Allocation patterns and phenology in wild and selected accessions of annual and perennial *Physaria* (*Lesquerella, Brassicaceae*)

Luciana González-Paleo · Damián A. Ravetta

Abstract Two processes that determine a good performance of plants in arid environments are phenology and resource allocation patterns. With a longer growing season and larger allocation to root, perennials achieve better access to resources and are more resilient to stresses than annuals. In traditional agricultural systems selection for optimal soil nutrient uptake has been a secondary breeding objective, because crops receive subsidies of water and fertilizers. However in arid lands, caution is required during domestication, to avoid changes in structural traits which may be the basis for sustainable production. Due to inherent differences in hierarchy among annual and perennial species, we propose that the changes in phenology and allocation brought about by selection will depend on the life cycle. We performed field studies comparing wild and selected accessions of annual and perennial species of *Physaria*. Life cycle determined the functional basis of seed yield. In annuals, selection resulted in early anthesis (1 week earlier), a lower allocation to roots and leaves (twofold lower), and an increase in harvest index (an increase of 62%). Selected perennials had higher biomass at maturity (45% higher), linked to a longer reproductive period (3 weeks longer) than their wild relatives. The vegetative allocation found in wild perennials

remained unchanged after selection. While annuals selected for seed yield could compromise the capacity for acquisition of resources, selection in perennials did not modify the allocation strategy responsible for their positive adjustment to low resource environments. We found a trade off between seed yield potential and yield stability that resulted in lower performance of selected accessions in low quality environments in relation to their wild relatives.

Keywords Harvest index · Root · Anthesis · Yield stability · Selection · Arid lands

Introduction

The world is facing serious shortages of fresh water which is also less available for agriculture. The majority of the more fertile lands are already under cultivation, so the expansion of agricultural potential must come largely from farming in marginal habitats such as the arid lands (Chapin 1980; McLaughlin 1985; Ravetta and Soriano 1998). However, in many of these marginal environments agricultural practices have threatened the long-term sustainability for crop production. The domestication of desert-adapted plants and the production of perennial crops have been suggested as two of the ways to prevent environmental degradation while increasing cultivation intensity in arid lands.

L. González-Paleo (✉) · D. A. Ravetta
CONICET-Museo Paleontológico Egidio Feruglio,
Trelew, Chubut, Argentina
e-mail: lgonzalezpaleo@mef.org.ar

Appropriate patterns of phenology and allocation are key determinants of crop adaptation in arid ecosystems (Passioura et al. 1993; Richards et al. 1993; Passioura 1996). Differences in both phenological and allocation patterns (and in the functional features derived from them), appear to be more important for many ecological questions relevant to the development of new crops (e.g. drought tolerance, competition avoidance, etc.) than differences in physiological mechanisms (amount of enzymes and light harvesting complexes per unit leaf area, or extra layers of palisade parenchyma, etc.; Schwinning and Weiner 1998; Poorter 2002). Phenology and allocation have been highly modified during domestication and selection of all main crops.

In general, the process of domestication of a new crop candidate starts with genotype selection based on seed yield (Thompson 1990; Ray et al. 2005). The increase in seed yield achieved in annual crops has been associated with the reallocation of photoassimilates (i.e. improved harvest index; Evans 1993; Slafer 1994), and early anthesis (Halse and Weir 1970; Austin et al. 1980). Both characters are linked to reduced allocation of assimilates to competing sinks, especially roots and leaves (Donald and Hamblin 1976). In turn, the decrease in allocation to roots can limit the plant's ability to access water and nitrogen (Foulkes et al. 2009) and thus compromise yield stability in low resource environments (Khan et al. 2010). Still, the selection of adequate root systems for nutrient uptake has secondary importance because nutrient acquisition can be improved by increasing soil fertility and water availability (but also increasing production costs). Similarly, the decrease in allocation to leaves found during domestication in some crops may be incompatible with increases in radiation use efficiency, needed in many cases to improve productivity (Foulkes et al. 2009). Thus, when developing new oilseed crops for arid environments some caution is required to avoid or limit changes in structural and functional traits which may be the basis for sustainable crop production under water limiting conditions.

Differences in hierarchy of allocation to alternative sinks are expected to be linked to the plant's life cycle (annual, biannual, or perennial). For example, annuals invest most of their carbohydrates on leaf growth and seeds, whereas herbaceous perennials prioritize carbon allocation to roots, vegetative reproduction and storage organs (e.g. rhizomes or rosettes; Schultz 1982; Tilman 1988). Due to the higher investment to root biomass and better access to water and nutrients, perennials maintain photosynthetically active leaf area during periods in which CO_2 assimilation and green leaf area of annual plants decrease, due to leaf senescence. Thus, perennials may overcompensate for a smaller total leaf area with a longer season of active carbon assimilation than that of annuals, and even accumulate more total biomass (DeHaan et al. 2007).

Even though the resource allocation pattern of perennials may imply a better adjustment to environments of low water availability (Cox et al. 2006), one of the challenges raised against the feasibility of high yielding perennial grain crops is an assumed trade off between resources devoted to seeds and to vegetative growth (roots and leaves; Moffat 1996). Increased seed allocation harvest index, (HI), brought about by selection in perennial species, has resulted in reduced allocation to perpetuation structures (Piper and Kulakow 1995) and has been proposed to reduce plant longevity (Pimm 1997). However, it has been reported for several perennials that vegetative organs are hierarchically important sinks, but this allocation strategy does not result in low seed hierarchy (Piper and Kulakow 1995; Ploschuk et al. 2005). Still, even if the trade off between reproduction and perenniality exists, an alternative way to increase seed yield in perennial crops that would not compromise vegetative allocation is to increase total biomass while maintaining allocation to perpetuation (Pimm 1997).

