

Morphological traits and allocation patterns related to stress-tolerance and seed-yield in wild and domesticated evening primrose (*Oenothera* L. Onagraceae)

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ABSTRACT

Wild evening primrose species (*Oenothera* spp.) native to Argentina, have been suggested as a new crop for irrigated valleys of semi-arid Patagonia. This paper describes patterns of biomass allocation, morphological traits related to stress-tolerance and seed-yield in four species of *Oenothera* grown in a common garden at three plant densities. Wild and domesticated species are compared. The effect of resource availability on those traits during three phenological stages (vegetative, reproductive and maturity) is described. Native species were characterized by traits related to stress-tolerance (high root allocation and low specific leaf area) during the vegetative stage. This suite of traits resulted in low biomass accumulation and low seed-yield. The domesticated *O. biennis* was characterized by a combination of traits related to stress-tolerance (low specific leaf area) and high productivity (high leaf allocation and leaf area ratio and low root allocation). Domesticated species accumulated more biomass than natives. Total biomass and total non-structural carbohydrates present in roots were positively correlated to seed-yield.

Oenothera biennis showed the highest seed-yield, although this species showed yield instability in response to changes in the environmental quality. No changes in seed-yield in response to plant density were recorded for either *O. lamarckiana* or native species. *Oenothera biennis* showed an optimum density of 20 plants m⁻² and yielded 260 g m⁻², a seed-yield similar to that reported in other countries. Low seed-yield of native species is major drawback that must be overcome. Improving seed-yield in these species could be possible by selection oriented to increase total biomass. Since no detrimental effect of density was found in *O. lamarckiana* and natives, a higher plant density might increase yield production per unit area.

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1. Introduction

Oenothera L. (evening primrose) is a relatively new, high-value oilseed crop for temperate regions. Seeds are characterized by an oil content of 20–30%, with 7–10% of γ -linolenic acid (Court et al., 1993; Christie, 1999), an essential fatty acid with proven applications as a nutritional and a medicinal supplement (Simpson, 1994; Murphy et al., 2004). The cultivation of evening primrose has been established in Northern and Eastern Europe, North America and Australasia (Simpson and Fieldsend, 1993). *Oenothera* species native to Argentina have been suggested as a new seed-oil crop for irrigated valleys of semi-arid Patagonia (Ravetta and Soriano, 1998). The need for diversification of crop systems has led to the search for and domestication of new crops that can perform well

in resource-limited environments (Cox et al., 2002). Particularly in the Patagonia steppe, erratic precipitations less than 300 mm, strong winds and low winter temperatures, result in low productivity (Paruelo et al., 1998) and limit opportunities for cultivation of traditional crops. Candidate crops for these environments need to be stress-tolerant in order to minimize the risk imposed by the severe ecological restrictions (McLaughlin, 1985). Usually, species native to resource-limited environments are characterized by a set of morphological and physiological traits that result in high performance under stressful conditions (Chapin, 1980). Therefore, the domestication of native species might be an alternative to find new tolerant crops for arid and semi-arid lands (Ravetta and Soriano, 1998).

Our objectives were (1) to characterize morphological and functional traits related to stress-tolerance in wild and domesticated species of *Oenothera* and (2) to assess the effect of resource availability on stress-tolerance traits and on seed-yield stability.

We performed a field plant density experiment in order to generate a resource gradient (Vega et al., 2000). Effects of plant density on biomass accumulation and allocation, leaf morphological

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traits, carbohydrates accumulation and seed-yield were described. Root allocation and low specific leaf area (SLA) were considered attributes associated with high performance under stressful conditions (Chapin, 1980; Poorter and Remkes, 1990). Traits related to high growth capacity, such as high SLA and high leaf allocation were associated with poor performance under stressful conditions (Chapin, 1980; Poorter and Garnier, 1999; Aerts and Chapin, 2000; Westoby et al., 2002). The potential use of these traits for breeding programs is discussed.

2. Material and methods

2.1. Plant material

We selected four taxa of *Oenothera*: two domesticated species (*Oenothera biennis* and *Oenothera lamarckiana*), and two native taxa of Argentina, widely distributed in the Monte Desert (*O. mendocinensis* and *O. odorata* × *O. mendocinensis*, a natural occurring hybrid). Bulk seeds of *O. mendocinensis* were collected in Tupungato, Mendoza (ID 738 33°03'158"S, 69°17'201"W, 2318 m a.s.l.); the hybrid was collected in Las Leñas, Mendoza (ID 756, 35°08'773"S, 70°04'690"W, 2256 m a.s.l.). Hereafter, these four taxa will be referred to as "species" for the sake of simplicity.

2.2. Study site and experimental conditions

A two factor (species and density) field experiment with a completely randomized design was performed in the Chubut River Valley, Patagonia Argentina (43°21'31"S; 65°38'39"W). In this area the mean annual precipitation is 179 mm, the mean low temperature of the coldest months (June and July) is 1 °C, the absolute minimum air temperature is −10.8 °C and the maximum temperature attain 40 °C (Cabrera, 1994).

Domesticated and native species were grown in a common garden at three different levels of plant densities: (1) low (20 plants m⁻²); (2) medium (50 plants m⁻²) and (3) high (100 plants m⁻²). Twenty plants per square meter is a density low enough to avoid intraspecific competition (Deng et al., 2001; Sekeroglu and Özgüven, 2006; Vilela et al., 2008a).

