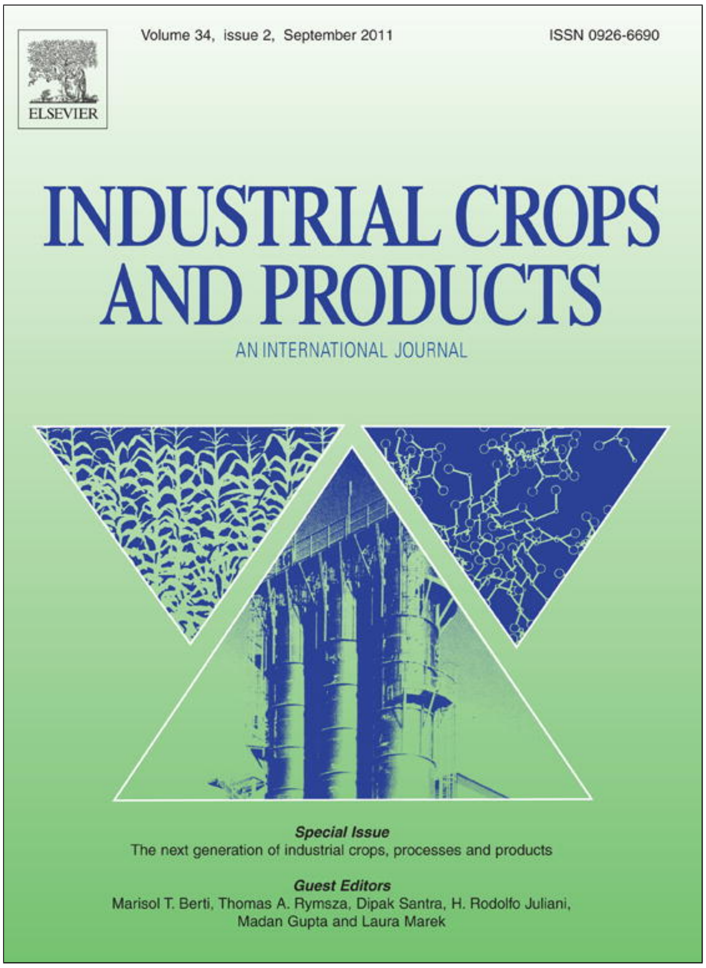

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Indirect changes associated with a selection program for increased seed-yield in wild species of *Lesquerella* (Brassicaceae): Are we developing a phenotype opposite to the expected ideotype?

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ABSTRACT

Seed-yield stability, frequently associated with drought-tolerance strategies, is one of the main breeding objectives for the development of crops for semi-arid mediterranean-type environments. Since breeding of new industrial crops targeted for arid lands is not appreciably different from that of traditional crops, higher yield is achieved by increased harvest-index, at the cost of losing traits associated with drought-tolerance and reduced seed-yield stability. Using *Lesquerella* as a model we compared selected and unselected accessions of annual (*L. gracilis* and *L. angustifolia*) and perennial (*L. pinetorum* and *L. mendocina*) genotypes grown in field experiments in Patagonia, Argentina. Our objective was to assess the effects of breeding for increased seed-yield on traits related to the main characteristics that define the most common ideotype for mediterranean-type environments: early vigor, conservative growth strategy post-anthesis and reserves storage. Our specific question was: Have any of the attributes associated with seed-yield stability been indirectly selected during the domestication process? Our results show that these characteristics were reduced or lost, in selected lines compared to their wild relatives. Early vigor was lower in selected accessions and was associated mainly with reduced relative growth rate and CO_2 assimilation. During the reproductive period the growth strategy was changed by selection towards a non-conservative and more acquisitive resource use strategy. Traits associated with this strategy were linked to higher water use efficiency and growth capacity (higher CO_2 assimilation rate, specific leaf area, and leaf allocation), but also with loss of structural adaptation to low resource environments (i.e. low specific leaf area), an increase in nutrient and water demands, and reduced nutrient use efficiency. Carbohydrates accumulation pre-anthesis was lower in selected accessions of all four species, and also, just in perennials we found lower reserves storage post-anthesis. These changes in the pattern of carbohydrates accumulation could be associated to lower seed-yield stability due to the loss of buffer capacity linked with the use of pre-anthesis reserves for seed filling. On the other hand, in perennial species lower reserves storage after seed harvest could reduce plant longevity and survival. We conclude that indirect changes occurred during the preliminary domestication of both annual and perennial species of Lesquerella used in our experiment. These changes were against those required if these species were to be developed as crops for semi-arid, mediterranean environments and should result in low seed-yield stability.

1. Introduction

Because most of the fertile lands of the world are already cultivated, expansion of agriculture must come largely from farming in environmentally marginal habitats (Chapin, 1980). In particular, crop production in arid lands requires large additions of water and fertilizer which increase production costs, often times to the point of precluding cultivation altogether (McLaughlin, 1985).

Under these circumstances the selection and cultivation of plant species native to arid environments, has been proposed as an alternative to reduce irrigation costs.

Although xerophytes tend to be more drought-tolerant, they have lower growth rates and seed-yield potential than mesophytes. On the other hand, they may serve as a form of insurance against environmental variability because they tend to have higher seed-yield stability (Jackson and Koch, 1997), and thus, have been linked to more sustainable production systems (Pimm, 1997). As a result, many species with a very low profile in modern agriculture are coming under considerable scrutiny in breeding and germplasm evaluation programs around of the world (Ravetta and Soriano, 1998).

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Among these arid adapted species, the genus *Lesquerella* (Brassicaceae) is considered a good candidate for domestication, because it contains hydroxy fatty-acids in its seeds (Dierig et al., 1993, 1996). *Lesquerella fendleri* (Gray) Wats. was identified as the most promising taxon for rapid domestication and commercialization in the Southwestern United States (Dierig et al., 1996). Results on selection and breeding for agronomic characters such as seed-yield, seed-oil and fatty acid yields have been reported by Dierig and Thompson (Thompson et al., 1992; Thompson, 1990; Dierig et al., 1993). Irrigation studies with bred cultivars of this species indicate that approximately 600–800 mm of water are required in Arizona for optimum seed-yields; this water requirement is similar to that for traditional winter wheat (Dierig et al., 1993).