The objective of this work was to compare potential differences in allocation and phenological patterns brought about by selection for seed yield in a group of annual and perennial species of *Physaria (Nuttall ex Torrey & Gray) Gray*; formerly *Lesquerella;* Brassicaceae), a new oil seed crop for arid lands (Thompson et al. 1989; Dierig et al. 1993; Ravetta and Soriano 1998). *Physaria* species contain a seed-oil with up to 55% of a 20-carbon long fatty acid that carries a single hydroxyl group and double bond. This fatty acid profile is similar to that of castor oil (Dierig et al. 1993). Large markets exist for these chemical feedstocks for lithium greases, polymers in paints and coatings, base stocks for lubricants, nylon-11, hydraulic fluids, and applications in the personal care industry (Dierig and Ray 2008). The hydroxyl group of this oil makes it a prime candidate as additives to diesel fuel to improve lubricity (Naughton 1992).

We propose that domestication of annual *Physaria* will result in strong selection for increased reproductive output (higher harvest index, number fruits per plant, number seeds per fruit, and/or seed weight) as found in many crops (Calderini et al. 1999; Abeledo et al. 2002; Garcia del Moral et al. 2003). Reallocation of resources to seeds will also result in a reduction of the biomass to vegetative organs (roots and leaves), and in early anthesis. In perennials the increase in seed yield will be associated with a longer growth cycle and larger total biomass accumulation, with small changes in allocation to vegetative organs.

We examined these hypotheses by means of field studies of total biomass, allocation patterns, seed yield and their components comparing wild and selected accession of annual and perennial species of *Physaria*. We also evaluated associated changes in phenological patterns.

Materials and methods

Field experiments

Two field experiments were carried out during 2006 (experiment 1) and 2007 (experiment 2) at the Chubut River Valley, Patagonia, Argentina (43°17′S, 65°29′W). In both experiments we tested two factors, species and breeding status in a completely randomized design. We compared four species of *Physaria*, two annuals, *P. gracilis* (origin Oklahoma and Texas) and *P. angustifolia* (origin Oklahoma and Texas), and two perennials, *P. pinetorum* (origin Arizona) and *P. mendocina* (origin La Pampa, Argentina). Breeding had two levels, wild and selected accessions. Wild accessions came from regeneration plots established from seed collected from native stands of each species (González-Paleo and Ravetta 2011). These same wild accessions were used to generate the selected accessions through mass selection. The selection criterium was individual plant seed yield (three top yielding plants). This criterium was applied to plants growing in stands of 160 plants per species, in introductory gardens in the Chubut River Valley (Table 1). The selection cycle was carried on for three generations. Seed from each year's selected plants was used to produce the next generation's stands. All *Physaria* species evaluated are self incompatible and cross pollinated (Dierig and Ray 2008). Thus, a desired trait

Table 1 Average baseline seed yield of selected and wild accessions of the four species of *Physaria* used in these experiments

Species	Wild (unselected) g seeds per plant	Selected
P. gracilis	0.93 ± 0.31	3.57 ± 0.85
P. angustifolia	1.61 ± 0.12	2.04 ± 0.28
P. pinetorum	1.03 ± 0.32	4.04 ± 0.74
P. mendocina	0.31 ± 0.10	4.30 ± 0.93

Data are means ± standard errors. The means of selected accessions are after three selection cycles

$N = 10$ individual plants

is more difficult to select because the seeds from a single plant are half instead of full siblings, as self pollination rarely occurs (Dierig et al. 2004).

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 transplanted to the field, 66 days after sowing (DAS; April 21, 2006), and 72 days after sowing (May 2, 2007), respectively.

Experimental units consisted of field plots (0.75 m * 0.70 m; 10 reps per species and breeding level). Each of the ten plots included eight plants, for a total of 80 plants per treatment (species and breeding level, eight treatments, 640 plants in total). 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 (Brahim et al. 1998). Plots were flood irrigated every 7 days. Weeds were removed manually. Fungal controls with etilen bis ditiocarbamate-zinc (zineb, 2 g/l) and 2-metoxicarbamoil-bencimidazol (carbendazim, 0.5 cm^3/l) were applied monthly.

Study site

The environment where the selection process and following experiments were carried out is semiarid with a Mediterranean climate (Koppen 1948; (FAO) 2010). The Mediterranean type climate has a notable wet season during fall and winter, while summers are hot and dry ((FAO) 2010). Limited supplemental irrigation is available in this area of Patagonia from late spring (September) until early fall (April).

Meteorological conditions differed during the two experiments (Table 2; Fig. 1). For experiment 1 (2006/2007 growing season), mean annual temperature and mean annual precipitation were similar to the average for this area. Experiment 2 (2007/2008) was held under much drier and cooler conditions than experiment 1.

Measurements

(a) Phenology The number of days from germination to anthesis and to maturity was recorded on eight plants (multiple measurements to generate one individual mean per plot) in each of the ten plots (replications). The criteria used to decide the time in which a species had reached anthesis was when 50% of the plants of a plot had at least one flower, and had

Table 2 Meteorological variables for the 2 years of experiments and historical values for the Chubut river Valley-Argentina

	Annual max. mean temp	Annual min. mean temp (°C)	Annual mean temp	Annual precipitation (mm)
Normal year	21.2	6.2	13.2	204.9
Driest and cooler year	19.2	3.6	11.7	157.5
Historical values	20.1	6.9	13.3	196

The growing season 2006/07 represented a normal year, while the growing season 2007/08 was a driest and cooler year. Historical values for the Chubut river Valley-Argentina were obtained using a 30-year period (1971-2000)