In late Winter (August) seeds were sown in germination trays filled with soil, peat moss and sand in equal proportion and maintained in a greenhouse, where they received 80% of outside light levels. Temperature ranged between 25 and 15 °C (average maximum daytime and average minimum nighttime temperature respectively). Seedlings were transplanted to the field 60 days after sowing (October).

Experimental units consisted of plots (4 reps per species and density treatment, for a total of 48 plots). Each plot contained 65 plants arranged as follows: (1) low plant density: 30 cm between rows and 15 cm between plants; (2) medium plant density: 14 cm between rows and plants; and (3) high plant density: 10 cm between rows and plants. Plants were flood irrigated every 15 days between September and May, when irrigation water is available in the valley. Weed control was done by hand-pulling and with licensed herbicides (Treflan, trifluralina: α , α , α alfa-trifluoro-2,6-dinitro-N,Ndipropil-p-toluidina), applied in recommended doses (1.5 l/ha; Berti personal communication).

2.2.1. Total biomass and allocation variables

Whole plants were harvested (3 plants/plot; 4 plots/species) at three phenological phases (vegetative, reproductive and maturity) and placed in an oven at 60 °C until constant weight. Plants were considered to be in vegetative growth phase during the period ranging from the time of seeding until bolting. Reproductive stage was considered from bolting until 50% of the individuals in each plot showed at least one flower in anthesis. Physiological maturity was

considered from the time at the 50% of the individuals in each plot showed at least one flower in anthesis until final harvest, at the end of the growing season. Plants in vegetative phase were harvested in late Fall and plants in reproductive stage were harvested in early Spring. Plant biomass was divided into leaves, stems, roots, fruits and seeds. Total plant biomass was measured and the proportions of biomass allocated to leaves (leaf mass ratio, LMR), stems (stem mass ratio, SMR) and roots (root mass ratio, RMR) were calculated as the ratio between organ dry weight (leaves, stems or roots) and total biomass. Stems were considered as a reproductive support-structure (Vilela et al., 2008b). Total seed-weight per plant (g plant⁻¹) was calculated as follows: individual seed dry weight × number of seeds per fruit × number of fruits per plant. Individual seed dry weight was calculated by measuring the weight of 25 seeds (5 reps per plant). The number of seeds per fruit corresponded to the mean number of 5 fruits per plant.

Carbohydrate storage was determined as the amount of total non-structural carbohydrates (TNC, starch and soluble sugars) present in roots. Root biomass samples (250 mg per plant) were digested in HCl (10%) at 100 °C for 1 h. TNC were determined by the anthrone method (Yemm and Willis, 1954). Differences among species in biomass accumulation and partitioning, morphological leaf traits, storage and seed-yield reported in Section 3.1, were recorded in plants growing under low density treatment.

2.2.2. Specific leaf area and leaf area ratio

Harvested plants were stored in plastic bags during transport to the lab and placed in water for rehydration (SLA > 10 m² kg⁻²; Garnier et al., 2001). Leaf area was determined using UTHSCSA Image Tool for Windows, Version 2.02. After scanning, leaves were oven-dried until constant weight. Leaf area ratio (LAR, leaf area per unit total plant mass) reflects the amount of leaf area a plant develops per unit total plant mass and, therefore, depends on the proportion of total plant biomass and was calculated as follows: LAR = LMR × SLA, where LMR is the proportion of biomass allocated to leaves (g g⁻¹) and SLA the specific leaf area (cm² g⁻¹, Lambers et al., 1998).

2.3. Statistical analyses

Biomass accumulation and partition and leaf morphological differences among species and the effect of plant density on those traits were analyzed using two-way ANOVA, with species (four levels) and density (three levels) as the factors. Comparison of means among species was assessed by Tukey's test. In order to achieve normal distributions and homogeneity of variance, arcsin transformation was applied to %TNC. Log transformation was applied to RMR, LMR, SLA and LAR and Ln was applied to total biomass. Effects of traits on seed-yield were assessed through linear regression models. Statistical analyses were done using InfoStat 2009.

3. Results

3.1. Biomass accumulation and partition, leaf morphological traits, storage and seed-yield

3.1.1. Vegetative stage

No differences in total biomass were recorded among species (Fig. 1A). Native species showed higher RMR ($F = 33.26$; $p < 0.0001$) and lower LMR ($F = 33.25$; $p < 0.0001$) than domesticated ones (Fig. 1B and C). Within the natives, the hybrid showed higher RMR and lower LMR than *O. mendocinensis*.

Rosettes of *O. lamarckiana* showed the highest LAR ($F = 14.06$; $p < 0.001$), SLA ($F = 4.21$; $p < 0.05$; Fig. 1E and F) and TNC content ($F = 5.89$; $p < 0.05$; Fig. 2).

