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# Growth pattern of *Bidens cernua* L.: relationships between relative growth rate and its physiological and morphological components

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## Abstract

Seedlings of *Bidens cernua* L. emerged when mean air temperature was 17.0±1.3 °C. The highest net photosynthetic rate ($P_N$), 13.8±0.8 μmol($CO_2$) $m^{-2}$ $s^{-1}$, was monitored during the vegetative period (May–August), decreasing on an average by 50 % during flowering (August–September) and during fruiting (September–November) phases. The senescence phase (October–November) was characterised by 79, 58, and 18 % decrease of $P_N$, chlorophyll content, and leaf area (LA), respectively, from the maximum values. The time span from seedling emergence to the end of fruiting phase was 202 d. The total plant biomass was 1.58±0.05 g of which 81 % was aboveground plant portion. The total dry mass relative growth rate averaged over the assimilation period was 0.0804±0.0002 kg $kg^{-1}$ $d^{-1}$, and it was correlated to both the net assimilation rate (NAR) and the leaf area ratio (LAR).

*Additional keywords*: chlorophyll content; dry mass; growth analysis; leaf area ratio; net assimilation rate; net photosynthetic rate; plant biomass; plant height; stomatal conductance.

## Introduction

Major factors involved in plant growth and reproduction are the acquisition and investment of resources; it is mainly the leaves which are involved in the vegetative growth of annual species, in general the stem comprising at most 2.5 % of the shoot dry mass, DM (Groeneveld 1998). Plant species with a high intrinsic capacity to grow possess structures to efficiently capture and process resources from the environment, *i.e.* a large leaf area per unit plant DM and a high photosynthetic capacity (Cornelissen *et al.* 1998). Relative growth rate (RGR) is a useful indicator of the extent to which a species is using its photosynthates for growth (Groeneveld 1998). RGR is a function of the net assimilation rate (NAR) and leaf area ratio (LAR). Since plant growth is an important determinant of survival and reproduction in nature, knowledge of RGR is of central importance in plant ecology (Tilman 1988, Westoby 1998, Almeida-Cortez *et al.* 1999, Shipley 2002, Montgomery 2004, Jach and Ceulemans 2006).

*Bidens cernua* L. (*Asteraceae*) is an annual hygro-nitrophilous species (Almeida-Cortez 1999, Brändel 2004, Wisskirchen 2006) growing on river banks, lakes, and in wet ditches and nutrient-rich muddy soils, usually with its roots submerged (Crowe and Parker 1981, Brändel 2004). It is distributed in Europe, Asia, and North America (Brändel 2004), and it is included in the red list of Italian Flora (Conti *et al.* 1997) as a critically endangered species for the *Latium* (Central Italy).

The main objective of this paper was to analyse the growth efficiency of *B. cernua*. Moreover, we determined the most important morphological and physiological factors driving its RGR.

## Materials and methods

**Study site**: Experiments were carried out at the Botanical Garden of Rome (41°53′ N, 12°28′ E; 53 m a.s.l.). Freshly-matured seeds of *B. cernua* were collected at the beginning of November 2005 from plants naturally

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*Abbreviations:* Chl – chlorophyll content; DM – dry mass; $g_s$ – stomatal conductance; H – plant height; LA – leaf area; LAR – leaf area ratio; LE – plant height growth efficiency; LMR – leaf mass ratio; NAR – net assimilation rate; $P_N$ – net photosynthetic rate; PAR – photosynthetically active radiation; RGR – relative growth rate; $RGR_{tot}$ – total dry mass relative growth rate; SLA – specific leaf area; TLA – total leaf area; TPB – total plant dry mass.

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growing at Posta Fibreno Lake (*Latium,* 41°41'71"N, 13°41'52"E; 290 m a.s.l. – Montelucci 1979, Conti *et al.* 2005).

One hundred twenty seeds were stratified in a cold chamber at 5 °C for 4 months: thirty seeds in each of 4 fine-mesh polyester cloth bags, and each bag was buried to a depth of 5 cm in moist sand in 9 cm diameter plastic pots with drainage holes (Brändel 2004). Seed germination was conducted in light and temperature controlled chambers (type *CC7*, *Amcota*, Italy), at 15–30 °C and 12-h daylength, equipped with cool-white fluorescent tubes providing a photon flux density of 80 μmol(photon) $m^{-2}$ $s^{-1}$. The seeds were tested for germination on the top of two filter paper discs, previously moistened with distilled water in 14-cm diameter glass Petri dishes (the germination criterion was the emergence of a root through the seed coat). Four replicates of 30 seeds were used.

