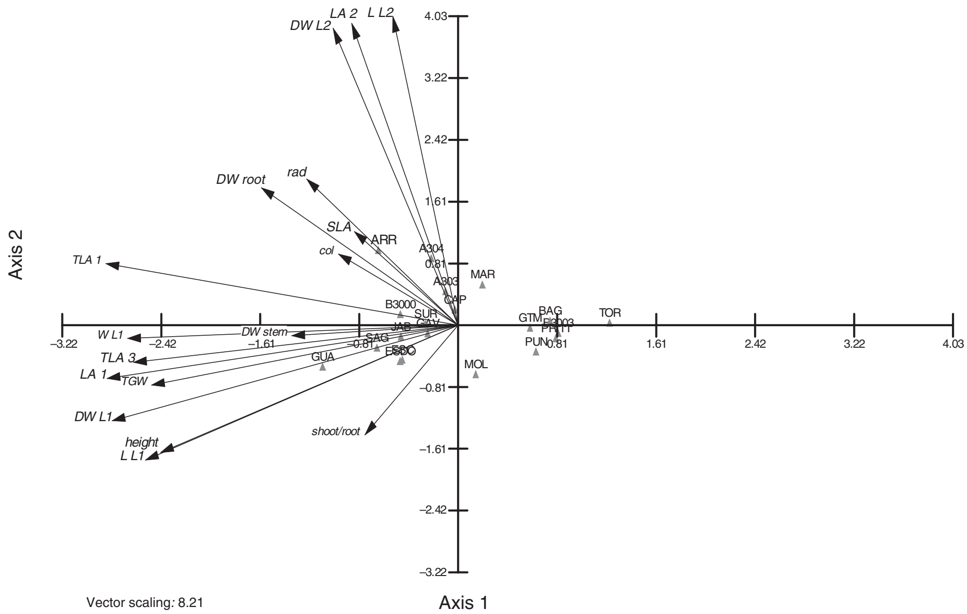

Fig. 4. Principal component analysis for parameters putatively related to early vigour in several cultivars of bread wheat (greenhouse, random seed size). In this analysis, the data for day 10 *v.* total leaf area of the seedling (TLA, i.e. early vigour) on day 31 are included. Abbreviations: col, coleoptile length; DW L1, DW L2, DW root, DW stem, dry weight of leaf 1, leaf 2, root, and stem; LA1, LA2, area of leaf 1 and leaf 2; LL1, LL2, length of leaf 1 and leaf 2; rad, length of radicle; shoot/root, shoot dry weight/ root dry weight; SLA, specific leaf area; TGW, 1000-grain weight; WL1, WL2, width of leaf 1 and leaf 2. For abbreviations of cultivars see Table 1.

Table 4. Multiple regression analysis of the relationship between seedling leaf area at 31 days after planting (i.e. early vigour) and several parameters in 20 cultivars of bread wheat (*Triticum aestivum* L.) grown in a glasshouse

For more details see *Material and methods* and Table 1. The equations, values of *P* and coefficients of determination (r^2) are shown. Abbreviations: $WL1_{10}$: width of leaf 1 at 10 days after planting; $WL1_{31}$ and $WL2_{31}$: width of leaves 1 and 2 at 31 days after planting; TGW, 1000-grain weight

Eqn no.	Equation of the regression	*P*	r^2
1	$5.604\ WL1_{31} - 5.37$	0.0001	0.76
2	$5.648\ WL2_{31} - 5.276$	0.0001	0.82
3	$0.0281\ TGW + 3.202\ WL1_{31} - 9.047$	0.0001	0.85
4	$0.0221\ TGW + 3.728\ WL2_{31} - 8.106$	0.0001	0.86
5	$5.446\ WL1_{10} - 4.54$	0.0001	0.73
6	$0.303\ TGW + 2.748\ WL1_{10} - 8.162$	0.0001	0.81

where NAR is the increase in plant weight per unit leaf area and unit of time, and LAR (leaf area ratio) is the leaf area per unit plant weight (Poorter 1989). The morphological component of this equation, i.e. LAR, is the product of SLA (leaf area per unit leaf weight) and leaf weight ratio (the fraction of dry mass invested in the leaves). The physiological component of this equation, NAR, is related to net photosynthetic rate, but it is also affected by biomass allocation to heterotrophic organs and carbon losses by respiration and exudation (e.g. ter Steege *et al.* 2005). In our results, changes in the ranking of early vigour of the cultivars between two successive sampling dates were associated with differences in NAR during that period. The