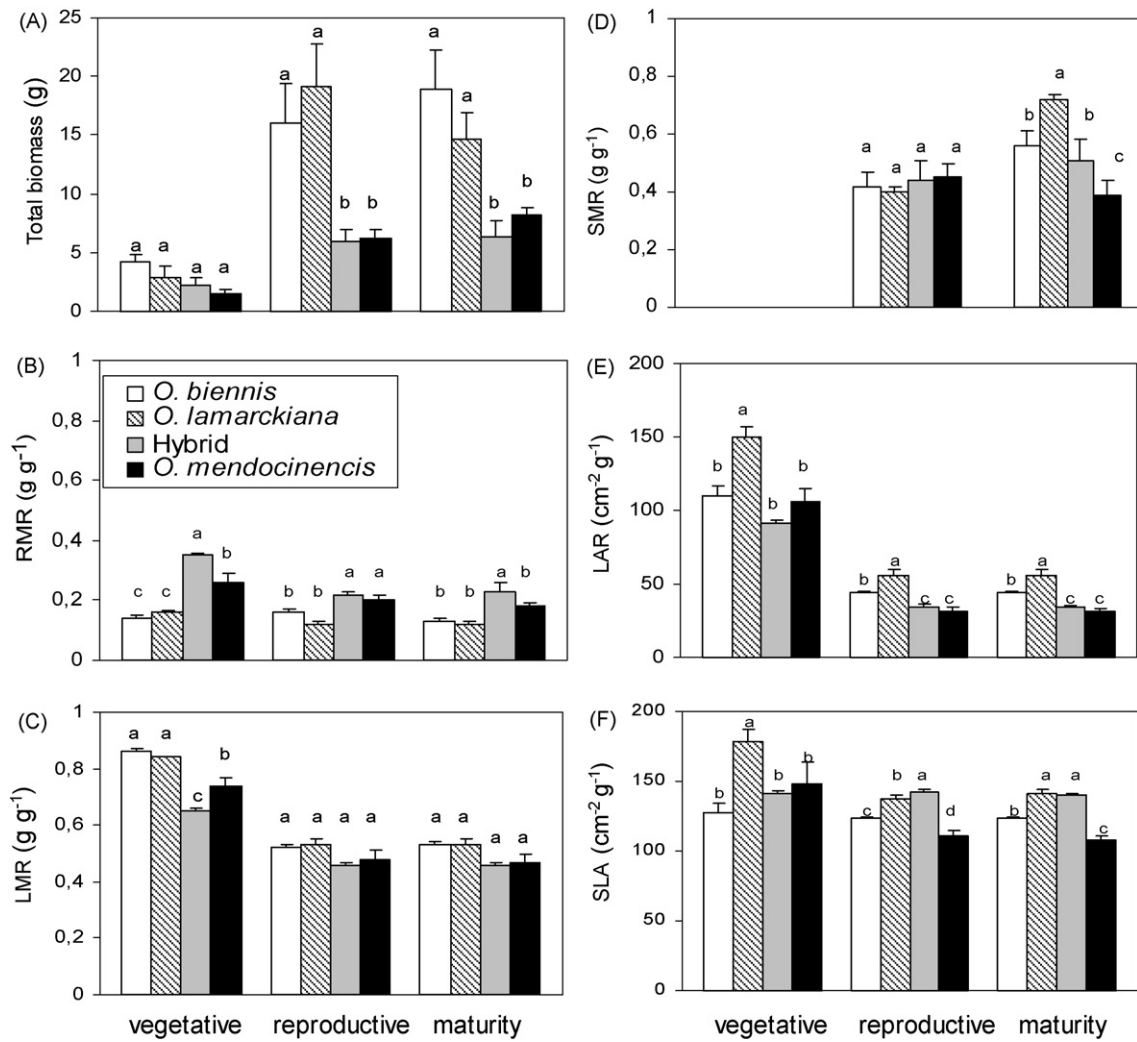


Fig. 1. Biomass accumulation and partition and morphological traits in four species of *Oenothera* cultivated in a common garden in the Chubut River Valley. Comparison of means among species was assessed by Tukey's test. Significant differences ($p < 0.05$) among species are indicated by different letters. RMR = root mass ratio, LMR = leaf mass ratio, SMR = stem mass ratio, LAR = leaf area ratio and SLA = specific leaf area.

3.1.2. Reproductive stage

Domesticated species accumulated more biomass than natives. As a result, at the end of this stage, total biomass was higher in the former than in the latter ($F = 30.33$; $p < 0.0001$; Fig. 1A). RMR continued to be higher in natives ($F = 11.59$; $p < 0.001$) than in domesticated species (Fig. 1B), while differences in LMR found in the vegetative stage, vanished in the reproductive phase (Fig. 1C).

SMR did not differ among species (Fig. 1D). LAR was higher in domesticated species than in natives. The highest LAR value was found in *O. lamarckiana* ($F = 27.3$; $p < 0.0001$; Fig. 1E). SLA was significantly different in the four species ($F = 382.89$; $p < 0.0001$). The highest SLA values were exhibited by the hybrid and *O. lamarckiana* (Fig. 1F). *O. lamarckiana* showed the lowest TNC content ($F = 4.86$; $p < 0.05$; Fig. 2).