One issue with *L. fendleri* has been the limitation of yields brought about by low winter temperatures (Ploschuk et al., 2003) and water stress during peak flowering and seed development (Hunsaker et al., 1998), particularly in mediterranean-type environments. As an alternative to overcome the limitations of annual *Lesquerella*, Ravetta and Soriano (1998) proposed the evaluation of the perennial *L. mendocina*, native to the Monte region in Argentina. Although potential seed-yield and harvest index are lower in perennials than in annuals (Moffat, 1996), physiological and phenological traits associated with the perennial habit have been deemed useful for increased crop performance, yield-stability and ecological sustainability (Glover, 2003) particularly under water limiting conditions (DeHaan et al., 2007). In these low-resource environments yields are often highly variable, and so the main breeding objective is seed-yield stability to minimize the likelihood of crop failures (Ceccarelli et al., 1991).

Despite our increased understanding of stress physiology in many cultivated species, the development of drought-tolerant cultivars has been slow for most crops (Fukai and Cooper, 1995). Although the ideotype of a drought tolerant crop is not always well defined (Blum, 2005), some morpho-physiological traits determinant of higher seed-yield stability have been identified by several authors. These attributes conform the most commonly cited ideotype for mediterranean-type environments (Araus et al., 2002; Slafer et al., 2005).

As a first step in the identification of an ideotype for semi-arid mediterranean environments, a conceptual model of seed-yield determination must be formulated. Given the nature of mediterranean-type environments, growth should be maximized at times when water is available and evaporative demand is at a minimum. This may require selecting for fast winter growth or high early vigor (Slafer et al., 2005). Early vigor is a complex trait related to a number of seedlings characteristics, such as leaf area index (LAI), leaf area ratio (LAR), specific leaf area (SLA), CO_2 assimilation and relative growth rate (RGR; López-Castañeda et al., 1996). Early vigor should increase total biomass at anthesis and seed-yield, because it increases soil water use early in the season and, as consequence, crop water use efficiency is also enhanced (Wright et al., 1996).

An alternative strategy for drought-tolerance is the tolerance to post-anthesis stress. In many crops seed filling depends, in part, on current photosynthesis and not only on re-traslocation of carbohydrates stored during pre-anthesis (Araus et al., 2002). Under conditions of terminal drought stress, net photosynthesis can decrease significantly and the proportion of translocated carbohydrate stores as a source of carbon for seed growth becomes larger. Under this scenario any increase in pre-anthesis carbohydrate storage could serve as a buffer to support seed-filling under unfavorable weather conditions during anthesis and seed-filling (Samonte et al., 2001).

On the other hand, under terminal drought, a conservative strategy through a more conservative use of water or higher water use efficiency can be beneficial (Kamoshita et al., 2009). This strategy is defined by a low relative growth-rate, low SLA, high leaf longevity and high allocation to roots and reserves (Jackson and Koch, 1997). While low SLA limits photosynthetic capacity, it provides higher resistance to biotic and abiotic stresses (e.g. herbivory; drought, frost, wind; Poorter et al., 2009) and higher water use efficiency (Turner and Kramer, 1980).

Although these strategies provide seed-yield stability, increase in seed-yield potential is frequently achieved by increases in the partitioning of crop biomass to the harvested products (higher harvest index; Gifford and Evans, 1981), reductions in allocation to vegetative structures and to reserve storage (Foulkes et al., 2009), and changes in leaf morphology (higher SLA, Pujol et al., 2008). These changes in allocation patterns and leaf morphology have been associated with a lower drought tolerance (Premachandra et al., 1994), and an impoverished plant defence system (Pujol et al., 2008). As a consequence, selection under relatively favorable environmental conditions usually translates into lower seed-yield in less favorable environments, which means an implicit reduction in seed-yield stability (Ceccarelli and Grando, 1991).

Breeding of new industrial crops targeted for arid lands is not appreciably different from that of the more established food, feed, and fiber crops (Thompson, 1990). Some of the wild characteristics that most often had been positively changed during domestication are shattering, dormancy, irregular germination, daylength sensitivity, branching habit, vegetative/reproductive ratio, and disease and insect susceptibility (Ray et al., 2005). The effects of breeding on attributes that determine seed-yield stability and drought-tolerance have not been extensively investigated. We know little on how those traits that conform the most common ideotype for mediterranean-type environments, other than those directly targeted, have evolved through breeding. Still, the understanding of these mechanisms is crucial when defining new criteria of selection in crops specifically targeted to arid land.

Using *Lesquerella* as a model we compared selected and unselected accessions of annual (*L. gracilis* (Hook.) Wats. and *L. angustifolia* (Nutt.)), and perennial (*L. pinetorum* Wooton y Standley and *L. mendocina* (Phil) Kurtz) genotypes. Our objective was to assess the effects of breeding for increased seed-yield on traits related to early vigor, a conservative growth strategy post-anthesis, and reserves storage. Our specific question was: Have any of the attributes that define the most common ideotype for mediterranean-type environments, which are associated with higher seed-yield stability, been indirectly selected during the domestication process?

2. Materials and methods

2.1. Field experiments

Field experiments were carried out during 2006 (experiment 1) and 2007 (experiment 2) at the Chubut River Valley, Argentina (43°17′S, 65°29′W). The target area has a mediterranean environment (Patagonian semi-desert), with cold, wet winters and dry summers, but with the possibility to irrigate from late Spring (September) until early Fall (April). In both experiments we tested two factors, species and breeding status, in a completely randomized design. We used four species of *Lesquerella*: two annuals, *L. gracilis* (origin Oklahoma and Texas) and *L. angustifolia* (origin Oklahoma and Texas), and two perennials, *L. pinetorum* (origin Arizona) and *L. mendocina* (origin La Pampa, Argentina). Breeding had two levels: wild and selected accessions (see Section 2.2).

For these experiments, seeds were initially sown in germination-trays filled with soil: peat moss (1:1 by volume) in February 14, 2006 and February 19, 2007, and maintained in a greenhouse until transplanting to the field, 66 days after sowing (DAS; April 21, 2006), and 72 days after sowing (May 2, 2007), respectively. Experimental units consisted on field plots (0.75 m × 0.70 m × 0.5 m deep; 10 reps) isolated from the ground with polyethylene lining. Plant density was 16 plants m^{-2}, with a spacing of 0.17 m between plants and rows 0.4 m apart. Density was low enough to avoid detrimental effects of competition on final biomass, probability of flowering, and seed-yield. Each of the ten experimental units (plots) included 8 plants per species and breeding level, for a total of 80 plants per treatment. Plots were flood-irrigated every 10 days. Weeds were removed manually. Etilen bis ditiocarbamate-zinc (T.N. zineb, 2 g/L) and 2-metoxicarbamoil-bencimidazol (T.N. carbendazim, 0.5 cm^3/L) were applied monthly to prevent fungal damage.