Thirty randomly selected seedlings were grown and cultivated in black polyethylene plastic pots (10 cm diameter, 15 cm height; 3 plants for each pot). The pots were filled with peat (pH 7) and placed in a pool (1 m in diameter and 0.30 m deep), filled with water and placed under local environmental conditions (Baskin *et al.* 1999).

**Climate**: The climate of Rome is of Mediterranean type, and most of the total annual rainfall (676 mm) occurred in autumn and winter (Meteorological Station of the Collegio Romano, data for the period 1995–2006) (Fig. 1*A*). The mean minimum air temperature of the coldest month (February) was 5.5 °C, the mean maximum air temperature of the hottest months (July–August) was 31 °C, and the annual mean air temperature was 16.9 °C. Dry period was from May to August (120 mm of total rainfall during that period).

During the study period (2006), the mean minimum air temperature of the coldest month (January) was 3.2 °C, and the mean maximum air temperature of the hottest month (July) was 32.6 °C (Fig. 1*B*). Total annual rainfall was 466 mm.

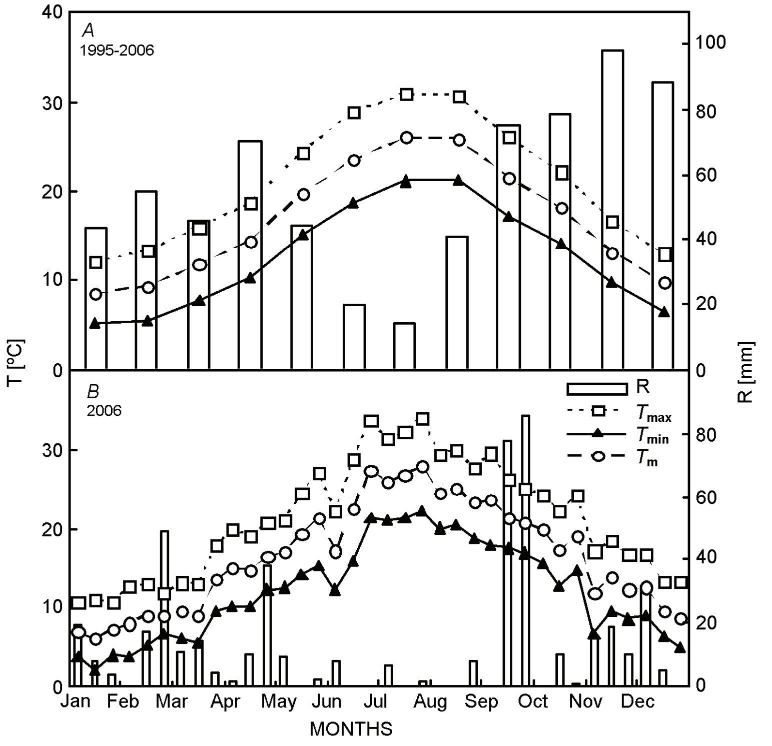

Fig. 1. The climatic diagram of Rome for the period 1995–2006 (*A*) and for the study year 2006 (*B*). In the climatic diagram of the year 2006 each point represents a decade. R – total monthly rainfall; $T_{min}$ – minimum air temperature; $T_{max}$ – maximum air temperature; $T_m$ – mean air temperature. Data provided by the Meteorological Station of the Collegio Romano.

**Phenology and growth dynamics**: Phenological observations (time of seedling emergence, stem elongation, flowering, fruiting, plant senescence) were carried out weekly on 30 selected plants, during the study period (May–December 2006). Growth parameters included plant height (H), total plant DM (TPB), mean leaf area (LA), and total leaf area per plant (TLA). TLA was calculated by multiplying the total leaf number per plant by the mean LA. LA was measured with the *Image Analysis System* (*Delta-T Devices,* UK) in six sampling occasions during the leaf expansion period on one leaf at the middle position of the stem (five plants per each sampling occasion). H was measured when its maximum was attained. Ten plants at the maximum H were harvested at soil level and then separated into stem+leaves and roots. DM of each fraction was recorded after oven drying at 80 °C to constant mass and TPB was determined.

The plant height growth efficiency (LE) was calculated as LE = ΔH×LAR, where LAR was measured as total LA to TPB according to Lambers and Poorter (1992).