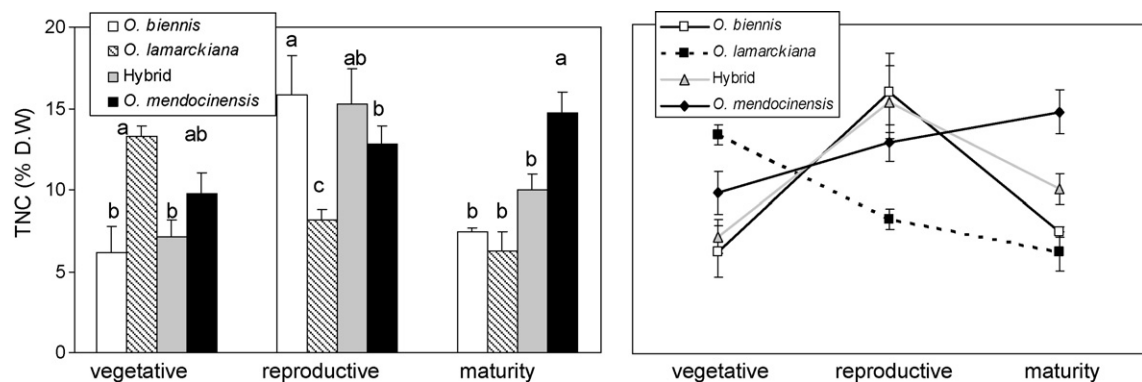


Fig. 2. Root non-structural carbohydrates content (TNC) of four species of *Oenothera* growing in a common garden in the Chubut River Valley over three phenological stages.

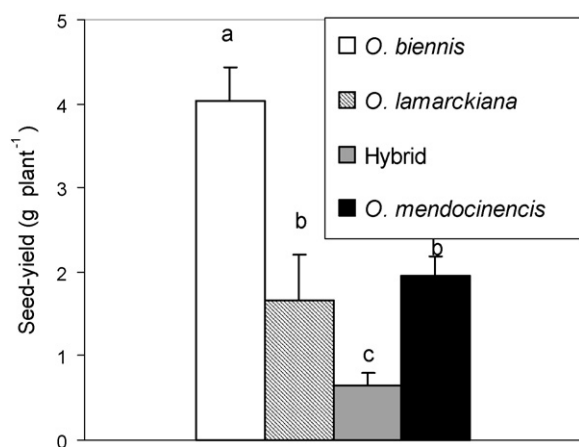


Fig. 3. Seed-yield per plant of two domesticated (*O. biennis* and *O. lamarckiana*) and two native (*O. mendocinensis* and a natural occurring hybrid) *Oenothera* species growing in a common garden in the Chubut River Valley, at low, medium and high plant densities.

3.1.3. Maturity stage

Total biomass continued to be higher in domesticated than in native species (Fig. 1A). RMR was significantly higher for the hybrid ($F=9$, $p<0.05$; Fig. 1B) than for the other species, while no differences in LMR were found among species. The highest SMR was recorded in *O. lamarckiana* and the lowest in *O. mendocinensis* (Fig. 1D). LAR showed the same pattern in maturity than in reproductive stage: domesticated species showed higher LAR than native ones (Fig. 1E). *Oenothera lamarckiana* and the hybrid showed the highest SLA ($F=335.12$; $p<0.0001$; Fig. 1F). No differences between these species were recorded (Fig. 1F). The highest value of TNC content was found in *O. mendocinensis* ($F=11.96$; $p<0.001$; Fig. 2). *Oenothera biennis* showed the highest seed-yield per plant and the hybrid showed the lowest ($F=15.68$; $p<0.001$). No differences among *O. lamarckiana* and *O. mendocinensis* were recorded (Fig. 3).

Table 1

One-way ANOVA for biomass accumulation and partition, leaf morphological traits and root carbohydrate storage in vegetative, reproductive and physiological maturity stages of *Oenothera biennis*, cultivated under low, medium and high plant densities. Comparison of means among density treatments was assessed by Tukey's test.

	<i>Oenothera biennis</i>		
	Low density	Intermediate density	High density
<i>Vegetative stage</i>			
Total biomass (g)	4.2 ± 0.6 a	1.8 ± 0.2 b	1.6 ± 0.3 c
RMR (g g ⁻¹)	0.1 ± 0.0 a	0.1 ± 0.0 a	0.14 ± 0.0 a
LMR(g g ⁻¹)	0.9 ± 0.0 a	0.9 ± 0.0 a	0.8 ± 0.0 a
Root %TNC	6.2 ± 1.6 a	6.5 ± 1.1 a	6.4 ± 2.2 a
LAR (cm ² g ⁻¹)	109.6 ± 6.6 a	149.5 ± 11 b	154.3 ± 10 b
SLA (cm ² g ⁻¹)	127.9 ± 6.2 a	172.3 ± 10 b	184.2 ± 12 b
<i>Reproductive stage</i>			
Total biomass (g)	16.1 ± 3.3 a	10.9 ± 1.1 b	8.5 ± 1.2 b
RMR (g g ⁻¹)	0.2 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a
SMR(g g ⁻¹)	0.4 ± 0.0 a	0.4 ± 0.0 a	0.4 ± 0.0 a
LMR(g g ⁻¹)	0.5 ± 0.0 a	0.5 ± 0.0 a	0.5 ± 0.0 a
Root %TNC	15.8 ± 2.4 a	13.1 ± 0.6 a	13.9 ± 0.6 a
LAR (cm ² g ⁻¹)	44.2 ± 0.8 a	44.3 ± 1.7 a	46.1 ± 2.9 a
SLA (cm ² g ⁻¹)	123.8 ± 0.9 a	125.3 ± 0.8 a	126.8 ± 0.7 a
<i>Maturity stage</i>			
Total biomass (g)	18.9 ± 3.1 a	9.3 ± 1.8 b	5.9 ± 0.6 b
RMR (g g ⁻¹)	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a
SMR(g g ⁻¹)	0.6 ± 0.0 a	0.5 ± 0.0 a	0.6 ± 0.0 a
LMR(g g ⁻¹)	0.5 ± 0.0 a	0.5 ± 0.0 a	0.5 ± 0.0 a
Root %TNC	7.4 ± 0.3 a	7.8 ± 0.4 a	7.7 ± 0.5 a
LAR (cm ² g ⁻¹)	45.8 ± 2.7 a	44.8 ± 0.5 a	47.2 ± 1.5 a
SLA (cm ² g ⁻¹)	125.3 ± 5.6 a	126.3 ± 0.9 a	122.8 ± 0.9 a
Seed-yield (g plant⁻¹)	4.1 ± 0.4 a	2.1 ± 0.5 b	1.5 ± 0.4 b