2.2. Selection process

Seed for the wild accessions came from seed-enhancement plots (one cycle of multiplication) which were established from seeds collected from native stands of each species. On these plots seed was bulk harvested. These same seed collected from native stands was used to generate selected accessions. The selection criterion was individual plant seed-yield applied on stands of plants growing in introductory gardens in the Chubut River Valley (160 plants per species). The selection cycle was carried on for three generations in which we selected the four most productive plants per generation. Seed from each year's selected plants was used to produce the next generation's stands. During all three selection cycles, fields were watered once every 7–10 days to water saturation.

2.3. Measurements

2.3.1. Phenology

The number of days from sowing to anthesis and to maturity was recorded on all eight plants in each of the ten plots. The criteria used to decide the time in which a species had reached anthesis or maturity was when 50% of the plants of a plot had at least one flower or 50% of the plants of a plot had at least 25% of the fruit present in the half part of the floral stem matures, respectively.

2.3.2. Gas exchange

Net photosynthesis rate ($A = \mu mol\, CO_2\, m^{-2}\, s^{-1}$) and transpiration rate ($E = mmol\, H_2O\, m^{-2}\, s^{-1}$) were measured on individual leaves of one plant per plot (only six of the ten plots per specie and breeding level) at bolting (September, 13) and anthesis (October, 10) using an IR gas analyzer (LCA-4, ADC, Hoddeson, England). The measurements were made on leaves fully exposed to direct sunlight, and maintained at right angles to incident solar radiation. We performed five daily measurements at 2–3 h intervals between 08:30 A.M and 20:00 P.M. (08:30, 11:00, 14:00, 17:30, and 20:00).

Accumulated assimilation (Ad, mmoles $m^{-2}\, day^{-1}$) was calculated integrating instantaneous values of CO_2 assimilation. Integrated water use efficiency (WUE, mmoles CO_2/moles H_2O) was derived by dividing A by E. These measurements were taken only in experiment 1 (2006/2007).

2.3.3. Growth analysis and allocation

For growth analysis, plants were harvested at five phenological stages: rosette (166 DAS for both annuals and perennials), floral bud (202 and 213 DAS for annuals and perennials, respectively), anthesis (237 and 242 DAS, for annuals and perennials, respectively), fructification (283 and 289 DAS for annuals and perennials, respectively) and maturity (329 and 339 DAS for annuals and perennials, respectively). This harvest methodology is appropriate to avoid the ontogenetic effects on relative growth rate and growth-related traits. At each harvest date six plant were uprooted (one plant per plot, 6 plots per specie and breeding status), and biomass was separated into roots, leaves, reproductive support structures (fruits and stems), and seeds. Biomass allocation was quantified as the proportion of total plant biomass allocated to roots (RMR), or to leaves (LMR). Leaf area was determined using UTSHCSA Image Tool for Windows, Version 2.0. After scanning, leaves were dried at 70 °C and weighed. Specific leaf area was calculated as the ratio leaf area: leaf mass. Leaf area ratio was calculated as LAR = SLA × LMR.

Relative growth rate (RGR), and net assimilation rate (NAR) were estimated on the basis of allocation-related traits and physiological traits, using the formulae given in Hunt et al. (2002). These variables were calculated for both, vegetative (rosette to anthesis), and post-anthesis period (anthesis to fructification).

Carbohydrate storage was determined as the amount of total non-structural carbohydrates present in roots (TNC). Root biomass samples (250 mg per plant) were milled and digested in HCl (10%) at 100 °C for 1 h, and filtered. The solubilized sugars were then analyzed colorimetrically (620 nm) following the Anthrone method (Yemm and Willis, 1954).

2.4. Statistical analysis

To test for differences in RGR between accessions for each species, we performed a two factor analysis of variance (Breeding and Time), with Ln transformed plant weight as the dependent variable. A significant Breeding × Time interaction indicates differences in RGR between accessions.

Analysis of variance (ANOVA) with two factors (Breeding and Year), were used to determine differences in the morpho-physiological traits measured between selected and wild accessions for each species, and among years of experiments (interaction Breeding × Year effects). Assumptions of ANOVA were calculated using Shapiro–Wilk's test for normality and Levene's test for homogeneity of variance. When these assumptions were violated, data were transformed with natural logarithm or ranking.

We used path analysis to determine how the morphological and physiological traits influenced seed-yield. This approach allowed the quantification of the degree to which each component contributed to seed-yield and the determination of the basis for seed-yield differences between wild and selected accessions of *Lesquerella*. Path coefficients in the model quantify the strength of the relationship between model variables and correspond to standardized partial regression coefficients.

3. Results

3.1. Seasonal pattern of growth

The seasonal pattern of growth was changed as a result of selection for seed-yield. Biomass at the rosette stage (initial biomass) did not differ among accessions in any species (Table 1). In general, selected accession had a lower RGRvegetative (RGRv) and lower total biomass at anthesis in both experiments, except for RGRv of *L. pinetorum* in experiment 1 and *L. mendocina* in experiment 2 (Table 1). RGRr (RGR post-anthesis) was higher in selected accession of all species, in both experiments (Table 1).