Source http://www.inta.gov.ar

Fig. 1 Pattern of weekly mean temperatures (°C) and rainfall (mm) for **a** the first experiment (2006/2007) and **b** the second experiment (2007/2008)
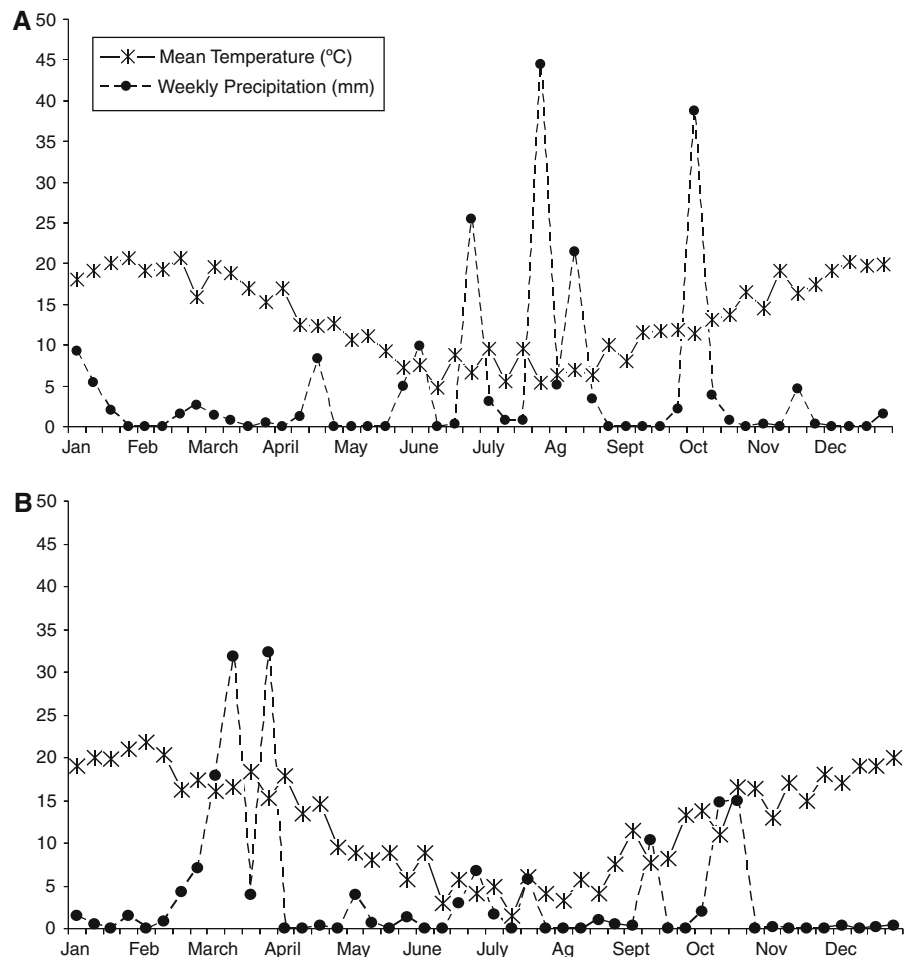
reached maturity when 50% of the plants of a plot had matured at least 25% of the fruits present at that time.

(b) Allocation pattern, seed yield and yield components For the analysis of allocation, plants were harvested at maturity (246 and 344 DAS for annuals and perennials, respectively). Ten plants were harvested including most root biomass (one plant per plot, 10 plots per replication- per species and breeding level), and biomass was separated into roots, leaves, reproductive structures and seeds.

Total seed weight per plant, individual seed weight, the number of seeds per fruit and the number of fruits per plant were determined on these plants. Individual seed weight was estimated using sub samples of 100 seeds. Harvest index was calculated as the ratio between seed yield and total biomass (including roots).

Allometric analysis

The pattern of allocation has been frequently described in terms of the fraction of the total biomass per plant allocated to each organ. This procedure has been criticized (Jasienski and Bazzaz 1999), since allocation patterns can change with plant size (Pearsall 1927), and a higher proportion of the changes in partitioning are consequence of changes in plant size (Coleman et al. 1994; McConnaughay and Coleman 1998). To separate those changes in allocation patterns due to treatment or to changes in plant size, we performed an allometric analysis. We obtained linear allometric functions for each biomass fraction and total biomass, following Coleman et al. (1994) for each species and breeding level.

F-tests were used to investigate proportionality of allocation and the influence of breeding level on these relationships (Samson and Werk 1986; Klinkhamer et al. 1990). This was done using biomass components as the dependent variable (Y), breeding level and year as fixed factors (X1 and X2), and total biomass as the covariate (Z), in a generalized linear model. The effect of breeding on the allocation coefficient for each biomass fraction (slope of the relationship) was analyzed as the interaction treatment * covariate. Non-significant interactions indicate that proportional allocation was not changed by breeding (parallel relationships).

Statistical analysis

We performed an Analysis of Variance (ANOVA) with three fixed factors (species, breeding and year), to determine differences in seed yield, total biomass, harvest index and phenological patterns. When the triple interaction was significant, we performed an ANOVA with two factors: species and breeding, for each year, separately. If in the new ANOVA species x breeding interaction was significant, we performed an ANOVA with one factor (breeding) for each species separately; to test differences between selected and wild accessions (Gomez and Gomez 1984).

Assumptions of ANOVA were tested 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 multiple linear regressions to calculate the path coefficients to establish direct and indirect relationships between the changes in the phenological and allocation patterns and the increase in seed yield in selected accession in relation to their wilds relatives. We performed a path model for both annuals and perennials to identify differences in the functional basis of the increase in yield associated with the plant's life cycle. The path analysis was performed using the program package AMOS (Arbuckle and Wothke 1999).

Results

Total biomass, harvest index, seed yield, and yield components

Differences in seed yield, harvest index, total biomass, and yield components between selected and wild accessions were in general dependent on the experiment analyzed (significant interaction for species x breeding x year, Tables 3 and 4). Because of this interaction we performed, for each experiment (year), a two-factor ANOVA (species and breeding), to test differences associated with life cycle in response to selection.