Significant differences ($p<0.05$) are indicated by different letters. RMR = root mass ratio, LMR = leaf mass ratio, SMR = stem mass ratio, TNC = total non-structural carbohydrates, LAR = leaf area ratio and SLA = specific leaf area.

3.2. Plant density

3.2.1. *Oenothera biennis*

The increase in plant density provoked a decrease in individual plant total biomass in every phase ($F=9.71$; $p<0.05$, $F=15.43$; $p<0.05$; $F=13.41$; $p<0.05$ respectively, Table 1). LAR increased at intermediate and high densities in the vegetative stage ($F=6.22$; $p<0.05$) due to a concomitant increase in SLA ($F=24.97$; $p<0.001$), the morphological component of LAR. LMR, the fraction of total plant weight allocated to leaves, did not differ among densities. Difference in SLA and LAR recorded in the vegetative stage vanished in the reproductive and maturity stages. Seed-yield was halved in *O. biennis* by the increase in plant density ($F=8.17$; $p<0.05$; Table 1).

3.2.2. *Oenothera mendocinensis*

Total biomass of individual plants decreased with increasing density in the reproductive and maturity stages ($F=10.4$; $p<0.05$; $F=8.58$; $p<0.05$ respectively, Table 2). An increase in SLA was observed in response to high plant density in this species ($F=5.12$; $p<0.05$ and $F=4.74$; $p<0.05$ for reproductive and maturity stages respectively).

3.2.3. *Oenothera lamarckiana* and the hybrid

Neither total biomass nor allocation or morphological variables were affected by plant density (Tables 3 and 4).

3.3. Relationship between seed-yield, morphological traits and biomass partition

A significant positive correlation between seed-yield and total biomass was found, considering the four species together ($r^2=0.67$; $F=84.43$; $p<0.001$; Fig. 4A). No other general correlations were found between seed-yield and morphological traits (LAR and SLA) or biomass allocation variables (LMR and RMR).

Table 2

One-way ANOVA for biomass accumulation and partition, leaf morphological traits and root carbohydrate storage in vegetative, reproductive and physiological maturity stages of *Oenothera mendocinensis*, cultivated under low, medium and high plant densities.

	<i>Oenothera mendocinensis</i>		
	Low density	Intermediate density	High density
<i>Vegetative stage</i>			
Total biomass (g)	1.5 ± 0.4 a	1.7 ± 0.2 a	1.8 ± 0.4 a
RMR(g g ⁻¹)	0.7 ± 0.0 a	0.8 ± 0.0 a	0.8 ± 0.0 a
LMR(g g ⁻¹)	0.3 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a
Root %TNC	9.7 ± 1.3 a	10.1 ± 1.4 a	10.5 ± 1.4 a
LAR(cm ² g ⁻¹)	106.6 ± 8.7 a	100.4 ± 4.8 a	102.9 ± 11 a
SLA (cm ² g ⁻¹)	147.6 ± 16 a	131.3 ± 7.5 a	140.2 ± 25 a
<i>Reproductive stage</i>			
Total biomass (g)	6.3 ± 0.7 a	3.6 ± 0.2 b	3.1 ± 0.4 b
RMR(g g ⁻¹)	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a
SMR(g g ⁻¹)	0.4 ± 0.0 a	0.4 ± 0.0 a	0.5 ± 0.0 a
LMR(g g ⁻¹)	0.5 ± 0.0 a	0.5 ± 0.0 a	0.5 ± 0.0 a
Root %TNC	12.7 ± 1.1 a	13.5 ± 1.1 a	14.1 ± 0.9 a
LAR(cm ² g ⁻¹)	31.3 ± 2.3 a	34.7 ± 1.3 a	29.8 ± 2.1 a
SLA (cm ² g ⁻¹)	111.2 ± 1.1 a	117.7 ± 1.7 a	122.4 ± 2.8 b
<i>Maturity stage</i>			
Total biomass (g)	8.2 ± 0.7 a	4.7 ± 0.7 b	4.6 ± 0.4 b
RMR(g g ⁻¹)	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a
SMR(g g ⁻¹)	0.4 ± 0.0 a	0.4 ± 0.0 a	0.5 ± 0.0 a
LMR(g g ⁻¹)	0.5 ± 0.0 a	0.5 ± 0.0 a	0.4 ± 0.0 a
Root %TNC	14.7 ± 1.3 a	13.4 ± 1.3 a	10.2 ± 1.1 a
LAR(cm ² g ⁻¹)	33.5 ± 3.6 a	41.5 ± 0.9 a	31.3 ± 1.4 a
SLA (cm ² g ⁻¹)	101.2 ± 0.6 a	111.7 ± 1.4 a	123.4 ± 1.7 b
Seed-yield (g plant ⁻¹)	1.9 ± 0.2 a	1.1 ± 0.5 a	1.5 ± 0.3 a

Comparison of means among density treatments was assessed by Tukey's test. Significant differences ($p < 0.05$) are indicated by different letters. RMR = root mass ratio, LMR = leaf mass ratio, SMR = stem mass ratio, TNC = total non-structural carbohydrates, LAR = leaf area ratio and SLA = specific leaf area.