$RGR_{tot}$ was calculated as:

$$\mathrm{RGR_{tot}} = \frac{\ln \mathrm{TPB_2} - \ln \mathrm{TPB_1}}{\mathrm{t_2} - \mathrm{t_1}}$$

where $\mathrm{TPB_1}$ and $\mathrm{TPB_2}$ were the TPB at times $t_1$ and $t_2$, respectively.

$\mathrm{RGR_{tot}}$ components were: specific leaf area (SLA), LAR, leaf mass ratio (LMR), and net assimilation rate (NAR). SLA was calculated as leaf area to leaf DM after oven drying at 80 °C to constant mass; LMR was calculated as total leaf DM to TPB; NAR was calculated by the equation: $\mathrm{RGR_{tot}} = \mathrm{NAR} \times \mathrm{LAR}$ (Lambers and Poorter 1992).

**Gas exchange and chlorophyll (Chl) content**: Gas exchange was measured from the beginning of May to the end of October 2006. Net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), and photosynthetically active radiation (PAR) were measured by an open infrared $CO_2$ gas analyzer (*ADC LCA4,* UK), equipped with a leaf chamber (*PLC*, *ADC*, UK). Measurements were made *in situ* on cloud-free days [PAR>1 000 μmol(photon) $m^{-2}$ $s^{-1}$] in the morning (09:30–12:30) to ensure that near-maximum daily photosynthetic rates were measured (Reich *et al.* 1995). On each sampling occasion, fully expanded leaves were used; measurements were carried out on five plants (three leaves per plant).

Chl was measured weekly by a *SPAD-502 meter* (*Minolta,* Japan). Measurements were carried out on fully expanded leaves after cleaning the surface dust from each leaf, and various points on the surface of each leaf sample were measured. Measurements were carried out on five plants (three leaves per plant) and three readings per leaf were averaged to account for within-leaf variation (Sadras *et al.* 2000).

**Statistics**: Differences in the considered variables were determined by the analysis of variance (ANOVA) and Tukey test for multiple comparisons. Simple regression analysis was carried out to examine the correlation between RGR and its components, and among the considered physiological variables. A multiple regression analysis was carried out using $\mathrm{RGR_{tot}}$ as the dependent variable and LAR, LMR, and NAR as independent variables. All statistical tests were performed using a statistical software package (*Statistica, Statsoft,* USA).

## Results

**Phenology and growth dynamics**: The phenological trend of *B. cernua* was characterised by the seedling emergence in early May, 15 d after sowing, when the daily mean air temperature was 17.0±1.3 °C and the mean minimum air temperature was 12.5±1.6 °C. Leaves were produced continuously from the middle of May to the beginning of August, when the daily mean air temperature ranged from 19.3 to 27.9 °C. Flowering started at the beginning of August and fruits developed from the end of September to the middle of November (daily mean air temperature of the period was 20.7–13.8 °C). Seed dispersion started in the middle of October; senescence phase started at the beginning of October, and stems and leaves were completely dry at the beginning of December. The length of the phenological cycle, from the seedling emergence to the end of fruiting, was 202 d; the length of the reproductive period (flowering and fruiting) was 106 d.

The maximum plant height (45.3±2.0 cm) was reached 90 d after the beginning of the vegetative activity (Fig. 2). *B. cernua* had a LE of 7.2±0.4 $m^3$ $kg^{-1}$. The leaf area expansion began in the middle of May, and 15 d afterwards the maximum leaf area (8.4±1.1 $cm^2$) was reached (Fig. 3). During the senescence phase leaf area was 18 % lower than the maximum. At the end of the vegetative period, total leaf area per plant was 277±13 $cm^2$; TPB was 1.58±0.05 g with 4.3 above/below DM ratio. $\mathrm{RGR_{tot}}$ was 0.0804±0.0002 kg $kg^{-1}$ $d^{-1}$; the physiological $\mathrm{RGR_{tot}}$ component (NAR) was 4.9± 0.3 g $m^{-2}$ $d^{-1}$, and the morphological one (LAR) was 18±1 $m^2$ $kg^{-1}$. SLA was 32.1±1.6 $m^2$ $kg^{-1}$ and LMR 0.47± 0.03 kg $kg^{-1}$.