3.2. Morpho-physiological traits associated to changes in growth

3.2.1. Pre-anthesis

The lower growth capacity of selected accessions was due mainly to the lower pre-anthesis CO_2 assimilation (Table 2) and to changes in morphological traits related to allocation to photosynthetic structures: allocation to leaves (LMR) and leaf area ratio

Table 1
Relative growth rate for the vegetative (RGRveg, from rosette to anthesis) and reproductive (RGRrep, from anthesis to fructification) periods, of selected and wild accessions of annuals (*L. gracilis* and *L. angustifolia*) and perennial (*L. pinetorum* and *L. mendocina*) species.*

	Experiment 1 (2006/2007)				Experiment 2 (2007/2008)			
	Biomass at rosette (g)	RGRveg ($mg\,g^{-1}\,day^{-1}$)	Biomass at anthesis (g)	RGRrep ($mg\,g^{-1}\,day^{-1}$)	Biomass at rosette (g)	RGRveg ($mg\,g^{-1}\,day^{-1}$)	Biomass at anthesis (g)	RGRrep ($mg\,g^{-1}\,day^{-1}$)
L. gracilis								
Selected	0.06 ± 0.02 ns	50 ± 1 a	0.86 ± 0.13 a	56 ± 3 b	0.06 ± 0.01 ns	40 ± 2 a	0.34 ± 0.17 a	59 ± 1 b
Wild	0.04 ± 0.01 ns	65 ± 2 b	1.64 ± 0.41 b	35 ± 2 a	0.05 ± 0.02 ns	51 ± 1 b	0.79 ± 0.13 b	30 ± 3 a
L. angustifolia								
Selected	0.14 ± 0.01 ns	25 ± 2 a	0.78 ± 0.30 a	24 ± 2 b	0.05 ± 0.01 ns	36 ± 2 a	0.36 ± 0.06 a	56 ± 3 b
Wild	0.10 ± 0.04 ns	40 ± 2 b	1.65 ± 0.44 b	15 ± 1 a	0.03 ± 0.01 ns	47 ± 2 b	0.45 ± 0.05 b	49 ± 2 a
L. pinetorum								
Selected	0.08 ± 0.01 ns	50 ± 2 ns	4.04 ± 0.61 a	47 ± 2 b	0.12 ± 0.01 ns	34 ± 2 a	1.76 ± 0.22 a	25 ± 1 b
Wild	0.09 ± 0.02 ns	55 ± 3 ns	5.31 ± 0.32 b	32 ± 3 a	0.10 ± 0.01 ns	50 ± 3 b	2.80 ± 0.18 b	15 ± 2 a
L. mendocina								
Selected	0.11 ± 0.02 ns	43 ± 2 a	4.01 ± 0.76 a	28 ± 2 b	0.07 ± 0.01 ns	29 ± 2 ns	0.37 ± 0.08 a	34 ± 2 b
Wild	0.13 ± 0.04 ns	54 ± 2 b	5.79 ± 0.64 b	14 ± 1 a	0.09 ± 0.01 ns	27 ± 3 ns	0.73 ± 0.09 b	25 ± 4 a

[*] Data is means ± S.E. Different letters indicate significant differences between accessions (selected and wild) for each species ($p < 0.05$). Comparison of means was assessed by Tukey's test.

(LAR). These traits were reduced in selected accessions of *L. angustifolia* (both experiments), and of *L. gracilis* and *L. mendocina* only in experiment 2 (Table 3), compared to wild accessions. On the other side, the higher SLA found in selected accessions in both perennials was associated with a lower CO_2 assimilation and thus, did not result in increases in RGRv as could be expected (Table 3). Selected accession of *L. angustifolia* also showed a lower allocation to roots (RMR, Table 3).

3.2.2. *Post-anthesis*

The morphological traits associated with the higher growth capacity of selected accession found during the reproductive period were a higher SLA in all species and, only in *L. angustifolia* and *L. mendocina*, higher LAR and LMR (Table 4). Allocation to roots post-anthesis was not changed with selection in any of the four species. In *L. pinetorum*, in general, morphological traits did not change with selection for increased seed-yield neither during the vegetative (Table 3) nor during the reproductive phases (Table 4).

The physiological traits that changed during the reproductive stage as consequence of the selection process were *A* and WUE, that were higher in selected than in wild accessions. No changes were found in *E*, so *A* was responsible for the increases found in WUE (Table 4).

3.2.3. *Seasonal pattern of carbohydrate accumulation*

Selection also caused changes in the seasonal pattern of carbohydrate accumulation. In both annuals, selected accession reached anthesis with lower carbohydrates storage (both experiments), although reserves did not differ after anthesis (Fig. 1a and b). Selected accessions of perennials had less carbohydrate reserves at anthesis and post-anthesis (fructification, Fig. 2a and b) than wild accessions.

Table 2
Physiological traits: CO_2 assimilation rate (*A*; $\mu mole\,CO_2\,m^{-2}\,s^{-1}$) daily CO_2 assimilation rate (Ad; $\mu mole\,CO_2\,m^{-2}\,day^{-1}$) net assimilation rate (NAR; $mg\,cm^{-2}\,day^{-1}$); transpiration rate (*E*, $mmole\,H_2O\,cm^{-2}\,s^{-1}$) and water use efficiency (WUE, $\mu mole\,CO_2\,mmoles\,H_2O^{-1}$) for selected and wild accession of four species of *Lesquerella*. The traits were measured at bolting (vegetative period) and anthesis (reproductive period) only in the experiment 1 (2006/2007).

	Vegetative period		Reproductive period	
	Selected	Wild	Selected	Wild
L. gracilis				
A	6.3 ± 0.7 a	8.7 ± 1.2 b	13.0 ± 1.4 b	6.2 ± 1.3 a
Ad	315.7 ± 21.8 a	402.4 ± 58.3 b	371.2 ± 30.0 b	288.1 ± 26.4 a
NAR	0.56 ± 0.03 a	0.92 ± 0.04 b	4.15 ± 1.05 b	1.3 ± 0.7 a
E	1.5 ± 0.1 ns	1.6 ± 0.1 ns	3.5 ± 0.3 ns	4.0 ± 0.6 ns
WUE	4.3 ± 0.7 ns	3.1 ± 0.5 ns	3.3 ± 0.6 b	2.0 ± 0.4 a
L. angustifolia				
A	9.0 ± 0.5 a	13.8 ± 1.2 b	14.5 ± 2.3 b	6.9 ± 1.2 a
Ad	369.1 ± 104.5 ns	314.6 ± 27.2 ns	416.7 ± 28.5 b	246.5 ± 35.1 a
NAR	0.44 ± 0.04 a	1.14 ± 0.06 b	3.10 ± 0.79 b	1.1 ± 0.38 a
E	1.3 ± 0.2 ns	2.1 ± 0.5 ns	4.8 ± 0.6 ns	4.7 ± 0.6 ns
WUE	7.2 ± 1.2 ns	6.5 ± 1.0 ns	3.1 ± 0.3 b	1.6 ± 0.3 a
L. pinetorum				
A	10.9 ± 1.7 ns	8.2 ± 1.1 ns	12.0 ± 1.1 b	6.7 ± 1.2 a
Ad	448.0 ± 65.4 ns	417.7 ± 38.5 ns	494.9 ± 33.1 b	369.2 ± 26.4 a
NAR	0.74 ± 0.23 ns	0.83 ± 0.12 ns	1.9 ± 0.2 b	0.73 ± 0.16 a
E	1.3 ± 0.2 ns	1.6 ± 0.2 ns	2.5 ± 0.4 ns	3.3 ± 0.5 ns
WUE	4.0 ± 2.4 ns	4.3 ± 0.8 ns	3.2 ± 0.4 b	1.3 ± 0.2 a
L. mendocina				
A	7.7 ± 1.4 ns	7.7 ± 1.1 ns	12.3 ± 2.1 b	4.9 ± 1.2 a
Ad	376.6 ± 27.6 a	412.2 ± 28.9 b	473.3 ± 49.7 b	321.1 ± 45.8 a
NAR	0.59 ± 0.10 a	0.91 ± 0.15 b	4.1 ± 0.44 b	0.34 ± 0.49 a
E	1.3 ± 0.2 ns	1.6 ± 0.2 ns	3.7 ± 0.4 ns	2.8 ± 0.5 ns
WUE	5.0 ± 2.0 ns	4.0 ± 0.7 ns	2.7 ± 0.5 b	0.3 ± 0.5 a