A significant positive correlation between seed-yield and TNC was found in the domesticated species ($r^2 = 0.65$; $F = 31.42$; $p < 0.0001$; Fig. 4B).

In *O. biennis*, a significant negative correlation among seed-yield and SLA and LAR ($r^2 = -0.67$; $F = 18.31$; $p < 0.001$ and $r^2 = -0.55$; $F = 12.4$; $p < 0.05$ respectively) was found.

4. Discussion

Screening for traits associated with stress-tolerance and agricultural performance in wild species of *Oenothera* will help identify a range of characteristics necessary for successful development of new industrial crops for production in arid lands. In this work

Table 3

One-way ANOVA for biomass accumulation and partition, leaf morphological traits and root carbohydrate storage in vegetative, reproductive and physiological maturity stages of *Oenothera lamarckiana*, cultivated under low, medium and high plant densities.

	<i>Oenothera lamarckiana</i>		
	Low density	Intermediate density	High density
<i>Vegetative stage</i>			
Total biomass (g)	2.9 ± 1.1	3.1 ± 0.4	1.4 ± 0.3 NS
RMR (g g ⁻¹)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0 NS
LMR (g g ⁻¹)	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0 NS
Root %TNC	13.3 ± 0.6	13.1 ± 0.3	14.2 ± 0.4 NS
LAR (cm ² g ⁻¹)	149.6 ± 7.3	152.6 ± 8.5	161.4 ± 14 NS
SLA (cm ² g ⁻¹)	178.2 ± 9.5	184.3 ± 11	192.8 ± 14 NS
<i>Reproductive stage</i>			
Total biomass (g)	19.2 ± 3.6	16.8 ± 1.7	14.6 ± 4.1 NS
RMR (g g ⁻¹)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0 NS
SMR (g g ⁻¹)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0 NS
LMR (g g ⁻¹)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0 NS
Root %TNC	8.2 ± 0.6	8.7 ± 0.4	8.5 ± 1.1 NS
LAR (cm ² g ⁻¹)	56.1 ± 3.3	48.1 ± 1.6	50.8 ± 6.4 NS
SLA (cm ² g ⁻¹)	136.9 ± 0.4	137.1 ± 0.2	137.7 ± 0.7 NS
<i>Maturity stage</i>			
Total biomass (g)	14.7 ± 2.4	9.7 ± 1.5	7.6 ± 1.8 NS
RMR (g g ⁻¹)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0 NS
SMR (g g ⁻¹)	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0 NS
LMR (g g ⁻¹)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0 NS
Root %TNC	6.2 ± 1.2	6.7 ± 1.1	6.3 ± 0.4 NS
LAR (cm ² g ⁻¹)	57.2 ± 4.3	52.8 ± 6.4	49.1 ± 16 NS
SLA (cm ² g ⁻¹)	136.9 ± 0.4	137.1 ± 0.2	137.7 ± 0.7 NS
Seed-yield (g plant ⁻¹)	1.7 ± 0.5	1.6 ± 0.5	0.8 ± 0.2 NS

Comparison of means among density treatments was assessed by Tukey's test. Significant differences ($p < 0.05$) are indicated by different letters. RMR = root mass ratio, LMR = leaf mass ratio, SMR = stem mass ratio, TNC = total non-structural carbohydrates, LAR = leaf area ratio and SLA = specific leaf area.

Table 4
One-way ANOVA for biomass accumulation and partition, leaf morphological traits and root carbohydrate storage in vegetative, reproductive and physiological maturity stages of Hybrid, cultivated under low, medium and high plant densities.

