Relationships between reproductive output, morpho-physiological traits and life span in Lesquerella (Brassicaceae)

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**ABSTRACT**

The development of perennial industrial crops could contribute to increase agriculture sustainability and yield stability in arid environments. Since perennial plants allocate resources preferentially to perpetuation and to structural and functional characters that provide drought tolerance, they tend to have lower reproductive output (yield) than their congeneric annuals. Four species of Lesquerella native to arid regions were evaluated to understand the relationships between reproduction, drought tolerance, and their association with the plant’s life span. We assessed the following set of characters (defined as plant strategies): phenology, gas exchange, specific leaf area, leaf area ratio, total biomass and biomass allocation. Annual (Lesquerella gracilis and Lesquerella angustifolia), and perennial (Lesquerella mendocina and Lesquerella pinetorum) species were compared under water limiting conditions. Within this set of species differences in structural and functional characters were observed. The annual, L. gracilis showed a plant strategy characterized by high reproductive output, harvest index (HI) and specific leaf area (SLA). L. mendocina (perennial), produced more total biomass, but had a low reproductive output, and also showed characters that provide drought tolerance (high allocation to roots-root mass ratio (RMR), and storage-total non structural carbohydrates (TNC) and low SLA). The annual L. angustifolia and the perennial L. pinetorum had intermediate plant strategies (i.e. intermediate values of traits typically related with annual or perennial life span). In the case of L. pinetorum we found some traits common to L. mendocina: long vegetative period, high biomass, CO₂ assimilation rate (A), and water use efficiency (WUE), and low SLA. The high reproductive output in L. pinetorum was a result of both, higher total biomass and longer growing season compared to the annuals, and of higher HI in relation to the other perennial. These differences in plant strategies among perennial Lesquerella show that there are different combinations of traits that could be used as criteria for the selection of a perennial crop in programs of domestication for Patagonia and others arid lands.

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1. Introduction

The need to diversify cropping systems, has lead to an interest in the development of new crops that can perform well in resource-limited environments, which are marginal for the cultivation of more traditional crops (Cox et al., 2002). Between the many wild species included in germplasm evaluation programs, Lesquerella (S. Watson, Brassicaceae) has been proposed as a potential new oil-seed crop (Thompson, 1985) because it contains hydroxy fatty acid in its seed oil (Dierig et al., 1993). This genus is of particular interest to understand the process of domestication because it has approximately 100 wild species native to arid and semiarid regions of America (Rollins and Shaw, 1973) differing in life span (annual, biannual, or perennial), which is ideal for studying structural and functional relationships between growth and traits associated with reproduction and drought tolerance in drought-prone environments (Hautekeete et al., 2001).

As of today, only one North-American species, Lesquerella fendleri A. Gray S. Watson (Thompson and Dierig, 1994; Dierig et al., 2004), and one South-American species, Lesquerella mendocina (Phil.) Kurtz, (Ploshchuk et al., 2005; Windauer et al., 2006; González-Paleo and Ravetta, 2011) have been extensively evaluated for agronomic potential, and show an incipient degree of domestication. L. mendocina has been the species of choice for irrigated valleys in Patagonia (Argentina) because this perennial species exhibits several morphological, physiological, and phenological traits that could contribute to increase ecological and economical sustainability (Ploshchuk et al., 2001; Paris, 2003; Cox et al., 2006).

Perennials crops offer an alternative to annuals, to alleviate some of the environmental problems frequently associated to

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agricultural practices required to grow annual crops on fragile ecosystems, such as greater nutrient and organic matter losses due to inefficiencies in internal nutrient cycling and soil erosion (Moffat, 1996; Glover, 2003). Also, perennials may increase yield stability compared to that of annual crops (Ploschuk et al., 2001). Still, a major drawback for the development of perennial crops that must be overcome is the trade-off between perenniality and reproductive structures, which results in low allocation to reproduction (Moffat, 1996), although the ecological literature suggests that this trade-off is not fixed (DeHaan et al., 2005).

Plant species can be characterized by a "plant strategy" (Craine, 2009), defined as a set of phenological, morphological, and physiological traits that lead to successful growth and reproduction in a particular environment. Among this suite of traits, photosynthates allocation, leaf morphology and gas exchange are the basis that determine the trade-off between high productivity when resources are abundant and the capacity to tolerate water and nutrient shortage (Chapin, 1980; Poorter et al., 2009). For example, species with high SLA have leaves that are thin, non-lignified, with high water and nitrogen content, and as result, high CO₂ assimilation rate and low resistance to abiotic factors such as drought, frost and wind (Poorter et al., 2009).