Data is means ± S.E. Different letters indicate significant differences between accessions (selected and wild) for each species ($p < 0.05$).

Table 3
Morphological traits pre-anthesis: leaf area ratio (LAR), leaf mass ratio (LMR), specific leaf area (SLA), and root mass ratio (RMR) for selected and wild accession of four species of *Lesquerella*. Data is means ± S.E. Different letters indicate significant differences between accessions (selected and wild) for each species ($p < 0.05$).

	Attributes	*p*-Value interaction Breeding × Year	Experiment 2006/2007		Experiment 2007/2008	
			Selected	Wild	Selected	Wild
L. gracilis	LAR ($cm^2\,g^{-1}$)[a]	0.04	83.9 ± 7.5 ns	90.7 ± 3.2 ns	68.3 ± 3.5 a	84.6 ± 8.4 b
	LMR ($g\,g^{-1}$)[a]	0.005	0.65 ± 0.01 ns	0.60 ± 0.02 ns	0.51 ± 0.02 a	0.62 ± 0.06 b
	SLA ($cm^2\,g^{-1}$)	ns	140.3 ± 3.9 ns	139.0 ± 9.9 ns	133.6 ± 1.3 ns	135.9 ± 1.4 ns
	RMR ($g\,g^{-1}$)	ns	0.11 ± 0.01 ns	0.1 ± 0.01 ns	0.1 ± 0.01 ns	0.14 ± 0.02 ns
L. angustifolia	LAR ($cm^2\,g^{-1}$)	ns	70.8 ± 1.8 a	83.3 ± 1.8 b	63.6 ± 1.8 a	70.83 ± 1.76 b
	LMR ($g\,g^{-1}$)	ns	0.79 ± 0.02 a	0.85 ± 0.02 b	0.66 ± 0.02 a	0.76 ± 0.03 b
	SLA ($cm^2\,g^{-1}$)[a]	ns	89.9 ± 5.9 ns	97.8 ± 4.1 ns	96.0 ± 0.4 a	99.1 ± 1.8 b
	RMR ($g\,g^{-1}$)	ns	0.21 ± 0.01 b	0.15 ± 0.02 a	0.34 ± 0.02 b	0.24 ± 0.03 a
L. pinetorum	LAR ($cm^2\,g^{-1}$)	ns	87.7 ± 8.3 ns	63.5 ± 6.9 ns	105.1 ± 4.3 ns	108.2 ± 12.7 ns
	LMR ($g\,g^{-1}$)	ns	0.68 ± 0.01 ns	0.71 ± 0.03 ns	0.79 ± 0.03 ns	0.81 ± 0.06 ns
	SLA ($cm^2\,g^{-1}$)[a]	0.02	129.0 ± 2.3 b	89.4 ± 8.6 a	114.2 ± 3.9 ns	113.3 ± 2.4 ns
	RMR ($g\,g^{-1}$)	ns	0.09 ± 0.01 ns	0.10 ± 0.01 ns	0.21 ± 0.03 ns	0.19 ± 0.06 ns
L. mendocina	LAR ($cm^2\,g^{-1}$)	ns	92.9 ± 19.2 ns	88.4 ± 4.3 ns	91.8 ± 7.2 ns	89.9 ± 6.7 ns
	LMR ($g\,g^{-1}$)[a]	0.046	0.63 ± 0.02 ns	0.71 ± 0.03 ns	0.43 ± 0.05 a	0.56 ± 0.06 b
	SLA ($cm^2\,g^{-1}$)[a]	0.005	157.9 ± 3.1 b	125.1 ± 2.3 a	117.0 ± 12.2 ns	116.2 ± 22.3 ns
	RMR ($g\,g^{-1}$)	ns	0.13 ± 0.01 ns	0.10 ± 0.01 ns	0.14 ± 0.02 ns	0.14 ± 0.02 ns

[a] Indicates variables log transformed. In the cases that the interaction among Breeding × Year (see *p*-value of interaction) was significant, the experiments were analyzed separated.

3.3. *Functional determinants of the increase in seed-yield*

According to the path analysis model, the main changes found in pre-anthesis stages (also associated with the variation in seed-yield) were CO_2 assimilation and allocation to roots (Fig. 3). The variation of these traits and their relationship with the RGRv (a positive and a negative relationship between RGRv and NAR, and RGRv and RMR, respectively), were determinants of the difference in growth capacity pre-anthesis among species and yield levels. Although differences in morphological traits such as LMR and LAR were evident between accessions (Table 3) these attributes were not associated with seed-yield (path coefficients were not significant), neither directly nor indirectly.

Seed-yield was linked positively to RGRr and to CO_2 assimilation at anthesis (Fig. 4). The variation in RGRr between accessions was, in turn, consequence of changes in both, morphological (SLA and LAR) and physiological traits (*A*; Fig. 4). Also, specific leaf area was positively linked to CO_2 assimilation.

The rate of CO_2 assimilation at bolting was determinant of the variation in carbohydrate accumulation found at anthesis. Although these pre-anthesis carbohydrates did not influence vegetative growth or seed-yield (Fig. 3), they were directly linked to TNCr content (TNC post-anthesis; Fig. 4). They were, in turn, linked with both RGRr and seed-yield, although in opposite ways: TNCr was directly and positively related with seed-yield, however, the indirect and negative effects of TNCr, through RGRr on seed-yield was more important (higher path coefficient; Fig. 4).