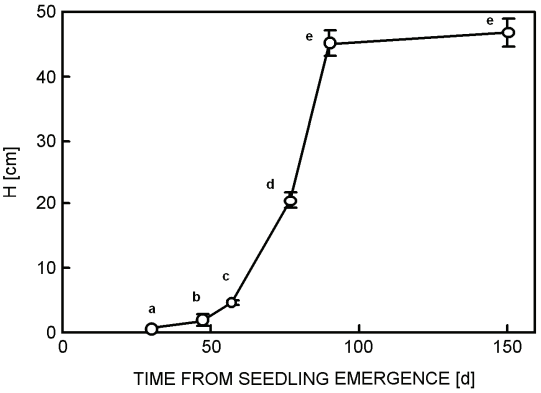

Fig. 2. Plant height (H) during *B. cernua* phenological cycle. Means with the same letters are not significantly different from each other (ANOVA, $p>0.05$). Errors represent standard error.

The results of the simple regression analysis among the considered growth parameters are shown in Fig. 4. There were significant ($p<0.001$) correlations between $\mathrm{RGR_{tot}}$ and NAR, and $\mathrm{RGR_{tot}}$ and LAR. NAR and LAR explained 55 and 49 %, respectively, of $\mathrm{RGR_{tot}}$ variation. Moreover, LAR was significantly ($p<0.001$) correlated to SLA.

Multiple regression analysis between $\mathrm{RGR_{tot}}$ and its components (Table 1) showed that the combination of LAR, LMR, and NAR explained 60 % of $\mathrm{RGR_{tot}}$ variation.

**Gas exchange and Chl content**: The highest $P_N$ [13.8±0.8 µmol(CO$_2$) m$^{-2}$ s$^{-1}$] was found in July, 75 d after seedling emergence, when the daily mean air temperature was in the range of 23.4 to 27.8 °C, decreasing by 43 % at 96 d after seedling emergence (beginning of August, 29.8 °C mean maximum air temperature), and by 61 % at 136 d after seedling emergence (20.3 °C mean air temperature) (Fig. 5). $g_s$ exhibited the same trend as $P_N$, showing a maximum [0.16±0.05 mol(H$_2$O) m$^{-2}$ s$^{-1}$] in July and decreasing by 73 % in August (Fig. 5).

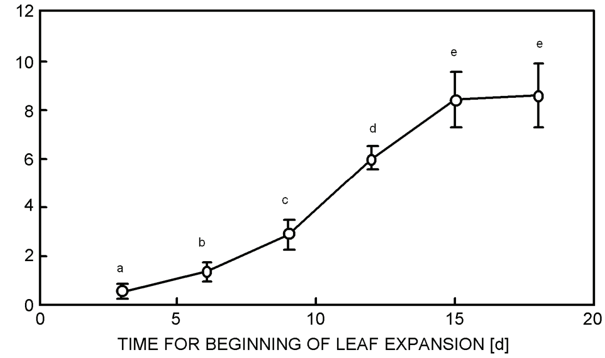

Fig. 3. Leaf area (LA) trend of *B. cernua* during the leaf expansion period. Means with the same letters are not significantly different from each other (ANOVA, $p > 0.05$). Errors represent standard error.

Table 1. Multiple regression analysis using the total dry mass relative growth rate (RGR$_{tot}$) as dependent variable and net assimilation rate (NAR), leaf area ratio (LAR), and leaf mass ratio (LMR) as independent variables. NAR and LMR were the only variables extract by the analysis. Multiple R value, intercept value, un-standardized (ß coefficient), standardized (B coefficient) regression coefficients, and significance level (*p*) of those coefficients are shown. N.S. = not significant.

| Independent variable | | NAR | LMR |
|---|---|---|---|
| Multiple R-value | 0.60 | | |
| Intercept | 0.078 | | |
| ß regression coefficient | | 0.57 | 0.25 |
| B regression coefficient | | 0.0003 | 0.001 |
| *p* | | 0.001 | 0.119 N.S. |

The highest Chl content (33.1±1.8 SPAD units) was found in July (Fig. 5) and a significant ($p < 0.05$) decrease was observed in August (16 %), during the reproductive phase. The lowest Chl content (13.8±1.0 SPAD units) was observed in October, at the beginning of the senescence phase.

There were significant correlations between Chl and $P_N$ ($p < 0.001$), and between $P_N$ and $g_s$ ($p < 0.01$) (Fig. 6).