Plant species seem to lie on a continuum between two extremes of resource allocation: annual (short-lived plants which mature rapidly and devote a large fraction of their stored energy to producing flowers, fruits, and seeds) and perennial (long-lived plants which have a prolonged vegetative stage and devote a small fraction of their photosynthates to reproduction; Barbour et al., 1980). For example, a plant strategy that can be found in perennials includes lower relative growth rate than annuals, as a result, of: (1) their allocation pattern: high allocation to roots and storage (Chapin, 1980), and a small fraction of photosynthates to reproduction (Barbour et al., 1980) and (2) their leaf morphology: low SLA that determines high WUE and low CO₂ assimilation rate (Canham et al., 1999).

High root and storage allocation (Chapin et al., 1993), low SLA (Coley et al., 1985; Poorter et al., 2009), and high WUE (Larcher, 1995) have been associated with drought tolerance. Also, a low dependence on external resource supply (Poorter, 1989), key to carbon and nutrient conservation, is a typical characteristic of perennial species which may tolerate environmental extremes and defend against herbivores and pathogens to persist and perpetuate (Bell et al., 2008). Because of this background we propose that perennial *Lesquerella* should perform better under drought conditions, than their annual counterparts.

Ecophysiologicial traits show a continuous variability among and within species (e.g. phenological traits, Verboom et al., 2004; leaf attributes, Vilela et al., 2008; and allocation to storage, Sasnová and Klimesová, 2009). This variability in individual traits creates the possibility for “intermediate plant strategies”: annual or perennial species that show plant strategies (combinations of morpho-physiological traits) different to those expected based on their life span.

In this study, structural and functional attributes known to provide either drought tolerance or high reproductive output (RO) were compared between annual and perennial congeneric species. Combinations of traits specific to each species and potentially different among species with a similar life span are expected. Within the context of the development of perennial crops for arid lands, species with intermediate plant strategies might combine ecophysiological traits associated to drought tolerance, high reproductive output and a perennial life span. The understanding of these relationships could then generate new breeding objectives for perennial oilseeds.

2. Materials and methods

2.1. Study site and experimental conditions

A one-factor (species) field experiment with four levels in a randomized completely block design, was conducted from February 23, 2004 to March 5, 2005 in Trelew, Patagonia, Argentina (−43°14′S, −65°18′).

Four species of *Lesquerella* (Brassicaceae), two perennials: *L. mendocina* (Phil.) Kurtz (La Pampa, Argentina) and *L. pinetorum* Wooton & Standley (Arizona), and two annuals: *L. angustifolia* Wats (Oklahoma) and *L. gracilis* Wats (Oklahoma) were evaluated. For each species bulked seeds harvested from four high-yielding individual plants were used. These plants belonged to an elite population formed after two cycles of selection for seed yield from an original population established with plants from wild, unselected germplasm. Selection was done in the same target environment, in introductory gardens in Patagonia.

For this experiment, seed was sown in germination trays filled with soil: peats moss (1:1) and maintained in a greenhouse until transplanting to the field, 67 days after sowing (May 1, 2004). Blocks consisted of plots (3.6 m x 1.5 m x 0.4 m deep; 5 replicates) isolated from the ground below with polyethylene lining with small holes to allow for drainage of excess water. The soil which filled the plots was the local clay-loam soil. Each of the five blocks had one experimental unit per species of 1.5 m x 0.9 m that included 30 plants (for a total of 150 plants per species). Plant density was 23 plants m⁻², with a spacing of 0.15 m between plants and 0.3 m between rows. Density was low enough to avoid detrimental effects of competition on final biomass, on the probability of flowering and on seed-yield (Brahim et al., 1998). Etielen bis ditiocarbamato-zinc (zineb, 2 g L⁻¹) and 2-metoxcarbamol-bencimidazol (carbendazim, 0.5 cm³ L⁻¹) were applied monthly to prevent fungal damage. Weeds were removed manually.

During the course of the experiment mean temperature was 12.1 °C and total precipitation was 156.6 mm. To allow the phenotypic expression of drought tolerance related traits, plots were irrigated until soil saturation, and allowed to dry up for about 20 days. Water stress cycles were imposed during the entire length of the reproductive phase (25 September to March) since this phenophase is the critical period for yield determination in at least another species of this genus (*L. fendleri*, Puppala et al., 2004) and is coincident with the dry season in this area. During these 20-day cycles, net CO₂ uptake was reduced to below 50% of that found the day after irrigation (data not shown, González-Paleo, 2010).

2.2. Sampling and analysis

2.2.1. Phenology

The number of days from sowing to anthesis and to maturity was recorded on all plants in each of the five plots (30 plants per plot). The criteria used to decide the moment in which a species had reached anthesis and maturity was when 50% of the plants of a plot had at least one flower or one mature fruit, respectively.