Overall, the physiological component, NAR post-anthesis, was the more important determinant of seed-yield.

4. Discussion

In low-resource environments crop yields are often highly variable, thus, seed-yield stability, which is frequently associated with drought-tolerance strategies (Blum, 2005), becomes one of the main breeding objectives (Ceccarelli et al., 1991). While, the development of drought tolerant cultivars has been slow in most crops (Fukai and Cooper, 1995), some desirable characteristics for mediterranean-type environments have been identified by several authors. Increased crop performance may be achieved through different strategies: (1) improvements in water use pre-anthesis through traits associated with a high early-vigor, (2) a conservative growth strategy and high water use efficiency post-anthesis, and/or, (3) improvements in harvest index and increases in the accumulation of carbohydrate reserves pre-anthesis and their later use to sustain seed-filling (Araus et al., 2002; Slafer et al., 2005). Our experiments provide evidence that a selection program using seed-yield as the main trait provoked, in *Lesquerella*, indirect changes in several of the characters that define the most common ideotype for mediterranean-type environments. The main changes were: lower early vigor, a non-conservative but acquisitive growth strategy

Table 4
Morphological traits post-anthesis: leaf area ratio (LAR), leaf mass ratio (LMR), specific leaf area (SLA), and root mass ratio (RMR) for selected and wild accession of four species of *Lesquerella*. Data is means ± S.E. Different letters indicate significant differences between accessions (selected and wild) for each species ($p < 0.05$).

	LAR ($cm^2\,g^{-1}$)[a]	LMR ($g\,g^{-1}$)	SLA ($cm^2\,g^{-1}$)[a]	RMR ($g\,g^{-1}$)
Selected				
L. gracilis	67.8 ± 5.9 ns	0.36 ± 0.03 ns	152.5 ± 9.0 b	0.05 ± 0.02 a
L. angustifolia	55.4 ± 5.6 b	0.34 ± 0.03 b	141.7 ± 12.9 b	0.10 ± 0.01 ns
L. pinetorum	84.3 ± 7.4 ns	0.51 ± 0.04 ns	148.0 ± 13.4 ns	0.06 ± 0.007 ns
L. mendocina	69.6 ± 5.2 b	0.34 ± 0.03 b	135.8 ± 9.2b	0.11 ± 0.02 ns
Wild				
L. gracilis	80.3 ± 6.3 ns	0.39 ± 0.05 ns	113.8 ± 10.6 a	0.09 ± 0.03 b
L. angustifolia	42.6 ± 2.0 a	0.26 ± 0.01 a	116.5 ± 5.1 a	0.09 ± 0.01 ns
L. pinetorum	76.6 ± 5.6 ns	0.57 ± 0.04 ns	124.9 ± 13.3 ns	0.06 ± 0.01 ns
L. mendocina	54.9 ± 5.2 a	0.26 ± 0.01 a	106.7 ± 7.2 a	0.10 ± 0.02 ns

[a] Indicates variables log transformed. The interaction Breeding × Year was not significant for any traits and the experiments were analyzed together.

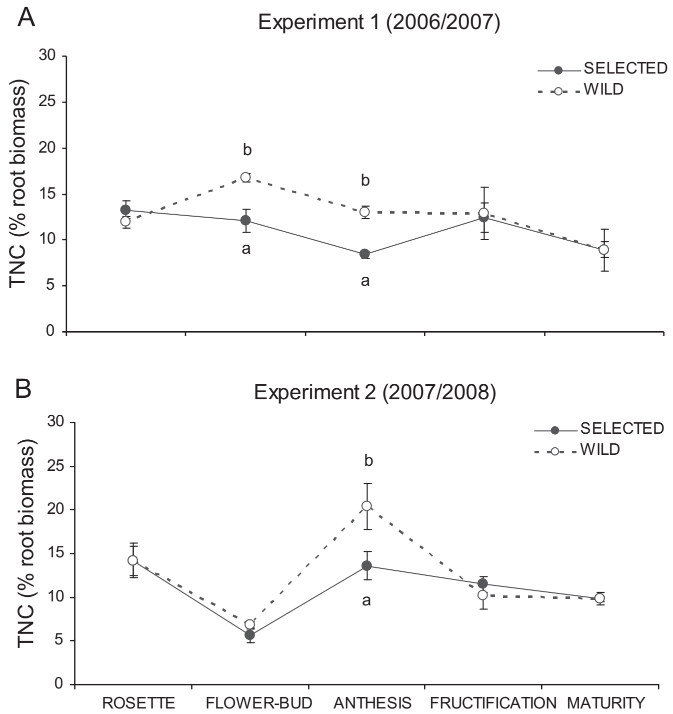

Fig. 1. Annual pattern of carbohydrates accumulation for selected and wild accessions of annual species pooled together (*L. gracilis* and *L. angustifolia*). Total non-structural carbohydrates were measured in five phenological stages. Data are the means ± S.E. of 12 plants per breeding level for the two annual species.

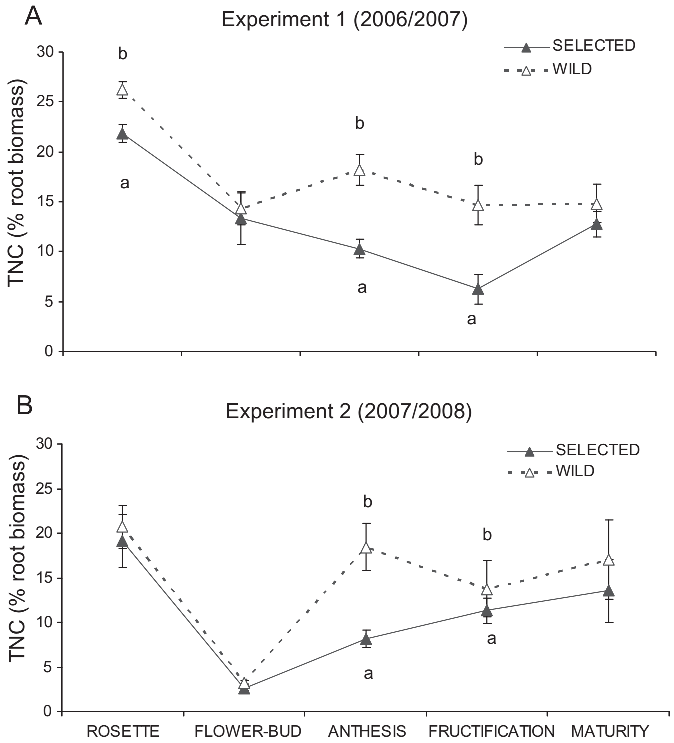

Fig. 2. Annual pattern of carbohydrates accumulation for selected and wild accessions of perennial species pooled together (*L. pinetorum* and *L. mendocina*). Total non-structural carbohydrates were measured in five phenological stages. Data are the means ± S.E. of 12 plants per breeding level for the two perennial species.