## Discussion

In the temperate climate, plant growth was largely limited by the length of the growing season (Kikuzawa 1995, Seiwa 1999), and air temperature was one of the most important environmental factors for vegetative activity (Gratani and Crescente 1997, Fenner 1998, Crescente *et al.* 2002, Parmesan 2007). *B. cernua* seedling emergence happened when mean daily air temperature reached 17.0±1.3 °C, in early May; leaf area expansion duration was 15 d. Maximum plant height was reached 90 d after seedling emergence; vegetative activity finished when air temperature was 27.9 °C. Ackerly (1999), Westoby *et al.* (2002), and Seiwa *et al.* (2006) underlined that leaf phenology evolved to maximize leaf photosynthetic assimilation and minimize leaf loss. $P_N$ and Chl content increase continually with plant growth according to Jiang *et al.* (2006), reaching the maximum values of 13.8± 0.8 µmol(CO$_2$) m$^{-2}$ s$^{-1}$ and 33.1±1.8 SPAD units, respectively. Flowering happened at the beginning of August, followed by fruit development until the middle of November. Plant senescence began in early October, documented by 79, 58, and 18 % decrease from maximum values of $P_N$, Chl, and LA, respectively. The time span from seedling emergence to the end of fruiting was 202 d. Fenner (1998) underlined that the timing and duration of bud burst, leaf expansion, maturity, and senescence were crucial to the fitness of plants; the high *B. cernua* LE (7.2±0.4 m$^3$ kg$^{-1}$) underlined its ability to finish the phenological cycle before air temperature could be a limiting factor.

Plant growth was the result of the interaction between environmental factors and biomass allocation parameters determining the potential RGR (McKenna and Shipley 1999, Galmés *et al.* 2005). Because plant size and growth were important determinants of survival and reproduction in nature, inter-specific differences in RGR were of central importance in plant ecology (Shipley 2002). The mean RGR$_{tot}$ averaged over the assimilation period (0.0804±0.0002 kg kg$^{-1}$ d$^{-1}$) was a useful trait to characterise *B. cernua* biomass accumulation (1.58±0.05 g). *B. cernua* RGR$_{tot}$ was in accordance with the value monitored by Almeida-Cortez *et al.* (1999) for the same species, and with McKenna and Shipley (1999) for annual angiosperms.

*B. cernua* RGR$_{tot}$ was correlated with its physiological (NAR) and morphological (LAR) components; nevertheless NAR, *i.e.* a measure of whole plant daily photosynthetic rate, weighted by the rate of change in plant carbon content (Poorter 1989, McKenna and Shipley 1999), was the most important factor driving RGR$_{tot}$, as confirmed by the significant ($p < 0.001$) correlation between the two variables. NAR largely reflected the rates of photosynthetic carbon gain minus carbon released by respiration (Lambers and Poorter 1992), underlining *B. cernua* capability to allocate most of its photosynthate to the above-ground plant portion (81 % of TPB).

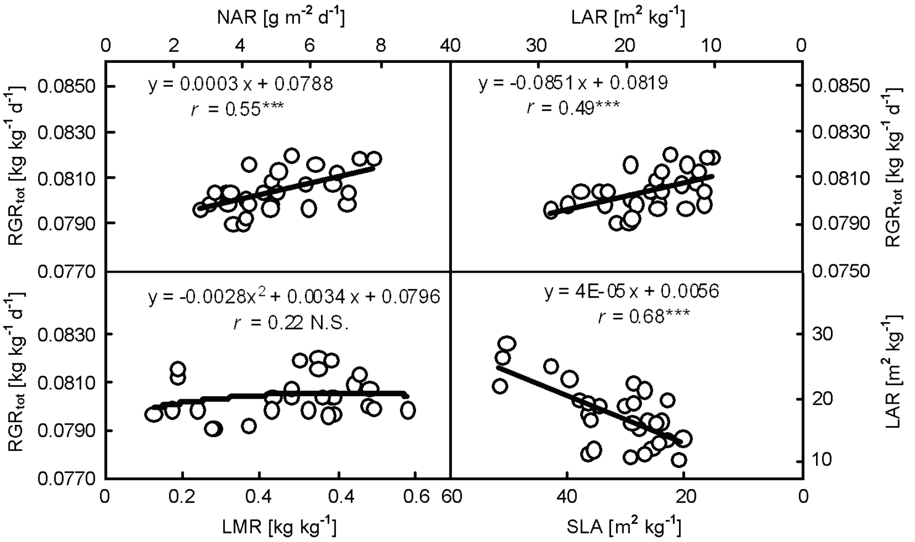

Fig. 4. Results of the simple regression analysis among growth parameters (LAR – leaf area ratio; LMR – leaf mass ratio; NAR – net assimilation rate; RGR$_{tot}$ – total dry mass relative growth rate; SLA – specific leaf area) at the end of the vegetative period. Regression equation and correlation coefficient (*r*) are shown. $^{***}p<0.001$, N.S. = not significant.