2.2.2. Gas exchange

CO₂ assimilation rate (A) and transpiration rate (E) were measured when plants reached anthesis, on individual leaves of six plants (sub samples) per plot using an infrared (IR) gas analyzer (LCA-4, ADC, Hoddesdon, England). Measurements were made on leaves fully exposed to direct sunlight, and maintained at right angles to the incident solar radiation. Measurements were taken between 10:00 A.M and 2:00 P.M. Values for water use efficiency (WUE) were derived by dividing A by E.
2.2.3. Specific leaf area and leaf area ratio (LAR)

One fully expanded leaf per plant (30 plant sub samples for each of the five plots) was clipped when plants set the first fruit, and stored in plastic bags during transport to the lab. Leaf area was determined using UTHSCSA Image Tool for Windows, Version 2.0. After scanning, leaves were oven-dried until constant weight. Specific leaf area was calculated as the ratio leaf area: leaf weight. Leaf area ratio was calculated as: LAR = SLA × LMR, where leaf mass ratio (LMR) is the fraction of the total plant biomass allocated to leaves (Lambers and Poorter, 1992). Root mass ratio was calculated as the fraction of total biomass allocated to roots.

2.2.4. Total biomass and allocation

Six plants (sub samples) per each of the five plots were harvested after physiological maturity (i.e. when fruits were dehiscent at touch and contained reddish in color seeds). Plant biomass was divided into vegetative shoots, roots, seeds, fruits, and support structures (structures of the reproductive organs other than seeds and fruits), dried at 70 °C for 48 h, and weighed. Reproductive output (g seed plant⁻¹), and yield components (individual seed weights and number of seeds) were measured (six plants – sub samples – for each of the five plots) after seeds reached physiological maturity. Individual seed weight was estimated using a sample of 100 seeds. Harvest index was calculated as the ratio among seed biomass and total biomass, and as the slope of the relationships among seed biomass and total biomass (coefficient of allocation to seed).

Carbohydrate storage at maturity 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 stabilized sugars were then analyzed colorimetrically (620 nm) following the Anthrone method (Yemm and Willis, 1954).

2.3. Data analysis

Data was analyzed using an analysis of variance for a randomized complete block design and Tukey’s multiple comparison test for post hoc comparisons between species. In order to achieve normal distributions and homogeneity of variance before performing parametric analysis, log transformation was applied to SLA, LMR, and LAR. Harvest index and root TNC (%) was analyzed using Kruskal–Wallis non-parametric test. Data was obtained as pooled values for all sub samples for each experimental unit, yielding a total of five replicates (plots) per species. Allometric analysis (Coleman et al., 1994) was used to correct allocation patterns for size (total biomass) differences among plants. Biomass allometric relationships between each biomass fraction and total plant weight were assessed through linear regression models. Partition coefficients were assessed as the slope of the linear regressions. Slopes were compared using one-way ANOVA between species means.

For the multivariate analysis, the data matrix was standardized before cluster analysis. From the standardized data matrix, a squared Euclidean matrix was computed for species and traits. Hierarchical agglomerative clustering with incremental sums of squares (Ward, 1963) as the fusion criterion was applied to matrices, i.e. in any part of the dendrogram, members or groups were joined to minimize the new within-group sums of squares. Dendrograms were constructed to examine similarities in patterns among species (in relation to traits). We estimated the principal components (PCs) of the two–way standardized matrix of 20 individual plants of each species of Lesquerella, and 14 traits: days to anthesis, A, E, WUER, LAR, SLA, LMR, RMR, root TNC, total biomass, HI, RO, seed weight (Seed Wt), and seed number (N Seeds). From this analysis, a bi-plot of the first two PCs of species and traits was con-
structured, and the species groups derived from cluster analysis were superimposed.

3. Results

3.1. Phenology and morpho-physiological traits

The two annual species reached all reproductive stages (anthesis and maturity) earlier than the perennials, and had shorter phases of both, vegetative \( (p < 0.01) \) and reproductive growth \( (p < 0.01) \). CO\(_2\) assimilation rates were significantly greater \( (p < 0.01) \) for the perennial species \( (L.\ pinetorum\ and\ L.\ mendocina) \) than for the annuals \( (L.\ gracilis\ and\ L.\ angustifolia) \). Transpiration rate did not differ among species \( (p > 0.05) \) and this resulted in lower WUE for annuals than for perennial \( (p < 0.01) \). Between perennial species, WUE was greater in \( L.\ pinetorum \) than in \( L.\ mendocina \) (Table 1).