	Hybrid		
	Low density	Intermediate density	High density
<i>Vegetative stage</i>			
Total biomass (g)	2.2 ± 0.6	1.8 ± 0.4	1.5 ± 0.2 NS
RMR (g g ⁻¹)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0 NS
LMR (g g ⁻¹)	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0 NS
Root %TNC	7.1 ± 1.1	9.3 ± 1.1	9.9 ± 0.4 NS
LAR (cm ² g ⁻¹)	91.4 ± 1.9	92.7 ± 2.8	92.3 ± 11 NS
SLA (cm ² g ⁻¹)	140.9 ± 2.9	132.9 ± 3.4	130.2 ± 10 NS
<i>Reproductive stage</i>			
Total biomass (g)	6.1 ± 0.9	4.4 ± 1.1	3.4 ± 0.9 NS
RMR (g g ⁻¹)	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0 NS
SMR (g g ⁻¹)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0 NS
LMR (g g ⁻¹)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1 NS
Root %TNC	15.2 ± 2.2	16.1 ± 2.4	12.8 ± 1.7 NS
LAR (cm ² g ⁻¹)	33.8 ± 1.5	38.2 ± 1.4	38.7 ± 7.4 NS
SLA (cm ² g ⁻¹)	142 ± 0.1	141.5 ± 0.3	141.1 ± 0.6 NS
<i>Maturity stage</i>			
Total biomass (g)	6.4 ± 1.3	4.3 ± 0.8	4.5 ± 0.8 NS
RMR (g g ⁻¹)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0 NS
SMR (g g ⁻¹)	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0 NS
LMR (g g ⁻¹)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0 NS
Root %TNC	10.1 ± 0.9	12.2 ± 0.5	10.1 ± 0.6 NS
LAR (cm ² g ⁻¹)	34.1 ± 1.7	45.2 ± 1.3	34.8 ± 3.5 NS
SLA (cm ² g ⁻¹)	143 ± 0.5	139.5 ± 0.6	142.9 ± 0.9 NS
Seed-yield (g plant ⁻¹)	1.1 ± 0.1	0.7 ± 0.1	0.6 ± 0.3 NS

Comparison of means among density treatments was assessed by Tukey's test. Significant differences ($p < 0.05$) are indicated by different letters. RMR = root mass ratio, LMR = leaf mass ratio, SMR = stem mass ratio, TNC = total non-structural carbohydrates, LAR = leaf area ratio and SLA = specific leaf area.

we identified differences in morphological traits and allocation patterns of four *Oenothera* species in three phenological phases. Morphological and functional variations among species are related to adaptation patterns to different environments and reflect fundamental differences in the way in which species acquire, invest and utilize resources (Lambers and Poorter, 1992; Aerts and Chapin, 2000).

Rosettes (vegetative stage) of native and domesticated species did not differ in total biomass, but in their allocation pattern the former showed higher root allocation (RMR) and lower leaf allocation (LMR) than the latter. These results are in agreement with Chapin's (1980) predictions: species coming from resource-limited environments (natives) allocated resources preferentially to root. This allocation pattern is associated with low growth potential and resource conservation and usually results in a high performance under resource-limited environments. On the other hand, domesticated species, selected for resource-rich environments, allocated

resources preferentially to leaf, which is associated with high growth potential and resource acquisition (Poorter and Garnier, 1999; Aerts and Chapin, 2000).

Rosettes of *O. lamarckiana* were characterized by high LAR and SLA. These traits are associated with high growth capacity (Poorter and Garnier, 1999) and resulted in the largest individual plants, from bolting to maturity (Fig. 1A). Alternatively, *O. biennis* and native species showed low SLA, which is determined by a greater dry matter fraction and higher cell wall carbon content, traits associated with drought tolerance and mechanical damage avoidance (Coley, 1983). Therefore, *O. lamarckiana* showed a fast-growing strategy during the vegetative phase while native species favored stress-tolerance. Rosettes of *O. biennis* showed an interesting combination of traits related to stress-tolerance (low SLA) and high productivity (high LMR and low RMR and LAR). This suite of traits might improve the establishment and productivity of the crop in semi-arid environments such as irrigated valleys of Patagonia,

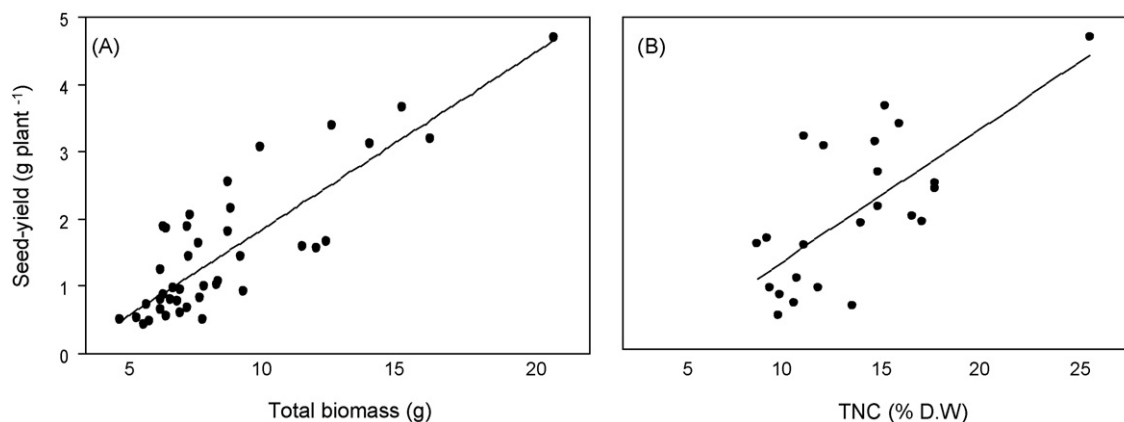


Fig. 4. Linear regression analysis between seed-yield and total biomass (A) of four species of *Oenothera* and TNC (B) of the domesticated *Oenothera* species growing in a common garden in the Chubut River Valley. Each dot corresponds to the mean value of three plants per plot.

where the environmental conditions impose severe limitations to growth (Paruelo et al., 1998).