In experiment 1 (held under average weather conditions for this area), the interaction species x breeding was significant (Table 3), thus differences in seed yield, HI, total biomass, n° fruits per plant, and n°

Table 3 Analyses of variance for yield related attributes (Total biomass, Harvest Index, Seed yield, N° fruits plant⁻¹, N° seeds fruit⁻¹, and Seed weight), and phenology (Anthesis, Maturity), of selected and wild accessions of annuals (*P. gracilis* and *P. angustifolia*) and perennials (*P. mendocina* and *P. pinetorum*) species of *Physaria*

Source of Variation	Total biomass	HI	Seed yield	N° fruits plant⁻¹	N° seed fruit⁻¹	Seed weight	Anthesis	Maturity
Triple interaction (species x breeding x year)	3.33 (144)	8.76 (144)	3.43 (144)	3.07 (144)	11.04 (144)	0.34 (*) (144)	10.67(144)	36.28 (144)
Double interaction (species x breeding)								
Year 06/07	2.86 (72)	2.64 (72)	2.10 (72)	6.44 (72)	4.07 (72)	0.36 (*) (72)	2.37 (*) (72)	3.98 (72)
Year 07/08	3.09 (72)	8.17 (72)	4.46 (72)	7.18 (72)	7.43 (*) (72)	0.68 (*) (72)	39.69 (72)	1.96 (*) (72)
Main effects								
Year	72. 56 (144)	6.66 (144)	51.88 (144)	5.08 (144)	14.39 (144)	14.14 (144)	596.41 (144)	100.69 (144)
Species	2.76 (144)	11.47 (144)	4.88 (144)	3.78 (144)	91.94 (144)	95.08 (144)	34.64 (144)	85.69 (144)
Breeding	11.74 (144)	3.97 (144)	13.87 (144)	8.87 (144)	0.08 (144)	0.11 (144)	0.70 (*) (144)	3.34 (*) (144)

Data are *F*-statistics and error degrees of freedom between parentheses

Non significant effects are shown by (*)

seeds per fruit among selected and wild accessions depended of species considered. The average seed yield increased 154% for selected accessions compared to their wild progenitors. The exception was *P. pinetorum* for which, no statistical difference in seed yield among accessions was detected. The lack of seed yield difference between wild and selected *P. pinetorum* was in part due to the large variability in seed yield among the individuals included in the wild accession (Table 4). Selected accessions of both annuals and the perennial *P. mendocina* had more total biomass and higher harvest index than their wild relatives (Table 4). The increase in HI in selected accessions was due to a higher number of fruits per plant, and, only in selected accessions of *P. angustifolia* and *P. gracilis* was there an increase in the number of seeds per fruit (Table 5). In selected *P. pinetorum* the increase in total biomass was accompanied by a decrease in harvest index (due to a lower number fruits per plant; Tables 4, 5).

In the experiment 2, we found a significant interaction species x breeding for seed yield, HI, total biomass, and n° fruits per plant (Table 3). The higher seed yield potential of selected accessions of three of the species tested (both perennials and *P. gracilis*) was not evident in experiment 2 (Table 4), in which yield was much lower than in the first experiment. The decrease in seed yield, HI, and total biomass compared to that of experiment 1, was proportionally larger in selected than in wild accessions. Only selected accessions of *P. angustifolia* maintained the higher proportional allocation to seeds (HI) and so, had higher seed yield in relation to wild accession, in spite of the unfavorable environmental conditions of the experiment 2.

Allocation Patterns

No departures from linearity were found for any of the tested allometric relationships (*P* < 0.05, data not shown), revealing that allocation patterns were determined by plant size. Differences between accessions for each species in allocation were dependent on the experiment (significant interaction species x breeding x year; *P* < 0.05). Only the changes brought about selection in allocation to roots, leaves, and seeds in *P. angustifolia* and allocation to leaves and seeds in *P. pinetorum* (Fig. 2) were not different among years. In experiment 1, differences between selected and unselected accessions were mainly due to changes in

Table 4 Total biomass, harvest index and seed yield in selected and wild accessions of annual (*P. gracilis* and *P. angustifolia*) and perennial (*P. pinetorum* and *P. mendocina*) species of *Physaria*

		Experiment 1 (2006/2007)			Experiment 2 (2007/2008)		
		Total biomass (g plant^{-1})	HI (%)	Seed yield (g plant^{-1})	Total biomass (g plant^{-1})	HI (%)	Seed yield (g plant^{-1})
Selected	*P. gracilis*	27.9 ± 3.5b	34.8 ± 3.5 b	9.9 ± 1.8 b	10.1 ± 3.4 ns	34.1 ± 5.6 ns	3.6 ± 1.7 ns
	P. angustifolia	12.7 ± 2.4 ns	43.7 ± 3.4 b	5.7 ± 1.2 b	5.1 ± 0.7 ns	34.7 ± 2.3 b	3.8 ± 0.3 b
	P. pinetorum	21.5 ± 2.2 b	18.0 ± 1.1 a	3.9 ± 0.5 ns	6.2 ± 1.4 a	16.0 ± 1.2 a	0.9 ± 0.2 a
	P. mendocina	28.9 ± 2.9 b	37.1 ± 3.2 b	11.6 ± 2.1 b	3.9 ± 0.9 ns	27.1 ± 3.2 ns	0.8 ± 0.2 ns
WILD	*P. gracilis*	16.9 ± 3.2 a	24.2 ± 3.7 a	3.6 ± 1.7 a	8.2 ± 1.4 ns	33.8 ± 3.5 ns	3.7 ± 0.7 ns
	P. angustifolia	7.6 ± 2.37 ns	24.2 ± 3.8 a	1.5 ± 0.4 a	3.7 ± 0.7 ns	23.4 ± 4.9 a	1.0 ± 0.3 a
	P. pinetorum	15.9 ± 2.9 a	23.2 ± 1.2 b	2.5 ± 0.9 ns	10.3 ± 1.6 b	22.0 ± 0.2 b	2.1 ± 0.6 b
	P. mendocina	18.5 ± 5.5 a	23.3 ± 2.0 a	6.1 ± 1.3 a	3.7 ± 1.8 ns	24.1 ± 4.5 ns	1.1 ± 0.4 ns