post-anthesis, and lower carbohydrate accumulation pre-anthesis in both annuals and perennials, and post-anthesis just in perennials. Some of these changes were opposite to those expected.

4.1. Early vigor and water-use pre-anthesis

An efficient use of the water available early in the season should increase total biomass and seed-yield (Slafer et al., 2005). Early vigor and related traits such as fast development of leaf area, high pre-anthesis CO_2 assimilation rates, and high relative growth rate (López-Castañeda et al., 1996), are characteristics that have been suggested as relevant in the development of high-yielding, drought tolerant crops for mediterranean environments.

Regardless their growth cycle (annual or perennial) on all four species of *Lesquerella* tested in these experiments, selection for increased seed-yield resulted in a lower early-vigor (lower biomass at anthesis and RGRvegetative) compared to unselected genotypes (Table 1). This reduced growth was mainly due to a lower photosynthetic capacity of selected accessions (Fig. 3). Although in some species we found differences in leaf traits between wild and selected accessions (e.g. lower LMR and LAR; Table 3), these changes were not related to the increase in seed-yield found in selected accessions (non-significant path coefficient, Fig. 3).

While the path model showed that the proportional allocation to root should also be responsible for the lower vegetative growth-rate of selected accessions (indirect effects of root allocation on

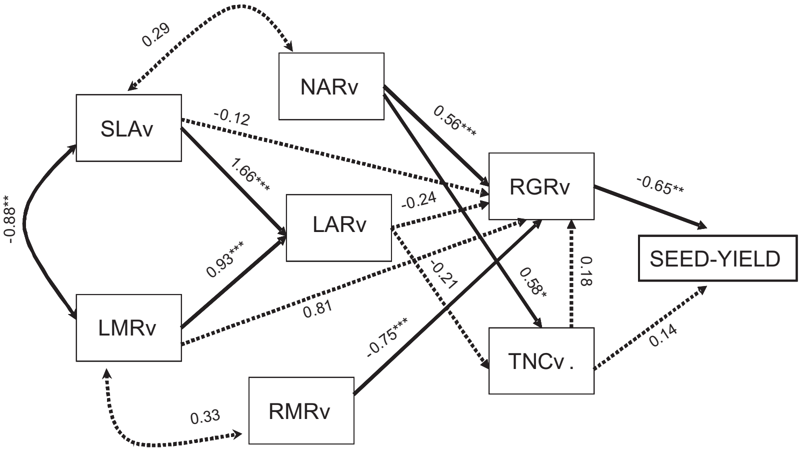

Fig. 3. Path diagram showing the direct and indirect effects of morphological and physiological traits pre-anthesis (vegetative period) on seed-yield. Numbers indicate direct path coefficients between traits. Leaf mass ratio (LMRV), specific leaf area (SLAV), leaf area ratio (LARV), root mass ratio (RMRV), net assimilation rate (NARV), relative growth rate (RGRV) and total non-structural carbohydrates (TNCV). Asterisks indicate significance of the coefficient: $^{***}p < 0.001$ and $^{**}p < 0.01$.

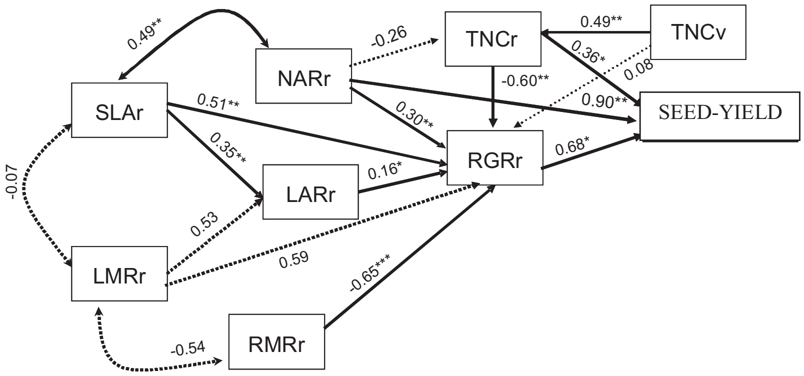

Fig. 4. Path diagram showing the direct and indirect effects of morphological and physiological traits post-anthesis (reproductive period) on seed-yield. Numbers indicate direct path coefficients between traits. Leaf mass ratio (LMRr), specific leaf area (SLAr), leaf area ratio (LARr), root mass ratio (RMRr), net assimilation rate (NARr), relative growth rate (RGRr), vegetative (TNCv) and reproductive (TNCr) total non-structural carbohydrates. Asterisks indicate significance of the coefficient: $^{***}p < 0.001$ and $^{**}p < 0.01$.

seed-yield through RGRv; Fig. 3), we only found significant differences in root allocation between accessions in *L. angustifolia* (higher RMR in selected accessions; Table 3).

4.2. Conservative growth strategy and WUE post-anthesis

Another strategy that has been suggested to improve crop performance in mediterranean-type environments is high water use efficiency post-anthesis (Araus et al., 2002; Slafer et al., 2005). High WUE could be achieved through a conservative resource use strategy during the reproductive phase. This strategy is associated with an overall high resource-use-efficiency (Westoby et al., 2002) and confers a more positive plant carbon balance when these resources are limiting (Sheriff et al., 1995). Traits that determine the conservative resource use strategy (i.e. low SLA and CO_2 assimilation, and high reserve storage) are typically found in species with low RGR, native to low-resource environments (Poorter, 1989), and geared towards the conservation of acquired resources (Poorter, 1989, Westoby et al., 2002), and are also found in some landraces from subsistence farming systems (Jackson and Koch, 1997).