The perennials and \( L.\ gracilis \) had higher LMR \( (p < 0.01) \) which resulted in higher LAR \( (p < 0.01) \). Specific leaf area was higher in annuals than in perennials (Table 1).
3.2. Total biomass and allocation

At maturity, both perennial species had accumulated more biomass than the annuals (p < 0.01, Table 1). Allocation to all fractions of biomass increased linearly with plant size (Fig. 1a–e). The allometric relationships showed a greater allocation to roots in perennials than in annuals, although L. mendocina had a higher partition coefficient to roots (assessed as the slope of the relationship, p < 0.01, Fig. 1a) than L. pinetorum. A similar pattern was observed for shoots, while there was no difference among species within the same life span (p > 0.05, Fig. 1b). No significant differences between the perennial species and L. gracilis were observed for the partition to reproductive support structures (Fig. 1c). The higher partition to vegetative fractions observed in the perennial species compared to the annuals was at the expense of a lower coefficient of partition to fruits and seeds (p < 0.01 and p < 0.05, respectively, Fig. 1d and e). The pattern of allocation to storage at maturity differed between annuals and perennials, as annuals had lower root TNC than perennials (p < 0.01, Table 1). Among perennials, L. mendocina had higher root TNC than L. pinetorum.

Reproductive output was higher for the perennial L. pinetorum and the annual L. gracilis (p < 0.01, Table 1), than for the other species.

3.3. Multivariate analysis

The first and second principal components (PC) accounted for 59% and 22% of the total variation, respectively. The species grouping derived from the cluster analysis are indicated by the circles in Fig. 2.

The PC1 axis appears to be associated with the expression of traits assembling species with similar life span (annual or perennial). Both perennial species had higher total biomass and allocated resources preferentially to growth (high LAR and LMR), storage and perpetuation (high allocation to root and to carbohydrate reserves). Oppositely, the annuals had high harvest index and specific leaf area. L. mendocina and L. pinetorum also showed high CO2 assimilation rate and WUE. The determinant traits of the species’ separation were confirmed by the strong correlation found between the first PC and total biomass (r² = −0.94), LAR (r² = −0.90), LMR (r² = −0.99), RMR (r² = −0.67), root TNC (r² = −0.98), A (r² = −0.89), WUE (r² = −0.89), SLA (r² = 0.71) and HI (r² = 0.99).

Principal component 2 grouped species with different life cycle: L. angustifolia and L. mendocina at the bottom, in association with lower reproductive output and higher seed-weight (r² = −0.75) and RMR (r² = −0.63). L. pinetorum and L. gracilis at the top of the diagram showed higher reproductive output and high WUE (r² = 0.99), SLA (r² = 0.67) and seed number (r² = 0.99).

4. Discussion

Morpho-physiological traits can be grouped to generate sets of traits that define plant strategies (Craine, 2009). Perennial plants tend to carry traits that have also been shown to provide drought tolerance (i.e. low biomass and seed production, low SLA, and high root and storage allocation). These traits make perenniality attractive in the development of new crops for low resource, erosion prone environments, as it could combine drought tolerance, and reduced soil tillage. Still, these traits have frequently been associated with low seed yield (Blum, 2005).

Our data shows that variation in morpho-physiological traits and reproductive output was not linked to life span. Four plant strategies defined by specific combinations of traits that regulate drought tolerance or productivity were observed. At one extreme, the annual L. gracilis was characterized by a set of traits that result in high productivity and low drought tolerance: short-lived plants that reached both anthesis and maturity rapidly, and had a high reproductive output brought about by high HI and leaf area allocation (SLA and LAR; Roumet et al., 2005; Verboom et al., 2004). At the other extreme, the perennial L. mendocina showed traits such as prolonged vegetative period and low allocation to reproduction.
The main barrier for the incorporation of perennial oilseed crops to cultivation is the expected low reproductive output and the set of morpho-physiological traits that characterize the plant strategy commonly associated with the perennial life span. However, these morpho-physiological traits showed in *Lesquerella* a continuous variation along a gradient, creating the possibility for intermediate plant strategies that combine structural and functional traits different to those expected based on their life span. This variation in morpho-physiological traits was not related to life span, and was species specific. Four plant strategies (defined by specific combinations of traits) were observed, which can be related with either high or low reproductive output and with annual or perennial life span. The plant strategy of the perennial *L. mendocina* was determined by characters that provide high drought tolerance and low reproductive output, such as high root mass and storage allocation, and also low SLA and HI, high LAR, CO₂ assimilation rate and total biomass. The annual *L. gracilis* was characterized by a high HI, high reproductive output and SLA, and fast development (early flowering time and maturity). *L. angustifolia* and *L. pinetorum* formed a group that was intermediate between *L. mendocina* and *L. gracilis. L. pinetorum* showed traits similar to the other perennial species (low SLA, and high LAR, A, and total biomass) but also traits with intermediate values among *L. gracilis* and *L. mendocina* (RMR, root TNC, HI), which determined a high reproductive output in this perennial. In the context of the development of a perennial crop of *Lesquerella* for arid lands, *L. pinetorum* could combine traits that provide drought tolerance, but also high reproductive output and perenniality.

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