Different letters indicate significant differences among accessions for each species and each year, since we found significant interaction species x breeding x year ($P < 0.001$), and significant interaction species x breeding ($P < 0.01$)

Table 5 Number fruits per plant, number of seeds per fruit, and seed weight (g) in selected and wild accessions of annual (*P. gracilis* and *P. angustifolia*) and perennial (*P. pinetorum* and *P. mendocina*) species of *Physaria*

		Experiment 1 (2006/2007)			Experiment 2 (2007/2008)		
		Fruits (n^o. fruits plant^{-1})	Seeds (n^o. seed fruit^{-1})	Seed weight (g)	Fruits (n^o. fruits plant^{-1})	Seeds (n^o. seed fruit^{-1})	Seed weight (g)
P. gracilis	Selected	696 ± 67 b	16.1 ± 1.2 b	0.08 ± 0.01 ns	510 ± 79 ns	12.8 ± 0.9 ns	0.04 ± 0.01 ns
	Wild	515 ± 28 a	12.2 ± 0.6 a	0.08 ± 0.01 ns	503 ± 92 ns	14.7 ± 0.9 ns	0.04 ± 0.01 ns
P. angustifolia	Selected	316 ± 52 b	4.7 ± 0.3 b	0.08 ± 0.01 ns	207 ± 39 ns	2.9 ± 0.09 ns	0.08 ± 0.01 ns
	Wild	128 ± 30 a	3.5 ± 0.2 a	0.08 ± 0.01 ns	266 ± 56 ns	2.8 ± 0.3 ns	0.08 ± 0.01 ns
P. pinetorum	Selected	272 ± 44 a	6.9 ± 0.3 ns	0.08 ± 0.01 ns	359 ± 58 ns	4.3 ± 0.3 ns	0.08 ± 0.01 ns
	Wild	477 ± 71 b	6.9 ± 0.3 ns	0.08 ± 0.01 ns	448 ± 79 ns	4.7 ± 0.7 ns	0.10 ± 0.02 ns
P. mendocina	Selected	436 ± 22 b	6.7 ± 0.3 ns	0.36 ± 0.08 ns	99 ± 26 ns	4.5 ± 0.4 ns	0.2 ± 0.02 ns
	Wild	290 ± 29 a	6.7 ± 0.6 ns	0.28 ± 0.04 ns	95 ± 27 ns	4.9 ± 0.8 ns	0.2 ± 0.02 ns

Different letters indicate significant differences between accessions for each species and each experiment ($P < 0.05$)

allocation to roots and seeds. We found a higher coefficient of allocation to seeds in selected accessions of both annuals and of *P. mendocina* (Fig. 2 a, b and d), while in *P. pinetorum* allocation to seeds did not differ between selected and wild accessions (Fig. 2c).

In both annuals this higher allocation to seed was accompanied by a lower allocation to roots (Fig. 2a, b), and in *P. gracilis* by a reduction in allocation to reproductive support structures (Fig. 2a). Allocation to leaves decreased only in selected accessions of annuals in relation to their wild relatives (Fig. 2b). In perennials we did not find changes in the general allocation pattern as consequence of the higher seed yield (Fig. 2c, d).

In experiment 2, the differences between selected and wild accessions were opposite to those found in experiment 1, particularly due to differences in allocation to roots and seeds between years. In three species (*P. gracilis* and both perennials) selected accessions were found to have a higher allocation to roots in relation to that of experiment 1, (at the expense of a lower seed allocation). As a result of these changes in allocation between experiments, we did not find differences (in *P. gracilis*) or we found a lower seed yield in selected (*P. mendocina*) in relation to wild accessions, in experiment 2 (Fig. 2a, d). For both accessions of *P. angustifolia* we found a decrease in root allocation in experiment 2 in relation with
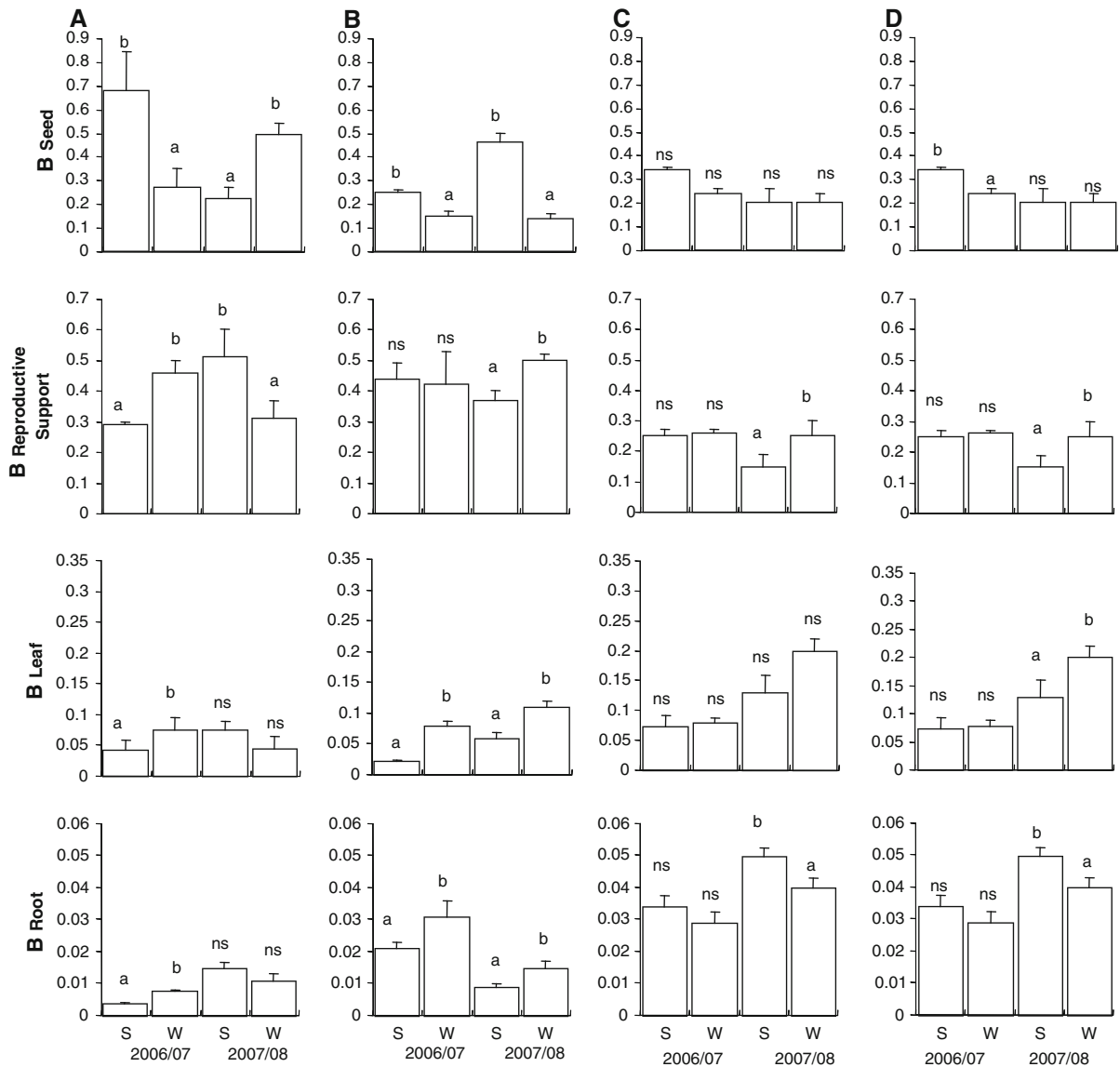
Fig. 2 Allometric coefficients (*B*) for the allocation to different structures (seed-B seed, reproductive support structures-B reproductive support, leaf-B leaf and root-B root) in annual: **a** *P. gracilis*; **b** *P. angustifolia*, and perennial: **c** *P. pinetorum* and **d** *P. mendocina*, species of *Physaria*. Different letters indicate significant differences between selected (*S*) and wild (*W*) accessions of each species and each experiment (experiment 1, 2006/07 and experiment 2, 2007/08)