The conservative strategy was evident in wild accessions of all four species we tested in our experiments, while selection for seed-yield provoked a change in resource-use strategy. The suite of traits we found in selected *Lesquerella* are those typical of species with high RGR, growing in environments that are relatively rich in water and nitrogen (sensu Poorter, 1989), and geared for high rates of resource acquisition (also including traits such as high SLA and CO_2 assimilation, and low carbohydrates storage, Table 5, Fig. 4). These traits confer high rates of biomass production but also an increase in nutrient and water demands, and reduced nutrient and water use efficiency (Chapin, 1980). Within this context, and considering that the seed-filling stage in *Lesquerella* is the most sensitive to water deficit for seed-yield determination (Puppala et al., 2004), selected accession should be less drought-tolerant than wild accessions.

The change in the seasonal pattern of growth found in selected accession compared to their wild predecessor also had important consequences on water use and water use efficiency. While WUE pre-anthesis measured at the leaf level (A/E) did not differ between selected and unselected genotypes (Table 3), at the crop level, a decrease in crop WUE, early in the season could be expected since the lower leaf area development and the lower CO_2 assimilation of selected accessions should decrease the ratio of crop transpiration/soil evaporation (Fischer, 1979). On the other side, despite the change in the post-anthesis resource use strategy to a more acquisitive and less drought-tolerant strategy, selected accession of *Lesquerella* had a higher WUE.

Against previous reports in other plant species (Blum, 2005), the increment in WUE was linked to higher A without significant changes in water use, since E was unchanged by selection. In this context, selection for high WUE post-anthesis in *Lesquerella* would not result in less growth (total biomass and leaf area), as what has happened in other crops (Blum, 2005), and could be used as a criteria for selection, to complement seed-yield.

4.3. Reserves storage

The third strategy suggested as the basis to increase performance and seed-yield in mediterranean-type environments is a large accumulation of pre-anthesis carbohydrates and their use post-anthesis. In these semi-arid mediterranean environments current photosynthesis is strongly reduced by spring/summer terminal drought, and as consequence, seed-yield is also reduced. An increase in the contribution of carbohydrate reserves may buffer reductions on current photosynthesis caused by the lack of water, and improve harvest index and seed-yield stability (Araus et al., 2002; Slafer et al., 2005). In our selected accessions of *Lesquerella* the main source of carbon for seed-filling was current photosynthesis and not carbohydrates stored pre-anthesis (a high path coefficient was found between NAR and seed-yield; Fig. 4), which makes seed-yield dependent on environmental conditions in late spring and summer.

Still, although pre-anthesis reserve storage was not directly associated with higher seed-yield, we found an indirect effect through TNCr and RGRr. We found a differential trade-off between reserve storage and growth in selected and wild accessions of *Lesquerella*. While unselected accessions allocated resources preferentially to reserves (which was associated with low growth capacity post-anthesis), selected accession allocated preferentially to structures relates to a high growth capacity (higher leaf allocation and SLA) at the expense of a lower accumulation of reserves. This allocation strategy of selected accession resulted in higher seed-yield compared to wild accessions (Fig. 4). This pattern of yield determination of selected accessions should result in a lower buffer capacity and lower seed-yield stability. Carbon storage is also necessary in perennials for re-growth, and to improve survival through unfavorable periods (e.g. cold temperature, drought) during which the plant remains dormant (Wyka, 2000). The consequence of allocating carbon to growth and away from storage is to increase the relative growth rate of the plant, and increase the compartment competent to manufacture carbohydrate for future seed production. In either case, seed set should tend to decrease storage and plant longevity (Jackson and Dewald, 1994).

Our experiments provide evidence that in perennial species of *Lesquerella*, improved seed-yield brought about by selection, reduced the amount of carbohydrates accumulated at the end of the annual cycle, which could, in turn compromise survival and plant longevity and perennial seed-yield stability. Although the chances of perennial crops are limited by a fixed negative relationship between seed-yield and longevity that could not be influenced by selection, mutation or environmental changes (Jackson and Dewald, 1994; Wagoner, 1990), it has also been shown that the trade off between seed-yield and perenniality is not fixed (DeHaan et al., 2007).

If perennials have an adequate CO_2 assimilation rate and carbon supply to support a high biomass production, the question becomes one of harvest index rather than a trade-off. In particular in perennial *Lesquerella*, there is still a need to evaluate seasonal patterns of carbohydrates reserves for longer than one growth cycle, especially in autumn, when plants have the potential to reestablish the storage reserves after seed maturity.

5. Conclusion

Our results show that selection for seed-yield in wild species of *Lesquerella* resulted in a lost of the main attributes that determine the more common characteristics that conform the ideotype for semi-arid, mediterranean-type environments. Early vigor, a conservative resource use strategy post-anthesis (linked to higher water use efficiency) and high pre-anthesis reserves (and their mobilization during seed-filling period) were reduced or lost, in selected lines compared to their wild relatives. All these characters are associated with better performance in low-resource environments and with higher seed-yield stability, one of the main breeding objectives for crops to be grown under these conditions.

Changes found in the seasonal pattern of growth (lower pre-anthesis growth and higher post-anthesis growth), and changes in structural and functional traits related to these capacities, had implications in water use and water use efficiency. While WUE pre-anthesis was unchanged, the lower CO_2 assimilation rate and reduce allocation to leaves, both responsible of the lower early vigor of selected accessions, should result in a decrease in the ratio crop transpiration/soil evaporation and should determine a reduction in crop water use and, thus, in water use efficiency. Changes found in leaf morphology in selected accession are also important, because although associated with higher leaf WUE post-anthesis, the high-SLA of selected accessions is linked to the loss of structural adaptation to low quality environments and is also associated with lower resource use efficiency and survival.

Selected accession had lower carbohydrate accumulation probably as a result of the trade-off between storage and growth. The importance for seed filling of current photosynthesis and relative growth rate during the reproductive period could result in low seed-yield stability in mediterranean-type environments, due to the dependence of seed-yield on environmental conditions in late spring and summer. Also reductions in carbohydrate reserves post-anthesis could be linked to lower survival and plant longevity in perennials selected for increased yield.

We conclude that indirect changes occurred during the initial stages of domestication of both annual and perennial species of *Lesquerella* used in our experiment. These changes were against those required if these species were to be developed as crops for semi-arid, mediterranean environments and should result in low seed-yield stability.

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