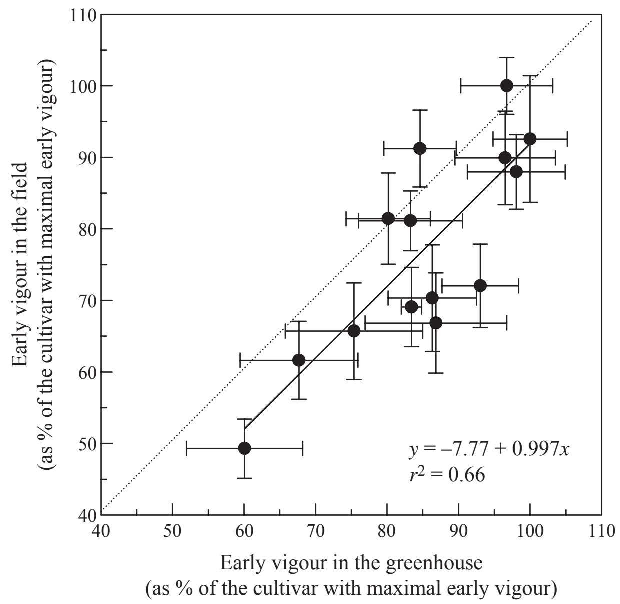

Fig. 5. Relationship between early vigour (i.e. seedling leaf area) of 14 cultivars of bread wheat grown in the greenhouse *v.* the field. Random-sized seeds were used in both experiments. For each cultivar, early vigour is expressed as a percentage of the cultivar with the largest seedling leaf area. Each point represents the mean of each cultivar ± s.e. ($n = 8$ in the greenhouse experiment, and $n = 15$ in the field). Equations and coefficient of determination (r^2) are shown. The broken line shows a hypothetical 1 : 1 relationship with identical early vigour in the greenhouse and the field.

importance of NAR (compared with SLA) in determining RGR has been questioned in previous studies (e.g. Poorter and Van der Werf 1998). More recently, other authors (e.g. Shipley 2006) have reported that the importance of NAR may be greater in studies where plants grow at higher (realistic) photosynthetic photon flux densities (PPFD), compared with studies conducted in growth chambers. The importance of SLA would be lower under those conditions (high PPFD), and our data are consistent with this idea. From our results, we cannot speculate whether cultivar differences in NAR are explained by differences in photosynthesis or carbon use. For instance, differences in carbon use at root level could be involved, because roots of more vigorous cultivars in Australia have lower respiration costs than less vigorous ones (see Palta 2007). In other words, regardless of root biomass, higher respiratory rates in roots can lead to a lower NAR in less vigorous cultivars. Further research is needed to answer this question.

A higher SLA, which is a negative indicator of leaf thickness and/or leaf density, has been associated with higher RGR (Poorter 1989) and higher early vigour (Rebetzke *et al*. 2004). One possible reason for the poor association (i.e. small coefficient of determination) that we found between SLA and early vigour could be the small variability in SLA in the present set of cultivars (see for instance Fig. 2*a*, *b*). In fact, variability in SLA seemed to be moderate (~400–550 $cm^2 mg^{-1}$), and this could underestimate (spuriously) the importance of the relationship between SLA and early vigour.

Morphological indicators of early vigour: an empirical approach

In this set of 20 cultivars of bread wheat, the width of the leaves was the morphological parameter better correlated with early vigour (Table 3), in agreement with data from researchers of CSIRO in Australia (e.g. Rebetzke and Richards 1999). According to those authors, leaf width is highly heritable, and its measurement is simple and fast (Richards *et al*. 2002). In order to predict early vigour 31 days after planting with data corresponding to 10 days after planting, we carried out a multiple regression analysis including several traits such as 1000-grain weight, SLA, and leaf width. The statistical fit was higher including 1000-grain weight and leaf width than for leaf width alone (compare Eqns 5 and 6 in Table 4). The inclusion of SLA did not improve the predictive value of the equations (not shown). The use of 1000-grain weight and leaf width in multiple regressions may be integrative, because 1000-grain weight represents seed reserves, and the width of leaf 1 has been reported as a good indicator of embryo size (Rebetzke *et al*. 2008).

Early vigour under field conditions

Several environmental constraints could limit the expression under field conditions of early vigour measured in a greenhouse environment (Botwright *et al*. 2002) and eventually eliminate the advantage of more vigorous cultivars. There was a close association (high coefficient of determination, i.e. $r^2 = 0.66$) between our greenhouse (day 31 after transplanting) and field experiments, demonstrating the good predictive value of the former experiment.

Concluding remarks

The main conclusions of this work can be summarised as follows: (*a*) there was a moderate–high variability in early leaf area in this set of 20 cultivars of bread wheat, opening an opportunity to improve plant establishment through selection for increased early vigour; (*b*) although seed mass was the parameter most closely related to early vigour, some residual variability in this trait should be explained by other causes; (*c*) SLA, a leaf trait related to high relative growth rate and early vigour in previous works, did not show a strong association with seedling leaf area, although this could be explained by the small variability in SLA in the present set of cultivars; (*d*) we did not find penalties of a larger seedling root on early vigour and *vice versa*; (*e*) the inclusion of several different parameters (e.g. seed mass and leaf width) in a multiple regression analysis provides an integrative framework to predict early vigour.

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