experiment 1, which was accompanied by an increase in seed allocation only in selected accessions (Fig. 2b).

Phenology

The date of anthesis was earlier in selected accession of three species (both annuals, $P < 0.05$; and in *P. mendocina,* Table 6) than in their wild relatives. The date of maturity did not change in selected

accessions of *P. angustifolia, P. mendocina* (both experiments), and *P. gracilis* (only in experiment 2) in relation to wild accessions. Only in experiment 1, selected accession of *P. gracilis* had an earlier date of maturity (Table 6) than wild accessions.

In *P. pinetorum*, we found a significant interaction Breeding * year in date of anthesis ($P < 0.05$) and date of maturity ($P < 0.05$). In experiment 1, selected accessions had a later date of anthesis and maturity, in

relation to wild accessions, while we did not find differences between accessions in experiment 2.

Relationships between phenological and allocation patterns and the increase in seed yield brought about by selection

The functional bases of the increase in seed yield were different for annuals and perennials. In annuals the increase in seed yield brought about by selection was determined by a change in the allocation pattern (positive relationships between HI and seed yield, Fig. 3a) and a change in phenology of selected accession (positive and negative relationships between the length of the vegetative period, and root and seed allocation, respectively; Fig. 3a). In perennials the change in phenology resulted in a change in total biomass of selected accessions (positive relationships between vegetative period length and total biomass; Fig. 3b) and not in the allocation pattern (the relationships were not significant, Fig. 3) or HI. Early anthesis of selected accession had two opposite effects on total biomass accumulation. On one side, the shorter vegetative period length resulted in a lower total biomass in selected accessions (Fig. 3b). However, the vegetative period length had also an indirect effect on total biomass, through a trade off with the length of the reproductive period (Fig. 3b). This indirect effect on total biomass was more important

than the direct effect (higher path coefficient, −0.47 versus 0.28, respectively).

Discussion

Due to meteorological differences we expected that predicted changes in phenological and allocation patterns brought about by selection would be different for the two experiments. In particular, the conditions found during experiment 2 should result in larger reductions in total biomass production and seed yield in relation to experiment 1 in selected accessions than in wild accessions (i.e. lower yield stability). This lower stability in selected accessions should be consequence of reduced allocation to vegetative organs in relation to wild accession.

Functional basis of the seed yield increase in selected accessions

Because of differences in the hierarchy of biomass allocation between annuals and perennials (Schultz 1982), we proposed that the life cycle of a plant should determine the way in which patterns of allocation change with selection for seed yield. In annuals, we expected the increase in yield would be brought about by an increase in harvest index, at the expense of a lower assignment to vegetative structures, and also
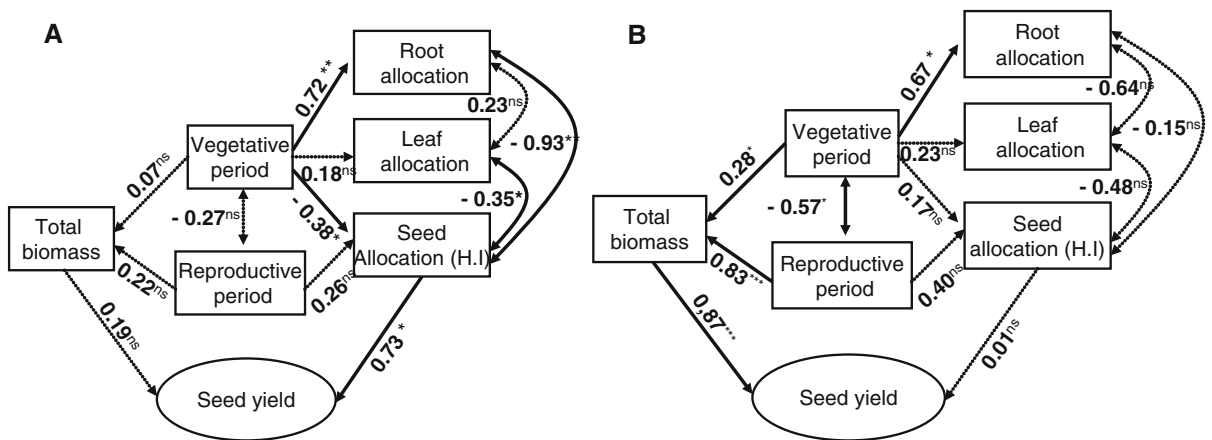