During the reproductive and maturity stages, domesticated species accumulated higher biomass than natives. Biomass accumulation in domesticated species was associated with seed-yield. RMR continued to be higher in natives than in their domesticated relatives. Because allocation to stress-tolerance traits and reproduction are assumed to be mutually exclusive functions (Chapin, 1980), increased survival frequently results in trade-offs with reproduction (Bazzaz, 1997). High root allocation found in wild *Oenothera* species, usually results in structural and functional limitations for reproduction (Vilela et al., 2008b), as confirmed in our experiment by low seed-yield of native species (Fig. 3). During the maturity stage, *O. mendocinensis* decreased allocation to roots to a level similar to that found in domesticated species, while the hybrid did not. According to Bazzaz (1997), this change could be related to an increase in allocation to reproduction. Our results are in agreement with this proposition, as indicated by the higher seed-yield found in *O. mendocinensis* compared with the hybrid.

Vilela et al. (2008b) described for *Oenothera* species a negative correlation between LAR and reproductive allocation. This was confirmed in domesticated species: *O. lamarckiana* showed in the reproductive and maturity stages a significantly higher LAR and lower seed-yield than *O. biennis*. Differences between natives in seed-yield could not be explained by differences in LAR.

Carbohydrate storage in roots is considered a stress-tolerance trait (Kobe, 1997; Myers and Kitajima, 2007). At the same time, in our experiment root TNC was associated with seed-yield in domesticated species (Fig. 4B). Consequently, the increase allocation to TNC observed in *O. biennis* during Winter (time between vegetative and reproductive harvest; Fig. 2) might be responsible, at least partially, for the high seed-yield of this species. In *O. lamarckiana* TNC reserves built up during past growing seasons might be a carbon source for respiration during wintertime, providing low temperature tolerance (Canham et al., 1999).

The expected positive association between TNC and seed-yield was not found in native species, suggesting a different destination for the TNC. High levels of root carbohydrates recorded at the end of the season are generally associated with perennality (Dina and Klikoff, 1974). Since native *Oenothera* species can behave as annuals or perennials (Vilela et al., 2008b) depending on environmental conditions, high root TNC found in *O. mendocinensis* could be associated to perennality.

According to Chapin (1980) and Chapin and Shaver (1991) species that are adapted to low or high-resource environments respond differentially to changes in resource availabilities. Species from resource-limited environments, with low-resource requirements usually have a low maximum growth rate and saturate at low-resource availabilities. When resources are available, low-resource species do not respond to a surplus of resources, while high-resource species respond strongly to additional resources (see examples in Vilela et al., 2003 and Valladares et al., 2000, among others). Native plants growing under high-resource availability (low density) were not able to take advantage of that surplus, increasing their relative growth rate and accumulating more biomass under high resource than under low-resource availability, as domesticated species did. Regarding other morphological traits, such as SLA and LAR, *O. biennis* and the hybrid responded as expected by this prediction, supporting the idea that a high-resource species (*O. biennis*) is more plastic in its morphological traits and allocation pattern than a low-resource (hybrid) one. The low responsiveness of native species to resources availability could be due either to low intrinsic phenotypic plasticity or to the possibility that resource limitation at high density treatment was not low enough to trigger responses (Valladares et al., 2000). The direc-

tion of changes observed in SLA and LAR indicates that plants might be in competence for light instead of soil resources. Many authors demonstrated that these growth components tend to decrease with decreasing soil resources availability (Werf van der et al., 1993; Fernández and Reynolds, 2000; Poorter et al., 2009). The increase in SLA and LAR with plant density observed in *O. biennis* and *O. mendocinensis* could be considered, according to Lambers et al. (1998) a functional adjustment to capture more light.

In *O. biennis*, seed-yield decreased with increasing plant density. This result is in agreement with the prediction that species selected under favorable conditions will result in seed-yield instability in resource-limited environments (Ceccarelli, 1996). Selection to improve yield potential through increasing harvest index has increased the sensitivity of most modern cultivars changes in the environmental quality (Donald, 2005).

Since seed-yield in the studied *Oenothera* species increased linearly with biomass accumulation, seed-yield improvement in native species could be possible by selection oriented to increase total biomass instead of harvest index. Nevertheless, breeding for increased biomass should be done within the constraints imposed by environmental conditions of the targeted environment, avoiding the generation of a large water-consuming biomass crop (McLaughlin, 1985) and the ecological problems associated with excessive and inefficient irrigation in arid lands (Deng et al., 2006).

5. Conclusion

Oenothera species are characterized by different combination of traits related to stress-tolerance and high productivity. Low specific leaf area and high allocation to roots resulted in stress-tolerant rosettes and might improve the establishment of *Oenothera* crop in arid environments. The high root allocation characteristic of native *Oenothera* species resulted in limitations for reproduction. Low seed-yield of native species is a major drawback that must be overcome. Seed-yield improvement in this species could be possible by selection oriented to increasing total biomass within the limits imposed by environmental restrictions. The domesticated *O. lamarckiana*, characterized by low stress-tolerance traits and low seed-yield has little to offer. *Oenothera biennis* showed an optimum density of 20 plants m^{-2} . At optimum plant density *O. biennis* yielded 260 $g m^{-2}$. This production is in accordance with that reported under favorable growing conditions in other countries (Sekeroglu and Özgüven, 2006; Ghasemnezhad and Hornermeier, 2008). Since no detrimental effect of density was found in *O. lamarckiana* and the native species, a higher plant density per unit area may increase seed-yield.

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