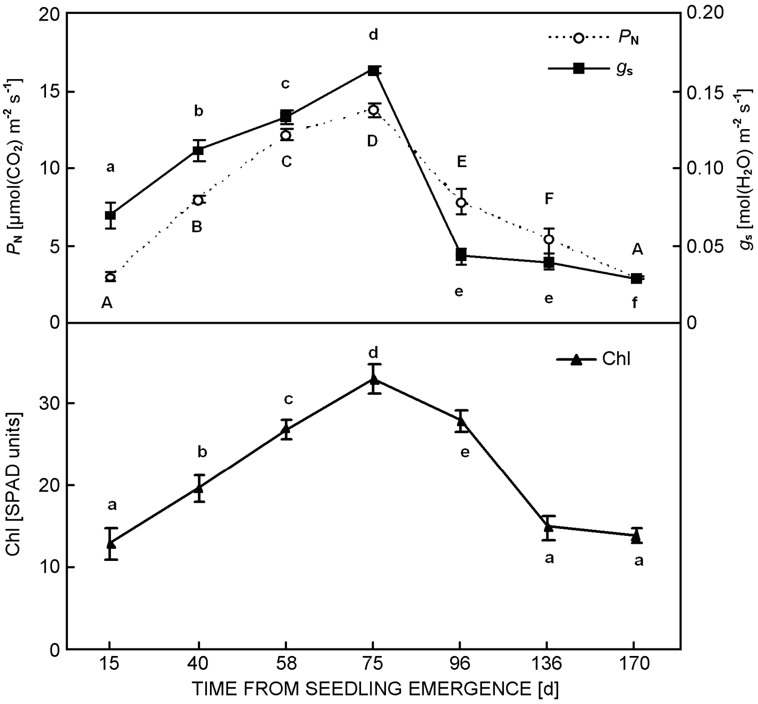

Fig. 5. Net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), and chlorophyll (Chl) content during *B. cernua* phenological cycle. Mean values with the same letters are not significantly different from each other (ANOVA, $p>0.05$). Errors represent standard error.

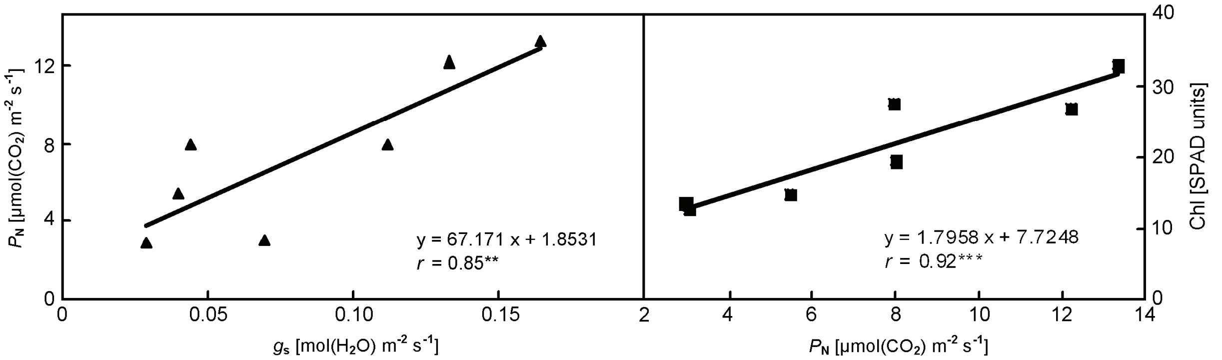

Fig. 6. Simple regression analysis among physiological parameters (Chl – chlorophyll content, $g_s$ – stomatal conductance, $P_N$ – net photosynthetic rate) during *B. cernua* phenological cycle. Regression equation and correlation coefficient (*r*) are shown. $^{**}p<0.01$; $^{***}p<0.001$.

LAR, an index of the plant leafiness (Loveys *et al.* 2002), was highly correlated to SLA, underlining *B. cernua* strategy to invest mostly in LA than in DM and indicating its high resource acquisition capability allowing the highest $P_N$ during the favourable period [12.8±0.5 μmol(CO$_2$) m$^{-2}$ s$^{-1}$, mean value of the period]. $g_s$ showed the same trend as $P_N$ confirmed by the significant ($p<0.01$) correlation between the two variables.

Knowing potential growth of wild species and their resource use capability is aimed at the conservation of native populations of threatened species to support *ex situ* conservation projects through plant reintroduction and habitat restoration.

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