Fig. 3 Path diagrams showing the effects of phenology (length of the vegetative and reproductive periods–days), allocation pattern (root, leaf and seed allocation, % of total biomass) and total biomass, on seed yield for **a** annuals (*P. gracilis* and *P. angustifolia*) and **b** perennials (*P. pinetorum* and *P. mendocina*) *Physaria* species. Numbers indicate the direct B path coefficients between attributes. *Asterisks* indicate significance of the coefficients: ***$P < 0.001$;**$P < 0.01$ and *$P < 0.05$

Table 6 Phenological patterns (days to anthesis and to maturity) in selected and wild accessions of annual (*P. gracilis* and *P. angustifolia*) and perennial (*P. pinetorum* and *P. mendocina*) species of *Physaria*

	Phenology (DAS*)	Experiment 1– 2006/2007		Experiment 2– 2007/2008	
		Selected	Wild	Selected	Wild
P. gracilis	Anthesis	227 ± 2 a	233 ± 2 b	247 ± 1 a	247 ± 2 b
	Maturity	308 ± 1 a	314 ± 2 b	314 ± 2 ns	316 ± 2 ns
P. angustifolia	Anthesis	227 ± 1 a	232 ± 1 b	243 ± 1 a	251 ± 1 b
	Maturity	302 ± 1 a	321 ± 1 b	318 ± 2 ns	317 ± 3 ns
P. pinetorum	Anthesis	247 ± 1 a	252 ± 1 b	254 ± 1 ns	257 ± 1 ns
	Maturity	344 ± 6 ns	331 ± 6 ns	325 ± 1 ns	322 ± 1 ns
P. mendocina	Anthesis	249 ± 1 a	255 ± 2 b	246 ± 1 a	255 ± 1 b
	Maturity	362 ± 8 ns	344 ± 4 ns	327 ± 2 ns	323 ± 2 ns

DAS days after sowing

(*) Duration of phenological phases is expressed as days after sowing (DAS)

Different letters indicate significant differences among accessions for each species and each experiment ($P < 0.05$)

early flowering and shorter growth cycle. In perennials the increase in seed yield would be associated with a longer growth cycle and an increase in total biomass, but not with changes in allocation.

As expected, the functional basis behind the increase in seed yield were related to the plant's life cycle (annual or perennial; Fig 3a, b). Similarly to what has been reported for other crops (e.g. maize, Rusell 1991; wheat, Evans 1993; sunflower, López-Pereira et al. 1999), selected accessions of all four *Physaria* species in our experiments had an early anthesis than their wild relatives, which resulted in a shorter vegetative period (Table 6). This change in phenological development brought about by selection had different consequences on allocation patterns, total biomass and seed yield in selected annuals or perennials.

In annuals, the shorter vegetative period resulted in a lower proportional allocation to roots (positive relationships between vegetative period and root allocation; Fig. 3a) and leaves (indirect path coefficient among vegetative period and leaf allocation through seed allocation; Fig. 3a) than in their wild relatives. This type of change in assignment to vegetative structures has been linked to a trade off between vegetative and reproductive allocation (Stearns 1992). The lower allocation to vegetative structures (Fig. 2a, b) was also linked to an increase in harvest index (Table 4, Fig. 3a) and to a higher number of fruits per plant and number of seeds per fruit (Table 5). In other Brassicaceae (Degenhart and

Kondra 1984; Ali et al. 2003) harvest index was also the main determinant of the increase in seed yield with selection. The number of fruits per plant has been shown to be the main determinant of seed yield in *Physaria* (Masnatta and Ravetta 2011), although in other Brassicaceae this character has a low heritability and, thus should be highly influenced by environmental conditions (Diepembrock 2000).

For both selected perennials, the main determinant of the increase in seed yield was total biomass, and not changes in harvest index or in vegetative allocation (Fig. 3b). The early anthesis of selected accessions resulted in a longer reproductive period, more total biomass accumulation, and higher seed yield than in their wild relatives (Table 4). In perennial *Physaria* dry matter production prior to anthesis would not represent an important fraction of the total biomass at maturity, contrary to what is common in annual crops (López-Castañeda et al. 1995). Biomass accumulation post-anthesis appears to be significant, reflecting that vegetative and reproductive growth occur simultaneously in these species. Vegetative growth by branching during the reproductive period contributes to the increase in the reproductive output in several forbs (Suzuki and Ohnishi 2006). The importance of the duration of the reproductive period for yield determination has already been reported for other forbs of the genus *Oenothera* (Vilela et al. 2008) and for another Brassicaceae (Diepembrock 2000).

Annuals and perennials differ in plasticity (Pujol et al. 2008) and intrinsic hierarchy of allocation

(Barbour et al. 1980), which could be the cause of the differential response to selection associated to the life cycle. It is expected that annuals should have higher vegetative allocation plasticity in response to resource variability, while maintaining their reproductive allocation fixed to its physiological maximum for the environment (Stearns 1992). Slow-growing perennials, may be able to change their reproductive allocation in response to environmental factors, such as the quantity of available resources (Hautekeete et al. 2001), but maintaining vegetative allocation as a hierarchical sink (Chapin et al. 1993). Thus, annuals and perennials should not respond in a similar way when selected in an environment with higher resource availability (resource rich selection environment) compared to their wild environment of origin. It is expected that selected annuals have a large decrease in vegetative allocation in relation to their wild relatives, while selected perennials should maintain the vegetative resource allocation pattern similar to their wild predecessors (i.e. lower plasticity). Our results for *Physaria* are in accord with these predictions.

The changes found in vegetative allocation patterns (leaves and roots) that we found after selection in annual *Physaria* could have different consequences on the plants ability to acquire resources. On one hand, reductions in allocation to leaves may be incompatible with the need to increase the photosynthetic capacity (i.e. higher leaf area surface and radiation use efficiency) and the supply of assimilates. However, the reduction in allocation to leaves in annual *Physaria* has been shown to be compensated by concomitant changes in leaf structure (higher specific leaf area in selected accessions, González-Paleo and Ravetta 2011), so as to maintain the plant's leaf area ratio. High plasticity in specific leaf area has been reported for other species as well (Foulkes et al. 2007).

The low allocation to root biomass is one of the main changes found in our improved germplasm of *Physaria* as well as in several grain crops (Loss and Siddique 1994; Waines and Ehdaie 2007). In agricultural systems in which production is subsidized with water and nutrients the development of a deep root system to efficiently acquire soil resources is not relevant (Shou-Chen et al. 2008). However, some caution is required when a reduction in root biomass takes place in crops developed for low resource production systems. Under these conditions, fewer roots could result in a limited ability to access water

and/or nitrogen, and potentially reduce drought tolerance of selected accession (Richards and Lukacs 2002). Several reports have shown that there is likely only a limited capacity to reduce partition to roots and reallocation of this carbon to seed production, because of the trade off with water and nitrogen capture required for future biomass gains (Foulkes et al. 2009). In particular, in annual *Physaria* there is a need to evaluate other relevant morphological traits for soil resource-acquisition (e.g. specific root length, depth, diameter, etc.), that will allow a better understanding of these changes brought about by selection.

The set of traits that provides the capacity of perenniation of a species is not well defined (DeHaan et al. 2007). However, there are many traits that differentiate perennials from annuals. Particularly important are characteristics such as better access to resources, longer reproductive period, and a conservative strategy in the use of nutrients, found in the former. These desirable characteristics found in wild relatives must be retained during the selection process for increased seed yield, if the breeding objective is the increase in allocation to seed without alteration of the resource allocation strategy typical of the perennial habit (DeHaan et al. 2005). Our results show that an increase in biomass accumulation is an appropriate strategy to improve yield without loosing the partition strategy of perennials (Fig. 2b), as suggested by other authors (Piper and Kulakow 1995; Pimm 1997).

Although in our experiments the partition of vegetative biomass was not changed in perennials, low root carbohydrate reserves have been reported in these selected accessions (González-Paleo and Ravetta 2011). It is possible that the missing carbohydrates were used as an alternative carbon source to support the higher yield in selected accessions, although its depletion could have a potential negative impact on plant longevity and survival. Carbohydrate storage provides a carbon buffer when physiological demands are not synchronized with photosynthesis (Kobe et al. 2010), and is used for the construction of leaves and fine roots at the start of new growing seasons (perenniation; Kozlowski 1992).

Although for most grain crops, seed yield is the main breeding objective, traits that determine drought-tolerance, such as vegetative allocation and phenological patterns, are also key characters in the domestication and breeding of new crops for arid and semi-arid lands. Because drought-tolerance is

measured by many characters (among which negative correlations with seed yield often exist), germplasm evaluation and selection must be based on multiple traits. The characterization of the changes on these characters, brought about by selection, provides the basis for the understanding of the functional mechanisms behind the increase in seed yield. This knowledge can contribute to choose adequate selection criteria and to design future breeding programs for new crops for arid lands.

Seed yield stability

It has been suggested that while breeding programs have clearly increased yield potential, they also might have increased the responsiveness of most modern cultivars to better environments (i.e. decrease seed yield stability; Cox et al. 1988; Perry and D'antuono 1989; Slafer and Kernich 1996). Despite their noticeably reduced yield stability, the yields of some modern cultivars can still be higher (or at least not lower) than that of older cultivars when grown in low resource environments (Calderini and Slafer 1999; Tollenaar and Lee 2002; Sinebo 2005).

Our results agree with the idea of the existence of trade off mechanisms between traits that allow for high yield potential, which are expressed under favourable environmental conditions, and those that provide a lower sensitivity to low quality environmental conditions (i.e. drought stress). Selected accessions of *P. gracilis* and of both perennial species had higher yield potential but lower interannual seed yield stability relative to that of their wild relatives (Table 4). This relationship was also supported by our results with selected accessions of *P. angustifolia,* whose yield was stable between years, although low.

Conclusion

In *Physaria,* life cycle determined the way in which patterns of allocation and phenology change with selection based only on seed yield. In annuals, seed yield increased as the result of an increase in harvest index at the expense of a lower allocation to leaves and roots. This change in allocation was a consequence of early anthesis of selected accessions. The lower root and leaf allocation of selected annuals could compromise their capacity for resource acquisition in low

resource environments. In perennials, the increase in seed yield resulted from an increase in total biomass, without changes in vegetative allocation. In spite of the higher harvest index of selected perennials, this component was not directly responsible for the improvement in seed yield. The increase in total biomass was determined by an extension of the length of the reproductive period of selected accessions. In conclusion, our results show that while annuals selected for seed yield could compromise the capacity for acquisition of soil resources, selection on perennials did not modify the resource allocation strategy responsible for their positive adjustment to environments with low resource availability.

Independently of the life cycle, the superior performance of bred accessions (i.e. higher yield potential of *P. gracilis* and both perennials) was expressed only under non-limiting resource conditions. We propose that this pattern could be consequence of a trade off between yield potential and yield stability. However the functional mechanism of this trade off in *Physaria* should be elucidated.

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